

1 **Dark diversity reveals importance of biotic resources and competition**
2 **for plant diversity across broad environmental gradients**

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10

11 **Abstract**

12 Species richness is the most commonly used metric to quantify biodiversity. However, examining dark
13 diversity, the group of missing species which can potentially inhabit a site, can provide a more thorough
14 understanding of the processes influencing observed biodiversity and help evaluate the restoration potential
15 of local habitats. So far, dark diversity has mainly been studied for specific habitats or largescale landscapes
16 while less attention has been given to variation across broad environmental gradients or as a result of local
17 conditions and biotic interactions. In this study, we investigate the importance of local environmental
18 conditions in determining dark diversity and observed richness in plant communities across broad
19 environmental gradients. We use the ecospace concept to investigate how abiotic gradients (defined as
20 position), availability of biotic resources (defined as expansion), spatiotemporal extent of habitats (defined as
21 continuity), as well as species interactions through competition, relate to these biodiversity measures.
22 Position variables were important for both plant richness and dark diversity, some with quadratic
23 relationships, e.g., plant richness showing a unimodal response to soil fertility corresponding to the
24 intermediate productivity hypothesis. Competition represented by community mean Grime C showed a
25 negative correlation with plant richness. Besides position, organic carbon was the most important variable
26 for dark diversity, indicating that in late succession habitats such as forests and shrubs, dark diversity is
27 generally low. The importance of Grime C indicate that intermediate disturbance, such as grazing, may
28 facilitate higher species richness and lower dark diversity. Comparing various biodiversity metrics and their
29 influencing factors might reveal important drivers of biodiversity changes and result in better conservation
30 decision-making.

31 **Keywords**

32 Beal's smoothing, community completeness, conservation, regional species pool, species co-occurrence,
33 vascular plants

34 **Introduction**

35 The global biodiversity crisis represents one of the most critical challenges in the 21st century, with up to
36 one million plant and animal species facing extinction and accelerating declines despite numerous
37 international agreements and management responses (Tittensor et al., 2014, Butchart et al., 2010, Díaz et al.,
38 2019). Achieving conservation goals and prioritizing efforts requires appropriate metrics to quantify
39 biodiversity and identify the factors driving the declines. The most commonly used measure is observed
40 species richness which traditionally depends on visual surveys to count the individual species. Although
41 observed diversity can provide valuable insights into the richness of species within a given site, it does not
42 account for the absent part of the species pool that could potentially inhabit that site based on suitable
43 environmental conditions and biogeographic history, i.e., the dark diversity (Pärtel et al., 2011). Identifying

44 this part of the biodiversity can provide a more thorough understanding of the processes influencing
45 biodiversity and help evaluate the restoration potential of local habitats (Lewis et al., 2016).

46 In contrast to observed diversity, dark diversity focuses on the portion of diversity potentially able to
47 occur in a particular habitat type. This metric can provide insight into the determinants of missing species by
48 helping us understand why certain species are missing more often than others and the characteristics of sites
49 typically missing many species that could potentially exist there. Quantifying dark diversity patterns, in
50 combination with observed diversity patterns, may allow researchers to better understand the mechanisms
51 and processes acting on individual populations or entire communities (Pärtel et al., 2017b). So far, the
52 potential value of dark diversity to guide conservation and restoration planning has been demonstrated for
53 mammals (Estrada et al., 2018), sharks (Boussarie et al., 2018), and fungi (Pärtel et al., 2017b, Pärtel et al.,
54 2017a). However, most of its potential as a conservation tool has been realized in plants and has been used in
55 conservation prioritization of plant communities across Europe (Moeslund et al., 2017) and determining the
56 relationship between dark diversity and the invasion potential of alien species in semi-natural grasslands
57 (Bennett et al., 2016). Dark diversity has also proven valuable in understanding plant diversity patterns, such
58 as determining that vascular plant dark diversity across Europe follows a latitudinal gradient (Ronk et al.,
59 2015). So far, the plant traits likely to increase a species' probability of being part of the dark diversity
60 include stress intolerance, tall, adaption to low light and nutrient levels, and producing fewer and heavier
61 seeds (Riibak et al., 2017, Moeslund et al., 2017, Riibak et al., 2015). Understanding the ecological processes
62 governing plant dark diversity is important since vascular plants can not only predict biodiversity across
63 environmental gradients and broad taxonomic realms, but are also related to the occurrence of regionally red-
64 listed species of other taxa (Brunbjerg et al., 2018). Furthermore, plants are bio-indicators of their abiotic
65 environment and anthropogenic impact (Bartelheimer and Poschlod, 2016), and they form the living and
66 dead organic carbon pools and biotic surfaces that are the niche space for all other taxonomic groups
67 (Brunbjerg et al., 2017b). Nevertheless, as a relatively new concept, more research is required to establish its
68 full potential and to understand the ecological processes governing dark diversity across plant communities.

69 Dark diversity can also be used to derive community completeness, a relativized biodiversity index,
70 which has been proposed as a valuable tool for facilitating biodiversity comparisons irrespective of regions,
71 ecosystems, and taxonomic groups (Pärtel et al., 2013). The community completeness index can be defined,
72 in general terms, as the proportion of species from the regional species pool which have dispersed to and
73 established at a site after abiotic and biotic filtering (Pärtel et al., 2013). Since patterns in observed species
74 richness may mimic patterns in dark diversity (e.g., exhibit a strong latitudinal gradient) (Aning, 2017, Ronk
75 et al., 2015, Pärtel et al., 2011, Zobel, 1997), community completeness can provide a better measure of
76 biodiversity as it accounts for the variation in species pool size and expresses biodiversity on a relative scale
77 (Pärtel et al., 2013). For instance, in previous studies, completeness exhibited no relationships to latitudinal
78 gradients, but strong relations to anthropogenic disturbance (higher completeness in areas with lower

79 disturbance) for fungi (Pärtel et al., 2017b), plants (Ronk et al., 2015, Ronk et al., 2016), and birds (Cam et
80 al., 2000). Comparing the environmental processes influencing these biodiversity measurements can provide
81 valuable information for better prioritization of resources and understanding patterns of biodiversity.
82 However, despite observed species richness and its determining factors being relatively established, dark
83 diversity and its completeness counterpart are new methodologies, and as such, have not been well
84 investigated and the factors influencing them are not fully understood.

