Grassland productivity in response to nutrient additions and herbivory is scale-dependent

Erica A.H. Smithwick Corresp., [1] , Douglas C. Baldwin [2] , Kusum J. Naithani [3]

[1] Department of Geography and Intercollege Graduate Degree Program in Ecology, The Pennsylvania State University, University Park, Pennsylvania, United States

[2] Department of Geography, The Pennsylvania State University, University Park, Pennsylvania, United States

[3] Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, United States

Corresponding Author: Erica A.H. Smithwick
Email address: smithwick@psu.edu

Vegetation response to nutrient addition can vary across space, yet studies that explicitly incorporate spatial pattern into experimental approaches are rare. To explore whether there are unique spatial scales (grains) at which grass response to nutrients is best expressed, we imposed a large (~3.75 ha) experiment in a South African coastal grassland ecosystem. In two of six 60 x 60 m grassland plots, we imposed a scaled sampling design in which fertilizer was added in replicated sub-plots (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 area. Three of the six plots were fenced to exclude herbivory. We calculated empirical semivariograms 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). We compared empirical semivariogram parameters to parameters from semivariograms derived from a set of simulated landscapes (neutral models). Empirical semivariograms showed spatial structure in plots that received multi-scaled nutrient additions, particularly at the 2 x 2 m grain. The level of biomass response was predicted by foliar P concentration, and to a lesser extent, N, with the treatment effect of herbivory having a minimal effect. Neutral models confirmed the length scale of the biomass response and indicated few differences due to herbivory. Overall, we conclude that interpretation of nutrient limitation in grasslands is dependent on the grain used to measure grass response and that herbivory had a secondary effect.

1   **Grassland productivity in response to nutrient additions and**

2   **herbivory is scale-dependent**

4   Erica A.H. Smithwick[1], Douglas C. Baldwin[2], Kusum J. Naithani[3]

6   [1] Department of Geography and Intercollege Graduate Degree Program in Ecology, The

7   Pennsylvania State University, University Park PA USA

8   [2] Department of Geography, The Pennsylvania State University, University Park PA USA

10   [3] Department of Biological Sciences, University of Arkansas, Fayetteville AR USA

11   Corresponding Author:

12   Erica Smithwick[1]

13   302 Walker Building, University Park, PA, 16802, USA

14   Email address: smithwick@psu.edu

17   **ABSTRACT**

18   Vegetation response to nutrient addition can vary across space, yet studies that explicitly

19   incorporate spatial pattern into experimental approaches are rare. To explore whether there are

20   unique spatial scales (grains) at which grass response to nutrients is best expressed, we imposed

21   a large (~3.75 ha) experiment in a South African coastal grassland ecosystem. In two of six 60 x

22   60 m grassland plots, we imposed a scaled sampling design in which fertilizer was added in

23   replicated sub-plots (1 x 1 m, 2 x 2 m, and 4 x 4 m). The remaining plots either received no

24   additions, or were fertilized evenly across the entire area. Three of the six plots were fenced to

25   exclude herbivory. We calculated empirical semivariograms for all plots one year following

26   nutrient additions to determine whether the scale of grass response (biomass and nutrient

27   concentrations) corresponded to the scale of the sub-plot additions and compared these results to

28   reference plots (unfertilized or unscaled). We compared empirical semivariogram parameters to

29   parameters from semivariograms derived from a set of simulated landscapes (neutral models).

30   Empirical semivariograms showed spatial structure in plots that received multi-scaled nutrient

31   additions, particularly at the 2 x 2 m grain. The level of biomass response was predicted by

32   foliar P concentration, and to a lesser extent, N, with the treatment effect of herbivory having a

33   minimal effect. Neutral models confirmed the length scale of the biomass response and

34   indicated few differences due to herbivory. Overall, we conclude that interpretation of nutrient

35   limitation in grasslands is dependent on the grain used to measure grass response and that

36   herbivory had a secondary effect.

39   **INTRODUCTION**

40   Nutrient limitation is known to constrain ecosystem productivity (Vitousek and Howarth

41   1991, LeBauer and Treseder 2008). In general, temperate systems are expected to have greater

42   levels of nitrogen (N) limitation on vegetation growth than sub-tropical or tropical systems,

43   whereas phosphorus (P) may be more limiting due to highly weathered soils (Vitousek and

44   Sanford 1986, Hedin 2004, Lambers et al. 2008, Domingues et al. 2010). Ecological inference is

45   dependent on observational scale, however, and our ability to infer ecosystem function from

46   patterns in nutrient availability rests on the grain and extent of the measurement (Dungan et al.

47   2002).   Indeed, the optimal grain (resolution) for diagnosing nutrient limitation, especially in

48   grassland ecosystems, is not known and may vary at fine scales (Klaus et al. 2016). Patchiness

49   in nutrient availability can be governed by variability in soil properties or terrain, spatial

50   variability in microbial community composition, or differential nutrient affinities across

51   functional groups that have different spatial or temporal distributions (Reich et al. 2003, Ratnam

52   et al. 2008). Perhaps as a result of this spatial heterogeneity, N, P, and N+P limitations on

53   vegetation productivity have all been documented in African savanna or grassland systems

54   (Augustine et al. 2003, Craine et al. 2008, Okin et al. 2008, Ngatia et al. 2015). This study asks

55   whether new approaches that actively test (sensu McIntire and Fajardo 2009) the scale of grass

56   response to nutrients and herbivory can aid understanding of nutrient limitation in grassland

57   ecosystems.

58   Herbivores influence nutrient availability and can further enhance or diminish spatial and

59   temporal variability in nutrient limitation (Senft et al. 1987, Robertson et al. 1993, Augustine and

60   Frank 2001, Okin et al. 2008, Liu et al. 2016). Herbivores affect spatial patterns of nutrient

61   availability directly through deposition of nutrient-rich manure or urine, which can lead to

62   heterogeneous patterns of primary productivity (Fuhlendorf and Smeins 1999). As animals

63   move across an area and rest in new locations, variability can be further enhanced (Auerswald et

64   al. 2010, Fu et al. 2013). On the other hand, consumption of nutrient-rich grasses may reduce

65   overall variance by reducing differences in biomass amounts compared to ungrazed areas.

66   Through model simulations, Gil et al. (2016) recently showed that herbivores may have a greater

67   influence on controlling biomass at fine versus broad extents, suggesting scale-dependence in

68   herbivore control of plant biomass. In a field experiment, van der Waal et al. (2016) concluded

69   that herbivore consumption of nutrient rich patterns eliminated the positive effects of fertilization

70   on the plant community and that patchiness itself (independent of the patch size) can affect the

71   outcome of trophic relationships in grassland and savanna ecosystems. Taken together,

72   understanding scale dependence (Sandel 2015), specifically the degree to which grass

73   productivity is governed by the grain and extent nutrient availability and herbivore activity, is

74   important for making inferences about ecosystem function in grasslands and requires new

75   methodological approaches for its study.

76   Incorporating spatial autocorrelation into ecological studies has augmented our

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

78   function, often at multiple, nested scales (Watt 1947, Turner et al. 2012). Understanding the

79   autocorrelation structure of key ecosystem properties is critical for determining optimal scales

80   for studying ecological systems, interpreting change in ecological communities, and assessing

81   landscape connectivity or ecosystem resilience. However, for any given study, the scale of this

82   autocorrelation structure and its implications for inferring ecological processes are not known in

83   advance. Select studies have employed experimental spatial designs *a priori* (Stohlgren et al.

84   1995) or have used computational simulations to explore the influence of space on ecosystem

85   properties (With and Crist 1995, Smithwick et al. 2003, Jenerette and Wu 2004). Geostatistical

86   analysis is commonly used (Jackson and Caldwell 1993b, Robertson et al. 1993, Smithwick et al.

87   2005, Jean et al. 2015) to describe the grain and extent of observed ecological patterns, while

88   other approaches may be more useful for predictive modeling of ecological processes through

89   space and time (Miller et al. 2007, Beale et al. 2010), though these, too, rest on an understanding

90   of autocorrelation structures.

