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5 Context-dependent assembly rules and the role of dominating grasses in semi-natural abandoned

6 sub-mediterranean grasslands

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1   Abstract

2   We investigated fine-scale patterns of trait-based community assembly in calcareous grasslands of

3   the Central Apennines, Italy. We used the habitat template of environmentally contrasting north-

4   facing and south-facing slopes of a mountain valley to understand mechanisms that contribute to

5   species coexistence (i.e. the persistence of diversity) after cessation of previous land use practices.

6   Firstly, we tested late successional dominating grasses (*Sesleria nitida, Brachypodium rupestre and*

7   *Bromopsis erectus*) for their ability to serve as biotic filtering effects on the diversity of subordinate

8   species in plant communities. Secondly, we analyzed fine-scale trait-based (i.e. species-level traits

9   related to competition, regeneration, establishment, dispersal, and flowering) community assembly

10 of subordinate species in absence of dominant grass. We found that assembly rules for traits related

11 to the same life-history process were mostly consistent within habitats. Further we established that

12 within habitats the traits related to different life-history processes can show different assembly

13 rules. For example, while generative regeneration traits (seed mass) may show convergence pattern,

14 divergence was inferred for the vegetative (clonal) regeneration traits. Depending on traits, the

15 assembly rules can be similar or contrasting in different habitats. We conclude that our finding of

16 non-random assembly in the majority of investigated traits emphasizes the importance of

17 hierarchical exclusion of strong biotic filters when searching for trait-based assembly rules in

18 abandoned grasslands. Thus, for nature conservation purposes, disturbance appears to be the

19 process that is most important in driving the survival of subordinate species by the exclusion of

20 biotic filters. Subsequently, a multitude of trait-based mechanisms allow for coexistence of the

21 subordinate species. These mechanisms depend on habitats and traits and thus may vary from

22 community to community, indicating that heterogeneous landscapes might support multiple

23 processes of coexistence.

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5   1. Introduction

6   European grasslands represent a wealth of immense conservation value. Despite the prevailing

7   secondary origin of this vegetation formation in Europe due to a long history of pastoralism and

8   agrarian colonization, their primary nuclei represent the westernmost fragmented fringe of the

9   Eurasian steppe and forest-steppe embedded within the temperate forest biomes (Walter and Straka,

10   1970; Ellenberg and Leuschner, 2010). These ecosystems support a rich flora and they may develop

11   a very high small-scale species density, mirroring intricate coexistence mechanisms (Wilson et al.,

12   2012). The sub-mediterranean grasslands of the Apennines, which are included in habitat 6210 by

13   the 92/43/EEC Directive as 'habitat of priority importance', characterize a typical pastoral

14   landscape where grazing was the dominant land use type. Preserving these ecosystems is an urgent

15   task to counteract possible negative effects of changes in land-use such as abandonment imposing

16   threats on local biodiversity (Collins et al., 1998; Poschlod and WallisDeVries, 2002; Balmford et

17   al., 2005; Catorci et al., 2011).

18   As a consequence of reduction of grazing pressure, some clonal tall grasses tend to prevail and

19   dominate the grassland community through specific plant traits such as tall canopies, extensive

20   lateral spread, litter deposition, and capacity to project shoots through litter and herbaceous cover

21   (Campbell et al., 1992; Catorci et al., 2011). It was reported, for instance, that highly competitive,

22   often late successional grass species of genera such as *Sesleria* and *Brachypodium* would negatively

23   influence species diversity in grasslands of Southern and Western Europe (Crofts and Jefferson,

24   1994, Sebastià et al., 2008; Catorci et al., 2011; 2013).

1   Although much research has been conducted on the effects of grazing pressure and abandonment on

2   vegetation patterns (e.g. Milchunas and Lauenroth, 1993; Biondini et al., 1998; Frank, 2005), the

3   knowledge of the nature of the relationship between dominants the subordinate co-occurring species

4   is scarce. Aiming to preserve species-rich grassland communities in times of abandonment of

5   traditional land use we need to understand how the subordinate species, which represent most of the

6   plant diversity, can sustain their existence under the new land-use conditions. New recent

7   developments in the study of trait-based assembly rules focus on revealing the background of

8   species coexistence and the strategy of species dealing with novel environmental challenges

9   (Wilson, 1999; Grime, 2006; Shipley et al., 2006; Schamp et al., 2008, 2011; Götzenberger et al.,

10   2012). Our study falls within this scope and aims to contribute to understanding the mechanisms of

11   diversity maintenance in abandoned sub-mediterranean grasslands. Taking into account the findings

12   of previous studies on the community assembly (Watkins and Wilson, 1992; Wilson, 1999; Bartha

13   et al., 2004), we establish that fine-scale spatial resolution offers the most appropriate scale of study

14   of community assembly rules because of the fine scale of interactions between plants.

15   In this paper we explore the fine-scale species co-occurrence in calcareous dry grasslands of the

16   *Festuco-Brometea* in a representative pastoral landscape of the Montagna di Torricchio Nature

17   Reserve located in the Central Apennines, Italy, where the pastures have been abandoned over 30

18   years ago. We use the topographical habitat template of a typical V-shaped valley to compare two

19   contrasting habitats – grasslands on north-facing *versus* south-facing slopes. These habitats differ in

20   site disturbance history as well as in water and nutrient supply (Wellstein et al., 2013).

