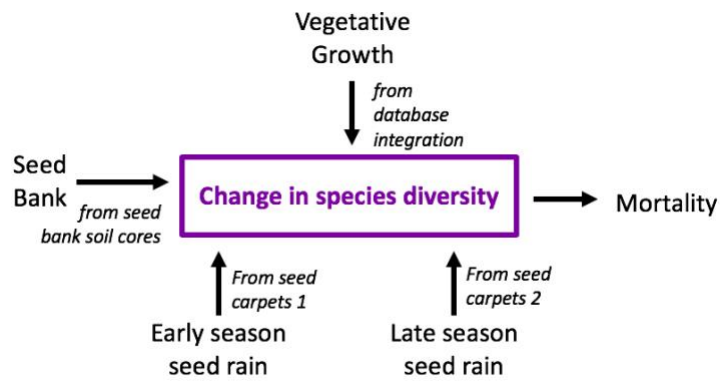


Seed Rain/Seed Bank Collection for DRAGNet Add-On

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ABSTRACT

How communities recover from disturbances and what factors influence both their transitory trajectories and recovered stable states remain open questions in community ecology. The goal of this DRAGNet add-on is to understand how communities recover from disturbance and the role of temporal versus spatial dispersal of seeds for recovery. We will quantify the soil seed bank (temporal dispersal) and seed rain (spatial dispersal) to understand how changes in diversity are reflected by these two processes that are key for community assembly. Additionally, we will examine how disturbance alters local seed rain, and the role of small-scale, local, and regional dynamics in yearly seed rain. This add-on will assist in mechanistically understanding *why* we observe given changes across treatments, and the relative importance of spatial versus temporal assembly processes (Figure 1). In brief, we will utilize seed traps (astroturf carpet squares) and



seed bank soil cores from the control, NPK μ , disturbance, and NPK μ + disturbance plots to partition the mechanisms behind changes in biodiversity across treatments and sites. **Participants that submit observational data (Year 0 data) by April 2022 can opt-in to all or some portions of this add-on study; multiple levels of commitment are available.**

Figure 1. Partitioning changes in species diversity.

SIGNIFICANCE

The proposed add-on study will add greatly to our understanding of why community change follows a specific trajectory across treatments and broad-scale geographic patterns that vary across sites. Specifically, the add-on aims to address the following questions:

1. *Do priority effects change the trajectory of community recovery as well as the final, equilibrium community?*
 - a. *Does this depend on treatment and/or land-use history?*
2. *How do nutrient additions and disturbance alter the relationship between species presence and abundance in the aboveground cover, vs seed bank, vs seed rain?*
3. *What is the relative importance of spatial versus temporal dispersal in community recovery?*

PARTICIPATION OPTIONS

We have a flexible, three option design to maximize buy-in from DRAGNet sites and for sites to choose their own level of commitment. All options, described below, will use the control, NPK μ , disturbance, and NPK μ + disturbance plots, for a total of 12 plots/year (for the proposed 3-block DRAGNet design).

If sites wish to be eligible for the opt-out paper using the seed rain/seed bank study, they can choose any of these three options, but must submit year Y⁻¹ and/or Y⁰ data to DRAGNet HQ by April 1, 2022. Thus, participants keen on contributing to the opt-out paper should probably establish their site and collect Y⁻¹ data no later than December 2020/January 2021, and apply the first round of disturbance & nutrient treatments by approximately December 2021/January 2022.

Option 1: Seed bank and pre- and post-treatment seed rain comparison. Sites that choose this option will delay their initial treatment for one year – i.e., add an additional year of observational data, here called year Y⁻¹. This allows for a comparison of seed rain pre- and post-treatment. **At the beginning of the growing season in Y⁻¹,** sites will place seed traps (defined as growing season traps) in plots. At Y⁻¹ peak biomass, sites will collect growing season traps and collect core DRAGNet data (cover, light, biomass, but not soil cores for soil chemistry – these will be collected in Y⁰ – or the year of treatment addition). Sites will place additional seed traps (defined as senescence traps) that will remain until the end of senescence and/or first frost. **At the beginning of the growing season in Y⁰,** sites will collect seed bank cores and repeat the growing season seed rain protocol. At Y⁰ peak biomass, sites will collect growing season seed traps, core DRAGNet data, and will apply treatments. Immediately after treatment application, sites will deploy Y⁰ senescence traps, to be collected at the end of senescence and/or first frost.

