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Grassland biodiversity: habitat types, ecological processes and conservation status

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Annex 1

Abstract

Grassland ecosystems, including pastures and (semi)-natural grasslands, and land dominated by forbs, mosses or lichens, are among the largest ecosystems in the world. Among grassland ecosystems, semi-natural dry grasslands represent an important land use in Europe covering more than a third of the European agricultural area. They have been created through traditional and low intensity agricultural practices, without using inorganic fertilizers or pesticides, and their maintenance over time is linked to human activity (mowing or livestock grazing), which intensity and extent, together with environmental conditions, determine semi-natural grassland structure and composition. The long history of low-intensity agricultural land use has created species-rich assemblages and semi-natural dry grasslands are among the most species-rich habitats in Europe. Beyond biodiversity, ecosystem services value of semi-natural dry grasslands is considerable. Semi-natural dry grasslands are recognized for providing regulating, provisioning, and cultural ecosystem services, thereby accomplishing an important role in biodiversity conservation and in ecosystem service supply. Despite the importance of semi-natural dry grasslands is largely recognized, they are affected by several pressures and threats in most European countries, and they are currently listed as Vulnerable in the European Red List of Habitats. The "Habitat Directive", that is based on a conservation approach to biodiversity, requires governments to provide monitoring, management, and all appropriate measures to maintain or restore, habitats at a "Favourable Conservation Status" (FCS). The concept of FCS is central to the EC Habitats Directive and means that a habitat's natural range and area are stable or increasing and the species structure and functions which are necessary for its long-term maintenance exist and are likely to continue to exist for the foreseeable future. Finally, the populations of its typical species are stable and self-maintaining. Several studies have devoted much effort to the development of methodologies for assessing the conservation status of habitats or ecosystems. Although proposed protocols are based on different approaches and different scales, they all suggest considering both quantitative and qualitative aspects, which refer to specific structures (physical components) and functions (ecological processes) necessary for the maintenance of the community. However, since the recognition of discrete thresholds and endpoints of the structural or functional decline of a habitat is mostly related to the decline of properties that involve species and interactions among species and between species and their environment, discrete thresholds and endpoints are difficult to detect. Moreover, since changes to ecological function are difficult to quantify, many protocols end up using qualitative criteria.

The main aim of this study was to assess semi-natural dry grassland conservation status. To this aim, the research focused on the identification of "proxies" that can be used to quantitatively evaluate

changes to ecological functions. The issue has been addressed from different perspectives; to define quantitative thresholds we considered both taxonomical and functional attributes of the community, and the associated ecosystem services (ES) associated to semi-natural dry grasslands. In this research, we first defined all possible semi-natural dry grassland communities in the study area and quantified four ecosystem services supplied by each community, using specifical proxy indicators. Then, the values of ES were used to define quantitative thresholds in the conservation status.

The study was carried out on semi-natural *Bromus erectus*-dominated dry grasslands in the pre-alpine and hill sector of Veneto region (NE Italy). Investigated dry grasslands are included in habitat 6210* "Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (*important orchid sites)" of the Habitat Directive 92/43/ECC.

A total of 179 1x1 m^2 plots were arranged in the study system through a stratified random sampling design. For each plot, we collected environmental data (e.g., altitude, slope inclination and aspect), community attributes (e.g., total vegetation cover, moss cover, mean vegetation height). We also recorded all vascular plants, and visually estimated their abundance using a continuous percentage scale. Moreover, we collected data on soil, biomass, and litter. Plant trait measurements were also performed to determine the three leaf traits necessary for the calculations of the CSR strategies (i.e., Specific Leaf Area -SLA, Leaf Dry Matter Content-LDMC, and Leaf Dry Weight-LDW). For each dry grassland community identified, we also measured four ecosystem services, namely Biodiversity, Productivity, Climate regulation and Pollination. The quantification of ES was made by using proxy indicators, respectively: i) species richness; ii) biomass weight; iii) carbon concentration in vegetation and soil and iv) the number of flowered species during the community's flowering season.

To characterise semi-natural dry grasslands and evaluate the influence of abiotic filters on community structure and composition, we built a matrix composed of the 179 surveyed plots and soil parameters, i.e., soil depth (cm), pH, moisture (g x kg-1), carbon content (C %) and nitrogen content (N %). The matrix of 179 plots by 5 soil variables was analysed by cluster analysis (PC-ORD 5.1 software), using Relative Sorensen as a distance measure and Flexibe Beta method algorithm for dendrogram construction. To summarise environmental variations among groups emerged by cluster analysis, at the same matrix of 179 plots by five soil variables it was applied a Principal Component Analysis (PCA). To investigate the relationships between environmental and community attributes, and between the four ecosystem services and data regarding both taxonomical and functional community attributes, we applied Spearman correlations.

We defined three different communities of semi-natural dry grassland based on taxonomical and functional community structure and composition and considering abiotic factors.

Results allowed to evidence a certain homogeneity among the three communities as far as some environmental parameters and community attributes are concerned. Altitude and soil N revealed as the most important environmental variables affecting plant community attributes. Semi-natural dry grasslands communities could be associated to the presence of groups of specialised species, which, together with environmental variables, allowed to distinguish three different communities, namely *Artemisia alba*, *Bromus erectus* and *Brachypodium rupestre* communities. The three communities supplied different values of the four ecosystem services (ES) studied. Among the three grasslands communities, *Bromus erectus* community turned out as the community with the highest multi-service value. results showed that not only the number but also identity of specialised species were important factors in determining semi-natural dry grasslands functioning and provision of ecosystem services. This approach allowed to identify threshold levels corresponding to the ideal, intermediate, and bad condition of the community as regards ecosystem service provision. With respect to *Bromus erectus* community, quantitative thresholds were defined. These thresholds were based on community attributes that governed ES, corresponding thus to different habitat conditions. These attributes can thus be used to define the "reference status", which includes the list of specialised (i.e., typical) species and the range of values of community attributes (e.g., range of vegetation cover, forb vs. grass species cover) that correspond to the "ideal" state. In this framework, threshold limits correspond to the values indicating a shift in habitat conditions, namely a change in the status of the habitat.

From a conservation point of view, the study showed that different communities of semi-natural dry grasslands provide different or different levels of the same ecosystem service. Thus, only the maintenance of different communities will assure a high provision of ES at local scale. A methodological framework that considers different and standardized indicators is useful to pinpoint better strategies to biodiversity conservation, in order to maximise ecosystem services provisioning to human well-being. Further studies should be implemented to better understand mechanisms involved in trade-offs between ecosystem services, to increase the potential of the concept of ES in adding value to current conservation approaches.

General introduction

Grasslands in the wider sense are among the largest ecosystems in the world. Their area is estimated at 40% of the terrestrial area (World Resources Institute, 2000, based on International Geosphere/Biosphere Program (IGBP) data) and the 10% is non-woody grassland. In its narrow sense, grasslands may be defined as ground covered by vegetation dominated by grasses, with little or no tree cover. UNESCO defines grasslands as “land covered with herbaceous plants with less than 10% tree and shrub cover” (White, 1983).

Grassland ecosystems include pastures and (semi)-natural grasslands and land dominated by forbs, mosses, or lichens. Annex I of the Habitats Directive (92/43/EEC) lists different types of grasslands in Europe that can be grouped in two broad categories: i) natural grasslands, which include grassland habitats that exist without direct human intervention and are limited by specific ecological, soil and climate conditions (e.g., alpine and subalpine siliceous and calcareous grasslands), and ii) semi-natural grasslands, which include grassland habitats that are to some extent managed.

Semi-natural grasslands, that include self-seeded herbaceous and shrub vegetation, can be defined as open habitats with a grass- and forb- dominated vegetation, with a low proportion of woody species (Crofts and Jefferson 1999). They are an important land use in Europe covering more than a third of the European agricultural area (Lesschen et al., 2014; Velthof et al., 2014). Semi-natural grasslands have been created through traditional and low intensity agricultural practices, without using inorganic fertilizers or pesticides (Hejcman et al. 2013). Their maintenance over time is thus linked to human activity (mowing or livestock grazing), and different semi-natural grassland communities are the result of different extent of human influence on the grassland species composition and environmental conditions. Specifically, without mowing and grazing, semi-natural grasslands naturally overgrow with shrubs and forest (Rūsiņa, 2017). Mowing and grazing promote gradual soil compaction, accumulation of plant root remains, branching of plant roots and lower levels of oxygen in the soil. Because of this, creeping grasses disappear and are succeeded by bunch grasses. This stage lasts for decades, but with the gradual accumulation of organic material, the inflow of oxygen is constantly reduced, resulting in the introduction of compact tussock grasses. In dry places, the compact tussock stage can last for a very long time (even several centuries) (Rūsiņa, 2017).

Semi-natural grasslands occur in different climates and on different soil types and moisture conditions; they can develop on wet, moist, or dry, fertile or nutrient-poor, acidic or calcareous soils. Three main types of semi-natural grasslands occur in Europe: i) neutral or mesotrophic grasslands, which usually occur on moderately moist mineral soils with neutral soil pH; ii) calcareous grasslands, which typically develop on dry soil on pervious calcareous bedrock; and iii) wet grasslands, which occur on soils with a high ground-water table for most of the year (Crofts and Jefferson 1999).

Different types of semi-natural grasslands, originated from different abiotic conditions and management types, contain distinct vegetation, and are characterized by different plant species; and many of these species are confined to a given habitat type, as they have adapted to and depend on these particular conditions (Tälle, 2018). Moreover, differences in environmental conditions determine suitable management approaches and options of their application (Calaciura and Spinelli, 2008; Rūsiņa (Ed.), 2017).

Among temperate semi-natural grassland types, dry grasslands are a unique ecosystem evolved by traditional agricultural activities. They are created by traditional farming practices and are mainly used for grazing by livestock or haymaking but might also be a successional stage of abandoned arable fields, characterizing a typical traditional agri-environmental landscape (Wellstein et al., 2014). The long history of low-intensity agricultural land use has created species-rich assemblages and semi-natural dry grasslands are among the most species-rich habitats in Europe (Wilson et al., 2012; Chytrý et al., 2015).

They harbour a high diversity of plants species, sometimes over 50 species per m^2 (Wilson et al., 2012). Besides plants, they also provide habitat for rare species from different taxonomic groups, including thousands of pollinator species (Bastian, 2013; Zulka et al., 2014). Beyond biodiversity, also ecosystem service values of semi-natural dry grasslands are considerable. They are recognized for providing regulating, provisioning, and cultural ecosystem services (World Resources Institute, 2005; Bastian, 2013; Dengler et al., 2014; Zulka et al., 2014).

Biodiversity itself can be considered as an ecosystem service, reflecting an intrinsic value. The unique semi-natural dry grasslands richness is not only valuable in itself, but it also underpins the other ecosystem services provided by grasslands since biological diversity at the level of genes and species directly contributes to some goods (Mace et al., 2012). Productivity is the primary function of grassland ecosystems, providing forage for livestock (Garnier et al., 2016; Garnier and Navas, 2012). Semi-natural grasslands also play an important role in carbon sequestration thereby contributing to the local climate regulation (Hungate et al., 2017), as accumulation rates are high and decomposition of organic material slow (Gibson, 2009). Moreover, the contribution of semi-natural grasslands to pollination service is also notable (de Bello et al., 2010; Clough et al., 2014; Fantinato et al. 2016; 2018; 2019) and the conservation of semi-natural habitats such as wildflower-rich grasslands have been suggested as one of the most effective measure in pollinator species conservation (Orford et al., 2016). Finally, grassland ecosystems also provide an attractive environment for leisure activities and recreation, representing valuable destinations for eco-tourists and a suitable landscape for environmental education.

Although the importance of semi-natural dry grasslands is largely recognised, they have declined and are affected by several pressures and threats in most European countries (Janssen et al., 2016). In Europe, semi-natural dry grassland communities are mostly included in the habitat 6210(*) “Semi-natural dry grasslands and shrubland facies on calcareous substrates (Festuco-Brometalia)” by the 92/43/EEC Directive as “habitat of priority importance” when they host populations of orchid species (European Commission, 2013). Despite this, they are currently listed as Vulnerable in the European Red List of Habitats (Janssen et al., 2016). At national level, the 3rd National Report on the conservation status of habitats and species (Genovesi et al., 2014), that is requested by Article 17 of “Habitat Directive” 92/43, substantially agrees in referring to semi-natural grasslands as one of the most threatened habitat types.

Over the last decades, semi-natural dry grasslands have been subjected to a rapid decline in quality and quantity mostly due to land-use changes (e.g., afforestation, conversion to cropland) and inappropriate management following the gradual vanishing of traditional rural cultures throughout Europe (Janišová et al., 2014; Stoate et al., 2001; Poschlod et al., 2005 Oppermann et al., 2012).

Intensification of management techniques can involve fertilization and herbicide use, but also overgrazing, which reduces species richness, concerning both plants and animal species. Intensified use has negative effects on this habitat type that is adapted to low nutrient levels; a greater availability of soil nutrients determines a greater productivity of some species to the detriment of less competitive species, thereby leading to a decline in species diversity (Fridley, 2002; Caroll et al., 2003).

On the other side, the abandonment of traditional land-uses, that is often part of wider demographic, socio-economic and cultural shifts across large parts of the European rural landscape, also results in the loss of species and leads to the development of dynamic phases dominated by shrubs and woody communities, often poorer in species. Furthermore, when grazing or mowing are ceased, litter accumulates very quickly (Török et al., 2011; Olmeda et al., 2019) as the biomass is not removed. The accumulation of litter has also a fertilising effect, thereby increasing the content of soil organic matter (Olmeda et al., 2019). Moreover, disturbance (biomass-destroying phenomena) contributes to the decrease in biomass production of some grass species that negatively influences species diversity (Bonanomi and Allegrezza, 2004; Croft and Jefferson, 1999; Sebastià et al, 2008; Bullock, 1994; Wilson and Tilman, 2002; Sochera et al., 2013).

The “Habitat Directive”, based on a conservation approach to biodiversity, requires governments to provide monitoring, management, and all appropriate measures to maintain or restore, habitats at a “Favourable Conservation Status” (FCS). The concept of FCS is central to the EC Habitats Directive and means that a habitat’s natural range and area are stable or increasing and the species structure and functions which are necessary for its long-term maintenance exist and are likely to continue to exist for the foreseeable future. Finally, the populations of its typical species are stable and self-maintaining (Jones, 2002).

European Member States are required to undertake surveillance of the conservation status of the natural habitats and species referred to in Article 2 of the Habitat Directive, with particular regard to priority habitat types and species. European Member States have developed methodologies for assessing the conservation status of habitat types and species of Community interest or are in the process of developing/improving such methods. These methods usually define variables and criteria for the key parameters as range, area, structure and function, that indicate whether the habitat type is in a favourable conservation status (FCS) (Olmeda et al., 2019).

Historically, the structure of a community has been described on the basis of quantitative aspects such as species presence and abundances, using species richness and other parameters that reflect the “taxonomic facet” of diversity (de Bello et al., 2010; Duelli 1997). Recent studies introduced other insights, highlighting the role of the functional diversity of a community in explaining the relationship between biodiversity and ecosystem functioning (Cadotte et al., 2011; Diaz et al., 2006; Gaucherand and Lavorel, 2007), and the use of a functional approach to study diversity is considered relevant to quantify ecosystem services (Cardinale et al., 2012; Garnier et al., 2016).

Since 1990s many works have demonstrated that biodiversity generally enhances many process rates, and the relationship between biodiversity and ecosystem functioning (B-EF) has received detailed scientific interest (Loreau et al., 2001; Reiss et al., 2009; Tilman et al. 1997; Vitousek et al., 1993). The role of biodiversity in supporting and maintaining ecosystem services has been largely demonstrated (Vitousek et al., 1993; Thompson and Starzomski, 2007), as well as the consequences of the loss of biodiversity on the functioning of ecosystems and in turn on human well-being (Balvanera et al., 2014; Bastian, 2013; Harrison et al., 2014).

Species diversity influences the stability and resistance of ecosystem functions against environmental perturbations. A more diverse ecosystem is likely to contain species that are adapted to a changed environment, thereby assuring stable ecosystem functions (Vannucchi and Bretzel, 2015; Gaujour et al., 2012; Willig, 2011) and stability under increasing pressure from several anthropogenic drivers, such as habitat degradation, over-exploitation, invasive alien species, pollution, and climate change. Therefore, only ecosystems and habitats in a good conservation status ensure the continued provision of ecosystem services (Brondizio et al., 2019; Westman, 1977).

Recently, a variety of frameworks has been proposed for assessing the conservation status of habitats or ecosystems (e.g., Evans and Arvela, 2011; Keith et al., 2013; New South Wales Scientific Committee, 2012; Nicholson et al., 2009; Rodríguez et al., 2011, 2012; Walker et al., 2006). Although protocols are based on different approaches and different scales, they all suggest considering both quantitative and qualitative aspects, which refer to specific structures (physical components) and functions (ecological processes) necessary for the maintenance of the community. The recognition of discrete thresholds and endpoints of the structural or functional decline of a vegetation type is mostly related to the decline of properties that involve species and interactions among species and between species and their environment; given that, discrete thresholds and endpoints are difficult to detect (Nicholson et al., 2009). Since changes to ecological function are difficult to quantify, many protocols end up using qualitative criteria (Del Vecchio et al., 2016).

Standardized biodiversity monitoring schemes are needed to build scientifically sound decision-making tools for biodiversity conservation and standardized indicators are essential to define the conservation status (Duelli and Obrist, 2003; Noss, 1990; Spangenberg, 2007). Therefore, there is the necessity to define and develop quantitative criteria and thresholds that will help to characterize the conservation status of habitats and ecosystems. Currently, a methodological framework for the assessment of plant community quality and functioning using quantitative standardized thresholds still lacks. However, such a methodological framework is expected to support a better monitoring, provide a better understanding of the dynamics of degraded ecosystems, and facilitate mitigation of degradation processes, and restoration actions (Andrade et al., 2015).

Objectives of the thesis

The general objective of this thesis was to define and test a methodological framework for the assessment of plant community quality and functioning using quantitative standardized thresholds. To this aim, the research focused on the identification of ‘proxies’ that can be used to quantitatively evaluate changes to ecological functions. Since no single indicator can capture all dimensions of biodiversity (Andrade et al., 2015; Briske et al., 2006; Brondizio et al., 2019; de Bello et al., 2010; Hobbs et al., 2009), different indicators have been integrated into a common framework. Specifically, to define quantitative thresholds we considered both taxonomical and functional attributes of the community, and the associated ecosystem services (ES). Indeed, although the benefits provided by semi-natural grasslands to society are widely acknowledged, the identification and the quantification of ES are still critical components of effective conservation plans (Maes et al., 2018). Methodologies to obtain information about ES are increasing (Bastian, 2013), but the potential of the concept of ES in adding value to current conservation approaches remains poorly explored (Maes et al. 2012). Furthermore, recent studies have increased attention towards the multiple relationships among ecosystem services (Bennet et al., 2009; Carpenter et al., 2009). Some studies mapped supply and demand of ES (van Jaarsveld et al., 2005), others assessed the current and potential future status of ecosystem services (Carpenter et al., 2006), and others also assessed the threats towards ecosystem services (MEA, 2005; Tilman et al., 2001). Despite this increasing attention, relatively little has been made to understand the relationship between community attributes and the provision of ecosystem services (Drius et al., 2019; Kremen and Ostfeld, 2005). Moreover, the ES study appears important and complex because of the trade-offs between them (Bennett et al., 2009). Further studies need to be conducted to understand mechanisms involved in trade-offs between ecosystem services and help land managers to optimize the available supplies of multiple ecosystem services (Pan et al., 2014).

In this research, we first identified all semi-natural dry grassland communities in the study area and quantified four ecosystem services (i.e., Biodiversity, Productivity, Climate regulation and Pollination) supplied by each community, using specifical proxy indicators. Then, we evaluated the relationships between the four ecosystem services and community taxonomical and functional attributes. Finally, the values of ES were used to define quantitative thresholds in community attributes that could help define the community conservation status.

Specifically, the main objective has been developed through the following steps:

I. Characterisation of semi-natural dry grasslands through the description of community attributes based on both a taxonomical and a functional approach, and considering environmental factors;

II. Assessment of ecosystem functioning through the evaluation of four different ecosystem services provided by semi-natural dry grasslands, namely biodiversity, productivity, climate regulation and pollination;

III. Definition of ecological thresholds in community attributes that could help define the community conservation status, using the values of ecosystem services.

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Study system

The study was carried out on semi-natural *Bromus erectus*-dominated dry grasslands in the pre-alpine and hill sector of Veneto region (NE Italy), in particular: Lessini Mounts, Euganei, Berici and Asolani Hills and floodplain of the Piave river (Fig. 1). These localities were designated as Special Protection Areas (SPA) and Sites of Community Interest (SCI) according to Directive 92/43/EEC, since they host numerous endangered or endemic species and habitats of community interest.

Pre-alpine and hilly reliefs in the northern part of Veneto region (respectively Lessini Mounts and Asolani Hills), were originated from an orogenic thrust front resulting from the collision of the Adriatic and the European plates at the end of the Cretaceous (Cuffaro et al., 2010), which caused the lift of marine sedimentary rocks of calcium carbonate. Berici and Euganei Hills, located in the southern part of the region, are of volcanic origin (from late Paleocene to late Oligocene; Macera et al., 2003) and between the volcanic deposits there are calcareous and clayey marine sediments (Kaltenrieder et al., 2010). Dry grasslands in the floodplain of the Piave river are situated in area called “grave” that are consolidated terraces made by alluvial sediment of coarse gravel but not affected by river’s overflow.

The landscape of the study area is historically a mosaic of arable fields, semi-natural grasslands, and deciduous woodlands; semi-natural dry grasslands establish on mid-altitude limestone slopes on sites characterized by poorly developed, shallow, and skeletal calcareous soils, characterized by low water retention capacity and high leaching rates (Bini, 2001; Fantinato et al., 2016). Bioclimatic classification, performed according to the Worldwide Bioclimatic Classification System (http://www.globalbioclimatics.org/), shows a Temperate-Oceanic type.

By the end of the Second World War, marginal, less productive areas of the hilly and pre-alpine relieves of the Veneto region had suffered a progressive decline. Nowadays, abandonment still results in the loss of landscape heterogeneity, leading to the development of dynamic phases dominated by shrubs and deciduous woodlands, which indeed represent the major land cover type (Cocca et al., 2012; Campagnaro et al., 2017).

Investigated dry grasslands are included in habitat 6210* “Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (*important orchid sites)” of the Habitat Directive 92/43/ECC. According to the Interpretation Manual of European Union Habitats (European Commission, 2013) and based on Terzi (2015) syntaxonomic revision, the habitat 6210 includes plant communities belonging to two orders within the phytosociological class *Festuco-Brometea* Br.-Bl. & Tx. ex Klika & Hadač 1944: the steppic or subcontinental grasslands (*Festucetalia valesiacae* Br.-Bl.

& Tüxen ex Br.-Bl. 1949 order) and the grasslands of more oceanic and sub-Mediterranean regions (*Festuco-Brometalia* Br.-Bl. & Tx ex Br.-Bl. 1950 order). In the latter, a distinction is made between primary dry grasslands of the alliance *Xerobromion* Br.-Bl. & Moor 1938, and secondary (semi-natural) dry grasslands of the alliance *Mesobromion* Braun-Blanquet & Moor 1938 (or *Bromion*) with *Bromus erectus* (Calaciura and Spinelli, 2008; European Commission, 2013).

