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Small mammal activity alters plant community composition and microbial activity in an old-field ecosystem

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Abstract. Herbivores modify their environment by consuming plant biomass and redistributing materials across the landscape. While small mammalian herbivores, such as rodents, are typically inconspicuous, their impacts on plant community structure and chemistry can be large. We used a small mammal exclosure experiment to explore whether rodents in a southeastern old field directly altered the aboveground plant species composition and chemistry, and indirectly altered the belowground soil community composition and activity. In general, when rodents were excluded, C_3 graminoids increased in cover and biomass, contributing toward a shift in plant species composition relative to plots where rodents were present. The plant community chemistry also shifted; plant fiber concentration and carbon : nitrogen were higher, whereas plant nitrogen concentration was lower in exclosure plots relative to access plots. While microbial community enzyme activity increased when rodents were excluded, no significant changes in the fungal : bacterial or potential nitrogen mineralization occurred between treatments. Our results show that rodents can rapidly influence aboveground plant community composition and chemistry, but their influence on belowground processes may require plant inputs to the soil to accumulate over longer periods of time.

Key words: aboveground; belowground; communities; ecosystem function; foliar chemistry; nitrogen mineralization; plant chemistry; plant–animal interactions; rodent herbivory; soil microbes; species composition.

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INTRODUCTION

Understanding how abiotic factors such as temperature and precipitation shape large-scale plant distributions, diversity patterns, and ecosystem function remains a focal interest of ecology (Whittaker 1960, Meentemeyer 1978). However, biotic factors, including plant–herbivore interactions (Bardgett and Wardle 2003), often shape local-scale diversity patterns and associated functions. Globally, herbivores consume 10–20% of net primary productivity in forests and over twice as

much in grasslands (Frank and McNaughton 1992, Augustine et al. 2003, Howe et al. 2006, Martin and Wilsey 2006, Borer et al. 2014). Thus, via their consumption of plants, herbivores influence the amount and quality of materials that enter the soil system, having large impacts on the functioning of ecosystems.

In addition to the removal of plant biomass, herbivores can selectively consume high-quality plants leading to temporal shifts in the nutrient content of plants in the community (Ritchie et al. 1998, van Wijnen et al. 1999). Further, herbivory can induce plant defenses that can bind nutrients into complexes that are difficult both for herbivores to digest and for decomposers to degrade (e.g., Schultz and Baldwin 1982, Agrawal et al. 1999). Lower-quality litter slows microbial decomposition and thus can slow ecosystem function (Pastor et al. 1993, Sirotnak and Huntly 2000). However, at larger scales, herbivores can also stimulate nutrient cycling and plant productivity (e.g., Bardgett and Wardle 2003, Clark et al. 2005) by redistributing nutrients on the landscape (Day and Detling 1990, Afzal and Adams 1992, Willot et al. 2000). In particular, small mammals can stimulate soil nutrient cycling through fecal deposition (Bakker et al. 2004) and by mixing soil and litter with their rooting and burrowing behaviors (e.g., Hole 1981, Brown and Heske 1990, Huntly and Reichman 1994).

Some of the characteristics that make plants generally more palatable to herbivores, such as a high nitrogen concentration, are also characteristics that make leaf material more labile to decomposers. Thus, herbivore-mediated changes in plant community composition should impact the decomposer community and its function in soils (Wardle et al. 2001, Sariyildiz et al. 2005, Cornwell et al. 2008, Bagchi and Ritchie 2010, Lessard et al. 2012). As the inputs to the soil system change to an altered chemical quality, the soil community may shift its function to produce enzymes that can degrade this new complex of molecules (Sinsabaugh et al. 2002) or change from being governed by fast-decomposing, bacterial-dominated to slower-decomposing, fungal-dominated assemblages (e.g., Ritchie et al. 1998, Bardgett and Wardle 2003). Thus, via direct changes in plant composition (quality) and plant material inputs (quantity), herbivores can indirectly alter belowground communities, processes, and ecosystem functioning (Wardle et al. 2001, Bagchi and Ritchie 2010, Veen et al. 2010, Niwa et al. 2011, Lessard et al. 2012).

Clearly, the influence of herbivores on ecosystems can be complex and variable (e.g., Huntly 1991). To explore how rodents alter the above- and belowground composition and function of an old-field ecosystem, we used a rodent exclusion experiment and measured the response of aboveground (plant community structure and composition, standing green plant biomass, and litter mass chemistry) and belowground (soil fungal and bacterial gene copy numbers, extracellular enzyme activity [microbial activity], and potential N mineralization [nutrient cycling and an index of soil nitrogen available for plant uptake]) variables. We predicted that excluding rodents from an ecosystem would stimulate ecosystem function. Specifically, we predicted that rodent exclusion would (1) directly increase aboveground plant biomass as well as cause a shift in plant community composition toward more palatable nitrogen fixers and C_3 grasses, thereby increasing plant community chemical quality, and (2) indirectly lead to an increase in soil bacteria relative to fungi, extracellular enzymatic activity, and nutrient cycling.

