

1 **Incorporating spatial autocorrelation in rarefaction methods:**
2 **implications for ecologists and conservation biologists**

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21 **Abstract**

22 Recently, methods for constructing Spatially Explicit Rarefaction (SER) curves have been introduced
23 in the scientific literature to describe the relation between the recorded species richness and sampling
24 effort and taking into account for the spatial autocorrelation in the data. Despite these methodological
25 advances, the use of SERs has not become routine and ecologists continue to use rarefaction methods
26 that are not spatially explicit. Using two study cases from Italian vegetation surveys, we demonstrate
27 that classic rarefaction methods that do not account for spatial structure can produce inaccurate results.
28 Furthermore, our goal in this paper is to demonstrate how SERs can overcome the problem of spatial
29 autocorrelation in the analysis of plant or animal communities. Our analyses demonstrate that using a
30 spatially-explicit method for constructing rarefaction curves can substantially alter estimates of relative
31 species richness. For both analyzed data sets, we found that the rank ordering of standardized species
32 richness estimates was reversed between the two methods. We strongly advise the use of spatially-
33 explicit rarefaction methods when analyzing biodiversity: the inclusion of spatial autocorrelation into
34 rarefaction analyses can substantially alter conclusions and change the way we might prioritize or
35 manage nature reserves.

36

37 **Keywords:** Biodiversity, Coastal Dune Vegetation, Conservation, Rarefaction Curves, Reserve
38 Selection, Site of Community Importance, Spatial Autocorrelation, Spatially Explicit Rarefaction.

39

40 **Abbreviations:** RC – Rarefaction Curve; SA – Spatial Autocorrelation; SER – Spatially Explicit
41 Rarefaction; SCI – Site of Community Importance.

42 INTRODUCTION

43 Biogeography and ecology are deeply permeated by the spatial nature of their data (Legendre, 1993).
44 Several types of spatial analysis and statistics are routinely used to determine how spatial structure
45 affects the movement of individuals, species distributions, the structure and composition of species
46 assemblages, and to predict the consequences of spatial heterogeneity (Fortin et al. 2012). Geo-
47 referenced data are increasingly available (e.g., Martellos and Attorre 2012) and are being used to
48 address pressing planetary challenges from climate change and increased human-driven land use. Such
49 uses require spatio-temporal analyses that take into account the spatial and temporal extent and grain of
50 the data (Fortin et al. 2012, Bacaro et al. 2012).

51 Traditionally, the analysis of species richness at relatively large extents has relied on the use of
52 standardized sampling at smaller extents combined with the use of statistical estimators for
53 extrapolating to larger extents (D'Alessandro and Fattorini 2002; Chiarucci et al. 2003; Chiarucci et al.
54 2011). However, robust methods for such extrapolation are not routinely used. The development of
55 methods for such sampling and extrapolation offers new challenges and opportunities (Palmer et al.
56 2002; Engemann et al. 2015). Rarefaction curves (RCs) have been extensively used to compare species
57 richness among very different types of habitat and biota (e.g., Heilmann-Clausen and Christensen 2004;
58 Schneider and Culver 2005; Sogin et al. 2006; Roesch et al. 2007; Koellner et al. 2004; Chiarucci et al
59 2008b; Acosta et al. 2008, Bacaro et al. 2012). Rarefaction allows comparisons of species richness
60 among data sets by standardizing estimates to an equal-effort basis. Species richness estimates increase
61 with the number of sampling units (e.g., plots, traps; Fairbanks and McGwire 2004). Therefore, a
62 suitable comparison of species richness estimated from data sets of different sample sizes should be
63 done only after rarefying each to the an equal sampling effort, such as area or number of sampling units
64 (Gotelli and Colwell 2001). Depending on the units used to express sampling effort, i.e., the number of
65 individuals sampled or the number of sampling units, it is possible to calculate either individual- or

66 sample-based rarefaction curves.

67 Formally, given a collection of n sampling units, sample-based rarefaction provides the expected
68 number of accumulated species as the number of sampling units increases from 1 to n . This is often
69 obtained by repeatedly resampling the pool of n sampling units at random without replacement and
70 plotting the average number of species recorded by 1, 2, . . . , n sampling units (Gotelli and Colwell
71 2001).