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22   In our study we target two aims, (1) Firstly, we are asking whether there is *spatially-explicit*

23   *dependence between the three late successional, potentially dominating grasses (Sesleria nitida,*

24   *Brachypodium rupestre and Bromopsis erectus) and the subordinated species in the respective*

25   *studied grasslands.* (2) Secondly, we are *seeking assembly rules at fine spatial scales for*

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1 *subordinate species coexisting in grassland communities under exclusion of the dominating*

2 *grasses.*

3 Ecological theory of assembly rules and evidence from recent field studies show two contradictory

4 streams of reasoning regarding competition-related traits. On one hand, the increase in competition

5 for light, which usually occurs under favourable conditions, could promote divergence in

6 competition-related traits such as specific leaf area (SLA) or plant height and hence demonstrate a

7 mechanism of limiting similarity (*e.g.* Spasojevic and Suding, 2011). On the other hand, following

8 Keddy (1990), Grime (2006), and supported by recent studies by Violle et al. (2009) and Bernard-

9 Verdier et al. (2012), more favourable conditions (implying enhanced availability of resources) can

10 lead to an assembly of functionally similar species with respect to traits related to the life-history

11 processes of competition and establishment. This phenomenon was also described as trait

12 convergence through mechanisms of equalizing fitness (Chesson, 2000). In comparison, the absence

13 of competitive filters in unfavorable habitats would then allow for trait divergence (see Stubbs and

14 Wilson, 2004). However, one could also argue that functional divergence under such circumstances

15 would be due to other reasons such as facilitation (e.g. Spasojevic and Suding, 2011) or habitat

16 heterogeneity. Some recent studies, however, suggest that both convergence or divergence may be

17 detected for competition- and establishment-related traits associated with a single aspect of life

18 history (e.g. Stubbs and Wilson, 2004; Mason et al., 2011; Gross et al., 2013). Based on these

19 findings and the controversial evidence on the role of traits in grassland assembly, we *predict*

20 *random pattern (i.e. neither divergence nor convergence) in the competition- and establishment-*

21 *related traits in either of the investigated habitats* (H1).

22 As the regeneration traits are more important in environments with frequent disturbance (Klimešová

23 and Klimeš, 2007) we predict *convergence in regeneration traits on the south-facing slope*

24 *characterized by higher erosion rates* as a consequence of the open grassland canopy and increased

25 evaporation enhancing the localized drought stress (H2). Following Wright and Westoby (1999)

5

1   and Pakeman et al. (2008) larger seed mass offers an advantage or is more common under drier

2   environmental conditions owing to its reserve effect under drought stress. We therefore expect the

3   constraints on the regeneration niche to be larger on the drought prone south-facing slope and

4   consequently predict that the *seed mass would show trait convergence* (H3).

5   Further, because of general lack of evidence of the role of regeneration-related belowground traits

6   as well as of flowering phenology in grassland assembly, we shall ask if there are ecologically

7   meaningful patterns for these traits.

8

9   2. Materials and methods

10

11   2.1 Study area and site selection

12   Our field sampling was carried out in the Montagna di Torricchio Nature Reserve near Camerino

13   (Province of Macerata, Marche Region, Italy) in the Central Apennines, Italy (Fig. 1) – an area of

14   317 ha which has been under strict protection since 1970. Previously, the grasslands were managed

15   as pastures. Mean annual precipitation reaches 1250 mm and mean annual temperature is around 11

16   °C (Halassy et al., 2005). Jurassic-Cretaceous limestone (Ital. 'scaglia rosata') prevails in the area.

17   For this study, we selected two sites with an area of about eight (north-facing slope) and five (south-

18   facing slope) hectare representing the contrasting environmental conditions of the north- and south-

19   facing slopes (Fig. 2; Table. 1). The north-facing slope is covered with a dense, late-successional

20   dry grassland community assigned to the *Seslerio nitidae-Brometum erecti* (Venanzoni and

21   Kwiatkowski, 1995). This association has its core area in the upper montane belt of the Central

22   Apennines. This secondary grassland replaces beech forests which used to cover the north-facing

23   mesic slopes. The dense grassland canopy is interrupted by a mosaic of gaps created by solifluction

24   and presence of rocky outcrops. The south-facing slope hosts open pioneer dry grassland with a

25   scanty cover assigned to the *Asperulo purpureae-Brometum erecti* (Venanzoni and Kwiatkowski,

6

1   1995). This association has its core area in the lower montane belt of the Central Apennines; and it

2   is rich in species showing sub-mediterranean and Apennine distribution ranges. Due to higher

3   erosion rates the soils are poorly developed, shallow and skeletal (Kwiatkowski and Venanzoni,

4   1994). As indicated by the constituent species, the south-facing slopes could be seen as relatively

5   stable habitat where populations of steppe flora found suitable conditions during glacial periods of

6   the Quaternary and a regional refugium during the forest spread in the Holocene. Finally, the

7   environmental conditions characteristic of the north-facing slope (with favourable conditions of

8   water and nutrient supply) as opposed to south-facing slope (with stressful conditions of water

9   shortage and erosion), provide a well-defined contrasting habitat template to study context-

10   dependent trait-based assembly rules.

11

12   2.2 Data collection

13   In both grassland communities, presence of plant species was recorded along 52 m long

14   topologically circular belt transect of 1040 units of 0.05 x 0.05 m contiguous sampling units (see

15   Bycroft et al., 1993; Palmer and van der Maarel, 1995). Transects were randomly placed within a

16   representative, quasi-homogeneous grassland area of each slope (see also Fig. 1). In order to avoid

17   an effect of pseudo-replications, we considered a minimum distance of 80 m among the sampled

18   transects within a slope. The distances of transects among slopes vary from 400 m to 700 m. Two

19   transects were established in the grassland community of the north-facing slope. In order to account

20   for the larger heterogeneity of the open pioneer grassland, three transects were established in the

21   south-facing slope. The presence/absence of the rooting species of vascular plants (both individuals

22   and/or ramets) in each sampling unit was recorded. The large number and small size of micro-

23   quadrates ensure the precise estimation of the frequency of species (see sources on line-intercept

24   sampling; Stampfli, 1991; Murphy and Lodge, 2002; Kercher et al., 2003). The stands were

25   sampled in 2010 (N1, S1, S2) and in 2012 (N2, S3), always in early July.

