

1 This is the accepted, but not typeset version of the paper:

2 Ladouceur E., Bonomi C., Bruelheide H., Klimešová J., Burrascano S., Poschlod P., Tudela-  
3 Isanta M., Iannetta P., Mondoni A., Amiaud B., Cerabolini B.E.L., Cornelissen J.H., Craine J.,  
4 Louault F., Minden V., Öllerer K., Onipchenko V., Soudzilovskaia N.A., Jiménez-Alfaro B.  
5 2019. The functional trait spectrum of European temperate grasslands.  
6 Journal of Vegetation Science 30: 777-788. <https://doi.org/10.1111/jvs.12784>

7

## 8 **Title**

9 The functional trait spectrum of European temperate grasslands

10

## 11 **Running head**

12 Functional traits of European grasslands

13

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75 **Printed Journal page estimate:** 5822 words (7.2 pages), Figures 3 pages, Tables 1.5  
76 pages, Data sources 3288 words (1 table & second references list, 3.8 pages).

77

78 **Keywords:** clonality, functional traits, germination, grasslands, regeneration niche, seed  
79 traits, specialist species, species pool, trait spectrum

80

81 **Nomenclature:** The Plant List (<http://www.theplantlist.org/>, accessed on 22 June,  
82 2015).

83 **Funding Statement:** EL, BJA, MTI, AM, PI and CB acknowledge the research leading to  
84 these results has received funding from the People Programme (Marie Curie Actions) of  
85 the European Union's Seventh Framework Programme FP7/2007-2013/ under REA  
86 grant agreement n°607785- as a part of the Native Seed Science TEchnology and  
87 Conservation (NASSTEC) Initial Training Network (ITN). BJA was further funded by the  
88 Marie Curie Clarín-COFUND program of the Principality of Asturias and the European  
89 Union (ACB17-26). BJA, and HB acknowledge support from the German Centre for  
90 Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig funded by the German  
91 Research Foundation (DFTG FZT 118) through the sPlot research platform. PI  
92 acknowledges support from the Rural & Environment Science & Analytical Services  
93 Division of the Scottish Government. KÖ thanks R01567-IBB03/2018 for financial  
94 support.

95

96 **eTOC:** In specialist species of alpine grassland habitat types of continental Europe, non-  
97 regeneration traits seem to be filtered across habitat gradients, and most regeneration  
98 traits demonstrate multiple strategies within each habitat type, indicating possible  
99 variable trait strategies with trait groups associated with different processes.

100 **Abstract**

101 **Questions:** What is the functional trait variation of European temperate grasslands and  
102 how does this reflect global patterns of plant form and function? Do habitat specialists  
103 show trait differentiation across habitat types?

104 **Location:** Europe.

105 **Methods:** We compiled 18 regeneration and non-regeneration traits for a continental  
106 species pool consisting of 645 species frequent in five grassland types. These grassland  
107 types are widely distributed in Europe but differentiated by altitude, soil bedrock and  
108 traditional long-term management and disturbance regimes. We evaluated the  
109 multivariate trait space of this entire species pool and compared multi-trait variation  
110 and mean trait values of habitat specialists grouped by grassland type.

111 **Results:** The first dimension of the trait space accounted for 23% of variation and  
112 reflected a gradient between fast-growing and slow-growing plants. Plant height and  
113 SLA contributed to both the first and second ordination axes. Regeneration traits mainly  
114 contributed to the second and following dimensions to explain 56% of variation across  
115 the first five axes. Habitat specialists showed functional differences between grassland  
116 types mainly through non-regeneration traits.

117 **Conclusions:** The trait spectrum of plants dominating European temperate grasslands  
118 is primarily explained by growth strategies which are analogous to the trait variation  
119 observed at the global scale, and secondly by regeneration strategies. Functional  
120 differentiation of habitat specialists across grassland types is mainly related to  
121 environmental filtering linked with altitude and disturbance. This filtering pattern is  
122 mainly observed in non-regeneration traits, while most regeneration traits demonstrate  
123 multiple strategies within the same habitat type.

124

## 125 INTRODUCTION

126 At the global scale, the variation in plant functional traits in multidimensional trait-  
127 space is determined by two axes related to plant size and leaf area (Diaz et al. 2016).  
128 These two dimensions are mainly linked with ecological trade-offs on a uniformly fast,  
129 medium, or slow growth strategy gradient (Reich 2014; Salguero-Gómez et al. 2016);  
130 and along a leaf construction economics spectrum (Wright, Reich, Westoby, Ackerly &  
131 Baruch 2004). The multidimensional trait perspective helps to understand evolutionary  
132 constraints of functional diversity for plant species, but linking this trait variation with  
133 ecological drivers is still needed (Bruehlheide et al. 2018). Functional plant strategies can  
134 be explained by traits filtered in biogeographic regions and in local ecological  
135 communities as a response to historical and environmental conditions (de Bello et al.  
136 2006; de Bello et al. 2012; Poschlod et al. 2013). However, linking local filtering with  
137 regional and continental processes remains a major challenge (Pärtel et al. 2016), and  
138 new approaches in functional-trait ecology are needed to better understand these  
139 patterns and processes in plant community ecology.

140 Within different historical and environmental contexts, some species are more  
141 influenced by ecological filters than others, resulting in some level of species sorting in  
142 different habitats (Leibold & Chase 2018). While some species may be restricted to one  
143 habitat as specialists (Fridley et al. 2007), other species can plastically respond to  
144 different environmental conditions (Vellend 2016) and occur commonly across  
145 environmental gradients as generalists. The presence of generalists in local  
146 communities weakens the importance of environmental filtering within habitat types,  
147 and the predictability of environment-trait relationships in favour of non-niche  
148 processes such-as dispersal limitation (Fridley et al. 2007). In contrast, plant specialists

149 consistently associated with local environmental conditions are expected to present  
150 specific traits that make these species a strong competitor in a given habitat. Studying  
151 the trait variation of plant specialists may therefore help to detect environmental  
152 filtering within the species pool, or the species which can potentially occur at a site  
153 (Pärtel et al. 2011). Identification of functional species pools is a pre-requisite to  
154 differentiate functional patterns produced by abiotic filters (de Bello et al. 2012), and  
155 separating specialists from generalists within species pools could perhaps strengthen  
156 this approach.

