



Cross-site comparison of herbivore impact on nitrogen availability in grasslands: the role of plant nitrogen concentration

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Herbivores may influence the nitrogen (N) recycling rates and consequently increase or decrease the productivity of grasslands. Plant N concentration emerged as a critical parameter to explain herbivore effects from several conceptual models, which predict that herbivores decrease soil N availability when plant N concentration is low whereas they increase it when plant N concentration is high (Hobbs 1996, Ritchie et al. 1998, Pastor et al. 2006). However, a broader cross-site comparison among published studies to test these predictions is hampered by the different methodologies used to measure soil N availability or a proxy thereof, and a lack of measurements of plant N concentration. Therefore it remains unclear whether these model predictions are generally valid across a range of grasslands.

We tested whether there is a relationship between plant N concentration and herbivore impact on soil N availability (measured with resin bags) with a study of replicate 6–8 year old exclosures (with an unfenced control) of vertebrate herbivores (>1 kg) established at each of seven grassland sites in North America and Europe. Contrary to model predictions, we found a negative relationship between the effect of herbivores on resin bag soil N availability and plant N concentration. Our study confirms the importance of plant N concentration as a predictor of herbivore effect on soil N availability across grasslands, but contradicts the models. A possible explanation may be that the results represent a transient situation as the exclosures were relatively young whereas the models may refer to an equilibrium state. Simultaneous measurements of both plant N concentration and herbivore effect on soil N availability from more grassland sites, preferably with contrasting plant N concentrations and including exclosures of different ages, should resolve the contrast between model predictions and our field measurements.

Herbivorous mammals can have profound effects on the sustainability and productivity of grasslands through their effects on nutrient cycling (Milchunas and Lauenroth 1993, McNaughton et al. 1997, Frank et al. 2000). In many grasslands plant productivity is limited by nutrients, especially N (Schlesinger 1991, Vitousek and Howarth 1991). The impact of herbivores on soil N cycling can range from being positive to negative. Field tests conducted in grasslands grazed or browsed by a diverse array of local vertebrate herbivores have accumulated evidence for both outcomes: herbivores have been found to accelerate N cycling (Holland and Detling 1990, McNaughton et al. 1997, Frank and Groffman 1998) and also to decelerate N cycling (Ritchie et al. 1998, Van Wijnen et al. 1999, Bakker et al. 2004), sometimes even both within the same study site (Augustine et al. 2003).

Several conceptual models have been developed to explain these contrasting results and identify under which conditions herbivores may increase or decrease soil N availability (Hobbs 1996, Ritchie et al. 1998, Wardle et al. 2004, Pastor et al. 2006). In these models plant nitrogen concentration has been identified as a key parameter, which

may explain an increase or decrease in N availability under grazing or browsing. Plant N concentration may modify both the direct and indirect effects of herbivores on N cycling, which has also been termed the fast and slow cycle of nutrient return (Ritchie et al. 1998, Belovsky and Slade 2000, Bakker et al. 2004).

Direct effects occur when herbivores consume plants and return plant nutrients through the input of waste products to the soil, which is called the fast cycle of nutrient return. The return of nutrients through excreta may result in an acceleration of N cycling (Bazely and Jefferies 1985, Frank et al. 1994, McNaughton et al. 1997). With increasing plant N concentration herbivores increase the proportion of nitrogen excreted as urea, which is more readily available to plants than many of the N compounds in feces (Hobbs 1996, Pastor et al. 2006). Therefore, herbivores are predicted to increase soil N availability with increasing plant N concentration through the direct effects of nutrient excretion (Hobbs 1996).

The rest of the nutrients are returned through plant litter, or the slow cycle (Knops et al. 2002). Both plant N

concentration and plant palatability are positively related to decomposition rates in grassland plants (Grime et al. 1996, Diaz et al. 2004). Herbivores can modify the slow cycle when they alter the quality (plant tissue N concentration) of N input through litter (Bardgett and Wardle 2003, Semmartin et al. 2004, Garibaldi et al. 2007), resulting in indirect herbivore effects on soil N availability. Whether herbivores increase or decrease N availability depends on herbivore selectivity and plant recovery after grazing (Ritchie et al. 1998, Wardle et al. 2004). When most plants contain a high N concentration and low defenses, herbivores may graze unselectively and increase litter quality when grazed plants re-grow and the fresh re-growth ultimately results in more nutrient rich and decomposable plant litter (Olofsson and Oksanen 2002), leading to an increase in N cycling (Belovsky and Slade 2000). Alternatively when average plant quality is low due to low N concentrations or high defenses, herbivores selectively feed on poorly defended N rich plant species, reducing average plant litter quality when these N rich plants can not recover after grazing, resulting in slow decomposition and thus reduced N cycling (Pastor et al. 1993, Ritchie et al. 1998, Sirotiak and Huntly 2000). The increase of N cycling through indirect herbivore effects on plant litter quality are thought to be associated with fertile habitats, whereas a decreasing effect is associated with unfertile habitats (Wardle et al. 2004, Pastor et al. 2006).

