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35TH CYCLE

EFFECT OF LAND USE CHANGE ON THE INVASION PROCESSES OF BRACHYPODIUM SPECIES FROM A TAXONOMIC AND FUNCTIONAL POINT OF VIEW IN SUB-MEDITERRANEAN GRASSLANDS

PHD STUDENT
FABIO TOLU

SUPERVISOR
PROF. ANDREA CATORCI

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1 Introduction

Grasslands are very important biodiversity hotspots, even at small scale (Wilson et al. 2012), characterized by a specific floristic composition. The definition given to this type of ecosystem has seen many different versions through time. In this work, we use as definition of grassland the one given by Janišova et al. (2011), which defines these ecosystems as "herbaceous communities dominated by grasses (*Poaceae*) or other graminoids (*Cyperaceae, Juncaceae*)".

Present since the Pleistocene era, natural grasslands have covered most of Europe during the glaciations whereas, during the interglaciations, they retreated immensely in smaller areas, as a consequence of forest expansion (Pärtel et al. 2005).

Grasslands can be of two main types: natural and semi-natural (primary and secondary). The difference between these two categories lies mainly in the effects of the pastoral agriculture and anthropogenic landscape modifications that the latter faced (Alan Hopkins 2009).

At European landscape level, this ecosystem constitutes the primary element and, although present mainly in the semi-natural form (Janišova et al. 2011), it is considered one the most species-rich ecosystems worldwide (Chytrý et al. 2015) presenting around 18% of the European endemic vascular plant species (Hobohm & Brunchmann 2009). For this reason, these grasslands are considered priority habitats for European biodiversity (92/43/EEC Directive), due to the great biodiversity heritage they host (Catorci et al. 2013). A significant number of plant and animal species hosted are of community interest and are included in the threatened categories of the International Union for Conservation of Nature (Giarrizzo et al. 2017).

Nowadays, these ecosystems are threatened by land abandonment, overgrazing, and intensive agriculture (Cramer et al. 2007; Kahmen et al. 2008), having strong impacts on both the flora and fauna that they host. Understanding these modifications is an important aspect for conservational, restoration and social viewpoints (Cramer et al. 2007).

The cessation of semi-extensive farming or underutilization of grasslands is leading to vegetation changes throughout Europe, as well as in semi-natural sub-Mediterranean mountains. Because of their importance for European biodiversity conservation, several Mediterranean semi-natural grassland communities have been considered priority habitats by the European Union (92/43/EEC Directive) and judged worthy of conservation. However, as in the rest of Europe, owing to their low agricultural productivity, these managed pastures are in strong decline and threatened by abandonment. In fact, it was widely documented that over the last several decades, the Mediterranean region has been subjected to major changes in land use/cover (Bracchetti et al. 2012; Malavasi et al.

2018). The socio-economic changes that occurred after World War II triggered the abandonment of mountain areas and, in turn, induced changes at both landscape and plant community scale. The most common effects at the landscape level have been the expansion of shrub-lands and forests. At the community level, abandonment or decrease in forage exploitation typically induce the invasion of coarse tall grasses with competitive stress-tolerant strategies (Poschlod et al. 1998; Louault et al. 2002). In this regard, throughout the Italian peninsula, a cardinal species is *Brachypodium rupestre,* a competitive, stress tolerant, coarse grass, 40-70 cm-tall, whose flowering peak is in late spring-early summer. *B. rupestre* grows on clay and calcareous soils (Dowgiallo & Lucchese 1991), occurring in a wide elevation range, up to 1,200-1,300 m a.s.l., where it is replaced by *Brachypodium genuense* (Camiz et al. 1991).

The competitive behavior of perennial *Brachypodium* species is chiefly related to their high tiller density and branching frequency (Pottier & Evette 2010), as well as to their capacity for clonal growth and clonal integration strategy (de Kroon & Bobbink 1997). Moreover, Tardella et al. (2017) proved that, at least *B. genuense* has a great Specific Leaf Area variability, underlining the ability to adapt its strategy to different environmental conditions, and allowing individuals to maintain the coordination between resource acquisition and conservation and to sustain dominance in a wide range of environmental conditions. *Brachypodium* perennial species have silica-rich and hairy leaves and herbivores feed on them only in overstocking condition (Catorci et al. 2014) or in small clumps enclosed in a multi-taxa community (Canals, et al. 2017). Recurrent defoliation by herbivores makes these species more palatable and richer in nutrients, which in turn increases their attractiveness to herbivores (Catorci et al. 2014). In response to this increased export of biomass, the plants direct resources to the regeneration of aboveground leaves and reduce the level of reserves allocated to the belowground organs (Canals et al. 2017). Instead, the cessation of herbivory fosters the enhancement of a vigorous belowground system; likewise, the lack of leaf consumption leads to the accumulation of a thick litter layer, as well as a set of micro-scale variations of site conditions, such as light amount at the ground level, soil temperature, moisture and acidity (Catorci et al. 2011a). All these characteristics and properties allow the rapid spread of clonal tussocks, which, in the final phases of invasion, often form nearly mono-dominant stands by coalescence of different patches (de Kroon and Bobbink, 1997). This leads to a modification of the coenological composition of pasture by the decrease of the grassland specialist taxa (early flowering taxa, small-sized plants, taxa with storage organs, etc.) and the increase of those typical of fringe communities and of nitrophilous habitats (tall herbs with late flowering strategies, species with runners and ability to form patches, etc.) (Catorci et al. 2011b). In addition, the spread of *Brachypodium* reduces the overall feed value of pastures (Vitasović-Kosić et al. 2014), thus negatively affecting the economic sustainability of mountain

farming (Catorci et al. 2014a). Similarly, it has been shown that *B. genuense* encroachment reduces habitat suitability for wild herbivores (Corazza et al. 2016).

Because of such profound impact on grassland ecosystem services and the great diffusion in the last decades of the *Brachypodium*-dominated communities restoration of grasslands is becoming a paramount issue and one of the major international research topics.

2 Research aims

The cessation of semi-extensive farming or underutilization of grasslands is leading to vegetation changes in semi-natural sub-Mediterranean mountains and throughout Europe. At the community level, these changes foster the spread of unpalatable tall grasses, mostly with competitive stress-tolerant strategies (*Brachypodium* sp. pl. in the Italian peninsula), affecting species diversity and decreasing the nutrient value of grassland ecosystems. The research aims are to:

1. understand the implications of abandonment of pastoral systems on plant communities' taxonomical and functional diversity along environmental gradients at different spatial scales;
2. evaluate species and functional composition recovery in grassland communities invaded by *Brachypodium* after mowing management in the sub-Mediterranean climate;
3. understand how the species and functional composition and diversity of the community change over time in mown and abandoned meadows and how they change along long-term disturbance gradients.

3 Expected outcomes

We expected to get a more profound understanding of the response mechanisms to disturbance of both *Brachypodium* and the grassland community, and to acquire knowledge for the definition of management plans and recovery of pastoral systems with important effects on the economic sustainability of the semi-extensive farming in sub-Mediterranean areas. Thus, the expected outcomes were:

1. modelling the effect of environmental conditions on species and functional diversity of grassland communities after long-term abandonment at different spatial scales;
2. disentangling the effects of disturbance from those of dominant tall grass features in driving the functional variation of restored grasslands;

3. modelling the change of community's species and functional composition and diversity along disturbance gradients and over time.

4 Working plan

Understanding the implications of abandonment of pastoral systems on plant communities' taxonomical and functional diversity along environmental gradients at different spatial scales

Preliminary activities

- 1st year. Bibliographic search aimed at the definition of the scientifical background concerning the issue of tall grass species invasion after abandonment of traditional management.
- 1st year. Definition of the state of the art concerning perennial *Brachypodium* species in central Italy, plant community changes caused by the spread of tall grass species.

Data collection

- 1st year. Analysis of plant samples that had been collected during the previous summer fieldwork in the Montagna di Torricchio Nature Reserve, in 5 m x 5 m plots. Plant functional characteristics (plant trait) were collected at the growing peak (summer) and measured during winter. Leaf samples were and preserved using the standardized procedures described in Cornelissen et al. (2003).
- 1st year. Laboratory activities. Scan of *Brachypodium rupestre* leaves and drying in the oven to measure the dry weight. Leaf area measurement using a software and calculation of Specific leaf area, indicator of resource exploitation strategies. Categorical traits will be obtained by field observations and consultation of on-line databases.

Statistical elaboration

- 1st year. Calculation of taxonomic and functional alpha and beta-diversity indices (de Bello et al., 2010)
- 2nd year. Data processing using cluster analysis, Redundancy Analysis (RDA) (Borcard et al., 2011), Indicator Species Analysis (ISA), ANOVA, etc. (de Cáceres et al., 2012; Dufrêne & Legendre, 1997)

Evaluation of species and functional composition recovery in grassland communities invaded by Brachypodium after mowing management in the sub-Mediterranean climate

Preliminary activities

- 1st year. Review of scientific papers at the European scale, with a focus on central Italy, on the restoration of semi-natural grasslands invaded by invasive *Brachypodium* species.

Data collection

- Species presence-absence data had been collected at a small spatial scale. The sampling units were 20 cm x 20 cm plots.
- To understand how *Brachypodium* affects the functional composition of the community, some traits related to space occupation (e.g. plant height, horizontal and vertical architecture, and vegetative propagation), reproductive strategies (e.g. seed mass and vegetative propagation), temporal niche occupation (e.g. life span, leaf span) and resource storage strategies (e.g. storage organs, leaf anatomy) were selected.

Statistical elaboration

- 1st year. Participation to the elaboration of the data set using generalized mixed-effects models (Bolker et al., 2009).

Understanding how the species and functional composition and diversity of the community change over time in mown and abandoned meadows and how they change along long-term disturbance gradients

Preliminary activities

- 2nd year. Literature search on the effect of perennial *Brachypodium* species on diversity.
- 2nd year. Selection of a study area in the pastoral landscape of central Apennines (Marche region) to record the variation of species and functional composition along a spatial disturbance intensity gradient. The study area of Prati di Ragnolo (Bolognola, Macerata, central Italy), located at 1400 m a.s.l. in the northern part of the Sibillini Mountains National Park. This area has been marked by pastoral practices for centuries, but in the last 35-40 years some areas have been abandoned and invaded by *Brachypodium genuense*. Nowadays, continuously mowed, discontinuously mowed, and abandonment grasslands are present. This area was chosen because a database of phytosociological relevés carried out in 2008 is available.

- 2nd year. Preparation of the database of relevés carried out in 2008 for the following statistical elaborations.
- 2nd year. Planning the sampling design and statistical elaborations.

Data collection

- 2nd year. Selection, using a random stratified approach, of three areas with different management, which are characterized by different disturbance intensities: continuously mowed grassland, discontinuously mowed grassland, and abandoned grassland. In each type of management, a number of plots (10 m x 10 m) proportional to the extent of each management type were laid in the same environmental conditions (north aspect, similar slope and altitude values), for a total of 55 plots. In each plot, species identity and species cover were be recorded using percentage visual estimation. Species were associated to some indicator traits of disturbance.
- 3rd year. Relevés carried out in areas with different disturbance intensities were compared to assess the taxonomic and functional response of plant community and the effect on *Brachypodium genuense*. Data collected in the survey were compared with those collected by Catorci et al. (2011) in the same study area. As plots coordinates were not available, following Giarrizzo et al. (2017), a semi-permanent approach will be adopted.

Statistical elaboration

- 3rd year. Calculation of Ellenberg Indicator Values (EIVs) (Ellenberg et al., 1991)., Community Weighted Means (CWMs), taxonomic and functional diversity (FD) (Botta-Dukát, 2005) indices and comparison between management types along a disturbance gradient and between years
- 3rd year. Data processing using modified permutation tests for EIVs (Zelený & Schaffers, 2012), CWMs and FD indices (de Bello et al., 2010; Ricotta & Moretti, 2011; Zelený, 2018), and Indicator Species Analysis (ISA). (De Cáceres et al., 2012; Dufrêne & Legendre, 1997)

5 Scientific background

5.1 Invasion ecology of tall grasses

5.1.1 Ecological strategies and functional traits supporting tall grasses invasiveness

Competition is an interspecific interaction, naturally and continuously present in nature. There are a high number of definitions regarding this phenomenon, but it, in essence, involves adverse effects on the net reproductive rates of two or more species involved (Bullock 1996). The definition given by Begon et al. (1990) stated: "competition is an interaction between individuals, brought by a shared requirement for a resource in limited supply, and leading to a reduction in the survivorship, growth and/or reproduction of the competing individuals concerned" (Bullock 1996).

Competitors are strong and large perennial plants with high potential growth, meaning high rates of tissue turnover. Reproduction early in their life cycle and mainly iteroparous, these plants highly invest in reproduction both through seeds or through clonal strategies. This is hypothesized to result in greater abilities to compete for resources. Both great competitors for light, thanks to their rapidly ascending and expanding canopy and also for soil resources with their rapidly spreading roots and shoot systems (Bullock 1996).

The fact that competitors are such good at acquiring resources shows that they are unable to tolerate situations with low levels of resources. Furthermore, these plant species are not able to tolerate well disturbance as a consequence of their perennialism and tall growth form (Bullock 1996).

Competition is function of the area, the activity, and the distribution, both in space and time, of the surfaces through which resources are captured. The success of a species over another depends mainly on the combination of its plant traits (Grime 2001).

A significant number of traits is involved in a plant's competitive success. One of the most important is plant height. This trait is strongly linked to the quality and quantity of radiation, but also the supply of energy, the structural materials obtainable from the storage organs, the current photosynthesis and on the morphology of the shoots (Grime 2001).

Another important trait is the lateral spread capacity. Thanks to this strategy, it is possible for plants to reach a significant height without having to capture a bigger portion of resources present in its habitat. In herbaceous species, and particularly in grasses, tall stature is associated to a growth form in which the lateral spread is marked by a high density of shoots and roots. The main advantage of this strategy seems to be the pre-emptive occupation of space and acquiring of resources.

All these aspects are linked to the annual production of large quantities of photosynthate, fundamental aspect for these competitive species that determine their high rate of success. It is guessed that a

precondition for the coexistence of species in herbaceous vegetation are all those factors which are able to limit the expression of dominance (Grime 2001).

All dominant tall grass species, stress-tolerant competitors, are perennial species which have a strong capacity for vegetative spread, mainly by the use of rhizomes and/or expanding tussocks. By this strategy, these plants are able to quickly expand and suppress the growth of the smaller neighbours (Grime 2001). Their invasive behavior causes a change in community structure and species composition (Bonanomi et al. 2006; Catorci et al. 2011, 2014). Overall, the increase of tall-grass species leads to a marked drop of species richness and species diversity (Catorci et al. 2011; Lepš 2014; Filibeck et al. 2020), by a process called "weaker-competitor exclusion" (Chesson 2000). This process suggests that the prevalence of hierarchical differences in the competitive ability between species should favor strong competitors which remove all those species with less competitive abilities (Mayfield & Levine 2010; Lepš 2014), leading to a community characterized by few ecologically similar species. In addition, tall-grass invasion may influence the temporal niche partitioning of coexisting species by selecting a set of species able to cope with environmental conditions (community structure, litter deposition, soil feature modification, etc.) determined by dominant plants (Catorci et al. 2014).

5.1.2 Main invasive tall grasses in Europe

In Europe, two different and opposite trends that endanger these ecosystems can be distinguished: land abandonment, more common in the sub-Mediterranean area, and land use intensification, in north-western Europe (Plantureux et al. 2005). These two phenomena both lead to the alteration of plant communities, caused mainly by the reduction of the landscape complexity and the promotion of the natural secondary succession process (Galvánek and Lepš 2012). Studies have demonstrated a progressive increase in the dominance of a small number of very competitive tall grass species (e.g. *Brachypodium* sp. pl.*, Calamagrostis epigejos, Deschampsia cespitosa* and *Molinia* sp. pl.) which can suppress small and short-lived plants. By specific traits such as tall canopies, extensive lateral spread, and litter deposition, these species tend indeed to dominate the plant community, causing and a change in community composition. As a consequence, we observe a decrease in species diversity, richness and evenness (Bonanomi et al. 2013). Furthermore, it has been demonstrated that not only the community does change but there are significant modifications also at single plant functional trait level (Peco et al. 2012), i.e. a shift from functional strategies devoted to grazing avoidance and tolerance to those devoted to competition for light and resource acquisition (Grime, 2001).

A short overview of the invasive species in Europe is reported below.

- *Northern Europe*

In England (Castel Hill National Nature Reserve, East Essex) Hurst et al. (1999) reported a considerable increase in the dominance of *Brachypodium pinnatum* with the decrease of grazing intensity. This species has demonstrated to be an invasive and aggressive plant species typical of chalk grasslands. Earlier experiments, carried out mainly in the Dutch region, have shown that the competitive ability of this species greatly increased in eutrophication conditions, especially regarding the nitrogen concentration (Bobbink, 1991). The general response observed in the study was the increase of the *B. pinnatum* dominance and the decrease in the diversity and species richness. Furthermore, it was observed that *B. pinnatum* itself did not have that strong of an impact on productivity and biomass. The authors concluded that, from a conservation point of view, the removal of this species does not guarantee a return to the typical chalk grassland environment, mainly because of the substantial abiotic modifications that it causes to the ecosystem.

- *Eastern Europe*

In Hungary the species *Calamagrostis epigejos* (L.) Roth was studied by Hazi et al. (2011). This species is a tall perennial clonal grass (Prach & Pyšek 1994) with a broad distribution range in Europe, which spreads successfully in abandoned areas (Prach & Pyšek 2001). It has been seen to have a strong tendency in invading secondary grasslands slowing down or blocking the secondary succession natural process. After a 11-years study, Hazi et al. (2011) showed that multiyear mowing could decrease *C. epigejos* cover and increase species richness of the community. This phenomenon was also observed in the controlled area but with less rapidity. Although a significant difference in the species richness was observed after five years of mowing, another important observation was the increase in diversity after eight years, caused probably by the high presence of storage organs.

In Slovakia, as reported by Jendrišáková et al. (2011), grassland abandonment determines the fast spread of *Deschampsia caespitosa* (L.) P. Beauv., *Calamagrostis epigejos* (L.), *Brachypodium pinnatum* (L.) P. Beauv., and *Cirsium eriophorum* (L.) Scop., characterized by a poor palatability. Furthermore, these species can reduce the growth of other high value species due to their competitive ability.

- *Mediterranean mountains*

Canals et al. (2017) studied the effect of the *B. pinnatum* and its invasion in northern Spain (Aezkoa valley, Western Pyrennes). This area is characterized mainly by the intensification of fire used to control ungrazed vegetation invaded by *B. pinnatum*. The main consequence of this invasion was the loss of sympatric species and the rise of extremely degraded, species-poor communities. Where mainly the plant community richness was low, the presence of *B. pinnatum* was higher than 50%, whereas in the opposite case the presence of the species was less than 50%. Finally, the peculiarity of this species is that it is able to halt the ordinary succession towards shrub and tree encroachment.

Brachypodium genuense frequently develops on gravelly soils where the deposits are stable or only slightly mobile. Such conditions allow *B. genuense* to spread. The high dominance of this species and of some other grasses with high vegetation cover, confers to the pasture a dense coverage. The pastoral value of mountain grasslands of Monti Sibillini grazed by sheep is affected by a high abundance of *B. genuense* (D'Ottavio et al 2005). The *Brachypodium* species can also affect the physical condition of grazing sheep with a significant increase in the epithelial keratinisation degree and a decrease of Body Condition Score and body weight (Scocco et al 2013).

In high-mountain and subalpine undisturbed grasslands growing on South and East-facing slopes of Monti Reatini, grasslands are dominated by *B. genuense* and *Festuca paniculata* (Abbate et al 1994).

In secondary grassland (Festuco-brometea) dominated by B. rupestre located at Gran Bosco di Salbertrand Natural Park (NW-Italian Alps), the most effective practice to reduce B. rupestre cover was mowing coupled with mineral fertilization. Leading also to an increase of nutrient-demanding plant species, therefore to a higher forage quality. (Pittarello et al., 2022)

Regarding Brachypodium rupestre communities of southern Italy, only few associations are described, the *Polygalo mediterraneae-Brachypodietum* which was proposed (only as provisional) for the Lucanian Apennines (Di Pietro et al., 2014). In Sicily, it is present in typical limestone substrates of the mountains surrounding Palermo and Trapani and in the metamorphic substrates on the north-eastern side of the island. (Gianguzzi et al., 2018).

5.1.3 The invasive behaviour of *Brachypodium* species

The competitive success of perennial *Brachypodium* species in grasslands is related to their tall canopies, high tiller density and branching frequency (Pottier and Evette, 2010), as well as to their capacity for clonal growth and clonal integration strategy (de Kroon and Bobbink, 1997). The latter allows transport of resources from original ramets growing in non-stressful micro-habitats to subsequent ramets developing in harsher conditions, through a connecting rhizome or stolon (Alpert, 1999). These features allow to maintain the coordination between resource acquisition and conservation, through the rapid spread of clonal tussocks. In the final phases of invasion, coalescence of different clonal patches often forms nearly mono-dominant stands (de Kroon and Bobbink, 1997). In addition, the ability to modulate the resources exploitation by leaf traits allows individuals to sustain dominance in a wide range of environmental conditions (Tardella et al. 2017). The silica-rich, tough and hairy leaves of *Brachypodium* species make the plant poorly palatable to domestic herbivores, minimizing the loss of resources (Canals et al., 2017). The lack of leaf consumption due to the cessation of herbivory fosters the enhancement of a vigorous belowground system; likewise, leads to the accumulation of a thick litter layer, which protects the young shoots arising in spring.

Brachypodium species can also induce the exclusion of weaker competitors changing micro-scale environmental conditions. For example, the spread of dense *B. genuense*-dominated stands was found to be correlated to several micro-scale environmental modifications (Catorci et al., 2011). *B. genuense* individuals, which reach their maximum height and cover value in summer, reduce the light radiation at the ground level up to 90% of the total incident radiation (Catorci et al., 2012). A further important change is due to the high litter accumulation (Catorci et al., 2011). Litter deposition and the shadow of *B. genuense* leaves likely lower soil temperature (Beatty and Sholes, 1988). In fact, Catorci et al. (2011) recorded a temperature constantly lower of about 2 °C during the growing season, except in early spring, under the cover of *B. genuense* tussocks. Moreover, litter accumulation is likely responsible of several changes of soil features (i.e. nitrogen enrichment) and water dynamics (Beatty and Sholes, 1988; Knapp and Seastedt, 1986). Soil moisture values did not differ significantly between invaded and non-invaded communities in spring, probably because in this season soil is saturated by snow melt and spring rainfall; instead, in late spring and summer, moisture was higher in the non-invaded condition (Catorci et al., 2011). These findings are probably related to the high photosynthetic activity of *B. genuense*; in fact, in these periods *B. genuense* individuals are mostly in the "vegetative growth" and "flowering" phenological phases. Thus, it is probable that a large amount of water is drawn from the soil while a progressive drought stress is acting in the system because of the decrease in rainfall in late spring and summer, typical of the Mediterranean climate.

Regarding changes in soil chemical parameters, Catorci et al. (2011) observed an increase in organic matter and a slight soil acidification related to *Brachypodium* invasion, in comparison with mown meadows, where the quantity of organic matter tends to be constant over time. As expected, litter deposition enriches the soil with nutrients (Pastor et al. 1993, Zobel et al. 1996, Huhta et al. 2001); indeed, in *Brachypodium*-invaded grasslands a significant increase of potassium concentration, as well as a slight increase of both nitric and mineral nitrogen have been detected.

5.2 Biology, taxonomy and chorology of perennial *Brachypodium* species in sub-Mediterranean grasslands

5.2.1 The *Brachypodium* genus: taxonomic placement and chorological aspects

Brachypodium P. Beauv. is a genus of plants (including the only *Brachypodieae* Harz tribe) belonging to the *Poaceae* Barnhart family (*Poales* Small order, *Commelinidae* Takht. subclass and *Magnoliopsida* Brongn. class, Thorne & Reveal 2009).

The generic name comes from the Greek "*brachys*" (short) and "*podon*" (foot) with reference to its sub-sessile spikelets.

This genus is widespread across much of Africa, Eurasia, and Latin America (Catalán et al. 2016) and contains ca. 17 species distributed worldwide (Schippmann 1991; Catalán and Olmstead 2000; Catalán et al. 2012), three of which are annual (*B. distachyon*, *B. stacei* and *B. hybridum*) and 14 perennial taxa (Catalán et al. 2016).

The annual species are distributed in the Mediterranean region (Catalán et al. 2012; López-Alvarez et al. 2012, 2016). Among the perennial species, *B. sylvaticum* and *B. pinnatum* have a wide Eurasian distribution; *B. rupestre* is a European species, *B. retusum* is Mediterranean, *B. phoenicoides* West-Mediterranean, *B. genuense* Central Mediterranean, *B. glaucovirens* East-Mediterranean-South/West Asian, *B. boisseri* is distributed in South Spain, *B. arbuscula* in the Canary isles, *B. bolusii* in South Africa, *B. flexum* in tropical and South Africa, *B. madagascariense* in Madagascar, *B. kawakamii* in Taiwan, *B. mexicanum* in central and South America (Catalán et al. 2016).

5.2.2 The *Brachypodium* genus in Italy: overview of taxa and their morphological characterization

In Italy, the *Brachypodium* genus consists of ten species: *B. distachyon* (L.) P. Beauv.; *B. genuense* (DC.) Roem. & Schult.; *B. glaucovirens* (Murb.) T. Durand & B.D. Jacks.; *B. hybridum* Catalán, Joch. Müll., Hasterok & Jenkins; *B. phoenicoides* (L.) Roem. & Schult.; *B. pinnatum* (L.) P. Beauv.; *B. retusum* (Pers.) P. Beauv.; *B. rupestre* (Host) Roem. & Schult.; *B. stacei* Catalán, Joch. Müll., Hasterok & Jenkins; and *B. sylvaticum* (Huds.) P. Beauv. (Bartolucci et al. 2018).

The annual species are characterized by short life cycle, ephemeral habit and self-fertility (Catalán & Olmstead 2000; Catalán et al. 2012); instead, most of the perennial taxa show long rhizomes and self-incompatibility (Catalán et al. 1995; Khan and Stace 1999).

Among the perennial rhizomatous species, *B. retusum* bears branched and woody stems and long-lasting innovations; *B. pinnatum*, *B. rupestre* and *B. phoenicoides* show erect panicles; the xerophilous species *B. phoenicoides* is glabrous and presents partially inrolled leaves, semi-patent twisted spikelets and awnless lemmas (Catalán et al. 2016). The mesophilous species *B. pinnatum* and *B. rupestre* have short awns and bright green leaves. *B. rupestre*, once considered a subspecies of *B. pinnatum*, differs from it in its glabrous leaves and spikelets and in leaf epidermal traits (Schippmann 1991). *B. genuense* departs from these two species for genetic reasons (Catalán et al. 2016) and few morphological differences (Valdés & Scholz 2009). *B. sylvaticum* is characterized by its nodding panicle, densely hairy habit and long-awned lemma. *B. glaucovirens* shows intermediate features between *B. sylvaticum* (short rhizome and long awn) and *B. pinnatum* (bright green leaves, broad leaf ribs and erect panicle) (Catalán et al. 2016).

5.2.3 The *Brachypodium* genus in Italy: distribution area and habitat

Among the annual species, *B. distachyon* is the most widespread, occurring in all the Italian regions, except Valle d'Aosta and Piemonte, while *B. stacei* is restricted to Sicily and *B. hybridum* is indicated in Apulia, Sicily and Sardinia (Bartolucci et al. 2018).

Among the perennials, *B. sylvaticum* occurs in the whole Italian territory and is the only *Brachypodium* species exclusively linked to forest habitats; *B. rupestre* in all the regions, except Sardinia; *B. genuense* in Piemonte, Lombardia, Liguria and all the regions of the Italian peninsula, except Apulia; *B. glaucovirens* in Valle d'Aosta, Lombardia, Veneto, Lazio, Abruzzo, Molise, Calabria; *B. pinnatum* is distributed in the Alpine chain (Aeschimann et al. 2004) and Apulia. *B. phoenicoides* in Piemonte, central and Southern Italy, including Sicily; *B. retusum* mostly in central and southern Italy and islands; *B. hybridum* in Apulia, Sicily and Sardinia (Bartolucci et al. 2018).

5.2.4 The invasive *Brachypodium* species in the mountain grasslands of the Italian peninsula: morphological characters and distribution

Two of the *Brachypodium* species, namely *B. rupestre* and *B. genuense*, are widespread in the secondary grasslands of the Italian peninsula. Both have traits typical of dominant species, such as large dimensions, strong capacity of vegetative reproduction (with remarkable lateral spreading), growth from basal meristems, and high phytomass production, which confer them a high competitive ability (Lucchese 1987, Camiz et al. 1991). Because their leaves have silica and hairs, they also are

poorly palatable species (Roggero et al. 2002) and thus are not eaten by sheep and cattle in under-grazed farming systems. This promotes their invasion in calcareous grasslands, especially in the abandoned ones (Catorci et al. 2011a,b).

These two species have quite distinct distribution areas, the former being distributed in the sub-Mediterranean hills, below 1200-1300 m a.s.l., the latter mainly in the Apennine ridge, usually over 1300-1400 m. However, from the taxonomic viewpoint they are very similar (Lucchese 1989). A description of the two species is reported below, together with chromosome number, distribution and dormancy period. Table 1 reports the diagnostic characters between the two species.

Brachypodium rupestre (Host) Roem. & Schult. (published in: *Systema Vegetabilium* 2: 736, 1817)

Chromosome number – 2n = 18, 2n = 28

Distribution – B. rupestre is a south-European-sub-Atlantic species, occurring in Pyrenées (Spain and France), Jura, Central Massif and Corse (France), French, Italian, Swiss, and Austrian Alps, Apennines, Sicily, Dinarids and Carpathian Mountains (Aeschimann et al. 2004). In Italy, it occurs in all the regions, except Sardinia (Bartolucci et al. 2018).

Morphological characters – Perennial grass, with long rhizomes forming loose and spaced tussocks, ending with 2-3 straight non-hooked innovations, 35-60(-70) cm tall. Culms thick (0.7-1.2 mm), with 2-3 pubescent nodes. Basal leaves with maximum width at 1/3 from the ligule, 7(-)30 cm x (4.5)-6-(8) mm, acuminate. Leaves on the stem 25 cm x 4.5-(7) mm; fresh leaves rigid and flat, dry leaves convoluted; abaxial face little rough, adaxial face glabrous; sparse irregular hairs only on leaf margin; main veins well visible and distinct; leaves pale green or glaucous, ligules of innovations with convex margin, sometimes eroded, 1-1.5 mm x 1.6-2 mm, those of the culm 1.5-1.8 x 1.5-1.7 mm; sheats glabrous, sometimes hairy. In transversal section, leaf veins have flat upper margin and apparently flat profile (bulliform cells not deepened). The abaxial surface of leaves has regularly distributed stomata, adaxial epidermis, long hairs absent, asperities absent or very rare.

The inflorescence is a raceme composed of spikelets more or less spaced along the inflorescence axis, straight, (4-)10(-15) cm long, with (4-)6-8(-10) spikelets, (1.5)-2.5-(3) cm x 2-2.5 mm; the glumes are lanceolate, mucronate, the lower 6-7-(8) x 1 mm, 4-5-veined, the upper 7-8-(9) x 1.4-(2) mm, 5-7-veined, glabrous or pubescent. Lemmas lanceolate (8)-9-(11) x 1.8-2-(2.3) mm, 5-veined; awn (2)-3-(6) mm. Palea 8.5 x 1.8 mm, truncated in the apical part, generally shorter than or equalling lemma. Lodiculae lanceolate (1.5)-1.7 x 0.35 mm with apex and margins very hairy in the distal half. Anthers 3.5-4.3 mm. Caryopsis 7.2 x 2.2 mm. *Dormancy period* – Summer or winter.

Brachypodium genuense (DC.) Roem. & Schult. (published in: *Systema Vegetabilium* 2: 742, 1817) *Chromosome number* – 2n = 18 *Distribution* – This species occurs in Piemonte, Lombardia, Liguria and in all the regions of the Italian peninsula, except Apulia (Bartolucci et al. 2018). *Morphological characters* – Perennial grass, densely caespitose with short rhizomes and numerous emerging hook-shaped innovations, 25-(40)-60 cm tall. Culms attenuated under the inflorescence (diameter 0.5-0.7 mm) with 2-3-(4) pubescent nodes. Basal leaves flat or narrowly convoluted in the dry, (10)-20-(30) cm x 3.5 mm; penultimate leaf of the culm convoluted, 5-10 x 2-3 mm; hairs 0.3-1 mm long, regularly distributed on the leaf margins and abundant on the adaxial surface, scattered and sparse on the abaxial surface; sheats glabrous; ligule truncated 0.2-0.5 x 2-2.5 mm or smaller. In transversal section veins have upper margin with convex and undulating profile (bulliform cells deepened). The abaxial epidermis without stomata or with sporadic stomata, not homogeneously distributed. The epidermis of the adaxial face has long hairs.

The inflorescence is a raceme (3.5-)6(-7.5) cm long, with (2-)4-6(-8) spikelets, sub-sexile or on peduncules 0.5-1-(4) mm; sometimes 1-2 aborted spikelets in the lowest part of the raceme. Spikelets lanceolate or ventricose when ripen, 1.3-2.2 cm x 2.3-3-(4) mm, 5-8-(13) flowers; glumes are lanceolate, shortly mucronate, glabrous, the lower 6.2-8 x 1.3 mm, 5-veined, the upper 8-10 x 1.9-2 mm, 5-7-veined, both 1/3 longer than the spikelet. Lemmas lanceolate, glabrous, shiny and membranous, 8.5-10 x 2.3-2.5 mm, (3)-5-6-veined; awn 1.8-3-(5.2) mm, often sinuate. Palea (5.6)-7-8 x (1.3)-1.5-(2.5) mm, longer than lemma when ripen, with apex eroded and smarginated. Lodiculae ovoidal, ciliate on the borders and on the surface 1.2-1.5 x 0.3-0.5 mm. Anthers 4-4.8 mm. Caryopsis 7.1 x 1.8 mm. *Dormancy period* – Winter.

Table 1- Diagnostic characters of Brachypodium rupestre and B. genuense

Character	***B. rupestre***	***B. genuense***
Rhizomes	Long and thick, originating small spaced tussocks	Short, originating single and robust tussocks
New shoots (innovations)	Non hooked	Hooked
Culms	Uniform thickness	Thickness reduced below the inflorescence
Leaf hairs	Few or absent	Margins and leaf faces
Abaxial epidermis	Stomata present and homogeneously distributed	Absent or sporadic stomata, not homogeneously distributed
Adaxial epidermis	Long hairs absent	Long hairs present
Leaf veins (cross section)	Flat upper margin and apparently flat profile (bulliform cells not deepened)	Upper margin with convex and undulating profile (bulliform cells deepened).
Ligule	Rounded	Truncated
Spikelets length	> 20 mm	< 15 mm

6 Ph.D. activities

6.1 Understanding the implications of abandonment of pastoral systems on plant communities' taxonomical and functional diversity along environmental gradients at different spatial scales

Anthropogenic mountain grasslands undergo major changes in their composition and structure after abandonment of their use as pastures or hay meadows. Distinct plant community-types may develop in habitats that differ in environmental conditions, such as soil water availability; however, few studies have addressed this issue in abandoned anthropogenic grasslands.

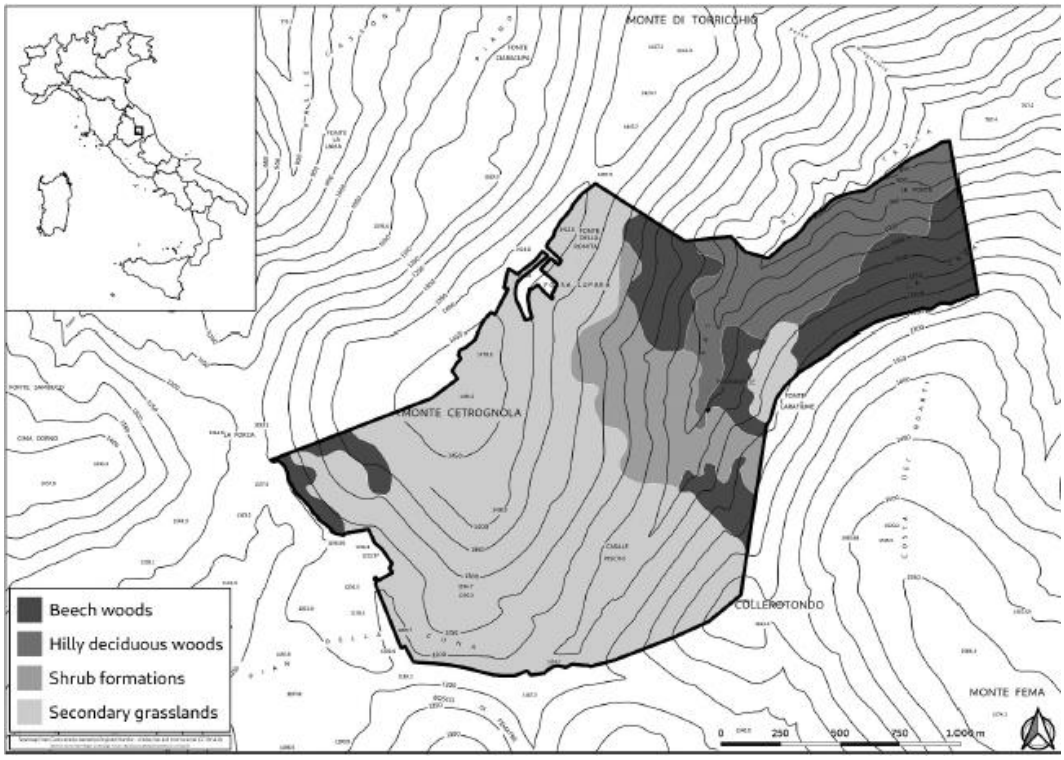
Scientific paper:

Bricca A., Tardella F.M., Ferrara A., Xinfang X., Tolu F., Catorci A, 2021. Environmental heterogeneity compensates the potential homogenising effect of abandonment of grazing in a sub-Mediterranean mountain landscape. **PLANT ECOLOGY & DIVERSITY 14(5-6): 223–243.** https://doi.org/10.1080/17550874.2022.2039314

Research aim - Understanding the impact of the cessation of traditional land-use management on grassland communities' taxonomic and functional diversity along environmental gradients at different spatial scales.

Study area - Montagna di Torricchio Nature Reserve (central Apennines, Italy).

Figure 1 - Map of the study site in the Montagna di Torricchio Nature Reserve, central Apennines, Italy, where grasslands between 1,150 and 1,350 m a.s.l. on the south-east and north-west facing slopes were studied.



Data collection - We used a proportional stratified random sampling design. We limited our study to the elevation range between 1150 and 1350 m considered two opposite slope categories as two distinct units. To obtain a sample as much representative as possible, we divided the elevation range into two strata, i.e., 1,150–1,250 m, with average slope angles of 15–20°, and 1,251–1,350 m, with average slope angles of 20–30°. Thus, we distinguished four main sampling units: east-south-eastto south-east-facing areas between 1,150 and 1,250 m (Unit 1); east-south-east- to south-east-facing areas between 1,251 and 1,350 m (Unit 2); north-west-facing area between 1,150 and 1,250 m (Unit 3); and north-west-facing areas between 1,251 and 1,350 m (Unit 4). We selected the number of plots (5 m × 5 m) proportional to the extent of each unit. We sampled a total of 52 plots (24 plots in Unit 1; 12 plots in Unit 2; 8 plots in Unit 3; 8 plots in Unit 4). During the growing season of 2019 (from mid- June to early July), we recorded in each plot the cover of each vascular species (percent values, visually estimated), slope angle (vertical degree), aspect (azimuth degrees), elevation (m. a.s.l.), soil depth (average of five measurements per plot), cover of litter, bare soil, debris and outcropping rock (percent values visually estimated). Finally, we clipped the above-ground phytomass at 0.5– 1.0 cm from ground level, oven-dried and weighed it (g m−2 of dry matter). We harvested aboveground phytomass in an area of 0.5 m × 0.5 m in the lower left-hand corner of each plot. The functional dimension of the plant communities was characterized by assigning to each

species a set of traits capturing different functions, Plant height (H) (size-asymmetric competition), Specific leaf area (SLA) (spatial resource exploitation strategies), Leaf phenology (LP) (temporal resource exploitation strategies) (Klotz et al. 2002) Vegetative propagation (VP) (spatial propagation) and Storage organ (SO) (resource conservation strategy) (Klimešová et al. 2017). Scan of *Brachypodium rupestre* leaves and drying in the oven to measure the dry weight. Leaf area measurement using a software and calculation of Specific leaf area, indicator of resource exploitation strategies. Plant functional characteristics were collected at the growing peak (summer) and measured during winter. Leaf samples were and preserved using the standardized procedures described in Cornelissen et al. (2003). Categorical traits have been obtained by field observations and consultation of on-line databases.

