



## Species Loss Due to Nutrient Addition Increases with Spatial Scale in Global Grasslands

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# **Species Loss Due to Nutrient Addition Increases with Spatial Scale in Global Grasslands**

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**Abstract** Effects of human-induced changes to nutrient supplies and herbivore density on species diversity vary with spatial scale, because coexistence mechanisms are scale dependent. This scale dependence may alter the shape of the species-area relationship (SAR), which can be described by changes in species richness (S) as a power function of the sample area (A):  $S=cA^z$ , where c and z are constants. We analyzed the effects of experimental manipulations of nutrient supply and herbivore density on species richness across a range of scales (0.01 – 75 m<sup>2</sup>) at 30 grasslands in 10 countries. We found that nutrient addition reduced the number of species that could coexist locally, indicated by the SAR intercepts (log c), but did not affect the SAR slopes (z). As a result, proportional species loss due to nutrient enrichment was largely unchanged across sampling scales, while total species loss increased over threefold across our range of sampling scales.



## Introduction

What determines the number of species at a location? This question lies at the core of community ecology. The answer is inherently scale dependent (Arrhenius 1921; Godwin 1923; Gleason 1926; MacArthur & Wilson 1967; Grace *et al.* 2011; Chase *et al.* 2018), because different mechanisms influence diversity at different spatial scales (Chesson 2000; Leibold *et al.* 2004; Hart *et al.* 2017; Leibold & Chase 2017; Thompson *et al.* 2020). For example, non-spatial coexistence mechanisms that depend on tradeoffs (e.g., in resource use efficiency or susceptibility to consumers) or temporal variability (e.g., temporal storage effects) can lead to coexistence at very small spatial scales (Hutchinson 1961; Tilman 1982; Holt *et al.* 1994; Chesson 2000). In contrast, coexistence mechanisms that depend on spatial variability, such as dispersal limitation or competition-colonization tradeoffs, influence diversity at larger spatial scales (Godwin 1923; Gleason 1926; MacArthur & Wilson 1967; Hastings 1980; Chesson 2000; Leibold *et al.* 2004; Vellend 2010). Furthermore, the size of individuals and the spatial heterogeneity of the environment will determine the scales at which species interact and the minimum possible scale of coexistence (Goldberg & Miller 1990; Oksanen 1996; Seabloom *et al.* 2005). For these reasons, differences in diversity observed in field studies, across space or in response to environmental changes induced by ecological and anthropogenic drivers, will reflect both sampling scale and the scale-dependence of coexistence mechanisms. Nevertheless, most empirical studies sample and compare diversity at a single scale, leading to an incomplete understanding of diversity responses to ecological drivers (Chalcraft *et al.* 2008; Lan *et al.* 2015; Chase *et al.* 2018).

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110 A variety of approaches have been used to incorporate scale explicitly into measures of  
111 diversity, the most canonical of which is the species-area relationship (SAR)(Arrhenius 1921;  
112 MacArthur & Wilson 1967; Flather 1996; Chase *et al.* 2018). While a wide variety of SAR models  
113 exist (Flather 1996; Dengler *et al.* 2020), a simple model which has been found to be applicable  
114 in many systems is

115 
$$S = cA^z \tag{1a}$$

116 or equivalently

117 
$$\log(S) = \log(c) + z\log(A) \tag{1b}$$

118 where S is the number of species and A is the area sampled (Arrhenius 1921; Flather 1996;  
119 Fridley *et al.* 2005; Drakare *et al.* 2006; Dengler *et al.* 2020).  
120 In this formulation, the y-intercept, log (c), reflects a measure of local diversity (proportional to  
121  $\alpha$  diversity when A=1), and the slope (z) is a measure of spatial heterogeneity in community  
122 composition (proportional to some measures of  $\beta$  diversity)(Crist & Veech 2006; Grace *et al.*  
123 2011; Scheiner *et al.* 2011). While the SAR only attains a true asymptote at a global scale  
124 (Williamson et al. 2001), saturation within sampling confines can be taken as a measure of the  
125 available species pool ( $\gamma$  diversity)(Grace *et al.* 2011; Chao *et al.* 2014). The x-intercept indicates  
126 the Minimal Insular Area ( $A_{min}$ ) (sensu, Heatwole 1975), the area at which only a single species  
127 is found (S=1 or log(S)=0) and is a nonlinear function of c and z:

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$$A_{min} = c^{\frac{-1}{z}} \tag{2}$$

