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

Erica A.H. Smithwick, Douglas C. Baldwin, Kusum J. Naithani

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 multi-scalar ecological patterns and this hybrid approach should be used more widely in ecological studies.

1 **Quantifying spatial patterns of grass response to nutrient**

2 **additions using empirical and neutral semivariogram models**

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17   **ABSTRACT**

18   Disturbances influence vegetation patterns at multiple scales, but studies that isolate the effect of

19   scale are rare, meaning that scale and process are often confounded. To explore this, we

20   imposed a large (~3.75 ha) experiment in a South African coastal grassland ecosystem to

21   determine the spatial scale of grass response to nutrient additions. In two of six 60 x 60 m

22   grassland plots, we imposed nutrient additions using a scaled sampling design in which fertilizer

23   was added in replicated sub-plots of varying sizes (1 x 1 m, 2 x 2 m, and 4 x 4 m). The

24   remaining plots either received no additions, or were fertilized evenly across the entire plot area.

25   We calculated empirical semi-variograms for all plots one year following nutrient additions to

26   determine whether the scale of grass response (biomass and nutrient concentrations)

27   corresponded to the scale of the sub-plot additions and compared these results to reference plots

28   (unfertilized or unscaled). In addition, we calculated semi-variograms from a series of simulated

29   landscapes generated using random or structured patterns (neutral models) and compared the

30   semivariogram parameters between simulated and empirical landscapes. Results from the

31   empirical semivariograms showed that there was greater spatial structure in plots that received

32   additions at sub-plot scales, with range values that were closest to the 2 x 2 m grain. These

33   results were in agreement with simulated semivariograms using neutral models, supporting the

34   notion that our empirical results were not confounded by random effects. Overall, our results

35   highlight that neutral models can be combined with empirical semivariograms to identify multi-

36   scalar ecological patterns and this hybrid approach should be used more widely in ecological

37   studies.

40 **INTRODUCTION**

41   The importance of spatial autocorrelation for understanding vegetation distributions and

42 processes is well-known (Turner 1989; Watt 1947) and these insights have augmented our

43 understanding of how spatial structure of soils, plants, and climate can regulate ecosystem

44 function, often at multiple, nested scales (Turner et al. 2012). However, for any given study, the

45 scale of this autocorrelation structure and its implications for inferring ecological processes are

46 often not known in advance. Select studies have employed experimental spatial designs *a priori*

47 (Stohlgren et al. 1995) or have used computational models to explore the influence of space on

48 ecosystem properties (Jenerette & Wu 2004; Smithwick et al. 2003; With & Crist 1995).

49 Geostatistical analysis of ecological patterns is also implemented widely (Jackson & Caldwell

50 1993b; Robertson et al. 1993; Smithwick et al. 2005b) but is often used for observations on

51 treatment response rather than to design experimental studies in advance. In the majority of

52 experimental ecological studies, spatial autocorrelation is accounted for *post hoc* through

53 statistical segregation of error terms (Beale et al. 2010).

54   Understanding the scale of ecological processes may help unravel a number of complex

55 ecological questions. For example, disturbances generate structural patterns that may influence

56 ecological processes at many scales (Schoennagel et al. 2008; Turner et al. 2007). However,

57 developing predictive models about the scale of these responses is more difficult because

58 disturbance processes can have cascading effects across scales (Falk et al. 2007; Peters et al.

59 2007). The emergence of bark beetles in the Western U.S. is another multi-scalar process that

60 can only be understood through an unraveling of processes that scale from the tissue-level to the

61 landscape-level (Raffa et al. 2008). Similarly, in savanna science, determining the factors that

62 govern the distribution of grassland and woodland ecosystems is a central focus of many studies

63 (House et al. 2003; Scholes & Archer 1997) and multiple spatial scales are likely needed to

64 explain complex grass-tree interactions (Mills et al. 2006; Okin et al. 2008; Pellegrini 2016;

65 Wang et al. 2010). Herbivore grazing or fire disturbances may each influence savanna nutrient

66 cycling but the scale at which these systems respond is unclear given that the spacing of

67 individual plants, soil properties, terrain, and herbivore assemblages can each contribute to

68 spatial patterns in nutrient availability (Augustine & Frank 2001; Liu et al. 2016; Okin et al.

69 2008; Robertson et al. 1993; Senft et al. 1987; Smithwick et al. 2005b).

70  New approaches are needed to characterize how ecosystems re-organize in response to

71 disturbance and to determine whether there are specific scale or scales of response that are most

72 relevant. In so doing, it may be possible to reduce bias in empirical studies due to scale, thus

73 deepening insights into ecological processes controlling those patterns and minimizing the

74 potential for scale mismatches (Cumming 2011; Ludwig et al. 2000). Implications of such

75 understanding can be significant. For example, Gil et al. (2016) recently found that increasing

76 the scale of nutrient enrichment weakens herbivore control of primary producers, challenging

77 understanding of ecosystem resilience to ecosystem eutrophication. In a more applied setting, it

78 has been shown that the spatial scale of grazer movement can influence nutrient additions in an

79 agricultural field, which could be used to more efficiently target agricultural management

80 activity (Fu et al. 2013). Unraveling such bias or, more optimistically, incorporating scale into

81 ecological prediction and application, requires better experimental approaches to explicitly test

82 for the influence of scale on an ecological pattern.

83  To address this, our overarching goal was to develop a novel methodology to directly test

84 for multi-scalar patterns in primary production following nutrient addition. Our first objective

85 was to determine: at what scale does vegetation respond to nutrient additions? Understanding

86 nutrient limitation to grass productivity is an important question in its own right (Craine et al.

87   2008; Wakeling et al. 2010) but often neglects questions related to the scale of the response. We

88   hypothesized that the grass response would differ between three subplots scales (1 x 1 m, 2 x 2

89   m, and 4 x 4 m). These scales were chosen correspond to ecosystem processes that might govern

90   nutrient uptake, including the spacing of grass tufts, trees or shrubs, and plot-level topography,

91   respectively, which have been identified as critical sources of variation in soil biogeochemistry

92   (Ettema & Wardle 2002; Jackson & Caldwell 1993a; Rietkerk et al. 2000; Smithwick et al.