Methods

Study site

Our study site was located on Freels Bend, part of the Oak Ridge National Environmental Research Park near Oak Ridge, Tennessee (35°58′ N, 84°17′ W). Agricultural activities on the site were discontinued in 1943, and the field surrounding the experimental site has been maintained as wildlife habitat with variable mowing regimes since 2000; however, the field containing our exclosures was not mowed during the duration of the experiment. The experimental site was burned with a low-intensity fire on 22 March 2008 prior to establishing the experiment. The soil is classified as a Typic Hapludult (Phillips et al. 2001). Precipitation is evenly distributed throughout the year with an annual mean of 1360 mm, while mean daily temperatures range from 3°C in January to 31°C in July. Common plant species included tall goldenrod (*Solidago altissima*), blackberry (*Rubus* sp.), white crownbeard (*Verbesina virginica*), trumpet creeper (*Campsis radicans*), sericea

(*Lespedeza cuneata*), brome grass (*Bromus* sp.), yellow crownbeard (*Verbesina occidentalis*), clovers (*Trifolium* spp.), broomsedge (*Andropogon virginicus*), and orchard grass (*Dactylis glomerata*). The most common rodent at our site was the hispid cotton rat (*Sigmodon hispidus*), although woodland vole (*Microtus pinetorum*), eastern harvest mice (*Reithrodontomys humulis*), and deer mice (*Peromyscus* spp.) were also present.

Experimental design

In March 2008, we constructed twenty 4 × 8 m rectangular plots in an old-field community and randomly assigned 10 plots as rodent exclosure treatment plots and 10 plots as access plots (the control plots for the experiment; Fig. 1A). Plot perimeters consisted of a galvanized hardware cloth (122 cm width, 0.64 cm mesh) fence sunk 40 cm into the soil profile and extended 82 cm above the soil surface. Fencing depth was sufficient to exclude burrowing rodents. We installed aluminum flashing (36 cm width) on the upper portion of the fence to exclude climbing rodents. Ten holes (15 × 30 cm) were cut at ground level around the perimeter of the access plots to allow for passive entry of rodent-sized animals, while the exclusion plots remained unaltered (Fig. 1).

To monitor the effectiveness of the exclusion and access plots, we surveyed the rodent community across the entire field site twice annually (March and July) inside and outside plots from 2008 to 2010, using Sherman live traps in a 10 × 10 square grid with traps spaced 10 m apart. Low recapture rates precluded estimation of densities for the species captured, so we report minimum number known alive, averaged between trapping periods within years. *Microtus pinetorum* was absent in 2008 but trapped in 2009 and 2010 (seven and 17 individuals, respectively). *Reithrodontomys humulis* and *S. hispidus* showed similar patterns (0, 10, 3; 0, 3, 40, respectively). *Peromyscus* species were found in 2008 (seven) as well as in 2009 (three) and 2010 (seven). In addition, we set two traps inside each of the access and exclusion plots during each trapping period to monitor the effectiveness of the rodent barriers. Although captures of rodents were low in the access plots, rodent signs in the form of runways, burrows, feces, and herbivory were obvious. We never caught rodents or observed signs of rodent activity in any of the exclusion plots, suggesting that the barriers were effective. To check

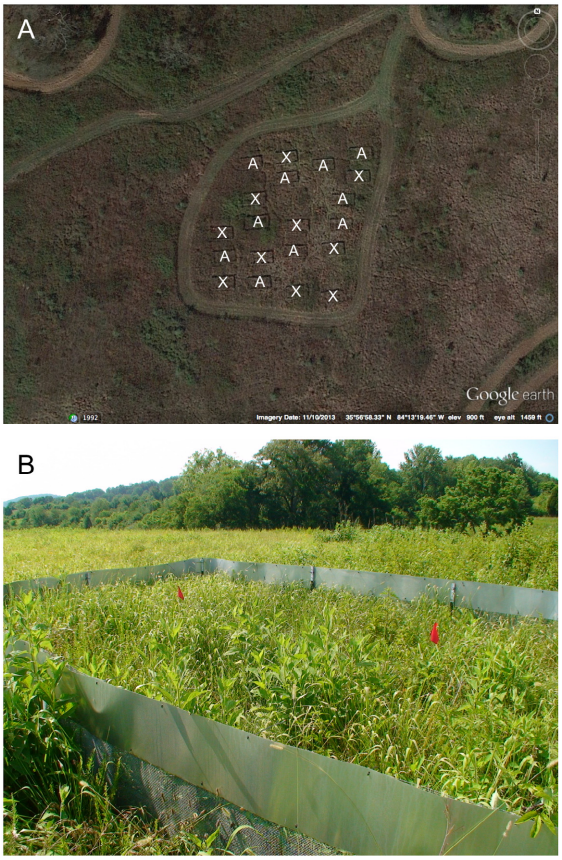

Fig. 1. (A) Google Earth image of Freels Bend study site showing aerial image of access ("A") and exclosure ("X") plots. Area containing plots within mowed perimeter is roughly 60 × 60 m. (B) Photograph of one of the twenty 4 × 8 m rectangular rodent manipulation plots established in an old-field community. Plot perimeters consisted of a galvanized hardware cloth fence sunk 40 cm into the soil profile and extending 82 cm above the soil surface. We installed aluminum flashing on the upper portion of the fence to exclude climbing rodents. Holes were cut in half of the plots at ground level for rodent-sized animal access.