Data analysis - To identify the plant communities, we ran a cluster analysis using the complete linkage method on the similarity matrix, based on Euclidean distance, calculated on the Hellinger-transformed species data matrix (plot × species cover expressed in percentage values) To identify the indicator species of the clusters emerged from cluster analysis, we undertook an Indicator Species Analysis (ISA) on the 'plot × species cover' matrix and the plot membership in the clusters was treated as grouping factor. Calculation of taxonomic and functional alpha and beta-diversity indices. Data processing using cluster analysis, Redundancy Analysis (RDA), Indicator Species Analysis (ISA), ANOVA.

Results - We identified four community-types whose distribution was related to topographically determined soil water availability Cluster I dominated by *Sesleria nitida*, Cluster II grouped grasslands dominated by *Bromopsis erecta* with the presence of *Sesleria nitida*, Cluster III included plots of grasslands dominated by *Bromopsis erecta*, with the scattered occurrence of *Brachypodium rupestre*, Cluster IV grouped grasslands dominated by *Bromopsis erecta*, with a noteworthy cover of *Brachypodium rupestre*.

The species composition of drought-tolerant communities on south-eastern slopes with shallow soils was largely similar to that of anthropogenic grasslands. Resource-poor conditions were related to enhanced α-TD and β-TD and reduced α-FD for traits related to temporal niche differentiation and resource acquisition strategies. Species coexistence was mainly promoted by strategies linked to the exploitation of resources in space and time. Topographic conditions (slope orientation and angle) act as the main drivers in determining the function-based mechanisms of community assembly.

Conclusion - This research confirms how in grassland the landform factors act as important drivers in determining the coenological composition and the diversity faces of community's types.

6.2 Evaluation of species and functional composition recovery in grassland communities invaded by *Brachypodium* after mowing management in the sub-Mediterranean climate

Restoration of pastures after tall-grass invasion is a paramount issue because of this profound impact on biodiversity and ecosystem services. This requires a deep understanding of the mechanisms behind the coenological recovery processes that occur after disturbance events.

It is generally assumed that a higher number of plant strategies and species are present under disturbed conditions (Grime 2006) since disturbance decreases the chance of competitive exclusion of taxa with traits associated with a low competitive ability (i.e., weaker competitor exclusion) (Chesson 2000; Mayfield and Levine 2010; Lepš 2014). Mowing is considered one of the most appropriate management types for ensuring the high conservation value of grasslands (Oroian et al. 2014; Tälle et al. 2014). It is a non-selective disturbance (Köhler et al. 2005) that, increasing light availability, promotes the survival of light-demanding seedlings, low-growth species (Overbeck et al. 2003; Ilmarinen and Mikola 2009) and germination rates of subdominant plants (Collins et al. 1998; Hautier et al. 2009). Moreover, it modifies the functional composition of plant communities by enhancing specific avoidance and tolerance strategies (Huhta et al. 1998; Klimešová et al. 2008; Valkó et al. 2012; Halassy et al. 2019). In particular, it was proven that vascular plant species richness was highest with two cuts per year (Socher et al. 2013).

Notwithstanding such scientific advancement, there is still a need to understand better the effect of disturbance activities (i.e., mowing) and disentangle such effects from those related to the processes of plant-plant interaction (dominant vs subordinate species) (Grime 2001). Abiotic interactions (i.e., disturbance through mowing) could be an important driver in species coexistence at a small-scale, with different effects depending on the type and intensity of the disturbance regime, the frequency in the disturbance events (de Bello et al. 2013), but also depending on the considered time scale (Halassy et al. 2019). However, biotic interactions (i.e., plant-plant interactions) are considered the major driver at a small-scale. Indeed, dominant species may remove from the community all weaker competitors (i.e., weaker competitor exclusion), acting as a biotic filter, which leads to the coexistence of more functionally similar species.

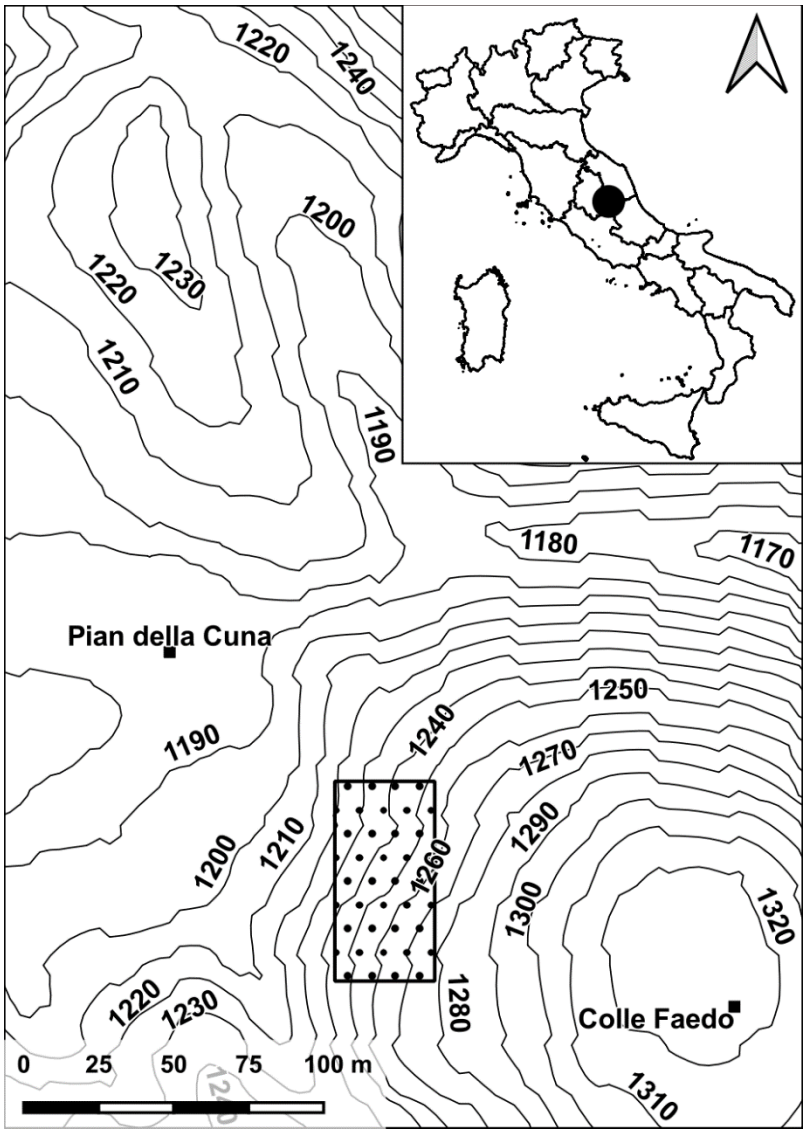
Scientific paper:

Bricca A., Tardella F.M., Tolu F., Goia I., Ferrara A., Catorci A. 2020. Disentangling the Effects of Disturbance from Those of Dominant Tall Grass Features in Driving the Functional Variation of Restored Grassland in a Sub-Mediterranean Context. **DIVERSITY 12 (11)** https://doi.org/10.3390/d12010011

Research aim – Disentangling the effects of twice-a-year mowing from those of *Brachypodium rupestre*'s features in driving the functional variation in grasslands invaded by *B. rupestre*.

Study area – The study area was located in the central Apennines, near the Montagna di Torricchio State Nature Reserve, in an area in which, for at least 30 years before the start of the experiment, vast grassland areas, especially the steeper ones, had been undergrazed and are undergoing dynamic processes.

Figure 2 - Map of the study site near the Montagna di Torricchio Nature Reserve, central Apennines, Italy.



Data collection – The experimental site (two hectares) was established and fenced in February 2010, within each block, half of the surface, i.e. 0.5 ha (50 x 100 m), was mown twice a year (end of June, namely, just before the *B. rupestre* flowering, and at the end of October), two cm above the soil level; hay and litter were removed after each mowing event. The other half was left unmown, without

litter removal. Relevés were performed from 2010 to 2016.

We randomly selected the coordinates of the lower left-hand corners of 20 plots (0.2 m × 0.2 m) in the mowed and unmowed area, for a total of 40. In each plot, we recorded: species occurrence and *B. rupestre* plant height (since plant height is related to size-asymmetric competition). We clipped the aboveground phytomass (2 cm above the soil level) and we separated the phytomass of *B. rupestre* from that of the other plants. We oven-dried the samples at 70 °C for 96 h and weighed them. To understand how the functional composition of the community on a fine scale was affected by the variation of *B. rupestre*'s traits, we selected some traits related to space occupation (e.g. plant height, horizontal and vertical architecture, and vegetative propagation), reproductive strategies (e.g. seed mass and vegetative propagation), temporal niche occupation (e.g. life span, leaf span) and resource storage strategies (e.g. storage organs, leaf anatomy).

Data analysis – We performed linear general mixed-effects models to investigate the effect of *B. rupestre* cover and twice-a-year mowing on functional composition of the rest of the community, calculated as "plot × trait state" matrix (number of species sharing the same trait state in each plot), with sample identity as random effect.

Results – Our results indicated that twice-a-year mowing altered the functional composition of the community, fostering the spread of species characterized by avoidance strategies (e.g. annual life span) and tolerance strategies (e.g. clonal strategy). We observed that the effect of mowing on the functional variation of the community was more effective than that related to plant-plant interactions and that different drivers mostly acted on different trait states.

Conclusion – Mowing was the main driver in the recovery process, acting as an abiotic filter (enhancement of tolerance-avoidance strategies). Furthermore, the reduction of weaker competitor exclusion processes fostered the increase of functional variation between coexisting species. Both drivers acted on different plant traits (e.g., mowing on life span, vegetative propagation types and plant height, mowing and *B. rupestre* features on space occupation types, seed mass and leaf anatomy), generally enhancing the extent of functional strategies related to resource acquisition and storage, reproduction, space occupation and temporal niche exploitation.

6.3 Understanding how the species and functional composition and diversity of the community change over time in mown and abandoned meadows and how they change along long-term disturbance gradients

6.3.1 Introduction

Considerable evidence supports the hypothesis that co-existence of plants is conditioned by stress and disturbance intensities (Grime, 2001). Mowing is considered one of the most appropriate disturbance types for ensuring the high conservation value of grasslands (Oroian et al., 2014; Tälle et al., 2014; Tardella et al., 2020). More specifically, it is a non-selective disturbance type (Kohler et al., 2005) that reduces above-ground phytomass, prevents litter accumulation and increases species diversity (Bonanomi et al., 2006; Tardella et al., 2020).

Several studies have shown that mowing fosters traits that allow resource allocation to leaves or underground storage organs (e.g. Klimeš and Klimešová, 2002; Craine, 2005) and reduces the abundance of perennial tall grasses in favour of short-lived species (Bonanomi et al., 2022). Because of these effects and since it reduces above-ground biomass and prevents litter accumulation (when hay is removed), mowing promotes the survival of light-demanding seedlings and low-growth species in seminatural grasslands (Overbeck et al., 2003; Ilmarinen and Mikola 2009), and leads to an increase in species richness and diversity, at least in productive conditions (Huhta et al., 2001; Bonanomi et al., 2006, 2022; Ruprecht et al., 2010; Hejcman et al., 2011; Valko et al., 2012). It is more destructive to the taller species because a larger proportion of their phytomass is removed (Klimešová et al., 2011; Lepš, 2014). Moreover, mowing fosters species that are not vulnerable to impact at the cutting date, e.g. species that have completed flowering and seed production before a mowing event, leading to a complex trait-determined use of different temporal reproductive niches (Catorci et al., 2017).

Conversely, the absence of disturbance related to grassland abandonment promotes the spread of tall grasses (*Brachypodium rupestre* and *B. genuense* in Apennines' seminatural grasslands), leading to a marked drop of species richness and species diversity (Catorci et al., 2011; Lepš, 2014), by a process called "weaker-competitor exclusion" (Chesson, 2000). This process suggests that the prevalence of hierarchical differences in the competitive ability between species should favour strong competitors which remove all those species with less competitive abilities (Mayfield & Levine 2010; Lepš, 2014), leading to a community characterized by few ecologically similar species. In addition, tall grass invasion may influence the temporal niche partitioning of coexisting species by selecting a set of species able to cope with environmental conditions (community structure, litter deposition, soil features modifications, etc.) determined by dominant plants (Catorci et al., 2014). Finally,

Brachypodium species are also unpalatable, reducing the nutrient value of pastures (Vitasović Kosić, et al., 2014).

Few studies have employed both taxonomic and functional methodologies for a multi-approach evaluation of the effect of disturbance in grasslands and the continuity of its effect over time. In addition, short temporal scales have been mainly considered, focusing therefore only on the effect of mowing vs. abandonment in regard to taxonomic / functional composition and diversity, but not on the changes of mowed and abandoned grasslands over time (Halassy et al., 2019; Klimešová et al., 2011).

We hypothesized that along a disturbance gradient, i) absence of disturbance reduced species and functional diversity, fostering traits of dominant species, linked to competitive ability and resource acquisition (tall plants with rhizomes, and upright forbs); ii) discontinuous disturbance increased species diversity, fostering coexistence of species with traits related to different space occupation strategies (plant vegetative height, horizontal and vertical space occupation) and temporal niche partitioning (leaf phenology, life span), and augmenting functional diversity of these traits; iii) regular disturbance fostered traits related to avoidance and tolerance strategies (creeping species, runners, rosettes, storage organs, annual, and small-sized species), increasing functional diversity of these traits, compared to absence-of-disturbance condition, but reducing it compared to discontinuous disturbance condition. Moreover, we hypothesized that mowing maintained the species composition and diversity over time, enhancing the plant traits devoted to ensure coexistence, and that prolonged abandonment leads to further decrease of taxonomic and functional diversity values, fostering competitive strategies and, thus, exacerbating the effects of weaker competitor exclusion.

The research aims were to: i) understand how the species and functional composition and diversity of the community change over time in mown and abandoned meadows; and ii) how they change along long-term disturbance gradients.

The research questions were: i) How do species and functional composition and diversity change along long-term disturbance gradients? ii) How do species and functional composition and diversity change over time in mown and abandoned meadows?

6.3.2 Materials and methods

6.3.2.1 Study area

The "Prati di Ragnolo" study area is located at about 1450 m a.s.l. (43°01'16''N, 13°13'36''E) in the northern part of the Sibillini Mountains National Park (central Italy) and is distinguished by limestone bedrock (Regione Marche 1991); the morphologies have intermediate relief-energy in northern-facing slopes. Soils, of a total depth of 40-50cm, are classifiable as typic/lithic haplustoll loamy mesic and mollic leptosol, with OiAC profile; they are acid or sub-acid (pH: 5.0-6.0) and have a clayey-sandy texture with fine siliceous skeleton. The soil water-regime is ustic/udic (Pieruccini 2007).

Figure 3 - Map of the study site in the "Prati di Ragnolo", central Apennines, Italy.

From a bioclimatic point of view, this area is encompassed within temperate region, in the upper supratemperate bioclimatic belt, lower hyper-humid ombrotype, and is characterized by four months with mean minimum temperatures below 0 °C, with possibility of strong frosts from October to the end of March. There is a short period of water scarcity (drought stress) in July and the plant-growing season lasts 120-150 days, between May and September. Annual rainfall is about 1400 mm, while the summertime average is 240 mm; mean annual temperature is 7.3 °C (Catorci et al. 2007a).

The productivity of this ecosystem, intended as above-ground phytomass and dry matter, is respectively of 1000-1100 g/m^2 and 300-400 g/m^2 (Gatti et al. 2007b). Flowering time occurs between the first days of May and the end of July, while the highest peak is within the first ten days of July; there is no grass growth between late July and late August (Gatti et al. 2007a).

This area has been marked by pastoral practices for centuries. Traditional pastoral activities were mowing at the end of June, followed by sheep and cattle grazing. In the last 25-30 years, these practices underwent a collapse, and today only mowed or abandoned meadows are present.

6.3.3 Sampling design and data collection

i) How do species and functional composition and diversity change along long-term disturbance gradients?

In the months of June and July of 2021, 55 phytosociological relevés were carried out in 10 m x 10 m plots using a proportional random stratified sampling design, with disturbance intensity (continuously and homogeneously mown meadows; discontinuously and heterogeneously mown meadows – hereafter intermediate disturbance intensity; and abandoned meadows) as stratifying criterion. Plots were laid in the same environmental conditions (north aspect, similar slope and altitude values). Twenty, 15, and 20 relevés, respectively, were carried out inside the three disturbance intensity classes.

For each plot we recorded the date, the altitude (m a.s.l.), the geolocation, the surface of the plot (m^2), the total vegetation cover (percentage values, visually estimated), the list of plant species and their cover values (percentage values, visually estimated). Species nomenclature followed Bartolucci et al. (2018) and Del Guacchio and Caputo (2020), was checked using the Portal of the Flora of Italy (available at http:/dryades.units.it/floritaly, accessed 15/01/2023).

The plant samples of *taxa* undetermined in the field were collected and then identified and stored at the *Herbarium Universitatis Camerinensis* (CAME).

ii) How do species and functional composition and diversity change over time in mown and abandoned meadows?

To highlight possible vegetation changes over time, an approach based on the resampling of historical vegetation plots was chosen. It is the result of a compromise between the availability of historical data and the reliability of their reallocation, as it proved to be a valid approach to investigating to semi-natural grasslands (e.g., Bennie et al., 2006; Wesche et al., 2012).

We gathered the phytosociological relevés carried out in 2008 by Catorci et al. (2011) in mown and abandoned grasslands, in which species abundance was expressed using the Braun-Blanquet scale. In 2021 the relevés carried out in 2008 were repeated in the same grassland patches that maintained the

same management conditions using a percent abundance scale. To ensure comparability between the two data sets, the former values (2008) were converted into the average cover values of Braun-Blanquet classes: + (< 1%) – 0.5 %; 1 (1–5%) – 3 %; 2 (5–25%) – 15%; 3 (25–50%) – 37.5%; 4 (50–75%) – 62.5%; 5 (75–100%) – 87.5%. *r* (rare species) were attributed 0.1%. Then, we transformed the percentage cover values of the species collected in 2021 into the Braun-Blanquet scale and then in the average cover values of the Braun-Blanquet classes.

6.3.4 Data analysis

Changes in environmental characteristics

To assess the differences in environmental conditions between 2008 and 2021 in mown and abandoned conditions, and along the disturbance intensity gradient (abandoned vs. intermediate disturbance; intermediate disturbance vs. mowing; and abandoned vs. mowing), we used the Ellenberg's Indicator Values (EIVs; Ellenberg et al., 1991), adapted to Italian flora (Pignatti, 2005; Guarino et al., 2012), since EIVs proved to be useful in analyzing the drivers of vegetation change (i.e. McCollin et al., 2000; Klaus et al., 2012), especially when considered for comparison on a regional/local scale (Godefroid & Dana, 2007).

We computed mean EIVs at plot level, weighted on presence/absence species data, using the function provided by Zelený & Schaffers (2012), and performed the basic descriptive statistics for each group.

We tested the differences in EIVs between relevés groups using the modified permutation test elaborated by Zelený & Schaffers (2012), based on a null model obtained using mean randomized EIVs (i.e. calculated from species EIVs randomized among species) instead of randomized mean EIVs, in order to separate information on compositional similarity possibly present in mean EIVs. Prior to this analysis, the species cover data were transformed into presence-absence data. The modified permutation test was run using the *summary.aov.iv* R-function provided by Zelený & Schaffers (2012).

All the analyses were run using R, version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org).

Changes in species composition

To identify the indicator species of the pairs of relevés groups, we performed an Indicator Species Analysis (ISA) on the 'plot × species cover' matrix and the relevés groups (year or disturbance condition) had been carried out, where year or disturbance condition were treated as grouping factor (Dufrêne and Legendre 1997). We tested the statistical significance ($P < 0.05$) of the observed maximum indicator value (IV) using permutation tests with 999 runs, and we discarded from the list of significant species, those species whose component of relative abundance was lower than 0.6 or whose component of relative frequency was lower than 0.25 (de Cáceres et al. 2012). ISA was made with the multipatt function ('indicspecies' package, version 1.7.9). To interpret the changes in species composition, for each species highlighted by the ISA, we attributed the phytosociological placement following Mucina et al. (2016) and Biondi and Blasi (2016).

Changes in taxonomical diversity

We calculated species richness (S), Pielou Evenness (J), Shannon index (H), and Gini-Simpson index (G). We calculated H and G using the *diversity* function in the *vegan* R-package, version 2.4-4, and J dividing H by the natural logarithm of species richness. To assess the differences in taxonomic diversity indices between 2008 and 2021 in mown and abandoned conditions, and along the disturbance intensity gradient (abandoned vs. intermediate disturbance; intermediate disturbance vs. mowing; and abandoned vs. mowing), we used the Wilcoxon-Mann-Whitney test and applied the Holm's correction for multiple comparisons). This test was made with the wilcox.test function ('stats' package, version 3.4.1).

Changes in functional traits' composition

To assess the changes in plant functional traits' composition between 2008 and 2021 in mown and abandoned conditions, and along the disturbance intensity gradient, we selected a set of traits that represent different functions, namely plant vegetative height – size- asymmetric competition; horizontal and vertical plant architecture, vegetative propagation – space occupation. related to the competitive capacity and the acquisition of resources (Díaz et al. 2004; de Bello et al. 2005; Lavorel et al. 2007; Klimešová et al. 2017); leaf phenology (Klotz et al. 2002) and life span – temporal resource exploitation strategies; storage organ and leaf anatomy – resource conservation strategy (e.g. Klimešová et al. 2017); palatability (Roggero et al., 2002).

We computed the community weighted mean values (hereafter, CWM) of pairs of relevés groups

as follows:

$$CWM = \sum_{i=1}^{S} p_i x_i$$

where CWM is the relative contribution of each trait state (for categorical traits), S is the number of species, p_i is the relative abundance of species *I* (*i* = 1,2, …, S), and x_i is the trait value for species *i*. We calculated *CWMs* using the *cwm* function in the *weimea* package. We tested the significance of differences between groups of relevés using the test_cwm function (method = aov, based on row-based permutation test with 999 permutations, Zelený, 2018) in the *weimea* package. Finally, we performed the basic descriptive statistics for each group.

Changes in functional diversity

To characterise and assess the variation between relevés groups in α-functional diversity (FD) of the selected traits, we used Rao's Quadratic Entropy (Q), which provides a robust framework to compare different aspects of diversity (de Bello et al. 2010).
The α-diversity is calculated as follows:

$$\alpha Q = \sum_{i,j}^{S} d_{ij} p_i p_j \qquad (2)$$

where *S* is the number of species, $d_{ij}$is the distance or dissimilarity between the *i*-th and *j*-th species, p_i and p_j are the proportions of *i*-th or *j*-th species in the sampling unit (plot). Taxonomic diversity (αTD) assumes that the distance between the *i*-th and *j*-th species is fixed (d_{ij} = 1 for all pairs of species) unless *i=j* (thus, d_{ij} = 0). In this case, αTD corresponds to 1-Simpson (Ricotta 2005). Similarly, for αFD, d_{ij} may vary from 0 (two species have the same trait values or trait state combination) and 1 (two species have completely different trait values or trait state combination). We used the Gower distance as a measure of distance for FD, because it provides a standardisation of distances between 0 and 1 (Pavoine et al. 2009; de Bello et al. 2010). As any variation in FD could be driven by a variation in TD (Devictor et al. 2010), we checked the presence of collinearity using Pearson correlation coefficients between αTD and αFD. Pearson correlation coefficients (p) were highly positive and significant for all traits. We inferred how functional diversity was affected by the predictors without the influence of TD by regressing αFD against αTD. Then, we adopted the residuals of these regressions as response variables (αFD_{RES}) in the following analyses. and we assessed differences in αFD_{RES} among relevés groups using ANOVAs. We carried out ANOVAs using the *aov* function, Pearson correlation was made by using with *cor.test* function in the 'stats' package, linear regressions were made with the *lm* function ('stats' package).

6.3.5 Results

i) How do species and functional composition and diversity change along long-term disturbance gradients?

We did not find any significant change in EIVs along the disturbance gradient (Figure 4).

The 44% of indicator species of the abandoned condition belonged to *Festuco-Brometea* or *Seslerietea* classes; the 33% to *Fagetea sylvaticae* or *Trifolio-Geranietea*, while the 23% to *Molinio-Arrhenatheretea* and/or *Nardetea strictae* (Table 2). In the intermediate disturbance condition, 46% of indicator species belonged to *Festuco-Brometea* or *Seslerietea*, while 31% to *Molinio-Arrhenatheretea* and/or *Nardetea strictae* (Table 2). In mown meadows, 64% of indicator species belonged to *Festuco-Brometea* or *Seslerietea* classes, and 21% to *Molinio-Arrhenatheretea* or *Nardetea strictae* (Table 2).

All the taxonomic diversity indices differed significantly, except for species richness between abandoned and grasslands characterized by discontinuous disturbance, with the highest values in the mown condition (Figure 5).

Several traits' CMWs changed along the disturbance gradient, especially when comparing mown and abandoned grasslands (Table 3). In particular, it was observed an increasing trend from abandonment to mowing in annual species, pleiocorm, no horizontal architecture, sedge, prostrate forbs, hemirosulate upright forbs, bulbs as storage organs and vegetative propagation type, tap roots, spring green and overwintering green leaves, mesomorphic leaves, 80-100 cm-tall species (Table 3). The opposite trend was observed in perennial species, caespitose, grass, erosulate upright forbs, and runners (Table 3). In intermediate disturbance intensity, plant species with only sexual reproduction, persistent green leaves, succulent leaves, palatable species recorded a maximum, while reptant, summer green leaves, tuber as vegetative propagation type and storage organ, and unpalatable species a minimum (Table 3).

As far as functional diversity is concerned (Table 4), we detected the only differences in vegetative propagation and horizontal architecture, between intermediate-disturbance and abandoned grasslands.

Figure 4 - Boxplots of Ellenberg indicator values (EIVs) under abandonment (A), intermediate disturbance (Int), and mowing (M). Equal letters indicate no significant differences in the pairwise comparisons between A, Int, and M.

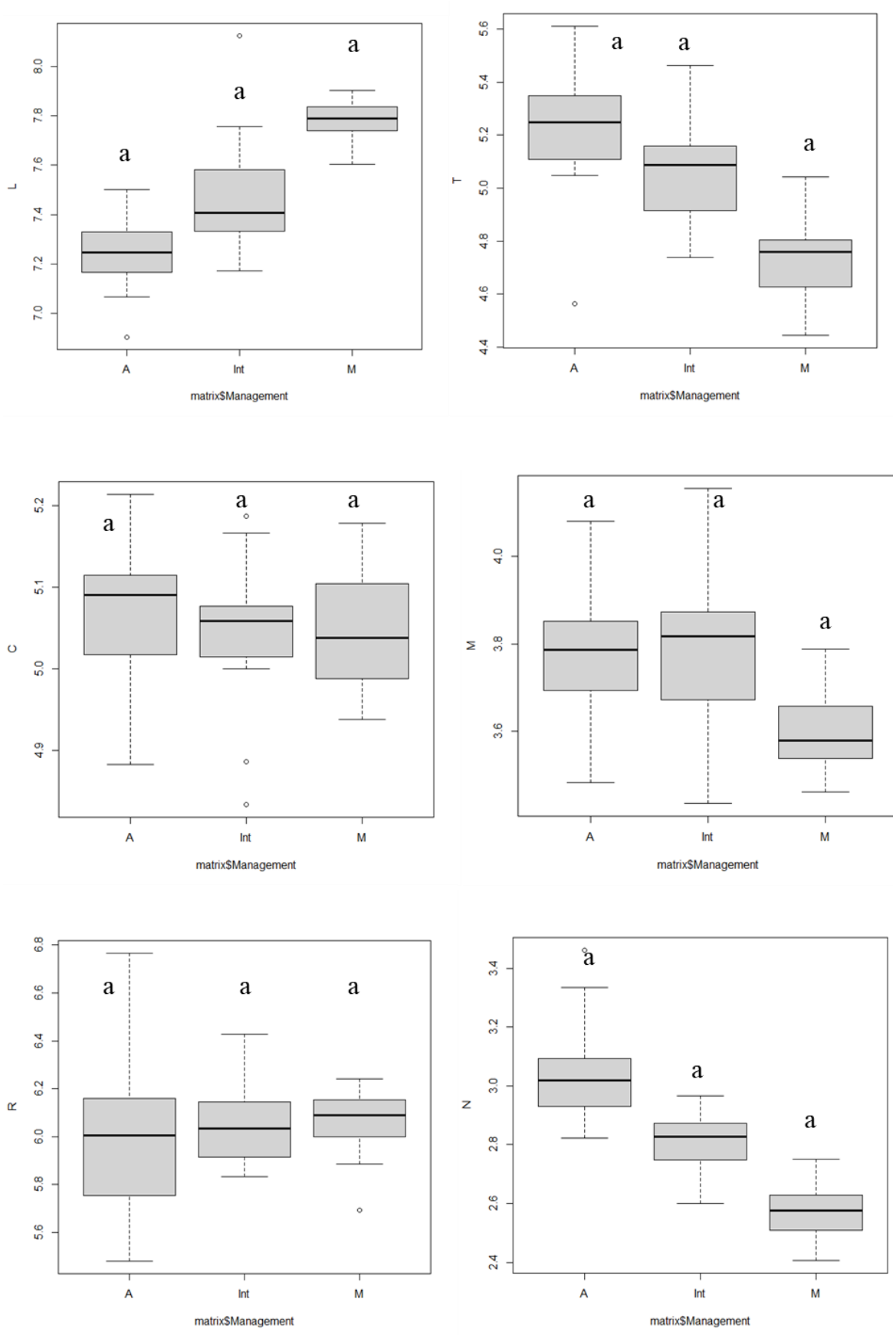


Table 2- Indicator species of the relevé groups of abandoned meadows (A), discontinuously mown meadows (I), and continuously mown meadows (M) as performed by Indicator Species Analysis. Coenological group, relative abundance, relative frequency, square root of Indicator Value (Stat), and significance value (P) are reported for each species whose A was ≥ 0.6 and B ≥ 0.25.

Management	Species	Coenological group	Relative abundance	Relative frequency	Stat	P	
A	Dianthus hyssopifolius L.	FES	1.000	1.000	1.000	0.001	***
	Campanula micrantha Bertol.		0.949	1.000	0.974	0.001	***
	Cruciata glabra (L.) C.Bauhin ex Opiz	GER	1.000	0.800	0.894	0.001	***
	Campanula glomerata L.	FES	0.827	0.750	0.788	0.001	***
	Carlina acaulis L. subsp. caulescens (Lam.) Schubl. & G. Martens	SES	0.948	0.600	0.754	0.001	***
	Thesium linophyllon L.	FES	0.854	0.650	0.745	0.003	**
	Brachypodium genuense (DC.) Roem. & Schult.	FES	0.513	1.000	0.716	0.001	***
	Alchemilla glaucescens Wallr.	MOL/NAR	0.979	0.500	0.700	0.001	***
	Festuca nigrescens	MOL/NAR	0.546	0.850	0.681	0.007	**
	Avenella flexuosa (L.) Drejer subsp. flexuosa	NAR	1.000	0.400	0.632	0.002	**
	Nardus stricta L.	NAR	1.000	0.400	0.632	0.001	***
	Senecio doronicum (L.) L. subsp. doronicum	SES	0.694	0.450	0.559	0.043	*
	Hypericum perforatum L. subsp. perforatum	GER	0.811	0.300	0.493	0.040	*
	Primula veris L. subsp. columnae (Ten.) Maire & Petitm.	FAG	0.927	0.250	0.481	0.039	*
	Helianthemum nummularium (L.) Mill. subsp. obscurum (Čelak.) Holub	FES	1.000	0.200	0.447	0.036	*
	Lilium bulbiferum L. subsp. croceum (Chaix) Jan.	GER	1.000	0.200	0.447	0.030	*
	Lilium martagon L.	FAG	1.000	0.200	0.447	0.026	*
I	Helleborus viridis L. subsp. bocconei (Ten.) Peruzzi	PUB	0.978	0.800	0.885	0.001	***
	Trifolium ochroleucon Huds.	FES	0.783	0.867	0.824	0.001	***
	Arabis hirsuta (L.) Scop.	FES	0.709	0.867	0.784	0.001	***
	Knautia calycina (C.Presl) Guss.	FES	0.653	0.933	0.780	0.001	***
	Koeleria splendens C.Presl	FES	0.800	0.667	0.730	0.002	**
	Cynosurus cristatus L.	MOL	0.859	0.533	0.677	0.003	**
	Rumex nebroides Campd.	SES	0.685	0.667	0.676	0.001	***
	Petrosedum rupestre (L.) P.V.Heath	SED	0.685	0.667	0.676	0.016	*
	Anthoxanthum nipponicum Honda	TRI	0.993	0.400	0.630	0.002	**
	Cynanchica pyrenaica (L.) P.Caputo & Del Guacchio subsp. cynanchica (L.) P.Caputo & Del Guacchio	FES	0.661	0.600	0.630	0.001	***
	Rhinanthus alectorolophus (Scop.) Pollich subsp. alectorolophus	MOL	0.690	0.400	0.525	0.019	*
	Genista tinctoria L.	GER/NAR	0.964	0.267	0.507	0.006	**
	Agrostis capillaris L. subsp. capillaris	MOL/NAR	0.949	0.267	0.503	0.008	**
M	Narcissus poëticus L.	MOL	0.914	0.950	0.932	0.001	***

Biscutella laevigata L. subsp. laevigata	SES	1.000	0.800	0.894	0.001	***
Hippocrepis comosa L. subsp. comosa	FES	0.984	0.800	0.887	0.001	***
Gentiana lutea L. subsp. lutea	SES	0.825	0.950	0.885	0.001	***
Senecio scopolii Hoppe & Hornsch. subsp. scopolii	-	0.864	0.900	0.882	0.001	***
Rumex acetosa L. subsp. acetosa	MOL	0.964	0.750	0.850	0.001	***
Plantago media L. subsp. media	MOL	0.863	0.800	0.831	0.001	***
Galium anisophyllon Vill.	SES	0.854	0.800	0.826	0.001	***
Bellardiochloa variegata (Lam.) Kerguélen subsp. variegata	TRI	0.893	0.750	0.819	0.001	***
Thymus longicaulis C.Presl subsp. longicaulis	FES	0.758	0.850	0.803	0.001	***
Centaurea triumfettii All.	GER	0.678	0.950	0.803	0.001	***
Trifolium pratense L. subsp. pratense	MOL	0.800	0.800	0.800	0.001	***
Dactylorhiza sambucina (L.) Soó	FES	0.896	0.700	0.792	0.001	***
Pilosella cymosa (L.) F.W.Schultz & Sch.Bip.	FES	0.691	0.900	0.789	0.002	**
Ranunculus pollinensis (N.Terrac.) Chiov.	NAR	0.940	0.650	0.781	0.001	***
Lotus corniculatus L. subsp. corniculatus	MOL	0.585	0.950	0.746	0.004	**
Rhinanthus wettsteinii (Sterneck) Soó		0.911	0.600	0.739	0.002	**
Potentilla rigoana Th.Wolf	FES	0.834	0.650	0.736	0.003	**
Carex macrolepis DC.	SES	0.559	0.900	0.709	0.001	***
Bromopsis erecta (Huds.) Fourr.	FES	0.466	1.000	0.682	0.003	**
Briza media L.	FES	0.547	0.850	0.682	0.043	*
Helictochloa praetutiana (Parl. ex Arcang.) Bartolucci, F.Conti, Peruzzi & Banfi subsp. praetutiana	FES	0.606	0.750	0.674	0.017	*
Armeria gracilis Ten. subsp. gracilis	FES	0.546	0.800	0.661	0.015	*
Polygala alpestris Rchb. subsp. alpestris	SES	1.000	0.400	0.632	0.002	**
Gymnadenia conopsea (L.) R. Br.	MOL	0.906	0.350	0.563	0.005	**
Cruciata pedemontana (Bellardi) Ehrend.	FES	1.000	0.300	0.548	0.005	**
Pedicularis tuberosa L.	TRI	1.000	0.300	0.548	0.008	**
Siler montanum Crantz subsp. stabianum	GER	0.993	0.300	0.546	0.013	*
Aster alpinus L. subsp. alpinus	SES	1.000	0.250	0.500	0.009	**
Poa alpina L. subsp. alpina	SES	0.923	0.250	0.480	0.035	*
Anthyllis vulneraria L. subsp. weldeniana (Rchb.) Cullen	FES	0.876	0.250	0.468	0.045	*
Anthyllis vulnearia L. subsp. polyphylla (DC.) Nyman	FES	1.000	0.200	0.447	0.029	*
Festuca stricta Host subsp. sulcata (Hack.) Patzke ex Pils	FES	1.000	0.200	0.447	0.030	*
Ziziphora granatensis (Boiss. & Reut.) Melnikov subsp. alpina (L.) Bräuchler & Gutermann	SES	1.000	0.200	0.447	0.032	*

*** $P < 0.001$; ** $P < 0.01$; * $P \leq 0.05$

Figure 5 - Boxplots of diversity indices species richness (S), Shannon-Wiener index (H), Simpson (G) and Pielou's evenness (J) under abandonment (A), intermediate disturbance (Int), and mowing (M). Different letters indicate significant differences in the pairwise comparisons between A, Int, and M.

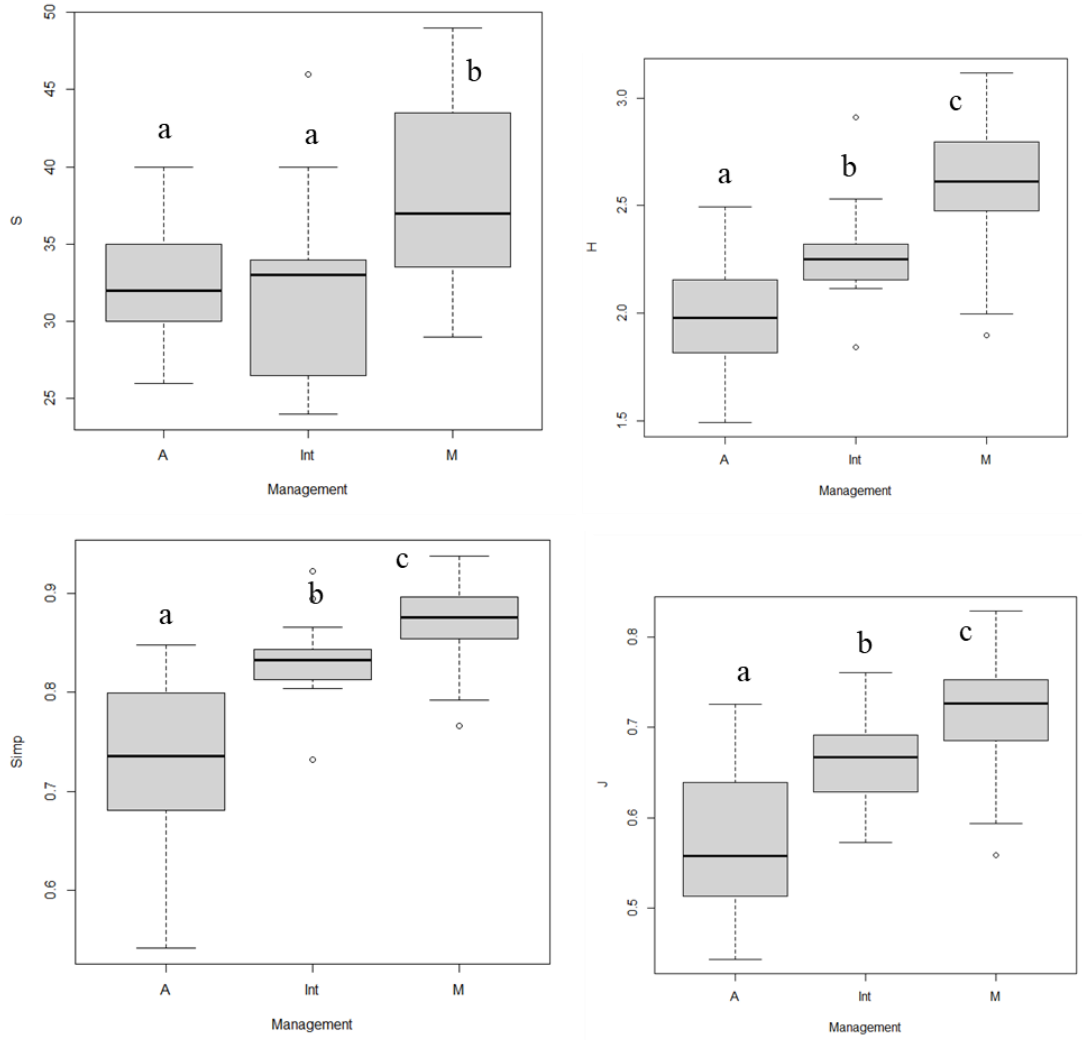


Table 3 - Mean, Standard deviation (SD), median of trait attributes and significance values (P) of the pairwise comparison between abandonment (A), intermediate disturbance (I), and mowing (M). Significant P values (after Holm correction) are in bold.