The investigated community is characterised by a wide variety of grasses and forbs; some species are associated with tall-growing vegetation, others with woodland fringes and gaps, and other species are more typical of open grasslands with both tall and short vegetation. Accordingly, the species pool is rather heterogeneous and can be dominated by species as *Bromus erectus* Huds., *Artemisia alba* Turra, *Bothriochloa ischaemum* (L.) Keng, *Dorycnium pentaphyllum* Scop., *Koeleria pyramidata* (Lam.) P. Beauv., followed by others of less abundance as *Scabiosa triandra* L., *Helianthemum nummularium* subsp. *obscurum* (Čelak.) Holub, *Euphorbia cyparissias* L. and *Globularia bisnagarica* L.; moreover, sometimes dry grasslands of the study area host several orchid species (Fantinato et al. 2016; 2019).

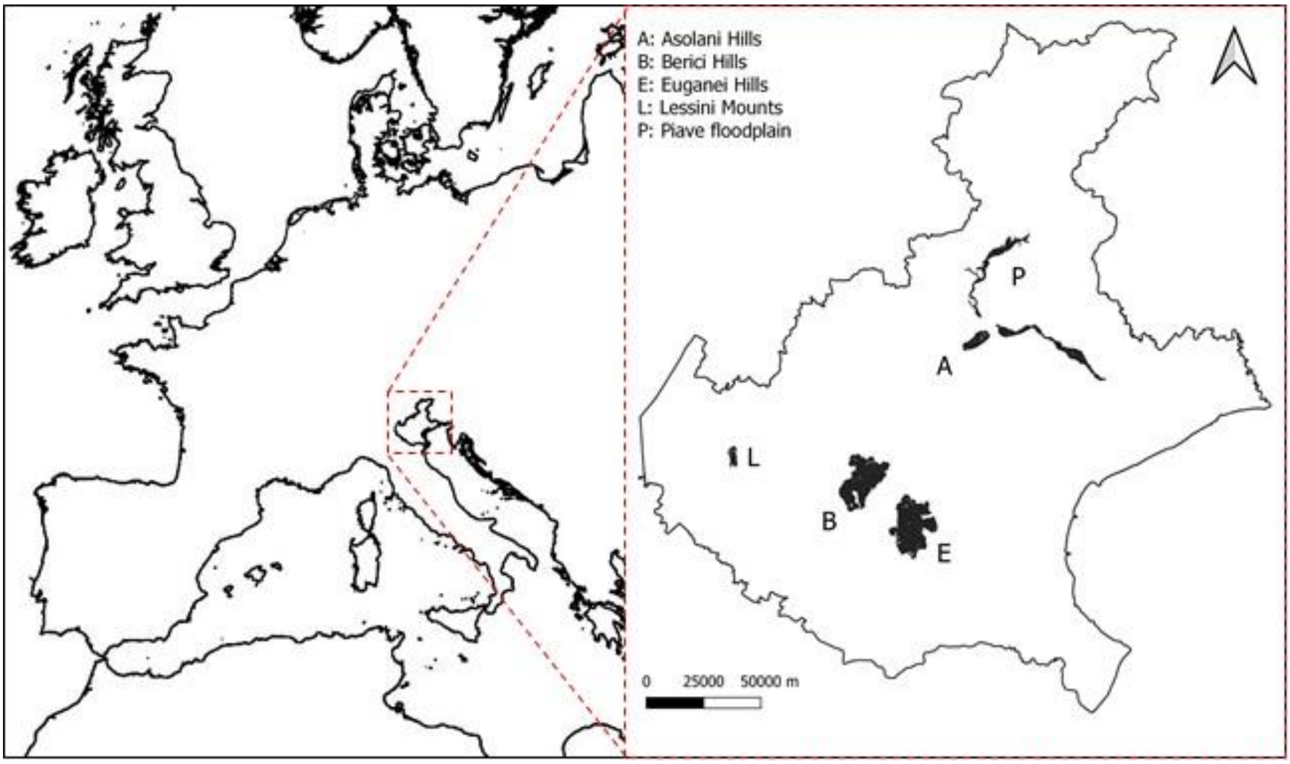

Figure 1: Study area of the semi-natural dry grasslands investigated, in the pre-alpine and hill sector of Veneto region (NE Italy). L: Lessini Mounts; E: Euganei Hills; B: Berici Hills; A: Asolani Hills; P: floodplain of the Piave river

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Chapter I. Characterisation of semi-natural dry grasslands through the description of community attributes based on both a taxonomical and a functional approach, and considering environmental factors

Introduction

Being subjected to diverse perturbations, both natural and human induced, that vary in their spatial extents, periods, durations, frequencies and intensities, plant communities can be found in several possible states. DeMalach et al. (2018) and Fukami and Nakajima (2013) defined these alternative states as communities that vary in structure (e.g., species composition and diversity) and/or function (e.g., total biomass and carbon flux), with shifts from a state to another occurring at critical thresholds (Dent et al., 2002; Holling, 1973; Knowlton, 1992; May, 1977; Scheffer et al., 2001; Sutherland, 1974).

Species composition of semi-natural grassland is highly variable being affected by numerous factors (Janisovà et al., 2010). The EU Habitat Interpretation Manual (Europena Commission, 2013) recommends a rather wide interpretation of the habitat, also including some woody species, which develop with the relaxation of management. Overall, the differences in species composition could be the result of abiotic factors acting directly as a filter, or of biotic interactions or even a combination of these (Filibeck et al., 2020; Keddy, 2005).

Among abiotic factors, landscape topography, expressed by elevation, slope inclination and exposure, affects the quantity of incoming solar radiation (Bennie et al., 2004) and have been recently proved to influence the conservation status of semi-natural dry grasslands (Büchler et al., 2020). Soil characteristics as fertility, chemistry parameters and physical properties, are also considered very important in shaping community richness and composition (Moeslund et al., 2013); for example, great soil moisture or high nutrient contents may allow fast-growing species to achieve high cover or biomass (Filibeck et al., 2020). N-deposition seems to have a significant impact on semi-natural dry grasslands since it negatively affect species richness, especially in oligo- to mesotrophic soils (Dengler and Tischew, 2018; Dengler et al., 2020). Bobbink et al. (1998) found that eutrophication of soil through nitrogen inputs from the air influences species composition, as dominant grasses and nitrophytic species become dominant, displacing characteristic species. Soil depth can also affect community species richness, with richness increasing with increasing soil depth (Fridley et al., 2011; Palpurina et al., 2015).

Concerning biotic factors, trade-offs between plant stress tolerance, adaptation to disturbance and competitive ability is often assumed to exist (e.g., Grime, 1977); accordingly, highly competitive, and fast-growing plants are expected where stress (phenomena limiting plants growth) and disturbance (biomass-destroying phenomena) remain low (Grime et al., 2007). However, high levels of cover or biomass of fast-growing species are known to negatively impact species richness (Czarniecka-Wiera et al., 2019) since slower-growing or smaller species are excluded through light interception and high litter accumulation that can suppress the germination of other species (Garnier et al., 2007). Another crucial species trait is the height of an individual, which confers a competitive advantage through prior access to light and is also central to a species' carbon gain (King 1990; Westoby et al. 2002). Furthermore, a high reproductive height also improves pollination and/or efficiency of seed dispersal in herbaceous species (Lortie and Aarssen 1999; Soons et al. 2004), explaining the large investment of many grassland plant species, especially rosette plants, in stem length (Bazzaz et al. 2000).

Over the years, researchers have identified several mechanisms that regulate species patterns (Götzenberger et al., 2012). Most current quantifications deal with the "taxonomic facet" of diversity. Accordingly, the structure of a community has been investigated through the analysis of species richness and the distribution of their abundances (Garnier et al., 2016), assumed as attributes easy to obtain, and cost-effective for quantifying and interpreting data from a given species assemblage (Cadotte et al., 2010). Therefore, the species richness, as the total number of vascular species, and evenness index (J), which are among the most popular diversity indices in vegetation analysis (Gurevitch et al. 2002; Ricotta, 2005), are frequently used as synthetic descriptors of the community structure. Despite their frequent use, the relationships between the two indices still remain unclear. Some studies suggested a direct positive relationship between species evenness and species richness (e.g., Alatalo, 1981; Hill, 1973); while other recent studies revealed that the relationship between species richness and evenness is not always positive (Manier and Hobbs, 2006; Stirling and Wilsey, 2001). Species evenness and richness also differ in their responses to local habitat factors (Lundholm and Larson, 2003; Ma, 2005), suggesting that the two descriptors may vary independently and can be influenced by different ecological processes. For example, Wilsey and Stirling (2007) showed that, while the richness was influenced by the availability of seed sources in the surroundings, the evenness component was more sensitive to changes in the intensity of competition.

The diversity of organisms is also related to other differences which transcend taxonomic boundaries (Lavorel et al., 2008). One of these relates to the diversity of functions carried out by different organisms in relation to the environments that they occupy, i.e., the "functional facet" of diversity. The functional component of organisms is essential to understand the functioning of ecological systems (Lavorel and Garnier, 2002), and the determination of how the observed functional patterns relate to environmental conditions may be useful to clarify the relationships between community structure and ecosystem function (Grime 2006; Diaz et al., 2007).

The functional diversity can be defined as the distribution of trait values in a community (Díaz and Cabido 2001; Tilman 2001), where the different characteristics used to describe plants are called “traits” (McGill et al., 2006; Violle et al., 2007). Research based on the utilization of traits increased over the last three decades (Garnier and Navas, 2012; Naeem and Bunker, 2009; Pierce et al., 2013; Wright et al., 2005), and plant traits have been identified as strong candidates to quantify ecosystem service delivery given their effects on underlying ecosystem processes (de Bello et al., 2010; Diaz et al., 2006; Lavorel and Grigulis, 2012).

Numerous classification systems based on various morphological and/or functional criteria have been proposed during the twentieth century. Among these, the development of Grime’s adaptive strategies model continues to be influential in plant ecology (Grime and Pierce, 2012). The Competitor-Stress-Ruderal (CSR) classification of plant functional types (Grime, 1974; 1977; 2001) is frequently used to described variations in the vegetation through its functional composition (Cerabolini et. al, 2010) and the application of the CSR theory led to the development of CSR classification methods (Grime, 1988; Hodgson et al., 1999; Pierce et al., 2017). The system predicts the occurrence of three plant strategies corresponding to different investment of resources: i) ability to compete with neighbours (Competitor - C), ii) tolerate stress (Stress tolerator - S) and iii) survive biomass destruction or disturbance (Ruderal plant - R). Plant species can exhibit different proportions of the characteristics related to each of the three primary strategies (C-, S- and R-). C-strategy plants are adapted for a rapid uptake of resources and a long-term occupation; S-strategy plants have small stature with longer lifespan, an investment in durable and well-defended structures; R-strategy plants have often short stature, with short lifespans as an adaptation for rapid development and reproduction. Besides the three primary strategies, four secondary (CR, SR, SC, and CSR) and twelve intermediate strategies exist within the CSR system, each exploiting a different intermediate combination of stress and disturbance.

The study of functional patterns to understand assembly processes can be also approached using indices useful to describe how species and communities respond to biotic and abiotic factors (Lavorel and Garnier 2002; Mason et al., 2005). Functional diversity (FD) quantifies how dissimilar the coexisting species are within a community and is widely used to detect assembly rules (Botta-Dukat 2005; Laliberté and Legendre 2010). Patterns of trait divergence (overdispersion, i.e., the set of species in a given plot would be less similar to each other than expected at random) are expected wherever communities are constrained by assembly rules (Diamond 1975) such as limiting similarity, which states that the niche similarity of coexisting species is restricted by the interactions’ controls

(MacArthur and Levins 1967). Conversely, trait convergence patterns (underdispersion, i.e., species in a given plot would be more similar to each other than expected at random) are expected to arise from constraints imposed by environmental filters (Keddy 1992, Weiher and Keddy 1995).

Considering what stated above, the aim of this study was to characterise semi-natural dry grasslands through the description of community attributes (i.e., community structure and species composition) based on both a taxonomical and a functional approach and considering environmental factors.

This approach will allow to identify main taxonomical, functional, and environmental gradients of the studied vegetation.

Materials and Methods

Sampling design and data collection

A total of 179 1x1 m^2 plots were arranged through a stratified random sampling design. 28 patches included in the hilly sector (altitude < 500 m a.s.l.) and categorized as habitat 6210* of the Habitat Directive 92/43/ECC in the Natura 2000 Veneto cartography (available at "http://www.regione.veneto.it") were selected in GIS environment (QGIS 3.6.0), and the QGIS tool "random point inside polygons" was used to obtain random plots. Plots were sampled from April to July in 2018 and 2019, when the vegetation was at the period of peak standing biomass.

For each 1x1 m^2 plot, we collected environmental data as altitude (m), slope inclination (degree), slope aspect (degree), community attributes as the percentage of total vegetation cover, the percentage of vascular species cover, the percentage of moss cover, and the mean vegetation height (cm), measured at 4 points within each 1x1 m^2 plot and averaged. Each 1x1 m^2 plot has been surveyed using a wooden frame divided into 100 squares of 10x10 cm^2. For each plot, all vascular plants were recorded, and their abundance was visually estimated, determining their projected cover and using a continuous percentage scale. Species nomenclature follows The Plant List (2013).

Soil parameters, biomass and litter

Five soil samples were collected in a homogeneous area of about 3x3 m^2 around each plot; samples were collected one at each corner and one at the centre of the area, in the uppermost 10-15 cm depth using a little shovel. The five samples were than mixed to obtain one single soil sample for each plot. Moreover, in correspondence with each soil collection point, the soil depth (i.e., the depth from surface to bedrock) was measured using a metre and averaged to obtain a single value per plot. Collected samples were appropriately labelled and packed in plastic bags. On arrival at the laboratory, samples were air-dried, disaggregated, and passed through a 2-mm sieve. Following the procedures in “Metodi ufficiali di analisi chimica del suolo” (Ministero per le Politiche Agricole,1999), soil pH was measured in a soil/water suspension (1:2.5) by electrode and meter, and soil moisture was determined weighting 20 gr of air-dried soil samples before and after oven-dried at 105° C for at least 16 hours.

Within each 3x3 m^2 area around each plot, samples of aboveground standing biomass and litter were collected in one sub-quadrat area of 0.5 x 0.5 m^2, to be representative of the vegetation pattern; samples were clipped at about 1 cm from ground level, during the sampling period for aboveground biomass and autumn (September-October) for litter. Clipped samples were kept in plastic bags and weighed in the laboratory using a digital scale to determine the fresh weight; lastly, samples were oven-dried at 70° for 48 hours and weighed (Garnier et al.,2016).

Oven-dried samples of aboveground biomass, litter and soil were used for C and N analysis. Concentrations of the two elements were measured in a CHNS analyser instrument (Vario Micro Cube, CHNS, *Elementar*, Fisher). Samples were grinded using a shaker vibration to obtain a homogenised powder, freezed and then lyophilised to remove all water content; a sample quantity of approximately 2 mg was weighed with an analytical balance on an aluminium capsule of 0,5 cm^2, and 2 mg of tungsten (for the protection of the instrument) are added. The standard chosen was Organic Analytical Standard (OAS) (C: 1,86%; N: 0,12%; S: 0,012%). The instrument, which uses a continuous flow of helium and an instantaneous flow of oxygen to combustion reaction, allows to obtain the area of the peak concentration of C and N by passing the sample through a combustion column at 1200 ° C (reaction combustion) and a reduction column at 800 ° C (reduction reaction). In the final phase a column (trap) divides the gases obtained from the previous reactions and an analyser detects the area of the peaks of the various elementary components. Finally, the area of the peaks was determined manually.

Species traits measurements

To characterise communities through their functional composition, the classification of plant species was based on their biological attributes. Plant trait measurements were performed to determine the three leaf traits necessary for the calculations of the CSR strategies (i.e., Specific Leaf Area -SLA, Leaf Dry Matter Content-LDMC, and Leaf Dry Weight-LDW) following Pierce et al. (2017).

To calculate leaf traits, for each ramet of the 46 species collected, 4 young fully expanded and undamaged herbaceous leaves (including the petiole) were cut. The set of species was composed of the most abundant species, and their percentage cover, i.e., standing biomass, represented approximately 80% of the total species cover, thereby ensuring an adequate description of overall properties. Leaf fresh weight (LFW) was measured using a digital analytical scale (Radwag/AS220.R2, Poland, with maximum of 220 g and a precision of 0.001 g). The fresh leaves were scanned using a digital scanner (CanoScan LiDE 220, Vietnam) and the images were saved as a bitmap file for the calculation of the Leaf Area (LA) using Leaf Area Measurement V-1.3 (A.P. Askew 2003, The University of Sheffield-UK). The leaf dry weight (LDW) was then determined with the same analytical scale of LFW after 24 h of drying in an oven at 105 °C.

The Specific Leaf Area (SLA) values for each leaf replicate were calculated by using the following equation:

$$\mathrm{SLA} = \frac{\text{Leaf area (mm}^2\text{)}}{\text{Leaf dry weight (mg)}}$$

The percentage of the dry matter content of leaf replicates was calculated using the following formula:

$$\mathrm{LDMC} = \frac{\text{Leaf dry weight (mg)}}{\text{Leaf fresh weight (mg)}}$$

Values of LA, LDMC and SLA of 46 species were used for the species classification into CSR strategy categories. The classification tool, the CSR triangulator VP provided by Pierce et al. (2013), allows the classification of species within a triangular space corresponding to Grime’s theoretical CSR triangle. The CSR classification analyses the trade-off between the traits LA, LDMC and SLA to calculate the relative proportion of C-, S- and R-strategy of species and classifies species according to their CSR-strategy. For all those species not directly measured (43 species), we retrieved data available at BiolFlor ("https://www.ufz.de/biolflor/index.jsp") (Annex 1).

Plant height (H) was determined for the species that were picked up for leaf traits determination; following the methodology in Perez-Harguindeguy et al. (2013), plant height was measured as the shortest distance (cm; using a meter) between the upper boundary of leaves and soil surface. For all those species not directly measured, plant height was assigned following Pignatti (1982).

Data analysis

To characterise semi-natural dry grasslands and evaluate the influence of abiotic filters on community structure and composition, we built a matrix composed of the 179 surveyed plots and soil parameters, i.e., soil depth (cm), pH, moisture (g x kg^{-1}), carbon content (C %) and nitrogen content (N %).

The matrix of 179 plots by 5 soil variables was analysed by cluster analysis (PC-ORD 5.1 software), using Relative Sorensen as a distance measure and Flexibe Beta method algorithm for dendrogram construction. To summarise environmental variations among groups emerged by cluster analysis, at the same matrix of 179 plots by five soil variables it was applied a Principal Component Analysis (PCA). This analysis allowed to describe the major environmental gradient (the first principal component). Moreover, to detect which of the five soil variables mostly contributed to the dissimilarity between groups we performed an analysis of similarity percentages (SIMPER analysis) using Bray Curtis dissimilarity metric (Clarke, 1993). Both the PCA and the SIMPER analysis were performed using Past Software (Hammer et al., 2001).

For each group of plots emerged by the clustering, we calculated the mean value of environmental parameters: (i) slope inclination (°); (ii) slope exposure (°); (iii) altitude (m); (iv) soil depth (cm), pH, moisture (g x kg^{-1}), carbon content (%) and nitrogen content (%). Moreover, for each group of plots we calculated the mean value of some community attributes as (i) total vegetation cover (%); (ii) vascular species cover (%); (iii) moss cover (%); (iv) mean species height (cm); (v) the percentage cover of growth forms, considering grasses (G; herbaceous plants with narrow leaves growing from the base, graminoids), forbs (F; herbaceous flowering plants that are not graminoids) and shrubs (S; woody plants, including: a) dwarf shrubs, with usually multiple, ascending, woody stems less than 0.5 m tall; b) shrubs, woody plants between 0.5 m and 5 m tall, with canopy typically carried by several trunks that are usually thinner and younger than typical mature tree trunks; c) trees, woody plants usually >5 m tall; only seedling trees were found in sampling plot). Growth form categories were determined through field observation following Cornelissen et al. (2003); (vi) species richness (S), i.e., the number of species encountered per plot; (vii) Evenness index J’, calculated as J’=H’/ln S, where H' is the Shannon index (H'= - ∑pi ln pi), S is the number of species encountered in each plot, and the lnS is the natural logarithm of the number of species encountered in a plot (Magurran, 1988); (viii) pollination mode as the percentage cover and the richness of entomophilous and anemophilous species; information about the pollination mode was retrieved by BiolFlor classification (https://www.ufz.de/biolflor/index.jsp); (ix) the percentage cover of C-, S- , R- and CSR- strategy of species; (x) biomass weight (g/m^2), biomass carbon and nitrogen content (%); (xi) litter weight (g/m^2), litter carbon and nitrogen content (%).

To describe the functional structure of the community we used the community-weighted mean (CWM; Garnier et al., 2004; Ricotta and Moretti, 2011) that reflects the dominant trait value within a community. CWM and FD are often analysed in parallel (Dainese et al., 2012; de Bello et al., 2010). CWM corresponds to the average trait value in a community, weighted by the relative abundance of the species carrying each value (Garnier et al., 2004). The Functional diversity (FD; Villéger et al., 2008; Laliberté and Legendre 2010) quantifies the degree of (i) functional richness (FRic) that represents the amount of functional trait space filled by species in the community, (ii) functional evenness (FEve) that describes the evenness of the distribution of abundance in a functional trait space (Mason et al. 2005) and (iii) functional divergence (FDiv) that relates to how abundance is distributed within the volume of the functional trait space occupied by species (Villéger et al., 2008). Both CWM and FD indices were computed with the *dbFD* function in the R package "FD" (Laliberté et al., 2014). Using trait values of Specific Leaf Area (SLA), Leaf Dry Matter Content (LDMC) and Plant height (H) (determined from the species collected for leaf trait measurement), we computed for each trait in each plot the community-weighted mean value (CWM) and the functional diversity (FD), i.e., (i) the community weighted mean of height (CWM H), specific leaf area (CWM SLA) and leaf dry matter content (CWM LDMC); (ii) the functional richness (FRic), evenness (FEve) and divergence (FDiv).

To detect differences in the parameters among the groups, we performed one-way PERMANOVA test (Permutational Multivariate Analysis of Variance; 9999 randomizations; Past Software; Hammer et al. 2001) and Tukey Test using the groups of the cluster analysis as independent variable (Anderson and Ter Braak, 2003).

To define species specialisation to a given group of plots we performed an Indicator Species Analysis (ISA; Dufrêne and Legendre, 1997), using the *multipatt* function in R package "indicspecies" (De Cáceres and Legendre, 2009; De Cáceres et al., 2010) and choosing 'r.g' as the statistical value to identify species fidelity to the cluster analysis' groups. Only species with P-values < 0.1 were considered specialized for a given group of plots (Hart and Chen, 2008; Kumar et al., 2017).

Finally, to investigate the relationships between environmental and community attributes, we applied Spearman correlation (Past software; Hammer et al. 2001) to a matrix of 179 plots by 37 variables, regarding environmental variables (slope inclination (°), slope aspect (°), altitude (m), soil depth (cm), pH, moisture (g x kg -1), carbon content (C %) and nitrogen content (N %)) as well as community attributes (both taxonomical and functional). Significance was determined at $p < 0.05$.

