1   D. Ciccarelli[1], G. Bacaro[2]

2

3   **Quantifying plant species diversity in coastal dunes: A help from Spatially Constrained Rarefaction**

4

5   [1]Department of Biology, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy

6   [2]Department of Life Sciences, University of Trieste, Via L. Girgieri 10, 34127 Trieste, Italy

7

8   Corresponding author: Daniela Ciccarelli, e-mail: daniela.ciccarelli@unipi.it

9   Tel: +39 (0)50 2211327, Fax: +39 (0)50 2211309

1   **Quantifying plant species diversity in coastal dunes: A help from Spatially Constrained Rarefaction**

2

3   **Abstract**

4   Since coastal dunes are one of the most vulnerable landscapes in Europe, their maintenance requires

5   specific conservation and monitoring programs. In this paper, the coastal dune systems of two natural

6   parks located in central Italy were analysed aiming at: (1) assessing diversity patterns of all vascular

7   species, endemic and alien taxa in plant communities along the coast-to-inland gradient; (2) comparing

8   these patterns between coastal sections characterised by different dynamical processes (accreting, stable

9   and erosive coasts); (3) testing the differences induced by the methodological approach used to

10   characterise these patterns. Twenty-one transects were randomly positioned perpendicular to the shoreline

11   on the whole coastal area (30 km in length) and the full spectrum of plant communities was sampled.

12   Patterns of plant diversity was assessed using spatially explicit methods, namely Spatial Constrained

13   Rarefaction (SCR), able to avoid the confounding effect of spatial autocorrelation. Results showed that

14   species richness varied significantly between plant communities along the coast-to-inland gradient with

15   the highest values at level of mobile dunes and transition dunes. Species richness was significantly higher

16   in stable coastal dunes than those found in accreting and in erosive dunes. In fact, sand dynamics

17   (accumulation as well as erosion) creates periodic vegetation disturbances affecting composition

18   variability and succession. SCR methodology avoided overestimation in species richness when compared

19   to classical rarefaction curves. Our findings pinpointed that coastal plant communities create a highly

20   spatially structured mosaic in which mobile dunes represent the highest compositional heterogeneity.

21   Local managers are encouraged to consider these results for planning adequate conservation strategies.

29

Introduction

31   The term biodiversity is currently used in the literature to cover both the number of different populations

32   and species that exist and the complex interactions that occur among them. Its measurement at all levels is

33   really important for any conservation biology purposes (Pullin 2002). The effects of biodiversity loss or

34   changes in plant community composition on the functioning of ecosystems have been the focus of much

35   ecological research (Schulze and Mooney 1993; Kinzig et al. 2002; Loreau et al. 2002). Species

36   composition, richness, evenness, and interactions all respond to and influence ecosystem properties and

37   stability. Unfortunately, the ever-growing impact of human activities is causing biodiversity loss of

38   natural habitats (Hawksworth and Bull 2008).

39   Coastal sand dune systems are dynamic and heterogeneous habitats characterised by complex

40   environmental gradient which determines a characteristic coast-to-inland plant community zonation

41   (Ranwell 1972; Doing 1985; Psuty 2004; Wiedemann and Pickart 2004; Frederiksen et al. 2006; Acosta

42   et al. 2007). They contain a high ecological diversity in terms of plant community composition, species

43   rarity and endemism (Van der Maarel and Van der Maarel-Versluys 1996; Van der Maarel 2003;

44   Martínez et al. 2004; Ciccarelli et al. 2014). Unfortunately, coastal dune environments are one of the most

45   vulnerable landscapes in Europe, prone to many stress and disturbance factors, such as erosion, global

46   warming and anthropogenic pressure (Cori 1999; Brown and McLachlan 2002; Van der Maarel 2003;

47   Coombes et al. 2008; Carboni et al. 2009; Gornish and Miller 2010; Miller et al. 2010; Ciccarelli et al.

48   2012; Ciccarelli 2014; 2015). Quantifying the number of species that occur along a coast-to-inland

49   ecological gradient could make a valuable contribution to managing the ecosystem.

50   It should be considered that the examination of species distribution patterns through space can help

51   us to better understand the effects of ecological and environmental pressures. To quantify the relative

52   importance of natural and anthropogenic pressures at appropriate spatial scales, both spatial and spatial-

53   temporal analyses are required. The species' spatial distribution and their relationship with the

54   environmental heterogeneity should be explored using specifically developed techniques. Among these,

55   individual and sample-based rarefaction curves are widely recognised powerful tools, also used to

56   evaluate the effectiveness of sampling and to compare species richness in different habitats (Gotelli and

57   Colwell 2001; Moreno and Halffter 2001; Koellner et al. 2004; Crist and Veech 2006; Chiarucci et al.

58   2008a-b; Acosta et al. 2009; Bacaro et al. 2012a; Chiarucci et al. 2012). Even if this method offers an

59   elegant solution to the interpolation of the number of species observed as a function of sample size, it is

60   affected by various spatial components. However, after Chiarucci et al. (2009) and Bacaro et al. (2012a),

61   the problem related to the lack of independence in the sampling units between samples has been

62   considered thanks to spatially constrained rarefaction (SCR) that incorporates the autocorrelated structure

63   of biological communities into sample-based rarefaction. More specifically, this method addressed the

64   problem of spatial autocorrelation by building the rarefaction curve based on the adjacency of the

65   sampling units. Nevertheless, the use of SCRs has not yet become routine (the only recent ecological

66   application can be found in Janisova et al. (2014) and is expected to be particularly useful in those

67   situations where the spatial structure of biological communities is high, as is the case in coastal dune

68   systems.

69   Erosion has a serious impact on some 20% of the coastline of Europe (Doody 2013). In Italy, 42%

70   of coasts are largely affected by erosion processes, and in particular the Tuscan coastline reflects this

71   scenario, with 37% of its sand dune systems being affected by erosion (Gruppo Nazionale per la Ricerca

72   sull'Ambiente Costiero 2006). Although it has been assessed that shoreline erosional processes are one of

73   the major threats for coastal sand dune systems worldwide, little is known regarding plant diversity and

74   coastline erosion (Roman and Nordstrom 1988; Avis and Lubke 1996; García-Mora et al. 1999; Lubke

75   and Hertling 2001; da Silva et al. 2008; Ciccarelli et al. 2012; Ciccarelli 2014).