Option 2: Seed bank and post-treatment seed rain. Option 2 is similar to Option 1, but does not delay treatment application. **At the beginning of Y⁰,** sites collect the seed bank cores and deploy growing season seed traps. At Y⁰ peak biomass, sites will collect growing season traps, core DRAGNet data (including soil chemistry), and will apply treatments. Immediately after treatment application, sites will deploy Y⁰ senescence traps, to be collected at the end of senescence and/or first frost.

Option 3: Seed bank only. In this option, sites collect only the seed bank cores at the beginning of the growing season during Y⁰—the same year they disturb.

Note: Sorting of seeds from the seed carpets will be done by the Sullivan lab, while germination of seed bank samples will be completed by each individual site (see below for detailed protocol – used in a previous NutNet add-on).

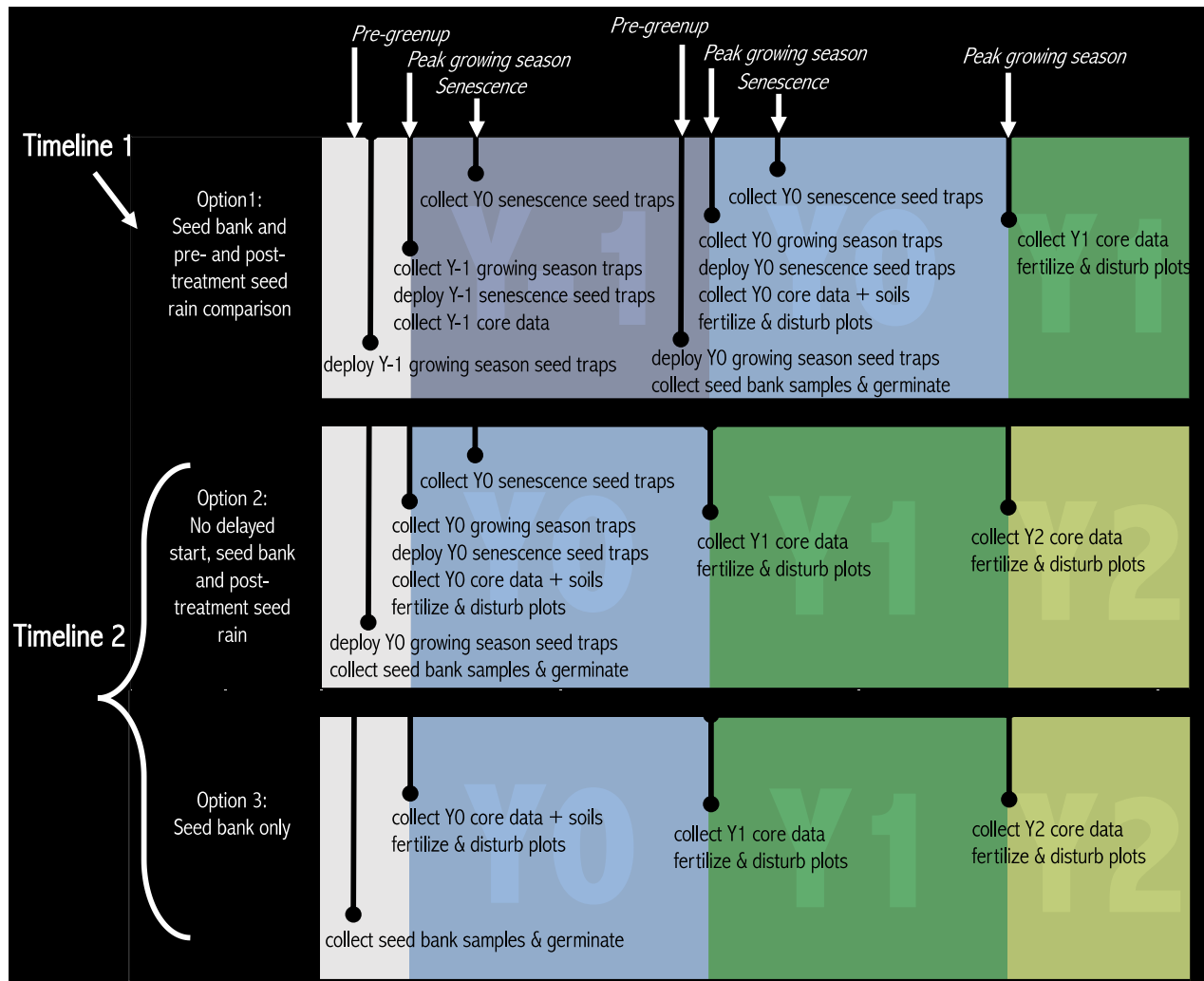


Figure 2. Add-on study timeline. Seed bank/seed rain measures should start as close to the beginning of the growing season as possible, and end as close to the end of the growing season as possible, with the switch of the carpets at peak biomass.