Results

Interpretation of the results of cluster analysis clearly indicated three groups of plots (Fig. 1). PERMANOVA test evidenced significant differences among the three groups (F=17,53; p=0,0001). The first principal component (Component 1) of the PCA analysis (Fig. 2), captured ca. 34% of the total variation in the data and was highly correlated with soil carbon content (C %), and pH. The second principal component (Component 2), explaining the 23% of the variance, represented soil moisture

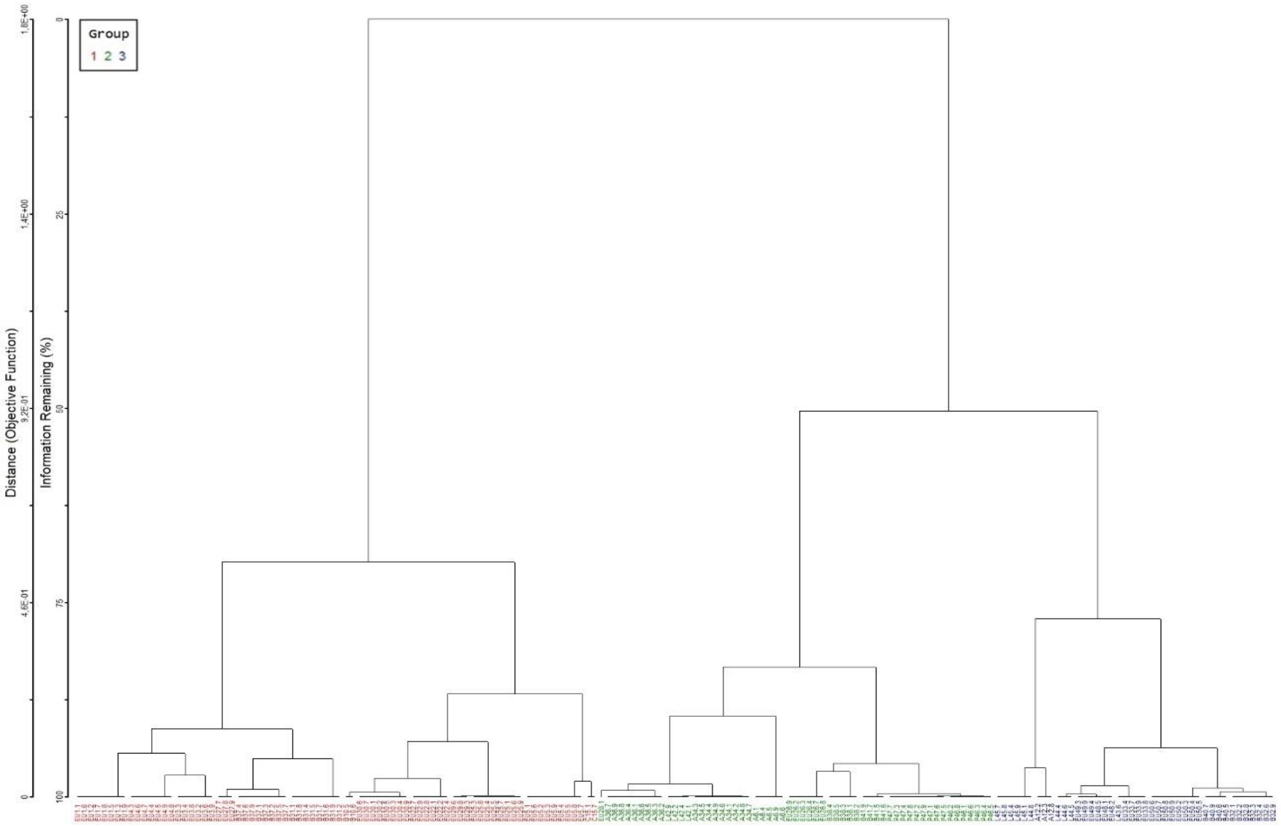

Figure 1: Cluster dendrogram of the 179 sampling plots. Groups resulted from the cluster analysis are painted in different colours according to the legend of the figure. Red = Group 1; Green = Group 2; Blue = Group 3

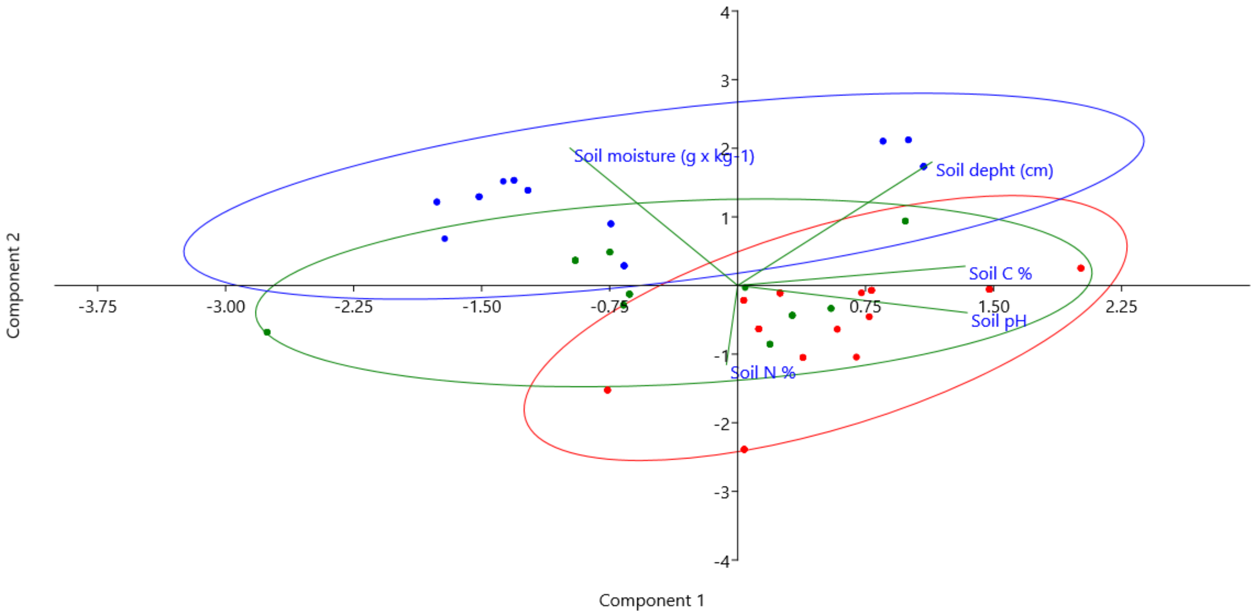

Figure 2: Principal Component Analysis (PCA) The first principal component (Component 1) captured ca. 34% of the total variation in the data and was highly correlated with soil carbon content (C %), and pH. The second principal component (Component 2), explaining the 23% of the variance, represented soil moisture. Red dots and circle indicate plots of group 1, green dots and circle for plots of groups 2 and blue dots and circle for plots of groups 3.

(g x kg^{-1}) and soil depth (cm). Overall, the ordination showed that plots of Group 1 (red dots in Fig. 2) were characterised by a higher soil carbon content (C%) and soil nitrogen content (N%), while high values of soil pH were shared by plots of Group 1 and Group 2. Group 3 (blue dots) contained plots that were clearly distinguished by soil moisture (g x kg^{-1}) and soil depth (cm).

SIMPER analysis of the soil data revealed that soil moisture on average explained the 86% of dissimilarity. Soil moisture together with soil carbon content explained a cumulative percentage of dissimilarity of about 90% between Groups 1 and 2 (cumulative percentage of 89,61; Tab. 1A) and between Groups 2 and 3 (cumulative percentage of 92,62; Tab. 1C). Group 1 differed from group 3 mainly due to soil moisture (91,71 % contribution of dissimilarity; Tab 1B) and soil depth (3,44%); these two variables together displayed a cumulative dissimilarity of 95,15%. The percent contribution of the other soil variables, as soil pH and soil nitrogen content, was quite low, contributing on average of 2% and 1% respectively (Tab. 1).

Table 1: SIMPER analysis identifying the % contribution of each soil variable to the Bray Curtis dissimilarity metric between group 1 and 2 (A), group 1 and 3 (B) and between group 2 and 3 (C).

A

Overall average dissimilarity group 1 and 2: 28,8%

Soil variables	**Average dissimilarity**	**Contribution %**	**Cumulative %**	**Mean abundance 1**	**Mean abundance 2**
Soil moisture (g x kg-1)	23,41	81,29	81,29	30,8	65,9
Soil C %	2,395	8,316	89,61	12,8	9,86
Soil depth (cm)	1,815	6,3	95,91	6,69	5,58
Soil N %	1,01	3,506	99,41	1,92	0,819
Soil pH	0,1689	0,5866	100	7,7	7,7

B

Overall average dissimilarity group 1 and 3: 44,95%

Soil variables	**Average dissimilarity**	**Contribution %**	**Cumulative %**	**Mean abundance 1**	**Mean abundance 2**
Soil moisture (g x kg-1)	41,22	91,71	91,71	30,8	116
Soil depth (cm)	1,548	3,445	95,15	6,69	7,9
Soil C %	1,15	2,558	97,71	12,8	11,5
Soil N %	0,8617	1,917	99,63	1,92	1,25
Soil pH	0,1673	0,3722	100	7,7	7,58

C

Overall average dissimilarity group 2 and 3: 24,02%

Soil variables	**Average dissimilarity**	**Contribution %**	**Cumulative %**	**Mean abundance 1**	**Mean abundance 2**
Soil moisture (g x kg-1)	20,86	86,85	86,85	65,9	116
Soil C %	1,387	5,774	92,62	9,86	11,5
Soil depth (cm)	1,315	5,476	98,1	5,58	7,9
Soil N %	0,3096	1,289	99,38	0,819	1,25
Soil pH	0,148	0,6161	100	7,7	7,58

PCA (Fig. 2), the post hoc test “Tukey Test” (Fig. 3, 4, 5, 6,7) and the Indicator Species Analysis (Tab. 2), allowed to differentiate the three groups of plots with differences regarding both site conditions and community attributes (Tab. 3).

Plots of the three Groups were evenly distributed across the study area, i.e., there was not a concentration of plots of a given group in a specific geographic area. Group 1 was made of 78 plots from Euganei and Berici Hills and Piave river; Group 2 included 59 plots from Euganei, Berici and Asolani Hills, Lessini Mounts and Piave river; Group 3 contained 42 plots of Euganei, Berici and Asolani Hills and Lessini Mounts.

According to the Indicator Species Analysis (Tab. 2), the three groups had in common some widespread species typical of dry grasslands as *Bromus erectus*, *Scabiosa columbaria*, *Allium sphaerocephalon*, *Euphorbia cyparissias*. However, they also exhibited a set of almost exclusive species that allowed to distinguish them according to their species composition, structure, and environmental parameters.

Group 1 - *Artemisia alba* community. This group was characterized by a high frequency and cover of *Artemisia alba, Fumana procumbens, Globularia bisnagarica*. Several species were only found in this group of plots: *Bothriochloa ischaemum*, *Briza media*, *Ononis natrix*, *Potentilla hirta*, and *Anacamptis pyramidalis.*

Group 2 - *Bromus erectus* community. Group 2 had the most numerous set of specialised species, including exclusive species as *Carex caryophyllea*, *Alopecurus pratensis*, *Anthericum liliago*, *Anthyllis vulneraria*, and *Salvia pratensis*. Despite being in common with all groups, other species characterised the community with their high cover: *Bromus erectus*, *Peucedanum cervaria*, *Galium verum*, *Teucrium montanum*, *Geranium purpureum*, *Potentilla pusilla*, and *Helianthemum nummularium* subsp. *obscurum*.

Group 3 - *Brachypodium rupestre* community. This group of plots was characterised by a set of species as *Brachypodium rupestre* and *Melica ciliata*, which reached here the highest frequency and percentage cover, and ruderal and edge species such as *Hypericum perforatum*, *Geranium sanguineum*, *Fragaria viridis*, *Peucedanum oreoselinum*, and *Orlaya grandiflora.*

Table 2: List of the species (frequency and mean cover ± sd) significantly specialised to the nine groups according to the Indicator Species Analysis (ISA). In bold species that resulted significantly associated only to the group that they belong to, in black species specialised for more than only one group and in light grey species that belong to the group but are not significant according to ISA. Annex 2 for the complete list of species.

		Group 1		**Group 2**		**Group 3**	
Plant species	P values	freq%	cov% ± sd	freq%	cov% ± sd	freq%	cov% ± sd
Artemisia alba Turra	**0,001**	**84,62**	**16,75 ± 14,51**	50,85	6,91 ± 17,65	59,52	3,24 ± 4,69
Fumana procumbens (Dunal) Gren. & Godr.	**0,001**	**64,1**	**2,29 ± 2,87**	52,54	0,68 ± 0,9	21,43	0,21 ± 0,67
Globularia bisnagarica L.	**0,006**	**56,41**	**2,65 ± 4,48**	59,32	1,3 ± 2,28	47,62	0,79 ± 1,25
Koeleria pyramidata (Lam.) P.Beauv.	**0,001**	**52,56**	**3,72 ± 6,1**	33,9	0,69 ± 1,38	23,81	0,09 ± 0,2
Carex flacca Schreb.	**0,001**	**26,92**	**0,42 ± 0,79**	1,69	0,01 ± 0,07	-	-
Chrysopogon gryllus (L.) Trin.	**0,002**	**20,51**	**9,28 ± 19**	40,68	4,62 ± 7,45	9,52	0,36 ± 1,61
Ononis natrix L.	**0,001**	**20,51**	**0,13 ± 0,42**	-	-	-	-
Thlaspi perfoliatum L.	**0,022**	**20,51**	**0,04 ± 0,19**	-	-	-	-
Anacamptis pyramidalis (L.) Rich.	**0,001**	**17,95**	**0,08 ± 0,19**	-	-	-	-
Medicago minima (L.) L.	**0,039**	**15,38**	**0,09 ± 0,26**	3,39	0,02 ± 0,09	11,9	0,03 ± 0,09
Cleistogenes serotina (L.) Keng	**0,014**	**14,1**	**0,92 ± 3,68**	1,69	0,05 ± 0,39	7,14	0,11 ± 0,41
Bothriochloa ischaemun (L.) Keng	**0,001**	**14,1**	**0,12 ± 0,37**	-	-	-	-
Ononis spinosa L.	**0,013**	**12,82**	**0,14 ± 0,45**	3,39	0,03 ± 0,2	-	-
Briza media L.	**0,010**	**10,26**	**0,39 ± 1,84**	-	-	-	-
Prunella vulgaris L.	**0,002**	**10,26**	**0,29 ± 0,97**	-	-	-	-
Quercus pubescens Willd.	**0,013**	**7,69**	**0,21 ± 0,73**	3,39	0,02 ± 0,13	-	-
Potentilla hirta L.	**0,031**	**7,69**	**0,07 ± 0,37**	-	-	-	-

Table 2: Continued

Plant species	P values	**Group 1**		**Group 2**		**Group 3**	
		freq%	cov% ± sd	freq%	cov% ± sd	freq%	cov% ± sd
Peucedanum cervaria (L.) Cusson ex Lapeyr.	**0,001**	28,21	0,44 ± 1,07	**66,1**	**2,01 ± 2,33**	23,81	0,74 ± 1,49
Teucrium montanum L.	**0,001**	25,64	0,35 ± 1,06	**64,41**	**1,25 ± 2,15**	23,81	0,27 ± 0,59
Helianthemum nummularium subsp. obscurum Holub	**0,033**	55,13	0,78 ± 1,19	**57,63**	**1,3 ± 1,56**	47,62	0,73 ± 1,08
Galium verum L.	**0,001**	29,49	0,63 ± 1,68	**49,15**	**5,24 ± 8,03**	40,48	1,54 ± 2,96
Carex caryophyllea J.F.Gmel.	**0,001**	-	-	**37,29**	**1,07 ± 1,62**	-	-
Asperula cynanchica L.	**0,001**	5,13	0,02 ± 0,08	**32,2**	**0,55 ± 0,95**	21,43	0,02 ± 0,04
Inula hirta L.	**0,001**	20,51	0,4 ± 0,92	**28,81**	**0,82 ± 1,47**	-	-
Silene vulgaris (Moench) Garcke	**0,023**	10,26	0,19 ± 0,85	**27,12**	**0,42 ± 0,9**	4,76	0,03 ± 0,16
Dactylis glomerata L.	**0,005**	8,97	0,08 ± 0,3	**20,34**	**0,63 ± 1,61**	14,29	0,18 ± 0,49
Lotus corniculatus L.	**0,002**	5,13	0,02 ± 0,09	**20,34**	**0,53 ± 1,57**	2,38	0,05 ± 0,31
Alopecurus pratensis L.	**0,001**	-	-	**20,34**	**0,38 ± 0,96**	-	-
Potentilla pusilla Host	**0,029**	2,56	0,05 ± 0,4	**18,64**	**0,19 ± 0,47**	4,76	0,03 ± 0,16
Plantago media L.	**0,004**	2,56	0,02 ± 0,12	**13,56**	**0,11 ± 0,33**	-	-
Trifolium campestre Schreb.	**0,006**	5,13	0,01 ± 0,08	**11,86**	**0,41 ± 1,52**	7,14	0,02 ± 0,08
Anthericum liliago L.	**0,001**	-	-	**11,86**	**0,11 ± 0,35**	-	-
Geranium purpureum Vill.	**0,005**	-	-	**8,47**	**0,12 ± 0,42**	-	-
Salvia pratensis L.	**0,010**	-	-	**8,47**	**0,12 ± 0,46**	-	-
Anthyllis vulneraria L.	**0,006**	-	-	**8,47**	**0,01 ± 0,04**	-	-
Erigeron annuus (L.) Pers.	**0,024**	-	-	**6,78**	**0,19 ± 0,78**	-	-

Table 2: Continued

		Group 1		Group 2		Group 3	
Plant species	P values	freq%	cov% ± sd	freq%	cov% ± sd	freq%	cov% ± sd
Brachypodium rupestre (Host) Roem. & Schult.	**0,001**	26,92	1,5 ± 5,67	23,73	0,84 ± 2,03	**57,14**	**5,45 ± 8,82**
Convolvulus cantabrica L.	**0,001**	10,26	0,1 ± 0,38	5,08	0,46 ± 2,07	**52,38**	**1,98 ± 3,87**
Stachys recta L.	**0,001**	8,97	0,09 ± 0,38	1,69	0,02 ± 0,13	**52,38**	**0,75 ± 1,23**
Geranium sanguineum L.	**0,001**	5,13	0,07 ± 0,43	1,69	0,01 ± 0,07	**45,24**	**1,98 ± 3,6**
Bupleurum baldense Turra	**0,007**	23,08	0,07 ± 0,16	10,17	0,02 ± 0,05	**45,24**	**0,13 ± 0,24**
Melica ciliata L.	**0,001**	-	-	6,78	1,44 ± 5,6	**40,48**	**6,96 ± 10,2**
Dorycnium herbaceum Villar	**0,013**	20,51	0,79 ± 1,91	44,07	0,82 ± 1,33	**33,33**	**1,81 ± 3,13**
Hypericum perforatum L.	**0,001**	2,56	0,003 ± 0,02	3,39	0,07 ± 0,37	**30,95**	**0,25 ± 0,47**
Orlaya grandiflora (L.) Hoffm.	**0,001**	-	-	1,69	0,01 ± 0,07	**16,67**	**0,38 ± 1,01**
Galium lucidum All.	**0,002**	5,13	0,03 ± 0,16	-	-	**11,9**	**0,24 ± 1,1**
Torilis arvensis (Huds.) Link	**0,001**	-	-	-	-	**11,9**	**0,08 ± 0,24**
Eryngium amethystinum L.	**0,010**	3,85	0,03 ± 0,14	5,08	0,15 ± 0,69	**9,52**	**0,74 ± 2,47**
Peucedanum oreoselinum (L.) Moench	**0,011**	-	-	-	-	**7,14**	**0,36 ± 1,32**
Fragaria viridis Weston	**0,011**	-	-	-	-	**7,14**	**0,05 ± 0,22**
Bromus erectus Huds.	0,001	100	21,59 ± 20,48	100	52,75 ± 19,84	100	39,79 ± 15
Ononis reclinata L.	0,026	24,36	0,14 ± 0,29	8,47	0,04 ± 0,13	28,57	0,18 ± 0,37
Scabiosa columbaria L.	0,011	1,28	- ± 0,03	11,86	0,15 ± 0,47	26,19	0,29 ± 0,72
Euphorbia cyparissias L.	0,003	8,97	0,02 ± 0,07	35,59	0,16 ± 0,32	26,19	0,17 ± 0,34
Allium sphaerocephalon L.	0,015	-	-	18,64	0,02 ± 0,04	11,9	0,02 ± 0,05

Concerning site conditions, plots of Group 2 were located at significantly higher altitude compared to plots of Group 1 and 3 (mean altitude of 251,30±73,31, 120,85±87,75 and 150,22±87,80, respectively; difference not significant between Group 1 and 3; Fig. 3) and significantly higher slope inclination (5,86±5,91) (Fig. 3; Tab. 3), while slope exposure did not show differences among groups. Soil characteristics highly contributed to differentiating the three groups. Three parameters (soil moisture, soil depth and soil carbon) were significantly different across the three groups of plots (Tab.1).

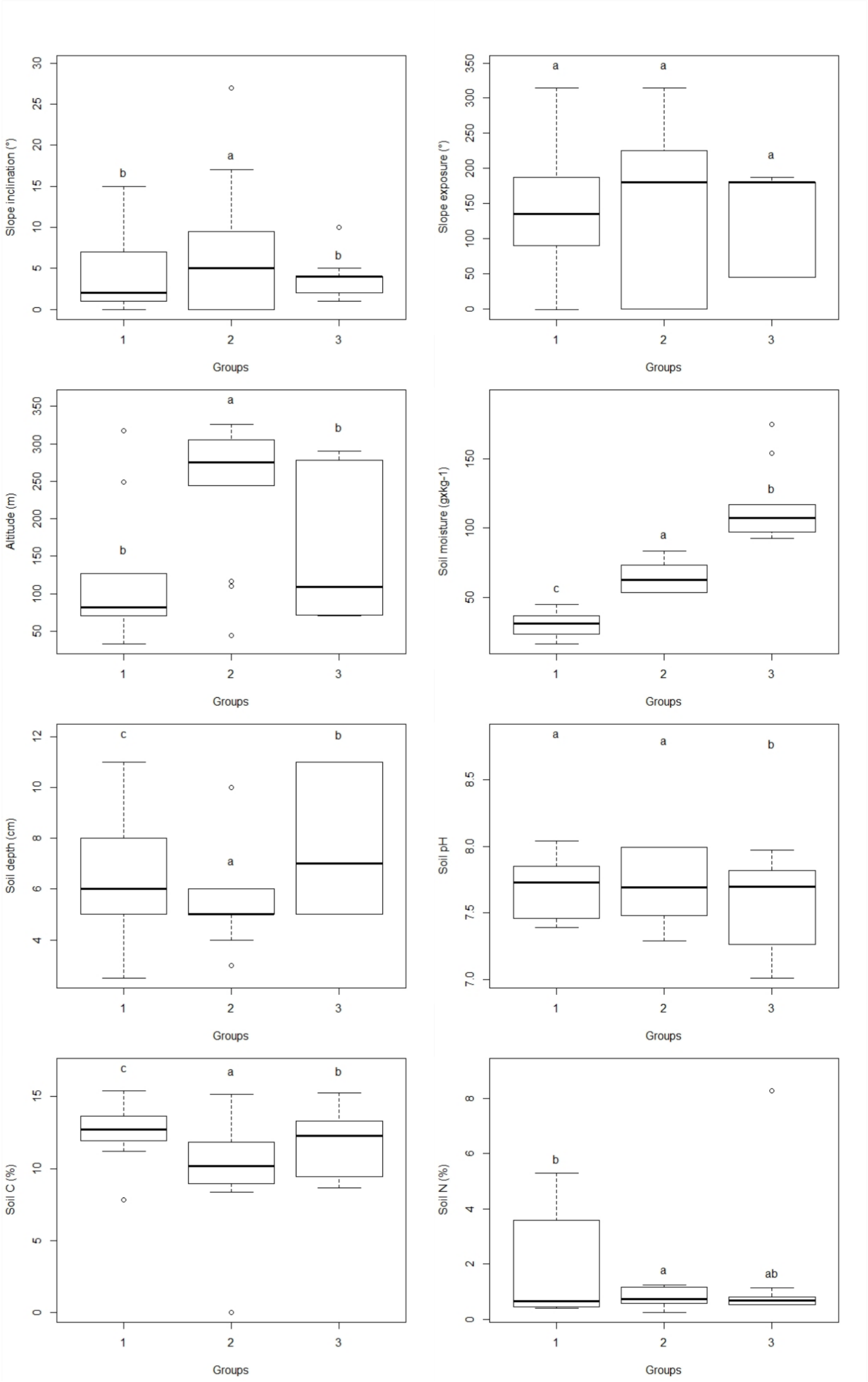

Fig. 3: Box plot describing environmental parameters (Slope inclination, Slope exposure, Altitude; Soil moisture, Soil Soil depth, Soil pH, Soil Carbon content and Soil Nitrogen content) in the three groups resulted from cluster analysis (median and percentiles are shown; outliers are represented by white circles). Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

Specifically, Group 3 had the highest moisture and soil depth, while Group 1 had a significantly higher soil C. Soil pH resulted significantly different in the third group (7,58±0,34) compared to the other groups, while soil N was higher in Group 1 (not significant) (Tab. 3).

