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Diversity loss, recruitment limitation, and ecosystem functioning: lessons learned from a removal experiment

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A five-year removal experiment in which plant functional group diversity was manipulated found strong limitation of ecosystem functioning caused by the differing abilities of remaining functional groups to recruit into space left unoccupied by the plants removed. We manipulated functional group diversity and composition by removing all possible combinations of zero, one, or two plant functional groups (forbs, C_3 graminoids, and C_4 graminoids), as well as randomly chosen biomass at levels corresponding to the functional group removals, from a prairie grassland community. Although random biomass removal treatments showed no significant effect of removing biomass in general on ecosystem functions measured ($P > 0.05$), the loss of particular functional groups led to significant differences in above- ($P < 0.001$) and belowground ($P < 0.001$) biomass, rooting-zone ($P = 0.001$) and leached ($P = 0.01$) nitrogen, nitrogen mineralization ($P < 0.001$), and community drought resistance ($P = 0.002$). Many of these differences stemmed from the marked difference in the ways remaining functional groups responded to the experimental removals. Strong recruitment limitation of C_4 graminoids resulted in large areas of open ground, high nutrient leaching, and high community drought resistance in plots containing just this functional group. In contrast, rhizomatous C_3 graminoids quickly colonized space and used soil resources made available by the removal of other groups, leading to lower soil nitrate in plots containing C_3 graminoids. These effects of recruitment limitation on ecosystem functioning illustrate possible effects of diversity loss not captured by synthetic experiments in which diversity gradients are created by adding high densities of seeds to bare soil.

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Over the last decade, a flurry of research investigating the relationship between biodiversity and ecosystem functioning has led to increased understanding of how the number and types of organisms in an ecosystem influence ecosystem properties and processes. Some experiments have shown that, on average, productivity and nutrient capture increase as species richness increases (Naeem et al. 1994, 1996, Tilman et al. 1996, 1997, Symstad et al. 1998, Hector et al. 1999). The interpretation of some of these results has been vigorously debated, however (e.g. Aarssen 1997, Huston 1997, Tilman 1997b, Wardle et al. 1997, Lawton et al. 1998, Huston et al. 2000, Hector et al. 2000). One of the items of contention is that diversity gradients in some of these studies were created by randomly drawing species from a designated species pool to construct the experimental communities (Huston 1997). This method reduces the problem of composition confounding diversity levels and is the best method to test the hypothesis of whether diversity, in general, influences ecosystem functioning. However, it is unclear whether ecological communities are assembled this way in na-

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ture and therefore raises the issue of how relevant the results of these studies are to natural systems (Huston 1997). In addition, most studies to date investigating the relationship between biodiversity and ecosystem functioning have relied upon synthetic communities in which diversity gradients were created from a limited species pool by adding high densities of seeds or organisms to bare substrate (Naeem et al. 1994, Tilman et al. 1996, 1997, Hooper and Vitousek 1997, McGrady-Steed et al. 1997, Naeem and Li 1997, Symstad et al. 1998, Hector et al. 1999, Wardle et al. 2000).

In 1993 we began an experiment to investigate the effects of plant functional group richness and composition on ecosystem functioning and the mechanisms behind any effects. Specifically, the experiment was designed to test the hypotheses that increasing the functional group diversity of the plant community would positively affect primary productivity, nitrogen (N) retention, and community drought resistance in a prairie grassland. In this experiment, problems associated with assembling communities from a limited species pool were avoided by removing plant functional groups from a naturally assembled community instead of planting seeds into bare substrate. This method of creating a diversity gradient may better mimic the effects of losing diversity from ecosystems, such as when disease virtually eliminated the American chestnut (*Castanea dentata*) and American elm (*Ulmus americana*) from the forests of North America. Another motivation for using the removal method was the expectation that it would establish quasi-equilibrium communities faster than would synthesized communities. This expectation was incorrect. Each of the three functional groups in our experiment (C_3 graminoids, C_4 graminoids, and forbs) reacted differently to the disturbance of the removal of competitors, causing some treatments to be much slower than others at approaching a new quasi-equilibrium density. As a result, we suggest that differences among organisms in their local recruitment abilities will be important for determining at least the transient effects of diversity loss on ecosystem functioning, and that such transient effects can last many years.

Methods

Study site

This experiment was conducted in an abandoned agricultural field, (Field C, Tilman 1987) last cultivated in 1934, in east-central Minnesota, USA at Cedar Creek Natural History Area. The soil is in the Zimmerman series, an excessively drained, fine sand formed in glacial outwash sediments (Grigal et al. 1974). The nutrient-poor, sandy soils make N the limiting nutrient to net primary productivity except during drought years, when water is limiting (Tilman 1990, Tilman and El Haddi 1992). At the outset of the experiment, the dominant species in the field, in decreasing order of cover, were the sand prairie/grassland species *Schizachyrium scoparium* (Michx.) Nash (25% absolute cover), *Ambrosia psilostachya* DC (15%), *Poa pratensis* L. (12%), *Helianthus laetiflorus* Pers. (7%), *Solidago nemoralis* Aiton (7%), and *Artemisia ludoviciana* Nutt (5%).

