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Quantifying spatial patterns of grass response to nutrient additions using empirical and neutral semivariogram models

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ABSTRACT

Disturbances influence vegetation patterns at multiple scales, but studies that isolate the effect of scale are rare, meaning that scale and process are often confounded. To explore this, we imposed a large (~3.75 ha) experiment in a South African coastal grassland ecosystem to determine the spatial scale of grass response to nutrient additions. In two of six 60 x 60 m grassland plots, we imposed nutrient additions using a scaled sampling design in which fertilizer was added in replicated sub-plots of varying sizes (1 x 1 m, 2 x 2 m, and 4 x 4 m). The remaining plots either received no additions, or were fertilized evenly across the entire plot area. We calculated empirical semi-variograms for all plots one year following nutrient additions to determine whether the scale of grass response (biomass and nutrient concentrations) corresponded to the scale of the sub-plot additions and compared these results to reference plots (unfertilized or unscaled). In addition, we calculated semi-variograms from a series of simulated landscapes generated using random or structured patterns (neutral models) and compared the semivariogram parameters between simulated and empirical landscapes. Results from the empirical semivariograms showed that there was greater spatial structure in plots that received additions at sub-plot scales, with range values that were closest to the 2 x 2 m grain. These results were in agreement with simulated semivariograms using neutral models, supporting the notion that our empirical results were not confounded by random effects. Overall, our results highlight that neutral models can be combined with empirical semivariograms to identify multiscale ecological patterns and this hybrid approach should be used more widely in ecological studies.
The importance of spatial autocorrelation for understanding vegetation distributions and processes is well-known (Turner 1989; Watt 1947) and these insights have augmented our understanding of how spatial structure of soils, plants, and climate can regulate ecosystem function, often at multiple, nested scales (Turner et al. 2012). However, for any given study, the scale of this autocorrelation structure and its implications for inferring ecological processes are often not known in advance. Select studies have employed experimental spatial designs *a priori* (Stohlgren et al. 1995) or have used computational models to explore the influence of space on ecosystem properties (Jenerette & Wu 2004; Smithwick et al. 2003; With & Crist 1995).

Geostatistical analysis of ecological patterns is also implemented widely (Jackson & Caldwell 1993b; Robertson et al. 1993; Smithwick et al. 2005b) but is often used for observations on treatment response rather than to design experimental studies in advance. In the majority of experimental ecological studies, spatial autocorrelation is accounted for *post hoc* through statistical segregation of error terms (Beale et al. 2010).

Understanding the scale of ecological processes may help unravel a number of complex ecological questions. For example, disturbances generate structural patterns that may influence ecological processes at many scales (Schoennagel et al. 2008; Turner et al. 2007). However, developing predictive models about the scale of these responses is more difficult because disturbance processes can have cascading effects across scales (Falk et al. 2007; Peters et al. 2007). The emergence of bark beetles in the Western U.S. is another multi-scalar process that can only be understood through an unraveling of processes that scale from the tissue-level to the landscape-level (Raffa et al. 2008). Similarly, in savanna science, determining the factors that govern the distribution of grassland and woodland ecosystems is a central focus of many studies (House et al. 2003; Scholes & Archer 1997) and multiple spatial scales are likely needed to
explain complex grass-tree interactions (Mills et al. 2006; Okin et al. 2008; Pellegrini 2016; Wang et al. 2010). Herbivore grazing or fire disturbances may each influence savanna nutrient cycling but the scale at which these systems respond is unclear given that the spacing of individual plants, soil properties, terrain, and herbivore assemblages can each contribute to spatial patterns in nutrient availability (Augustine & Frank 2001; Liu et al. 2016; Okin et al. 2008; Robertson et al. 1993; Senft et al. 1987; Smithwick et al. 2005b).

New approaches are needed to characterize how ecosystems re-organize in response to disturbance and to determine whether there are specific scale or scales of response that are most relevant. In so doing, it may be possible to reduce bias in empirical studies due to scale, thus deepening insights into ecological processes controlling those patterns and minimizing the potential for scale mismatches (Cumming 2011; Ludwig et al. 2000). Implications of such understanding can be significant. For example, Gil et al. (2016) recently found that increasing the scale of nutrient enrichment weakens herbivore control of primary producers, challenging understanding of ecosystem resilience to ecosystem eutrophication. In a more applied setting, it has been shown that the spatial scale of grazer movement can influence nutrient additions in an agricultural field, which could be used to more efficiently target agricultural management activity (Fu et al. 2013). Unraveling such bias or, more optimistically, incorporating scale into ecological prediction and application, requires better experimental approaches to explicitly test for the influence of scale on an ecological pattern.

To address this, our overarching goal was to develop a novel methodology to directly test for multi-scalar patterns in primary production following nutrient addition. Our first objective was to determine: at what scale does vegetation respond to nutrient additions? Understanding nutrient limitation to grass productivity is an important question in its own right (Craine et al.
but often neglects questions related to the scale of the response. We hypothesized that the grass response would differ between three subplot scales (1 x 1 m, 2 x 2 m, and 4 x 4 m). These scales were chosen to correspond to ecosystem processes that might govern nutrient uptake, including the spacing of grass tufts, trees or shrubs, and plot-level topography, respectively, which have been identified as critical sources of variation in soil biogeochemistry (Ettema & Wardle 2002; Jackson & Caldwell 1993a; Rietkerk et al. 2000; Smithwick et al. 2005b). We surmised that insights gained about the specific scale of response could be used in future studies to understand the scale-specific ecological mechanisms that might be operating at that scale.

