Within-community environmental variability drives trait variability in species-rich grasslands

Jodi Price^{1,2,*}, Riin Tamme^{3,4}, Antonio Gazol^{3,5}, Francesco de Bello^{6,7}, Krista Takkis³, Jaime Uria Diez⁸,

Liis Kasari³, Meelis Pärtel³

1. Institute of Land, Water and Society, Charles Sturt University, Albury, NSW, Australia, 2640

2. School of Plant Biology, University of Western Australia, Crawley, Perth, WA, Australia, 6009

3. Institute of Ecology and Earth Sciences, University of Tartu, Lai 40, Tartu, Estonia, 51005

4. Evolution & Ecology Research Centre, School of Biological, Earth and Environmental
Sciences, University of New South Wales, Sydney, NSW, Australia, 2052

5. Instituto Pirenaico de Ecología (CSIC), Avda. Montañana 1005, Zaragoza, Spain, 50059

6. Department of Botany, Faculty of Sciences, University of South Bohemia, Na Zlate Stoce 1,
CZ-370 05 České Budějovice, Czech Republic, 37982

7. Institute of Botany, Czech Academy of Sciences, Třeboň, Czech Republic, 37982

8. Department of Forest Resource Management, Swedish University of Agricultural Sciences
(SLU), Faculty of Forest Sciences, Skogsmarksgränd, SE-901 83 Umeå, Sweden

* Corresponding author. Email: joprice@csu.edu.au

Tamme, R. (Riin.Tamme@ut.ee)

Gazol, A. (agazol@ipe.csic.es)

de Bello, F. (fradebello@ctfc.es)

Takkis, K. (krista.takkis@gmail.com)

Uria Diez, J. (jaime.uria.diez@slu.se)

Kasari, L. (Liis.Kasari@ut.ee)

Pärtel, M. (Meelis.Partel@ut.ee)

Abstract

Aims: Spatial environmental heterogeneity has been considered an important coexistence mechanism, because environmental variation enables different species to co-occur. We predict if functional differences are important for coexistence, then both species and functional diversity should be positively related to environmental heterogeneity.

Location: 33 dry calcareous grassland sites in Estonia.

Methods: In each site, we established a transect (10 x 0.1 m), consisting of 100 quadrats (10 x 10 cm). In each quadrat, we recorded species richness and composition, and measured soil depth, moisture, and light availability. We collected data on 8 traits from most of the species found across the sites. We calculated functional diversity (FD) at the quadrat scale, and compared the observed-FD to that expected at random using two null models. The first null model used all the species that occurred in the transect in the randomisations, to determine environmental filtering from the transect to the quadrat. The second null model restricted the species used in the randomisations to those species whose trait range was within the range of the observed values in the quadrat, to detect evidence for niche partitioning. Quadrat mean trait values and variability in functional composition were then related to small-scale mean environmental conditions and heterogeneity respectively.

Results: We found convergent patterns in biomass, specific leaf area, specific root length, and clonality compared to randomised communities that included all species occurring in the transect, and we found divergence in height and leaf area using the range restricted null model. Hence, we found patterns consistent with environmental filtering and niche partitioning depending on the traits considered. Quadrat mean traits were significantly correlated with the measured environmental variables, with the main trend being a positive relationship between size-related traits and soil resources, and a negative relationship between these traits and light availability. Trait variability in height, leaf area, leaf dry matter content, specific root length and clonality was positively related to

soil depth heterogeneity. There was a trend for species richness to be negatively related to environmental heterogeneity.

Conclusions: Co-occurring species were more similar in several traits partly due to micro-environmental filtering in response to variability in soil depth. While environmental heterogeneity increased trait variability, negative relationships with species richness were observed. Hence, niche partitioning does not appear to be important for the maintenance of high small-scale species richness in these grasslands.

Keywords Community assembly; Convergence; Divergence; Functional diversity; Grasslands; Heterogeneity; Niche partitioning; Trait dispersion

Abbreviations: FD = functional diversity; H:B ratio = height:biomass ratio; LDMC = leaf dry matter content; MPD = mean pairwise difference; SES = standardized effect size; SLA = specific leaf area; SRL = specific root length