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3 129  $A_{min}$  can be thought of as the minimal area of coexistence, the area above which more than one  
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6 130 species can co-occur (Heatwole 1975; Connor & McCoy 1979; Seabloom *et al.* 2005).  
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9 131 Understanding the patterns and determinants of diversity across scales has gained increased  
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11 132 relevance as human domination of the biosphere has altered many of the controls on species  
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14 133 diversity, leading to scale-dependent changes in diversity (Chase *et al.* 2019). For example,  
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16 134 humans have increased the supply of biologically-limiting nutrients and have changed the  
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19 135 density of herbivores in many ecosystems (Foley *et al.* 2005; Ripple *et al.* 2015; Steffen *et al.*  
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21 136 2015), both of which can alter plant diversity at a range of spatial scales (Chaneton & Facelli  
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24 137 1991; Olff & Ritchie 1998; Crawley *et al.* 2005; Bakker *et al.* 2006; Hillebrand *et al.* 2007;  
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26 138 Chalcraft *et al.* 2008; Leps 2014; Lan *et al.* 2015).  
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29 139 The effects of environmental change on diversity may shift with spatial scale, and this scale-  
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31 140 dependence may be reflected in diversity-scaling relationships such as the parameters of the  
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34 141 SAR (Chaneton & Facelli 1991; Olff & Ritchie 1998; Bakker *et al.* 2006; Chalcraft *et al.* 2008; Lan  
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36 142 *et al.* 2015; Chase *et al.* 2018). For example, in grassland ecosystems, increasing the supply  
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39 143 rates of biologically limiting nutrients like nitrogen (N) and phosphorus (P) often leads to  
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42 144 reduced plant diversity at local scales leading to lower  $\log c$  and higher  $A_{min}$  (Figure 1) by  
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44 145 reducing the opportunity for coexistence through tradeoffs in soil resource use efficiency and  
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47 146 increasing competition for light (Goldberg & Miller 1990; Dybzinski & Tilman 2007; Harpole &  
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49 147 Tilman 2007; Hautier *et al.* 2009; Borer *et al.* 2014b; Harpole *et al.* 2016; Midolo *et al.* 2019).  
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52 148 While the effects of nutrient addition and herbivory on local coexistence in grasslands have  
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54 149 been examined experimentally, it remains unclear how these effects will change with spatial  
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3 150 scale. Importantly, if environmental changes alter the shape of the SAR, diversity change  
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6 151 measured at a single scale may overestimate or underestimate diversity change at larger spatial  
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8 152 scales (Figure 1)(Lan *et al.* 2015).  
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11 153 Changes in the scaling relationship will depend on the specific coexistence mechanisms (e.g.,  
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13 154 spatial versus non-spatial mechanisms) affected by nutrient enrichment or herbivore exclusion.  
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16 155 Furthermore, different mechanisms will be associated with the total biomass, light availability,  
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18 156 and size of the species pool (Bakker *et al.* 2006; Chalcraft *et al.* 2008; Borer *et al.* 2014b; Lan *et*  
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20 157 *al.* 2015; Harpole *et al.* 2016). For example, nutrient enrichment or herbivore exclusion may  
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22 158 increase biomass, which can lead to thinning due to light competition and in turn reduce the  
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24 159 SAR slope and intercept, because there are fewer larger individuals in each sample (Lan *et al.*  
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26 160 2015). These treatments also may increase the dominance of a few species, which would  
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28 161 reduce evenness and decrease the SAR intercept but increase its slope (Lan *et al.* 2015)(Figure  
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30 162 1E). Treatments also may cause the extinction of specific species, leading to a smaller species  
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32 163 pool and lower SAR slope (Figure 1A) (Lan *et al.* 2015).  
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39 164 We have more specific expectations for cross-scale effects of nutrient addition than for  
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41 165 herbivore exclusion, because nutrient addition has more consistent effects on grassland plant  
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43 166 diversity across scales (Chalcraft *et al.* 2008; Borer *et al.* 2014b; Lan *et al.* 2015). For example, if  
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45 167 nutrient-induced light limitation reduces coexistence opportunities for a consistent set of  
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47 168 species through non-spatial processes such as reduced niche dimensionality (Harpole & Tilman  
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49 169 2007; Harpole *et al.* 2016), then the local diversity loss ( $\log c \downarrow$ ) will be accompanied by a  
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51 170 reduced total species pool ( $\gamma$  diversity  $\downarrow$ ) (Harpole & Tilman 2007; Chalcraft *et al.* 2008; Lan *et*  
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3 171 *al.* 2015)(Figure 1C & D). These effects also would be associated with increased biomass and  
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6 172 reduced light availability. In this case, the effects on the SAR slope depend on the relative rate  
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8 173 of species loss across scales (Lan *et al.* 2015). If there is a constant proportional loss of species  
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11 174 with increasing area, then the slope of the SAR would remain constant (Figure 1C & D). In  
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13 175 contrast, the slope would decline if there is greater proportional loss at larger spatial scales  
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15 176 ( $z \downarrow$ )(Figure 1A & B), resulting in a more spatially homogeneous community. In a less extreme  
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18 177 case, nutrient addition could increase the dominance of species that favor high nutrient  
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20 178 conditions without causing species extinctions. In this case, nutrient addition would increase  
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23 179 the SAR slope ( $z \uparrow$ ) due to local diversity loss ( $\log c \downarrow$ ), but the total species pool would remain  
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25 180 unchanged (Figure 1A & B). This effect also would be reflected in reduced species evenness (Lan  
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28 181 *et al.* 2015).

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31 182 Nutrient addition also could influence spatial coexistence mechanisms, such as competition-  
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34 183 colonization tradeoffs or mass effects by reducing dispersal and local recruitment (Hastings  
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36 184 1980; Tilman *et al.* 1994; Leibold *et al.* 2004; Vellend 2010). Reduced dispersal and colonization  
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39 185 would lower local diversity ( $\log c \downarrow$ ) but leave the total species pool unchanged, resulting in an  
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41 186 increased SAR slope ( $z \uparrow$ ) in communities with low to moderate dispersal (Mouquet & Loreau  
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44 187 2003; Lan *et al.* 2015)(Figure 1A & B), although very high rates of dispersal may reduce both  
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46 188 local richness and the total species pool (Mouquet & Loreau 2003). Finally, nutrient addition  
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49 189 could increase individual plant size (Goldberg 1987; Oksanen 1996), thereby reducing local  
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51 190 diversity ( $\log c \downarrow$ ). As with reduced dispersal, increased plant size would not affect the total  
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54 191 species pool, but would increase the SAR slope ( $z \uparrow$ ) (Lan *et al.* 2015) (Figure 1E & F).

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192 In any of these cases, a constant SAR slope indicates constant proportional change in species  
193 with increasing spatial scale, whereas a change in the SAR slope indicates an increasing or  
194 decreasing proportional change in the numbers of species with increasing spatial scale (Lan *et al.* 2015). Importantly, if there is a constant proportional loss (or gain) of species, there will be  
196 an increase in the total number of species lost (or gained) at larger spatial scales (Lan *et al.* 2015) (Figure 1C & D).

198 In summary, as we expect nutrient addition to decrease local diversity in grasslands ( $\log c \downarrow$ )  
199 (Borer *et al.* 2014b; Midolo *et al.* 2019), the SAR slope will either increase ( $z \uparrow$ ) or decrease ( $z \downarrow$ )  
200 depending on the change in diversity at larger scales (Chalcraft *et al.* 2008; Lan *et al.* 2015)(Figure 1). While there are few specific predictions for the minimal area of coexistence  
202 ( $A_{min}$ ), we expect this to be negatively correlated with local diversity ( $\log c$ ) via the relationship  
203 in Equation 2, such that the expected nutrient-induced reduction in  $c$  should lead to an increase  
204 in  $A_{min}$  depending on the change in  $z$ .

205 While the predictions for herbivore effects on diversity scaling are less well developed, the  
206 effects should be mediated through changes in the same core processes governing nutrient  
207 effects. For example, herbivores may reduce diversity if the community becomes dominated by  
208 a few unpalatable species that are resistant to grazing ( $\log c \downarrow$ ), or they may increase diversity if  
209 they reduce the abundance of dominant, competitive, or fast-growing species ( $\log c \uparrow$ ) (Olff &  
210 Ritchie 1998; Viola *et al.* 2010; Lind *et al.* 2013; Koerner *et al.* 2018). Herbivores also may  
211 increase diversity by increasing availability of a limiting resource (e.g., light) or increasing seed  
212 dispersal and colonization rates (Olff & Ritchie 1998; Borer *et al.* 2014b). The presence of

herbivores also may change environmental variation, for example through localized deposition of feces or urine (Olff & Ritchie 1998), which may increase the SAR slope ( $z\uparrow$ ). As is the case with nutrient effects, we expect herbivore effects on plant diversity to be related to evenness, light availability, and plant biomass, such that herbivores will likely have positive effects on diversity at light-limited, productive sites that are dominated by a few plant species (Bakker *et al.* 2006; Borer *et al.* 2014b; Koerner *et al.* 2018).