85 Since biodiversity varies greatly across ecosystems and are highly dependent on the habitat and region of
86 interest, dark diversity and completeness aims to reconcile the role of local (biotic interactions, abiotic filters,
87 dispersal, stochastic events) and large-scale processes (species diversification and historic migration
88 patterns) underlying biodiversity patterns (Pärtel, 2014, Pärtel et al., 2011). Determining the set of species
89 which can theoretically inhabit a site, the species pool, is typically estimated using species co-occurrence
90 patterns with Beal's smoothing which assumes species with shared ecological requirements and
91 biogeographical history will have similar likelihoods of being present at a particular site (de Bello et al.,
92 2012, Lewis et al., 2016, Beals, 1984, Münzbergová and Herben, 2004, McCune, 1994). This approach also
93 assumes to account for prevalent competitive interaction which is a major factor influencing species
94 occurrence patterns (de Bello et al., 2012, Cornell and Harrison, 2014), especially in plant communities
95 (Riibak et al., 2015). Despite co-occurrence being successfully implemented as a proxy for species
96 ecological requirements and competition, this assumption has not been examined. Another potential issue is
97 that many ecological processes are scale dependent with different spatial scales inherently including varying
98 amounts of environmental heterogeneity (Scott et al., 2011). However, most of dark diversity research has
99 ignored the variability between types of habitats and have mostly been restricted to narrow sets of variables
100 and specific habitats (Riibak et al., 2015) or largescale landscapes (Ronk et al., 2016, Ronk et al., 2015), with
101 no studies examining how dark diversity varies across large environmental gradients or the importance of
102 local conditions and biotic interactions. One way to consider the roles these factors play in dark diversity
103 measurements can be provided with the recently developed ecospace framework which recognizes the
104 influence of environmental gradients (defined as position), availability of biotic resources (defined as
105 expansion), and the spatiotemporal extent of habitats (defined as continuity) in determining biodiversity
106 (Brunbjerg et al., 2017b). This framework can help us better quantify and determine the different aspects that
107 each contribute and how they relate to the various diversity metrics.

108 In this study, we investigate the importance of local environmental conditions that determine dark
109 diversity and completeness in plant communities across broad environmental gradients and compare these
110 measures with the observed richness. We use the ecospace concept to investigate how abiotic condition,
111 biotic resources and spatiotemporal processes relate to these biodiversity measures. To examine the
112 assumption that co-occurrence can be a proxy for competitive exclusion, we used the community mean of
113 plant competitive scores (Grime, 1979) to quantify the importance of local interspecific competition for

114 establishment of species. We discuss how dark diversity can contribute with new aspects for informed
115 conservation and management.

116 **Materials and Methods**

117 *Study sites*

118 Our data stems from Biowide, a nationwide survey of biodiversity in Denmark (Brunbjerg et al., 2017a).
119 A total of 130 study sites (40 m × 40 m) were evenly distributed across five geographic regions in Denmark
120 with a minimum distance of 500 m between sites (Fig 1a). Each site is sampled in four 5 m circle plots (Fig
121 1b). Sampling was designed with the purpose of evaluating the ecospace framework, stating that biodiversity
122 varies according to abiotic conditions, build-up and diversification of organic resources and spatio-temporal
123 continuity (Brunbjerg et al., 2017b). The sites were stratified according to environmental gradients and 30
124 sites were allocated to represent cultivated habitats and 100 sites to natural and semi-natural habitats. The
125 cultivated subset was stratified according to major land-use types and the natural subset was stratified
126 according to soil fertility, soil moisture and successional stage. Saline and fully aquatic habitats were
127 deliberately excluded, but temporarily inundated depressions, as well as wet mires and fens were included.
128 The final set of 24 environmental strata consisted of the following six cultivated habitat types: Three types of
129 fields (rotational, grass leys, set aside) and three types of plantations (beech, oak, spruce). The remaining 18
130 strata were natural and semi-natural habitats, constituting all factorial combinations of: fertile and infertile;
131 dry, moist, and wet; open, tall herb/scrub, and forest. All 24 strata were replicated in each of the five
132 geographical regions. For the purpose of the present study, we exclude all agricultural fields, resulting in a
133 dataset of 115 sites. All field work and sampling was conducted in accordance with Responsible Research at
134 Aarhus University and Danish law. For a thorough description of site selection and stratification procedures,
135 see Brunbjerg et al. (2017a).

136 *Data*

137 *Plant species richness*

138 Vascular plant species richness was inventoried by trained botanists during the summer 2014 and spring
139 2015 to account for variations in phenology. The reference dataset of plant inventories in 5 m circles from
140 the national monitoring program (Danish Nature Agency, 2016) was also inventoried by trained botanists.
141 We removed all subspecies, hybrids, variations, and neophytes (i.e. species that are not considered a natural
142 part of the vegetation given their history and dispersal ability, see appendix tables 6–8 in (Buchwald et al.,
143 2013). Plant species nomenclature was obtained from the species checklist Denmark database from
144 <https://allearter-databasen.dk> to match the two datasets and account for different synonyms.