Summarizing, current conceptual models predict that herbivores decrease soil N availability when plant N concentration is low whereas they increase it when plant N concentration is high both through direct and indirect effects of herbivores on N cycling (Hobbs 1996, Ritchie et al. 1998, Pastor et al. 2006). However, whereas plant N concentration is recognized as an important parameter, it is frequently not measured or not reported in published studies. Additionally, N availability or a proxy thereof can be measured in many different ways which makes a comparison among studies difficult. Therefore it remains unclear whether these model predictions are generally valid across a range of grasslands.

In this study we test whether herbivore impact on soil N availability changes with plant N concentration in a cross-site comparison. We performed a large-scale field experiment for which we built replicate exclosures (with an unfenced control) of vertebrate herbivores within seven grassland sites in North America and Europe. We sampled soil N availability and plant tissue N concentration in a standardized manner in our sites after six to eight years of herbivore exclusion.

Material and methods

Experimental design

We selected seven grassland sites in North America and Europe which represented a ten-fold gradient of above ground plant production, strong differences in total soil nitrogen concentration and rainfall and each supported a varied assemblage of mammalian herbivores (Bakker et al. 2006, Table 1). Detailed descriptions of each site can be found in Supplementary material Appendix 1. We designed

our experiment to maximize the number of sites in our study resulting in a limited amount of replicates within each site to provide a robust test of the interrelationships among our target parameters. Between 1994 and 1996 we established similar sets of exclosures and unfenced controls on 400–900 m² plots at a minimum of three locations (blocks) at each of the seven sites. At each set of plots, one plot was fenced to exclude all herbivores (> 1 kg), and one plot remained an unfenced control. At the Montane prairie, pocket gophers *Thomomys talpoides* were excluded because the poultry netting prevented them from tunneling beneath the snow-pack into plots in winter. Small rodents (>0.001 kg) were excluded at the shortgrass steppe. At all other sites, small rodents (mice, voles, pocket gophers) could potentially enter all exclosures, as could all insects.

Standing crop, productivity and grazing intensity

In 2002, six to eight years after establishment of the exclosures, aboveground standing crop was sampled in each grazing treatment at peak biomass in late June – mid August by clipping an area of 0.25 m² (0.25 × 1 m). The litter layer was collected separately. Litter was defined as the laying dead material and was collected from the same area after clipping and removal of the standing crop. Standing crop biomass samples include both standing live and dead material and were sorted into grasses, forbs, legumes and woody plants (including woody forbs). Aboveground annual net primary production (ANPP) was estimated during the growing season in 2002 using temporary cages (1.0 × 1.5 m wide and 1.0 m high, mesh 2.5 cm) that were placed in the grazed treatment in early spring. Due to logistic reasons of working on multiple sites we did not move the cages throughout the growing season. Our measurement of production in the grazed treatments is thus a conservative estimate of plant production. The cages were sampled at peak biomass following the same methods as for standing crop sampling. For shrubs only newly developed shoots (e.g. current-year growth) were collected to estimate annual production. We estimated the amount of biomass removed through grazing by comparing the production in the temporary cage with the standing crop biomass in the quadrat open to grazing (i.e. removal over the growing season). As we did not move the cages and harvested at peak standing crop, the percent biomass removal through grazing is an estimate. For the shortgrass steppe 2002 was an extreme drought year, yielding very low plant production. Therefore data for 2001, which were collected in a similar manner, were used for this site. The number of quadrats clipped within each grazing treatment and block varied from 2 to 10 among sites according to the local monitor program. All data were pooled within a grazing treatment in each block before further calculation.

C and N concentration of plants and soil and soil bulk density

We used the total soil N concentration (g N kg⁻¹ dry soil) and soil C:N ratio as an index of soil fertility at each site (Table 1). Separate soil samples were taken in the strips where above ground biomass was clipped, immediately after

Table 1. Characteristics of the study sites. All values represent means (± 1 SE). Precipitation is given as long-term average, ranging from 18–97 years of measurements (see site descriptions). The precipitation from September 2001–September 2002, the full year previous to biomass sampling in 2002, is displayed between brackets. The percentage biomass removed indicates the grazing intensity at each site. Proportion dissimilarity indicates the difference in plant species composition between grazed and ungrazed plots, using Whittaker’s index of dissimilarity. Full site descriptions, including sources for climatic data, can be found in Supplementary material Appendix 1.

