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

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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 and herbivory 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) and to plots with and without herbivory. 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 influence. 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.

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2 **herbivory is scale-dependent**

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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 and herbivory is best

21   expressed, we imposed a large (~3.75 ha) experiment in a South African coastal grassland

22   ecosystem. In two of six 60 x 60 m grassland plots, we imposed a scaled sampling design in

23   which fertilizer was added in replicated sub-plots (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 area.

25   Three of the six plots were fenced to exclude herbivory. We calculated empirical semivariograms

26   for all plots one year following nutrient additions to determine whether the scale of grass

27   response (biomass and nutrient concentrations) corresponded to the scale of the sub-plot

28   additions and compared these results to reference plots (unfertilized or unscaled) and to plots

29   with and without herbivory. We compared empirical semivariogram parameters to parameters

30   from semivariograms derived from a set of simulated landscapes (neutral models). Empirical

31   semivariograms showed spatial structure in plots that received multi-scaled nutrient additions,

32   particularly at the 2 x 2 m grain. The level of biomass response was predicted by foliar P

33   concentration and, to a lesser extent, N, with the treatment effect of herbivory having a minimal

34   influence. Neutral models confirmed the length scale of the biomass response and indicated few

35   differences due to herbivory. Overall, we conclude that interpretation of nutrient limitation in

36   grasslands is dependent on the grain used to measure grass response and that herbivory had a

37   secondary effect.

40 **INTRODUCTION**

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

42 1991, LeBauer and Treseder 2008, Fay et al. 2015). In general, temperate systems are expected

43 to have greater levels of nitrogen (N) limitation on vegetation growth than sub-tropical or

44 tropical systems, whereas phosphorus (P) may be more limiting due to highly weathered soils

45 (Vitousek and Sanford 1986, Hedin 2004, Lambers et al. 2008, Domingues et al. 2010), although

46 co-limitation and the role of other nutrients is also acknowledged to be important (Fay et al.

47 2015). Ecological inference is dependent on the observational scale of the measurements,

48 however, and as such, our ability to infer ecosystem function from patterns in nutrient

49 availability rests on the grain and extent of the measurement (Dungan et al. 2002).   In a spatial

50 context, grain reflects the finest level of resolution (precision of measurement) whereas extent

51 refers to the size of the study area, and the choice of these dimensions offer differ among studies

52 (Turner and Gardner 2015). In the case of nutrient limitation, the optimal grain for diagnosing

53 nutrient limitation, especially in grassland ecosystems, is not known and may vary at fine scales

54 (Klaus et al. 2016). Patchiness in nutrient availability can be governed by variability in soil

55 properties or terrain, spatial variability in microbial community composition, or differential

56 nutrient affinities across functional groups that have different spatial or temporal distributions

57 (Reich et al. 2003, Ratnam et al. 2008). Perhaps as a result of this spatial heterogeneity, N, P,

58 and N+P limitations on vegetation productivity have all been documented in African savanna or

59 grassland systems (Augustine et al. 2003, Craine et al. 2008, Okin et al. 2008, Ngatia et al.

60 2015). This study asks whether new approaches that actively test (sensu McIntire and Fajardo

61 2009) the scale of grass response to nutrients and herbivory can aid understanding of nutrient

62 limitation in grassland ecosystems.

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

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

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

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

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

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

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

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

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

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

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

74   that herbivore consumption of nutrient rich patches eliminated the positive effects of fertilization

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

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

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

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

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

80   methodological approaches for its study.

81   Incorporating spatial autocorrelation into ecological studies has augmented our

82   understanding of how spatial structure of soils, plants, and climate regulates ecosystem function,

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

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

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

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

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

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

89   1995) or have used computational simulations to explore the influence of space on ecosystem

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

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

92   2005, Jean et al. 2015) to describe the grain and extent of observed ecological patterns, while

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

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

95   of autocorrelation structures.

96   Understanding these spatial structures is often elusive because ecological patterns

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

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

99   spatial scales (Mills et al. 2006). Disturbances further create structural patterns that may

100   influence ecological processes at many scales (Turner et al. 2007, Schoennagel et al. 2008).