76   In this paper, a systematic sampling design based on belt transects was used to sample the full

77   spectrum of coastal dune plant communities. Considering that biodiversity loss is a critical issue in

78   coastal environments, we analysed the diversity patterns of vascular plant species across the coastal dune

79   systems of two natural parks in Tuscany (Italy) in order to correctly characterise occurring diversity

80   patterns. In detail, we specifically aimed at (1) assessing diversity patterns of all vascular species,

81   endemic and alien taxa in plant community types along the coast-to-inland gradient; (2) comparing these

82   patterns between coastal sections characterised by different dynamical processes (accreting, stable and

83   erosive coasts) and, finally; (3) testing the influence of the methodological approaches used (e.g., regular

84   rarefaction vs. spatially constrained rarefaction) for diversity comparisons purposes. This latter point has

85   major implications when conservation actions should be planned and, for this reason, deserves particular

86   attention.

87

88

89   **Materials and methods**

90   *Study Area*

91   The present study was conducted in the coastal sand dunes of two protected areas along the Tuscan

92   littoral (Italy): Migliarino-San Rossore-Massaciuccoli Regional Park (MSRM) and Maremma Regional

93   Park (MP; Fig. 1).

94   The Migliarino-San Rossore-Massaciuccoli Regional Park is 142 km^2 wide and located near Pisa,

95   in the North of Tuscany. The Park hosts 40 km^2 of coastal forest, a sandy beach and inland marshes. This

96   area is characterised by a Mediterranean sub-humid climate, with a mean annual temperature >15°C and a

97   mean annual rainfall of 800-900 mm (Rapetti 2003).

98   The Maremma Regional Park is located on the coast near Grosseto, in the southern part of

99   Tuscany. The Park is 90 km^2 and offers a diverse array of landscape types: coastal dunes, inland salt

100   marshes, rocky vegetated foothills, coastal pinewood, and farming areas. This area is characterised by a

101   C1 type of climate, Mediterranean sub-arid, with an average annual temperature of 15.6 °C and an

102   average annual rainfall of 618 mm (Pinna 1985).

103   The coastal dune systems of both parks belong to the Natura 2000 network and include the

104   following Sites of Community Importance (SCIs): "Coastal sand dunes of Torre del Lago" and "Coastal

4

105   sand dunes of Uccellina", respectively. Moreover, these two protected areas are a geomorphological

106   reference system for investigating variability of species richness related to coastal erosion and accretion,

107   because sections which belong to the same physiographic unit experience very different and localised

108   dynamical processes (Gruppo Nazionale per la ricerca sull'ambiente costiero 2006; Anfuso et al. 2011).

109

110   *Vegetation sampling*

111   We analysed plant communities on coastal dunes along the Ligurian and Tyrrhenian coast of Italy (Fig.

112   1). In each study area, the whole coastal system (20 km and 10 km in length for MSRM and MP park,

113   respectively) was divided into sections of 1 km and one random transect was selected within each section.

114   Several sections were excluded from this study because there were practically no more foredunes, as a

115   result of coastal erosion. In May-June 2010 and in May-June 2011, a total of 21 transects (14 for MSRM

116   and 7 for MP park) were positioned orthogonal to the seashore; their length varied depending on dune

117   morphology and width. Along each transect, all vascular plant species were recorded and the cover

118   percentage of each species was recorded in contiguous 1 m x 1 m plots. According to the guidelines of

119   Biondi et al. (2009), all plots were assigned to the coastal habitats (see Ciccarelli 2014 for details on

120   sampling procedure and habitat classification) included in the Habitats Directive of the Council of

121   European Communities 92/43/EEC (EEC 1992). For this study, we arranged the plots into three main

122   plant community types that are most related to coastal dune zonation (Table 1): (i) upper beach and

123   embryo dunes, which included annual vegetation of drift lines (Habitat code: 1210), and embryonic

124   shifting dunes (Habitat code: 2110); (ii) mobile dunes, identified as shifting dunes along the shoreline

125   with *Ammophila arenaria* (Habitat code: 2120); (iii) transition dunes, which included *Crucianellion*

126   *maritimae* fixed beach dunes (Habitat code: 2210), and *Malcolmietalia* dune grasslands (Habitat code:

127   2230). In order to fine-tune the resulting data, we decided to consider as a single group both communities

128   of foredunes - upper beach and embryo dunes – and both assemblages of transition dunes because they

129   tend to grow in a mosaic pattern.

130

131   *Data analysis*

132   The presence or absence of vascular plant species recorded in each plot was used for data analysis. The

133   first step was to analyse species richness within each plant community type along the coast-to-inland

134   zonation. Secondly, to study the relationships between species richness and coastal dynamics, all plots

135   were classified in dependence to the dynamical processes of the coastal section to which they belonged.

136   The following dynamic classes were considered: CD1 (accreting coastline), CD2 (stable coastline), and

137   CD3 (erosive coastline). These trends of shoreline change were determined using aerial photographs

138   (Gruppo Nazionale per la ricerca sull'ambiente costiero 2006; Anfuso et al. 2011).

139   Species richness values were calculated at the plot scale for each group of samples (plant

140   community types and coastal dynamic classes), and significant differences were tested using a non-

141   parametric Kruskal-Wallis test, with Bonferroni correction for multiple comparisons.

142   For each plant community and coastal dynamic class spatially constrained sample-based

143   rarefaction curves (Chiarucci et al. 2009; Bacaro et al. 2012a) were calculated. In order to calculate SCR,

144   the following framework was used: let s be the set of N plots defined in the d-coordinates space (in this

145   specific case, d equals 2, i.e., the set of projected coordinates of each plot in the Euclidean space). Given a

146   dataset containing the plot identity (label) and the coordinates of each sampling unit, the proposed routine

147   (the R code is available in Bacaro et al. 2012a) calculates the distance between the sampling units, and for

148   each unit, it orders all the others by increasing distance. For each ordered sequence of plots, an

149   accumulation curve is then calculated and the SCR is obtained as the average of all the accumulation

150   curves. 95% IC are also computed. Generally, rarefaction curves enable to compare two or more data sets

151   considering the same sampling effort – in this case the same number of grid cells (Gotelli and Colwell

152   2001; Collins and Simberloff 2009). Spatially constrained rarefaction is suitable for comparing areas of

153   different size, because in the sets of samples collected across larger areas the rarefaction curves would

154   increase more steeply due to the distance decay of similarity (for details see Chiarucci et al. 2009). Bacaro

155   et al. (2012a) developed "pointpattern" and "SCR" routines in the R environment for calculating SCR,

156   making this technique readily available. In order to compare SCRs with classical rarefaction curves

157   (RCs), we used the specaccum function in the vegan R package to calculate unconstrained and classic

158   rarefaction curves (using the analytical formula proposed by Kobayashi 1974).