Overall, plots of the *Artemisia alba* community (Group 1) showed a significantly higher cover of the moss layer (26,58±29,68), while plots of *Bromus erectus* community (Group 2) displayed a higher vascular species cover (86,83±13,18) than the other two groups. *Brachypodium rupestre* community (Group 3) had a significantly lower total vegetation cover (78,64±13,86) (Fig. 4; Tab. 3).

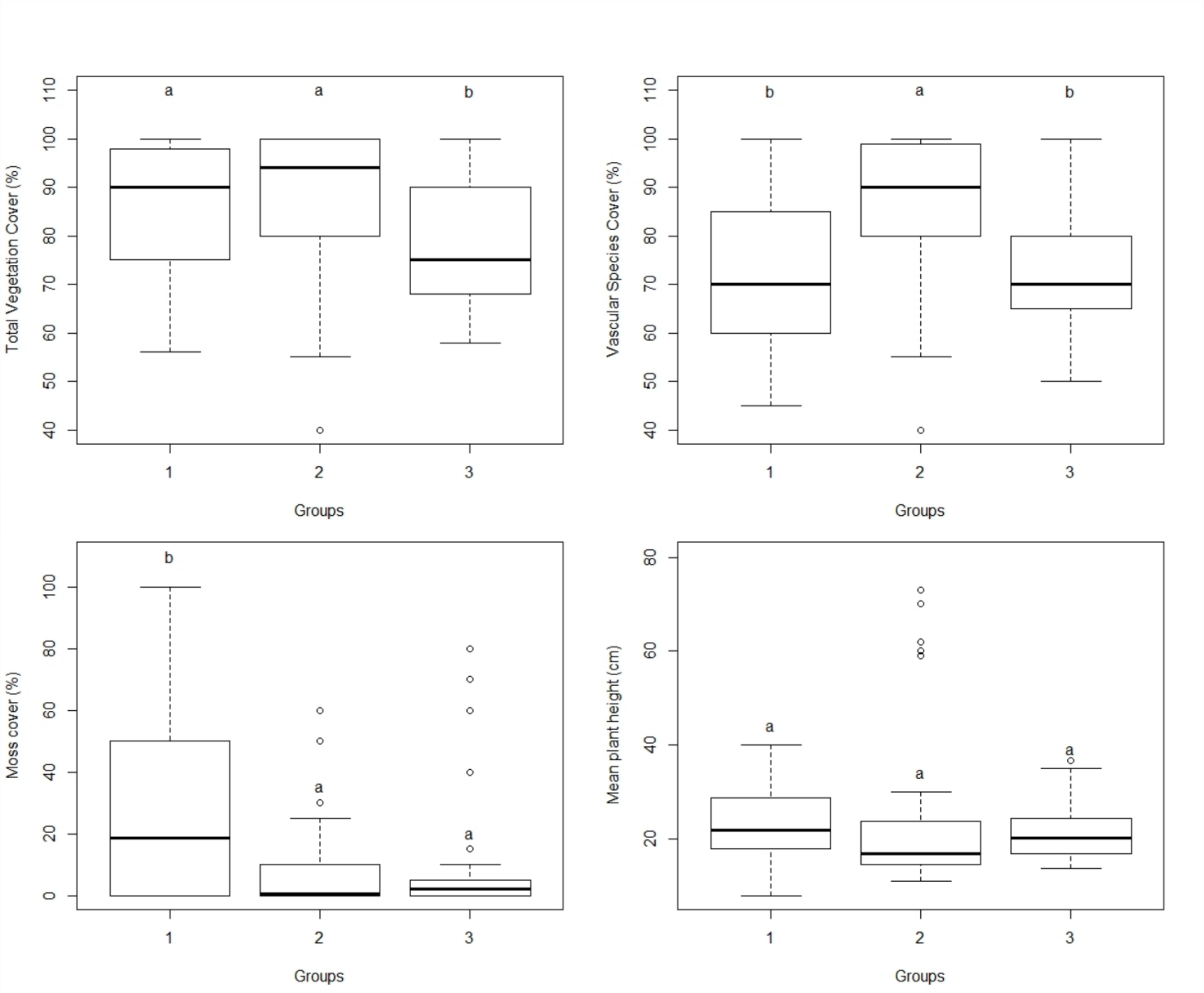

Fig. 4: Box plot describing Total vegetataion cover, Vascular species cover, Moss cover, Mean species hight, in the three groups resulted from cluster analysis (median and percentiles are shown; outliers are represented by white circles). Different letters indicate significant differences according to Tukey's test (p < 0.05).

Mean species richness per plot was not significantly different among the three groups, ranging from 12,49 ± 2,59 of *Artemisia alba* community to 13,64 ± 3,69 of *Bromus erectus* community (Tab. 3). Conversely, Evenness index J displayed a significantly higher value in Group 3 (0,59±0,09) (Fig. 5; Tab. 3).

Anemophilous species were higher both in number (not significantly) and percentage cover in plots of Group 2 (Fig. 5; Tab. 3). Although not significantly, Group 2 also showed a higher cover of entomophilous species, followed by plots of Group 2 and Group 1. Another notable difference regarded dominant growth forms; while plots of Group 1 were dominated by dwarf shrub species (mean cover of 22,46±16,24), plots of Group 2 and 3 showed a clear dominance of grasses (mean cover of 62,18±18,90 and 53,65±19,22), and forbs (mean cover of 17,09±8,01 and 15,49±11,98) (Fig. 5; Tab. 3).

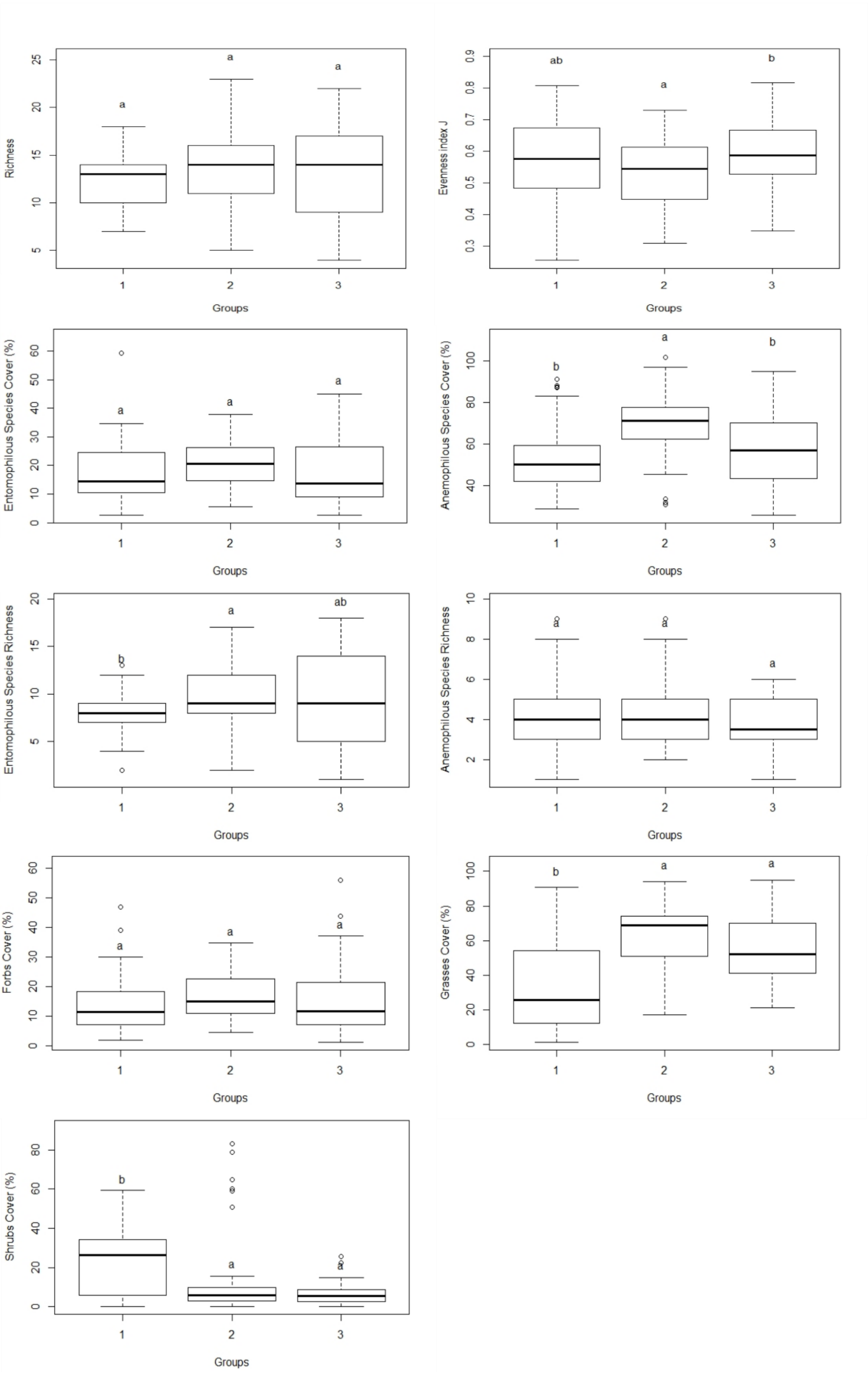

Fig. 5: Box plot describing Richenss, Evenness index J, Entomophilous species cover, Anemophilou species cover, Entomophilous species richness, Anemophilous speciess richness, Forbs cover, Grasses cover, Shrubs cover, in the three groups resulted from cluster analysis (median and percentiles are shown; outliers are represented by white circles). Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

Concerning biomass and litter characteristics, only litter weight did not result significantly different among the three groups (Fig. 6). Plots of Group 1 displayed on average the highest biomass weight and carbon content (respectively 180,8±65,69, and 43,00±3,39) and the lowest biomass nitrogen content (2,32±0,92) (Fig. 6). Concerning litter, Group 3 showed the lowest value of litter carbon content (33,61±7,91) and Group 1 showed the lowest value of litter nitrogen content (1,64±0,53) (Fig. 6; Tab. 3).

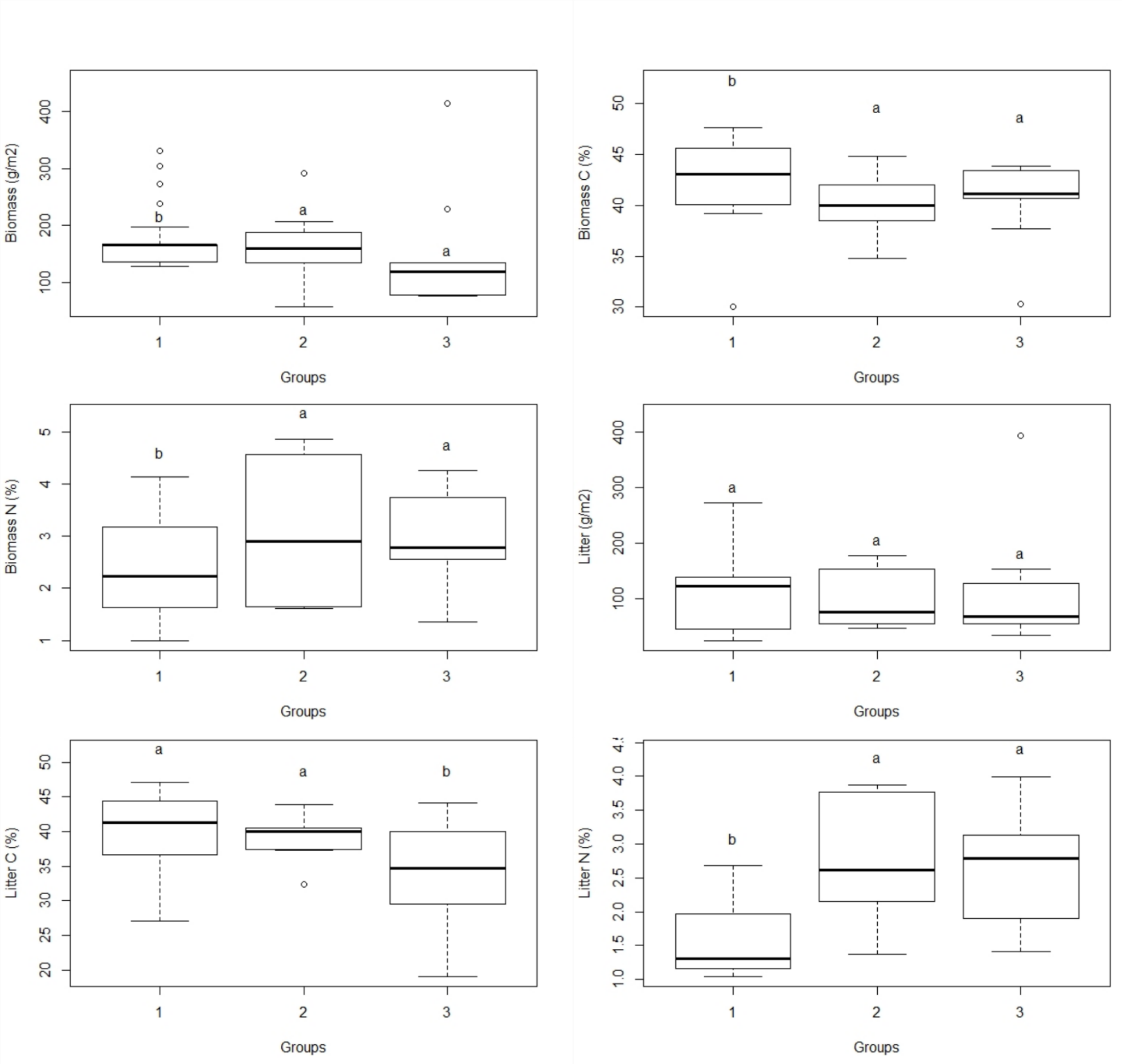

Fig. 6: Box plot describing Biomass weight, Biomass carbon content, Biomass nitrogen content, Litter weight, Litter carbon content and Litter nitrogen content, in the three groups resulted from cluster analysis (median and percentiles are shown; outliers are represented by white circles). Different letters indicate significant differences according to Tukey's test ($p < 0.05$).

The different set of species that characterised the three groups had repercussions on the CSR-strategy. Stress tolerant species always dominated, although their cover percentage significantly increased in plots of *Bromus erectus* community where overall they almost reached the 80%. Conversely, in Group 1, stress tolerant species dropped to about 50%, with an increase in ruderal species which significantly characterises the *Artemisia alba* community (Fig. 7; Tab. 3). The set of competitive species was always very small, and species exhibited only negligible percentage covers.

Finally, from the functional point of view the three groups displayed differences in the community weighed mean of leaf dry matter content (CWM LDMC). In accordance with the distribution of the CSR strategy, CWM LDMC evidenced a significant decreasing gradient from *Bromus erectus* community, to *Brachypodium rupestre* community, and the *Artemisia alba* community. Plant heigh (CWM H; heigh determined from species picked up for leaf trait measurement) allowed to significantly differentiate *Artemisia alba* community from *Bromus erectus* community, with *Brachypodium rupestre* community in intermediate position. CWM SLA did not show any significant difference among groups (Fig. 7).

Components of functional diversity differently varied among the three groups. Functional divergence (FDiv) resulted the most significant component with a decreasing trend from Group 2 to Group 1, with Group 3 in intermediate position. The other two components had a different behaviour; functional richness (FRic) contributed to significantly differentiate plots of Group 3 (with the lowest value of 4,34±2,40) (Tab. 3); while functional evenness (FEve) separated plots of Group 2 from the other two groups, due to the significantly higher value (Fig. 7; Tab. 3).

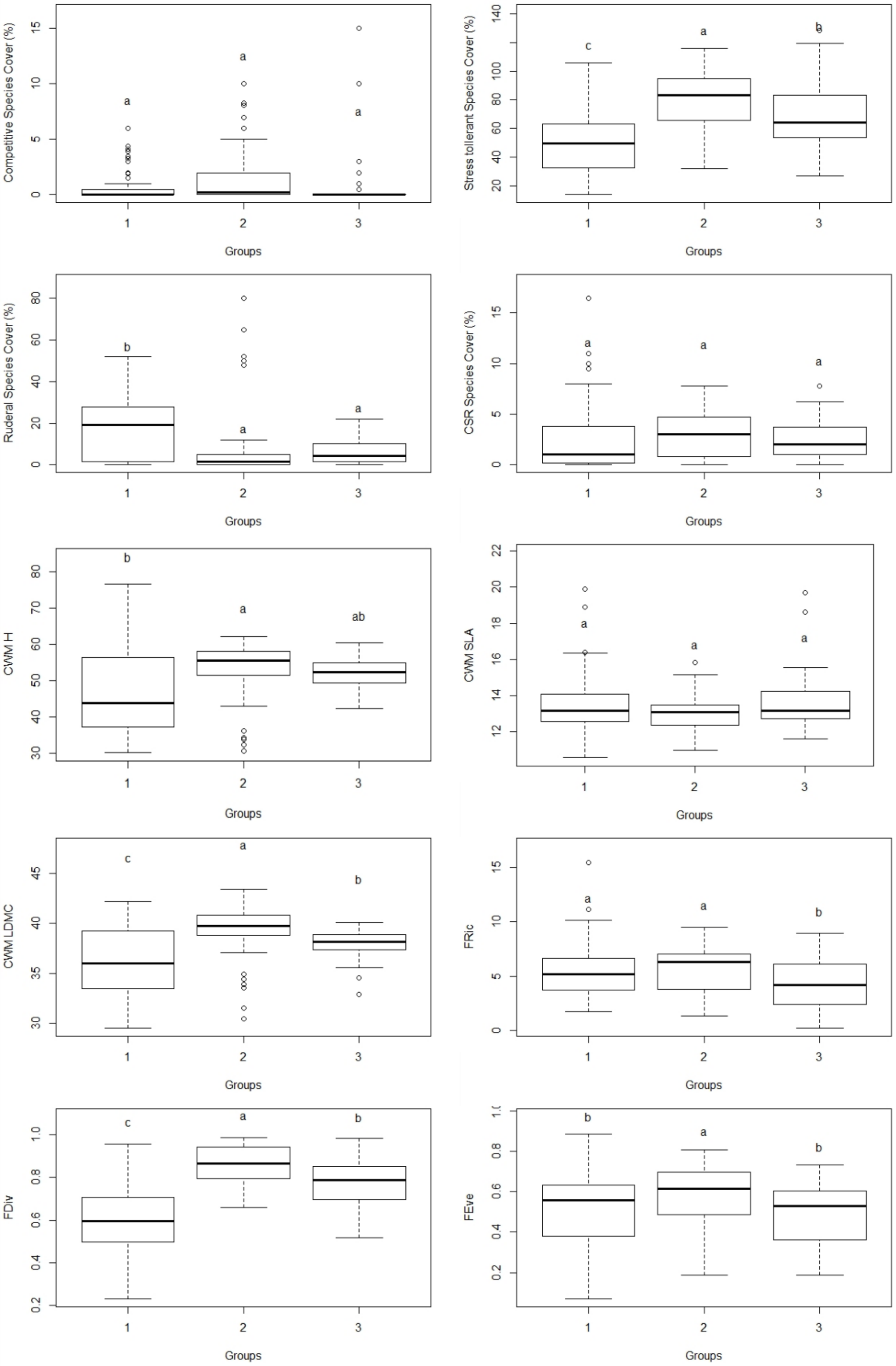

Figure *7: Box plot describing Competitive species cover, Stress tolerant species cover,Ruderal species cover, Competitive-Stress tolerant-Ruderal species cover, CWM H = Community Weighted Mean Heigh, CWM SLA = Community Weighted Mean Specific Leaf Area, CWM LDMC = Community Weighted Mean Leaf Dry Matter Content, FRic = Functional Richness, FEve = Functional Evenness and FDiv = Functional Divergence in the three groups resulted from cluster analysis (median and percentiles are shown; outliers are represented by white circles). Different letters indicate significant differences according to Tukey's test (p < 0.05).*

Table 3: Variables analysed in each group (mean ± sd). C = Competitive species; S = Stress tolerant species; R = Ruderal species; CSR = Competitive-Stress tolerant-Ruderal species; CWM H = Community Weighted Mean Heigh; CWM SLA = Community Weighted Mean Specific Leaf Area; CWM LDMC = Community Weighted Mean Leaf Dry Matter Content; FRic = Functional Richness; FEve = Functional Evenness; FDiv = Functional Divergence.

