University of Minnesota

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All Methods

Methods for Experiment 249 -

Diversity Treatments

The diversity levels are maintained via hand weeding multiple times each growing season from scaffolding above the plots to avoid trampling. Plots are burned annually to mimic the natural fire regime of the prairie. Because of this, fall biomass harvests are good indicators of seasonal net primary productivity.

Experiment Design

The BAC experiment uses a subset of 38 plots from the Biodiversity II (e120) experiment which began in 1994. See: Tilman et al. (2001) Diversity and productivity in a long-term grassland experiment. Science 294, 843?845. doi:10.1126/science.1060391 Biodiversity II is contained within a block of 342 plots laid out in a grid. Each plot was established as a 13 m x 13 m square, but only the central 9 m x 9 m is actively maintained to contain the specified species and level of plant diversity.

BAC plots consist of fourteen monoculture plots, nine plots planted to 4 species, nine plots planted to 16 species and six plots planted to 32 species. Plant species are from 4 different functional groups (4 species, each, of C3 grasses, C4 grasses, legumes and non-legume forbs) that are likely to respond differently to changes in temperature and water availability. The diversity by warming treatments are a fully factorial split-plot design, with each of high, low, and control heat treatment subplots nested within each of 1, 4, 16 and 32 species diversity treatment whole-plots.

Heat Lamps

Kalglo Electronics, Bethlehem, PA, USA

High Intensity: Model MRM-2412 240V w/ mod ref 1200W 5.0A 50/60Hz 1Ph 30’ cord
Ser.#: 20071771

Low Intensity: Model MRM-2406 240V w/ mod ref 600W 2.5A 50/60Hz 1Ph 50’ cord
Ser.#: 20071651

Warming Treatments

Warming treatments are applied to 2.5 x 3 m subplots via infrared lamps (Kalglo Electronics, Bethlehem, PA, USA). The three warming treatments are control (metal shade above plot to simulate shading effects of lamps), low heat (600 W lamp), and high heat (1200 W lamp). Pre-experiment bare-ground trials of the infrared lamps indicated that the low heat treatment would approximately warm the soil by +1.5C while the high heat treatment would exhibit an approximate warming of +3C. Lamps are turned on from March to November.

acde249 - Legume shoot N15 13C Isotopes

Legume shoot N15 13C Isotopes - Instrumentation

Isotopic composition of samples was measured with a PDZ Europa ANCA-GSL elemental anlyzer coupled to a PDZ Europa 20-20 isotope ratio mass spectrometer at the Stable Isotope Facility at the University of California, Davis. Final delta values are expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively.

Legume shoot N15 13C Isotopes - Tissue sampling and preparation

All aboveground tissues (stems and leaves) were collected and analyzed for each individual. Individuals collected were found in the biomass harvest strip. Two individuals of each legume species were harvested in early August 2009 and 2010 from each subplot of selected plots. Three individuals were harvested in each subplot of the monocultures of the four legumes. In plots not containing legumes, aboveground tissue of non-legume individuals was also harvested to act as reference species for nitrogen fixation calculations.

Tissue was dried at 65 degrees C and then finely ground using a ball mill grinder. Grinding equipment was thoroughly with EtOH between each sample.

acee249 - Net nitrogen mineralization

Net nitrogen mineralization

Soil incubation: For each incubation period, two tubes 2cm in diameter were inserted 18cm into the soil of each subplot. One tube was removed immediately while the other tube was capped and removed one month later. Soil from the tubes was placed on ice until extraction later the same day. The start of the three incubation periods occurred in the middle of May, July, and September.

Soil moisture of extracted soil was measured gravimetrically.
Nitrogen was extracted from the soil with 2 molar KCl.
Nitrogen quantification: NH_4+ and NO_3- in the KCl extracts were measured colorimetrically.

Net nitrogen mineralization - Instrumentation

Nitrogen quantification. Absorbance was measured with a SpectraMax Plus spectrophotometer.

adde249 - Normalized Difference Vegetation Index (NDVI)

NDVI Instrumentation

Reflectance spectra were measured with an UniSpec single channel spectrometer (PP Systems International, Amesbury, MA, USA) fitted with a straight fiberoptic cable.

NDVI calculation

Standardized reflectance was calcuated by dividing raw reflectance of the patch by the reflectance of the white standard at the same wavelength. Normalized difference vegetation index (NDVI) was calculated by dividing the difference of standardized reflectance values at 800nm and 660nm by the sum of the reflectance values at those two wavelengths. NOTE: After calculation, it was found that the white standard reference measurements had saturated at the relevant wavelengths.