Running head: Environmental variability drives trait variability

Introduction

Trait dissimilarity among co-occurring plant species is commonly used to determine the relative importance of abiotic and biotic processes driving community assembly (e.g. Götzenberger et al. 2012; Mason & de Bello 2013). Increased similarity (i.e. trait convergence) among species is usually attributed to either abiotic filtering, reflecting shared adaptations to environmental conditions (Weiher & Keddy 1995; Cornwell & Ackerly 2009), or to competitive hierarchies, whereby species with trait values associated with low competitive ability are excluded, or species are more similar in competitive ability making exclusion unlikely (Mayfield & Levine 2010; de Bello et al. 2012b; Kunstler et al. 2012). Increased dissimilarity (i.e. trait divergence) is usually attributed to limiting similarity, based on the idea that species must be functionally different to coexist (e.g. Macarthur & Levins

1967; Stubbs & Wilson 2004). However, trait dispersion studies have been criticised as both convergence and divergence can be due to either abiotic or biotic processes, and trait patterns alone cannot separate these processes (Cavender-Bares et al. 2009; Mayfield & Levine 2010; HilleRisLambers et al. 2012; Kunstler et al. 2012; Spasojevic & Suding 2012; Adler et al. 2013; Gerhold et al. 2015).

A multi-scale approach has been proposed as one 'fix' to separate the influence of biotic and abiotic processes, and a shift from abiotically driven convergence to biotically driven divergence with decreasing spatial scale has been found (Bernard-Verdier et al. 2012; de Bello et al. 2013; Laliberté et al. 2013). Functional divergence has been more often detected at small spatial scales (reviewed in Götzenberger et al. 2012), attributed to limiting similarity because co-occurring species should be directly competing for resources, and environmental variation should be minimised (Stubbs & Wilson 2004). There is no doubt that environmental filtering is important at large spatial scales, and is responsible for much of the variation in species distributions (together with dispersal). At large spatial scales, evidence for environmental filtering comes from changes in community weighted mean trait values along environmental gradients (Cornwell & Ackerly 2009; Fortunel et al. 2014; Baastrop-Spohr et al. 2015; Jager et al. 2015), and this approach is also suitable for detecting environmental filtering within communities (i.e. micro-environmental filtering). However, the influence of environmental variation on trait patterns within communities is largely unknown, and may explain the large variation in traits that is often reported within communities (Wright et al. 2004, Mudrák et al. 2015). Trait dispersion studies may be used to infer evidence for environmental filtering, but environmental variables must be directly measured and related to trait patterns, rather than indirectly inferred based on spatial scale.

Spatial environmental heterogeneity has long been considered an important coexistence mechanism because environmental variation enables *different* species to co-occur (i.e. niche partitioning, Reynolds et al. 1997; Lundholm 2009; Stein et al. 2014). Hence, heterogeneity should be positively related to both species and functional diversity, but the relationship between

functional diversity and heterogeneity has rarely been addressed (Schwilk & Ackerly 2005; Willis et al. 2010; Price et al. 2014). Moreover, negative heterogeneity-species diversity relationships have been found at fine-spatial scales where plant individuals interact, through altering competitive interactions among species (Lundholm 2009; Tamme et al. 2010). This can occur if some species are better able to access patchily distributed resources (Gazol et al. 2013, Tamme et al. 2016), for example where tall, clonal species occur in the community (Eilts et al. 2011). In a simulation study, increased divergence of traits that were directly associated with resource acquisition, led to a decrease in species richness (Herben and Goldberg 2014).

We explored functional trait patterns in dry calcareous species-rich grasslands in Estonia, Northern Europe. These grasslands are internationally recognized for their very high small-scale species richness (Wilson et al. 2012); maximum species richness at the 10 x 10 cm scale was 20 species (mean = 9, Gazol et al. 2012). Hence, they are a good model system to understand the links between functional trait patterns and species coexistence in communities where many species interact. The sites are characterised by small-scale heterogeneity in soil depth, which effects species richness patterns by determining the spatial heterogeneity of soil resources (Gazol et al. 2012). We measured a number of plant traits that were expected, based on the literature (Table 1), to be important for community assembly processes, including whole plant traits, leaf and root traits. We aim to improve the inference of mechanisms driving functional trait variability by examining the effect of small-scale environmental heterogeneity on functional diversity and species richness.

Specifically we address:

1. Is there evidence for non-random trait-based community assembly in species-rich grasslands? Are co-occurring species more similar, or more different than expected from random?

2. Micro-environmental filtering. We examine the relationship between quadrat-level mean traits and quadrat-level environmental variables. Significant relationships support micro-environmental filtering, i.e. species are filtered according to small-scale environmental conditions.

3. Niche partitioning. We examine the relationship between heterogeneity of environmental variables, species richness and functional diversity. A positive relationship between heterogeneity and FD supports the concept of niche partitioning. If niche partitioning is important for coexistence then species richness should also be positively related to heterogeneity.