Site	Habitat type	Geographic position	Total soil N (g N kg ⁻¹ dry soil) ungrazed	Total soil N (g N kg ⁻¹ dry soil) grazed	C:N ratio in soils ungrazed	C:N ratio in soils grazed	Precipitation (mm year ⁻¹)	Above ground ANPP (g m ⁻²) 2002	% Biomass removed	Proportion dissimilarity in species composition	No. of replicates	Mammalian herbivores >1 kg
Deseret Sage (Utah US)	Sagebrush steppe	41°11'N 111°5'W	1.51 \pm 0.19	1.57 \pm 0.06	14.5 \pm 2.0	10.7 \pm 0.7	305 (261)	47 \pm 5	36 \pm 3	0.23 \pm 0.03	3	cattle, elk, mule deer, pronghorn rabbits, prairie dogs
Shortgrass Steppe (Colorado US)	Shortgrass steppe	40°49'N 104°46'W	1.43 \pm 0.29	1.37 \pm 0.26	10.7 \pm 0.2	10.3 \pm 0.4	322 (160)	97 \pm 9 ^a	11 \pm 5 ^b	0.26 \pm 0.11	3	cattle, pronghorn, rabbits
Deseret Low (Utah US)	Bunchgrass steppe	41°21' N 111°5'W	1.14 \pm 0.24	1.07 \pm 0.07	9.8 \pm 0.1	17.3 \pm 5.8	305 (261)	125 \pm 17	37 \pm 5	0.26 \pm 0.03	3	cattle, elk, mule deer, pronghorn, rabbits, prairie dogs
Deseret High (Utah US)	Montane grassland	41°25'N 111°25'W	1.51 \pm 0.30	1.52 \pm 0.32	12.1 \pm 0.4	12.2 \pm 0.6	635 (532)	203 \pm 30	30 \pm 15	0.44 \pm 0.07	3	sheep, elk, rabbits
Cedar Creek (Minnesota US)	Oak savanna	45°5'N 93°10'W	0.84 \pm 0.06	1.03 \pm 0.12	17.9 \pm 0.3	16.7 \pm 0.4	825 (840)	222 \pm 19	15 \pm 7	0.48 \pm 0.05	6	white-tailed deer
Konza (Kansas US)	Tallgrass prairie	39°08'N 96°62'E	3.03 \pm 0.18	3.01 \pm 0.31	13.5 \pm 0.2	13.1 \pm 0.9	835 (676)	302 \pm 37	49 \pm 5	0.45 \pm 0.04	3	bison, white-tailed deer
Junner Koeland (Netherlands)	Floodplain grassland	52°32'N 6°29'E	2.76 \pm 0.34	3.28 \pm 0.66	11.3 \pm 0.2	11.9 \pm 0.3	758 (835)	489 \pm 19	20 \pm 9	0.58 \pm 0.10	5	cattle, rabbits

^aMeasured in 2001, due to extreme drought at this site in 2002.

^bGrazing intensity is targeted at 40% of primary production, but cages underestimate consumption due to compensatory re-growth in shrub-grassland short grass communities (Milchunas et al. 2004, Morgan et al. 2004).

clipping. In each treatment samples (1–3 cores of 5.5–8.5 cm diameter) from the upper 10 cm soil were collected. Plant and soil samples were dried for 48 h at 70°C and weighed to determine biomass dry weight for plants and bulk density for the soil samples. Samples were ground and analyzed for C and N concentration through dry combustion on a C/N analyzer. Samples were pooled and averaged within each block before data analysis.

Climatic data

We collected precipitation data from existing sources for each site, which can be found in the site descriptions in Supplementary material Appendix 1. We present both long-term data and the precipitation from September 2001–September 2002, the full year previous to biomass sampling in 2002.

Measurements of nitrogen availability

Nitrogen availability was measured with resin bags (Binkley and Matson 1983, DiStefano and Gholz 1986). Resin bags were composed of undyed nylon hose filled with 4 g anion (Dowex 1-X8) and 4 g cation (Dowex 50W-X8) resins, both 20–50 mesh. The bags were placed in the soil at a depth of 10 cm. At all sites the bags were buried in May 2001 and recovered in October 2001. At the shortgrass steppe thin colored wire was tied to the bags and a couple inches of the wire left protruding from the soil surface to aid in locating, which resulted in cattle ripping out most of the bags. Therefore a new batch of resin bags was buried in the same period in 2002 without the wires, which was successful. These data from 2002 were used for further analysis for the shortgrass steppe. In each grazing treatment, ten bags were placed in two rows of five bags each, approximately 2 m apart. Retrieved bags were thoroughly rinsed in distilled water, extracted for 12 h in 1 M KCl and subsequently analyzed for NO_3^- and NH_4^+ content on a continuous flow analyzer. We used the accumulated N on the resin bags as an index of N availability.

Not all ten replicates could be used for the analysis since some bags could not be found in the field and others were damaged and subsequently discarded for analysis. At least five and on average 9.1 resin bags were analyzed per grazing treatment per block. All values were pooled within a block within a grazing treatment before further calculation; therefore the number of blocks per site represents the number of replicates per site. Unfortunately the resin bags from the grazed treatment in one block at the shortgrass steppe and the montane prairie were all lost.

Species composition of the vegetation

At peak standing crop at each site (from late June–mid-August 2001) we recorded plant species composition. Species cover was estimated visually at each site as the percentage of surface area covered by each plant species (with a maximum of 100% total cover). The amount of plots sampled per block and treatment and size of the plots varied per site according to the local monitoring program:

45 plots of 0.2×0.5 m at the shortgrass steppe, 20 plots of 0.5×1.0 m at the tallgrass prairie, the montane grassland, bunchgrass steppe and sagebrush steppe, ten plots of 0.5×1.0 m at the oak savanna and two plots of 2.0×2.0 m at the floodplain grassland. Data of all subplots were pooled per block per grazing treatment before further calculation. Shifts in plant community composition under grazing are expressed as proportion dissimilarity using Whittaker's index of dissimilarity (Whittaker 1952).

