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CORSO DI DOTTORATO DI RICERCA IN SCIENZE AGRARIE

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Ciclo XXXV

Sardinian grasslands: from environmental, management and biotic factors interconnection to a regional thematic database

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General introduction

My doctoral thesis embarks on a research journey that spans from comprehending Mediterranean grasslands' environmental aspects, management, and biotic interactions. Within an interdisciplinary context, in Sardinia, where Mediterranean grasslands have historically played a pivotal role, the acquisition of accurate data becomes imperative to address current challenges, achieve a deeper understanding of these ecosystems, and provide concrete tools for their management and conservation, thus reinforcing their relevance in contemporary society.

Mediterranean grasslands have historically played a crucial role in human societies (Jouven et al., 2010), providing a wide range of habitats that support high biological diversity and hosting many plant and animal species (Giallonardo et al., 2019). They are important in rural areas, offering valuable ecosystem services (Bagella and Caria, 2011; Torralba et al., 2016), including the provision of food and biodiversity conservation (Bagella et al., 2020); fundamental forage production for livestock farming (Porqueddu et al., 2016; Seddaiu et al., 2018); the regulation of the carbon cycle (Moreno et al., 2018), and hence the potential for climate change mitigation (Lal, 2020). Grassland vegetation comprises highly heterogeneous natural plant communities, typically grasses, legumes, and other plants, with occasional woody species (Porqueddu et al., 2016). These plant communities hold significant conservation value, often thriving in challenging environments and supporting wildlife or domestic grazing (Jouven et al., 2010; Wang et al., 2020). Various factors influence the diversity of plant species in pastures, the actual species present, their relative abundance, and the vegetative structure of grasslands (Hopkins and Holz, 2005). These factors, including intensive grazing management, the abandonment of marginal grasslands (Klimek et al., 2007), and climate change (Dibari et al., 2021), have led to a decrease in the area and quality of grasslands, presenting a severe conservation issue (Klimek et al., 2007). Due to the substantial variability in abiotic and biotic factors, different systems, such as agro-silvopastoral systems, are practised in Mediterranean grassland ecosystems (Porqueddu et al., 2016).

Inter-annual variability in floristic composition is an intrinsic feature of Mediterranean grasslands, and understanding this variability is crucial for sustainable pasture management and ensuring an adequate supply of nutrient resources for livestock.

Climate is one of the primary factors controlling the structure and function of grassland ecosystems worldwide (Philip A. Fay et al., 2003). In Mediterranean regions, which are sensitive to climate change (Noto et al., 2023), the characteristic climatic factors are intense summer droughts and strong intra- and inter-annual fluctuations in precipitation (Fernández-Moya et al., 2011), especially during spring and summer (Catorci et al., 2021). This leads to significant year-to-year differences in productivity, species composition, and diversity (Dibari et al., 2021).

While these grasslands are adapted to seasonal drought, they are vulnerable to changes in precipitation, as water availability serves as a limiting factor for most terrestrial ecosystems (Catorci et al., 2021; Wang et al., 2023). In recent decades, the Mediterranean region has become increasingly arid, with projections indicating a potential 20% decrease in annual precipitation by 2050 and increased interannual variation (Kafle and Bruins, 2009; Golodets et al., 2013). Furthermore, inter-annual precipitation variation is expected to increase, with years of exceptionally wet conditions alternating with drought years in the Mediterranean region (Golodets et al., 2013). These precipitation fluctuations pose significant challenges for various sectors, including agriculture, water supply, and ecosystem stability. Consequently, it is becoming increasingly crucial to develop adaptation strategies and global plans to address the impacts of these changing climatic conditions (Golodets et al., 2013). As recently highlighted by Slabbert et al. 2022, abiotic factors play a more significant role than land management and biotic interactions in shaping vascular plants.

The first part of the research focuses on the intra-interannual variability of the floristic composition of Mediterranean grasslands. We investigate yearly variations within precipitation variability within a typical silvopastoral system in the Mediterranean basin in Central-Western Sardinia, Italy. This study aims to comprehend and document the composition of plant species from year to year. Analyzing these fluctuations in plant composition enhances our knowledge, providing insights for better land management and conservation practices in the Mediterranean region. Surveys were carried out over five years twice each year: the first in winter, following the annual vegetative growth before the winter vegetative dormancy period, and the second in spring, at the peak of the vegetation season. This seasonal approach comprehensively evaluates year-to-year variations in plant composition, offering valuable data for understanding changes in plant communities within the Mediterranean silvopastoral system (**Chapter 1**).

Profound changes in grasslands and agro-silvopastoral systems worldwide, involving both intensification and abandonment, can reduce plant diversity and lower productivity (Bagella et al., 2013).

The Mediterranean region has faced significant human-induced disruptions for millennia, making it one of the most transformed areas globally (Falcucci et al., 2007). Some regions of Italy have been identified as European hotspots of land abandonment and declining agricultural areas. This abandonment, coupled with the loss of agro-silvopastoral farms, has adverse effects, including economic decline, depopulation, and the rewilding of agropastoral areas (Salis et al., 2022). Maintaining sustainable levels of grazing livestock is increasingly recognized as essential for preserving plant species and associated habitats in pastures (Farris et al., 2013).

While climate is the primary factor influencing grassland plant composition, studying biotic interactions and different management practices is vital for sustainable pasture management and nature conservation (Niamir- Fuller and Huber-Sannwald et al., 2020; Sternberg et al., 2000).

Identifying key taxonomic groups and their potential relationships and land-use patterns can help standardize effective tools for biodiversity assessment and monitor the effects of land-use abandonment (Larrieu et al., 2018). Vascular plants are recognized as suitable indicators for monitoring and conservation and are well-known from a taxonomic perspective. However, many arthropod groups are considered good land-use change or abandonment indicators. Among these, ants are efficient environmental indicators in nearly all terrestrial habitats due to their broad range of climatic niches and their ability to cope with variable environmental conditions (Andersen and Majer, 2004; Jiménez-Carmona et al., 2020), dynamically responding to changes in habitat structure (Farji-Brener et al., 2002; Gallé et al., 2016). A multispecies approach can provide a better assessment of overall diversity in an area, more accurately reflect diversity changes caused by habitat modification, and offer more comprehensive information to combat biodiversity loss (Hevia et al., 2016). Studying how different management methods influence other taxa is valuable, as illustrated in our research in Verdinelli et al. (2022).

The second part of the thesis analyses the reliability of plant functional groups, ant traits and ant functional groups as indicators of land abandonment use changes in mountainous Sardinia and Piedmont regions (Italy). The research covered four sites across three biogeographic regions (Mediterranean, Continental, and Alpine). The goals are: examining vascular plant and ant community patterns in response to land abandonment; evaluating the reliability of using functional plant groups, ant traits, and ant functional groups as indicators of significant changes in community composition, regardless of the biogeographic region; exploring overall congruence between ants and vascular plant communities in terms of species and functional composition, investigating whether ants can serve as surrogates for biodiversity assessment (**Chapter 2**).

Given the crucial structural and functional role that vegetation plays in terrestrial ecosystems, vegetation databases are essential tools for vegetation classification, analyzing species' spatial and temporal distribution concerning environmental factors, assessing the conservation status of species and habitats, and monitoring environmental changes, including those related to climate change and the spread of alien species (Landucci et al., 2012; Janssen et al., 2023).

The third part of the thesis describes a new thematic database for Sardinia's grasslands called SaGRA. It was created by collecting and standardising vegetation plots from ten research projects across various locations in Sardinia. Each plot was georeferenced in Qgis and associated with environmental and management data. In particular, the database contains information on plant species, vegetation structure, and environmental variables such as altitude, slope, exposure, vegetation series, geological types, and land use, providing a comprehensive overview of Sardinian grasslands. By creating this database, we would support current research and lay a foundation for future investigations. Shifting from detailed to regional data acquisition will enhance our understanding of Sardinia grasslands. The database is still in the assembly phase and will require additional steps for release, maintenance, server management, and third-party use (**Chapter 3**).

The proposed project for my doctoral thesis aligns with the scientific debate on Mediterranean grasslands and aims to contribute to a comprehensive understanding of these ecosystems, specifically focusing on their ecological dynamics and the factors influencing them. This research aims to enhance our knowledge of Mediterranean grasslands, shedding light on the response of vegetation to environmental changes and human activities and ultimately providing valuable insights for their effective management and conservation.

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Abstract

This doctoral thesis explores Mediterranean grasslands in Sardinia, Italy, focusing on environmental factors, land management, and species interactions. Grasslands support a high level of biodiversity and provide several ecosystem services. Moreover, they are historically and culturally significant, and precise data collection is essential for understanding and preserving them. The main goal is to increase the knowledge of these ecosystems by studying how they respond to environmental changes and human activities to aid in their conservation.

The first part of the research analyses the intra and inter-annual variability of their floristic composition and investigates how they depend on rainfall. The study was conducted on a farm representative of Mediterranean silvopastoral systems in the central-western part of Sardinia, Italy. The study took place from 2018 to 2022, with surveys in both spring and winter, using the point quadrat method along eight permanent transects. Significant variations in plant composition between winter and spring seasons affect pasture quality and biodiversity. Rainfall affects different plant species differently.

The second study underscores the importance of considering species interactions and land management for sustaining grasslands and conserving the ecosystem. A multispecies approach helps understand plant community diversity and prevent biodiversity loss due to changes in habitat. It examines how plant and ant communities respond to pasture abandonment, showing that ants can be effective indicators of biodiversity. This approach simplifies complex taxonomy and aids in the early detection of environmental changes and biodiversity loss in mountain habitats.

The third study describes a georeferenced regional database created for Sardinia's grasslands named SaGRA. The database collects vegetation data from projects conducted between 2011 and 2021. Each survey is associated with environmental and management variables. The surveys were performed in 685 plots, mainly in central-western and north-eastern Sardinia. Despite some limitations, such as uneven survey site distribution, SaGRA represents a step forward in scientific research and natural resource management in Sardinia, facilitating broader-scale research.

With these three studies, the doctoral thesis aims to contribute to understanding, managing, and preserving Mediterranean grasslands in Sardinia.

Keywords: Biodiversity Conservation, Databases, Environmental Factors, Funcional traits, Grassland management, Mediterranean grassland vegetation, multitaxon analysis, vegetation dynamics.

