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Integrated responses of grassland biodiversity and ecosystem properties to hay management: A field experiment

Bryan L. Foster[1], Irene S. Khavin[2], Cheryl A. Murphy[1], Val H. Smith[1], Matthew E. Ramspott[3], Kevin P. Price[4] and Kelly Kindscher[5]

1. *Department of Ecology and Evolutionary Biology, Haworth Hall, 1200 Sunnyside Avenue, University of Kansas, Lawrence, Kansas 66045-7534 USA, bfoster@ku.edu 785-864-4361, FAX: 785-864-5860.*
2. *School of Medicine, Translational Research Laboratories, 125 South 31st Street, University of Pennsylvania, Philadelphia, Pennsylvania 19104-3403 USA*
3. *Department of Geography, Gunter Hall, 101 Braddock Road, Frostburg State University, Frostburg, Maryland 21532-2303 USA*
4. *Department of Geography, 230 Lindley Hall, University of Kansas, Lawrence, Kansas 66045-7534 USA*
5. *Kansas Biological Survey, Higuchi Hall , 2101 Constant Avenue, University of Kansas, Lawrence, Kansas 66047-3759 USA*

We examined effects of fertilization and haying on grassland biodiversity, primary production and other ecosystem properties in a hay management experiment conducted in northeastern Kansas. We examined relationships between biodiversity and ecosystem productivity across our experimental landscape and evaluated relationships of biodiversity and ecosystem properties to a remotely-sensed Normalized Difference Vegetation Index (NDVI). Fertilization increased production, measured as standing crop biomass and seasonally integrated NDVI and altered a range of ecosystem traits above- and belowground. Shifts at the ecosystem level in response to fertilization were accompanied by declines in plant and arthropod diversity, and negative relationships of both to gradients of standing crop and NDVI, the magnitude of which was altered by haying disturbance in the case of plants. Bacterial diversity showed no response to fertilization, but was increased by haying. In contrast to plants and arthropods, bacterial diversity showed a positive relationship to productivity, although this association was obscured by opposing influences of fertilization on plant productivity and soil pH. Correlations of biodiversity and ecosystem traits to NDVI indicate that it is feasible to develop predictive models using remote-sensing to monitor management influences and landscape patterns of plant and arthropod biodiversity and ecosystem function in these cool-season grasslands.

Keywords: Grassland biodiversity, ecosystem properties, hay management

Introduction

In natural, semi-natural and intensively-managed grassland ecosystems, nutrient supply and disturbance are among the most important factors affecting biodiversity and ecosystem function (Pickett and White 1985; Wilson and Tilman 1991; Pimentel et al. 1992; Huston 1994; Matson et al. 1997; Collins et al. 1998; Smith et al. 2000). Plant production, which is an integrative measure of overall ecosystem function, is frequently limited by nitrogen (N) as illustrated by correlations between productivity and N fertility along environmental gradients and by responses to nutrient enrichment (Vitousek and Howarth 1991; Schlesinger 1997). Disturbances such as fire, grazing, and haying often affect primary production directly by removing photosynthetic biomass, but a disturbance can also have various indirect and feedback effects on production and other ecosystem processes

depending upon the type and intensity of disturbance (McNaughton 1985; Knapp and Seastedt 1986; Dyer et al. 1991).

Plant diversity in grassland landscapes often declines in response to nutrient enrichment and may either increase or decrease in response to disturbance, depending on the frequency or intensity of disturbance and the level of soil fertility (Grime 1979; Tilman 1988; Huston 1994; Collins et. al. 1998; Grace 1999; Wilson and Tilman 2002). Across a landscape, plant diversity is often correlated with primary production, frequently exhibiting a unimodal relationship (Al-Mufti et al. 1977; Grime 1979; Tilman and Pacala 1993; Huston 1999). It is unlikely that this well known "hump-shaped" curve results solely from direct influences of productivity on plant diversity, but also from direct and indirect influences of soil properties and disturbance regimes which interact to produce the complex landscape gradients in resource supply, primary production and species composition to which plant diversity is often correlated (Grime 1979; Huston 1979; Grace 1999; Huston 1999).

In this paper we examine the effects of fertilization and annual haying regime on components of grassland biodiversity and ecosystem properties in the initial years of a long-term hay management experiment in eastern Kansas (USA). We also evaluate relationships that have developed between indices of biodiversity and plant productivity across our experimental landscape. Indices of productivity and ecosystem function used in this study include the peak standing crop of plants (hay yield) and a seasonally-integrated, vegetation index derived from remotely-sensed spectral reflectance data: TINDVI (time-integrated, normalized difference vegetation index).

Most studies examining effects of grassland management (grazing, haying, burning, fertilization) on biological diversity have focused on a single group of organisms, most frequently the terrestrial plants, but less frequently arthropods (Haddad 2000). Very few studies have examined responses across multiple taxonomic groups and fewer still have included soil microorganisms. The omission of soil microorganisms is unfortunate because this group is intimately involved in mediating important plant-soil interactions and ecosystem processes such a nutrient cycling. This lack of attention is understandable given that the techniques required to estimate soil microbial diversity are still in the developmental stages. In this study we examine effects of hay management on the communities of plants, arthropods and soil bacteria. We ask if indices of diversity for these three groups respond in a similar fashion to manipulations of fertility, to haying and to resulting spatial gradients in primary productivity that have developed across our experimental grassland landscape. If groups of organisms as different as plants, arthropods and soil bacteria jointly respond to these factors in a predictable manner, it may be feasible to develop monitoring approaches for predicting patterns of diversity of the larger biotic community across managed grassland landscapes using readily-measured environmental correlates such as plant biomass or remotely-sensed vegetation indices as predictors.

The Normalized Difference Vegetation Index (NDVI), derived from remotely-sensed spectral reflectance data, is frequently used as an indicator of photosynthetically-active biomass and primary production, and thus represents a synthetic index of ecosystem function (Dyer et al. 1991; Skidmore et al. 2003). In recent years, NDVI has also been employed to evaluate regional and landscape patterns of biodiversity (Gould 2000; Fairbanks and McGwire 2004; Levin et al. 2007). The use of NDVI for biodiversity assessment rests on the assumption that robust relationships exist between biodiversity and biophysical characteristics of the landscape that are discriminated by NDVI. If such relationships can be consistently found, this would allow development of predictive

models and remotely-sensed monitoring schemes to evaluate biodiversity, ecosystem function and links between them across entire landscapes. Here we investigate the response of TINDVI to fertilization and haying, and evaluate relationships of TINDVI across our experimental landscape to biomass production, above-and below-ground ecosystem properties and grassland biodiversity.