72 An analytical formulation for the calculation of the rarefaction curve was first developed by Shinozaki
73 (1963), and later independently rediscovered by a number of authors during the 1970s (Kobayashi
74 1974; Holthe 1975; Engen 1976; Smith et al. 1979) and in the last decade (Ugland et al. 2003; Colwell
75 et al. 2004). Chiarucci et al. (2008a) described the history of the multiple discoveries of sample-based
76 rarefaction as a classical example of geographical and linguistic bias in scientific literature. If \mathbf{G}
77 denotes the set of species observed in the collection of n sampling units, S_n denotes the total number of
78 observed species, and n_k denotes the number of sampling units containing at least one individual of
79 species $k \in \mathbf{G}$, then, the expected number of species S_i is:

$$80 \quad S_i = S_n - \binom{n}{i}^{-1} \sum_{k \in \mathbf{G}} \binom{n - n_k}{i} \quad i = 1, \dots, n \quad (1)$$

81 This equation describes the expectation of S_i when i samples are randomly resampled without
82 replacement (Chiarucci et al. 2008a). This estimator is unbiased if the spatial distribution of individuals
83 is random (Kobayashi 1982; Gotelli and Colwell 2001; Collins and Simberloff 2009) and it can be used
84 for comparisons among data sets if sample sizes are sufficient and the data sets were sampled in a
85 similar way (Abele & Walters 1979). The latter two requirements are easy to control and or adjust for
86 either in the initial sampling design or in choosing which data sets to compare. However, individuals
87 are almost never randomly distributed in space, either due to heterogeneity of environmental factors or
88 non-random dispersal of individuals. Thus, it is necessary to develop rarefaction methods that account

89 for such non-random distributions.

90 Recently, Chiarucci et al. (2009) defined a new type of rarefaction curve, termed Spatially Constrained
91 Rarefaction (SCR) that accounts for the spatially-autocorrelated structure of biological communities. In
92 this paper we refer to this method by the somewhat more accurate name of Spatially Explicit
93 Rarefaction (SER). This method addressed the problem of spatial autocorrelation by building the
94 rarefaction curve based on the adjacency of the sampling units (see Chiarucci et al. 2009 for a full
95 description of the rationale and method). More recently, Bacaro et al. (2012a) developed “pointpattern”
96 and “SCR” routines in the R environment for calculating a SER, making this technique readily
97 available. To our knowledge, however, the use of SERs has not become routine (a recent ecological
98 application can be found in Janisova et al. 2014). Conversely, a plethora of recent studies (e.g.,
99 Hardersen and Corezzola 2014; Sieglöcher et al. 2014; Brazeal et al. 2014; Jung et al. 2014; Giesecke et
100 al. 2014, Xu et al. 2014a; Xu et al. 2014b) and statistical software (see, for example, Oksanen et al.
101 2015; Cardoso et al. 2015) continue to use nonspatially-explicit rarefaction methods. In this paper, we
102 aim at demonstrating how SERs can overcome the problem of spatial autocorrelation in the analysis of
103 plant or animal communities.

104

105 **Case Studies**

106 To illustrate how the two methods (RCs and SERs) differ in practice and why spatially-explicit
107 rarefactions should be preferred, we re-analyzed two published vegetation datasets (Ciccarelli 2014;
108 Chiarucci et al. 2008a). The examples differ both in sampling strategies and vegetation type, and
109 demonstrate the generality of our conclusions.