CHAPTER 1

Exploring dynamics of floristic composition in Mediterranean grasslands: a case study from Sardinia, Italy

Abstract

Mediterranean grasslands are biodiversity hotspots because they provide many habitats supporting high biological diversity, characterized by inter-annual variability of floristic composition. This study aims to assess the inter-annual dynamics of the floristic composition in a grassland within a typical silvopastoral system in the Mediterranean basin in the framework of rain variability. The study was performed on a farm representative of Mediterranean silvopastoral systems in Central-Western Sardinia (Italy). Vegetation surveys started in the spring of 2018 and concluded in the spring of 2022. They were conducted annually in the spring (S) and winter (W) seasons, with the point quadrat method along eight permanent transects. The dynamics of floristic composition were evaluated inside and between years in terms of variability of specific percentage contribution (CSP) of each plant species. The results showed substantial differences in the composition of plant assemblages between the two seasons (W vs S) and between surveys within each season. *Anthemis arvensis*, *Lolium rigidum*, *Festuca ligustica*, and *Medicago polymorpha* were the main plant species that discriminated between seasons. Considering the discriminant species between the four winter surveys, we found a positive effect of August rain on *Cynodon dactylon* CSP and a negative one on *Trifolium subterraneum* CSP. Considering the spring surveys, we found a positive effect of January rain on *L. rigidum* and a negative effect on *Plantago lanceolata*.

The rain influences the inter-annual dynamics of floristic composition in the Mediterranean grasslands. Other factors, such as temperature, remain to be investigated. Moreover, a more extended data series may allow us to strengthen our results.

Keywords: Climate change, false breaks, inter-annual variability, Mediterranean grasslands, seed bank, vegetation dynamics.

1. Introduction

Mediterranean grasslands are biodiversity hotspots because they provide many habitats supporting high biological diversity, including many plant and animal species (Gigante et al., 2018; Chytrý et al., 2015; Shi et al., 2014; D'Aniello et al., 2011). They are secondary formations culturally significant due to their ancient human history (Catorci et al., 2021), which provide several ecosystem services, including nutrient cycling, carbon sequestration, and water cycle regulation (Grenke et al., 2022; Seddaiu et al., 2018; Ribeiro et al., 2014) and agricultural goods (Unger and Jogen, 2014). However, they are facing significant challenges, including intensive grazing management, marginal grassland abandonment (Klimek et al., 2007), and climate change (Dibari et al., 2021). These factors have led to declining surface area and quality of grasslands, presenting a severe conservation problem (Klimek et al., 2007). The floristic composition and vegetation structure in grasslands result from complex interactions involving various biological and environmental factors (Puerto and Rico, 1992). Livestock grazing is a crucial factor that affects vegetation structure in grasslands. The intensity and timing of grazing can significantly impact plant diversity and abundance (Ribeiro et al., 2014).

Inter-annual variability of floristic composition is an intrinsic feature of Mediterranean grasslands. Understanding this variability is crucial for the sustainable management of pastures and ensuring they provide nutritious resources for livestock effectively. The most prominent climatic factors shaping grasslands are the presence of intense summer droughts, unpredictable autumn rains and significant intra- and inter-annual fluctuations in precipitation (Fernández-Moya et al., 2011), particularly during the spring and summer months (Catorci et al., 2021). These conditions result in substantial differences in productivity, species composition, and overall diversity of these ecosystems from one year to the next (Carmona et al., 2015). The timing and variability of rainfall within a year influence the growth and performance of plant species (Suttle et al., 2007). Annual species, which evolved specific adaptations to survive under such conditions, dominate these communities. These species spend the summer months in seed form, effectively avoiding the harsh conditions of high temperatures and water scarcity during this period. Autumn is a critical period for the renewal of plant life in these ecosystems: the first rains initiate germination and regrowth of many species.

Some models suggest that regions experiencing a significant increase in temperature accompanied by a simultaneous decrease in precipitation are particularly vulnerable to biodiversity loss (Engler et al., 2011). In such areas, there is a likelihood of an increase in drought-resistant and poorly palatable species (Sebastià et al., 2008), and drought is considered the main factor controlling forage quality and palatability (Dibari et al., 2021). Additionally, research has demonstrated that grasslands with a high level of biodiversity are less susceptible to the adverse effects of drought events, showing a more rapid recovery following periods of drought. Indeed, biodiversity plays a crucial role in enhancing the resilience of plant communities to extreme weather events and contributes to the overall stability of ecosystems (Isbell et al., 2015; Hautier et al., 2015).

Climate conditions in the Mediterranean basin have been experiencing a trend toward increased aridity in recent decades (Kafle and Bruins, 2009). This shift towards drier conditions is expected to persist, with projections indicating a potential 20% decrease in total annual precipitation by 2050 (Black 2009; Evans 2009). Intra-seasonal variation in rainfall is expected to become more extreme, with fewer but more intense rainfall events within a season. Furthermore, inter-annual variation in rainfall will increase, leading to years of exceptionally wet conditions alternating with years of drought in the Mediterranean region. These fluctuations in precipitation can pose significant challenges for various sectors, including agriculture, water supply, and the stability of ecosystems. As a result, it is becoming increasingly crucial to develop adaptation strategies and comprehensive plans to address the impacts of these changing climatic conditions (Golodets et al., 2013).

This study aims to assess the inter-annual dynamics of the floristic composition in a grassland within a typical silvopastoral system in the Mediterranean basin in the framework of rainfall variability.

Surveys were carried out over five years twice each year: the first in winter, following the annual vegetative growth before the winter vegetative dormancy period, and the second in spring, at the peak of the vegetation season. This seasonal sampling approach allows for comprehensively assessing inter-annual variability in floristic composition, capturing vegetation dynamics at two critical points in the year and providing valuable data for understanding fluctuations in plant communities within the Mediterranean silvopastoral system.

2. Materials and methods

2.1. Study area

The study site is located within a private farm in Central-western Sardinia, Italy, at coordinates 40° 8' 47.48" N and 8° 42' 17.00" E, with an elevation of 500 meters above sea level (Fig. 1), characterized by calcifugal, meso-supramediterranean cork oak series, specifically *Violo dehnhardtii - Querco suberis* Σ (Bacchetta et al., 2009). The underlying geological substrate consists of Plio-Pleistocene and Oligo-

Miocene volcanic rocks. The landscape in this region is diverse and includes various land uses associated with agro-silvopastoral production activities.

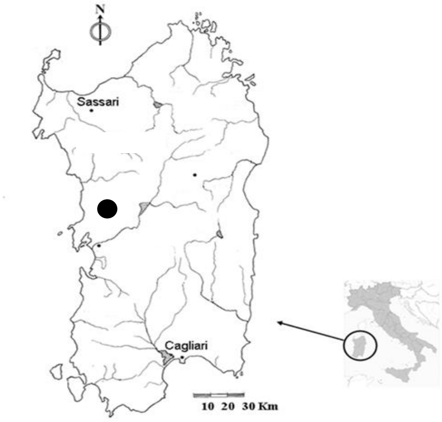

Fig. 1 – Study site location.

This farm serves as a representative example of Mediterranean silvopastoral systems. Livestock grazing on the farm comprises two breeds: Charolais and Sardo-Modican. The animals' diet combines direct grazing on available forage in permanent grasslands and hay produced on the farm. Additionally, external feed supplements are used to meet the nutritional needs of the animals, with feeding strategies adapted to the seasonal availability of grazing herbage and the specific dietary requirements of the animals at different physiological stages.

The farmer employs a vertical transhumance system, which involves the seasonal movement of livestock from mountainous areas to valley regions. Grazing primarily occurs in winter and spring, following a continuous scheme with a stocking rate of 1.5 LSU (Livestock Unit) per hectare per year.

2.2. Data collection

Vegetation surveys were conducted using the "point quadrat" method (Bullock, 1996). The survey areas were determined by eight permanent transects measuring 50 meters long, randomly located in 2018. Along each transect, points were marked at regular intervals of 1 m, and all plant species that came into contact with a needle at these points were recorded to assess the frequency occurrence of each species (Daget and Poissonet, 1971). In addition, since the less frequent species are likely missed using this method, a complete list of plant species within a buffer zone of 1 meter to the right and 1 meter to the left of the transect line was compiled (Verdinelli et al., 2022).

These transects' starting and ending points were recorded using a Garmin Montana 610 handheld GPS device, allowing for precise mapping of their positions.

Plant nomenclature and life form associated with each species follow https://dryades.units.it/floritaly/.

Vegetation surveys started in the spring of 2018 (survey 0) and concluded in the spring of 2022 (survey 8). The surveys were conducted annually in two seasons: spring (S) and winter (W) (Table 1).

Tab. 1 – Year and season of each survey.

Season	Year	Survey
Spring	2018	0
Winter	2018	1
Spring	2019	2
Winter	2019	3
Spring	2020	4
Winter	2020	5
Spring	2021	6
Winter	2021	7
Spring	2022	8

Meteorological data for the years 2017-2022 (daily Tmax, tmin, and rainfall) were provided by ARPAS (Agenzia regionale per la protezione dell'Ambiente della Sardegna) from the meteorological station of Macomer, 14 km from the study site. The study period showed inter-annual rainfall fluctuations in total quantity and monthly distribution. Annual rain varied between 1221 mm (2018) and 752 mm (2020), and the rainiest month was November 2021 (239 mm). The average annual Tmax ranged between 19.8°C (2018) and 20.6°C (2021); the warmest month was August 2012 (32.5°C). The average yearly

Tmin went between 10.4°C (2017) and 11.0°C (2018), and the coldest month was February 2018 (3.1°C).

2.3. Data analysis

The dynamics of floristic composition were evaluated within and between years in terms of variability of specific percentage contribution (CSP) of each plant species,

The frequency of occurrence of each plant species (FS_i) recorded along each transect was converted into CSP_i using the formula:

$$\mathrm{CSP}_i = \frac{\mathrm{FS}_i}{\sum \mathrm{FS}_i} 100$$

All species exclusively recorded within the buffer area were assigned a CSP of 0.3 according to Pittarello et al., (2019) and Tasser and Tappeiner (2005). The CSP of each species for each transect was used to build a species/cover matrix for data analysis.

To calculate the dissimilarity between pairs of samples, the Bray–Curtis dissimilarity measure was applied to square-root transformed data, resulting in a distance matrix. Non-parametric multidimensional scaling (nMDS) was employed as the ordination method to visualize differences in the composition of plant assemblages (Clarke and Gorley, 2006). The floristic composition of each sample was compared among season (W vs S) and years of surveys (T) using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001), using a nested design, with T nested in W or S. Taxa responsible for differences among samples in different surveys were identified by similarity percentages for species contributions analysis (SIMPER) (Clarke, 1993). Species that contributed at least 5% dissimilarity for any comparisons were considered significant discriminators.

The multivariate statistical analysis were carried out using the PRIMER software package version 7, with the PERMANOVA add-on developed by Anderson et al. (2008).

To evaluate the possible effects of rain on the CSP of the taxa responsible for the inter-annual differences in the composition of plant assemblages in winter, we considered the rainfall before the winter surveys, i.e August to November, as single months or cumulated. To evaluate the possible effects of rain on the CSP of the taxa responsible for the inter-annual differences in the composition of plant assemblages in spring, we considered the rainfall before the spring surveys, i.e February to May, as a single month or cumulated. Pearson correlation coefficients (r_p) were calculated to describe the linear relationships between CSP values and rain.