93   2005b). We surmised that insights gained about the specific scale of response could be used in

94   future studies to understand the scale-specific ecological mechanisms that might be operating at

95   that scale.

96   In addition to testing the within-plot scale of vegetation response to fertilization, our

97   second objective was to test whether there would be stronger spatial structure (enhanced

98   patchiness) across plots in which nutrients were added at multiple sub-plot scales, compared to

99   homogenously fertilized or unfertilized reference plots. To avoid issues of pseudoreplication,

100   these comparisons were made between empirical semivariograms and neutral semivariograms

101   (computer-simulated landscapes that mimic hypothesized patterns). Empirical and neutral

102   semivariograms were compared for six conditions that received, or were artificially modeled to

103   simulate, different nutrient addition patterns (no fertilizer, homogenously fertilized, or fertilized

104   in sub-plot scales) and levels of grazing (fenced or unfenced). We hypothesized that the ranges

105   (autocorrelation distances, or length scales) calculated in the neutral models would be

106   comparable to the ranges calculated from empirical data, and that they would show greater

107   spatial structure in plots that received heterogeneous fertilizers compared to reference plots.

108   In addition, we used these neutral models to explore the influence of herbivory on the

109   spatial structure of fertilized or reference plots. Following nutrient additions, herbivores can

110   influence patterns of primary productivity through enhanced consumption of nutrient-rich

111   patches and/or through enhanced deposition of new nutrients via manure or urine from animals

112   entering the fertilized area, which may have variable effects on spatial heterogeneity depending

113   on the scale of the observations (Fuhlendorf & Smeins 1999). Consumption of nutrient-rich

114   patches may reduce overall variance by reducing differences in biomass amounts compared to

115   ungrazed areas but new nutrient additions by animals also occur when animals move across the

116   plot, or rest in new locations, thus enhancing variability (Auerswald et al. 2010). Therefore, in

117   this study we tested the hypothesis that herbivory increases spatial heterogeneity (Liu et al. 2016)

118   such that patchiness would be highest. i.e., range scales would be smaller, in plots that received

119   both manure additions and scaled nutrient additions (unfenced and heterogeneously fertilized),

120   relative to plots that received only grazing or only homogeneous nutrients.

121   Our third objective was to estimate the degree to which grass biomass was related to

122   patterns in foliar nitrogen content (% N) and phosphorus content (% P), while accounting for

123   random effects due to scale and treatment. Generally, temperate systems are expected to have

124   greater levels of N limitation on vegetation growth than sub-tropical or tropical systems, where P

125   may be more limiting due to highly weathered soils (Domingues et al. 2010; Hedin 2004;

126   Lambers et al. 2008; Vitousek & Sanford 1986). Yet, N, P, and N+P limitations on vegetation

127   productivity have all been documented in African savanna or grassland systems (Augustine et al.

128   2003; Craine et al. 2008; Ngatia et al. 2015; Okin et al. 2008), indicating that an understanding

129   of nutrient limitations on biomass productivity in these systems is still unclear. Complexity in

130   understanding differences in nutrient limitations has been attributed to, for instance, differential

131   nutrient affinities across functional groups (Ratnam et al. 2008; Reich et al. 2003) as well as

132   complex herbivory feedbacks (Augustine et al. 2003), both of which are compounded by issues

133   of scale (Ekblom & Gillson 2010; Fuhlendorf & Smeins 1999). While a complete test of these

134   factors was beyond the scope of this study, we had the opportunity to explore the effect of foliar

135   nutrient concentrations on biomass responses under a dual nutrient addition experiment, while

136   treating the levels of our experimental design (e.g. plot, subplot, treatment) as separate random

137   effects. By so doing, we were able to explore whether foliar N, P or N and P together explained

138   patterns in biomass, beyond the given variability in our study design, and to determine the level

139   (scale) at which these random effects were most pronounced.

140

141   **METHODS**

142   **Study area.** This study was conducted in Mkambathi Nature Reserve, a 7720-ha protected area

143   located at 31° 13′ 27″ S and 29° 57′ 58″ E along the Wild Coast region of the Eastern Cape

144   Province, South Africa. The Eastern Cape is at the confluence of four major vegetative

145   groupings (Afromontane, Cape, Tongaland-Pondoland, and Karoo-Namib) reflecting

146   biogeographically complex evolutionary histories. It is located within the Maputaland-

147   Pondoland-Albany conservation area, which bridges the coastal forests of Eastern Africa to the

148   north, and the Cape Floristic Region and Succulent Karoo to the south and west. The

149   Maputaland-Pondoland-Albany region is the second richest floristic region in Africa, with over

150   8,100 species identified (23 % endemic), and 1,524 vascular plant genera (39 endemic).

151   Vegetation in Mkambathi is dominated by coastal sour grassveld ecosystems, which dominate

152   about 80 % of the ecosystem (Kerley et al. 1995; Shackleton et al. 1991), with small pockets of

153   forest along river gorges, wetland depressions, and coastal dunes. Dominant grasses in the

154   Mkambathi reserve include the coastal *Themeda triandra – Centella asiatica* grass community,

155   the tall grass *Cymbopogon validas – Digitaria natalensis* community in drier locations, and the

156   short-grass *Tristachya leucothrix-Loudetia simplex* community (Shackleton 1990). Grasslands in

157   Mkambathi have high fire frequencies, and typically burn biennially. Soils are generally derived

158   from weathered Natal Group sandstone and are highly acidic and sandy with weak structure and

159   soil moisture holding capacity (Shackleton et al. 1991). Climate is generally considered warm

160   temperate, where average monthly minimum and maximum temperature in East London is 14

161   and 23 ºC respectively, and precipitation is 921 mm yr^{-1} (1961 – 1990 average, S.A. Weather

162   Service).

163   We established a large-scale experimental site that included six 60 x 60 m plots arranged

164   in a rectangular grid (Eastern Cape Parks and Tourism Agency Permit RA0081). The site was

165   surrounded by a fuel-removal fire-break and each plot was separated by at least 10 m for a total

166   size of 3.75 ha for the entire site. To account for grazing, a fence was constructed around three

167   of these plots.