Methods

Study site

The study was conducted in a former cool-season hayfield at the University of Kansas Field Station which is located in the deciduous forest-tallgrass prairie ecotone of northeastern Kansas (USA). Soils are clay loams, formed from glacial till (Kettle and Whittemore 1991). Mean annual precipitation is 930 mm and mean annual temperature is 12.9˚C. The field site has a long history of cultivation, but more recently was used for cool-season hay production. The site may have been fertilized as recently as 1987, but after that point in time the site was maintained solely by periodic mowing until 1997 or 1998 when mowing was ceased. At the start of this study in 2000, the site was dominated by introduced C_3 grasses previously planted for cool-season hay: *Bromus inermis* Leyss and *Lolium arundinaceum* Schreb.

Experimental design

March 2000 we implemented a 49 x 101 m experimental landscape (Fig. 1) by establishing a 4 x 4 grid of sixteen 10m x 20 m plots separated by 3 m buffer strips. We further divided each plot into two 10 m x 10 m subplots, and assigned a 2 x 2 factorial set of treatments to all 32 subplots in a split-plot design: two levels of nutrient fertilization (fertilized; not fertilized) applied as the whole-plot factor and two levels of haying (hayed; not hayed) applied as the split-plot factor.

In April of each year we spread NPK fertilizer (29-3-4) at 14-16 g N/m^2 per year. These rates are at the high end of what is typically applied to cool-season hayfields of this region. From 2001 through 2004 appropriate subplots were hayed once annually in mid-June at the time of peak standing crop as is typical for cool-season hay management in our region.

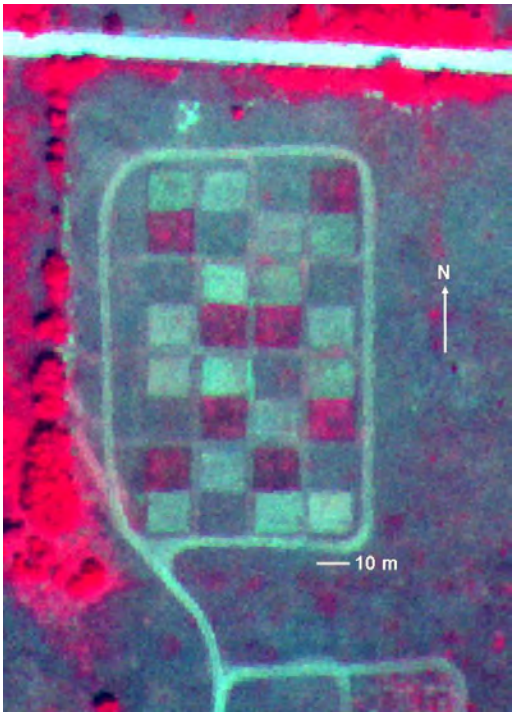

Figure 1. Multi-spectral image of the experimental landscape taken 3200 m ASL in June 2003 with a DuncanTech, MS 3100 digital multi-spectral camera mounted in a single engine light aircraft. The experimental landscape is comprised of 16, 10 x 20 m plots, each divided into two 10 x 10 subplots.

Vegetation sampling and processing

In mid-June 2003 and 2004, we sampled aboveground plant biomass, prior to haying. With electric clippers we harvested two 0.8 x 2 m strips of biomass located randomly in each subplot. We clipped biomass to ground level and collected litter in each strip. We separated each sample into live and litter fractions, and sorted live fractions to species. Biomass was dried to constant mass at 74˚C and weighed. In 2003, the live fraction was ground and analyzed for carbon (C) and nitrogen (N) content using a CHN Combustion Analyzer

(Carlo Erba, Milan Italy). Also, in mid-June 2003 (prior to haying), we recorded all plant species in each subplot to provide a robust estimate of subplot species richness.

Arthropod sampling and processing
We collected arthropod samples mid-June 2003, just prior to haying. Arthropods were sampled with a sweep net along six transects in each subplot (25 sweeps per transect; each sweep approximately 2 m in length). Arthropods were transferred to a jar with ethyl acetate as a killing agent. Later, samples were sorted to species or morphospecies (Oliver and Beattie, 1994) and enumerated.

Soil sampling and processing
In mid June 2003, we collected soil for analyses of pH, total soil C and N content and P availability. For each sub-plot, three soil cores were taken to a depth of 15 cm with a tube sampler (diameter = 2.54 cm). Cores were mixed to generate one composite sample per sub-plot. Litter and root material were removed from the samples before soils were homogenized and air-dried for analysis. C and N were analyzed using a LECO CN dry combustion analyzer (Yeomans and Bremner 1991). Soil available P (Bray P) was analyzed colorimetrically (Bray and Kurtz 1945). Soil pH was measured with a pH meter from a 1:1 slurry of soil and deionized water. Soil texture was measured using the hydrometer method.

Prior to haying in early June 2004, additional soil samples were collected for analysis of bacterial communities using the same sampling approach described above. Samples were shipped on ice to Los Alamos National Laboratory. Soil bacterial communities were analyzed using Terminal-Restriction Fragment Length Polymorphism (T-RFLP) Analysis, a technique that measures variation in small-subunit (16S) ribosomal DNA sequences. For each sample, two sub-samples of DNA were extracted using the UltraClean DNA Isolation Kit (MoBio Lab, Inc.) and DNA yields quantified using EtBr stained agarose gels. Polymerase Chain Reaction (PCR) was used to amplify bacterial 16S rDNA using the FAM-labeled forward primer 27F.1 (5'-AGRGTTTGATCMTGGCTCAG-3') and un-labeled reverse primer 787Rb (5'GGACTAcNRGGGTATCTAAT-3'). Each 50 microliter reaction contained 30 mMTris, 50 mM KCl, 1.5 mM MgCl2, 50 uM each dNTP, 50 pmol each primer, 0.75 units Taq polymerase (AmpliTaq LD; Perkin Elmer, Foster City, CA) and 200 pg soil DNA. Cycling conditions were: 4 minutes denaturation at 94C, 35 cycles of 45 s at 55C, 1 min at 72C, 30 s at 94C, and a final cycle of annealing at 55C for 45 s and extension at 72C for 5 min. Three reactions were performed for each sample and combined. PCR products were purified from gels using a Millipore Montage DNA gel extraction kit and pooled. PCR products were digested with restriction enzymes RsaI and samples run on acrylamide gels using an ABI 377 sequencer. Each sample was run on each of three different gels to control for potential gel-to-gel variability. Peaks between standard markers 37 and 827 were digitized and the three technical reps were averaged as described in Kuske et al. (2002). Standardization of T-RFLP profiles was conducted according to Dunbar et al. (2001).