continued exclosure efficacy, we used track plates to measure rodent activity in 2012. We created track plates using acetate paper painted with a graphite solution and stapled to sheets of aluminum flashing (see Connors et al. 2005). Two plates were placed within each mammal access and exclosure plot and collected after 48 h. At collection, we took pictures of each plate. In the laboratory, we used WinFolia 2009a (Regent Instruments Inc., Canada) to scan each

photograph. This program differentiates contrast differences between leaves and background color and is often used to measure percentage of leaf herbivory. We set the parameters of the program to distinguish black and white contrast and recorded the number of black and white pixels using white pixels as a proxy of disturbance. Disturbance included smudges from incidental vegetation movement (e.g., wind) and mammal activity. We found that disturbance events on the plates were significantly higher under mammal access (64.61 [8.72]; 34.38 [6.24] mean [standard error]; $F = 9.85$, $P < 0.05$). Further, we saw numerous rodent prints on plates in the access plots, but never saw indication of rodent activity on plates from exclusion plots.

Aboveground structure and composition

We assessed plant community structure in our treatments by measuring plant foliar cover and harvesting plant aboveground biomass in two randomly selected 0.5-m^2 subplots at peak growing season in September 2009. We measured species-specific foliar cover with a modified Braun-Blanquet cover class scale (Braun-Blanquet 1932) with six categories: 1 = <1%; 2 = 1–5%; 3 = 5–25%; 4 = 25–50%; 5 = 50–75%; and 6 = 75–100%. We used the median of each foliar cover category value as an estimate of species-specific abundance per plot, averaged across the two 0.25-m^2 subplots. We calculated the Shannon diversity index (H') from foliar cover data using the median of each cover class category as our values of abundance. We then calculated the proportional cover of each species and then summed across proportions. We calculated evenness (J') as $H'/\ln S$ (where S is species richness). Functional group (e.g., forbs, C_3 graminoids, C_4 graminoids, nitrogen fixers, and woody) foliar cover was calculated as summed species-specific foliar cover within each functional group. Finally, to determine aboveground biomass, we clipped all individuals within each 0.5-m^2 subplot, sorted them into forbs, C_3 graminoids, C_4 graminoids, nitrogen fixers, and woody, and then oven-dried them at 60°C for at least 48 h to calculate oven-dry mass.

Plant and litter chemistry

During September 2010, we harvested samples of aboveground plants and plant litter from each plot to understand how rodent exclusion influenced the relative abundance and chemical composition of plant functional groups and litter inputs. We harvested all aboveground standing green plant biomass within two randomly located 0.5-m^2 subplots per plot by clipping at ground level. Standing green biomass was sorted into functional groups (woody, C_4 graminoids, C_3 graminoids, nitrogen fixers, and forbs) for further analysis. Litter mass (i.e., senesced plant material) was harvested from standing biomass (suspended litter) and from the soil surface (surface litter). Suspended litter mass was collected by gently lifting it out from standing green biomass by moving two open hands slowly up from the base to the top of the plant canopy. Surface litter mass was collected from the soil surface after standing litter mass and aboveground biomass was removed. Prior to further analysis, suspended and surface litter samples were combined into a single litter sample for each subplot. Directly after harvest, we dried aboveground green biomass and litter mass samples at 60°C for approximately 48 h. We quantified functional group and litter abundance as oven-dried mass (g), after which a portion of each sample was ground in a Wiley mill in preparation for foliar chemical analysis.

In an attempt to understand how rodent-mediated changes in the plant community might influence ecosystem processes, we assayed each plant functional group and litter sample separately for foliar chemical properties related to resistance to herbivory and decomposition. These properties were carbon (C), nitrogen (N), fiber, and lignin. C and N were quantified via combustion analysis using a Thermo Finnigan Flash 1112 elemental analyzer (Thermo Finnigan, San Jose, California, USA). Fiber (cellulose and lignin) and lignin were quantified as acid detergent fiber (ADF) and acid detergent lignin (ADL), respectively, via sequential extraction in hot acid–detergent using an Ankom 200 Digester (ANKOM Technology, Fairport, New York, USA).

For each replicate plot, we linked functional group biomass with foliar chemical properties by calculating an index we call community chemistry (CC). The CC of foliar chemical property j for each plot was calculated as

$$\mathrm{CC}_j = \sum_{i=1}^{n} B_i P_i$$

where B_i is the proportional biomass relative to total biomass of functional group i, and P_i is the

assayed value of the foliar chemical property (e.g., % ADF) of functional group *i*.

