DRAGNet Experimental Protocol

Brief overview

In brief, this experiment will consist of a set of 3-5 blocks (spatial replicates), each with five, 5 x 5 m plots receiving either: long-term nutrient addition; a physical disturbance (annual removal of standing vegetation, litter, rhizomes and tilling of the soil); nutrient addition combined disturbance; short-term nutrient addition; or no treatments (controls). Response variables will be the same as in the NutNet experiment (plant species composition, aboveground standing crop, light intercepted by the plant canopy, and soil chemistry), but will also include an optional seed bank and seed rain study.

Site Selection

Each site selected for the observational study/experiment needs to be relatively homogeneous (i.e., not encompassing large gradients), dominated by herbaceous vegetation, and representative of a particular ecosystem (e.g., shortgrass steppe, tallgrass prairie). The site also needs to be large enough to accommodate a footprint of about 600 m^2. Natural disturbances, such as fire, do not need to be excluded from the site – in fact, if the network is able to recruit sites from a range of natural disturbance regimes, this could work to our advantage. Regardless, a good record of the disturbance regime (frequency, approximate date of last major disturbance) is required.

Timeline Summary

We expect to run this experiment for ten years; however, sites can participate for shorter lengths of time. At the bare minimum is contributing observational (pre-treatment, Year 0) data, and never performing the experimental treatments. In NutNet, these observational data have been extremely valuable, and are sometimes the sole source of data in publications. For experimental data, the bare minimum is to maintain the experiment for one year, i.e., to disturb the plots only once, and contribute pre-treatment (Year 0) and first post-treatment year (Year 1) data. After five years of treatment (Year 5), we will re-assess whether annual sampling is needed for our experiment. If this experiment is anything like NutNet, we expect the treatment effects and science to become more interesting over time. We strongly encourage participants to commit to at least five, and ideally ten, years of treatments.

Sites may join at any time, but to be eligible for the first round of "opt-out" papers (papers that include all participants by default), sites must submit their baseline (Year 0) data no later in than April 2022 (see Project Timeline for Opt-Out Paper Eligibility). Thus, sites should probably be established, and first disturbance/nutrient treatments applied, no later than January 2022.

See the following page (Figure 1) for a general experiment timeline.