Here we analyze species richness data from 30 grasslands or low-stature shrublands (hereafter grasslands) spanning spatial scales of three orders of magnitude (0.01 – 75 m<sup>2</sup>; Figure S1) in the context of the Nutrient Network Distributed Experiment (NutNet, [www.nutnet.org](http://www.nutnet.org)), a globally-replicated experiment manipulating nutrient supply and herbivore density (Borer *et al.* 2014a; Borer *et al.* 2017). The sites for this study are located in 10 countries on five continents and represent a wide range of environmental conditions and ecosystem types including annual grasslands, deserts, tundra, montane meadows, semi-arid and mesic grasslands, and old fields. We use these data to address the long-standing gap in our understanding of how environmental drivers affect diversity across spatial scales (Chalcraft *et al.* 2008; Chase *et al.* 2018). Specifically, we quantify variability among sites in the slope and intercept of the species-area relationship (SAR) and test the effects of nutrient addition and herbivore exclusion on the SAR. In addition, we test whether among-site differences in the SAR are correlated with evenness, light availability, plant biomass, the size of the total species pool, and whether these covariates affect the strength of the nutrient or consumer reduction treatments (Chalcraft *et al.* 2008; Lan *et al.* 2015).

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**Materials and Methods**

*Experimental Design and Data Collection*

We include data from 30 sites in 10 countries which are part of the Nutrient Network (NutNet) distributed experiment (Borer *et al.* 2014a; Borer *et al.* 2017) (Table S1). Sites were dominated by herbaceous or low-statured vegetation and spanned wide gradients in elevation (6 to 3500 m), latitude (52° S to 69° N), mean annual precipitation (249 to 1877 mm yr<sup>-1</sup>), mean annual temperature (-3 to 23 °C), and mean aboveground live biomass (34 to 900 g m<sup>-2</sup>). Local richness (4 to 43 species m<sup>-2</sup>) and total site richness (18 to 142 species site<sup>-1</sup>) were highly variable among sites. We used data from control plots at 30 sites and from two experiments at subsets of the sites: the Multiple-nutrient experiment (21 sites) and the Consumer-nutrient experiment (16 sites). Experimental duration at the time of sampling varied from 3-11 years (Table S1); inclusion of duration in statistical models did not qualitatively change results.

**Multiple-nutrient Experiment:** This experiment factorially combined three nutrient-addition treatments each at two levels (Control or Fertilized): Nitrogen addition (10 g N m<sup>-2</sup> yr<sup>-1</sup> as timed-release urea), Phosphorus addition (10 g P m<sup>-2</sup> yr<sup>-1</sup> as triple-super phosphate), and Potassium and Micronutrient addition (10 g K m<sup>-2</sup> yr<sup>-1</sup> as potassium sulfate and 100 g m<sup>-2</sup> yr<sup>-1</sup> of a micronutrient mix (6% Ca, 3% Mg, 12% S, 0.1% B, 1% Cu, 17% Fe, 2.5% Mn, 0.05% Mo, and 1% Zn). N, P, and K were applied annually, and the micronutrient mix was applied once at the start of the study.



**Consumer-nutrient Experiment:** This experiment factorially combines nutrient addition (Control or Fertilized) and vertebrate consumer presence (Control or Fenced). To do this, we combine the unfenced control and unfenced plots with all nutrients added from the Multiple Nutrient Experiment with two additional treatments using herbivore fencing: Fenced with no nutrients added and Fenced with all nutrients added. Fences were 2.1 m tall and excluded aboveground, non-climbing, vertebrate herbivores. The lower 0.9 m was composed of 1 cm woven wire mesh with a 0.3 m outward-facing flange stapled to the ground to exclude digging animals. The top 1.2 m was composed of five rows of wire. Minor variations in fence design are described by Borer et al (2014a).

**Data Collection:** We estimated SARs using data collected at five spatial scales: 0.01, 1, 6.25, 25, and 75 m<sup>2</sup> (sampling scheme illustrated in Figure S1). We recorded the presence of all species in each 5 x 5 m plot (25 m<sup>2</sup>), a 2.5 x 2.5 m subplot nested within each 5 x 5 m plot (6.25 m<sup>2</sup>), a 1 x 1 m subplot nested within each 2.5 x 2.5 m plot (1 m<sup>2</sup>), and four 0.1 x 0.1 m subplots placed at the corners of the 1 m<sup>2</sup> subplot (4 by 0.01 m<sup>2</sup>). We aggregated species lists across the three replicate 5 x 5 m plots to estimate species richness at the 75 m<sup>2</sup> scale. We also sampled plant species abundances in the 1 m x 1 m subplot by visually estimating the areal cover of each species, allowing us to calculate Simpson's evenness at the 1 m<sup>2</sup> scale (Smith & Wilson 1996).

We used the mean richness of the four 0.01 m<sup>2</sup> subplots in our analyses, and we excluded a small number of 0.01 m<sup>2</sup> subplots with a mean species richness of zero as log richness was undefined (0.1% of samples). We note here that the 75 m<sup>2</sup> richness estimate is based on non-nested data and includes among block variability, as it is composed of three spatially separate

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3 274 25 m<sup>2</sup> plots. Nested and non-nested SAR's typically have similar slopes in non-forested  
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6 275 terrestrial habitats (Drakare *et al.* 2006). Furthermore, when we only used the fully nested  
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8 276 samples with a maximum area of 25 m<sup>2</sup>, our results were qualitatively similar. One site did not  
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10 277 collect species richness data at the 75 m<sup>2</sup> scale (chilcas.ar) and had a maximum area of 18.75 m<sup>2</sup>  
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12 278 (area of three 2.5 m<sup>2</sup> plots). Inclusion or exclusion of this site did not qualitatively change  
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15 279 results. We calculated site richness (i.e., site species pool) as the total number of species found  
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18 280 across all sampled plots and years at the site (Table S1).  
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21 281 We sampled aboveground plant biomass by clipping all aboveground biomass (live and dead) in  
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24 282 two 0.1 m x 1 m strips, sorting current year's biomass (live biomass) from previous year's  
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26 283 biomass (dead biomass), drying the biomass to a constant mass at 60 °C, and weighing it to the  
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29 284 nearest 0.01 g. Within each 1 m<sup>2</sup> quadrat, we measured the proportion of photosynthetically  
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31 285 active radiation (PAR) reaching the ground level and above the canopy.  
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34 286 *Statistical Analyses*