157         Plant species may be particularly sensitive to environmental filtering at the  
158 regeneration stage and this might be more important than other life-history stages as  
159 species could be totally excluded from a habitat due to inappropriate environmental  
160 conditions for germination or successful establishment (Grub 1977). However, our  
161 knowledge of plant trait ecology is largely focused on few traits concerning  
162 aboveground vegetative growth and morphology, and very few studies have assessed  
163 the potential role of regeneration traits (Poschlod et al. 2013; Jiménez-Alfaro et al.  
164 2016; Larson & Funk 2016; Saatkamp et al. 2018). Regeneration traits have been long  
165 acknowledged as relevant to the natural maintenance of biodiversity (Grubb 1977), and  
166 have been found to be important for both species coexistence and species sorting  
167 (Bernard-Verdier et al. 2012; Pierce et al. 2014; Fernández-Pascual et al. 2017).  
168 Processes captured by regeneration traits including flowering, seed production, clonal  
169 growth, dispersal, germination, and growth rates are relevant to community assembly,  
170 species turnover, survival and persistence (Pohl et al. 2011; Poschlod et al. 2013;  
171 Klimešová et al. 2016). When combined with non-regeneration traits, regeneration  
172 traits might add new dimensions to the plant trait spectrum (Laughlin 2013; Pierce et  
173 al. 2014; Salguero-Gómez et al. 2016; Herben et al. 2016), providing a better

174 understanding of the role of environmental filtering in plant communities and on  
175 different types of traits.

176         Here, we study the functional trait variation of a species pool representative of  
177 European temperate grasslands. Our case study consists of the most frequent and  
178 dominant species occurring in widely distributed grasslands types, with similar growth  
179 and life forms but differing in species composition and environmental conditions along  
180 gradients of altitude and traditional long-term disturbance regimes (Ellenberg 2009;  
181 Körner 2003; Nagy et al. 2003; Dainese et al. 2012). By combining non-regeneration  
182 traits and less commonly studied regeneration traits, our first aim was to describe the  
183 trait spectrum of the species pool of European temperate grasslands, and to test  
184 whether this spectrum reflects the main dimensions observed at the global scale. Our  
185 second aim was to test whether habitat specialists of each grassland type show trait  
186 differentiation that might explain environmental filtering. Despite the marked  
187 dominance of few life- and growth- forms in European grasslands, we expect habitat  
188 specialists to exhibit trait combinations that may allow us to functionally characterize  
189 vegetation types, possibly with more subtle detail than at a global scale across disparate  
190 biomes.

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194

195 **METHODS**

## 196 **Species data**

197 We focused on five grassland types representing wide ecological variation in altitude,  
198 soil and disturbance (Table 1) as described in the classification of European habitats  
199 (Galvanek & Janak 2008; Garcia-Gonzalez 2008; Calaciura & Spinelli 2008; European  
200 Environment Agency 2012). We obtained the species list of constituent species from a  
201 continental review (Schaminée et al. 2016) based on the European Vegetation Archive  
202 (Chytrý et al. 2015) and over 1 million field surveys to report species frequencies in  
203 these European habitat types. We removed rare species with <5% frequency of  
204 occurrence in each grassland type at the continental scale. We identified as specialists  
205 those species with a significantly ( $P < 0.05$ ) higher frequency of occurrence in one  
206 grassland type than any other using a Fisher's exact test (Agresti 2002). Most of these  
207 specialists are generally described as characteristic or dominant species of the study  
208 grassland types in Europe (see habitat descriptions and references in Table 1). All other  
209 species were labelled as generalists. We note that our definition of specialists applies  
210 exclusively to the five grassland types compared here, assuming the association of  
211 species with one grassland type is mainly due to ecological preferences.

## 212 **Traits selection and data collection**

213 The majority of trait data was compiled from existing datasets contributed to the TRY  
214 Plant Trait Database (see Data Use and Table 2) (Kattge et al. 2011). A request to TRY  
215 for relevant datasets returned 8,655,033 records and 96,493 unique species names  
216 across 95 trait categories in 104 datasets. Taxonomic synonyms were made consistent  
217 using the Plant List Project (Missouri Botanical Gardens & Royal Botanic Gardens Kew  
218 2013). Traits were selected to represent different aspects of plant organs or whole plant  
219 properties and their functional significance and life history (Laughlin 2013; Jiménez-



220 Alfaro et al. 2016). Data were then extracted by matching accepted plant names and  
221 known synonyms from the target species list into subsets. Eleven traits were used from  
222 TRY, across 47 TRY datasets, and 10 from other sources (Table 2), selected from a  
223 larger list of traits of interest. A trait was used as long as there were at least three data  
224 points for each grassland type. Trait units of measurement were standardised across  
225 datasets, and where multiple values existed, we used the mean of all individual traits of  
226 each species. While this approach does not account for intraspecific trait variation, it is  
227 expected that the influence of this variation takes place mostly within each grassland  
228 type, representing less than 20% of total variation (Siefert et al. 2015).

## 229 **Data Analysis**

230 All statistical analyses and plotting were conducted using the R Studio language and  
231 environment for statistical computing and graphics (version 3.4.0). Each R package used  
232 is referenced as each approach is explained.

233 We used a Principal Component Analysis (PCA) to describe the multivariate trait  
234 spectrum and to identify the contribution of individual traits. We used the R package  
235 missMDA (Husson & Josse 2018) to replace missing values in the numerical traits for  
236 the PCA and to estimate parameters based on existing values within the dataset (Josse &  
237 Husson 2012; Josse & Husson 2016). Out of 9,002 (643 rows of numerical trait data x 14  
238 columns) possible records in the PCA, 48.5% of data was missing and replaced with  
239 predicted values from the observed data. Categorical traits were excluded from the  
240 multivariate analysis. Missing data was unevenly distributed across traits, with less-  
241 commonly studied regeneration traits missing more data than others, but averages  
242 between non-regeneration and regeneration traits overall were the same, and all traits  
243 were considered regardless (Data Quality, Table S1). The implication of this gap-filling

244 method is that the variance in the estimators is underestimated (Josse & Husson 2012;  
245 Josse & Husson 2016). The gap-filled data was then log transformed as data were not  
246 normally distributed. The loadings of each trait and the scores of each species were  
247 extracted for the first five axes. Results were plotted using ggplot2 (Wickham 2009),  
248 FactoMineR (Husson, Josse & Mazet 2018), and factoextra (Kassambara & Mundt 2017).  
249 The collinearity of traits in the PCA was evaluated in a correlation matrix using the  
250 package corrplot (Wei & Simko 2017).