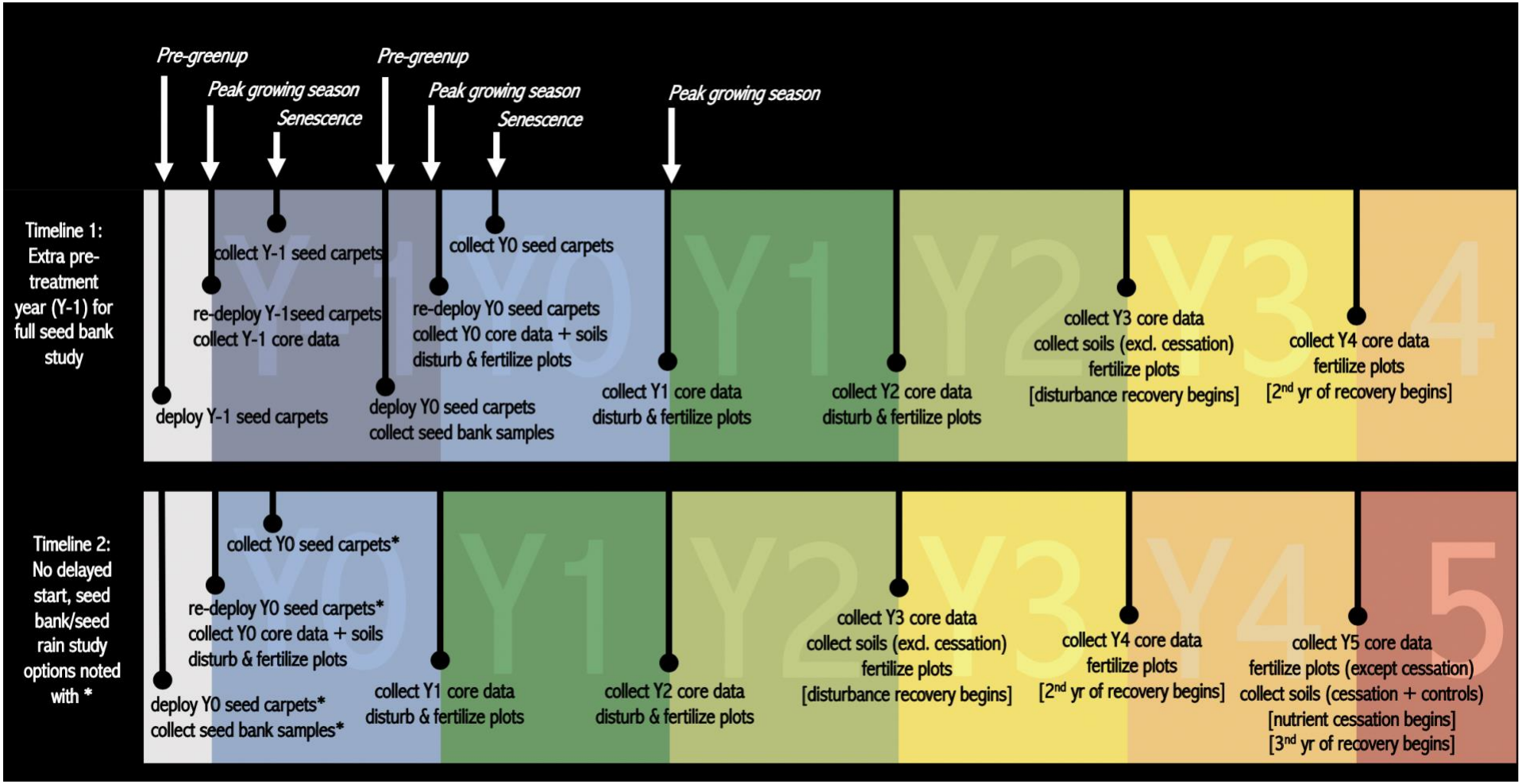

Figure 1. A generalized DRAGNet experiment timeline for the first 4-5 years of treatments. Years are named for the length of time the experiments have been running. For example, Y5 = treatments have been running for five years; Y0 = the year before treatments are applied. Treatments will be applied, and core response variables will be measured, at peak growing season (specific to each site). Note that DRAGNet has two experiment timeline options. Timeline 1 has an extra pre-treatment year ("Y-1") to collect baseline data on the seed bank and seed rain at each site. If a delayed start to treatments is not possible or not desired, sites may follow "Timeline 2." Certain portions of the seed bank/seed rain study are still available to those following Timeline 2; these optional (but encouraged) measures are noted in Year 0 with asterisks *.

Treatment Descriptions and Objectives

This experiment will consist of four treatments (besides unmanipulated controls): nutrient addition (NPKμ), physical disturbance, disturbance crossed with nutrient addition, and a short-term (3-year) nutrient addition followed by cessation.

Nutrient Addition (NPKμ)

To assess nutrient limitation at each site, we will apply a combination of Nitrogen (N), Phosphorus (P), Potassium (K) and micronutrients (μ). We will add nutrients in the same rates (10 g m^{-2} each of N, P and K) and in the same forms as in the "NPKμ" treatment in NutNet.

Disturbance

To create the conditions for community re-assembly to occur, we will kill all adult plants including perennials and disturb the soil from pre-treatment conditions in a subset of plots. This will be accomplished *non-chemically* by clipping and removing all standing biomass; tilling the soil; and removing large rhizomes.[1] Unlike the nutrient addition treatment, which is defined by its application rates, the disturbance treatment is defined by its desired effects on the standing plant community. We are less concerned about the exact methods used to produce the disturbance, as these will be determined by the logistical constraints posed at each site. The disturbance will be repeated annually for three years.

NPKμ + Disturbance

To determine whether nutrient addition affects the rate or trajectory of community assembly, a subset of plots will receive both nutrients and a disturbance treatment (see above). In these plots, disturbance will only be applied for the first three years, while nutrients will be applied annually for the duration of the experiment immediately after the disturbance.

NPKμ Cessation – starting in treatment Y5:

To evaluate the resilience of grassland plant communities to eutrophication and to compare resilience of plant communities to nutrient addition and disturbance, a subset of plots will receive nutrients for only the first five years of the experiment.