7

Considering that rare species may have a strong stochastic effect on the detected patterns (Tóthmérész and Erdei, 1992), they were omitted from the analyses. The frequency of 0.0075 in at least two transects was taken as critical limit for rarity. According to this criteria, for the analysis of functional diversity, we selected 23 species contributing to more than 93% of total presences based on the transect data (see Electronic Appendix A).

Environmental parameters and soil nutrient availability, such as electric conductivity (µS/cm), K (mg/100g), Mg (mg/100g), P (mg/100g), NH_4 (mg/100g), NO_3 (mg/100g), and C/N ratio were analysed and/or calculated for 120 random points within each grassland community. Differences in the respective mean values between the north- and the south-facing slopes were reported in Wellstein et al. (2013), and are also presented in Electronic Appendix B.

2.3 Selection and measurements of plant functional traits

Following Lepš et al. (2006), for the selected species we chose a set of 17 aboveground and belowground easy-to-measure functional traits, related to the life-history processes competition, regeneration, establishment, dispersal, and flowering phenology (Table 2). These traits are likely to be useful for predicting vegetation response to disturbance and a broad range of other environmental factors (Weiher et al., 1999). The targeted traits (see Table 2 for technical details) were:

- *specific leaf area*, a good surrogate for ability to use light efficiently;
- *canopy height*, an indirect indicator of ability to compete for light;
- *seed mass*, having implications for the space/time dispersal ability and indicative of seedling survivorship;
- *life form*, based on position of perennating buds being informative about stress avoidance;
- *stem woodiness*, an indication of plant persistence capacity;
- *tap root presence*, an advantage in reaching nutrients pools under nutrient poor conditions;

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1   - *vegetative mobility*, pertinent to rate of space occupancy;

2   - *perennial aboveground/belowground bud bank* and *position of aboveground buds*, both

3   informing on resprouting ability after disturbance (e.g. grazing);

4   - *clonal growth organ*, as indication of regeneration potential, space acquisition, and nutrient

5   conservation strategy;

6   - *flowering start* and *flowering length*, both being linked to stress and disturbance avoidance;

7   - *architectural category*, a surrogate for plant structure and space occupancy; and

8   - *leaf hairiness*, *leaf persistence,* and *leaf anatomy*, all fundamental indicators of stress

9   tolerance and leaf longevity.

10 Trait values were obtained from own field measurements (ca. 20% of all attributes) using

11 standardized sampling measurement protocols (Cornelissen et al., 2003), and from existing database

12 and literature sources (ca. 80% of all attributes) (Pignatti, 1982; Barkman, 1988; Lindacher et al.,

13 1995; Klimeš et al., 1997; Klotz et al., 2002; Kleyer et al., 2008; Royal Botanic Gardens, 2008;

14 Wellstein and Kuss, 2011). Information was available for all considered species and traits.

15

16 2.4 Data collation and analyses

17 2.4.1 *Patterns of spatial relationships*

18 Local species richness of subordinated species was calculated for each 5 x 5 cm micro-quadrat.

19 Subsequently, micro-quadrats were classified according to the number of subordinate species (S).

20 Three classes were distinguished: micro-quadrats where no subordinate species is present (S=0),

21 where one subordinate species is present (S=1), and micro-quadrats where more than one

22 subordinate species is present (S>1). Pairwise spatial associations (I(A,B)) between dominant

23 grasses and classes of subordinate species richness were assessed by the mutual information

24 between two variables (Juhász-Nagy and Podani, 1983).

1 Three types of randomizations were used. 1) Complete Randomization Model (Site Model; Wilson

2 et al., 1987) separates the effects of frequencies from other structural constraints. 2) Random Shifts

3 Model (Palmer and van der Maarel, 1995; Bartha and Kertész, 1998; Roxburgh and Chesson, 1998)

4 keep the spatial pattern of each individual variable, but randomize the relative positions of the

5 variables by shifting (rotating) them randomly along the circular transect. Thus random shifts

6 randomize only the interspecific patterns and therefore intraspecific autocorrelations (aggregations)

7 will not influence the results (cf. Roxburgh and Chesson, 1998). 3) Patch Model (Watkins and

8 Wilson, 1992) randomizes with a short spatial range and separates the effects of larger scale

9 heterogeneity in the sample. All associations were tested in spatial-series analyses (Podani, 1992;

10 Bartha and Kertész, 1998). Because the results remained consistent within a range of plot sizes, we

11 present here only the results found at 5 x 5 cm scale.

12 Because there are many clonal species in our grasslands, the finest resolution 5 x 5 cm might not

13 represent well the local species structure and composition. Therefore larger sampling units of 5 x 25

14 cm micro-quadrats were chosen for analyses related to the functional diversity and the local

15 frequency of species was approximated by the number of presences (with scores changing between

16 0 and 5 according to the local presence) in these sampling units.

17 We used computerized sampling (Podani, 1987) and sampled 5 x 25 cm micro-quadrats from

18 random positions under the conditions of *S. nitida* being excluded. Because the micro-quadrats can

19 be autocorrelated and spatial autocorrelations might affect our results, we calculated a distance

20 decay function (using Bray-Curtis similarity) and removed spatially dependent micro-quadrats (for

21 the summary of the analyses see Electronic Appendix C).