Trait	Trait attribute	Label	Mean			SD			Median			P		
			A	I	M	A	I	M	A	I	M	A vs I	M vs I	M vs A
Horizontal architecture	None	ha_n	0.047	0.070	0.105	0.023	0.074	0.051	0.038	0.055	0.098	0.178	0.112	**0.001**
	Caespitose	ha_c	0.744	0.718	0.634	0.079	0.090	0.127	0.730	0.711	0.622	0.381	0.041	**0.002**
	Pleiocorm	ha_p	0.137	0.176	0.212	0.051	0.079	0.073	0.136	0.161	0.203	0.071	0.169	**0.001**
	Reptant	ha_re	0.609	0.316	0.322	0.098	0.136	0.057	0.603	0.303	0.314	**0.001**	0.880	**0.001**
Vertical architecture	Sedge	va_se	0.010	0.052	0.060	0.020	0.043	0.036	0.000	0.058	0.056	**0.001**	0.563	**0.001**
	Grass	va_gr	0.730	0.666	0.564	0.083	0.095	0.137	0.724	0.679	0.541	**0.040**	**0.013**	**0.001**
	Hemirosulate upright forb	va_huf	0.113	0.177	0.253	0.047	0.085	0.087	0.111	0.163	0.261	**0.006**	**0.013**	**0.001**
	Erosulate upright forb	va_euf	0.123	0.083	0.073	0.061	0.081	0.037	0.118	0.055	0.068	0.092	0.659	**0.003**
	Prostrate forb	va_pf	0.001	0.003	0.011	0.001	0.003	0.010	0.000	0.004	0.006	**0.002**	**0.014**	**0.001**
Types of vegetative propagation	Only sexual reproduction	vp.n	0.131	0.282	0.162	0.061	0.129	0.056	0.132	0.262	0.158	**0.001**	**0.001**	0.109
	Tuber	vp_tu	0.025	0.019	0.057	0.027	0.019	0.030	0.013	0.009	0.060	0.501	**0.001**	**0.001**
	Bulb	vp_b	0.001	0.001	0.005	0.001	0.001	0.007	0.000	0.000	0.003	0.505	**0.003**	**0.001**
	Runner	vp_r	0.650	0.283	0.284	0.068	0.121	0.067	0.645	0.271	0.287	**0.001**	0.962	**0.001**
Leaf persistence	Persistent green leaves	lp_p	0.322	0.525	0.472	0.069	0.125	0.130	0.324	0.513	0.519	**0.001**	0.226	**0.001**
	Summer green leaves	lp_su	0.677	0.473	0.516	0.068	0.125	0.128	0.676	0.487	0.476	**0.001**	0.291	**0.001**
	Spring green leaves	lp_sp	0.000	0.001	0.005	0.000	0.001	0.007	0.000	0.000	0.003	0.189	**0.003**	**0.001**
	Overwintering green leaves	lp_o	0.001	0.001	0.003	0.001	0.002	0.006	0.000	0.000	0.002	0.988	0.083	**0.017**
Leaf anatomy	Succulent	la_s	0.000	0.005	0.002	0.000	0.008	0.003	0.000	0.004	0.000	**0.001**	0.202	**0.003**
	Mesomorphic	la_m	0.243	0.288	0.334	0.066	0.093	0.088	0.248	0.271	0.307	0.108	0.146	**0.001**
	Scleromorphic-mesomorphic	la_sm	0.634	0.574	0.562	0.117	0.099	0.086	0.638	0.592	0.575	0.141	0.698	0.028
Plant vegetative height	<20cm	h_a	0.094	0.061	0.063	0.061	0.080	0.042	0.081	0.026	0.054	0.173	0.942	0.040
	21-40cm	h_b	0.269	0.339	0.333	0.069	0.129	0.074	0.258	0.295	0.309	0.043	0.940	**0.007**
	41-60cm	h_c	0.626	0.591	0.566	0.102	0.150	0.093	0.635	0.644	0.555	0.414	0.530	0.059
	80-100cm	h_e	0.004	0.005	0.030	0.010	0.011	0.032	0.000	0.001	0.027	0.894	**0.005**	**0.001**
Life span	annual	ls_a	0.000	0.003	0.009	0.002	0.003	0.011	0.000	0.003	0.005	0.004	**0.034**	**0.001**
	biennial	ls_b	0.002	0.004	0.001	0.004	0.008	0.002	0.000	0.003	0.000	0.318	0.082	0.964
	perennial	ls_pe	0.998	0.993	0.990	0.005	0.009	0.012	1.000	0.995	0.993	0.031	0.401	**0.003**
Storage organs	absent	so_n	0.097	0.153	0.114	0.074	0.093	0.060	0.081	0.139	0.102	0.058	0.142	0.417
	rhizomes	so_rh	0.748	0.683	0.711	0.094	0.138	0.069	0.759	0.728	0.720	0.097	0.464	0.158
	bulb	so_b	0.001	0.001	0.006	0.001	0.001	0.007	0.000	0.000	0.003	0.795	**0.003**	**0.001**
	tuber	so_t	0.001	0.000	0.005	0.001	0.001	0.007	0.000	0.000	0.002	0.860	**0.013**	**0.002**
	tap root	so_tr	0.170	0.185	0.235	0.062	0.081	0.078	0.172	0.166	0.228	0.551	0.080	**0.007**
Palatability	unpalatable	pa_a	0.706	0.421	0.556	0.081	0.127	0.102	0.697	0.425	0.570	0.001	**0.002**	**0.001**
	palatable	pa_b	0.294	0.578	0.443	0.081	0.128	0.102	0.303	0.575	0.430	0.001	**0.001**	**0.001**

Table 4 - Mean, Standard deviation (SD), median of α-functional diversity (residuals), and significance values (P) of the pairwise comparison between abandonment (A), intermediate disturbance (I), and mowing (M). Significant P values (after Holm correction) are in bold.

α -functional diversity (residuals)	Mean			SD			Median			P		
	A	I	M	A	I	M	A	I	M	A vs I	M vs I	M vs A
Life span	-0.000081	0.000108	0.000003	0.000187	0.000350	0.000476	-0.000147	0.000060	-0.000111	0.048	0.956	0.188
Types of vegetative propagation	-0.000010	0.000014	-0.000033	0.000801	0.000693	0.000444	0.000061	-0.000053	-0.000026	0.926	0.672	0.801
Storage organs	-0.000025	0.000034	0.000001	0.001175	0.001103	0.000888	0.000191	-0.000167	-0.000263	0.881	0.992	0.774
Leaf persistence	0.000080	-0.000107	0.000030	0.000535	0.000483	0.000805	0.000082	0.000063	0.000232	0.293	0.764	0.613
Leaf anatomy	0.000126	-0.000168	-0.000054	0.000877	0.000709	0.000367	0.000157	-0.000347	-0.000091	0.296	0.453	0.057
Plant vegetative height	0.000400	-0.000533	0.000038	0.000571	0.000886	0.000664	0.000322	-0.000630	-0.000031	0.026	0.417	0.740
Vertical architecture	0.000009	-0.000012	0.000060	0.000548	0.000587	0.000894	0.000091	0.000023	0.000301	0.913	0.622	0.770
Horizontal architecture	0.000197	-0.000263	0.000128	0.000513	0.000657	0.001136	0.000228	-0.000218	0.000097	**0.001**	0.725	0.027
Palatability	-0.000257	0.000342	0.000147	0.002047	0.001584	0.001399	0.000049	-0.000190	0.000283	0.354	0.471	0.087

ii) How do species and functional composition and diversity change over time in mown and abandoned meadows?

In mown meadows we found a significant change in light intensity EIV, which increased from 2008 to 2021 (Figure 6). Forty percent of species highlighted by Indicator species analysis in 2008 belonged to *Festuco-Brometea*, *Ononidetea*, and *Seslerietea*, 30% to *Molinio-Arrhenatheretea* and *Nardetea*, and 5% to *Quercetea pubescentis*, while 57% species highlighted in 2021 belonged to *Festuco-Brometea* and *Seslerietea*, 27% to *Molinio-Arrhenatheretea* and *Nardetea*, and 5% to *Trifolio-Geranietea* (Table 5). Among the taxonomic diversity indices, species richness differed significantly between the two years, increasing in 2021 (Figure 7). Biennial, less than 20 cm-tall, with runners, reptant species and erosulate upright forbs underwent a reduction, whereas caespitose and sedge habit, fragmentation and roots with adventitious buds as vegetative propagation, and spring green leaves increased (Table 6). Instead, we did not detect any significant difference in functional diversity (Table 7).

In abandoned meadows we did not find any significant change in EIVs between 2008 and 2021 (Figure 8). Twentynine percent of species highlighted by Indicator species analysis in 2008 belonged to *Festuco-Brometea* and *Seslerietea*, 50% to *Molinio-Arrhenatheretea* and *Nardetea*, while 67% of species highlighted in 2021 belonged to *Festuco-Brometea* and *Seslerietea*, and 25% to *Molinio-Arrhenatheretea* and *Nardetea* (Table 8). Species richness and Shannon-Wiener index differed significantly, reducing their values from 2008 to 2021 (Figure 9).

Several traits' CMWs changed significantly: rosulate, <20 cm, only sexual reproduction, absence of storage organs, species with persistent green, succulent, and scleromorphic leaves increased; perennial species, hemirosulate upright forbs, prostrate forbs, 21-40 cm and 60-80 cm-tall, without horizontal architecture, tubers, bulbs, and rhizomes (both vegetative propagation and storage organs), spring, summer, and overwintering green leaves, succulent-hygromorphic, mesomorphic, and mesomorphic-hygromorphic leaves decreased (Table 9). As far as functional diversity is concerned, we detected the only difference in storage organs (Table 10).

Figure 6 - Boxplots of Ellenberg indicator values (EIVs) under mowing in 2008 and 2021. Different letters indicate statistically significant difference.

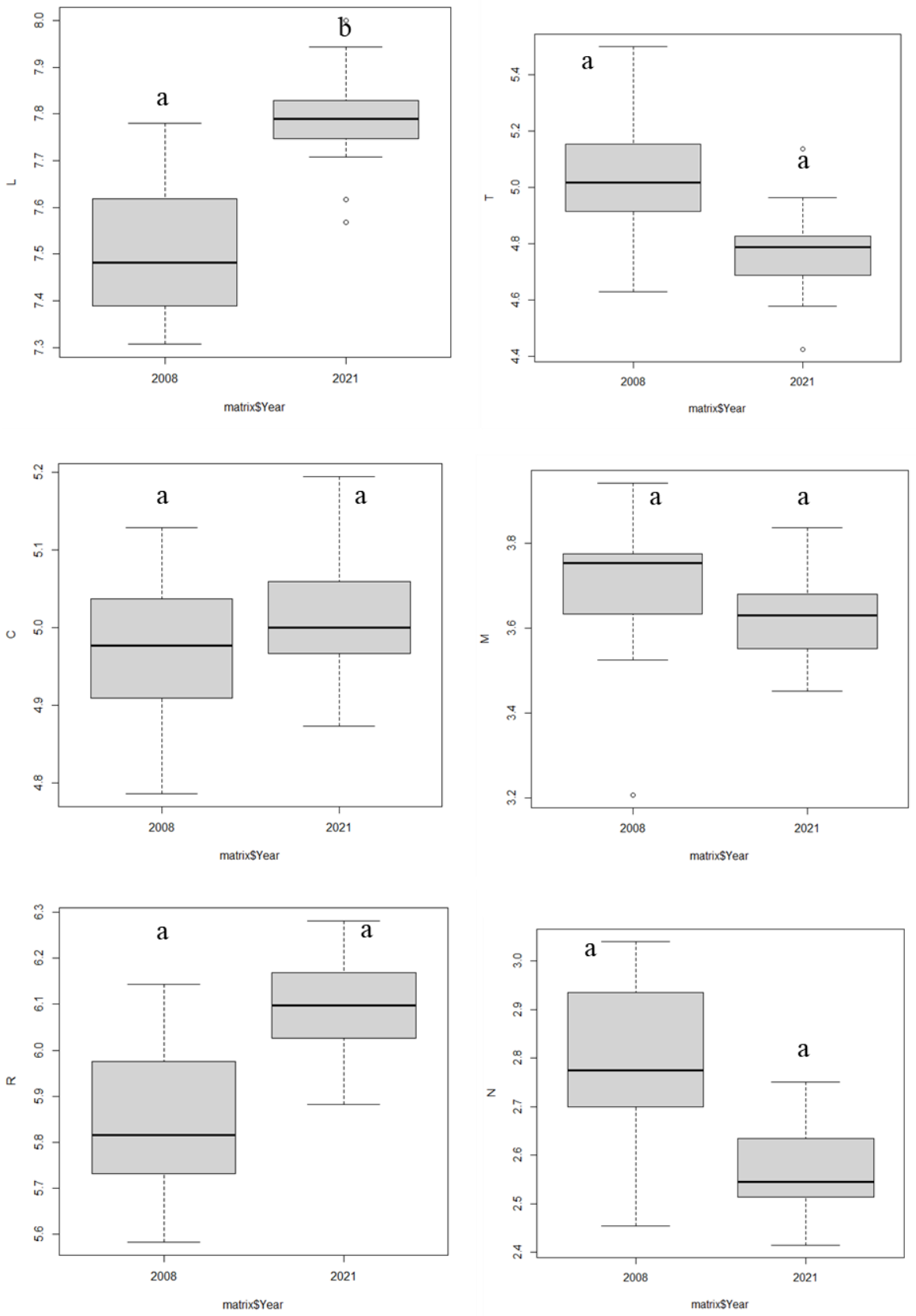


Table 5 - Indicator species of the relevé groups of 2008 and 2021 in mown meadows as performed by Indicator Species Analysis. Coenological group, relative abundance (A), relative frequency (B), square root of Indicator Value (Stat), and significance value (P) are reported for each species whose A was ≥ 0.6 and B ≥ 0.25.

Year	Species name	Coenological group	A	B	Stat	P	
2008	Dianthus hyssopifolius L.	FES	0.979	1.000	0.990	0.001	***
	Cerastium arvense L. subsp. suffruticosum (L.) Ces.	ONO	0.918	1.000	0.958	0.001	***
	Nardus stricta L.	NAR	1.000	0.864	0.929	0.001	***
	Campanula glomerata L.	FES	0.948	0.909	0.928	0.001	***
	Agrostis capillaris L. subsp. capillaris	MOL/NAR	0.993	0.818	0.901	0.001	***
	Campanula micrantha Bertol.	-	1.000	0.727	0.853	0.001	***
	Cynanchica pyrenaica (L.) P.Caputo & Del Guacchio subsp. cynanchica (L.) P.Caputo & Del Guacchio	FES	0.932	0.682	0.797	0.001	***
	Anthoxanthum odoratum L.	BUL	0.737	0.773	0.754	0.038	*
	Trifolium ochroleucon Huds.	FES	1.000	0.546	0.739	0.001	***
	Luzula campestris (L.) DC. Subsp. campestris	FES	0.637	0.818	0.722	0.025	*
	Cynosurus cristatus L.	MOL	1.000	0.500	0.707	0.001	***
	Galium corrudifolium Vill.	FES	0.761	0.636	0.696	0.006	**
	Helleborus viridis L. subsp. bocconei (Ten.) Peruzzi	PUB	1.000	0.455	0.674	0.001	***
	Leucanthemum vulgare (Vaill.) Lam. subsp. vulgare	MOL	1.000	0.455	0.674	0.001	***
	Rhinanthus minor L.	MOL	0.820	0.546	0.669	0.016	*
	Achillea millefolium L. subsp. millefolium	MOL	1.000	0.364	0.603	0.002	**
	Festuca laevigata Gaudin subsp. laevigata	SES	1.000	0.273	0.522	0.021	*
	Luzula sylvatica (Huds.) Gaudin subsp. sieberi (Tausch) K.Richt.	-	1.000	0.227	0.477	0.042	*
2021	Senecio scopolii Hoppe & Hornsch. subsp. scopolii	-	0.958	0.900	0.928	0.001	***
	Biscutella laevigata L. subsp. laevigata	SES	1.000	0.850	0.922	0.001	***
	Lotus corniculatus L. subsp. corniculatus	MOL	0.823	1.000	0.907	0.001	***
	Hippocrepis comosa L. subsp. comosa	FES	0.958	0.850	0.902	0.001	***
	Narcissus poëticus L.	MOL	0.854	0.950	0.901	0.001	***
	Centaurea triumfettii All.	GER	0.851	0.950	0.899	0.001	***
	Pilosella cymosa (L.) F.W.Schultz & Sch.Bip.	FES	0.894	0.900	0.897	0.001	***
	Festuca circummediterranea Patzke	FES	0.800	1.000	0.895	0.001	***
	Plantago media L. subsp. media	MOL	0.940	0.850	0.894	0.001	***
	Briza media L.	FES	0.872	0.900	0.886	0.001	***
	Rumex acetosa L. subsp. acetosa	MOL	0.923	0.850	0.886	0.001	***
	Trifolium pratense L. subsp. pratense	MOL	0.977	0.800	0.884	0.001	***
	Gentiana lutea L. subsp. lutea	SES	0.809	0.950	0.877	0.002	**
	Bromopsis erecta (Huds.) Fourr.	FES	0.767	1.000	0.876	0.001	***
	Carex macrolepis DC.	SES	0.842	0.900	0.870	0.001	***
	Brachypodium genuense (DC.) Roem. & Schult.	FES	0.717	1.000	0.847	0.001	***
	Bellardiochloa variegata (Lam.) Kerguélen subsp. variegata	TRI	0.940	0.750	0.840	0.007	**
	Tragopogon pratensis L.	FES	0.849	0.800	0.824	0.001	***
	Knautia calycina (C.Presl) Guss.	FES	0.737	0.900	0.815	0.041	*
	Armeria gracilis Ten. subsp. gracilis	FES	0.819	0.800	0.809	0.004	**
	Festuca rubra L. subsp. commutata (Gaudin) Markgr.-Dann.	MOL/NAR	1.000	0.650	0.806	0.001	***
	Luzula multiflora (Ehrh.) Lej. subsp. multiflora	NAR	1.000	0.650	0.806	0.001	***

Rhinanthus wettsteinii (Sterneck) Soó	-	0.897	0.700	0.792	0.007	**
Galium anisophyllon Vill.	SES	0.765	0.800	0.782	0.003	**
Leucanthemum adustum (W.D.J.Koch) Gremli subsp. adustum	SES	1.000	0.600	0.775	0.001	***
Thesium linophyllon L.	FES	0.746	0.650	0.696	0.004	**
Pedicularis tuberosa L.	TRI	1.000	0.450	0.671	0.001	***
Gymnadenia conopsea (L.) R. Br.	MOL	0.846	0.500	0.650	0.042	*
Linum catharticum L. subsp. catharticum	FES	0.908	0.450	0.639	0.004	**
Aster alpinus L. subsp. alpinus	SES	1.000	0.400	0.632	0.002	**
Cruciata pedemontana (Bellardi) Ehrend.	FES	1.000	0.400	0.632	0.003	**
Poa molinerii Balb.	FES/SES	1.000	0.400	0.632	0.002	**
Siler montanum Crantz subsp. stabianum	GER	1.000	0.350	0.592	0.005	**
Euphrasia stricta D.Wolff ex J.F.Lehm.	FES	1.000	0.300	0.548	0.013	*
Onobrychis viciifolia Scop.	MOL	1.000	0.300	0.548	0.009	**
Ajuga reptans L.	MOL	1.000	0.250	0.500	0.022	*
Veronica orsiniana Ten.	FES	1.000	0.250	0.500	0.020	*

*** $P < 0.001$; ** $P < 0.01$; * $P \leq 0.05$

Figure 7- Boxplots of diversity indices species richness (S), Shannon-Wiener index (H), Simpson (G) and Pielou's evenness (J) under mowing in 2008 and 2021. Different letters indicate statistically significant difference.

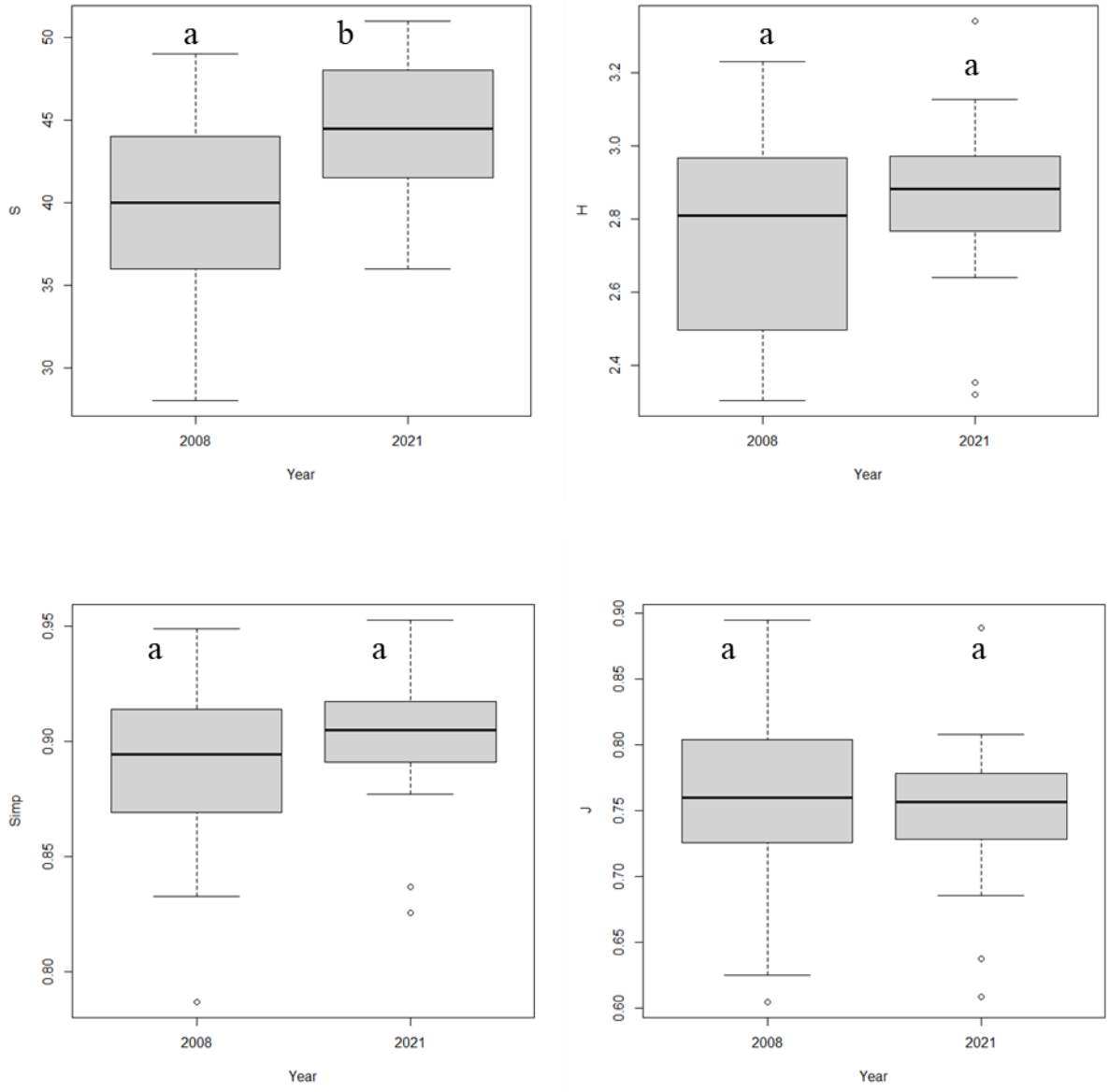


Table 6 – Descriptive statistics of trait attributes, and significance values (P) of the comparison between meadows in 2008 and 2021 under mowing.

Trait	Trait attribute	Label	Mean		SD		Min		1st quartile		Median		3rd quartile		Max		P	
			2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021		
Horizontal architecture	None	ha_n	0.122	0.126	0.060	0.054	0.043	0.038	0.076	0.097	0.105	0.126	0.152	0.159	0.260	0.251	0.828	
	Caespitose	ha_c	0.466	0.572	0.156	0.122	0.153	0.354	0.374	0.488	0.509	0.558	0.549	0.685	0.726	0.815	0.028	*
	Pleiocorm	ha_p	0.266	0.243	0.107	0.058	0.110	0.138	0.189	0.206	0.229	0.244	0.380	0.283	0.458	0.349	0.412	
	Reptant	ha_re	0.395	0.301	0.142	0.059	0.138	0.231	0.268	0.255	0.446	0.284	0.481	0.327	0.638	0.438	0.004	**
	Rosulate	ha_ro	0.024	0.042	0.017	0.055	0.000	0.002	0.015	0.012	0.019	0.023	0.033	0.033	0.080	0.173	0.178	
Vertical architecture	Sedge	va_se	0.032	0.055	0.036	0.034	0.000	0.000	0.004	0.017	0.020	0.065	0.054	0.072	0.133	0.124	0.039	*
	Grass	va_gr	0.432	0.503	0.163	0.128	0.093	0.215	0.333	0.431	0.476	0.485	0.543	0.597	0.682	0.735	0.135	
	Rosette forb	va_rf	0.026	0.049	0.018	0.059	0.004	0.008	0.016	0.019	0.022	0.025	0.033	0.033	0.090	0.185	0.106	
	Hemirosulate upright forb	va_huf	0.252	0.281	0.109	0.079	0.073	0.137	0.163	0.234	0.222	0.282	0.337	0.335	0.450	0.433	0.320	
	Erosulate upright forb	va_euf	0.230	0.099	0.094	0.041	0.086	0.038	0.154	0.061	0.219	0.098	0.289	0.118	0.438	0.183	0.001	***
	Prostrate forb	va_pf	0.028	0.014	0.057	0.014	0.000	0.000	0.000	0.003	0.004	0.013	0.033	0.017	0.254	0.064	0.401	
Types of vegetative propagation	Only sexual reproduction	vp.n	0.211	0.188	0.087	0.057	0.091	0.081	0.142	0.143	0.210	0.193	0.264	0.234	0.397	0.289	0.324	
	Tuber	vp_tu	0.046	0.068	0.052	0.028	0.004	0.026	0.011	0.043	0.029	0.067	0.063	0.086	0.191	0.127	0.130	
	Roots with adventitious buds	vp_rab	0.000	0.007	0.000	0.007	0.000	0.000	0.000	0.002	0.000	0.003	0.000	0.014	0.000	0.020	0.001	***
	Bulb	vp_b	0.005	0.008	0.006	0.007	0.000	0.000	0.000	0.002	0.004	0.003	0.008	0.014	0.020	0.020	0.148	
	Runner	vp_r	0.358	0.261	0.146	0.058	0.120	0.148	0.220	0.231	0.389	0.247	0.473	0.285	0.596	0.413	0.009	**
	Fragmentation	vp_fr	0.000	0.003	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.043	*
	Rhizome	vp_rh	0.694	0.669	0.143	0.061	0.314	0.554	0.608	0.625	0.726	0.682	0.802	0.705	0.885	0.761	0.469	
Leaf persistence	Persistent green leaves	lp_p	0.487	0.463	0.110	0.123	0.280	0.207	0.417	0.335	0.491	0.530	0.557	0.545	0.711	0.630	0.508	
	Summer green leaves	lp_su	0.505	0.519	0.109	0.118	0.283	0.363	0.437	0.437	0.502	0.458	0.575	0.614	0.712	0.773	0.713	
	Spring green leaves	lp_sp	0.004	0.008	0.004	0.007	0.000	0.000	0.000	0.002	0.004	0.004	0.006	0.014	0.011	0.020	0.011	*
	Overwintering green leaves	lp_o	0.004	0.004	0.004	0.005	0.000	0.000	0.000	0.002	0.004	0.002	0.007	0.004	0.017	0.016	0.838	
Leaf anatomy	Succulent	la_s	0.005	0.004	0.004	0.004	0.000	0.000	0.001	0.002	0.006	0.003	0.008	0.004	0.018	0.014	0.236	
	Mesomorphic	la_m	0.318	0.375	0.117	0.069	0.137	0.241	0.209	0.336	0.325	0.367	0.425	0.436	0.506	0.489	0.078	.
	Mesomorphic-hygromorphic	la_mh	0.012	0.002	0.032	0.002	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.002	0.133	0.006	0.176	
	Scleromorphic	la_scl	0.111	0.104	0.084	0.042	0.024	0.035	0.052	0.081	0.088	0.106	0.125	0.137	0.305	0.182	0.764	
	Scleromorphic-mesomorphic	la_sm	0.545	0.504	0.130	0.062	0.299	0.403	0.465	0.458	0.523	0.497	0.620	0.543	0.828	0.657	0.215	
	<20cm	h_a	0.169	0.090	0.094	0.043	0.049	0.025	0.095	0.067	0.158	0.080	0.217	0.104	0.398	0.200	0.001	***

Plant vegetative height	21-40cm	h_b	0.328	0.377	0.118	0.064	0.146	0.241	0.233	0.344	0.312	0.368	0.403	0.420	0.527	0.485	0.115	
	41-60cm	h_c	0.447	0.493	0.173	0.078	0.184	0.346	0.297	0.444	0.477	0.484	0.582	0.525	0.764	0.657	0.305	
	60-80cm	h_d	0.025	0.009	0.045	0.018	0.000	0.000	0.000	0.000	0.008	0.001	0.025	0.006	0.196	0.060	0.158	
	80-100cm	h_e	0.030	0.031	0.049	0.036	0.000	0.002	0.004	0.005	0.007	0.017	0.043	0.029	0.208	0.122	0.990	
Life span	annual	ls_a	0.022	0.015	0.038	0.015	0.000	0.002	0.000	0.005	0.006	0.013	0.024	0.018	0.147	0.066	0.483	
	biennial	ls_b	0.007	0.002	0.010	0.001	0.000	0.000	0.000	0.000	0.004	0.002	0.007	0.002	0.047	0.004	0.005	**
	perennial	ls_pe	0.971	0.983	0.042	0.014	0.848	0.934	0.960	0.980	0.989	0.985	1.000	0.993	1.000	0.998	0.260	
Storage organs	absent	so_n	0.139	0.129	0.088	0.057	0.023	0.038	0.069	0.100	0.139	0.120	0.188	0.157	0.336	0.282	0.692	
	rhizomes	so_rh	0.626	0.659	0.145	0.064	0.254	0.537	0.585	0.612	0.666	0.677	0.700	0.700	0.845	0.746	0.342	
	bulb	so_b	0.006	0.009	0.006	0.007	0.000	0.002	0.000	0.002	0.004	0.004	0.008	0.015	0.020	0.024	0.115	
	tuber	so_t	0.019	0.008	0.043	0.009	0.000	0.000	0.004	0.002	0.007	0.004	0.010	0.007	0.179	0.029	0.363	
	tap root	so_tr	0.301	0.272	0.135	0.065	0.131	0.153	0.210	0.229	0.270	0.279	0.372	0.303	0.678	0.386	0.408	
Palatability	unpalatable	pa_a	0.577	0.559	0.099	0.098	0.397	0.397	0.506	0.477	0.581	0.552	0.636	0.635	0.766	0.735	0.558	
	palatable	pa_b	0.423	0.439	0.099	0.099	0.234	0.263	0.364	0.361	0.419	0.448	0.494	0.522	0.603	0.603	0.627	

*** $P < 0.001$; ** $P < 0.01$; * $P \leq 0.05$

Table 7 – Descriptive statistics of α-functional diversity (residuals), and significance values (P) of the comparison between meadows in 2008 and 2021 under mowing.

α-functional diversity residuals	Mean		SD		Min		1st quartile		Median		3rd quartile		Max		P
	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	
Life span	0.000041	-0.000045	0.000836	0.000624	-0.000592	-0.000741	-0.000427	-0.000438	-0.000274	-0.000107	0.000158	0.000072	0.002375	0.002170	0.386
Types of vegetative propagation	-0.000060	0.000066	0.000358	0.000456	-0.000963	-0.000606	-0.000319	-0.000286	-0.000015	0.000022	0.000231	0.000417	0.000435	0.000801	0.320
Storage organs	-0.000027	0.000029	0.000598	0.000753	-0.001654	-0.001129	-0.000206	-0.000640	-0.000011	0.000117	0.000251	0.000712	0.001004	0.001118	0.789
Leaf persistence	0.000009	-0.000010	0.000312	0.000730	-0.000605	-0.002284	-0.000137	-0.000248	-0.000008	0.000163	0.000174	0.000366	0.000695	0.001261	0.916
Leaf anatomy	-0.000038	0.000042	0.000502	0.000395	-0.001369	-0.000633	-0.000072	-0.000350	0.000067	0.000178	0.000201	0.000277	0.000750	0.000731	0.569
Plant vegetative height	0.000042	-0.000046	0.000423	0.000644	-0.000987	-0.000909	-0.000108	-0.000574	0.000027	-0.000164	0.000267	0.000382	0.000986	0.001405	0.818
Vertical architecture	0.000020	-0.000022	0.000217	0.000808	-0.000406	-0.001729	-0.000053	-0.000520	0.000043	0.000197	0.000156	0.000516	0.000392	0.001162	0.817
Horizontal architecture	0.000028	-0.000030	0.000287	0.001142	-0.000676	-0.001914	-0.000133	-0.000846	-0.000015	0.000025	0.000199	0.000378	0.000647	0.002233	0.600
Palatability	0.000052	-0.000057	0.000633	0.001341	-0.001311	-0.002720	-0.000266	-0.000929	0.000126	0.000334	0.000308	0.000767	0.001504	0.002176	0.734

Figure 8- Boxplots of Ellenberg indicator values (EIVs) under abandonment in 2008 and 2021. Equal letters indicate no significant difference.

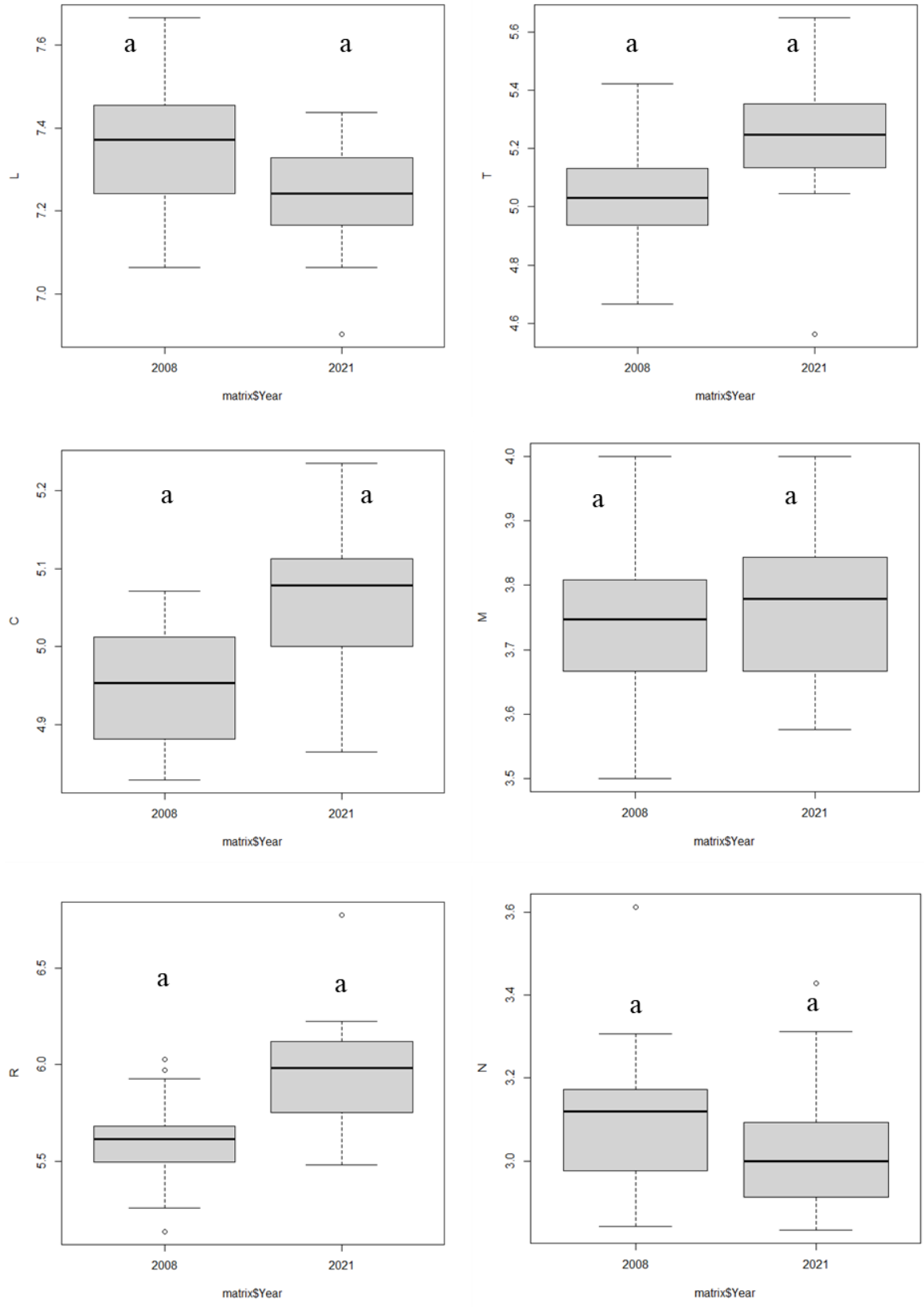


Table 8 - Indicator species of the relevé groups of 2008 and 2021 in abandoned meadows as performed by Indicator Species Analysis. Coenological group, relative abundance (A), relative frequency (B), square root of Indicator Value (Stat), and significance value (P) are reported for each species whose A was ≥ 0.6 and B ≥ 0.25.