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

102   observed patchiness in soil moisture at three unique scales (0.5 m, 1.8 m and 2.8 m) in response

103   to herbivore impacts. Following fire in the Greater Yellowstone Ecosystem (Wyoming, U.S.A.),

104   Turner et al. (2011) observed variation in soil properties at the level of individual soil cores, and

105   Smithwick et al. (2012) observed autocorrelation in post-fire soil microbial variables that ranged

106   from 1.5 to 10.5 m. Patchiness in soil resources at the level of individual shrubs and trees has

107   been demonstrated by several studies (Liski 1995, Pennanen et al. 1999, Hibbard et al. 2001,

108   Lechmere-Oertel et al. 2005, Dijkstra et al. 2006).   In savanna systems, multiple spatial scales

109   are needed to explain complex grass-tree interactions (Mills et al. 2006, Okin et al. 2008, Wang

110   et al. 2010, Pellegrini 2016) and it is likely that these factors are nested hierarchically with

111   spatial scale (Pickett et al. 2003, Rogers 2003, Pellegrini 2016).

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

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

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

115   dependence of grass biomass to nutrient additions and herbivory using a novel experimental

116   design. Our objectives were to: (1) quantify the grain size at which vegetation biomass and

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

118   level of biomass response to plant nutrient concentrations and herbivory and (3) assess the

119   degree to which herbivory and nutrient treatments explained the spatial structure of grass

120   productivity through comparison of empirical semivariograms and neutral models (simulated

121   semivariogram models based on prescribed landscape patterns).

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

123   subplots scales at which fertilizer was added (1 x 1 m, 2 x 2 m, and 4 x 4 m). These patch sizes

124   were chosen to correspond to ecosystem processes that might govern nutrient uptake, including

125   the spacing of individual plants, plant groupings, or plot-level topography, respectively, which

126   have been identified as critical sources of variation in soil biogeochemistry (Jackson and

127   Caldwell 1993a, Rietkerk et al. 2000, Ettema and Wardle 2002). We posited that, at the finest

128   sampling grain (1 x 1 m), grass biomass and nutrient concentrations would likely reflect

129   competition for nutrient resources among individuals of a given species, or between occupied

130   and unoccupied (open) locations (Remsburg and Turner 2006, Horn et al. 2015). At the

131   intermediate grain (2 x 2 m), we expected that biomass and nutrient concentrations would reflect

132   the outcome of competitive exclusion among grass clumps comprised of different species (Grime

133   1973, Schoolmaster et al. 2014, Veldhuis et al. 2016). At the largest grain (4 x 4 m), we

134   expected that abiotic processes such as variability in hydrology or soil properties would strongly

135   determine the response of grass biomass and nutrient concentrations in addition to competitive

136   processes among individuals and species (Ben Wu and Archer 2005, Mills et al. 2006). Half of

137   the plots were fenced to exclude herbivory to determine whether there were differences the scale

138   of the response due to animal activity. We used a semivariogram model developed from

139   empirical data and used model parameters to estimate the spatial structure of biomass and

140   nutrient concentrations. We expected that biomass and vegetation nutrient concentrations would

141   have range parameters from empirical semivariograms that corresponded to the hypotenuse

142   distances of the subplot scales (i.e., 1 m, 2.83 m, and 5.66 m hypotenuse distances for the 1 x 1

143   m, 2 x 2 m, and 4 x 4 m subplots, respectively). We expected that patchiness would be highest.

144   i.e., range scales would be smaller, for the unfenced, heterogeneously fertilized plot because

145   these areas would have received nutrient additions in the form of manure and urine from animal

146   activity in addition to nutrient additions (Liu et al. 2016).

147   For Objective 2, we hypothesized that biomass responses to nutrient additions at the plot

148   level would best explained by foliar N and P concentrations, given previous work indicating the

149   importance of coupled nutrient limitation to grassland productivity (Craine et al. 2008, Craine

150   and Jackson 2010, Ostertag 2010, Fay et al. 2015). We expected that herbivory would have

151   limited effects on biomass productivity relative to the influence of nutrients.

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

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

154   semivariogram models, based on computer-simulated landscapes that mimic hypothesized

155   patterns due to known ecological processes (Fajardo and McIntire 2007). This approach allowed

156   us to compare empirical patterns across a set of null models in which the patterns were known.