91   Understanding these spatial structures is often elusive because ecological patterns

92   develop from complex interactions among individuals across variable abiotic gradients (Jackson

93   and Caldwell 1993a, Rietkerk et al. 2000, Ettema and Wardle 2002) and manifest at multiple

94   spatial scales. Disturbances further create structural patterns that may influence ecological

95   processes at many scales (Turner et al. 2007, Schoennagel et al. 2008). Resultant patchiness in

96   ecological phenomena is common. For example, Rietkerk et al. (2000) observed patchiness in

97   soil moisture at three unique scales (0.5 m, 1.8 m and 2.8 m) in response to herbivore impacts.

98   Following fire in the Greater Yellowstone Ecosystem (Wyoming, U.S.A.), Turner et al. (2011)

99   observed variation in soil properties at the level of individual soil cores, and Smithwick et al.

100   (2012) observed autocorrelation in post-fire soil microbial variables that ranged from 1.5 to 10.5

101   m. Patchiness in soil resources at the level of individual shrubs and trees has been demonstrated

102   by several studies (Liski 1995, Pennanen et al. 1999, Hibbard et al. 2001, Lechmere-Oertel et al.

103   2005, Dijkstra et al. 2006).   In savanna systems, multiple spatial scales are needed to explain

104   complex grass-tree interactions (Mills et al. 2006, Okin et al. 2008, Wang et al. 2010, Pellegrini

105   2016) and it is likely that these factors are nested hierarchically with spatial scale (Pickett et al.

106   2003, Rogers 2003, Pellegrini 2016).

107   In the absence of understanding the scale at which ecosystems are nutrient-limited, nor

108   the causal mechanisms underlying this scale-dependence, the ability to extrapolate nutrient

109   limitations to broader areas is hindered. Here we report on a study in which we tested the grain-

110   dependence of grass biomass to nutrient additions using a novel experimental design. Our

111   objectives were to (1) quantify the grain size at which vegetation biomass and nutrient

112   concentrations respond to nutrient additions in fenced and unfenced plots, (2) relate the level of

113   biomass response to plant nutrient concentrations and herbivory and (3) assess the degree to

114   which herbivory and nutrient treatments explained the spatial structure of grass productivity

115   through comparison of empirical semivariograms and neutral models (simulated semivariogram

116   models based on prescribed landscape patterns).

117   For Objective 1, we hypothesized that the grass response would differ between three

118   subplots scales at which fertilizer was added (1 x 1 m, 2 x 2 m, and 4 x 4 m). These scales were

119   chosen to correspond to ecosystem processes that might govern nutrient uptake, including the

120   spacing of individual plants, plant groupings, or plot-level topography, respectively, which have

121   been identified as critical sources of variation in soil biogeochemistry (Jackson and Caldwell

122   1993a, Rietkerk et al. 2000, Ettema and Wardle 2002). Half of the plots were fenced to exclude

123   herbivory to determine whether there were differences the scale of the response due to animal

124   activity. We used a semivariogram model developed from empirical data and used model

125   parameters to estimate the spatial structure of biomass and nutrient concentrations. We thus

126   expected that biomass and vegetation nutrient concentrations would have range parameters from

127   empirical semivariograms that corresponded to the hypotenuse distances of the subplot scales

128   (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

129   subplots, respectively). We expected that patchiness would be highest. i.e., range scales would

130   be smaller, for the unfenced, heterogeneously fertilized plot because these areas would have

131   received nutrient additions in the form of manure and urine from animal activity in addition to

132   nutrient additions (Liu et al. 2016).

133   For Objective 2, we hypothesized that expressions of biomass responses to nutrient

134   additions at the plot level would best explained by patterns in foliar N and P concentrations,

135   given previous work indicating the importance of coupled nutrient limitation to grassland

136   productivity (Craine et al. 2008, Craine and Jackson 2010, Ostertag 2010). We expected that

137   herbivory would have limited effects on biomass productivity relative to the influence of

138   nutrients at the plot level.

139   To test the robustness of our empirical results against a broader set of prescribed

140   landscape patterns (Objective 3), we compared the empirical semivariogram models with neutral

141   semivariogram models, computer-simulated landscapes that mimic hypothesized patterns due to

142   known ecological processes (Fajardo and McIntire 2007). This approach allows us to compare

143   empirical patterns across a set of null models in which the patterns are known and to avoid issues

144   of pseudoreplication so that we could test a replicated set of artificial landscapes in which we

145   imposed herbivory and nutrient patterns. The null assumption is that ranges (autocorrelation

146   distances, or length scales) calculated in the neutral models would be similar to the ranges

147   calculated from empirical data. Similarity of model parameters between empirical and neutral

148   models would provide confidence that observed patterns reflect known ecological processes. We

149   hypothesized that that there would be greater spatial structure in plots that received

150   heterogeneous fertilizers compared to reference plots. In homogenously fertilized plots or

151   unfertilized plots, spatial structure would be observed at scales other than scales of the subplots

152   (or not at all) and we would expect to see lower levels of spatial structure explained by the model

153   relative to random processes (higher nugget:sill, described below).

154

155 **METHODS**

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

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

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

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

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

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

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

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

164 8,100 species identified (23 % endemic), and 1,524 vascular plant genera (39 endemic) (CEPF

165 2010). Vegetation in Mkambathi is dominated by coastal sour grassveld ecosystems, which

166 dominate about 80 % of the ecosystem (Shackleton et al. 1991, Kerley et al. 1995), with small

167 pockets of forest along river gorges, wetland depressions, and coastal dunes. Dominant grasses

168 in the Mkambathi reserve include the coastal *Themeda triandra – Centella asiatica* grass

169 community, the tall grass *Cymbopogon validas – Digitaria natalensis* community in drier

170 locations, and the short-grass *Tristachya leucothrix-Loudetia simplex* community (Shackleton

171 1990). Grasslands in Mkambathi have high fire frequencies, and typically burn biennially. Soils

172 are generally derived from weathered Natal Group sandstone and are highly acidic and sandy

173 with weak structure and soil moisture holding capacity (Shackleton et al. 1991).

174   Annual precipitation in Mkambati Reserve averaged 1165 mm yr^{-1} between 1925 and

175 2015 and 1159 mm yr^{-1} between 2006 and 2015. June is typically the driest month (averaging

176 30.8 mm 1996-2015) and March is typically the wettest month (averaging 147.6 mm 1996-

177   2015). For nearby Port Edward, where data was available, the maximum temperatures is highest

178   in February (26.7 ºC), averaging 23.7 ºC annually, while minimum temperature is coolest in July

179   (average 13.0 ºC, averaging 17.4 ºC annually). During the years of this study (2010-2012),

180   annual temperature averaged 17.4 ºC (min) to 23.7 ºC (max), well within the historical average.

181   The year 2010 was one of the driest years on record (656.6 mm yr^{-1}), whereas 2011 and 2012

182   (1413.6 and 1766.3 mm yr^{-1} respectively) were wetter years than average, although within the

183   historical range (652.8 – 2385.9 mm yr^{-1}). All climate data were obtained from the South

184   African Weather Service.

185

186   **Nutrient Addition Experiment**. We established a large-scale experimental site that included six

187   60 x 60 m plots arranged in a rectangular grid (Eastern Cape Parks and Tourism Agency Permit

188   RA0081). The site was surrounded by a fuel-removal fire-break and each plot was separated by

189   at least 10 m for a total size of 3.75 ha for the entire site. To account for grazing, a fence was

190   constructed around three of these plots to exclude herbivores. Nutrient additions were applied to

191   four plots whereas two plots received no fertilizer additions; plot treatment was random. Of the

192   four plots that received fertilization, two received nutrients evenly across the entire 60 x 60 m

193   plot ("homogenous plots") and the other two fertilized plots received nutrient additions within

194   smaller subplots in a heterogeneous design ("heterogeneous plots"). Within heterogeneous plots,

195   fertilizer was applied within subplots of three different sizes (1 x 1 m, 2 x 2 m, and 4 x 4 m) that

196   were replicated randomly across each plot (**Fig. 1**). Location of individual subplots was

197   determined prior to field work using a Latin Hypercube random generator that optimizes the

198   variability of lag distances among sampling plots and is ideal for geostatistical analysis (Xu et al.

199   2005). There were a total of 126 subplots per plot that received fertilizer in the heterogeneous

200   plots. All sampling locations were geo-referenced with a GPS (Trimble 2008 Series GeoXM; 1

201   m precision) and flagged. The number of sub-plot units at each scale was determined so as to

202   equalize the total fertilized area at each sub-plot scale (i.e., six 4 x 4 m plots and 24, 2 x 2 m

203   plots). To ensure aboveground grass biomass would respond to nutrient additions, we employed

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

205   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

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

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

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

209   number of treatments, thus increasing sample size. Towards the end of the summer wet season

210   (February), we applied fertilizer to subplots in the two heterogeneous plots and evenly across the

211   two homogeneous plots. The amount of fertilizer received was equal on a per unit area basis

212   among plots and subplots.