168   **Nutrient additions**. Nutrient additions were applied to four plots whereas two plots received no

169   fertilizer additions. Of the four plots that received fertilization, two received nutrients evenly

170   across the entire 60 x 60 m plot ("homogenous plots") and the other two fertilized plots received

171   nutrient additions within smaller subplots in a heterogeneous design ("heterogeneous plots").

172   Within heterogeneous plots, fertilizer was applied within subplots of three different sizes (1 x 1

173   m, 2 x 2 m, and 4 x 4 m) that were replicated randomly across each plot (**Fig. 1**). Location of

174   individual subplots was determined prior to field work using a Latin Hypercube random

175   generator that optimizes the variability of lag distances among sampling plots and is ideal for

176   geostatistical analysis (Xu et al. 2005). There were a total of 126 subplots per plot that received

177   fertilizer in the heterogeneous plots. All sampling locations were geo-referenced with a GPS

178 (Trimble). The number of sub-plot units at each scale was determined so as to equalize the total

179 fertilized area at each sub-plot scale (i.e., six 4 x 4 m plots and 24, 2 x 2 m plots).

180   To ensure aboveground grass biomass would respond to nutrient additions, we employed

181 a dual (nitrogen (N) + phosphorus (P)) nutrient addition experiment. Additional N was added as

182 either ammonium nitrate (230 g kg^{-1} N) or urea (460 g kg^{-1}) at a rate of 10 g m^{-2} yr^{-1} in a single

183 application, following the protocols of Craine et al. (2008). Additional P was added as

184 superphosphate (105 g kg^{-1} P) at a rate of 5 g m^{-2} yr^{-1}. Dual addition (N+P) was chosen to

185 increase the likelihood of treatment response and increase geostatistical power by reducing the

186 number of treatments, thus increasing sample size. Per unit area, levels of nutrient additions

187 were constant among plots and subplots.

188 **Vegetation and Soil Sampling**. One year following nutrient additions, a subset of subplots was

189 sampled for soil and vegetation nutrient concentrations and biomass. Sub-plots to be sampled

190 were randomly selected prior to being in the field using the Latin Hypercube approach. The

191 approach allowed us to specify a balanced selection of subplots within each subplot size class

192 (four 4 x 4 m, eight 2 x 2 m, and thirty-two 1 x 1 m). Within each subplot location that was

193 revisited, we randomly selected locations for biomass measurement and vegetation clippings.

194 Two locations were identified and flagged from within the 1 x 1 m subplots (center coordinate

195 and a random location 0.5 m from center), four samples were identified and flagged from within

196 the 2 x 2 m subplots, and eight samples were identified and flagged from within the 4 x 4 m

197 subplots. At each flagged location within sampled subplots, productivity was measured as grass

198 biomass using a disc pasture meter (DPM; Bransby and Tainton, 1977) and grab samples of grass

199 clippings were collected for foliar nutrient analysis, using shears and cutting to ground-level.

200 Calibration of the DPM readings was determined using ten random 1 x 1 m subplots in each plot

201 (n = 60 total) that were not used for vegetation or soil harvesting, in which the entire biomass

202 was harvested to bare soil. Soil samples from the top 0 – 10 cm soil profile depth were collected

203 adjacent to vegetation samples. Due to logistical and financial constraints, these samples were

204 collected in fenced plots only. The A horizon of the Mollisols was consistently thicker than 10

205 cm, so all samples collected were drawn from the A horizon. Soil samples were shipped to

206 BEMLab (Strand, South Africa) for nutrient analysis.

207 **Laboratory Analysis.** Biomass samples were separated into grasses and forbs, weighed, dried

208 for 24 h at 60 °C, and reweighed. Vegetation nutrient samples were dried, ground with a 40 mm

209 grinding mesh, and then shipped to the Penn State Agricultural Analytical Laboratory

210 (University Park, Pennsylvania; USDA Permit PDEP11-00029). Grass P concentration was

211 analyzed using a hot block acid digestion approach (Huang & Schulte 1985) and grass N

212 concentration was measured with a Combustion-Elementar Vario Max method (Horneck &

213 Miller 1998). Soil N and C concentrations were determined on a LECO elemental analyzer

214 (Leco Corporation, St. Joseph, MI). Soil P was analyzed using acid extraction following the

215 method of Wolf and Beegle (1995). Soil pH was estimated using KCl extraction following

216 Eckert and Sims (1995).

217

218 **Calculations and Statistics.** Aboveground grass biomass was estimated from DPM

219 measurements, using linear regression to relate DPM estimates with harvested biomass at

220 calibration subplots. DPM estimates were significantly correlated with total grass biomass (R^2 =

221 0.76, p < 0.0001) (**Supplmentary Material, Fig S1**) and the resulting equation was then used to

222 estimate biomass at the remaining 606 locations.

223   To test the scale of grass biomass response to fertilization (Objective 1), we used a

224   maximum likelihood approach to quantify the spatial structure of biomass separately for each of

225   the six empirical plots. The approach assumes that the data ($Y_1 \ldots Y_n$) are realizations of an

226   underlying spatial process, and that the distribution of the data follows a Gaussian multivariate

227   distribution:

228    $\quad Y \sim N(\mu 1, C \Sigma + C_0 I)$ (1)

229   where μ is the mean of the data multiplied by an n-dimensional vector of 1's, C is the partial sill

230   (total sill = $C_0 + C$), Σ is an n x n spatial covariance matrix, C_0 is the nugget effect, and I is an n

231   x n identity matrix. The i,jth element of Σ is calculated with a spatial covariance function $\rho(h_{ij})$,

232   where h_{ij} is the Euclidean distance between measurement points i and j. An exponential

233   covariance model was chosen for its relative simplicity. The full equation for summarizing the

234   second order moment for an element i,j is:

235    $\gamma(h_{ij}) = C_0 + C \left[\exp\left(\frac{-h_{ij}}{\phi}\right) \right]$ (2)

236   where $\gamma(h_{ij})$ is the modeled spatial covariance for measurements i and j, ϕ is the range parameter,

237   and $3 * \phi$ is the range of spatial autocorrelation. The underlying spatial mean μ may be held

238   constant or estimated with a linear model across all locations. We used the plot-level mean of

239   the data for μ (Table 1).