	Group 1	**Group 2**	**Group 3**
No. of plots (n)	78	59	42
Slope inclination (°)	3,74 ± 3,97	5,86 ± 5,91	3,55 ± 2,24
Slope exposure (°)	136,9 ± 91,21	146,31 ± 108,32	136,25 ± 60,66
Altitude (m)	120,85 ± 87,75	251,3 ± 73,31	150,22 ± 87,79
Soil moisture (g x kg-1)	30,75 ± 7,93	65,89 ± 11,41	116 ± 25,36
Soil depth (cm)	6,69 ± 2,66	5,58 ± 1,76	7,9 ± 2,68
Soil pH	7,7 ± 0,21	7,7 ± 0,23	7,58 ± 0,34
Soil carbon content (%)	12,75 ± 1,42	9,86 ± 3,55	11,47 ± 2,17
Soil nitrogen content (%)	1,92 ± 1,89	0,82 ± 0,32	1,24 ± 1,98
Total vegetation cover (%)	86,17 ± 13,57	88,41 ± 13,44	78,64 ± 13,86
Vascular species cover (%)	73,01 ± 15,78	86,83 ± 13,18	74,07 ± 13,69
Moss cover (%)	26,58 ± 29,68	7,05 ± 12,87	10,79 ± 21,76
Mean species height (cm)	23,42 ± 6,95	22,17 ± 14,09	21,46 ± 5,77
Species richness	12,49 ± 2,59	13,64 ± 3,69	13,17 ± 4,86
Evenness index J	0,57 ± 0,12	0,54 ± 0,11	0,59 ± 0,09
Entomophilous species (% cover)	17,51 ± 9,67	21,32 ± 8,52	17,94 ± 11,79
Anemophilous species (% cover)	52,6 ± 16,06	69,91 ± 15,13	57,75 ± 17,61
Entomophilous species (richness)	8,09 ± 2,2	9,54 ± 3,37	9,33 ± 5,03
Anemophilous species (richness)	4,4 ± 1,72	4,1 ± 1,36	3,83 ± 1,29
Forbs (% cover)	13,8 ± 8,95	17,09 ± 8,01	15,49 ± 11,98
Grasses (% cover)	33,83 ± 25,44	62,18 ± 18,9	53,65 ± 19,22
Shrubs (% cover)	22,47 ± 16,25	11,94 ± 19,07	6,54 ± 5,61
Biomass weight (g/m2)	180,8 ± 65,69	151,43 ± 49,01	134,89 ± 88,12
Biomass carbon content (%)	43 ± 3,39	39,97 ± 2,89	40,08 ± 4,38
Biomass nitrogen conten (%)	2,32 ± 0,92	2,95 ± 1,3	2,93 ± 0,87
Litter weight (g/m2)	105,37 ± 64,11	101,46 ± 50,59	100,84 ± 90,5
Litter carbon content (%)	40,11 ± 5,25	39,49 ± 3,09	33,61 ± 7,91
Litter nitrogen content (%)	1,64 ± 0,53	2,73 ± 0,78	2,71 ± 0,8
C (% cover)	0,58 ± 1,21	1,38 ± 2,35	0,87 ± 2,78
S (% cover)	50,12 ± 23,19	79,34 ± 20,91	67,14 ± 23,33

R (% cover)	17,61 ± 15,11	7,86 ± 17,44	6,2 ± 5,9
CSR (% cover)	2,49 ± 3,11	2,95 ± 2,33	2,37 ± 1,94
CWM H	48,12 ± 12,94	53,51 ± 7,19	52,46 ± 3,95
CWM SLA	13,42 ± 1,5	13,03 ± 0,96	13,62 ± 1,56
CWM LDMC	36,12 ± 3,49	39,35 ± 2,53	37,98 ± 1,47
FRic	5,61 ± 2,65	5,69 ± 2,14	4,35 ± 2,4
FEve	0,51 ± 0,18	0,59 ± 0,14	0,49 ± 0,14
FDiv	0,6 ± 0,16	0,86 ± 0,09	0,78 ± 0,12

Spearman correlations evidenced significant relationships among environmental variables and community attributes (Tab. 4). Altitude and soil N revealed as the most important environmental variables affecting plant community attributes.

Overall, altitude had a positive effect on vascular species cover, and both anemophilous species cover and richness. As it could be expected, also the cover of grasses significantly increased with altitude. The cover of both competitive and stress-tolerant species increased with altitude, while ruderal species resulted linked to lower altitudes. Interestingly, while altitude had no (species richness) or a negative effect on taxonomic attributes, it was positively correlated with the functional component (especially, FEve and FDiv). Soil N had the highest number of negative relationships, affecting both the structure (vascular species cover, plant height, species richness and evenness, entomophilous species cover) and composition in terms of growth forms (forbs) and strategy (competitive and stress-tolerant species cover). Moss cover, shrub cover, and the cover of ruderal species were among the community attributes positively associated with soil N, together with litter weight, litter C content, and biomass C content.

Soil moisture had positive relationships with anemophilous species cover and the cover of grasses, while it had a negative effect on the shrub cover. It had also a negative effect on the production of biomass (biomass weight), while it had a positive relationship with litter accumulation (litter weight).

Soil pH had a negative relationship with species height. It positively affected C and N biomass content, while for litter it has a negative relation with the C content and no relation with N. Soil pH also resulted positively correlated with the moss cover, and the cover of shrubs.

The shrub cover was also positively correlated with soil C content, together with mean species height, and the cover of ruderal species.

Soil depth was the variable with the lowest number of relationships, with negative effects on the richness of anemophilous richness, litter weight, and litter C content.

Table 4: Spearman correlation matrix. P values above the diagonal; significative values ($p<0,05$) are highlighted in yellow; green values indicate positive relationship, while red values negative relationship. R values under the diagonal.

	Slope inclination (°)	Slope exposure (°)	Altitude (m)	Soil moisture (g x kg-1)	Soil depht (cm)	Soil pH	Soil C content (%)	Soil N content (%)	Total vegeattion cover (%)	Vascular species cover (%)	Moss cover (%)	Mean species height (cm)	Species richness	Evenness index J	Entomophilous species cover (%)	Anemophilous species cover (%)	Entomophilous species (richness)	Anemophilous species (richness)	Forbs (% cover)	Grasses (% cover)	Shrubs (% cover)	Biomass weight (g/m2)	Biomass C content (%)	Biomass N content (%)	Litter weight (g/m2)	Litter C content (%)	Litter N content (%)	C cover (%)	S cover (%)	R cover (%)	CSR cover (%)	CWM H	CWM SLA	CWM LDMC	FRic	FEve	FDiv
Slope inclination (°)		1,9845E-07	0,0011159	0,003159	0,15017	0,0099065	0,18031	0,096945	0,21293	0,12285	0,016623	0,28765	0,88715	0,61186	0,17102	0,16112	0,1255	0,00028937	0,30747	0,25487	0,54322	1,9702E-06	0,30362	1,4598E-13	1,5306E-09	0,5922	0,0013861	0,00019484	0,027743	0,040147	2,5615E-09	0,094439	0,80017	0,0048017	0,3062	0,0011184	0,19734
Slope exposure (°)	0,37693		0,17933	0,0088709	0,29378	4,8589E-08	0,2184	0,0036251	0,81979	0,93824	0,0415	0,0064323	0,6011	0,0064865	0,11379	0,89905	0,54898	0,11815	0,24631	0,56594	0,88734	0,00029213	1,1249E-11	0,061266	0,0083739	0,00086316	0,58018	0,00012949	0,5938	0,69592	9,8799E-05	0,26571	0,47677	0,014199	0,30823	0,081419	0,041309
Altitude (m)	-0,2417	0,10082		0,00021936	0,0022709	0,7731	0,00016922	0,07804	0,21532	0,0032007	6,5354E-12	0,17987	0,079126	0,0022206	0,39295	3,7282E-05	0,94393	3,7444E-05	0,87528	1,8718E-11	7,8893E-10	0,46048	2,3943E-10	2,6802E-15	5,4588E-06	0,00033851	4,0827E-10	2,07E-06	1,8127E-08	2,3579E-14	0,00011224	1,909E-16	0,066282	4,6665E-17	0,037854	0,0003961	2,6086E-11
Soil moisture (g x kg-1)	0,21947	0,19509	0,27287		0,81131	8,2693E-06	2,9141E-05	0,78277	0,015149	0,65922	3,9306E-06	0,10546	0,063112	0,60192	0,95018	0,0087668	0,088439	0,68243	0,75804	2,4494E-07	1,3817E-09	9,6406E-06	7,732E-08	0,011455	0,026458	0,00010032	1,1818E-16	0,22732	3,5968E-06	1,4791E-05	0,80152	2,6866E-06	0,97908	7,1485E-05	0,19609	0,6518	5,0746E-10
Soil depht (cm)	0,10799	-0,078901	-0,22675	-0,017968		0,00062629	1,468E-06	0,3011	0,060769	0,10722	0,85722	0,062562	0,07753	0,91207	0,32827	0,25081	0,22544	0,0060657	0,07885	0,67789	0,16327	0,72166	0,29494	0,3761	0,016329	6,404E-08	0,33375	0,097027	0,47448	0,58803	0,01097	0,21058	0,45741	0,1965	0,23204	0,97202	0,21013
Soil pH	-0,19231	-0,39397	0,0217	-0,32633	0,25322		0,069187	0,0037945	0,098541	0,1094	0,00109	0,0086962	0,41024	0,00010042	0,58556	0,98656	0,61998	0,01002	0,98837	0,42439	0,015729	0,56167	0,0001206	0,01598	7,2015E-05	0,044163	0,47659	0,23795	0,52782	0,30121	0,52572	0,0030288	0,45219	0,062608	0,56556	0,22221	0,25213
Soil C content (%)	-0,10059	-0,092444	-0,27751	-0,30701	0,35088	0,13614		1,4231E-06	0,051674	0,26249	0,1405	0,043993	0,62669	0,40112	0,45617	0,24079	0,68894	0,46506	0,28465	0,0010455	0,00010036	0,46684	0,11956	0,032138	0,0060127	5,5586E-06	0,1666	0,92286	0,0034536	0,014464	0,79847	0,0012221	0,0765	0,0011539	0,97469	0,15569	0,00020896
Soil N content (%)	0,12445	-0,21637	-0,13206	-0,020751	-0,077719	0,21533	-0,3513		0,097461	0,0032588	1,9124E-05	2,936E-09	0,0017021	0,0023801	0,0020289	0,075006	0,1754	0,0038332	0,00061559	0,1091	0,048876	0,40196	0,001052	0,00020539	9,9875E-05	2,1635E-14	0,13884	0,028004	0,0071919	0,025261	0,0043578	0,0023129	0,35754	0,0056472	0,71772	0,019245	0,45869
Total vegeattion cover (%)	0,093549	-0,017146	-0,093064	-0,1813	-0,14045	0,12387	-0,14568	0,12426		4,7638E-21	2,5203E-06	0,3273	0,84497	0,0041384	0,0002417	2,7882E-09	0,11529	0,00061998	0,0025228	0,0008927	0,1939	0,048586	0,20188	0,0078789	0,56434	0,013422	0,3249	0,27646	1,9155E-05	0,88813	0,070767	0,26243	0,46065	0,67058	0,26073	0,36129	0,093165
Vascular species cover (%)	0,11575	-0,0058321	0,21918	0,033185	0,1208	0,12006	-0,084193	-0,21877	0,62828		0,00032683	0,23485	0,17021	0,0038514	1,6922E-09	3,4152E-28	0,01906	0,0022083	3,895E-12	2,4529E-20	0,0013944	9,6515E-05	0,0012765	0,93068	0,96143	0,0050618	0,0081881	0,32256	3,0965E-32	1,6595E-07	0,001711	3,9535E-05	0,32898	1,3612E-10	0,44448	0,32631	1,3166E-06
Moss cover (%)	-0,17881	-0,15254	-0,48422	-0,33715	-0,013542	0,24218	0,1106	0,31362	0,34343	-0,26558		2,3156E-05	0,001509	0,63345	0,53046	0,00062161	0,24403	0,022057	0,033253	3,972E-13	1,3587E-15	0,032625	6,5253E-09	0,074363	1,6016E-08	7,0665E-05	0,010946	0,0057331	7,1345E-13	2,2605E-20	0,10217	2,4387E-21	0,60234	1,5986E-23	0,79185	0,2771	0,0011587
Mean species height (cm)	0,079907	0,20297	-0,10069	-0,12141	0,13949	-0,19558	0,15074	-0,42534	-0,073632	0,08924	-0,31063		0,15748	2,1263E-05	0,6713	0,29612	0,92434	0,012013	0,073924	0,26255	0,60992	0,0095417	0,79927	0,018653	0,40239	0,29882	4,675E-05	0,24468	0,4097	0,80508	0,10159	0,40417	0,09901	0,87684	0,44264	0,053258	0,00020381
Species richness	0,010681	-0,039337	0,13158	0,1392	-0,13228	-0,061924	0,036599	-0,23293	-0,014718	0,10296	-0,23546	0,1061		0,023762	6,443E-08	0,038307	3,8984E-62	0,00013254	7,0501E-06	0,81079	0,24595	0,37233	0,20707	0,39863	0,49929	0,23255	0,68618	0,00014984	0,09092	0,0014363	0,019304	0,58198	0,84883	0,015311	8,8868E-17	0,0017653	0,85653
Evenness index J	-0,03818	0,20276	-0,22724	0,039249	-0,0083115	-0,28661	0,063135	-0,22573	-0,21334	-0,21499	0,035884	0,31197	0,16896		8,9892E-09	2,9943E-12	0,15527	0,040324	5,6935E-08	6,0266E-10	0,0052661	0,97725	0,02012	0,027896	5,5675E-06	0,69445	0,30009	0,93269	3,8966E-05	0,00035121	0,35945	2,3231E-05	0,011784	0,00017308	0,035869	0,50671	3,2321E-06
Entomophilous species cover (%)	0,10277	0,11861	-0,064236	-0,0047027	0,073486	-0,041026	-0,056045	-0,22918	0,27112	0,43114	-0,047191	0,031934	0,39063	0,41322		0,78606	1,7647E-14	1,2111E-05	1,2121E-67	0,77909	0,073084	0,020352	0,081531	0,0032793	0,0021483	0,5718	0,76977	0,96097	1,1891E-05	0,26331	0,19481	0,0082148	0,57247	0,17552	5,3625E-11	0,15602	0,89524
Anemophilous species cover (%)	0,10519	-0,0095486	0,30306	0,19538	0,086279	-0,0012683	-0,088123	-0,13341	0,42589	0,70482	-0,25336	-0,078521	-0,15498	-0,491	-0,020429		0,067792	0,17398	0,47727	8,0985E-45	3,9892E-07	0,023864	0,00028136	0,032544	0,010348	0,0018887	1,7754E-05	0,62275	8,5218E-33	3,0637E-07	0,008185	1,3043E-11	0,001589	1,7083E-11	0,0072001	0,70841	2,0736E-10
Entomophilous species (richness)	0,11494	-0,045086	-0,0052937	0,12771	-0,091053	0,037312	-0,030124	-0,10173	0,11812	0,1751	-0,08752	0,0071485	0,88959	0,10667	0,53219	-0,13683		0,12094	1,6461E-09	0,39023	0,010127	0,6201	0,95356	0,024458	0,072379	0,97233	0,33049	0,23474	0,069	0,061398	0,16006	0,060282	0,89765	0,24754	6,9336E-24	0,017513	0,78353
Anemophilous species (richness)	-0,26783	0,11721	0,30299	-0,030789	-0,20437	-0,19203	0,054946	-0,2151	-0,25341	-0,22736	-0,17105	0,18739	0,28181	0,15342	-0,32062	-0,10206	-0,11634		0,00031348	0,68704	0,5574	0,58826	0,01206	0,023064	0,30976	0,44892	0,61152	2,4888E-07	0,050132	0,66487	0,053492	0,0010422	0,7302	0,14793	0,17876	0,036349	0,77613
Forbs (% cover)	0,076705	0,087099	-0,011814	0,023185	0,1317	-0,0010976	-0,080405	-0,25355	0,22445	0,48873	-0,15923	0,13391	0,32869	0,3921	0,90509	0,053456	0,43143	-0,26635		0,35444	0,53776	0,00077384	0,0066748	0,0037377	0,0098152	0,15972	0,17856	0,56756	8,1032E-08	0,028553	0,21564	0,49507	0,92711	0,014276	6,1866E-07	0,33206	0,748
Grasses (% cover)	0,085547	-0,043188	0,47485	0,3743	-0,031256	-0,060075	-0,24303	-0,12017	0,24621	0,61929	-0,50787	-0,084182	-0,018019	-0,44172	-0,021111	0,82032	-0,064605	-0,030318	0,069617		2,4764E-31	0,35914	3,0935E-06	0,00026416	2,0966E-05	0,00017289	7,832E-12	0,025306	2,7648E-69	4,8087E-31	0,0053469	4,8674E-36	0,12126	2,85E-33	0,012372	0,27362	2,4627E-16
Shrubs (% cover)	-0,045737	0,010663	-0,439	-0,43325	0,10465	0,1803	0,28662	0,14745	0,097554	-0,23711	0,55085	-0,038388	0,087165	0,20773	0,1343	-0,3681	0,19176	-0,044141	-0,046357	-0,73215		0,91077	5,5446E-06	3,2308E-05	4,7551E-06	0,00038778	5,6243E-12	0,030223	1,1661E-20	6,6109E-37	0,017166	1,2391E-42	0,79487	3,4634E-25	7,7032E-05	0,70534	2,0091E-08
Biomass weight (g/m2)	0,34685	0,26765	-0,055511	-0,32405	0,02681	-0,043663	-0,054729	0,063023	0,14764	0,28728	-0,15979	0,19326	-0,067075	0,0021468	0,17329	0,16884	-0,0373	-0,040731	0,24906	0,068941	0,0084357		0,35071	1,2673E-08	1,5413E-08	0,034175	7,734E-05	0,6363	0,076979	0,15648	0,9597	0,055365	0,6137	0,012254	0,038031	0,089809	0,068752
Biomass C content (%)	-0,077315	-0,47942	-0,45088	-0,38845	0,078712	0,28345	0,11677	0,2429	0,095841	-0,23894	0,41674	-0,019139	-0,094754	-0,17361	-0,13055	-0,26834	0,0043832	-0,18728	-0,20207	-0,34055	0,3322	-0,070157		0,32941	0,051653	1,5217E-06	7,7292E-14	0,51787	7,4366E-08	6,3234E-08	0,78215	8,3833E-08	0,0072075	3,7778E-11	0,14776	0,79711	6,4635E-07
Biomass N content (%)	-0,5159	-0,14018	0,54602	0,18861	-0,066549	0,17987	0,16024	-0,27406	-0,19803	0,0065476	-0,13371	-0,17569	-0,06347	-0,16437	-0,21863	0,15987	-0,16814	0,1698	-0,21567	0,2695	-0,30536	-0,4094	-0,073313		0,11752	6,7268E-09	4,2682E-13	0,0025081	0,08455	0,010449	3,8864E-08	0,0029907	0,41325	0,041994	0,00027755	0,00079366	4,1869E-06
Litter weight (g/m2)	0,43219	0,19652	0,33242	0,1659	-0,17929	-0,29224	-0,20458	0,2867	-0,043366	0,0036402	-0,40677	-0,062966	-0,05082	-0,33214	-0,22795	0,19121	-0,13462	0,076343	-0,19255	0,31219	-0,33442	0,4072	-0,1457	-0,11741		0,094129	0,14099	0,74568	0,0011475	6,0059E-09	0,5144	5,6524E-12	0,57421	1,9114E-10	0,36079	0,019217	0,07952
Litter C content (%)	0,040303	-0,24689	-0,26493	-0,28662	-0,39071	-0,15062	-0,33216	0,53066	0,1845	-0,20866	0,29256	-0,078085	-0,089679	0,029562	-0,042539	-0,23072	-0,0026111	-0,056951	-0,10554	-0,27713	0,26239	0,15842	0,35039	-0,41641	0,12551		5,8707E-08	0,40446	1,3512E-05	4,8999E-05	0,44966	3,1196E-05	0,12669	0,00055638	0,0052776	0,029965	4,4258E-05
Litter N content (%)	-0,23723	0,041616	0,44562	0,5676	0,07266	0,053539	-0,10383	-0,11107	-0,073997	0,19708	-0,18978	-0,29939	-0,030406	-0,07788	0,022027	0,31477	-0,073151	0,038216	0,10099	0,48263	-0,48554	-0,29104	-0,5209	0,50729	-0,11047	-0,39174		0,042824	6,6969E-12	2,0731E-08	0,83216	3,3747E-09	0,52394	1,3634E-08	0,061308	0,0010452	4,4248E-14
C cover (%)	-0,275	-0,28222	0,34616	-0,090687	-0,12442	0,088653	-0,0072881	-0,16426	-0,081781	0,074356	-0,20572	0,0874	0,27966	0,0063574	0,0036832	0,037017	0,08926	0,3741	0,043009	0,16717	-0,16204	-0,035583	-0,048643	0,22458	-0,02441	0,06269	0,15157		0,071689	0,00039496	0,00085197	0,0001114	0,30601	0,0013609	0,3523	0,018815	0,57125
S cover (%)	0,16453	0,040128	0,40537	0,33842	0,053796	-0,047494	-0,21747	-0,20026	0,31359	0,73944	-0,50307	-0,061995	0,12674	-0,30235	0,32089	0,74384	0,13624	-0,14665	0,38789	0,90926	-0,6234	0,13253	-0,38892	0,12929	0,24113	-0,31896	0,48401	0,13495		3,7147E-34	0,15369	1,3506E-25	0,16346	2,6926E-40	0,95256	0,046322	1,1118E-14
R cover (%)	-0,15356	-0,029412	-0,53001	-0,31758	0,040756	0,077701	0,18253	0,16722	0,010588	-0,37915	0,61974	0,018574	-0,2365	0,26424	-0,084048	-0,37147	-0,14011	-0,032599	-0,1637	-0,72977	0,77358	-0,10636	0,39086	-0,19096	-0,41764	0,29862	-0,40384	-0,26204	-0,75417		0,18221	4,1032E-41	0,076445	2,8519E-56	0,78205	0,0093569	8,6615E-09
CSR cover (%)	-0,42679	-0,28688	0,28469	0,01892	-0,18972	-0,047736	0,019217	-0,21215	0,13539	0,23282	-0,12256	0,12277	0,17475	0,068897	0,097356	0,19709	0,10545	0,14458	0,092999	0,20737	-0,17795	0,003803	0,020812	0,39659	-0,049047	0,056857	0,015951	0,24715	0,10707	-0,10016		0,39916	0,34773	0,44883	0,44956	0,26867	0,10081
CWM H	0,12539	0,083629	0,56438	0,34253	-0,094028	-0,22041	-0,23984	-0,22635	-0,084204	0,30212	-0,63187	0,062729	0,041419	-0,31058	-0,197	0,4781	-0,14071	0,24309	-0,051321	0,76766	-0,80861	0,14347	-0,38748	0,22069	0,48549	-0,30592	0,42386	0,28482	0,67956	-0,79996	0,063399		0,59341	1,184E-39	0,02517	0,13755	1,7482E-07
CWM SLA	-0,019051	-0,053517	-0,13758	0,0019741	-0,055892	-0,056541	-0,13274	0,069171	-0,05549	-0,073379	0,039204	0,12369	-0,014347	0,18788	0,042465	-0,23438	-0,0096822	0,025954	0,0068861	-0,11624	0,019568	-0,037983	0,20021	-0,061528	-0,042273	0,11458	-0,047941	-0,076935	-0,10461	0,13277	0,070591	-0,040171		0,045895	0,0098641	0,033181	0,8515
CWM LDMC	0,2099	0,18302	0,57373	0,29236	-0,09699	-0,13947	-0,24102	-0,20608	0,032008	0,45635	-0,65741	0,011665	0,18102	-0,27711	0,1017	0,47567	0,086875	0,10859	0,18287	0,74751	-0,67534	0,18687	-0,46843	0,15218	0,45307	-0,25551	0,40858	0,23762	0,79512	-0,87009	0,056961	0,79122	-0,14942		0,83248	0,021939	2,3548E-13
FRic	0,077343	-0,077021	-0,15622	-0,097629	-0,090288	-0,043477	-0,0024015	-0,027362	0,084984	0,057837	0,019976	-0,05807	0,57215	0,15785	0,46752	-0,20135	0,66421	-0,10152	0,36434	-0,18767	0,2927	0,15608	0,10925	-0,27007	-0,069098	0,20884	-0,14095	0,070326	-0,0045032	0,02094	0,057194	-0,16827	0,1935	-0,016012		0,089363	0,0030671
FEve	-0,243	-0,13134	0,26343	-0,034151	0,0026555	0,09221	0,10716	-0,17581	-0,069025	0,074204	-0,082138	-0,14554	0,23345	-0,050232	0,10708	-0,028306	0,1784	0,15745	0,073329	0,082732	-0,028618	-0,12789	-0,01946	0,24993	-0,17586	-0,16321	0,24438	0,17644	0,14998	-0,19484	0,083588	0,11206	0,16019	0,17217	0,12807		0,00094093
FDiv	0,097358	0,15354	0,4742	0,44571	-0,094656	0,086529	-0,27525	-0,056051	0,12659	0,35424	-0,24227	-0,2757	-0,013686	-0,34176	0,0099677	0,45458	0,020795	-0,021524	-0,024317	0,5653	-0,40631	-0,13713	-0,36376	0,33806	0,13216	-0,30192	0,52775	-0,042843	0,53821	-0,41578	0,12374	0,38051	0,014171	0,51459	-0,22136	0,24651	

Discussion

Results allowed to evidence a certain homogeneity among surveyed grasslands as far as some environmental parameters and community attributes are concerned. Semi-natural dry grasslands were differentiated into three main groups, which were characterised through the description of environmental factors (topographical and soil characteristics) and community attributes (i.e., community structure and species composition) based on both a taxonomical and a functional approach.