159   Moreover, aiming at testing differences in beta diversity among sets of plots belonging to different

160   plant community types, the simple analytical procedure proposed by Bacaro et al. (2012b; 2013) was

161   applied. This procedure consists of shuffling within-community dissimilarities among community and

162   disregarding between-community dissimilarities. By repeating this operation many times (999 for the

6

163 performed analyses), a distribution of the test statistics under the null hypothesis of no differences in the

164 mean plot-to-plot dissimilarities within groups was obtained. This analysis was performed for each

165 natural park separately and by pooling in a unique set plots from the two areas in order to test

166 independence of beta diversity patterns to the specific park considered. The "betadispersion2" function

167 was here used (freely available in Bacaro et al. 2013). All the statistical analyses were performed using

168 the R 3.2.0 software (2015).

169

170 **Results**

171 *Species richness patterns*

172 A pooled species list of 63 vascular plants belonging to 23 Families was obtained from the 980 sampled

173 plots (Appendix I). Of these, Poaceae, Asteraceae, Caryophyllaceae, Apiaceae and Fabaceae showed the

174 largest number of species (16, 11, 5 and 4, respectively). One Tuscan coastal dune endemic species

175 (*Solidago littoralis*) was recorded in 38 plots, representing about 4% of all sampled plots. In contrast,

176 seven alien species - *Arundo donax*, *Cuscuta scandens*, *Elaeagnus angustifolia*, *Erigeron canadensis*,

177 *Oenothera biennis, Pinus pinaster* (dubitative alien in Tuscany, see Arrigoni and Viegi 2011), and

178 *Xanthium orientale* subsp. *italicum* - were recorded on the foredunes of the protected areas (they were

179 present in 236 plots equals to 24% of the sampled plots).

180 Species richness varied significantly between the three plant community types for all vascular

181 species (with Kruskal-Wallis statistic of 42.1 and $p < 0.001$), and for both endemics (with K-W = 53.9

182 and $p < 0.001$), and alien species (with K-W = 26.2 and $p < 0.001$). Regarding all species, upper beach-

183 embryo dunes showed the significant lowest mean value of species richness per plot and the lowest

184 pooled number of species (Table 2). In contrast, mobile dunes and transition dunes have similar mean

185 numbers of species per plot and the pooled number of species (Table 2). The highest number of endemics

186 per plot was found in mobile dunes, while upper beach-embryo dunes showed the highest number of alien

187 species per plot (Table 2), where the most abundant aliens were *Xanthium orientale* subsp. *italicum* and

188 *Oenothera biennis* with a frequency of 19% and 10%, respectively.

189 With respect to the coastal dynamic processes, species richness varied significantly for all vascular

190 species (K-W = 31.0 and $p < 0.001$), and for both endemics (with K-W = 99.2 and $p < 0.001$), and alien

191 species (with K-W = 145.8 and $p < 0.001$) between the dunes located on littorals affected by different

192   sedimentary dynamics. Stable dunes (CD2) exhibited the highest mean value of species richness per plot,

193   the highest pooled number of species, and the highest mean value of endemics per plot (Table 3),

194   resulting significantly different from both accreting (CD1) and erosive dunes (CD3). In contrast, erosive

195   dunes showed the lowest number of alien species per plot (Table 3), with the predominance of *Arundo*

196   *donax* (about 3%). While accreting dunes and stable dunes have similar mean numbers of alien species

197   per plot, stable dunes showed the highest pooled number of alien species (Table 3). In particular, the most

198   abundant aliens were *Xanthium orientale* subsp. *italicum* and *Oenothera biennis*, which reached a

199   frequency, respectively, of 21% and 20% in accreting dunes.

200

201   *Species rarefaction curves and beta diversity*

202   The rarefaction curves obtained by the pooled sample of plots classified by plant community types

203   showed different patterns of species richness (Fig. 2). For a low number of sampled plots ($n < 100$), no

204   appreciable differences emerged between plant communities. When the number of plots increased, the

205   species RC of mobile dunes was steeper and higher than the one of transition dunes, which was

206   intermediate, and curve of upper beach-embryo dunes, which was the lowest. All curves showed an

207   asymptotic pattern (Fig. 2). Considering beta diversity, we observed the highest values for upper beach-

208   embryo dunes (for both the MP and MSRM nature areas), while mobile and transition dunes showed

209   comparatively less compositional differentiation (Table 4). Even when plots from the two parks were

210   merged together, the previous observed patters did not change (as expected, however, the mean beta

211   values increased). Interestingly, tests for beta dispersion resulted significant at both the parks and the

212   whole aggregate dataset scales, indicating a substantial difference in plant assemblages occurring in these

213   habitats, independent by the specific area analysed.

214   The rarefaction curves obtained by the pooled sample of plots classified by the coastal dynamics

215   (Fig. 3) showed no differences between accreting (CD1) and erosive (CD3) sand dune systems. Instead,

216   the curve of stable coastal units (CD2) was the steepest, indicating the highest value of species richness

217   and of compositional heterogeneity. In fact, the curves of CD1 and CD3 were flatter than that of CD2. In

218   both Fig. 2 and Fig. 3, the SCR curves increased less steeply than the RC curves resulting in lower

219   estimates of species richness at a given extent.

220

Discussion

In line with existing literature (Acosta et al. 2009; Peyrat and Fichtner 2011; Ciccarelli et al. 2012; Ciccarelli 2014), the total species richness recorded in this study cannot be considered high. In fact, it is well known that in coastal dunes species composition and abundance is reduced by extreme abiotic factors (Ranwell 1972; Roman and Nordstrom 1988; Clark 1977; Hesp 1991; Sykes and Wilson 1991; Maun 1997; Stallins 2002; Stallins and Parker 2003; da Silva et al. 2008; Miller et al. 2010; Ciccarelli et al. 2014).

Both RC and SCR curves calculated for each community type showed an asymptotic pattern (Fig. 2), suggesting that almost all species have been recorded in the study area. This is a good result, because as seen in other studies (Chiarucci et al. 2008a- b; 2012; Acosta et al. 2009), the question of "sampling effort" is crucial to quantify species richness in a study area. If under-reporting occurred, then many rare and endangered species may not have been recognised, which could have important implications for conservation programs.

