The functional trait spectrum of European temperate grasslands

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Title

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Running head

Functional traits of European grasslands

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

Abstract

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

Location: Europe.

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

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

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

INTRODUCTION

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

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

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

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

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

Data Analysis

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

We used a Principal Component Analysis (PCA) to describe the multivariate trait spectrum and to identify the contribution of individual traits. We used the R package missMDA (Husson & Josse 2018) to replace missing values in the numerical traits for the PCA and to estimate parameters based on existing values within the dataset (Josse & Husson 2012; Josse & Husson 2016). Out of 9,002 (643 rows of numerical trait data x 14 columns) possible records in the PCA, 48.5% of data was missing and replaced with predicted values from the observed data. Categorical traits were excluded from the multivariate analysis. Missing data was unevenly distributed across traits, with less-commonly studied regeneration traits missing more data than others, but averages between non-regeneration and regeneration traits overall were the same, and all traits 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 (Oksansen 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 χ^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

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

RESULTS

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

Multivariate trait spectrum

The first, second and third axis of the PCA based on 14 numerical traits explained 23%, 9.2 % and 9.1% of the total variance, respectively (Fig. 1). All non-regeneration traits, including radial growth rate, SLA and plant height had relatively high loadings on the first axis of the PCA (Fig. 1a, Table 3). The regeneration traits with the highest 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, R2=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, R2
310 0.098, P=<0.001, Table S6f) than for non-regeneration traits (F= 10.071, R2= 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

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

DISCUSSION

The trait spectrum of European temperate grasslands

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

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

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

Trait differences between habitat specialists

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

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

CONCLUSION

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

Author Contributions

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

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Data Accessibility

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

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684

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

688

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. 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)
Nardus-dominated	1400-1800m	81	e1.7 Closed non-Mediterranean dry acid & neutral grassland	Mountain acid grassland Species Rich Nardus 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)

695

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	**0.29**	**-0.30**	0.05	0.07	0.06
Plant height	0.27	**0.49**	0.09	0.19	0.25
Radial growth rate	**0.30**	-0.04	0.07	0.1	-0.07
Flowering onset	**-0.29**	**0.36**	**0.33**	0.05	-0.06
Flowering duration	**0.31**	**-0.32**	-0.24	**-0.27**	0.25
Clonal spread	0.28	0.18	0.26	-0.19	**-0.40**
Clonal index	0.26	-0.07	**0.38**	-0.19	**-0.46**
Clonal cyclicity	-0.25	0.1	0.22	**-0.37**	0.08
Seed mass	0.16	**0.34**	**-0.4**	0.5	-0.2
Seed # /ramet	0.24	**0.34**	0.27	-0.17	**0.51**
Soil seed bank longevity	**0.33**	-0.04	0.08	-0.01	0.11
T_{min}	-0.18	-0.23	**0.33**	**0.41**	-0.15
T_o	-0.05	**-0.3**	**0.43**	**0.43**	**0.37**
T_{max}	**0.37**	-0.04	0.13	0.18	-0.12