Detailed Treatment Descriptions

Nutrients

Timing of application

Nitrogen, Phosphorus, and Potassium will be applied annually, with the first application occurring after pre-treatment sampling and prior the start of the next growing season. Micronutrients will only be applied in the first year of nutrient addition. We prefer that nutrients

[1] Treatment application in peak-summer increases the chance of solarization effects where the heat of summer sun helps kill any plants surviving the cultivation. We recognized that shade-clothing could also be an effective kill-agent, but because of side effects re: soil microbial feedbacks that require investigation, we chose not to use this method. Anyways, trust us! We debated the exact form of this disturbance extensively.

are added immediately after the biomass harvest, starting after Year 0 core data are collected. This application coincides with peak biomass at each site, ensuring the maximum potential uptake of nutrients by plants and thereby reducing losses via leaching and volatilization. However, site PIs know their sites best, so it is also acceptable to apply nutrients at the beginning of the growing season if there is concern of nutrient loss during a dormant period.

Application rates and specific forms of N, P, and K allowed

Detailed application instructions are available at https://nutnet.org/nutrients. Use the worksheet to determine the amounts of fertilizer to add. Fertilizer amounts will vary by fertilizer type, but will always result in the addition of 10 g m^{-2} each of N, P, and K.

Important note about micronutrients

You can use Scott's Micromax or ICL Specialty Fertilizers Micromax Premium, which has a similar (but not identical) formula. Please contact the network coordinator if you are unable to source or afford (it's almost $250 Canadian a bag, probably much more in other countries) either micronutrient blend, as "home brew" recipes are available. We are nothing if not resourceful!

Disturbance

Timing of application

Disturbance plots will receive an annual disturbance treatment for the first three years of the experiment, with the first disturbance occurring after initial sampling, at and before the next growing season. Disturbance and nutrients should be applied concurrently, preferably immediately after pre-treatment sampling, though some sites prefer to do this at the beginning of the growing season. In any case, treatments should be initiated prior to green-up of year 1. Timing of disturbances and nutrient application should be reported with data submission.

Disturbance step 1: clipping and removing all standing biomass

We recommend using a string trimmer or similar tool for this step. Try to minimize moving soils, rocks, and seeds to neighboring plots (or to your face!). Sites with deep moss mats (i.e., tundra and alpine sites) should remove the moss layer to the organic horizon, using hands or a pair of clippers. For the purposes of moss removal, we define the organic horizon as the point where distinct moss structures are no longer visible to the naked eye (so, you will remove both green and brown layers of moss, but not "soil"). Rake and remove the clipped biomass from the plots.

Figure 2. *Sorghum halepense* stolons, © Harry Rose (http://commons.wikimedia.org) CC BY 2.0. Sorry everybody!!!

Disturbance step 2: removing rhizomes

We apologize in advance for this part. As part of our treatment goal of removing adult plants and allowing new

plants, especially annuals, to colonize the plots, we ask that participants remove large, visible stolons and rhizomes (> 20cm long, and > 1 cm in diameter) from the first 15 cm of soil. Sites that have high dominance of rhizomatous plants may wish to remove these rhizomes before tilling, or potentially even before clipping, so that locations of rhizomatous plants are apparent. At this stage, plants may be removed using a tool like a stirrup or loop hoe. After tilling, any additional large visible rhizomes should be removed using a rake and by hand. Sites may use discretion as to whether additional tilling would be helpful for the breakup of small rhizomes into smaller pieces. Note this should only be large effort in the first year.

Disturbance step 3: tilling the soil

We recommend using some combination of an earth auger, sod-cutter, roto-tiller or heavy-duty raking tool such as the McLeod Rake for this step. The specific goals of this treatment are to disturb and homogenize the soil to the shallower of 15 cm, rooting depth, or rock layer (for very shallow soils); and to break up the soil into clumps no larger than 10 cm in diameter (slightly smaller than a tennis ball).

Option 1: Roto-tillers

Figure 3. The DR Pro XLDRT Rear Tine Rototiller. Doesn't this look fun?