Reflectance Measurements

The canopy reflectance spectra of two 36cm diameter patches of ground were measured in all subplots of 4, 16, and 32 species plots throughout the 2011 growing season. Measurements were taken within one hour of solar noon on sunny days. The reflectance spetra of a white standard was measured frequently during each measurement period to serve as a reference.

adee249 - Phenology of individuals

Phenology of individuals

Individual tagging: In all 1- and 16-species plots and two 32-species plots, up to three individuals of each species were tagged in each subplot at the start of each growing season. Tagged individuals were restricted to a 40cm wide strip in the center of the subplot.

Phenology Observations: Once a week during the 2009, 2010, and 2011 growing seasons, all tagged individuals were observed and the presence of buds, open flowers, and/or fruits was recorded. The height of each tagged individual was also meaured at the time of observation.

GDD Calculation: Cumulative growing degree days (GDD) were calculated using Cedar Creek weather data available online. For each day with an average temperature over 10?? C, the number of degrees above 10 was calcuated. These growing degrees were then summed until the day of interest.

adfe249 - Population level phenology

Population level phenology

Phenology Observations: During the 2009, 2010, and 2011 growing seasons, all subplots of 1- and 16-species plots and two 32-species plots were observed once a week, and the proportion of individuals of each species that were flowering in each subplot was estimated. Observations were restricted to a 40cm wide strip in the center of each subplot.

GDD calculation: Cumulative growing degrees days were calculated using Cedar Creek weather data (available online) using 10 degrees Celsius as the base temperature. For each day with an average temperature above 10 degrees, the number of degrees over 10 was calculated. These growing degrees were then summed until the day of interest.

adse249 - Soil microbial functions and enzyme activity

Soil microbial functions and enzyme activity

Soil sampling
Soil samples were taken in August 2011 and 2012 (peak of the growing season) for soil microbial measurements and enzyme assays. From each subplot, nine (2011) or six (2012) soil samples were taken to a depth of 20 cm using a metal corer (diameter 2 cm) and pooled per subplot in a plastic bag. The samples were sieved (2 mm) to remove large stones, roots, earthworms and other invertebrates >2 mm, then stored at -20?C until measurement of soil microbial properties. Soil temperature was measured in each subplot using iButtons (Maxim Integrated Products, Sunnyvale, California) that recorded soil temperatures at 1 cm and 10 cm depths every hour throughout the 2012 growing season (April ? September).
Soil microbial measurements

Approximately 4.5 g soil fresh weight was used to measure soil microbial biomass and growth after addition of different substrates containing potentially limiting elements, such as nitrogen and phosphorus. Using an automated respirometer based on electrolytic O2 microcompensation (Scheu 1992), soil microbial biomass was determined from the maximum initial respiratory response (MIRR) within the first 10 hours after the

addition of D-glucose using the substrate-induced respiration method (SIR; Anderson and Domsch 1978). For this purpose the catabolic enzymes of soil microorganisms were saturated by adding 4 mg glucose g-1 soil dry weight as an aqueous solution. Gravimetric soil water content was determined by drying the soil samples previously used for SIR measurements overnight at 60?C to constant weight and calculating the difference in weight between fresh and dried soil.

Furthermore, soil microbial growth was measured after glucose and nitrogen (CN) addition (supplied as $(NH_4)2SO4$; ammonium sulfate) at a C:N ratio of 10:2 (Anderson and Domsch 1978). An aqueous solution (500 ?l; 4 mg glucose g-1 soil dry weight and 2.6 mg ammonium sulfate g-1 soil dry weight) was added to each sample to adjust to equal water content in each sample. Additionally, soil microbial growth was examined after adding glucose and phosphorus (CP) (supplied as Na2HPO4; sodium phosphate) at a C:P ratio of 10:1 (4 mg glucose g-1 soil dry weight and 0.9 mg sodium phosphate g-1 soil dry weight) as well as the combination of glucose, ammonium sulfate and sodium phosphate (CNP) (C:N:P; 10:2:1). Microbial growth rate was determined using the lowest reading of MIRR and highest value within the first 10 h (Eisenhauer et al. 2010). The respiration rates were log-transformed to account for the exponential growth of soil microorganisms after nutrient addition. Transformed respiration rates were plotted against time, and the slope of microbial growth was determined by linear regression (Eisenhauer et al. 2010).