Methods

Study site

This study was carried out in dry calcareous semi-natural grasslands (known as alvars) located on the islands of Saaremaa and Muhu in Estonia, Northern Europe (Fig. 1). The climate on these islands is maritime with a mean temperature of 16.5°C in summer (July) and -3°C in winter (January). Mean annual precipitation is ~600 mm (Estonian Meteorological and Hydrological Institute). Alvar grasslands occur on thin soils (usually <20 cm depth), on Ordovician or Silurian limestone bedrock. The thin soil layer means that alvars can completely dry out in summer, and can flood temporarily after heavy rains and snow melt (Pärtel et al. 1999). The vegetation is characterised by a low biomass herbaceous layer (~170 g/m² in our study sites during peak biomass, unpublished data), with sparsely distributed *Juniperus communis* shrubs. We found 122 species across the 33 sites; the most common species were *Thymus serpyllum*, *Pilosella officinarum*, *Helictotrichon pratense*, *Carex caryophyllea*, *Festuca ovina*, *Galium verum* (a list of the most common species and their mean frequency can be found in Appendix S1).

Data collection

We selected 33 grassland sites, representing almost all of the remaining good condition alvars in the region (Gazol et al. 2012). In each site, a transect of 10 x 0.1 m was placed in a central location representing typical site environmental conditions, and as much as possible away from the influence of encroaching shrubs. Transects were divided into 100 quadrats (10 x 10 cm) to measure small-scale species richness and composition, and environmental variables (Fig. 1). Species presence was recorded as species which were rooted in the quadrat. Soil depth, moisture, and light availability were also measured in each quadrat. Sampling was conducted in 2010 during the peak floristic season from the 33 study sites (total of 3300 quadrats). We used a metal rod (40 cm in length) to measure soil depth until bedrock. Surface soil moisture (depth of 10 cm) was measured using a WET-2 sensor (Delta-T Devices, Cambridge, UK). We also measured soil pH, which does not vary much at this scale (average pH is 7.3, Gazol et al. 2012). Soil depth is the main proxy for resource availability in these grasslands because total nutrient concentration is quite uniform, and soil resources are distributed in a thin layer above bedrock. Hence, it is the soil depth interacting with resource concentration that gives a better indication of total amount of nutrients when soil depth is heterogeneous (Pärtel et al. 1999). Light availability was measured using a LI-190SA quantum sensor and LI-250A light meter (LI-COR, Lincoln, USA) and expressed as the ratio between photosynthetically active radiation below and above the vegetation. Light measurements were taken around mid-day, on relatively cloud free days and within each transect the measurements were collected within a 1 hr time frame.

In 2012, we collected plant trait data from 15 of the 33 sites to incorporate between-site variability (Fig. 1). We selected 8 vegetative traits that are associated with key aspects of plant ecological strategies (Table 1). In each site, we collected trait data for all species occurring in >10% of the quadrats, so only rare species were not sampled. We produced a species x trait matrix that contained at least 10 measures per species, and up to a total of 150 samples if a species occurred in all 15 study sites. This sampling effort obtained trait data for ~ 70 species from a total of 122 found across the 33 sites.

Traits measured were plant height, specific leaf area (SLA), leaf area, leaf dry matter content (LDMC), shoot biomass, height:biomass ratio, specific root length (SRL), and clonal index (Table 1). Traits were measured using standardized protocols (Table 1). Plant height was measured as the length from ground level to the highest photosynthetically active tissue. On the same plants used for height measurements, whole plants (aboveground material) were collected for calculations of biomass and height:biomass ratio. For leaf traits, we collected fully formed adult leaves and stored them in moist plastic bags until they could be weighed (fresh weight for LDMC) and scanned (to measure leaf area and SLA), the same leaves were used for all leaf traits. Leaves were then dried for 48 hr at 70°C and weighed. Leaf area was measured as the one-sided projected surface area of a whole leaf (mm²). Leaf area was calculated from leaf scans using Image-J (Rasband 2007). For SRL, we collected fine root samples from 5-10 individuals per species in total across the study sites. Roots were carefully washed and scanned in water at 800 dpi using dual-lens scanner (Epson Perfection V700 Photo; Epson, Suwa, Japan). The images were analysed using WinRhizo 2008 (Regent Instruments Inc, Quebec, Canada). Root samples were dried for 48 hr at 60 °C and weighed. Clonal index was computed using the approach of Johansson et al. (2011), based on two clonal-related traits, lateral spread and number of annual offspring. Clonal traits were taken from CLO-PLA database (Klimešová & de Bello 2009). We looked at correlations between all pairs of the measured continuous traits and found some significant relationships (Appendix S2).