In soil with low clay, rock and dense root content, a roto-tiller should be sufficient to disturb the soil, and may require less physical strength than the earth auger. For sites with very loose soil, a "tiller cultivator" style roto-tiller, which has rotating blades on the front of the machine, should be sufficient. Examples of tiller cultivators available in the US in 2019 include the Mantis 4-Cycle Tiller Cultivator 7490, which (perhaps over-optimistically) advertises maximum tilling depths of 25 cm and costs approximately $300 US. For sites with denser soil and roots, a "garden tiller" with dual-rotating or counter-rotating blades located on the rear of the machine will be a better option. At about $800 US, these tend to be costlier than the tiller cultivators, but are much more powerful. Potential brands of garden tillers available in the US include the Cub Cadet RT45 18" 208 cc counter-rotating rear-tine tiller, which advertises maximum tilling depths of 15 cm.

If using a roto-tiller, please do at least 4 overlapping perpendicular passes across the plot, or until the largest soil clumps and root mats are smaller than 10 cm in diameter (slightly smaller than a tennis ball). For most roto tillers, you will know you have reached 15 cm depth when the blades are completely submerged in loose soil as they pass through the plot, and meet little resistance.

Option 2: Earth Augers

Earth augers work effectively in any type of soil. They are typically used for creating post holes or for planting trees, but can be effectively used to disturb soil in a much larger area than the bit size by applying it in a repeated circular motion, and at angles into the ground, until the desired

area is disturbed sufficiently. Some strength is required to apply pressure in harder soils, and if there are a lot of rocks caution is required. Spending some time to learn the safety features of your particular auger is important. Safety features of the Stihl machines mean the drill will shut off if it gets caught; then, rocks can be removed by hand with a shovel. Engine and bit combined weigh approximately 10 kg, but these components can be divided for easier transport over longer distances. We recommend the Stihl BT 131 Earth Auger, which is internationally available and costs approximately $700 US. Participants can mark the 15 cm disturbance depth on the bit with a sharpie or spray paint. If dense root mats, small rhizomes, and soil clumps remain, an additional pass or two with a roto-tiller (below) may be necessary.

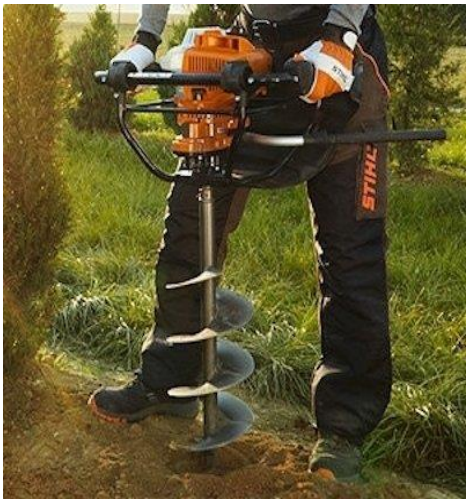

Figure 4. The Stihl BT 131 Earth Auger. Extra points for wearing Stihl-brand chaps in the field.

See some videos, and note some of them think there's no need for the safety bar -- don't follow this advice!

https://youtu.be/12mxpJz0hHw
https://youtu.be/g5sUNvu79LQ
https://youtu.be/ImUr4wplgug

OPTION 3: MCLEOD RAKE, SHOVEL, STIRRUP HOE, ETC.

We salute you, DRAGNetters who have chosen to disturb soil by manual means alone. We offer you no specific advice, except please stay hydrated and wear strong boots.

Experimental Design

Brief Overview

The DRAGNet design and sampling scheme will be identical in many ways to the original NutNet experiment. Five core treatments will be applied: Control, NPKμ, Disturbance, Disturbance + NPKμ, and NPKμ Cessation (Figure 4; however, the experiment may be reduced or expanded to suit site-specific constraints and participant interests). As with the Nutrient Network, sites will be precluded from opt-in papers and analyses that require data from treatments not applied at their site; however, site PIs will still have the option to contribute to these opt-in papers in other capacities (see Authorship Requirements on the Nutrient Network website).

Experiment Design Types

In order of increasing effort, participants may establish the following types of sites:

Observational – 1-time sampling: Sites may do a 1-time sampling of 10-30 plots using standard NutNet/DRAGNet methods, and apply no treatments at all. We require at least 10 replicate

observations (plots) from a site, but to be compatible with previous observational studies in NutNet, 30 plots are preferred.