213

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

215   sampled for soil and vegetation nutrient concentrations and biomass. Subplots to be sampled

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

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

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

219   randomly selected locations for biomass measurement and vegetation clippings: two locations

220   were identified and flagged from within the 1 x 1 m subplots (center coordinate and a random

221   location 0.5 m from center), four samples were identified and flagged from within the 2 x 2 m

222   subplots, and eight samples were identified and flagged from within the 4 x 4 m subplots.

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

224 biomass using a disc pasture meter (DPM) (Bransby and Tainton 1977) and grab samples of

225 grass clippings were collected for foliar nutrient analysis, using shears and cutting to ground-

226 level. Calibration of the DPM readings was determined using ten random 1 x 1 m subplots in

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

228 biomass was harvested to bare soil. Linear regression was used to relate DPM estimates with

229 harvested biomass at calibration subplots ($R^2 = 0.76$, p < 0.0001; **Supplementary Material, Fig**

230 **S1**) and the resulting equation was then used to estimate biomass at the remaining 606 locations.

231  Soil samples from the top 0 – 10 cm soil profile depth were collected adjacent to

232 vegetation samples. Due to logistical and financial constraints, these samples were collected in

233 fenced plots only. The A horizon of the Mollisols was consistently thicker than 10 cm, so all

234 samples collected were drawn from the A horizon. Soil samples were shipped to BEMLab

235 (Strand, South Africa) for nutrient analysis.

236

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

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

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

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

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

242 concentration was measured with a Combustion-Elementar Vario Max method (Horneck and

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

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

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

246   Eckert and Sims (1995).

247

248   **Empirical semivariograms.** Semivariogram models were fit to empirical data and model

249   parameters were used to test Objective 1. The range parameter was used to estimate the scale of

250   autocorrelation; the sill parameter was used to estimate overall variance; and the nugget

251   parameter was used to represent variance not accounted for in the sampling design. A maximum

252   likelihood approach was used to quantify the model parameters. This approach assumes that the

253   data ($Y_1 ... Y_n$) are realizations of an underlying spatial process, and that the distribution of the

254   data follows a Gaussian multivariate distribution:

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

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

257   (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

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

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

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

261   second order moment for an element *i,j* is:

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

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

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

265   constant or estimated with a linear model across all locations and in this case we used the plot-

266   level mean of the data for μ (Table 1).

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

268   distributions, which violated the assumption of Gaussian stationarity within the underlying

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

270   plot using a box-cox transformation (Box and Cox 1964):

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

272    $\hat{Y}_i = \log\left(Y_i\right)$ if $\lambda = 0$

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

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

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

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

277   that maximized a normal multivariate log-likelihood function (Diggle et al. 2003). To

278   approximate a sampling distribution of each parameter, a bootstrapping algorithm was used

279   where a randomly sampled subset of data was input into the same maximum likelihood approach

280   for 1000 iterations. This provided a population of fitted parameters and models that was used to

281   analyze the approximate distributions of each parameter for each plot. The maximum likelihood

282   optimization was cross-validated by removing a random sub-sample of measurements from the

283   optimization and then using the optimized model to make predictions at locations where

284   measurements were removed. Observed vs. predicted values from the cross-validation procedure

285   were then analyzed at each plot separately.

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

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

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

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

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

291

292   **Mixed Model**. To relate these patterns in biomass to vegetation nutrient concentrations

293   (Objective 2), we used a linear mixed modeling approach. Experimental factors such as

294   herbivory, fertilizer type (i.e., heterogeneous, homogenous, and unfertilized), plot treatment, and

295   subplot size were included as random effects to manage non-independence of data and avoid

296   issues of pseudoreplication (Millar and Anderson 2004). Multiple combinations of random

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

298   biomass, and model error was assumed to be Gaussian. A normal likelihood function was

299   minimized to estimate optimal regression coefficients for each mixed model formulation. To

300   identify a mixed model that estimated biomass closely to observations, while also having the

301   fewest possible parameters, we used the Akaike's Information Criterion (AIC) and Bayesian

302   Information Criterion (BIC), which decrease with a negative log-likelihood function but increase

303   with the number of parameters used in the model (Burnham and Anderson 2002). The model

304   with the lowest BIC was chosen as best representing the tradeoff of parsimony and prediction

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

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

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

308   modeling was conducted with the R package lme4 (R Development Team, 2014).

309

310   **Simulated semivariograms**. The neutral semivariogram models were constructed for six

311   simulated landscapes (**Fig. 2**) to represent alternative landscape structures in response to nutrient

312   addition and grazing: (**a**) fenced-unfertilized (biomass was assumed to be randomly distributed

313   around the mean of the biomass from the fenced, unfertilized experimental plot), (**b**) fenced-

314   heterogeneous (biomass of (a) was doubled for selected subplots, following the same subplot

315   structure that was used in the field experiments), (**c**) fenced-homogenous (biomass of (a) was

316   doubled at every grid cell to mimic an evenly distributed fertilization response), (**d**) unfenced-

317   unfertilized (biomass of (a) was increased by 50 % in response to a combined effect of biomass

318   loss by grazing and biomass gain by manure nutrient additions by herbivores; the increase

319   occurred at a subset of sites to mimic random movement patterns of herbivores), (**e**) unfenced-

320   heterogeneous (biomass equaled biomass of herbivory only, fertilizer only, or herbivory +

321   fertilizer), and (**f**) unfenced-homogenous (biomass of (d) was doubled at all grid cells to mimic

322   the additive effects of herbivores and homogenous fertilizer additions).

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

324   likelihood approach as described for empirical models and data was not transformed. The mean

325   (μ) was estimated using a constant trend estimate. Given that the magnitude of observed and

326   simulated biomass can change the amount of spatial variance, we scaled the nugget and sill

327   parameters by dividing these parameters by the maximum calculated spatial autocorrelation in

328   the data according to the 'modulus' method (Cressie 1993).

329

330   **RESULTS**

331   Vegetation biomass varied by 50% across plots, with the highest biomass found for

332   heterogeneously fertilized plots (**Table 1**). Vegetation nutrient concentrations increased, and

333   N:P ratios declined, following fertilization (**Table 1**). Vegetation N concentration averaged 0.60

334   ± 0.01 % in unfertilized plots, 0.72 ± 0.02 % in heterogeneously fertilized plots, and 0.77 ± 0.02

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

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

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

338   increase of 34 and 35 %, respectively. The vegetation N:P ratios ranged from a high of 17.9 in

339   the fenced-unfertilized plot to 12.1 in the unfenced-homogenously fertilized plot. Vegetation C

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

341   fertilization in the fenced plots, where these variables were measured (**Supplementary Table 1**).

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

343   unfertilized plot and 4.08 in fertilized plots. Confirming reference conditions, pH measured in a

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

345   Empirical semivariogram models show that there was a statistically significant patch

346   structure at scales corresponding to the scale of the subplots in the fenced and unfenced,

347   heterogeneously fertilized plots (Objective 1; **Fig. 3b,f**). Also confirming expectations, in

348   unfertilized (reference) or homogenously fertilized plots the range scale was significantly longer

349   or shorter (**Fig. 3; Supplementary Table 2**). The sampling distributions of the semivariogram

350   range values for vegetation biomass determined from the maximum likelihood and bootstrapping

351   analysis show that the range value most closely resembles that of the hypotenuse of the 2 x 2 m

352   subplot, relative to the other subplots (**Fig. 3d,h**). Higher spatial structure in the heterogeneous

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

354   These maps also highlight the higher mean levels of biomass in fertilized subplots relative to

355   areas outside of subplots or relative to other plots. These hotspots contributed to the higher than

356   average biomass values for heterogeneously fertilized plots as a whole.

357   Normalized nugget/sill ratios represent the ratio of noise-to-structure in the

358   semivariogram model, and thereby provide an estimate of the degree to which the overall

359   variation in the model is spatially random. Nugget/sill ratios were highest in the unfenced,

360   homogeneously fertilized plot (3.89), suggesting more random variation in the overall model

361   variance, whereas ratios were lower (0-0.02) for heterogeneously fertilized or fenced treatments,

362   suggesting that there was little contribution of spatially random processes in the overall model.