Belowground structure and composition

Given the strong plant responses to herbivore exclusion reported in the literature, we predicted that litter inputs to the soil system would also have changed. Thus, we followed up the plant community work with soil measurements in July of 2010. Plots were visually divided into three equal sections and soil cores (0–15 cm, 5 cm diameter) were taken from the middle of each section, to minimize edge effects. We combined and homogenized all three cores taken per plot. A subsample was frozen for molecular analysis and the rest of the samples were kept cool (4°C) until analysis within 24 h. Soils not used in molecular analyses were sieved to 2 mm, and gravimetric water content was determined by drying a subsample (105°C for 48 h). Relevant data are shown on a dry mass basis.

We assessed the composition and activity of the soil community in three ways. First, we estimated the relative abundance of fungi and bacteria using quantitative polymerase chain reactions (qPCR). To amplify 16S and 18S rRNA genes from bacteria and fungi, respectively, we performed PCR analyses using primers 63f and 1087r8 for 16s rRNA genes and ITS1f and ITS4r for 18s rRNA genes on a 96-well T-gradient thermocycler (Biometra, Goettingen, Germany; see Cregger et al. 2012). Next, we assessed microbial potential extracellular enzyme activity by assaying phenoloxidase, peroxidase, β-glucosidase, cellobiohydrolase, β-xylosidase, α-glucosidase, *N* acetylglucosaminidase (NAGase), phosphatase, and sulfatase. We measured activity using substrates L-3,4-dihydroxyphenylalanine (L-DOPA), 4-MUB-β-D-glucoside, 4-MUB-β-D-cellobioside, 4-MUB-β-D-xyloside, 4-MUB-α-D-glucoside, 4 MUB-*N*-acetyl-β-D-glucosaminide, 4-MUB-phosphate, and 4-MUB-sulfate, respectively. Phenoloxidase and peroxidase are involved in lignin degradation. β-Glucosidase, cellobiohydrolase, β-xylosidase, and α-glucosidase break down carbohydrates and polysaccharides. NAGase mineralizes nitrogen from chitin, phosphatase releases inorganic phosphorus, and sulfatase is involved in inorganic sulfur release. We suspended one gram of soil from each sample in 125 mL of sodium acetate buffer (pH 5) by mixing the slurry on a stir plate for 2 min. We used eight replicate 96-well plates (clear for phenoloxidase and peroxidase, black for other enzymes) in the following ways: Clear plates had negative substrate controls and negative sample controls; black plates used a similar well set up but also used eight replicate wells for reference standards and quench controls. We incubated the plates in a dark environment at room temperature and read them using BioTek Gen 5 software (BioTek Instruments, Inc., Winooski, Vermont, USA) on a BioTek Synergy HT multi-mode microplate reader (BioTek Instruments, Inc., Winooski, Vermont, USA) according to activity. We stopped reactions in black plates using 25 μL of NaOH prior to reading (see Saiya-Cork et al. 2002). Finally, we measured the ability of the microbial community to mineralize nitrogen (NO_3^- and NH_4^+) with a potential net nitrogen mineralization incubation (see Robertson et al. 1999). We removed a subsample (~20 g) of soil from each plot and brought it up to field water-holding capacity. We incubated all samples in a mason jar (25°C in the dark) for 28 d. We extracted samples, 0 and 28 d, with 2 mol/L KCl and determined soil nitrate (NO_3^-) and ammonium (NH_4^+) concentrations on an autoanalyzer (SmartChem 200; Unity Scientific, Brookfield, Connecticut, USA).

Statistical analyses

We used a series of one-way analyses of variance (ANOVA) to examine the impact of rodents on plant community structure (biomass, richness, evenness, diversity, and plant functional groups), plant and litter chemistry (fiber, lignin and nitrogen concentrations, C:N, and fiber : N), soil community structure (fungal : bacterial), and soil community potential function (extracellular enzyme activity, nitrogen mineralization). Response variables that did not meet normality assumptions were log-transformed. P-values < 0.05 were considered statistically significant, and values between 0.05 and 0.10 were considered marginally significant.

To determine the effects of rodents on plant functional group composition and to explore whether the composition of belowground enzymes contributed to overall differences in activity between treatments, we conducted a permutational multivariate analysis of variance (PERMANOVA; Anderson et al. 2006). The PERMANOVA tested whether the observed variability in plant functional group and extracellular enzyme composition between treatments differed from expected