Spectral reflectance and light interception
We obtained remotely-sensed, spectral reflectance data from over-flights of the experimental landscape on ten different dates (April 12 - October 28) during the 2003 growing season. Over-flights were conducted approximately every 20 days at an altitude of 3200 m ASL. Imagery was captured using a DuncanTech, MS 3100 digital multi-spectral camera mounted in a single engine light aircraft. The camera captures data in the red (630-690 nm) and near-infrared (760-900 nm; NIR) spectral bands. These data were used to calculate the Normalized Difference Vegetation Index (NDVI; Rouse et al., 1973) using the formula NIR - Red/NIR + Red. To optimize the consistency of our data set, all images were flown on clear days between 10:00 AM and 3:00 PM.

In late May 2003, we measured canopy interception of photosynthetically active radiation (PAR) in all subplots using a 0.8 m Accupar Ceptometer probe (Decagon Devices, Pullman, Washington). Multiple measurements below and above the plant canopy were made at two locations within each subplot. PAR interception was calculated as a percentage of full sun ([1-(PAR below canopy/PR above canopy)] x 100).

Data Analyses

Diversity Indices - We calculated taxonomic richness (*S*), evenness (*E*) and Shannon diversity index (*H´*) for each taxonomic group (plants, arthropods and bacteria). We evaluated plant richness as the total number of species recorded in a subplot (using biomass data) and arthropod richness as the total number of species or morphospecies collected in a sweep net sample. Bacterial richness was calculated as the total number of phylotypes present in the T-RFLP profile. *H´* was calculated for each taxonomic group as $-\Sigma\, p_i \times \log (p_i)$ where p_i is the proportional abundance represented by a given species, morphospecies or bacterial phylotype. Arthropod diversity (*H´*) was calculated using the relative density for each species and morphospecies in a subplot. Bacterial diversity (*H´*) was calculated using standardized phylotype peak areas (Fierer and Jackson 2006).

Time-integrated NDVI – Using the NDVI data time series, we computed the seasonal time-integral of NDVI (TINDVI) for each subplot using a trapezoidal approximation (Weideman 2010).

Analysis of variance (ANOVA) – We used split-plot ANOVA to evaluate treatment effects on diversity indices and ecosystem variables. Repeated measures ANOVA (RMANOVA) was used to evaluate seasonal changes in NDVI and interactions with experimental treatments. All NDVI and biomass data were $\log_{10}$ transformed to meet assumptions of ANOVA.

Regression analyses - We used linear regression to examine the relationship between standing crop biomass and TINDVI and to examine the dependence of organismal diversity on indices of production (standing crop and TINDVI). We performed one set of regressions to evaluate relationships across all 32 subplots. We performed a second set in some cases to evaluate relationships separately for non-hayed and hayed subplots. We conducted separate regressions for independent variables that exhibited a standing crop x haying interaction or TINDVI x haying interaction in an analysis of covariance (ANCOVA).

We used backward elimination multiple regressions to evaluate the contribution of multiple predictors of organismal diversity across our landscape. Independent variables chosen for entry into the regression models varied with the organismal group under consideration. We expected plant diversity to be influenced by productivity (standing crop biomass and TINDVI), litter biomass, light interception, and aspects of the soil physical and chemical environment (soil texture, pH, soil N and P). For arthropod diversity we expected productivity, litter, plant tissue C:N ratio, plant diversity and plant composition to be important. For bacterial diversity we expected productivity, litter, plant diversity, the soil physical and chemical environment (soil texture, pH, soil C, N and P) and plant composition to be important. The influence of plant composition was evaluated by entering Principal Components of plant composition into the regressions. Two components, generated by Principal Components Analysis (PCA), explained a total of 77% of the variation in composition within the plant data set.

Because standing crop and TINDVI were collinear, we conducted two multiple regression procedures for predicting plant and arthropod diversity: one entering TINDVI as an index of productivity and another entering standing crop. Because the results were similar for both approaches, we present results only

Table 1. Treatment effects on plant, arthropod and bacterial diversity (evaluated with ANOVA).
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Community Measure	-Fert -Hay (mean)	-Fert +Hay (mean)	+Fert -Hay (mean)	+Fert +Hay (mean)	†Significant sources of variation
Plants (2003)					
Richness	31.38	34.13	18.00	28.00	F***, H**, F x H**
Evenness	0.66	0.47	0.43	0.54	F x H**
Shannon Diversity (*H'*)	1.25	1.11	0.64	0.89	F***, F x H**
Arthropods (2003)					
Richness	44.13	53.38	46.75	47.50	ns
Evenness	0.81	0.74	0.60	0.58	F***
Shannon Diversity (*H'*)	1.33	1.28	1.01	0.97	F***
Bacteria (2004)					
Richness	16.62	26.75	17.87	23.25	H*
Evenness	0.94	0.96	0.83	0.95	ns
Shannon Diversity (*H'*)	2.47	3.13	2.32	2.91	H*

† F= effect of fertilization; H = effect of haying; F x H = interaction of fertilization and haying

from the regression models using TINDVI. For bacterial diversity, which was measured in 2004, we entered 2004 standing crop biomass as the index of productivity. Statistical analyses were performed using SPSS (version 12.0).

