How do fertilizers and herbivory affect functional traits in herbaceous communities?

Proposal by:

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Functional traits research has developed with the aim of finding general patterns in how the function of plant assemblages changes to different land-uses. **Functional effect traits** are characteristics of a plant species that alter resources such as nutrient and light availability. **Functional response traits** are characteristics that allow a species to respond to changes or perturbations. Functional traits provides information on how the presence and abundance of different species may affect ecosystem functioning. General patterns in community level aggregates of functional traits are useful for predicting how a community will change when subjected to anthropogenic disturbance and for guiding restoration efforts for degraded sites. Few studies have examined changes in functional trait diversity within an experimental framework (exception Suding et al. 2005) and none have examined quantitatively measured traits in an experimental framework. Most studies have compared sites across regions where land-use histories varied, but there has been little control over the intensity of disturbances applied. The Nutrient Network experiment, therefore, presents a unique opportunity to examine the response and effect of functional traits across grassy ecosystems characterised by a diverse range of climatic conditions and subjected to the same set of nutrient addition treatments and a grazing/no grazing treatment.

Characteristics of physiological leaf traits such as, specific leaf area (SLA, mm^2/mg, fresh leaf area/oven-dry mass), and leaf dry matter content (LDMC, mg/g, oven dry mass/water-saturated fresh mass) correlate with carbon acquisition strategies known to influence ecosystem functioning (Diaz et al., 2004;Wright et al., 2004;Westoby and Wright, 2006). SLA is the investment a plant makes in growing light-capturing area per dry mass content (Westoby, 1998;Cornelissen et al., 2003). Species with a relatively high SLA tend to have a higher rate of return on the resources invested into making tissue (cheaper leaves in terms of energy and resources needed to produce them) when compared to species with a lower SLA (more expensive leaves to produce). LDMC is representative of the average leaf density, with a high LDMC reflecting a tough and longer-lived leaf and a lower LDMC a softer, shorter-lived leaf (Cornelissen et al., 2003). Ecosystems low in resource availability are generally comprised of slow-growing plant species better at resource conservation (low SLA and high LDMC); while, ecosystems high in resource availability tend to contain fast growing plant species better at resource acquisition (high SLA, and low LDMC) (Lavorel and Garnier, 2002;Diaz et al., 2004;Wright et al., 2004;Wright et al., 2005).

Leaf traits have also been used to compare the impact of anthropogenic disturbances on ecosystem functioning across sites, including grazing and nutrient levels (Lavorel and Garnier, 2002;Diaz et al., 2004;McIntyre and Lavorel, 2006;Diaz et al., 2007;Quetier et al., 2007;McIntyre, 2008). In a meta-analysis of community trait responses to grazing, Diaz et al. (2007) found that certain generic functional traits were favoured by grazing pressure including: annual over perennial, short over tall, unpalatable over palatable, and stoloniferous and rosette over tussock architecture. McIntyre (2008) found that grazed or fertilized sites were characterised by higher SLA and lower LDMC values, indicating a predominance of resource acquisition specialists.

Data on functional traits will be collected at just one census time, after three or four years of treatment.

Paper 1: The main aim of this paper will be to quantify how changes in nutrient availability and grazing alter the functional traits of species over the short-term. We hypothesize that grazing and fertilizer treatments at the cross-site level will follow trends found in other global initiatives such as the Global Plant Traits Network. With fertilizer addition, SLA values should increase, LDMC decrease, and leaf nitrogen and phosphorus levels increase. The magnitude of these effects should be greater in the fertilized/grazing exclusion treatment. In the grazed treatments, it is likely the fast growing species will be grazed more frequently because of increased palatability. We will also explore whether these differences occur because of: 1) changes in species identity, 2) changes in species abundance, and/or 3) within species variation in leaf traits at the site level. We will then examine whether these trends are consistent across sites. We hypothesize that the magnitude of change in quantitative functional traits between treatments will depend on land-use history and climatic conditions, i.e. precipitation.

In the methods section below, there is a list of effect and response functional traits to be measured for the five most abundant plant species found within each of the treatment types, including the control treatment.

Paper 2: This paper will explore the hypothesis that invasive species are more plastic in terms of functional traits than native species. In order to do this, we need to measure the same functional traits as above for species on a "Most Wanted Species List". The species on this list are ones from the population observational paper where we have collected abundance data in their introduced and native range are included. The same functional traits as above should be measured for these species, even if not in the top five most abundant species, as per paper 1. This data will allow us to explore the trait plasticity of invasive species and also investigate the commonly held idea that intra-specific variability in plant traits is not an important consideration in cross-site comparisons. Several recent studies have found that invasive plant species are more plastic in their response to perturbations, but not fundamentally different in how they capture resources (Funk, 2008; Leishman et al., 2010). The following species are introduced species, some invasive others ubiquitous weeds, that have been measured at NutNet sites within both their native and introduced range.

