**Slide 1:**

* Hello, my name is Evan Batzer and I’m here to discuss the scale-dependence of community change in response to N enrichment.

**Slide 2:**

* Nitrogen enrichment is a major threat to biodiversity worldwide.
* Changes to biogeochemical cycling through atmospheric deposition, fertilizer runoff, and land use change have dramatically changed resource budgets in plant ecosystems.
* The effect of nitrogen enrichment on biodiversity is well-explored. Chief among hypothesized mechanisms of diversity loss is a change in availability of limiting resources that results in an increase in light limitation and shifts in community richness and relative abundance.
* Beyond this change in abundance, we also recognize that there are spatial mechanisms that may drive this change. N enrichment may reduce spatial heterogeneity in limiting resources, reducing the number of opportunities for spatial coexistence.
* Increasingly, we understand that our ability to detect these changes depends on the spatial scale of sampling

**Slide 3:**

* Concerns of scale are persistent in ecology, and recent scholarship has suggested that the impacts of human-induced environmental changes may depend on the extent of sampling.
* Effects may stay constant, change in magnitude, or even invert depending on the spatial scale at which we sample.
* However, much of our understanding of how nitrogen enrichment impacts plant diversity comes from relatively small-scale experiments that evaluate change in a limited spatial context, often 1m2 or less.
* Rather than focusing on a single scale, attempting to incorporate scale-dependence into analysis can give nuanced insights into the mechanisms of N-driven biodiversity change, particularly related to changes in species richness, relative abundance, and spatial pattern.

**Slide 4:**

To understand how cross-scale comparisons can provide insight into these effects, I will first guide you through a framework for this analysis, and how we can assess multiple outcomes of biodiversity change.

As an example, let’s say we have two communities, one of which has been subjected to experimental nutrient addition.

* Control community contains 6 species, a nitrogen enriched community contains just 4.
* In response to nutrient enrichment, this community also exhibits a more random spatial pattern.

**Slide 5:**

* Classically, we could evaluate this change using a single sampling scale – picking some subplot area and taking a measure of average alpha diversity values. In this case, we would detect relatively little difference in our communities.
* We could also incorporate a measure of the entire sampled community into our analysis – constructing a value of gamma diversity between the two communities. In this case, we find that our effect is magnified, and we detect substantial decline in response to treatment.
* Taking the ratio of these two measures can also give us a sense of community turnover, or beta diversity. If our effects diverge at different scales, we can attribute this to a change in patterns of turnover in species identity or composition.

**Slide 6:**

- However, this sort of analysis only gives us information on two coarse sampling scales. More nuanced information can come from decomposition of these relationships into sample-based rarefaction curves.

* One method in doing so is to construct spatially-explicit sample curves, where diversity measures are accumulated by always including new samples based on proximity to some starting point. Doing so allows us to examine how diversity is encountered in the system and where threshold points of change exist in sampling.
  + Here, we see that our observed difference in communities is nonlinear, and there’s a threshold sample size where interpretation of change flips.
* To understand how spatial patterns affect this interpretation, we can also accumulate samples at random. This nonspatial rarefaction will generate two separate curves that ignore spatial patterns.
  + If communities exhibit more spatial aggregation (clumping), we’ll see that this random accumulation will be much more rapid than its spatially structured counterpart.
  + Comparing observed differences between spatial and nonspatial curves can be used to quantify aggregation – in this case, our observed diversity change is more negative with random rarefaction, indicating that aggregation reduces our observed effect.
* For all of these metrics, we can use species richness (as in this example) or any other scale-invariant diversity metric, such as the inverse simpson index (equivalent to diversity at order 2, for those of you familiar with the hill numbers framework), which also incorporates changes in species cover. Doing so may contrast effects when purely focusing on either species identity or relative abundance.

**Slide 7:**

With this framework in mind, California grasslands are an ideal system within which to examine how these drivers of biodiversity change following nutrient enrichment.

To begin, California grasslands exhibit very high diversity despite their history of invasion – 10 or more species per square meter. Unique geologic history and high spatial heterogeneity of soil resources may be key to the maintenance of community diversity.

Current understanding often suggests that fertilization enhances the growth of exotic grasses at the expense of native forb and legume species.

**Slide 8:**

Using this framework, we asked a set of questions:

1. How do choices of sampling scale affect our interpretation of nitrogen-driven biodiversity change?
   1. Do effects increase, decrease, or stay with same as a function of spatial scale?

2. To what degree is the scale-effect relationship dependent on changes in species richness and relative abundance?

1. Are these changes primarily mediated by loss of species from the system, or changes in their relative abundance that interact with sampling effects.

3. Does N enrichment have a spatially homogenizing effect on diversity?

* 1. Is there potential loss in relevant nitrogen variation with treatment that may play a role in the spatial structure of communities? At what scale is this effect most apparent?