3. Results

3.1. Dynamics of floristic composition

In total, 179 plant species were identified, with 166 present in spring and 102 in winter. The most abundant species belonged to the Fabaceae family (36%), followed by Poaceae (32%) and Asteraceae (11%). When considering biological forms, Therophytes were strongly dominant, followed by Hemicryptophytes (Fig. 2).

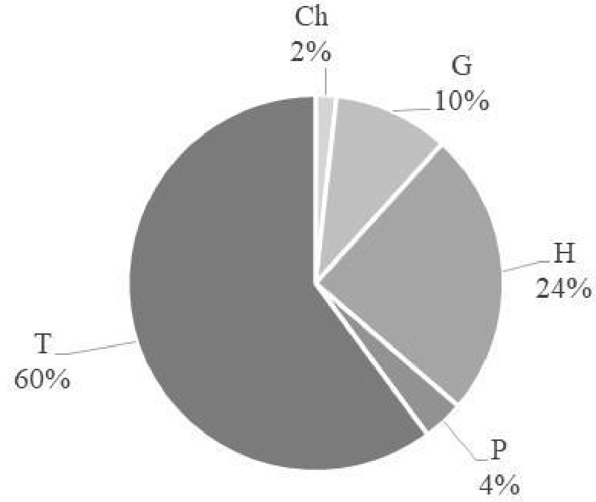

Fig. 2 – Biological spectrum (Ch=Chamaephytes; G=Geophytes; H=Hemicryptophytes; P=Phanerophytes; T=Theropytes).

The results obtained from nMDS analysis (Fig. 3) highlighted substantial differences in the composition of plant assemblages between the two seasons (W vs S) and between surveys (0-8) within each season for all *a posteriori* comparisons, as confirmed by the PERMANOVA analysis (Pperm=0.001).

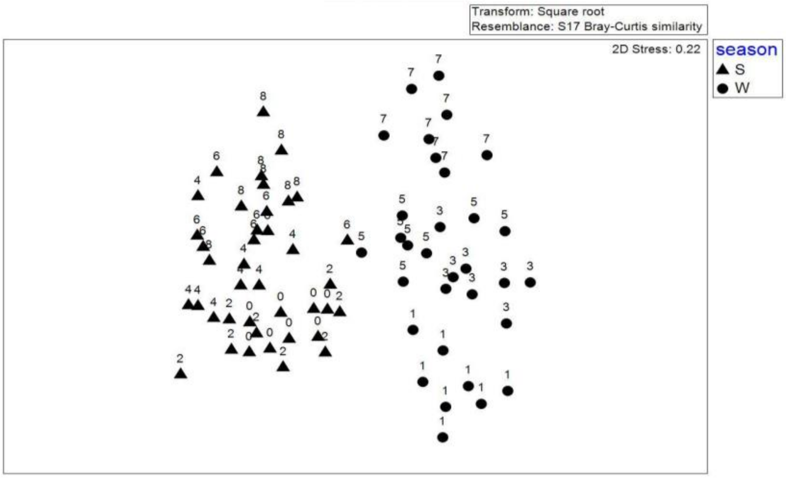

Fig. 3 - Two-dimensional nonmetric multidimensional scaling ordination (sMDS) of plant assemblage composition in the two seasons (S=spring; W=winter) and surveys (0-8).

As identified by SIMPER, the main discriminating plant species between seasons were four (Fig. 4). The most abundant species in winter was *Anthemis arvensis*, and in spring *Lolium rigidum*. The analysis highlighted the marked seasonality of *Festuca ligustica* and *Medicago polymorpha* among the other discriminating species, showing higher frequency values in spring.

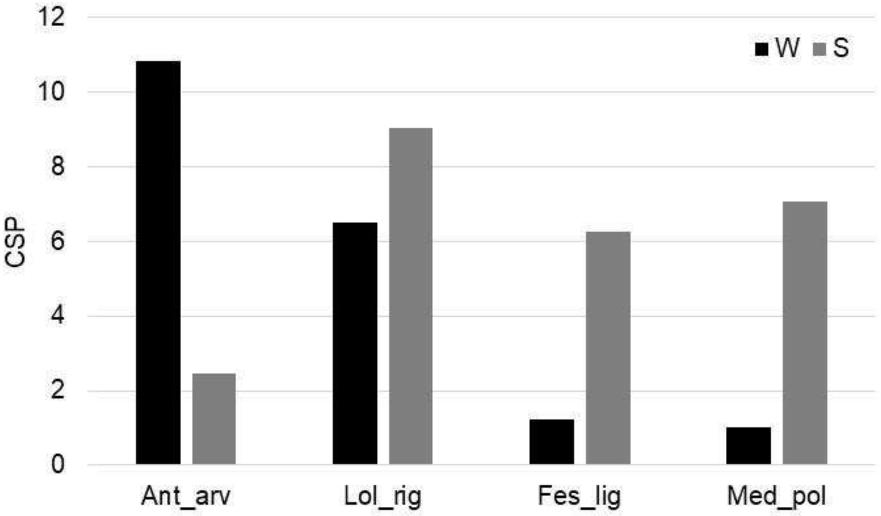

Fig. 4 - Average abundance (CSP) of the four species contributing at least 5% to the dissimilarity the comparisons between plant assemblages in spring (S) and winter (W) according to SIMPER (Ant_arv=*Anthemis arvensis*, Lol_rig=*Lolium rigidum*, Fes_lig=*Festuca ligustica*, Med_pol=*Medicago polymorpha*).

Poaceae and Fabaceae were more abundant in spring, while Asteraceae cover was similar in the two seasons (Fig. 5).

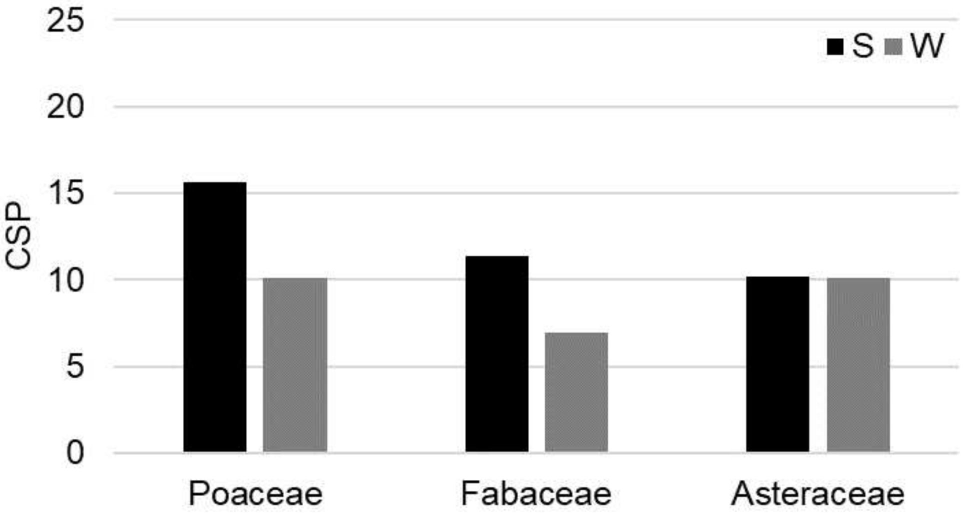

Fig. 5 - Average abundance (CSP) of the three dominant families in spring (S) and winter (W).

Comparing the four winter surveys, the main discriminating species, as identified by SIMPER, were five (Fig. 6). Among them *A. arvensis, Cynodon dactylon* and *T. subterraneum* showed a high CSP variability year by year while *Bromus hordeaceus* and *L. rigidum* were more stable.

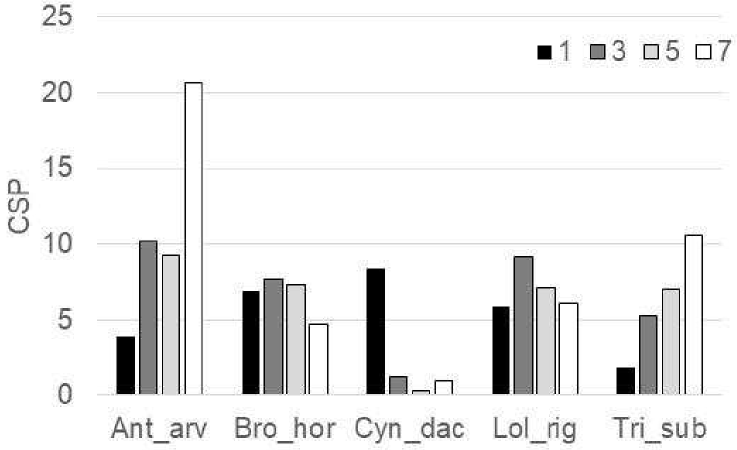

Fig. 6 - Abundance (CSP) of the four species contributing at least 5% to the dissimilarity of the comparisons between plant assemblages in winter surveys (1,3,5,7) according to SIMPER (Ant_arv=*Anthemis arvensis*, Bro_hor=*Bromus hordeaceus*, Cyn_dac=*Cynodon dactylon*; Lol_rig=*Lolium rigidum*, Tri_sub=*Trifolium subterraneun*).

Comparing the five spring surveys, the main discriminating species identified by SIMPER, were six (Fig. 7): *Avena barbata*, *F. ligustica*, *L. rigidum*, *M. polymorpha*, *Plantago lanceolata* and *T.*

subterraneum. Some of them, such as *M. polymorpha* and *P. lanceolata*, showed a very high CSP variability year by year.

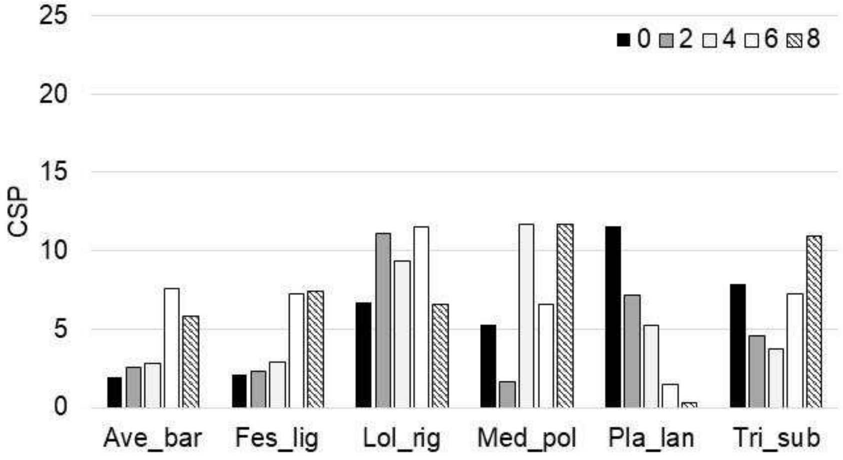

Fig. 7 - Abundance (CSP) of the six species contributing at least 5% to the dissimilarity of the comparisons between plant assemblages in spring surveys (0,2,4,6,8) according to SIMPER (Ave_bar=*Avena barbata*, Fes_lig=*Festuca ligustica*, Lol_rig=*Lolium rigidum*, Med_pol=*Medicago polymorpha,* Pla_lan=*Plantago lanceolata,* Tri_sub=*Trifolium subterraneun*).