22

23 2.4.2 *Functional diversity (FD)*

24 Among several methods purposed to estimate FD of single traits, the Rao's coefficient

25 demonstrated to be one of the most efficient functional diversity indices (Botta-Dukát, 2005; Lepš

10

1 et al., 2006). It is a generalized form of the Simpson's diversity index. If the proportion of the i-th

2 species in a community is p_i and the dissimilarity of species i and j is d_{ij}, the Rao's coefficient has

3 the form:

4

5 $$FD = \sum_{i=1}^{s} \sum_{j=1}^{s} d_{ij}\, p_i\, p_j \qquad\qquad\qquad\qquad \text{Eq. (1)}$$

6

7 where S is the number of species in the community, p_i and p_j represent the relative frequencies (here

8 based on scores found in 5 x 25 cm micro-quadrats) of species i and j, and d_{ij} is the functional

9 dissimilarity between the species pair i and j; $d_{ii} = 0$, i.e. dissimilarity of each species to itself is

10 zero. If $p_{ij} = 1$ for any pair of species (so each pair of species is completely different), then the FD is

11 the Simpson's index of diversity expressed as 1 minus the Simpson index of dominance (Botta-

12 Dukát, 2005). Because multiple traits might neutralize each other in result (Lepš et al., 2006), we

13 tested each trait separately. First the local FD was calculated for the 5 x 25 cm micro-quadrats, then

14 the average FD was calculated at the level of each transect. We used an established null model

15 (Schamp et al., 2008, 2011) to test for deterministic (non-random) assembly. In this null model,

16 traits are assigned randomly to species, however, associations between different traits are

17 conserved, because all traits together are assigned to another species. This algorithm conserves also

18 both marginals of the species x micro-quadrats matrix. To generate this random reference, and in

19 order to avoid an artificial increase of the range of trait values found in each given transect, and

20 thereby the likelihood of detecting significant assembly rules, we used a separate species pool for

21 the transects of each of the studied habitats (slopes). Distributions of each community-level test

22 statistic were generated from 9999 randomizations of the trait matrix.

23 For FD, the standardized effect sizes were calculated as follows:

24

25 $$SES = (M_{obs} - M_{sim})/\sigma_{sim} \qquad\qquad\qquad\qquad \text{Eq. (2)}$$

1

2   where M_{obs} and M_{sim} are the mean FD values for the observed and simulated community data

3   respectively, and σ_{sim} is the standard deviation of the metrics of the simulated data. Significant

4   differences were calculated comparing field FD with respect to the null hypothesis (that is the min-

5   max range obtained by 9999 randomisation runs). As we used several transects, we adjusted for

6   multiple testing by dividing the commonly used 0.05 threshold of the p value by 3 in the south-

7   facing slope (three transects) and by 2 in the north-facing slope (two transects) that yielded the

8   adjusted thresholds of 0.016 and 0.025, respectively.

9

10   3. Results

11

12   3.1 Effect of the dominating grasses

13   Significant spatial associations were found between the dominating grasses and the patterns of

14   species richness classes (Table 3). *S. nitida* showed consistent positive association with micro-

15   quadrats where no subordinate species were present (S=0 class) and negative associations with S=1

16   and S>1 richness classes, hence those with micro-quadrates where one or more subordinate species

17   were present. These patterns were the same in both open (southern exposition) and closed (northern

18   exposition) grasslands and were consistent across transects within each grassland type (Table 3).

19   We expected that the other two dominant grass species *B. rupestre* and *B. erectus* would produce

20   similar relationships, but they showed the opposite pattern − negative association with empty

21   microsites and positive association with classes *S*=1 and *S*> 1. Significant negative spatial

22   associations were found between *S. nitida* and the other two dominating grasses, while positive

23   spatial associations appeared between *B. rupestre* and *B. erectus*. These results remained consistent

24   in all spatial-series analyses (not shown) and in all used types of randomizations (Table 3).

25

1   3.2 Traits assembly in gaps

2   In gaps without the dominant grass *S. nitida,* significant differences to random assembly were

3   detected in more than 2/3 of the investigated traits (12 out of 17 traits) at least for one of the

4   investigated transects (Table 4). Significant trait under-dispersion indicates trait

5   convergence/clumping, while significant trait over-dispersion is indicative of trait

6   divergence/spread.

7   Significant results of traits related to competition showed trait divergence on both the north- and the

8   south-facing slopes. Seed mass, a trait related to dispersal and establishment, showed trait

9   convergence only on the south-facing slope. Regenerative traits showed convergence on the north-

10   facing slope, and, by contrast, trait divergence on the south-facing slope. However, the regenerative

11   trait 'position of aboveground buds' deviated from this pattern by showing convergence also on the

12   south-facing slope. Significant results for traits related to flowering phenology showed convergence

13   in grasslands on the north-facing slope, while they were inconclusive on the south-facing slope.

14

15   4. Discussion

16   In previous trait-based studies, especially on grasslands, increased or decreased local functional

17   diversity (FD) related to null models was often interpreted in contrasting ways (Mason et al., 2011;

18   2012; Bernard-Verdier et al., 2012; Münkemüller et al., 2012). In order to to shed more light on this

19   multifaceted picture, we present in our study a novel hierarchical approach in form of a two-step

20   analysis, where in the fist step (Aim 1) we set off to clarify whether a strong biotic filter effect

21   exists due to a dominant grass. Consequently, in the second step (Aim 2 and the associated

22   hypotheses H1 through H3) we focused only on the subordinate species dwelling in gaps after

23   filtering out the effect of the dominating species.