110

111 *Example 1: coastal dune plant communities*

112 The first example was a vegetation survey carried out on coastal dune plant communities. Dune

113 ecosystems are diverse in terms of both environmental heterogeneity and species composition (Van Der
114 Maarel 2003; Martínez and Psuty 2004). Furthermore, the dynamic nature of sandy coastal habitats,
115 together with the strong zonation patterns exhibited by the vegetation make dune communities the
116 focus of several national and international conservation efforts and policies. We analyzed a set of plots
117 collected in the coastal sand dunes of two Protected Areas (PA) along the Tuscan littoral of Italy (Fig.
118 1): Migliarino-San Rossore-Massaciuccoli Regional Park (MSRM) and Maremma Regional Park (MP).
119 The coastal dune ecosystems of both parks are part of the Natura 2000 network. Plant species data were
120 collected using a systematic sampling design. In each protected area, the entire coastal system (20 km
121 and 10 km in length for MSRM and MP parks, respectively) was divided into sections of 1 km. Within
122 each section (13 for MSRM and 7 for MP) a transect was randomly located orthogonal to the seashore.
123 The lengths of the transects varied depending on dune morphology and width. Along each transect,
124 species presences were recorded in contiguous 1 m × 1 m plots. Sampling occurred between May 2010
125 and August 2011; for further details, see Ciccarelli (2014). For each park, classic rarefaction curves
126 (RCs) and spatially-explicit rarefaction curves (SERs) were calculated. For RCs we used the
127 “specaccum” function within the “vegan” R package (Oksanen et al. 2015). For SERs we used the R
128 routines “pointpattern” and “SCR” (Bacaro et al. 2012a). From a practical point of view, SERs are
129 constructed by accumulating species of nearest plots for the given n number of plots sampled. This is
130 done by firstly ordering plots according to their minimum distance and then performing an
131 accumulation curve for each ordered sequence. The rarefaction curve (SER) was then calculated as the
132 mean of the n accumulation curves.

133 As expected, the larger area sampled in the MSRM compared to the MP area resulted in a higher
134 observed species richness (53 vs. 39 species, respectively; Table 1). Figure 2 compares the RCs and
135 SERs for the two parks. As expected, the SERs increased less steeply than the RCs resulting in lower
136 estimates of species richness at a given extent. Notably, at any given extent, the relative ranking of the

137 two parks was reversed for the two methods and, overall the RC method always estimated higher
138 species richness than the SER method (Table 1). Typically in rarefaction analyses, species richness
139 estimates are standardized at the sample size of the smaller data sets, which was 305 plots for these
140 data. At that extent, the RC method estimated a species richness of 44.9 for MSMR compared to the
141 total of 39 species observed for MP. In contrast, the SER method estimated only 34.6 species for the
142 MSMR. Thus by accounting for spatial structure, we can conclude that the MP is more diversified than
143 the MSMR. This example shows how the inclusion of spatial autocorrelation into rarefaction analyses
144 can alter conclusions and eventually even change the way we might prioritize or manage nature
145 reserves.

146

147 *Example 2: Sites of Community Importance*

148 The second example was a survey of plant species occurrence within the Sites of Community
149 Importance (SCIs) of the Province of Siena, Italy. The complete survey is described in Chiarucci et al.
150 (2012) and a subset of these data was previously analyzed in Chiarucci et al. (2008b). The dataset
151 consists of 604 vegetation plots collected across the entire network of 17 SCIs (Figure 3). A grid of 1
152 km x 1 km cells were laid across the network and a random point selected within each; plant presences
153 were recorded for a 10 x 10 m plot centered at each point. For this paper, we analysed data from three
154 SCIs (Table 1): Montagnola Senese (MNS), Crete dell'Orcia e del Formone (FOR), and Castelvecchio
155 (CAS). The three SCIs differ for main land-cover types: thermophilous oak forests, mesophilous oak
156 forests and *Castanea sativa* dominated forests (MNS), agricultural areas, dry grasslands, pastures,
157 badland vegetation, shrublands and riverbed vegetation (FOR), and thermophilous deciduous forest and
158 evergreen forests (CAS).

159 SERs and RCs were calculated as in the first example. Similar to the other example, the rank orderings
160 of the curves produced by the two methods were reversed (Figure 4). For the estimate of species

161 richness at the common sampling effort (11 plots), using RCs the species richness ranking was FOR
162 (201.3 species), MNS (195.5 species) and CAS (191 species); SERs produced exactly the opposite
163 ordering, CAS (191 species), MNS (187.8 species) and FOR (167.4 species). Again, rarefaction
164 methods were shown to be susceptible to spatial autocorrelation.