In brief, seed traps are 10 cm x 10 cm astroturf carpet squares that will be deployed to catch seeds that disperse into the plot; seed trap protocol has been preliminarily tested at Tyson, MO. We will determine diversity of the seed bank by germinating seeds present in 10cm x 10 cm soil cores. Cores will be germinated in participating sites' greenhouses. Seed bank protocol is based on the original NutNet add-on from Anu Eskelinen and Lauren Sullivan.

All sites that opt-in to the initial add-on study will have the ability to opt-in to future seed bank and seed rain add-ons, allowing us to look at change through time in seed rain and below- and above-ground species pools. We will work with the sub-group that opts-in to determine the best timing for any potential future sampling. Our initial thoughts are for repeated measuring of the seed bank and seed rain the first and third year after disturbance treatments end (Y^0 and Y^3 of recovery). **Opting-in to this add-on does not, however, require any future sampling other than described here.**

Seed Bank Detailed Sampling Protocol:

OVERVIEW

In this add-on experiment, we will sample only the fertilization x disturbance factorial – thus only sampling the control, disturbed, NPK μ , NPK μ + disturbed plots (12 plots for all three blocks). **Sites should be sampled right before the growing season begins, prior to growth occurring at the site. This will allow for the samples to be representative of the seeds that have survived the winter, have undergone site-specific dormancy breaking protocols, and have the potential to germinate and grow that year.** Field work includes taking soil samples from 12 plots and should not take much time. The most labor-intensive and long-lasting effort comes from germinating and growing seedlings from soil samples, which would make for an excellent undergraduate project. Participating sites need to follow the seed bank samples for 1 year (with the possible option of sites that initially opt-in to resample after disturbance treatments are complete).

IN THE FIELD

Work Summary:

- Taking four 10cm x 10cm square soil samples from 12 plots: Control, NPK, disturbance, NPK + disturbance from all three blocks (locations marked in Figure 3 with gray pentagons).
- Sampling timing: in the early growing season, as growth at the site is beginning.
- Time consumption: taking soil samples from 12 plots should not take more than 2-3 hours.

Equipment list:

- At least 12 plastic bags (or more if you have more than 3 blocks and you want to sample them all, and it's good to have some extra). Choose bags that can be closed (ziploc bags) of at least 3 liters/1 gallon in size.
- Trowel – it's helpful if this has a sharp edge. A butter knife or bread knife can also be used for a straighter line
- Frame created to be 10x10cm². This can be constructed for the needs of the site. Some suggestions include 4 plastic bendy straws taped together if your soils are sandier because they can handle a bit of chopping with the trowel, or a thick metal frame if your soils are rockier and you need to do some serious digging.
- Ruler
- Permanent marker
- 12 large paper bags
- Large backpack or bag to carry samples in. Please note soil samples can be heavy so be prepared if you need to hike into your site.