24

25   4.1 Effect of the dominating grasses

1   Only *S. nitida* was found to have a profound effect on the spatial associations and enhance

2   constraints on patterns of species diversity, and hence presumably controlling processes shaping

3   patterns of persistence and coexistence of the subordinate species. The other two investigated late-

4   successional grasses, *B. erectus* and *B. rupestre*, were negatively associated with *S. nitida* and they

5   were found to behave like subordinate species. They locally co-occur with other species and do not

6   constrain diversity. Their subordinate role may go on the account of their growth architecture –

7   hence inability to form high-density tussock composed of ramets as demonstrated by *S. nitida*. They

8   produce less dense clones leaving more space for other plants. Our results of significantly negative

9   correlations between the occurrence of the dominant species *S. nitida* and the subordinate species,

10   including the other grasses, support the assumption that *S. nitida* is a main driver of the local

11   diversity acting as a biotic filter in both habitats. In a previous study undertaken in the same habitats

12   (Wellstein et al., 2013), this grass showed a higher capacity of space occupancy on the north-facing

13   slope, where it forms dense carpets. The clear increase of the species' cover, aboveground

14   phytomass, and possibly also litter decomposition processes are the main supressing species

15   coexistence – a pattern consistent with several other studies on clonal grasses possessing similar

16   traits (e.g. Campbell et al., 1992; Sebastià et al., 2008; Catorci et al., 2011; 2013).

17   On the south-facing slope, under conditions of low resources availability and permanent disturbance

18   due to ongoing erosion, *S. nitida* occurs in another phenotype; instead of forming a dense carpet, it

19   builds only single, well-isolated tussocks (Wellstein et al., 2013). Therefore, on the south-facing

20   slope, a vast space is available to other species.

21

22   4.2 Trait-based assembly in assemblages of subordinate species

23   *Traits related to competition and establishment (H1)*

24   Although it is often assumed that a lower functional diversity in traits (and hence more pronounced

25   habitat filtering effect) should occur in productive and thus competitive environments (Grime,

1   2006), we found, in absence of the biotic filter *S. nitida*, evidence for increased functional trait

2   diversity (hence mechanisms of niche differentiation) for traits related to competition in the

3   investigated communities in both productive as well as nutrient-poor environments. Some literature

4   sources (Cornwell and Ackerly, 2009; Mason et al., 2011) suggest, however, that habitat filtering

5   and niche differentiation are not mutually exclusive, despite their apparent opposite effects on

6   community-level trait distributions. Thus it is likely that niche differentiation for the same trait can

7   be inferred also in contrasting communities. Co-variance of traits along different axes of

8   specialization, defining different tradeoffs for plants to compete with other plants through

9   acquisition and use of local resources (Suding et al., 2003) can be seen as lending support to this

10   view. Within plant communities, independent axes of trait specialization have been characterized,

11   each supporting different major dimensions of the niche space (Gross et al., 2013). Consequently,

12   significant under-dispersion can select for species along a particular set of traits whereas

13   overdispersion can be detected in other independent traits (Spasojevic and Suding, 2012; Gross et

14   al., 2013). Grasslands on north-facing slopes were more diverse in terms of *stem woodiness* and

15   *architectural categories* than expected. This result might be interpreted in terms of gaps becoming

16   more 'crowded' (support high density of individuals) and thus increased partitioning of space may

17   occur (Mason et al., 2011). This hypothesis, however, remains to be tested. The expectation that

18   trait diversity increases in more productive environments (Mason et al., 2011) does not consider the

19   possibility for strong competitive interactions and resource partitioning for below-ground resources

20   (nitrogen, water) in resource-poor and drought prone grasslands as the south-facing slope in our

21   study, where complex trade-offs among nitrogen and water acquisition and tolerance strategies may

22   allow for increased coexistence (Spasojevic and Suding, 2011). However, the significantly larger

23   variability of the traits *canopy height* and *life form* on the south-facing slope also may suggest that

24   tall, large-leaved species may facilitate the persistence of other, small-sized species, possibly due to

25   protection from wind or cold exposure by reducing evapotranspiration losses (Spasojevic and

Suding, 2011). The *specific leaf area (SLA)* is known to correlate strongly with temperature, irradiance and water availability (Poorter et al., 2009). In the north-facing slope grassland SLA does not show significant assembly signal, while on the south-facing slope, a tendency toward trait over-dispersion ($p=0.021$) is corroborated by similar studies focusing on stressed (drought) environments (e.g. Spasojevic and Suding, 2011).

In summary, we have falsified our hypothesis (H1) since we have found complex, yet non-random pattern in the competition- and establishment-related traits in either of the investigated habitats.

Traits related to regeneration converge in the south-slope grasslands

Contrary to our expectation (H2), the presence or absence of a *taproot* as well as of *perennial bud bank aboveground or belowground,* appeared to be more divergent rather than random. The presence of the ongoing disturbance by erosion may, coupled with insufficient water supply, lead to frequent aboveground biomass destruction, triggering a need for subsequent replacement of plant organs (Klimešová and Klimeš, 2007). Plants that lack *perennial bud bank aboveground* follow other strategies – either belowground regeneration or having annual life cycle. We detected trait convergence in *position of aboveground buds*. Probably, the presence of frequent disturbance on the south-facing slope imposes constraints selecting for similar patterns in the position of aboveground buds. For example, after the destruction of the aboveground part of the biomass, plants have to regenerate from buds below the location of damage (e.g. Vesk and Westoby, 2003).