Disturbance only – 4+ years of sampling: Sites may contribute data from only control plots and plots that receive the three-year disturbance treatment.

All core treatments – 4+ years of sampling: The standard design: all four treatments applied, plus controls.Other treatments: DRAGNet may also be expanded to include additional control plots, or other treatments, e.g., the other NutNet experimental treatments, a Disturbance + NPKμ Cessation treatment, simulated or real herbivore grazing, drought manipulation, seed addition, or litter addition.

Spatial Design

We have R code for randomizing plot and subplot locations for initial site setup that is available on the project website. In all cases, we require a minimum of 3 replicates of each DRAGNet treatment, with treatments spatially nested within blocks. For participants who hope to write papers about their site alone, we strongly recommend five or more blocks.

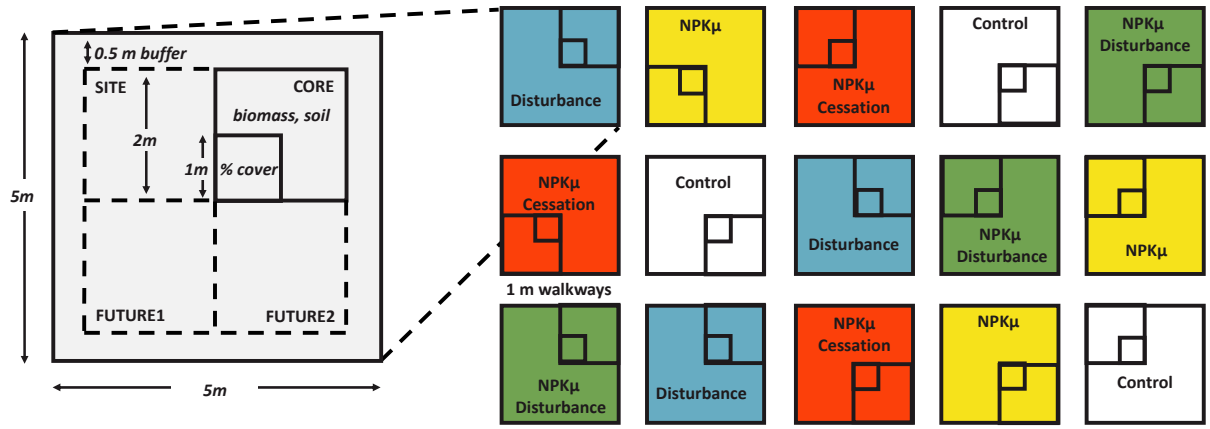

Figure 4. Schematic of the experiment, showing a hypothetical random arrangement of plots and subplots.

Plots will be 5 x 5 m in size, separated by 1 m walkways. This will require 19 x 31 m = 589 m^2. Thus, the standard size of this experiment will be about half the size of the original NutNet experiment. Because a secondary aim of the experiment is to evaluate whether responses to NPKμ are determined by initial conditions, sites that have an existing NutNet experiment should place the new plots near the original experiment in similar vegetation. Sites with existing NutNet plots, or those establishing both a new NutNet and a DRAGNet experiment, may share control plots across the two experiments if space is limited.

Each 25 m^2 plot will have a 0.5 m buffer on all four edges where no sampling will occur. Plots will be divided into four equal-sized 2 x 2 m subplots, with one dedicated to the core sampling (see below), one to additional site-specific studies, and the remaining two for future network-level research (Fig. 3). Subplots should be randomized within plots.

Plots should be permanently marked (i.e., with rebar stakes or similar) at all four corners, and one stake to mark the center of the plot. For sites with tall or dense vegetation, we recommend placing one more stake to mark the other diagonal corner of the 1 m^2 cover quadrat.