Data analysis

We calculated quadrat mean trait values for all the individual traits by averaging the trait values of the species present in the quadrat (for which trait data were available). We calculated trait dissimilarity between each pair of species within a transect for individual traits using Euclidean distance. We used a mean trait value in the calculations for all species based on trait values from all sites where the species occurred (and was collected, >10 % of quadrats). We standardised the dissimilarities between transects by dividing the values with the maximum pair-wise dissimilarity found in a transect. Data for leaf area, biomass, height:biomass ratio and height were ln-

transformed prior to analysis. Functional diversity was then calculated for each trait, using the mean pairwise distance (MPD) in trait values between species within a quadrat. We used this index because it is independent of species diversity (de Bello et al. 2006, 2016). We have also compared the results using the MNTD index, and the results are similar (not shown), because the indexes are correlated when species richness values are low.

Within each transect, we used null-models to assess if observed quadrat-FD was different from that expected by chance (Question 1, Fig. 1). We used two null models to test for different aspects of community structure: 1. to examine evidence for micro-environmental filtering, and 2. to examine evidence for niche partitioning. First, randomised communities were generated using a species pool that included all of the species that occurred in each transect (i.e. 33 site-specific species pools). This null model was used to examine environmental filtering from the species pool to the quadrat. We used the randomization approach of Stubbs and Wilson (2004), where species richness in each quadrat was fixed, and species frequencies partially fixed. Species richness in the 10 x 10 cm quadrats was kept the same as the field observations, this assumes different carrying capacities of individual quadrats and follows the most common approach in randomization tests (Stubbs & Wilson 2004; Götzenberger et al. 2012). To take into account species frequencies, species were divided into two groups; species whose occurrences were above the median in the transect (common species), and species whose occurrences were below the median (uncommon). We kept species frequencies partially fixed to avoid giving too much weight to traits associated with uncommon species, and to minimise the potential effects of environmental filtering among sites that may make some species more locally abundant than others. However, species frequencies were not completely constrained, which allows competitive hierarchies to be modified (following Mason et al. 2011 and Mudrák et al. 2015). Additionally, fixing both species richness and species frequency reduces the number of possible randomizations. Randomizations were run 999 times for each transect. The second null model was similar to the first (richness fixed and frequency partially fixed), but randomised communities for each quadrat consisted of species whose trait values (species

minimum and maximum) fit the range of mean trait values for species observed in the quadrat (referred to as the 'range restricted model'). This approach was not used for clonality since each species only had one trait value. By reducing the species pool used in randomisations to only species that can occur within the observed trait range (from the site-specific species pool), we can then determine if species are more different than expected within the environmental constraints of the quadrat. To detect trait convergence (i.e. coexisting species are more functionally similar than expected) and trait divergence (i.e. coexisting species are more functionally dissimilar than expected), the standardized effect size (SES) was computed for each transect and trait as:

$$SES = \frac{MPD_{obs} - MPD_{exp}}{SD(MPD_{exp})}$$

where MPD_{obs} is the MPD observed in all 100, 10 x 10 cm quadrats (mean of the transect). MPD_{exp} is the mean MPD expected by the null model for each transect. SD (MPD_{exp}) is the standard deviation of the mean MPD_{exp} values per transect. The null hypothesis (random assembly) is valid when the SES-MPD approaches zero, i.e. when trait values of species co-occurring at the 10 x 10 cm scale can be expected by the random combination of species from the transect. To estimate the trait convergence/divergence across all transects we used one-sided t-test to evaluate if the SES values of 33 transects are significantly different from 0.

For each trait, we correlated the quadrat trait means with each of the measured environmental variables (soil moisture and depth were ln-transformed prior to analysis) to test for micro-environmental filtering (Question 2, Fig. 1). We used correlations rather than a regression model because the direction of the effect was unknown, biotic causing abiotic or vice versa, and therefore we examine patterns. The correlations were made for each transect separately and the overall effect determined using a meta-analytical approach. We used the 33 correlation coefficients as effect sizes and implemented the Olkin-Pratt fixed-effect meta-analysis. This method estimates an

unbiased mean effect size (G) using the correlation coefficients. We used a fixed-effect model since all of our sampling was conducted using the same protocol (Gurevitch 2013). Quadrat mean data for leaf area, biomass, height:biomass ratio and height were ln-transformed before the analysis.