Several studies provided evidence of a correlation between species diversity and the coast-to-inland environmental gradient: total species richness generally increases as one moves from the annual communities of the upper beach (more instable habitats) towards the fixed dunes (more stable environments) along the psammophilous sequence (Foster and Tilman 2000; Acosta et al. 2009; Kuiters et al. 2009; Miller et al. 2010; Isermann 2011; Peyrat and Fichtner 2011; Vaz et al. 2013). The present study showed both the lowest values of species richness and pooled number of species for upper beach-embryo dunes confirming the most stressful conditions close to the shoreline. On the other hand, mobile dunes and transition dunes are characterised by higher richness as already shown by Acosta et al. (2009) for coastal dune systems in central Italy. In particular, the highest frequency of *Solidago litoralis* - the only endemic and threatened species recorded in this study - along mobile dunes can be explained by the preference of this plant to live in association with *Ammophila arenaria* communities that are typical of mobile dunes (Vagge and Biondi 1999). In literature it is known that sandy Tuscan coasts are characterised by the presence of three endemics – *Centaurea aplolepa* subsp. *subciliata*, *Limonium etruscum*, and *Solidago litoralis* (see Ciccarelli et al. 2014): only *Solidago litoralis* has been found in our study, because it is distributed along foredunes where transects were placed. Conversely, *C. aplolepa* subsp. *subciliata*, which is present only in MSRM park, has not been recorded because it lives in

250   backdune areas; and *L. etruscum*, a rare endemic taxon occurring in MP park, is typical of humid dune

251   slacks, not included in this study.

252   Alien species – especially *Xanthium orientale* subsp. *italicum* – seem to prefer upper beach-

253   embryo dunes habitat, where *X. orientale* subsp. *italicum* competes against *Cakile maritima* which is the

254   native psammophilous species of these environments (EEC 1992). This alien species could take

255   advantage of the local enrichment in organic matter and nitrogen content – caused by natural or human

256   factors - in the pioneer habitats of the foredune zone, which are generally characterised by poor soils. In

257   fact, previous studies performed in the Mediterranean region on coastal sand dunes highlighted the link

258   between soil modifications of poor substrates and alien species invasions (Santoro et al. 2011; Del

259   Vecchio et al. 2013). Future research on this topic would greatly benefit from a comparison between

260   diversity patterns for the different species groups here considered (total, endemic and alien taxa, for an

261   example see Bacaro et al. 2015), as it would allow identifying those species groups that are facilitated,

262   mediated or inhibited by environmental factors (such as disturbance regime, stress gradients) associated

263   with coast coarse scale variation.

264   When analysing species rarefaction curves obtained by the plots classified as plant community

265   types (Fig. 2), differences emerged especially between mobile dunes and the other plant assemblages. In

266   particular, when the number of accumulated plots increased, the SCR curve of mobile dunes was the

267   steepest and highest, while the SCR curve of upper beach-embryo dunes was the flattest (Fig. 2). These

268   results suggest that plant communities of upper beach-embryo dunes are characterised by a lower number

269   of species and by a homogeneous species composition within the different coastal sections. In contrast,

270   plant communities of mobile dunes showed a higher species richness whose composition is not always

271   the same, but it depends on the coastal section considered. These results are in accordance with Jiménez-

272   Alfaro et al. (2015) who found that space was the strongest factor influencing metacommunity structure

273   in shifting dunes (identified as habitat 2120 = shifting dunes along the shoreline with *Ammophila*

274   *arenaria*) across the Iberian coastline.

275   Interestingly, at the plot level, patterns of beta diversity were substantially different: when pairwise

276   dissimilarities were averaged, in fact, upper beach-embryo dunes showed the highest beta diversity values

277   (for both parks and also for the pooled set of plots, see Table 4). The observed patterns might be

278   explained by the intrinsic nature of these patchily fragmented and very sparse plant communities: on

279   average, smaller sampling units can exhibit (by chance) either higher or lower similarity than the actual

280   similarity characterising the whole community composition at larger spatial scales, thus introducing a

281   type of stochastic noise (Bacaro et al. 2012c). Generally speaking, smaller sampling units "will have only

282   a subset of the possible species and will contain identical species lists only a portion of the time" (Nekola

283   and White 1999) and will exhibit, on average, higher pairwise dissimilarity. From an ecological

284   perspective, observed patterns express the high environmental heterogeneity that vary dynamically within

285   the coastline area, resulting in a highly differentiated local plant community composition and,

286   consequently, characterised by a very diverse structural and functional vegetation types.

287   As suggested by Honrado et al. (2009), beta diversity may act as reliable indicator of disturbance

288   in foredune vegetation, especially under transgressive dynamics which characterise dune fields where

289   sand is blowing over vegetated to semi-vegetated terrain (Hesp and Walker 2013). In our study area, the

290   presence of intermediate levels of stress and/or disturbance could promote complex variations of

291   environmental conditions at fine scales (see Lomba et al. 2008; Vaz et al. 2013; Ciccarelli 2015).

292   Moreover, our research found evidence that species richness was higher in stable coastal dunes

293   than in accreting and erosive dunes (Table 3, Fig. 3). In fact, it is widely accepted that in dunes, sand

294   dynamics (accumulation as well as erosion) creates periodic vegetation disturbances affecting

295   composition variability and succession (Jungerius et al. 1995; Isermann 2011). These results are partially

296   reflected in the findings of Gallego-Fernández and Martínez (2011), who observed that species richness

297   and diversity were significantly lower on accreting foredunes than on stable or erosive foredunes along

298   the Gulf of Mexico. Recently, Honrado et al. (2009) found that under transgressive dynamics, the species

299   *Ammophila arenaria* becomes dominant along the northern coastline of Portugal, not only because of its

300   ability to tolerate deep sand burial (Maun 1997; Levin et al. 2008), but also for the reduction or

301   disappearance of other species. Similarly, Ciccarelli et al. (2012) showed that coastal tracts of the

302   Migliarino-San Rossore-Massaciuccoli Regional Park affected by high levels of erosional processes were

303   characterised by instable plant communities with the predominance of *Ammophila arenaria*. In this study,

304   the highest mean number of endemics per plot and the highest pooled number of alien species in stable

305   dunes could suggest that shoreline accretion as well as erosional processes may disturb both endemic

306   survival and alien plant invasion. Interestingly, the most frequent alien species found in erosive dunes was

11

307 *Arundo donax*, which is an invasive tall perennial cane (Arrigoni and Viegi 2011), highly resistant to

308 disturbance.