variability generated from permutational shuffling (10,000 iterations). Functional group foliar cover and microbial extracellular enzyme activity were used in the permutational shuffling that generated pseudo-*F*-ratios. Permutational multivariate analysis of variance tests were conducted on Bray–Curtis similarity triangular matrices (Bray and Curtis 1957) generated from transformed ($\log x + 1$) functional group-specific relative foliar cover and on extracellular enzyme-specific relative activity. A significant pseudo-*F*-ratio from a PERMANOVA indicates between-treatment differences in location of functional group or extracellular enzyme composition. Likewise, within-treatment differences in dispersion of functional group or extracellular enzyme composition in multivariate space could also contribute to a significant pseudo-*F*-ratio. As a result, we followed PERMANOVA with a permutational analysis of multivariate dispersions (BETADISPER) to test whether, in addition to differences in compositional location, there were any differences in community dispersion (i.e., variability) within treatments. Finally, we used a principal coordinate (PCO) approach to explore how plant functional groups (2009 and 2010) and specific enzymes described access and exclosure communities. Principal coordinate was performed on the Bray-Curtis similarity matrix, which was based on log-transformed ($\log x + 1$) functional group-specific relative foliar cover, biomass, and extracellular enzyme-specific relative potential activity. Plant functional group and extracellular enzyme vectors were overlaid to represent their association with the PCO axes and associated rodent treatments. We used R version 3.2.2 (R Development Core Team 2013) and JMP versions 9 and 11.1 (SAS Institute Inc., Cary, North Carolina, USA) for statistical analyses.

Results

When rodents were excluded, total aboveground plant biomass in 2009 was slightly (19%) higher than in plots where rodents were present, a marginally significant effect. Rodent exclusion resulted in 2.6× greater foliar cover of C_3 graminoids, while woody foliar cover was 4.4× greater in access plots and there were no changes in foliar cover of nitrogen fixers, forbs, or C_4 graminoid plant species (Table 1, Fig. 2A). We found no difference between rodent treatments in plant richness, evenness, and diversity (Table 1). However, the effect of rodents on the plant community was significant in 2010; C_3 biomass was 672% greater in exclosure plots compared to access plots, while forb cover was 140% greater in access plots but C_4, woody, and nitrogen fixer biomass did not differ between treatments. We find similar patterns between years in composition as well. We found that rodent exclusion only marginally influenced the compositional similarity of the plant functional group community in 2009 (Fig. 3A, pseudo-$F = 2.86$, P [perm] = 0.07). However, in 2010, we found a stronger pattern of rodent exclusion on compositional dissimilarity (Fig. 3B, pseudo-$F = 0.259$, P [perm] < 0.05). Plant CC also changed when rodents were removed. Litter fiber concentration and C:N were 4.6% and 24.7% higher, respectively, in exclosure plots relative to access plots, while litter nitrogen concentration was 23.6% higher in access plots compared to exclosure plots (Table 1).

While we found no change in the soil fungal-to-bacterial ratio between our treatments (Table 1), when rodents were excluded enzyme activity tended to increase. Specifically, in exclusion plots, phosphatase activity was 97% higher, phenoloxidase was 35% higher, cellobiohydrolase was 118% higher, and α-glucosidase was 80% higher relative to access plots (Table 1, Fig. 2B). Sulfatase activity was marginally significantly higher in exclusion relative to access plots, whereas xylosidase, β-glucosidase, and peroxidase activities were not significantly different between exclusion and access plots. Compositional similarity of the belowground community function (microbial extracellular enzyme activities) differed between treatments (Fig. 3, pseudo-$F = 4.06$, P [perm] = 0.02). Potential net nitrogen mineralization and nitrification rates were not significantly different between the treatments; however, ammonium immobilization was marginally higher in exclusion plots (Table 1).

Finally, we found no effects of rodent treatments on over-dispersion (2009 plant functional group BETADISPER: $F = 0.04$, P [perm] = 0.85; 2010 plant functional group BETADISPER: $F = 0.36$, P [perm] = 0.98; extracellular enzyme BETADISPER: $F = 0.57$, P [perm] = 0.46). This result indicates that the compositional dissimilarity between rodent treatments was a function of rodent treatment effects on compositional location (e.g., lack

Table 1. Means, standard errors, *F*, and *P*-values for all above- and belowground responses in small mammal access and exclosure plots.