Experimental design and establishment

For this experiment, we divided species into three functional groups – C_3 graminoids (C3s), C_4 graminoids (C4s), and forbs (non-graminoid herbaceous plants), which together comprised approximately 15%, 52%, and 32% of the biomass and 12%, 53%, and 34% of the plant cover, respectively, in this field. Our groupings were based on the fact that the characteristics that determine how organisms interact with their environment also determine how they affect ecosystem processes (e.g. Whittaker 1956, 1975, Grime 1974, 1977, Huston 1979, 1985, Solbrig 1993). Based on their morphological and phenological differences, we expected that these three functional groups would play different roles in N cycling and productivity, especially during drought. C3s, dominated by *Poa pratensis*, *Panicum oligosanthes* Schultes, and *Agropyron repens* (L.) Beauv., grow primarily in cool conditions (April to mid-June and September to November). We expected that their generally lower water-use efficiency (Pearcy and Ehleringer 1984) and lower allocation to belowground biomass (Tilman and Wedin 1991) would cause C3s to be less resistant to drought than the C4s. C4s, dominated by *Schizachyrium scoparium* and *Sorghastrum nutans* (L.) Nash, are mostly native, warm-season plants that grow from late May through early September. They have lower tissue N concentrations and are generally more efficient in using N than the C_3 plants (Brown 1985, Marschner 1995). All the forbs in this experiment have the C_3 photosynthetic pathway, but they differ from graminoids in their growth form, rooting depth, and tissue nitrogen concentration. Because legumes were uncommon ($< 1\%$ of plant cover), we did not distinguish them from other forbs.

During the summer of 1993, treatments were established in 4×8 m plots in a completely randomized design. The nine treatments consisted of all possible combinations of zero, one, or two plant functional groups removed at a time, plus two extra control treatments in which 25% and 55% of plant biomass was removed. Abbreviations for the treatments, which refer to the functional group(s) *remaining* in the plots are: Forbs only = F; C3s only = C3; C4s only = C4; forbs and C3s = F/C3; forbs and C4s = F/C4; C3s and C4s = C3/C4. Unmanipulated controls (CONT) and 25% and

55% random biomass removal plots (RBL for "low" and RBH for "high", respectively) contained all three functional groups. The random biomass removal treatments were included to assess the general effects of removing biomass. Biomass in these treatments was removed from the appropriate number of randomly located 10 × 10 cm squares in a plot. The values of biomass removed approximate the amount killed to establish the one- and two-functional group treatments, based on cover estimates done in June 1993. Each treatment had four replicates, except for CONT, which had six, yielding a total of 38 plots.

Initially (1993), biomass was removed with herbicides. Glyphosate (formulated as Round-up) was hand-painted on leaves of individual plants to kill only C3s or C4s or to kill biomass in random spatial patterns. Selective herbicides were sprayed with a backpack sprayer at the manufacturers' recommended rates to remove all forbs (2,4-D formulated as Amine 4) or all graminoids (sethoxydim formulated as Poast Plus). Effects of these herbicides on non-target plants were not detected, and both have minimal or no effects on soil microflora and fauna (Tomlin 1994). Hand-painting was done twice during the 1993 growing season, and spraying was done three times. After some selective herbicide use in the spring of 1994, all treatments were maintained for the duration of the experiment by hand weeding from elevated, movable walkways.

At the beginning of the experiment in 1993, soil samples for initial total N were taken. Eight 2.5-cm-diameter × 18-cm-deep cores from each plot were pooled, homogenized, dried, and sieved through a 1-mm mesh to remove roots. A subsample from each plot was ground and analyzed for total N with a Carlo-Erba NA1500 Analyzer (Carlo-Erba, Milan, Italy). All plots were burned in early May, 1994. This burn removed the aboveground litter of plants killed by herbicides during the previous growing season, therefore reducing its impact on N cycling. Plots were burned again in late April, 1996. Although herbivores were not excluded from the experimental plots, mounds of dirt occasionally created by pocket gophers (*Geomys bursarius*) were removed and attempts were made to remove the animals.

Ecosystem functioning measurements

This experiment took place over five years (1993–1997), with some aspects of ecosystem functioning measured each year and others designed to address questions specific to a year. Each plot contained two 4 × 4-m subplots that were treated and sampled identically until 1996, when we created an experimental drought in one subplot of each plot. In late July–mid-August of each year, the same person estimated the cover of each plant species, bare ground, and litter in one 1 × 1-m area per subplot. Repeatability and objectivity of values obtained by this method were assured by comparing plant cover to cards of known cover over a range of sizes.

Productivity and N capture – 1994 and 1995

Aboveground biomass was sampled in mid-August, the time of peak aboveground biomass in this system, of both years. Vegetation from one 0.1 × 3-m strip in each subplot was clipped at ground level, sorted to species, dried, and weighed. Belowground biomass was sampled in late August–early September, 1995 by taking three, 5-cm-diameter cores from 0–20 cm, 20–40 cm, and 40–60 cm depths inside the clip strip in each subplot. Soil was rinsed from belowground biomass under running water over a 1-mm screen and biomass dried and weighed. Belowground biomass was not sorted.

Reproductive output of the dominant C4, *Schizachyrium scoparium*, was measured in early September 1994 and 1995. Ten individual plants (bunches) were randomly selected in each plot containing C4s, except random biomass removal treatments in 1995. For each plant, the number of reproductive stems was counted and basal diameter measured. To account for differences in bunch size, stem number was normalized by dividing by bunch diameter. Comparisons of reproductive output among treatments used the average stem number per bunch diameter for each plot.