165

166 **Discussion**

167 Our analyses demonstrate that using a spatially-explicit method for constructing rarefaction curves can
168 substantially alter estimates of relative species richness. It is just as important to consider spatial
169 autocorrelation as it is to note data extent and grain for such comparisons. The analysis of how the
170 spatial configuration of sampling units influences species richness estimates has become an important
171 issue (Kühn 2007) because species richness is one of the simplest and most popular diversity measures,
172 with intuitive mathematical and statistical properties (Chao and Jost 2012; Chiarucci et al., 2011). Our
173 results do not indicate that all previous analyses that used or compared RCs are flawed. However, as
174 stated by Kühn (2007), “if spatial autocorrelation is ignored we simply do not know if we can trust the
175 results at all.” Therefore, the presence of residual spatial autocorrelation should always be tested for in
176 spatial ecology and appropriate methods should be used if there is evidence of a significant spatial
177 autocorrelation (Cressie 1993; Fortin and Dale 2009, Fortin et al. 2012). Spatially-explicit methods
178 should routinely be used in rarefaction analyses and be included in the development of new methods.
179 Recently, both functional (Ricotta et al. 2012) and phylogenetic rarefactions (Chao et al. 2015) have
180 been proposed, but neither of those two new methods considered the spatial structure of the data. If the
181 effects of spatial autocorrelation were included, we predict similar results as seen here.

182 Just accounting for spatial autocorrelation of samples may not address all of the effects of spatial
183 patterning, which are needed to compare different datasets. The shape of a sample-based rarefaction
184 curve is controlled by several factors, including the extent, grain and number of sampling units (Weins

185 1989; Palmer and White 1994; Nekola and White 1999; Dungan et al. 2002). The other key factor is
186 summarized by the first law of geography (“Everything is related to everything else, but near things are
187 more related than distant things”, Tobler 1970). This law is apparent as distance-decay patterns at both
188 global and local scales that result in a decrease of compositional similarity with increasing distance
189 (Palmer 2005; Nekola and White 1999; Nekola and Brown 2007; Soininen et al. 2007; Bacaro et al.
190 2012b). From this pattern, we expect sample-based rarefaction curves to increase faster when sampling
191 units are farther apart (Condit et al. 1996; Palmer et al. 2002; Chiarucci et al. 2008a; Hui 2008) because
192 greater habitat heterogeneity is likely to lead to greater total species richness (Diamond 1988; Palmer et
193 al. 2002). Because of this effect, differences in species richness among data sets could be at least partly
194 due to difference in the extent of the sampling units. (Palmer 2007, McGlenn and Palmer 2011).
195 Similarly, we predict that deviations between RCs and SERs to increase as a direct function of the
196 spatial autocorrelation among sampling units. Methods that account for such effects still need to be
197 developed.

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199

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210 Table 1. The total number of sampled plots and diversity values (alpha and gamma) for the Nature 2000
 211 areas compared in this study (for both the worked examples). The common sampling effort is the area
 212 of the least sampled data set within a comparison.
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Nature Reserve/SCI	Number of plots	Total species richness (gamma)	Mean plot species richness (alpha)	Richness at the common sampling effort – SER	Richness at the common sampling effort – RC
Migliarino San Rossore					
Massaciuccoli Regional Park (MSRM)	675	53	4.15	34.62	44.94
Maremma Park (MP)	305	39	3.52	39.00	39.00
Montagnola Senese (MNS)	137	570	32.85	187.76	195.54
Crete dell'Orcia e del Formone (FOR)	86	472	33.37	167.44	201.35
Castelvechio (CAS)	11	191	42.27	191	191

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221 **Figure Captions**

222 Figure 1. The locations of the Migliarino-San Rossore-Massaciuccoli Regional Park (MSRM) and the
223 Maremma Regional Park (MP) in the northern and southern parts of Tuscany, Italy, respectively.