Results

Biodiversity responses

Plant richness and Shannon diversity declined with fertilization in both the non-hayed and hayed subplots, but to a lesser extent in hayed subplots (Fert x Hay interaction; Table 1). Haying increased plant species richness and Shannon diversity only in fertilized subplots. Plant community evenness was reduced by fertilization only in non-hayed subplots (Fert x Hay interaction). Haying reduced plant community evenness in non-fertilized subplots, but increased evenness in fertilized subplots (Fert x Hay interaction). Arthropod species richness was unaffected by the experimental manipulations. However, both arthropod community evenness, and Shannon diversity were reduced by fertilization, but unaffected by haying (Table 1). Among all experimental plots, arthropod diversity (*H'*) was positively correlated with plant diversity ($r = 0.52$. $P < 0.05$). No measure of bacterial diversity was significantly affected by fertilization. However, bacterial phylotype richness and Shannon diversity were both significantly increased by haying (Table 1). Bacterial diversity was not significantly correlated with any measure of plant or arthropod diversity ($P > 0.05$).

Ecosystem responses

NDVI varied significantly in response to fertilization, haying, season of measurement and all possible interactions (Fig. 2; $P < 0.001$ for all sources of variation in RMANOVA). NDVI exhibited two seasonal peaks: one in spring prior to haying (April-May); and the other in the fall (September-October). On the four spring measurement dates prior to haying (spring peak), NDVI was significantly greater in fertilized than non-fertilized plots ($P < 0.001$) and was significantly greater in hayed than non-hayed plots ($P < 0.001$). NDVI declined strongly in mid-June, as the hottest and driest period of the year approached. On July 24 and August 19, NDVI was significantly reduced in hayed compared to non-hayed plots. After the August minimum, NDVI increased rapidly into the cooler fall months, but particularly in the hayed plots. During the fall, NDVI was significantly greater in fertilized than non-fertilized plots ($P < 0.001$) and significantly greater in hayed than non-hayed plots ($P < 0.001$). However, the positive effects of haying on fall NDVI were of greater magnitude under fertilization (Fert x Hay interaction: $P < 0.001$).

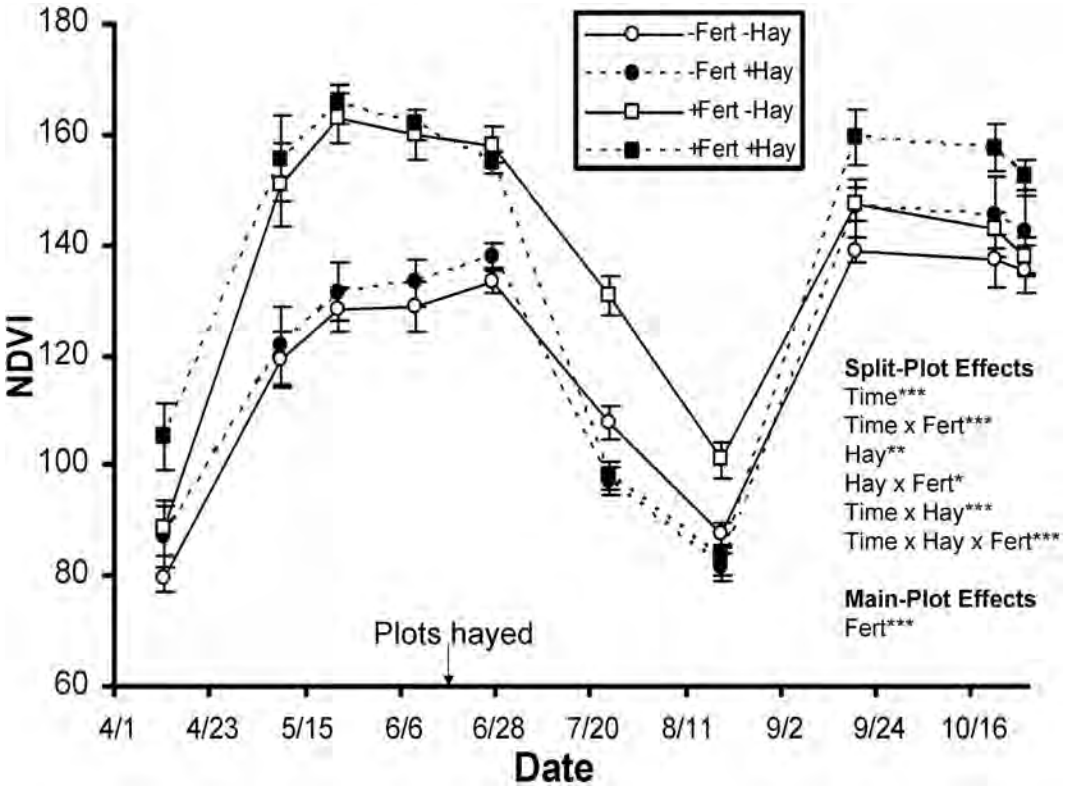

Figure 2. NDVI time series for the 2003 growing season as affected by fertilization and haying (mean ± 1 SD). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Time-integrated NDVI (TINDVI) and standing crop were increased significantly by fertilization (Table 2). TINDVI was unaffected by haying, but standing crop was increased by haying in fertilized plots (Fert x Hay interaction). Litter was decreased by haying in both non-fertilized and fertilized subplots, but increased by fertilization in the non-hayed subplots only (Fert x Hay interaction). TINDVI explained 85% of the variation in 2003 standing crop biomass when assessed across all 32 subplots ($r^2 = 0.85$, $P < 0.001$; Fig. 3A). This positive relationship reflects both within and between treatment covariation of standing crop and TINDVI. Standing crop and TINDVI were significantly correlated across replicate subplots within three of the four unique treatment combinations (+Fert/-Hay: $r^2 = 0.93$, $P < 0.01$; -Fert/+Hay: $r^2 = 0.92$, $P < 0.01$; +Fert/+Hay: $r^2 = 0.95$, $P < 0.01$). The slope of the standing crop-TINDVI relationship was significantly greater across hayed than non-hayed subplots (TINDVI x Hay interaction: $F_{1,28} = 22.92$, $P < 0.001$). TINDVI explained 91% and 93% of the variation in standing crop in the non-hayed and hayed subplots respectively. TINDVI, which was measured in 2003, was also a significant predictor of standing crop biomass measured one year later (2004), explaining 83% of the variation ($r^2 = 0.83$ $P < 0.001$; Fig. 3B). There was no TINDVI x hay interaction in 2004.