Net N mineralization and extractable, inorganic N were measured monthly throughout the 1995 growing season from early May to late October. Mineralization was measured with in situ soil incubation using covered, 1.9-cm-diameter plastic (PVC) tubes sunk to 18 cm. One pre- and post-incubation sample from each subplot was homogenized, extracted in 1 M KCl, shaken for 0.5 h, settled overnight at 4°C, and analyzed for NO_3^--N and NH_4^+-N on an Alpkem autoanalyzer (O. I. Analytical, College Station, Texas). A subsample from each pre-incubation sample was also extracted at 0.01 M KCl to measure rooting-zone extractable NO_3^--N and NH_4^+-N (henceforth rooting-zone N) to estimate N available for plant uptake. Monthly mineralization rates were summed over the growing season and converted from mg N/kg dry soil to g N/m^2 based on the bulk density of the soil in the subplot. Rooting-zone N was averaged over the entire growing season for each plot.

Comparative leaching losses of inorganic N were estimated with porous cup lysimeters ("Slim Tube Soil Water Samplers" with 10^5 Pa porous cups, Soilmoisture Equipment Corporation, Santa Barbara, California). One lysimeter was installed in the center of each subplot at 50 cm depth (> 95% of belowground biomass is in the top 40 cm of soil in this system) by 24 May 1995. They were sampled weekly from 6 June to 22 September and once on 13 October, but the first sample was not included in any analyses. Contents of individual lysimeters were frozen, then later combined into one

sample per plot on each date and analyzed for concentrations of NO_3^--N and NH_4^+-N. We present leachate data only as total N concentration ([NO_3^--N] + [NH_4^+-N]) because the lysimeters used do not accurately sample soil moisture (Wagner 1962) and because analyses of leaching losses using concentration × volume values yielded results qualitatively similar. Only values averaged over the entire growing season are presented.

Community drought resistance – 1996

In May 1996, a 3 × 3-m rain shelter was constructed in one randomly chosen subplot of each plot. Greenhouse-grade, 6-mm-thick plastic was permanently attached to the shelters' A-shaped frames so that there was approximately 45 cm of open space between the ground and the bottom of the shelter. Although this plastic reduced light levels under the shelters by approximately 18%, up to 70% light reduction has been shown to have no effect on productivity at Cedar Creek (Tilman 1990). Each shelter was sloped so that rainwater flowed into two holes (approximately 75 cm × 30 cm, 50 cm deep) at two corners outside of the shelter. Because of the high permeability of the sandy soil at this site, there was very little surface flow of water from unsheltered to sheltered areas and little accumulation of water in the drainage holes during rainfall. The shelters excluded all rainfall from 20 May to 12 August 1996. In contrast, the unsheltered half of the plots received 19.6 cm of rainfall in this period. The duration of the drought was chosen to approximate a severe drought that occurred in this area in 1988, when aboveground productivity was approximately half that of normal years (Tilman and Downing 1994).

Cover estimates in this year were done in the center of the rain shelter (drought subplot) and at least 2 m from the edge of the shelter in the non-drought subplot. Aboveground biomass was sampled on 5–6 August 1996 by clipping the vegetation from two, 0.1 × 1-m strips at ground level just outside each cover estimate area, making a 0.2-m^2 sample for each subplot. (The smaller clip strips compared to previous years were necessitated by the rain shelter size.) The clipped vegetation was sorted into three categories [live (green), standing dead (no green tissue), and litter (previous years' growth)], dried, and weighed. Total aboveground biomass was calculated by summing the "live" and "standing dead" categories, which represents all aboveground productivity of 1996. Community drought resistance was calculated as the ratio of total aboveground biomass of the drought subplot to that of the non-drought subplot. This index is based on Pimm's (1984) definition of resistance, modified to incorporate the split-plot design of the experiment.

Belowground biomass in each subplot was sampled with one 5-cm-diameter × 30-cm deep core per clip strip, treated as in 1995. Gravimetric soil moisture content was measured in each subplot on 17 July and 12 August 1996. At each sampling time, four 2.5-cm diameter × 18-cm cores were taken in each subplot, pooled, homogenized, and weighed, then dried for 24 h at 100°C and weighed again.

1997

Symstad (2000) discusses the results of adding seeds of "invaders" to a portion of each non-drought subplot. Aboveground biomass and cover data for this invasion study were collected in the non-drought subplots in the same manner as in 1996 and are reported here. Invader biomass and cover were quite small and are not included in the present analyses.

Data analysis

Because of the drought imposed on half of each plot in 1996, plant cover and productivity data for 1996 and 1997 are from the non-drought subplots only unless noted otherwise. Preliminary statistical analyses indicated that pre-experimental soil heterogeneity, measured by initial total soil N, did not significantly affect any measured properties. Thus, we eliminated it from further analyses. We used multivariate analysis of variance (MANOVA) to compare functional group composition among the three control treatments, one-way analyses of variance (ANOVA) followed by Tukey comparison of means to compare measured properties among all treatments, and backwards elimination multiple regressions to investigate relationships among measured properties. Multiple regressions were checked for problems of multicollinearity using the criteria in Freund and Littell (1991). No problems were found. All data met equal variance and normality assumptions of the ANOVA except leached N and 1995 *Schizachyrium* reproductive output, which were log-transformed to better fit the assumptions. Statistical analyses were performed with SYSTAT 8.0 for Windows (SPSS Inc. 1998).