**Slide 9:**

To assess scale-dependent patterns of nitrogen enrichment on plant diversity, we established a nested plot experiment at three University of California reserves.

In each reserve we established 4 blocks, each consisting of 2 paired plots

Each subplot was then divided into an 8x8 grid containing .5m x .5m subplots spaced every 1m.

We then randomly assigned one plot out of each pair to an experimental fertilization treatment, consisting of a slow-release urea fertilizer applied at a rate of 10g N/m2 in winter months.

**Slide 10:**

- We then collected data on three response variables:

- The first, community diversity and percent areal cover, was collected in all subplots at three times in each year, corresponding to peak phenological periods to ensure that all representative species were sampled.

- Light was measured using a 10-cell PAR sensor placed above and below the grass canopy in 10 random locations within each plot.

- We also measured changes in plant productivity using clipped strips of aboveground biomass in the margins between individual subplots.

**Slide 11:**

Consistent with prior studies of nutrient enrichment, nitrogen addition produced significant increases the amount of light interception in both years, indicating an increased demand for light as a limiting resource.

**Slide 12:**

Commensurate with an increase in light limitation was some evidence of increase in plant productivity. While there was a trend towards increased biomass in response to treatment, we didn’t find significant results in our first two years of sampling, perhaps due to the variability of these measures.

**Slide 13:**

Despite this increase in light limitation, permutation-based tests of significance indicate that communities showed no significant change in either species richness, or abundance-weighted diversity in our first year of sampling. This is not surprising – California grasslands, as an annual system, are likely to take more than one year for nutrient enrichment to affect community assembly.

**Slide 14:**

After two years of treatment, however, nutrient enrichment showed strong effects, though our interpretation differed significantly depending on the choice of metric used.

When purely focused on species richness, comparison of alpha-beta-gamma relationships indicate that nitrogen enrichment results in a substantial decline in alpha-scale diversity (individual subplots), though did not appear to cause consistent reductions in the total number of species present within the entire spatial extent of sampling. This change corresponds with an interpreted increase in beta diversity – the turnover in species richness found within subsamples in each plot.

Incorporating abundance-weighted metrics into analysis, on the other hand, demonstrates declines in diversity that are consistent at both the individual sampling unit and whole plot scale, with no significant change in the turnover of these communities.

**Slide 15:**

Further decomposition of species richness change into its constituent accumulation curves indicates that response change with sampling effort was highly nonlinear – even small increases in sample accumulation dramatically reduced effects of nutrient enrichment. What we see here is the estimated biodiversity loss at the two endpoints, and estimated change as we accumulate samples in a spatial fashion. Threshold response at ~10 sample.

When this spatial accumulation curve is compared to its random counterpart, we see that diversity loss was larger when ignoring spatial structure. The lines plotted here show This change suggests that our control communities exhibit a somewhat higher degree of spatial aggregation (clumping) at small sampling scales.

**Slide 16:**

Use of abundance-weighted metrics to calculate diversity change effects indicates a far more linear response as a function of sampling effort – regardless of the number of samples taken, nutrient enrichment effects on the relative abundances of different species appears roughly consistent.

Moreover, the spatial aggregation effect in control treatments is far more pronounced when incorporating abundance into estimates of diversity change, and is detectable at far greater sampling efforts.

**Slide 17:**

In summary – we found evidence for scale-dependence of N-enrichment effects, but they depended on the metric used. When focusing purely on species richness, we found that N enrichment reduced species diversity in our individual sampling subplots, but failed to do so at broader spatial scales. This change was also highly nonlinear – relatively minor changes in sampling effort had really dramatic effects on interpreted responses to nutrient enrichment.

When focusing on abundance-based metrics, however, we found little evidence for scale-dependent change. What this suggests is that while species are rarely being lost from the system, the relative abundance of species within the community has become less even. As a result, increases in sampling effort are likely to pick up these rare species, eventually reaching a similar overall species pool.

When comparing the shape of our random and spatial rarefaction curves, we also find evidence for spatial homogenization in response to nitrogen enrichment. This finding suggests that N enrichment reduces the presence of aggregation (clustering) of species in space.

**Slide 18:**

What these findings suggest is that attempting to extrapolate the results of small-scale Nutrient addition experiments using species richness as a response may overestimate species loss at larger scales. At least in the relatively short-term, scale-invariant metrics of relative abundance change may be a more effective measure.

This analysis may also shed light on the role of spatial processes that may act independently of biodiversity change. Large changes in intraspecific aggregation, for example, may affect spatial coexistence mechanisms, the role of storage effects, and response to disturbance within a system.

Cumulatively, cross-scale analyses may lead to deeper insights into patterns and mechanisms of biodiversity change in response to nutrient enrichment. In particular, we can use this framework to better understand how changes in biodiversity at the small scales of our experimental studies may translate to the broader contexts at which conservation and land management are conducted.