145 *Explanatory variables*

146 We used the ecospace concept to investigate how abiotic condition (i.e., position), biotic resources (i.e.,
147 expansion), and spatiotemporal extent of habitats (i.e., continuity) explain plant richness, dark diversity and
148 community completeness (Table 1). The position variables included in the model were soil moisture index
149 (SMI) and soil fertility index (SFI). For each site SFI represents the predicted value from the best linear
150 model (of all sites) of site mean Ellenberg N (plant-based bioindication of nutrient status; (Ellenberg et al.,
151 1991)) as a function of soil Ca, leaf N, leaf NP and soil type. We calculated a soil moisture index for each
152 site using the predicted values from the best linear model (of all sites) of mean Ellenberg F (plant-based
153 bioindication of soil moisture; Ellenberg et al., 1991) as a function of mean precipitation in 2001-2010 (10
154 km × 10 km grid resolution) and measured site soil moisture (trimmed mean of 16 measures pr. site taken
155 with a FieldScout TDR 300 Soil Moisture Meter in May 2016) (Brunbjerg et al., in prep). Position also
156 included soil pH measured on 4 pooled soil samples from 0-10 cm depth and light measured as light intensity
157 (Lux) using HOBO Pendant® Temperature/Light 8K Data Loggers installed at the ground as detailed in
158 Brunbjerg et al. (2017a). The expansion variables included: 1) Bare soil percent coverage as a subjective
159 estimate, 2) Litter mass (g/m² of four litter samples within a 21 cm × 21 cm frame pr. site). The four
160 samples from each site were pooled, taken to the laboratory, dried (60° for 48 hours) and mass (g/m²) was
161 registered, 3) Soil organic matter as a percentage of the 0-10 cm soil core that was categorized as organic
162 soil, and 4) soil organic carbon as % soil C in 0-10 cm soil layer (g/m² average of four soil samples taken in
163 each site) as described in Brunbjerg et al. (2017a). Expansion also included structural heterogeneity from
164 variation in the digital elevation model (DEM6) (40 cm × 40 cm resolution) from the digital surface model
165 (40 cm × 40 cm resolution) to create a grid representing the above-ground vegetation height. From this the
166 structural heterogeneity was calculated as the sum of variability in the shrub layer and canopy layer,
167 measured as the variance of the 90th percentile for returns > 3 m within the site reflecting the variability of
168 the height of mainly trees and the variance of the 90th percentile for returns 30 cm - 3 m reflecting the
169 variability of the height of the shrub layer (Brunbjerg et al., in prep). Landscape characteristics were
170 represented by two co-variables: 1) share of intensive fields within a 500 m buffer and 2) the share of natural
171 habitats in a 1 km × 1 km grid from a national mapping , interpolated using Spline in ArcGIS 10.2.2 (weight
172 0.5, number of points 9, (Ejrnæs et al., 2014)). Temporal continuity was estimated as time since major land
173 use change within the 40 m × 40 m site. For each site, a temporal sequence of aerial photos and historical
174 maps was inspected starting with the most recent photos (photos from 2014, 2012, 2010, 2008, 2006, 2004,
175 2002, 1999, 1995, 1968, 1954, 1945) and ending with historical maps reflecting land use in the period 1842-
176 1945. Temporal continuity (the year in which a change could be identified) was reclassified into a numeric 4-
177 level variable: 1: 1-14 years, 2: 15-44 years, 3: 45-135 years, 4: >135 years (Brunbjerg et al., 2017a). Lastly,
178 to examine the importance of species interactions and the assumption that co-occurrence can be a proxy for
179 competitive exclusion, we quantified local interspecific competition by calculating an index for the intensity

180 of plant competition (Grime C) using the community mean plant competitive score (Grime, 1979) which is
181 thought to reflect plant species' adaptation to interspecific competition (Grime et al., 2014). The original C-
182 S-R species strategies were recoded into numeric mean site C values (Ejrnæs and Bruun, 2000).

183 *Data analysis*

184 *Regional pool, dark diversity and completeness*

185 All statistical analyses were performed in R version 3.5.3 (R Core Team, 2019). To calculate regional
186 pools, we used 5 m circle plots of observed plant species from both datasets. We did not include species-poor
187 plots, i.e., those with less than five observed species, i.e., resulting in 448 plots from Biowide and 52362
188 plots from the reference data set. The regional pool was calculated using Beals index (Beals, 1984), as
189 recommended by (Lewis et al., 2016). The Beals index represents the probability that a particular species
190 will occur within a given site based on the assemblage of co-occurring species (Beals, 1984, Münzbergová
191 and Herben, 2004, McCune, 1994). We calculated Beals index using the 'beals' function in the 'vegan'
192 package (Oksanen et al., 2017). The threshold for including a particular species in the regional species pool
193 is recommended to be the 5th percentile of the Beals index value for the species in question (Gijbels et al.,
194 2012, Ronk et al., 2015). Preceding the calculation of each threshold, the lowest Beals index value among
195 plots with occurrence of the species in question was identified, and all plots having values below that
196 minimum were not considered.

197 Analyses were done at the site level (n=115) by creating a site regional pool combining the four plot
198 regional pools at each site. Observed species in the site, but not included in the regional pools (n=2) were
199 added to the regional pools to ensure that site regional pool included all observed species. Then, dark
200 diversity was calculated for each plot as the difference between the regional pool and the observed species
201 richness (Pärtel et al., 2011) and completeness was calculated following Pärtel et al. (2013) using the formula
202 $\ln(\text{observed richness}/\text{dark diversity})$. Because dark diversity is relative based on habitat types and may not be
203 suitable for comparison across habitats (Scott et al., 2011), completeness is suggested as possible alternative
204 (Pärtel et al., 2013). Completeness should have been suitable for investigations across habitat types, but in
205 this study we found that completeness was highly correlated with observed species richness (Figure 2).
206 Therefore, we obtained a dark diversity measure corrected for habitat differences by using the residuals of a
207 model of dark diversity as a function of the habitat type, which we termed Bio-stratum, a classification of the
208 sites using all inventoried species data, i.e., plants, fungi, insects, etc., resulting in eight classes spanning
209 gradients in succession (Early, Late), moisture (Wet, Dry) and nutrients (Rich, Poor). The habitat types
210 explained 51% of the variation in dark diversity.