251         We examined the distribution of specialists and generalists in the trait space by  
252 plotting the PCA scores of both species groups for each grassland type. The PCA results  
253 were used to compare the trait space of different grassland types, plotted using  
254 packages ade4 (Dray, Dufour & Thioulouse 2018) and adegraphics (Dray & Siberchiot  
255 2018). Differences in PCA scores between grassland types were tested with a  
256 permutational analysis of variance (PERMANOVA) using vegan (Oksanen et al. 2018)  
257 and with a post-hoc pairwise PERMANOVA statistical tests using RVAideMemoire  
258 (Hervé 2018). Both tests were based on 999 permutations. To identify the traits that  
259 best separated the different grassland types, we employed discriminant analysis using  
260 the gap-filled data. A stepwise forward variable model selection was performed based  
261 on Wilk's Lambda criterion using the packages klaR (Roever 2018), and mda (Hastie et  
262 al. 2017), which minimizes within-class distances and contextually maximises class  
263 discrimination (Bianco et al. 2016).

264         We tested for differences in mean trait values among grassland types using a  
265 post-hoc pairwise Kruskal-Wallis  $\chi^2$  test from the package PMCMR (Pohlert 2018). Raw  
266 data were plotted for single traits, with no gap-filling or transformation taking place.  
267 Categorical traits were plotted in the package ggplot2 (Wickham 2018). The numerical

268 traits and individually plotted PCA axis coordinates were visualized as RDI plots (Raw  
269 data, descriptive and inference statistics) created using the package yarr (Phillips  
270 2017), to effectively visualise the breadth of each trait within categories, as well as for  
271 visual comparison between them. This allowed for individual investigation of trait  
272 patterns found in the multivariate space without gap-filling.

273

## 274 **RESULTS**

275 We compiled a list of 645 species in total (excluding 44 species with missing data),  
276 including 257 generalists and 388 specialists, 52 taxonomic families and 244 genera  
277 (Table S1, Supporting Information). Overall, we identified 56 specialists in meadows,  
278 75 in dry grasslands, 77 in *Nardus*-dominated grasslands, 133 in calcareous alpine  
279 grasslands, and 47 in acidic alpine grasslands for which there was available trait data  
280 (Table S1). Both the specialists and generalists represent > 83% of the species detected  
281 in the study habitats and in the context of this study we refer to them as the continental  
282 species pool.

283

284

### 285 **Multivariate trait spectrum**

286 The first, second and third axis of the PCA based on 14 numerical traits explained 23%,  
287 9.2 % and 9.1% of the total variance, respectively (Fig. 1). All non-regeneration traits,  
288 including radial growth rate, SLA and plant height had relatively high loadings on the  
289 first axis of the PCA (Fig. 1a, Table 3). The regeneration traits with the highest  
290 contribution with the first axis were  $T_{max}$ , soil seed bank longevity, flowering duration

291 and flowering onset. Plant height and SLA also contributed to the second axis, together  
292 with flowering onset, seed mass, seed number per ramet, and flowering duration (Fig.  
293 1a, b, Table 3, Table S2). On the third axis, regeneration traits ( $T_o$ , seed mass, clonal  
294 index, flowering onset and  $T_{min}$ ) contributed the most variation (Fig. 1 b, Table 3).

295 Most of the traits (85%) were significantly correlated among each other (Table  
296 S4). The strongest correlations were between flowering onset and flowering duration  
297 (Pearson  $r = -0.463$ ); between  $T_{max}$  and duration of seed bank longevity (0.358); and  
298 between plant height and seed number (0.345).  $T_o$  was negatively correlated with plant  
299 height and seed number per ramet  $T_{min}$ , clonal shoot cyclicity and flowering onset were  
300 negatively correlated with all traits except  $T_o$ , seed mass and each other. Seed mass was  
301 negatively correlated with seed number per ramet and clonal index, and positively  
302 correlated with all other traits except  $T_o$ . The other traits had positive correlations.

### 303 **Trait variation of habitat specialists**

304 The groups of habitat specialists were differentiated among each other in the trait space  
305 (PERMANOVA  $F=13.43$ ,  $R^2=0.095$ ,  $P<0.001$ , Fig. 2 a, Table S6b,c). Main differences  
306 between habitats were related to the first axis of variation (Fig. S1 b, Kruskal-Wallis  $s^2=$   
307  $127.4$ ,  $P<0.001$ , Table S5). On axis 2, only acidic alpine grasslands were differentiated  
308 from *Nardus*-dominated grasslands (Fig. S1 b,  $s^2= 12.029$ ,  $P= 0.03$ , Table S5). The  
309 variability between group means (F-value) was larger for regeneration ( $F= 13.77$ ,  $R^2$   
310  $0.098$ ,  $P<0.001$ , Table S6f) than for non-regeneration traits ( $F= 10.071$ ,  $R^2= 0.073$ ,  
311  $P<0.001$ , Table S6e). The most important traits in differentiating specialist groups  
312 according to the discriminant analyses were plant height, flowering duration, seed bank  
313 longevity, SLA, and flowering onset (Table 3,i; Table S7; Fig. S2 ). There was an overall  
314 misclassification error of 51%, which was mainly brought about by a strong overlap in

315 predictions for *Nardus*-dominated grasslands with calcareous and acidic alpine  
316 grasslands (Table S8a). Predictions were similar when non-regeneration traits were  
317 analysed separately, better characterising certain grassland specialists, while other  
318 specialists were better characterised by regeneration traits (Table S8bc). However, the  
319 discriminative power was more accurate when all traits were used together, rather than  
320 separately (Table S8).

321         Acidic alpine grasslands had the lowest SLA (Kruskal-Wallis  $s^2= 73.28$ ,  $P=$   
322  $<0.001$ , Table S1), shortest plant height ( $s^2= 95.59$ ,  $P= <0.001$ , Table S10), and a slow  
323 radial growth rate ( $s^2= 28.56$ ,  $P= <0.001$ , Table S10) with a late ( $s^2= 51.38$ ,  $P= <0.001$ ,  
324 Table S10) and short flowering duration ( $s^2= 74.87$ ,  $P= <0.001$ , Table S10) (Fig. 3, Fig.  
325 4). *Sempervivum arachnoideum*, with its far-reaching runners was the only acidic alpine  
326 specialist with a high clonal spread rate. Human assisted dispersal was proportionally  
327 less present in calcareous alpine and acidic alpine grassland specialists than in other  
328 grassland types (Fig. S3 d). The traits of *Nardus*-dominated grassland specialists were  
329 similar to alpine specialists in terms of their non-regeneration traits, i.e. short height,  
330 low SLA and slow radial growth. They start flowering at a similar time to acidic,  
331 calcareous alpine, dry grasslands and generalists, but flowering for a similarly short  
332 length as both alpine grassland types, and much shorter time than dry grassland  
333 specialists (Table S9,  $P=0.01$ ).