309　　Finally, regarding the method for calculating species rarefaction curves, we observed a general

310 overestimation of species richness by the traditional way to calculate rarefaction (Fig. 2-3): observed

311 divergences between classic rarefactions and SCRs represent the manifest effects of the spatial

312 dependence in the distribution of individuals in the space. While RCs are based on too restrictive

313 statistical assumptions (i.e., the spatial distribution of individuals in the environment is random- for a

314 complete list of assumption see also Gotelli and Colwell 2001), their spatially explicit counterparts allow

315 ecologists and conservation biologists to produce curves which compare actual patterns of species

316 richness and composition. Too often rarefaction curves have been used to compare biodiversity between

317 areas in order to plan conservation strategies but, if their spatial component are not explicitly measured,

318 their use could completely reverse the ranking of protected areas based on species richness value obtained

319 by their calculation, leading to an erroneous prioritisation of sites. In our analyses, once the spatial

320 autocorrelation in the distribution of data was taken in account, we observed that the MP resulted more

321 diversified than MSMR (see Bacaro et al. submitted for a specific discussion on this and other examples).

322 From a practical point of view, this example shows how the inclusion of spatial autocorrelation into

323 rarefaction analyses can alter conclusions and eventually even change the way we might prioritise or

324 manage nature reserves. Similar patterns were also observed by Kühn (2007) analysing data on a study

325 that explored the relationship between plant species richness and environmental correlates in Germany: a

326 dramatic change in sign (from positive to negative) effect was observed when a spatial simultaneous

327 linear error model was applied to describe the relationship between the altitudinal gradient and species

328 richness.

329

330 **Conclusions**

331 The present study highlights that quantitatively comparing the species diversity of different plant

332 communities along the coast-to-inland zonation and of coastal sections affected by different dynamics by

333 using spatial constrained rarefaction techniques is a useful method to assess biodiversity for coastal dune

334 ecosystems. In particular, for a successful biodiversity conservation program in these coastal ecosystems,

335 it is recommended to preserve all the habitats of the whole coast-to-inland succession because all of them

336   contribute to its functioning. The fact that several plant communities are characterised by a higher beta

337   diversity suggests that conservation programs need to consider local scale variability in order to maintain

338   regional diversity. Moreover, from this study emerges that erosion is an important factor of disturbance

339   that causes the loss of biodiversity. Hence, managers are encouraged to find the most appropriate

340   techniques to protect the eroding foredunes. Lastly, we can conclude that it is fundamental to preserve the

341   natural dynamics of these ecosystems in order to maintain their biodiversity and sustain ecosystem

342   services, such as coastal protection (Martínez et al. 2004; 2006; Provoost et al. 2004).

343

346

347 **References**

348 Acosta A, Carranza ML, Izzi CF (2009) Are there habitats that contribute best to plant species diversity in

349   coastal dunes? *Biodivers Conserv* 18: 1087–1098

350 Acosta A, Ercole S, Stanisci A, De Patta Pillar V, Blasi C (2007) Coastal vegetation zonation and dune

351   morphology in some Mediterranean ecosystems. *J Coast Res* 23: 1518-1524

352 Anfuso G, Pranzini E, Vitale G (2011) An integrated approach to coastal erosion problems in northern

353   Tuscany (Italy): Littoral morphological evolution and cell distribution. *Geomorphology* 129: 204–

354   214

355 Arrigoni PV, Viegi L (2011) *La flora vascolare spontaneizzata della Toscana.* Regione Toscana,

356   Florence, Italy (in Italian)

357 Avis AM, Lubke RA (1996) Dynamics and succession of coastal dune vegetation in the Eastern Cape,

358   South Africa. *Landscape Urban Plan* 34: 347–354

359 Bacaro G, Gioria M, Ricotta C (2012b) Testing for differences in beta diversity from plot-to-plot

360   dissimilarities. *Ecol Res* 27: 285-292

361 Bacaro G, Gioria M, Ricotta C (2013) Beta diversity reconsidered. *Ecol Res* 28: 537-540

362 Bacaro G, Rocchini D, Duprè C, Diekmann M, Carnesecchi F, Gori V, Chiarucci A (2012c) Absence of

363   distance decay in the similarity of plots at small extent in an urban brownfield. *Comm Ecol* 13: 36-

364   44

365 Bacaro G, Rocchini D, Ghisla A, Marcantonio M, Neteler M, Chiarucci A (2012a) The spatial domain

366   matters: Spatially constrained species rarefaction in a free and open source environment. *Ecol*

367   *Complex* 12: 63-69

368 Bacaro G, Altobelli A, Camelletti M, Ciccarelli D, Martellos S, Palmer MW, Ricotta C, Rocchini D,

369   Scheiner SM, Chiarucci A (2015) Incorporating spatial autocorrelation in rarefaction methods:

370   implications for ecologists and conservation biologists (submitted)

371 Bacaro G, Maccherini S, Chiarucci A, Jentsch A, Rocchini D, Torri D, Gioria M, Tordoni E, Martellos S,

372   Altobelli A, Escudero CE, Arevalo JR, Otto R, Fernandez-Lugo S, Fernandez-Palacios JM, Arevalo

373   JM (2015) Distributional patterns of endemic, native and alien species along a roadside elevation

374   gradient in Tenerife, Canary Island. *Comm Ecol* (in press)

375   Biondi E, Blasi C, Burrascano S, et al (2009) *Manuale Italiano di Interpretazione degli Habitat della*

376   *Direttiva 92/43/CEE. Società Botanica Italiana*. Ministero dell'Ambiente e della tutela del

377   Territorio e del Mare, Direzione Protezione della Natura. Available at: http://vnr.unipg.it/habitat/

378   Brown AC, McLachlan A (2002) Sandy shore ecosystems and the threats facing them: some predictions

379   for the year 2025. *Environ Conserv* 29: 62-77

380   Carboni M, Carranza ML, Acosta A (2009) Assessing conservation status on coastal dunes: A multiscale

381   approach. *Landscape Urban Plan* 91: 17-25

382   Chiarucci A, Bacaro G, Filibeck G, Landi S, Maccherini S, Scoppola A (2012) Scale dependence of plant

383   species richness in a network of protected areas. *Biodivers Conserv* 21: 503-516