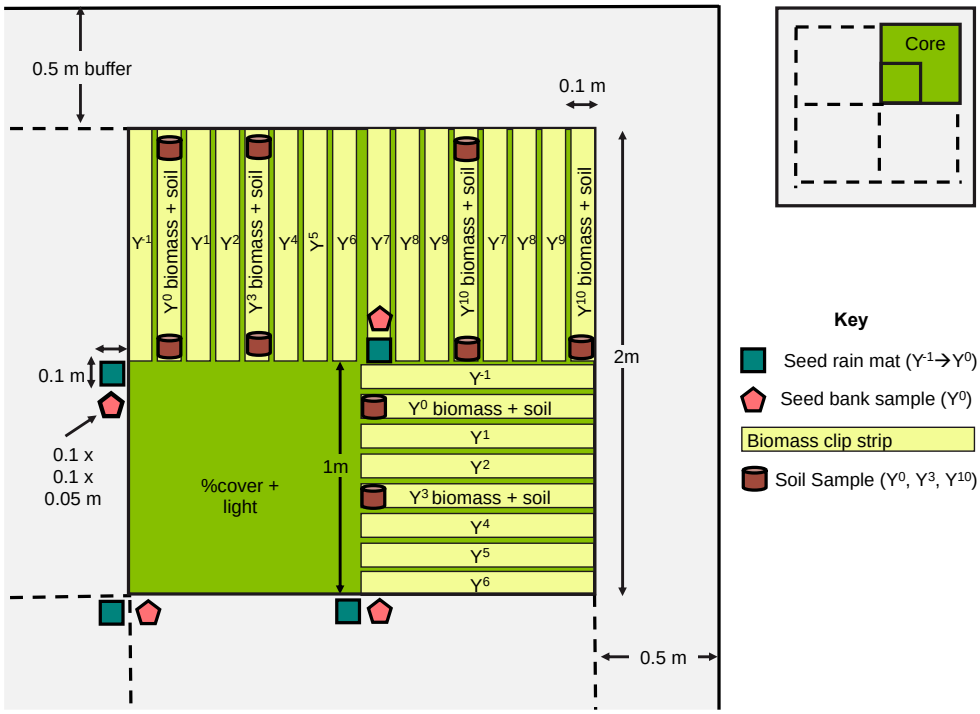

Figure 5. Sampling locations within the core subplot.

Where to Sample: the "Core Subplot"

The core sampling 2 x 2 m subplot will be divided into four 1 x 1 m permanent subplots. The 1-m^2 subplot closest to the center of the plot will be designated for plant species composition sampling and light measures; the other three will be designated for destructive sampling (aboveground biomass and soil cores). All four core measurements will be collected from all plots immediately before the disturbance and nutrient addition treatments, and in each year of the experiment, using the same methodology for all sites. However, soil samples will only be collected from the plots for nutrient analyses prior to initiation of the experiment, in Year 3 (just after treatments are applied), and in Year 10 (the final year of the experiment).

Response variables

Optional/strongly encouraged pre-treatment measurement instructions

Seed bank and seed rain study (measured in Years Y^{-1} and Y^0)

Information about the existing seed bank and arriving seeds provide important data for understanding site-level mechanisms behind recovery trajectories and for stronger tests of dispersal and spatial theory (e.g. Bolker, Pacala, Neuhauser 2003; Wisnosk et al. accepted).

We do not anticipate all sites will be able to collect these data, but by including this description here, we hope to encourage as many sites as possible to collect these data.

These additional measurements are for PI's eager to find out from where the plants arise in their bare ground plots—they must come from somewhere, either from the seed bank within the plot, or dispersed from seeds outside the plot. Collecting seed bank and seed rain data will allow us to connect patterns in community recovery with underlying dispersal and storage processes of seeds. Detailed protocols are provided in the add-on documentation; however below is a general overview of the protocol:

The seed bank and seed rain add-on will use the control, NPK, disturbance, and NPK + disturbance plots, for a total of 12 plots/year (for the proposed 3-block DRAGNet design). To collect seed rain data, deploy four "seed carpets"[2] in each plot, once early in the growing season and again late in the growing season, *in the year prior to the first DRAGNet treatment implementation (Year Y -1).* Carpets will be collected, air dried, and sent to the University of Missouri for separation and quantification. Along with your seed carpets, also submit a seed guide for your site with examples of seeds of the most common species in your plots identified. Seed carpets will also be deployed in a similar manner in the first year of treatment application. Finally, seed bank samples will be collected at the beginning of the Y0 growing season, i.e. seed bank samples are collected at the beginning of the growing season the same year as the first disturbance and fertilization. These samples will need to be spread onto trays and germinated in a greenhouse at your site over the course of 6 months to a year. See the extended protocol for more detail.