334         *Nardus* grassland specialists had the smallest seed mass by a large margin ( $s^2=$   
335  $28.30$ ,  $P= <0.001$ , Table S10); a soil seed bank characterised by short-term persistent  
336 seeds (Table 2); and a high  $T_{\min}$ , but only differentiated from the very low  $T_{\min}$  of  
337 meadows ( $P=0.03$ , Table S11). There was higher proportion of seed shedding in late  
338 summer in *Nardus*-dominated (70%), calcareous alpine (79%) acidic alpine grasslands

339 (79%) (Fig. S3 c), which was consistent with the marginally narrower window of  
340 germination temperatures available to specialists of these grassland types (Fig. 4 g-i).  
341 Finally, both meadows and dry grasslands had the highest SLA, tallest plants, the fastest  
342 radial growth rates, and the earliest flowering onset and longest flowering duration  
343 compared to the other three, high-altitude habitats. Meadows also had a faster clonal  
344 lateral spread rate than calcareous alpine specialists ( $s^2= 13.53$ ,  $P= 0.018$ , Table S11).  
345 Specialists of dry grasslands had a notably larger presence of physical dormancy (PY)  
346 (24%, Fig. S3 a) than other habitat types.

347

## 348 **DISCUSSION**

### 349 **The trait spectrum of European temperate grasslands**

350 The main trait dimension of the temperate grasslands analyzed in this study is similar  
351 to the 'fast-slow' continuum described globally (Reich 2014; Salguero-Gómez et al.  
352 2016) and to the latitude-driven first dimension of the global spectrum of vascular  
353 plants (Westoby 1998; Diaz et al. 2016). The main gradient between fast-growing plants  
354 with high regeneration rates (on the right side of the PCA) and slow-growing plants  
355 with shorter flowering duration (on the left side) is likely driven by ecological  
356 differences in seasonal length and climate along the altitudinal range in which these  
357 species occur. We further found that regeneration traits including annual radial growth  
358 rate, clonal lateral spread,  $T_{max}$ , and seed bank longevity also contribute to the fast-slow  
359 strategy.

360 The second axis of trait variation of European temperate grasslands suggests a  
361 leaf economic spectrum driven by environmental gradients, which is mainly explained  
362 by the contribution of SLA. Plant height, flowering onset and seed mass also contributed

363 largely to the second axis, while on the third, fourth and fifth axes major contributions  
364 were related to regeneration traits such as  $T_o$ ,  $T_{min}$ , flowering duration, clonal shoot  
365 cyclicity, clonal index, clonal spread, and seed number. Similarly, Pierce et al. (2014)  
366 found that reproduction traits affect plant survival independently from primary  
367 strategies, while Salguero-Gómez (2016) found that regeneration traits, such as degree  
368 of iteroparity and net reproductive rate, were perpendicular to the leaf economics  
369 spectrum. Other studies found weak correlation or even a lack of correlation between  
370 seed and clonal traits across a whole flora (Herben et al. 2012; Herben et al. 2016). Our  
371 results therefore suggest that reducing the species pool to a unique formation  
372 (temperate grasslands) removes functional variation of distinct habitat types (such as  
373 forests, wetlands, etc.) that may confound the interpretation of habitat-specific trait  
374 dimensions. In addition, the use of traits representing different life-stage processes  
375 (regeneration and non-regeneration traits) provides a more comprehensive explanation  
376 of the observed functional patterns.

377

### 378 **Trait differences between habitat specialists**

379 Our results support the idea that environmental filtering and disturbance governs the  
380 functional composition of plant specialists related to temperate grassland types in  
381 Europe. Specialists of low-altitude disturbed and managed habitats are characterised by  
382 'fast' traits such as greater height, SLA, longer flowering duration and increased radial  
383 growth rate. In contrast, the 'slow' traits stand out in specialists of harsh alpine habitats  
384 with less disturbance. The combination of all available traits discriminated grassland  
385 types better than considering non-regeneration or regeneration traits separately,  
386 supporting the multidimensional nature of plant traits for understanding environmental

387 relationships (Laughlin 2013). Nevertheless, we found weak functional differentiation  
388 between the high-altitude acidic and calcareous alpine grasslands, despite having  
389 distinct differences in soil conditions and species composition. Trait-based differences  
390 between these habitats have been found in germination traits related specifically to pH  
391 and water availability that require more experimental data (Tudela-Isanta et al. 2017)  
392 than is currently available for this entire species pool. Despite the comprehensive set of  
393 traits used in this study, the lack of more specific traits, reflecting e.g. physiological  
394 species responses, makes it possible to differentiate up to three major functional  
395 groups: (i) high-altitude grasslands (both acidic and calcareous grasslands); (ii) low-  
396 altitude grasslands from disturbed habitats (dry grasslands and meadows); and (iii)  
397 mid-altitude *Nardus*-dominated grasslands (differentiated from (i) or (ii) depending on  
398 which trait is being examined).

399         The explanatory power of traits for differentiating habitat types was mainly  
400 related to non-regeneration traits and flowering, which are in general more under-  
401 dispersed than would be expected randomly within each habitat, suggesting  
402 environmental filtering may be taking place on these traits. The regeneration traits of  
403 specialists showed more over-dispersion than expected randomly, varying more within  
404 than among grassland types, supporting the idea that a multitude of regeneration niches  
405 may coexist within the same grassland type (Grubb 1977). This suggests that  
406 regeneration traits may have a different ecological role than non-regeneration traits,  
407 such as the competitive niches of species, or coexistence mechanisms (Mayfield &  
408 Levine 2010; HilleRisLambers et al. 2012). However, we found differentiation of soil  
409 seedbank longevity across habitats, which is likely influenced by traditional long-term-  
410 management practices such as regular mowing or grazing in meadows (Bekker et al.  
411 1998; Fenner & Thompson 2005). In addition, human dispersed seeds are less



412 represented among alpine grassland specialists, demonstrating the importance of  
413 traditional management for seed dispersal (Poschlod et al. 1998; Auffret 2011) and the  
414 possible complementary effect of long-term disturbance regimes for understanding  
415 functional differentiation in these habitats (Louault et al. 2005; Kahmen & Poschlod  
416 2008). Our results also suggest that clonal traits could be affected by abiotic and biotic  
417 filters such as disturbance and soil, which perhaps are clearer in wider gradients than  
418 explored here (Fujita et al. 2013), so the extent of environmental filtering at different  
419 scales and gradients on these traits (Klimešová et al. 2013; Klimešová & Herben 2014)  
420 is worth investigating further.