Data analysis

First we present soil resin-available N, soil bulk density, standing crop, litter biomass, plant tissue N and C:N ratio (from standing crop) and litter N concentration and C:N ratio in the presence and absence of herbivores for each site. The effects of herbivores were tested with an Anova with site and grazing treatment as fixed factors. Data were log-transformed to improve homogeneity of variances. We used a type III model for most tests and a type VI model to analyze a design with missing cells (StatSoft Inc. 2008), which was the case for the data on soil resin-N availability. We tested the relationship between plant C:N ratio and plant %N in standing crop and litter with a Pearson correlation, using all data available per block, site and grazing treatment, resulting in $n=52$. We tested the relationship between the abiotic variables soil N concentration and rainfall over the previous year and resin-available N, plant production and plant N concentration with multiple regressions. All regression analyses that we performed use the average of the measured parameter in each site as a data point, resulting in $n=7$. We weighted the data by the number of replicates for each site. All data used in the regression analyses were normally distributed and therefore no transformations were applied. Subsequently, we tested the relationship between standing crop N concentration and C:N ratio in the ungrazed and grazed plots and herbivore effect on resin-available N, using regression analysis. We calculated herbivore effect on resin-available N by subtracting resin-available N in the enclosures from resin-available N in the presence of herbivores and divided the difference by resin-available N in the enclosures for each block within a site. The effect of herbivores on resin-available N is therefore the proportional effect of herbivores, which we expressed as a percentage. In a multiple regression analysis we analyzed the effect of total soil N, rainfall and grazing intensity together with plant N concentration as these parameters may have affected the impact of herbivores on resin-available N at our sites. Statistical analyses were done with Statistica 8.0.

Results

Resin-available N, total soil N concentration and soil bulk density

The amount of resin-available N differed strongly among sites (Fig. 1A, Table 2). Soil N concentration differed more than three-fold across sites (Table 1). Resin-available N was not significantly related to total soil N concentration

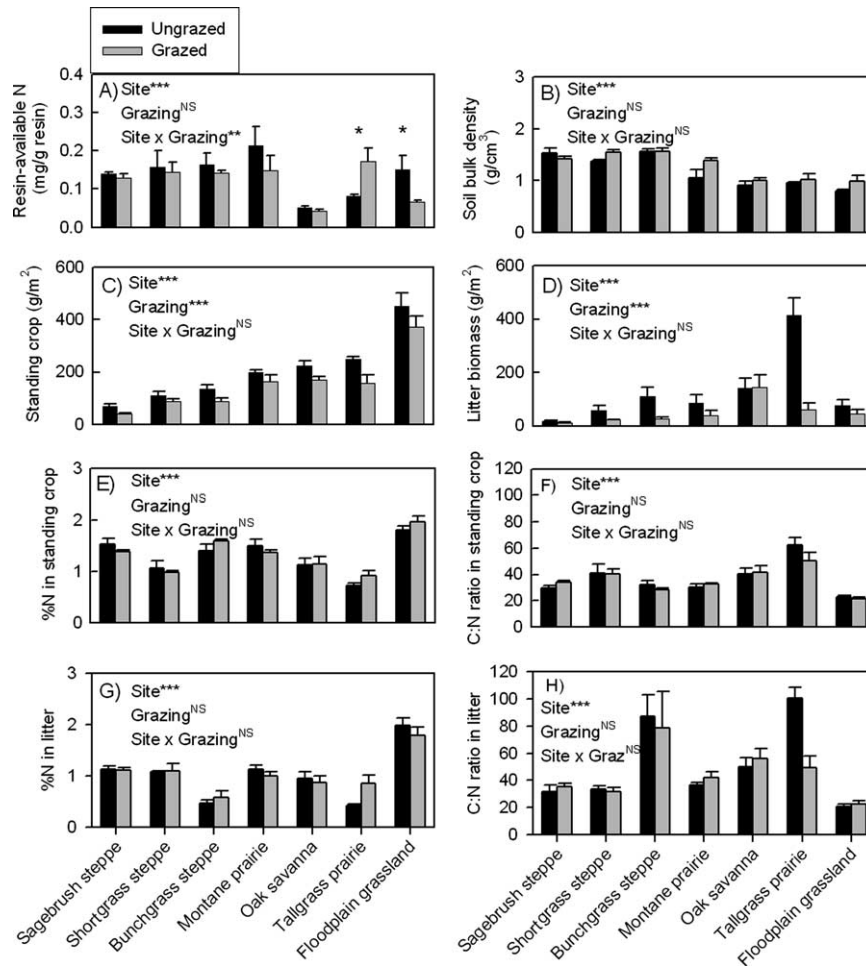


Figure 1. Total amount of resin-available N (A), soil bulk density (B), standing crop (C), litter biomass (D) and standing crop N concentration (E) and C:N ratio (F) and litter N concentration (G) and C:N ratio (H) in the ungrazed and grazed plots at each of the seven sites. Data are means \pm SE. Results of a two-way Anova are given in each panel, see Table 2 for corresponding F-values. ^{NS} $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