240   The measured soil and plant variables exhibited varying degrees of non-normality in their

241   distributions, which violates the assumption of Gaussian stationarity within the underlying

242   spatial data generating process. To uphold this assumption, we transformed variables at each

243   plot using a box-cox transformation (Box & Cox 1964):

244    $\hat{Y}_i = \left(Y_i^\lambda - 1\right)/\lambda \qquad\qquad$ if $\lambda \neq 0$ (3)

245 $\hat{Y}_i = \log(Y_i)$ if $\lambda = 0$

246 where Y_i is an untransformed variable (e.g., biomass) at location i, $\hat{Y}_i$ is the transformed variable,

247 and λ is a transformation parameter. We optimized the three spatial covariance model

248 parameters and the transformation parameter (C_0, C, ϕ, λ) with the maximum likelihood

249 procedure. A numerical finite-difference approximation algorithm selected the set of parameters

250 that maximized a normal multivariate log-likelihood function (more details in Diggle et al,

251 2003). To approximate a sampling distribution of each parameter, a bootstrapping algorithm was

252 used where a randomly sampled subset of data was inputted into the same maximum likelihood

253 approach for 1000 iterations. This provided a population of fitted parameters and models that

254 was used to analyze the approximate distributions of each parameter for each plot. The

255 maximum likelihood optimization was cross-validated by removing a random sub-sample of

256 measurements from the optimization and then using the optimized model to make predictions at

257 locations where measurements were removed. Observed vs. predicted values from the cross-

258 validation procedure were then analyzed at each plot separately.

259   We used ordinary kriging (Cressie 1988) with the optimized spatial covariance model

260 from the maximum likelihood analysis to estimate biomass across all plots. Ordinary kriging is

261 useful in this case, because we detected spatial structure in the biomass data when considering all

262 biomass data at once (see Results). The geoR package (Ribeiro Jr. & Diggle 2001) in the R

263 statistical language (R Development Team, 2014) was used for all spatial modeling and kriging.

264   To compare spatial structure across empirical or simulated patterns (Objective 2), we

265 compared the empirical semivariograms with neutral semivariogram models. Essentially this

266 approach allows us to compare empirical patterns across a set of null models in which the

267 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).

280   The spatial structure of simulated landscapes was analyzed using the same maximum

281   likelihood approach as described above and data was not transformed. The mean (μ) was

282   estimated using a constant trend estimate. To compare neutral (simulated data) and empirical

283   (observed data) semivariogram models, we scaled the nugget and sill model parameters by

284   dividing these parameters into the maximum observed semivariance value within each plot.

285   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

291   and fixed effects were tested, where foliar N and P represented fixed effects upon biomass, and

292   model error was assumed to be Gaussian. A normal likelihood function was minimized to

293   estimate optimal regression coefficients for each mixed model formulation.

294   Our objective was to identify a mixed model that estimated biomass closely to

295   observations, while also having the fewest possible parameters. The Akaike's Information

296   Criterion (AIC) and Bayesian Information criterion (BIC) were used to compare different

297   models, since they decrease with a negative log-likelihood function but increase with the amount

298   of parameters used in the model (Burnham and Anderson, 2004). After all model formulations

299   were fitted to observed biomass and both AIC and BIC were calculated for each model, we chose

300   the model with the lowest BIC as best representing the tradeoff of parsimony and prediction

301   skill. The BIC associated with all other models was subtracted into the lowest available BIC,

302   and models with a difference in BIC > 2 were deemed significantly less favorable at estimating

303   biomass and representing random effects than the model with the lowest BIC. All mixed

304   modeling was conducted with the R package lme4.

305

306   **RESULTS**

307   Across plots, vegetation biomass ranged from 376 ± 6.0 g m^{-2} (mean $\pm$ 1 Standard Error

308   (SE), n = 124) in the unfenced, homogenously fertilized plot to 563 ± 18.6 g m^{-2} (n = 128) in the

309   unfenced, heterogeneously fertilized plot (**Table 1**). Forb biomass was approximately 18 % of

310   total biomass, with the remaining 82 % represented by grass. Vegetation nutrient concentrations

311   increased, and N:P ratios declined, following fertilization (**Table 1**). Vegetation N concentration

312   ranged from 0.58 ± 0.01 % to 0.75 ± 0.04 %, averaging 0.60 ± 0.01 % in unfertilized plots versus

313   an average of 0.72 ± 0.02 % in heterogeneously fertilized plots and 0.77 ± 0.02 % in

314   homogenously fertilized plots, an increase of 20 % and 28 %, respectively. Vegetation P

315   concentration averaged 0.037 ± 0.001 mg g^{-1} in unfertilized plots, 0.056 ± 0.002 mg g^{-1} in

316   heterogeneously fertilized, and 0.057 ± 0.002 mg g^{-1} in homogeneously fertilized plots, an

317   increase of 34 and 35%, respectively. The N:P ratios ranged from a high of 17.9 in the fenced-

318   unfertilized plot to 12.1 in the unfenced-homogenously fertilized plot. Vegetation C content

319   averaged 44.6 ± 0.13 % across all six plots. Soil P and N were also higher following fertilization

320   in the fenced plots, where these variables were measured (**Supplementary Table 1**). Soil C

321   ranged from 2.49 ± 0.01 % to 2.55 ± 0.01 % across plots. Soil pH was 4.27 in the unfertilized

322   plot and 4.08 in fertilized plots. Confirming reference conditions, pH measured in a single

323   control plot in 2011 prior to fertilization was 4.21 ± 0.01.

324   We had expected that biomass and vegetation nutrient concentrations following

325   fertilization would be spatially autocorrelated at scales corresponding to the scale of the

326   fertilization additions (Objective 1). In heterogeneously fertilized plots this would be indicated

327   by range distances from empirical semivariograms that corresponded to the hypotenuse distances

328   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,

329   and 4 x 4 m subplots, respectively). In homogenously fertilized plots or unfertilized plots, spatial

330   structure would be observed at scales other than scales of the subplots (or not at all) and we

331   would expect a higher degree of nugget variance (spatial structure expressed at scales finer than

332   those that were measured). Results from the empirical semi-variogram model of the

333   heterogeneous plots show that there was a statistically significant patch structure at scales similar

334   to the scale of the subplots in both of the heterogeneously fertilized plots (**Fig. 3b,f**). Also

335   confirming these expectations, in unfenced and fenced plots (unfertilized or homogenously

336   fertilized) the range scale was significantly longer or shorter (**Fig. 3; Supplementary Table 2**).