157   We also could avoid issues of pseudoreplication associated with the limited set of replications in

158   the field by developing a set of artificial landscapes in which we imposed known herbivory and

159   nutrient patterns. Using this approach, the null assumption is that ranges (autocorrelation

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

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

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

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

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

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

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

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

168

169   **METHODS**

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

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

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

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

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

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

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

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

178   8,100 species identified (23 % endemic), and 1,524 vascular plant genera (39 endemic) (CEPF

179   2010). Vegetation in Mkambathi is dominated by coastal sour grassveld ecosystems, which

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

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

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

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

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

185   1990). Grasslands in Mkambathi have high fire frequencies, and typically burn biennially. Soils

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

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

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

189   2015 and 1159 mm yr^{-1} between 2006 and 2015. June is typically the driest month (averaging

190   30.8 mm 1996-2015) and March is typically the wettest month (averaging 147.6 mm 1996-

191   2015). For nearby Port Edward, South Africa, where data was available, the maximum

192   temperatures is highest in February (26.7 ºC), averaging 23.7 ºC annually, while minimum

193   temperature is coolest in July (average 13.0 ºC), averaging 17.4 ºC annually. During the years of

194   this study (2010-2012), annual temperature averaged 17.4 ºC (min) to 23.7 ºC (max), well within

195   the historical average. The year 2010 was one of the driest years on record (656.6 mm yr^{-1}),

196   whereas 2011 and 2012 (1413.6 and 1766.3 mm yr^{-1} respectively) were wetter years than

197   average, although within the historical range (652.8 – 2385.9 mm yr^{-1}). All climate data were

198   obtained from the South African Weather Service.

199

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

201   60 x 60 m plots arranged in a rectangular grid (Eastern Cape Parks and Tourism Agency Permit

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

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

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

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

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

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

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

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

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

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

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

213   2005). There were a total of 126 subplots that received fertilizer in each heterogeneous plot. All

214   sampling locations were geo-referenced with a GPS (Trimble 2008 Series GeoXM; 1 m

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

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

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

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

219   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

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

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

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

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

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

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

226   among plots and subplots.

227

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

249   (Strand, South Africa) for nutrient analysis.

250

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

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

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

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

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

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

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

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

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

260   Eckert and Sims (1995).

261

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

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

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

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

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

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

268   data follows a Gaussian multivariate distribution:

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

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

271   (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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

299   were then analyzed at each plot separately.

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

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

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

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

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

305

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

322   modeling was conducted with the R package lme4.

323

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

343

344   **RESULTS**

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

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

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

348   ± 0.01 % in unfertilized plots, 0.72 ± 0.02 % in heterogeneously fertilized plots, and 0.77 ± 0.02

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

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

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

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

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

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

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

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

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

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

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

360 structure at scales corresponding to the size of the subplots in the fenced and unfenced,

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

378 nutrient addition, especially at the 2 meter scale.

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

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

381   fenced, heterogeneously fertilized plot, where herbivores were absent. However, higher or lower

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

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

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

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

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

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

388   **Table 3**).

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

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

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

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

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

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

395   subplot size as random effects.

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

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

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

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

400   length scales in unfenced plots.

401

402   **DISCUSSION**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

418   response to nutrient additions.

419

420   **Inferring the scale of grass response to nutrient additions**

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

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

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

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

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

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

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

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

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

430   2 x 2 m scale. Studies have found finer-grain spatial structure in grassland soil properties

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

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

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

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

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

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

437   in species composition or plant groupings rather than soil characteristics, suggesting a possible

438   influence of competitive exclusion, at least in fenced plots where soil nutrients were sampled.

439   Although vegetation responses were stronger at the 2 x 2 m grain, all subplots in the

440   heterogeneous plots responded to nutrient additions, as observed in the kriged maps. As a result,

441   the heterogeneous plots had greater average biomass than plots which were fertilized

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

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

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

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

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

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

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

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

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

451   lifespan, rooting structures, or uptake rate to maximize nutrient supply (Robinson 1994, Hodge

452   2004). In turn, initial advantages afforded by plants in nutrient-rich locations may result in

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

454   (Casper et al. 2000).

455

456   **Implications for understanding nutrient limitations**

457   The goal of our study was to determine the scale of grass response to nutrient additions

458   and herbivory but our results also convey some general lessons about the role of nutrient

459   limitation in grassland ecosystems. First, our study supports the notion of coupled N and P

460   limitation in grasslands (Craine and Jackson 2010), including the subtropics (Klaus et al. 2016).

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

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

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

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

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

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

467   1996, Reich and Oleksyn 2004), although there are limits to this approach (Townsend et al.

468   2007, Ostertag 2010). Using this index, our N:P ratios of vegetation in reference plots would

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

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

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

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

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

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

475   fertilized plots.

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

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

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

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

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

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

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

483   2005). In the case of heterogeneous nutrient supply, species competitive relationships across

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

485   1982, Wijesinghe et al. 2005), although other studies have found little evidence to support this

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

487   vegetation N and P concentrations that we observed. Shifts in species composition were likely

488   minimal in this study given the short-term nature of the study (one year), but patchiness in

489   biomass responses indicate size differences that could have modified competitive relationships in

490   the future (Grime 1973). Unfortunately, the site burned one year following the experiment,

491   precluding additional tests of these relationships.