698

Figures

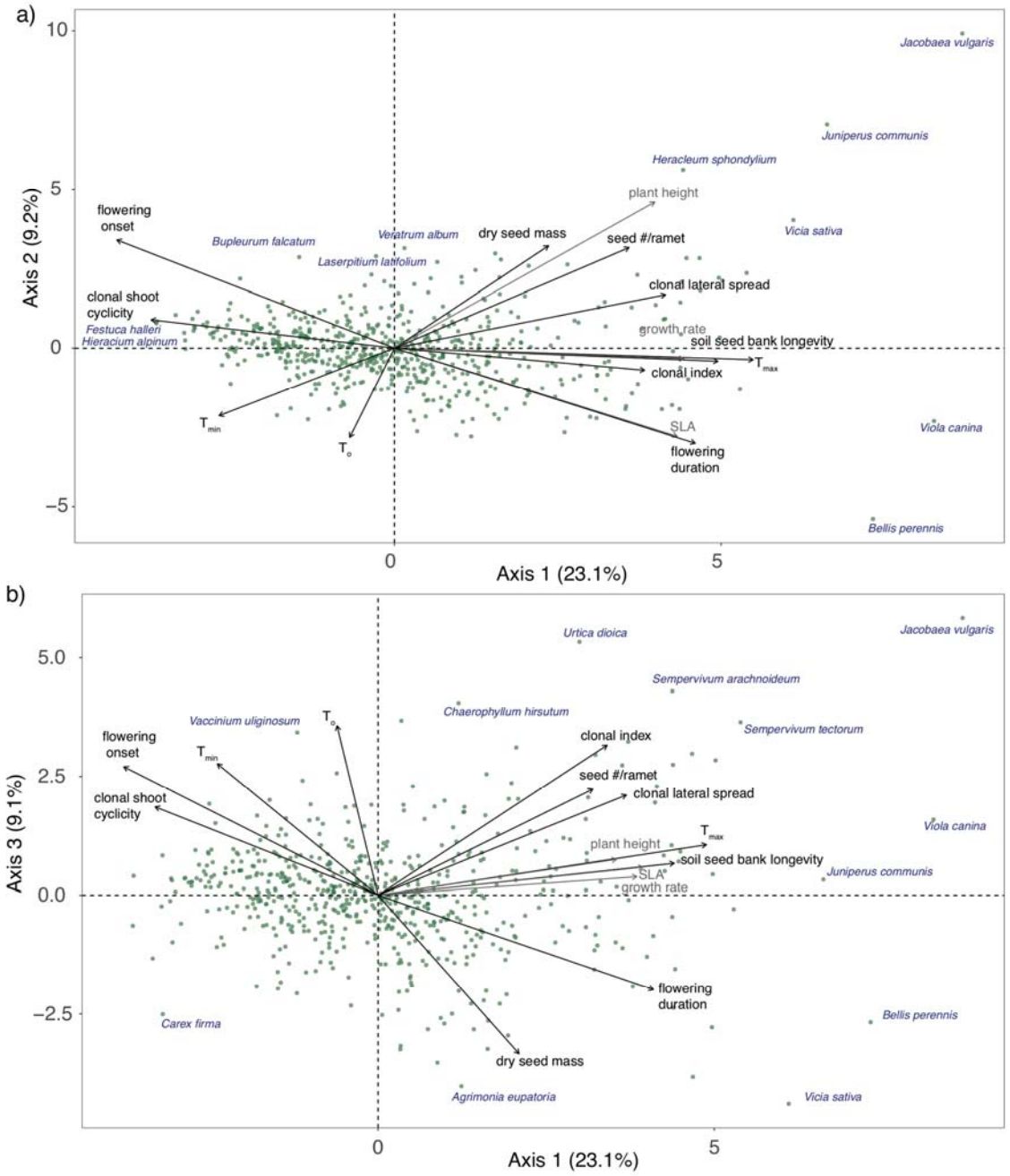


Figure 1: Trait space of the functional species pool of European temperate grasslands. Sample scores representing 645 plant species in a Principal Component Analysis (PCA) based on numerical plant traits and a correlation matrix. Each point represents a species, arrows represent the visualisation of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