In addition to testing the within-plot scale of vegetation response to fertilization, our second objective was to test whether there would be stronger spatial structure (enhanced patchiness) across plots in which nutrients were added at multiple subplot scales, compared to homogenously fertilized or unfertilized reference plots. To avoid issues of pseudoreplication, these comparisons were made between empirical semivariograms and neutral semivariograms (computer-simulated landscapes that mimic hypothesized patterns). Empirical and neutral semivariograms were compared for six conditions that received, or were artificially modeled to simulate, different nutrient addition patterns (no fertilizer, homogenously fertilized, or fertilized in subplot scales) and levels of grazing (fenced or unfenced). We hypothesized that the ranges (autocorrelation distances, or length scales) calculated in the neutral models would be comparable to the ranges calculated from empirical data, and that they would show greater spatial structure in plots that received heterogeneous fertilizers compared to reference plots.

In addition, we used these neutral models to explore the influence of herbivory on the spatial structure of fertilized or reference plots. Following nutrient additions, herbivores can
influence patterns of primary productivity through enhanced consumption of nutrient-rich patches and/or through enhanced deposition of new nutrients via manure or urine from animals entering the fertilized area, which may have variable effects on spatial heterogeneity depending on the scale of the observations (Fuhlendorf & Smeins 1999). Consumption of nutrient-rich patches may reduce overall variance by reducing differences in biomass amounts compared to ungrazed areas but new nutrient additions by animals also occur when animals move across the plot, or rest in new locations, thus enhancing variability (Auerswald et al. 2010). Therefore, in this study we tested the hypothesis that herbivory increases spatial heterogeneity (Liu et al. 2016) such that patchiness would be highest. i.e., range scales would be smaller, in plots that received both manure additions and scaled nutrient additions (unfenced and heterogeneously fertilized), relative to plots that received only grazing or only homogeneous nutrients.

Our third objective was to estimate the degree to which grass biomass was related to patterns in foliar nitrogen content (% N) and phosphorus content (% P), while accounting for random effects due to scale and treatment. Generally, temperate systems are expected to have greater levels of N limitation on vegetation growth than sub-tropical or tropical systems, where P may be more limiting due to highly weathered soils (Domingues et al. 2010; Hedin 2004; Lambers et al. 2008; Vitousek & Sanford 1986). Yet, N, P, and N+P limitations on vegetation productivity have all been documented in African savanna or grassland systems (Augustine et al. 2003; Craine et al. 2008; Ngatia et al. 2015; Okin et al. 2008), indicating that an understanding of nutrient limitations on biomass productivity in these systems is still unclear. Complexity in understanding differences in nutrient limitations has been attributed to, for instance, differential nutrient affinities across functional groups (Ratnam et al. 2008; Reich et al. 2003) as well as complex herbivory feedbacks (Augustine et al. 2003), both of which are compounded by issues
of scale (Ekblom & Gillson 2010; Fuhlendorf & Smeins 1999). While a complete test of these factors was beyond the scope of this study, we had the opportunity to explore the effect of foliar nutrient concentrations on biomass responses under a dual nutrient addition experiment, while treating the levels of our experimental design (e.g. plot, subplot, treatment) as separate random effects. By so doing, we were able to explore whether foliar N, P or N and P together explained patterns in biomass, beyond the given variability in our study design, and to determine the level (scale) at which these random effects were most pronounced.

METHODS

Study area. This study was conducted in Mkambathi Nature Reserve, a 7720-ha protected area located at 31° 13′ 27″ S and 29° 57′ 58″ E along the Wild Coast region of the Eastern Cape Province, South Africa. The Eastern Cape is at the confluence of four major vegetative groupings (Afromontane, Cape, Tongaland-Pondoland, and Karoo-Namib) reflecting biogeographically complex evolutionary histories. It is located within the Maputaland-Pondoland-Albany conservation area, which bridges the coastal forests of Eastern Africa to the north, and the Cape Floristic Region and Succulent Karoo to the south and west. The Maputaland-Pondoland-Albany region is the second richest floristic region in Africa, with over 8,100 species identified (23% endemic), and 1,524 vascular plant genera (39 endemic). Vegetation in Mkambathi is dominated by coastal sour grassveld ecosystems, which dominate about 80% of the ecosystem (Kerley et al. 1995; Shackleton et al. 1991), with small pockets of forest along river gorges, wetland depressions, and coastal dunes. Dominant grasses in the Mkambathi reserve include the coastal *Theseda triandra* – *Centella asiatica* grass community, the tall grass *Cymbopogon validas* – *Digitaria natalensis* community in drier locations, and the
short-grass *Tristachya leucothrix-Loudetia simplex* community (Shackleton 1990). Grasslands in Mkambathi have high fire frequencies, and typically burn biennially. Soils are generally derived from weathered Natal Group sandstone and are highly acidic and sandy with weak structure and soil moisture holding capacity (Shackleton et al. 1991). Climate is generally considered warm temperate, where average monthly minimum and maximum temperature in East London is 14 and 23 °C respectively, and precipitation is 921 mm yr\(^{-1}\) (1961 – 1990 average, S.A. Weather Service).