211 *Statistical analyses*

212 Soil pH, litter mass, organic carbon, organic matter and shrub and canopy height variation were log-
213 transformed and all explanatory variables were standardized. We conducted model selection by first testing

214 for multicollinearity, which could affect p-values and model validity using the Variance Inflation Factor
215 (VIF). We then removed canopy height variation and organic matter resulting in a maximum VIF of 2.9. The
216 remaining variables were used as explanatory variables (linear terms) in a set of generalized linear models
217 (GLM) using the MASS package (Venables and Ripley, 2013) with Poisson distribution for the count data
218 for dark diversity and plant richness, and normal distribution for residual dark diversity. However, since
219 models for plant richness were over-dispersed we chose negative binomial models for this response instead.
220 To allow for non-linear relationships for position variables corresponding to the intermediate disturbance
221 hypothesis (Townsend et al., 1997, Connell, 1978) and intermediate productivity hypothesis (Fraser et al.,
222 2015), we used AIC (Burnham and Anderson, 2002) to evaluate if inclusion of quadratic terms for the
223 variables SMI, SFI, Light, soil pH and bare soil improved the model fit, in which case relevant quadratic
224 terms were added. Subsequently, we checked the parameter estimates against plausible ecological
225 hypotheses (Table 1) and excluded ecologically implausible responses (Burnham and Anderson, 2002). We
226 then dropped remaining variables sequentially based on AIC using the lme4 package (Bates et al., 2007). The
227 final models were tested for over-dispersion and evaluated by visual inspection of residual plots and for
228 spatial autocorrelation using Moran's I in the ape package (Paradis et al., 2004).

229 **Results**

230 We found a total of 580 species of vascular plants in the 115 sites spanning from open habitats to shrubs
231 and late succession forests. The species richness per site ranged from 8 to 127 species and dark diversity
232 ranged from 84 to 243 species. Completeness and plant species richness were highly positively correlated,
233 whereas dark diversity was less correlated with plant species richness (Figure 2). The final models explained
234 between 14 and 65 % of the variation in dark diversity, residual dark diversity and species richness (Table 2).
235 We found position variables to be important for dark diversity and plant species richness (Figure 3, 5). SMI
236 invoked a unimodal response in dark diversity but a bimodal response in species richness. We observed a
237 positive effect of SFI and soil pH on dark diversity and unimodal relationships with species richness. Light
238 had a negative linear and unimodal relationship with dark diversity and species richness, respectively. No
239 position variables were found to be important for the residual dark diversity, as would be expected. Organic
240 carbon was important for all measured responses with a linear negative relationship across response variables
241 (Figure 3-5). Grime C had a positive linear relationship with dark diversity and a negative linear relationship
242 with plant species richness (Figure 3, 5), while natural landscapes had a linear negative relationship with
243 residual dark diversity (Figure 4).

244 **Discussion**

245 Completeness, thought to be less dependent on habitat types (Pärtel et al., 2013), was highly correlated
246 with plant species richness and therefore added no new information to our study of plant community

247 diversity aspects. Therefore, we explore residual dark diversity, another measure independent of habitat type.
248 Position variables were important for both plant richness and dark diversity. Not surprisingly, once
249 differences between habitat types were accounted for, i.e. residual dark diversity, the position variables were
250 no longer significant. Plant richness was highest at intermediate conditions of soil fertility and pH,
251 corresponding to the intermediate productivity hypothesis, which states that few species can tolerate the
252 environmental stresses at low productivity and a few highly competitive species dominate at high
253 productivity (Fraser et al., 2015). Species richness increased with pH, possibly corresponding to the
254 generally large regional species pool in calcareous habitats, and aligns with previous research indicating that
255 plant diversity has a strong positive association with soil pH in temperate and boreal regions (Pärtel, 2002,
256 Pärtel et al., 2004). The unimodal relationship between dark diversity and soil moisture may be due to
257 communities at the extremes being more distinct than communities at intermediate soil moisture, i.e.,
258 specific adaptations for waterlogged and very dry soil are required (Ernst, 1990). Therefore, fewer
259 coincidental species may appear in these extreme habitats compared to habitats of intermediate moisture,
260 resulting in a low estimated regional pool and lower dark diversity at the extremes.

261 Regarding the expansion variables, variation in shrub height was negatively correlated with dark
262 diversity and positively correlated with species richness, indicating that vegetation heterogeneity (and
263 resulting local variation in light, temperature, etc.) increases the establishment and survival of species (Stein
264 et al., 2014). Previous research has shown that shrub height can increase species richness in competitive
265 environments as variance in heights can ameliorate conditions to neighboring plants and cause biomass to be
266 distributed in vertical space, thereby reducing competition for space (Bråthen and Lortie, 2016). We also
267 found bare soil had a negative effect on species richness as would be expected since more bare soil will by
268 definition have fewer observed individuals and species. The only expansion variable to influence both dark
269 diversity measures (and the only expansion variable influencing the residual dark diversity) and species
270 richness was soil organic carbon, which had a negative relationship with both dark diversity and species
271 richness. Previous studies have also indicated that native and exotic richness is lower with higher soil organic
272 carbons (Perelman et al., 2007). These results may be due to temporal continuity and the effect on the
273 various successional levels on the accumulation of soil organic carbon. For example, more established and
274 complete communities (e.g., old forests) accumulate greater organic carbon but exhibit lower species
275 diversity (Garnier et al., 2004). This may also be true for species poor acidic habitats like mires, bogs and
276 heaths, which were represented in this study. Competition plays a large role with early successional species
277 able to modify the environment through rapid growth and inhibit the success of species currently present,
278 while later stage communities are typically composed of the most competitive species and replace earlier
279 succession species (Connell and Slatyer, 1977). Therefore, the potential species that can inhabit the area may
280 be restricted by competitive species. This competitive advantage inherit in late successional communities
281 results in greater dark diversity, corresponding to our result that Grime C is important for plant dark