Response variables	Access	Exclosure	*F*	*P*
Plant community 2009				
Woody cover (%)	9.30 ± 3.23	2.10 ± 1.47	3.63	*0.07*
C_4 graminoid cover (%)	37.55 ± 1.50	42.58 ± 5.97	0.48	0.50
C_3 graminoid cover (%)	1.55 ± 1.50	3.95 ± 1.87	3.23	*0.09*
Nitrogen fixer cover (%)	12.83 ± 3.92	21.88 ± 6.60	1.39	0.25
Forb cover (%)	37.75 ± 4.06	41.13 ± 5.94	0.22	0.65
Total cover (%)	98.98 ± 7.52	111.63 ± 8.45	1.25	0.28
Standing biomass	354.71 ± 22.29	420.50 ± 25.59	3.76	*0.07*
Species diversity	1.79 ± 0.05	1.72 ± 0.06	0.65	0.43
Species evenness	0.75 ± 0.02	0.74 ± 0.02	0.30	0.59
Species richness	11.00 ± 0.45	10.60 ± 0.60	0.29	0.60
Plant community 2010				
Woody biomass (g)	17.07 ± 5.49	12.31 ± 3.19	0.53	0.48
C_4 biomass (g)	104.09 ± 39.39	56.83 ± 19.64	0.07	0.79
C_3 biomass (g)	38.97 ± 14.35	301.0 ± 39.90	24.11	**<0.05**
N-fixer biomass (g)	180.40 ± 66.50	64.31 ± 25.66	1.68	0.21
Forb biomass (g)	102.17 ± 37.19	42.68 ± 17.82	5.41	**<0.05**
Total biomass (g)	442.70 ± 44.57	477.27 ± 41.86	0.32	0.58
Litter biomass (g)	197.69 ± 29.77	186.31 ± 20.27	0.10	0.76
Green leaf chemistry 2010				
ADF (cellulose + lignin, %)	43.10 ± 0.73	45.09 ± 0.59	4.35	**0.05**
ADL (lignin, %)	13.05 ± 0.99	11.47 ± 0.59	1.77	0.20
N (nitrogen, %)	1.57 ± 0.10	1.27 ± 0.11	4.31	**0.05**
C (carbon, %)	46.85 ± 0.84	45.51 ± 1.32	0.96	0.34
C:N	30.64 ± 1.59	38.21 ± 3.37	4.42	**0.05**
ADL : N	8.42 ± 0.66	9.43 ± 0.64	1.21	0.29
Microbial community (gene copy number/g soil)				
Fungal : bacterial	0.81 ± 0.13	0.83 ± 0.15	0.53	0.48
Fungal abundance	1.89 (10^5) ± 2.77 (10^4)	2.50 (10^5) ± 3.54 (10^4)	2.10	0.16
Bacterial abundance	2.54 (10^5) ± 3.69 (10^4)	3.12 (10^5) ± 2.25 (10^4)	2.91	0.11
Enzyme activity (nmol·h^{-1}·g $soil^{-1}$)				
Phenoloxidase	465.86 ± 44.94	626.88 ± 51.33	5.57	**0.03**
Peroxidase	1058.21 ± 156.89	1011.80 ± 146.42	0.05	0.83
Beta-glucosidase	23.50 ± 8.41	35.92 ± 6.03	2.53	0.14
Cellobiohydrolase	1.81 ± 1.27	3.95 ± 2.48	5.27	**0.04**
Xylosidase	6.59 ± 3.49	8.34 ± 1.50	1.5	0.24
Alpha-glucosidase	0.88 ± 0.23	1.58 ± 0.33	4.85	**0.04**
NAGase	30.21 ± 5.78	43.28 ± 5.53	2.69	0.12
Phosphatase	55.70 ± 11.67	109.77 ± 17.00	6.88	**0.02**
Sulfatase	3.94 ± 0.67	5.58 ± 0.63	3.18	*0.09*
Total enzyme activity	1502.92 ± 206.93	1829.03 ± 281.92	0.12	0.74
Potential nitrogen availability (mg·kg^{-1}·d^{-1})				
Total (NH_4 + NO_3)	0.41 ± 0.11	0.37 ± 0.15	0.03	0.86
Nitrate (NO_3)	14.92 ± 2.96	18.57 ± 6.11	0.05	0.83
Ammonium (NH_4)	−2.66 ± 1.09	−7.35 ± 2.22	3.52	*0.08*
Soil moisture (GWC)	0.20 ± 0.01	0.23 ± 0.01	6.52	**<0.05**

Notes: ADF, acid detergent fiber; ADL, acid detergent lignin; NAGase, *N* acetylglucosaminidase. Significant *P*-values (<0.05) are bolded. Marginal *P*-values (0.05–0.10) are shown in italics.