We established a large-scale experimental site that included six 60 x 60 m plots arranged in a rectangular grid (Eastern Cape Parks and Tourism Agency Permit RA0081). The site was surrounded by a fuel-removal fire-break and each plot was separated by at least 10 m for a total size of 3.75 ha for the entire site. To account for grazing, a fence was constructed around three of these plots.

**Nutrient additions.** Nutrient additions were applied to four plots whereas two plots received no fertilizer additions. Of the four plots that received fertilization, two received nutrients evenly across the entire 60 x 60 m plot (“homogenous plots”) and the other two fertilized plots received nutrient additions within smaller subplots in a heterogeneous design (“heterogeneous plots”). Within heterogeneous plots, fertilizer was applied within subplots of three different sizes (1 x 1 m, 2 x 2 m, and 4 x 4 m) that were replicated randomly across each plot (Fig. 1). Location of individual subplots was determined prior to field work using a Latin Hypercube random generator that optimizes the variability of lag distances among sampling plots and is ideal for geostatistical analysis (Xu et al. 2005). There were a total of 126 subplots per plot that received fertilizer in the heterogeneous plots. All sampling locations were geo-referenced with a GPS.
The number of sub-plot units at each scale was determined so as to equalize the total fertilized area at each sub-plot scale (i.e., six 4 x 4 m plots and 24, 2 x 2 m plots).

To ensure aboveground grass biomass would respond to nutrient additions, we employed a dual (nitrogen (N) + phosphorus (P)) nutrient addition experiment. Additional N was added as either ammonium nitrate (230 g kg\(^{-1}\) N) or urea (460 g kg\(^{-1}\) N) at a rate of 10 g m\(^{-2}\) yr\(^{-1}\) in a single application, following the protocols of Craine et al. (2008). Additional P was added as superphosphate (105 g kg\(^{-1}\) P) at a rate of 5 g m\(^{-2}\) yr\(^{-1}\). Dual addition (N+P) was chosen to increase the likelihood of treatment response and increase geostatistical power by reducing the number of treatments, thus increasing sample size. Per unit area, levels of nutrient additions were constant among plots and subplots.

**Vegetation and Soil Sampling.** One year following nutrient additions, a subset of subplots was sampled for soil and vegetation nutrient concentrations and biomass. Sub-plots to be sampled were randomly selected prior to being in the field using the Latin Hypercube approach. The approach allowed us to specify a balanced selection of subplots within each subplot size class (four 4 x 4 m, eight 2 x 2 m, and thirty-two 1 x 1 m). Within each subplot location that was revisited, we randomly selected locations for biomass measurement and vegetation clippings. Two locations were identified and flagged from within the 1 x 1 m subplots (center coordinate and a random location 0.5 m from center), four samples were identified and flagged from within the 2 x 2 m subplots, and eight samples were identified and flagged from within the 4 x 4 m subplots. At each flagged location within sampled subplots, productivity was measured as grass biomass using a disc pasture meter (DPM; Bransby and Tainton, 1977) and grab samples of grass clippings were collected for foliar nutrient analysis, using shears and cutting to ground-level. Calibration of the DPM readings was determined using ten random 1 x 1 m subplots in each plot.
(n = 60 total) that were not used for vegetation or soil harvesting, in which the entire biomass
was harvested to bare soil. Soil samples from the top 0 – 10 cm soil profile depth were collected
adjacent to vegetation samples. Due to logistical and financial constraints, these samples were
collected in fenced plots only. The A horizon of the Mollisols was consistently thicker than 10
cm, so all samples collected were drawn from the A horizon. Soil samples were shipped to
BEMLab (Strand, South Africa) for nutrient analysis.

Laboratory Analysis. Biomass samples were separated into grasses and forbs, weighed, dried
for 24 h at 60 °C, and reweighed. Vegetation nutrient samples were dried, ground with a 40 mm
grinding mesh, and then shipped to the Penn State Agricultural Analytical Laboratory
(University Park, Pennsylvania; USDA Permit PDEP11-00029). Grass P concentration was
analyzed using a hot block acid digestion approach (Huang & Schulte 1985) and grass N
concentration was measured with a Combustion-Elementar Vario Max method (Horneck &
Miller 1998). Soil N and C concentrations were determined on a LECO elemental analyzer
(Leco Corporation, St. Joseph, MI). Soil P was analyzed using acid extraction following the
method of Wolf and Beegle (1995). Soil pH was estimated using KCl extraction following

Calculations and Statistics. Aboveground grass biomass was estimated from DPM
measurements, using linear regression to relate DPM estimates with harvested biomass at
calibration subplots. DPM estimates were significantly correlated with total grass biomass (R² =
0.76, p < 0.0001) (Supplementary Material, Fig S1) and the resulting equation was then used to
estimate biomass at the remaining 606 locations.
To test the scale of grass biomass response to fertilization (Objective 1), we used a maximum likelihood approach to quantify the spatial structure of biomass separately for each of the six empirical plots. The approach assumes that the data \((Y_1, \ldots, Y_n)\) are realizations of an underlying spatial process, and that the distribution of the data follows a Gaussian multivariate distribution:

\[
Y \sim N(\mu 1, \Sigma + C_0 I)
\]