or rainfall in the previous year (for ungrazed: $R^2 = 0.39$, $p = 0.37$, for grazed: $R^2 = 0.65$, $p = 0.12$, both multiple regressions). The impact of herbivores on resin-available N depended on the site studied (significant interaction site \times grazing, Table 2). Resin-N was significantly affected by grazing treatment in both the tallgrass prairie and the floodplain grassland, but not in the other sites. At the tallgrass prairie herbivores significantly increased resin-available N, whereas they significantly decreased resin-available N in the floodplain grassland (Fig. 1A). Soil bulk density differed significantly among sites (Fig. 1B, Table 2). Soil bulk density tended to be higher in the presence of herbivores in most sites, but this was not significant (Table 2, $p = 0.096$). There was no significant relationship between the effect of herbivores on soil bulk density and herbivore effect on resin-available N ($R^2 = 0.12$, $p = 0.44$).

Above ground plant biomass and grazing intensity

Above ground plant production varied about ten-fold among the sites (Table 1). Plant production was positively related to rainfall over the previous year ($p = 0.025$) and to total soil N concentration in the grazed plots ($p = 0.035$;

whole model $R^2 = 0.90$, $p = 0.010$, multiple regression). Above ground standing crop was significantly reduced under grazing (Fig. 1C, Table 2) and the exclusion of herbivores resulted in a significant accumulation of litter (Fig. 1D). Grazing intensity, expressed as the percentage of biomass removed by herbivores, ranged from 11% to 49% across sites (Table 1).

Plant N concentration and C:N ratio

The tissue N concentration and C:N ratio in standing crop and litter differed among sites, but were not significantly affected by grazing (Fig. 1E–H, Table 2). Plant tissue N concentration in standing crop was strongly correlated with the plant C:N ratio, as expected (Pearson correlation: in standing crop: $r = -0.93$, $p < 0.001$; in litter: $r = -0.80$, $p < 0.001$, both $n = 52$). The N concentrations in standing crop and litter were significantly positively correlated (Pearson correlation: $r = 0.71$, $p < 0.001$, $n = 52$). Because of these strong correlations, we focus on plant tissue N concentration in standing crop as our plant N parameter. For the main tests of the relationship between herbivore effect on resin-N availability in the soil we additionally use

Table 2. Results from a two-way Anova on the effect of grassland site and grazing treatment on resin-available N, soil bulk density, plant standing crop, litter biomass, standing crop and litter N concentration and C:N ratio. The degrees of freedom for the resin-N measurements are smaller due to two missing data points (Methods). NS: $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

	DF	Resin-available N (mg g^{-1} resin)	DF	Soil bulk density (g cm^{-3})	Standing crop (g m^{-2})	Litter biomass (g m^{-2})	Plant tissue N concentration (%)	Litter N concentration (%)	Plant C:N ratio	Litter C:N ratio
Site	6,36	F = 18.96***	6,38	F = 25.70***	F = 63.83***	F = 5.37***	F = 18.87***	F = 27.76***	F = 17.99***	F = 25.23***
Grazing	1,36	F = 1.56 ^{NS}	1,38	F = 2.91 ^{NS}	F = 26.66***	F = 14.23***	F = 0.15 ^{NS}	F = 2.40 ^{NS}	F = 0.13 ^{NS}	F = 1.65 ^{NS}
Site \times Grazing	6,36	F = 3.42**	6,38	F = 0.31 ^{NS}	F = 0.57 ^{NS}	F = 0.34 ^{NS}	F = 0.62 ^{NS}	F = 2.14 ^{NS}	F = 0.63 ^{NS}	F = 2.15 ^{NS}

the C:N ratio in standing crop. Plant tissue N concentration was not significantly related to soil N concentration or rainfall (multiple regression, whole model: $R^2 = 0.025$, $p = 0.95$ for ungrazed plots; $R^2 = 0.018$, $p = 0.68$ for grazed plots).

Species composition of the vegetation

The shift in plant community composition upon enclosure of herbivores ranged from 0.23 to 0.58 proportional dissimilarity between grazed and ungrazed plots across sites (Table 1). Dissimilarity increased significantly with plant production ($R^2 = 0.32$, $p = 0.003$). The study sites were dominated by graminoids (mainly grasses) apart from the sagebrush steppe, which was dominated by the woody forb big sagebrush *Artemisia tridentata* (see Fig. S1 and site descriptions in Supplementary material Appendix 1).