282 diversity. The positive effect of Grime C on dark diversity indicates that there are more species missing from
283 communities dominated by competitive species. The effect on plant species richness was opposite, likely
284 because dominant competitive species exclude other species, thereby reducing the overall plant species
285 richness. It seems that competition does not affect the species pool, otherwise suitable species would become
286 locally extinct due to dominant species, but they still remain in dark diversity and can be restored if
287 competition is controlled (e.g. disturbance). Spatial continuity, in this case natural landscape, was only
288 important for residual dark diversity, whereas competition was not important. One possibility is that the
289 habitat types account not only for variation in position but also the inherent competitive strategies of the
290 species in the habitat types e.g., late successional stages dominated by competitive species, possibly
291 explaining why competition is not important for residual dark diversity. Natural landscapes may decrease
292 dark diversity by influencing local processes, i.e., landscapes with high nature density are likely to have a
293 higher local pool of species, increased dispersal, increased species survival, metapopulation structures, and
294 less negative edge-effects of intense land use (Brunbjerg et al., 2017b). Another explanation why natural
295 landscape was only significant for residual dark diversity may be that the effect of habitat type and position
296 variables is large and masks all other effects in both dark and observed diversity. This effect is only visible
297 when these variables are accounted for.

298 This study shows that there are many different methodologies to measure biodiversity, and that they
299 contribute with different aspects to better understand drivers of diversity, with their applicability depending
300 on the desired objectives and goals. For example, if looking at the effects of organic carbon on species
301 richness one may conclude that carbon capture and storage may lead to loss of species richness, however
302 dark diversity indicates that carbon storage could increase habitat completeness. Just like there are dozens of
303 different ways to measure species diversity (Lyashevskaya and Farnsworth, 2012), there are also many ways to
304 calculate dark diversity. Besides the typical co-occurrence measure, dark diversity can also be calculated as
305 community completeness (Pärtel et al., 2013), based on ecological requirements (Lewis et al., 2016, Bello et
306 al., 2016) or species distribution (Bello et al., 2016), and probabilistic measurements such as hypergeometric
307 distributions (Carmona et al., 2019). Here, we compared dark diversity and completeness, but found that
308 completeness was highly correlated with observed species richness. We therefore advocate to compare and
309 analyze different diversity measures as both dark diversity and completeness are still relatively new metrics.
310 Furthermore, applying dark diversity within one habitat type, e.g., as seen for grasslands (Riibak et al., 2015)
311 may produce adequate results, however, when applying dark diversity across habitat types or broad
312 environmental gradients, correcting for these differences through a residual dark diversity measure may
313 provide more interpretable results.

314 With global biodiversity rapidly disappearing, it is vital to understand the drivers of biodiversity to
315 prioritize conservation and make management more efficient. In this study, besides the ecospace position
316 variables, competition seems to be the greatest driver of plant richness. Conservation management focusing

317 on intermediate disturbance such as grazing can disrupt competitive communities making room for more
318 species. Besides ecospace position, organic carbon was the most important variable for both dark diversity
319 measures indicating that advanced succession and possibly temporal continuity may increase completeness
320 or decrease dark diversity. Examining the influencing factors of different measures of biodiversity can lead
321 to better decision-making in the future conservation of the world's biodiversity.

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483

484 **Tables**

485 Table 1. Explanatory variables, their affiliation to ecospace elements and their hypothesized association with
486 response variables. Question marks indicate that hypotheses are either ambiguous or unknown.

Explanatory variable	Ecospace dimension	Dark Diversity	Plant Richness
Soil moisture index (SMI)	Position	?	Unimodal
Soil fertility index (SFI)	Position	?	Unimodal
Soil pH	Position	?	Positive
Light	Position	?	Positive
Litter	Expansion	Positive	Negative
Bare soil	Expansion	Negative	Positive
Organic carbon (OrgC)	Expansion	?	?
Organic matter	Expansion	?	?
Shrub height variation	Expansion	Negative	Positive
Canopy height variation	Expansion	Negative	Positive
Intensive field	Continuity	Positive	Negative
Natural landscape	Continuity	Negative	Positive
Temporal continuity	Continuity	Negative	Positive
Competition (Grime C)	Interactions	Positive	Negative