To examine the relationship between environmental heterogeneity and functional diversity, we correlated the coefficient of variation (CV; mean/SD) in environmental variables (of 3 quadrat blocks) with the coefficient of variation in quadrat mean traits (of 3 quadrat blocks, Question 3, Fig. 1). We used partial correlations to account for the effect of the mean environmental conditions in the three-quadrat blocks (mean soil moisture and depth were ln-transformed). The overall effect was determined using the meta-analytical approach described above. The analyses were conducted in the R environment (R development core team 2015). We used the *mpd* function in the *picante* package for FD analysis (Kembel et al. 2010). We used the *metacor.OP* function from *metacor* library (Laliberté 2011) for the meta-analysis and *pcor* function from the *ggm* library (Marchetti et al. 2015) for partial correlations.

Results

Question 1. Grassland functional diversity patterns

We found convergence in biomass, specific leaf area, specific root length and clonality compared to randomised communities where all species in the transect were used in species pool (no traits were significantly divergent, Fig. 2). Using the range restricted model, we found height and leaf area were significantly divergent, suggesting co-occurring species are more different in these traits when some environmental filtering is accounted for (Fig. 2). Biomass, SLA and SRL were also significantly convergent using the range restricted null model (Fig. 2).

Question 2. Small-scale environmental conditions and quadrat mean trait values

We found a clear functional response to the within-community environmental gradient as evidenced by significant correlations between mean trait values and small-scale environmental variables (Fig.

3). The main trend was a positive relationship between size-related traits and increased soil resources (moisture and depth). Height, biomass, leaf area and clonality were positively related to soil depth, and biomass and leaf area were positively related to soil moisture (Fig. 3). In addition, size-related traits (height, biomass, leaf area and clonality) were negatively related to light availability (Fig. 3), suggesting larger plants are favoured in high resource patches which thereby reduce light availability. H:B ratio, SLA and SRL were negatively related to soil depth (Fig. 3). Clonality was negatively related to soil moisture (Fig. 3). LDMC was the only trait that was not significantly associated with any of the mean environmental variables (Fig. 3).

Question 3. Small-scale environmental heterogeneity, species richness and functional diversity
Functional diversity (CV of mean quadrat traits) for height, leaf area, LDMC, SRL and clonality were positively related to heterogeneity in soil depth demonstrating environmental variability in soil depth increases trait variability (Fig. 4). Functional diversity in LDMC was also positively related to heterogeneity in soil moisture. There was a trend towards a negative relationship between heterogeneity in all environmental variables and species richness (Fig. 4). There were no significant relationships between heterogeneity in light availability and functional diversity in any of the traits (Fig. 4).

Discussion

Species-rich grasslands in Estonia were characterised by functionally similar co-occurring species for many traits. Functional convergence is related to micro-environmental filtering, as all traits (except LDMC) were significantly correlated with at least one environmental variable. Trait variability was mostly a response to variability in soil depth (as a proxy for resource availability), because soil moisture and light availability can be influenced by the plants themselves (and had less significant relationships). Additionally, we found trait divergence in height and leaf area using a range restricted null model, consistent with the patterns expected by niche partitioning. Hence, we found patterns

that were consistent with environmental filtering (for biomass, SLA, SRL and clonality) and niche partitioning (for height and leaf area).

The grassland communities showed a clear functional response to the within-community environmental gradient as evidenced by significant correlations between most traits and mean environmental variables. We found increased soil resources (depth and moisture) were positively associated with size-related traits, i.e. species had larger leaf area, more biomass and height, and had lower SLA, SRL, and height:biomass ratio. Low specific root length in high resource patches might be because plants do not invest energy in increasing root length, as they do not need to forage for resources (Pérez-Harguindeguy et al. 2013). In addition, we found a negative relationship between light availability and size-related traits, indicating that in higher resource patches larger species are favoured, which then reduce light availability. Hence, species are sorted according to small-scale variation in soil conditions, which has been reported in other grasslands at similar scales (Reynolds et al. 1997; Fridley et al. 2011; Luzuriaga et al. 2015; Martorell et al. 2015).