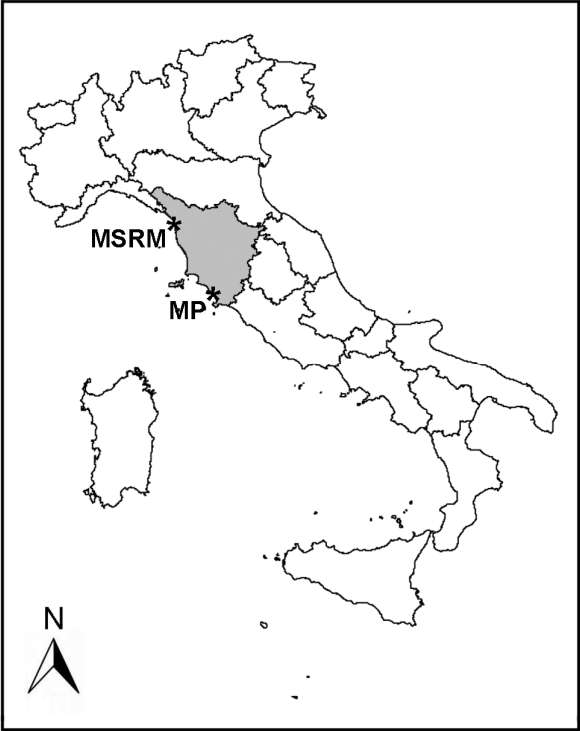
224 Figure 2. Classic and spatially-explicit rarefaction curves for the two coastal dune parks. For the SERs,
225 the Maremma Park (MP) shows on average more species than those collected in an equal-sampled
226 area in the Migliarino San Rossore Masaciuccoli Park (MSRM), the reverse of the pattern of the
227 RCs.

228 Figure 3. The locations of the Natura 2000 Network in Siena Province and the three SCIs included in
229 example 2 (MNS, FOR and CAS)

230 Figure 4. Rarefaction curves for the three SCIs (A). SER curves for the three SCIs (B). The rank
231 ordering of the latter curves is the reverse of the former curves.

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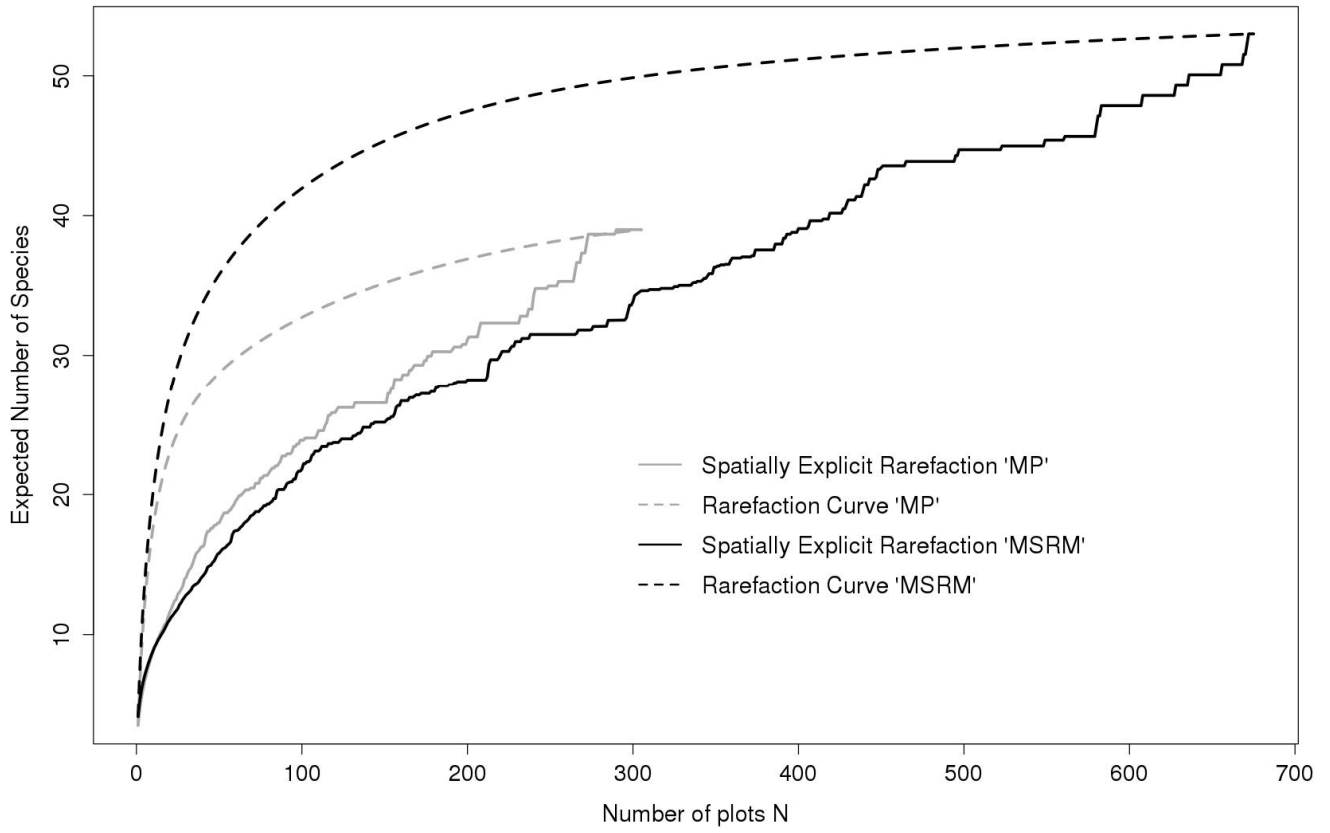
246 **Figure 1**



