The very easy leaf damage protocol

Research Plan:
Aims:
The objectives of this add-on study are to understand **(1)** how N and P addition influence herbivory and disease, **(2)** how herbivory and disease vary with climate, functional group and species traits, and **(3)** whether enemy release of exotic species depends on N and P addition and explains home-away differences in abundance (see abstracts, below). Preliminary data from three sites suggest that we will find interesting variation across treatments, sites, and species.

Time required:
Based on preliminary tests, the full protocol for seven plant species can be completed by two people in three to seven hours. Working with two people, one person should estimate damage for all leaves and species to avoid bias. Each site will be sampled only once, and to make it easier for more sites to participate, sampling can occur in 2015 or 2016.

Approach and species selection:
We will measure leaf damage on the most common 5-10 plant species at each site within three reps of the following treatments (12 plots in all): **Control/N+/P+/NP+**. Because species will serve as replicates for many analyses, we ask that observers include as close to 10 species as possible. Lists of potential species will be sent to each participating site, however these lists will be based on average abundance in cover plots, and therefore represent only a small section of each plot. Consequently, it will usually be necessary to adjust these lists based on which species are common in the larger 5 m * 5 m plots at the time of sampling. Before sampling, we recommend visiting each of the 12 plots and determining which species are present in all or most of the plots. Any species present in at least 10 of the 12 plots is worth considering for inclusion. If many species are available, choose a subset that covers as many as possible of the following categories: grasses, forbs, legumes, native species, introduced species, and species used in other NutNet studies (Traits, Home-and-Away). If some of the species under consideration are relatively uncommon, it is helpful to mark individuals with pin flags while conducting the initial survey, to avoid having to search for them twice. Note that the initial survey stage can be skipped if you know which species can be reliably found.

Measurements:
In each plot, 5 leaves (or as many as available if there are fewer than 5) of each species will be evaluated for damage. Because herbivory and disease vary seasonally, measurements will be conducted near to the time of peak biomass (please note exceptions). Measurements can be conducted in any portion of the 5*5 m plot EXCEPT for the core plots (to minimize disturbance there). For identification of types of damage, it is important to have magnification, such as a hand lens or a portable microscope (we will supply toy microscopes that have worked better for us than hand lenses).

Plant selection: Where possible, measure one leaf from each of five well-spaced individual plants. To minimize bias towards larger plants, scan subsets of the whole plot carefully, choosing the first plants encountered. To minimize disturbance to the plot, start by walking around the edge of the plot, scanning the area easily visible from the edge, and then continue into the center of the plot as needed. Where possible, however, avoid sampling within 0.25 m2 of the plot edge. For species that are less common or otherwise difficult to find, it is not necessary to search exhaustively for five

individuals. Rather, we suggest first reducing the number of individual plants and measuring more than one leaf per individual, and if that is not sufficient, measuring less than five leaves. Where very few plants are available, it is OK to measure all fully expanded leaves on individual plants.
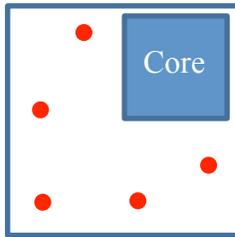

Figure 1 – Ideal locations of sampled plants (red dots).

Leaf selection: Because damage depends strongly on leaf age, it is important to standardize leaf age as much as possible. Choose fully expanded leaves of moderate age. For plants with stems, choose leaves approximately half-way up the stem. For plants without stems, choose leaves approximately half-way between the center and edge of the plant. Within those constraints, try to choose leaves as randomly as possible, and without reference to the amount of damage on the leaves. We recommend removing leaves to allow for easier inspection, at least for common species for which the impact of removing 5 leaves per 25 m^2 is likely to be negligible.

Herbivory/pathogen estimation: For each leaf, we differentiate between the following damage types:
- **chewing damage:** holes that go all the way through the leaf, including tissue removal at leaf margins
- **invertebrate damage:** damage that does not go all the way through the leaf (e.g., mining, or rasping damage
- **fungal damage:** rust damage, powdery mildew damage, other/unknown fungal damage
- **vertebrate damage:** evidenced by relatively straight cuts, and adjacent leaves cut in the same location

Note that some damage is present only on the underside of leaves, so remember to check both sides of the leaf for damage. For each type of damage, enter a value in the associated column if present, otherwise leave blank. Please categorize the level of chewing damage (Chew), mining damage (Mine), rust damage (Rust), powdery mildew damage (Mildew), other/unknown fungal damage (Fungi Oth.) and vertebrate damage (Vert) separately using five categories (0%, 1-5%, 6-25%, greater than 25%). Examples for the different damage types and categories are given in the Appendix. We recommend bringing copies of the appendix to the field to help standardize damage estimates.