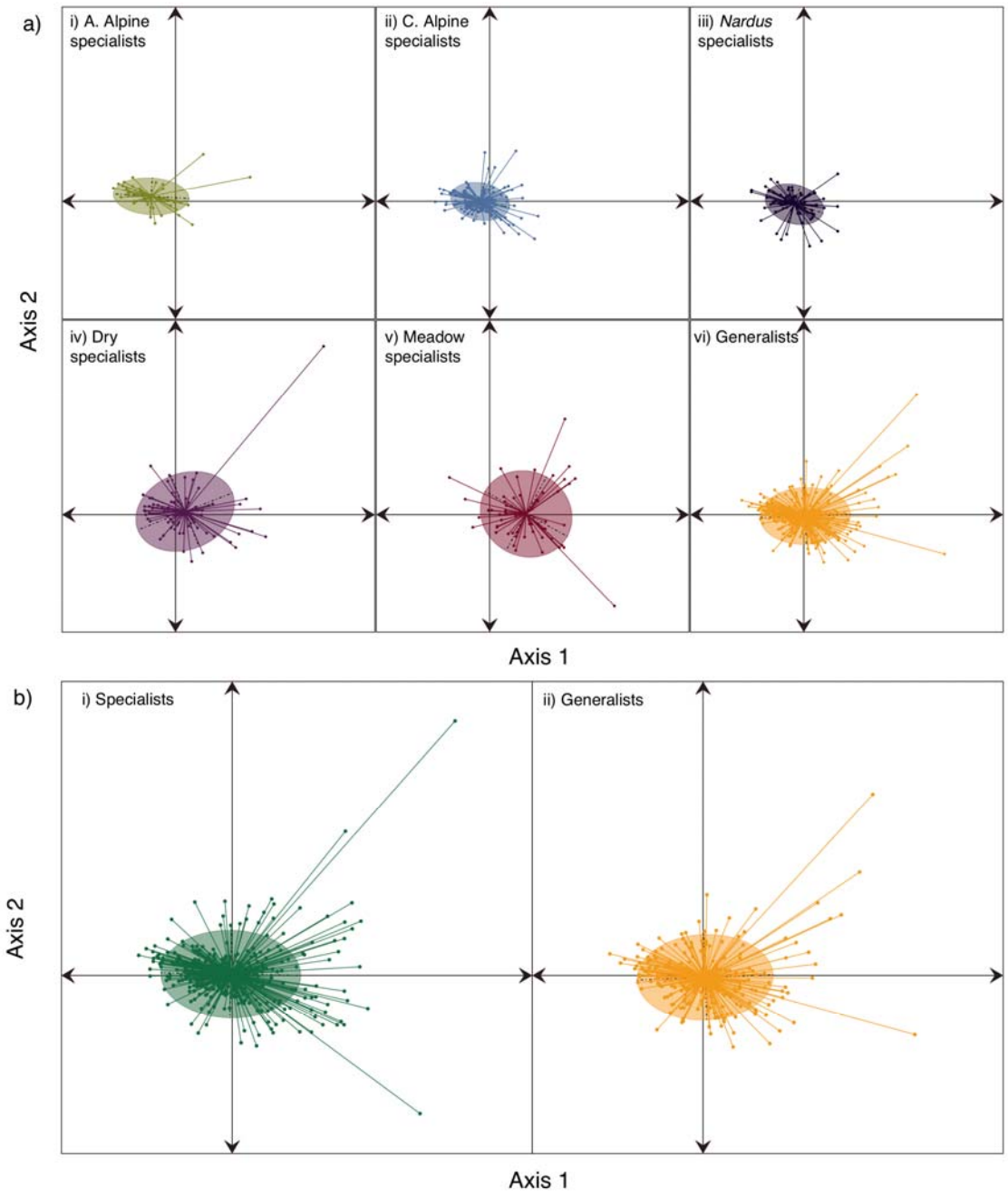


Figure 2: Trait space of habitat specialists grouped in five grassland types and generalists. Sample scores representing 645 plant species in a Principal Component Analysis (PCA) for axis 1 & 2 based on numerical plant traits and a correlation matrix. Each point represents a species, and each line shows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