363   These results support the expectation of strong spatial structure in biomass response to nutrient

364   addition, especially at the 2 meter scale.

365   The semivariogram range values for vegetation % N and % P (**Supplementary Table 3**)

366   were comparable to subplot scales of nutrient additions (% P, ~ 4.9 m, % N, ~ 5.8 m) in the

367   fenced, heterogeneously fertilized plot, i.e., where herbivores were absent. However, higher or

368   lower range values were found for the other plots. Similar to results for biomass, the nugget:sill

369   ratio in semivariogram models of vegetation % N and % P was highest in the unfertilized plots,

370   suggesting a larger degree of spatially random processes contributing to overall variance. In

371   turn, this indicates higher spatial structure captured in models of the fertilized treatments, relative

372   to random processes. Semivariogram parameters of soil carbon and nutrients showed few

373   differences among treatments where these were measured (fenced plots, only) (**Supplementary**

374   **Table 3**).

375   Mixed models used to predict biomass levels from N or P foliar concentrations, while

376   treating plot and treatment as random effects, showed that biomass was best predicted by levels

377   of foliar P, relative to foliar N alone or foliar N x P (Objective 2; **Table 2**). Although foliar P

378   alone did better than foliar N alone as a fixed effect, the difference was marginal (< 2 BIC). The

379   'best' model used only plot treatment type as a random effect, which outperformed model

380   formulations using herbivory or fertilizer type and those with nested structures incorporating

381   subplot size as random effects.

382   The spatial structure of heterogeneous plots was estimated to be similar between neutral

383   and empirical semivariogram models and generally matched subplot scales (Objective 3; **Fig. 5**).

384   Interestingly, the neutral models estimated higher range values (longer length scales) in fenced

385   plots compared to unfenced plots, whereas empirical semivariogram models estimated longer

386   length scales in unfenced plots.

387

388   **DISCUSSION**

389   Although scale-dependence is known to be critical for inferring ecological processes

390   from ecological pattern (Levin 1992, Dungan et al. 2002, Sandel 2015), and although nutrient

391   limitation and herbivory are known to influence grassland productivity at multiple scales

392   (Fuhlendorf and Smeins 1999, House et al. 2003, Pellegrini 2016, van der Waal et al. 2016), our

393   study is the first to our knowledge to impose an experimental design that directly tests the scale

394   at which grass responds to nutrient additions. By imposing the scale of nutrient additions *a*

395   *priori* we were able to discern, using semivariograms based on empirical data, greater biomass

396   response at the 2 x 2 m grain compared to finer (1 x 1 m) or broader (4 x 4 m) grain sizes.

397   Comparisons to neutral models based on simulated landscapes with known patterns, supported

398   our expectations that herbivore activity and nutrient additions can contribute to the spatial

399   structure found in our empirical results. Mixed model results further indicated that foliar nutrient

400   concentrations accounted for the majority of observed patterns in the level of biomass response,

401   with limited influence of herbivory.   Overall, these results yield data on the spatial scale of the

402   nutrient-productivity relationship in a grassland coastal forest of the Eastern Cape, South Africa,

403   and support the assertion that ecological processes are likely multi-scaled and hierarchical in

404   response to nutrient additions.

405

Inferring the scale of grass response to nutrient additions

407   This study provided an opportunity experimentally test the scale at which nutrient

408 limitation is most strongly expressed, providing an alternative to studies in which spatial

409 autocorrelation is observed post-hoc. Detecting the autocorrelation structure of an ecological

410 pattern is a critical but insufficient approach for inferring an ecological process. A preferred

411 approach, such as tested here, is to impose a pattern at a certain (set of) scale(s) and determine if

412 that process responds at that scale(s). The benefit to this approach is a closer union between

413 observed responses (biomass) and ecological processes (nutrient limitation) and the ability to

414 compare responses across scales. Our results indicate that biomass responded to nutrient

415 additions at all subplot scales, with spatial autocorrelation of the biomass response highest at the

416 2 x 2 m scale. Studies have found finer-grain spatial structure in grassland soil properties

417 (Jackson and Caldwell 1993a, Rietkerk et al. 2000, Augustine and Frank 2001) while others have

418 observed biomass responses to nutrient additions or herbivory at finer (Klaus et al. 2016) or

419 broader (Lavado et al. 1995, Augustine and Frank 2001, Pellegrini 2016) scales, or a limited

420 effect of scale altogether (van der Waal et al. 2016).   Indeed, we observed high nugget variance

421 for soil nutrients and carbon under heterogeneous fertilization, implying variation below the

422 scale of sampling. The response of biomass at the 2 x 2 m scale may thus reflect spatial patterns

423 in species composition or plant groupings rather than soil characteristics, at least in fenced plots

424 where soil nutrients were sampled.

425   Although response was quantified to be stronger at the 2 x 2 m grain, all subplots in the

426 heterogeneous plots responded strongly to nutrient additions, as observed in the kriged maps. As

427 a result, the heterogeneous plots had greater average biomass than plots which were fertilized

428 homogeneously, despite the fact that fertilizer was added equally on a per area basis for both

429 treatments. Several other studies have found higher biomass following heterogeneous nutrient

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

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

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

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

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

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

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

437 lifespan, rooting structures and uptake rate to maximize nutrient supply (Robinson 1994, Hodge

438 2004). In turn, initial advantages afforded by plants in nutrient-rich locations may result in

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

440 (Casper et al. 2000).

441

442 **Implications for understanding nutrient limitations**

443   The goal of our study was not to determine the nature and extent of nutrient limitation to

444 grass productivity, as has been studied previously (Craine et al. 2008, Klaus et al. 2016), as much

445 as it was to infer the scale of this response. Yet, our results do convey some lessons about the

446 role of nutrient limitations in these grasslands. First, our study supports the notion of coupled N

447 and P limitation in grasslands (Craine and Jackson 2010), including the subtropics (Klaus et al.

448 2016). Ostertag (2010) also showed that there was a preference for P uptake in a nutrient limited

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

450 variability in P availability. We found that P was the variable that explained most of the

451   variation in the level of biomass response across all plots, followed by N. In addition, we saw a

452   strong difference in N:P ratios between reference and fertilized plots. Many studies have used

453   stoichiometric relationships of N and P to infer nutrient limitation (Koerselman and Meuleman

454   1996, Reich and Oleksyn 2004), although there are limits to this approach (Townsend et al.

455   2007, Ostertag 2010). Using this index, our N:P ratios of vegetation in reference plots would

456   indicate co-limitation for N and P prior to fertilization (N:P > 16). Addition of dual fertilizer

457   appeared to alleviate P limitation more than N, with N:P ratios reduced one year following

458   treatment, indicating N limitation or co-limitation with another element (N:P < 14). Grazing

459   may also preferentially increase grass P concentrations in semi-arid systems in South Africa

460   (Mbatha and Ward 2010) and thus the cumulative impacts of preferential plant P uptake and P

461   additions from manure may explain the high spatial structure observed in our grazed and

462   fertilized plots.

463   Relating biomass response to nutrient limitation using *in situ* data is complicated by

464   processes such as luxury consumption (Ostertag 2010), initial spatial patterns in soil fertility

465   (Castrignano et al. 2000), root distribution, signaling and allocation (Aiken and Smucker 1996),

466   species and functional group shifts (Reich et al. 2003, Ratnam et al. 2008), or species'

467   differences in uptake rates or resorption (Townsend et al. 2007, Reed et al. 2012). Spatial

468   patterns of finer-scale processes such as microbial community composition have also been

469   explored and are known to influence rates of nutrient cycling (Ritz et al. 2004, Smithwick et al.

470   2005). In the case of heterogeneous nutrient supply, species competitive relationships across

471   space may be enhanced (Du et al. 2012) and may result in increases in plant diversity (Fitter

472   1982, Wijesinghe et al. 2005), although other studies have found little evidence to support this

473   claim (Gundale et al. 2011). Together, these factors may explain any unexplained variance of

474   vegetation N and P concentrations that we observed. On the other hand, effects of species

475   composition shifts on plant biomass were likely minimal in this study given the short-term nature

476   of the study (one year), but patchiness in biomass responses indicate size differences that are

477   likely to modify competitive relationships in the future. Unfortunately, the site burned one year

478   following the experiment, precluding additional tests of these relationships.