Among tested environmental parameters, slope exposure did not allow to significantly distinguish the groups evidenced by the cluster analysis; conversely, slope inclination and particularly altitude significantly influenced the three groups. Semi-natural dry grasslands have been recently proved to be influenced by site conditions topography, as for example in Büchler et al. (2020). In a study on calcareous grassland in England (Balme, 1953), it has been shown that a reduction in slope inclination caused a corresponding reduction in species richness. In our results, even if no significant relationship were displayed, on average a higher slope inclination corresponded to a higher species richness. Furthermore, in accordance with the results of Büchler et al. (2020) where stress tolerant species were more likely to grow on steep slopes, we found a positive relation between slope inclination and the cover of stress tolerant species.

Soil characteristics such as soil N content, soil moisture, soil depth, also showed a clear trend, thereby proving helpful in characterizing dry grassland communities, with effects on both structure and composition. Many studies have focused on the factors influencing plant diversity in species-rich grasslands, and specifically the relation between the soil characteristics and plant diversity. For instance, the ability of a species to withstand low levels of soil moisture has been proven to be inversely related to its capacity to rapidly dominate and displace other species (Gurevitch, 1986). The ability to achieve dominance is connected to functional traits such as for example biomass production, which allows plants to outcompete smaller or slower-growing species (Cerabolini et al., 2016). This trend is confirmed by our data, which showed a correlation between soil moisture and the mean value of SLA (CWM SLA), suggesting that higher water availability favours faster growth rates (Bernard-Verdier et al., 2012; Wellstein et al., 2013). Higher levels of available soil water also corresponded to a much higher soil depth as well as soil pH. With respect to these three variables, results evidenced that intermediate values of water availability, soil depth and pH allow the coexistence of a richer and more diverse community, as in correspondence with *Bromus erectus* community, although none of the three variables alone showed a significant correlation with species richness. It can be thus hypothesised an indirect effect on other community attributes such as biomass or litter production that in turn either enhance or limit species richness. Interestingly, soil moisture was positively correlated to the functional divergence (FDiv) of communities; the shift towards more mesophilous condition of the site allows species not typical to dry grasslands to enrich the community and the functional divergence.

In addition to environmental parameters, identified dry grasslands differed from each other in the presence of specific groups of species resulting from the Indicator Species Analysis. The concept of “indicator species” can be similar to the concept of “typical species” (species which occur regularly in a habitat type) according to the Habitat Directive. In the Habitat Directive, a habitat type can be considered at a ‘Favourable Conservation Status’ (FSC) when its “typical species” are at FCS, although no clear definition of “typical species” is provided (Evans and Arvela 2011). In this study, indicator species, which were those that exhibited high specialisation within a group, allowed a clear differentiation between dry grasslands dominated by *Artemisia alba*, those dominated by *Bromus erectus* and those by *Brachypodium rupestre*. This analysis thus allowed to recognise different aspects within the same habitat type (dry grassland), characterised by diversified pools of species, structure, and functions. Interestingly, although there was a pool of species in common among the three aspects (e.g., *Bromus erectus*, *Koeleria pyramidata*, *Galium verum*, *Artemisia alba*, *Eryngium amethystinum*), these species showed different abundances as a response to shifts in environmental variables, thereby allowing to recognise the small-scale variability of dry grasslands.

As observed with environmental parameters and Indicator Species analysis, also the analysis of plant strategies allowed a clear differentiation between the three groups. Studied grasslands were strongly dominated by stress tolerant species, although values changed across groups, with differences linked to topographic variables, particularly slope inclination and altitude, and soil chemical factors. Specifically, we found a significant relationship between the cover of ruderal species and soil nitrogen content. This is consistent with previous studies that evidenced the presence of ruderal species in nutrient-rich environments (Pierce et al., 2017) and in disturbed environments (Sochera et al., 2013) probably because of biomass-destroying phenomena, as mowing, grazing, or trampling. As suggested in e.g., Bobbink et al. (1998), in grasslands, soil N is generally recognized as one of the most growth-limiting nutrients responsible for variation in floristic composition and biomass production. Consistently with other studies (Bobbink et al., 1998; De Schrijver et al., 2011; Roth et al., 2013) our results showed that high values of soil N are linked to lower values of species richness and lead to confirm that eutrophication of oligo- to mesotrophic environments is expected to determine an impoverishment of species richness and changes in community evenness (Büchler et al., 2020).

Various studies confirmed that species richness in grasslands is negatively associated with high cover or biomass of a dominant species (Czarniecka-Wiera et al., 2019), such as for example *Bromus* *erectus* or *Brachypodium rupestre* (Bonanomi and Allegrezza 2004; Wellstein et al., 2014), or by the grass functional group as a whole (Li et al., 2016). The presence of grass species with high cover or high biomass likely leads to a decline in species richness by reducing light reaching the ground (Grytnes, 2000). High cover or biomass of a dominant species excludes smaller species through light interception, while high litter accumulation can limit or suppress the possibility of germination of other species (Garnier et al., 2007). Our results did not show significant differences in species richness among the three groups. However, the highest value of species richness was observed in *Bromus erectus* community, in correspondence with the highest grass cover and intermediate values of biomass weight. Grime (1997) suggested that the relationship between herbaceous biomass and richness often has a hump shape with a peak in species richness at a low to intermediate level of biomass. Probably in *Bromus erectus* community, characterised by high species richness and high grass cover, richness had not reached the point of decrease.

The need to use both taxonomic and functional species characteristics for the definition of different groups was evident when considering the relationships between plants traits and environmental parameters. As affirmed in Diaz et al. (2004), nutrient-rich environments are characterised by fast growing species that usually have high SLA and low LDMC. However, the two leaf traits demonstrated to be useful to evidence differences also within nutrient-poor environments as dry grasslands. In our case, even a small increase in soil N or soil moisture corresponded to an increase in CWM SLA, confirming that when nutrients and water are available plants investment is in growth as affirmed in Ellenberg (1988). Another functional characteristic tested was plant height. Plant height has been associated with the competitive ability for light interception in herbaceous plant communities; the general idea is that competition for light, and so plant height, becomes more intense when aboveground biomass increases (Hautier et al., 2009; Pierce et al., 2013). Overall, our results were consistent showing a positive trend between plant height and biomass.

Functional diversity (FD) patterns were also explained considering the influence of abiotic parameters on community attributes. According to Cornwell (2006), functional richness (FRic) is affected by increasing pressure of environmental filters and is directly proportional to species richness. As a consequence, low values of FRic suggest that the functional volume of the community decreases and environmental filters limit the spatial range of species traits (Mason et al., 2008). Manson et al. (2008) affirmed that when environmental characteristics show extreme values, FRic displays low values; conversely, in correspondence with intermediate values of environmental characteristics, FRic shows high values. Our results agree with this hypothesis since *Bromus erectus* community, that had intermediate values of all soil characteristics, except for soil pH, showed the highest value of FRic.

As for the other two components of functional diversity, Mason et al. (2005) stated that FEve describes the evenness of abundance distribution in a functional trait space. High values of FEve indicate that trait abundances are evenly distributed among the community (Villeger et al., 2008). FDiv measures the abundance-weighted functional differences between the species within a community (Mason et al., 2005). Values of FDiv are low when the most abundant species have functional traits that are close to the center of the functional trait range; conversely, when the most abundant species has extreme functional trait values, the divergence is high. The pattern we obtained, in which *Bromus erectus* community showed the highest FDiv and FEve, confirms that in dry grasslands of the study area, both taxonomic diversity and the components of functional diversity reach their maximum values in correspondence of intermediate values of environmental parameters and soil characteristics. Every shift to harsher (*Artemisia alba* community) or milder (*Brachypodium rupestre* community) conditions involves a loss of diversity.

The aim of this study was to characterise semi-natural dry grasslands through the description of community attributes and environmental parameters. Overall, results showed the importance of soil characteristics, as especially soil moisture, soil depth and soil nitrogen content, in determining different communities in semi-natural dry grasslands. Moreover, the description of both taxonomical and functional community attributes could also be applied to diversify semi-natural dry grasslands, especially in relation to plant strategies. Although stress tolerant species were the most abundant species in the studied grasslands, even small differences in topographic or edaphic characteristics were evidenced by shifts in the percentage cover of other plant strategies. Finally, through the utilization of the Indicator Species Analysis, that can be related to the concept of "typical species" used in Habitat Directive, it was possible to divide the studied semi-natural dry grasslands into three groups, associated mainly to the presence of *Artemisia alba*, *Bromus erectus* and *Brachypodium rupestre*. In general, semi-natural dry grasslands appear complex habitats differentiating on the basis of soil composition, community structure and most abundant and high specialised species. A better understanding of the dynamics presented in this habitat could help to mitigate degradation processes and anthropic pressures, and to increase the conservation of this important habitat.

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Chapter II. Assessment of ecosystem functioning through the evaluation of different ecosystem services provided by semi-natural dry grasslands

Introduction

Semi-natural dry grasslands provide numerous and essential benefits to society (Garnier et al., 2016). Indeed, besides having a high conservation value, grasslands may also be important contributors of ecosystem services (ES) (Sala and Paruelo 1997; Pilgrim et al., 2010; Bullock et al., 2011; Lemaire et al., 2011; Lavorel et al., 2013; Werling et al., 2014; Burrascano et al., 2016). A wide range of different ES has been studied for grasslands (reviewed in Bengtsson et al., 2019). They supply “regulating services” such as different aspects of the water cycle or phenomena such as the climate, through the process of carbon sequestration (Bullock et al., 2011; Hungate et al., 2017; Klumpp et al., 2017; Lal, 2004). Grasslands can storage large amounts of carbon in the soil, much more than croplands and sometimes as much as forest soils (Burrascano et al., 2016). Though carbon stocks of grasslands per unit area are lower than those of forest ecosystems, they play an important role in global carbon storage because of their wide distribution (MEA 2005). Another “regulating service” provided by grasslands is pollination (de Bello et al., 2010; Fantinato et al., 2016; 2018; 2019; Kremen et al., 2007), and Öckinger and Smith (2007) suggested that preservation of the remaining semi-natural grasslands or recreation of flower-rich grasslands are essential to sustain the abundance and diversity of insect pollinators.

Grasslands are also recognised for their “supporting services” (e.g., nutrient cycling, biomass production) that, although not directly used by humans, are necessary for ecosystem functioning. Among the supporting services, a primary function of grassland ecosystems is certainly represented by primary production (Garnier et al., 2016; Garnier and Navas, 2012). Being categorized as a supporting ecosystem service, primary production relates to other provisioning and regulating services (Millennium Ecosystem Assessment, MEA, 2005). The relationship between primary production and biodiversity potentially affects some ecosystem services such as supply of habitat for wild species (Costanza et al., 2007). The relationship between plant species richness and above-ground plant biomass (a proxy for productivity; Palpurina et al., 2015) has been a central theme in ecology for decades (Axmanová et al., 2013; Grime, 1979; Gillman and Wright, 2006; Mittelbach et al., 2001). Primary production can be also related to supplies of soil organic carbon, which in turn affect the carbon sequestration service of the ecosystem (Wang et al., 2011). Moreover, primary productivity is believed to be a good surrogate for ecosystem function (Tilman et al., 1997). Interestingly, Imhoff et al. (2004) chose human appropriation of net primary production, not counting the primary production remaining for other species to use, as an indicator for identifying the impact of human consumption on ecosystems.

Grasslands also provide “cultural services” corresponding to the use of ecosystems for recreation, aesthetic, or spiritual values. In this regard, research evidenced that the unique grassland biodiversity is not only valuable in itself as the so-called “existence value of biodiversity” (e.g., Drius et al., 2016; Stanisci et al., 2014), but it also underpins other ecosystem services such as climate regulation or pollination (Mace et al., 2012; Kremen et al., 2007) acting as a “provisioning service”.

Although numerous studies have investigated grasslands ES (reviewed in Bengtsson et al., 2019; Peciña et al., 2019), grasslands have remained underestimated in the framework of ES (Frelichova et al., 2014) and sometimes, they have been combined with other types of rangelands, e.g., shrublands and savannas (e.g., Sala et al., 2017). Moreover, they are not as frequently discussed in terms of systems of multiple ecosystem service deliveries as in other production systems (e.g., cropland; Robertson et al., 2014) or natural ones (e.g., forests; Hönigova et al., 2011; Lemaire et al., 2011; Lavorel et al., 2013; Werling et al., 2014; Modernel et al., 2016; Burrascano et al., 2016). Grasslands have also been neglected in global policy discussions concerning ES (e.g., IPBES, 2019; Diaz et al., 2015; Pascual et al., 2017; see also Parr et al., 2014; Bond 2016), even though grasslands not only have a local importance for the maintenance of biodiversity, but they also affect ecological processes at landscape (e.g., pollination), regional (e.g., water regulation, recreation), and global scales (e.g., climate regulation) (Bengtsson et al., 2019).

The quantification and implementation of ecosystem goods and services have been among the biggest challenges of current ecosystem science (Wallace, 2007), and the explicit quantification of ES are considered one of the main requirements for the implementation of the ES concept into environmental institutions and decision making (Daily and Matson, 2008).

The principal challenges in managing ES are that they are not independent of each other (Rodríguez et al., 2006). Since most processes and functions of ecosystems involve complex relationships, in ecosystems supplying multiple ecosystems services we can expect trade-offs or possibly synergies (Pan et al., 2014), especially between provisioning and regulating services (Bennett et al., 2009), as well as between provisioning and cultural services at the landscape scale (Wu et al., 2012). Interactions among ecosystem services occur when different services react to the same driver of change or when interactions themselves cause changes in one service and alter the provision of another (Bennett et al., 2009). Ecosystem service trade-offs arise when the provision of one service is improved to the detriment of the provision of another service; while ecosystem service synergies arise when multiple services are simultaneously enhanced (Raudsepp-Hearne et al., 2010).

Research on ecosystem service trade-offs mostly focused on production systems. Globally, increasing food provisioning have determined losses of carbon stocks on agricultural land (West et al., 2010). In forested landscapes, timber production has been increased at the cost of carbon sequestration and crop pollination (Olschewski et al., 2010). In such production systems, ecosystem service trade-offs normally arise from humans' management choices, which can change the type, magnitude, and relative mix of services provided by ecosystems through land use change, fertilization, or the creation or expansion of settlements (Bennett et al., 2009). In semi-natural ecosystems, it can be expected that trade-offs also exist due to environmental (e.g., soil features) or biotic (e.g., species richness, diversity, primary production) factors that can either limit or foster ecosystem service supply. Moreover, studies of trade-offs related to ecosystem services can support an improved use and management of ecosystems.

The present chapter aims at a) providing a quantification of four ecosystem services, namely biodiversity, productivity, climate regulation and pollination, in semi-natural dry grasslands, , and b) evaluating the relationships between these services and community attributes in terms of trade-offs and synergies. Extensively borrowing from the field of conservation planning, we have adopted the concept of surrogates (van Jaarsveld et al., 1998). Specifically, we identified proxy indicators (i.e., "indicator" parameters) of ecosystem service supply and for each group identified in the previous chapter (Chapter I), we assessed the supply capacity for each ES.

Materials and methods

Ecosystem services' proxy indicators

The selection of the ecosystem services to be measured was based on literature review on the most relevant ecosystem services provided by grassland ecosystems (e.g., Garnier et al., 2016; de Bello et al., 2010). Four ES were selected as significative, two provisioning (Biodiversity and Productivity) and two regulating (Climate regulation and Pollination) (Tab. 1). Ecosystem service proxy indicators were based on literature review (review in Burkhard et al., 2009; Garnier et al., 2016; MEA, 2005) and data availability.

Biodiversity

Since functional community attributes turned out to be more significantly variable across the three identified communities, to evaluate the biodiversity ecosystem service, we chose the components of functional diversity (i.e., functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv); see Chapter I for detailed description of functional components) (Drius et al., 2019; Mace et al., 2012; Mason et al., 2005). Firstly, the values of the three components were standardised and then averaged to obtain a single value for each plot. Afterwards, we assigned to each of the three groups of plots identified through the cluster analysis in Chapter I (i.e., Group 1 *Artemisia alba* community, Group 2 *Bromus erectus* community and Group 3 *Brachypodium rupestre* community) a single value as the median value. Median values were considered as representative values of each group and used as proxy indicator (Tab. 1).

Productivity

To evaluate the ecosystem service of productivity in relation to forage production, we evaluated the aboveground standing biomass as property of the service (Palpurina et al., 2017; Garnier et al., 2016). We considered the amount of standing biomass (g/m^2) collected during field sampling for each plot (methodologies in Chapter I). Afterwards, we assigned to each of the three groups of plots the median standing biomass (g/m^2) and used it as proxy indicator (Tab. 1).

Climate regulation

Carbon content values for biomass, litter and soil were derived from field sampling and laboratory analysis. Carbon content was computed from carbon concentration (mg/g) determined as described in the previous chapter (Chapter I) for each sampling plot. The total carbon content of each plot was computed by summing the carbon concentration in biomass, litter, and soil. The median total carbon content for each of the three groups was calculated and used as proxy indicator of climate regulation (Tab. 1).

Pollination

The assessment of pollination service considered the pollinator provision as the property of the service (Tab. 1). As proxy indicator for the service, we considered the flowering period of animal-pollinated species found in sampled plots. Data about species flowering period was retrieved from previous studies (Fantinato et al., 2016; 2018; 2019), where the flowering period was defined based on phenological surveys carried out every ten days throughout the flowering season (April – September). Data allowed to build the “community flowering curve”, by pooling together the flowering periods of all animal-pollinated species observed in a plot. Specifically, the flowering curve is a cumulative curve, in which the x-axis reports the surveys and the y-axis the number of flowering species recorded in each survey. The area of the curve has been quantified by summing the flowering periods of the animal-pollinated species occurring in the plots. The median area value for each of the three groups of plots identified in Chapter I was then used as proxy indicator for pollination service (Tab. 1).

Table 1: Ecosystem services, properties, and proxy indicators selected for this study. Ecosystem service categories follow the Millennium Ecosystem Assessment (2005); P: provisioning; R: regulating. FRic = Functional Richness; FEve = Functional Evenness; FDiv = Functional Divergence.

Ecosystem services	***Properties***	***Proxy indicators***
Biodiversity (P)	Existence of biodiversity value	Median value of FRic, FEve and FDiv
Productivity (P)	Accumulation of standing biomass	Biomass weight (g/m^2)
Climate regulation (R)	Carbon content in vegetation and soil	Total carbon concentration (mg/g) in vegetation biomass, litter and soil
Pollination (R)	Pollination provision	Area of the flowering curve

Ecosystem services scoring

To evaluate multiple ecosystem services (ES) in the same framework, ES' proxy indicators have been reduced to the same scale with common units (Nardo et al.,2005; Drius et al., 2019). Standardisation (or z-scores) approach was employed according to the continuous nature of the ES indicators, with respect to the range of indicator values. As suggested in Wolff et al. (2015), Nedkov and Burkhard (2012) and Burkard et al. (2012), to quantify ES we used a scoring approach. Standardised values of the four ES' proxy indicator were assigned to each plot. Considering all plots, we selected the minimum and maximum values of each proxy indicator, and the range between the minimum and maximum values of each proxy indicator was divided into five equal intervals: 1: very low, 2: low, 3: medium, 4: high, and finally 5: very high. Plots were then split into the three groups, and for each groups the median values of each proxy indicator were calculated. Each median value of proxy indicators was then assigned to a rank from 1 to 5. To visually express results, we assigned a colour to each rank as follows: 1: red, 2: orange, 3: yellow, 4: green, 5: dark green.

The four ES were represented in a spider chart for each group. Spider charts have been commonly used to demonstrate changes and typology of trade-offs between various ecosystem services (Raudsepp-Hearne et al., 2010). Charts were built as two-dimensional chart type designed to plot more series of values over multiple quantitative variables. Each ES has its own axis, and all axes are joined in the centre of the figure that represents the 0 value of the ordinal rank, while the more external part of the chart represents the 5 values of the rank. The ranked values of the four ES were inserted in the spider plots, and they were linked drawing a four-sided shape; the wider is the area (A) in the spider plot, the higher is the multi-service value of the site (Drius et al., 2019).

Finally, to evaluate the relationships between the four ecosystem services (i.e., Biodiversity, Productivity, Climate regulation and Pollination) and data regarding both taxonomical and functional community attributes (Chapter I), we applied a Spearman correlation to a matrix of 179 plots by 29 variables (Past software; Hammer et al. 2001). Significance was determined at $p < 0,05$.

Results

ES proxy indicators' values varied among the three groups, ranking from very low (red colour) to very high values (dark green colour) (Table 2).

Table 2: List of ES proxy indicator' values for each group resulted from the cluster analysis. For each proxy indicator the median value is reported. Colours represent levels of the ES proxy values, ranging from 1: red (very low), 2: orange (low), 3: yellow (medium), 4: green (high) to 5: dark green (very high). Group 1: Artemia alba community; Group 2: Bromus erectus community; Group three: Brachypodium rupestre community.

Ecosystem services	***Proxy indicators***	***Group 1***	***Group 2***	***Group 3***
Biodiversity	Mean value of FRic, FEve and FDiv	2,06	2,53	1,84
Productivity	Biomass weight (g/m^2)	165,60	159,88	119,36
Climate regulation	Carbon concentration (mg/g)	988,23	885,26	879,90
Pollination	Area of the flowering curve	50,00	64,00	52,00

Biodiversity

Based on the ranking, the three groups had high to very high values, increasing from *Brachypodium rupestre* community to *Bromus erectus* community (Tab. 2). Biodiversity mostly resulted positively correlated to the cover of forbs and, accordingly, of entomophilous species; conversely, biodiversity evidenced a negative correlation with the moss cover, the mean species height, and the cover of ruderal species. Overall, a significant and positive relationship was also evidenced with both biomass and litter nitrogen content (Tab. 4).