384   Chiarucci A, Bacaro G, Rocchini D (2008a) Quantifying plant species diversity in a Natura 2000

385   network: Old ideas and new proposals. *Biol Conserv* 141: 2608–2618

386   Chiarucci A, Bacaro G, Rocchini D, Ricotta C, Palmer MW, Scheiner SM (2009) Spatially Constrained

387   Rarefaction, incorporating the autocorrelated structure of biological communities into sample-based

388   rarefaction. *Comm Ecol* 10: 209-214

389   Chiarucci A, Bacaro G, Vannini A, Rocchini D (2008b) Quantifying species richness at multiple spatial

390   scales in a Natura 2000 network. *Comm Ecol* 9: 185–192

391   Ciccarelli D (2014) Mediterranean coastal sand dune vegetation: Influence of natural and anthropogenic

392   factors. *Environ Manage* 54: 194-204

393   Ciccarelli D (2015) Mediterranean coastal dune vegetation: Are disturbance and stress the key selective

394   forces that drive the psammophilous succession? *Estuar Coast Shelf Sci* doi:

395   10.1016/j.ecss.2015.05.023

396   Ciccarelli D, Bacaro G, Chiarucci A (2012) Coastline dune vegetation dynamics: Evidence of no stability.

397   *Folia Geobot* 47: 263-275

398   Ciccarelli D, Di Bugno C, Peruzzi L (2014) Checklist della flora vascolare psammofila della Toscana.

399   *Atti Soc Tosc Sci Nat, Mem, Serie B* 121: 37-88 (in Italian)

400   Clark JR (1977) *Coastal environment management*. Wiley & Sons, Chichester

401   Collins M, Simberloff D (2009) Rarefaction and nonrandom spatial dispersion patterns. *Environ Ecol Stat*

402   16: 89-103

403 Conti F, Abbate G, Alessandrini A, Blasi C (2005) *An annotated checklist of the Italian vascular flora.*

404   Palombi Editors, Rome, Italy (in Italian)

405 Conti F, Alessandrini A, Bacchetta G, et al (2007) Integrazioni alla checklist della flora vascolare italiana.

406   *Natura Vicentina* 10: 5-74 (in Italian)

407 Coombes EG, Jones AP, Sutherland WJ (2008) The biodiversity implications of changes in coastal

408   tourism due to climate change. *Environ Conserv* 35: 319–330

409 Cori B (1999) Spatial dynamics of Mediterranean coastal regions. *J Coast Conserv* 5: 105-112

410 Crist TO, Veech JA (2006) Additive partitioning of rarefaction curves and species-area relationships:

411   unifying alpha-, beta- and gamma-diversity with sample size and habitat area. *Ecol Lett* 9: 923-932

412 da Silva MG, Hesp P, Peixoto J, Dillenburg SR (2008) Foredune vegetation patterns and alongshore

413   environmental gradients: Moçambique Beach, Santa Catarina Island, Brazil. *Earth Surf Proc Land*

414   33: 1557–1573

415 Del Vecchio S, Acosta A, Stanisci A (2013) The impact of *Acacia saligna* invasion on Italian coastal

416   dune EC habitats. *CR Biol* 336: 364-369

417 Doing H (1985) Coastal fore-dune zonation and succession in various parts of the world. *Vegetatio* 61:

418   65–75

419 Doody JP (2013) Sand dune conservation, management and restoration. Springer-Verlag, Dordrecht,

420   Heidelberg, New York, London

421 EEC (1992) Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of

422   wild fauna and flora. *Off J Eur Comm, Series L* 206: 7-50

423 Foster BL, Tilman D (2000) Dynamic and static views of succession: testing the descriptive power of the

424   chronosequence approach. *Plant Ecol* 146: 1-10

425 Frederiksen L, Kollmann J, Vestergaard P, Bruun HH (2006) A multivariate approach to plant community

426   distribution in the coastal dune zonation of NW Denmark. *Phytocoenologia* 36: 321–342

427 Gallego-Fernández JB, Martínez ML (2011) Environmental filtering and plant functional types on

428   Mexican foredunes along the Gulf of Mexico. *Ecoscience* 18: 52-62

429 García-Mora MR, Gallego-Fernández JB, García-Novo F (1999) Plant functional types in coastal

430   foredunes in relation to environmental stress and disturbance. *J Veg Sci* 10: 27–34

431   Gornish ES, Miller TE (2010) Effects of storm frequency on dune vegetation. *Global Change Biol* 16:

432   2668–2675

433   Gotelli NJ, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and

434   comparison of species richness. *Ecol Lett* 4: 379-391

435   Gruppo Nazionale per la ricerca sull'ambiente costiero (2006) Lo stato dei litorali italiani. *Studi Costieri*

436   10: 1–174 (in Italian)

437   Hawksworth DL, Bull AT (2008) *Biodiversity and Conservation in Europe*. Springer-Verlag, Dordrecht,

438   Heidelberg, New York, London

439   Hesp PA (1991) Ecological processes and plant adaptations on coastal dunes. *J Arid Environ* 21: 165–191

440   Hesp PA, Walker IJ (2013) Coastal dunes. In Shroder J (Editor in Chief), Lancaster N, Sherman DJ, Baas

441   ACW (eds) *Treatise on Geomorphology*. Academic Press, San Diego, CA, vol. 11, Aeolian

442   Geomorphology, pp. 328-355

443   Honrado J, Vicente J, Lomba A, Alves P, Macedo JA, Henriques R, Granja H, Caldas FB (2009) Fine-

444   scale patterns of vegetation assembly in the monitoring of changes in coastal sand-dune landscapes.

445   *Web Ecol* 10: 1-14

446   Isermann M (2011) Patterns in species diversity during succession of coastal dunes. *J Coast Res* 27: 661-

447   671

448   Janisova M, Michalcova D, Bacaro G, Ghisla A (2014) Landscape effects on diversity of seminatural

449   grasslands. *Agric Ecosys Environ* 182: 47-58

450   Jiménez-Alfaro B, Marcenò C, Guarino R, Chytry M (2015) Regional metacommunities in two coastal

451   systems: spatial structure and drivers of plant assemblages. *J Biogeogr* 42: 452-462

452   Jungerius PD, Koehler H, Kooijman AM, Mücher HJ, Graefe U (1995) Response of vegetation and soil

453   ecosystem to mowing and sod removal in the coastal dunes "Zwanenwater", the Netherlands. *J*