Results

Functional group responses to the experimental manipulation

In general, the removal of biomass in 1993 led to decreased plant cover in 1994. In this year, total plant cover was significantly, negatively related to biomass removal level (0, 1, or 2 functional groups or the random biomass equivalent removed), both when all treatments were considered together (simple linear regression $F = 32.1$, df = 1, 36, $P < 0.0001$) or when only the control treatments (CONT, RBL, and RBH) were considered (simple linear regression $F = 33.6$, df = 1, 12, $P < 0.0001$). This relationship disappeared after 1994 (Fig. 1A).

The functional groups responded to the initial removal of biomass differently, which was most evident in the treatments with just one functional group remaining. *Schizachyrium scoparium* reproductive output was significantly greater in the C4 treatment than in all but one other treatment in 1994 ($F = 6.02$, df = 5, 20, $P = 0.001$; C3/C4 treatment not significantly different) and all other treatments in 1995 ($F = 13.2$, df = 3, 14, $P < 0.001$). This allocation to reproductive stems led to high aboveground biomass in these years, but the strength of this reaction decreased over time (Fig. 1B). Despite *Schizachyrium*'s increased reproductive output, C4 recruitment in the C4 treatment was low. Spaces created by the removal of competitors remained open, as seen by this treatment's consistently low total plant cover (Fig. 1A) and large areas of open ground even four years after the initial removal. In contrast, in the C3 treatment, from which C4s and forbs were removed, C3s quickly filled in open ground via rhizomes. Although this functional group was a relatively minor component of the original grassland community (average 12% of total plant cover in 1995 CONT plots), total plant cover in the C3 treatment was similar to or greater than that in the CONT treatment after 1993 (Fig. 1A). Unlike in the C4 treatment, plant cover was relatively evenly distributed and aboveground biomass low (Fig. 1B). Total plant cover in the F treatment, with just forbs, tended to be lower than in the C3 treatment, but higher than in the C4 treatment (Fig. 1A).

Despite the strong differences in the responses of the two graminoid groups to the experimental manipula-

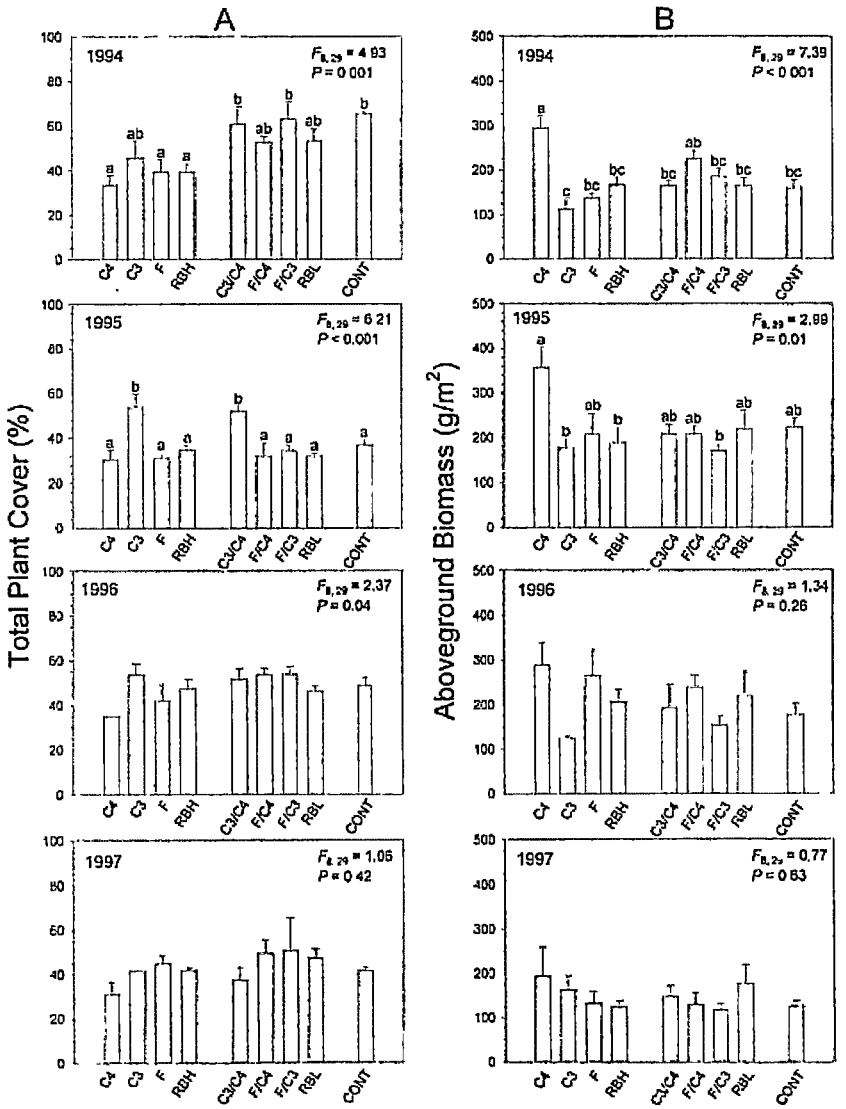

Fig. 1. Total plant cover (A) and aboveground biomass (B) for each treatment over the four years of the experiment. Values for 1996 and 1997 are for the non-drought subplots only. *F*- and *P*-values from one-way ANOVAs on all treatments are shown for each year. Significant differences among treatments within a year are indicated by different letters. In this and the following figures, treatment abbreviations indicate the functional groups *remaining* in a treatment or the random biomass removal level and are as follows: F = Forbs only; C3 = C3s only; C4 = C4s only; F/C3 = forbs and C3s; F/C4 = forbs and C4s; C3/C4 = C3s and C4s; RBH = high random biomass removal; RBL = low random biomass removal; CONT = no biomass removal (control). Treatments are grouped by biomass removal level (C4, C3, F, RBH = 2; C3/C4, F/C4, F/C3, RBL = 1, CONT = 0). Bars are treatment means, and error bars are 1 se.