Relationship between plant N concentration and C:N ratio in standing crop and herbivore impact on soil resin-available N

The herbivore effect on resin-available N was significantly negatively related to plant N concentration from ungrazed plots (Fig. 2A; $B = -0.85$, $R^2 = 0.73$, $p = 0.014$) and just not significantly to plant N concentration from grazed plots (Fig. 2B; $B = -0.75$, $R^2 = 0.56$, $p = 0.053$). The relationship between plant C:N ratio in standing crop and herbivore effect on resin-available N was significantly positive both for the C:N ratio in plants from ungrazed plots (Fig. 2C; $B = 0.93$, $R^2 = 0.86$, $p = 0.003$) and grazed plots (Fig. 2D; $B = 0.80$, $R^2 = 0.64$, $p = 0.031$). The relationship between plant nutrient concentration and herbivore effect on soil N availability that we found was a little bit stronger when using the C:N ratio in standing crop, but as current models use plant N concentration we also focus on this parameter.

In a multiple regression analysis we tested which parameters were related to herbivore impact on resin-available N when total soil N concentration (from ungrazed plots), rainfall in the year previous to measurements, plant N concentration and grazing intensity were considered collectively. We analyzed these relationships separately using total soil N concentration and plant N concentration in standing crop in the ungrazed and grazed plots. The four predictors were not significantly correlated with each other ($p = 0.38$ or larger for parameters from ungrazed plots, $p = 0.24$ or larger for grazed plots). Collectively, rainfall, total soil N concentration and plant N concentration in standing crop of ungrazed plots and grazing intensity significantly affected herbivore impact on resin-available N (total model $R^2 = 0.99$, $p = 0.008$). Plant N concentration in standing crop in the ungrazed plots was the most significant parameter, showing a negative relationship with herbivore effect on soil resin-N availability ($B = -0.87$, $p = 0.003$) and total soil N concentration in ungrazed plots ($B = 0.45$, $p = 0.015$) and rainfall in the previous year ($B = -0.23$, $p = 0.047$) also contributed significantly to the model, whereas the effect of grazing intensity was not significant ($B = 0.12$, $p = 0.17$). Using data from the grazed plots resulted in a model that was not significant (total model

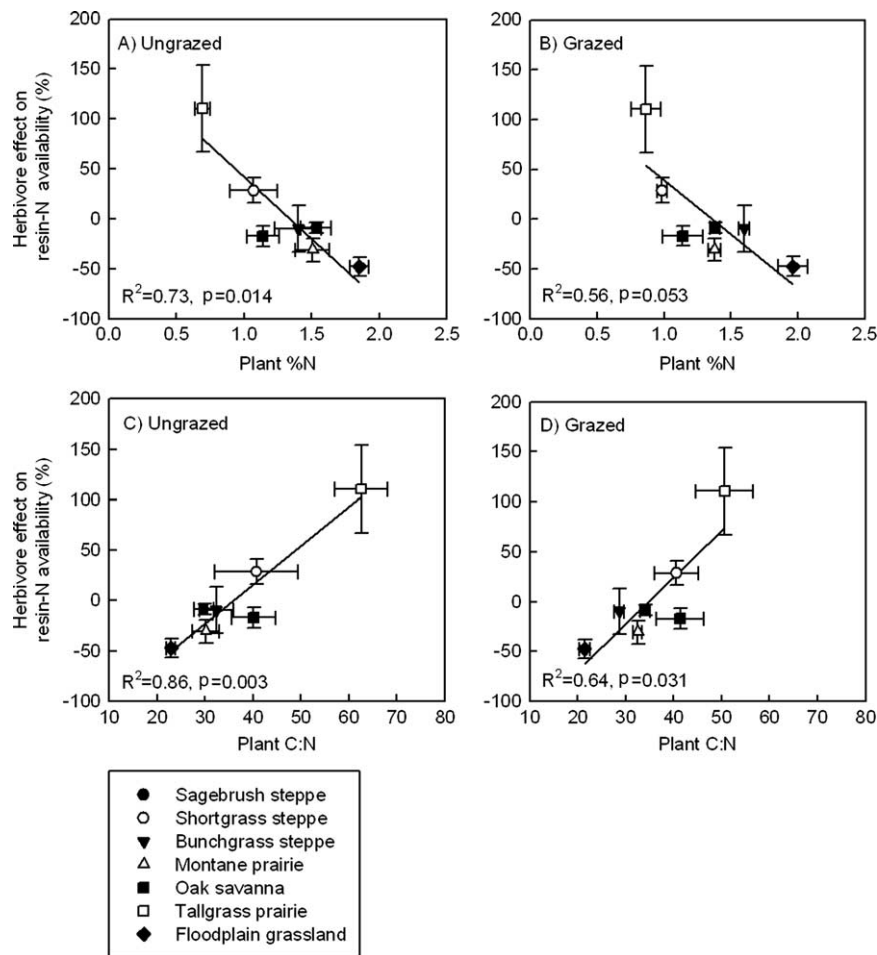


Figure 2. The relationship between plant tissue N concentration and C:N ratio (in standing crop) in ungrazed (A, C) and grazed plots (B, D) and herbivore impact on soil resin-available N. Herbivore effect is given as the percentage increase or decrease of soil resin-N in grazed plots relative to ungrazed plots. The relationship is tested with linear regression analysis, with $n = 7$ sites and corrected for the amount of replicates per site. Data are means \pm SE.