454   *Coast Conserv* 1: 3-16

455   Kinzig A, Pacala SW, Tilman D (2002) *The Functional Consequences of Biodiversity.* Princeton

456   University Press, Princeton, NJ

457   Kobayashi S (1974) The species-area relation I. A model for discrete sampling. *Res Popul Ecol* 15: 223-

458   237

459   Koellner T, Herperger AM, Wohlgemuth T (2004) Rarefaction method for assessing plant species

460   diversity on a regional scale. *Ecography* 27: 532-544

461   Kühn I (2007) Incorporating spatial autocorrelation may invert observed patterns. *Diversity Distrib* 13:

462   66-69

463   Kuiters AT, Kramer K, Van der Hagen HGJM, Schaminée JHJ (2009) Plant diversity, species turnover

464   and shifts in functional traits in coastal dune vegetation: Results from permanent plots over a 52-

465   year period. *J Veg Sci* 20: 1053-1063

466   Levin N, Kidron GJ, Ben-Dor E (2008) A field quantification of coastal dune perennial plants as

467   indicators of surface stability, erosion or deposition. *Sedimentology* 55: 751-772

468   Lomba A, Alves P, Honrado J (2008) Endemic sand dune vegetation of the Northwest Iberian Peninsula:

469   Diversity, dynamics, and significance for bioindication and monitoring of coastal landscapes. *J*

470   *Coast Res* 24: 113-121

471   Loreau M, Naeem S, Inchausti P (2002) *Biodiversity and Ecosystem Functioning: Synthesis and*

472   *Perspectives.* Oxford University Press, Oxford

473   Lubke RA, Hertling UM (2001) The role of European marram grass in dune stabilization and succession

474   near Cape Agulhas, South Africa. *J Coast Conserv* 7: 171–182

475   Martínez ML, Maun AM, Psuty N (2004) The fragility and conservation of the world's coastal dunes:

476   geomorphological, ecological and socioeconomic perspectives. In Martínez ML, Psuty N (eds)

477   *Coastal dunes: ecology and conservation*. Springer-Verlag, Berlin, Heidelberg, New York, pp. 355–

478   369

479   Martínez ML, Gallego-Fernández JB, García-Franco JG, Moctezuma C, Jiménez CD (2006) Assessment

480   of coastal dune vulnerability to natural and anthropogenic disturbances along the Gulf of Mexico.

481   *Environ Conserv* 33: 109–117

482   Maun MA (1997) Adaptations of plants to burial in coastal sand dunes. *Canad J Bot* 76: 713–738

483   Miller TE, Gornish ES, Buckley H (2010) Weather and coastal vegetation: effects of storms and drought.

484   *Plant Ecol* 206: 97–104

485   Moreno CE, Halffter G (2001) On the measure of sampling effort used in species accumulation curves. *J*

486   *Appl Ecol* 38: 487-490

487   Nekola JC, White PS (1999) The distance decay of similarity in biogeography and ecology. *J Biogeogr*

488   26: 867-878

489   Peyrat J, Fichtner A (2011) Plant species diversity in dry coastal dunes of the southern Baltic coast.

490   *Comm Ecol* 12: 220-226

491   Pinna M (1985) Contributi di Climatologia. *Mem Soc Geog Ital* 39: 147-167 (in Italian)

492   Provoost S, Ampe C, Bonte D, Cosyns E, Hoffmann M (2004) Ecology, management and monitoring of

493   grey dunes in Flanders. *J Coast Conserv* 10: 33–42

494   Psuty N (2004) The coastal foredune: a morphological basis for regional coastal dune development. In

495   Martínez ML, Psuty N (eds) *Coastal dunes: ecology and conservation*. Springer-Verlag, Berlin,

496   Heidelberg, New York, pp. 11–27

497   Pullin AS (2002) *Conservation Biology*. Cambrige University Press, Cambridge

498   R Development Core Team (2015) R: A language and environment for statistical computing. R

499   Foundation for Statistical Computing. Vienna, Austria. Available at: http://cran.r-project.org/

500   Ranwell DS (1972) *Ecology of salt marshes and sand dunes*. Chapman & Hall, London, Great Britain

501   Rapetti F (2003) Il clima. In AAVV (eds) *Atlante tematico della Provincia di Pisa*. Pacini Editor, Pisa,

502   Italy, pp. 16-17 (in Italian)

503   Roman CT, Nordstrom KF (1988) The effects of erosion rate on vegetation patterns of an east coast

504   barrier island. *Estuar Coast Shelf Sci* 26: 233–242

505   Rossi G, Montagnani C, Gargano D, et al (2013) *Lista Rossa della Flora Italiana. 1. Policy Species e*

506   *altre specie minacciate*. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del

507   Territorio e del Mare. Stamperia Romana, Roma, Italy

508   Santoro R, Jucker T, Carranza ML, Acosta ATR (2011) Assessing the effects of *Carpobrotus* invasion on

509   coastal dune soils. Does the nature of the invaded habitat matter? *Comm Ecol* 12: 234-240

510   Schulze ED, Mooney HA (1993) *Biodiversity and Ecosystem Function*. Springer-Verlag, Dordrecht,

511   Heidelberg, New York, London

512   Stallins JA (2002) Dune plant species diversity and function in two barrier island biogeomorphic systems.

513   *Plant Ecol* 165: 183-196

514   Stallins JA, Parker AJ (2003) The influence of complex systems interactions on barrier island dune

515   vegetation pattern and process. *Ann Assoc Amer Geogr* 93: 13–29

516   Sykes MT, Wilson JB (1991) Vegetation of a coastal sand dune system in southern New Zealand. *J Veg*

517   *Sci* 2: 531–538

518   Vagge I, Biondi E (1999) La vegetazione delle coste sabbiose del Tirreno settentrionale italiano.

519   *Fitosociologia* 36: 61-95 (in Italian)

520   Van der Maarel E (2003) Some remarks on the functions of European coastal ecosystems.

521   *Phytocoenologia* 33: 187–202

522   Van der Maarel E, Van der Maarel-Versluys M (1996) Distribution and conservation status of littoral

523   vascular plant species along the European coasts. *J Coast Conserv* 2: 73-92

524   Vaz AS, Macedo JA, Alves P, Honrado JP, Lomba A (2013) Plant species segregation in dune

525   ecosystems emphasises competition and species sorting over facilitation. *Plant Ecol Divers* doi:

526   http://dx.doi.org/10.1080/17550874.2013.843210

527   Wiedemann AM, Pickart A (2004) Temperate zone coastal dunes. In Martínez ML, Psuty N (eds) *Coastal*

528   *dunes: ecology and conservation*. Springer-Verlag, Berlin, Heidelberg, New York, pp. 53–65

529

530 **Figures captions**

531 **Fig. 1** Location of the Migliarino-San Rossore-Massaciuccoli (MSRM) Regional Park and the Maremma