3.2. Variations in rainfall and their effects

The rainfall pattern has shown significant variability between years in all months of the year (Fig. 8).

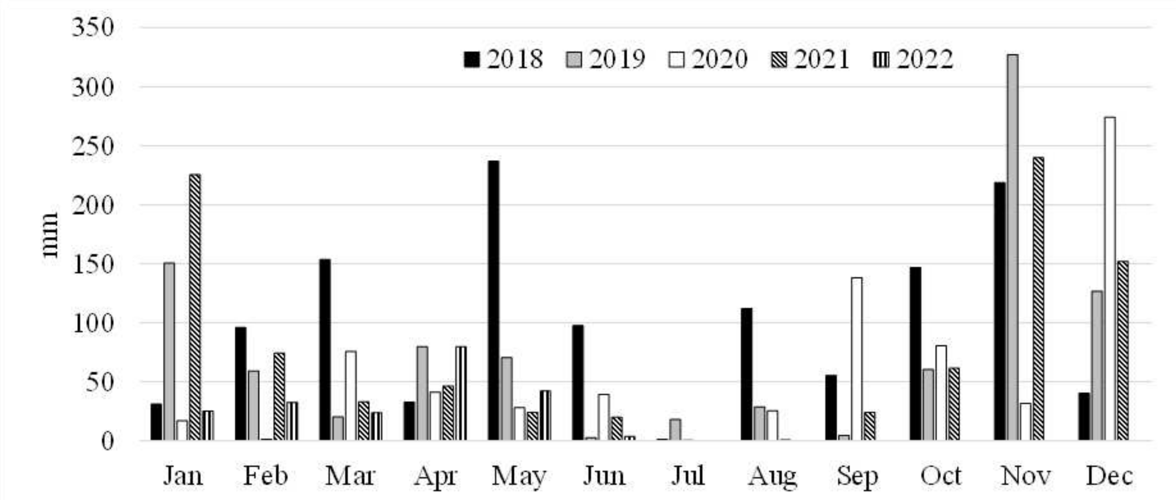

Fig. 8 - Monthly rainfall amount (mm) during the survey period.

Pearson correlation coefficients (r_p) between CSP of the taxa responsible for the inter-annual differences in the composition of plant assemblages in winter and the rainfall in the previous months were significant only for two species. We found a positive effect of August rainfall on *C. dactylon* (rp=0.95) and a negative effect on *T. subterraneum* (r_p=0.92).

Pearson correlation coefficients (r_p) between CSP of the taxa responsible for the inter-annual differences in the composition of plant assemblages in spring and the rainfall in the previous months were significant only for two species. We found a positive effect of January rainfall on *L. rigidum* (rp=0.84) and a negative effect May rainfall on *P. lanceolata* (r_p=0.84).

4. Discussion

The pastures in the study area exhibit typical Mediterranean pasture characteristics. They are indeed very species-rich pastures, as already observed in similar areas (Bagella et al., 2013; Farris et al., 2010; Tárrega et al., 2009), which is related to the traditional land use (Bagella et al., 2016). The dominance of Therophytes is another aspect typical of Mediterranean grasslands (Pitt and Heady, 1978; Díaz-Villa et al., 2003; Tárrega et al., 2009; Fernández-Moya et al., 2011;) because this life form is particularly well suited to disturbance tolerance (Grime, 1977) and soil disturbance by trampling favoured seedlings recruitment (Noy-Meir et al., 1989). Fabaceae and Poaceae play a fundamental role in these grasslands. Fabaceae, including some excellent forage species such as *T. subterraneum* and *M. polymorpha*, ensure the supply of proteins to the grazing animal during the entire grazing season, improve soil fertility with natural N fixation (Lucas et al., 2010) and support C cycling and the productivity and persistence of these secondary grasslands (Bagella et al., 2020). Poaceae support the forage production during the coldest months the excellent forage species *L. rigidum* with the contribution of *A. barbata* and *F. ligustica*. Moreover, they contribute to limiting soil erosion (Bagella et al., 2020).

According to the vertical transhumance system employed in the farm, we monitored the floristic composition in winter and spring when grazing primarily occurs in the area. As expected in the two seasons under comparison, the plant assemblage composition was different.

The spring vegetative cover was generally characterized by higher floral diversity and a more significant presence of forage grasses and legumes compared to winter. The bulk of primary production and biodiversity occurs in this season (Moreno and Pulido, 2009), extending through late spring and early summer, during which plants undergo seed formation and eventual senescence. Specifically, during the spring season, *L. rigidum*, *M. polymorpha*, and *F. ligustica*, with an average coverage percentage close to 10% for *L. rigidum* and slightly lower for *M. polymorpha* and *F. ligustica*, represent a significant portion of the vegetation cover. *L. rigidum* and *M. polymorpha* are considered excellent and good forage plants, respectively, and *F. ligustica* is considered fair (Bagella et al., 2013). Therefore, the presence and substantial coverage of these species constitute the primary support for pasture quality during the spring season.

On the other hand, the winter vegetation cover was dominated by *A. arvensis*, which is not consumed by grazing animals (Bagella et al., 2013). However, even in this season, though in limited quantities, other plant species are available.

Plants grow in response to the initial autumn rain events (Pitt and Heady, 1978). In the case of annual species, the rainfall that induces seed germination is termed the break of season. If this event occurs too soon, it can induce germination of pasture species that do not survive and are termed false breaks (Chapman and Asseng, 2001; Turner et al., 2001). In our study, we observed an effect of the August rainfall in the development of two species that most characterize the floristic composition during the winter season is related to the rainfall in this month more than in the following months. However, the two species on which these effects are most evident, *C. dactylon* and *T. subterraneum*, show an opposite response, positive in the former case and negative in the latter, which should be related to their different adaptation strategies. *C. dactylon* is a perennial stolonifera C4 plant which grows mainly in summer under maximal light availability and high temperatures. The C4 strategy in the Mediterranean grassland species is unusual, typically dominated by annual C3 grasses and legumes that are active during the wet period of the year (Aires et al., 2008). Using this adaptation *C. dactylon* avoids competition for light, water and nutrients with other annual species whose seeds germinate later. The observed positive effect of August rainfall on the CSP of this species in the winter could be explained by the efficient use of water by a species with photosynthetic efficiency at high temperatures and radiation intensities (Galiano, 1985). *T. subterraneum* is an annual early-season species that starts growing slowly in autumn or winter if the weather is mild, grows rapidly in the spring and sets seed by the end of the spring (Papanastasis, 1981). It was instead negatively affected by break season rainfall (Turner et al., 2001). The negative effect could probably be related to a false break due to a combination of a germination-inducing rainfall event followed by a period of drought determining a widespread death of seedlings, as already observed in Australian grasslands (Chappman and Asseng, 2001). In the study area, competition for water with *C. dactylon*, which has higher evapotranspiration enhancing soil water depletion, exacerbates the situation (Aires et al., 2008).

Concerning the floristic composition in spring, we found a direct effect of the January rainfall on the cover of two species: *L. rigidum* and *P. lanceolata*, which CSP was positively and negatively affected, respectively. *P. lanceolata* is a perennial species which produces rosettes and reproduces by seed production and clonal propagation (Cavers et al., 1980). It consistently improved under high temperatures and drought conditions (Rasmussen et al., 2020). *L. rigidum* is an annual species whose seeds germinate during autumn and winter (Steadman et al, 2003). Seed dormancy prevents false breaks before this time due to sporadic summer rains but allows germination when rainfall is sufficient to sustain plant growth and development (Steadman et al., 2004). This should be why abundant rains in January favoured seed germination and seedling growth. The less demanding *P. lanceolata* was favoured in the year with less rainy January. These observations confirm that in an environment where water is scarce, the species abundance is regulated by competition-driven water depletion (Tsialtas et al., 2001).

Rainfall is a crucial determinant of production and composition in arid and semi-arid systems (Dudney et al., 2017), and extreme events, such as extended autumn droughts, are forecasted to increase in Mediterranean regions under climate change scenarios (Nogueira et al., 2017). Such changes, particularly those responsible for false breaks, may also adversely affect the soil seed bank, which is strategic for conserving and regenerating Mediterranean grasslands.

In this paper, we explored the inter-annual dynamics of floristic composition in the Mediterranean grasslands, considering the rain as a key factor for this dynamic. Other factors, such as temperature, remain to be investigated. Moreover, a more extended data series may allow us to strengthen our results.

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CHAPTER 2

CONGRUENT RESPONSES OF VASCULAR PLANT AND ANT COMMUNITIES TO PASTORAL LAND-USE ABANDONMENT IN MOUNTAIN AREAS THROUGHOUT DIFFERENT BIOGEOGRAPHIC REGIONS

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Congruent responses of vascular plant and ant communities to pastoral land-use abandonment in mountain areas throughout different biogeographic regions

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Abstract

Background: There is a long-term trend towards the abandonment of agro-pastoral activities in the mountain areas of Europe: the following encroachment process of semi-natural grasslands by shrubs is one of the main severe threats to the conservation of biodiversity in mountain environments. To better understand the impact of land abandonment, we analysed the reliability of plant functional groups, ant traits, and ant functional groups as indicators of land use changes. We carried out the research in Italy at four sites along a latitudinal/altitudinal gradient in three biogeographic regions (Mediterranean, Continental, Alpine). We identified three stages of a chronosequence at each site as representative of the plant succession in response to pastoral land-use abandonment.

Results: As expected, both the plant and ant assemblages considerably differed across sites at the species level and, within each site, among the three stages. This trend was found also using ant traits, functional groups of ants, and plant functional groups. Ant and plant communities were related in terms of composition and functionality. Harvester ants and ants with collective foraging strategy were associated with annual legumes and grasses (Therophytes); ants with a strictly individual foraging strategy with Phanerophytes. Ant traits and plant functional groups indicated significant differences among the three stages of the chronosequence. However, ant functional groups could not discriminate between the stages represented by secondary grasslands currently grazed and shrub-encroached grasslands ungrazed.

Conclusion: Despite some limitations of ant functional groups in explaining the succession stages of land abandonment, our results suggest that ants are a good surrogate taxon and might be used as bioindicators of land-use changes and ecosystem functioning. Furthermore, our findings indicate that the functional group approach should be applied to other European ecosystems. Finally, reducing the taxonomic complexity could contribute to developing predictive models to detect early environmental changes and biodiversity loss in mountain habitats.