where \(\mu\) is the mean of the data multiplied by an \(n\)-dimensional vector of 1’s, \(C\) is the partial sill (total sill = \(C_0 + C\)), \(\Sigma\) is an \(n \times n\) spatial covariance matrix, \(C_0\) is the nugget effect, and \(I\) is an \(n \times n\) identity matrix. The \(i,j\)th element of \(\Sigma\) is calculated with a spatial covariance function \(\rho(h_{ij})\), where \(h_{ij}\) is the Euclidean distance between measurement points \(i\) and \(j\). An exponential covariance model was chosen for its relative simplicity. The full equation for summarizing the second order moment for an element \(i,j\) is:

\[
\gamma(h_{ij}) = C_0 + C \left[ \exp\left( -\frac{h_{ij}}{\phi} \right) \right]
\]

where \(\gamma(h_{ij})\) is the modeled spatial covariance for measurements \(i\) and \(j\), \(\phi\) is the range parameter, and \(3 \times \phi\) is the range of spatial autocorrelation. The underlying spatial mean \(\mu\) may be held constant or estimated with a linear model across all locations. We used the plot-level mean of the data for \(\mu\) (Table 1).

The measured soil and plant variables exhibited varying degrees of non-normality in their distributions, which violates the assumption of Gaussian stationarity within the underlying spatial data generating process. To uphold this assumption, we transformed variables at each plot using a box-cox transformation (Box & Cox 1964):

\[
\hat{Y}_i = \left( Y_i^{\lambda} - 1 \right) / \lambda \quad \text{if} \quad \lambda \neq 0
\]
\[
\hat{Y}_i = \log(Y_i) \quad \text{if } \lambda = 0
\]

where \(Y_i\) is an untransformed variable (e.g., biomass) at location \(i\), \(\hat{Y}_i\) is the transformed variable, and \(\lambda\) is a transformation parameter. We optimized the three spatial covariance model parameters and the transformation parameter \((C_0, C, \phi, \lambda)\) with the maximum likelihood procedure. A numerical finite-difference approximation algorithm selected the set of parameters that maximized a normal multivariate log-likelihood function (more details in Diggle et al, 2003). To approximate a sampling distribution of each parameter, a bootstrapping algorithm was used where a randomly sampled subset of data was inputted into the same maximum likelihood approach for 1000 iterations. This provided a population of fitted parameters and models that was used to analyze the approximate distributions of each parameter for each plot. The maximum likelihood optimization was cross-validated by removing a random sub-sample of measurements from the optimization and then using the optimized model to make predictions at locations where measurements were removed. Observed vs. predicted values from the cross-validation procedure were then analyzed at each plot separately.

We used ordinary kriging (Cressie 1988) with the optimized spatial covariance model from the maximum likelihood analysis to estimate biomass across all plots. Ordinary kriging is useful in this case, because we detected spatial structure in the biomass data when considering all biomass data at once (see Results). The geoR package (Ribeiro Jr. & Diggle 2001) in the R statistical language (R Development Team, 2014) was used for all spatial modeling and kriging.

To compare spatial structure across empirical or simulated patterns (Objective 2), we compared the empirical semivariograms with neutral semivariogram models. Essentially this approach allows us to compare empirical patterns across a set of null models in which the patterns are known. The neutral semivariogram models were constructed for six simulated
268 landscapes (Fig. 2) to represent alternative landscape structures in response to nutrient addition
269 and grazing: (a) fenced-unfertilized (biomass was assumed to be randomly distributed around the
270 mean of the biomass from the fenced, unfertilized experimental plot), (b) fenced-heterogeneous
271 (biomass of (a) was doubled for selected subplots, following the same subplot structure that was
272 used in the field experiments), (c) fenced-homogenous (biomass of (a) was doubled at every grid
273 cell to mimic an evenly distributed fertilization response), (d) unfenced-unfertilized (biomass of
274 (a) was increased by 50 % in response to a combined effect of biomass loss by grazing and
275 biomass gain by manure nutrient additions by herbivores; the increase occurred at a subset of
276 sites to mimic random movement patterns of herbivores), (e) unfenced-heterogeneous (biomass
277 equaled biomass of herbivory only, fertilizer only, or herbivory + fertilizer), and (f) unfenced-
278 homogenous (biomass of (d) was doubled at all grid cells to mimic the additive effects of
279 herbivores and homogenous fertilizer additions).

The spatial structure of simulated landscapes was analyzed using the same maximum
280 likelihood approach as described above and data was not transformed. The mean (µ) was
281 estimated using a constant trend estimate. To compare neutral (simulated data) and empirical
282 (observed data) semivariogram models, we scaled the nugget and sill model parameters by
283 dividing these parameters into the maximum observed semivariance value within each plot.

The relationship between biomass and foliar N and P (Objective 3) was investigated
286 using a linear mixed modeling approach, where herbivory, fertilizer type (i.e., heterogeneous,
287 homogenous, and unfertilized), plot treatment, and subplot size were all included as random
288 effects. Accounting for experimental factors as random effects is a desirable approach for
289 managing non-independence in data collected across different treatments that are not replicated
290 (i.e., pseudoreplication) (Millar & Anderson 2004). Multiple combinations of random effects
and fixed effects were tested, where foliar N and P represented fixed effects upon biomass, and
model error was assumed to be Gaussian. A normal likelihood function was minimized to
estimate optimal regression coefficients for each mixed model formulation.