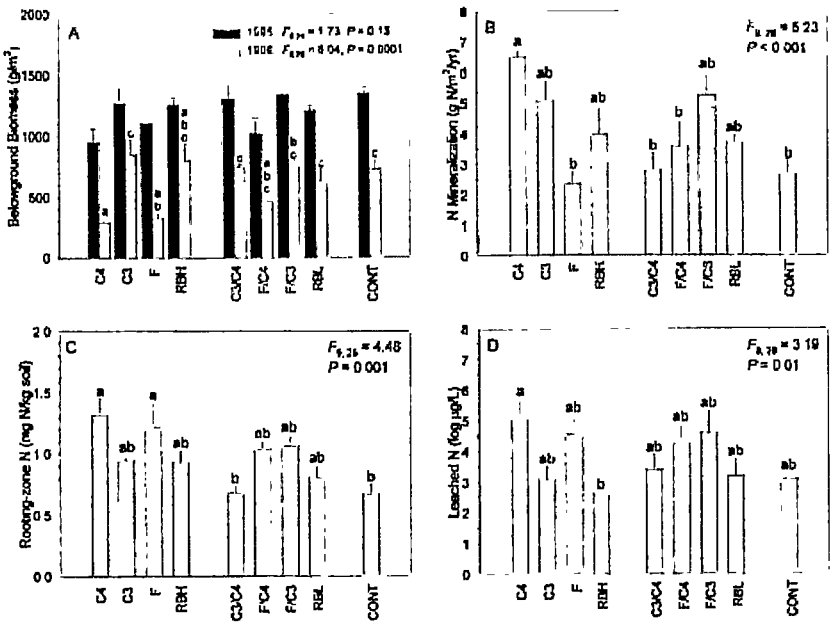

Fig. 2. (A) Belowground biomass in 1995 and 1996. Values for 1995 are from 0–60 cm depth, averaged over the two subplots, while those from 1996 are only from 0–30 cm depth in the non-drought subplot. (B, C, D) Aspects of N cycling by treatment in 1995, averaged over the two subplots. F- and P-values from one-way ANOVAs on all treatments are shown. Significant differences among treatments within a year are indicated by different letters. Bars are treatment means, and error bars are 1 se.

tions, MANOVAs showed that there was little change in the abundance of each functional group in the random biomass removal treatments. Forb biomass was significantly higher in the RBH treatment than in the CONT treatment in 1994 (Pillai's Trace = 1.01, $F = 3.39$, df = 6, 20, $P = 0.018$), but this effect was transient. In 1995, there was no difference in distribution of biomass or percent cover of the three functional groups among the RBL, RBH, and CONT treatments (biomass: Pillai's Trace = 0.04, $F = 0.61$, df = 6, 20, $P = 0.72$; percent cover: Pillai's Trace = 0.53, $F = 1.20$, df = 6, 20, $P = 0.35$). This lack of difference ($P > 0.10$) among control treatments' plant composition continued in 1996 and 1997, when the abundance of individual functional groups was measured by percent cover only.

Ecosystem functioning

Productivity and N cycling

There were no significant differences among the three control treatments for any of the productivity or N cycling properties except total plant cover in 1994 (Figs 1, 2). All of these properties did show significant differences among the nine treatments in at least one of the years in which they were measured, however. Aboveground productivity in the C4 treatment was significantly higher than other treatments in 1994 and 1995, but this difference declined through time (Fig. 1B). Relative values of belowground biomass among treatments were consistent in the two years in which this property was measured, but treatments differed significantly only in 1996 (Fig. 2A). The F, C4, and F/C4 treatments had relatively low belowground biomass compared to other treatments. N mineralization, a measure of N supply to plants, was greatest in the C4 treatment (Fig. 2B). As would be expected, relative values among treatments were similar for rooting-zone and leached N (Fig. 2C, D). N in the rooting zone, a measure of N availability to plants, was significantly higher in the F and C4 treatments than in the C3/C4 and CONT treatments, and leached N was significantly higher in the C4 treatment than in the RBH treatment.

The relatively high levels of rooting-zone and leached N in the C4 treatment and the low level of plant cover in this treatment in 1995 suggested that these properties might be related. However, N supply and plant productivity could also affect N availability. Multiple regression analysis with backwards elimination showed that rooting-zone N was negatively related to total plant cover ($F = 5.44$, df = 1, 35, $P = 0.03$) and positively related to N mineralization ($F = 7.42$, df = 1, 35, $P = 0.01$), but not related to above- or belowground biomass ($P > 0.2$ for both).