Productivity

Productivity proxy indicator values varied across the groups from low to very low (Tab. 2). As it could be expected, the aboveground biomass increased with increasing total and vascular species cover (Tab. 4). Interestingly, among growth forms, only the forb cover had a significant, positive relationship with this service. On the other hand, productivity had a clearly negative relationship with moss cover, and both biomass and litter nitrogen content (Tab. 4).

Climate regulation

Overall, climate regulation proxy indicator values evidenced good levels of carbon accumulation in vegetation and soil across groups, with standing biomass always playing a primary role compared to soil and litter (Tab. 3). *Artemisia alba* community revealed as the most efficient in carbon sequestration considering the carbon percentage in soil, biomass, and litter (Tab. 3). Overall, higher carbon concentration was related to an increased percentage cover of shrubs and mosses (Tab. 4), while the cover of both forbs and grasses had a negative relationship with the service. Analogously to productivity, climate regulation service was negatively associated to the increasing percentage of nitrogen content in biomass and litter (Tab. 4).

Table 3: Soil carbon content (%), Biomass carbon content (%) and Litter carbon content (%) (Mean ± sd) in the three groups (Group 1: Artemia alba community; Group 2: Bromus erectus community; Group three: Brachypodium rupestre community). Different letters indicate significant differences according to Tukey's test (p < 0,05) in Chapter I.

	Group 1		**Group 2**		**Group 3**	
Soil carbon content (%)	12,75 ± 1,42	c	9,86 ± 3,55	a	11,47 ± 2,17	b
Biomass carbon content (%)	43 ± 3,39	b	39,97 ± 2,89	a	40,08 ± 4,38	a
Litter carbon content (%)	40,11 ± 5,25	a	39,49 ± 3,09	a	33,61 ± 7,91	b

Pollination

The “community flowering curve” (Fig. 1), showed a similar trend for each group. The highest mean number of flowering species was evidenced between the sixth survey and the tenth survey, which correspond to late spring (between the second week of May and the third week of June), in correspondence with the peak of precipitations in the study area. *Brachypodium rupestre* community (Group 3) was highly variable, showing high standard deviations.

Pollination proxy indicator evidenced low to medium values. The higher value has been found for *Bromus erectus* community (Group 2); while both *Artemisia alba* community (Group 1) and *Brachypodium rupestre* community (Group 3) ranked low. A clear relationship existed between the pollination service and species richness, and the functional richness (FRich) and evenness (FEve); being mostly made of entomophilous species, also the cover of forb species had a positive relationship with pollination (Tab. 4).

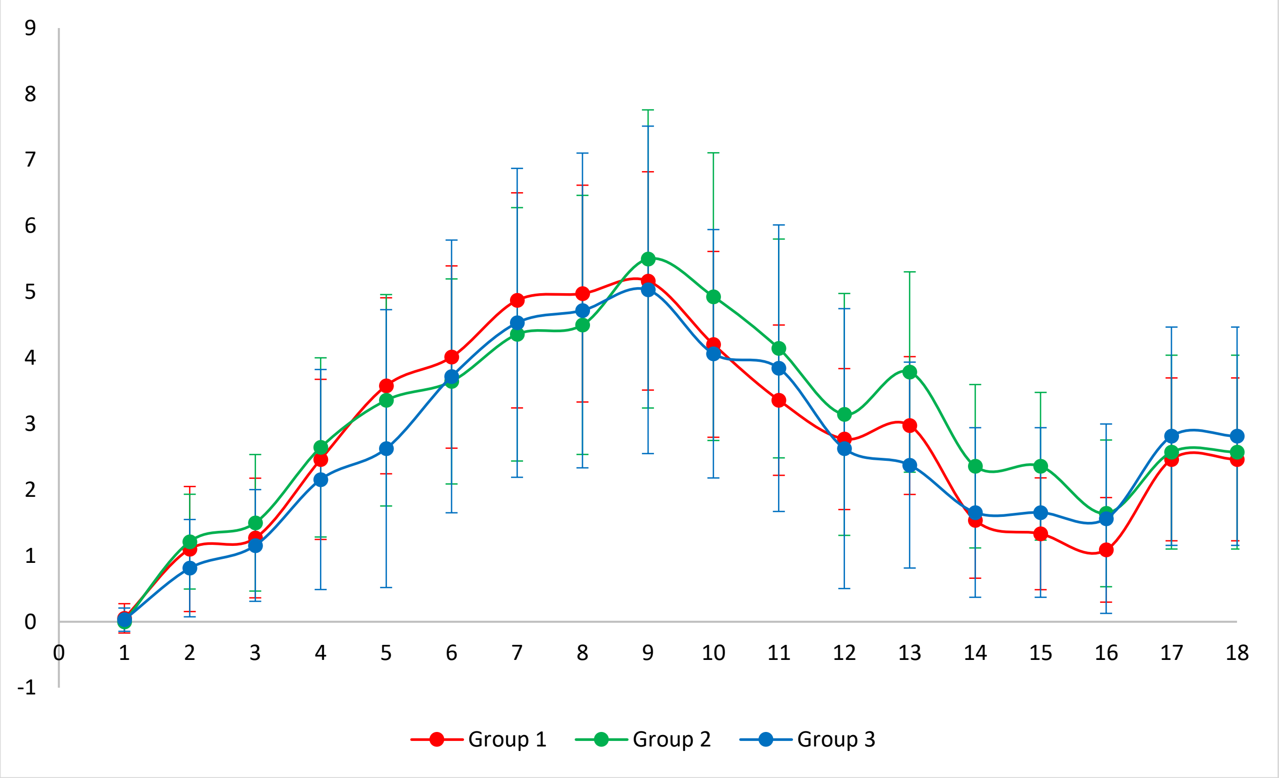

Figure 1: Community flowering curves for the three groups (Group 1 Artemisia alba community, Group 2 Bromus erectus community and Group 3 Brachypodium rupestre community). The x-axis reports the surveys and the y-axis the mean number of flowering species recorded in each survey. Surveys carried out every ten days; the first survey was the last week of March, the last survey was the second week of September.

Table 4: Spearman correlations. Significative P values (p<0,05) are highlighted in yellow; green values indicate positive relationships, while red values negative relationships.

	Biodiversity	**Productivity**	**Climate regulation**	**Pollination**
Total vegetation cover (%)	0,335330	0,048586	0,164690	0,009289
Vascular species cover (%)	0,000114	0,000097	0,000091	0,002424
Moss cover (%)	0,021461	0,032625	0,000000	0,834200
Mean species height (cm)	0,006916	0,009542	0,485830	0,029412
Species richness	0,000000	0,372330	0,675440	0,000000
Evenness index J	0,150080	0,977250	0,719550	0,660890
Entomophilous species cover (%)	0,000005	0,020352	0,184700	0,000000
Anemophilous species cover (%)	0,123750	0,023864	0,000070	0,705710
Entomophilous species (richness)	0,000000	0,620100	0,831810	0,000000
Anemophilous species (richness)	0,504950	0,588260	0,718790	0,049188
Forbs (% cover)	0,000885	0,000774	0,013341	0,000002
Grasses (% cover)	0,000529	0,359140	0,000000	0,855960
Shrubs (% cover)	0,254060	0,910770	0,000000	0,013819
Biomass weight (g/m2)	0,572150	0,000000	0,766500	0,071908
Biomass C content (%)	0,042743	0,350710	0,000000	0,913610
Biomass N content (%)	0,025141	0,000000	0,023497	0,703120
Litter weight (g/m2)	0,164020	0,000000	0,134830	0,046793
Litter C content (%)	0,053462	0,034175	0,000000	0,901180
Litter N content (%)	0,000004	0,000077	0,000000	0,601790
C cover (%)	0,047781	0,636300	0,017888	0,051416
S cover (%)	0,000000	0,076979	0,000000	0,014065
R cover (%)	0,000000	0,156480	0,000001	0,017945
CSR cover (%)	0,055066	0,959700	0,158480	0,009748
CWM H	0,007023	0,055365	0,000000	0,059449
CWM SLA	0,061209	0,613700	0,415820	0,135110
CWM LDMC	0,000000	0,012254	0,000000	0,118130
FRic	0,000000	0,038031	0,023582	0,000000
FEve	0,000000	0,089809	0,316860	0,000777
FDiv	0,000000	0,068752	0,000000	0,223680

Overall, productivity and the climate regulation were mostly linked, either positively or negatively, to attributes regarding the percentage cover of the community (either total or regarding specific species pools). Biomass and litter N content had a negative effect on both productivity and the climate regulation service. Conversely, biodiversity (as expected) and pollination were mostly affected by attributes related to species richness, with forbs (mostly animal-pollinated species) playing a crucial role in both cases. Neither the anemophilous species cover nor their richness had effects on the biodiversity service. Among attributes regarding the cover, it is worth mentioning the contrasting role of stress tolerant and ruderal species cover; while stress tolerant species were positively correlated to both services, the increase in cover of the ruderal species seemed to have a detrimental effect, more marked for biodiversity.

The multi-service value of the three groups was expressed by the spider diagrams reported in Figure 2. Overall, biodiversity showed the maximum number of high values (4 and 5), while productivity was the ES with the highest number of low values (1 and 2). Climate regulation showed two out of three medium values (3) and an outstanding value for Group 1. Finally, pollination was situated in intermediate position with two low values and one medium value of the proxy indicator.

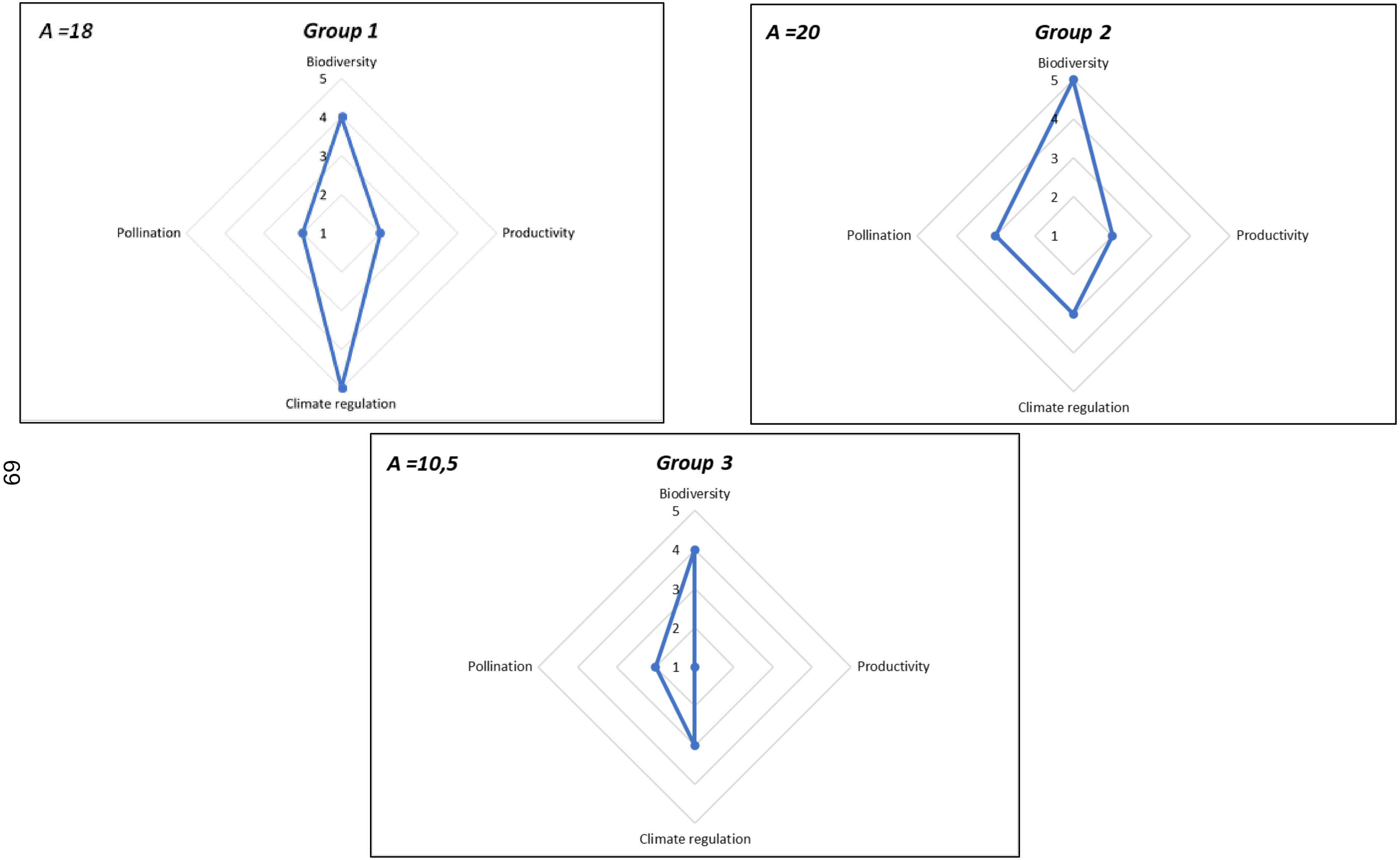

Figure 2: Ecosystem service values of the three groups (Group 1: Artemisia alba community; Group 2: Bromus erectus community; Group 3: Brachypodium rupestre community). Spider plots of each group are built using the ranked values for each ES, specifically: Biodiversity, Productivity, Climate regulation and Pollination. For each spider plot the area (A) of the figure is reported.

Comparing the area of the four-shaped figure of each spider plot, it emerged that the distribution of the multi-service value was different among the three communities, ranging from the minimum value of *Brachypodium rupestre* community (Group 3; $A = 10{,}5$) to the maximum value of *Bromus erectus* community (Group 2 and 3; $A = 20$). *Artemisia alba* community (Group 1) showed intermediate value of the four-shaped figure of the spider plot ($A = 18$). Overall, none of the communities was a hotspot for all the four services investigated. For example, *Artemisia alba* community (Group 1) had the highest value of climate regulation but dropped to low values for both productivity and pollination. *Bromus erectus* community (Group 2) and similarly *Brachypodium rupestre* community (Group 3) ranked very high and high for biodiversity, to the detriment of productivity that showed low and very low values, respectively. The area of the spider charts allowed to rank communities along an axis of ES supply in descending order (Tab. 5).

Table 5: Spider plots chart area corresponding to the three communities defined in Chapter I.

Spider chart area	***Community***
20	*Bromus erectus* community
18	*Artemisia alba* community
10,5	*Brachypodium rupestre* community

Discussion

Grassland ecosystem services refer to all the benefits provided by biodiversity and ecosystem structure and function of grasslands to meet the needs of human survival, life, and well-being (Sala and Paruelo 1997). In this research, we have evaluated the multi-service value of ES supply in semi-natural dry grasslands, using measured proxy indicators. Ecosystem service supply varied in the three groups driven by the different community attributes that were identified for each group in the previous chapter (Chapter I), implying that communities more important for one ecosystem service are less important for another.

Results about biodiversity ecosystem service evidenced an overall high value of the service. Research has focused on factors that influenced biodiversity, and largely recognised the importance of forb species in the maintenance of grasslands biodiversity (Rusina, 2017). Furthermore, also the presence of animal-pollinated species was proved to be correlated with high grassland biodiversity (Fantinato et al., 2016; 2018). Consistently with previous studies, our results highlighted a general positive relationship between biodiversity ecosystem service and entomophilous and forb species cover, although the two values are often correlated since forb species are normally animal-pollinated species. Higher biodiversity is in general predicted to increase biomass production (Tilman, 2001); Cardinale et al. (2012) affirmed that species diversity in grasslands enhanced the production of fodder but had mixed or even detrimental effects on many other services. As a matter of fact, the relationship between biodiversity and other ecosystem services in grasslands needs to be better clarified (Zhao et al., 2020). Contrary to what is stated in Cardinale et al. (2012), in our case, high values of biodiversity ES corresponded to low values of productivity ES. Sonkoly et al. (2019) suggested that besides diversity, dominance effects and the so-called mass ratio hypothesis (Grime, 1998) may also play a key role in explaining biomass production. Indeed, with increasing plant production, diversity first increases, then after a certain point starts to decrease (Grime, 1998; Mittelbach et al., 2001). However, interesting results arise when considering the different components of biodiversity, both taxonomical and functional. Species richness was positively correlated to both biodiversity and pollination, while it did not show any correlation with productivity and the climate regulation service. Conversely, functional richness, that is the amount of functional trait space filled by species in the community, had positive correlations with all investigated ES. It can thus be argued that the relationship between diversity and productivity depends on the specific component of diversity used.

The importance of species traits is evidenced by studies on the dynamics of grassland habitats. As reported in previous studies (Campagnaro et al., 2017; Cocca et al., 2012; European Commission, 2013; Rusina, 2017) when grasslands overgrow with shrubs, biodiversity decreases, but standing biomass, the proxy we used for productivity, increases due to the presence of woody species and the increase in total vegetation cover.

In addition, it was notable the relationship between the increase in productivity of grasslands and the decrease of nitrogen content in biomass and litter. Probably, as reported in Bobbink et al. (1998), De Schrijver et al. (2011) and Roth et al. (2013), eutrophication of the environment leads to a decrease of aboveground biomass production.

Grassland ecosystems have also attracted scientific and policy interest because of their potential role as sinks or sources for atmospheric carbon dioxide; the processes of carbon sequestration and carbon storage, as soil organic matter, and fluxes of greenhouse gases in grasslands are intimately linked to each other (Bengtosson et al., 2019; Török et al., 2020). Pan et al. (2014) suggested that overall, increasing forage production of grassland ecosystems may lead to a reduction in regulating services such as carbon sequestration. Our results underlined a different win-win situation: increasing values of productivity corresponded to increasing values of climate regulation. The outcome evidenced by our results, probably derived from the relationship between the high cover of shrub species that simultaneously contribute to increase biomass weight and C sequestration in soil, biomass and litter. Since the quantification of climate regulation service derived from the carbon concentration in aboveground biomass, litter and soil, the three groups showed a different level of the service, precisely because of the different characteristics of the communities that allowed a different accumulation of carbon in the three considered carbon pools (details about carbon concentration in biomass, litter and soil in Chapter I).

Grasslands are also important for thousands of pollinator species, such as bees, flies, beetles, butterflies (Bastian, 2013; Zulka et al., 2014; Hegland and Boeke 2006; Holland et al. 2017), and pollination service by many wild plants are suggested as unique to grasslands and cannot be performed by other ecosystems in the same way (Zhao et al., 2020). In this regard, it is interesting to note that the positive evaluation of the service could be linked to the value of the biodiversity service. Confirming our results about the link between pollination and biodiversity, numerous studies reported that dry grasslands can crucially contribute to biodiversity and pollination services (Fantinato et al., 2016; 2018; Van Swaay, 2002). However, in our case, only the *Bromus erectus* community (Group 2) showed a relatively high value for pollination, while the other two communities ranked low, in a certain way contradicting what stated by e.g., Zhao et al. (2020). With respect to this, literature proved that different grassland communities support different local pools of plant and pollinator species (Öckinger et al., 2007; Valkó et al., 2012), and Fantinato et al. (2019) suggested that rather than a single community, it is the mosaic of different grasslands that mostly contribute to the long-lasting provision of this ecosystem service by increasing pollination interactions and improving the resistance and resilience of the pollination networks.

The aim of this study was to provide a quantification of some of the most important ecosystem services in semi-natural dry grasslands (biodiversity, productivity, climate regulation and pollination) together with the evaluation of the relationships between them and community attributes. Overall, this study showed the importance of all the four different studied ecosystem services, in particular biodiversity. The three communities performed differently, and evidenced trade-offs among the investigated ES as a response to community attributes. *Bromus erectus* grassland turned out as the community with the highest multi-service value; however, results also highlighted the importance of the maintenance of different semi-natural dry grasslands characterised by different structure and species pools.

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Chapter III. Can the value of ecosystem services be used to define ecological thresholds in habitat conservation status?

Introduction

Biodiversity conservation is largely recognized as an important target for humankind (Cafaro and Primack, 2014), as shown by the variety of regional, national, and international agreements aiming at slowing down or preventing biodiversity loss (see e.g., CITES, 1973; European Commission, 2011; United Nations, 1976, 1992).

Over the last decades, the International Union for Conservation of Nature (IUCN) developed quantitative criteria that allow for objective and repeatable assessments of species extinction risk (IUCN, 2012, 2013; Mace et al., 2008). By ranking species at risk of extinction, the IUCN Red Lists provide a global indication on the state of one, basic level of biodiversity (Baillie et al., 2004; Butchart et al., 2004; McCarthy et al., 2008).

More recently, the scientific community, conservationists and institutions are increasingly concerned with biodiversity assessments concerning higher levels of biological organisation (Izco, 2015; Keith, 2009; Keith et al., 2013, 2015; Kontula and Raunio, 2009; IUCN, 2015; Nicholson et al., 2009; Rodríguez et al., 2011, 2012, 2015), and specifically, habitats and ecosystem. In fact, these levels are expected to more efficiently represent the biological diversity as a whole; act as a surrogate for those species yet undescribed or poorly known (Cowling and Heijnis, 2001; Nicholson et al., 2009); allow to incorporate further information, such as the role of species richness/diversity, offering precious tools both for species and habitat prioritization (see, e.g., Berg et al., 2014; Lindenmayer et al., 2008; Pärtel et al., 2005).

In Europe, the European Directive 92/43/EEC on the Conservation of natural habitats and of wild fauna and flora (the Habitats Directive) sets the framework for the European Union's policies on nature conservation. It gives the common objective for the Member States ‘to ensure the maintenance or restoration, at favourable conservation status, of the natural habitats and species of wild fauna and flora of Community interest’ (Art. 2). In the Habitat Directive, the conservation status of a habitat is defined as “the effect of all the influences acting on a natural habitat as well as its typical species that may affect its long-term natural distribution, its structure and functions as well as the long-term survival of its typical species” (Art. 1). Specifically, according to the Habitat Directive, the assessment of the conservation status of a habitat is based on four major parameters: area, range, structure and functions, and future prospects, i.e., deteriorations it undergoes and evolutions of its area. However, the implementation of the Habitat Directive has been problematic as fundamental concepts (e.g., Favourable reference values) and the four parameters used to assess Conservation Status are still unclear (Delbosc et al., 2021).

The first problem regards the definition of "typical species". The definition quoted above implies that to assess the conservation status of a habitat we need to evaluate the conservation status of its components, i.e., "typical species". The conservation status of a habitat becomes favourable when these elements contribute to the survival of the habitat over time and its stability or expansion in space (Maciejewski et al., 2016). However, despite "typical species" and their conservation status are considered as one of the main criteria for assessing the status of structure and functions, the Habitats Directive does not provide a clear and unambiguous definition, and this notion is often questioned (Gigante et al., 2018). Despite several approaches can be used to define the typical species pool, a central approach is that of "diagnostic" species, strictly linked to a given habitat, and based on the concept of fidelity (Chytrý et al., 2002; Dengler et al., 2008). Although informative, there are many habitats with wide floristic variability within their distribution range. Thus, as stated by Gigante et al. (2016), lists of "typical" species (in the sense of "diagnostic" species), risk to have a diagnostic value, rather than a value as descriptors of a good conservation status. In addition, the concept of typical species developed by Evans and Arvela (2011) did not strictly focus on the species diagnostic value, but rather on identifying those species having the role of synthetic indicators of the conservation status of a habitat. In this sense, another possible approach is the use of the concept of "ecological specialization" which refers to a restricted ecological niche breadth for a given species (Carboni et al., 2016). Specialist species are those strictly linked to a specific combination of environmental conditions and that can only tolerate a limited range of conditions. Conversely, generalist species have wide environmental tolerances, successfully growing in several different habitats. These patterns should reflect at community-scale, namely communities found in different stressful environments are expected to contain different specialized species. Thus, we can hypothesise that the specialised species pools will vary across the three different communities identified, and within the same community type according to current site environmental conditions, namely according to the variability of environmental parameters. Consequently, a community which hosts only or the highest number of specialised species, can become the reference status for the system under analysis.