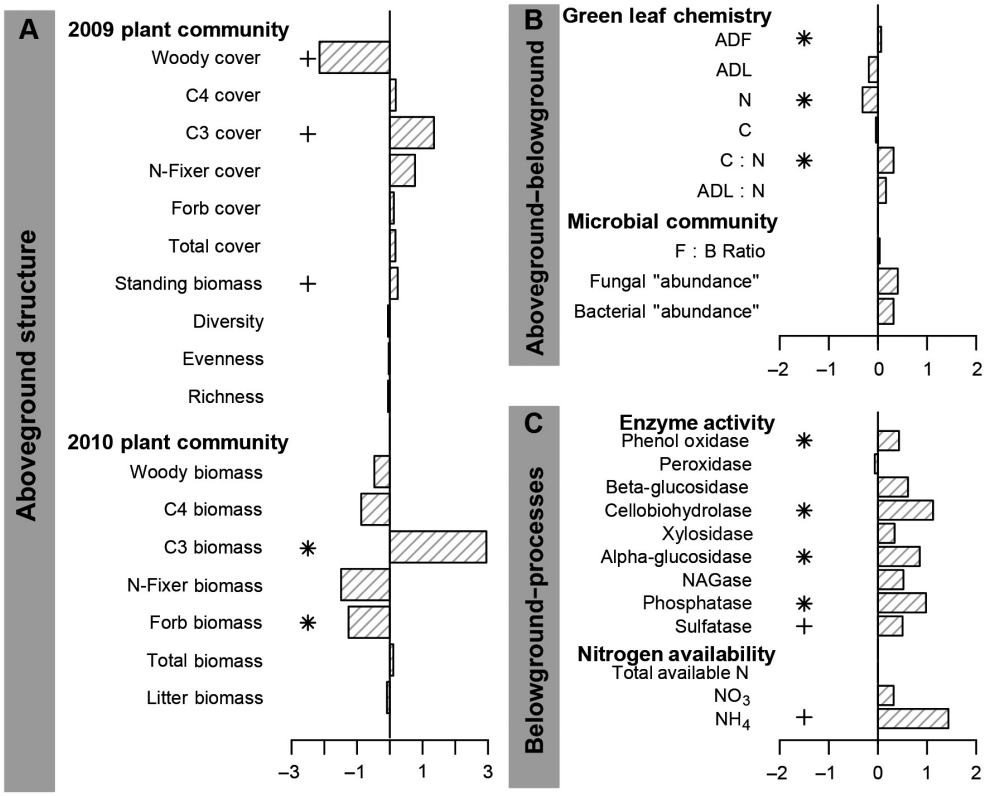

Fig. 2. Effects of rodent absence/presence on (A) 2009 and 2010 aboveground community structure; (B) plant leaf chemistry and belowground structure; and (C) ecosystem process and function expressed as log (2) of the ratios of means from rodent exclusion to access plots. Values below 0 indicate stimulation under rodent access; values above 0 indicate stimulation under rodent exclusion. $^{*}P < 0.05$, + indicates $0.05 < P < 0.10$. Values for actual means ± standard error and ANOVA results are given in Table 1.

of overlap between plant or enzyme composition in rodent present vs. rodent removal plots) rather than on compositional variability (e.g., the amount of over-dispersion of composition in rodent present vs. rodent removal plots).

Discussion

After three years of rodent experimental manipulation, plant community structure and composition shifted as we predicted—toward higher biomass (in 2009) and a community with more C_3 graminoids in our small mammal exclusion treatments. In 2009 and 2010, the cover and biomass of C_3 grasses were higher in the exclosure plots than in the access plots, a pattern that became stronger in the second year. In 2009, C_3 cover was 155% higher in exclosure plots, and in 2010, C_3 biomass was 672% higher in exclosure plots than in access plots. This large increase in C_3 grasses suggests the plant community is shifting toward a newly C_3-dominated community composition (Fig. 3). In addition, the standing stock of aboveground plant biomass was 19% higher when rodents were excluded in the short term (2009). Our findings support previous work showing that rodents can significantly alter plant communities. For example, when meadow voles (*Microtus pennsylvanicus*) were given access to previously enclosed prairie grassland communities, both a legume and C_3 grass species were eliminated within 48 months (Howe and Lane 2004). Similarly, exclusion of small mammals in an annual grassland system in northern California led to a 47% increase in aboveground plant biomass and a 90% increase in primarily C_3 grasses (Peters 2007).

Given that plant functional group composition shifted toward higher C_3 graminoid cover and

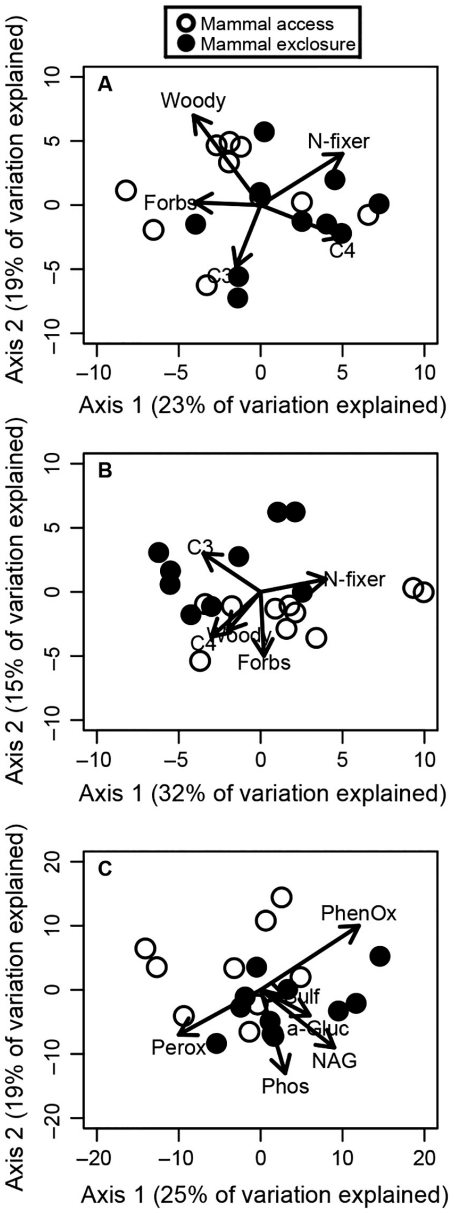

Fig. 3. Principal coordinate (PCO) axes illustrating 2009 plant functional group (A), 2010 plant functional group (B), and extracellular enzyme composition (C) with small mammal access (clear circles) and rodent exclusion (filled circles) plots. Principal coordinate was performed on Bray-Curtis similarity matrix, which was based on log-transformed (log $x + 1$) functional group-specific relative foliar cover and extracellular enzyme-specific relative potential activity. Plant functional group and extracellular enzyme vectors were overlaid to represent their association with the PCO axes and associated rodent treatments.