Community drought resistance

Experiment-wide, aboveground biomass was 45% lower in drought subplots than in non-drought subplots ($t = 7.43$, df = 35, $P < 0.0001$). Aboveground biomass in three treatments was not significantly affected by the imposed drought; t-tests showed that the drought resistance indices for the C4, F, and F/C3 treatments were not significantly different from 1. For the C4 treatment, which had only C4s remaining, and the F/C3 treatment, from which C4s were the only group removed, drought resistance was consistently high so that aboveground

productivity was not significantly different between drought and non-drought sides of the plots. On the other hand, large variability in drought resistance among plots in the F treatment caused the lack of a statistically significant drought effect, even though mean drought resistance was relatively low (Fig. 3). Aboveground productivity in all other treatments was significantly, negatively affected by the drought.

Drought resistance differed significantly among the nine treatments, with the F/C3 treatment having significantly higher resistance than four of the eight other treatments and the C4 treatment having similarly high resistance (Fig. 3). Although the C4 and C3 treatments had relatively high drought resistance, the treatment with both of these functional groups (C3/C4) had the lowest resistance of all. There were no significant differences among random biomass treatments and the unmanipulated control (RBL, RBH, and CONT; Fig. 3). Community drought resistance was unrelated to drought-side belowground biomass or soil moisture in either month that moisture was measured ($P > 0.5$ in multiple regressions).

Discussion

This experiment was designed to investigate the effects of removing functional groups on ecosystem functioning. At the outset, we anticipated simple increases in productivity, nutrient capture, and drought resistance as functional diversity increased. Statistical analyses (not shown) of these a priori hypotheses confirmed only the nutrient capture-diversity prediction. Productivity was unrelated, and drought resistance negatively related, to functional group richness. On the other hand, we observed strong effects of the removal of particular functional groups on ecosystem functioning. These effects seemed to stem from an unanticipated consequence of the loss of taxa: recruitment limitation. Variance in recruitment ability among functional groups left treatments within and among our functional group richness levels at different levels of establishment, making the analysis of our results in a functional group richness context problematic. However, as we discuss below, the results yielded valuable insights regarding how recruitment limitation and functional group interactions influence the effects of losing taxa from an established community.

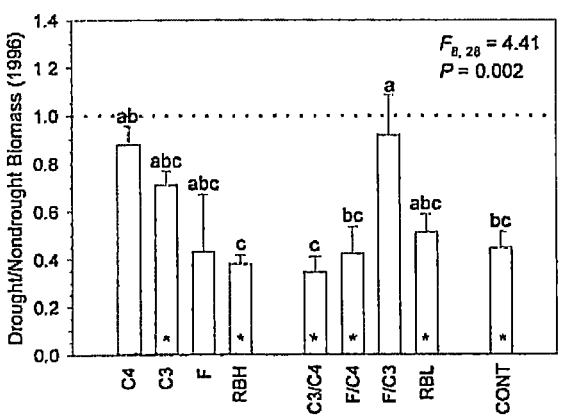

Fig. 3. Mean (+1 se) drought resistance of experimental treatments, where drought resistance of a plot is the ratio of drought-side to nondrought-side aboveground biomass. F- and P-values are from a one-way ANOVA on all treatments. Significant differences in resistance among treatments are indicated by different letters above a bar. Dotted line at $y = 1$ indicates no drought effect. An asterisk in a bar indicates that the treatment mean resistance is significantly different from 1.

Evidence of recruitment limitation

Three lines of evidence show that recruitment limitation, or the lack of new individuals becoming established, of at least one functional group occurred in this experiment. First, the lack of effects of random biomass removal on ecosystem properties demonstrates that it was not just removal of biomass, but removal of particular functional groups, that caused the observed treatment effects. An initial difference in total plant cover among control treatments lasted just one year. Thus, when all functional groups remained in a plot, it did not take long for the plant community to fill in open space. Treatments with one functional group remaining, however, varied considerably in the amount of time it took to recover from the original disturbance, suggesting that individual functional groups responded differently to the removal of competing functional groups.

Second, the high levels of rooting-zone and leached N, as well as low levels of belowground biomass, in the C4 treatment were inconsistent with previous studies in which recruitment was not limited. In high-density, seeded monocultures of the most abundant grasses in this ecosystem, *Schizachyrium scoparium* (C_4), *Poa pratensis* (C_3), and *Agropyron repens* (C_3), *Schizachyrium* had greater belowground biomass and lower rooting-zone N than *Poa* and *Agropyron* (Wedin and Tilman 1990, Tilman and Wedin 1991). Thus, we expected that treatments containing C4s would have lower rooting-zone and leached N than the treatments with just C3s or forbs. This was not the case, however, because of the low densities of C4s in the C4 only plots.

Finally, differences among treatments in aboveground productivity and total plant cover decreased over time. This, along with the difference in spatial distribution of individuals in the C3 versus C4 treatments, suggests that the C4 treatment, and perhaps others, had not recovered from the 1993 biomass removal even after five growing seasons.

Differences in functional group responses affected ecosystem functioning

Many of our results stem at least partly from the different response of the dominant C3s and C4s to release from competition with other functional groups. The rhizomatous growth form of the dominant C3s allowed them to spread quickly into openings. The significant relationship between total plant cover and rooting-zone N, but not between belowground biomass and rooting-zone N, suggests that it was this colonization of open space, not allocation to roots, that determined nitrogen uptake in this experiment. Although rhizomes do not take up nutrients, their ability to put plants in resource-rich areas probably contributed to the lower levels of rooting-zone and leached N in treatments with C3s compared to treatments without this functional group (Fig. 2A).