337   Examining the sampling distributions of the semivariogram range values for vegetation

338   biomass determined from the maximum likelihood and bootstrapping analysis, it can be seen that

339   the range value most closely resembles that of the hypotenuse of the 2 x 2 m subplot, relative to

340   the other subplots (**Fig. 3d,h**). Higher spatial structure in the heterogeneous versus

341   homogeneous or unfertilized plots can also be seen in the kriged plots of biomass (**Fig. 4**). These

342   maps also demonstrated the higher mean levels of biomass in fertilized subplots relative to areas

343   outside of subplots or relative to other plots. Normalized nugget/sill ratios were highest in the

344   unfenced, homogeneously fertilized plot (3.89) with lower ratios (0-0.02) for heterogeneously

345   fertilized or fenced treatments.

346   Consistent with the biomass results, semivariogram parameters for vegetation % N and %

347   P (**Supplementary Table 3**) generally supported expectations, at least for the heterogeneously

348   fertilized and fenced plot, in which range scales were comparable to subplot scales in the (% P, ~

349   4.9 m, % N, ~ 5.8 m); however, other plots showed higher or lower ranges. The proportion of

350   nugget variance in semivariogram models of vegetation % N and % P was highest in the

351   unfertilized plots, which supports expectations of higher spatial structure in fertilized treatments,

352   as for biomass, but differences were variable among individual plots. However, semi-variograms

353   of soil carbon and nutrients (**Supplementary Table 3**) showed few differences among model

354   parameters among treatments where these were measured (fenced plots, only). In sum,

355   differences in spatial structure that could be attributed to treatments were most evident for

356   biomass, somewhat evident for vegetation % N and % P, and least evident for soil C or nutrients.

357   To compare mean differences among plots (Objective 2), we compared normalized

358   spatial model fits of neutral and empirical semi-variograms, which allowed us to quantify the

359   relative influence of treatment responses to random processes not accounted for in the

360   experimental design. Results indicated that the range of autocorrelation in heterogeneous plots

361   was similar to that found in the empirical semi-variograms and generally matched subplot scales

362   (**Fig. 5**). Interestingly, range scales were higher, and proportional nugget variance was greater in

363   fenced plots, compared to unfenced plots. This was an opposite pattern than that observed in

364   empirical plots, in which grazing resulted in longer range scales and higher nugget variance.

365   These results likely reflect processes not included in the neutral model. Particularly, the neutral

366   model was designed to mimic patterns in biomass using assumptions about herbivore movement,

367   grazing, and manure additions, assuming they were additive to the effects of fertilization,

368   whereas empirical results likely reflect complex interactions between grazing and fertilization.

369   In this case, longer observed range scales in grazed versus ungrazed plots may reflect enhanced

370   homogenization of biomass through grazing, an effect that was apparently greater than biomass

371   enhancement from nutrient addition. Nonetheless, the fact that heterogeneous plots were similar

372   between fenced and unfenced plots indicates the strong scalar influence of nutrient additions

373   relative to nutrient-herbivore interactions.

374   Addressing Objective 3, mixed models used to estimate biomass from N or P foliar

375   concentrations, while treating plot and treatment as random effects, showed that biomass was

376   best predicted by levels of foliar P, relative to foliar N alone or foliar N x P (**Table 2**). Although

377   foliar P alone did better than foliar N alone as a fixed effect, the difference was marginal (< 2

378   BIC). The 'best' model uses only plot treatment type as a random effect, which outperformed

379   model formulations using herbivory or fertilizer type and those with nested structures

380   incorporating subplot size as random effects.

381

382   **DISCUSSION**

383   Scale issues confound a complete understanding of ecosystem response to perturbations

384   (Turner 2010). This is particularly important in grassland or savanna systems in which the

385   relative importance of factors affecting grass-woodland ecosystems are debated (e.g., Mills et al.

386   2006; Sankaran et al. 2004; Scholes & Archer 1997), with some suggesting that these factors are

387   nested hierarchically with spatial scale (Pellegrini 2016; Pickett et al. 2003; Rogers 2003).

388   Results of this experiment yield data on the spatial scale of the nutrient-productivity relationship

389   in a grassland coastal forest of the Eastern Cape, South Africa, and support the assertion that

390   ecological processes are multi-scaled and hierarchical in response to nutrient additions.

391   Specifically, we observed immediate (one year following fertilization) biomass response at the

392   plot level due to nutrient addition and fencing. Using spatial modeling, we discerned strong

393   autocorrelation structure in biomass at subplot scales associated with scales of nutrient addition.

394   The strongest evidence for spatial structure was observed at the 2 x 2 m scale. Comparing this

395   spatial structure between neutral and empirical semivariogram models, we were able to infer the

396   relative importance of treatment conditions (nutrients and grazing) to random, spatially

397   structured processes. Traditional ecosystem-level approaches to quantify spatial pattern have

398   focused on understanding extant patterns, in which processes are necessarily inferred or

399   correlated. However, by examining spatial patterns under experimental conditions in which scale

400   and treatment are controlled, we were able to relate ecological responses to known scales of

401   perturbation.

402   Subplots responded very strongly to nutrient additions at subplot scales, as evident from

403   the kriged maps, showing that most of the increase in average plot biomass was due to large

404   increases in subplot biomass. One surprising result of our study was the detection of average

405   greater biomass in plots that received heterogeneous fertilizer applications compared to plots that

406 received homogeneous fertilizer, despite the fact that fertilizer was added equally on a per area

407 basis. Several other studies have found higher biomass following heterogeneous nutrient

408 applications. For example, Day et al. (2003) observed that heterogeneous spatial patterns of

409 nutrient supply in early stages of grassland development led to enhanced nutrient acquisition and

410 biomass productivity. Similarly, Du et al. (2012) observed increased plant biomass following

411 heterogeneous nutrient fertilization in old-field communities in China. Mechanisms for

412 enhanced productivity following heterogeneous nutrient supply are not clear but may include

413 shifts in root structure and function or shifts in species dominance, which were not analyzed

414 here. For example, roots may respond to patchiness in nutrient availability by modifying root

415 lifespan, rooting structures and uptake rate to maximize nutrient supply (Hodge 2004; Robinson

416 1994). In turn, initial advantages afforded by plants in nutrient-rich locations may result in

417 larger plants and advantages against competitive species, potentially via enhanced root growth

418 (Casper et al. 2000).