479

480   **Herbivory-Nutrient interactions**

481   Our study indicates a strong scalar influence of nutrient additions relative to nutrient-herbivore

482   interactions. First, we found that the significant length scale was similar between unfenced and

483   fenced plots, indicating that herbivory did not alter the grain of biomass response to nutrient

484   limitation. In addition, herbivory was not significant in final mixed effects models, relative to

485   the inclusion of foliar nutrient variables, suggesting that nutrients had a greater influence on the

486   level of biomass response. However, our study was not designed to unravel the multivariate

487   influence of herbivores on grasslands, which may influence vegetation biomass through biomass

488   removal, movement activity, and manure additions (Milchunas and Lauenroth 1993, Adler et al.

489   2001, van der Waal et al. 2016). Interestingly, our empirical semivariogram model indicates

490   longer range scales where herbivores were present compared to simulated semivariogram

491   models, which may reflect homogenization of biomass through grazing and thus a greater top-

492   down approach of herbivory on ecosystem productivity than previously appreciated (van der

493   Waal et al. 2016), or other complex interactions between grazing and fertilization not accounted

494   for in the current study.

495

496   **Uncertainties**

497   There are several key uncertainties and caveats in applying our methodological approach

498   more broadly. First, the experimental design described herein was labor-intensive, requiring

499   both precision mapping of locations for nutrient additions and post-treatment vegetation

500   sampling, as well as extensive replication of treatments that would respond to broader ecological

501   patterns, i.e., grazing. This necessitated a trade-off between sampling effort across scales

502   (subplots, plots). Important processes at scales above and below the extent and grain of

503   sampling used here were likely important but were not included. Second, our neutral models

504   assumed additive effects of herbivore activity and fertilization; in contrast, empirical results

505   likely reflect complex, potentially non-additive, interactions between grazing and fertilization.

506   Third, recent work has suggested that both nutrient patchiness and the form of nutrient limitation

507   (e.g., N vs. P) may change seasonally (Klaus et al. 2016), which was not assessed here.

508   Moreover, annual variation in precipitation, in our case a dry year followed by a wet year, may

509   have influenced the level of biomass response to nutrient additions.

510

511   **Conclusions**

512   Understanding the factors that regulate ecosystem productivity, and the scales at which

513   they operate, is critical for guiding ecosystem management activities aimed at maintaining

514   landscape sustainability. New approaches are needed to characterize how ecosystems are

515   spatially structured and to determine whether there are specific scale or scales of response that

516   are most relevant. In South Africa, grasslands cover nearly one-third of the country and maintain

517   the second-highest levels of biodiversity but are expected to undergo significant losses in

518   biodiversity in coming decades due to increasing pressure from agricultural development and

519   direct changes in climate (Biggs et al. 2008, Huntley and Barnard 2012). We employed a neutral

520   model approach to test for ecological process, an approach that has been advocated for decades

521   (Turner 1989) but which is rarely imposed (but see With 1997, Fajardo and McIntire 2007). We

522   conclude that these grasslands express nutrient limitation at intermediate scales (2 x 2 m) and

523   exhibit relatively strong nutrient limitations for both N and P, with a more limited influence of

524   herbivory. By extending this approach to other areas and other processes, specifically by

525   imposing experimental studies to test for the influence of scale on other ecological processes, it

526   may be possible to reduce bias in empirical studies, minimize the potential for scale mismatches,

527   and deepen insights into ecological pattern-process interactions.

528

529   **ACKNOWLEDGEMENTS**

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

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

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

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

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

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

536   and laboratory work.

537   REFERENCES CITED

538   Adler, P. B., D. A. Raff, and W. K. Lauenroth. 2001. The effect of grazing on the spatial
539   heterogeneity of vegetation. Oecologia **128**:465-479.
540   Aiken, R. M., and A. J. M. Smucker. 1996. Root system regulation of whole plant growth.
541   Annual Review of Phytopathology **34**:325-346.
542   Auerswald, K., F. Mayer, and H. Schnyder. 2010. Coupling of spatial and temporal pattern of
543   cattle excreta patches on a low intensity pasture. Nutrient Cycling in Agroecosystems
544   **88**:275-288.
545   Augustine, D. J., and D. A. Frank. 2001. Effects of migratory grazers on spatial heterogeneity of
546   soil nitrogen properties in a grassland ecosystem. Ecology **82**:3149-3162.
547   Augustine, D. J., S. J. McNaughton, and D. A. Frank. 2003. Feedbacks between soil nutrients
548   and large herbivores in a managed savanna ecosystem. Ecological Applications **13**:1325-
549   1337.
550   Beale, C. M., J. J. Lennon, J. M. Yearsley, M. J. Brewer, and D. A. Elston. 2010. Regression
551   analysis of spatial data. Ecology Letters **13**:246-264.
552   Biggs, R., H. Simons, M. Bakkenes, R. J. Scholes, B. Eickhout, D. van Vuuren, and R.
553   Alkemade. 2008. Scenarios of biodiversity loss in southern Africa in the 21st century.
554   Global Environmental Change **18**:296-309.
555   Box, G. E. P., and D. R. Cox. 1964. An analysis of transformations. Journal of the Royal
556   Statistical Society Series B-Statistical Methodology **26**:211-252.
557   Bransby, D., and N. Tainton. 1977. The disc pasture meter: Possible applications in grazing
558   management. Proclamations of the Grassland Society of South Africa **12**:115-118.
559   Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a
560   practical information-theoretic approach. Springer-Verlag, New York, New York.
561   Casper, B. B., J. F. Cahill, and R. B. Jackson. 2000. Plant competition in spatially heterogeneous
562   environments. Pages 111-130 *in* M. J. Hutchings, E. A. John, and A. J. A. Stewart,
563   editors. Ecological Consequences of Environmental Heterogeneity. Blackwell Science,
564   Oxford, UK.
565   Castrignano, A., L. Giugliarini, R. Risaliti, and N. Martinelli. 2000. Study of spatial relationships
566   among some soil physico-chemical properties of a field in central Italy using multivariate
567   geostatistics. Geoderma **97**:39-60.
568   Craine, J. M., and R. D. Jackson. 2010. Plant nitrogen and phosphorus limitation in 98 North
569   American grassland soils. Plant and Soil **334**:73-84.
570   Craine, J. M., C. Morrow, and W. D. Stock. 2008. Nutrient concentration ratios and co-limitation
571   in South African grasslands. New Phytologist **179**:829-836.
572   Cressie, N. 1988. Spatial prediction and ordinary kriging. Mathematical Geology **20**:405-421.
573   Cressie, N. 1993. Statistics for Spatial Data. John Wiley & Sons, Inc., New York, NY.
574   Day, K. J., M. J. Hutchings, and E. A. John. 2003. The effects of spatial pattern of nutrient
575   supply on the early stages of growth in plant populations. Journal of Ecology **91**:305-315.
576   Diggle, P. J., P. J. Ribeiro Jr, and O. F. Christensen. 2003. An introduction to model-based
577   geostatistics. Pages 43-86 *in* J. Möller, editor. Spatial statistics and computational
578   methods. Springer.
579   Dijkstra, F., K. Wrage, S. Hobbie, and P. Reich. 2006. Tree Patches Show Greater N Losses but
580   Maintain Higher Soil N Availability than Grassland Patches in a Frequently Burned Oak
581   Savanna. Ecosystems **9**:441-452.