Under the circumstances of open gaps in the dense *Sesleria* carpet of the north-facing slope, a certain type of *clonal growth*, namely 'shoot-derived clonal growth', might be favored, most likely because of the obvious advantage of efficient space occupancy (see *e.g.* Wellstein and Kuss, 2011) assured by growth of dense rhizome plexus filling the restricted space in the gaps among the tussocks of the dominating grass. These findings give support to a suggestion (Klimešová et al.,

1   2011) that, depending on the ecological circumstances, clonal traits may be involved in species

2   sorting – a hypothesis worth exploring in rigorous manner.

3

4   *Seed mass converges on the south-slope*

5   Our findings support the hypothesis (H3) predicting the convergence of *seed mass* on south-facing

6   slopes, hence under conditions of environmental adversity. As seed mass is known to influence

7   seedling establishment (*e.g.* Wright and Westoby, 1999), a constraint of its trait attributes may

8   suggest the advantage of a specific regeneration strategy. Here filters in form of conditions adverse

9   to seed regeneration, (e.g. increased run-off and enhanced occurrence of drought spells, increased

10   evapotranspiration due to open canopy), are possibly driving the selection for similar seed mass.

11   These findings are supported by evidence from recent studies (see Pakeman et al., 2008 and

12   references therein). Franzen (2004), who also found evidence for seed size under-dispersion

13   (convergence) in a semi-natural grassland, suggested that the differences in trait under- and over-

14   dispersion may also be a result of different biotic conditions triggered by system productivity. In

15   our system, possibly, biotic interactions in the more productive north-facing slope could affect

16   seedling establishment by lowering light availability and space for root anchoring (Wellstein, 2012)

17   and thus favor trait convergence as larger seed size enhances seeding fitness under stressful

18   (competition for light) conditions (Pakeman et al. 2008). However, no such patterns were found in

19   the more productive north-facing slope.

20

21   *Traits related to flowering phenology*

22   Regarding these traits, our results suggest that assembly rules are differentiated locally on the south-

23   facing slope, but are more likely to be subject to a filter on the north-facing slope. The flowering

24   pattern of a plant community is driven by functional differentiation of species that in turn is filtered

25   by environmental stress/disturbance type and intensity (Primak, 1985; DeBussche et al., 2004;

1   Catorci et al., 2012). In a sub-mediterranean mountain grassland, Catorci et al. (2012) compared the

2   flowering phenology in two sites, highlighting significant differences mediated/filtered by

3   environmental combinations. In particular, they emphasized, also according to Ansquer et al.

4   (2009), that subordinated species living in small gaps tend to avoid the flowering co-occurrence

5   with the dominant, more competitive grasses, showing a tendency to bloom during periods at the

6   edge of the vegetative season. This is consistent with our findings of convergence in flowering

7   length in the north-facing slope, where the pressure and competitive ability of *S. nitida* are quite

8   evident. Moreover, a recent study conducted in a homogeneous and mown old-field (Dante et al.,

9   2013) found a convergence in flowering time suggesting an interspecific facilitation in terms of

10   pollinator attraction.

11

12   *Differences between transects*

13   The use of several transects within a habitat was motivated by possible variability of trait-assembly

14   patterns. While highly significant results appeared sometimes in only one of the tested transects, the

15   findings were in all cases consistent between transects within a habitat, suggesting consistent

16   mechanisms underpinning the assembly rules. Only in case of *flowering duration* we found

17   inconsistent patterns.

18

19

20   5. Conclusions and management implications

21   While previous grassland studies sometimes found random pattern of multiple traits (see

22   Götzenberger et al. 2012), we detected multiple cases of non-random trait-driven community

23   assembly. Our findings highlight the importance of hierarchical exclusion of strong biotic filters and

24   the relevance of fine spatial scales in studies of community assembly. Using contrasting

25   environmental situation created highly productive framework to study and understand mechanisms

1 underpinning trait-based assembly rules of coexistence in species-rich vegetation systems, such as

2 semi-natural grasslands. Most importantly, we were able to demonstrate that assembly rules are

3 strongly context-dependent. Our findings suggest that grasslands species diversity might be

4 dependent on gaps created by natural disturbance regimes, such as solifluction or soil erosion.

5 However, under such circumstances, functional diversity of regenerative traits and flowering

6 duration is decreased by convergence due to possible selection for certain highly-fitted traits. In

7 habitats with naturally low productivity with shallow soils and disturbance due to drought and

8 erosion, as in the case of the grasslands occupying the south-facing slope habitats, the functional

9 diversity of many traits increases possibly either because competitive filters would be lacking or

10 due to facilitative effects and/or environmental heterogeneity.

11 Disturbance appears to be the process that is most important in driving the survival of subordinate

12 species by the exclusion of biotic filters such as strongly dominating, matrix-forming species.

13 Subsequently, a multitude of trait-based mechanisms allow for coexistence of the subordinate

14 species. These mechanisms are context-dependent and may vary from location to location

15 indicating that heterogeneous environments might support multiple processes of coexistence.

16 Successful management of secondary (semi-natural) grasslands for species and functional diversity

17 (largely resting on occurrence and coexistence of subordinate species) has to take into account the

18 effect of biotic filters such as dominant grasses.