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38 287 All analyses were conducted using R version 4.0.2 (R Development Core Team 2010). We fit a  
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40 288 SAR for each treatment at each site using Equation 1b (Figures S2 – S5) with the *lmList* function  
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42 289 in the *lmer4* R library (version 1.1-23), which fits linear models to subgroups of data (e.g.,  
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44 290 different sites). We used the site-level estimates of *z*, *c*, and *A<sub>min</sub>* in subsequent analyses. *A<sub>min</sub>*  
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46 291 was natural log transformed due to a highly skewed distribution. We tested whether among  
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48 292 site differences in the SAR parameters were correlated with evenness, light availability, plant  
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50 293 biomass, the size of the total species pool. While we focus on the SAR model in Equation 1,  
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52 294 there are a wide range of potential models for SARs (e.g., Flather 1996). We found that a  
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295 general three parameter model (Equation S1) did not provide a better fit to the data than the  
296 two parameter model (Appendix S1) (Flather 1996).

297 For the Multiple-Nutrient or Consumer Nutrient experiments, we tested whether experimental  
298 treatments altered the SAR parameters with Mixed Effects Models (MEMs) using the lmer  
299 function in the lme4 R library with p-values generated using Satterthwaite's degrees of freedom  
300 method using the lmerTest R library (version 3.1-2). We included site as a random effect in  
301 these models, and model specifications are included in Tables S2-S4. Inclusion of experimental  
302 duration as a random effect in these models did not change any results and occasionally  
303 prevented models from fitting due to singularities. We tested for effects of differential errors  
304 associated with the estimates of  $c$  and  $z$  at each site using weighted regression in which weights  
305 were the inverse of the standard error of site-level parameter estimates. Weighted regressions  
306 were nearly identical to unweighted regressions and did not change interpretation of any  
307 results presented here. Here we present results of unweighted regressions for simplicity.

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309 In testing for interactions between treatment effects and covariates (evenness, light availability,  
310 plant biomass, the size of the total species pool), we used site level treatment mean of  
311 evenness, light availability, and plant biomass, while the total species pool has only a single  
312 measurement per site. Evenness ( $1 \text{ m}^2$ ), light ( $1 \text{ m}^2$ ), and plant biomass ( $0.2 \text{ m}^2$ ) were measured  
313 at different scales, and the total species pool is the summed number of species across 30  $1 \text{ m}^2$   
314 plots. We used a multi-model approach to model selection using the dredge and model.avg  
315 functions in the MuMIn library (version 1.43.17)(Grueber *et al.* 2011). We standardized the

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316 input variables using the arm library (version 1.11-1) and included all models within 4 AIC<sub>c</sub> units  
317 of the best model.

318 **Results**

319 Across all sites, there was more variability in the SAR intercept (c) than in the SAR slope (z)  
320 (Figure S2). The coefficient of variation (CV = standard deviation / mean) for z (0.19) was about  
321 3 times lower than for c (0.58). Across all sites (N=30) under ambient conditions (Control Plots),  
322 SAR slopes ranged from 0.12 to 0.33 (mean=0.23 log(species)/log(m<sup>2</sup>)) and the intercept ranged  
323 from 2.6 to 33.0 species m<sup>-2</sup> (mean=10.7). The x-intercept (*A<sub>min</sub>*) had a mean of 1.2 \* 10<sup>-3</sup> m<sup>2</sup> (12  
324 cm<sup>2</sup>) and was highly variable ranging from near 0 to 0.02 m<sup>2</sup> (200 cm<sup>2</sup>) with a CV of 2.5. We  
325 used natural log transformed values of *A<sub>min</sub>* in our analyses, which ranged from -14.5 to -4.6  
326 log(m<sup>2</sup>) (Figures 2 & 3).

327 In the control plots, the SAR slope (z) and (log c) were uncorrelated (r=0.11, p=0.548), slope (z)  
328 and x-intercept (log(*A<sub>min</sub>*)) were positively correlated (r=0.51, p=0.004), and (log c) and x-  
329 intercept (log(*A<sub>min</sub>*)) were negatively correlated (r=-0.73, p<0.001). As would be expected, the  
330 SAR intercept was highly correlated with species richness at 1 m<sup>2</sup> when log(A)=0 (r=0.97,  
331 p<0.001).

332 Among site variability in the SAR slope increased with site richness but was unrelated to any  
333 other of our covariates in the observational data (evenness, live biomass, or proportion of light  
334 at ground level) (Table S2; Figure 4). As predicted, local richness (log c) increased with light  
335 availability and total site richness (Table S2; Figure 4), and the minimal area of coexistence

336 ( $A_{\min}$ ) declined with light availability (Table S2; Figure 4). There were no significant correlations  
337 among the site means of the covariates ( $p > 0.05$ ).

338 Nutrient addition reduced local diversity ( $\log c$ ) and increased the minimal coexistence area  
339 ( $A_{\min}$ ) but did not affect the slope of the SAR (Figures 2 & 3; Tables S3 & S4). The lack of a  
340 treatment effect on the SAR slope may reflect either a lack of change in the slope or high  
341 variability in the estimates. As noted above, slopes did not vary widely among sites, and  
342 variance among slope estimates among sites was not large within treatments (Figures 4 & 5).  
343 For example, in our analyses of the experimental data, the standard error in the slope estimates  
344 and treatment effects were close to 0.01 (Tables S3 & S4), suggesting that we could detect  
345 small differences in slopes among sites and treatments.

346 Because slopes remained constant and species loss was proportional across scales, addition of  
347 all nutrients in combination caused more absolute species loss at the largest scale (mean of 3.2  
348 species lost at 75 m<sup>2</sup>) than at the smallest scale (mean of 0.9 species lost at 0.01 m<sup>2</sup>). The  
349 nutrient effect on species loss was driven by the effects of N addition (Figure 3; Table S4).  
350 Fencing did not have a consistent effect on any of the SAR parameters (Figure 2; Table S3).

351 There were significant interactions between experimental treatments and the environmental  
352 covariates. For example, the interaction between site richness and the effects of nutrient  
353 enrichment on local richness ( $\log c$ ), was such that sites with more species had higher rates of  
354 species loss in the experimental plots (Figure 4, Table S5). Fencing effects on local richness were  
355 strongly affected by light transmission, with higher species loss at sites in which fencing  
356 reduced light availability (Figure 4, Table S5).