Keywords: Biodiversity conservation, Bioindicators, Cross-taxon congruence, Functional groups, Land abandonment, Monitoring, Mountain pastures

Background

Agro-pastoral activities in mountain areas are crucial for maintaining the viability of rural traditions in Europe and are particularly relevant for resource and nature

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conservation (Gómez Sal and González García 2007). However, the abandonment of mountain environments due to socio-economic changes after the Second World War led to a drastic reduction of agro-pastoral activities and changed land uses (MacDonald et al. 2000; Tasser and Tappeiner 2002). Pastoral activities have shaped the landscape into a mosaic of plant communities supporting different levels of biodiversity and providing different ecosystem services (Yapp et al. 2010; Bagella et al. 2013; 2020; Pittarello et al. 2020). On the other hand, the cessation or the limitation of grazing management for several years has led to natural vegetation succession processes (Garbarino et al. 2013). Dwarf shrubs first, and trees later, were responsible for the encroachment of broad areas of semi-natural grasslands, which is considered one of the main threats to biodiversity conservation in mountain environments (Laiolo et al. 2004; Koch et al. 2015; Pittarello et al. 2016).

Identifying key taxonomic groups and possible relationships among them and land use patterns can help standardise efficient tools for biodiversity assessment and monitor the effects of land-use abandonment (Larrieu et al. 2018). Vascular plants are recognized as particularly suitable indicators in monitoring and conservation: they provide the physical structure for other organisms, including many habitat specialists distributed across broad environmental gradients, and are well known from a taxonomic point of view. For these reasons, vascular plants are traditionally considered one of the key surrogate taxonomic groups to select areas of concern in biodiversity conservation as they can reflect the diversity of other significant and less easily detectable taxa (Pharo et al. 2000; Burrascano et al. 2011; Bagella 2014; Bagella et al. 2014). However, many arthropod groups are considered good land-use change or abandonment indicators. Among them, ants are effective environmental indicators in almost all terrestrial habitats because of their wide range of climatic niches and their ability to cope with varying environmental conditions (Andersen and Majer 2004; Jiménez-Carmona et al. 2020). Besides, they play multiple roles in food webs: herbivores, decomposers, predators, parasites, seed dispersers, and pollinators (Hölldobler and Wilson 1990). As they are influenced by a more complex suite of environmental variables than plants, ants can provide useful information on short-term environmental changes in highly dynamic and vulnerable ecosystems (Zina et al. 2021).

Simple metrics as species richness have proved to be unreliable tools in biodiversity monitoring (Andersen and Majer 2004; Wan Hussin et al. 2012). Indeed, ecosystem processes are affected by the functional traits of organisms involved, and the taxonomic identity is not suitable to make functional comparisons among different biogeographical regions (Westoby et al. 2002; Garnier et al. 2004; Segar et al. 2013). Since functional traits mediate the response of organisms to the environment and influence ecosystem functioning (Chelli et al. 2019), they may help predict how communities might respond to landscape changes. Likewise, functional groups can be helpful and cost-effective to detect congruent and robust patterns of community structure between regions by transcending the effects of historical factors on species composition (Hoffmann 2010; Laureto et al. 2015). The definition of functional groups, based on specific traits depending on the aims and scale of the research (Smith and Huston 1989; Wilson 1999), has a long history in ecology, especially for plants (e.g. Raunkiaer 1934). The impact of pastoral land-use abandonment on vegetation has been investigated, and previous studies showed that plant traits provide insight on key processes driving vegetation changes (Prévosto et al. 2011). Plant functional groups were also successfully used for measuring the recovery of vegetation after cropland abandonment (Heydari et al. 2020). Ant functional groups have a relatively recent history (Andersen 1995). Although Andersen's scheme (1995) is still questionable outside Australia, it could be a useful generalisation in broad studies of functional trait variation in Europe (Gómez et al. 2003; Azcarate and Peco 2012; Satta et al. 2012; Verdinelli et al. 2017).

Despite the importance of ant-seed dispersal in conserving semi-open habitats of European pastures (Travers et al. 2020), information on ant-plant relationships for the European mountain areas is minimal (Hevia et al. 2016; Heuss et al. 2019). In addition, a multispecies approach can provide a better assessment of the overall diversity of an area, reflect more accurately changes in diversity caused by habitat modification, and provide more complete information for halting the biodiversity loss (Hevia et al. 2016). Therefore, we aimed to investigate the possibility of using very different taxa as bioindicators by analysing the effect of land abandonment on ant and plant communities in Italian mountain areas. For this purpose, we followed the space for time substitution approach (Pickett 1989) in different sites, characterised by a shared history of land-use, we identified three different stages of a vegetation succession following a pastoral land-use abandonment. Moreover, to examine the effect of geographical variation, we considered a biogeographic gradient, i.e. Mediterranean, Continental, and Alpine (EEA 2016). The specific objectives of the research were: (i) to analyse vascular plant and ant community patterns in response to pastoral land-use abandonment; (ii) to evaluate if vascular plant functional groups, ant traits, and ant functional groups were reliable indicators of the main changes of community composition, regardless of the biogeographic region, and (iii) to verify a possible overall cross-taxon congruence between ant and vascular plant communities, at the level of species and functional composition, and investigating whether ants can be used as surrogates of biodiversity of the studied taxa.

Materials and methods

Study sites and experimental design

The research was carried out in Italy at four sites located along a latitudinal/altitudinal gradient, in three biogeographic regions (Fig. 1; Table 1). On a physiognomic basis, we identified three dynamic stages of a chronosequence at each site (Table 1), representing the plant succession from pastoral use to abandonment. Stage 1 was represented by secondary grasslands currently grazed (grasslands dominated by grasses and other herbaceous species, e.g. forbs, legumes, etc.). Shrub-encroached grasslands ungrazed for 20–30 years (grasslands dominated by shrub and fern species, with a sporadic presence of trees. The herbaceous layer was mainly dominated by grasses and other herbaceous species, e.g. forbs, legumes, etc.) were the dominating vegetation in stage 2. Stage 3 was represented by woodlands, abandoned for at least 50 years (woodlands with a close canopy cover). The overstorey layer was dominated by tree species, whereas the understorey by shrubs or ferns. The understorey herbaceous layer had a low cover.

A number of 3 to 9 sampling units (hereafter 'plots') were established for each stage of each site for sampling vascular plants and ants, for 60 plots (Table 1). In Val Vogna, being located above the treeline, only stage 1 and stage 2 were represented.

Data collection

Surveys on vascular plants (hereafter 'plants') were performed from May to July 2019, at the peak blooming period, to identify all species more easily. Along with four transects corresponding to four perpendicular radii of each plot, we applied the vertical point-quadrat method (Daget and Poissonet 1971) to assess the frequency of

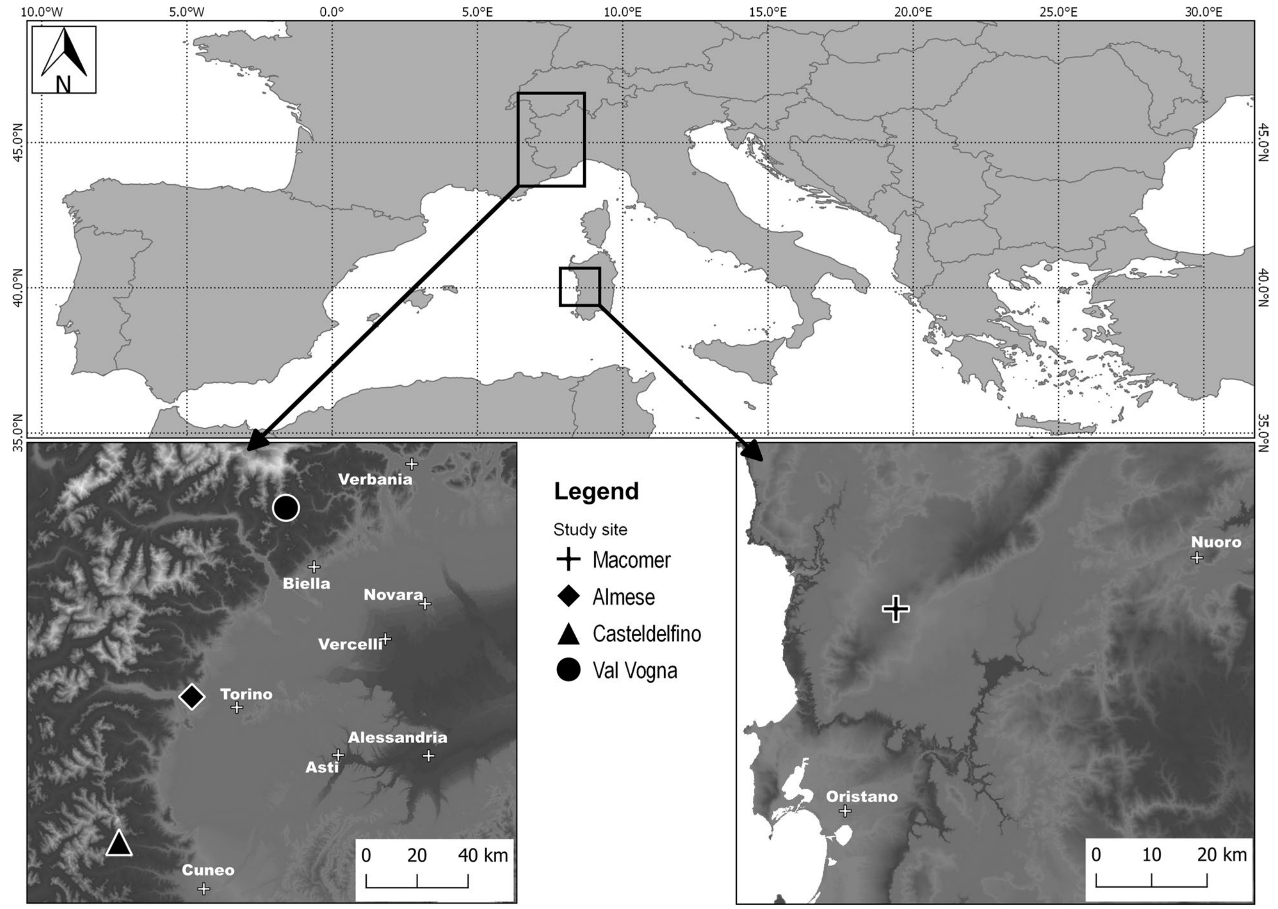


Fig. 1 Map of the four study sites in Italy. Each site is in a specific biogeographic region: Mediterranean (Macomer), Continental (Almese), and Alpine (Casteldelfino and Val Vogna)

Table 1 Coordinates, bedrock, topographic features, biogeographic region, and number of sampling units per each chronosequence stage of the four study sites located in Italy