Year	Species name	Coenological group	A	B	Stat	P	
2008	Agrostis capillaris L. subsp. capillaris	MOL/NAR	1.000	1.000	1.000	0.001	***
	Senecio scopolii Hoppe & Hornsch. subsp. floccosus (Bertol.) Greuter	NAR	1.000	1.000	1.000	0.001	***
	Rumex acetosa L. subsp. acetosa	MOL	0.902	1.000	0.950	0.001	***
	Galium anisophyllon Vill.	SES	0.972	0.917	0.944	0.001	***
	Narcissus poëticus L.	MOL	0.985	0.833	0.906	0.001	***
	Ranunculus pollinensis (N. Terracc.) Chiov.	NAR	0.971	0.833	0.899	0.001	***
	Lotus corniculatus L. subsp. corniculatus	MOL	0.752	1.000	0.867	0.002	**
	Dactylorhiza sambucina (L.) Soó	FES	0.816	0.917	0.865	0.001	***
	Cerastium arvense L. subsp. suffruticosum (L.) Ces.	ONO	0.796	0.917	0.854	0.002	**
	Campanula micrantha Bertol.	-	0.706	1.000	0.840	0.005	**
	Gymnadenia conopsea (L.) R. Br.	MOL	0.938	0.750	0.839	0.001	***
	Alchemilla glaucescens Wallr.	NAR	0.830	0.833	0.832	0.015	*
	Filipendula vulgaris Moench	FES	0.769	0.833	0.801	0.022	*
	Crocus neglectus Peruzzi et Carta	-	0.956	0.667	0.798	0.001	***
	Carex macrolepis DC.	SES	0.638	0.917	0.765	0.046	*
	Ajuga reptans L.	MOL	1.000	0.583	0.764	0.001	***
	Pedicularis tuberosa L.	TRI	1.000	0.583	0.764	0.001	***
	Luzula sylvatica (Huds.) Gaudin subsp. sieberi (Tausch) K.Richt.	-	0.952	0.583	0.745	0.002	**
	Galium verum L. subsp. verum	FES	0.823	0.667	0.741	0.030	*
	Veronica officinalis L.	NAR	1.000	0.500	0.707	0.001	***
	Dactylis glomerata subsp. glomerata	MOL	0.948	0.500	0.689	0.002	**
	Viola eugeniae Parl. subsp. eugeniae	-	1.000	0.417	0.645	0.002	**
	Genista tinctoria L.	NAR	0.870	0.333	0.538	0.029	*
	Saxifraga bulbifera L.	FES	0.870	0.333	0.538	0.028	*
2021	Luzula multiflora (Ehrh.) Lej. subsp. multiflora	NAR	0.970	0.900	0.934	0.001	***
	Briza media L.	FES	0.872	1.000	0.934	0.001	***
	Cruciata glabra (L.) C.Bauhin ex Opiz	FES	0.850	0.850	0.850	0.004	**
	Trifolium montanum L. subsp. rupestre (Ten.) Nyman	FES	0.668	0.950	0.797	0.041	*
	Thesium linophyllon L.	FES	0.932	0.650	0.778	0.005	**
	Carlina acaulis L. subsp. caulescens (Lam.) Schubl. & G. Martens	SES	0.847	0.600	0.713	0.017	*
	Cynosurus cristatus L.	MOL	0.839	0.550	0.679	0.029	*
	Senecio doronicum (L.) L. subsp. doronicum	SES	1.000	0.450	0.671	0.001	***
	Cynanchica pyrenaica (L.) P.Caputo & Del Guacchio subsp. cynanchica (L.) P.Caputo & Del Guacchio	FES	1.000	0.400	0.632	0.001	***
	Senecio scopolii Hoppe & Hornsch. subsp. scopolii	-	1.000	0.400	0.632	0.002	**
	Nardus stricta L.	NAR	0.807	0.400	0.568	0.035	*
	Helictochloa praetutiana (Parl. ex Arcang.) Bartolucci, F.Conti, Peruzzi & Banfi subsp. praetutiana	FES/SES	0.878	0.350	0.554	0.031	*

*** $P < 0.001$; ** $P < 0.01$; * $P \leq 0.05$

Figure 9 - Boxplots of diversity indices species richness (S), Shannon-Wiener index (H), Simpson (G) and Pielou's evenness (J) under abandonment in 2008 and 2021. Different letters indicate statistically significant difference.

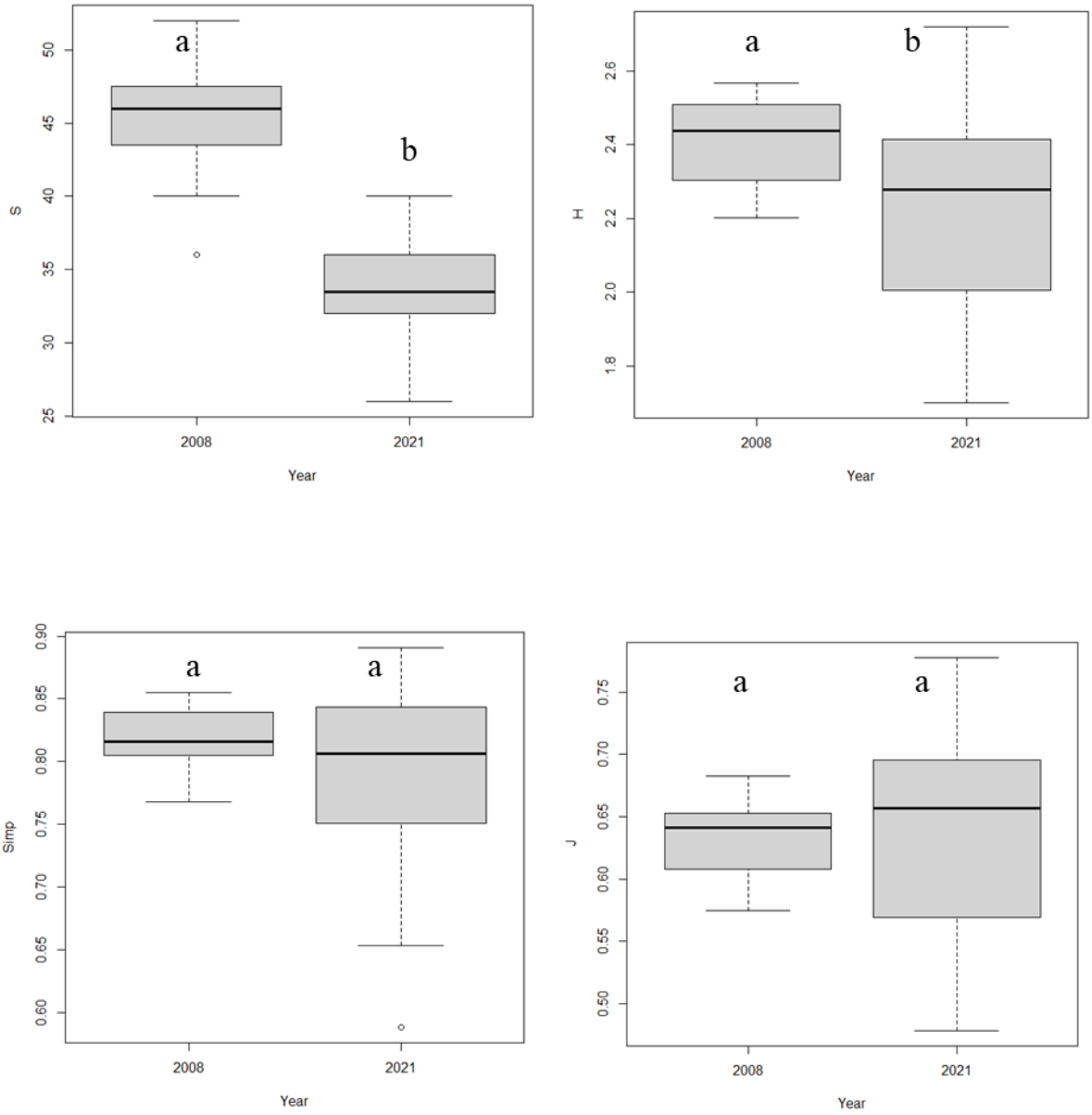


Table 9 – Descriptive statistics of trait attributes, and significance values (P) of the comparison between meadows in 2008 and 2021 under abandonment.

Trait	Trait attribute	Label	Mean		SD		Min		1st quartile		Median		3rd quartile		Max		P	
			2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021	2008	2021		
Horizontal architecture	None	ha_n	0.166	0.059	0.038	0.018	0.091	0.032	0.139	0.048	0.169	0.057	0.202	0.064	0.212	0.104	**0.001**	***
	Caespitose	ha_c	0.663	0.695	0.069	0.076	0.535	0.557	0.627	0.659	0.666	0.691	0.716	0.740	0.799	0.863	0.158	
	Pleiocorm	ha_p	0.126	0.154	0.048	0.059	0.042	0.049	0.098	0.108	0.124	0.151	0.164	0.213	0.208	0.249	0.086	
	Reptant	ha_re	0.612	0.573	0.060	0.112	0.485	0.366	0.579	0.482	0.600	0.587	0.662	0.676	0.740	0.739	0.150	
	Rosulate	ha_ro	0.012	0.032	0.009	0.034	0.004	0.000	0.006	0.009	0.009	0.022	0.014	0.030	0.042	0.102	**0.005**	**
	Climber	ha_cl	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.210	
	Shrub	ha_sh	0.001	0.000	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.013	0.004	0.309	
	Prostrate	ha_pr	0.000	0.001	0.001	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.016	0.738	
Vertical architecture	Sedge	va_se	0.023	0.015	0.028	0.029	0.000	0.000	0.003	0.000	0.010	0.001	0.037	0.009	0.084	0.095	0.342	
	Grass	va_gr	0.641	0.675	0.075	0.084	0.473	0.505	0.602	0.632	0.639	0.667	0.684	0.715	0.791	0.863	0.161	
	Rosette forb	va_rf	0.018	0.031	0.010	0.034	0.002	0.000	0.009	0.007	0.019	0.022	0.021	0.030	0.040	0.102	0.077	.
	Hemirosulate upright forb	va_huf	0.204	0.140	0.044	0.047	0.120	0.052	0.175	0.103	0.208	0.144	0.233	0.176	0.305	0.227	**0.001**	***
	Erosulate forb	va_euf	0.111	0.137	0.042	0.054	0.042	0.025	0.083	0.106	0.104	0.140	0.142	0.160	0.184	0.230	0.087	.
	Climber	va_cl	0.001	0.000	0.001	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.192	
	Shrub	va_sh	0.001	0.000	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.013	0.004	0.323	
	Prostrate forb	va_pf	0.002	0.001	0.001	0.002	0.000	0.000	0.002	0.000	0.002	0.000	0.003	0.003	0.005	0.004	**0.033**	*
	Upright forb	va_uf	0.315	0.277	0.072	0.075	0.167	0.114	0.266	0.232	0.310	0.275	0.358	0.320	0.459	0.412	0.102	
Types of vegetative propagation	Only sexual reproduction	vp.n	0.097	0.149	0.037	0.072	0.052	0.036	0.064	0.106	0.091	0.147	0.119	0.181	0.178	0.307	**0.006**	**
	Tuber	vp_tu	0.054	0.030	0.026	0.031	0.019	0.000	0.025	0.013	0.063	0.018	0.072	0.043	0.101	0.104	**0.012**	*
	Bulb	vp_b	0.008	0.002	0.006	0.002	0.000	0.000	0.002	0.000	0.008	0.002	0.013	0.003	0.017	0.007	**0.001**	***
	Runner	vp_r	0.613	0.612	0.058	0.081	0.483	0.428	0.579	0.569	0.601	0.593	0.661	0.685	0.740	0.723	0.958	
	Rhizome	vp_rh	0.826	0.733	0.050	0.099	0.708	0.527	0.800	0.680	0.835	0.729	0.861	0.791	0.900	0.925	**0.002**	**
Leaf persistence	Persistent green leaves	lp_p	0.300	0.345	0.044	0.075	0.223	0.112	0.275	0.306	0.295	0.364	0.320	0.400	0.398	0.434	**0.011**	*
	Summer green leaves	lp_su	0.686	0.644	0.045	0.075	0.584	0.563	0.669	0.593	0.689	0.624	0.712	0.679	0.767	0.881	**0.022**	*
	Spring green leaves	lp_sp	0.011	0.001	0.007	0.002	0.002	0.000	0.005	0.000	0.013	0.000	0.015	0.002	0.028	0.004	**0.001**	***
	Overwintering green leaves	lp_o	0.003	0.001	0.003	0.002	0.000	0.000	0.002	0.000	0.002	0.000	0.002	0.003	0.014	0.004	**0.008**	**
	Succulent	la_s	0.001	0.001	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.002	0.004	**0.038**	*

	Succulent-hygromorphic	la_sh	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.002	**0.026**	*
	Mesomorphic	la_m	0.394	0.283	0.056	0.066	0.286	0.183	0.364	0.227	0.392	0.284	0.425	0.354	0.527	0.384	**0.001**	***
Leaf anatomy	Mesomorphic-hygromorphic	la_mh	0.004	0.001	0.004	0.002	0.000	0.000	0.002	0.000	0.003	0.000	0.005	0.002	0.016	0.007	**0.003**	**
	Scleromorphic	la_scl	0.036	0.124	0.027	0.091	0.005	0.013	0.018	0.040	0.024	0.111	0.046	0.202	0.091	0.261	**0.001**	***
	Scleromorphic-mesomorphic	la_sm	0.560	0.589	0.057	0.109	0.426	0.396	0.519	0.529	0.557	0.576	0.599	0.665	0.688	0.761	0.297	
	<20cm	h_a	0.048	0.107	0.024	0.047	0.017	0.018	0.032	0.083	0.042	0.110	0.057	0.140	0.104	0.173	**0.001**	***
	21-40cm	h_b	0.355	0.300	0.056	0.081	0.254	0.160	0.325	0.239	0.345	0.285	0.388	0.371	0.490	0.415	**0.016**	*
Plant vegetative height	41-60cm	h_c	0.566	0.578	0.046	0.105	0.449	0.415	0.552	0.480	0.568	0.568	0.594	0.635	0.635	0.766	0.646	
	60-80cm	h_d	0.024	0.007	0.029	0.008	0.000	0.000	0.000	0.000	0.007	0.003	0.057	0.014	0.074	0.022	**0.012**	*
	80-100cm	h_e	0.008	0.006	0.005	0.010	0.002	0.000	0.004	0.000	0.006	0.003	0.010	0.006	0.017	0.040	0.531	
	annual	ls_a	0.000	0.001	0.001	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.007	0.116	
Life span	biennial	ls_b	0.000	0.002	0.001	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.018	0.081	
	perennial	ls_pe	1.000	0.998	0.001	0.005	0.996	0.982	1.000	0.997	1.000	1.000	1.000	1.000	1.000	1.000	**0.032**	*
	absent	so_n	0.038	0.117	0.023	0.090	0.012	0.014	0.022	0.049	0.033	0.101	0.044	0.157	0.097	0.353	**0.001**	***
	rhizomes	so_rh	0.812	0.712	0.049	0.104	0.704	0.510	0.778	0.651	0.828	0.720	0.837	0.777	0.894	0.902	**0.001**	***
Storage organs	bulb	so_b	0.008	0.002	0.006	0.002	0.000	0.000	0.002	0.000	0.008	0.002	0.013	0.003	0.017	0.010	**0.001**	***
	tuber	so_t	0.008	0.002	0.006	0.002	0.000	0.000	0.004	0.000	0.005	0.002	0.008	0.003	0.022	0.006	**0.001**	***
	tap root	so_tr	0.169	0.192	0.049	0.067	0.081	0.088	0.131	0.135	0.166	0.197	0.205	0.249	0.274	0.303	0.193	
Palatability	unpalatable	pa_a	0.668	0.685	0.064	0.077	0.545	0.539	0.630	0.631	0.671	0.685	0.709	0.746	0.794	0.824	0.434	
	palatable	pa_b	0.332	0.314	0.065	0.077	0.204	0.176	0.291	0.254	0.329	0.315	0.370	0.367	0.455	0.461	0.442	

*** $P < 0.001$; ** $P < 0.01$; * $P \leq 0.05$

Table 10 – Descriptive statistics of α-functional diversity (residuals), and significance values (P) of the comparison between meadows in 2008 and 2021 under abandonment.

α-functional diversity residual	Mean		SD		Min		1st quartile		Median		3rd quartile		Max		P	
	2008	**2021**	**2008**	**2021**	**2008**	**2021**	**2008**	**2021**	**2008**	**2021**	**2008**	**2021**	**2008**	**2021**		
Life span	-0.000004	0.000004	0.000041	0.000106	-0.000045	-0.000077	-0.000027	-0.000049	-0.000018	-0.000031	0.000005	-0.000002	0.000140	0.000373	0.739	
Types of vegetative propagation	-0.000070	0.000084	0.000460	0.000378	-0.001005	-0.000529	-0.000497	-0.000134	-0.000001	0.000092	0.000207	0.000185	0.000715	0.000820	0.238	
Storage organs	-0.000186	0.000224	0.000670	0.000651	-0.001240	-0.000958	-0.000643	-0.000162	-0.000263	0.000083	0.000397	0.000726	0.000899	0.001378	**0.047**	*
Leaf persistence	-0.000067	0.000080	0.000444	0.000414	-0.000964	-0.001200	-0.000378	-0.000038	-0.000088	0.000137	0.000199	0.000266	0.000881	0.000807	0.265	
Leaf anatomy	-0.000084	0.000100	0.000267	0.000406	-0.000626	-0.000858	-0.000221	-0.000195	-0.000050	0.000142	0.000069	0.000466	0.000396	0.000720	0.079	
Plant vegetative height	-0.000046	0.000056	0.000246	0.000289	-0.000429	-0.000553	-0.000288	-0.000054	-0.000022	0.000043	0.000169	0.000209	0.000397	0.000558	0.446	
Vertical architecture	-0.000012	0.000015	0.000436	0.000364	-0.001043	-0.000872	-0.000381	-0.000187	0.000013	0.000070	0.000220	0.000209	0.000706	0.000628	0.824	
Horizontal architecture	0.000042	-0.000050	0.000439	0.000331	-0.000856	-0.000583	-0.000199	-0.000320	0.000149	-0.000008	0.000296	0.000115	0.000865	0.000804	0.213	
Palatability	0.000179	-0.000215	0.001159	0.001191	-0.002126	-0.002648	-0.000469	-0.001018	0.000234	-0.000168	0.000953	0.000708	0.002054	0.002057	0.274	

** $P < 0.01$; * $P \leq 0.05$

6.3.6 Discussion

How do species and functional composition and diversity change along long-term disturbance gradients?

No significant EIVs change was detected along the disturbance gradient, underlining that different disturbance intensities did not cause a dramatic change in environmental conditions within the community. From the coenological viewpoint, on the less disturbed end of the gradient, we found a higher occurrence of indicator species referred to *Fagetea sylvaticae* and *Trifolio-Geranietea* classes, characterized by higher abundance and frequency values. This may be interpreted as a step forward forest recovery, of which *Brachypodium*-dominated communities represent a dynamic stage (Endresz et al. 2005). This stage usually hosts several species moderately shade-tolerant and without avoidance/tolerance defence against disturbance factors (Vitasović Kosić et al. 2011) like *Fagetea sylvaticae* and *Trifolio-Geranietea* species.
On the other extreme of the gradient, indicator species belonged to *Molinio-Arrhenateretea* and *Festuco-Brometea* classes, in line with the composition of managed grasslands in central Apennines (Catorci et al. 2010).

The significant higher taxonomic diversity values (richness and diversity indices) toward the mown condition are consistent with several studies in European grasslands (e.g. Catorci et al., 2011, 2012; Tardella et al. 2020). The increasing trend of species richness along the gradient suggests that the maximum disturbance experienced by the community was not so intense to negatively impact on richness, according to the Grime's (1977) intermediate disturbance theory.

Our results indicated that mowing promoted four types of growth forms (pleiocorm, sedge, hemirosette forb, and prostrate forb), referred to two space occupation strategies. The spread of sedges and species with pleiocorms may be understood as the establishment of strategies aimed at enabling fast re-occupancy of the canopy gaps after mowing, thanks to a large number of reserve buds (Klimeš and Klimešová 2002). In particular, pleiocorms have multiple stems arising from adventitious buds near the stem base, whose growth is fostered by cutting, ensuring a certain degree of horizontal spread. The hemirosette and prostrate growth forms are escape strategies that allow plants to survive disturbance and exploit the newly available spatial niches, taking advantage of shorter canopy height and lower competition for light (Grime et al., 2001; Tardella et al. 2020).
Runners, clonal growth form allowing horizontal spread, were negatively influenced by mowing, probably because of the reduction of *Brachypodium genuense* (see Tardella et al. 2020). These

species, indeed, are positively selected by the need to find and exploit new gaps when within the community there is strong competition for resources, especially due to competitive, dominant species (Catorci et al., 2012), while they would lose their adaptive meaning in communities in which competition is not so intense.
Storage organs (bulbs and tap roots), which are related to spring and overwintering green leaves, are part of a double strategy, typical of sub-Mediterranean climate, aimed at tolerating non-selective disturbance (mowing) by their resprouting ability after defoliation and avoiding the summer drought stress period (Tardella et al. 2016).
The higher FD values of horizontal architecture in the unmown than in the intermediate disturbance condition may be explained considering the wider range of space occupation types needed to avoid competition with the dominant species (i.e. *Brachypodium genuense*).

How do species and functional composition and diversity change over time in mown and abandoned meadows?

As far as the diachronic comparison within management types is concerned, as expected, species richness has been increasing under mowing; this trend coincided with that of light intensity, probably due to the opening of sward gaps; conversely it has shown the opposite trend under abandonment. This result is consistent with a wide body of literature (e.g. Maron et al. 2001; Piseddu et al. 2021). This trend reflects on the number of indicator species, which increased under mowing, and decreased under abandonment. The increase of indicator species in mown meadows over time seems to be mostly associated to that of *Festuco-Brometea*, *Ononidetea*, and *Elyno-Seslerietea* classes, while the decrease of indicator species in abandoned meadows was mostly linked to a marked impoorishment in *Molinio-Arrhenatheretea* and *Nardetea* species.

As far as functional composition is concerned, runners, reptant species, plants less than 20 cm tall were negatively influenced by mowing over time, confirming what observed along the spatial disturbance gradient, namely, they are positively filtered by the need to find and exploit new gaps to coexist with competitive species (Catorci et al., 2012), while under prolonged disturbance conditions they would lose their functional meaning.

Similarly, we observed under abandonment the decrease in abundance of species with storage organs (bulbs, tubers, and rhizomes), spring-green and overwintering green leaves, traits aimed at tolerating non-selective disturbance (mowing) and avoid drought stress in sub-Mediterranean grasslands (Tardella et al. 2016). This trend reflects over time the spatial trend observed under decreasing disturbance intensities.

6.3.7 Conclusion

In this research, the trends detected in previous research at a European level were confirmed, such as the one according to which the reduction of disturbance and the abandonment of traditional management of mown grasslands causes a reduction in species richness. However, some aspects regarding the variation in functional composition will need to be further investigated. For instance, an interesting aspect that needs to be further explored is that some functional trends detected along spatial disturbance gradients have also been detected along the temporal gradient, i.e. the persistence of disturbance conditions over time shows an effect similar to the increase in the intensity of the disturbance on a spatial scale.

7 General conclusion

7.1 Main remarks

The outcomes of the research activities conducted in sub-Mediterranean mountain grasslands may be summarized as follows.

On a landscape scale, the spatial heterogeneity (i.e. landform factors) acts as an important driver in maintaining a certain value of taxonomic and functional diversity for a long time after abandonment of a pastoral system. Dryer conditions were more suitable to preserve the presence of the species set typical of grazed grasslands and were characterised by higher α-taxonomic diversity, confirming that in sub-Mediterranean climate resource-poor environments are less impacted by abandonment. Instead, less resource-limited habitats, while maintaining in some cases higher functional diversity levels, linked to the need of differentiating the strategies for the acquisition and use of resources, were more prone to the invasion of *Brachypodium* species, which tend, on the one hand, to constitute the suitable environment for successional or fringe habitat species, and, on the other hand, to exclude weaker competitors.

Focusing on an abandoned pastoral system invaded by *Brachypodium rupestre* on a small spatial scale, we observed that disturbance (long-term twice-a-year mowing) had a negative effect on *Brachypodium rupestre*, allowing other species to coexist through the differentiation of spatial and temporal niches and by different strategies in use/acquisition of resources. This involves an increasing progressive adaptation (i.e., efficiency in resource use) of the plant community during the recovery processes to local environment features and resource fluctuation. Disturbance and biotic interactions (reduction of weaker competitor exclusion) contributed independently to the functional changes, each

affecting a specific set of trait states. Mowing was more effective in driving the recovery processes, weakening of the importance of processes related to dominant species.

On a plant community scale, mowing strongly shaped the mountain meadows diversity and composition, fostering taxonomic diversity and the abundance of species typical of managed mountain grasslands. Conversely, abandonment resulted in a reduction in species richness and the occurrence of successional species, indicators of a progression toward forest recovery, where *Brachypodium*-dominated communities represent a dynamic stage in this process. Overall, mowing generally enhanced the range of functional strategies associated with resource acquisition and storage, reproduction, space occupation, and temporal niche exploitation. Some functional trends detected along spatial disturbance gradients have also been detected along the temporal gradient, i.e. the persistence of disturbance conditions over time shows an effect similar to the increase in the intensity of the disturbance on a spatial scale.

These findings enhance our understanding of the role of management conditions in shaping species diversity and functional trait composition of sub-Mediterranean grassland communities at different spatial scales. Changes are mediated by the effect of disturbance on *Brachypodium* species dominance, with important consequences in defining appropriate management strategies and in the use of European funds for the conservation of sub-Mediterranean grasslands.

8 References

Abbate, G., Piera Di Marzio, and M. P. Gigli. "Dynamics between Nardus stricta L. grassland and Vaccinium myrtillus Willd. communities in the Monti Reatini (Central Italy)." Fitosociologia 26 (1994): 93-98.

Aeschimann, D., Lauber, K., Moser, D. M., & Theurillat, J.P. (2004). Flora alpina: ein Atlas sämtlicher 4500 Gefässpflanzen der Alpen.

Alpert, P. (1999). Effects of clonal integration on plant plasticity in Fragaria chiloensis. Plant Ecology, 141, 99-106.

Bartolucci, F., Peruzzi, L., Galasso, G., Albano, A., Alessandrini, A., Ardenghi, N. M. G., ... & Barberis, G. (2018). An updated checklist of the vascular flora native to Italy. Plant Biosystems 152(2): 179-303.

Beatty, S. W., & Sholes, O. D. (1988). Leaf litter effect on plant species composition of deciduous forest treefall pits. Canadian Journal of Forest Research, 18(5), 553-559.

Begon M., Townsend C. R., Harper J.L. (1990) Ecology: from individuals to ecosystems. 4th ed.

Bennie, J., Hill, M. O., Baxter, R., & Huntley, B. (2006). Influence of slope and aspect on long-term vegetation change in British chalk grasslands. Journal of ecology, 94(2), 355-368.https://doi.org/10.1111/j.1365-2745.2006.01104.x

Biondi, E., Blasi, C., Burrascano, S., Casavecchia, S., Copiz, R., Del Vico, E., ... & Zivkovic, L. (2016). Manuale Italiano di interpretazione degli habitat della Direttiva 92/43/CEE (Italian interpretation manual of the 92/43/EEC habitats directive). vnr. unipg. it/habit at/. Accessed, 1.

Bobbink, R. (1991). Effects of nutrient enrichment in Dutch chalk grassland. Journal of applied ecology, 28-41.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H., White, J.S.S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. Trends Ecol. Evol. 24: 127–135.

Bonanomi, G., Caporaso, S., & Allegrezza, M. (2006). Short-term effects of nitrogen enrichment, litter remo-val and cutting on a Mediterranean grassland. Acta Oecologica, 30(3), 419-425.

Bonanomi, G., Idbella, M., Abd-ElGawad, A. M., Motti, R., Ippolito, F., Santorufo, L., ... & Zotti, M. (2022). Impact of prescribed burning, mowing and abandonment on a Mediterranean grassland: A 5-year multi-kingdom comparison. Science of the Total Environment, 834, 155442.

Bonanomi, G., Incerti, G., & Allegrezza, M. (2013). Assessing the impact of land abandonment, nitrogen enrichment and fairy-ring fungi on plant diversity of Mediterranean grasslands. Biodiversity and conservation, 22, 2285-2304.

Borcard, D., Gillet, F., Legendre, P. (2011). Numerical Ecology with R. Springer, New York, US.

Botta-Dukát, Z. (2005). Rao's quadratic entropy as a measure of functional diversity based on multiple traits. Journal of Vegetation Science, 16(5): 533-540.

Bracchetti L., Carotenuto L., Catorci A. 2012. Land-cover changes in a remote area of central Apennines (Italy) and management directions. Land Urban Planning 104: 157–170. https://doi.org/10.1016/j.landurbplan. 2011.09.005

Braun-Blanquet J (1964) Pflanzensoziologie. 3rd edn. Springer, Wien–New York, 865 pp.

Bullock, J. M., Silvertown, J., & Hill, B. C. (1996). Plant demographic responses to environmental variation: distinguishing between effects on age structure and effects on age-specific vital rates. Journal of Ecology, 733-743.

Camiz S., Dowgiallo G., Lucchese F. (1991). Edaphic characters of Brachypodium communities on the Alps and the Apennines. Ecologia Mediterranea 17: 33-49.

Canals, R. M., San Emeterio, L., Durán, M., & Múgica, L. (2017). Plant-herbivory feedbacks and selective allocation of a toxic metal are behind the stability of degraded covers dominated by Brachypodium pinnatum in acidic soils. Plant and Soil, 415(1-2), 373-386.

Catalán, P., López-Álvarez, D., Díaz-Pérez, A., Sancho, R., & López-Herránz, M. L. (2016). Phylogeny and evolution of the genus Brachypodium. In: Vogel J.P. (ed.) Genetics and genomics of Brachypodium. Springer, Cham. pp. 9-38.

Catalán, P., Müller, J., Hasterok, R., Jenkins, G., Mur, L. A., Langdon, T., ..., López-Alvarez, D. (2012). Evolution and taxonomic split of the model grass Brachypodium distachyon. Annals of Botany 109(2): 385-405.

Catalán, P., Olmstead, R.G. (2000). Phylogenetic reconstruction of the genus Brachypodium P. Beauv. (Poaceae) from combined sequences of chloroplastndhF gene and nuclear ITS. Plant Systematics and Evolution 220(1-2): 1-19.

Catalán, P., Shi, Y., Armstrong, L., Draper, J., & Stace, C. A. (1995). Molecular phylogeny of the grass genus Brachypodium P. Beauv. based on RFLP and RAPD analysis. Botanical Journal of the Linnean Society 117(4): 263-280.

Catorci, A. (2007): Lineamenti storico-economici del paesaggio pastorale maceratese. – Braun-Blanquetia 42: 39–46.

Catorci, A., Antolini, E., Tardella, F.M., Scocco, P., (2014). Assessment of interaction between sheep and poorly palatable grass: a key tool for grassland management and restoration. J. Plant Interact. 9(1), 112-121

Catorci, A., Cesaretti, S., Gatti, R., Ottaviani, G., 2011a. Abiotic and biotic changes due to spread of Brachypodium genuense (DC.) Roem. & Schult. in sub-Mediterranean meadows. Community Ecol. 12, 117–125.

Catorci, A., Ottaviani, G., & Cesaretti, S. 2011c. Functional and coenological changes under different long-term management conditions in Apennine meadows (central Italy). Phytocoenologia, 41(1), 45.

Catorci, A., Ottaviani, G., Ballelli, S., Cesaretti, S., 2011b. Functional differentiation of Central Apennine grasslands under mowing and grazing disturbance regimes. Pol. J. Ecol. 59, 115–128.

Chesson, P. (2000). Mechanisms of maintenance of species diversity. Annual review of Ecology and Systematics, 31(1), 343-366.

Chesson, P. Mechanisms of maintenance of species diversity. Annu. Rev. Ecol. Syst. 2000, 31, 343–366.

Chytrý, M., Dražil, T., Hájek, M., Kalníková, V., Šibík, J., Ujházy, K., ... & Mládek, J. (2015). The most species-rich plant communities in the Czech Republic and Slovakia (with new world records). Preslia, 87(3), 217-278.

Collins S. and Beaufoy G., 2012. Improving the targeting, monitoring and management of semi-natural grasslands across Europe – essential steps to achieving EU Biodiversity Strategy targets on farmland. EFNCP report

Collins, S.L.; Knapp, A.K.; Briggs, J.M.; Blair, J.M.; Steinauer, E.M. Modulation of diversity by grazing and mowing in native tallgrass prairie. Science 1998, 280, 745–747.

Corazza, M., Tardella, F.M., Ferrari, C., Catorci, A., 2016. Tall grass invasion after grassland abandonment influences the availability of palatable plants for wild herbivores: insight into the conservation of the Apennine Chamois Rupicapra pyrenaica ornata. Env. Manag. 57, 1247–1261. https://doi.org/ 10.1007/s00267-016-0679-1

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., ... & Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. Australian journal of Botany, 51(4), 335-380

Craine, J. M. (2005). Reconciling plant strategy theories of Grime and Tilman. Journal of ecology, 93(6), 1041-1052.

Cramer GR, Ergul A, Grimplet J, Tillett RL, Tattersall EA, Bohlman MC, Vincent D, Sonderegger J, Evans J, Osborne C, Quilici D, Schlauch KA, Schooley DA, Cushman JC (2007) Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Funct Integr Genomics 7:111–134

de Bello, F., Lavergne, S., Meynard, C.N., Lepš, J., Thuiller, W. (2010). The partitioning of diversity: showing Theseus a way out of the labyrinth Journal of Vegetation Science 21: 992–1000. https://doi.org/10.1111/j.1654-1103.2010.01195.x.

de Bello, F., Lepš, J., & Sebastiá, M. T. (2005). Predictive value of plant traits to grazing along a climatic gradient in the Mediterranean. Journal of applied Ecology, 42(5), 824-833.https://doi.org/10.1111/j.1365-2664.2005.01079.x

de Bello, F.; Vandewalle, M.; Reitalu, T.; Lepš, J.; Prentice, H.C.; Lavorel, S.; Sykes, M.T. (2013). Evidence for scale-and disturbance-dependent trait assembly patterns in dry semi-natural grasslands. J. Ecol., 101, 1237–1244.

de Cáceres, M., Legendre, P., Wiser, S. K., & Brotons, L. (2012). Using species combinations in indicator value analyses. Methods in Ecology and Evolution, 3(6), 973-982.

de Kroon, H., Bobbink, R., 1997. Clonal plant dominance under elevated nitrogen deposition, with special reference to Brachypodium pinnatum in chalk grassland, in: de Kroon, H., van Groenendael, J. (Eds.), The ecology and evolution of clonal plants. Backhuys Publishers, Leiden, pp. 359–379.

Del Guacchio, E., Innangi, M., Giaco, A., Peruzzi, L., & Caputo, P. (2020). Taxa endemic to Campania (southern Italy): nomenclatural and taxonomic notes. Phytotaxa, 449(3), 217-231.

Devictor, V., Mouillot, D., Meynard, C., Jiguet, F., Thuiller, W., & Mouquet, N. (2010). Spatial mismatch and congruence between taxonomic, phylogenetic and functional diversity: the need for integrative conservation strategies in a changing world. Ecology letters, 13(8), 1030-1040.

Di Pietro R., Conte A.L. & Iamonico D., 2014. La vegetazione di San Martino D'Agri (Monografia e carta della Vegetazione). Comune di San Martino D'Agri. 180 pp. Grafica Mente (ed.), Sant'Arcangelo (PZ)

Diaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H., Jalili, A., ... & Zak, M. R. (2004). The plant traits that drive ecosystems: evidence from three continents. Journal of vegetation science, 15(3), 295-304. https://doi.org/10.1111/j.1654-1103.2004.tb02266.x

D'Ottavio P., Scotton M., Ziliotto U. Pastoral value of mountain pastures of Monti Sibillini (Central Apennines, Italy) grazed by sheep. In : Molina Alcaide E. (ed.), Ben Salem H. (ed.), Biala K. (ed.), Morand-Fehr P. (ed.). Sustainable grazing, nutritional utilization and quality of sheep and goat products. Zaragoza: CIHEAM, 2005. p. 93-98 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 67)

Dowgiallo, M. G., & Lucchese, F. (1991). Studio dei rapporti tra suolo e distribuzione di due specie di Brachypodium: B. rupestre e B. genuense. Annali della Facoltà di Agraria, Campobasso, 2, 67-82.

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological monographs, 67(3), 345-366.

Ellenberg, H., Arndt, U., Bretthauer, R., Ruthsatz, B., & Steubing, L. (1991). Biological monitoring. Signals from the environment.

Filibeck, G., Cancellieri, L., Bartolucci, F., Becker, U., Conti, F., Maestri, S., ... & Becker, T. (2020). Festuca valesiaca Schleich. ex Gaudin newly discovered in the Central Apennines (Italy): a further example of steppe relict in the Abruzzo “dry valleys”. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 154(5), 593-600.

Galvánek, D., & Lepš, J. (2012). The effect of management on productivity, litter accumulation and seedling recruitment in a Carpathian mountain grassland. Plant Ecology, 213, 523-533.

Gatti, R., Carotenuto, L. & Catorci, A. (2007a): Sinfenologia di alcuni syntaxa prativi dell’Appennino umbro-marchigiano (Italia centrale). – Braun-Blanquetia 42: 179–202.

Gatti, R., Vitanzi, A., Cesaretti, S. & Catorci, A. (2007b): Contributo alla quantificazione della fitomassa epigea di alcuni pascoli dell’Appennino umbro-marchigiano (Italia centrale). – Braun-Blanquetia 42: 255–266.

Gianguzzi, L., Caldarella, O., & Di Pietro, R. (2018). A phytosociological analysis of the Brachypodium rupestre (Host) Roem. & Schult. communities of Sicily. Plant Sociology, 55(1), 65-88.

Giarrizzo, E., Burrascano, S., Chiti, T., de Bello, F., Lepš, J., Zavattero, L., & Blasi, C. (2017). Re-visiting historical semi-natural grasslands in the Apennines to assess patterns of changes in species composition and functional traits. Applied Vegetation Science, 20(2), 247-258.

Godefroid, S., & Dana, E. D. (2007). Can Ellenberg's indicator values for Mediterranean plants be used outside their region of definition? Journal of Biogeography, 34(1), 62-68. https://doi.org/10.1111/j.1365-2699.2006.01582.x

Grime, J.P. Plant Strategies, Vegetation Processes, and Ecosystem Properties, 2nd ed.; Wiley: Chichester, UK, 2001.

Grime, J.P. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. J. Veg. Sci. 2006, 17, 255–260.

Guarino, R., Domina, G. & Pignatti, S. 2012. Ellenberg’s Indicator values for the Flora of Italy-first update: Pteridophyta, Gymnospermae and Monocotyledoneae. Flora Mediterranea 22: 197–209. https://doi,org/10,7320/FlMedit22,197

Halassy, M.; Botta-Dukát, Z.; Csecserits, A.; Szitár, K.; Török, K. Trait-based approach confirms the importance of propagule limitation and assembly rules in old-field restoration. Restor. Ecol. 2019, 27, 840–849.

Hautier, Y.; Niklaus, P.A.; Hector, A. Competition for light causes plant biodiversity loss after eutrophication. Science 2009, 324, 636–638.

Házi, J., Bartha, S., Szentes, S., Wichmann, B., & Penksza, K. (2011). Seminatural grassland management by mowing of Calamagrostis epigejos in Hungary. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 145(3), 699-707.

Hobohm, C. & Brunchmann, I. 2009. Endemische Gef€aßpflan-zen undihre Habitate in Europa: Pl€adoyer f€ur den Schutz derGrasland-€Okosysteme. [Endemic vascular plants and theirhabitats in Europe-A plea for the protection of grasslandecosystems].Berichte der Reinhold-T€uxen- Gesellschaft 21, 142–161.

Hopkins, A. (2009, May). Relevance and functionality of semi-natural grassland in Europe–status quo and future prospective. In International workshop of the SALVERE-Project (pp. 9-14).

Huhta, A. P., Rautio, P., Tuomi, J., & Laine, K. (2001). Restorative mowing on an abandoned semi-natural meadow: short-term and predicted long-term effects. Journal of vegetation science, 12(5), 677-686.

Huhta, A.-P.; Rautio, P. Evaluating the impacts of mowing: A case study comparing managed and abandoned meadow patches. Ann. Bot. Fenn. 1998, 35, 85–99.

Hurst, A., & John, E. (1999). The biotic and abiotic changes associated with Brachypodium pinnatum dominance in chalk grassland in south-east England. Biological Conservation, 88(1), 75-84.

Ilmarinen, K.; Mikola, J. Soil feedback does not explain mowing effects on vegetation structure in a semi-natural grassland. Acta Oecol. 2009, 35, 838–848.

Janisová, M., Bartha, S., Kiehl, K. & Dengler, J. 2011: Advances in the conservation of dry grasslands. Introduction to contributions from the 7th European Dry Grassland Meeting. Plant Biosystems 145: 507–513.

Jendrišáková, S., Jančová, M., & Kizeková, M. (2011). Agri-environmental management of grassland habitats. Agri-environmental management of grassland habitats.

Kahmen, S., & Poschlod, P. (2008). Effects of grassland management on plant functional trait composition. Agriculture, Ecosystems & Environment, 128(3), 137-145.

Khan, M. A., Stace, C. A. (1999). Breeding relationships in the genus Brachypodium (Poaceae: Pooideae). Nordic Journal of Botany 19(3): 257-269.

Klaus, V. H., Kleinebecker, T., Boch, S., Müller, J., Socher, S. A., Prati, D., ... & Hölzel, N. (2012). NIRS meets Ellenberg's indicator values: Prediction of moisture and nitrogen values of agricultural grassland vegetation by means of near-infrared spectral characteristics. Ecological Indicators, 14(1), 82-86.https://doi,org/10,1016/j, ecolind,2011,07,016

Klimeš, L., & Klimešová, J. (2002). The effects of mowing and fertilization on carbohydrate reserves and regrowth of grasses: do they promote plant coexistence in species-rich meadows?. In Ecology and Evolutionary Biology of Clonal Plants: Proceedings of Clone-2000. An International Workshop held in Obergurgl, Austria, 20–25 August 2000 (pp. 141-160). Springer Netherlands.