Extracellular enzyme activities
The activities of seven soil enzymes were measured to get insights into microbial community function. The selected enzymes were, cellobiohydrolase (EC 3.2.1.91), β-1,4-glucosidase (EC 3.2.1.21), 1,4,-β-N-acetylglucosaminidase (EC 3.2.1.52), acid phosphatase (EC 3.1.3.2), urease (Amidohydrolase) (EC 3.5.1.5), phenol oxidase (Phenolox) (EC 1.10.3.2), and peroxidase (Perox) (EC 1.11.1.7). All of those enzymes are involved in cellulose and lignin decomposition and N and P mineralization in the litter layer and mineral soil horizons (Baldrian 2008). Four hydrolytic enzyme activities were measured using a fluorimetric method based on the release of methylumbelliferone (MU) from MU-labeled substrates. The enzyme cellobiohydrolase transforms cellulose into cellobiose, which is hydrolyzed by β-1,4-glucosidase to glucose. 1,4-β-N-acetylglucosaminidase hydrolyses N-acetyl-D-hexosamine residues in N-acetyl-β-D-hexosaminides such as chitin, and acid phosphatase acts on organic bound monophosphates. The absorbance of colorimetric enzyme reaction products of the oxidative enzymes phenol oxidase and peroxidase, both involved in lignin degradation, were measured spectrophotometrically using L-3,4-dihydroxy-phenylalanine as substrate. Urease hydrolyzing urea into carbon dioxide, and ammonia was measured by the use of urea as substrate. For fluorimetric enzyme assays (adapted to Saiya-Cork et al. 2002), a sample suspension was prepared from 1 g soil and 125 ml of 50 mM sodium acetate buffer (pH 5.0) and homogenized using an ultrasonic bath for 1 min. According the pH-value of the soil samples the pH of 5.0 was chosen to maintain field conditions. We set up three replicates of sample wells with substrate, three replicate blank wells with buffer, six negative ambient wells including substrate and buffer as well as a calibration curve for each sample with standard concentrations of 0 ?M, 2.5 ?M, 5 ?M, 10 ?M and 20 ?M per assay. After incubation at 20?C in the dark for 2 h the fluorescence was measured at 365 nm excitation and 450 nm emission using a fluorescence microplate reader (SpectraMAX Gemini EM, Molecular Devices, Sunnyvale, California, USA).

Absorbance of the oxidative enzymes was determined using 96-wells plates including three replicates of sample wells, six replicates of negative ambient wells and three replicates of blank wells. The activities of phenol oxidase and peroxidase were measured at 450 nm and of urease at 610 nm after an incubation period of 18 h in the dark at 20?C using an absorbance microplate reader (SpectraMAX 340, Molecular Devices, Sunnyvale, California, USA). All soil enzyme activities were then expressed in nano moles per hour per gram of dry soil (nmol h-1 g-1). Additionally, mass-specific enzyme activity (nmol activity h-1 ?g-1 microbial biomass carbon) was calculated by dividing the potential extracellular enzyme activity (EEA) by microbial biomass carbon (MBC) (Steinweg et al., 2013).

See: Steinauer, Katja; Tilman, G. David; Wragg, Peter Douglas; Cesarz, Simone; Cowles, Jane M.; Pritsch, Karin; Reich, Peter B.; Weisser, Wolfgang W.; Eisenhauer, Nico; Plant diversity effects on soil microbial functions and enzymes are stronger than warming in a grassland experiment; Ecology; 2015; DOI 10.1890/14-0088.1 2015

aene249 - Root ingrowth biomass

Protocol - Root ingrowth biomass

A root corer was used to create the root ingrowth sampling area in the soil. The core went to a depth of approximately 35 cm. A screen made of hardware cloth was molded around the root corer and inserted into the hole created by the first core. Soil from the edge of each plot was sieved of rocks, live plants and root mass (which was discarded) and then placed in the hole. The soil was packed into the hole until it was level with the surrounding soil and approximately 1 cm of the hardware cloth in the hole was left above soil level.

In order to sample the coring area an initial core was taken to the appropriate depth in cm and the contents were put into a labeled plastic bag. Roots were washed, dried at 40 degrees C and weighed.

aexe249 - Ground level light

Instrumentation - Ground level light

We used an Accupar LP80 light meter (Decagon, Pullman, WA).

Protocol - Ground level light

We measured light penetration to the soil surface as a proportion of ambient light within two hours of solar noon every 2-3 weeks from May through September, from 2010-2013. We inserted an 80 cm light sensor wand along the ground from the north or south edge of each subplot (whichever edge the heater was nearest), and used the distal 60 cm (well within the subplot) to avoid any edge effect in the proximal 20 cm. We did this at two locations per subplot 40 cm apart (20 cm west and 20 cm east of the centerline of the heater), and report the average of the two measurements.

aeye249 - Soil moisture near the surface measured by Thetaprobe

Thetaprobe soil moisture - instrumentaion

Surface soil moisture was measured using a Thetaprobe with 6 cm long probes (ML2x 2009-2011, SM300 2012-2013; Delta-T Devices, Cambridge, England).