We found a positive relationship between soil depth heterogeneity and functional diversity for many traits, as expected by niche partitioning. However, heterogeneity in environmental variables was negatively associated with species richness, consistent with a previous study from these sites (Gazol et al. 2012). Heterogeneity, by promoting functional differentiation among species, might reduce richness through increasing competitive ability differences (Kunstler et al. 2012; Herben & Goldberg 2014). Our results are consistent with patterns found in a large-scale study examining competition in European forest trees (Kunstler et al. 2012), and European grassland species (Herben & Goldberg 2014). Our results show that taxonomic and functional diversity can have contrasting patterns at fine spatial scales, inconsistent with the predictions based on niche partitioning.

In conclusion, co-occurring alvar grassland species are characterised by functional trait similarity in response to small-scale soil environmental variability. Coexistence of a high number of functionally similar species suggests that species co-exist when species differences are small. Mean

quadrat traits were significantly related to mean soil depth, supporting micro-environmental filtering driving trait convergence. Small-scale soil heterogeneity increased functional diversity, but had a negative effect on species richness, which is not consistent with the predictions of niche partitioning. Exploring the relationship between environmental variability, functional diversity, and taxonomic diversity will enable better inference of mechanisms facilitating species coexistence.

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Appendix 1. Common species in the 33 grasslands and average frequency

Appendix 2. Trait correlations

Table 1. Description of the functional traits, and their related functions, based on the literature and following the cited protocols.

Trait	Units	Functions	References
Plant height	cm	Competitive ability, light capture	Westoby et al. 2002
Shoot biomass	g	Competitive ability	
Height:biomass ratio (H:B ratio)	cm/g	Spatial occupancy and growth strategy, competitive ability	de Bello et al. 2012a
Specific leaf area (SLA)	mm ² mg ⁻¹	Resource acquisition and relative growth rate (RGR)	Pérez-Harguindeguy et al. 2013
Leaf area (LA)	mm ²	Competitive ability, light capture, leaf energy	Pérez-Harguindeguy et al. 2013
Leaf dry matter content (LDMC)	mg g ⁻¹	Leaf tissue density, stress tolerance	Pérez-Harguindeguy et al. 2013
Specific root length (SRL)	cm mg ⁻¹	Nutrient and water uptake, RGR	Pérez-Harguindeguy et al. 2013
Clonality	Semi-quantitative index	Spatial occupancy, persistence	Johansson et al. 2011 Klimešová and De Bello 2009

Question 1: Small-scale FD patterns

Are co-occurring species (10x10 cm) more similar or different than expected from null models?

Question 2: Micro-environmental filtering

Are quadrat-level mean trait values correlated with quadrat-level environmental variables?

Question 3: Niche partitioning

Is there a positive relationship between environmental heterogeneity (CV of environmental measures in 3 quadrats) and CV of quadrat means and SR (3 quadrats)?

Fig. 1. Diagrammatic representation showing the location of the 33 grassland sites on the islands of Saaremaa and Muhu in Estonia, and one of the grassland sites (photo). Species composition data and environmental variables were measured in one hundred 10 x 10 cm quadrats, positioned along the 10 m transects. The grey shaded boxes represent spatial environmental variability. **Question 1.** Observed values were compared to values obtained from null models to determine if trait patterns differ from that expected at random. **Question 2.** Small-scale mean traits were correlated with quadrat-level environmental variables. **Question 3.** Environmental heterogeneity (measured as the CV of 3 10 x 10 cm quadrats) was correlated with the CV of mean traits of 3 quadrats and species richness.

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Fig. 2. Standardised Effect Size (SES) values for all the individual traits across the 33 grassland sites using MPD (blue) and the range restricted null model (green). The SES values were used to determine if trait patterns were convergent (negative value) or divergent (positive value). Significant results are indicated by * <0.05, and · <0.1. Trait codes are H:B = height:biomass ratio, LA = leaf area, SLA = specific leaf area, LDMC = leaf dry matter content, SRL = specific root length. Note: clonality was not used in the range restricted model as trait ranges were not available.

Fig. 3. The relationship between small-scale environmental variables and mean traits based on a meta-analysis using correlation coefficients from each transect as an effect size. Significant results are indicated by * <0.05, and • <0.1. Trait codes are H:B = height:biomass ratio, LA = leaf area, SLA = specific leaf area, LDMC = leaf dry matter content, SRL = specific root length.

Fig. 4. The relationship between small-scale heterogeneity and the coefficient of variation in quadrat mean traits, and species richness (10 x 30 cm) based on a meta-analysis using partial correlation coefficients from each transect as an effect size. Significant results are indicated by * <0.05, and · <0.1. Codes are SR = species richness, H:B = height:biomass ratio, LA = leaf area, SLA = specific leaf area, LDMC = leaf dry matter content, SRL = specific root length.