419   Observing vegetative response to nutrient additions is complicated by processes such as

420 luxury consumption (Ostertag 2010), initial spatial patterns in soil fertility (Castrignano et al.

421 2000), root distribution, signaling and allocation (Aiken & Smucker 1996), species and

422 functional group shifts (Ratnam et al. 2008; Reich et al. 2003), or species' differences in uptake

423 rates or resorption (Reed et al. 2012; Townsend et al. 2007). Spatial patterns of finer-scale

424 processes such as microbial community composition have also been explored and are known to

425 influence rates of nutrient cycling (Ritz et al. 2004; Smithwick et al. 2005a). In the case of

426 heterogeneous nutrient supply, species competitive relationships across space may be enhanced

427 (Du et al. 2012) and may result in increases in plant diversity (Fitter 1982; Wijesinghe et al.

428 2005), although other studies have found little evidence to support this claim (Gundale et al.

429   2011). Together, these factors may explain the high unexplained variance of vegetation N and P

430   concentrations that we observed and may account for the nugget variance in our empirical spatial

431   models. However, effects of species composition shifts on plant biomass were likely minimal in

432   this study given the short-term nature of the study (one year), although the patchiness in biomass

433   in gridded fertilizer plots indicates size differences that are likely to modify competitive

434   relationships in the future. Unfortunately, the site burned one year following the experiment,

435   precluding additional tests of these relationships.

436   Understanding the length scales of key ecosystem properties is critical for determining

437   optimal scales for studying ecological systems, interpreting change in ecological communities,

438   and assessing landscape connectivity and ecosystem resilience (Johnson 2009; Turner et al.

439   2012). Autocorrelation structure may reflect influences of spatial interactions among

440   individuals, patches, or abiotic gradients (Ettema & Wardle 2002; Jackson & Caldwell 1993a;

441   Rietkerk et al. 2000; Smithwick et al. 2005b). Our study showed that biomass responded

442   strongly at all scales in which nutrients were added, but was strongest at the 2 x 2 m scale.

443   Rietkerk et al. (2000) observed patchiness in soil moisture at three unique scales (0.5 m, 1.8 m

444   and 2.8 m) in response to herbivore impacts. Following fire in the Greater Yellowstone

445   Ecosystem (Wyoming, U.S.A.), Turner et al. (2011) observed variation in soil properties at the

446   level of individual soil cores, and Smithwick et al. (2012) observed autocorrelation in post-fire

447   soil microbial variables that ranged from 1.5 to 10.5 m. In savanna and boreal ecosystems,

448   patchiness in soil resources at the level of individual shrubs and trees has been demonstrated by

449   several studies (Dijkstra et al. 2006; Hibbard et al. 2001; Lechmere-Oertel et al. 2005; Liski

450   1995; Pennanen et al. 1999). Obviously, other contingent factors (e.g., herbivory, historical fire,

451   land use) are important for explaining grass species distributions and productivity patterns at

452 broader temporal and spatial scales, but our study suggests that intermediate length scales (<4

453 m²) reflect ecological processes important for understanding patterns in grassland nutrition.

454   Many studies have used stoichiometric relationships of N and P to infer nutrient

455 limitation (Koerselman & Meuleman 1996; Reich & Oleksyn 2004), although there are limits to

456 this approach (Ostertag 2010; Townsend et al. 2007). Using this index, our N:P ratios of

457 vegetation in reference plots would indicate co-limitation for N and P prior to fertilization (N:P >

458 16). Addition of dual fertilizer appeared to alleviate P limitation more than N, with N:P ratios

459 reduced one year following treatment, indicating N limitation or co-limitation with another

460 element (N:P < 14). In addition, P was most strongly correlated with biomass in linear mixed

461 effects modeling across all plots, after accounting for random effects of the experimental design.

462 Ostertag (2010) also showed that there was a preference for P uptake in a nutrient limited

463 ecosystem in Hawaii and suggested that foliar P accumulation may be a strategy to cope with

464 variability in P availability. Grazing may also preferentially increase grass P concentrations in

465 semi-arid systems in South Africa (Mbatha & Ward 2010). The cumulative impacts of

466 preferential plant P uptake and P additions from manure may explain the high spatial structure

467 observed in our grazed and fertilized plots.

468   Spatial heterogeneity may be critical for influencing ecosystem resilience to stressors by

469 mediating disturbance spread (i.e., fire, invasive species dispersal) (Yang et al. 2008), explaining

470 patterns in disease emergence (Wu et al. 2015), or promoting recovery and regeneration through

471 maintenance of legacy structures and functions (Turner et al. 2012). Understanding the

472 importance of spatial pattern for maintaining soil fertility is increasingly relevant for agricultural

473 management that incorporates precision technology as well as for ecosystem management

474 activities that prioritize monitoring and restoration. In South Africa, grasslands cover nearly

475   one-third of the country and maintain the second-highest levels of biodiversity, behind the Cape

476   Floristic Region. Grasslands of Mkambati are representative of ancient grasslands that are under

477   threat globally, yet posses high levels of species endemism and provide significant ecosystem

478   goods and services (Bond 2016). Grasslands in southern Africa are expected to undergo

479   significant losses in biodiversity in coming decades due to increasing pressure from agricultural

480   development and direct changes in climate (Biggs et al. 2008; Huntley & Barnard 2012). For

481   example, losses in critical African bird species in fynbos and grassland biomass are expected to

482   be 30 – 40 % lower by 2085 as a result of projected climate change, with range reductions > 60

483   % in many cases. As a result of these pressures, understanding the factors that regulate

484   ecosystem productivity, and the scales at which they operate, is critical for guiding ecosystem

485   management activities aimed at maintaining landscape sustainability.