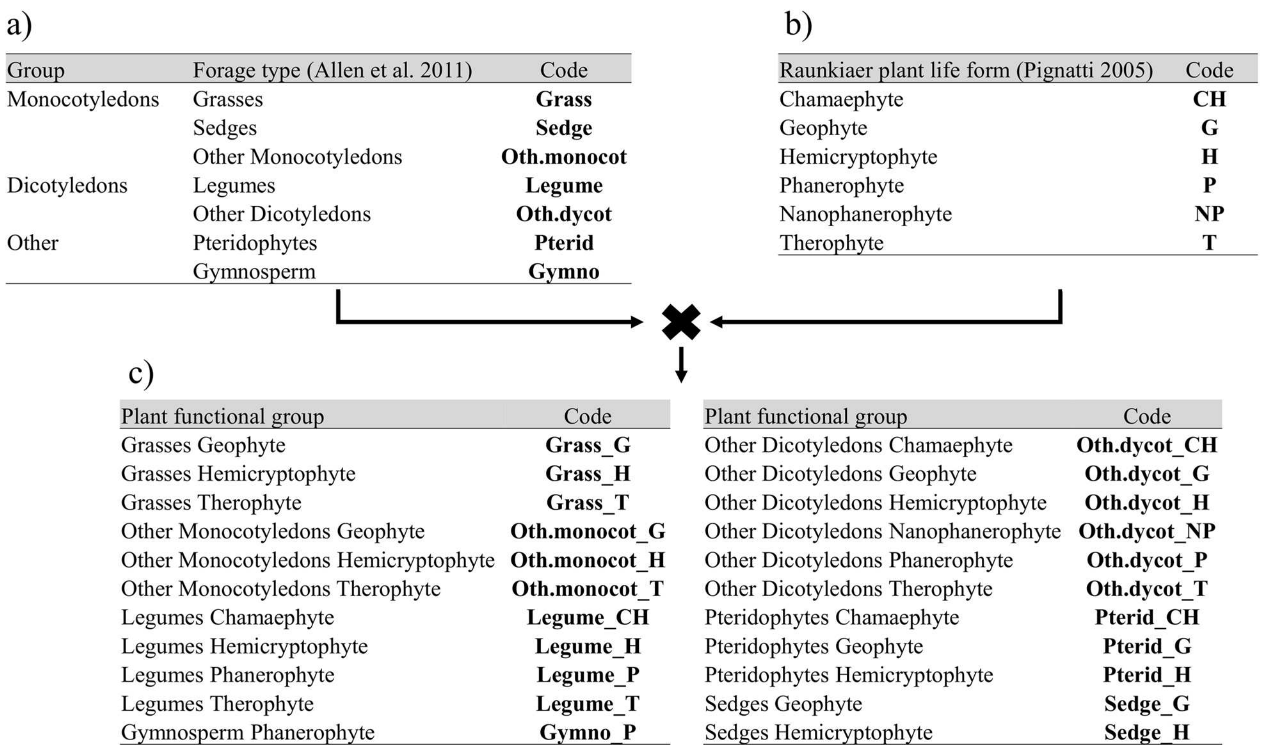
	Study sites			
	Macomer	**Almese**	**Casteldelfino**	**Val Vogna**
Coordinates	40°14′05.9"N, 8°42′08.8"E	45°06′25.2"N, 7°26′32.9"E	44°35′14.0"N, 7°05′39.2"E	45°46′51.5"N, 7°54′02.7"E
Bedrock	Basalt	Serpentinite	Serpentinite	Gneiss
Elevation (m a.s.l.)	670	480	1380	1760
Slope angle (degree)	5	21.2	26.3	18.6
Altitudinal zonation	Colline	Colline	Subalpine	Alpine
Biogeographic region	Mediterranean	Continental	Alpine below the tree line	Alpine above the tree line
Chronosequence stage	Number of sampling units			
1	9	3	3	3
2	9	6	6	6
3	9	3	3	0

occurrence of each plant species. Plot radius varied from 3 to 15 m, depending on the complexity of vegetation mosaic (Braun-Blanquet 1932). Plot size does not often influence patterns of vegetation composition (Otýpková and Chytrý 2006), and the variable sampling effort is considered suitable for analyses of changes that do not involve a comparison of alpha diversity (Chytrý and Otýpková 2003; Di Pietro et al. 2021). At regular intervals (12.5 cm to 50 cm, depending on the radius length), the plant species touching a vertical steel needle were identified and recorded (Perotti et al. 2018). Since the less frequent species are likely missed using this method, a complete list of plant species found within each circular plot was recorded. A schematic representation of the survey methodology is available in Additional file 1. Finally, in a 20 m radius buffer centred on the plot, the tree species percentage cover was detected from a photointerpretation of the most recent orthophotographs available from the Sardinian and Piedmont Region public repositories (Regione Piemonte 2018; Sardegna 2019). Nomenclature follows Pignatti et al. (2017).

The frequency of occurrence of each plant species recorded along the transects of each plot was converted to 100 measurements, i.e. species percentage cover (hereafter '%SC'). A %SC = 0.3 was attributed to all rare species (Pittarello et al. 2019).

Plant species were grouped into functional groups derived from their life form (Raunkiaer 1934) and forage type (Allen et al. 2011). Then, life forms following Pignatti (2005) (Fig. 2b) and forage types (Fig. 2a) were attributed to each plant species and combined to identify 22 plant functional groups (Fig. 2c). The sum of the %SC of the species belonging to each functional group was calculated afterwards for each plot.

Ant sampling was performed at the same time as the vegetation survey. Four pitfall traps (7-cm-diameter, 11.8-cm-deep polypropylene vials), sunk into the soil and partially filled with a solution of water and monopropylene glycol (10/1; v/v), were placed at the corners of a 10-m side square centred on each vegetation plot. A scheme of each ant sampling unit is available in Additional file 1. After one week, all traps were removed and the ants were identified at the laboratory. The abundance of ant workers for each group of four traps (sampling unit) was transformed in presence-absence for subsequent statistical analyses. The specimens were identified to species level (see Additional file 1: S3 for the list of dichotomous keys available in the literature and used in this work), and the nomenclature follows AntWiki (2021). All the ant species were scored for ten traits (Table 2 and Additional file 1: S4), to characterise better the different dimensions of their functional niche (Arnan et al. 2014). The traits were obtained from databases present in the available literature (Seifert 2007; Arnan et al. 2012, 2014, 2017; Retana et al. 2015; Scupola 2018; Boet et al. 2020) and personal observations (see Table 2 and Additional file 1 for full details). In addition to the trait-based approach, we followed the functional group approach to reduce the complexity of ant taxonomy further. Ants were then classified following a global model of ant community dynamics, based on functional groups in relation to environmental stress (factors limiting ant productivity) and disturbance (factors removing ant biomass), proposed by Andersen (1995) for Australian ant communities: Subordinate Camponotini (Cm), Hot-climate Specialists (HCS), Cold-climate Specialists (CCS), Cryptic Species (Cr), Opportunists (Op), and Generalised Myrmicinae (GM). Such a functional group scheme could be



a)

Group	Forage type (Allen et al. 2011)	Code
Monocotyledons	Grasses	**Grass**
	Sedges	**Sedge**
	Other Monocotyledons	**Oth.monocot**
Dicotyledons	Legumes	**Legume**
	Other Dicotyledons	**Oth.dycot**
Other	Pteridophytes	**Pterid**
	Gymnosperm	**Gymno**

b)

Raunkiaer plant life form (Pignatti 2005)	Code
Chamaephyte	**CH**
Geophyte	**G**
Hemicryptophyte	**H**
Phanerophyte	**P**
Nanophanerophyte	**NP**
Therophyte	**T**

c)

Plant functional group	Code	Plant functional group	Code
Grasses Geophyte	**Grass_G**	Other Dicotyledons Chamaephyte	**Oth.dycot_CH**
Grasses Hemicryptophyte	**Grass_H**	Other Dicotyledons Geophyte	**Oth.dycot_G**
Grasses Therophyte	**Grass_T**	Other Dicotyledons Hemicryptophyte	**Oth.dycot_H**
Other Monocotyledons Geophyte	**Oth.monocot_G**	Other Dicotyledons Nanophanerophyte	**Oth.dycot_NP**
Other Monocotyledons Hemicryptophyte	**Oth.monocot_H**	Other Dicotyledons Phanerophyte	**Oth.dycot_P**
Other Monocotyledons Therophyte	**Oth.monocot_T**	Other Dicotyledons Therophyte	**Oth.dycot_T**
Legumes Chamaephyte	**Legume_CH**	Pteridophytes Chamaephyte	**Pterid_CH**
Legumes Hemicryptophyte	**Legume_H**	Pteridophytes Geophyte	**Pterid_G**
Legumes Phanerophyte	**Legume_P**	Pteridophytes Hemicryptophyte	**Pterid_H**
Legumes Therophyte	**Legume_T**	Sedges Geophyte	**Sedge_G**
Gymnosperm Phanerophyte	**Gymno_P**	Sedges Hemicryptophyte	**Sedge_H**

Fig. 2 Identification pattern of the plant functional groups: **a** Forage type (according to Allen et al. 2011), **b** Raunkiaer life forms (according to Pignatti 2005), and **c** Plant functional groups

adapted to European ant assemblages and provide a good understanding of functional community structure and responses to land-use changes at a biogeographical scale.

For each plot, the number of species belonging to each qualitative trait and functional group and the average values of quantitative traits were computed.

Statistical analyses

Plants and ants as indicators

Non-metric multidimensional scaling (NMDS) was performed to examine the variation in the plant and ant community composition at the species level, across sites and chronosequence stage. Bray–Curtis measure was used to obtain all similarity matrices of plant %SC (Wildi 2010). Jaccard similarity index was used for ant presence absence data, as it is most suitable for the similarity calculation based on sparse binary data (Sanders et al. 2003).

Three NMDS analyses on plant and ant functional groups and ant traits were performed to test the hypothesis that community composition (and thus the placement of points on the ordination) is lacking in systematic structure across all study areas. Bray–Curtis similarity matrices were used for ordination analyses of all functional group data. As ant trait data derived from qualitative and quantitative traits, the dissimilarity matrix was computed with a Gower distance on standardised values (Pavoine et al. 2009). Distance matrices and NMDS were computed using *'vegdist'* and *'metaMDS'* functions, respectively, in Vegan R-package (Oksanen et al. 2019) of R ver. 3.6.0 (www.r-project.org).

Three Permutational Multivariate Analyses of Variance (PERMANOVA), each followed by multiple comparisons, were carried out based on 999 permutations using the 'adonis2' function in the Vegan R-package. PERMANOVAs were performed to test for differences among the three stages. Distance matrices and data transformation used in PERMANOVA were the same adopted in the NMDS of each functional group or trait. In addition, multiple comparisons with Bonferroni p-values correction were carried out with the 'pairwise.perm.manova' function of RVAideMemoire R-package (Hervè 2020).