Our objective was to identify a mixed model that estimated biomass closely to
observations, while also having the fewest possible parameters. The Akaike’s Information
Criterion (AIC) and Bayesian Information criterion (BIC) were used to compare different
models, since they decrease with a negative log-likelihood function but increase with the amount
of parameters used in the model (Burnham and Anderson, 2004). After all model formulations
were fitted to observed biomass and both AIC and BIC were calculated for each model, we chose
the model with the lowest BIC as best representing the tradeoff of parsimony and prediction
skill. The BIC associated with all other models was subtracted into the lowest available BIC,
and models with a difference in BIC > 2 were deemed significantly less favorable at estimating
biomass and representing random effects than the model with the lowest BIC. All mixed
modeling was conducted with the R package lme4.

RESULTS

Across plots, vegetation biomass ranged from 376 ± 6.0 g m\(^{-2}\) (mean ± 1 Standard Error
(SE), n = 124) in the unfenced, homogenously fertilized plot to 563 ± 18.6 g m\(^{-2}\) (n = 128) in the
unfenced, heterogeneously fertilized plot (Table 1). Forb biomass was approximately 18 % of
total biomass, with the remaining 82 % represented by grass. Vegetation nutrient concentrations
increased, and N:P ratios declined, following fertilization (Table 1). Vegetation N concentration
ranged from 0.58 ± 0.01 % to 0.75 ± 0.04 %, averaging 0.60 ± 0.01 % in unfertilized plots versus
an average of 0.72 ± 0.02 % in heterogeneously fertilized plots and 0.77 ± 0.02 % in
homogenously fertilized plots, an increase of 20 % and 28 %, respectively. Vegetation P concentration averaged \(0.037 \pm 0.001 \text{ mg g}^{-1}\) in unfertilized plots, \(0.056 \pm 0.002 \text{ mg g}^{-1}\) in heterogeneously fertilized, and \(0.057 \pm 0.002 \text{ mg g}^{-1}\) in homogeneously fertilized plots, an increase of 34 and 35%, respectively. The N:P ratios ranged from a high of 17.9 in the fenced-unfertilized plot to 12.1 in the unfenced-homogenously fertilized plot. Vegetation C content averaged 44.6 ± 0.13 % across all six plots. Soil P and N were also higher following fertilization in the fenced plots, where these variables were measured (Supplementary Table 1). Soil C ranged from 2.49 ± 0.01 % to 2.55 ± 0.01 % across plots. Soil pH was 4.27 in the unfertilized plot and 4.08 in fertilized plots. Confirming reference conditions, pH measured in a single control plot in 2011 prior to fertilization was 4.21± 0.01.

We had expected that biomass and vegetation nutrient concentrations following fertilization would be spatially autocorrelated at scales corresponding to the scale of the fertilization additions (Objective 1). In heterogeneously fertilized plots this would be indicated by range distances from empirical semivariograms that corresponded to the hypotenuse distances of the subplot scales (i.e., 1 m, 2.83 m, and 5.66 m hypotenuse distances for the 1 x 1 m, 2 x 2 m, and 4 x 4 m subplots, respectively). In homogenously fertilized plots or unfertilized plots, spatial structure would be observed at scales other than scales of the subplots (or not at all) and we would expect a higher degree of nugget variance (spatial structure expressed at scales finer than those that were measured). Results from the empirical semi-variogram model of the heterogeneous plots show that there was a statistically significant patch structure at scales similar to the scale of the subplots in both of the heterogeneously fertilized plots (Fig. 3b,f). Also confirming these expectations, in unfenced and fenced plots (unfertilized or homogenously fertilized) the range scale was significantly longer or shorter (Fig. 3; Supplementary Table 2).
Examining the sampling distributions of the semivariogram range values for vegetation biomass determined from the maximum likelihood and bootstrapping analysis, it can be seen that the range value most closely resembles that of the hypotenuse of the 2 x 2 m subplot, relative to the other subplots (Fig. 3d,h). Higher spatial structure in the heterogeneous versus homogeneous or unfertilized plots can also be seen in the kriged plots of biomass (Fig. 4). These maps also demonstrated the higher mean levels of biomass in fertilized subplots relative to areas outside of subplots or relative to other plots. Normalized nugget/sill ratios were highest in the unfenced, homogeneously fertilized plot (3.89) with lower ratios (0-0.02) for heterogeneously fertilized or fenced treatments.

Consistent with the biomass results, semivariogram parameters for vegetation % N and % P (Supplementary Table 3) generally supported expectations, at least for the heterogeneously fertilized and fenced plot, in which range scales were comparable to subplot scales in the (% P, ~4.9 m; % N, ~ 5.8 m); however, other plots showed higher or lower ranges. The proportion of nugget variance in semivariogram models of vegetation % N and % P was highest in the unfertilized plots, which supports expectations of higher spatial structure in fertilized treatments, as for biomass, but differences were variable among individual plots. However, semi-variograms of soil carbon and nutrients (Supplementary Table 3) showed few differences among model parameters among treatments where these were measured (fenced plots, only). In sum, differences in spatial structure that could be attributed to treatments were most evident for biomass, somewhat evident for vegetation % N and % P, and least evident for soil C or nutrients.