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Live biomass and light transmission were affected by experiment treatments. Nutrient addition, primarily N & P addition, reduced light transmission and increased live biomass (Figure 4; Tables S6 & S7). Evenness was unaffected by the experimental treatments (Figure 4; Tables S6 & S7). The covariate, site richness is measured at the site scale, so does not vary among plot or treatments within a site.

**Discussion**

We found that experimental addition of nutrients, and nitrogen in particular, reduced the SAR intercept ( $\log c$ ), but did not have a consistent effect on the SAR slopes ( $z$ ) across sites. As a result, proportional species loss was unchanged across spatial scales, while total species loss increased over 3.6-fold with spatial scale within individual sites (Figure 1C & D, 3, & 4). Furthermore, we found that nutrient-induced loss of species was highest at sites with larger species pools (i.e., site-level species richness)(Harpole *et al.* 2016), and that the effects of fencing were mediated by light availability (Borer *et al.* 2014b)(Figure 5; Table S5). While our maximum sample area was rather small relative to other studies, the SAR slopes in our data (mean=0.23) were similar to those spanning much larger spatial scales in other terrestrial, non-forested habitats (Drakare *et al.* 2006; Dengler *et al.* 2020).

Our study allowed us to examine small-scale patterns as reflected in the intercept of the SAR ( $\log c$ ) and the minimal area of coexistence ( $A_{min}$ ). At this scale, our results showed wide variation in local diversity (as estimated by  $\log c$ ) and the minimal area of coexistence ( $A_{min}$ ) among sites. This among-site variation was related to light availability and total site richness. Across all sites, nutrient addition reduced local diversity ( $\log c \downarrow$ ) (see also Borer *et al.* 2014b;

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3 378 Harpole *et al.* 2016; Midolo *et al.* 2019) and increased the minimal area of coexistence ( $A_{min}$ ),  
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6 379 which has not been previously reported. Nutrient induced changes in local richness were  
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8 380 strongest at sites with a larger number of species, as shown by Harpole *et al.* (2016) using many  
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11 381 of these same sites. Finally, we found no consistent relationship in the influence of herbivore  
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13 382 exclusion on local diversity measures, which in itself is consistent with other studies that find  
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15 383 that herbivore effects on diversity depend on site context (Proulx & Mazumder 1998; Borer *et*  
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17 384 *al.* 2014b; Koerner *et al.* 2018). In our case, the effects of herbivores depended on light  
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20 385 transmission, supporting the evidence for light as an important mechanism underpinning  
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23 386 herbivore effects on richness (Borer *et al.* 2014b).

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26 387 Contrary to the local-scale patterns, we found much less variation in the scaling relationships,  
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28 388 measured by SAR slopes ( $z$ ). While local diversity varied widely in unmanipulated control plots,  
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31 389 SAR slopes were similar across sites. It is important to note that a wide array of processes  
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34 390 govern SARs, and the consistent slopes do not necessarily indicate that the same processes  
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36 391 govern diversity across these sites. We found mixed results in our tests of local environmental  
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38 392 conditions predicted by theory to mediate SAR slopes. While we did not find the expected  
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41 393 negative correlation between SAR slope and evenness in our larger observational data set (30  
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43 394 sites), we found this in the subset of 16 sites at which we conducted the consumer-nutrient  
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46 395 experiment. We did find some evidence for increased SAR slope at sites with large numbers of  
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48 396 species in the observational data set. These mixed results mirror the literature. Some studies  
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51 397 have found relationships between environmental parameters (e.g., productivity) and slopes of  
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53 398 the SAR (e.g., Chiarucci *et al.* 2006; Moradi *et al.* 2020), while other multi-site studies failed to  
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56 399 find strong relationships between SAR parameters and environmental characteristics (e.g.,  
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DeMalach *et al.* 2019; Dengler *et al.* 2020). Furthermore, relationships between diversity and environmental factors may vary in complex ways across scales and among different diversity metrics (Chalcraft *et al.* 2004; Chalcraft *et al.* 2008; Chalcraft *et al.* 2009). In summary, while slopes varied among sites, these scaling relationships were less variable than local diversity and minimal area of coexistence.

Changes in the scaling relationship (i.e., the slope of the SAR) due to external factors, such as nutrient addition or herbivory, also can provide important insights into the nature by which biodiversity responds to experimental treatments (reviewed in Chase *et al.* 2018). Across our study sites, nutrient addition did not systematically change the SAR slope ( $z$ ), suggesting a constant proportional loss of species, and as a result, total species loss increased with area (Figure 1C & D). For example, addition of all nutrients in combination caused a mean loss of 0.9 species at the smallest scale (0.01 m<sup>2</sup>) and 3.2 species at the largest scale (75 m<sup>2</sup>). This is consistent with results from other studies that have manipulated nutrients (Lan *et al.* 2015), but others have shown either increases or decreases in  $z$  (and in some cases,  $\beta$  diversity, which is related to  $z$ ) with nutrient addition (Chalcraft *et al.* 2008; Sandel & Corbin 2012; Leps 2014; Lan *et al.* 2015; Zhou *et al.* 2018). Likewise, we found no effect of herbivore exclusion on the scaling of diversity with area ( $z$ ), whereas other studies have found positive, negative or neutral effects (Bakker *et al.* 2006; de Bello *et al.* 2007; Fernández-Lugo *et al.* 2011; Li *et al.* 2015; Godo *et al.* 2017). Taken in total, the variable results from other studies and the lack of a consistent directional shift in the SAR slope in our analysis indicates that nutrients and herbivore effects on diversity scaling are highly variable and context dependent.



Changes in the SAR slope (or lack thereof) can be influenced by at least three different features in a regional community (He and Legendre 2002, McGill 2011, Chase and Knight 2013, Chase *et al.* 2018): (1) the density or abundance of individuals (including their size), (2) the relative abundance (evenness) of species in the community, (3) the spatial clustering of species in the region (i.e., intraspecific aggregation). Furthermore, each of these can be altered by a number of environmental features, as well as experimental manipulations (e.g., nutrients and herbivory), but not necessarily with the same effect on  $z$ . For example, nutrient addition might simultaneously increase the size of individual plants, which may lead to higher  $z$  by reducing local diversity (Lan *et al.* 2015), while concurrently reducing the size of species pool resulting in a lower  $z$  (Lan *et al.* 2015); with the net result being no effect. Likewise, grasslands differ considerably in their ambient conditions of the three components that may influence  $z$  (DeMalach *et al.* 2019), and thus experimentally changing the biotic or abiotic environment could, for example, lead to higher or lower spatial clustering depending on where they started, which may obscure a general influence of experimental treatments on  $z$ . We did not collect the spatially explicit abundance data needed to fully resolve the importance of these processes (Powell *et al.* 2013; McGlinn *et al.* 2019); making these measurements at a large number of locations would provide greater mechanistic insight into the processes we describe here.