582 Domingues, T. F., P. Meir, T. R. Feldpausch, G. Saiz, E. M. Veenendaal, F. Schrodt, M. Bird, G.
583  Djagbletey, F. Hien, H. Compaore, A. Diallo, J. Grace, and J. Lloyd. 2010. Co-limitation
584  of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. Plant
585  Cell and Environment **33**:959-980.
586 Du, F., X. X. Xu, X. C. Zhang, M. G. Shao, L. J. Hu, and L. Shan. 2012. Responses of old-field
587  vegetation to spatially homogenous or heterogeneous fertilisation: implications for
588  resources utilization and restoration. Polish Journal of Ecology **60**:133-144.
589 Dungan, J. L., J. N. Perry, M. R. T. Dale, P. Legendre, S. Citron-Pousty, M. J. Fortin, A.
590  Jakomulska, M. Miriti, and M. S. Rosenberg. 2002. A balanced view of scale in spatial
591  statistical analysis. Ecography **25**:626-640.
592 Eckert, D., and J. T. Sims. 1995. Recommended Soil pH and Lime Requirement Tests.
593  Agricultural Experiment Station, University of Delaware, Newark, DE.
594 Ettema, C. H., and D. A. Wardle. 2002. Spatial soil ecology. Trends in Ecology & Evolution
595  **17**:177-183.
596 Fajardo, A., and E. J. B. McIntire. 2007. Distinguishing microsite and competition processes in
597  tree growth dynamics: An a priori spatial modeling approach. American Naturalist
598  **169**:647-661.
599 Fitter, A. H. 1982. Influence of Soil Heterogeneity on the Coexistence of Grassland Species.
600  Journal of Ecology **70**:139-148.
601 Fu, W. J., K. L. Zhao, P. K. Jiang, Z. Q. Ye, H. Tunney, and C. S. Zhang. 2013. Field-scale
602  variability of soil test phosphorus and other nutrients in grasslands under long-term
603  agricultural managements. Soil Research **51**:503-512.
604 Fuhlendorf, S. D., and F. E. Smeins. 1999. Scaling effects of grazing in a semi-arid grassland.
605  Journal of Vegetation Science **10**:731-738.
606 Gil, M. A., J. Jiao, and C. W. Osenberg. 2016. Enrichment scale determines herbivore control of
607  primary producers. Oecologia **180**:833-840.
608 Gundale, M. J., A. Fajardo, R. W. Lucas, M.-C. Nilsson, and D. A. Wardle. 2011. Resource
609  heterogeneity does not explain the diversity-productivity relationship across a boreal
610  island fertility gradient. Ecography **34**:887-896.
611 Hedin, L. O. 2004. Global organization of terrestrial plant-nutrient interactions. Proceedings of
612  the National Academy of Sciences of the United States of America **101**:10849-10850.
613 Hibbard, K. A., S. Archer, D. S. Schimel, and D. W. Valentine. 2001. Biogeochemical changes
614  accompanying woody plant encroachment in a subtropical savanna. Ecology **82**:1999-
615  2011.
616 Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New
617  Phytologist **162**:9-24.
618 Horneck, D. A., and R. O. Miller. 1998. Determination of total nitrogen in plant tissue.*in* Y. P.
619  Kalra, editor. Handbook and Reference Methods for Plant Analysis. CRC Press, New
620  York.
621 House, J. I., S. Archer, D. D. Breshears, and R. J. Scholes. 2003. Conundrums in mixed woody-
622  herbaceous plant systems. Journal of Biogeography **30**:1763-1777.
623 Huang, C.-Y. L., and E. E. Schulte. 1985. Digestion of Plant Tissue for Analysis by ICP
624  Emission Spectroscopy. Commun. in Soil Sci. Plant Anal. **16**:943-958.
625 Huntley, B., and P. Barnard. 2012. Potential impacts of climatic change on southern African
626  birds of fynbos and grassland biodiversity hotspots. Diversity and Distributions **18**:769-
627  781.

628 Jackson, R. B., and M. M. Caldwell. 1993a. Geostatistical patterns of soil heterogeneity around
629   individual perennial plants. Journal of Ecology **81**:683-692.
630 Jackson, R. B., and M. M. Caldwell. 1993b. The scale of nutrient heterogeneity around
631   individual plants and its quantification with geostatistics. Ecology **74**:612-614.
632 Jean, P. O., R. L. Bradley, J. P. Tremblay, and S. D. Cote. 2015. Combining near infrared spectra
633   of feces and geostatistics to generate forage nutritional quality maps across landscapes.
634   Ecological Applications **25**:1630-1639.
635 Jenerette, G. D., and J. Wu. 2004. Interactions of ecosystem processes with spatial heterogeneity
636   in the puzzle of nitrogen limitation. Oikos **107**:273-282.
637 Kerley, G. I. H., M. H. Knight, and M. DeKock. 1995. Desertification of subtropical thicket in
638   the Eastern Cape, South Africa: Are there alternatives? Pages 211-230.
639 Klaus, V. H., S. Boch, R. S. Boeddinghaus, N. Holzel, E. Kandeler, S. Marhan, Y. Oelmann, D.
640   Prati, K. M. Regan, B. Schmitt, E. Sorkau, and T. Kleinebecker. 2016. Temporal and
641   small-scale spatial variation in grassland productivity, biomass quality, and nutrient
642   limitation. Plant Ecology **217**:843-856.
643 Koerselman, W., and A. F. M. Meuleman. 1996. The vegetation N:P ratio: A new tool to detect
644   the nature of nutrient limitation. Journal of Applied Ecology **33**:1441-1450.
645 Lambers, H., J. A. Raven, G. R. Shaver, and S. E. Smith. 2008. Plant nutrient-acquisition
646   strategies change with soil age. Trends in Ecology & Evolution **23**:95-103.
647 Lavado, R. S., J. O. Sierra, and P. N. Hashimoto. 1995. Impact of grazing on soil nutrients in a
648   pampean grassland. Journal of Range Management **49**:452-457.
649 LeBauer, D. S., and K. K. Treseder. 2008. Nitrogen limitation of net primary productivity in
650   terrestrial ecosystems is globally distributed. Ecology **89**:371-379.
651 Lechmere-Oertel, R. G., R. M. Cowling, and G. I. H. Kerley. 2005. Landscape dysfunction and
652   reduced spatial heterogeneity in soil resources and fertility in semi-arid succulent thicket,
653   South Africa. Austral Ecology **30**:615-624.
654 Levin, S. A. 1992. The problem of pattern and scale in ecology. Ecology **73**:1943-1967.
655 Liski, J. 1995. Variation in soil organic carbon and thickness of soil horizons within a boreal
656   forest stand - effects of trees and implications for sampling. Silva Fennica **29**:255-266.
657 Liu, C., X. X. Song, L. Wang, D. L. Wang, X. M. Zhou, J. Liu, X. Zhao, J. Li, and H. J. Lin.
658   2016. Effects of grazing on soil nitrogen spatial heterogeneity depend on herbivore
659   assemblage and pre-grazing plant diversity. Journal of Applied Ecology **53**:242-250.
660 Mbatha, K. R., and D. Ward. 2010. The effects of grazing, fire, nitrogen and water availability
661   on nutritional quality of grass in semi-arid savanna, South Africa. J.Arid Environ.
662   **74**:1294-1301.
663 McIntire, E. J. B., and A. Fajardo. 2009. Beyond description: the active and effective way to
664   infer processes from spatial patterns. Ecology **90**:46-56.
665 Milchunas, D. G., and W. K. Lauenroth. 1993. Quantitative effects of grazing on vegetation and
666   soils over a global range of environments Ecological Monographs **63**:327-366.
667 Millar, R. B., and M. J. Anderson. 2004. Remedies for pseudoreplication. Fisheries Research
668   **70**:397-407.
669 Miller, J., J. Franklin, and R. Aspinall. 2007. Incorporating spatial dependence in predictive
670   vegetation models. Ecological Modelling **202**:225-242.
671 Mills, A. J., K. H. Rogers, M. Stalmans, and E. T. F. Witkowski. 2006. A framework for
672   exploring the determinants of savanna and grassland distribution. Bioscience **56**:579-589.