biomass in the exclosure plots, it is not unexpected we would find both fiber and C:N ratios to be higher in the exclosure plots relative to access plots. Furthermore, the higher plant leaf nitrogen in the access plots may be a consequence of rodents preferentially avoiding plants that are greater in nitrogen concentration because they may also be rich in unpalatable secondary compounds—such as alkaloids—which we did not measure. Alternatively, changes in the plant community composition may alter carbon allocation to the soil community via root exudation, a process that can increase microbial activity, nitrogen mineralization, and plant available nitrogen, but we did not observe this increase in our study (Wardle et al. 2003, 2004, Ladygina and Hedlund 2010).

We predicted that declines in the quality of plant inputs to the soil would lead to an increase in the fungal relative to bacterial gene copy numbers; however, we did not see differences in fungal and bacterial gene copy numbers. While gene copy numbers give insights to what organisms are present in the soil community, they also capture the inactive microbial pool (Strickland and Rousk 2010). Thus, these measurements are a rather coarse-scale measure of microbial community composition and may not have captured changes in the active community. At the same time, the community may have stayed constant, but shifted its activity with the changing plant inputs. Finally, old-field ecosystems can be relatively nutrient rich (Blue et al. 2011); thus, bacteria may still dominate the decomposition pathway even if litter entering the system is of lower quality.

While the coarse-scale composition of the microbial community remained unchanged, the enzyme activity of the soil community was higher in exclosure relative to access plots. Rodent exclosures had higher cellulose (cellobiohydrolase)-, starch (alpha-glucosidase)-, organic phosphorus (phosphatase)-, and lignin (phenoloxidase)-degrading enzyme activity. Whether enzyme activity reflects what nutrients are available (substrate supply) vs. what microbes are seeking (microbial demand) remains unknown. However, nutrient additions via herbivores or changes in plant communities can increase enzyme activities (Riggs and Hobbie 2016). The addition of labile carbon substrates to nutrient-rich ecosystems can stimulate enzyme activity by alleviating microbial carbon limitation (Asmar et al. 1994). When herbivores were removed from the

plots, the plant community shifted and the carbon inputs to the soil also likely shifted (e.g., Ritchie et al. 1998, Sirotnak and Huntly 2000). It may be that the aboveground chemical quality declined in the exclosure plots, which led to an increase in enzyme activity to break down the more recalcitrant litter inputs—with the higher phenoloxidase activity in exclosure plots possibly providing further support for this hypothesis.

Rodents in our system had little impact on potential soil nitrogen mineralization and nitrification rates; however, this lack of directional response has been observed in other studies (Sirotnak and Huntly 2000, Bakker et al. 2004). Potential nitrogen mineralization could be high in the access and the exclosure plots for different reasons: Bioturbation of the soil or deposition of fecal material by rodents may increase mineralization in the access plots to the same extent that changes in the plant composition may increase mineralization in the exclosure plots. Bioturbation by pocket gophers increased nitrification rates by 186% in an alpine system (Litaor et al. 1996). Herbivores in the access plots could have mixed the soil, leading to a release of plant available nutrients that we were unable to measure with our potential mineralization assays. An increase in mineralization due to soil mixing could lead to an increase in plant chemical quality when rodents were present. Alternatively, total soil carbon and nutrient pools are large and thus can be slow to respond to short-term (4–10 yr of experimental manipulation) changes in plant inputs (e.g., Hungate et al. 1996, Smith 2004). For example, deer exclosures in a boreal ecosystem impacted soil nitrogen mineralization, but only after 10 yr of manipulation (Harrison and Bardgett 2004). Thus, changes in the nitrogen mineralization may increase between our treatments over time as the influence of changes in biomass inputs and chemistry compounds.

Overall, our study shows that rodents can directly and indirectly impact above- and belowground ecosystem properties, even over short two- to three-year time scales. These data contribute to a growing body of work demonstrating that vertebrate consumers, both large and small, are important components of ecosystems and that their impacts on ecosystem function can extend beyond the consumption of plant biomass (Bardgett et al. 1998, Bardgett and Wardle 2003, Wardle et al. 2004, Habeck and Meehan 2008). However, consumers and their effects are often excluded or ignored in large-scale ecosystem manipulations that aim to understand how ecosystems will function under a variety of global changes (but see Borer et al. 2014). If ecologists are to better describe and predict what factors will structure ecosystems and their functions across landscapes and over time, rodent consumers should be included in both manipulations and models.

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