The fertilization and haying treatments influenced other ecosystem-level variables (Table 2). PAR interception increased with fertilization and decreased with haying. Leaf C and N increased with fertilization, while leaf C:N ratios declined with fertilization. Total soil C and N increased significantly with fertilization, but there were no treatments effects on soil C:N ratio. Fertilization and

Table 2. Treatment effects on ecosystem traits (evaluated with ANOVA) and the association of these traits to TINDVI. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Ecosystem Trait	-Fert -Hay (mean)	-Fert +Hay (mean)	+Fert -Hay (mean)	+Fert +Hay (mean)	†Significant sources of variation	Association with TINDVI (r^2)
TINDVI	118.19	120.01	137.11	135.40	F***	NA
Standing Crop (g m^{-2})	155.89	140.72	396.10	491.88	F***, F x H*	0.83***
Litter Biomass (g m^{-2})	175.57	61.08	343.21	45.42	F***, H***, F x H**	0.62***
Light Interception (%)	44.8	32.4	79.5	76.4	F**, H***	0.79***
Leaf C (%)	41.3	41.1	42.9	42.9	F***	0.70***
Leaf N (%)	1.33	1.36	1.76	1.72	F***	0.50***
Leaf C:N	31.20	30.21	24.46	25.24	F***	0.46***
Soil C (%)	1.50	1.52	1.75	1.72	F***	0.31**
Soil N (%)	0.13	0.14	0.16	0.16	F***	0.34**
Soil C:N	11.1	11.1	10.9	11.0	ns	0.01^{ns}
Soil P (ppm)	1.62	2.12	2.62	3.12	F***, H*	0.44***
Soil pH	5.94	6.02	5.69	5.70	F***	0.42***

† F= effect of fertilization; H = effect of haying; F x H = interaction of fertilization and haying

haying increased soil P availability, and fertilization led to a significant decline in soil pH. Litter mass, light interception, leaf C, leaf N, soil C, soil N and soil P were all positively correlated with TINDVI (Table 2). Leaf C:N and soil pH were negatively correlated with TINDVI.

Relationships between biodiversity and ecosystem variables

Across all 32 subplots, and regardless of treatment, plant species richness and Shannon diversity were negatively correlated with two key indices of plant productivity: standing crop biomass and TINDVI (Fig. 4). Across all 32 subplots, standing crop explained 42% ($r^2 = 0.42$ $P < 0.001$) and 39% ($r^2 = 0.39$ $P < 0.001$) of the variation in plant richness and Shannon diversity respectively. TINDVI was a better predictor than standing crop, explaining 53% ($r^2 = 0.53$ $P < 0.001$) and 54% ($r^2 = 0.54$ $P < 0.001$) of the variation in plant richness and Shannon diversity respectively. However, haying significantly altered the diversity-productivity relationship as indicated by significant Hay x standing crop interactions (richness: $F_{1,28} = 11.41$, $P < 0.01$; evenness: $F_{1,28} = 8.51$, $P < 0.05$; Shannon Diversity: $F_{1,28} = 15.44$, $P < 0.001$) and significant Hay x TINDVI interactions (richness: $F_{1,28} = 6.01$, $P < 0.05$; evenness: $F_{1,28} = 7.33$, $P < 0.05$; Shannon Diversity: $F_{1,28} = 4.39$, $P < 0.05$) in ANCOVA. In the case of evenness, relationships to standing crop and TINDVI were significant only in non-hayed subplots (Fig. 4 C and D). For both species richness and Shannon diversity the slopes of these relationships, and the variance explained, were reduced by haying (Fig. 4). When the backwards-elimination multiple regression was performed, we found that in addition to a strong negative association with TINDVI, plant diversity (Shannon diversity) was also negatively associated with light interception and positively associated with soil pH and soil sand content (Table 3).

Across all 32 subplots, arthropod evenness and Shannon diversity were both negatively correlated with standing crop and TINDVI (Fig. 5). Standing crop explained 60% and

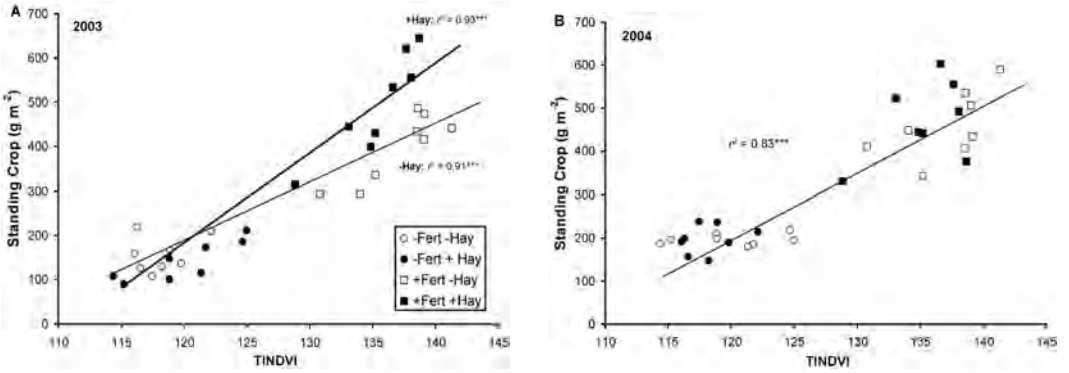

Figure 3. Relationships of plant standing crop biomass (A: 2003 and B: 2004) to TINDVI (2003). In the first panel (panel A), regression lines are drawn separately for non-hayed and hayed plots to illustrate the significant TINDVI x Hay interaction in 2003. *** $P < 0.001$.

65% of the variation in arthropod evenness and diversity respectively. TINDVI was a better predictor, explaining 70% and 74% of the variation in arthropod evenness and diversity respectively. Unlike for plants, the relationships of arthropod evenness and Shannon diversity to standing crop and TINDVI did not differ in slope between non-hayed and hayed subplots (Fig. 5). When the backwards-elimination multiple regression was performed, we found that the only predictors of arthropod diversity (Shannon diversity) retained were TINDVI and the first principle component of community composition (PC1), both with negative associations (Table 3).

No measure of bacterial diversity was significantly correlated with standing crop or TINDVI alone. However, when the backward-elimination multiple regression was performed, bacterial diversity (measured as Shannon diversity) did show a significant positive association with productivity (measured as 2004 standing crop) after taking into account correlations with other predictor variables (Table 3). Bacterial diversity in the multiple regression also showed a significant positive relationship to soil pH and the first principal component of plant community composition and showed a negative association with soil sand content.