492

493   **Herbivory-Nutrient interactions**

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

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

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

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

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

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

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

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

502 2001, van der Waal et al. 2016). Interestingly, our empirical semivariogram model indicates

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

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

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

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

507 for in the current study.

508

509 **Uncertainties**

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

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

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

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

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

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

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

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

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

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

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

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

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

523

524   **CONCLUSIONS**

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

540   and deepen insights into ecological pattern-process interactions.

541

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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.

2

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.
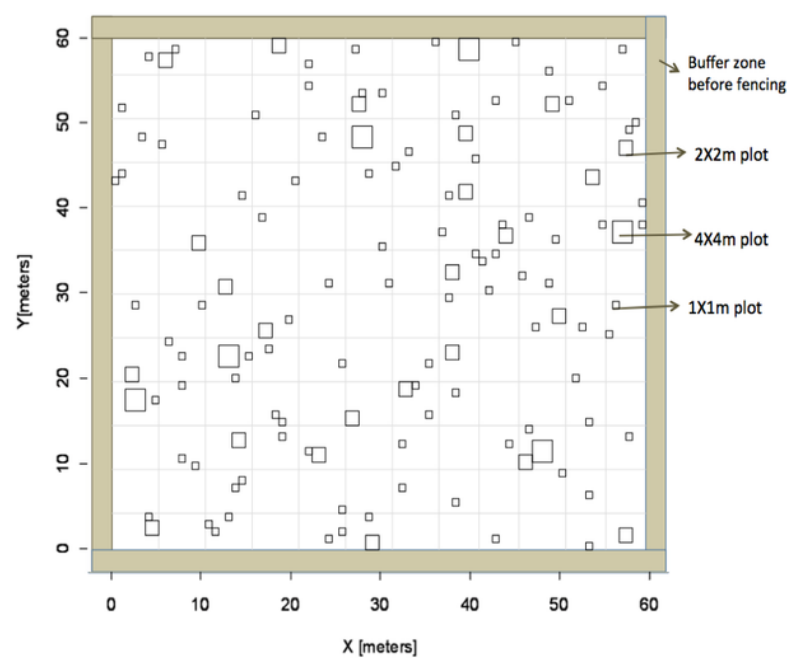
Figure 2

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.
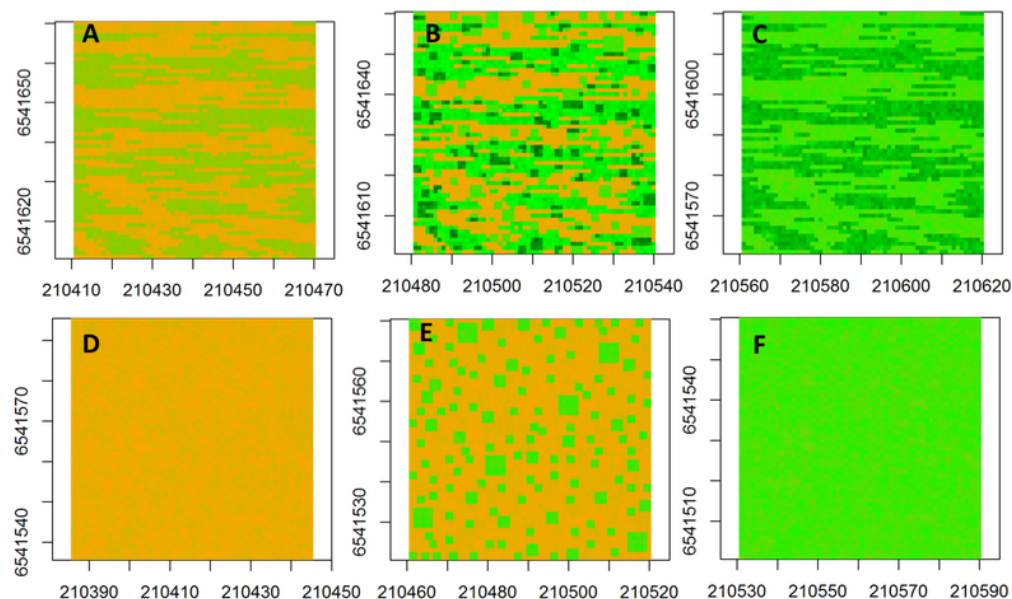
Figure 3