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488

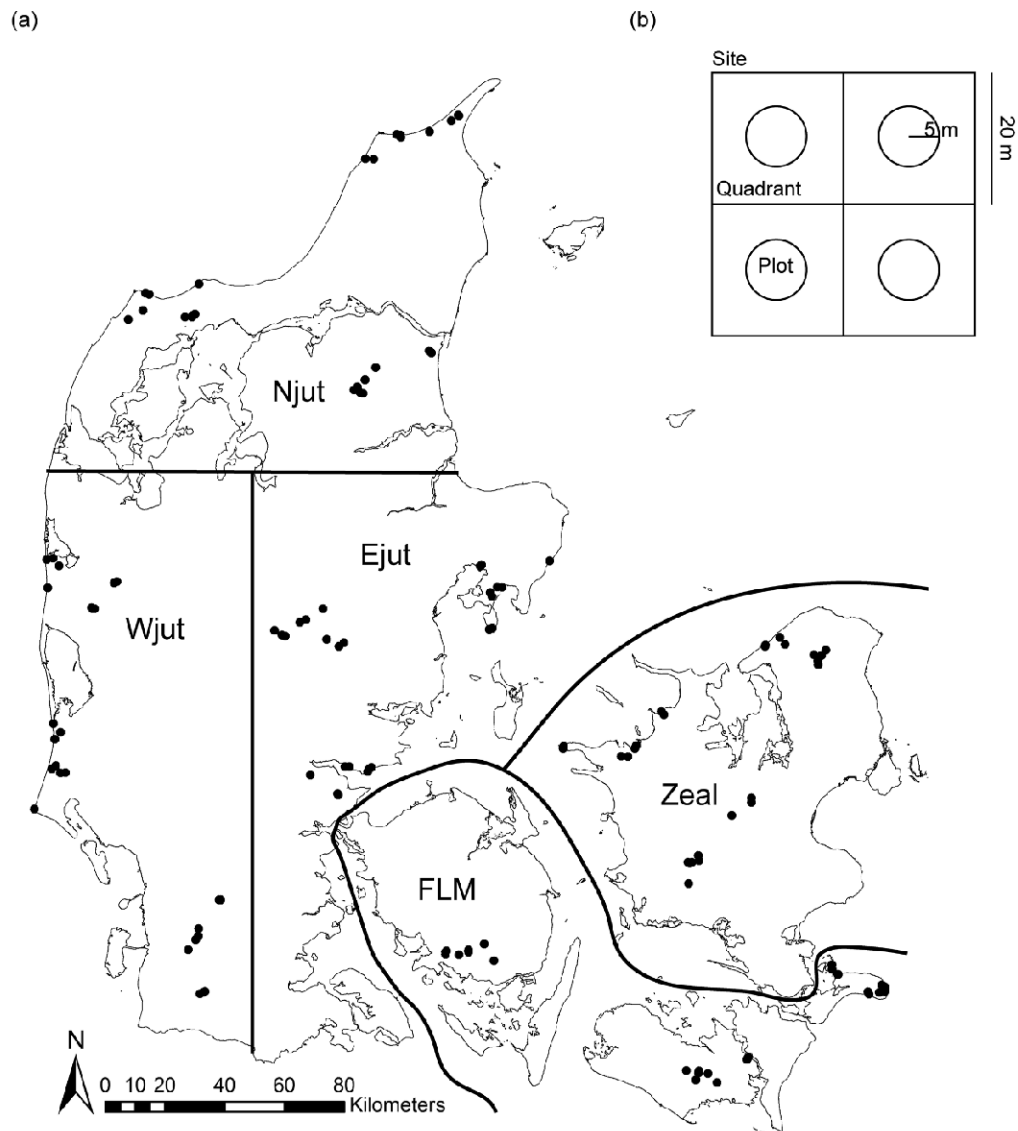
489 Table 2. Regression results for dark diversity (DD) using Poisson, residual dark diversity for habitat types
 490 using ordinary least squares and plant species richness (PlantRich) with negative binomial. R^2 is calculated
 491 as $1 - (\text{model deviance} / \text{model null deviance})$ for dark diversity and plant species richness for residual dark
 492 diversity (DD) we report the multiple R^2 . Parentheses gives the standard errors.
 493 * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$.

Ecospace	Variables	DD	Residual DD	Plant richness
	Intercept	5.090 ^{***} (0.014)	-0.000 (0.136)	3.762 ^{***} (0.081)
Position	Soil moisture index (SMI)	-0.020 [*] (0.011)	n.s.	0.190 ^{***} (0.054)
Position	SMI ²	-0.025 ^{**} (0.012)	n.s.	0.150 ^{***} (0.053)
Position	Soil fertility index (SFI)	0.069 ^{***} (0.011)	n.s.	0.233 ^{***} (0.055)
Position	SFI ²	n.s.	n.s.	-0.145 ^{***} (0.029)
Position	Soil pH	0.030 ^{***} (0.009)	n.s.	0.269 ^{***} (0.071)
Position	Soil pH ²	n.s.	n.s.	-0.060 [*] (0.034)
Position	Light	-0.042 ^{***} (0.010)	n.s.	0.102 (0.066)
Position	Light ²	n.s.	n.s.	-0.086 [*] (0.047)
Expansion	Litter (log)	n.s.	n.s.	-0.105 [*] (0.056)
Expansion	Organic carbon (OrgC)	-0.045 ^{***} (0.011)	-0.465 ^{***} (0.137)	-0.126 ^{**} (0.050)
Expansion	Bare soil	n.s.	n.s.	-0.079 (0.050)
Expansion	Shrub height variation	n.s.	n.s.	0.161 ^{***} (0.048)
Interaction	Competition (Grime C)	0.037 ^{***} (0.009)	n.s.	-0.192 ^{***} (0.048)
Continuity	Natural Landscape	n.s.	-0.326 ^{**} (0.137)	n.s.
Multiple R^2		0.65	0.14	0.65

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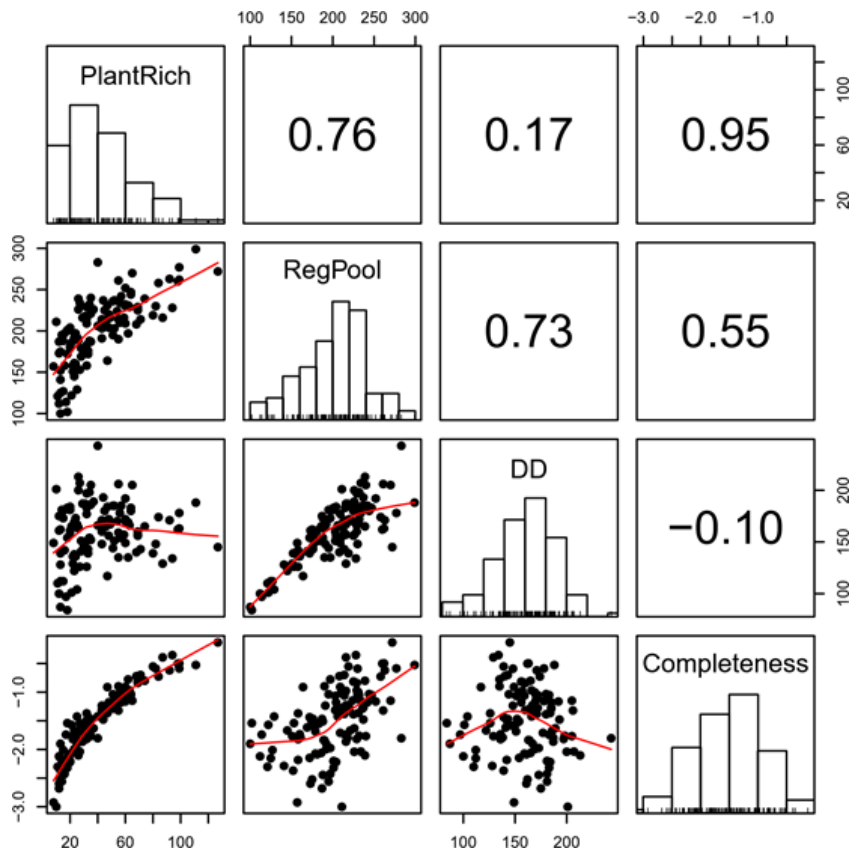
496 **Figures**



497

498 **Figure 1.** (a) Map of Denmark showing the 130 surveyed sites and the regions. (b) 40 m x 40 m site with the

499 four quadrants and 5m circle plots.