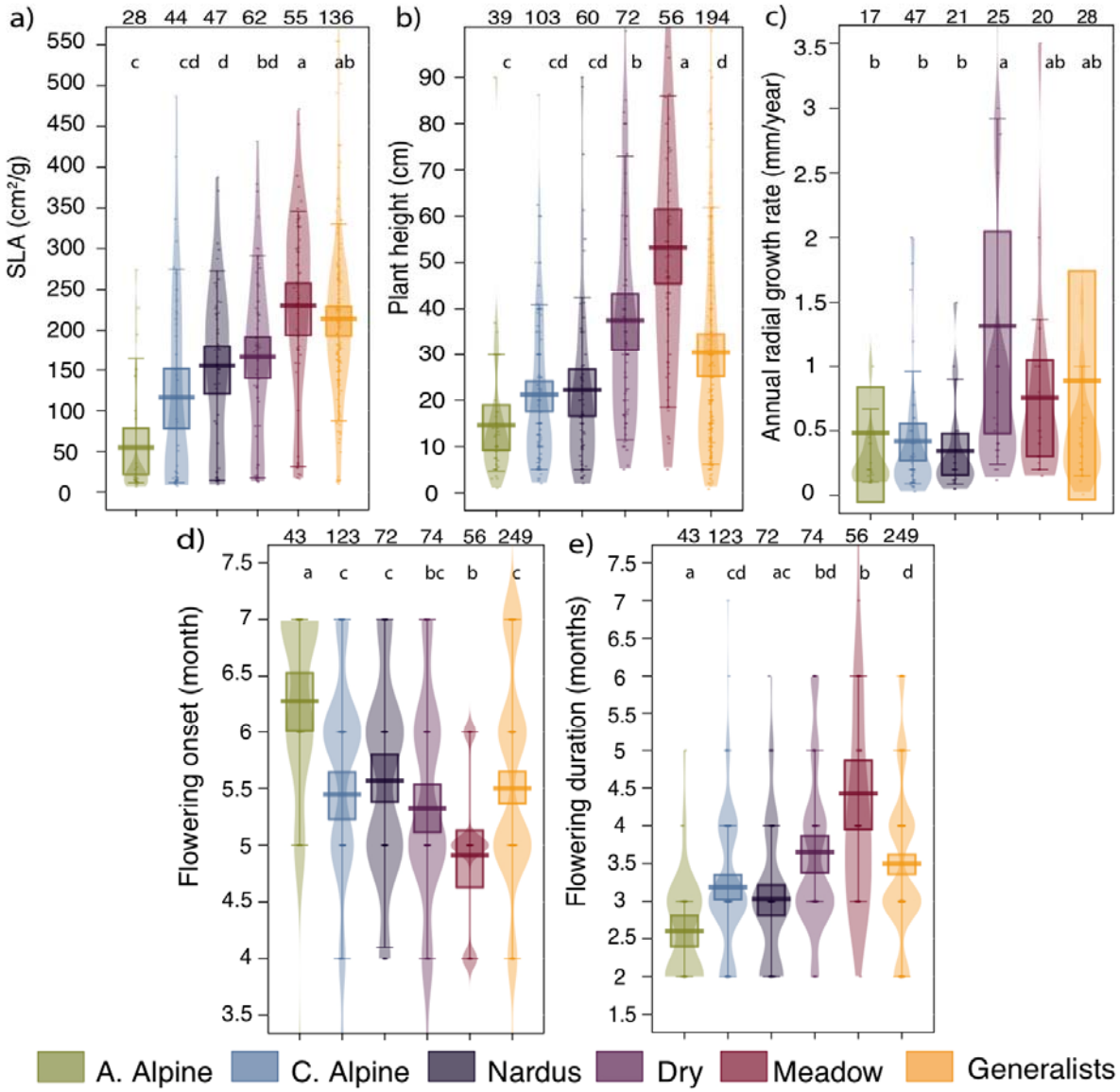
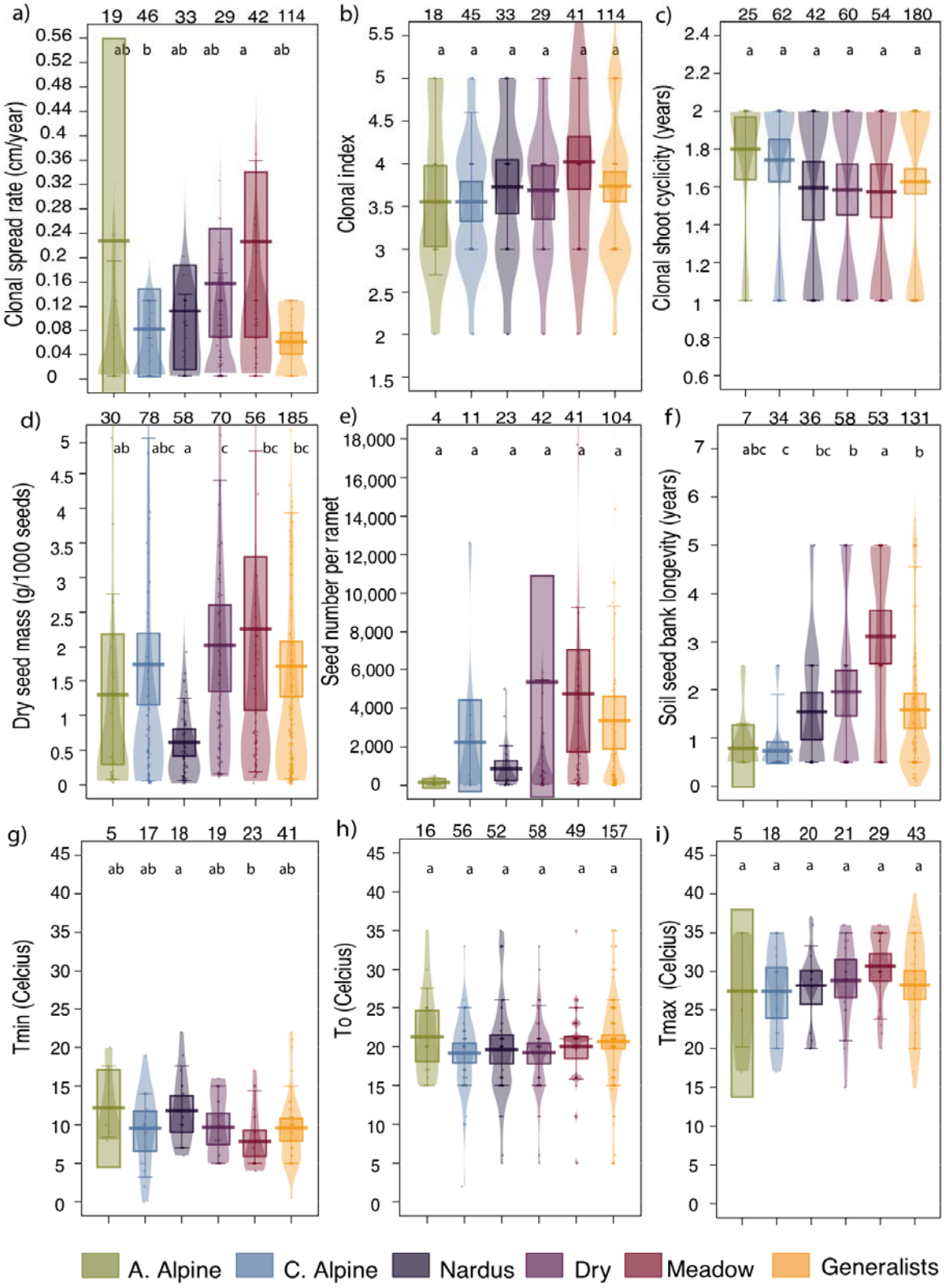


Figure 3: Non-regeneration traits across habitats. a) SLA, b) plant height, c) annual radial growth rate, d) flowering onset, e) flowering duration. RDI plots (Raw data, Descriptive and Inference statistics) show jittered points of raw data, centre bars indicate the mean of the data, beans outline the 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.


a)
Clonal spread rate (cm/year)
19 46 33 29 42 114
ab b ab ab a ab
b)
Clonal index
18 45 33 29 41 114
a a a a a a
c)
Clonal shoot cyclicity (years)
25 62 42 60 54 180
a a a a a a
d)
Dry seed mass (g/1000 seeds)
30 78 58 70 56 185
ab abc a c bc bc
e)
Seed number per ramet
4 11 23 42 41 104
a a a a a a
f)
Soil seed bank longevity (years)
7 34 36 58 53 131
abc c bc b a b
g)
Tmin (Celcius)
5 17 18 19 23 41
ab ab a ab b ab
h)
To (Celcius)
16 56 52 58 49 157
a a a a a a
i)
Tmax (Celcius)
5 18 20 21 29 43
a a a a a a
A. Alpine
C. Alpine
Nardus
Dry
Meadow
Generalists

725

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