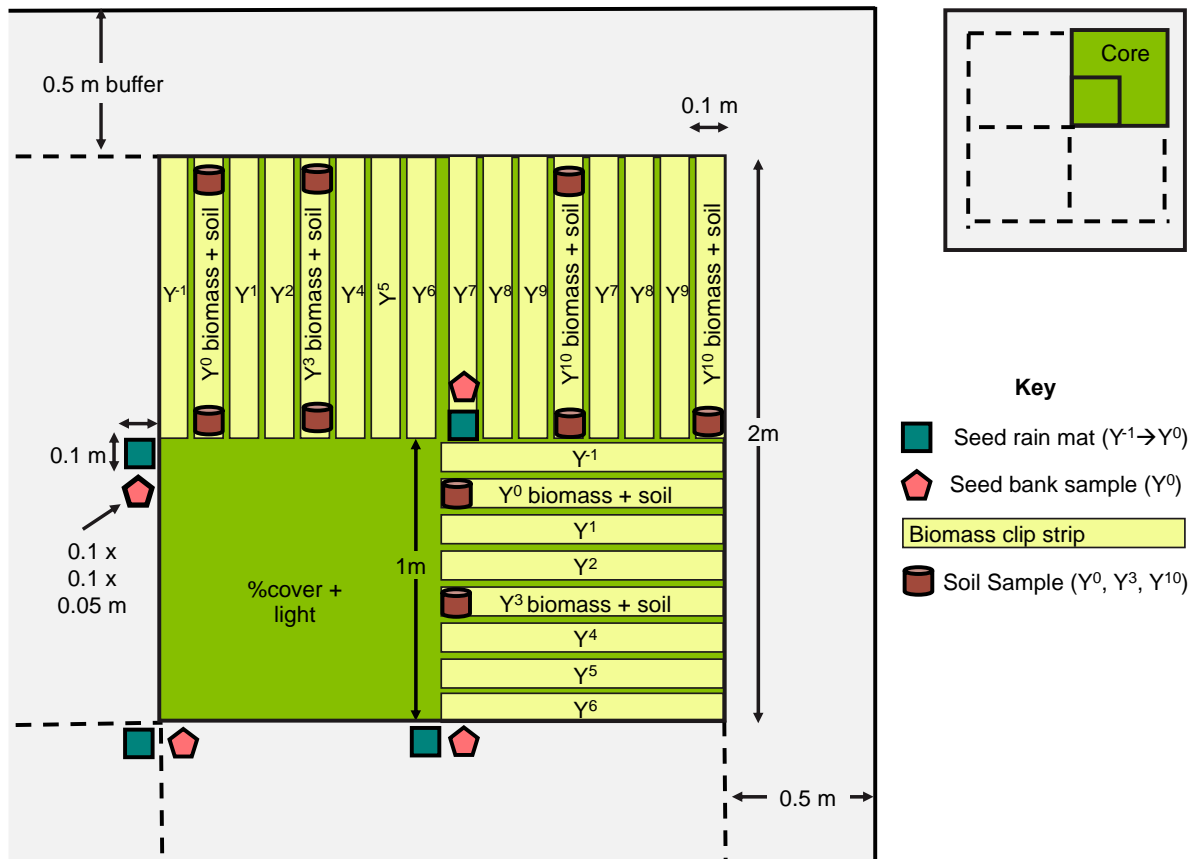


Figure 3. Placement of seed bank cores and seed rain mats.

Sampling:

- At the beginning of the growing season (as soon as you can at the start of the season, before seeds begin to germinate naturally in the field), take 4 soil cores in each plot—one at each corner outside of the permeant percent-cover quadrat (Figure 3, gray pentagons).
- When taking the soil samples, choose a place without big rocks right under the soil surface.
- 4 soil cores (10 x 10 x 5 cm deep, 500 cm³) will be taken from each corner (corresponds to an area of 20 × 20 cm², or a volume of 0.1m³).
- Each core is removed by placing the metal frame on the ground and removing the soil from the 10x10cm² area to a depth of 5cm with the trowel. Basically, you first use the metal frame to indicate the size of the area, and you cut the soil along the edges of the frame with trowel. Then you can remove the frame and remove the soil inside the cut area. Use a ruler to measure 5 cm depth, or mark your trowel or knife to 5 cm to help with cutting.
- Place the soil in a bag labelled with the plot information (It is ok to combine soil from the four cores in a given plot).
- Air dry soil samples in paper bags (for standardization purposes and ease of transport and rhizome removal).
- Once dry, germinate seeds as soon as possible.

IN THE GREENHOUSE

Work Summary:

- The soil samples collected from the experimental plots should be spread on individual seed germination trays (details provided below).
- Space requirements for the greenhouse is: 2 – 3 m².
- The plots need to be watered 2 – 3 times per week (so that the soil never dries).
- Time consumption in a greenhouse: This will be the most time-consuming part of the study and especially at the beginning a student helper would be valuable. During the first few months, the trays filled with soil should be checked 2-3 times for emerging new seedlings. After this time, checking should not need to be done more than once a week, and once germination has slowed (~3-5 months), it can be done every second week gradually going down to once every month. Overall, the greenhouse experiment requires ~6-12 months. Timing is very site dependent.

Equipment list:

- Trays (one or two for each plot sampled = 12 - 24), size: 50 cm × 25 cm (20" × 10") (<https://www.amazon.com/Durable-Black-Plastic-Growing-Trays/dp/B00K6B6PCA>), with holes cut in the bottom for drainage (if holes are not already present).
- Tray liners to allow water to pass through, but not soil. Use garden cloth like this, so long as it's not coated with anything and is permeable. http://www.homedepot.com/p/Scotts-Weedout-3-ft-x-50-ft-10-Year-Landscape-Fabric-209015/206790368?cm_mmc=Shopping%7cTHD%7cG%7c0%7cG-BASE-PLA-D28O-OutdoorGarden%7c&gclid=Cj0KEQjwioHIBRCes6nP56Ti1IsBEiQAxxb5G-n9kEtLbQHmFRPtD5YAJ_ygrTFkEsd4H916l5M2gUaAhQ78P8HAQ&gclidsrc=aw.ds
- Seed bank samples from the field that have been air dried.
- Sterile potting soil with perlite.