The lack of a consistent change in the diversity-scaling relationships (i.e., the slope of the SAR) to nutrient addition and herbivore exclusion could arise if these treatments primarily reduce non-spatial (local) rather than spatial coexistence mechanisms (but see Chalcraft *et al.* 2008). Non-spatial mechanisms, such as tradeoffs among different resource use efficiency or susceptibility to consumers, should have consistent effects across scales. For example, nutrient

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443 addition has frequently been implicated in a loss of non-spatial coexistence mechanisms; the  
444 addition of limiting resources leads to a reduction in niche dimensions or switch to single factor  
445 limitation such as light (Goldberg & Miller 1990; Dybzinski & Tilman 2007; Hautier *et al.* 2009;  
446 Borer *et al.* 2014b; Harpole *et al.* 2016). With respect to spatial mechanisms, a lack of  
447 consistent response in the slope of the SAR could be due to one of three possibilities. First,  
448 spatial coexistence mechanisms may not be particularly strong in these systems, at least at the  
449 scales as which we sampled ( $< 75 \text{ m}^2$ ). Second, nutrient additions may not have a strong effect  
450 on these mechanisms. For example, dispersal limitation and local feedbacks may be more likely  
451 to structure coexistence of rare species, and these dynamics may be less strongly influenced by  
452 the effect of dominant species. Finally, there may be a lag in diversity responses at larger spatial  
453 scales, as has been shown in models with a tradeoff between competition and dispersal (e.g.,  
454 the extinction debt; Tilman *et al.* 1994).

455 We note here that the SAR concept arose out of empirical and theoretical work at  
456 biogeographical scales (Arrhenius 1921; Godwin 1923; MacArthur & Wilson 1967), especially  
457 focused on variation among islands or large habitat patches; however, these relationships have  
458 been conceptually useful across a range of other spatial scales (Drakare *et al.* 2006; Dengler *et al.*  
459 *al.* 2020). Here, our focus is on smaller scale variation within contiguous habitat, and our total  
460 species diversity represents the species pool in a single grassland. Nevertheless, the slopes we  
461 estimated are quite similar to SARs reported in similar habitats spanning much larger spatial  
462 scales (Drakare *et al.* 2006; Dengler *et al.* 2020). Despite this similarity in slopes, processes  
463 governing diversity scaling at larger regional and biogeographic scales are different than those  
464 acting at the scales we address here.

Understanding and measuring diversity is inherently scale-dependent (Godwin 1923; Gleason 1926; MacArthur & Wilson 1967; Vellend 2010; Grace *et al.* 2011; Chase & Knight 2013; Chase *et al.* 2018), and we have shown that in grassland ecosystems this scaling is remarkably robust to environmental gradients and experimental manipulations of nutrient supplies and herbivore pressure. This result does not contradict the many studies that have shown the strong impacts of nutrients in particular on grassland diversity (Borer *et al.* 2014b; Harpole *et al.* 2016; Midolo *et al.* 2019). Rather, we build on this insight in showing that nutrient-induced diversity loss often causes constant proportional loss across spatial scales. As a result, more species will be lost at larger spatial scales, and existing estimates of nutrient-induced diversity loss are likely too low, because they are typically based on a single, relatively small scale of sampling. More generally, embracing the scaling of diversity and its change in response to environmental change is critical if we are to understand the impacts human activities on the biodiversity of the Earth's ecosystems.

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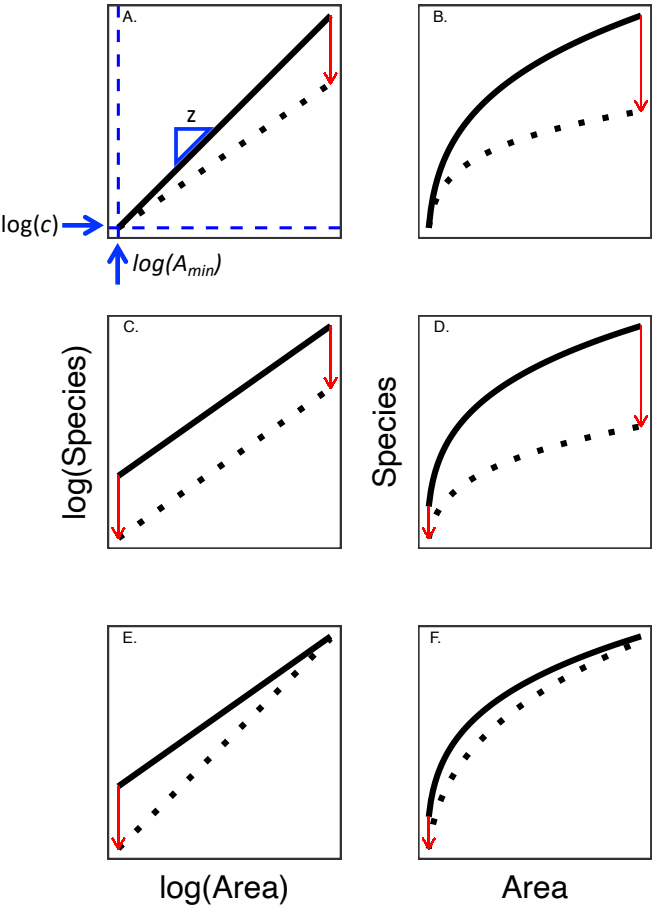
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Review Only

**Figure 1:** Hypothetical effects of experimental treatments (e.g., nutrient addition or herbivore exclusion) on species richness across spatial scales. The solid black line is constant in all panels and shows the species-area relationship (SAR) in control plots plotted in logged and untransformed units. Panel A shows the slope ( $z$ ), y-intercept ( $\log c$ ), and x-intercept ( $A_{min}$ ), which are the focus of the analyses in this paper. The black dotted line shows the SAR in the treated plots, and the red arrows show the change in richness at small or large scales. The blue dashed lines show the x and y intercepts in log space ( $\log(A)=0$  and  $\log(S)=0$ ). Left panels show  $\log(\text{Area})$  and right panels show the same relationship with area untransformed. The dotted lines in panels A and B show the effects of a reduced species pool, which reduces the slope but leaves the y-intercept unchanged. In this case, species loss increases with increasing spatial scale. Panels C and D show the effects of a constant proportional loss of species with increasing area (C), which leads to increasing total species loss with area (D). In this case, the y-intercept is reduced, while the slope is held constant. Panels E and F show the effects of reduced local richness without a reduction in the total species pool. In this case, the y-intercept is decreased but the slope is increased, such that species loss declines with area.