673 Ngatia, L. W., B. L. Turner, J. T. Njoka, T. P. Young, and K. R. Reddy. 2015. The effects of
674   herbivory and nutrients on plant biomass and carbon storage in Vertisols of an East
675   African savanna. Agriculture Ecosystems & Environment **208**:55-63.
676 Okin, G. S., N. Mladenov, L. Wang, D. Cassel, K. K. Caylor, S. Ringrose, and S. A. Macko.
677   2008. Spatial patterns of soil nutrients in two southern African savannas. Journal of
678   Geophysical Research-Biogeosciences **113**.
679 Ostertag, R. 2010. Foliar nitrogen and phosphorus accumulation responses after fertilization: an
680   example from nutrient-limited Hawaiian forests. Plant and Soil **334**:85-98.
681 Pellegrini, A. F. A. 2016. Nutrient limitation in tropical savannas across multiple scales and
682   mechanisms. Ecology **97**:313-324.
683 Pennanen, T., J. Liski, V. Kitunen, J. Uotila, C. J. Westman, and H. Fritze. 1999. Structure of the
684   microbial communities in coniferous forest soils in relation to site fertility and stand
685   development stage. Microbial Ecology **38**:168-179.
686 Pickett, S. T. A., M. L. Cadenasso, and T. L. Benning. 2003. Biotic and abiotic variability as key
687   determinants of savanna heterogeneity at multiple spatiotemporal scales. Pages 22-40 *in*
688   S. R. Du Toit, K. H. Rogers, and H. C. Biggs, editors. The Kruger Experience: Ecology
689   and Management of Savanna Heterogeneity. Island Press, Washington DC.
690 Ratnam, J., M. Sankaran, N. P. Hanan, R. C. Grant, and N. Zambatis. 2008. Nutrient resorption
691   patterns of plant functional groups in a tropical savanna: variation and functional
692   significance. Oecologia **157**:141-151.
693 Reed, S. C., A. R. Townsend, E. A. Davidson, and C. C. Cleveland. 2012. Stoichiometric
694   patterns in foliar nutrient resorption across multiple scales. New Phytologist **196**:173-
695   180.
696 Reich, P. B., C. Buschena, M. G. Tjoelker, K. Wrage, J. Knops, D. Tilman, and J. L. Machado.
697   2003. Variation in growth rate and ecophysiology among 34 grassland and savanna
698   species under contrasting N supply: a test of functional group differences. New
699   Phytologist **157**:617-631.
700 Reich, P. B., and J. Oleksyn. 2004. Global patterns of plant leaf N and P in relation to
701   temperature and latitude. Proceedings of the National Academy of Sciences of the United
702   States of America **101**:11001-11006.
703 Ribeiro Jr., P. J., and P. J. Diggle. 2001. geoR: A package for geostatistical analysis. R News
704   **1**:14-18.
705 Rietkerk, M., P. Ketner, J. Burger, B. Hoorens, and H. Olff. 2000. Multiscale soil and vegetation
706   patchiness along a gradient of herbivore impact in a semi-arid grazing system in West
707   Africa. Plant Ecology **148**:207-224.
708 Ritz, K., W. McNicol, N. Nunan, S. Grayston, P. Millard, D. Atkinson, A. Gollotte, D.
709   Habeshaw, B. Boag, C. D. Clegg, B. S. Griffiths, R. E. Wheatley, L. A. Glover, A. E.
710   McCaig, and J. I. Prosser. 2004. Spatial structure in soil chemical and microbiological
711   properties in an upland grassland. FEMS Microbiology Ecology **49**:191-205.
712 Robertson, G. P., J. R. Crum, and B. G. Ellis. 1993. The spatial variability of soil resources
713   following long-term disturbance. Oecologia **96**:451-456.
714 Robinson, D. 1994. The response of plants to nonuniform supplies of nutrients. New Phytologist
715   **127**:635-674.
716 Rogers, K. H. 2003. Adopting a heterogeneity paradigm: Implications for management of
717   protected savannas. Pages 41-58 *in* S. R. Du Toit, K. H. Rogers, and H. C. Biggs, editors.

718   The Kruger Experience: Ecology and Management of Savanna Heterogeneity. Island
719   Press, Washington DC.
720 Sandel, B. 2015. Towards a taxonomy of spatial scale-dependence. Ecography **38**:358-369.
721 Schoennagel, T., E. A. H. Smithwick, and M. G. Turner. 2008. Landscape heterogeneity
722   following large fires: insights from Yellowstone National Park, USA. International
723   Journal of Wildland Fire **17**:742-753.
724 Senft, R. L., M. B. Coughenour, D. W. Bailey, L. R. Rittenhouse, O. E. Sala, and D. M. Swift.
725   1987. Large herbivore foraging and ecological hierarchies. Bioscience **37**:789-797.
726 Shackleton, C. M. 1990. Seasonal changes in biomass concentration in three coastal grassland
727   communities in Transkei. Journal of Grassland Society of Southern Africa **7**:265-
728   269.
729 Shackleton, C. M., J. E. Granger, B. McKenzie, and M. T. Mentis. 1991. Multivariate analysis of
730   coastal grasslands at Mkambati Game Reserve, north-eastern Pondoland, Transkei.
731   Bothalia **21**:91-107.
732 Smithwick, E. A. H., M. E. Harmon, and J. B. Domingo. 2003. Modeling multiscale effects of
733   light limitations and edge-induced mortality on carbon stores in forest landscapes.
734   Landscape Ecology **18**:701-721.
735 Smithwick, E. A. H., M. C. Mack, M. G. Turner, F. S. Chapin, J. Zhu, and T. C. Balser. 2005.
736   Spatial heterogeneity and soil nitrogen dynamics in a burned black spruce forest stand:
737   distinct controls at different scales. Biogeochemistry **76**:517-537.
738 Smithwick, E. A. H., K. J. Naithani, T. C. Balser, W. H. Romme, and M. G. Turner. 2012. Post-
739   Fire Spatial Patterns of Soil Nitrogen Mineralization and Microbial Abundance. PLoS
740   ONE **7**:e50597.
741 Stohlgren, T. J., M. B. Falkner, and L. D. Schell. 1995. A Modified-Whittaker Nested Vegetation
742   Sampling Method. Vegetatio **117**:113-121.
743 Townsend, A. R., C. C. Cleveland, G. P. Asner, and M. M. C. Bustamante. 2007. Controls over
744   foliar N : P ratios in tropical rain forests. Ecology **88**:107-118.
745 Turner, M. G. 1989. Landscape ecology: The effect of pattern on process. Annual Review of
746   Ecology and Systematics **20**:171-197.
747 Turner, M. G., D. C. Donato, and W. H. Romme. 2012. Consequences of spatial heterogeneity
748   for ecosystem services in changing forest landscapes: priorities for future research.
749   Landscape Ecology.
750 Turner, M. G., W. H. Romme, E. A. H. Smithwick, D. B. Tinker, and J. Zhu. 2011. Variation in
751   aboveground cover influences soil nitrogen availability at fine spatial scales following
752   severe fire in subalpine conifer forests. Ecosystems **14**:1081-1095.
753 Turner, M. G., E. A. H. Smithwick, K. L. Metzger, D. B. Tinker, and W. H. Romme. 2007.
754   Inorganic nitrogen availability after severe stand-replacing fire in the Greater
755   Yellowstone Ecosystem. Proceedings of the National Academy of Sciences of the United
756   States of America **104**:4782-4789.
757 van der Waal, C., H. de Kroon, F. van Langevelde, W. F. de Boer, I. M. A. Heitkönig, R. Slotow,
758   Y. Pretorius, and H. H. T. Prins. 2016. Scale-dependent bi-trophic interactions in a semi-
759   arid savanna: how herbivores eliminate benefits of nutrient patchiness to plants.
760   Oecologia **181**:1173-1185.
761 Vitousek, P., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: how can it
762   occur? Biogeochemistry **13**:87-115.

763 Vitousek, P. M., and R. L. Sanford. 1986. NUTRIENT CYCLING IN MOIST TROPICAL
764   FOREST. Annual Review of Ecology and Systematics **17**:137-167.
765 Wang, L. X., P. D'Odorico, L. R. O'Halloran, K. Caylor, and S. Macko. 2010. Combined effects
766   of soil moisture and nitrogen availability variations on grass productivity in African
767   savannas. Plant and Soil **328**:95-108.
768 Watt, A. S. 1947. Pattern and process in the plant community. Journal of Ecology **35**:1-22.
769 Wijesinghe, D. K., E. A. John, and M. J. Hutchings. 2005. Does pattern of soil resource
770   heterogeneity determine plant community structure? An experimental investigation.
771   Journal of Ecology **93**:99-112.
772 With, K. A. 1997. The application of neutral landscape models in conservation biology.
773   Conservation Biology **11**:1069-1080.
774 With, K. A., and T. O. Crist. 1995. Critical Thresholds in Species Responses to Landscape
775   Structure. Ecology **76**:2446-2459.
776 Wolf, A. M., and D. B. Beegle. 1995. Recommended Soil Testing Procedures for the
777   Northeastern United States. Agricultural Experiment Station, University of Delaware,
778   Newark, DE.
779 Xu, C., H. S. He, Y. Hu, Y. Chang, X. Li, and R. Bu. 2005. Latin hypercube sampling and
780   geostatistical modeling of spatial uncertainty in a spatially explicit forest landscape
781   model simulation. Ecological Modelling **185**:255-269.
782