To compare mean differences among plots (Objective 2), we compared normalized spatial model fits of neutral and empirical semi-variograms, which allowed us to quantify the relative influence of treatment responses to random processes not accounted for in the
experimental design. Results indicated that the range of autocorrelation in heterogeneous plots was similar to that found in the empirical semi-variograms and generally matched subplot scales (Fig. 5). Interestingly, range scales were higher, and proportional nugget variance was greater in fenced plots, compared to unfenced plots. This was an opposite pattern than that observed in empirical plots, in which grazing resulted in longer range scales and higher nugget variance. These results likely reflect processes not included in the neutral model. Particularly, the neutral model was designed to mimic patterns in biomass using assumptions about herbivore movement, grazing, and manure additions, assuming they were additive to the effects of fertilization, whereas empirical results likely reflect complex interactions between grazing and fertilization. In this case, longer observed range scales in grazed versus ungrazed plots may reflect enhanced homogenization of biomass through grazing, an effect that was apparently greater than biomass enhancement from nutrient addition. Nonetheless, the fact that heterogeneous plots were similar between fenced and unfenced plots indicates the strong scalar influence of nutrient additions relative to nutrient-herbivore interactions.

Addressing Objective 3, mixed models used to estimate biomass from N or P foliar concentrations, while treating plot and treatment as random effects, showed that biomass was best predicted by levels of foliar P, relative to foliar N alone or foliar N x P (Table 2). Although foliar P alone did better than foliar N alone as a fixed effect, the difference was marginal (< 2 BIC). The ‘best’ model uses only plot treatment type as a random effect, which outperformed model formulations using herbivory or fertilizer type and those with nested structures incorporating subplot size as random effects.

**DISCUSSION**
Scale issues confound a complete understanding of ecosystem response to perturbations (Turner 2010). This is particularly important in grassland or savanna systems in which the relative importance of factors affecting grass-woodland ecosystems are debated (e.g., Mills et al. 2006; Sankaran et al. 2004; Scholes & Archer 1997), with some suggesting that these factors are nested hierarchically with spatial scale (Pellegrini 2016; Pickett et al. 2003; Rogers 2003).

Results of this experiment yield data on the spatial scale of the nutrient-productivity relationship in a grassland coastal forest of the Eastern Cape, South Africa, and support the assertion that ecological processes are multi-scaled and hierarchical in response to nutrient additions. Specifically, we observed immediate (one year following fertilization) biomass response at the plot level due to nutrient addition and fencing. Using spatial modeling, we discerned strong autocorrelation structure in biomass at subplot scales associated with scales of nutrient addition. The strongest evidence for spatial structure was observed at the 2 x 2 m scale. Comparing this spatial structure between neutral and empirical semivariogram models, we were able to infer the relative importance of treatment conditions (nutrients and grazing) to random, spatially structured processes. Traditional ecosystem-level approaches to quantify spatial pattern have focused on understanding extant patterns, in which processes are necessarily inferred or correlated. However, by examining spatial patterns under experimental conditions in which scale and treatment are controlled, we were able to relate ecological responses to known scales of perturbation.

Subplots responded very strongly to nutrient additions at subplot scales, as evident from the kriged maps, showing that most of the increase in average plot biomass was due to large increases in subplot biomass. One surprising result of our study was the detection of average greater biomass in plots that received heterogeneous fertilizer applications compared to plots that
received homogeneous fertilizer, despite the fact that fertilizer was added equally on a per area
basis. Several other studies have found higher biomass following heterogeneous nutrient
applications. For example, Day et al. (2003) observed that heterogeneous spatial patterns of
nutrient supply in early stages of grassland development led to enhanced nutrient acquisition and
biomass productivity. Similarly, Du et al. (2012) observed increased plant biomass following
heterogeneous nutrient fertilization in old-field communities in China. Mechanisms for
enhanced productivity following heterogeneous nutrient supply are not clear but may include
shifts in root structure and function or shifts in species dominance, which were not analyzed
here. For example, roots may respond to patchiness in nutrient availability by modifying root
lifespan, rooting structures and uptake rate to maximize nutrient supply (Hodge 2004; Robinson
1994). In turn, initial advantages afforded by plants in nutrient-rich locations may result in
larger plants and advantages against competitive species, potentially via enhanced root growth
(Casper et al. 2000).

Observing vegetative response to nutrient additions is complicated by processes such as
luxury consumption (Ostertag 2010), initial spatial patterns in soil fertility (Castrignano et al.
2000), root distribution, signaling and allocation (Aiken & Smucker 1996), species and
functional group shifts (Ratnam et al. 2008; Reich et al. 2003), or species’ differences in uptake
rates or resorption (Reed et al. 2012; Townsend et al. 2007). Spatial patterns of finer-scale
processes such as microbial community composition have also been explored and are known to
influence rates of nutrient cycling (Ritz et al. 2004; Smithwick et al. 2005a). In the case of
heterogeneous nutrient supply, species competitive relationships across space may be enhanced
(Du et al. 2012) and may result in increases in plant diversity (Fitter 1982; Wijesinghe et al.
2005), although other studies have found little evidence to support this claim (Gundale et al.
Together, these factors may explain the high unexplained variance of vegetation N and P concentrations that we observed and may account for the nugget variance in our empirical spatial models. However, effects of species composition shifts on plant biomass were likely minimal in this study given the short-term nature of the study (one year), although the patchiness in biomass in gridded fertilizer plots indicates size differences that are likely to modify competitive relationships in the future. Unfortunately, the site burned one year following the experiment, precluding additional tests of these relationships.