Thetaprobe soil moisture - measurment protocol

Soil moisture was measured in the surface 6 cm of soil every two weeks in 2009 and 2013, and weekly in 2010-2012, from May through September. We measured 6-10 (depending on the year) systematically located, permanently marked points and present the mean of these measurements for each subplot on each date. Measurements could not be taken on rainy days; to minimize bias, if rain was forecast for the systematically planned measurement day then measurements were instead taken either the previous or following day, alternating between the previous and following day. Measuring all subplots took an entire day. To standardize for drying of surface soil through the day, a set of 3 reference plots (one of each diversity treatment) was measured repeatedly through the day and the average linear drying trend in these plots was used (when significant) to adjust the other measurements; we present the estimated soil moisture for each subplot as if they had all been measured at the same time of day (at the mean measurement time).

Thetaprobe soil moisture calibration methods

We verified that Thetaprobe soil moisture values were correctly calibrated against a dry-down sequence of gravimetric soil moisture measurements in the lab of soil samples from plots of each diversity. These Thetaprobe soil moisture measurements were also tightly correlated with gravimetric soil moisture of 0-20 cm soil cores taken in 2011 and 2012 (r = 0.92).

aeze249 - Soil nitrate, ammonium, and gravimetric moisture

Soil nitrate, ammonium, and gravimetric moisture instrumentation

Nitrate and ammonium were analyzed colorimetrically using a QuickChem 8500 series 2 Flow Injection Analysis System (Lachat, Loveland, CO) at the Ecosystems Analysis Laboratory at the University of Nebraska.

Soil nitrate, ammonium, and gravimetric moisture protocols

These soil nitrate and moisture measurements were performed on soil composited into a single sample for each subplot on each date. 6 cores, 0-20 cm deep, were composited together from each subplot. Cores were distributed systematically through the central part of the subplot used for biomass clipping (1.5 m x 1 m in 2011, 2 m x 1 m in 2012). Cores were 2 cm in diameter in 2011, and 1 cm in diameter in 2012. Each sampling event occurred a few days after rain.

Nitrate and ammonium were extracted using 0.01 M KCl. Soil samples were placed in 0.01 M KCl and shaken vigorously for 30 minutes, and then allowed to settle overnight. The supernatant was analyzed colorimetrically for nitrate and ammonium.

Soil moisture proportion was calculated as (difference between fresh and oven-dry sample masses) / (fresh sample mass).

afhe249 - Daily mean iButton soil and air temp, air relative humidity

iButton methods

To quantify the efficacy and abiotic effects of our treatments, soil temperature, air temperature, and air relative humidity were measured throughout the growing season. Soil temperature was measured at least hourly via iButton data loggers (Maxim, Sunnyvale, CA, USA) at 1 cm depth in all years, with multiple depths measured in 2012 (1 and 10 cm), 2013 (1 and 10 cm), and 2014 (1, 10, 20, and 30 cm). iButtons deeper than 1 cm were affixed
to a wooden stake inserted into the soil. Relative air humidity and air temperature were measured hourly from 2012 to 2014 in each subplot using iButton data loggers attached to wooden stakes and sheltered from direct sunlight and rainfall under white plastic cups elevated 10 and 25 cm above the soil surface to assess microclimate conditions at various points in the
canopy.

All measurements in this dataset were taken at the same position in the center of each subplot to allow them to be compared among subplots and plots. However, in interpreting these data, please be aware: 1) surface soil was sometimes moved by wind or rain, resulting in ibuttons that were nominally at 1 cm depth being exposed to direct sunlight or conversely more deeply buried; this is responsible for at least some anomalies in the surface temperatures. 2) Methods of installing the soil ibuttons at consistent depths became more refined through time, so inter-annual variation in temperatures at a given nominal depth may be due in part to differences in methods of installation.

ple249 - Plant aboveground biomass data

Plant aboveground biomass

Aboveground biomass was sampled in 0.1 meter wide clip strips in early August. The clipped biomass was sorted to species, dried, and weighed.

roote249 - Root biomass data

2012 Root Sampling

Two 2" cores were taken and divided in to two depths (0-15 cm and 15-30 cm). Replicate 1 (labeled "center" on the field sheets) was always the northwest corner (66 cm from the N in the western strip). Replicate 2 (one labeled "edge" on the field sheets) was always the south east corner (66 cm from the S in the eastern strip).

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