421

## 422 **CONCLUSION**

423 This study is, to our knowledge, the first attempt to investigate the trait variation of a  
424 continental species pool within a particular vegetation type. We found that the trait  
425 spectrum of European temperate grasslands is related to the main trade-offs observed  
426 at the global scale. However, our analyses also reveal new contributions of traits with a  
427 functional role in our study system, details that might be lost when analyzing the  
428 functional variation across distinct vegetation types. Since temperate grasslands are by  
429 definition dominated by grasses and forbs, the analysis of functional variation within  
430 this relatively homogeneous system allows us to focus on patterns and drivers linked  
431 with the specific differentiation of grassland types. Non-regeneration traits related to  
432 plant growth were mainly related to environmental gradients and disturbance across  
433 grasslands, while regeneration traits demonstrated a multitude of regeneration  
434 strategies existing within grassland types. We conclude that functional characterization  
435 of habitat specialists within species pools may be a promising approach for

436 understanding the role of environmental filtering on trait-based ecology and vegetation  
437 diversity across large scales. However, this approach is also limited by the quality of  
438 plant traits available. Besides the integration of regeneration and non-regeneration  
439 traits, future research will need to explore traits with a stronger physiological impact on  
440 species ecological responses.

441

442 **Author Contributions**

443 BJA conceived the idea. EL, CB and BJA designed the methodology, and arranged  
444 acquisition of data. BA, SB, BC, JHC, JC, JK, FL, VM, AM, KÖ, VO, PP, NS, MTI, donated  
445 substantial amounts of data. EL lead data analysis and writing of the manuscript. BJA,  
446 HB, JK, SB, PP, MTI, PI, AM and CB contributed critical feedback to data interpretation  
447 and initial drafting of the manuscript. All authors made intellectual contributions and  
448 provided essential feedback. The first nine authors, and last author are ordered by their  
449 relative contribution, the others are ordered alphabetically.

450

451 **Acknowledgements**

452 EL, BJA, MTI, AM, PI and CB acknowledge the research leading to these results has  
453 received funding from the People Programme (Marie Curie Actions) of the European  
454 Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement  
455 n°607785- as a part of the NAtive Seed Science TEchnology and Conservation  
456 (NASSTEC) Initial Training Network (ITN). BJA was further funded by the Marie Curie  
457 Clarín-COFUND program of the Principality of Asturias and the European Union  
458 (ACB17-26). BJA, and HB acknowledge support from the German Centre for  
459 Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig funded by the German  
460 Research Foundation (DFG FZT 118) through the sPlot research platform. PI  
461 acknowledges support from the Rural & Environment Science & Analytical Services  
462 Division of the Scottish Government. KÖ thanks RO1567-IBB03/2018 for financial  
463 support.

464 **Data Accessibility**

465 Most data were collected from donated existing databases within the TRY Global Plant  
466 Trait Database (Table 2, & Supporting Information Appendix S2). Mean values of all trait  
467 data are detailed in Supporting Information Appendix S3 Table S1. Each individual  
468 dataset which was used to calculate the mean value of each trait is referenced  
469 individually in the extended version of Table 2, in Supporting Information Appendix S2.

470

471

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### 685 **Supporting Information**

686 Additional supporting information may be found in the online version of this article at

687 <https://onlinelibrary.wiley.com/doi/abs/10.1111/jvs.12784>

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689 **Table 1: Description of the five grassland types investigated.** Altitude shows estimated ranges above the sea level.  
690 Number of specialist species describes the total number of specialists identified for each grassland type. EUNIS and ANNEX  
691 I habitats and their management practices according to [www.eunis.eea.europa.eu/habitats](http://www.eunis.eea.europa.eu/habitats). Soil pH and moisture values  
692 are derived from the Ellenberg indicator values of each species (Fitter & Peat 1994; Sanda et al. 2003; Ciocârlan 2009;  
693 Moretti & Legg 2009; Ellenberg 2010; Hill et al.; Kattge; Öllerer), expressed as the mean of those specialists (Table 2). VPL=  
694 vegetation period length (Aeschimann et al. 2004). Data sources listed in Appendix S2.

Grassland type	Altitude	# of specialist species	European Nature Information System (EUNIS) habitat classification	ANNEX I habitats of EU Habitat Directive	Traditional management practice	Soil pH	Soil moisture	VPL
Meadows	0-1000m	57	E2.2 Low & Medium altitude meadow E2.3 Mountain hay meadows	Lowland Hay Meadows 6510 + High Altitude Hay Meadows 6520	Mowing, (Grazing)	6.9 (neutral)	4.78	March-October (8 months)
Dry Grasslands	500-1400m	77	E1.2 Perennial calcareous grassland and basic steppes	Semi-natural Dry Grasslands (Festuco-Brometalia) 6210	Mowing & Grazing	7.3 (calcareous)	3.2	March-October (8 months)
<i>Nardus</i> -dominated	1400-1800m	81	e1.7 Closed non-Mediterranean dry acid & neutral grassland	Mountain acid grassland Species Rich <i>Nardus</i> Grasslands 6230	Grazing	4.39 (acidic)	5.8	May-September (5 months)
Calcareous Alpine	1800-2700m	149	E4.4 Calcareous alpine and subalpine grassland	Alpine & subalpine calcareous grasslands 6170	None	7.7 (calcareous)	4.68	June-August (3 months)
Acidic Alpine	>2700m	52	4.3 Acid alpine and subalpine grassland	Acidic Alpine grasslands 6150	None	2.3 (acidic)	4.5	June-August (3 months)