Understanding the length scales of key ecosystem properties is critical for determining optimal scales for studying ecological systems, interpreting change in ecological communities, and assessing landscape connectivity and ecosystem resilience (Johnson 2009; Turner et al. 2012). Autocorrelation structure may reflect influences of spatial interactions among individuals, patches, or abiotic gradients (Ettema & Wardle 2002; Jackson & Caldwell 1993a; Rietkerk et al. 2000; Smithwick et al. 2005b). Our study showed that biomass responded strongly at all scales in which nutrients were added, but was strongest at the 2 x 2 m scale. Rietkerk et al. (2000) observed patchiness in soil moisture at three unique scales (0.5 m, 1.8 m and 2.8 m) in response to herbivore impacts. Following fire in the Greater Yellowstone Ecosystem (Wyoming, U.S.A.), Turner et al. (2011) observed variation in soil properties at the level of individual soil cores, and Smithwick et al. (2012) observed autocorrelation in post-fire soil microbial variables that ranged from 1.5 to 10.5 m. In savanna and boreal ecosystems, patchiness in soil resources at the level of individual shrubs and trees has been demonstrated by several studies (Dijkstra et al. 2006; Hibbard et al. 2001; Lechmere-Oertel et al. 2005; Liski 1995; Pennanen et al. 1999). Obviously, other contingent factors (e.g., herbivory, historical fire, land use) are important for explaining grass species distributions and productivity patterns at
broader temporal and spatial scales, but our study suggests that intermediate length scales (<4 m²) reflect ecological processes important for understanding patterns in grassland nutrition.

Many studies have used stoichiometric relationships of N and P to infer nutrient limitation (Koerselman & Meuleman 1996; Reich & Oleksyn 2004), although there are limits to this approach (Ostertag 2010; Townsend et al. 2007). Using this index, our N:P ratios of vegetation in reference plots would indicate co-limitation for N and P prior to fertilization (N:P > 16). Addition of dual fertilizer appeared to alleviate P limitation more than N, with N:P ratios reduced one year following treatment, indicating N limitation or co-limitation with another element (N:P < 14). In addition, P was most strongly correlated with biomass in linear mixed effects modeling across all plots, after accounting for random effects of the experimental design. Ostertag (2010) also showed that there was a preference for P uptake in a nutrient limited ecosystem in Hawaii and suggested that foliar P accumulation may be a strategy to cope with variability in P availability. Grazing may also preferentially increase grass P concentrations in semi-arid systems in South Africa (Mbatha & Ward 2010). The cumulative impacts of preferential plant P uptake and P additions from manure may explain the high spatial structure observed in our grazed and fertilized plots.

Spatial heterogeneity may be critical for influencing ecosystem resilience to stressors by mediating disturbance spread (i.e., fire, invasive species dispersal) (Yang et al. 2008), explaining patterns in disease emergence (Wu et al. 2015), or promoting recovery and regeneration through maintenance of legacy structures and functions (Turner et al. 2012). Understanding the importance of spatial pattern for maintaining soil fertility is increasingly relevant for agricultural management that incorporates precision technology as well as for ecosystem management activities that prioritize monitoring and restoration. In South Africa, grasslands cover nearly
one-third of the country and maintain the second-highest levels of biodiversity, behind the Cape Floristic Region. Grasslands of Mkambati are representative of ancient grasslands that are under threat globally, yet possess high levels of species endemism and provide significant ecosystem goods and services (Bond 2016). Grasslands in southern Africa are expected to undergo significant losses in biodiversity in coming decades due to increasing pressure from agricultural development and direct changes in climate (Biggs et al. 2008; Huntley & Barnard 2012). For example, losses in critical African bird species in fynbos and grassland biomass are expected to be 30 – 40% lower by 2085 as a result of projected climate change, with range reductions > 60% in many cases. As a result of these pressures, understanding the factors that regulate ecosystem productivity, and the scales at which they operate, is critical for guiding ecosystem management activities aimed at maintaining landscape sustainability.

CONCLUSIONS

This study provided an opportunity for direct hypothesis-testing of the influence of spatial pattern on ecosystem processes, providing an alternative to studies in which spatial autocorrelation is observed post-hoc or otherwise confounds complex ecosystem dynamics. Specifically, spatial models were used to test the scale at which vegetation responded to nutrient additions within plots and we compared these model parameters to those determined from model parameters from simulated landscapes using replicated neutral models. Biomass responded to nutrient additions, with spatial autocorrelation of the biomass response highest at the 2 x 2 m scale. Comparison of empirical and simulated neutral models, confirmed that this length scale accounted for the majority of the spatial structure in the observations. Vegetation P concentrations were elevated more than vegetation N suggesting plant P preference or luxury
consumption, although vegetation nutrient concentrations exhibited lower spatial structure than that observed for biomass. Finally, this study was undertaken in a nature reserve in which grasslands are being actively managed to meet multiple management objectives including wildlife management and biodiversity. These results indicate strong coupling between soil nutrients and aboveground function which should be considered in vulnerability assessments that consider grassland responses to shifts in climate and management activities.