On the other hand, the C_4 perennial bunch grass *Schizachyrium scoparium*, which does not colonize vegetatively (Tilman and Wedin 1991), responded to the removal of other functional groups by increasing reproductive output. Despite this allocation to reproduction, *Schizachyrium*'s recruitment was low even though ample resources for plant growth existed in the C4 plots, as evidenced by their high invasibility both by weeds and by seeds purposely added in a small part of each plot (Symstad 2000). This lack of recruitment prevented the C4s from reaching a state in which they could have the effect on N use seen in other high-density experimental monoculture plots (Wedin and Tilman 1990, Tilman and Wedin 1991). C_4 graminoids that did colonize the C4 treatment, the annual grasses *Setaria glauca* (L.) P. Beauv. and *Panicum capillare* L. and the short-rhizomed sedges, *Cyperus filiculmis* Vahl. and *C. schweinitzii* Torr., were rare in the undisturbed controls. This suggests that these disturbance-adapted, early successional species are probably poor competitors for nitrogen (Gleeson and Tilman 1990) and therefore would not have decreased rooting-zone N levels in the C4 treatment to a great extent. N supply, measured by N mineralization, also seemed to be affected by the responses of the two graminoid functional groups, but whether it caused or resulted from differences in N availability is difficult to determine.

Recruitment limitation of C4s may have also played a role in the high drought resistance of the C4 treatment. Based on the physiological and morphological traits we used to separate plants into functional groups, we expected that C4s would fare well compared to the other functional groups during this drought. This was true when C4s were alone (C4 treatment), but all other treatments containing this functional group had relatively low drought resistance. We believe this disparity was caused in part by the relatively low density of plants in the C4 treatment. The open ground in these plots meant that individual plants had less competition from neighbors than would be expected in the absence of recruitment limitation. Those individuals that remained seemed quite adept at using the soil water available, however, since aboveground productivity in the C4 treatment was similar in drought and non-drought subplots. Since C3s were not slow to recruit into open ground, it is unlikely that the relatively high drought resistance of the C3 and F/C3 treatments (Fig. 3) was an artifact of the removal manipulation.

Functional group interactions

Although differences in recruitment ability among functional groups were a major factor in our experiment, interactions between remaining functional groups also affected some aspects of ecosystem functioning, especially drought resistance. Our experimental drought did not yield the positive relationship between plant diversity and community drought resistance seen in this ecosystem during a natural drought in 1987–1988 (Tilman and Downing 1994, Tilman 1996; Fig. 3). This may be because our diversity gradient was not confounded by nitrogen fertilization, a factor that some have suggested caused the diversity-stability relationship in the earlier study (Givnish 1994, Huston 1997; but see Tilman et al. 1994, Tilman 1999). Moreover, it may be that species diversity, which we did not manipulate, is a more important determinant of stability than functional group diversity.

However, we believe the main difference in results between the two studies comes from a difference in the dominant type of interaction between species or functional groups during the drought. Tilman (1996) proposed that the diversity-stability relationship during the natural drought resulted partly from compensatory growth of sub-dominant species released from competition when dominant species were negatively affected by the drought in 1987 and 1988. In our experiment, however, such compensatory growth apparently did not occur. Instead, competition between at least two functional groups remained strong, perhaps because of the shorter duration of our drought.

Though not statistically significant, the relatively low drought resistance of the four treatments containing both graminoid functional groups compared to resistance of the C3 and C4 treatments is striking. Although some of this disparity is likely due to the density effect in the C4 treatment discussed earlier, we suspect that differences in the growing seasons of these two functional groups was also important. Unlike the 1987–1988 natural drought, in which the water table was low and soils were dry at the beginning of the 1988 growing season, the drought in this experiment began relatively late in the C3s' growing season. Thus, their seasonal productivity may not have been as limited by the rain shelters as was the productivity of the C4s. In treat-

ments with both types of graminoids, the C3s may have depleted the soil water enough so that there was little for the C4s to use when they began growing, suggesting competition instead of complementation in the more diverse treatments. Such an effect was seen during the 1930s "Dust Bowl", when *Agropyron smithii*, a C_3 grass, increased in abundance at the expense of C_4 species in the grasslands of the central United States (Weaver 1954, Bazzaz and Parrish 1982). Direct competition between C_3 and C_4 grasses, despite their small overlap in growing season, has also been shown in a grassy field by Fowler (1981). Because C3s comprised a relatively minor component of aboveground plant biomass in treatments with both graminoids in our experiment, their water use may have been disproportionately high compared to their contribution to biomass and therefore community drought resistance. Unfortunately, we have no early season soil moisture data to directly test this hypothesis.

In contrast to this possible competitive interaction between C3s and C4s, the high drought resistance of the F/C3 treatment suggests a complementary interaction between forbs and C3s. Since the dominant C3s in this ecosystem are shallowly rooted, they may have not depleted deeper water resources available to some forbs through their tap roots. Thus, spatial partitioning of resources, in addition to the C3s' early season water use, could explain this treatment's high drought resistance.

If interactions between different functional groups differed as we suggest here, caution should be used when trying to predict the response of a whole ecosystem to a disturbance from the reaction of its individual components to that same disturbance. How competition and complementarity affect ecosystem functioning depends strongly on the identity and interactions of the organisms present (Hooper 1998, Wardle et al. 2000).