Klimešová, J., Danihelka, J., Chrtek, J., de Bello, F., & Herben, T. (2017). CLO-PLA: a database of clonal and bud-bank traits of the Central European flora.

Klimešová, J.; Latzel, V.; de Bello, F.; van Groenendael, J.M. Plant functional traits in studies of vegetation changes in response to grazing and mowing: Towards a use of more specific traits. Preslia 2008, 80, 245–253.

Klotz, S., Kühn, I. & Durka, W. (2002) BiolFlor – eine datenbank mit biologisch-ökologischen merkmalen zur flora von Deutschland. Schriftenreihe für Vegetationskunde, 38, 1– 333.

Knapp, A. K., & Seastedt, T. R. (1986). Detritus accumulation limits productivity of tallgrass prairie. BioScience, 36(10), 662-668.

Köhler, B.; Gigon, A.; Edwards, P.J.; Krüsi, B.; Langenauer, R.; Lüscher, A.; Ryser, P. Changes in the species composition and conservation value of limestone grasslands in Northern Switzerland after 22 years of contrasting managements. Persp. Plant Ecol. Evol. System. 2005, 7, 51–67.

Lavorel, S., Díaz, S., Cornelissen, J. H. C., Garnier, E., Harrison, S. P., McIntyre, S., ... & Urcelay, C. (2007). Plant functional types: are we getting any closer to the Holy Grail?. Terrestrial ecosystems in a changing world, 149-164. https://doi.org/10.1007/978-3-540-32730-1_13

Lepš, J. (2014). Scale-and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment. Journal of Applied Ecology, 51(4), 978-987.

López-Alvarez, D., López-Herranz, M. L., Betekhtin, A., & Catalán, P. (2012). A DNA barcoding method to discriminate between the model plant Brachypodium distachyon and its close relatives B. stacei and B. hybridum (Poaceae). PLoS one 7(12), e51058.

López-Alvarez, D., Manzaneda, A. J., Rey, P. J., Giraldo, P., Benavente, E., Allainguillaume, J., ... & Ezrati, S. (2015). Environmental niche variation and evolutionary diversification of the Brachypodium distachyon grass complex species in their native circum-Mediterranean range. American Journal of Botany 102(7): 1073-1088.

Louault, F., Soussana, J.F., Perrodin, M., (2002). Long-term effects of a reduced herbage use in a semi-natural grassland: I – plant functional traits and plant response groups. Grassl. Sci. Eur. 7, 338–339.

Lucchese, F. (1987). Ruolo di alcune specie del genere Brachypodium nelle associazioni prative e forestali. Notiziario Fitosociologico 23: 173-188.

Lucchese, F. (1989). La distribuzione dei complessi Brachypodium pinnatum e B. rupestre nelle Alpi orientali e Dinariche. Atti del simposio della Società Estalpino-dinarica di fitosociologia. Feltre 29/6 - 3/7/1988: 147-160.

Malavasi M., Carranza M. L., Moravec D., Cutini M. (2018). Reforestation dynamics after land abandonment: a trajectory analysis in Mediterranean mountain landscapes. Regional Environmental Change: 1-11.

Maron, J.L., Jefferies, R.L. (2001) Restoring Enriched Grasslands: Effects of Mowing on Species Richness, Productivity, and Nitrogen Retention. Biological Sciences Faculty Publications. 344. https://scholarworks.umt.edu/biosci_pubs/344

Mayfield, M. M., & Levine, J. M. (2010). Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecology letters, 13(9), 1085-1093.

McCollin, D., Moore, L., & Sparks, T. (2000). The flora of a cultural landscape: environmental determinants of change revealed using archival sources. Biological Conservation, 92(2), 249-263. https://doi.org/10.1016/S0006-3207(99)00070-1

Mucina, L., Bültmann, H., Dierßen, K., Theurillat, J. P., Raus, T., Čarni, A., ... & Tichý, L. (2016). Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. Applied vegetation science, 19, 3-264.

Oroian, S., Sămărghiţan, M., Popa, R., & Calalb, T. (2014). The conservation status of grasslands habitats identified in the" Hârtibaciu-Târnava Mare-Olt" Natura 2000 site. Contributii Botanice, 49.

Overbeck, G.; Kiehl, K.; Abs, C. Seedling recruitment of Succisella inflexa in fen meadows: Importance of seed and microsite availability. Appl. Veg. Sci. 2003, 6, 97–104.

Pärtel, M., Bruun, H. H., & Sammul, M. (2005). Biodiversity in temperate European grasslands: origin and conservation. Grassland science in Europe, 10(1), 14.

Pastor, J., Dewey, B., Naiman, R. J., McInnes, P. F., & Cohen, Y. (1993). Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. Ecology, 74(2), 467-480.

Peco, B., Carmona, C. P., De Pablos, I., & Azcárate, F. M. (2012). Effects of grazing abandonment on functional and taxonomic diversity of Mediterranean grasslands. Agriculture, ecosystems & environment, 152, 27-32.

Pieruccini, P. (2007): Suoli e geomorfologia delle praterie montane nell'Appennino Umbro-Marchigiano. – Braun-Blanquetia 42: 19 38.

Pignatti, S. (2005). Valori di bioindicazione delle piante vascolari della flora d'Italia. Dipt. di Botanica ed Ecologia dell'Università Camerino.

Pipenbaher, N., Kaligarič, M., Mason, N. W., & Škornik, S. (2013). Dry calcareous grasslands from two neighboring biogeographic regions: relationship between plant traits and rarity. Biodiversity and conservation, 22, 2207-2221.

Piseddu, F., Bellocchi, G. & Picon-Cochard, C. (2021) Mowing and warming effects on grassland species richness and harvested biomass: meta-analyses. Agron. Sustain. Dev. 41, 74. https://doi.org/10.1007/s13593-021-00722-y

Pittarello, M., Gorlier, A., Ravetto E., S., Lonati, M., & Lombardi, G. (2022). Long-term effects of mowing and mineral fertilization for the restoration of a Brachypodium rupestre-invaded grassland in Western Alps. In - (pp. 1-1).

Plantureux, S., Peeters, A., & McCracken, D. (2005). Biodiversity in intensive grasslands: Effect of management, improvement and challenges. Agronomy research, 3(2), 153-164.

Poschlod, P., Kiefer, S., Tränkle, U., Fischer, S. & Bonn, S. (1998) Plant species richness in calcareous grasslands as affected by dispersability in space and time. Applied Vegetation Science, 1, 75–90.

Pottier, J., & Evette, A. (2010). On the Relationship Between Clonal Traits and Small-Scale Spatial Patterns of Three Dominant Grasses and its Consequences on Community Diversity. Folia Geobotanica, 45(1), 59-75. Retrieved from http://www.jstor.org/stable/23064588

Prach, K., & Pyšek, P. (1994). Clonal plants—what is their role in succession?. Folia Geobotanica, 29, 307-320.

Ricotta C, Moretti M. (2011). CWM and Rao's quadratic diversity: a unified framework for functional ecology. Oecologia. 167:181–188. https://doi.org/10.1007/s00442-011-1965-5.

Roggero, P. P., Bagella, S., & Farina, R. (2002). Un archivio dati di Indici specifici per la valutazione integrata del valore pastorale. Rivista di Agronomia, 36(2), 149-156.

Schippmann, U. (1991). Revision der europaischen Arten der Gattung Brachypodium Palisot de Beauvois (Poaceae). Boissiera 45: 5-250.

Scocco, P., Mercati, F., Brusaferro, A., Ceccarelli, P., Belardinelli, C., & Malfatti, A. (2013). Keratinisation degree of rumen epithelium and body condition score in sheep grazing on Brachypodium rupestre. Vet. Ital, 49, 211-217.

Socher, S.A.; Prati, D.; Boch, S.; Müller, J.; Baumbach, H.; Gockel, S.; Hemp, A.; Schöning, I.; Wells, K.; Buscot, F.; et al. Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. Basic Appl. Ecol. 2013, 14, 126–136.

Soreng, R.J., Peterson, P.M., Romschenko, K., Davidse, G., Zuloaga, F.O., Judziewicz, E.J., Filgueiras, T.S., Davis, J.I., Morrone, O. (2015). A worldwide phylogenetic classification of the Poaceae (Gramineae)". Journal of Systematics and Evolution 53(2): 117-137. doi:10.1111/jse.12150.

Stevens, C. J., Ceulemans, T., Hodgson, J. G., Jarvis, S., Grime, J. P., & Smart, S. M. (2016). Drivers of vegetation change in grasslands of the Sheffield region, northern England, between 1965 and 2012/13. Applied vegetation science, 19(2), 187-195.https://doi.org/10.1111/avsc.12206

Tälle, M., Bergman, K. O., Paltto, H., Pihlgren, A., Svensson, R., Westerberg, L., ... & Milberg, P. (2014). Mowing for biodiversity: grass trimmer and knife mower perform equally well. Biodiversity and conservation, 23, 3073-3089.

Tardella, F. M., Bricca, A., Piermarteri, K., Postiglione, N., & Catorci, A. (2017). Context-dependent variation of SLA and plant height of a dominant, invasive tall grass (Brachypodium genuense) in sub-Mediterranean grasslands. Flora, 229, 116-123.

Tardella, F. M., Piermarteri, K., Malatesta, L., & Catorci, A. (2016). Environmental gradients and grassland trait variation: insight into the effects of climate change. *Acta Oecologica*, *76*, 47-60.

Thorne, R.F., Reveal, J.L. (2007). An Updated Classification of the Class Magnoliopsida ("Angiospermae")". The Botanical Review. 73: 67–181. doi:10.1663/0006-8101(2007)73[67:AUCOTC]2.0.CO;2

Tropicos.org. Missouri Botanical Garden. 12 Oct 2018 <http://www.tropicos.org/Name/40030673>

Valdés, B., Scholz, H., von Raab-Straube, E., & Parolly, G. (2009). Poaceae (pro parte majore). Euro+ Med PlantBase–the information resource for Euro-Mediterranean plant diversity.–Published at http://ww2. bgbm. org/euroPlusmed/[accessed 8 Nov 2014, 19 Dec 2014].

Valkó, O.; Török, P.; Matus, G.; Tóthmérész, B. Is regular mowing the most appropriate and cost-effective management maintaining diversity and biomass of target forbs in mountain hay meadows? Flora 2012, 207, 303–309.

Vitasović-Kosić I., Tardella, F.M., Grbeša, D., Škvorc, Ž., Catorci, A. 2014. Effects of abandonment on the functional composition and forage nutritive value of a North Adriatic dry grassland community (Ćićarija, Croatia). Appl. Ecol. Environ. Res. 12, 285–299.

Vogel, J.P. (ed.) (2016). Genetics and Genomics of Brachypodium. Plant Genetics and Genomics: Crops and Models 18. Springer.

Watson, L., Dallwitz, M.J. (2008). "The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references". The Grass Genera of the World. Retrieved 2009-08-19.

Wesche, K., Krause, B., Culmsee, H., & Leuschner, C. (2012). Fifty years of change in Central European grassland vegetation: Large losses in species richness and animal-pollinated plants. Biological Conservation, 150(1), 76-85.https://doi.org/10.1016/j.biocon.2012.02.015

Wilson, J. B., Peet, R. K., Dengler, J., & Pärtel, M. 2012. Plant species richness: the world records. Journal of Vegetation Science, 23(4), 796-802.

Zelený (2018). Which results of the standard test for community-weighted mean approach are too optimistic? Journal of Vegetation Science, 29: 953-966. https://doi,org/10.1111/jvs.12688

Zelený, D., & Schaffers, A. P. (2012). Too good to be true: Pitfalls of using mean E llenberg indicator values in vegetation analyses. Journal of Vegetation Science, 23(3), 419-431.https://doi,org/10,1111/j,1654- 1103,2011,01366,x

Zobel, M., Suurkask, M., Rosén, E., & Pärtel, M. (1996). The dynamics of species richness in an experimentally restored calcareous grassland. Journal of Vegetation Science, 7(2), 203-210.

9 List of the Ph.D. research outputs

9.1 Published scientific papers.

Bricca A., Tardella F.M., Tolu F., Goia I., Ferrara A., Catorci A. 2020. Disentangling the Effects of Disturbance from Those of Dominant Tall Grass Features in Driving the Functional Variation of Restored Grassland in a Sub-Mediterranean Context. Diversity 12, 11; https://doi.org/10.3390/d12010011 [published paper – see **Appendix S1**].

Bricca A., Tardella F.M., Ferrara A., Xinfang X., Tolu F., Catorci A. 2022. Environmental heterogeneity compensates the potential homogenising effect of abandonment of grazing in a sub-Mediterranean mountain landscape, Plant Ecology & Diversityhttps://doi.org/10.1080/17550874.2022.2039314 [published paper – see **Appendix S2**].

9.2 Posters and oral presentations:

Tolu F., Bricca A., Tardella F.M., Catorci A. 2021. How biyearly mowing and *Brachypodium rupestre*'s features affects plant community. Atti riunioni scientifiche 1st Conference of Young Botanists. Genova 6-7 February 2020. Notiziario della Società Botanica Italiana, 5 (2): 135.

Bricca A., Tardella F.M., Ferrara A., Panichella T., Tolu F. 2021. Multifaced approach to analyse the effect of multi-year mowing on sub-Mediterranean invaded grasslands. 29th Conference of European Vegetation Survey, online conference, 6–7 September 2021. Abstracts. https://doi.org/10.5281/zenodo.5171736

9.3 Other Ph.D. activities

***Attendance at Conferences/Workshops/Seminars*:**

Alternative proteins from a consumer & industry perspective' with Prof Maeve Henchion (Teagasc) and Martin Fleming (Enterprise Ireland)

Another break in the (cell) wall: plant matrix and nutrients digestion. Edoardo Capuano, Associate Professor at Food Quality and Design, Wageningen University & Research

Biological BIG-DATA: leveraging "-omics" sciences towards a more reproducible and reliable scientific approach.

China Jiliang University, with a short introduction by prof. MonikaMortimer and Presentations from the School of Biosciences and Veterinary Medicine, UNICAM, with a short introduction by prof. Cristina Miceli.

Ciclo di incontri "Ambiente, Sostenibilità e Lavoro: Natura e turismo sostenibile: "Slow Tourism" e aree montane Marta Zarelli Guida ambientale, -- Tecniche e problematiche della reintroduzione

di specie estinte. Il caso del camoscio appenninico Alessandro Rossetti Biologo del Parco Nazionale Monti Sibillini. –Minimizzare l'impronta ambientale dell'alimentazione. Il gran Tour delle marche e la "challenge" con l'app scelta dalle Nazioni Unite contro il cambiamento climatico. Dott. Alberto Monachesi, coordinatore di Tipicità Marche.

Early blood-based biomarkers to tackle Alzheimer's disease management: From basic research to a validated test Prof. Daniela Uberti Dept. Molecular and Translational Medicine University of Brescia

Energy and Sustainability. Transizione energetica e sostenibilità: esperienze e prospettive. Workshop.

Genome-Wide Association Studies (GWAS): an unbiased approach in genomics to discover the genetic bases of any biological trait, from environmental to medical sciences – a practical guide to beginners.

How to present one year research work in 15 minutes. Prof. Guido Favia,Prof. Attilio Fabbretti, School of Biosciences and Veterinary Medicine

In vitro experimental models: from 2D to organoids. Prof. Stefano Lorenzetti Dip.to di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria (SANV) Istituto Superiore di Sanità, Roma.

Insights and drawbacks of the publishing process. Prof. Alessandro Di Cerbo, School of Biosciences and Veterinary Medicine University of Camerino

Intestinal microbiota: our tailored suit shaping physiology and nutrition. Dr. Ludovica Marinelli Center for Microbial Ecology and Technology. Ghent University

Mutant p53-dependent alterations of cellular metabolism and signaling pathways in cancer disease.

Scarti da costruzione e demolizione (CDW): opportunità per l'economia circolare e la ricostruzione. Workshop. G. Leoni, S. Mazzoli, E. Paris UNICAM, V. Grandinetti Progetto NUOVA VITA, M. De Vincentiis ANPAR e e-lab srl, G. Iezzi UNICH, F. Radica UNICH, F. Volpintesta UNICAM, E. Ossoli UNICAM, P. Stabile UNICAM, C. Santulli UNICAM G. Loffredo Subcommissario Ricostruzione Sisma 2016 G. Giampaoli COSMARI

seminar and workshop on Open Science. Paola Masuzzo, Elena Giglia, Yasemin Türkyilmaz-van der Velden

The nutritional role of dietary (poly)phenols. Prof. Daniele Del Rio and Prof. Pedro Mena, PhDs Laboratory of Phytochemicals in Physiology (Human Nutrition Unit) Department of Food and Drugs, University of Parma.

Theoretical and Practical course of MARKETING. Speaker Dr. Umberto Filippi

Young researchers bilateral meeting. Presentations from the Institute of Environmental and Health Sciences.

diversity

MDPI

Article

Disentangling the Effects of Disturbance from Those of Dominant Tall Grass Features in Driving the Functional Variation of Restored Grassland in a Sub-Mediterranean Context

Alessandro Bricca [1], Federico Maria Tardella [2], Fabio Tolu [1], Irina Goia [3], Arianna Ferrara [1,*] and Andrea Catorci [1]

[1] School of Biosciences and Veterinary Medicine, University of Camerino, via Pontoni 5, 62032 Camerino, Italy; alessandro.bricca@unicam.it (A.B.); fabio.tolu@studenti.unicam.it (F.T.); andrea.catorci@unicam.it (A.C.)

[2] Herbarium Universitatis Camerinensis, School of Biosciences and Veterinary Medicine, University of Camerino, via Pontoni 5, 62032 Camerino, Italy; dtfederico.tardella@unicam.it

[3] Department of Taxonomy and Ecology, Faculty of Biology and Geology, Babeş-Bolyai University, 42 Republicii Street, 400015 Cluj-Napoca, Romania; irina.goia@ubbcluj.ro

* Correspondence: arianna.ferrara@studenti.unicam.it

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Abstract: Land abandonment in sub-Mediterranean grasslands causes the spread of tall-grasses, affecting biodiversity and ecosystem functioning. Mowing allows the recovery of the coenological composition after invasion, but the mechanisms acting at the fine-scale are poorly investigated. Since 2010 in the Central Apennines, we fenced a grassland invaded by *Brachypodium rupestre*, divided it into two areas, half of each was mowed biyearly and half remained unmown. In 2017 we selected ten 20 × 20 cm experimental units per half-area, collecting data on species occurrences, plant traits, *B. rupestre* height and phytomass. We used generalized linear mixed-effect modelling to disentangle the role of mowing from the impact of *B. rupestre* features in driving the community functional variations. Mowing was the main driver in the recovery process, acting as an abiotic filter (enhancement of tolerance-avoidance strategies). Furthermore, the reduction of weaker competitor exclusion processes fostered the increase of functional variation between coexisting species. Both drivers acted on different plant traits (e.g., mowing on life span, vegetative propagation types and plant height, mowing and *B. rupestre* features on space occupation types, seed mass and leaf anatomy), generally enhancing the extent of functional strategies related to resource acquisition and storage, reproduction, space occupation and temporal niche exploitation.

Keywords: *Brachypodium rupestre* (Host) Roem. & Schult.; ecosystem functioning; weaker competitor exclusion; mowing; plant traits; abiotic filtering

1. Introduction

Mediterranean semi-natural grasslands, hosting a very rich flora with numerous endemic species, are considered priority habitats by the European Union (92/43/EEC Directive) and judged worthy of conservation. However, as in the rest of Europe, these ecosystems are in strong decline and are threatened by land abandonment, due to socio-economic changes that occurred after World War II and are still ongoing [1–3].

At a community level, abandonment and/or decrease in forage exploitation typically induce the spread of coarse tall-grasses with competitive stress-tolerant strategies, which trigger processes of biodiversity loss whose species identity is geographic-context dependent [4,5]. In the Italian mountain

landscapes, this group of species is well characterized by the *Brachypodium* genus. In particular, *Brachypodium rupestre* (Host) Roem. & Schult. (*Poaceae* Barnhart) dominates the low/hilly mountains up to an elevation of 1300–1400 m a.s.l., while *B. genuense* (DC.) Roem. & Schult. (a more nitrophilous, acidophilous and mesophilous species) dominates the higher mountains up to 2400 m a.s.l. The competitive behavior of perennial *Brachypodium* species is mainly related to the high tiller density and branching frequency [6], as well as to their capacity for clonal growth and clonal integration strategy [7]. These characteristics and properties, together with leaf plasticity [8], allow the rapid spread of clonal tussocks. These species are often able to form, through the coalescence of different patches, nearly mono-dominant stands [7]. This, in turn, leads to a modification of the coenological composition of pastures caused by the decrease of the grassland specialist taxa (early flowering, small-sized plants, with storage organs, etc.) and the increase of those typical of fringe communities and of nitrophilous habitats (i.e., tall herbs with late flowering strategies, species with runners and ability to form patches, etc.) [9,10], with an overall decrease in species richness and diversity [9]. Moreover, *Brachypodium* spread reduces the overall feed value of pastures [11], thus negatively affecting mountain farming economic sustainability [12]. Because of this profound impact on biodiversity and ecosystem services, restoration of pastures after tall-grass invasion (*B. rupestre* in the study case) is a paramount issue. This requires a deep understanding of the mechanisms behind the coenological recovery processes that occur after disturbance events [13,14].

It is generally assumed that a higher number of plant strategies and species are present under disturbed conditions [15] since disturbance decreases the chance of competitive exclusion of taxa with traits associated with a low competitive ability (i.e., weaker competitor exclusion) [16–18]. Generally, grazing and mowing are the principle management practices for grasslands. Grazers tend to avoid feeding on *Brachypodium* species for the presence of unpalatable leaves [19]. For this reason the feeding on this species results in harm to domestic herbivores and their welfare. This type of diet, in experimentations regarding sheep grazing, demonstrated how, feeding on this forage type, increased the rumen keratinization [12] and lowered the absorption of nutrients. For this reason, it is not feasible to use sheep grazing as a method for grassland recovery [20,21] and mowing should be the best practice in the restoration of *Brachypodium*-invaded grasslands [12]. Mowing is considered one of the most appropriate management types for ensuring the high conservation value of grasslands [22,23]. It is a non-selective disturbance [24] that, increasing light availability, promotes the survival of light-demanding seedlings, low-growth species [25,26] and germination rates of subdominant plants [27,28]. Moreover, it modifies the functional composition of plant communities by enhancing specific avoidance and tolerance strategies [29–32]. In particular, it was proven that vascular plant species richness was highest with two cuts per year, however the effect may differ in relation to the type of plant community, the timing of cutting and the biogeographic context [33]. This suggests that there is no best universal mowing frequency to promote plant species richness, however, mowing regimes need to be experimented and adapted to the regional and local environmental context.

Regarding *Brachypodium*-dominated communities, disturbance enhances plant biodiversity by reducing its abundance and preventing litter deposition [10,34]. In particular, recurrent mowing on *B. rupestre*-dominated grasslands fostered species of *Festuco-Brometea* and *Festuco-Ononidetea*, although species of *Molinio-Arrhenatheretea* partly replaced these groups after three/four years of mowing activities [35].

Notwithstanding such scientific advancement, there is still a need to understand better the effect of disturbance activities (i.e., mowing) and disentangle such effects from those related to the processes of plant-plant interaction (dominant vs subordinate species) [36]. Abiotic interactions (i.e., disturbance through mowing) could be an important driver in species coexistence at a small-scale, with different effects depending on the type and intensity of the disturbance regime, the frequency in the disturbance events [37], but also depending on the considered time scale [32]. However, biotic interactions (i.e., plant-plant interactions) are considered the major driver at a small-scale. Indeed, dominant species may remove from the community all weaker competitors (i.e., weaker competitor exclusion), acting

as a biotic filter, which leads to the coexistence of more functionally similar species. Otherwise, it is possible that in the absence of dominant species, biotic interactions may lead to coexistence of functionally dissimilar species [32,38].

It should also be considered that past research concerning the functional composition of *Brachypodium*-dominated communities arose from studies that used species abundance weighted data, while investigation with unweighted data (i.e., considering species presence/absence) were largely overlooked. The type of data used may affect the observed trait pattern, and its ecological interpretation [39] since species occurrence and their abundance may be connected to different processes [18,37]. Consequently, we used unweighted species data to investigate the dominant-subordinate plant-plant interactions and the effect of mowing, trying to disentangle their relative filtering effects on species assemblage at a fine scale.

We hypothesized that grassland restoration in a sub-Mediterranean climate context was the result of multiple assembly processes acting with different intensities. In particular, we expected that multiyear mowing, as opposed to vegetation successional processes, drove species turnover from competitive to stress-tolerant/ruderal strategies [36]. Additionally, we expected that multiyear mowing reduced the size of dominant tall grasses and fostered the shift from biotic filters (i.e., weaker competitor exclusion) to abiotic filters (i.e., tolerance and/or avoidance of disturbance) as drivers of the assembly rules. Finally, the weakening of the dominant species may change plant-plant (dominant vs. subordinate) interactions, leading to processes chiefly related to limiting the similarity among interacting individuals.

The specific research questions were: (i) do mowing and the variation of *B. rupestre*-dominance ability act on different species traits at a fine-scale? (ii) what is the relative importance of mowing, which halts the dynamic processes, and of *B. rupestre* features' changes in driving the functional variation of the plant community? (iii) how do such processes affect strategies of resource acquisition and storage, reproduction, space occupation, and temporal niche exploitation? (iv) can mowing decrease the effect that the dominant tall-grass *B. rupestre* has in shaping the functional community composition?

2. Materials and Methods

2.1. Study Area

The study area was located in the central Apennines (337,398.00 m E, 4757,200.00 m N, WGS 84 coordinate system, UTM zone 33 N) and encompassed north facing slopes, with limestone bedrock. The climate was sub-Mediterranean [40], characterized by the alternation of winter cold stress and summer drought stress. The mean annual rainfall was around 1000 mm, and the average annual temperature was 10–11 °C.

Managed grasslands belong to the *Brizo mediae-Brometum erecti brizetosum mediae* Biondi, Allegrezza & Zuccarello 2005 and the 6210* habitat [41]. It is a highly diverse, thick turf community, with aboveground productivity ranging from 170 to 200 $g \cdot m^{-2}$ of dry matter [41]. Beech woods of the *Fagetalia sylvaticae* order constituted the actual potential vegetation.

The study area had a centuries-old history of extensive grazing by sheep and, more recently, cattle. Traditionally, grasslands are grazed by flocks between late spring and early autumn; meadows are mown in early summer in the least steep or semi-flat slopes [41]. However, for at least the last 30 years, vast grassland areas, especially the steeper ones, have been undergrazed and are undergoing dynamic processes. Such processes foster the spread of *B. rupestre*'s clonal tussocks which form nearly mono-dominant stands by coalescence of different patches, through different modes (e.g., facilitation performed by thorny plants, accumulation of dead and senescent leaves, which protect the new shoots) [10].

The experimental site (two hectares) was established and fenced in February 2010. The site had homogeneous ecological conditions, i.e., altitude (1200–1250 m a.s.l.), aspect (west-northwest facing slope) and slope angle (20–25°), an average cover value of *B. rupestre* that exceeded 60%

and homogeneous land-use history. The fenced area had a soil (typic/lithic Haplustoll) [42] depth ranging from 30 to 40 cm and a neutral-sub-acid pH. Soil texture was characterized by sand (8–10%), silt (32–35%), and clay (50–60%). Skeleton amount ranged from 45 to 50%, while total nitrogen concentration was 6 to 7 $g \cdot kg^{-1}$ [43]. The surface of the experimental sites was divided into two areas (blocks) of 1 ha (100 m × 100 m). Since 2010, within each block, half of the surface, i.e., 0.5 ha (50 × 100 m), was mown twice a year (end of June, namely, just before the *B. rupestre* flowering, and at the end of October), two cm above the soil level; hay and litter were removed after each mowing event. The other half was left unmown, without litter removal.

2.2. Sampling Design and Data Collection

In 2017 we randomly selected the coordinates of the lower left hand corners of 10 experimental units (20 cm × 20 cm) for each half block, for a total of 40. In each experimental unit, we recorded the list of vascular species (Table S1).

Since plant height is related to size-asymmetric competition, it is the best candidate to detect the presence of biotic filters exerted by dominant species [18,36]. Thus, we measured the *B. rupestre* plant height in each experimental unit, according to the protocol described by Pérez-Harguindeguy et al. [44], namely, the shortest distance between the upper boundary of the main photosynthetic tissues on a plant (i.e., the top of the general canopy of the plant excluding inflorescences) and the ground level. We measured the available individuals (which were always less than 25, being the experimental unit area 400 cm^2) and averaged the measurements per experimental units. Finally, we clipped the aboveground phytomass (2 cm above the soil level) and separated that of *B. rupestre* from that of the other plants. We oven-dried the samples of *B. rupestre* aboveground phytomass at 70 °C and weighed them.

To investigate the functional processes behind the variation of species assemblage, for each recorded species, we gathered plant traits related to space occupation (plant height, vegetative propagation, horizontal and vertical architecture), reproductive strategies (seed mass and vegetative propagation), temporal niche exploitation (life span, leaf span) and resource storage strategies (storage organs, leaf anatomy) (Table S2). A description of each trait, with a list of the respective states and data sources, is reported in Table S3. We treated all traits as categoric variables.

2.3. Statistical Analysis

To assess if the management type (two level categorical variable, treated as fixed effect) exerted a significant influence on *B. rupestre* plant height and aboveground phytomass, following Bolker, et al. [45], we ran linear mixed-effects models when the response variable was normally distributed (*lmer* function of the lme4 R-package, version 1.1-21) [46], and a penalized quasi-likelihood modelling approach when it was not normally distributed and could not be normalized (*glmmPQL* function, family = gaussian, link = log, of the MASS R-package, version 7.3-51.1) [46]. We accounted for the sampling design using the block identity as a random effect. Significance values were obtained using the Type II Wald Chi-square test and the *Anova* function (car R-package, version 2.1-5) [46].

To analyze the effect of management type and *B. rupestre* aboveground traits in filtering the functional strategies displayed by subordinate species [36], we combined the "experimental unit × species (presence/absence)" matrix with the "species × trait state (presence/absence)" matrix obtaining a "experimental unit × trait state" matrix (number of species sharing the same trait state in each experimental unit). We ran a generalized linear mixed-effects model per trait state, using the Poisson distribution. Fixed effects were *B. rupestre* plant height, *B. rupestre* aboveground phytomass (both quantitative variables) and the type of management (two level categorical variable; mowed vs. unmowed). We accounted for the sampling design using block identity as a random effect. We executed these analyses using the *glmer* function and the lme4 R-package. Then, using an Akaike information criterion (AIC)-based model selection procedure applied to the full model, we selected the best fitting model for each response variable. We used the corrected AIC (AICc), since in our study the ratio between the number of observations and the number of fitted parameters was less than 40 [47]. This

model selection was performed using the *dredge* function of the MuMIn R-package (version 1.15.6) [46]. We accounted for possible within-group spatial autocorrelation of errors [48] in each reduced model, by refitting this model using the penalized quasi-likelihood GLMM (*glmmPQL* function, MASS package, version 7.3-51.1, family = Poisson) and then applying one by one the correlation structures AR1, CompSymm, Exp, Gaus, and Ratio, available in the nlme R-package library [49]. Then, for each model with a different correlation structure, we calculated the Spearman's correlation coefficient (*cor.test* function in stats package, version 3.5.3) between the model's residuals, ordered along the gradient depicted by the predictor variable(s), excluding the first residual of the series, and the residuals of the previous value along the series. Among all the fitted models, including the model without correlation structure, we selected that with the least correlated residuals (i.e., with the lowest and not statistically significant correlation coefficient).

3. Results

Mown and unmown experimental units differed significantly ($p < 0.001$) in plant height (15.4 vs. 30.9 cm) and aboveground phytomass of *B. rupestre* (1.9 vs. 11.3 g). Descriptive statistics of *B. rupestre* aboveground phytomass and height are shown in Table 1.

Table 1. Descriptive statistics of aboveground phytomass and plant height of *Brachypodium rupestre* in mown and unmown experimental units.

***Brachypodium rupestre* Features**	**Treatment**	**Min.**	**Max.**	**Mean**	**Standard Deviation**
Aboveground phytomass (g)	Unmown	5.40	20.30	11.32	4.27
	Mown	0.60	5.20	1.88	1.11
Plant height (m)	Unmown	0.22	0.47	0.31	0.06
	Mown	0.10	0.25	0.15	0.04

The variation in the three considered factors (management, *Brachypodium* aboveground phytomass and height) affected the frequency (i.e., the number of species sharing the same trait) of most of the traits considered (Table 2). In detail, the frequencies of the following trait states were the most positively influenced only by mowing: annual and perennial life span; presence and absence of vegetative propagation as well as runner and rhizome; presence of belowground storage organs, rhizome and tap root; persistent and summer green leaves; mesomorphic and scleromorphic/mesomorphic green leaves; absence of horizontal architecture and reptant species; erosulate upright forbs; plant height ≤ 20 and between 21–40 cm; seed mass class 0.51–1.00 and 1.01–2.00 mg (Table 2). Instead, other trait states were influenced only by the changes of *B. rupestre* features, whereby the *B. rupestre* aboveground phytomass was more important than plant height. In particular, the traits that were the most negatively influenced by *B. rupestre* aboveground phytomass were root tuber/stem tuber/root splitter; rosulate forbs; pleiocorm species; rosette and hemirosulate upright forbs; and seed mass ≤ 20 mg, while species with succulent leaves were negatively affected by *B. rupestre* plant height (Table 2).

Sedge growth form and spring green leaves were the only trait states positively influenced, besides mowing also by *B. rupestre* features (aboveground phytomass, the former; plant height, the latter, Table 2). Finally, some traits did not respond to any of the considered factors (Table 2).

Table 2. Effect of management (mowing/abandonment), *Brachypodium rupestre* plant height and aboveground phytomass (fixed effects) on the traits of species in the plant community (frequencies). A preliminary model selection was run on the full models fitted using generalized linear mixed-effect models with Poisson error distribution and block identity as the random intercept, through an Akaike Information Criterion-based (AICc) procedure. The reduced models were fitted using penalized quasi-likelihood generalized mixed-effect modelling with the same error distribution and random structure, accounting for autocorrelation of residuals. Model parameters (intercept and fixed effect estimates) were obtained after reduced models refitting using different correlation structures to account for autocorrelation, and selecting the model with the least correlated residuals. The correlation structures are indicated with a superscript. Trait states whose model residuals are in any case correlated are in italics.

Response Variable		Intercept Estimate	Fixed Effect Estimate			AICc	logLik
			Management Mown	*B. rupestre's* Phytomass	*B. rupestre's* Plant Height		
Life span	Annual [a]	−2.3030 ***	2.6027 ***			72.6	−33.0
	Perennial	2.0395 ***	0.6303 ***			219.1	−106.2
Vegetative propagation	Presence of vegetative propagation	1.6653 ***	0.7392 ***			203.2	−98.2
	Absence of vegetative propagation [a]	0.8329 **	0.6257 ***			151.4	−72.3
	Bulbils	−30.3026 n.s.	28.6932 n.s.			28.9	−11.1
	Root tuber/stem tuber/root splitter	0.2574 n.s.		−0.1046 **		86.5	-39.9
	Runner	1.0296 ***	0.7020 ***			160.8	−77.1
	Rhizome	1.5742 ***	0.6776 ***			185.4	−89.4
Storage organs	Presence of belowground storage organ [a]	1.6771 ***	0.8840 ***			214.0	−103.7
	Absence of belowground storage organ [a]	0.9272 ***				136.8	−66.8
	Rhizome	1.4400 ***	0.8507 ***			190.4	−91.9
	Bulb	−7.2559 n.s.	3.9508 n.s.		12.7530 n.s.	45.1	−18.0
	Root tuber/stem tuber/shoot tuber	−2.9957 ***				20.3	−8.0
Leaf persistence	Tap root	0.3498 n.s.	1.1100 ***			153.1	−73.2
	Persistent green leaves	1.3238 ***	0.6391 ***			174.2	−83.8
	Summer green leaves [a]	1.3863 ***	0.7419 ***			178.9	−86.1
	Spring green leaves [b]	−13.9086 **	8.6920 *	0.3188 n.s.	20.1435 *	45.2	−16.7
	Overwintering green leaves [c]	−0.3882 n.s.		−2.2784 n.s.		18.4	−3.3
Leaf anatomy	Succulent leaves [a]	2.1662 n.s.			−18.9194 *	44.5	−18.9
	Succulent/hygromorphic leaves	−30.3026 n.s.	28.4055 n.s.			24.0	-8.7
	Mesomorphic/hygromorphic leaves [a]	−1.7431 ***				45.5	−20.6
	Mesomorphic leaves [a]	0.7885 **	1.1062 ***			166.9	−80.1
	Scleromorphic leaves	0.0953 n.s.				101.4	−48.5
	Scleromorphic/mesomorphic leaves [b]	1.5005 ***	0.4672 ***			174.3	−83.8

Table 2. *Cont.*

Response Variable		Intercept Estimate	Fixed Effect Estimate			AICc	logLik
			Management Mown	*B. rupestre's* Phytomass	*B. rupestre's* Plant Height		
Horizontal space occupation	*Absence of horizontal architecture*	−0.7985 *	1.8101 ***			116.9	−55.1
	Caespitose [a]	1.6363 ***				161.8	−78.8
	Pleiocorm [a]	1.3798 ***		−0.1141 ***		143.0	−68.2
	Reptant [c]	0.7867 ***	0.9151 ***			149.2	−71.3
	Rosulate [a]	1.1130 ***		−0.1698 ***		113.6	−53.4
	Sedge	−8.6652 ***	7.5229 ***	0.3727 ***		52.3	−21.6
Vertical space occupation	Grass [a]	1.5265 ***				157.8	−76.7
	Rosette forb	1.0707 ***		−0.1616 ***		113.3	−53.3
	Hemirosulate upright forb	1.5479 ***		−0.1051 ***		146.2	−69.7
	Erosulate upright forb	0.2383 n.s.	1.1486 ***			138.9	−66.1
	Prostrate forb	−1.2138 n.s.		−0.2321 n.s.		33.9	−13.6
Plant height	≤20 cm [d]	0.1440 n.s.	1.5126 ***			149.7	−71.5
	21–40 cm	1.1800 ***	0.6781 ***			165.3	−79.3
	41–60 cm [a]	1.2025 ***				154.3	−75.0
	81–100 cm	−0.9186 *				67.4	−31.6
Seed mass	≤0.20 mg	1.5283 ***		−0.2310 ***		112.0	−52.6
	0.21–0.50 mg [a]	0.7659 ***				124.5	−60.1
	0.51–1.00 mg	−0.0813 n.s.	1.0986 ***			136.2	−64.8
	1.01–2.00 mg	0.9933 **	0.6457 ***			161.3	−77.3
	2.01–4.00 mg	0.2231 **				97.5	−46.6
	4.01–10.00 mg	−0.1625 n.s.				101.6	−48.7
	>10.00 mg	1.1543 n.s.			−22.0392 n.s.	23.7	−8.5

a—compound symmetry structure corresponding to a constant correlation; b—Gaussian spatial correlation; c—autoregressive process of order 1; d—exponential spatial correlation. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. not significant, $p \geq 0.05$

4. Discussion

Our results were consistent with previous findings on the negative effect of disturbance on *Brachypodium* and, as a consequence, in stopping the successional processes [34,35]. In particular, we observed a significant decrease in both aboveground phytomass and plant height. With regard to this, Canals et al. [19] stated that recurrent defoliation led to the decrease of reserves allocated to the belowground organs, which, in turn, reflected in the reduction of the aboveground phytomass [50] and cover value [35,51] of individuals. Moreover, leaf removal leads to the decrease in leaf area, leaf dry matter and lignin content, besides the increase in leaf nitrogen content, thus improving leaf palatability and enhancing the probability of leaf consumption by herbivores [12].

Regarding the variation in the functional features of the plant community at fine-scale, our results showed that, in general, disturbance and biotic interactions contributed independently to the functional changes, each affecting a specific set of trait states, overlapping only in a few cases.