532 Regional Park in the northern and southern parts of Tuscany (Italy), respectively. On the right a picture of

533 Italy with Tuscany

534 **Fig. 2** Plot-based rarefaction curves for the pooled sample of plots classified by plant community type

535 (see Table 1). The SCR curves show a lower amount of accumulated species given the same number of

536 sampled plots, with respect to the traditional exact-based method (RC)

537 **Fig. 3** Plot-based rarefaction curves for the pooled sample of plots classified by the three coastal dynamic

538 types: CD1 (accreting coastline), CD2 (stable coastline) and CD3 (eroding coastline). The SCR curves

539 show a lower amount of accumulated species given the same number of sampled plots, with respect to the

540 traditional exact-based method (RC)

Table 1 Plant communities used in this study: distribution along the sea-inland gradient, description of plant communities, habitat code according to the Directive of the Council of European Communities 92/43/EEC (EEC 1992), and main diagnostic species

Coastal dune zonation	Upper beach and Embryo dunes	Mobile dunes	Transition dunes
Plant community description	Formations of annual plants occupying upper beach, and pioneer perennial assemblages representing the first stages of dune construction	Mobile dunes forming the seaward cordon or cordons of dune systems of the coasts	Perennial communities of the inland side of mobile dunes, dominated by chamaephytic species forming a mosaic with annual grasses
Habitat code according to the Directive 92/43/EEC	1210 – Annual vegetation of drift lines 2110 - Embryonic shifting dunes	2120 – Shifting dunes along the shoreline with *Ammophila arenaria* (white dunes)	2210 – *Crucianellion maritimae* fixed beach dunes 2230 – *Malcolmietalia* dune grasslands
Main diagnostic species	*Cakile maritima*, *Salsola kali*, *Elymus farctus* subsp. *farctus*, *Sporobolus virginicus*	*Ammophila arenaria*	*Crucianella maritima*, *Pycnocomon rutifolium*, *Seseli tortuosum*, *Malcolmia ramosissima*, *Silene canescens*, *Vulpia fasciculata*

Table 2 Species richness, number of endemics and alien species per plot, and their pooled numbers in each plant community type. Means followed by the same letters are not significantly different at 5% according to the non-parametric Kruskal-Wallis one-way ANOVA after the Bonferroni correction for multiple comparisons. SD = standard deviation

Plant community type	Number of plots	Number of species per plot (mean ± SD)	Pooled number of species	Number of endemics per plot (mean ± SD)	Pooled number of endemics	Number of alien species per plot (mean ± SD)	Pooled number of alien species
Upper beach and Embryo dunes	429	3.52 ± 1.50a	46	0.01 ± 0.10a	1	0.32 ± 0.49b	5
Mobile dunes	250	4.44 ± 1.92b	47	0.12 ± 0.32b	1	0.18 ± 0.38a	4
Transition dunes	301	4.18 ± 1.90b	47	0.02 ± 0.13a	1	0.18 ± 0.39a	4

Table 3 Species richness. number of endemics and alien species per plot, and their pooled numbers in each coastal dynamic type. Means fo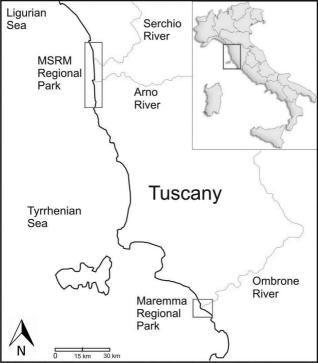llowed by the same letters are not significantly different at 5% according to the non-parametric Kruskal-Wallis one-way ANOVA after the Bonferroni correction for multiple comparisons. SD = standard deviation
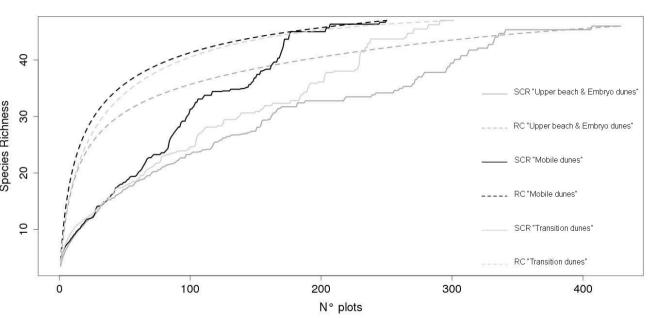
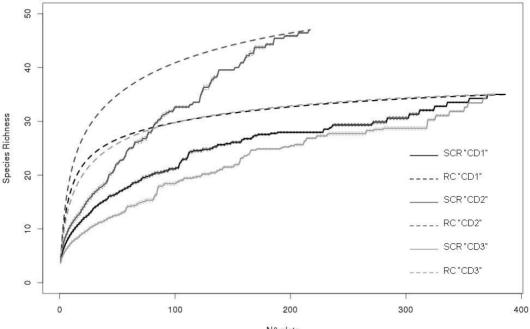| Coastal dynamic type	Number of plots	Number of species per plot (mean ± SD)	Pooled number of species	Number of endemics per plot (mean ± SD)	Pooled number of endemics	Number of alien species per plot (mean ± SD)	Pooled number of alien species
CD1 - Accreting	386	3.94 ± 1.70a	34	0.01 ± 0.11a	1	0.41 ± 0.50b	2
CD2 - Stable	217	4.46 ± 1.71b	46	0.16 ± 0.36b	1	0.40 ± 0.56b	6
CD3 – Erosive	377	3.71 ± 1.85a	34	0.00 ± 0.00a	1	0.05 ± 0.21a	3

Table 4 Permutational analysis of variance for significant differences in beta diversity between dune habitats in MP (Maremma Park), MSRM (Migliarino – San Rossore - Massaciuccoli Park) and in both parks. The Jaccard dissimilarity for presence/absence data was used for calculating plot-to-plot dissimilarity. p-values were obtained by permutation of within-group dissimilarities (999 permutations)

Dataset	Average Beta Upper beach-Embryo Dunes	Average Beta Mobile Dunes	Average Beta Transition Dunes	F model	p-value
MP	0.768	0.612	0.652	580.34	0.001
MSRM	0.711	0.684	0.622	1103.1	0.001
Full Dataset	0.784	0.725	0.652	5138	0.001

Ligurian Sea

Serchio River

MSRM Regional Park

Arno River

Tuscany

Tyrrhenian Sea

Ombrone River

Maremma Regional Park

N

0   15 km   30 km