Defining meaningful functional groups

The functional groups we used in this experiment were defined based on characteristics suspected to be related to the ecosystem functions of interest. The results of the experiment show at least two shortcomings of our functional group definitions, however. First, our results suggest that a functional group consisting of all forbs was too broad, since this group did not react to the removal of other groups in as distinct a manner as did the two graminoid groups. Its diversity was reflected in the large variation in drought resistance among plots within the F treatment (Fig. 3) and in its high species richness (nearly 70% of the 68 species observed in the experimental plots were forbs). Because this group encompassed plants ranging from small, annual, early season plants such as *Hedeoma hispidum* Pursh. to robust, perennial, deeply rooted, late season species such as *Artemisia ludoviciana*, the high variation among plots within the F treatment is not surprising. Since the beginning of our experiment, other authors have cautioned against using broad life-form categories as plant functional groups (Lavorel et al. 1997).

Second, our results suggest that functional group definitions need to be based on more than characteristics directly related to the function of interest. In our case, the functions of nutrient uptake and drought resistance suggested differences in rooting structure and growing season should be key elements in defining functional groups. However, the removal method we used to establish our experimental treatments revealed the importance of life history characteristics to ecosystem functioning. Considerable effort and progress has been made regarding the concept of functional groups since we began this experiment (e.g. Wilson and Roxburgh 1994, Lavorel et al. 1997, Smith et al. 1997, Wilson 1999), however, and much of it has yielded results similar to ours. Since functional groups are generally used to simplify ecological complexity for the purpose of predicting ecosystem responses to environmental changes, reproductive methods and colonization ability are now regularly considered in categorizing plants into functional groups (Grime et al. 1988, McIntyre et al. 1999 and references therein).

Understanding how diversity loss affects ecosystem functioning

Through this removal experiment, we found that diversity loss could affect ecosystem functioning in ways not yet shown in experiments with synthesized communities. The results of a similar removal experiment (Wardle et al. 1999), though not designed to address the diversity-ecosystem functioning question directly, were mostly consistent with ours. In both experiments, competition between functional groups was illustrated by the compensatory growth of the remaining plants when a functional group was removed. Indeed, in both studies, the removal of one or more functional groups stimulated the growth of a remaining species, at least temporarily. In Wardle et al. (1999) the removal of C_3 grasses resulted in greater cover of *Trifolium repens* during the cooler part of the growing season, and in our experiment, removing C_3 graminoids stimulated the reproductive output, and therefore aboveground biomass, of *S. scoparium* for the first two years after the initial removal. Compensation was not complete in either experiment, however, as shown by the low root biomass in treatments lacking C_3 graminoids in both experiments, as well as by low warm-season productivity in plots lacking C_4 grasses in Wardle et al. (1999) and somewhat low, patchy plant cover in the treatment containing just C_4 graminoids in the present study. The main difference in results between the two experiments

lies in the effects of removing functional groups on ecosystem properties of soil nutrients. Wardle et al. (1999) found generally very little effect of their removals on soil NO_3^- and NH_4^+ or other soil properties measured, which they attributed to the slow turnover time of some of these properties and to the possible buffering effect of the high amount of organic material in the soil. In contrast, we found a significant effect of the removal of C_3 graminoids on rooting-zone and leached NO_3^- and NH_4^+, which we attribute to the recruitment limitation of the remaining functional groups, particularly C_4 graminoids. Thus, diversity loss could have different effects on ecosystem functioning depending on the abilities of remaining species to occupy spaces left by taxa lost from a system (Grime 1998). These recruitment abilities were apparently good in all functional groups of the Wardle et al. study but were variable in our experiment.

Many others have shown that recruitment limitation can affect plant population dynamics and community structure, composition, and diversity (e.g. Cowles 1899, Platt and Weis 1977, Gross and Werner 1982, Howe et al. 1985, Huston and Smith 1987, Eriksson and Ehrlén 1992, Tilman 1990, 1994, 1997a, Ribbens et al. 1994, Clark et al. 1998, Hubbell et al. 1999, Lepš 1999). These in turn can influence ecosystem functioning. Therefore, understanding the role of recruitment limitation in ecosystems is important for predicting the short- and long-term effects of declining diversity. When a species is lost from an ecosystem, perhaps through climate change (Solomon and Kirilenko 1997), nitrogen deposition (Vitousek 1994), or the introduction of a pathogen, the immediate response of the ecosystem depends on the recruitment abilities of the species remaining. The transient response would be for a lost species to be replaced by one with high recruitment levels. If there is, on average, a tradeoff between recruitment ability and competitive ability (Horn and MacArthur 1972, Hastings 1980), this would mean that the loss of a dominant species would first lead to increased abundances of poor competitors that were better colonizers. Only after sufficient time for better competitors to colonize had passed would some or most of the lost functional capabilities of a given species be replaced by those of other species, assuming that such species were in that region. As such, recruitment limitation and the less efficient resource use that it could cause may be major responses to anthropogenic ecosystem simplification. This study showed how these responses occurred on local scales – scales of meters – for which five growing seasons was insufficient time to overcome them. Models of forest response to rapid climate change offer some examples – decreased productivity and diversity (Sykes and Prentice 1996) and reduced carbon storage (Solomon and Kirilenko 1997) – of the consequences of recruitment limitation on larger scales. Thus, the possibility that the loss of diversity might have long-lasting, though transient, effects because of recruitment limitation merits further exploration.

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