The definition of "Favourable reference values" represents the second problem in assessing the conservation status of a habitat. The word "favourable" by definition refers to some "ideal" conservation level of a habitat, with which the extant situation has to be compared. However, the ideal conservation status needs to be defined. In addition, we need to decide how large shifts from the “ideal” condition of e.g., distribution area, structure, species composition, can be allowed without losing the “favourable status” (Mehtälä and Vuorisalo, 2007). This involves identifying critical thresholds, i.e., potentially irreversible shifts in habitat attributes, beyond which the risk of serious or irreversible changes is supposed to substantially increase (Mehtälä and Vuorisalo, 2007; Zhang et al., 2018). Several approaches have been used to establish a “reference status”; e.g., undisturbed by human activity, historical condition, expert-based definition (Stoddard et al., 2006), or the best reachable status in areas where man is considered a part of the ecosystem, as is the case of semi-natural dry grasslands (Maciejewski et al., 2016).

Finally, a problem also arises from the difficulty to directly evaluate ecosystem functions. As a consequence, since habitat composition and structure are expected to reflect habitat functions, they have often been used as proxy indicators (Benson, 2006; Keith et al., 2013; Lindgaard and Henriksen 2011).

In this regard, the quantification of ecosystem services and the evaluation of the relationships between services and community attributes can be used as a tool for the assessment of the conservation status. Furthermore, we hypothesise that the integration of community specialisation and the values of ES supplied by different community will allow us to define thresholds and benchmarks of habitat conservation status.

In this work we considered as “typical species” those that exhibited high specialisation within a community, and we defined them as “specialised species”. Specialised species identified by Indicator Species Analysis were used as the reference species pool of a given community (i.e., *Artemisia alba* community, *Bromus erectus* community and *Brachypodium rupestre* community). We assumed that community with only specialised species or with the highest number of specialised species, can become the reference status for the studied grasslands. Furthermore, since high values of ecosystem services (ES) could represent habitat in a good conservation status (Brondizio et al., 2019; Westman, 1977), we analysed the relationship between community specialisation and the values of the four ecosystem services (biodiversity productivity, climate regulation and pollination), to define thresholds for habitat conservation status.

Data analysis

Specialised species identified through the Indicator Species Analysis in Chapter I (ISA; Dufrêne and Legendre, 1997) can be used as the reference species pool of a given group of plots (i.e., Group 1 *Artemisia alba* community, Group 2 *Bromus erectus* community, Group 3 *Brachypodium rupestre* community, identified in Chapter I). Therefore, at the plot level, any deviations from the reference status (i.e., the community state in which only specialised species can be found) can be quantified as the relative number of specialised species on the total number of species recorded.

In order to test whether deviations from the reference state can affect the provision of different ecosystem services, we compared the relative number of specialised species per plot with values of four ecosystem services: biodiversity sustainment (biodiversity), primary productivity (productivity), climate regulation and animal-mediated pollination (pollination). For each plot, biodiversity was quantified by averaging standardized values of functional richness (FRich), evenness (FEve) and divergence (FDiv); productivity was quantified as the plant biomass dry weight; climate regulation was quantified as the carbon storage in plant biomass, litter and soil; while pollination was quantified as the area of the plot flowering season (see Chapter II for a detailed description of chosen ecosystem services).

We used separate generalised linear models (GLM, R version 3.4.3) to explore the relationship between the relative number of specialised species and the four ecosystem services. We performed GLMs for each of the three groups of plots identified through the cluster analysis in Chapter I (i.e., Group 1 *Artemisia alba* community, Group 2 *Bromus erectus* community and Group 3 *Brachypodium rupestre* community) by using gaussian error-distribution and log link function for the ecosystem service “biodiversity”, gamma error-distribution and log link function for the ecosystem services “productivity” and “climate regulation”, and poisson error-distribution and log link function for the ecosystem service “pollination”.

Finally, with respect to *Bromus erectus* community which resulted the one that provided the highest values of almost all ecosystem services, when possible, we defined thresholds in community attributes that correspond to the provision of different values of ES. To define thresholds, we used the results obtained from Spearman correlations calculated in Chapter II. For each correlation, threshold values corresponded to natural breaks in data distribution. Specifically, we identified two threshold levels in data distribution that defined the range of values corresponding to the ideal, intermediate, and bad condition of the community.

Results

Overall, values of relative number of specialised species showed to differently vary among the three plant communities (i.e., *Artemisia alba* community, *Bromus erectus* community and *Brachypodium rupestre* community). Specifically, the relative number of specialised species varied from 0,11 to 0,67 (mean = 0,39; SD = 0,12) for *Artemisia alba* community, from 0,14 to 1,00 (mean = 0,51; SD = 0,20) for *Bromus erectus* community, and from 0,28 to 0,83 (mean = 0,49; SD = 0,14) for *Brachypodium* *rupestre* community.

The relationship between the relative number of specialised species and the ecosystem service "biodiversity" was significantly positive for *Bromus erectus* grasslands (z-value = 2,138; P-value = 0,037), while significantly negative for *Brachypodium rupestre* community (z-value = -4,491; P-value < 0,001) (Tab. 1). No significant relationships were found for *Artemisia alba* community. "Productivity" showed a significant relationship only with the relative number of specialised species of *Brachypodium rupestre* community (Tab. 1); specifically, as the relative number of specialised species increased, the primary productivity increased as well (z-value = 3,442; P-value = 0,001). The relationship between the relative number of specialised species and the ecosystem service "climate regulation" was significantly positive for *Bromus erectus* community (z-value = 4,049; P-value < 0,001), while no significant relationships were found for *Artemisia alba* and *Brachypodium rupestre* communities (Tab. 1). Lastly, the ecosystem service "pollination" showed a significantly positive relationship with the relative number of specialised species of *Bromus erectus* grasslands (z-value = 2,161; P-value = 0,030), while a significantly negative relationship emerged for *Artemisia alba* grasslands (z-value = -14,50; P-value < 0,001) and *Brachypodium rupestre* grasslands (z-value = -5,36; P-value < 0,001).

*Table 1: Results of generalised linear models (GLM) to explore the relationship between the relative number of specialised species for Artemisia alba, Bromus erectus and Brachypodium rupestre communities and the four ecosystem services. * = significant relationship.*

	Biodiversity		**Productivity**		**Climate regulation**		**Pollination**	
	z-value	P-value	z-value	P-value	z-value	P-value	z-value	P-value
Artemisia alba community	-1,199	0,234	-1,61	0,111	-0,154	0,878	-5,36	<0,001*
Bromus erectus community	2,138	0,037 *	-0,86	0,394	4,049	<0,001*	2,161	0,030 *
Brachypodium rupestre community	-4,491	<0,001*	3,442	0,001*	-1,32	0,194	-14,50	<0,001*

Natural breaks in data distribution, allowed the identification of thresholds in *Bromus erectus* community attributes responsible for different levels of ecosystem service provision (Tab. 2). Vascular species cover assured high values of productivity when higher than 87%; while when the vascular species cover decreased below 65%, the community only supplied low level of the service.

Forb cover positively influenced both productivity and pollination (see Chapter II), however it evidenced different threshold levels for the two services. While the upper level for productivity was at 17%, to assure high levels of the pollination service, forb cover had to be higher than 30%. Similarly, the entomophilous species cover that enhanced productivity was above 23%, but to obtain the highest values of the pollination service, entomophilous species cover had to increase to percentages higher than 30%. The most interesting case regarded the cover of grasses. According to Spearman correlations, grass cover was positively correlated to biodiversity and negatively correlated to the climate regulation service. Although with opposite effects, threshold levels were the same; namely, when the grass cover was equal to or higher than 80%, *Bromus erectus* community supplied the highest value of biodiversity and the lowest values of climate regulation. To have a win-win condition, grass cover should be included in the range between 40 and 80%.

Table 2: Thresholds levels for the four ecosystem services in Bromus erectus community. Where possible, threshold lower and upper limits were reported. Collinearity between ecosystem service proxy indicators and community attributes were specified. Significant P values (p<0,05) of Spearman correlations (Chapter II) are highlighted in yellow; green values indicate positive relationships, while red values negative relationships.

	Biodiversity			**Productivity**			**Climate regulation**			**Pollination**		
	P-value	Lower threshold limit	Upper threshold limit	P-value	Lower threshold limit	Upper threshold limit	P-value	Lower threshold limit	Upper threshold limit	P-value	Lower threshold limit	Upper threshold limit
Total vegetation cover (%)	0,335330			0,048586	60%	87%	0,164690			0,009289		
Vascular species cover (%)	0,000114			0,000097	65%	87%	0,000091			0,002424		
Moss cover (%)	0,021461	2%	40%	0,032625			0,000000	5%	25%	0,834200		
Mean species height (cm)	0,006916	20cm	30cm	0,009542			0,485830			0,029412		
Species richness	0,000000			0,372330			0,675440			0,000000	collinear	collinear
Evenness index J	0,150080			0,977250			0,719550			0,660890		
Entomophilous species cover (%)	0,000005			0,020352	13%	23%	0,184700			0,000000	20%	30%
Anemophilous species cover (%)	0,123750			0,023864			0,000070	40%	80%	0,705710		
Entomophilous species (richness)	0,000000			0,620100			0,831810			0,000000	collinear	collinear
Anemophilous species (richness)	0,504950			0,588260			0,718790			0,049188		
Forbs (% cover)	0,000885			0,000774	10%	17%	0,013341	10%	20%	0,000002	13%	30%
Grasses (% cover)	0,000529	40%	80%	0,359140			0,000000	40%	80%	0,855960		
Shrubs (% cover)	0,254060			0,910770			0,000000	3,50%	20%	0,013819		
Biomass weight (g/m2)	0,572150			0,000000	collinear	collinear	0,766500			0,071908		
Biomass C content (%)	0,042743	35	43	0,350710			0,000000	collinear	collinear	0,913610		
Biomass N content (%)	0,025141			0,000000			0,023497	2%	4%	0,703120		
Litter weight (g/m2)	0,164020			0,000000	80g/m2	110g/m2	0,134830			0,046793		

Table 2: continued

	Biodiversity			**Productivity**			**Climate regulation**			**Pollination**		
	P-value	Lower threshold limit	Upper threshold limit	P-value	Lower threshold limit	Upper threshold limit	P-value	Lower threshold limit	Upper threshold limit	P-value	Lower threshold limit	Upper threshold limit
Litter C content (%)	0,053462			0,034175	40%	43%	0,000000	collinear	collinear	0,901180		
Litter N content (%)	0,000004			0,000077			0,000000			0,601790		
C cover (%)	0,047781			0,636300			0,017888	1%	5%	0,051416		
S cover (%)	0,000000			0,076979			0,000000	77%	105%	0,014065		
R cover (%)	0,000000	2%	10%	0,156480			0,000001			0,017945	1%	9%
CSR cover (%)	0,055066			0,959700			0,158480			0,009748		
CWM H	0,007023	40cm	52cm	0,055365			0,000000			0,059449		
CWM SLA	0,061209			0,613700			0,415820			0,135110		
CWM LDMC	0,000000	35%	40%	0,012254	35%	40%	0,000000			0,118130		
FRic	0,000000	collinear	collinear	0,038031			0,023582	6,5	9	0,000000	4	7,5
FEve	0,000000	collinear	collinear	0,089809			0,316860			0,000777		
FDiv	0,000000	collinear	collinear	0,068752			0,000000			0,223680		

Discussion

The role of biodiversity in supporting human well-being and maintaining ecosystem services has been largely demonstrated (Balvanera et al., 2014; Harrison et al., 2014; Thompson and Starzomski, 2007). In Europe, the Habitat Directive, which is based on a conservation approach to biodiversity, requires governments to provide all appropriate measures to maintain or restore habitats at a “Favourable Conservation Status” (FCS). The concept of FCS is central to the Habitats Directive and the identification of “typical species” is required to define the FCS (Jones, 2002).

Analysing the three communities identified in Chapter I (*Artemisia alba* community, *Bromus erectus* community and *Brachypodium rupestre* community), our study evidenced that each community was characterised by a different pool of specialized species, both in type and number.

Bromus erectus community had the highest number of specialised species, either in terms of fidelity (frequency within the group of plots) or higher cover (see Table 2 in Chapter I, for the detailed list of specialised species). Interestingly, *Bromus erectus* community was also the most variable community in terms of relative number of specialised species, evidencing a correspondent variability in site environmental conditions which reflect on species’ response in terms of occurrence.

The relationship between the relative number of specialised species and the selected ecosystem service (biodiversity, productivity, climate regulation and pollination) evidenced interesting results. Overall, ecosystem service values changed in relationship with the number of different specialised species of the three communities. *Artemisia alba* community showed the lowest number of significant relationships between the number of specialised species and the provision of ES. This community only showed a negative relationship with the pollination service. This result can be explained by some traits of the specialised species of this community, which are mostly anemophilous species such as *Artemsia alba* and the several grasses characterising the community, e.g., *Koeleria pyramidata, Carex flacca*, *Bothriochloa ischaemum* and *Chrysopogon grillus*. Being the pollination service linked to forb species cover and, of course, the richness and cover of entomophilous species, each time the number or the cover of specialised species increased, the value of this service decreased, as already evidenced in other works (Villani et al., 2016). This reasoning can also be applied and enlarged when considering *Brachypodium rupestre* community. When community attributes shift towards a dominance of grasses to the detriment of forb species, the value of the pollination service correspondingly decreases. On the other hand, these same species pools possibly have a positive effect on other ES, as in our results that evidenced a positive relationship between the increase of specialised species of *Brachypodium rupestre* community and the productivity service. Numerous studies have confirmed the importance of communities with *Brachypodium rupestre* in terms of productivity (Bonanomi and Allegrezza, 2004). At the same time, different studies (Bonanomi and Allegrezza, 2004; Bobbink and Willens, 1991) also recognised the detrimental effect of *Brachypodium rupestre* communities on biodiversity and the pollination service. More in general, results concerning *Brachypodium rupestre* community confirm the trade-offs between different ES. Specifically, the productivity–biodiversity relationship; species pools that increase productivity also make services like biodiversity and pollination decrease.

These issues evidenced that the relative number of specialised species entailed the provision of ecosystem services but is the species identity that governed the provision of a specific ES. Indeed, in our case, *Bromus erectus* community and *Artemisia alba* community had a similar number of specialised species, but the two communities were hot spots of different ecosystem services.

Although the issue needs further in-depth analysis, our approach allowed the identification of threshold levels corresponding to the ideal, intermediate, and bad condition of the community with respect to ecosystem service provision. We only analysed *Bromus erectus* community since it resulted the one that provided the highest values of almost all ecosystem services; however, results are encouraging. In particular, the possibility to quantify ES values and link them to community attributes allowed not only to identify those community attributes that are crucial in governing ES provision, but also to quantitatively define values of community attributes corresponding to different habitat conditions. These attributes can thus be used to define the "reference status", which includes the list of specialised (i.e., typical) species and the range of values of community attributes (e.g., range of vegetation cover, forb vs. grass species cover) that correspond to the "ideal" state. In this framework, threshold limits correspond to the values indicating a shift in habitat conditions, namely a change in the status of the habitat.

The aim of this study was to evaluate the relationship between semi-natural dry grasslands and the value of provided ecosystem services. The identification of this relationship helped to define ecological threshold. Overall, results showed that not only the number but also identity of specialised species were important factors in determining semi-natural dry grasslands functioning and provision of ecosystem services. The identification of appropriate threshold levels for community attributes to determine whether the conservation status is favourable or not, is still under discussion at European level and represent an important scientific challenge for the next future.

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General conclusion

Under the global change scenario, it is increasingly important to monitor habitat and ecosystem attributes to prevent their loss and degradation. While different approaches have been proposed to achieve this goal, little effort has been devoted in linking the monitoring of habitat attributes with the ecosystem services provided.

The present research was aimed at filling this gap by assessing the relationship between environmental and biotic attributes of grassland communities and the provisioning of four ecosystem services (i.e., biodiversity, productivity, climate regulation and pollination). Our approach revealed that different community attributes can be related to the supply of different ecosystem services. Specifically, it was possible to identify threshold values in the community attributes (e.g., vascular species cover and richness) corresponding to different values of ecosystem services.

The proposed approach provided a methodological framework which could be applied for the assessment of habitat conservation status and functionality. According to the article 17 of Habitat Directive, each Member State has to report to the European Commission on the conservation status of habitats of European interest. The application of the approach proposed in the present research at a broader geographical scale could provide more objective insights on habitat conservation status and harmonize different approaches used by different States. Moreover, it could facilitate the reporting by integrating existing information on habitat area and range with overlooked information on habitat structure and function and on the ecosystem services provided. In this regard, for a habitat type to be in favourable conservation status, both its structure and functions and its typical species should be in favourable conservation status. In this research indicator species were informative on plant community structure, functions and on the ecosystem services provided. An ecological approach in the identification of indicator species allowed to differentiate the small-scale variability (i.e., different states) of different communities within the same habitat type, corresponding to different small scale environmental shifts. Therefore, indicator species may be used with diagnostic and characteristic species as typical species for the assessment of a habitat conservation status.

Lastly, the identification of quantitative thresholds of the community attributes is a diriment issue in habitat and biodiversity conservation. Indeed, thresholds can be used to speed up monitoring activities and data collection; but, most importantly, they can be valuable reference values to be used as early alert indicators of habitat changes.

Annex 1. *Ternary strategy, Growth form and Pollination mode for species recorded. Ternary stragegy: C = competitive species; S = stress-tolerant species; R = ruderal species. * = Ternary strategy for species collected (10 ramets for each species, 4 leaves for each ramet (total leaves n = 1840). For species not directly measured ternary strategy available at BioFlor ("https://www.ufz.de/biolflor/index.jsp"). Growth form: F= forb; G = grass; S = shrub. Pollination mode: E = entomophilous species; A = anemophilous species.*

Plant species	***Tertiary strategy***	***Growth form***	***Pollination mode***
Allium sphaerocephalon L.	CSR	F	E
Alopecurus pratensis L.	C	G	A
*Anacamptis pyramidalis (L.) Rich. **	C	F	E
Anthericum liliago L.	CSR	F	E
Anthyllis vulneraria L.	CSR	F	E
Arenaria serpyllifolia L.	R	F	E
*Artemisia alba Turra **	R/SR	S	A
Asparagus acutifolius L.	CS	S	A
*Asperula cynanchica L. **	S	F	E
*Bothriochloa ischaemon (L.) Keng **	S/SR	G	A
*Brachypodium rupestre (Host) Roem. & Schult. **	S/SC	G	A
Briza media L.	CSR	G	A
*Bromus erectus Hudson **	S/SC	G	A
*Bupleurum baldense Turra **	S	F	E
Carex caryophyllea Latourr.	CSR	G	A
Carex flacca Schreb.	CSR	G	A
Carex liparocarpos Gaudin	CSR	G	A
Carpinus betulus L.	C	S	A
*Catapodium rigidum (L.) C.E. Hubb. **	S/SR	G	A
Centaurea scabiosa L.	C	F	E
*Centaurium erythraea Rafn **	S	F	E
Cerastium brachypetalum Desp. ex Pers.	SR	F	E
Cerastium semidecandrum L.	R	F	E
Chrysopogon gryllus (L.) Trin.	SR	G	A
*Cleistogenes serotina (L.) Keng **	S/SR	F	A
Convolvulus arvensis L.	CR	F	E
*Convolvulus cantabrica L. **	S/SR	F	E
Cotinus coggygria Scop.	C	S	E
*Crepis sancta (L.) Bornm. **	C/CR	F	E
*Crepis vesicaria L. **	SC/CSR	F	E
Cynodon dactylon (L.) Pers.	CS	G	A
Dactylis glomerata L.	C	G	A
Delphinium peregrinum L.	-	F	E
Diplotaxis tenuifolia (L.) DC.	CR	F	E
*Dorycnium herbaceum Vill. **	S	F	E
Erigeron annuus (L.) Desf.	CR	F	E
*Eryngium amethystinum L. **	SC	F	E
Eryngium campestre L.	CS	F	E
*Euphorbia cyparissias L. **	S	F	E
Festuca rupicola Heuff.	CS	G	A
Filipendula vulgaris Moench	CSR	F	E
Fragaria viridis Duchesne	CSR	F	E
Fraxinus ornus L.	C	S	A
*Fumana procumbens (Dunal) Gren. & Godr. **	S	S	E
*Galatella linosyris (L.) Rchb.f. **	S	F	E
*Galium lucidum All. **	S	F	E
*Galium verum L. **	S	F	E
Geranium purpureum Vill.	CSR	F	E
*Geranium sanguineum L. **	S/SC	F	E
*Globularia bisnagarica L. **	S/SC	F	E
Haplophyllum patavinum (L.) G.Don	-	F	E
*Helianthemum nummularium subsp. obscurum Holub **	S	S	E
Hieracium pilosella L.	CSR	F	E
*Hippocrepis comosa L. **	S/SC	F	E
*Hypericum perforatum L. **	S	F	E
*Inula hirta L. **	S	F	E
*Koeleria pyramidata (Lam.) Domin **	S/SR	F	E
*Linum tenuifolium L. **	S	F	E
*Lotus corniculatus L. **	S/SR	F	E
*Medicago minima (L.) L. **	S/SR	F	E
*Melampyrum barbatum Waldst. & Kit. **	SR	F	E
Melica ciliata L.	CS	G	A
*Odontites luteus (L.) Clairv. **	S	F	E
*Ononis natrix L. **	S	F	E
Ononis reclinata L.	CSR	F	E
*Ononis spinosa L. **	S/SR	S	E
Ophrys sphegodes Mill.	CSR	F	E
Orlaya grandiflora (L.) Hoffm.	R	F	E
Peucedanum cervaria (L.) Cusson ex Lapeyr.	CS	F	E
Peucedanum oroselinum (L.) Moench	CS	F	E
Pistacia terebinthus L.	C	S	E
Plantago media L.	CSR	F	A
*Potentilla hirta L. **	S/SC	F	E
*Potentilla pusilla L. **	S	F	E
Prunella grandiflora (L.) Scholler	CSR	F	E
Quercus pubescens Willd.	C	S	A
*Salvia pratensis L. **	SC	F	E
*Sanguisorba minor Scop. **	S/SC	F	A
Scabiosa columbaria L.	CSR	F	E
*Scabiosa triandra L. **	S/SC	F	E
Silene vulgaris (Moench) Garcke	CSR	F	E
*Spartium junceum L. **	S	S	E
*Stachys recta L. **	S/SC	F	E
*Teucrium chamaedrys L. **	S/SR	S	E
*Teucrium montanum L. **	S	S	E
Thlaspi perfoliatum L.	SR	F	E
*Thymus oenipontanus Heinr.Braun **	S	S	E
*Thymus pulegioides L. **	R	S	E
Torilis arvensis (Huds.) Link	CS	F	E
*Trifolium campestre Schreb. **	SR/CSR	F	E
Trifolium scabrum L.	SR	F	E