19

24

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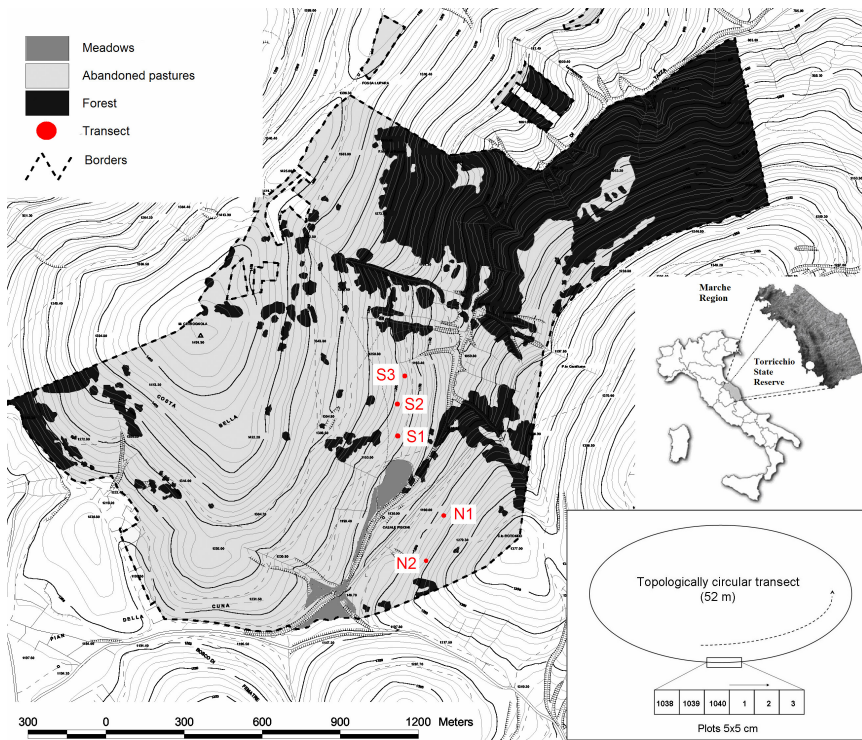
4　Fig. 1. Land use types, topography and position of the sampled transects in the north-facing slope

5　(transects N1, N2) and south-facing slope (transects S1, S2, S3) of the study area Montagna di

6　Torricchio State Reserve (Central Apennines, Marche Region, Italy).

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2 Fig. 2. Physiognomy of the dense and closed grassland on the north-facing slope (left) and the open

3 grassland on shallow and eroding soil on the south-facing slope (right).

1 **Table 1.** Topography, soil, and vegetation characteristics of the sampled sites. (Both associations belong to the order *Brometalia erecti* W. Koch 1926, class *Festuco-Brometea*

2 Br.-Bl. & Tx. in Br.-Bl. 1949)

3

	North-facing slope	South-facing slope
Coordinates	42°57'21.8''N – 13°01'10.1''E	42°57'35.1''N – 13°01'07.4''E
Altitude (m)	1200	1165
Aspect	320° N	100° N
Slope (°)	35	17
Soil	Deep calcareous lithosol over scaglia rosata	Shallow calcareous lithosol over scaglia rosata
Erosion rate	Low	High
Vegetation unit	*Seslerio nitidae - Brometum erecti*	*Asperulo purpureae – Brometum erecti*

1 **Table 2.** Summary of the studied plant traits and their presumed relevance to ecological processes (Comp: competition; Est: establishment; Dis:

2 dispersal; Reg: regeneration; BG=below ground; AG=above ground; Flo: flowering).

3

Process	Plant trait	Description	Data type	Data source
Comp	Canopy height	Distance between the highest photosynthetic tissue and the base of plant (Kleyer et al., 2008)	Numerical	Own data; Kleyer et al. (2008); Pignatti (1982)
Comp	Life form	Classification according to the position of perennating buds (Kleyer et al., 2008)	Categorical	Pignatti (1982)
Comp	Stem woodiness	Estimation of stem tissue density (Kleyer et al., 2008)	Ordinal	Kleyer et al. (2008); Pignatti (1982)
Comp	Leaf persistence	Leaf seasonality, considering the period in which leaves are green (Lindacher, 1985)	Categorical	Klotz et al. (2002); Lindacher (1985)
Comp	Leaf anatomy	Leaf structure according to water storage and gas exchange (Lindacher, 1985)	Categorical	Klotz et al. (2002); Lindacher (1985)
Comp	Architectural category	Classification according to plant architecture, with particular regard to stem and leaf position (Barkman, 1988)	Categorical	Barkman (1988)

Comp, Est	Leaf hairieness	Presence of hairiness on leaf lamina	Binary	Pignatti (1982)
Comp, Est	Specific leaf area	One sided area of a fresh leaf divided by its oven-dry mass (Kleyer et al., 2008)	Numerical	Own data; Kleyer et al. (2008)
Dis, Est	Seed mass	Oven-dry mass of an average seed of a species (Cornelissen et al., 2003)	Numerical	Own data; Royal Botanic Gardens (2008)
Reg (BG)	Tap root presence	Persistence of a tap root (CLOPLA1, Klimeš et al., 1997)	Binary	Wellstein and Kuss (2011); CLOPLA1, Klimeš et al. (1997)
Reg (BG)	Perennial bud bank (belowground)	Bud bearing organs persisting for 2 and more years are classified and as perennial (CLOPLA1, Klimeš et al., 1997)	Binary	Wellstein and Kuss (2011); CLOPLA1, Klimeš et al. (1997)
Reg (AG)	Perennial bud bank (aboveground)	Bud bearing organs persisting for 2 and more years are classified and as perennial (CLOPLA1, Klimeš et al., 1997)	Binary	Wellstein and Kuss (2011); CLOPLA1, Klimeš et al. (1997)
Reg (AG)	Position of aboveground buds	Position of the buds at disposal for vegetative regeneration (CLOPLA1, Klimeš et al., 1997)	Ordinal	Wellstein and Kuss (2011); CLOPLA1, Klimeš et al. (1997)
Reg	Clonal growth organ	Origin of clonal growth organ (stem or root) (CLOPLA1, Klimeš et al., 1997)	Categorical	Wellstein and Kuss (2011); CLOPLA1, Klimeš et al. (1997)
Reg	Vegetative mobility	Lateral spread per year (CLOPLA1, Klimeš et al., 1997)	Ordinal	Wellstein and Kuss (2011); CLOPLA1,

				Klimeš et al. (1997)
Flo	Flowering start	Starting of the flowering period (month of year)	Ordinal	Pignatti (1982)
Flo	Flowering length	Length of the flowering period (months)	Ordinal	Pignatti (1982)

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Table 3. Fine scale associations between the dominant grasses and local diversity of other subordinate species (s). Values: 1 = positive ($p < 0.05$), -1

= negative ($p < 0.05$), 0 = non significant. Randomization type: 1 = complete randomization, 2 = random shifts, 3 = patch model. Null model tests

were based on 999 randomisations.