Discussion

Biodiversity

A primary goal of our study was to evaluate the extent to which components of diversity for three different major taxonomic groups were similar in their response to fertilization and haying, as well as to landscape gradients in production that developed across our experimental landscape in response to management. Plant and arthropod diversity exhibited similar responses to fertilization and to gradients in productivity. In contrast, bacterial diversity was unaffected by fertilization, and its relation to productivity was opposite to that of plants and arthropods. Plant and bacterial richness both showed a tendency to increase in response to haying however.

Plant diversity - Declines in plant diversity with fertilization are common and have been attributed to a variety of mechanisms (Grace 1999). Increased plant biomass can reduce diversity by limiting light and excluding low-growing species (Goldberg and Miller 1990; Foster and Gross 1998). The multiple regression results support the role of light limitation. The regression model also indicated a negative association of plant diversity to soil pH, suggesting that soil acidification resulting from N fertilization may have contributed to observed diversity loss.

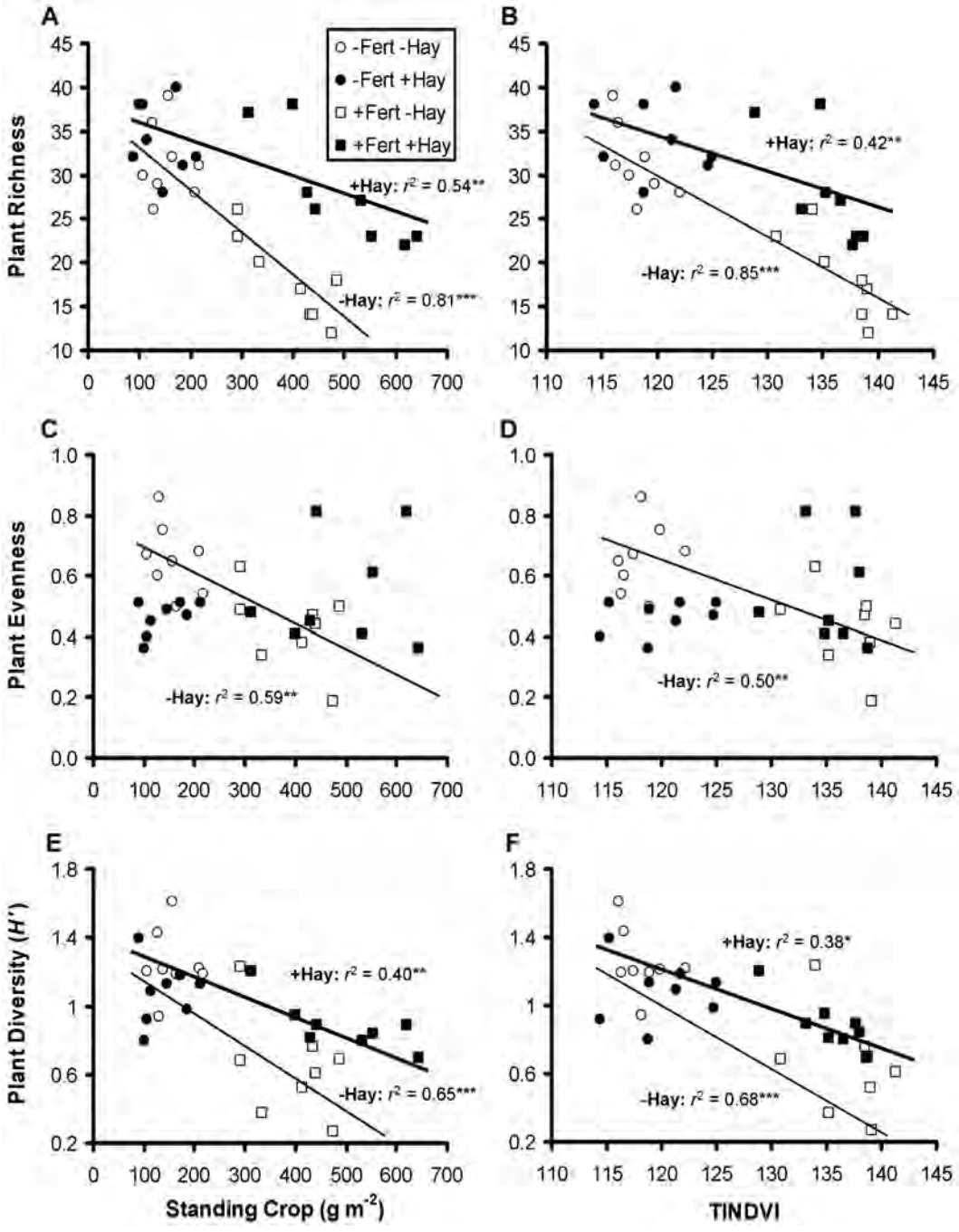

Figure 4. Relationships of plant species richness, evenness and Shannon diversity to standing crop biomass and TINDVI. For plant richness and Shannon diversity regression lines are drawn separately for non-hayed and hayed plots to illustrate significant TINDVI x Hay interactions.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table. 3. Results of backward elimination multiple regressions. Values for retained independent variables are partial correlation coefficients. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Independent Variables	Plant Diversity (H') Model $r^2 = 0.72$***	Arthropod Diversity (H') Model $r^2 = 0.78$***	Bacterial Diversity (H') Model $r^2 = 0.48$**
Productivity (TINDVI)	-0.78**	-0.83***	-
Productivity (Stand. crop)	-	-	0.47*
Litter Biomass	Entered/Excluded	Entered/Excluded	Entered/Excluded
Light Interception	-0.39*	-	-
Plant Diversity	-	Entered/Excluded	Entered/Excluded
Leaf C:N	-	Entered/Excluded	-
Soil pH	0.48*	-	0.50**
Soil C	-	-	Entered/Excluded
Soil N	Entered/Excluded	-	Entered/Excluded
Soil P	Entered/Excluded	-	Entered/Excluded
Soil Sand	0.46*	-	-0.50**
Plant Composition (PC1)	-	-0.42*	0.45*
Plant Composition (PC2)	-	Entered/Excluded	Entered/Excluded

Haying clearly tempered the negative influence of fertilization on plant diversity in our study, and weakened the relationship of plant diversity to productivity. Our finding that haying increased plant diversity at high fertility, but reduced evenness at low fertility is consistent with community models of Grime (1979), Huston (1979, 1994) and Kondoh (2000), which all predict that the effects of disturbance on diversity depend on levels of productivity or resource supply. At high productivity, disturbance is predicted to enhance diversity by freeing resources, reducing competition and promoting colonization. In line with this prediction we found that haying increased light and soil P availability and reduced the litter layer. These findings correspond with a variety of other studies illustrating interactive effects of fertility and disturbance on plant diversity (Hobbs et al., 1988; Carson and Pickett 1990; Wilson and Tilman 1991; Burke and Grime 1996; Collins et al. 1998).