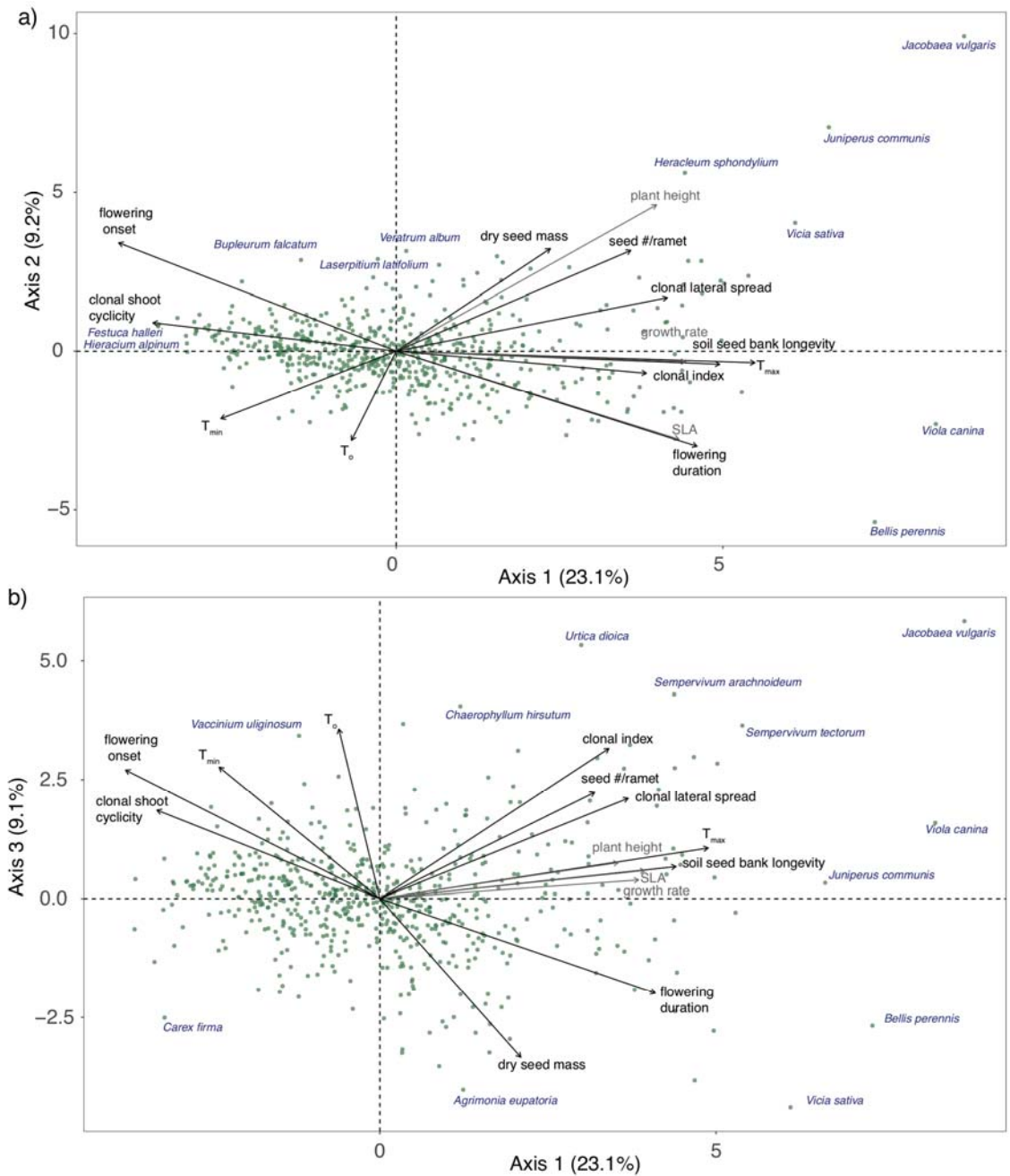
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696 **Table 2:** Loadings of the plant trait values for the first three axes of the PCA for: all traits and all species. Bold numbers  
 697 indicate the top three (3)- five (5) highest loadings on each axis.