Slope		South-facing									North-facing					
Transect		**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**N1**	**N1**	**N1**	**N2**	**N2**	**N2**
Randomization type		**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Pairs of the tested variables																
Sesleria nitida	s=0 subordinate spp.	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1
Sesleria nitida	s=1 subordinate spp.	-1	-1	0	-1	-1	-1	-1	0	0	-1	-1	-1	0	0	0
Sesleria nitida	s>1 subordinate spp.	-1	-1	-1	-1	-1	0	-1	-1	0	-1	-1	0	-1	-1	-1
Bromopsis erectus	s=0 subordinate spp.	-1	-1	-1	-1	-1	-1	-1	-1	-1	0	0	0	0	0	0

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Bromopsis erectus	s=1 subordinate spp.	0	0	0	1	1	1	1	1	0	0	0	0	1	1	0
Bromopsis erectus	s>1 subordinate spp.	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
Brachypodium rupestre	s=0 subordinate spp.	-1	0	0	-1	-1	0	0	0	0	-1	-1	0	0	0	0
Brachypodium rupestre	s=1 subordinate spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brachypodium rupestre	s>1 subordinate spp.	1	0	0	1	1	0	0	0	0	1	1	0	0	0	0
Sesleria nitida	Bromus erectus	-1	-1	0	-1	-1	0	-1	-1	0	-1	-1	-1	-1	-1	0
Sesleria nitida	Brachypodium rupestre	0	0	0	-1	-1	0	-1	-1	0	-1	-1	-1	-1	-1	0
Brachypodium rupestre	Bromopsis erectus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

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Table 4 . Standard effect sizes (SES) results of the traits-assembly analysis on species-level traits. The analyses used presence-absence data from five transects (considering only sampling units without the presence of *Sesleria nitida*). P-values indicate whether plots were over-dispersed (+ SES), or under-dispersed (- SES) for the trait of interest. Bold values indicate significant results (as correction for multiple tests of two transects (N1, N2) p ≤ 0.025 and of three transects (S1, S2, S3) p ≤ 0.016).

		N1		N2		S1		S2		S3	
Process	**Trait**	**SES**	**Sig.**	**SES**	**Sig.**	**SES**	**Sig.**	**SES**	**Sig.**	**SES**	**Sig.**
Competition	Canopy height	- 0.138	0.432	0.782	0.210	- 1.533	0.056	1.264	0.104	**6.982**	**<0.001**
	Life form	0.133	0.457	0.803	0.211	**2.642**	**0.004**	- 1.065	0.148	**2.328**	**0.008**
	Stem woodiness	- 0.790	0.220	**2.243**	**0.012**	1.351	0.087	- 0.778	0.217	**3.745**	**<0.001**
	Leaf persistence	1.030	0.149	- 1.493	0.073	- 0.571	0.288	1.537	0.063	- 0.493	0.304
	Leaf anatomy	0.598	0.279	- 1.778	0.042	0.652	0.259	- 0.076	0.481	- 1.515	0.064
	Architectural categories	**1.859**	**0.019**	- 1.226	0.115	2.024	0.017	1.116	0.133	1.681	0.042
Competition,	Leaf hairiness	- 1.557	0.059	1.790	0.036	- 0.644	0.258	0.105	0.459	1.990	0.021
Establishment	Specific leaf area	0.096	0.449	- 1.598	0.047	- 0.149	0.444	1.752	0.044	- 0.600	0.276
Dispersal, Establishment	Seed mass	0.200	0.412	0.478	0.305	1.565	0.061	**- 3.281**	**<0.001**	- 0.899	0.184
Regeneration	Tap root presence	**- 2.405**	**0.010**	1.413	0.074	**2.240**	**0.011**	0.884	0.185	**2.991**	**0.001**

(Below ground)	Perennial bud bank belowground	1.437	0.075	- 0.946	0.172	- 1.182	0.119	**2.400**	**0.008**	**4.511**	**<0.001**
Regeneration	Perennial bud bank aboveground	- 0.627	0.270	0.119	0.436	- 0.964	0.168	1.353	0.089	**2.915**	**0.003**
(Above ground)	Position aboveground buds	- 0.825	0.204	- 0.749	0.225	0.514	0.303	**- 4.997**	**<0.001**	- 0.028	0.492
Regeneration	Clonal growth organ	0.008	0.475	**- 2.172**	**0.008**	0.778	0.217	- 1.501	0.061	0.592	0.275
	Vegetative mobility	0.717	0.241	1.561	0.052	1.218	0.112	- 0.498	0.307	1.473	0.069
Flowering	Flowering start	- 1.566	0.056	1.311	0.098	- 0.038	0.480	**- 2.566**	**0.006**	- 1.238	0.106
	Flowering length	**- 3.450**	**<0.001**	1.454	0.068	**3.409**	**<0.001**	0.524	0.306	**- 4.994**	**<0.001**

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