Core DRAGNet measurement instructions

These core measurements should provide the basic information needed to assess the effects of nutrient limitation, disturbance and their interactive effects on community structure and productivity. Additional measures and "add-on studies" are welcome. Participants should contact the Coordinator with proposals. Coordination among the sites with these measurements will be encouraged.

Site Survey (at beginning of experiment only)

Upon site selection, participants will complete a questionnaire and submit it to the Network Coordinator. We will collect information about the abiotic conditions and disturbance regime at each site; a description of the habitat type; ground photographs that characterize the landscape and plant community; satellite photos (e.g., Google Earth images), if available; and the spatial coordinates of the site, block and plots. We ask all new sites to complete the Evolutionary History of Grazing Survey, currently being used within the Nutrient Network to quantify grazing pressure and grazer species richness at each site.

[2] Seed carpets: These are pieces of AstroTurf -- fake lawn grass used in outdoor patios/football fields. These will be provided to PIs by L. Sullivan and L. Shoemaker, leads on the seed rain/seed bank study.

Plant species composition (measured annually)

Areal percent cover will be estimated visually for each plant species rooted within the plot, and for woody overstory, litter, bare soil, animal disturbance, and rocks to the nearest 1%. Note that total cover will typically exceed 100% because species cover is estimated independently for each species. Thus, the most important aspect of these cover measures is that the percentages should be a good representation of the species abundances *relative* to other species and non-living categories within that the plot, with consistent methodology across years. Participants should attempt to standardize cover measures made within their site by "calibrating" observers on a few plots before recording data each year.

Within-season sampling frequency may need to be adjusted for individual ecosystems, based on the phenology of the component species in order to capture the maximum cover of each species, which will be used in subsequent analyses. For example, in the tallgrass prairie, species composition will be measured in the spring (late-May) and again in the fall (late-Aug) to capture maximum relative cover of early-season C3 forb and grass species and late-season C4 forb and grass species, respectively. Multiple sampling dates are not required.

Light availability (measured annually)

Light availability will be measured using a light meter (e.g., 1-m length Apogee MQ-301 with 10 sensors) capable of integrated measures of photosynthetically active radiation (PAR, mmol m^{-2} sec^{-1}). Light availability will be measured at the same time and in the same 1 m^2 subplot used for the species composition measurements. For each subplot, two light measurements at ground level (at opposite corners of the 1-m2 plot, diagonal to each other) and one above the canopy will be taken. Light availability will be calculated as the ratio of PAR below and above the canopy. If you use a point sensor, record the mean of at least 10 readings in different locations (this is done automatically with the linear sensors).

Ideally, light readings should be taken on a cloudless, windless day as close to solar noon as possible (i.e., 11 am to 2 pm). If no such day is available during your field season, a bright but completely and consistently cloudy day (i.e., high, gray clouds) close to solar noon is the next best option. We would prefer to have light measures of dubious quality than no light measures at all; the mean and variance in the ambient light readings among plots provide a measure of data quality. Please do measure light even if conditions are not perfect, and alert us to potential issues when you submit your data.

Aboveground standing crop (measured annually)

Aboveground biomass will be estimated destructively by clipping at ground level all plants rooted within a 0.2 m^2 area comprised of two, 10 x 100 cm "clip strips." Biomass will be clipped within the 1 m^2 subplots designated for destructive sampling within the core sampling subplot. To keep edge effects consistent among years, clip strips should have one end touching the cover quadrat, and the other end as close to the plot edge as possible. Location of the clip strips should be noted on a map or marked permanently to prevent resampling the same area less than five years apart (see Figure 4 for an example of a sampling plan). For shrubs and

subshrubs rooted within the quadrat, leaves and current year's woody growth should be collected; or allometric equations should be used if available.