Cross-taxon congruence

Three Mantel tests with 999 permutations and Pearson correlation analyses were carried out on dissimilarity matrices to ascertain the multivariate correlation between ants and plants in terms of composition and functionality. The first Mantel test aimed at explaining the dependence of ants on vascular plant species. Jaccard distance and Bray–Curtis distance were used to compute

Table 2 List and description of the ant traits used in this study to analyse the response of ant communities to pastoral land use abandonment

Trait	Data type	Measure*	Unit	Description
Worker size (WS)	Quantitative	Continuous	mm	Distance from the tip of the mandibles to the tip of the gaster
Worker polymorphism (WP)	Quantitative	Continuous	mm/mm	Mean worker size divided by worker size range
Colony size (CS)	Quantitative	Continuous	ln-transformed	Number of workers per colony
Diet (D)	Qualitative	Ordinal	1	Generalist (Gen)
			2	Mainly liquid feeder (LF)
			3	Predator/liquid feeder (PLF)
			4	Strictly predator (P)
			5	Seed harvester (SH)
Behavioural dominance (BD)	Qualitative	Binary	1	Dominant (Dom)
			2	Subordinate (Sub)
Diurnality (Di)	Qualitative	Binary	1	Not strictly Diurnal (nD)
			2	Strictly Diurnal (D)
Foraging strategy (FS)	Qualitative	Ordinal	1	Strictly group (FSG)
			2	Strictly collective (FSC)
			3	Both group and collective (FSGC)
			4	Strictly individual (FSI)
Number of nests per colony (nN)	Qualitative	Ordinal	1	Monodomy (Md)
			2	Both monodomy and polydomy (MPd)
			3	Polydomy (Pd)
Number of queens (nQ)	Qualitative	Ordinal	1	Monogyny (Mg)
			2	Both monogyny and polygyny (MPg)
			3	Polygyny (Pg)
Colony foundation (CF)	Qualitative	Ordinal	1	Dependent (Fd)
			2	Both dependent and independent (Fdi)
			3	Independent (Fi)

*These traits can be continuous, ordinal or binary, and the data are treated differently before all multivariate analyses

the ant and vascular plants dissimilarity matrices, respectively. The second aimed at finding the relationship between ant traits and plant functional groups. As ant data derived from both qualitative and quantitative traits, the dissimilarity matrix was computed with a Gower distance on standardised values (i.e. the sum within each survey sum up to 1). The dissimilarity matrix for plant functional groups was calculated with Bray–Curtis distance. The third was carried out to test the dependence of ant functional groups on plant functional groups. Both dissimilarity matrices were computed with Bray–Curtis distance. Mantel tests were carried out using the '*mantel*' function in Vegan R-package (Oksanen et al. 2019).

Results

Plants and ants as indicators

Across the four study areas, 417 plant species (Additional file 1: S5) and 50 ant species (Additional file 1: S4) were found. NMDS provided a good ordination of plant and ant assemblages at the species level, as confirmed by low-stress values (0.14 and 0.10, respectively) (Fig. 3).

The four study sites were ordered and well separated along a biogeographic gradient (i.e. MDS1 axis), from the Mediterranean to Alpine. The three stages were well separated within the convex hull characterising each study site (i.e. along the MDS2 axis) from a taxonomic point of view.

The NMDS of plant functional groups proficiently discriminated amongst the three stages, and we also achieved consistent results when considering ant traits and functional groups (stressplot: 0.15, 0.21, and 0.18, respectively) (Fig. 4).

Furthermore, the PERMANOVA results confirmed the NMDS: the pairwise comparisons indicated significant differences amongst the three stages for both taxonomic groups, except for ant functional groups between stage 1 and stage 2 (p-value = 0.08) (Table 3).

Hemicryptophytes (grasses, legumes, and other dicotyledons) and Therophytes (both grasses and legumes) were mainly associated with stage 1 (Fig. 4A). Hemicryptophytes (both grasses and sedges) were associated with stage 2 as well, but a clear correspondence of

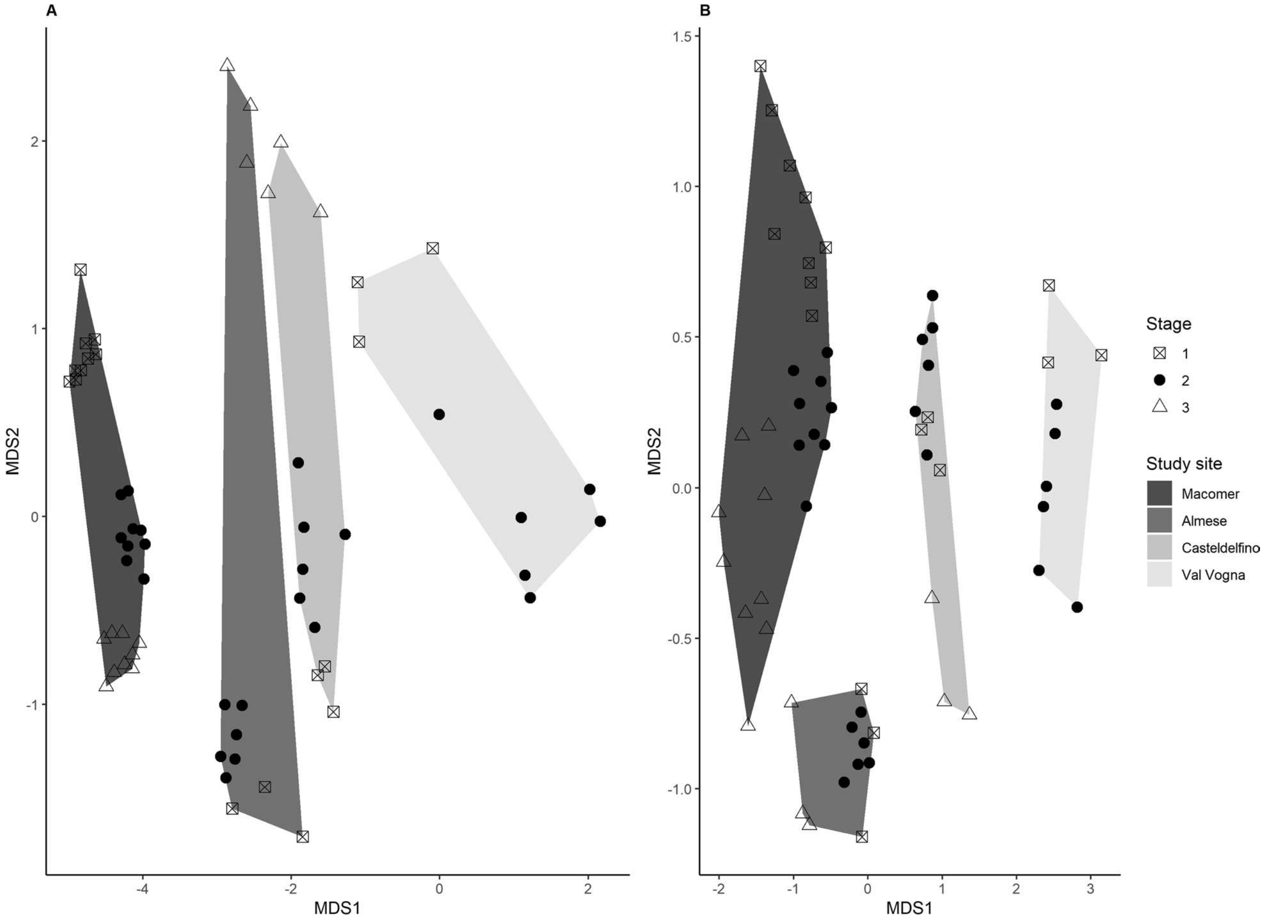


Fig. 3 Non-metric Multidimensional Scaling (NMDS) of plant species percentage cover (**A**) and ant presence-absence (**B**). Sample plot symbols are classified according to the chronosequence stages (1, 2 and 3), whereas convex hulls are coloured based on the study area

Nanophanerophytes (other dicotyledons) was also evident. Phanerophytes were strongly associated with stage 3.

The discrimination of the three stages by ants, both in terms of traits and functional groups, fairly reflected vegetation patterns. Indeed, the sequence of the three stages, from 1 to 3, detectable through the convex hull centroids in the NMDS of vegetation, was observable in the NMDS plots of ant traits and functional groups as well (Fig. 4B). At the level of traits and functional groups for ants, data groupings were fairly well distinguishable

(See figure on next page.)

Fig. 4 NMDS of plant functional groups (**A**), ant traits (**B**), and ant functional groups (**C**). Convex hulls and centroids of the distribution of grouped data Labels codes (centred on scores): **A** Grass_G: Grasses Geophytes; Grass_H: Grasses Hemicryptophytes; Grass_T: Grasses Therophyte; Gymno_P: Gymnosperm Phanerophytes; Legume_CH: Legumes Chamaephyte; Legume_H: Legumes Hemicryptophyte; Legume_P: Legumes Phanerophyte; Legume_T: Legumes Therophyte; Oth.dycot_CH: Other Dicotyledons Chamaephyte; Oth.dycot_G: Other Dicotyledons Geophyte; Oth.dycot_H: Other Dicotyledons Hemicryptophyte; Oth.dycot_NP: Other Dicotyledons Nanophanerophyte; Oth.dycot_P: Other Dicotyledons Phanerophyte; Oth.dycot_T: Other Dicotyledons Therophyte; Oth.monocot_G: Other Monocotyledons Geophyte; Oth.monocot_H: Other Monocotyledons Hemicryptophyte; Oth.monocot_T: Other Monocotyledons Therophyte; Pterid_CH: Pteridophytes Chamaephyte; Pterid_G: Pteridophytes Geophyte; Pterid_H: Pteridophytes Hemicryptophyte; Sedge_G: Sedges Geophyte; Sedge_H: Sedges Hemicryptophyte. **B** D: diurnal; nD: not strictly diurnal; FSG: strictly group foraging strategy; FSC: strictly collective foraging strategy; FSGC: both group and collective foraging strategy; FSI: strictly individual foraging strategy; Mg: monogyny; MPg: both monogyny and polygyny; Pg: polygyny; Md: monodomous; MPd: monodomous and polydomous; Pd: polydomous; Fd: dependent; Fdi: both dependent and independent; Fi: independent; Sub: subordinate; Dom: dominant; CS: colony size; WS: worker size; WP: worker polymorphism; Gen: generalist; LF: mainly liquid feeder; PLF: predators/liquid feeder; P: strictly predator; SH: seed harvester. **C** Op: opportunists; Cr: cryptic species; Cm: subordinate camponotini; GM: generalised myrmicinae; CCS: cold climate specialists; HCS: hot climate specialists