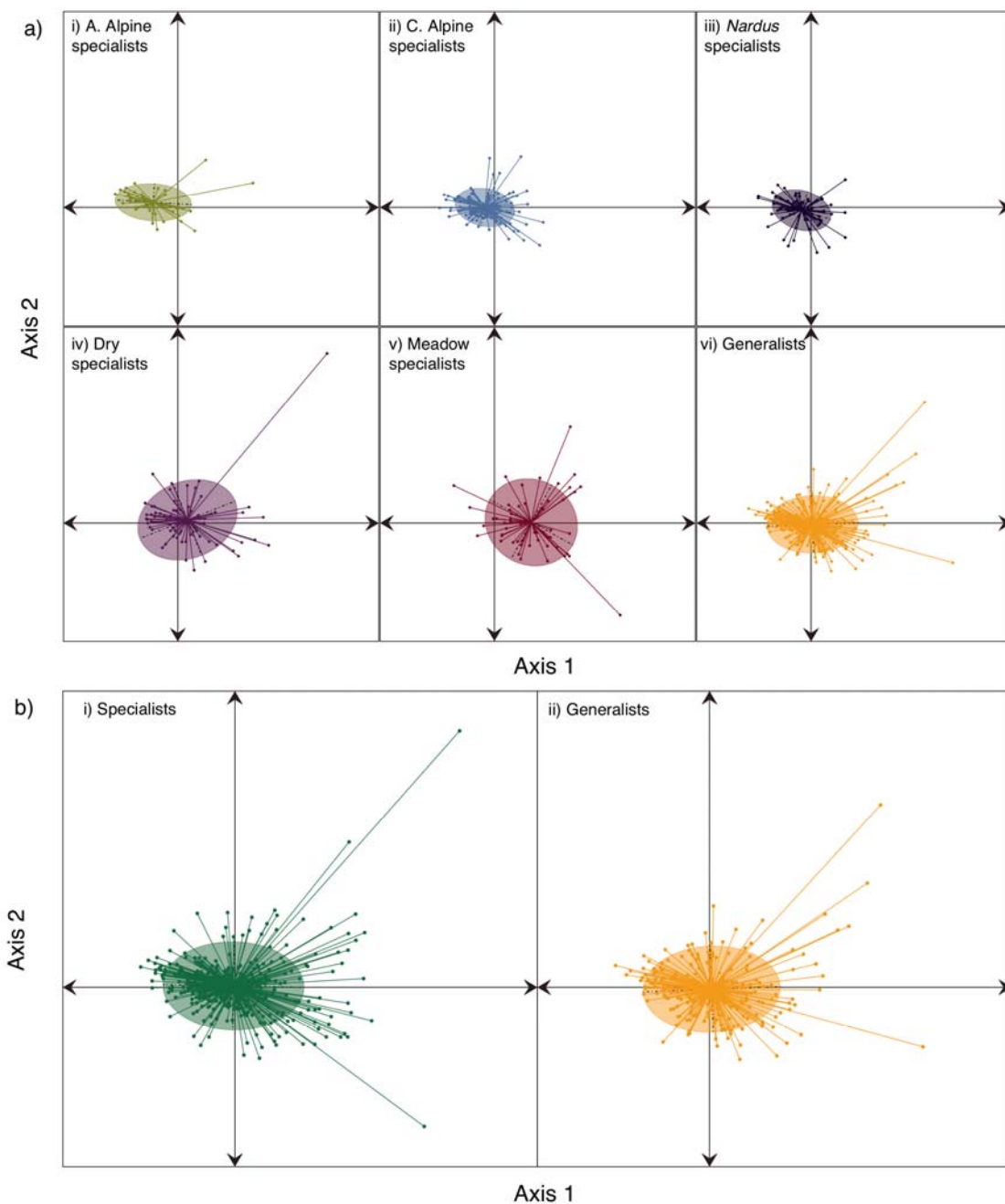
a) All Traits	i) All species (Main Analysis)				
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Variance Explained	23.1	9.15	9.08	7.84	6.83
Eigenvalue	3.23	1.28	1.27	1.1	0.96
SLA	<b>0.29</b>	<b>-0.30</b>	0.05	0.07	0.06
Plant height	0.27	<b>0.49</b>	0.09	0.19	0.25
Radial growth rate	<b>0.30</b>	-0.04	0.07	0.1	-0.07
Flowering onset	<b>-0.29</b>	<b>0.36</b>	<b>0.33</b>	0.05	-0.06
Flowering duration	<b>0.31</b>	<b>-0.32</b>	-0.24	<b>-0.27</b>	0.25
Clonal spread	0.28	0.18	0.26	-0.19	<b>-0.40</b>
Clonal index	0.26	-0.07	<b>0.38</b>	-0.19	<b>-0.46</b>
Clonal cyclicity	-0.25	0.1	0.22	<b>-0.37</b>	0.08
Seed mass	0.16	<b>0.34</b>	<b>-0.4</b>	0.5	-0.2
Seed # /ramet	0.24	<b>0.34</b>	0.27	-0.17	<b>0.51</b>
Soil seed bank longevity	<b>0.33</b>	-0.04	0.08	-0.01	0.11
T <sub>min</sub>	-0.18	-0.23	<b>0.33</b>	<b>0.41</b>	-0.15
T <sub>o</sub>	-0.05	<b>-0.3</b>	<b>0.43</b>	<b>0.43</b>	<b>0.37</b>
T <sub>max</sub>	<b>0.37</b>	-0.04	0.13	0.18	-0.12

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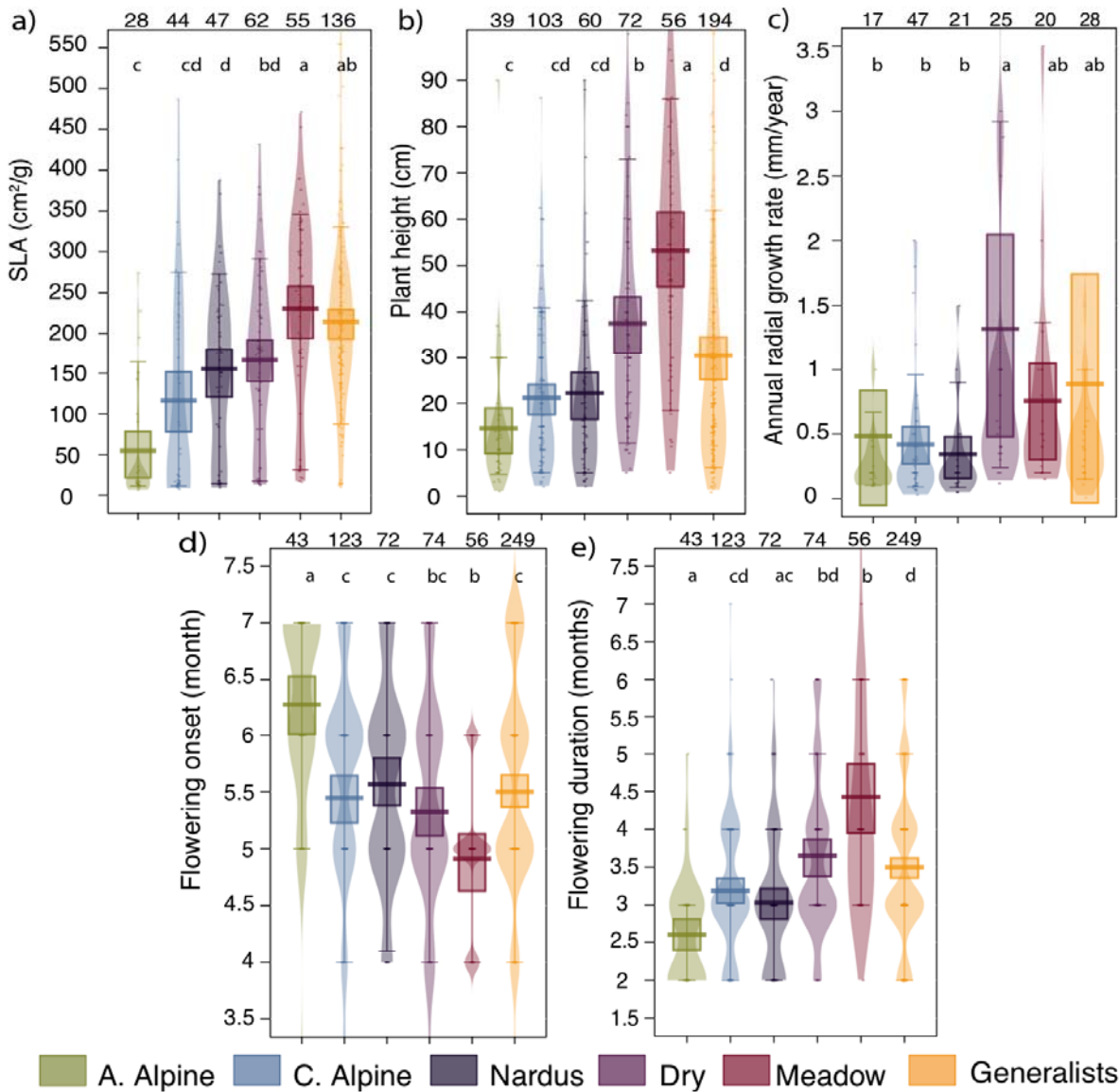
699 **Figures**