486

487   **CONCLUSIONS**

488   This study provided an opportunity for direct hypothesis-testing of the influence of

489   spatial pattern on ecosystem processes, providing an alternative to studies in which spatial

490   autocorrelation is observed post-hoc or otherwise confounds complex ecosystem dynamics.

491   Specifically, spatial models were used to test the scale at which vegetation responded to nutrient

492   additions within plots and we compared these model parameters to those determined from model

493   parameters from simulated landscapes using replicated neutral models. Biomass responded to

494   nutrient additions, with spatial autocorrelation of the biomass response highest at the 2 x 2 m

495   scale. Comparison of empirical and simulated neutral models, confirmed that this length scale

496   accounted for the majority of the spatial structure in the observations. Vegetation P

497   concentrations were elevated more than vegetation N suggesting plant P preference or luxury

498   consumption, although vegetation nutrient concentrations exhibited lower spatial structure than

499   that observed for biomass. Finally, this study was undertaken in a nature reserve in which

500   grasslands are being actively managed to meet multiple management objectives including

501   wildlife management and biodiversity. These results indicate strong coupling between soil

502   nutrients and aboveground function which should be considered in vulnerability assessments that

503   consider grassland responses to shifts in climate and management activities.

504

505   **ACKNOWLEDGEMENTS**

506   We are deeply grateful to the support of Jan Venter, the Eastern Cape Parks and Tourism Board,

507   and especially the Mkambathi Nature Reserve for allowing the establishment of the "Little

508   Pennsylvania" study area. We are grateful to the hard work of the Parks and People 2010, 2011,

509   and 2012 research teams, which included undergraduate students from The Pennsylvania State

510   University who helped in all aspects of field research. Special thanks to the dedication of Sarah

511   Hanson, Warren Reed, Shane Bulick, and Evan Griffin who assisted tirelessly with both field

512   and laboratory work.

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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.

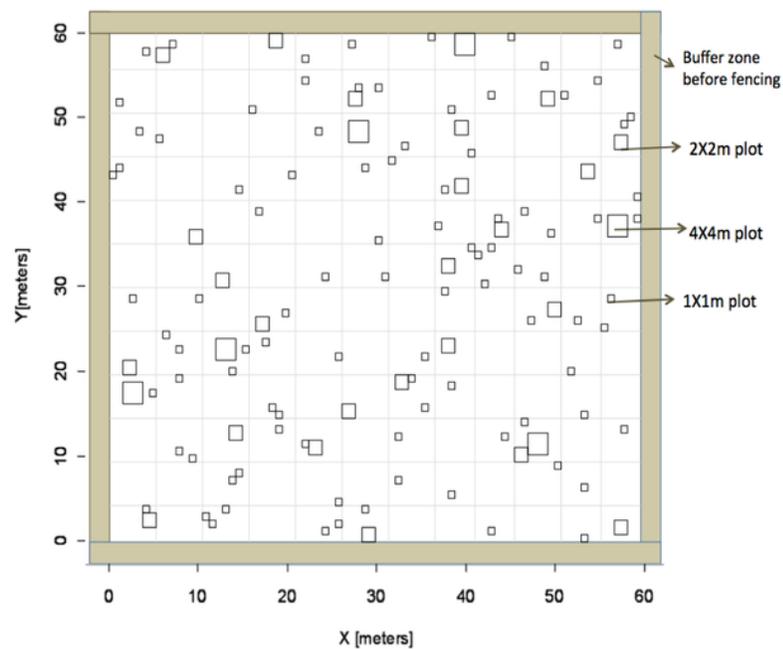
Treatment	Average Biomass ± 1 SE (g m^{-2})	Average N ± 1 SE (%)	Average P ± 1 SE (%)	N:P	n
Fenced Unfertilized	411.9 ± 9.75	0.646 ± 0.024	0.036 ± 0.001	17.9	134
Heterogeneous	542.4 ± 15.05	0.747 ± 0.041	0.048 ± 0.002	15.6	120
Homogeneous	456.2 ± 8.28	0.710 ± 0.014	0.054 ± 0.002	13.2	117
Unfenced Unfertilized	483.6 ± 13.70	0.576 ± 0.011	0.038 ± 0.001	15.2	132
Heterogeneous	562.6 ± 18.60	0.775 ± 0.015	0.064 ± 0.002	12.1	128
Homogeneous	375.4 ± 5.96	0.722 ± 0.017	0.059 ± 0.002	12.2	124

Mixed model results comparing biomass to foliar nutrients.

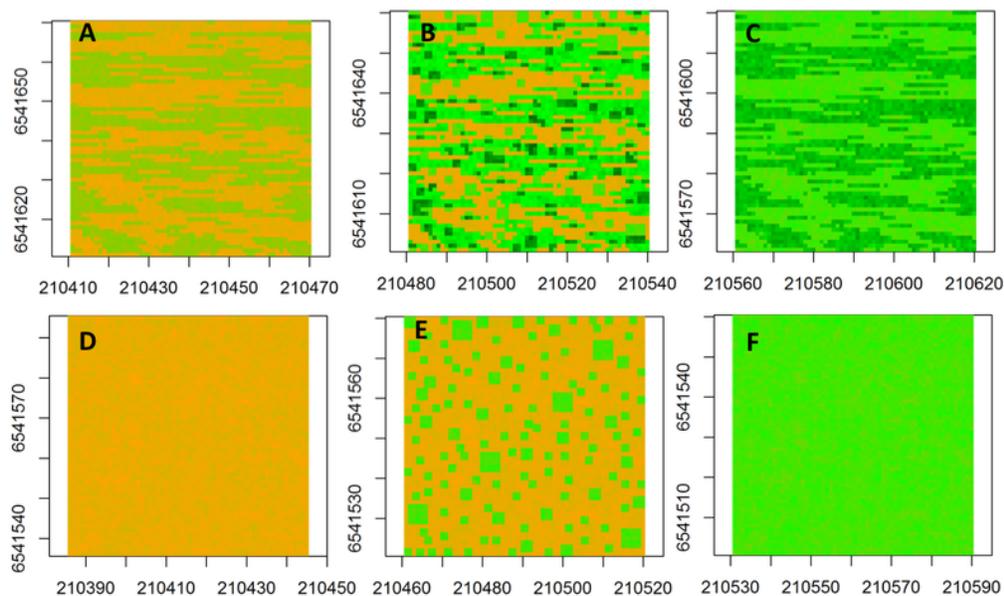
Results of the mixed model relating biomass to foliar nutrients, where herbivory, fertilizer type, plot treatment, and subplot size were all tested as random effects; foliar N and P represented fixed effects upon biomass, and model error was assumed to be Gaussian. A normal likelihood function was minimized 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.