4.1. Effects of Mowing

Consistently with previous research based on weighted abundance data [29–31], we observed that mowing chiefly affected the set of trait states linked to tolerance. In this sense, the expansion of perennial species, mainly with the ability of vegetative propagation, has proven to be fostered under management conditions [30]. These species can re-occupy the space very fast after mowing, also because they usually have belowground storage organs (tap roots, bulbs, rhizomes), which allow aboveground re-sprouting thanks to the mobilization of stored resources [36]. In addition, mowing affected traits related to avoidance strategies. In fact, we found that mowing promoted small-sized species (lower plant height classes, reptant strategy) [10,32] and fast growth strategies (i.e., annual life span) [34] or early flowering strategies, related to spring green and mesomorphic leaves, as stated by Catorci, et al. [52] for a sub-Mediterranean climate. It was argued that species with fast growth and only sexual reproduction (absence of vegetative propagation, in the study case) complete their life cycles before the growth of the dominant species [53] and the mowing events [54]. Contrariwise, the increase in species with persistent and tougher leaves (i.e., scleromorphic/mesomorphic leaves) is probably linked to the increase in light that reaches the ground level after mowing [27,55]. Finally, it is worth considering that small seed mass (0.51–2.00 mg) was affected positively by recurrent mowing in line with Kahmen, et al. [56], probably suggesting small seeds as by-products of early flowering strategies [57].

Therefore, our results confirmed the predicted shift towards avoidance and tolerance strategies due to mowing activities and a trend towards a more extensive number of strategies, particularly those linked to a temporal niche occupation. The differentiation of temporal niche occupation is a typical feature of disturbed grasslands and was proven to be related to time-specific trait patterns, driven by the interplay of the context dependent environmental conditions and the temporal fluctuation of resources [58].

4.2. Effects of Brachypodium rupestre Features

Regardless of the type of management, both the decrease in aboveground phytomass and plant height of *B. rupestre* influenced the functional composition of the plant community by reducing the number of functional features of the surrounding individuals, being aboveground phytomass (which is somehow a proxy for tussock dimension and indicates the range of horizontal space occupied by *B. rupestre* individuals) were more effective than plant height, maybe because the reduction rate of phytomass from an unmanaged to a mown condition was higher than that of plant height (−83% vs. −52%; see Table 1).

The decreasing value of *B. rupestre* aboveground phytomass also enhanced the increase of rosulate and hemirosulate upright forb species, with the main photosynthetic apparatus near the ground level. Contrariwise, sedge species, which shared a similar growth form with *B. rupestre*, showed

a positive relationship with *B. rupestre* aboveground phytomass. The weakening of competitive exclusion processes within the community seems to also be indicated by the increase in species with succulent leaves (i.e., *Sedum* species), linked to the decrease in *B. rupestre* plant height. Succulent plants have systems to store water inside leaves, so that they can grow and bloom later in the season when, in sub-Mediterranean climate, water shortage affects the systems and species with stored water can use a specific and not so crowded temporal niche for reproduction [59]. Moreover, *Sedum* species are small sized plants that can hardly compete with large and tall tussocks of *B. rupestre*. Analogously, the decreasing *B. rupestre* dominance fostered species with small seeds, which benefit from the reduced competition [60]. These results seemed to suggest that the decrease in dominance ability of *B. rupestre* promoted a shift from processes (weaker competitor exclusion) aimed at decreasing the number of functional strategies to processes (limiting similarity) aimed at increasing the number of functional strategies.

An unexpected finding was that *B. rupestre* features did not influence the height of subordinate species. Indeed, as plant height is a trait related to size-asymmetric competition for light, we would have expected a filtering effect on plant community (i.e., weaker competitor effect), reducing shorter species or enhancing the taller ones [18]. Our results, based on categorical traits data and species absolute frequencies instead of abundances, can probably mask this process.

5. Conclusions

We observed that recurrent mowing acted both directly and through the reduction in size of the dominant tall grass, so that the final results of the recovery processes were due both to the effect of abiotic (spread of tolerance and/or avoidance strategies) and biotic filtering (shift from weaker competitor exclusion to limiting similarity). We observed that the effect of mowing on the functional variation of the community was more effective than that related to plant-plant interactions and that different drivers mostly acted on different trait states.

Such results support the idea that in the process of plant community recovery, multiple assembly rules (abiotic and biotic filtering processes and limiting similarity) act on different trait states [37,61]. Additionally, our results suggested that one of the main effects of disturbance was the weakening of the importance of processes related to dominant species, allowing other species to coexist through the differentiation of spatial and temporal niches and by different strategies in use/acquisition of resources. This involves an increasing progressive adaptation (i.e., efficiency in resource use) of the plant community during the recovery processes to local environment features and resource fluctuation. Finally, our results strongly support previous suggestions that species success under a management regime must be evaluated with a set of different traits [30].

Supplementary Materials: The following are available online at http://www.mdpi.com/1424-2818/12/1/11/s1, Table S1: Management type, *Brachypodium rupestre* phytomass and plant height and observed species presence/absence in experimental units, Table S2: "Observed species -by- trait states" matrix, Table S3: Traits and trait states analysed in the present study, description with data sources and references.

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References

1. Burrascano, S.; Chytrý, M.; Kuemmerle, T.; Giarrizzo, E.; Luyssaert, S.; Sabatini, F.M.; Blasi, C. Current European policies are unlikely to jointly foster carbon sequestration and protect biodiversity. *Biol. Conserv.* **2016**, *201*, 370–376. [CrossRef]
2. Giarrizzo, E.; Burrascano, S.; Chiti, T.; de Bello, F.; Lepš, J.; Zavattero, L.; Blasi, C. Re-visiting historical semi-natural grasslands in the Apennines to assess patterns of changes in species composition and functional traits. *Appl. Veg. Sci.* **2017**, *20*, 247–258. [CrossRef]
3. Malavasi, M.; Carranza, M.L.; Moravec, D.; Cutini, M. Reforestation dynamics after land abandonment: A trajectory analysis in Mediterranean mountain landscapes. *Reg. Environ. Chang.* **2018**, *18*, 2459–2469. [CrossRef]
4. Poschlod, P.; Kiefer, S.; Tränkle, U.; Fischer, S.; Bonn, S. Plant species richness in calcareous grasslands as affected by dispersability in space and time. *Appl. Veg. Sci.* **1998**, *1*, 75–90. [CrossRef]
5. Louault, F.; Soussana, J.F.; Perrodin, M. Long-term effects of a reduced herbage use in a semi-natural grassland: I–plant functional traits and plant response groups. *Grassl. Sci. Eur.* **2002**, *7*, 338–339.
6. Pottier, J.; Evette, A. On the Relationship between Clonal Traits and Small-Scale Spatial Patterns of Three Dominant Grasses and its Consequences on Community Diversity. *Folia Geobot.* **2010**, *45*, 59–75. [CrossRef]
7. de Kroon, H.; Bobbink, R. Clonal plant dominance under elevated nitrogen deposition, with special reference to *Brachypodium pinnatum* in chalk grassland. In *The Ecology and Evolution of Clonal Plants*; de Kroon, H., van Groenendael, J., Eds.; Backhuys Publishers: Leiden, The Netherlands, 1997; pp. 359–379.
8. Tardella, F.M.; Bricca, A.; Piermarteri, K.; Postiglione, N.; Catorci, A. Context-dependent variation of SLA and plant height of a dominant, invasive tall grass (*Brachypodium genuense*) in sub-Mediterranean grasslands. *Flora* **2017**, *229*, 116–123. [CrossRef]
9. Bonanomi, G.; Allegrezza, M. Effetti della colonizzazione di Brachypodium rupestre (Host) Roemer et Schultes sulla diversità di alcune fitocenosi erbacee dell'Appennino centrale. *Fitosociologia* **2004**, *41*, 51–69.
10. Catorci, A.; Ottaviani, G.; Ballelli, S.; Cesaretti, S. Functional differentiation of Central Apennine grasslands under mowing and grazing disturbance regimes. *Pol. J. Ecol.* **2011**, *59*, 15–128.
11. Vitasović Kosić, I.; Tardella, F.M.; Grebeša, D.; Skovrc, Z.; Catorci, A. Effects of abandonment on the functional composition and forage nutritive value of a north Adriatic dry grassland community (Ćićarija, Croatia). *Appl. Ecol. Env. Res.* **2014**, *12*, 285–299. [CrossRef]
12. Catorci, A.; Antolini, E.; Tardella, F.M.; Scocco, P. Assessment of interaction between sheep and poorly palatable grass: A key tool for grassland management and restoration. *J. Plant Interact.* **2014**, *9*, 112–121. [CrossRef]
13. Suding, K.N.; Gross, K.L.; Houseman, G.R. Alternative states and positive feedbacks in restoration ecology. *Trends Ecol. Evol.* **2004**, *19*, 46–53. [CrossRef] [PubMed]
14. Suding, K.N.; Hobbs, R.J. Threshold models in restoration and conservation: A developing framework. *Trends Ecol. Evol.* **2009**, *24*, 271–279. [CrossRef] [PubMed]
15. Grime, J.P. Trait convergence and trait divergence in herbaceous plant communities: Mechanisms and consequences. *J. Veg. Sci.* **2006**, *17*, 255–260. [CrossRef]
16. Chesson, P. Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **2000**, *31*, 343–366. [CrossRef]
17. Mayfield, M.M.; Levine, J.M. Opposing effects of competitive exclusion on the phylogenetic structure of communities. *Ecol. Lett.* **2010**, *13*, 1085–1093. [CrossRef] [PubMed]
18. Lepš, J. Scale-and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment. *J. Appl. Ecol.* **2014**, *51*, 978–987. [CrossRef]
19. Canals, R.M.; San Emeterio, L.; Durán, M.; Múgica, L. Plant-herbivory feedbacks and selective allocation of a toxic metal are behind the stability of degraded covers dominated by Brachypodium pinnatum in acidic soils. *Plant Soil* **2017**, *415*, 373–386. [CrossRef]
20. Scocco, P.; Mercati, F.; Brusaferro, A.; Ceccarelli, P.; Belardinellli, C.; Malfatti, A. Keratinisation degree of rumen epithelium and body condition score in sheep grazing on Brachypodium rupestre. *Vet. Ital.* **2013**, *49*, 211–217.
21. Scocco, P.; Mercati, F.; Tardella, F.M.; Catorci, A. Increase of forage dryness induces differentiated anatomical response in the sheep rumen compartments. *Microsc. Res. Tech.* **2016**, *79*, 738–743. [CrossRef]

22. Oroian, S.; Sămărghiţan, M.; Popa, R.; Calalb, T. The conservation status of grasslands habitats identified in the "Hârtibaciu-Târnava Mare-Olt" Natura 2000 site. *Contrib. Bot.* **2014**, *49*, 179–189.
23. Tälle, M.; Bergman, K.O.; Paltto, H.; Pihlgren, A.; Svensson, R.; Westerberg, L.; Wissman, L.; Milberg, P. Mowing for biodiversity: Grass trimmer and knife mower perform equally well. *Biodivers. Conserv.* **2014**, *23*, 3073–3089. [CrossRef]
24. Köhler, B.; Gigon, A.; Edwards, P.J.; Krüsi, B.; Langenauer, R.; Lüscher, A.; Ryser, P. Changes in the species composition and conservation value of limestone grasslands in Northern Switzerland after 22 years of contrasting managements. *Persp. Plant Ecol. Evol. System.* **2005**, *7*, 51–67. [CrossRef]
25. Overbeck, G.; Kiehl, K.; Abs, C. Seedling recruitment of *Succisella inflexa* in fen meadows: Importance of seed and microsite availability. *Appl. Veg. Sci.* **2003**, *6*, 97–104. [CrossRef]
26. Ilmarinen, K.; Mikola, J. Soil feedback does not explain mowing effects on vegetation structure in a semi-natural grassland. *Acta Oecol.* **2009**, *35*, 838–848. [CrossRef]
27. Collins, S.L.; Knapp, A.K.; Briggs, J.M.; Blair, J.M.; Steinauer, E.M. Modulation of diversity by grazing and mowing in native tallgrass prairie. *Science* **1998**, *280*, 745–747. [CrossRef]
28. Hautier, Y.; Niklaus, P.A.; Hector, A. Competition for light causes plant biodiversity loss after eutrophication. *Science* **2009**, *324*, 636–638. [CrossRef]
29. Huhta, A.-P.; Rautio, P. Evaluating the impacts of mowing: A case study comparing managed and abandoned meadow patches. *Ann. Bot. Fenn.* **1998**, *35*, 85–99.
30. Klimešová, J.; Latzel, V.; de Bello, F.; van Groenendael, J.M. Plant functional traits in studies of vegetation changes in response to grazing and mowing: Towards a use of more specific traits. *Preslia* **2008**, *80*, 245–253.
31. Valkó, O.; Török, P.; Matus, G.; Tóthmérész, B. Is regular mowing the most appropriate and cost-effective management maintaining diversity and biomass of target forbs in mountain hay meadows? *Flora* **2012**, *207*, 303–309. [CrossRef]
32. Halassy, M.; Botta-Dukát, Z.; Csecserits, A.; Szitár, K.; Török, K. Trait-based approach confirms the importance of propagule limitation and assembly rules in old-field restoration. *Restor. Ecol.* **2019**, *27*, 840–849. [CrossRef]
33. Socher, S.A.; Prati, D.; Boch, S.; Müller, J.; Baumbach, H.; Gockel, S.; Hemp, A.; Schöning, I.; Wells, K.; Buscot, F.; et al. Interacting effects of fertilization, mowing and grazing on plant species diversity of 1500 grasslands in Germany differ between regions. *Basic Appl. Ecol.* **2013**, *14*, 126–136. [CrossRef]
34. Bonanomi, G.; Sicurezza, M.G.; Caporaso, S.; Esposito, A.; Mazzoleni, S. Phytotoxicity dynamics of decaying plant materials. *New Phytol.* **2006**, *169*, 571–578. [CrossRef] [PubMed]
35. Tardella, F.M.; Malatesta, L.; Goia, I.G.; Catorci, A. Effects of long-term mowing on coenological composition and recovery routes of a *Brachypodium rupestre*-invaded community: Insight into the restoration of sub-Mediterranean productive grasslands. Rendiconti Lincei. *Sci. Fis. Nat.* **2018**, *29*, 329–341. [CrossRef]
36. Grime, J.P. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd ed.; Wiley: Chichester, UK, 2001.
37. de Bello, F.; Vandewalle, M.; Reitalu, T.; Lepš, J.; Prentice, H.C.; Lavorel, S.; Sykes, M.T. Evidence for scale-and disturbance-dependent trait assembly patterns in dry semi-natural grasslands. *J. Ecol.* **2013**, *101*, 1237–1244. [CrossRef]
38. Bricca, A.; Conti, L.; Tardella, M.F.; Catorci, A.; Iocchi, M.; Theurillat, J.P.; Cutini, M. Community assembly processes along a sub-Mediterranean elevation gradient: Analyzing the interdependence of trait community weighted mean and functional diversity. *Plant Ecol.* **2019**, *220*, 1139–1151. [CrossRef]
39. Cingolani, A.M.; Cabido, M.; Gurvich, D.E.; Renison, D.; Díaz, S. Filtering processes in the assembly of plant communities: Are species presence and abundance driven by the same traits? *J. Veg. Sci.* **2007**, *18*, 911–920. [CrossRef]
40. Rivas-Martínez, S.; Rivas Sáenz, S.; Penas, A. Worldwide bioclimatic classification system. *Glob. Geobot.* **2011**, *1*, 1–634.
41. Catorci, A.; Cesaretti, S.; Gatti, R. Biodiversity conservation: Geosynphytosociology as a tool of analysis and modelling of grassland systems. *Hacquetia* **2009**, *8/2*, 129–146. [CrossRef]
42. Soil Survey Staff. *Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys*, 2nd ed.; United States Department of Agriculture Handbook 436; Natural Resources Conservation Service: Washington, DC, USA, 1999; p. 869.
43. Catorci, A.; Ottaviani, G.; Kosić, I.V.; Cesaretti, S. Effect of spatial and temporal patterns of stress and disturbance intensities in a sub-Mediterranean grassland. *Plant Biosyst.* **2012**, *146*, 352–367. [CrossRef]

44. Pérez-Harguindeguy, N.; Díaz, S.; Garnier, E.; Lavorel, S.; Poorter, H.; Jaureguiberry, P.; Bret-Harte, M.S.; Cornwell, W.K.; Craine, J.M.; Gurvich, D.E.; et al. New handbook for standardised measurement of plant functional traits worldwide. *Austral. J. Bot.* **2013**, *61*, 167–234. [CrossRef]
45. Bolker, B.M.; Brooks, M.E.; Clark, C.J.; Geange, S.W.; Poulsen, J.R.; Stevens, M.H.H.; White, J.S.S. Generalized linear mixed models: A practical guide for ecology and evolution. *Trends Ecol. Evol.* **2009**, *24*, 127–135. [CrossRef]
46. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2019.
47. Burnham, K.P.; Anderson, D.R. *Model Selection and Multimodel Inference*; Spinger: New York, NY, USA, 2002.
48. Dormann, C.F.; McPherson, J.M.; Araújo, M.B.; Bivand, R.; Bolliger, J.; Carl, G.; Davies, R.G.; Hirzel, A.; Jeta, W.; Kissling, D.; et al. Methods to account for spatial autocorrelation in the analysis of species distributional data: A review. *Ecography* **2007**, *30*, 609–628. [CrossRef]
49. Package 'nlme'. Available online: https://cran.r-project.org/web/packages/nlme/nlme.pdf (accessed on 15 August 2019).
50. Endresz, G.; Zöld-Balogh, Á.; Kalapos, T. Local distribution pattern of Brachypodium pinnatum (*Poaceae*)-Field Experiments in xeric loess Grassland in N. Hungary. *Phyton* **2005**, *45*, 249–265.
51. Bonanomi, G.; Caporaso, S.; Allegrezza, M. Short-term effects of nitrogen enrichment, litter removal and cutting on a Mediterranean grassland. *Acta Oecol.* **2006**, *30*, 419–425. [CrossRef]
52. Catorci, A.; Cesaretti, S.; Tardella, F.M. Effect of tall-grass invasion on the flowering-related functional pattern of submediterranean hay-meadows. *Plant Biosyst.* **2014**, *148*, 1127–1137. [CrossRef]
53. Catorci, A.; Carotenuto, L.; Gatti, R. Flowering patterns in sub-Mediterranean grasslands: A functional approach. *Plant Ecol. Evol.* **2012**, *145*, 165–175. [CrossRef]
54. Fahrig, L.; Coffin, D.P.; Lauenroth, W.K.; Shugart, H.H. The advantage of long-distance clonal spreading in highly disturbed habitats. *Evol. Ecol.* **1994**, *8*, 172–187. [CrossRef]
55. Huhta, A.P.; Rautio, P.; Tuomi, J.; Laine, K. Restorative mowing on an abandoned semi-natural meadow: Short-term and predicted long-term effects. *J. Veg. Sci.* **2001**, *12*, 677–686. [CrossRef]
56. Kahmen, S.; Poschlod, P.; Schreiber, K.F. Conservation management of calcareous grasslands. Changes in plant species composition and response of functional traits during 25 years. *Biol. Conserv.* **2002**, *104*, 319–328. [CrossRef]
57. Völler, E.; Auge, H.; Prati, D.; Fischer, M.; Hemp, A.; Bossdorf, O. Geographical and land-use effects on seed-mass variation in common grassland plants. *Basic Appl. Ecol.* **2012**, *13*, 395–404. [CrossRef]
58. Catorci, A.; Piermarteri, K.; Penksza, K.; Házi, J.; Tardella, F.M. Filtering effect of temporal niche fluctuation and amplitude of environmental variations on the trait-related flowering patterns: Lesson from sub-Mediterranean grasslands. *Sci. Rep.* **2017**, *7*, 12034. [CrossRef]
59. Catorci, A.; Cesaretti, S.; Gatti, R.; Tardella, F.M. Trait-related flowering patterns in submediterranean mountain meadows. *Plant Ecol.* **2012**, *213*, 1315–1328. [CrossRef]
60. Leishman, M.R.; Wright, I.J.; Moles, A.T.; Westoby, M. The evolutionary ecology of seed size. In *Seeds: The Ecology of Regeneration in Plant Communities*; Fenner, M., Ed.; CABI International: Oxon, UK, 2000; pp. 31–47.
61. Lhotsky, B.; Kovács, B.; Ónodi, G.; Csecserits, A.; Rédei, T.; Lengyel, A.; Kertéz, M.; Botta-Dukát, Z. Changes in assembly rules along a stress gradient from open dry grasslands to wetlands. *J. Ecol.* **2016**, *104*, 507–517. [CrossRef]

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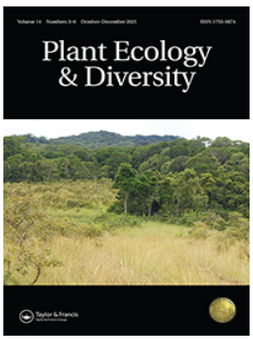

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Environmental heterogeneity compensates the potential homogenising effect of abandonment of grazing in a sub-Mediterranean mountain landscape

Alessandro Bricca, Federico M. Tardella, Arianna Ferrara, Xia Xinfang, Fabio Tolu & Andrea Catorci

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Environmental heterogeneity compensates the potential homogenising effect of abandonment of grazing in a sub-Mediterranean mountain landscape

Alessandro Bricca[a], Federico M. Tardella[b], Arianna Ferrara[a], Xia Xinfang[a], Fabio Tolu[c] and Andrea Catorci[a]

[a]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [b]Herbarium Universitatis Camerinensis, School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [c]School of Advanced Studies, University of Camerino, Camerino, Italy

ABSTRACT

Background: Anthropogenic mountain grasslands undergo major changes in their composition and structure after abandonment of their use as pastures or hay meadows. Distinct plant community-types may develop in habitats that differ in environmental conditions, such as soil water availability; however, few studies have addressed this issue in abandoned anthropogenic grasslands.

Aims: To quantify the effects of topographic factors and variables related to soil water availability on α and β taxonomic diversity (TD) and functional diversity (FD) to assess the role of landform heterogeneity in shaping vegetation structure.

Methods: We classified the vegetation of long-abandoned central Apennine pastures and related their species composition and diversity to environmental variables.

Results: We identified four community-types whose distribution was related to topographically determined soil water availability. The species composition of drought-tolerant communities on south-eastern slopes with shallow soils was largely similar to that of anthropogenic grasslands. Resource-poor conditions were related to enhanced α-TD and β-TD and reduced α-FD for traits related to temporal niche differentiation and resource acquisition strategies.

Conclusion: In long-abandoned sub-Mediterranean grasslands, landform heterogeneity guarantees a certain level of TD and FD at the landscape scale, contrasting the assumed role of abandonment of grazing on homogenisation of vegetation features.

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α-diversity; β-diversity; disturbance; functional diversity; grassland abandonment; taxonomic diversity

Introduction

European anthropogenic dry grasslands are ecosystems characterised by a rich flora (Dengler et al. 2014; Chytrý et al. 2015), hosting most of the species included in Europe in the threatened categories of the International Union for Conservation of Nature (EEA 2010). Most of these grasslands owe their existence to long-term continued management (i.e., grazing, cutting for hay and manuring) that has allowed their maintenance (Dengler et al. 2014). However, land use change that has taken place after the Second World War have had a large impact on these grasslands (Lasanta et al. 2017). Both land use intensification and their abandonment or reduced use have resulted in major changes in their composition and structure (Poptcheva et al. 2009; Peco et al. 2012). The Mediterranean mountains in particular have been affected by large-scale abandonment (Mazzoleni et al. 2004). As many of their grasslands are anthropogenic, their maintenance depends on the continuation of traditional land use activities (Dengler et al. 2014).

In the absence of disturbance that results from pasture and hay meadow management, the grasslands may revert to shrubland or forest (Bracchetti et al. 2012). However, woody species encroachment can be slow (e.g., Kahmen and Poschlod 2004; Köhler et al. 2005) or species composition can be stabilised after a rapid initial change, probably because of the absence of late-successional species in the neighbourhood or in the seedbank, preventing further changes (Marriott et al. 2009).

In general, impacts of disturbance on the species richness of grasslands seem to be in line with the intermediate disturbance hypothesis, IDH (Connell 1978). This hypothesis, albeit controversial (Fox 2013), implies that local diversity is greatest when disturbance is neither absent nor too intense/frequent, i.e., at intermediate levels of photosynthetic tissue removal intensities (Kaltsas et al. 2013). Recently, the IDH has been reformulated as the

CONTACT Alessandro Bricca alessandro.bricca@unicam.it
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'land use-moderated conservation effectiveness hypothesis' (Kleijn and Sutherland 2003; Kleijn et al. 2011), which asserts that species diversity can be maximised under moderate-intensity management. In addition to disturbance, environmental stress is another key driver that affects species richness of grassland communities. Thus, similarly to disturbance, the highest species richness is expected under intermediate levels of stress intensity (Grime 2001). Overall, in the absence of disturbance and in the presence of non-limiting resource availability, competitive species become dominant and tend to exclude weaker competitors from the community (i.e., weaker competitor exclusion; Lepš 2014) resulting in a decrease of plant diversity (Grime 2001). On the contrary, in the long run, frequent or high-intensity disturbance, as well as limiting resources (environmental stress), reduce competitive ability and thus enhance species diversity at the community level (Grime 2001).

Grazing is the most important disturbance that affects processes of plant community assembly (i.e., how species coexist within the community; de Bello et al. 2012) in grasslands globally (Diaz et al. 2007). Plants respond to herbivory both by compensating (tolerance strategy) and minimising (avoidance strategy) the loss of tissue (Briske 1996); however, the relative importance of these strategies is related to site productivity (Silvertown et al. 1994). In the presence of resource limitation (e.g., drought), tolerance strategies to grazing disturbance are less suitable than avoidance, because there are no sufficient resources for tissue lost due to herbivory to regrow (Maschinsky and Whitham 1989). As a result, graminoids, which are among the most preferred foods of large herbivores (O'Connor and Everson 1998) can be reduced in abundance by grazing in water-limited environments; the exclusion of grazing appears to increase the abundance of grasses (Catorci et al. 2013; Giarrizzo et al. 2017). Therefore, the species composition of grasslands strongly depends on the relationship between resource availability and disturbance intensity (Grime 2001).

All the above-mentioned considerations are especially pertinent for the sub-Mediterranean region, a transition zone between the Mediterranean and temperate bioclimatic regions (Pesaresi et al. 2017). This area is typically characterised by an alternation of cold winters (typical of temperate region) and dry summers (typical of Mediterranean region), modulated by elevation and landform factors (i.e., topographic factors; Somot et al. 2008). Topographic variables have proven to be effective predictors of species composition at the local scale, particularly in view of their relationship with microclimatic conditions (Burrascano et al. 2013) and their influence on soil water availability (Scocco et al. 2016). Topographic variables also proved to be good predictors of functional-type structure of communities (Tardella et al. 2016; Di Biase et al. 2021).

The abandonment of grazing, in general, has led to an increase in tall grass cover, which in turn has caused a drop in species diversity at the plot scale (α-diversity, e.g. Peco et al. 2006; Lepš 2014; Malatesta et al. 2019).

However, the topographic diversity of mountain landscapes results in a complex mosaic of different plant communities, and abandonment of pasturing may thus result in spatially different responses in community composition (Catorci et al. 2013; Tardella and Catorci 2015). Theoretically, in non-resource-limited conditions, competition by tall grass species may lead to high species turnover at the landscape scale and a reduction in species diversity (Catorci et al. 2013; Lepš 2014), whereas in resource-limited communities, competition is likely to be of less importance or intensity (Keddy 1992; Bertness and Callaway 1994; Brooker 2006) and little species turnover is expected (Bennie et al. 2006). Moreover, in semi-natural communities, the dominance of competitive species over wide areas in the absence of disturbance leads to declines in spatial β–diversity and to biotic homogenisation (Uchida et al. 2015).

The studies that have investigated the abandonment of Apennine grasslands focused on temporal changes in α-taxonomic diversity or on differences in α-functional diversity between grazed and abandoned grasslands. Catorci et al. (2013), assessing the changes in a grassland grazed until the 1980s (relevés recorded during the period of 1976–1980) after abandonment (resampling made in 2010), have found an increase in species richness and a decrease in evenness. Tardella and Catorci (2015) have compared grazed and abandoned grasslands, finding lower functional diversity in abandoned grasslands. Studies that analyse simultaneously within-plot (α) and between-plot (β) diversity, are of great importance to better understand the diversity patterns within the different community-types after abandonment.

In consideration of above and that the response to abandonment differs depending on the productivity of the grassland system

(Catorci et al. 2013; Janeček et al. 2013; Carmona et al. 2015), we studied the **α**- and β-diversity of plant community types in pastureland that has been abandoned for 50 years, using landform factors as proxies of a productivity (i.e., resource availability) gradient. We hypothesised that abandonment does not level off the differences in vegetation composition and structure (α- and β-taxonomic and functional diversity) between resource-limited vs. less resource-limited habitats and that landform diversity contrasts with the assumed role of abandonment of grazing on homogenisation of vegetation features.

To test our hypothesis, the main questions were as follows: i) which plant community types develop in a long-abandoned grassland system and what are the differences in species composition and ecological conditions across community types? ii) what are the differences in vegetation structure (α- and β-taxonomic and functional diversity) across community types? iii) which are the environmental drivers of α- and β-taxonomic and functional diversity?

Material and methods

Study area

The study was made in the Montagna di Torricchio Nature Reserve (MTNR; 337398 E, 4757200 N, UTM-WGS84), central Apennines, Italy (Figure 1). It covered an area of about 350 ha along a south-west to north-east oriented valley, from 800 to 1400 m a.s.l. The MTNR has been under strict protection since 1970, when grazing by livestock was excluded. Traditionally, grasslands were grazed by flocks of sheep and goats between late spring and early autumn; accessible areas were cut for hay in early summer (Catorci et al. 2009). In the last decades, few horses entered the reserve occasionally, exerting a very light disturbance. Wild grazers such as red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), and wild boar (*Sus scrofa*) are present in the Reserve in relatively low densities, thus their impact is by far less than the past disturbance caused by domestic livestock. In the last 50 years the area occupied by grasslands did not change significantly (Francalancia 1976, 1981; Venanzoni and Kwiatowski 1994). Regarding the composition of the pastures prior to the site become part of the MTNR, no data are available. There are

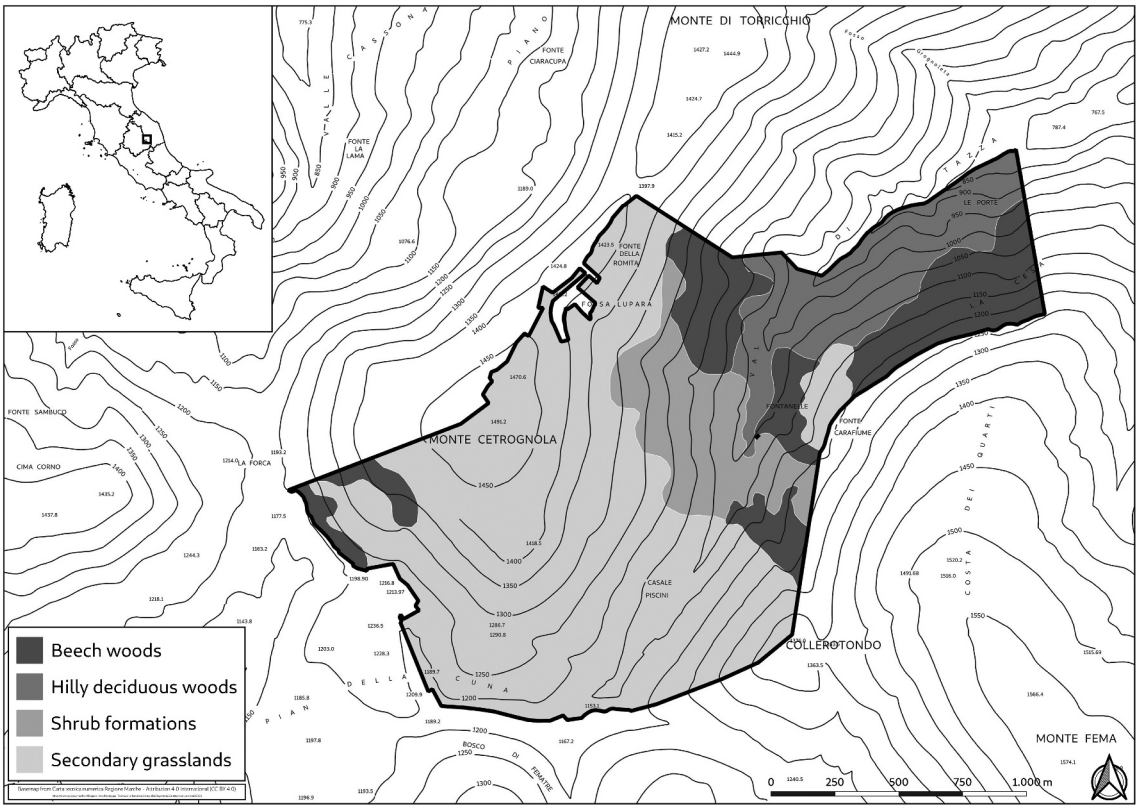

Figure 1. Map of the study site in the Montagna di Torricchio Nature Reserve, central Apennines, Italy, where grasslands between 1,150 and 1,350 m a.s.l. on the south-east and north-west facing slopes were studied.

data from four plots of hay-meadows (surveyed in 1976 and in 1992; Campanulo glomeratae – Cynosuretum cristati Francalancia et al. 1994, 1995) and from two 1-ha plots in *Seslera nitida*-dominated grasslands (Seslerio nitidae – Brometum erecti association; Ivan et al. 1994).

Grazed grasslands in the Reserve belong to the Cytiso spinescentis – Bromion erecti alliance, including sub-Mediterranean xeric calciphilous open grasslands in the submontane and lower montane belts of the central and southern Apennines (see Mucina et al. 2016), whose distribution is related to landform and elevation (see Catorci et al. 2009). They are included as habitat '6210* Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (*important orchid sites)', dominated or co-dominated by *Bromopsis erecta* (Huds.) Fourr., in the Habitats Directive (92/43/EEC).

The climate is sub-Mediterranean (*sensu* Rivas-Martínez et al. 2011). The mean annual rainfall is about 1,100 mm and the average annual temperature 10–11°C (Catorci et al. 2021). The average summer precipitation was 177 mm in the period 1971–2000 and 168 mm in the years 2001–2015 (Catorci et al. 2021). The soil is neutral to sub acidic, and its depth ranges from 10 cm (steep south-facing slopes) to over 100 cm (flat valley bottoms). Summer soil water deficit is usually present on both south- and north-facing slopes. South-facing slopes show a longer period of negative water balance (from the beginning of June to the end of September) and are covered by xeric plant communities rich in annual and chamaephytic species (Catorci et al. 2012; Scocco et al. 2016). North-facing slopes have a shorter and less intense soil water shortage from early/mid-July to the end of August and are covered by semi-mesophylous communities dominated by hemicryptophytes (Catorci et al. 2012; Scocco et al. 2016). Valley bottoms have hay-meadows dominated by hemicryptophytes (Catorci et al. 2012; Scocco et al. 2016). Aboveground primary production is characteristically bimodal, with the main peak in end of June – early July and a secondary peak in autumn, with a stagnation of the regrowth capacity in summer, sometimes associated with the drying of the grass cover (Cesaretti et al. 2009). In grazed systems, the average phytomass production at its peak, particularly low in south-facing slopes, i.e., ca. 50–110 g m^{-2} (Catorci et al. 2009; Cesaretti et al. 2009; Scocco et al. 2016), is comparable with the average values of Mediterranean grasslands (Smit et al. 2008). The peak phytomass ranges from 130 to 240 g m^{-2} on north-facing slopes and mountain tops (Catorci et al. 2009: Cesaretti et al. 2009; Scocco et al. 2016), in line with the average values of sub-Mediterranean grasslands (Smit et al. 2008). Only in mown hay-meadows of flat valley bottoms, not considered in this study, the peak phytomass ranges from 300 to 500 g m^{-2} (Catorci et al. 2009; Cesaretti et al. 2009; Scocco et al. 2016).

Data collection

We limited our study to the elevation range between 1150 and 1350 m to encompass the same elevation range on the south and north slopes of the valley studied. Grasslands on east -south-east- to south-east-facing slopes belong to the Scutellario columnae – Ostryo carpinifoliae sigmetum (in which *Ostrya carpinifolia* woodlands constitute the potential vegetation), while those on north-west-facing slopes belong to the Lathyro veneti – Fago sylvaticae sigmetum (in which *Fagus sylvatica* woodlands are the potential vegetation; Venanzoni and Kwiatowski 1994). In our sampling design, we considered the two opposite slope categories as two distinct units. To obtain a sample as much representative as possible, we divided the elevation range into two strata, i.e., 1,150–1,250 m, with average slope angles of 15–20°, and 1,251–1,350 m, with average slope angles of 20–30°. Thus, we distinguished four main sampling units: east-south-east- to south-east-facing areas between 1,150 and 1,250 m (Unit 1); east-south-east- to south-east-facing areas between 1,251 and 1,350 m (Unit 2); north-west-facing areas between 1,150 and 1,250 m (Unit 3); and north-west-facing areas between 1,251 and 1,350 m (Unit 4). Using a proportional stratified random sampling design and random points generated in Q-GIS software (QGIS Development Team 2019), we selected the number of plots (5 m × 5 m) proportional to the extent of each unit. We discarded plots falling on rocky outcrops, pathways, beech copses (ancient groups of centuries-old beech trees once used to shelter the herds in the hottest hours of the day), or heterogeneous in slope angle and aspect. We sampled a total of 52 plots (24 plots in Unit 1; 12 plots in Unit 2; 8 plots in Unit 3; 8 plots in Unit 4).

During the growing season of 2019 (from mid-June to early July), we recorded in each plot the cover of each vascular species (percent values, visually estimated), slope angle (vertical degree), aspect (azimuth degrees), elevation (m. a.s.l.), soil

depth (average of five measurements per plot), cover of litter, bare soil, debris and outcropping rock (percent values visually estimated). Finally, we clipped the above-ground phytomass at 0.5–1.0 cm from ground level, oven-dried and weighed it (g m^{-2} of dry matter). We harvested above-ground phytomass in an area of 0.5 m × 0.5 m in the lower left-hand corner of each plot; the harvested plot size is considered suitable for herbaceous vegetation in grasslands (Garnier et al. 2004). Species nomenclature, which followed Bartolucci et al. (2018) and Del Guacchio and Caputo (2020), was checked using the Portal of the Flora of Italy (available at http:/dryades.units.it/floritaly, accessed 15/07/2021).

To characterise the ecological conditions of the plant community types, we assigned to each species the Ellenberg Indicator Values (EIV; Ellenberg 1991) adapted to the Italian flora (Pignatti 2005): light intensity, air temperature, continentality, soil moisture, soil reaction and soil nutrients.

The functional dimension of the plant communities was characterised by assigning to each species a set of traits that represent different functions (Table S1), namely: plant height (H) – size-asymmetric competition; specific leaf area (SLA) – resource exploitation strategies; leaf phenology (LP) (Klotz et al. 2002) and flowering phenology (FP) – temporal resource exploitation strategies; vegetative propagation (VP) – spatial propagation (Klimešová et al. 2017); storage organ (SO) – resource conservation strategy (Klimešová et al. 2017); horizontal (HA) and vertical (VA) plant architecture – space occupation. For quantitative traits (H and SLA), we considered only the most abundant species, i.e., species whose relative cumulative cover (in decreasing order of abundance) reached 80% of the sum of species cover (Pakeman and Quested 2007). Whereas for qualitative categorical traits, since a species may show multiple states, setting to 1 the sum of the trait scores, each state was assigned a fractional score, i.e., 1/number of states (Pakeman 2004). For example, *Brachypodium rupestre,* which showed both short and long vegetative propagation, was attributed a score of 0.5 for each trait state. Data on SLA and H were drawn from the LEDA database (Kleyer et al. 2008). HA, VA, LP, and FP were drawn from Bricca et al. (2020) and Tardella et al. (2020) and implemented by visual inspection of the specimens in the *Herbarium Universitatis Camerinensis* (CAME). Data on VP and SO were adapted from the CLO-PLA database (Klimešová et al. 2017).

Statistical analyses

Identification and ecological characterisation of plant community-types

To identify the plant communities, we ran a cluster analysis using the complete linkage method on the similarity matrix, based on Euclidean distance, calculated on the Hellinger-transformed species data matrix (plot × species cover expressed in percentage values) (Borcard et al. 2011). Such combination is suitable for data containing many zeros being not influenced by double zeros and having as upper limit the $\sqrt{2}$ (Legendre and Legendre 2012).