700  
 701 **Figure 1: Trait space of the functional species pool of European temperate grasslands.**  
 702 Sample scores representing 645 plant species in a Principal Component Analysis (PCA) based on  
 703 numerical plant traits and a correlation matrix. Each point represents a species, arrows represent  
 704 the visualisation of the contributing variables (See Table 2 for trait abbreviations). Grey  
 705 arrows/labels represent non-regeneration traits and black arrows/labels regeneration traits. The  
 706 five species that contribute the most variation to each axis are labelled in blue and detailed in Table  
 707 S3. Percentages for the three main axes represent the explained variance.

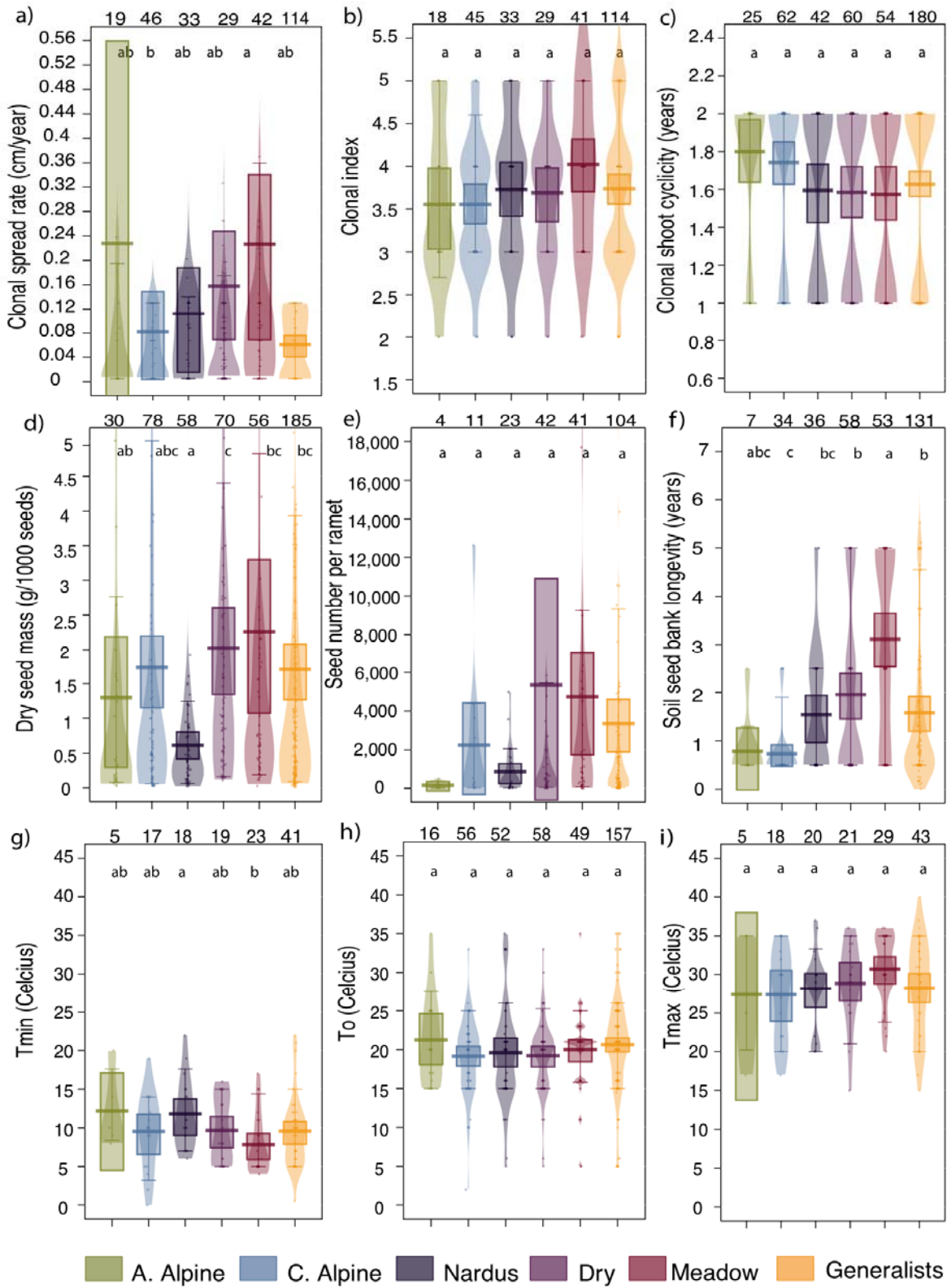


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 709 **Figure 2: Trait space of habitat specialists grouped in five grassland types and**  
 710 **generalists.** Sample scores representing 645 plant species in a Principal Component  
 711 Analysis (PCA) for axis 1 & 2 based on numerical plant traits and a correlation matrix. Each  
 712 point represents a species, and each line shows its distance from the centre point of the  
 713 data for each species group. a) Colored ellipses representing species belonging to each  
 714 specialist group are separated by facets, detailed in the key; b) pooled specialists vs.  
 715 generalists (See Table 1 for grassland type abbreviations). Degrees of freedom=5.



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**Figure 3: Non-regeneration traits across habitats.** a) SLA, b) plant height, c) annual radial growth rate, d) flowering onset, e) flowering duration. RDI plots (Raw data, Descriptive and Inference statistics) show jittered points of raw data, centre bars indicate the mean of the data, beans outline the smoothed density of the data, whiskers mark the 10% and 90% quartiles of the data, and inference bands show the Bayesian 95% High Density Interval inferential statistics for each group. Numbers at the top of each group indicates the number of data points for each trait, and letters show statistical differences between groups.



726 **Figure 4: Regeneration traits across habitats.** a) clonal lateral spread, b) clonal index  
727 (number of offspring + spread) and c) clonal shoot cyclicality, d) dry seed mass, e) seed  
728 number per ramet/plant, f) soil seed bank longevity, and seed germination traits g)  
729 minimum germination temperature, h) optimal germination temperature and i) maximum  
730 germination temperature. RDI plots as in Fig.3.