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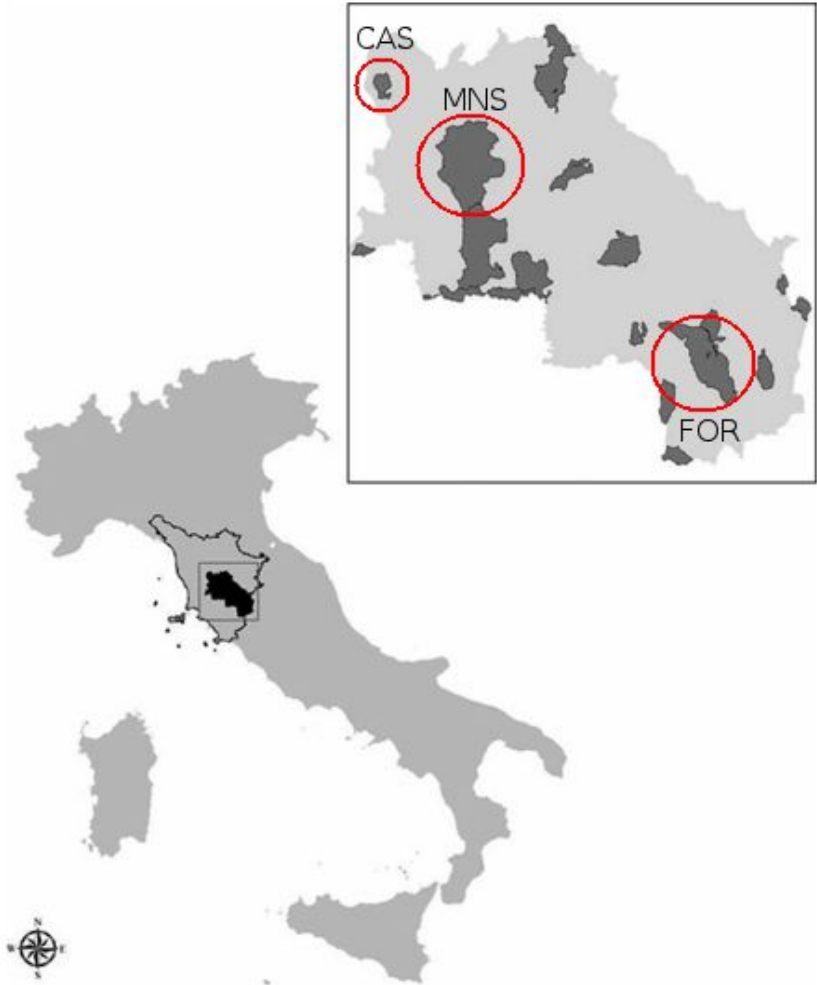
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249 **Figure 2**