To identify the indicator species of the clusters emerged from cluster analysis, we undertook an Indicator Species Analysis (ISA) on the 'plot × species cover' matrix and the plot membership in the clusters was treated as grouping factor (Dufrêne and Legendre 1997). We tested the statistical significance ($P < 0.05$) of the observed maximum indicator value (IV) using permutation tests with 999 runs, and we discarded from the list of significant species, those species whose component of relative abundance was lower than 0.6 or whose component of relative frequency was lower than 0.25 (de Cáceres et al. 2012). For each species highlighted by the ISA, we attributed the phytosociological placement following Mucina et al. (2016) and Biondi and Blasi (2016), and the indication of the habitat where it mostly grows in the central Apennines (Biondi et al. 1995).

All statistical analyses were made in R software, version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org). Cluster analysis was carried out using the *hclust* function while Hellinger transformation was made with *decostand*, both of the 'vegan' package (version 2.5-4). ISA was made with the *multipatt* function ('indicspecies' package, version 1.7.9).

To assess the variation in the ecological features of plant communities, we calculated the community unweighted mean values (CM) of EIV for each plot using presence/absence species data according to the following formula:

$$CM = \sum_{i=1}^{S} p_i x_i \quad (1)$$

where CM is the community unweighted mean value of a given EIV, S is the number of species, p_i is the relative abundance of species i (i = 1,2, . . ., S), and x_i is the Ellenberg indicator value for species i. We evaluated if the CM of EIV differed significantly among the clusters of the classification performing separate ANOVAs for each of the CM of EIV, followed by Tukey post-hoc tests. However, since the use of observed EIV inflates the Type I Error (Zelený and Schaffers 2012), we compared the observed adjusted R^2 of the ANOVA model to the adjusted R^2 of ANOVA models expected by chance, as suggested by Zelený (2018). This approach involved two independent randomisation procedures (one shuffling 999 times EIV across species and one shuffling 999 times the membership of each plot to the clusters) and the distributions of expected adjusted R^2 were compared to the observed adjusted R^2 values, separately for each randomisation. Type I Error is controlled only when observed adjusted R^2 values were significantly higher for both expected adjusted R^2 distributions (one-tailed t-test with α = 0.05) (for more details, see Zelený 2018).

Then, we carried out a Redundancy analysis (RDA) to relate environmental variables, i.e., elevation, aspect, slope angle, soil depth, bare soil cover, debris cover, and outcropping rock cover, to plant community species composition. Prior to running the RDA, we converted the aspect azimuth from the 0–360 compass scale to a linear scale (0–180) following a north-south gradient, with higher values towards the south and lower values towards the north (Warren 2008). We applied the Hellinger transformation on the 'plot × species cover' matrix (dependent variable matrix). We checked for possible multicollinearities of the predictors in the RDA model computing the variance inflation factors (VIF) of the variables and since none of the predictors showed any collinearity (VIF <4; Petraitis et al. 1996), we kept all of them in the RDA model (Borcard et al. 2011). The significances of the RDA model, of each predictor and of each axis, were assessed using permutation tests (999 iterations).

We characterised the clusters of the dendrogram with descriptive statistics (mean and standard deviation) for each measured environmental variable, and we tested the significance of differences running ANOVA models followed by Tukey's post-hoc tests.

RDA, Hellinger transformation and VIF analysis, were made with the *rda* (scaling = 2), the *decostand*, and the *vif.cca* functions ('vegan' package, version 2.5-4). The significance of the RDA models, of each predictor and the axis was tested using the *anova.cca* function ('vegan' package, version 2.5-4), using the *test.cwm* function (method aov, test max) in the 'weimea' package provided by Zelený (2020); the Tukey post-hoc test using the *TukeyHSD* function ('stats' package, version 3.5.2).

Taxonomic and functional α and β diversity of plant community types

To characterise and assess the variation between plant communities in taxonomic diversity (TD) and functional diversity (FD) at different scales (α, β), we used Rao's Quadratic Entropy (Q), which provides a robust framework to compare different aspects of diversity (de Bello et al. 2010).

The α-diversity is calculated as follows:

$$\alpha Q = \sum_{i,j}^{S} d_{ij} p_i p_j \quad (2)$$

where S is the number of species, d_{ij} is the distance or dissimilarity between the i-th and j-th species, p_i and p_j are the proportions of i-th or j-th species in the sampling unit (plot). Taxonomic diversity (α TD) assumes that the distance between the i-th and j-th species is fixed (d_{ij} = 1 for all pairs of species) unless $i = j$ (thus, d_{ij} = 0). In this case,α TD corresponds to 1-Simpson (Ricotta 2005). Similarly, for α FD, d_{ij} may vary from 0 (two species have the same trait values or trait state combination) and 1 (two species have completely different trait values or trait state combination). We used the Gower distance as a measure of distance for FD because it provides a standardisation of distances between 0 and 1 (Pavoine et al. 2009; de Bello et al. 2010). Then, we applied Jost's correction (Jost 2006) to α-diversity values for both taxonomic and functional diversity (de Bello et al. 2010) and we assessed differences in α-diversity among clusters that resulted from the dendrogram, by using ANOVAs, followed by Tukey post-hoc tests.

To calculate the β-diversity, we used the additive partitioning (that is, γ = mean α + β). However, to avoid misleading values of β-diversity we used the following steps as recommended by Villéger and Mouillot (2008) and by de Bello et al. (2010). First,

we calculated the mean α-diversity for each pair of plots of entire dataset considering both the weighted factor (f_k) (Villéger and Mouillot 2008) and Jost's correction. Weighted factor (f_k) corresponds to the relative contribution of the cover of each plot in the region, i.e., the cover of each plot divided by the total cover of plots in the region (which in our case equals each pair of plots). Second, we calculated the γ-diversity for each pair of plots using the same formula used for α-diversity (eq. 2). As for mean α-diversity, γ-diversity was calculated with Jost's correction and a weighted factor (f_i *sensu* de Bello et al. 2010), which in this case corresponds to the relative contribution of one species in the region, i.e., the total cover of one species in all plots divided by the total cover of all species in all plots (each pair of plots, in our case). Finally, we calculated β-additive diversity between each pair of plots as the difference between mean α-diversity and γ-diversity, expressing this difference as a proportion over γ-diversity (de Bello et al. 2010) as:

$$\beta\text{prop} = \frac{\gamma - \text{mean}\alpha}{\gamma} * 100 \qquad (3)$$

These calculations were applied to TD and FD, resulting in two beta diversity matrices that expressed the taxonomical and functional turnover between all possible pairs of plots.

Then, we investigated the variation of β-TD and β-FD diversity among the clusters by analysis of the multivariate dispersions (Anderson et al. 2006). This procedure is used to quantify variation in β-diversity by calculating for each plot its average distance (i.e., dispersion) to the centroid for each cluster in a multivariate space (Anderson et al. 2006). Then, we analysed if the distances (i.e., dispersions) of plots from the centroid were significantly different among the four clusters, by carrying out ANOVAs followed by Tukey post-hoc tests. The closer a plot to the centroid (low distance value), the lower the β-diversity of it (Anderson et al. 2006).

Model assumptions were evaluated visually (Zuur et al. 2010), while spatial autocorrelation in the model (Dormann et al. 2007) was checked with Moran's I test, which was applied to the residuals of significant ANOVA models. The matrix of weights used in the analysis has been generated as a Euclidean distance matrix of geographic coordinates.

We calculated all the above-mentioned indices for TD and FD using the *Rao* function provided by de Bello et al. (2010). We carried out ANOVAs using the *aov* function, Tukey post-hoc tests using the *TukeyHSD* function, and Pearson correlation by the *cor.test* function ('stats' package). The distance of each site to its centroid was calculated by the *betadisper* function ('vegan' package). Moran's I test was made with *Moran.I* function ('ape' package); the distance matrix was calculated using the *dist* function ('stats' package).

Since TD and FD can be, at least partially, correlated because they derive from a common species abundance matrix (Devictor et al. 2010), we checked the presence of collinearity using Pearson correlation coefficients between taxonomic and functional diversity, both for α diversity and β-prop diversity expressed as the distance of each plot from its common centroid. Pearson correlation coefficients (p) were highly positive and significant for all traits with the exception for β-functional diversity for storage organ and horizontal architecture (Table S2). The presence of strong correlation poses a problem when attempting to infer the mechanism through which plant communities were differentiated, because any variation in functional diversity could be driven by a variation in taxonomic diversity. To account for this issue, we regressed α and β-prop FD against α and β-prop TD, and then adopted the residuals of these regression as response variables (FD_{RES}) in the above-mentioned models, with the exception for β-functional diversity of storage organ and horizontal architecture. By determining what portion of the residual variance is explained by cluster variation, we were able to infer how clusters differed in the functional diversity without the influence of TD (Devictor et al. 2010).

Pearson correlation was made by using with *cor.test* function in the 'stats' package, linear regressions were made with the *lm* function ('stats' package).

Environmental drivers of α and β taxonomic and functional diversity

To interpret differences in taxonomic and functional diversity of plant community types in the light of variation of the environmental conditions, we ran two RDAs using α-FD and β-FD values of traits (or their residuals, when needed) as response variable matrices ('plot × α-FD' and 'plot × β-FD') and the environmental variables as predictors. We checked for possible multi-collinearities of the predictors in the RDA model computing the VIF of the variables as described above. We assessed the significance of the RDA models, of each predictor and of each axis using permutation tests (999 iterations). We carried out RDAs (equivalent to linear multiple regression analyses) to assess the relationship of

environmental variables with α and β taxonomic diversity and with α and β functional diversity of traits singly considered. Then, we applied to each full model a backward selection procedure using the *ordistep* function ('vegan' package). We assessed collinearity, significance of each model and of the selected environmental variables in each model, using the *vif.cca* and *anova* functions ('vegan' package). Finally, we fitted a linear model (*lm* function) to obtain the summary statistics *summary* function.

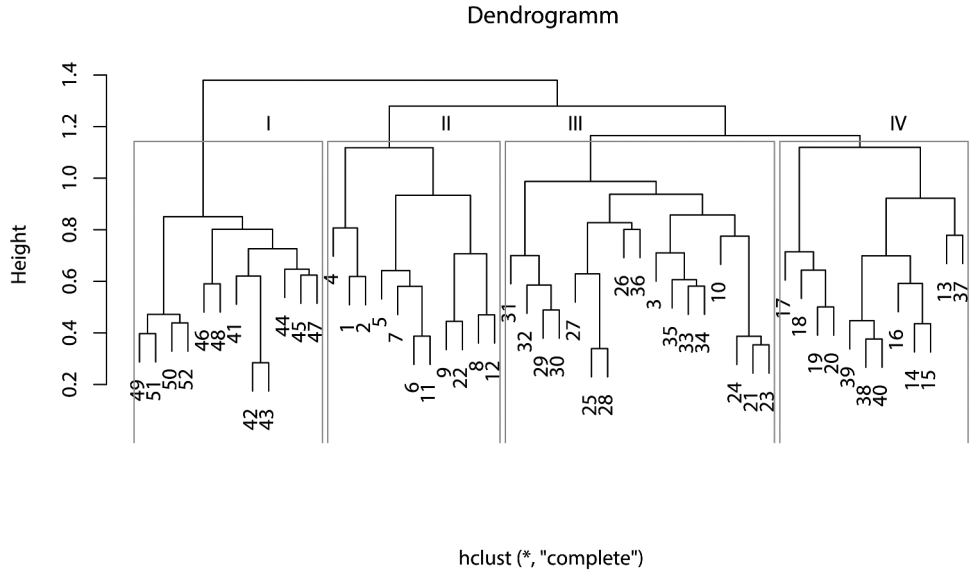

Figure 2. Cluster dendrogram of the plot x species cover matrix, Montagna di Torricchio Nature Reserve, central Apennines, Italy. I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta*-dominated community with *Brachypodium rupestre*; hclust refers to the function used to draw the dendrogram, while complete refers to the algorithm used. Height expresses the amount of dissimilarity between objects scaling up the trees, i.e., the height on y-axis is simply the value of this distance metric between clusters.

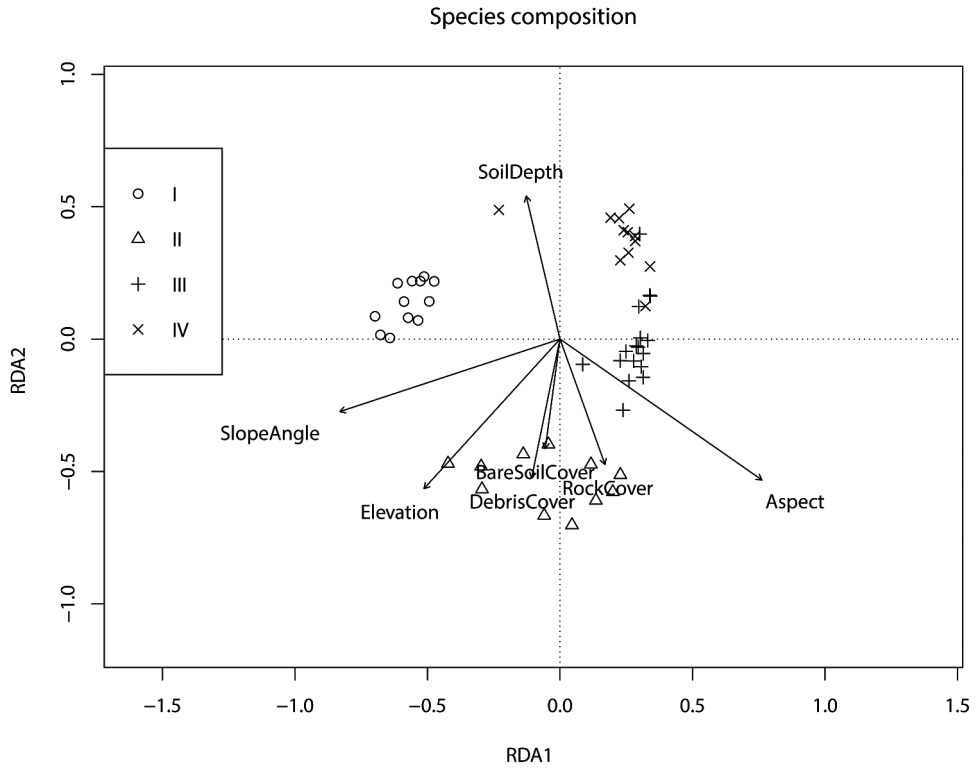

Figure 3. Ordination plot made by redundancy analysis on the plot × species cover data, constrained by the collected environmental variables, Montagna di Torricchio Nature Reserve, central Apennines, Italy. Circles – *Sesleria nitida*-dominated community (cluster I, in Figure 2); triangles – *Bromopsis erecta*-dominated community with *Sesleria nitida* (cluster II); plus signs – *Bromopsis erecta*-dominated community (cluster III); crosses – *Bromopsis erecta*-dominated community with *Brachypodium rupestre* (cluster IV).

Results

Characterisation of plant community types

The third-level division of the dendrogram obtained by cluster analysis (Figure 2) segregated four plant community types. Clusters I–IV included 12, 11, 17, and 12 plots, respectively. The RDA model (Figure 3) explained 48% (R^2) of the total variance (adj. R^2 40%; P = 0.001), partitioned into 48.5%, 28.7%, and 10.9% of variance explained by the first three RDA axes (P = 0.001). All the predictors were significant ($P < 0.05$), except debris cover.

Cluster I grouped grasslands dominated by *Sesleria nitida* subsp. *nitida*, growing on the steepest (33.3 ± 5.0°) north-west-facing slopes with relatively deep soils (24.7 ± 5.7 cm) (Table 1, Figure 3). These grasslands had high litter cover (60.3 ± 30.6%), the lowest mean value of phytomass (73.7 ± 44.3 g m^{-2}) and soil moisture EIV (3.3 ± 0.2), and an intermediate mean value for light intensity (8.0 ± 0.2) in comparison with the corresponding values of the other communities (Table 1, Table S3 and 1). Indicator species mostly belonged to the Festuco-Brometea class (Table 2; see Table S4 for definitions of vegetation classes).

Cluster II grouped grasslands dominated by *Bromopsis erecta* with the presence of *Sesleria nitida*, distributed on less steep (28.6 ± 7.6°) south-easterly slopes, with somewhat shallower soils (19.8 ± 7.1 cm) than those in Cluster I (Table 1, Figure 3). This community type had the lowest mean litter cover value (21.8 ± 10.8%), the highest mean value for bare soil (4.9 ± 4.5%), debris (8.9 ± 9.8%), and outcropping rock cover (1.5 ± 2.6%) (Table 1, Figure 3) of all communities. It was characterised by the highest mean value for light EIV (8.3 ± 0.2) and the lowest mean value for soil nutrients (2.7 ± 0.2) (Table 1). Indicator species were mostly xerophilous species of the Festuco-Brometea class, typical of open and rocky grasslands (Table 2).

Cluster III included plots of grasslands dominated by *Bromopsis erecta*, with the scattered occurrence of *Brachypodium rupestre*, growing on gentle slopes (20.4 ± 6.7°), not markedly exposed to the south with the shallowest soils (16.6 ± 2.9 cm) (Table 1, Figure 3). They had intermediate mean value of phytomass (80.6 ± 27.7 g m^{-2}) and litter cover (41.2 ± 17.6%) (Table 1). This plant community type showed intermediate mean value for EIV in terms of soil nutrients (2.9 ± 0.2), soil moisture (3.4 ± 0.1), and light intensity (8.1 ± 0.2) (Table 1, Figure S1). Indicator species were composed mostly of species growing on grasslands of the Festuco-Brometea class, with some nitrophilous species and meadow taxa (Table 2).

Cluster IV grouped grasslands dominated by *Bromopsis erecta*, with a noteworthy cover of *Brachypodium rupestre*, growing on gentle slopes (19.2 ± 5.4°), exposed to west – north-west and the east – south-east (Table 1). This cluster showed the highest soil depth values (34.3 ± 8.2 cm), absence of debris, outcropping rocks and negligible presence of bare soil (0.3 ± 0.9%) (Figure 3). This community had the highest value of phytomass (119.1 ± 36.6 g m^{-2}) and litter cover (61.7 ± 19.6%) (Table 1) and had the lowest light intensity values (7.6 ± 0.2) and the highest soil nutrients (3.4 ± 0.3) and moisture (3.7 ± 0.2) (Table 1, Figure S1). This cluster included the greatest number of indicator species belonging to the Molinio-Arrhenatheretea class of meadows and semi-mesophylous grasslands (Table 2).

Differences in α- and β-diversity among plant community types for taxonomic and functional facets

Values of α- and β-prop TD of Cluster I were significantly lower than those of the other three clusters (Figure 4). The greatest values of both indices were found in the Cluster II, whilst the lowest in the Cluster I.

Overall, we found a higher variation of FD_{RES} for the α-diversity component compared to the β-prop component (Table S5). In particular, α-diversity of leaf phenology and SLA was lower in Clusters II and III compared to those in Clusters I and IV (Figure 5 a,c). Leaf phenology was significantly different in the β-prop diversity values between Clusters II and III (Figure 5 b). There were no significant differences in taxonomic or functional diversity components of flowering phenology (Figure 6 a,b) and vegetative propagation (Figure 7 c-d). Alpha-diversity and β-prop diversity of storage organs showed a similar trend with lower values in Cluster II and higher values in Cluster IV (Figure 7 e-f). Alpha-diversity of plant height, vertical and horizontal architecture were significantly different across clusters. Regarding the first trait, Cluster I showed lesser values than those in Clusters III and IV (Figure 7 a), values of vertical architecture were less in Cluster IV than in the others, while those of horizontal architecture were greater in Clusters II and III than in Cluster IV (Figure 7 g).

Table 1. Mean (standard deviation) community unweighted mean (CM) values of Ellenberg Indicator Values, environmental descriptors and cover of dominant tall grasses for four groups resulting from a cluster analysis of vegetation data, Montagna di Torricchio Nature Reserve, central Apennines, Italy. Identical letters in a row indicate no statistically significant differences tested by ANOVA and Tukey's post-hoc test.

		Group			
		I	II	III	IV
Ellenberg indicator value (CM)	Light intensity **	8.00 (0.21) ^{a}	8.33 (0.16) ^{b}	8.10 (0.17) ^{a}	7.62 (0.24) ^{c}
	Air temperature $^{n.s.}$	5.77 (0.25) ^{a}	5.77 (0.18) ^{a}	5.90 (0.19) ^{a}	5.77 (0.16) ^{a}
	Continentality $^{n.s.}$	4.89 (0.21) ^{a}	4.95 (0.18) ^{a,b}	5.10 (0.11) ^{b}	5.10 (0.10) ^{b}
	Soil moisture *	3.33 (0.21) ^{a}	3.35 (0.18) ^{a}	3.42 (0.14) ^{a}	3.73 (0.15) ^{b}
	Soil reaction $^{n.s.}$	6.71 (0.24) ^{a}	6.69 (0.25) ^{a}	6.84 (0.18) ^{a}	6.65 (0.27) ^{a}
	Soil nutrients *	2.71 (0.39) ^{a}	2.70 (0.23) ^{a}	2.89 (0.23) ^{a}	3.41 (0.30) ^{b}
Environmental descriptor	Elevation (m a.s.l.) ***	1243 (41.67) ^{a}	1297 (48.10) ^{b}	1201 (43.36) ^{c}	1205 (20.67) ^{a,c}
	Slope (vertical degrees) ***	33.3 (4.99) ^{a}	28.6 (7.55) ^{a}	20.4 (6.73) ^{b}	19.2 (5.39) ^{b}
	Aspect (linear azimuth) ***	65.0 (9.50) ^{a}	131.0 (14.96) ^{b}	117.0 (11.39) ^{b,c}	98.3 (33.66) ^{c}
	Soil depth (cm) ***	24.7 (5.70) ^{a}	19.8 (7.05) ^{a,b}	16.6 (2.90) ^{b}	34.3 (8.20) ^{c}
	Oven-dried above-ground phytomass (g m^{-2}) **	73.7 (44.25) ^{a}	77.7 (31.97) ^{a}	80.6 (27.65) ^{a}	119.1 (36.57) ^{b}
	Total cover (%)***	97.4 (3.85) ^{a}	86.1 (12.38) ^{b}	97.6 (3.85) ^{a}	99. 7 (0.89) ^{a}
	Litter cover (%)***	60.3 (30.62) ^{a}	21.8 (10.79) ^{b}	41.2 (17.63) ^{a}	61.7 (19.57) ^{a}
	Bare soil cover (%)*	2.3 (3.39) ^{a,b}	4.9 (4.45) ^{b}	2.9 (3.11) ^{a,b}	0.3 (0.89) ^{a}
	Outcropping rock cover (%)*	0.0 (0.00) ^{a}	1.5 (2.58) ^{b}	0.4 (0.69) ^{a,b}	0.0 (0.00) ^{a}
	Debris cover (%)***	0.3 (0.87) ^{a}	8.9 (9.83) ^{b}	0.0 (0.00) ^{a}	0.0 (0.00) ^{a}
Tall grass cover	*Brachypodium rupestre* cover (%)	1.4 (2.32)	0.7 (1.47)	2.8 (7.15)	30.9 (12.48)
	Bromopsis erecta cover (%)	9.8 (6.12)	47.7 (24.33)	65.6 (16.09)	71.3 (21.22)
	Sesleria nitida cover (%)	79.2 (14.27)	16.0 (19.82)	0.3 (1.21)	3.8 (12.99)

**, $P < 0.01$; *, $P < 0.05$; n.s., not significant.
I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta*-dominated community with *Brachypodium rupestre*.

Detailed information on model parameters is reported in Table S5. None of the significant models were influenced by spatial autocorrelation (Table S6).

Environmental drivers of α and β taxonomic and functional diversity

We found that α-TD was positively related to soil depth, rock cover and aspect, and negatively to slope angle (Table 3). The overall RDA model significantly explained 30% (adj. R^2, $P = 0.001$) of the total variance of the α-FD data set. Soil depth, rock cover, and aspect were the variables most closely linked to RDA axis 1, the only significant axis ($P = 0.001$) (Figure 8). Soil depth was positively related to α-FD of SLA and leaf persistence, and negatively to vegetative propagation, vertical and horizontal architecture (Table 3). Bare soil cover was negatively related to leaf persistence; rock cover negatively influenced SLA and leaf persistence (Table 3). Among topographic factors, southerly slope orientation was positively related to α-FD of horizontal architecture and flowering phenology, and negatively to α-FD of storage organs and SLA (Table 3). Larger slope angle values were positively related to horizontal architecture and negatively to leaf persistence, while elevation was negatively related to the presence of storage organs (Table 3).

As far as β-diversity is concerned, β-TD was positively correlated with warm slope orientations and debris cover (Table 3). The overall RDA model did not significantly explain the effect of the environmental variables on β-FD (adj. R^2 0.0313, $P = 0.2$) and no environmental variable was significantly related to β-FD of any trait (Table 3).

Discussion

Species composition and ecological characterisation of grassland plant community types

As indicated by the RDA (Figure 3), topographic factors (mainly slope orientation and angle) and soil depth were related to the distribution of the different communities. Plant community types in Clusters I and IV were characterised by relatively less stressful conditions in terms of drought respect to the other two. Consistently with the Grime's model (see Grime 2001), Clusters I and IV were associated with a greater cumulative cover of tall grasses (*Sesleria nitida* and *Brachypodium rupestre*). Conversely, such species had lower frequency-abundance values in the two plant communities (Clusters II and III; Table 1) more prone to shortage of water and soil nutrients (see Figure S1).

Cluster I was characterised by *Sesleria nitida* (Table 1; Figure 2), while cluster IV grouped plots with a prominent cover of *Brachypodium rupestre*.

It has been widely demonstrated that the abandonment of grazing fosters the spread of tall grasses (Peco et al. 2006, 2012). In turn, their presence appears to foster the establishment of species of open woods, fringe vegetation and nitrophilous taxa (Allegrezza et al. 2016; Tardella et al. 2020), lowering the abundance of species characterising dry and semi-mesophylous grasslands (i.e., Festuco-Brometea species; Tardella et al. 2018). However, in our study, the very steep slopes for Cluster I and shallow soils and gentle slopes for Cluster IV apparently prevented tall grasses from achieving full dominance and, probably, allowed the permanence of a part of the set of species typical of grazed grasslands even after 50 years of abandonment. The large group of indicator species of this cluster, which are typical of semi-mesophylous grazed grasslands and meadows belonging to the Molinio-Arrhenateretea and Festuco-Brometea classes support this statement.

Communities growing in stressful conditions (Clusters II and III), mainly dominated by *Bromopsis erecta*, a typical dominant species of disturbed grasslands of the Apennines. These two clusters differed in the intensity of drought they are subject to in the summer. Cluster II grouped sample plots on steep and sunny slopes, with lower total vegetation cover, reflecting on higher mean values of light intensity and slightly lower values of soil moisture and soil nutrients than Cluster III (Table 1). Its indicator species, such as *Festuca inops, Helianthemum oelandicum* subsp. *incanum*, and *Ononis pusilla* subsp. *pusilla*, are typical of xerophilous grasslands, both in grazed and in abandoned conditions. Cluster III grouped plots of gentle slopes with shallow soils. The indicator species were mostly of dry and semi-mesophylous grasslands, mainly of the Festuco-Brometea class, with some species of Molinio-Arrhenatheretea and other vegetation classes (Table 2). The results are consistent with those by Klimeš et al. (2013) who argued that in dry habitats grassland species are little affected by the cessation of grazing.

Taxonomic and functional diversity of plant community types

The analysis of taxonomic and functional components of α- and β-diversity highlighted that species coexistence was mainly promoted by strategies linked to exploitation of resources in space and time, but not by the reproductive strategies, i.e. sexual reproduction and vegetative propagation (Figures 6 and 7).

The *Sesleria nitida*-dominated plant community type (Cluster I) was characterised by the lowest values of α-TD, β-proportional-TD and α-FD_{RES_H}, which were related to non-stressful conditions, i.e. north-facing slopes, low rock or debris cover. Such taxonomical and functional pattern suggested the presence of processes based on competitive exclusion (de Bello et al. 2012). In less stressful environments, dominant species equipped with competitive traits (i.e., plant height; Bernard-Verdier et al. 2012) exclude weaker competitors (de Bello et al. 2012), resulting in an assemblage of few species (i.e., lower taxonomic diversity; Lepš 2014) similar in size. The higher α-FD_{RES_VA} and α-FD_{RES_HA} values indicate that the outcome of competition for light was achieved through different space occupation strategies in accordance with Catorci et al. (2012). The α-FD_{RES_SLA} values, higher than in Clusters II and III, suggest that different strategies of resource exploitation are being used. More variable SLA values could be associated with higher variability in rates of relative growth and photosynthesis (Pérez-Harguindeguy et al. 2013), implying a wider range of strategies inside the community type. Although competition for light may force plants to converge in height (Gross et al. 2013), other axes of specialisation may allow competing species to coexist by using resources differently (Maire et al. 2012).

In the *Bromopsis erecta* and *Brachypodium rupestre* community type (Cluster IV), the higher abundance of tall caespitose grass species reflected in the lower α-FD_{RES_VA} and α-FD_{RES_HA} values. The higher α-FD_{RES_LP}, α-FD_{RES_SLA}, and α-FD_{RES_H} values indicate an assemblage of more dissimilar species in regard with competitive ability and timing of resource exploitation (de Bello et al. 2012).

The *Bromopsis erecta* community type (Cluster II) was found on dry south-facing steep slopes, characterised by much debris, outcropping rock, and bare soil cover. This community type was well distinguished in regard to the α-FD patterns, with the lowest α-FD_{RES_LP} and α-FD_{RES_SO} values, and lower α-FD_{RES_SLA} values than Clusters I and IV. Such functional patterns are associated with dryer conditions, i.e., shallow soils, large rock and bare soil cover, steep and sunny slopes. Alpha-FD_{RES_HA} and α-FD_{RES_VA}

Table 2. Indicator species of the groups identified by cluster analysis, Montagna di Torricchio Nature Reserve, central Apennines, Italy. Indicator Values (IndVal) and their significance level are reported. The phytosociological placement at the class level and the main type of Apennine plant community in which these species are found are indicated.

Cluster	Species	IndVal	Phytosociological class	Habitat
I	*Anthoxanthum odoratum*	0.60***	Molinio-Arrhenatheretea	semi-mesophylous grasslands
I	*Cynanchica pyrenaica* ssp. *cynanchica*	0.76***	Festuco-Brometea	dry and semi-mesophylous grasslands
I	*Pilosella officinarum*	0.57**	Festuco-Brometea	dry and semi-mesophylous grasslands
I	*Primula veris* ssp. *columnae*	0.78***	Carpino-Fagetea sylvaticae; Quercetea pubescentis	forest edge
I	*Sesleria nitida* ssp. *nitida*	0.90***	Festuco-Brometea	grassland and open dry forest
I	*Cerastium arvense* ssp. *suffruticosum*	0.65**	Festuco-Brometea	dry and semi-mesophylous grasslands
I	*Carex macrolepis*	0.60*	Elyno-Seslerietea	dry grasslands
II	*Anthyllis vulneraria* ssp. *polyphylla*	0.71***	Festuco-Brometea	dry grasslands
II	*Dianthus sylvestris* group	0.69*	Festuco-Brometea	dry grasslands
II	*Festuca inops*	1.00***	Festuco-Brometea	dry grasslands
II	*Helianthemum oelandicum* ssp. *incanum*	0.90***	Festuco-Brometea	dry grasslands
II	*Koeleria* sp.	0.54*	Festuco-Brometea	dry grasslands
II	*Ononis pusilla* ssp. *pusilla*	0.76***	Festuco-Brometea	dry grasslands
II	*Poa molinerii*	0.52*	Festuco-Brometea	dry grasslands
II	*Sabulina glaucina*	0.52*	Festuco-Brometea	dry grasslands
III	*Sedum sexangulare*	0.82**	Sedo-Schleranthetea	dry grasslands
III	*Onobrychis viciifolia*	0.78**	Molinio-Arrhenatheretea	semi-mesophylous grassland, meadows
III	*Erysimum pseudorhaeticum*	0.70**	Festuco-Brometea	dry grasslands
III	*Silene otites*	0.60*	Festuco-Brometea	dry grasslands
III	*Cynosurus echinatus*	0.60**	Stipo-Trachynietea distachyae	dry grasslands
III	*Rhinanthus alectorolophus* ssp. *alectorolophus*	0.82**	Molinio-Arrhenatheretea	semi-mesophylous grassland, meadows
III	*Poterium sanguisorba* ssp. *balearicum*	0.72**	Festuco-Brometea	dry and semi-mesophylous grasslands
III	*Alyssum simplex*	0.53*	Chenopodietea	dry grasslands
III	*Coronilla minima* ssp. *minima*	0.53*	Festuco-Brometea	dry grasslands
III	*Poa bulbosa* ssp. *bulbosa*	0.51*	Poetea bulbosae	semi-mesophylous grassland
III	*Scorzoneroides cichoriacea*	0.59*	Festuco-Brometea	semi-mesophylous grasslands
III	*Muscari neglectum*	0.71*	Festuco-Brometea	dry and semi-mesophylous grasslands
III	*Phleum hirsutum* ssp. *ambiguum*	0.79**	Festuco-Brometea	dry and semi-mesophylous grasslands
IV	*Brachypodium rupestre*	0.93***	Festuco-Brometea	semi-mesophylous grasslands
IV	*Cruciata laevipes*	0.65**	Epilobietea angustifolii	forest edge
IV	*Ononis spinosa* ssp. *spinosa*	0.62**	Festuco-Brometea	dry grasslands
IV	*Galium verum* ssp. *verum*	0.52*	Festuco-Brometea	semi-mesophylous grasslands
IV	*Trifolium ochroleucon*	0.64*	Festuco-Brometea	dry and semi-mesophylous grasslands
IV	*Senecio doronicum* ssp. *doronicum*	0.62**	Festuco-Brometea; Elyno-Seslerietea	dry and semi-mesophylous grasslands
IV	*Plantago media* ssp. *media*	0.57**	Molinio-Arrhenatheretea	semi-mesophylous grasslands, meadows
IV	*Centaurea arrigonii*	0.50*	Festuco-Brometea	dry and semi-mesophilous grasslands
IV	*Galium album* ssp. *album*	0.50*	Molinio-Arrhenatheretea	semi-mesophylous grasslands, meadows
IV	*Vicia cracca*	0.85***	Molinio-Arrhenatheretea	semi-mesophylous grasslands, meadows
IV	*Dactylis glomerata* ssp. *glomerata*	0.93***	Molinio-Arrhenatheretea	semi-mesophylous grassland, meadows
IV	*Achillea millefolium* ssp. *millefolium*	0.67**	Molinio-Arrhenatheretea	semi-mesophylous grassland, meadows
IV	*Poa pratensis* ssp. *pratensis*	0.50*	Molinio-Arrhenatheretea	semi-mesophylous grassland, meadows

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta*-dominated community with *Brachypodium rupestre*. Plant names follow Pignatti et al. (2017). Plant associations are after Mucina et al. (2016) and Biondi and Blasi (2016).

values overlap with those of Clusters I and III. The direction of these variations suggests a mechanism behind species coexistence, not dominated neither by competitive processes involving light-competition nor by niche partitioning for resource-use strategies.

The *Bromopsis erecta*-dominated community-type (Cluster III) showed FD values partly similar to those at less arid sites (higher FD_{RES_H} and FD_{RES_SO}) and partly to those at dryer ones (lower FD_{RES_SLA}), indicating the coexistence in the same community of a set of strategies aimed at differentiating the competitive ability to capture light and modulating the resource storage and use, however reducing the range of resource acquisition strategies at the leaf level.

Environmental drivers of α- and β-taxonomic and functional diversity

Alpha-TD was negatively related to less water-limited conditions, i.e., northerly steep slopes with low rock cover and moderate soil depth, which allow competitive species to become dominant, reducing the diversity inside the community due to the exclusion of weak

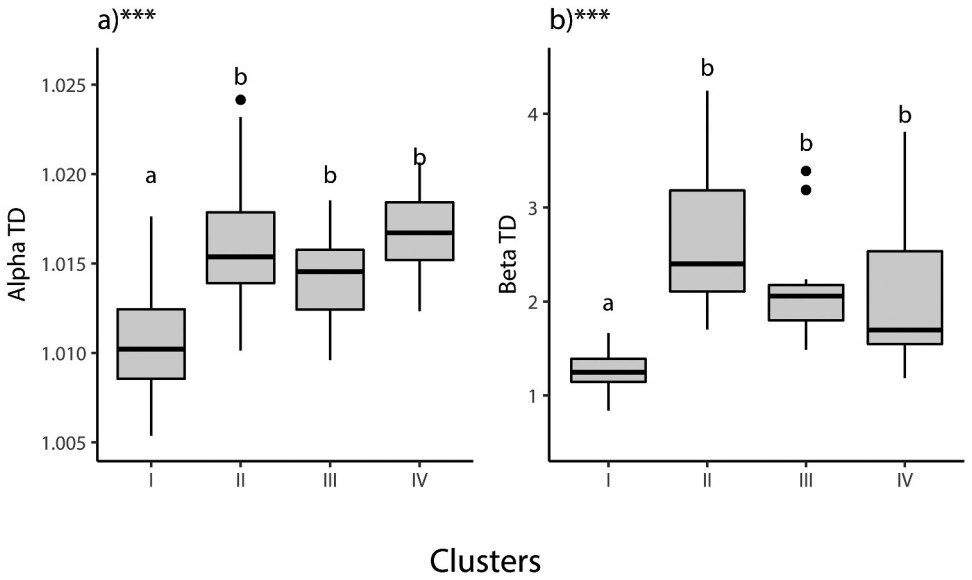

Figure 4. Distribution of the α- (a) and (b) β-proportional components of taxonomic diversity (TD) across the plant community types identified by cluster analysis, Montagna di Torricchio Nature Reserve, central Apennines, Italy. Identical letters above boxplots indicate no statistically significant difference between groups after Tukey's post-hoc test. Significance levels after ANOVA are reported (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $^{n.s}$, $P \geq 0.05$, not significant). I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta* and *Brachypodium rupestre*-dominated community.

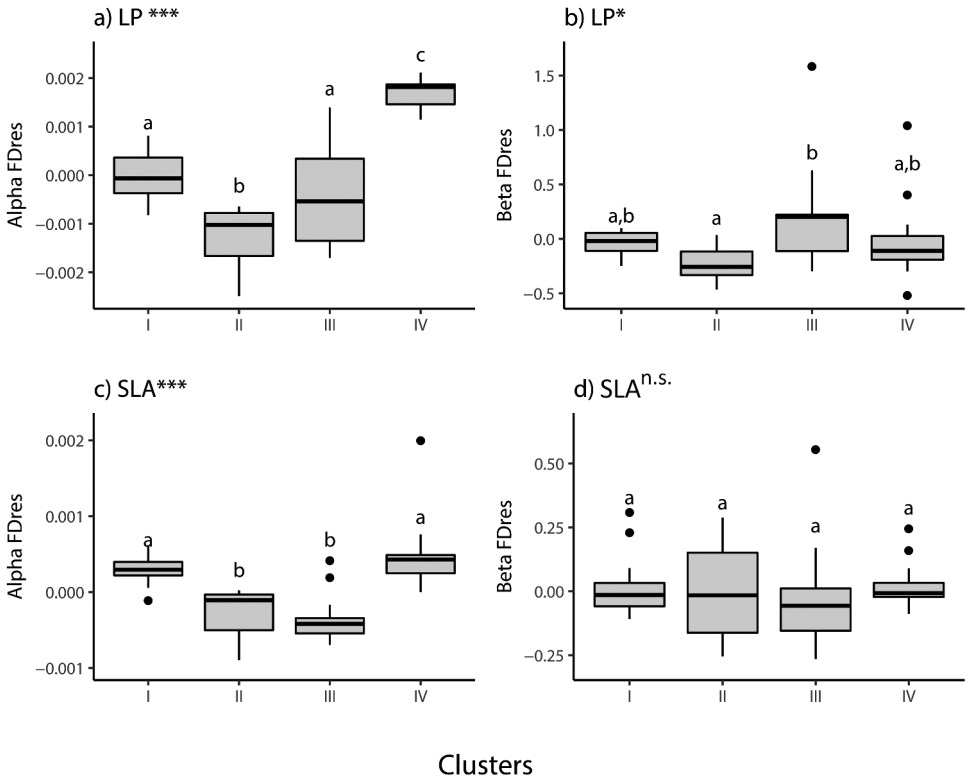

Figure 5. Distribution of α- and β-proportional functional diversity (FD_{RES}) for leaf traits across the plant community types identified by cluster analysis. α-FD_{RES} (a) and β-proportional-FD_{RES} (b) for leaf phenology (LP); α-FD_{RES} (c) and β-proportional-FD_{RES} (d) for specific leaf area (SLA), Montagna di Torricchio Nature Reserve, central Apennines, Italy. Identical letters above boxplots indicate no statistically significant difference between groups after Tukey's post-hoc test. Significance levels after ANOVA are reported (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $^{n.s}$, $P \geq 0.05$, not significant). I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta*-dominated community with *Brachypodium rupestre*.