Model	DF	AIC	BIC	Δ
Random Effects				
Plot	**5**	**1092.4**	**1114.2**	**0.0**
Herbivore	5	1190.1	1211.9	97.7
Fertilizer	5	1100.7	1122.5	8.3
Plot	Sub-Plot	6	1090.4	1116.5	2.3
Herbivore	Sub-Plot	6	1188.6	1214.7	100.5
Fertilizer	Sub-Plot	6	1102.7	1128.8	14.6
Fixed Effects				
N + P	5	1090.3	1112.1	5.3
P	**4**	**1089.8**	**1107.3**	**0.4**
N	4	1090.7	1108.2	1.3
N : P	6	1092.3	1118.5	11.6
N + P + Sub-Plot	6	1092.3	1118.5	11.6
N + P : Sub-Plot	8	1095.6	1130.5	23.6
$P + N^2$	5	1091.6	1113.4	6.6
$N + P^2$	5	1089.7	1111.5	4.7
$N^2 + P^2$	5	1091.1	1113.0	6.1
N^2	4	1093.3	1110.8	3.9
P^2	**4**	**1089.4**	**1106.9**	**0.0**

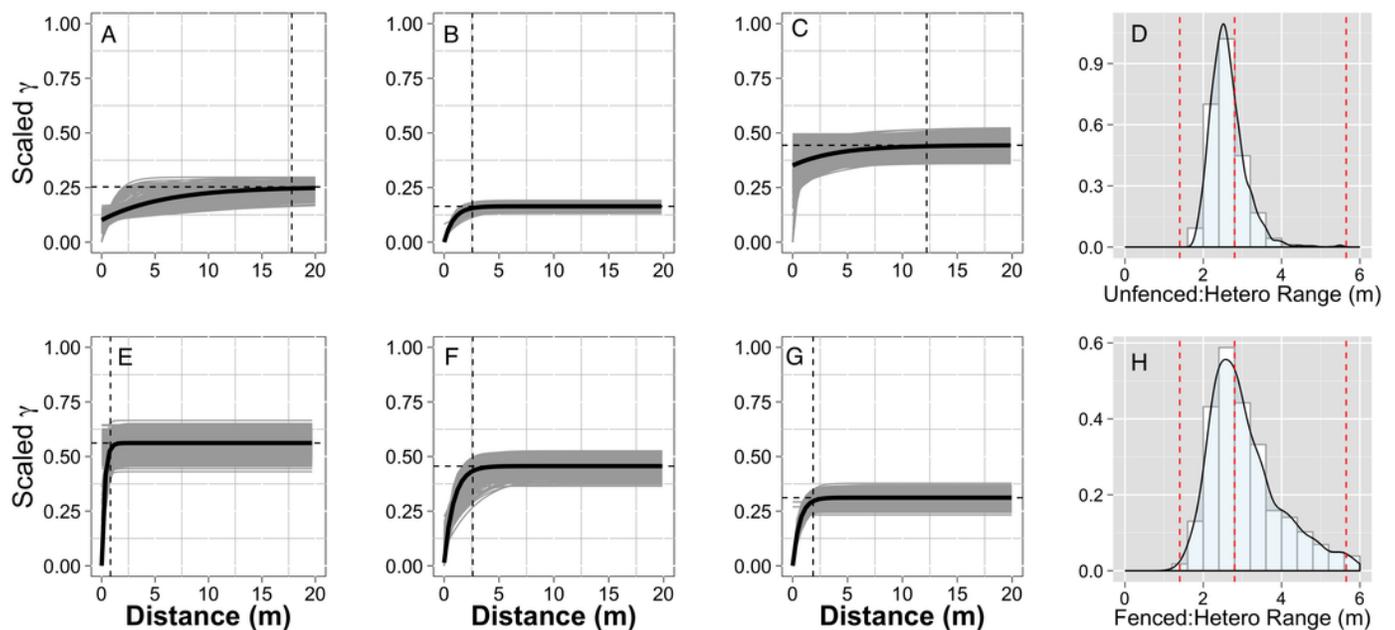
Experimental design

Overview of experimental design based on Latin Hypercube sampling used to identify subplot locations to receive fertilizer in the heterogeneous plots.

2

Spatial maps of neutral models

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.

3

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.

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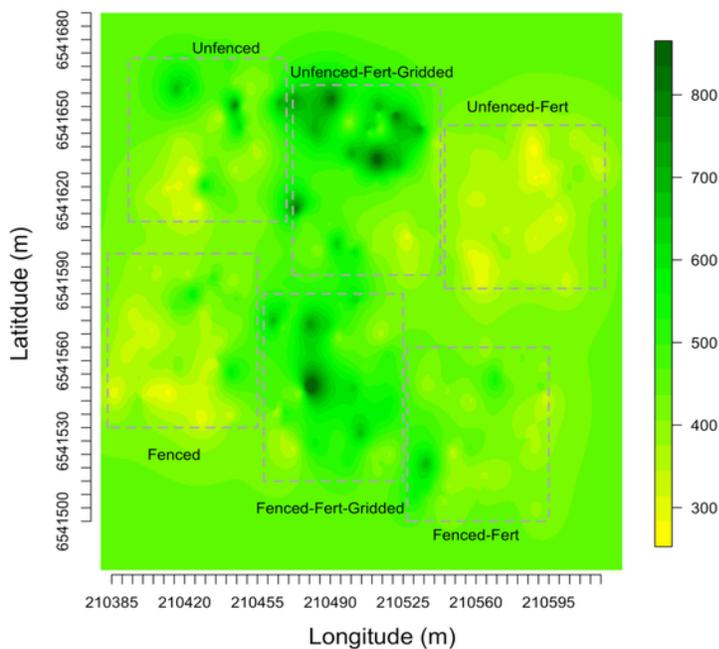
Kriged biomass map.

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.

5

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.