Biomass should be separated into the following six categories:

1. previous year's dead (litter and standing dead biomass)
2. current year's bryophytes (mosses and lichens)
3. current year's graminoid (grasses, sedges, rushes)
4. current year's legumes
5. current year's non-leguminous forbs
6. current year's woody growth

If time does not permit for this functional group-level sorting, at a minimum standing crop should be separated into the following categories: previous year's dead (litter), current year's bryophytes (green moss and live lichens), and current year's vascular plants. All biomass should be dried at 60°C for 48hrs prior to weighing to the nearest 0.01 g. Species-level biomass estimates, if made, should be aggregated to functional group level before submitting to the Coordinator.

Soil chemistry and texture: Years 0, 3, 5 and 10

Soils will be collected in all plots just prior to the start of the experimental manipulations (Year 0). After three years of manipulations, i.e., while Year 3 data are being collected in the field, soils will be collected from all plots *except the cessation treatment.* After five years of manipulations, soils will be collected in only cessation plots and controls. Finally, at the expected conclusion of the experiment (Year 10), soils will be collected from all plots.

For each plot, collect two to three soil cores (soil corer - 2.5cm x 10 cm) from each of the 2.5 x 2.5 m subplots. Litter and vegetation should be removed from the soil surface before collecting each sample. **Make this easy by doing your biomass harvest first, then going back to collect soil from the very same "clip strip" area.** Composite and homogenize these sub-samples into a single sample for each 5x5 m plot (total of 15 roughly 300 g samples). All soil samples should be double bagged in paper and allowed to air dry. Label each bag (with permanent marker, Sharpie preferred) with the following information: date of collection, name of collector, name of sampling site, and block/plot/treatment identification. Soils will be processed for nutrients at the University of Minnesota. Please view soil shipment protocols on nutnet.org or contact the Coordinator for instructions.

Add-on Studies

As in NutNet, studies that require additional observation or experimentation are welcome, and a great way to leverage the network for your own purposes! Please contact the Network Coordinator if you are interested in performing an add-on study. For some ideas, feel free to take a look at some of the NutNet add-on studies here: https://nutnet.org/methods

Data Submission, Management, and Access

Please submit your data in the standard spreadsheet for the core sampling data that we will provide. Copies of datasheets, electronic data and metadata should be sent to the Network Coordinator at the end of each growing season. These data will be integrated into the same relational database used by the Nutrient Network, and follow the same data management strategy (Lind 2016). All data, including NutNet data, will be available for collaborative analyses by network data contributors. Data used in network publications also will be published and freely available when a paper is published using these data.

Seed bank and seed rain datasheets will be sent to all participants in the add-on project and data will be compiled by Lauren Shoemaker and Lauren Sullivan, and synced with main NutNet database.

Time and financial costs

Time costs

One of the goals with this experiment – just as in the original NutNet experiment – was to design a project with a relatively low financial and time cost. However, we recognize that the time and equipment it will require to remove the plants and disturb the soil in a 5 x 5 m plots will vary widely across sites that vary in soil texture (clay and rock content), plant density and rhizome abundance. Our hope is that in the disturbance phase of the experiment, field work should take approximately **14 person-days**:

- 6 person-days for the disturbance treatment (one person-day for each disturbance plot)
- 2 person-days to sample biomass
- 2 person-days to sample cover
- 1 person-day to pre-weigh and add nutrients and measure light
- 2 person-days to measure and stake the plots (first year only)
- 2 person-days to collect seed bank and seed rain field data (Year -1 and Year 0 only)

Thus, a team of four could hopefully complete the field work in three full days. **Field work after the third year are expected to only require five person-days.** Sorting and weighing biomass to functional group, pre-processing soils and entering cover data should take approximately 2 person-days; this task is an excellent entry-level lab project for undergraduates. Seed bank germination takes considerably more time in the greenhouse, and seed rain samples can be sent off to Missouri for quantification upon collection (see detailed protocol).

Financial costs

Costs can be divided into one-time expenses, and annual maintenance costs. One-time expenses include:

- protective equipment, including gloves and safety glasses
- shovels and rakes
- hand-held clippers for biomass sampling
- string trimmer
- earth auger or roto-tiller
- light meter
- micronutrients
- soil nutrient analysis ($300 US, plus shipping)

Maintenance costs are primarily those of N, P and K fertilizers (approx. $50 US, but could vary widely by country and location within country).