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.
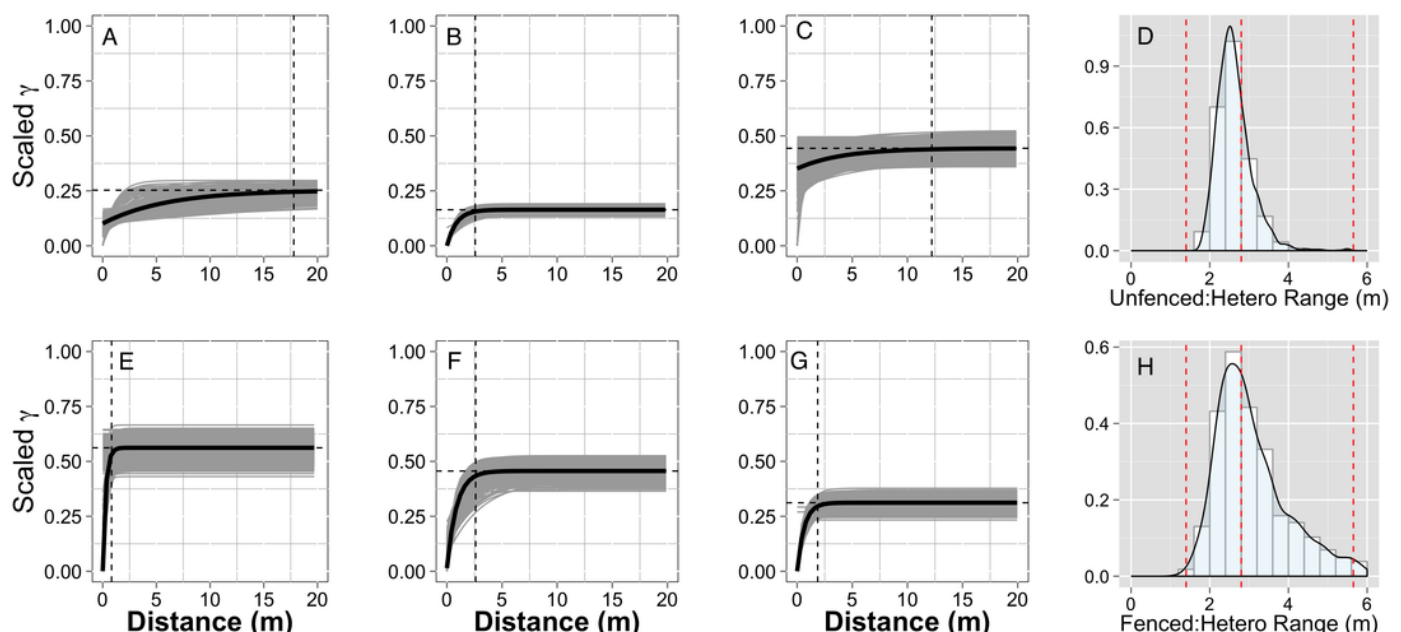
Figure 4

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.
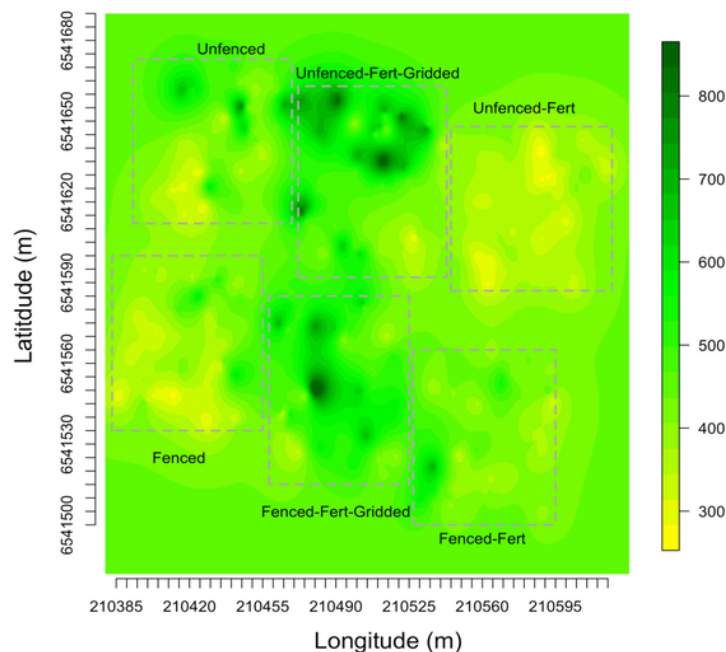
Figure 5

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.