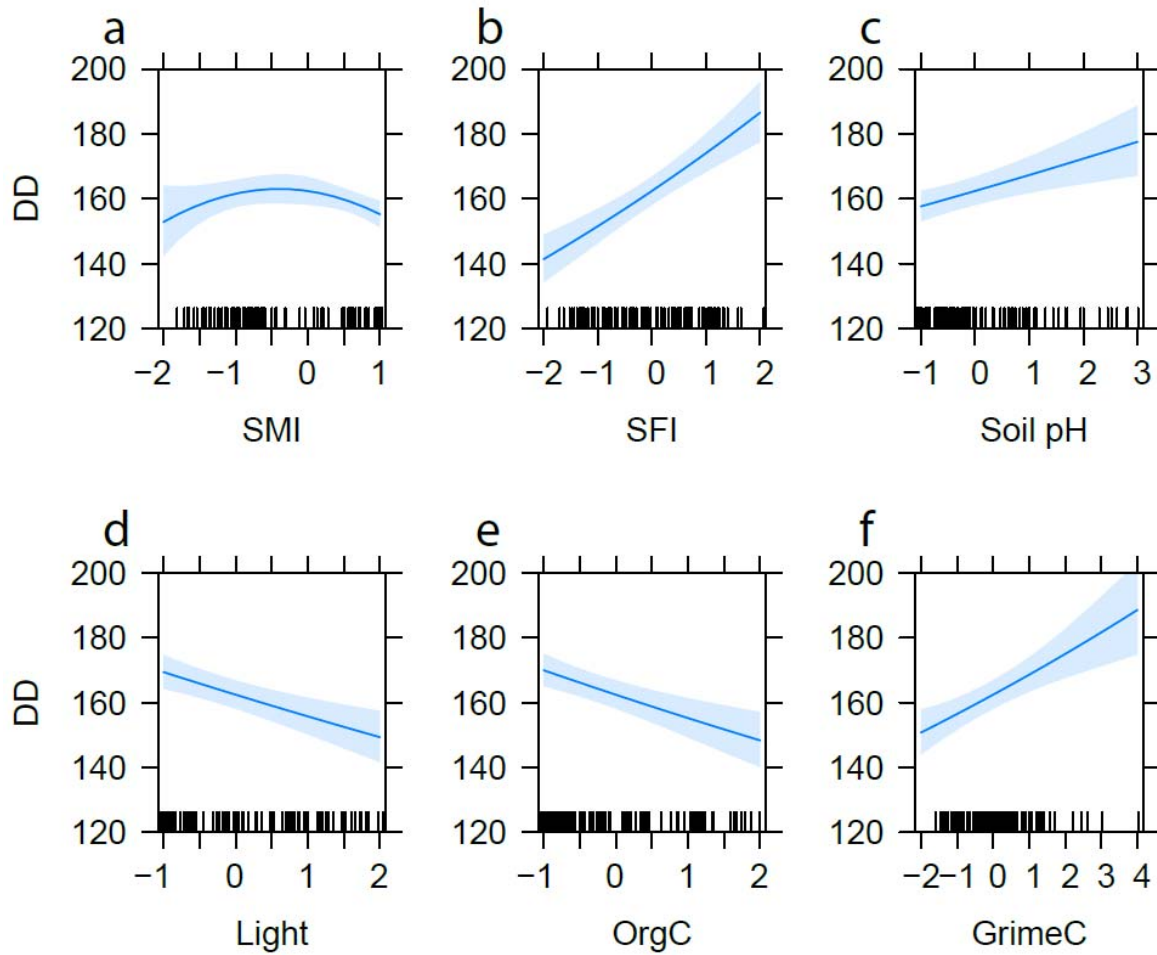


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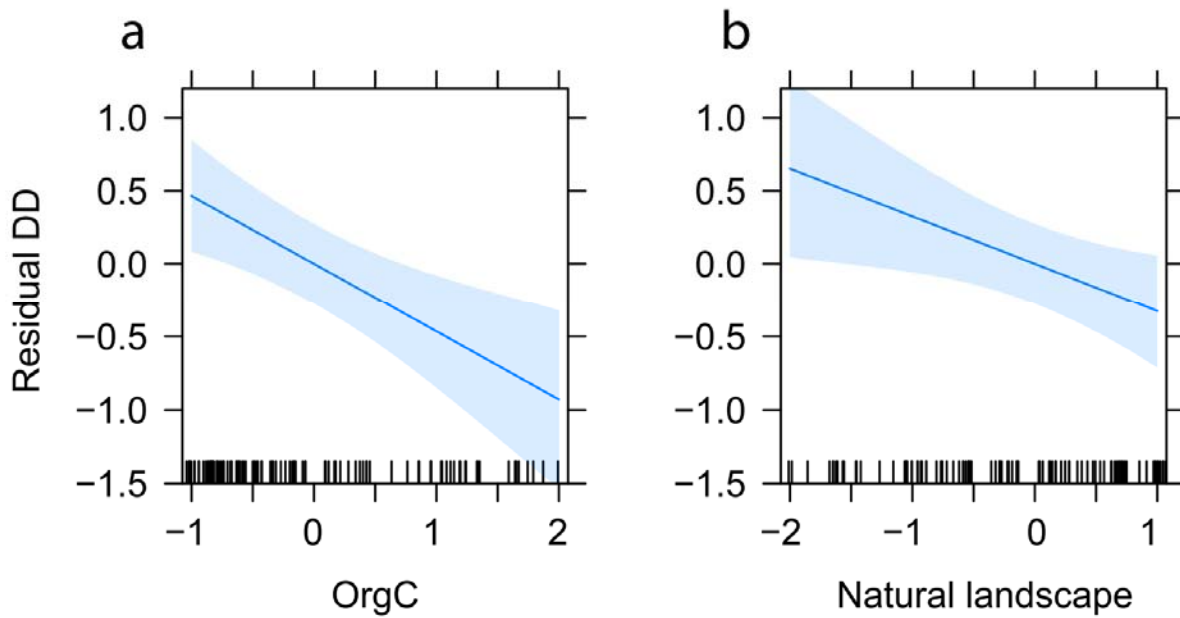
502