Arthropod diversity - Unlike for plants, the decline in arthropod diversity with fertilization and increased production found in this study is not as common in the literature. Haddad et al (2000) found a negative effect of N fertilization on arthropod diversity in Minnesota prairie. This trend was paralleled by an increase in plant production and tissue N content. Haddad et al. (2000) also found that declines in plant diversity played a direct role in reducing arthropod diversity. Consistent with this, we found a positive correlation between arthropod and plant diversity. However, multiple regression analysis indicated that this correlation was likely incidental and more directly related to shifts in plant composition and production.

In our experiment, the negative response of arthropod diversity to fertilization arose through changes in evenness, not through changes in species richness. Fertilization increased total arthropod density (data not shown), and resulted in increased dominance by several species of midge (*Diptera*) that specialize on grass seeds, and parasitoid wasps (*Hymenoptera*) that prey upon midge larvae residing in seeds. Seed production by the abundant grass, *Bromus inermis*, a known plant host for seed consuming midges (Knowles 1973), was significantly greater in fertilized subplots (data not shown). In fertilized subplots, wasp parasitoids in the genus *Tetrastichus* were particularly common.

Bacterial diversity – Modern molecular methods offer important new tools for the assessment of soil microbial communities. We used the T-RFLP, rDNA fingerprinting technique to evaluate bacterial phylogenetic diversity. This method has been used successfully to evaluate difference in bacterial diversity, including a recent study of

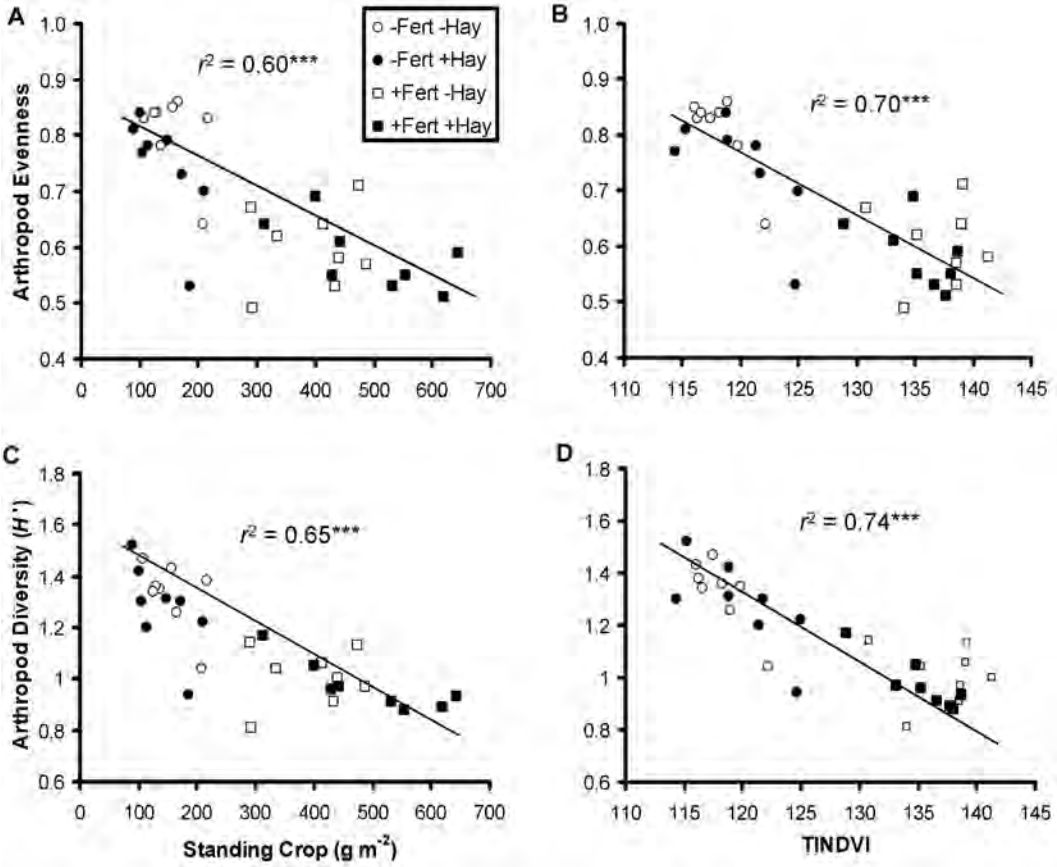

Figure 5. Relationships of arthropod density, evenness and Shannon diversity to standing crop biomass and TINDVI. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

biogeographical variation in soil bacteria across South, Central and North America (Fierer and Jackson 2005). The T-RFLP method does tend to underestimate the total bacterial diversity of a sample because it examines a limited number of bands per gel and because some taxa can share phylotypes. As in the study of Fierer and Jackson, another shortcoming of our approach was that assessments of bacterial diversity were based on soil samples taken at only one time period. Given that soil bacterial communities may change seasonally, we interpret our results with some caution.

Despite these limitations we documented a positive response of bacterial diversity to haying through an increase in phylotype richness. Although a variety of studies have documented effects of management on microbial communities (Bardgett et al. 1997; Wardle 2002; Langer and Klimanek 2006), little is known about the effects haying on grassland bacterial diversity. Haying undoubtedly altered a variety of factors that might influence microbial communities, many left unmeasured such as root biomass, root exudates and soil moisture. Soil P was increased by haying, but was not retained as a significant predictor of bacterial diversity in the regression analyses, suggesting that variation in bacterial diversity was not causally linked to shifts in P.