Species Most Wanted List:

1. *Achillea millefolium*
2. *Agrostis capillaris*
3. *Agrostis stolonifera*
4. *Alopecurus pratensis*
5. *Anthoxanthum odoratum*
6. *Arrhenatherum elatius*
7. *Bellis perennis*
8. *Cerastium fontanum*
9. *Cirsium arvense*
10. *Cirsium vulgare*
11. *Dactylis glomerata*
12. *Festuca rubra*
13. *Hieracium pilosella*
14. *Holcus lanatus*
15. *Lolium perenne*
16. *Myosotis discolour*
17. *Phleum pratense*
18. *Plantago lanceolata*
19. *Poa pratensis*
20. *Poa trivialis*
21. *Ranunculus repens*
22. *Prunella vulgaris*
23. *Rumex acetosella*
24. *Taraxacum officianle*
25. *Trifolium pratense*
26. *Trifolium repens*

Proposed method:

Table 1: Functional traits proposed to be measured and recorded for the five most abundant species within each of the treatment types, including the control treatment and any species found on the 'Most Wanted List'.

Effect trait	**Measured or [1]recorded**	**Response trait**	**Measured or recorded**
Specific leaf area	measured	Life form	recorded
Growth form	recorded	Dispersal mode	recorded
Height	measured	Seed mass	Measured or recorded
Leaf dry matter content	measured	Photosynthetic pathway	recorded
		Total leaf nitrogen and phosphorus content	[2]measured

[1] Refers to characteristics found and recorded from the literature
[2]this measurement will depend on funding

Measuring SLA and LDMC (these methods are taken directly from standardised protocols for functional trait measurements written by Cornelissen et al.(2003):

Specific leaf area is the one-sided area of a fresh leaf divided by its oven-dry mass, expressed in mm^2/mg.

Leaf dry matter content is the oven-dry mass (mg) of a leaf divided by its water-saturated fresh mass (g), expressed in mg/g.

- The NutNet experiment is comprised of three blocks, 10 plots and 10 treatment combinations.
- Species composition and abundance measurements are recorded twice yearly.
- At the census time in the peak of the growing season, leaves would need to be collected from the three most abundant plant species (and any additional species) in each plot.
- From each species 3 leaves from 5 individual plants should be sampled. It is recommended that whole twigs or stems be sampled and the leaves not removed until right before measurements are taken.
    - The individuals sampled should be healthy, adult plants that have their foliage exposed to full sunlight.
    - Because SLA can vary throughout the day, leaves should be sampled at least 2-3 hours after sunrise and 3-4 hours before sunset.
    - The same leaves will be used for measuring both the SLA and LDMC.
    - **Storage**: Once collected the leaf samples should be wrapped in moist paper and put into a sealed plastic bag so that they remain water-saturated. They should then be stored in a cool-box or refrigerator—if none is available it is better to store them without any additional moisture in a sealed plastic bag. If storage is to last for more than 24 hours than low temperatures (2-6ºC) are necessary to avoid rotting.
- Leaf area measurements should be taken as soon as possible, preferably within 48 hours of collection.
- Rehydration for at least 6 hours is essential in order not to underestimate SLA and LDMC. For rehydration, place the cut end of the stem in deionised water (e.g. test

tubes) in the dark (please see Garnier et al., 2001for more detailed rehydration methods).

- Each leaf should be cut from the stem and gently rubbed before measurements. Water saturated fresh weight measurements should be taken using scales.
- Area measurements can be taken with specialised equipment such as the Delta-T or with a regular flat bed scanner using free software developed by researchers at the University of Sheffield, called "Leaf Area", website: http://www.sheffield.ac.uk/~nuocpe/ucpe/leafarea.html
- After area measurements are taken, leaves should be placed in an oven at 60ºC for 72 hours and then weighed for dry mass.

Measuring Seed Mass (taken from Cornelissen et al.(2003)):

Seed mass is the over-dry mass of an average seed of a species, expressed in milligrams.

- From the same species, collect 20 seeds from 5 individual plants
    - As with leaves, collect seeds from healthy plants that have their foliage exposed to full sunlight.
    - The seeds should also be mature and alive.
    - Dry the seeds at 80ºC for 48 hours.
    - The 20 seeds collected count as one statistical observation for calculations of mean, standard deviation and standard error.

The other functional trait measurements can be found in the scientific literature or through functional trait databases such as:

- USDA trait database, http://www.plants.usda.gov
- LEDA trait database, http://www.leda-traitbase.org/LEDAportal/applications.jsp
- http://data.kew.org/sid/
- http://ucjeps.berkeley.edu/interchange.html
- http://linnaeus.nrm.se/welcome.html.en

Data Format

Excel spreadsheet for data collection provided.

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