Figure 2. Spearman rank correlations and dot plots of site regional pool, plant species richness, dark diversity and completeness. The red line in the plot shows a loess smoothing line.



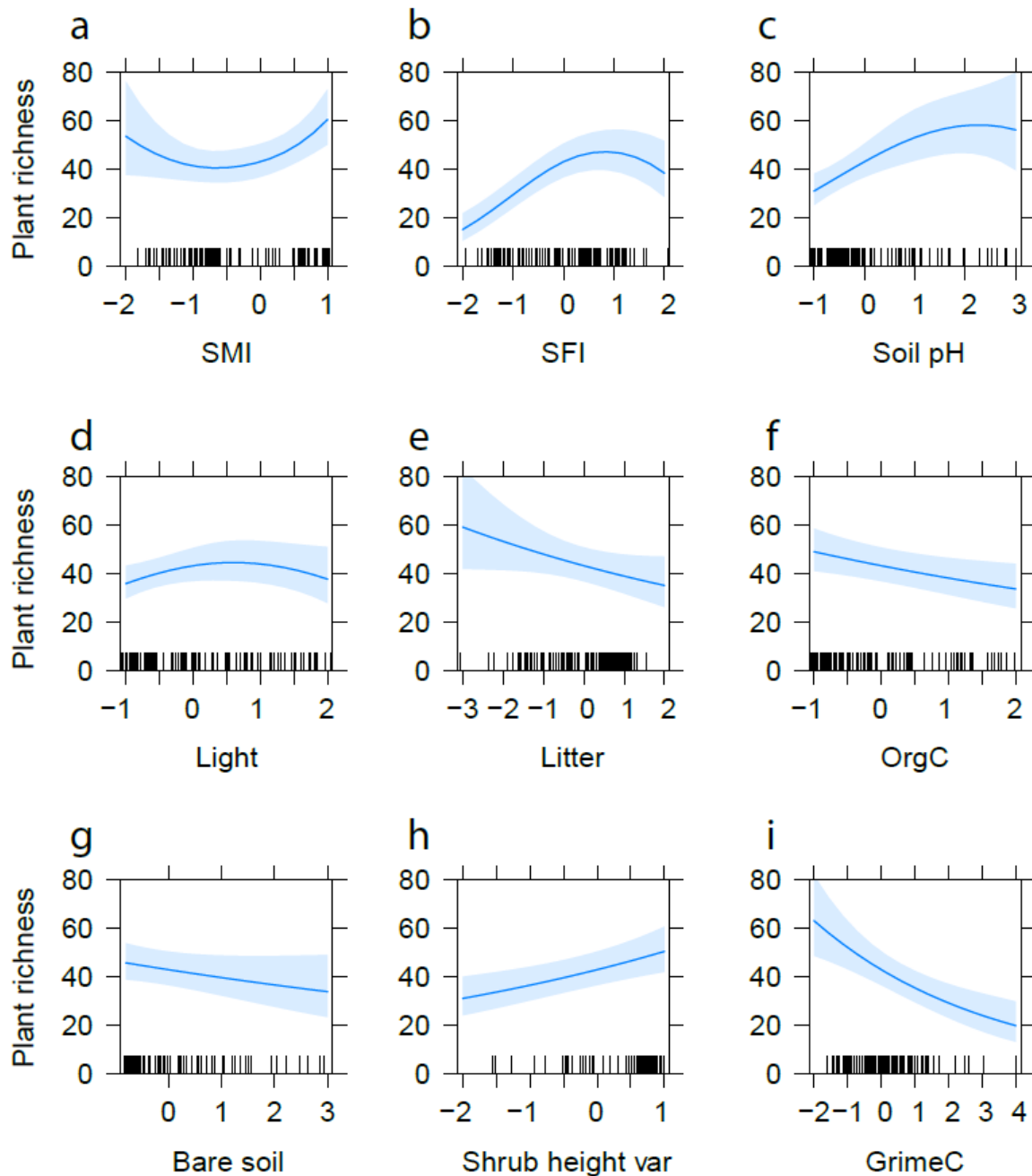
503

504 **Figure 3.** Parameter estimates with 95% confidence intervals from the significant environmental variables
505 predicting overall dark diversity. Relationships between the dark diversity and (a) soil moisture index (SMI),
506 (b) soil fertility index, (c) soil pH, (d) light, (e) organic matter, and (f) competition (GrimeC). Y-axis is
507 truncated.



508

509 **Figure 4.** Parameter estimates with 95% confidence intervals from the significant environmental variables
510 predicting residual dark diversity for habitat types. Relationships between residual dark diversity and (a)
511 organic carbon (OrgC), and (b) natural landscape.



512

513 **Figure 5.** Parameter estimates with 95% confidence intervals from the significant environmental variables
514 predicting plant species richness. Relationships between the plant species richness and (a) soil moisture
515 index (SMI), (b) soil fertility index (SFI) (c) soil pH, (d) litter mass, (f) organic carbon, (g) bare soil, (h)
516 shrub height variation, and (i) competition (GrimeC).

517