Bacterial diversity in our study was not correlated with the diversity of plants or arthropods. In addition, bacterial diversity

showed no response to fertilization, despite significant effects on plant productivity, plant composition and soil chemical properties. Not surprisingly, the influence of fertilization on microbial diversity and composition is strongly varied in the literature, exhibiting a variety of effects depending on the type of fertilizer used, the duration of treatment, the management context, the component of the microbial community examined (fungal versus bacterial) or the methodologies used (Bardgett et al. 1999; Sessitsch et. al. 2001; Diosma et al. 2006; Paterson et al. 2007). This suggests that biodiversity across fertility gradients may be less predictable for microbes than for macro-organisms.

For temperate grasslands a number of studies indicate that the size and activity of soil microbial communities are greater at low fertility than under conditions of N fertilization. Bardgett et al. (1999) suggested that shifts in microbial community structure in response to fertilization are indirect, arising through changes in plant composition and productivity. However, unlike the plants and arthropods, bacterial diversity in our study showed no simple response to nutrient enrichment. Although there were shifts in plant community composition in response to fertilization (data not reported), these changes were subtle and reflect changes in the relative abundance of several functionally similar dominant species (C_3 grasses). Three years of fertilization has not been long enough to cause a major turnover in plant species. We did find a significant association between bacterial diversity and plant composition, but this seems to reflect a response to variation in plant composition among plots that exists independent from effects of fertilization. We also observed a positive association of bacterial diversity to plant productivity. However, this positive relationship emerged independent from increases in plant production observed in response to fertilization and was detected only after accounting for the influence of other variables. This suggests that fertilization had opposing influences on the soil bacterial community. Although fertilization increased plant production, it also reduced soil pH, a variable positively associated with bacterial diversity in the multiple regression. That an association between productivity and bacterial diversity emerged only when pH was controlled for, suggests that any positive influence of fertilization on bacterial diversity emerging via increased production may have been countered by adverse effects of soil acidification. The relationship to pH observed in our study is consistent with the study of Fierer and Jackson (2005) who found that bacterial diversity in soils across North and South America using the T-RFLP method was positively correlated with pH, but was unrelated to other soil parameters and indices of productivity. Reductions in pH can stress many microorganisms and shifts in microbial composition and diversity have been linked to changes in pH in variety of ecosystems (Bardgett 1997; Hornstrom 2002; Bååth and Anderson 2003; Fierer and Jackson 2005)

The multiple regression analyses also found a significant negative relationship of bacterial diversity to soil sand content. This reflects correspondence between the bacterial community and underlying spatial variation in soil texture and perhaps soil moisture holding capacity across the experimental site and suggests greater diversity in plots with finer textured soils as also found by Sessitch et al. (2001).

Ecosystem properties

Fertilization and haying affected a number of ecosystem properties in this experiment. Of particular importance was the increase in primary production, measured as peak standing crop and TINDVI, observed in response to fertilization. Although TINDVI was unaffected by haying, NDVI was strongly affected by haying and by the interaction of fertilization and haying during particular periods of the year. NDVI was greatly reduced by haying immediately after haying and during the summer

months when C_3 grasses grow slowly. However, during the cool portions of the growing season (early spring and fall), haying increased NDVI, particularly in fertilized subplots, reflecting rapid compensation for lost biomass.

Effects of fertilization on plant biomass and NDVI were accompanied by changes in plant tissue chemistry: increases in C and N concentrations and a decline in C:N ratio. Such changes undoubtedly alter the quality of organic material entering the soil. This is reinforced by Billings et al (2006) that showed greater C and N soil inputs from root and leaf litter material in response to fertilization at our site. Accordingly, fertilization increased total soil C and N and available soil P. The increase in soil C was surprising given the short duration of our study and because organic C typically accumulates slowly in soils (Burke et al. 1995; Knops and Tilman 2000). However, although fertilization increased the organic inputs into these soils, additional work at the site has shown that most of the additional material was present in labile forms of C, which promoted CO_2 loss via microbial respiration rather than long-term C accumulation (Billings et al. 2006).

NDVI as an integrative predictor of biodiversity and ecosystem function

TINDVI proved to be an excellent predictor of plant biomass and light interception, justifying its use in this study as a comparative index of primary production. TINDVI explained 85% of the variation in plant biomass and over 90% of the variation in plant biomass when evaluating non-hayed and hayed plots separately. These findings suggest that NDVI can be useful for estimating production across larger areas of cool-season grassland in our region, but that the estimates would be more precise when modeled separately for frequently hayed and non-hayed or infrequently hayed areas.

In addition to its relationship to biomass, TINDVI was a significant predictor of a whole suite of interrelated ecosystem traits measured above and belowground, including plant tissue stoichiometry and soil nutrient pools. These findings highlight the potential of remote-sensing for monitoring the impacts of grassland management on the chemical properties of ecosystems over large areas.

Because of the close links between TINDVI and field-based measures of ecosystem function, it is not surprising that TINDVI was also found to be a significant predictor of plant and arthropod diversity. As found in several other studies, the link between plant biodiversity and NDVI observed at our site largely stems from the strong association of biodiversity to ecosystem productivity (Skidmore et al. 2003; Fairbanks et al. 2004). Surprisingly, TINDVI was actually a better predictor of plant and arthropod diversity than plant standing crop estimated from destructive harvests of vegetation. This may be because TINDVI integrates production throughout the entire growing season, providing a better indicator of actual primary production than a single biomass harvest at the time of peak standing crop.

Conclusion

This experimental study documents the effects of fertilization and haying management on the biodiversity and ecosystem functioning of a cool-season grassland in eastern Kansas. Fertilization increased primary production, measured as standing crop biomass and seasonally integrated NDVI (TINDVI), and altered a wide range of ecosystem level traits. These shifts at the ecosystem level in response to fertilization were accompanied by significant declines in both plant and arthropod diversity, and negative relationships of both to gradients in productivity, the magnitude of which was altered by haying disturbance only in the case of plants. Surprisingly, bacterial diversity showed no response to fertilization, but was increased by haying disturbance. In contrast to plants and arthropods, bacterial

diversity showed a positive relationship to gradients in productivity, although this association was obscured by the opposing influences of fertilization on plant productivity and soil pH. Correlations of biodiversity and ecosystem traits to TINDVI across our experimental landscape indicate that it may be feasible to develop predictive models using aerially-acquired spectral reflectance data to monitor landscape patterns of biodiversity and ecosystem function in these cool-season grasslands.

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