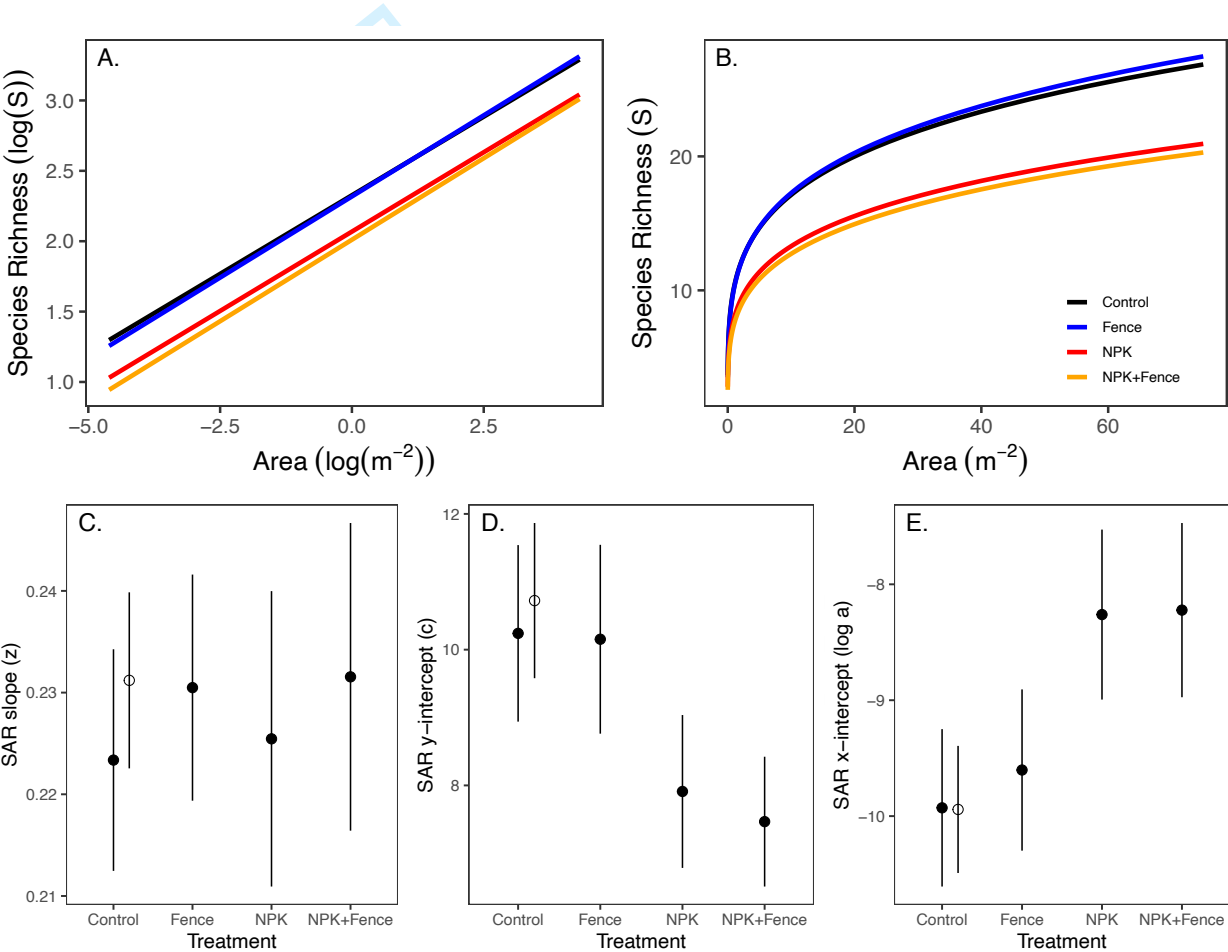
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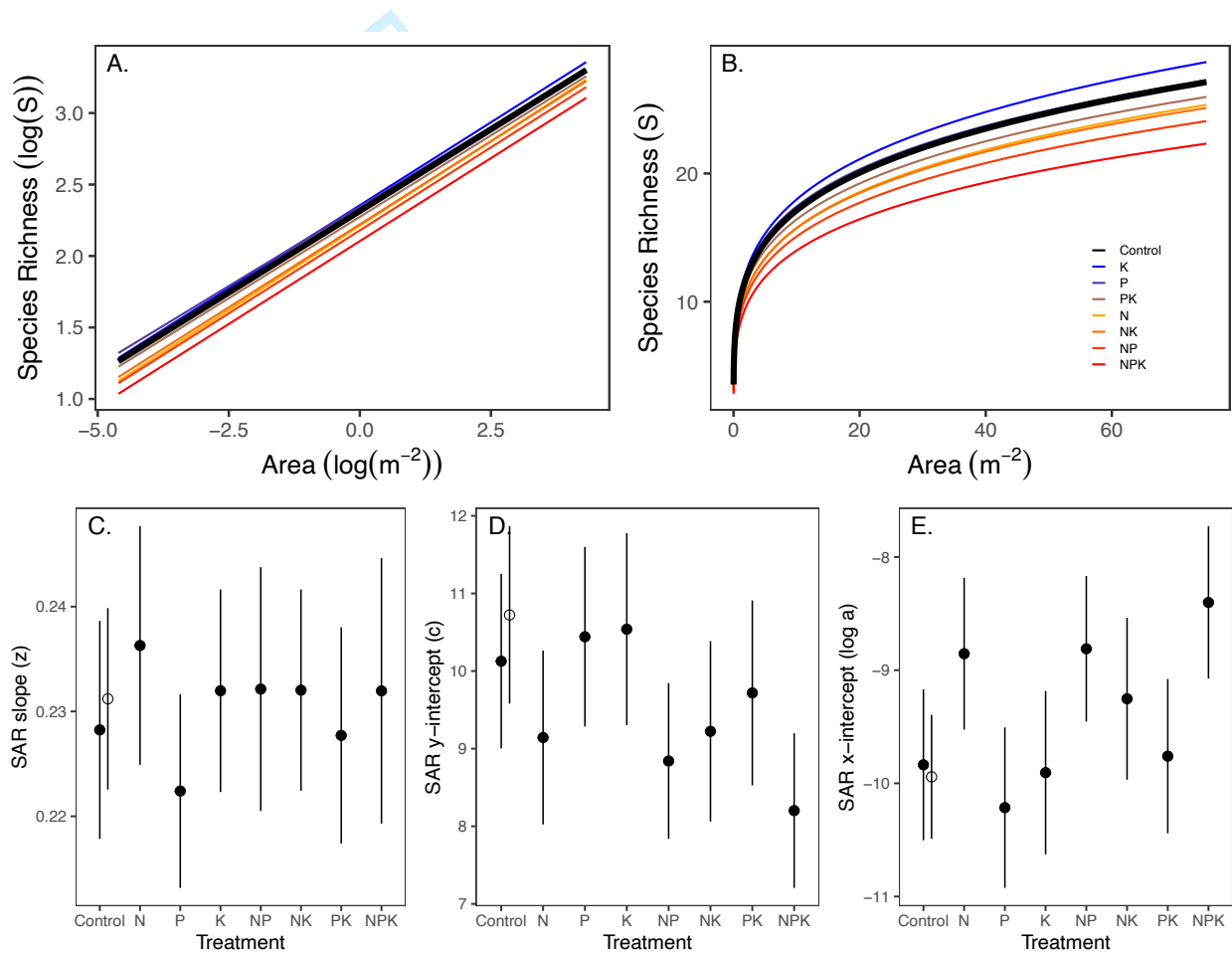
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**Figure 2:** Effects of consumers and nutrient addition on slope ( $z$ ), ( $\log c$ ), and  $x$ -intercept ( $a$ ) of the species-area relationships (SAR) in grasslands (16 sites). SAR plots (Panels A & B) use the mean parameter value for each treatment (Panels C - E). Open circle shows the values in control plots at the larger set of 30 observational sites which includes the 16 experimental sites (solid circles). Error bars represent 1 standard error of the mean (SEM). Full analysis is shown in Table S3, which includes estimates of treatment effects sizes and significance.