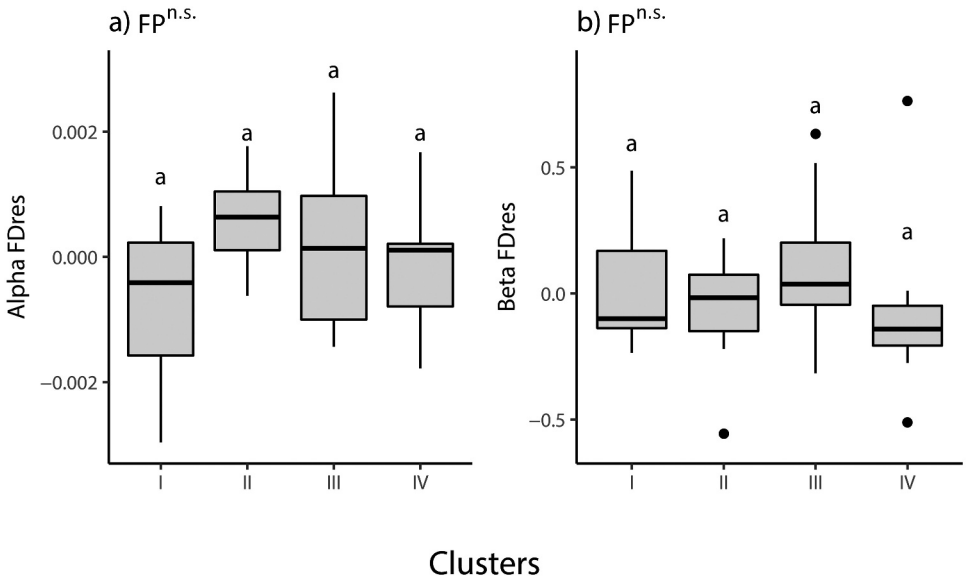

Figure 6. Distribution of α- and β-proportional functional diversity (FD_{RES}) for reproductive traits across the plant community types identified by cluster analysis. (a) α-FD_{RES} and (b) β-proportional-FD_{RES} for flowering phenology (FP), Montagna di Torricchio Nature Reserve, central Apennines, Italy. Identical letters above boxplots indicate no statistically significant difference between groups after Tukey's post-hoc test. Significance levels after ANOVA are reported (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $^{n.s}$, $P \geq 0.05$, not significant). I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta*-dominated community with *Brachypodium rupestre*.

competitors (de Bello et al. 2012; Lepš 2014). Similar environmental conditions (northerly slope orientations and low debris cover) showed a negative relationship also with β-TD, confirming the homogenising effect of competitive species both within and between plots. Instead, in sites characterised by lower water availability, the presence of heterogeneities due to the small-scale variations of vegetation structure (i.e., open-turf grassland with rock outcrops, debris and bare soil, lower mean soil depth, see Tardella and Catorci 2015) causes both higher species diversity within plots and a greater species turnover.

As with FD, soil depth was the main environmental driver of α-FD, being positively related to the diversity in resource acquisition strategies (i.e. SLA and leaf persistence) and negatively to the diversity in space occupation strategies (Table 3 and Figure 8). Higher variability in SLA, which was related to deeper soil, lower rock cover, and more shaded slope orientations, indicates the coexistence of plants with contrasting leaf strategies commonly found in less arid Mediterranean sites (Nunes et al. 2017). In contrast, a lower α-FD of SLA was attributed to a lower niche differentiation at dryer sites (Nunes et al. 2017; Stanisci et al. 2020). The observed relatively high α-diversity values of leaf persistence types under less stressful conditions, i.e., deep soil, gentle slopes, and low rock and bare soil cover is consistent with Tardella and Catorci (2015) who have attributed this trend to the extended growing period that resulted from low summer drought stress experienced by plants growing on deep soils. The higher α-FD of horizontal and vertical space occupation strategies in resource-limited conditions (shallower soils) is probably due to the more heterogeneous spatial distribution of soil resources, which fosters a wider range of adaptations to occupy and exploit the available resource niches.

None of the considered environmental factors affected β-FD of any trait (Table 3). This finding, along with the substantial homogeneity of β-FD values among clusters (Figures 5–7), probably indicates that plant community types identified by cluster analysis develop within homogeneous environmental contexts in which vegetation shows recurring patterns of strategies. This is consistent with Uchida et al. (2015), who have associated spatial homogenisation of β–diversity with land abandonment.

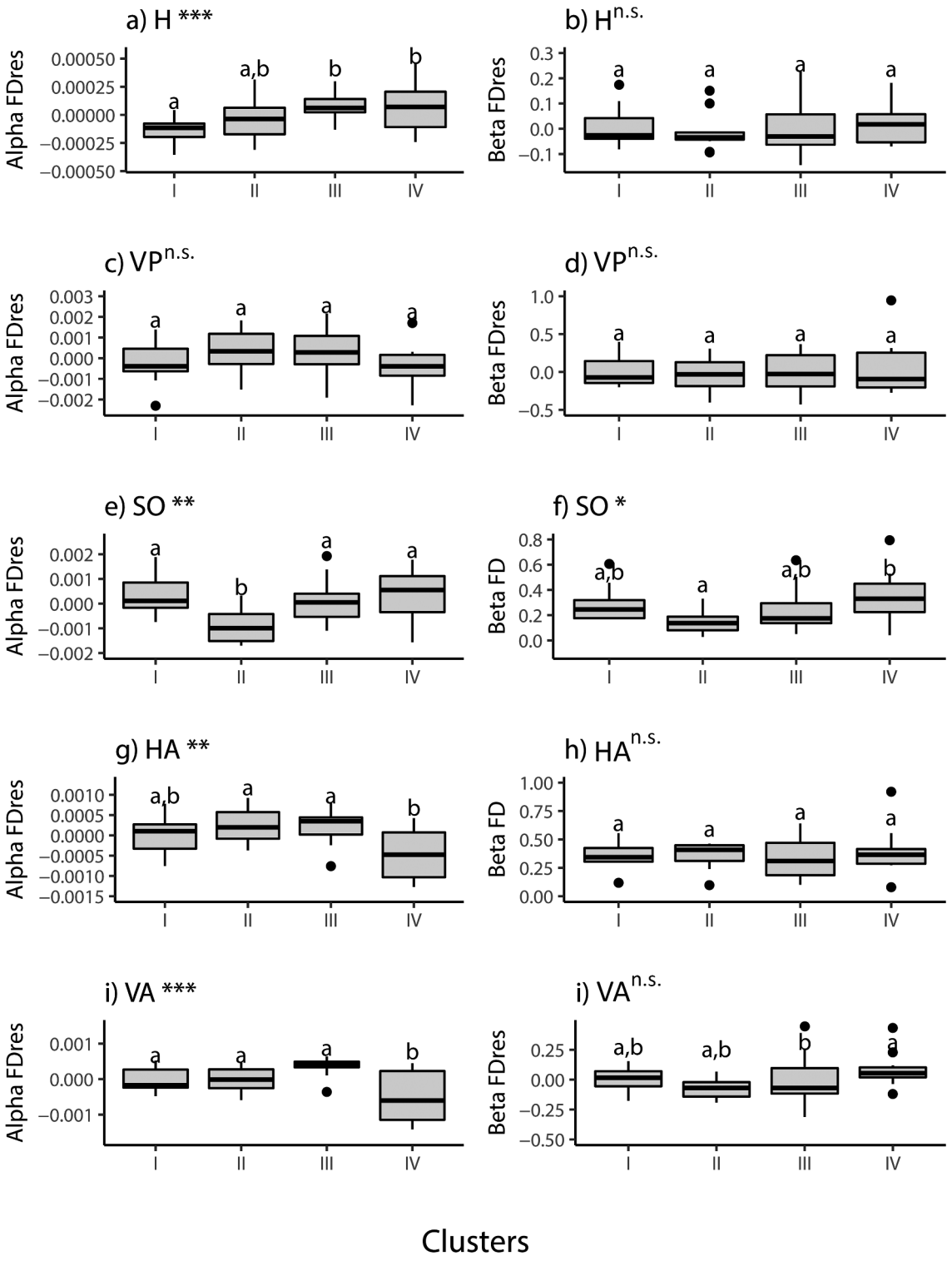

Figure 7. Distribution of α- and β-proportional functional diversity (FD_{RES}) for the complete set of plant traits across the plant community types identified by cluster analysis. (a) α-FD_{RES} and (b) β-proportional-FD_{RES} for plant height (H); (c) α-FD_{RES} and (d) β-proportional-FD for vegetative propagation (VP); (e) α-FD_{RES} and (f) β-proportional-FD for storage organs (SO); (g) α-FD_{RES} and (d) β-proportional-FD for horizontal architecture (HA); (i) α-FD_{RES} and (l) β-proportional-FD_{RES} for vertical architecture (VA), Montagna di Torricchio Nature Reserve, central Apennines, Italy. Identical letters above boxplots indicate no statistically significant difference between groups after Tukey's post-hoc test. Significance levels after ANOVA are reported (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; $^{n.s}$, $P \geq 0.05$, not significant). I – *Sesleria nitida*-dominated community; II – *Bromopsis erecta*-dominated community with *Sesleria nitida*; III – *Bromopsis erecta*-dominated community; IV – *Bromopsis erecta*-dominated community with *Brachypodium rupestre*.

Table 3. Results of the RDAs for α and β-proportional components of taxonomical diversity (TD) and residuals of functional diversity (FD_{RES}), Montagna di Torricchio Nature Reserve, central Apennines, Italy. Slope estimates and significance levels of variables in the reduced models are shown only for globally significant models.

Diversity index	Model *adj.* R^2	Soil depth	Debris cover	Bare soil cover	Rock cover	Elevation	Aspect	Slope angle
α-TD	0.4434***	$1.51 \times 10^{-4**}$			$9.71 \times 10^{-4**}$		$3.16 \times 10^{-5*}$	$-1.69 \times 10^{-4***}$
$\alpha\text{-FD}_{RES_VP}$	0.0842*	$-3.68 \times 10^{-5*}$						
$\alpha\text{-FD}_{RES_SO}$	0.2448***					$-5.60 \times 10^{-6***}$	$-1.02 \times 10^{-5*}$	
$\alpha\text{-FD}_{RES_VA}$	0.2555***	$-2.85 \times 10^{-5***}$						
$\alpha\text{-FD}_{RES_HA}$	0.2583*	$-2.05 \times 10^{-5**}$					$5.23 \times 10^{-6*}$	$1.89 \times 10^{-5*}$
$\alpha\text{-FD}_{RES_LP}$	0.6398***	$5.56 \times 10^{-5***}$		$-1.12 \times 10^{-4**}$	$-4.17 \times 10^{-4***}$			$-3.26 \times 10^{-5*}$
$\alpha\text{-FD}_{RES_SLA}$	0.6744***	$2.98 \times 10^{-5***}$			$-1.01 \times 10^{-4**}$	$1.52 \times 10^{-6\ n.s.}$	$-6.27 \times 10^{-6***}$	
$\alpha\text{-FD}_{RES_FP}$	0.1539**						$1.60 \times 10^{-5**}$	
β-TD	0.2916**		0.067***			$-3.87 \times 10^{-3\ n.s.}$	0.009*	
$\beta\text{-FD}_{RES_LP}$	0.0955*			$-0.02^{\ n.s}$		$-1.69 \times 10^{-3\ n.s.}$		
$\beta\text{-FD}_{RES_FP}$	0.0855*	$-7.10 \times 10^{-3\ n.s.}$	$-0.0121^{\ n.s.}$					

FP, flowering phenology; HA, horizontal architecture; LP, leaf persistence; H, plant height; SLA, specific leaf area; SO, storage organs; VP, vegetative propagation; VA, vertical architecture. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant

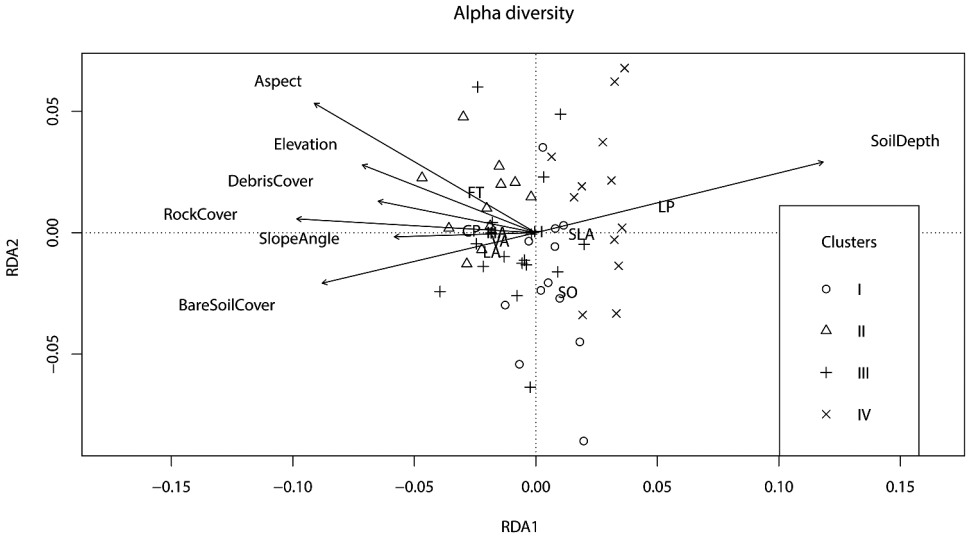

Figure 8. Ordination plot made by redundancy analysis on the plot × α-FD values of traits, constrained by the collected environmental variables, Montagna di Torricchio Nature Reserve, central Apennines, Italy. Circles – *Sesleria nitida*-dominated community (cluster I, Figure 2); triangles – *Bromopsis erecta*-dominated community with *Sesleria nitida* (cluster II); plus signs – *Bromopsis erecta*-dominated community (cluster III); crosses – *Bromopsis erecta*-dominated community with *Brachypodium rupestre* (cluster IV).FP, flowering phenology; HA, horizontal architecture; LP, leaf persistence; H, plant height; SLA, specific leaf area; SO, storage organs; VP, vegetative propagation; VA, vertical architecture.

General model

Our results confirm the hypothesis that, in a sub-Mediterranean context, abandonment does not level off the differences in vegetation composition and structure (α- and β-taxonomic and functional diversity). In fact, we have proved the existence of different plant communities whose distribution is correlated with the main landforms and soil depth, thus demonstrating that landform heterogeneity compensates the assumed role of abandonment of grazing on homogenisation of vegetation features. The analysis of taxonomic and functional components of α- and β-diversity highlighted that species coexistence was mainly promoted by strategies linked to the exploitation of resources in space and time, and that topographic conditions (slope orientation and angle) act as the main drivers in determining the function-based mechanisms of community assembly. More specifically, in resource-limited habitats, characterised by open-turf vegetation (south-facing slopes, shallow soils with lower water and nutrients availability), communities had higher α- and β-TD, but showed an assemblage of species characterised by a lower niche partitioning for resource acquisition at the leaf level and a similar timing of resource exploitation, but with dissimilar space occupation strategies. In less resource-limited habitats (north-facing slopes, with higher water and nutrients availability), where competitive tall grass species

reduced taxonomic diversity, higher α-FD values were mainly maintained by coexistence processes through niche partitioning of species with different resource acquisition but similar space occupation strategies.

Conclusions

Our results indicate that landform heterogeneity may represent, within the sub-Mediterranean mountains, a factor capable of guaranteeing a certain level of taxonomic and functional diversity at the landscape level for a long time after abandonment. Dryer conditions were more suitable to preserve the presence of the species set typical of grazed grasslands and are characterised by higher α-taxonomic diversity, confirming that in sub-Mediterranean climate resource-poor environments are less impacted by abandonment. Instead, less resource-limited habitats, while maintaining in some cases higher FD levels, linked to the need of differentiating the strategies for the acquisition and use of resources, are more prone to the invasion of potentially dominant species (i.e., tall grasses), which tend, on the one hand, to constitute the suitable environment for successional or fringe habitat species, and, on the other hand, to exclude weaker competitors.

These outputs could have important consequences in defining appropriate management guidelines and in the use of European funds for the conservation of the Apennine grasslands.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Notes on contributors

Alessandro Bricca is a post-doctoral fellow who studies plant–environment relationships and community assembly from a functional perspective.

Federico M. Tardella's fields of expertise include plant systematics, vegetation analysis, monitoring and mapping, plant ecology, ecosystem management, biodiversity conservation, geobotany applied to land planning, evaluation of the effects of plans, projects and activities on habitats of community interest in Natura 2000 sites, geobotanic quality maps.

Arianna Ferrara in her Master's thesis used functional traits in the study of plant community composition and assembly.

Xia Xinfang in her Master's thesis studied how context-dependent fine-scale variations affect coenological structure of un-managed sub-Mediterranean grasslands"

Fabio Tolu is a Ph.D. student whose project is focused on the use of taxonomic and functional diversity in the study of composition and assembly of grassland vegetation after land-use.

Andrea Catorci is an Associate Professor, with skills in geobotany, biodiversity conservation, ecology and ecosystems management, landscape ecology, applied botany and land planning, sustainable development of local communities, planning of wood-energy chains, management plans and conservation measures for Natura 2000 sites, evaluation of the effects of plans, projects and activities on plant communities and ecosystems.

ORCID

Alessandro Bricca http://orcid.org/0000-0003-0202-6776
Federico M. Tardella http://orcid.org/0000-0002-4319-9131
Arianna Ferrara http://orcid.org/0000-0002-8178-3910
Andrea Catorci http://orcid.org/0000-0001-9012-7635

References

Allegrezza M, Biondi E, Ballelli S, Tesei G, Ottaviani C, Zitti S. 2016. *Brachypodium rupestre* (Host) Roem. & Schult. herbaceous communities of heliophilous edge in the *Trifolio medii-Geranietea sanguinei* Müller 1962 class. Plant Sociol. 53:59–76. doi:10.7338/pls2016532/05.

Anderson MJ, Ellingsen KE, McArdle BH. 2006. Multivariate dispersion as a measure of beta diversity. Ecol Lett. 9:683–693. doi:10.1111/j.1461-0248.2006.00926.x.

Bartolucci F, Peruzzi L, Galasso G, Albano A, Alessandrini A, Ardenghi NMG, Astuti G, Bacchetta G, Ballelli S, Banfi E, et al. 2018. An updated checklist of the vascular flora native to Italy. Plant Biosyst. 152:179–303. doi:10.1080/11263504.2017.1419996.

Bennie J, Hill MO, Baxter R, Huntley B. 2006. Influence of slope and aspect on long-term vegetation change in British chalk grasslands. J Ecol. 94:355–368. doi:10.1111/j.1365-2745.2006.01104.x.

Bernard-Verdier M, Navas ML, Vellend M, Violle C, Fayolle A, Garnier E, Cornelissen H. 2012. Community assembly along a soil depth gradient: contrasting patterns of plant trait convergence and divergence in a Mediterranean rangeland. J Ecol. 100:1422–1433. doi:10.1111/1365-2745.12003.

Bertness MD, Callaway R. 1994. Positive interactions in communities. Trends Ecol Evol. 9(5):191–193. doi:10.1016/0169-5347(94)90088-4.

Biondi E, Ballelli S, Allegrezza M, Zuccarello V. 1995. La vegetazione dell'ordine *Brometalia erecti* Br.-Bl. 1936 nell'Appennino (Italia). Fitosociol. 30:3–45.

Biondi E, Blasi C 2016. Prodromo della Vegetazione Italiana. Check-list sintassonomica aggiornata di classi, ordini e alleanze presenti in Italia. Società Botanica Italiana (in Italian). [available online at http://www.prodromo-vegetazione-italia.org, accessed on 2020 May 15

Borcard D, Gillet F, Legendre P. 2011. Numerical Ecology with R. New York (US): Springer.

Bracchetti L, Carotenuto L, Catorci A. 2012. Land-cover changes in a remote area of central Apennines (Italy) and management directions. Landsc Urban Plan. 104 (2):157–170. doi:10.1016/j.landurbplan.2011.09.005.

Bricca A, Tardella FM, Tolu F, Goia I, Ferrara A, Catorci A. 2020. Disentangling the effects of disturbance from those of dominant tall grass features in driving the functional variation of restored grassland in a sub-Mediterranean context. Diversity. 12:11. doi:10.3390/d12010011.

Briske DD. 1996. Strategies of plant survival in grazed systems: a functional interpretation. In: Hodgson J, Illius AW, editors. The ecology and management of grazing systems. Wallingford: CAB International; p. 37–68.

Brooker RW. 2006. Plant–plant interactions and environmental change. New Phytol. 171(2):271–284. doi:10.1111/j.1469-8137.2006.01752.x.

Burrascano S, Anzellotti I, Carli E, Del Vico E, Facioni L, Pretto F, Sabatini FM, Tilia A, Blasi C. 2013. Drivers of beta-diversity variation in *Bromus erectus* semi-natural dry grasslands. Appl Veg Sci. 16:404–416. doi:10.1111/avsc.12021.

Carmona CP, Mason NW, Azcárate FM, Peco B. 2015. Inter-annual fluctuations in rainfall shift the functional structure of Mediterranean grasslands across gradients of productivity and disturbance. J Veg Sci. 26:538–551. doi:10.1111/jvs.12260.

Catorci A, Cesaretti S, Gatti R. 2009. Biodiversity conservation: geosynphytosociology as a tool of analysis and modelling of grassland systems. Hacquetia. 8:129–146. 10.2478/v10028–009–0010–2. doi:10.2478/v10028-009-0010-2.

Catorci A, Cesaretti S, Gatti R. 2013. Effect of long-term abandonment and spring grazing on floristic and functional composition of dry grasslands in a central Apennine farmland. Pol J Ecol. 61:505–518. http://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-27005050-ab21-4f62-971e-ea45a6309046 .

Catorci A, Lulli R, Malatesta TM, Tavoloni M, Tardella FM. 2021. How the interplay between management and interannual climatic variability influences the NDVI variation in a sub-Mediterranean pastoral system: insight into sustainable grassland use under climate change. Agric Ecosyst Environ. 314:107372. doi:10.1016/j.agee.2021.107372.

Catorci A, Ottaviani G, Vitasović Kosić I, Cesaretti S. 2012. Effect of spatial and temporal patterns of stress and disturbance intensities in a sub-Mediterranean grassland. Plant Biosyst. 146:352–367. doi:10.1080/11263504.2011.623192.

Cesaretti S, Castagna S, Montenegro B, Catorci A. 2009. Caratterizzazione ai fini zootecnici della vegetazione di un sistema pastorale come strumento per la conservazione della biodiversità: un caso studio nell'Appennino umbro-marchigiano. Inf Bot Ital. 41 (2):247–258.

Chytrý M, Dražil T, Hajek M, Kalníková V, Preislerova Z, Šibík J, Ujházy K, Axmanová I, Bernátová D, Blanár D, et al. 2015. The most species-rich plant communities in the Czech Republic and Slovakia (with new world records). Preslia. 87:217–278.

Connell JH. 1978. Diversity in tropical rain forests and coral reefs. Science. 199(4335):1302–1310. doi:10.1126/science.199.4335.1302.

de Bello F, Lavergne S, Meynard CN, Lepš J, Thuiller W. 2010. The partitioning of diversity: showing Theseus a way out of the labyrinth. J Veg Sci. 21:992–1000. doi:10.1111/j.1654-1103.2010.01195.x.

de Bello F, Price JN, Münkemüller T, Liira J, Zobel M, Thuiller W, Gerhold P, Götzenberger L, Lavergne S, Lepš J, et al. 2012. Functional species pool framework to test for biotic effects on community assembly. Ecol. 93:2263–2273. doi:10.1890/11-1394.1.

de Cáceres M, Legendre P, Wiser SK, Brotons L. 2012. Using species combinations in indicator value analyses. Methods Ecol Evol. 3:973–982. doi:10.1111/j.2041-210X.2012.00246.x.

Del Guacchio E, Caputo P. 2020. Splitting *Asperula* (*Rubiaceae*): a proposal for consistency purposes within sections *Cynanchicae, Thliphthisa* and *Hexaphylla*. Plant Biosyst. 154:766–-782. doi:10.1080/11263504.2020.1804008.

Dengler J, Janišová M, Török P, Wellstein C. 2014. Biodiversity of Palaearctic grasslands: a synthesis. Agric Ecosyst Environ. 182:1–14. doi:10.1016/j.agee.2013.12.015.

Devictor V, Mouillot D, Meynard C, Jiguet F, Thuiller W, Mouquet N. 2010. Spatial mismatch and congruence between taxonomic, phylogenetic and functional diversity: the need for integrative conservation strategies in a changing world. Ecol Lett. 13(8):1030–1040. doi:10.1111/j.1461-0248.2010.01493.x.

Di Biase L, Fattorini S, Cutini M, Bricca A. 2021. The role of inter-and intraspecific variations in grassland plant functional traits along an elevational gradient in a Mediterranean mountain area. Plants. 10(2):359. doi:10.3390/plants10020359.

Diaz S, Lavorel S, McIntyre SUE, Falczuk V, Casanoves F, Milchunas DG, Skarpe C, Rusch G, Sternberg M, Noymeir I, et al. 2007. Plant trait responses to grazing–a global synthesis. Glob Chang Biol. 13(2):313–341. doi:10.1111/j.1365-2486.2006.01288.x.

Dormann CF, McPherson JM, Araújo MB, Bivand R, Bolliger J, Carl G, Davies RG, Hirzel A, Jetz W, Kissling W, et al. 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography. 30(5):609–628. doi:10.1111/j.2007.0906-7590.05171.x.

Dufrêne M, Legendre P. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol Monogr. 67:345–366. doi:10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2.

EEA 2010. EU 2010 biodiversity baseline. EEA Technical report No 12/2010. Copenhagen: Publications Office of the European Union.

Ellenberg H. 1991. Zeigerwerte von pflanzen in Mitteleuropa. Scripta Geobot. 18:1–248.

Fox JW. 2013. The intermediate disturbance hypothesis should be abandoned. Trends Ecol Evol. 28:86–92. doi:10.1016/j.tree.2012.08.014.

Francalancia C. 1976. Carta della vegetazione della Riserva naturale di Torricchio. La Riserva naturale di Torricchio. 1:77–98.

Francalancia C. 1981. L'ortofotocarta della Riserva naturale di Torricchio. La Riserva naturale di Torricchio. 4:41–45.

Francalancia C, Galli P, Paradisi L. 1994. Aspetti floristici e dinamismo dei prati-pascoli a *Cynosurus cristatus* nella Riserva Naturale di Torricchio. La Riserva Naturale di Torricchio. 9:31–40.

Francalancia C, Galli P, Paradisi L. 1995. Variazioni nella composizione floristica dei prati a *Cynosurus cristatus* L. delle alte Val di Tazza di Fematre (Appennino marchigiano), in rapporto alle pratiche colturali. Fitosociol. 29:89–94.

Garnier E, Cortez J, Billès G, Navas ML, Roumet C, Debussche M, Laurent G, Blanchard A, Aubry D, Bellman A, et al. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology. 85(9):2630–2637. doi:10.1890/03-0799.

Giarrizzo E, Burrascano S, Chiti T, de Bello F, Lepš J, Zavattero L, Blasi C, Grytnes J-A. 2017. Re-visiting historical semi-natural grasslands in the Apennines to assess patterns of changes in species composition and functional traits. Appl Veg Sci. 20:247–258. doi:10.1111/avsc.12288.

Grime JP. 2001. Plant strategies, vegetation processes and ecosystem properties. 2nd ed. Chichester: Wiley.

Gross N, Börger L, Soriano-Morales SI, Le Bagousse-Pinguet Y, Quero JL, García-Gómez M, Valencia-Gómez E, Maestre FT. 2013. Uncovering multiscale effects of aridity and biotic interactions on the functional structure of Mediterranean shrublands. J Eco. 101 (3):637–649.

Ivan D, Donita N, Gafta D, Canullo R. 1994. La struttura dei pascoli a *Sesleria nitida* nella Riserva Naturale di Torricchio. La Riserva Naturale di Torricchio. 9:41–70.

Janeček Š, de Bello F, Horník J, Bartoš M, Černý T, Doležal J, Dvorský M, Fajmon K, Janečková P, Jiráská Š, et al. 2013. Effects of land-use changes on plant functional and taxonomic diversity along a productivity gradient in wet meadows. J Veg Sci. 24:898–909. doi:10.1111/jvs.12012.

Jost L. 2006. Entropy and diversity. Oikos. 113:363–375. doi:10.1111/j.2006.0030-1299.14714.x.

Kahmen S, Poschlod P. 2004. Plant functional trait responses to grassland succession over 25 years. J Veg Sci. 15:21–32.

Kaltsas D, Trichas A, Kougioumoutzis K, Chatzaki M. 2013. Ground beetles respond to grazing at assemblage level, rather than species-specifically: the case of Cretan shrublands. J Insect Conserv. 17:681–697. doi:10.1007/s10841-013-9553-0.

Keddy PA. 1992. Assembly and response rules: two goals for predictive community ecology. J Veg Sci. 3(2):157–164. doi:10.2307/3235676.

Kleijn D, Rundlöf M, Scheper J, Smith HG, Tscharntke T. 2011. Does conservation on farmland contribute to halting the biodiversity decline? Trends Ecol Evol. 26:474–481. doi:10.1016/j.tree.2011.05.009.

Kleijn D, Sutherland WJ. 2003. How effective are European agri-environment schemes in conserving and promoting biodiversity? J Appl Ecol. 40:947–969. doi:10.1111/j.1365-2664.2003.00868.x.

Kleyer M, Bekker RM, Knevel IC, Bakker JP, Thompson K, Sonnenschein M, Poschlod P, van Groenendael JM, Klimes L, Klimesová J, et al. 2008. The LEDA Traitbase: a database of life-history traits of Northwest European flora. J Ecol. 96:1266–1274. doi:10.1111/j.1365-2745.2008.01430.x.

Klimeš L, Hájek M, Mudrák O, Dančák M, Preislerová Z, Hájková P, Jongepierová I, Klimešová J. 2013. Effects of changes in management on resistance and resilience in three grassland communities. Appl Veg Sci. 16:640–649. doi:10.1111/avsc.12032.

Klimešová J, Danihelka J, Chrtek J, de Bello F, Herben T. 2017. CLO-PLA: a database of clonal and bud-bank traits of the Central European flora. Ecology. 98:1179–1179. doi:10.1002/ecy.1745.

Klotz S, Kuhn I, Durka W. 2002. BiolFlor. Eine Datebank zu biologischokologischen Merkmalen der Gefabpflanzen in Deutschland. Schriften fur Vegetationskunde, vol. 38. Bundesamt fur Naturschutz: Bonn. Available from http://www2.ufz.de/biolflor/index.jsp. Accessed 01 January 2020.

Köhler B, Gigon A, Edwards PJ, Krüsi B, Langenauer R, Lüscher A, Ryser P. 2005. Changes in the species composition and conservation value of limestone grasslands in Northern Switzerland after 22 years of contrasting managements. Persp Plant Ecol Evol System. 7:51–67. doi:10.1016/j.ppees.2004.11.003.

Lasanta T, Arnáez J, Pascual N, Ruiz-Flaño P, Errea MP, Lana-Renault N. 2017. Space–time process and drivers of land abandonment in Europe. Catena (Amst). 149:810–823. doi:10.1016/j.catena.2016.02.024.

Legendre P, Legendre L. 2012. Numerical Ecology. Third English ed. Amsterdam: Elsevier Science BV.

Lepš J. 2014. Scale-and time-dependent effects of fertilization, mowing and dominant removal on a grassland community during a 15-year experiment. J Appl Ecol. 51:978–987. doi:10.1111/1365-2664.12255.

Maire V, Gross N, Börger L, Wirth C, Proulx R, da Silveira Pontes L, Soussana J-F, Louault F. 2012. Habitat-filtering and niche differentiation jointly determine species relative abundance within grassland communities along fertility and disturbance gradients. New Phytol. 196:497–509. doi:10.1111/j.1469-8137.2012.04287.x.

Malatesta L, Tardella FM, Tavoloni M, Postiglione N, Piermarteri K, Catorci A, Feilhauer H. 2019. Land use change in the high mountain belts of the central Apennines led to marked changes of the grassland mosaic. Appl Veg Sci. 22(2):243–255. doi:10.1111/avsc.12416.

Marriott CA, Hood K, Fisher JM, Pakeman RJ. 2009. Long-term impacts of extensive grazing and abandonment on the species composition, richness, diversity and productivity of agricultural grassland. Agric Ecosyst Env. 134(3–4):190–200. doi:10.1016/j.agee.2009.07.002.

Maschinsky J, Whitham TG. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. Am Nat. 134:1–19. doi:10.1086/284962.

Mazzoleni S, Di Pasquale G, Mulligan M, Di Martino P, Rego F. 2004. Recent dynamics of the Mediterranean vegetation and landscape. Chichester: Wiley.

Mucina L, Bültmann H, Dierßen K, Theurillat J-P, Raus T, Čarni A, Šumberová K, Willner J, Dengler J, García RG, et al. 2016. Vegetation of Europe: hierarchical floristic classification system of vascular plants, bryophyte, lichen and algal communities. Appl Veg Sci. 19:3–264. doi:10.1111/avsc.12257.

Nunes A, Köbel M, Pinho P, Matos P, de Bello F, Correia O, Branquinho C. 2017. Which plant traits respond to aridity? A critical step to assess functional diversity in Mediterranean drylands. Agricultural and Forest Meteorology. 239:176–184. doi:10.1016/j.agrformet.2017.03.007.

O'Connor TG, Everson TM. 1998. Population dynamics of perennial grasses in African savanna and grassland. In: Cheplick GP, editor. Population biology of grasses. Cambridge: Cambridge University Press; p. 333–365.

Pakeman RJ. 2004. Consistency of plant species and trait responses to grazing along a productivity gradient: a multi-site analysis. J Ecol. 92(5):893–905. doi:10.1111/j.0022-0477.2004.00928.x.

Pakeman RJ, Quested HM. 2007. Sampling plant functional traits: what proportion of the species need to be measured? Appl Veg Sci. 10(1):91-–96. doi:10.1111/j.1654-109X.2007.tb00507.x.

Pavoine S, Vallet J, Dufour AB, Gachet S, Daniel H. 2009. On the challenge of treating various types of variables: application for improving the measurement of functional diversity. Oikos. 118(3):391–402. doi:10.1111/j.1600-0706.2008.16668.x.

Peco B, Carmona CP, de Pablos I, Azcárate FM. 2012. Effects of grazing abandonment on functional and taxonomic diversity of Mediterranean grasslands. Agric Ecosyst Environ. 152:27–32. doi:10.1016/j.agee.2012.02.009.

Peco B, Sánchez AM, Azcárate F. 2006. Abandonment in grazing systems: consequences for vegetation and soil. Agric Ecosyst Environ. 113:284–294. doi:10.1016/j.agee.2005.09.017.

Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, et al. 2013. New handbook for standardised measurement of plant functional traits worldwide. Aust J Bot. 61:167–234.

Pesaresi S, Biondi E, Casavecchia S. 2017. Bioclimates of Italy. J Maps. 13:955–960. doi:10.1080/17445647.2017.1413017.

Petraitis PS, Dunham AE, Niewiarowski PH. 1996. Inferring multiple causality: the limitations of path analysis. Funct Ecol. 421–431. doi:10.2307/2389934.

Pignatti S. 2005. Valori di bioindicazione delle piante vascolari della flora d'Italia. Braun-Blanquetia. 39:1–97.

Pignatti S, Guarino R, and La Rosa M. 2017. Flora d'Italia. Edagricole, Bologna.

Poptcheva K, Schwartze P, Vogel A, Kleinebecker T, Hölzel N. 2009. Changes in wet meadow vegetation after 20 years of different management in a field experiment (North-West Germany). Agric Ecosyst Environ. 134:108–114. doi:10.1016/j.agee.2009.06.004.

QGIS Development Team. 2019. QGIS geographic information system. Open Source Geospatial Foundation Project. version 2.18.11. http://qgis.osgeo.org. Accessed 10 February 2021.

Ricotta C. 2005. Additive partitioning of Rao's quadratic diversity: a hierarchical approach. Ecol Modell. 183(4):365–371. doi:10.1016/j.ecolmodel.2004.08.020.

Rivas-Martínez S, Sáenz SR, Penas A. 2011. Worldwide bioclimatic classification system. Glob Geob. 1:1–634. doi:10.5616/gg110001.

Scocco P, Piermarteri K, Malfatti A, Tardella FM, Catorci A. 2016. Increase of drought stress negatively affects the sustainability of extensive sheep farming in sub-Mediterranean climate. J Arid Environ. 128:50–58. doi:10.1016/j.jaridenv.2016.01.006.

Silvertown J, Dodd ME, McConway K, Potts J, Crawley M. 1994. Rainfall, biomass variation, and community composition in the park grass experiment. Ecology. 75:430–437. doi:10.2307/1940896.

Smit HJ, Metzger MJ, Ewert F. 2008. Spatial distribution of grassland productivity and land use in Europe. Agric Syst. 98:208–219. doi:10.1016/j.agsy.2008.07.004.

Somot S, Sevault F, Déqué M, Crépon M. 2008. 21st century climate change scenario for the Mediterranean using a coupled atmosphere-ocean regional climate model. Glob Planet Change. 63:112e126. doi:10.1016/j.gloplacha.2007.10.003.

Stanisci A, Bricca A, Calabrese V, Cutini M, Pauli H, Steinbauer K, Carranza ML. 2020. Functional composition and diversity of leaf traits in subalpine versus alpine vegetation in the Apennines. AoB Plants. 12(2). doi:10.1093/aobpla/plaa004.

Tardella FM, Bricca A, Goia I, Catorci A. 2020. How mowing restores montane Mediterranean grasslands following cessation of traditional livestock grazing. Agric Ecosyst Environ. 295:106880. doi:10.1016/j.agee.2020.106880.

Tardella FM, Catorci A. 2015. Context-dependent effects of abandonment vs. grazing on functional composition and diversity of sub-Mediterranean grasslands. Community Ecol. 16:254–266. doi:10.1556/168.2015.16.2.13.

Tardella FM, Malatesta L, Goia IG, Catorci A. 2018. Effects of long-term mowing on coenological composition and recovery routes of a *Brachypodium rupestre*-invaded community: insight into the restoration of sub-Mediterranean productive grasslands. Rend Lincei Sci Fis Nat. 29:329–341. doi:10.1007/s12210-018-0711-x.

Tardella FM, Piermarteri K, Malatesta L, Catorci A. 2016. Environmental gradients and grassland trait variation: insight into the effects of climate change. Acta Oecol. 76:47–60. doi:10.1016/j.actao.2016.08.002.

Uchida K, Ushimaru A. 2015. Land abandonment and intensification diminish spatial and temporal β-diversity of grassland plants and herbivorous insect within paddy terraces. J Appl Ecol. 52:1033e1043. doi:10.1111/1365-2664.12443.

Venanzoni R, Kwiatowski W. 1994. Carta delle serie di vegetazione della Riserva naturale di Torricchio (Appennino centrale). La Riserva naturale di Torricchio. 9:23–30.

Villéger S, Mouillot D. 2008. Additive partitioning of diversity including species differences: a comment on Hardy & Senterre (2007). J Ecol. 96:845–848. doi:10.1111/j.1365-2745.2007.01351.x.

Warren RJ. 2008. Mechanisms driving understory evergreen herb distributions across slope aspects: as derived from landscape position. Plant Ecol. 198:297–308. doi:10.1007/s11258-008-9406-1.

Zelený D. 2018. Which results of the standard test for community-weighted mean approach are too optimistic? J Veg Sci. 29(6):953–966. doi:10.1111/jvs.12688.

Zelený D 2020. weimea: weighted Mean Analysis. R package version 0.1.18.

Zelený D, Schaffers AP. 2012. Too good to be true: pitfalls of using mean Ellenberg indicator values in vegetation analyses. J Veg Sci. 23:419–431. doi:10.1111/j.1654-1103.2011.01366.x.

Zuur AF, Ieno EN, Elphick CS. 2010. A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol. 1(1):3–14. doi:10.1111/j.2041-210X.2009.00001.x.