783
784

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**

Figure 1

Experimental design

Overview of experimental design based on Latin Hypercube sampling used to identify subplot locations to receive fertilizer in the heterogeneous plots.
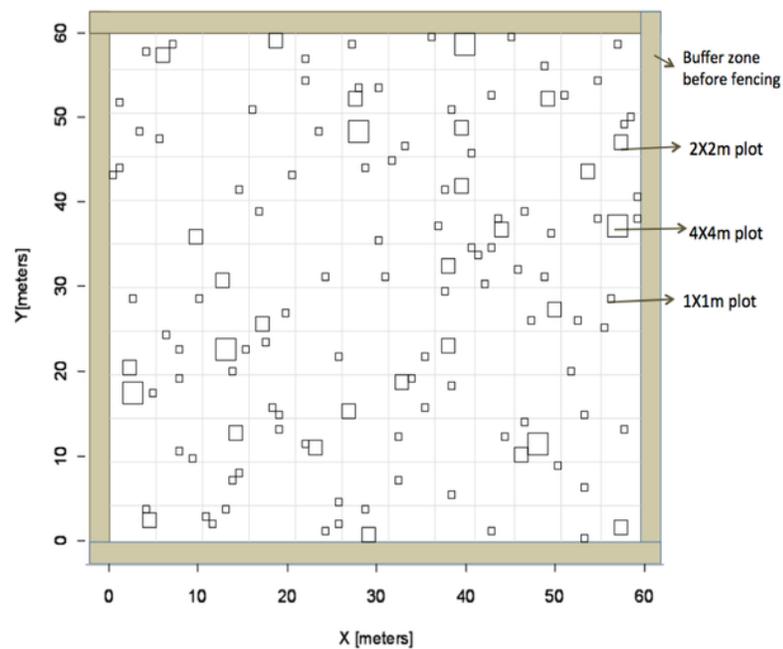
Figure 2

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.
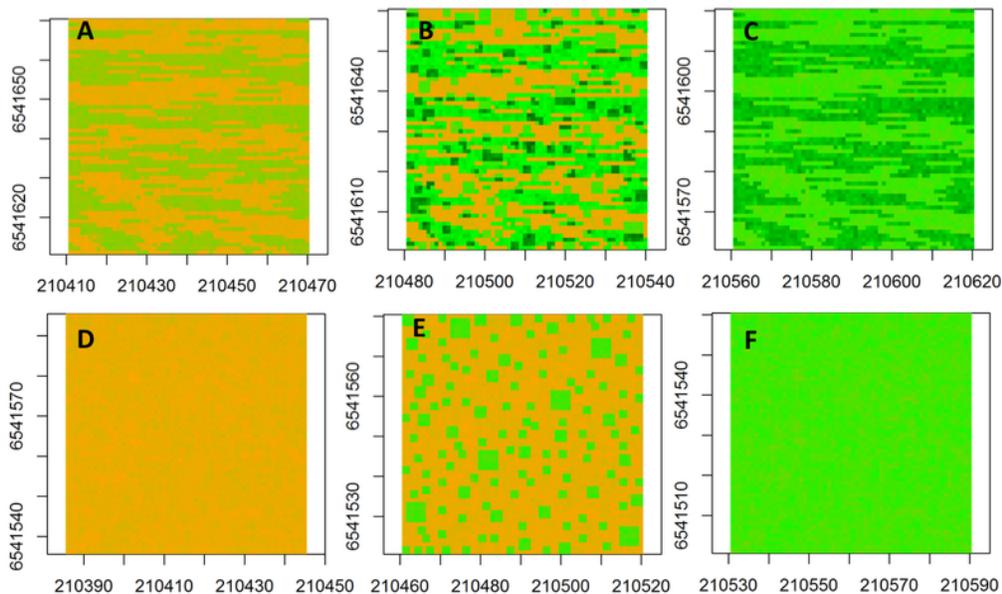
Figure 3

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.
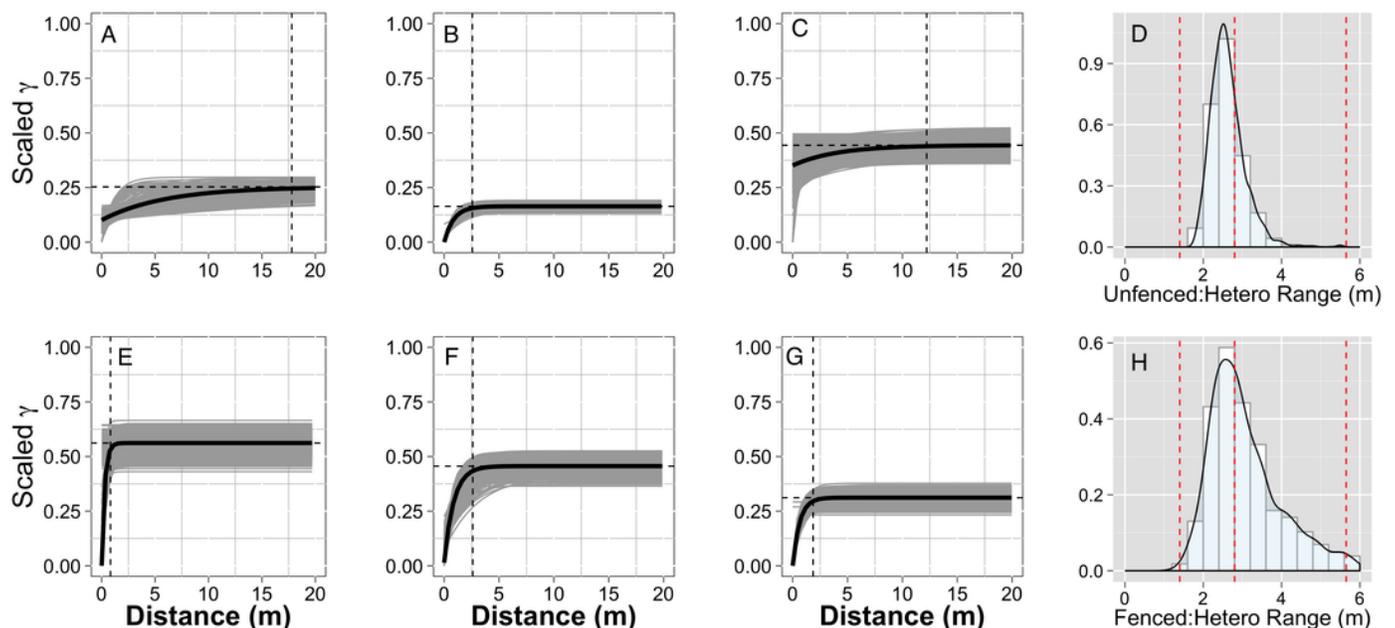
Figure 4

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.
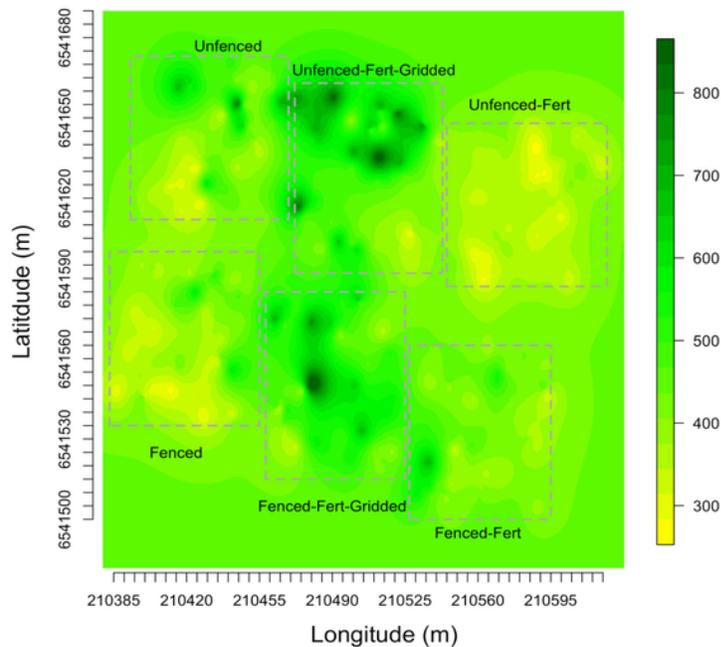
Figure 5

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.