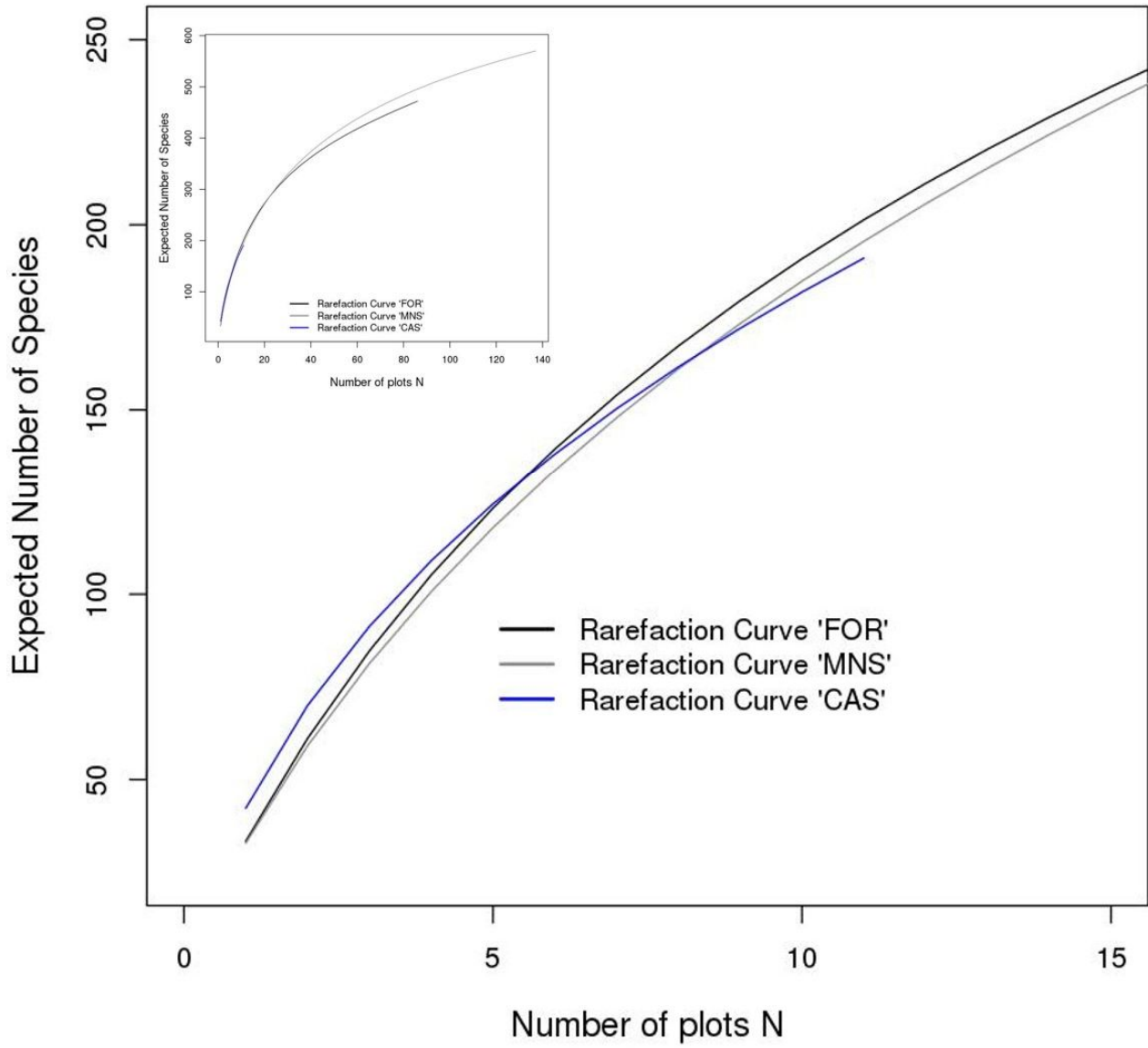
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254 **Figure 4**