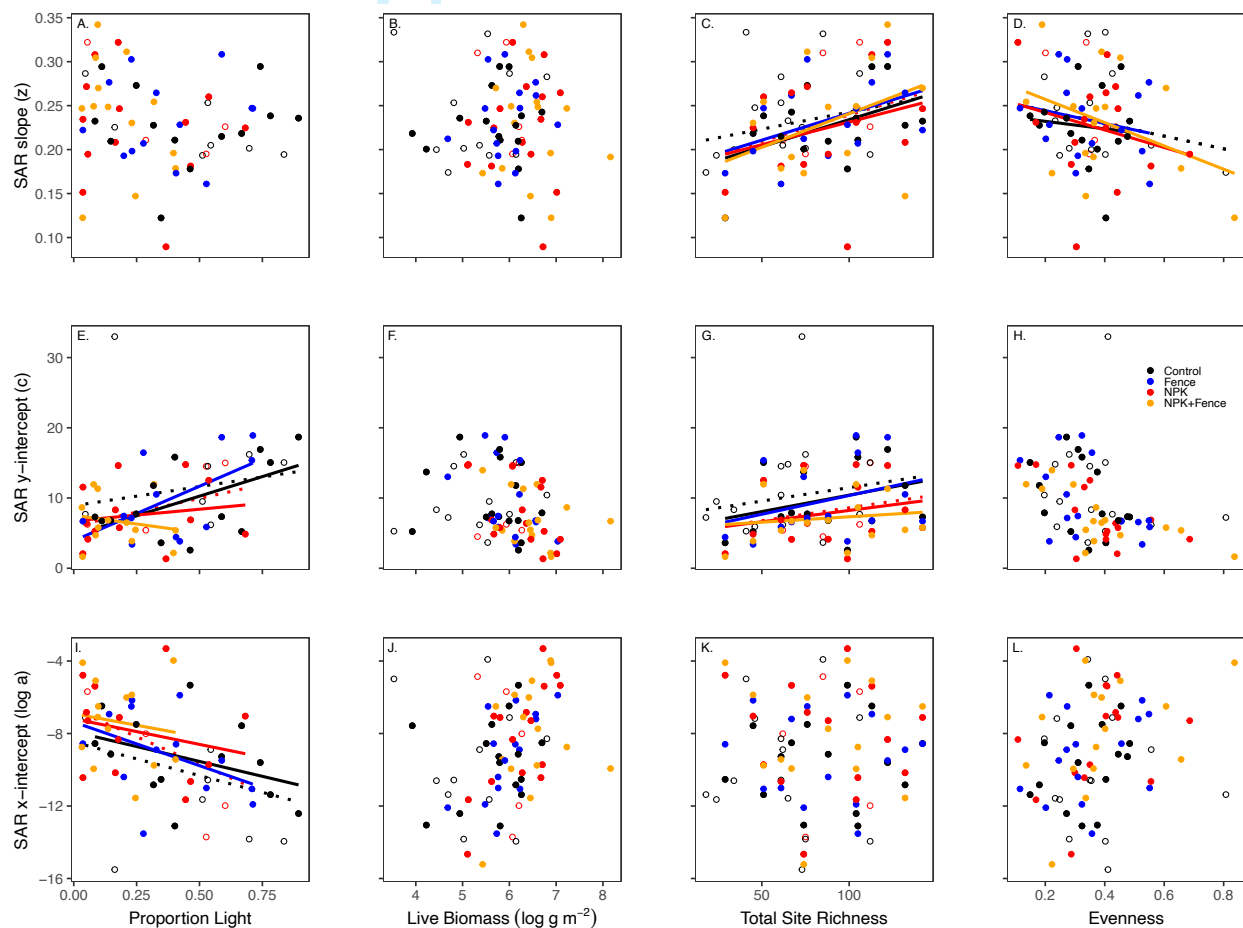


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4 689 **Figure 3:** Effects of nutrient addition on slope ( $z$ ), ( $\log c$ ), and x-intercept ( $a$ ) of the species-area  
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6 690 relationships (SAR) in grasslands (21 sites). SAR plots (Panels A & B) use the mean parameter  
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8 691 value for each treatment (Panels C - E). Open circles show the values in Control plots at the  
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10 692 larger set of 30 observational sites which includes the 21 experimental sites (solid circles). Error  
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12 693 bars represent 1 standard error of the mean (SEM). Full analysis is shown in Table S4, which  
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14 694 includes estimates of treatment effects sizes and significance.





**Figure 4:** Effects of proportion light transmission, aboveground live biomass, site richness, and evenness on the slope ( $z$ ), ( $\log c$ ), and  $x$ -intercept ( $a$ ) of the species-area relationships (SAR) in grasslands. Solid points show SAR parameters for the subset of sites with all fencing and nutrient addition treatments (16 sites). Open circles and dashed lines show SAR parameters from sites with control plots (black lines, 30 sites) and the subset of these with nutrient addition treatments but not fencing (red lines, 21 sites). Lines are shown only for significant regressions. Full analysis is presented in Table S5.



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