Sampling:

- Homogenize the seed bank sample by breaking up any clumps in the bag.
- Remove all rhizome/root/plant material and bigger rocks from the soil samples. Be sure to brush excess soil from these rhizomes/roots/rocks. Make sure to remove all plant material to prevent vegetative growth. We suggest using a sieve to remove roots and bigger rocks.
- Once you are ready to begin the germination trial, place the tray liner into the tray, and then fill each tray with ~2cm of potting soil.
- Spread the seed bank sample in a thin layer (~1 cm) across the whole tray.
- Water the samples.
- For the first few months (or until the initial germination flush is over), check germination 1-2 days/week, and water samples when dry (Figure 4).
 - Once seedlings have been ID'd to species, record on the data sheets, and remove the seedling.
- For the next few months, (assuming germination has slowed down), check germination 1 day/week, although samples will likely need to be watered more frequently.

- After germination has stopped and unknown plants have been removed, break up soil crusts and stir the collected soil (taking care to leave the potting soil behind when possible) by hand to allow better seed-soil placement and induce germination of new seeds.
- End experiment once no germination has occurred for 3 weeks (~6-12 months).



Figure 4. Seed bank samples germinating from a NutNet seed bank experiment.

Seed Rain Sampling Protocol:

OVERVIEW

As above, we will sample only the fertilization x disturbance factorial – thus only sampling the control, disturbed, NPK μ , NPK μ + disturbed plots. There will need to be 3 site visits per year for the first two years. As the growing season begins, when growth first starts to occur at the site, seed carpets will be placed out at the site. Then, at peak biomass, when the core DRAGNet sampling occurs, the first set of seed carpets will be removed and a second set will be set out. Finally, this second set of seed carpets will need to be collected at the end of the growing season, after senescence. While this protocol requires 2 or 4 extra trips to the field, time requirements for each trip are minimal.

In order to enforce standardization, seed carpets and ground staples will be provided to participating sites. Additionally, collected seed samples will be sent to the Sullivan lab for sorting. **Participating sites are greatly encouraged to make a seed key of the most prominent species at their site to help with the sorting process so we can sort the seeds to species.**

IN THE FIELD

Work Summary:

- For the first two trips per year, participants will be staking 4 10cm x 10cm Astroturf carpet soil squares in each of 12 plots: (control, disturbed, NPK μ , NPK μ + disturbed plots in all three blocks). On the second trip the first set of carpets will be replaced by the second one, and in the last trip the second set of carpets will be collected.
- Sampling timing: Visit 1: in the early growing season, as growth at the site is beginning. Visit 2: at peak biomass. Visit 3: at senescence, after most seeds have fallen from the plants.
- Time consumption: staking and collecting the seed carpets should take no more than 1 hour each trip.

Equipment list:

When placing carpets

- 4 10cm x 10cm carpet squares per plot – for 12 plots this will be 48 squares. These will be provided to the site.
- 96 ground staples (2 per carpet square). These will be provided to the site.
- Flags to mark where the carpets are placed.

When removing carpets

- At least 12 plastic bags (or more if you have more than 3 blocks and you want to sample them all, and it's good to have some extra). Choose bags that can be closed (ziploc bags) of at least 3 liters/1 gallon in size.
- Permanent marker
- A trowel or clippers to clear away the plants if necessary

Sampling:

When placing carpets

- At the outside corners of the % cover quadrats, place 4 carpet squares per plot (see Figure 3 for placement locations).
- Place a flag near each carpet square as it is easy to lose them when they become covered in litter (Figure 5).

When removing carpets

- Pull each carpet square gently so as not to lose seeds when removing the ground staples.
- Place the carpet square upside down in a bag labelled with the site name, plot number, date, and timing of seed carpet (first or second sampling). Taking care not to puncture the bag with the ground staples.
- Once the carpet is in the bag, gently remove the ground staples and save those to be re-used for the second set of carpet squares.
- Repeat for all carpet squares within a plot, binning all 4 squares per plot into the same bag. Seal the bags for transport.

IN THE LAB

Work Summary:

- The bags containing the seed carpet samples collected from the experimental plots should be opened so they air dry. Then the bags can be resealed and mailed to the Sullivan Lab using a permit (acquisition of permit is currently in process—details will be emailed to all participants).
- Time consumption in the lab: A few days to weeks to dry out the samples depending on moisture level.

Equipment list:

- Envelopes or box for mailing seed samples.
- Permit labels



Figure 5. Images of the seed carpets at work.