255 **A)**



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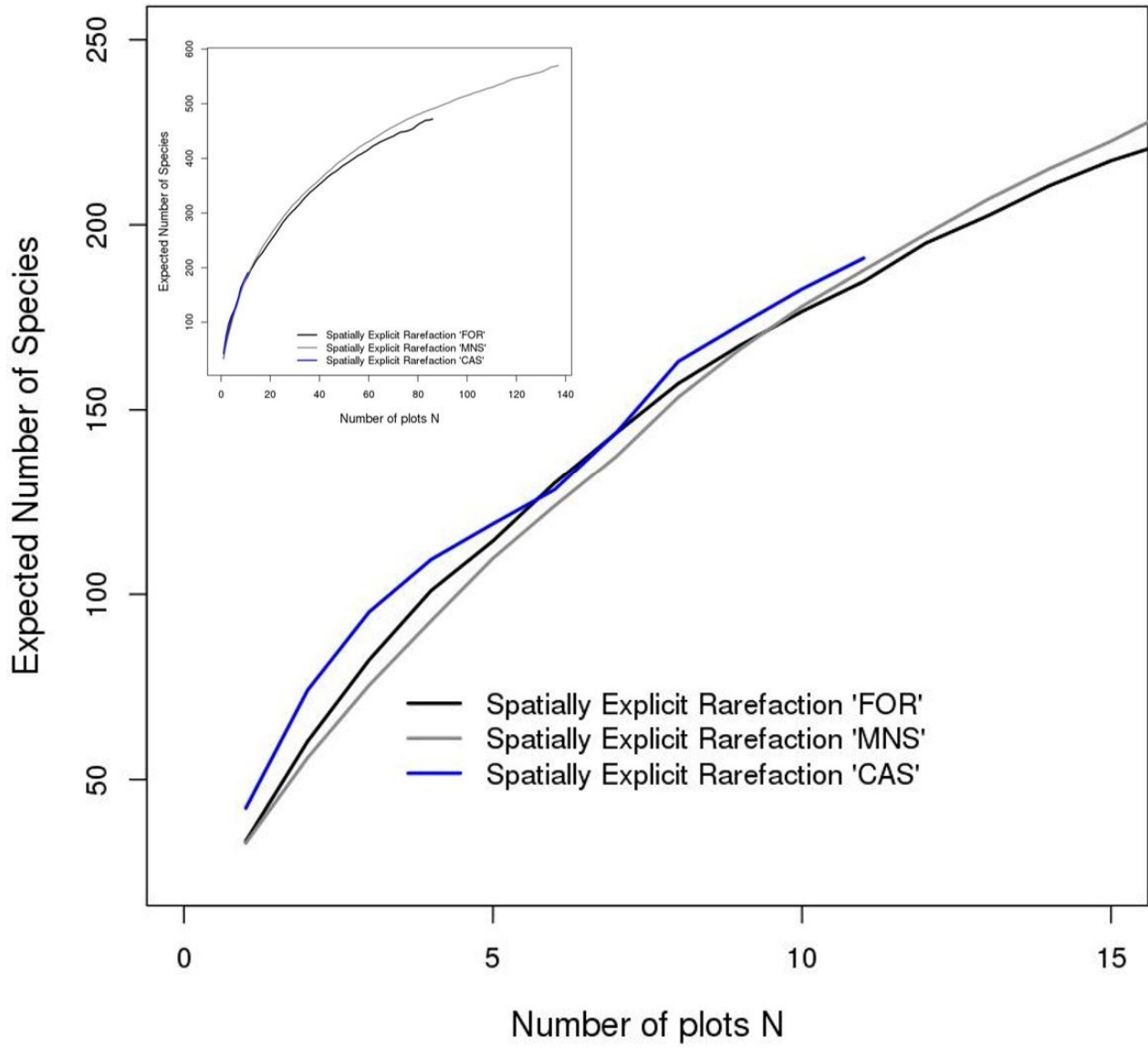
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261 **B)**



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