$R^2 = 0.93$, $p = 0.13$). Still standing crop plant N concentration in the grazed plots was the best predictor of herbivore impact on resin-available N concentration ($B = -0.90$, $p = 0.049$), but the other parameters total soil N concentration in grazed plots ($B = 0.52$, $p = 0.17$), rainfall ($B = -0.19$, $p = 0.48$) and grazing intensity ($B = 0.27$, $p = 0.33$) were not significant. The results of the analyses using plant standing crop C:N ratio were qualitatively similar (e.g. the same factors were significant) to the analyses using plant standing crop N concentration for both the ungrazed and grazed plots (data not shown).

Discussion

We found a relationship across all sites between plant N concentration and herbivore effect on soil N availability as predicted from theory. However, interestingly the relationship that we found was negative which is contrary to model predictions. The relationship between plant N concentration and herbivore effect on soil N availability is illustrated by the two sites with the highest soil N concentration, most rainfall and highest plant production, the tallgrass prairie

and floodplain grassland. Despite these similarities herbivores had strong but contrasting effects on soil N availability at these sites, which also contained plants of contrasting quality. Previous independent studies of N mineralization at both of these sites using a different methodology (in situ soil cores) showed a similar outcome: herbivores enhanced N mineralization at the tallgrass prairie (Knapp et al. 1999, Johnson and Matchett 2001), whereas they decreased N mineralization at the floodplain grassland (Bakker et al. 2004). Our results are therefore robust and not dependent on the methodology of using resin bags. These two sites with significant, but contrasting herbivore effects on soil N availability are at the extremes of the regression analysis between plant N concentration and herbivore effect on soil N availability and thus have a strong effect on the regression line. When we remove either of these sites from the analysis, the relationship is still significant when the floodplain grassland is removed ($p = 0.037$, $n = 6$) and shows a trend when the tallgrass prairie is left out of the analysis ($p = 0.068$, $n = 6$; both tested with plant N concentration in ungrazed plots) indicating that the relationship does not fully depend on the inclusion of both sites in the analysis.

At the site level, herbivore impact on soil N availability was not significant at the sites with a lower plant

production. This may be due to the small sample sizes per site, as our study was designed for a cross-site comparison favoring more study sites instead of more replicates per site. However, this also applies to the most productive sites where we did find a significant difference. The lack of difference in soil resin-N availability between the grazing treatments coincided with lower plant production and a limited shift in plant species composition when herbivores were excluded, as dissimilarity in community composition increased with plant production across sites. This generally limited response of both plant community composition and resin-N availability to herbivore exclusion may be the consequence of plant allocation patterns: plants at the less productive sites invest most of their biomass belowground to compete for water and nutrients (Craine et al. 2002), potentially limiting the quantitative effect of herbivores (Milchunas and Lauenroth 1993, Bakker et al. 2006). The changes in plant communities and soil N availability may also be slower in sites with a lower plant production. The exclosures were 6–8 years in place during our study and perhaps there were no differences between grazed and ungrazed plots yet. Herbivore effects on soil N availability may change over time (Pastor et al. 1993, Sirotnak and Huntly 2000, Olofsson et al. 2007). Perhaps the results of our study may represent a transient situation whereas the models may refer to an equilibrium state.

Plant N concentration and herbivore impact on soil N availability

We found a stronger relationship when we used plant N concentrations from the ungrazed plots than from the grazed plots. The plant N concentration from the ungrazed plots may better reflect the nutrient concentration of the plants in response to the abiotic conditions. The effect of herbivores may be best indicated by comparison with the vegetation that develops in their absence. The negative relationship between plant N concentration and herbivore impact on N availability that we found is in contrast with the conceptual models which predict a positive relationship. This may be the result of the relatively short duration of our study, which we can not exclude. Below we discuss whether there are any other mechanisms that may explain our results.

The impact of herbivores on N mineralization depends on the balance between the slow and fast cycles of nutrient return (Belovsky and Slade 2000). Herbivore consumption increases nutrient return through the fast cycle which is generally thought to increase N mineralization rates, whereas herbivore impact on the slow cycle can lead to an increase or decrease in N mineralization depending on the quantity and quality of nutrient input through litter. This means that herbivores can increase N availability through the slow and fast cycle simultaneously, but also decrease N availability through the slow cycle while increasing it through the fast cycle. Herbivores can influence N cycling through effects on litter biomass, as even if litter quality is improved, a reduction in litter biomass can greatly reduce N inputs through the slow, litter decomposition pathway (Pastor et al. 1993). When herbivores have the additional net effect of removing N by weight gain (Augustine et al.

2003), spatially concentrating N across the landscape (Frank et al. 2000, Augustine and Frank 2001, Bakker et al. 2004), or increasing N losses through leaching and ammonia volatilization (Ruess and McNaughton 1988), the net input of recycled N from herbivores through the fast cycle of waste products may not be sufficient to counteract the loss in inputs through the slow litter decomposition cycle. This has been suggested as an explanation for a reduction of N mineralization in the presence of rabbits which prevented the formation of large quantities of litter which had a stimulating effect on N mineralization inside exclosures (Olofsson et al. 2007). This effect would be most likely when litter N is high and contains easily decomposable N. Such a pattern was implicitly theoretically predicted by De Mazancourt et al. (1998). They show in a model analysis that herbivores increase soil nutrient availability and subsequently primary production when the loss of nutrients through the herbivore pathway is sufficiently smaller than the losses through the plant litter pathway (De Mazancourt et al. 1998). Consequently, herbivores decrease soil nutrient availability when the losses of nutrients are larger through the consumption pathway than through plant litter decomposition.