ACKNOWLEDGEMENTS

We are deeply grateful to the support of Jan Venter, the Eastern Cape Parks and Tourism Board, and especially the Mkambathi Nature Reserve for allowing the establishment of the “Little Pennsylvania” study area. We are grateful to the hard work of the Parks and People 2010, 2011, and 2012 research teams, which included undergraduate students from The Pennsylvania State University who helped in all aspects of field research. Special thanks to the dedication of Sarah Hanson, Warren Reed, Shane Bulick, and Evan Griffin who assisted tirelessly with both field and laboratory work.
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Table 1 (on next page)

Plot-level biomass and vegetation nutrient concentrations.

Mean (± 1 standard error (SE)) biomass, vegetation N concentration, vegetation P concentration, and N:P ratios across experimental plots in Mkambathi Nature Reserve, one year following nutrient fertilization.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Average Biomass ± 1 SE (g m⁻²)</th>
<th>Average N ± 1 SE (%)</th>
<th>Average P ± 1 SE (%)</th>
<th>N:P</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Fenced</td>
<td></td>
<td></td>
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<tr>
<td>Unfertilized</td>
<td>411.9 ± 9.75</td>
<td>0.646 ± 0.024</td>
<td>0.036 ± 0.001</td>
<td>17.9</td>
<td>134</td>
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<td>Heterogeneous</td>
<td>542.4 ± 15.05</td>
<td>0.747 ± 0.041</td>
<td>0.048 ± 0.002</td>
<td>15.6</td>
<td>120</td>
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<tr>
<td>Homogeneous</td>
<td>456.2 ± 8.28</td>
<td>0.710 ± 0.014</td>
<td>0.054 ± 0.002</td>
<td>13.2</td>
<td>117</td>
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<tr>
<td>Unfenced</td>
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<td></td>
<td></td>
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<tr>
<td>Unfertilized</td>
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<td>0.038 ± 0.001</td>
<td>15.2</td>
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<td>Heterogeneous</td>
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<td>0.775 ± 0.015</td>
<td>0.064 ± 0.002</td>
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<td>0.722 ± 0.017</td>
<td>0.059 ± 0.002</td>
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Table 2 (on next page)

Mixed model results comparing biomass to foliar nutrients.

Results of the mixed model relating biomass to foliar nutrients, where herbivory, fertilizer type, plot treatment, and subplot size were all tested as random effects; foliar N and P represented fixed effects upon biomass, and model error was assumed to be Gaussian. A normal likelihood function was minimized to estimate optimal regression coefficients for each mixed model formulation. Both Akaike’s Information Criterion (AIC) and Bayesian Information criterion (BIC) were used to compare different models. Delta (Δ) represents differences in BIC between the current model and the model with the lowest BIC.
<table>
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<tr>
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<th>AIC</th>
<th>BIC</th>
<th>Δ</th>
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<td>1114.2</td>
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<td>1211.9</td>
<td>97.7</td>
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<td>1112.1</td>
<td>5.3</td>
</tr>
<tr>
<td>P</td>
<td>4</td>
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<td>1107.3</td>
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</tr>
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<td>1.3</td>
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</table>
Experimental design

Overview of experimental design based on Latin Hypercube sampling used to identify subplot locations to receive fertilizer in the heterogeneous plots.
Spatial maps of neutral models used to simulate vegetation biomass for the following conditions: (a) unfenced, unfertilized, (b) Unfenced, heterogeneously fertilized, (c) Unfenced-homogeneously fertilized, (d) Fenced, unfertilized, (e) Fenced, heterogeneously fertilized, (f) Fenced, homogeneously fertilized.
Empirical semivariograms

Empirical semi-variograms of vegetation biomass for each plot: (A) Unfenced, unfertilized, (B) Unfenced, Heterogeneously Fertilized, (C) Unfenced, homogeneously fertilized, (E) Fenced, unfertilized, (f) Fenced, heterogeneously fertilized, (G) Fenced, homogeneously fertilized. Shaded lines represent semi-variogram models fitted during the bootstrapping procedure. Dashed vertical line represents the range value. Also shown: the sampling distribution of the range parameter for heterogeneously fertilized plots that were either (D) Unfenced, or (H) Fenced. The distribution was calculated with a bootstrapping approach with maximum likelihood optimization. Dashed vertical lines represent the hypotenuses of the 1x1 m (1.4), 2x2 (2.8), and 4x4 (5.7) sub-plots.
Kriged biomass map.

Kriged map of biomass using ordinary kriging with a spatial covariance model optimized by a maximum likelihood analysis: (A) Unfenced, unfertilized, (B) Unfenced, heterogeneously fertilized, (C) Unfenced, homogeneously fertilized, (D) Fenced, unfertilized, (E) Fenced-heterogeneously fertilized, (F) Fenced, homogeneously fertilized.
Semivariograms from neutral models.

Simulated semivariograms of vegetation biomass for each plot from neutral landscape models: (A) Unfenced, unfertilized, (B) Unfenced, heterogeneously fertilized, (C) Unfenced, homogeneously fertilized, (D) Fenced, unfertilized, (E) Fenced, heterogeneously fertilized, (F) Fenced, homogeneously fertilized. Shaded lines represent semi-variogram models fitted during the bootstrapping procedure. Dashed vertical line represents the optimal range value. Also shown: the sampling distribution of the range parameter for heterogeneously fertilized plots that were either (D) Unfenced, or (H) Fenced. The distribution was calculated with a bootstrapping approach with maximum likelihood optimization.