In our study plant tissue N concentration in standing crop and litter N concentrations were strongly correlated. The N concentration of plant litter plays an important role in whether N is mineralized or immobilized during decomposition (Bosatta and Staaf 1982). Plants with litter of high N concentration can enhance N mineralization rates (Wedin and Tilman 1990, Van der Krift and Berendse 2001) although this is not necessarily a direct relationship (Knops et al. 2002). Knops et al. (2002) proposed that there is a threshold of a concentration of 0.75% N in plant material below which immobilization is the dominant process whereas above it mineralization dominates. At the tallgrass prairie, where litter N concentration was less than 0.75% in the absence of herbivores, bison grazing increased litter quality strongly above this threshold, which might explain part of the strong increase in soil N availability under grazing at this site. Bison removed large quantities of poor quality litter, returned nutrients through excreta and litter quality improved under grazing. In this case the fast and slow cycle can enhance each other, resulting in a positive impact of herbivores on N availability.

However, when litter quality is already high in the absence of herbivores, it is questionable how much herbivores might be able to improve it. Grazing can enhance re-growth and improve litter quality (Olofsson and Oksanen 2002) in fast growing plants with a high N concentration (such as some grasses), but not all plants which have a high N concentration are grazing tolerant, for example legumes, but also forb species. Herbivores may decrease litter quality through removing nitrogen-rich grazing intolerant species, such as legumes, which in turn leads to a decline in N mineralization under grazing (Ritchie et al. 1998, Sirotnak and Huntly 2000). This might potentially offer an explanation of the observed reduction in N mineralization under grazing at the floodplain grassland, where grazers returned nutrients through excreta but at the expense of preventing the formation of high quality litter, which could be an example of inhibition between the fast and slow cycle. At the floodplain grassland,

standing crop is high, but litter biomass is low, indicating rapid turnover of litter.

Herbivore effect on N availability in relation to other factors

Although our study focused on the role of plant N concentration, we did measure other factors that are known to potentially affect herbivore impact on N availability, including soil N concentration, rainfall (Stark et al. 2002, Augustine and McNaughton 2006), grazing intensity (Shariff et al. 1994, McNaughton et al. 1997) and soil bulk density (Van Wijnen et al. 1999). Additionally to the negative relationship between herbivore effect on soil N availability and plant N concentration, total soil N concentration was positively related to herbivore effect on N availability in a multiple regression analysis, whereas the negative relationship with rainfall was just significant and with grazing intensity was not significant. Generally, a positive relationship between habitat fertility and herbivore effect on N availability is expected, as plant re-growth after grazing is better and average plant N concentration can be higher with increasing habitat fertility (Van der Wal et al. 2003, Wardle et al. 2004, Augustine and McNaughton 2006). However, this is not always the case, as plant N concentration depends positively on soil N concentration and negatively on rainfall (Olf et al. 2002) and may thus not be directly related to habitat fertility. In our study there was no significant relationship between plant N concentration and soil N concentration or rainfall. Plant N concentration was more important as predictor of herbivore effect on N availability than soil N concentration or rainfall which were only significant when plant N concentration was included in the model. Even though they had no general significant effect, the soil bulk density and grazing intensity can still have played a role as different parameters could be causing an effect at individual sites, while being unimportant at other sites, which we can not conclude from our data.

Conclusions

We found a negative relationship between plant N concentration and herbivore impact on soil resin-N availability. Therefore our study confirms the importance of plant N concentration as a predictor of herbivore effect on soil N availability. However, the relationship that we found is opposite of what current models predict. Simultaneous measurements of both plant N concentration and herbivore effect on soil N availability from more grassland sites, preferably with contrasting plant N concentrations, should resolve the contrast between model predictions and our field measurements.

Acknowledgements – We thank Heidi Hillhouse for collecting data at Cedar Creek and Konza. We thank the National Forest Service for permission to work on Junner Koeland and Julia Stahl and Marieke Hartevelde for field assistance there. We thank Cathleen McFadden for performing the chemical analyses. This study was supported with NSF-grant DEB95-27585 and DEB00-87255 for

cross-site studies. Additional support at the Shortgrass steppe site was provided through LTER DEB96-32852 and DEB02-17631, the help of Mark Lindquist, Nicole Kaplan, Judy Hendryx, the LTER field crew, and the USDA-ARS that maintains the long-term grazing treatment pastures. We thank John Pastor for comments on a previous version of this paper. This is publication 4561 of the NIOO-KNAW Netherlands Inst. of Ecology.

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Supplementary material (available online as Appendix O17199 at <www.oikos.ekol.lu.se/appendix>). Appendix 1.