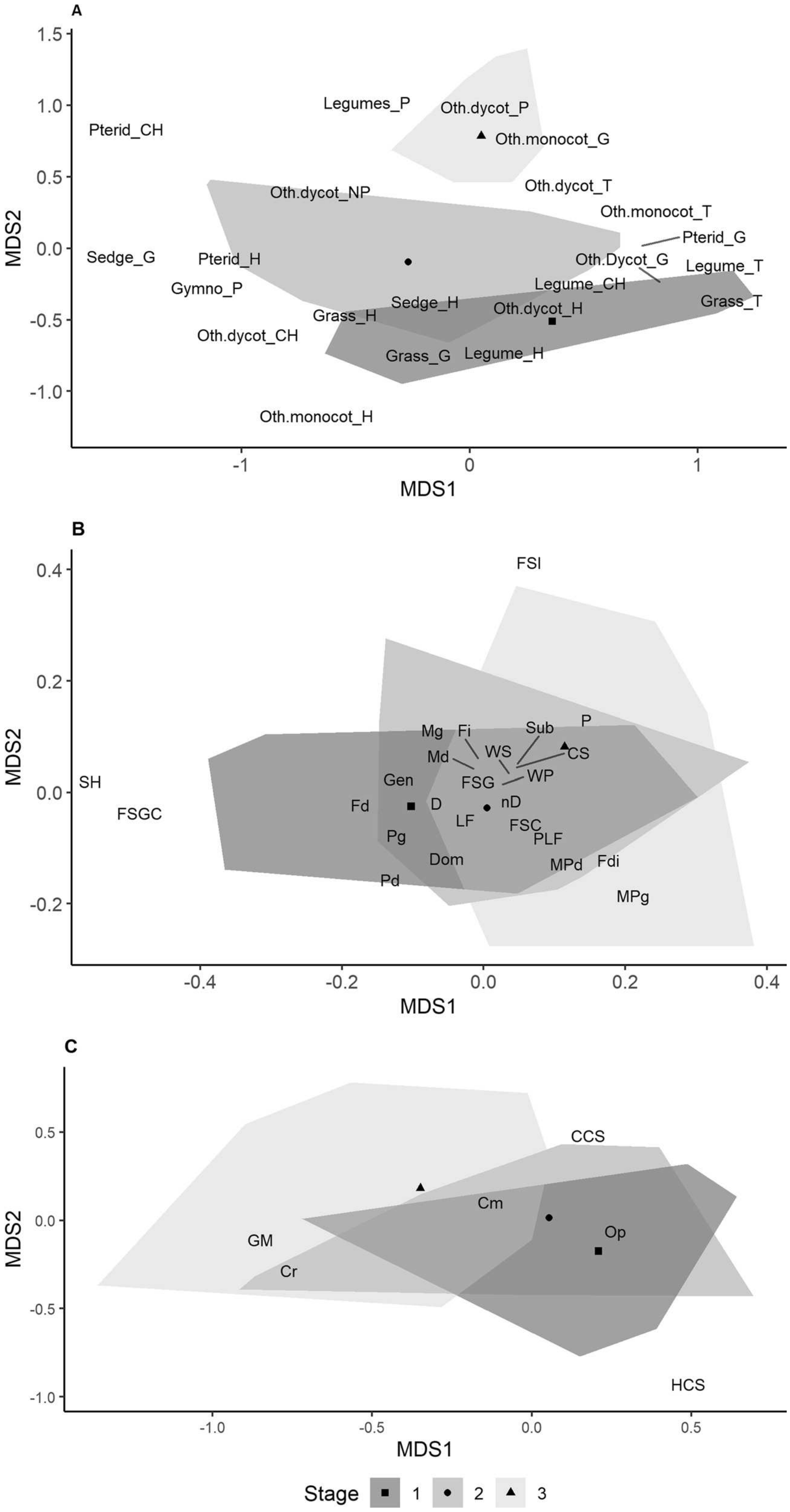


Fig. 4 (See legend on previous page.)

Table 3 Results of PERMANOVA and pairwise comparisons with Bonferroni *P*-value adjustments on the three stages based on plant functional groups, ant traits, and ant functional groups, respectively

	Df	SumOfSqs	R^2	*F*	*Pr* (>*F*)	Stage*		
						1	2	3
Plant functional groups	2	4.97	0.3	15	***	a	b	c
Ant traits	2	0.34	0.2	5.9	***	a	b	c
Ant functional groups	2	1.29	0.2	6.2	***	a	a	b

[*] Data within a row followed by the same letter do not differ at $P \geq 0.05$

within each phase, although the positions of plots in the multivariate space were partially overlapped. Stage 1 was characterised by seed harvester ants with a group and collective foraging strategy. Conversely, the woodlands of stage 3 harboured above all species with an individual foraging strategy. NMDS did not separate stages 1 and 3 from stage 2, which was characterised by an intermediate combination of ant traits. In terms of ant functional groups, the NMDS results showed a distribution of all sampling units similar to that obtained from the trait data (Fig. 4C). Cold-climate Specialists increased from stage 1 to 3, whereas Hot-climate Specialists followed the opposite trend. Stage 2 differed from stage 1 mainly for a higher contribution of Cold Climate Specialists and less importance of Hot Climate Specialists. Stage 3 was characterised by the absence of Hot Climate Specialists and the higher relative contribution of Cryptic Species.

Cross-taxon congruence

In plants and ants, the patterns of species composition revealed by NMDS were characterised by a gradual change along the ordination axes from secondary grasslands over woodlands, i.e. across the chronosequence stages following the pastoral land-use abandonment. The comparison of community similarity matrices performed using the overall dataset showed a strong and significant relationship between the two taxa at the species level (Mantel $r=0.75$, $p<0.001$). However, such a relationship between community structures was moderate at the functional level. Mantel test performed on similarity matrices obtained from ant traits and plant functional groups showed moderate relationships between the two taxa (Mantel $r=0.36$, $p<0.001$). A direct correspondence between ants and vascular plants was also confirmed at the functional group level, although the cross-taxon congruence was weaker (Mantel $r=0.25$, $p<0.001$).

Discussion

Plants and ants as indicators

At the species level, plant and ant communities showed a consistent pattern related to the biogeographic region. Stage 1 was the expression of secondary grasslands with a dominance of herbaceous species maintained by grazing livestock (Kahmen et al. 2002; Diaz et al. 2007). Secondary grasslands showed a strong differentiation, particularly at the opposite biogeographic positions, with different plant species: *Dasypyrum villosum* and *Avena barbata* in the Mediterranean site and *Festuca rubra* subsp. *commutata* and *Poa alpina* in the Alpine area above the treeline. At the two intermediate sites, species of secondary grasslands were mainly from the *Festuco-Brometea* class (Ciaschetti et al. 2020). The dynamic processes of grassland encroachment (stage 2) led to different communities depending on the biogeographical region of location (Bagella and Caria 2011). We found *Pteridium aquilinum* subsp. *aquilinum* in the Mediterranean, *Rubus ulmifolius* in the Continental, *Prunus spinosa* in the Alpine areas below the tree line and *Rhododendron ferrugineum* above the tree line. Woodlands vegetation (stage 3) was characterised by a floristic composition gradually more mesophilous (e.g. from *Quercus ichnusae* to *Fagus sylvatica*).

The ant communities in grasslands (stage 1) were typical of open habitats (Seifert 2007; Lentini and Verdinelli 2012; Scupola 2018; Verdinelli et al. 2017). In the Mediterranean region *Tapinoma simrothi*, *Messor ibericus*, *Aphaenogaster spinosa* commonly occurred in open grasslands, whereas in the Continental region *Pheidole pallidula* and other omnivorous species were frequent. In the Alpine regions, the community was represented by species seeking food both in the herbaceous and arboreal layers (Seifert 2007) and that were also well adapted to mountain area grasslands (Scupola 2018), with *Lasius alienus* and *Formica fusca* below the tree line and the boreal *Formica lemani, Myrmica sulcinodis* and *Manica rubida* above the tree line. At the stage 2 within the Alpine region, temporary parasitic and/or dulotic species (e.g. *F. exsecta* and *F. lugubris*) were sampled. Species commonly found under the trees or in shaded habitats of stage 3, such as *Aphaenogaster ichnusa* and *Myrmecina graminicola*, were shared with stage 2 as probably favoured by the abundance of shrubs and tall grasses (Verdinelli et al. 2017; AntWiki 2021).

In the Mediterranean area, annual legumes and grasses were dominant, as annual species are more tolerant to dry environments due to their fast growth rates and early and prolific seed set (Grime 1974). In Continental and Alpine areas Hemicryptophytes (mainly legumes, grasses, and sedges) are more abundant (Körner 2003) and grazing favoured their persistence (Illa et al. 2006). Stage 2 was defined by plant species typical of an early abandonment process, including tall and/or woody species (Pykälä 2005), which characterise the natural dynamic processes to dwarf shrub- and tree-dominated communities (Kesting et al. 2009). In the Mediterranean site we found tall Pteridophyte Hemicryptophyte species. Instead, in Continental and Alpine sites we found Nanophanerophyte dicotyledons. Stage 3 was entirely separated from stage 1 and stage 2, as the dominant species were Phanerophytes. Indeed, trees were sporadic in stages 1 and 2 as Phanerophytes are generally less frequent in grazing systems (Illa et al. 2006; Komac et al. 2013).

Also, ant traits and functional groups were good indicators of the time stages. Changes in ground cover may be important in determining the number of species able to use disturbed environments (Andersen 2019; Carvalho et al. 2020). These changes can have wide-ranging ecosystem effects given the role of ants as ecosystem engineers and their impact on many ecosystem functions (Folgarait 1998). The direct trophic relationship between ants and plants was clear in stage 1. Indeed, the seed diet, the collective foraging strategy, and other traits are associated with open habitats (Retana et al. 2015). Hot-climate Specialists include thermophilic taxa and specialist seed harvesters (Morton and Davidson 1988), whose occurrence was likely due to the combination of soil type and vegetation cover, which are the main drivers of ant assemblages (Bestelmeyer and Wiens 2001; Ríos-Casanova et al. 2006). Shrub-encroached grasslands offer moderate soil surface temperatures and substrate for potential prey and liquid food (i.e. extrafloral nectar and/or honeydew). These conditions favoured the Cold-climate Specialists and Cryptic Species (small to minute species, predominantly myrmicines and ponerines, that nest and forage primarily within soil, litter, and rotting logs), as a result of the decreasing insolation and increasing moisture (Arnan et al. 2012). The similarities between ant and plant communities' successional pathways are also remarkable in stage 3. Cold-climate Specialists and Cryptic Species increased their relative frequency in woodlands, where the thermal influence of canopy cover is stronger, and the insolation at ground level is minimal (Andersen 1995, 1997; Zina et al. 2021).

Cross-taxon congruence

Even though the plant and ant community composition differed among sites, the two groups responded to pastoral land-use abandonment in a similar way, both at taxonomic and functional levels. This finding was coherent with the general principle that cross-taxon congruence is strengthened at a large scale (Westgate et al. 2014). Although climatic or biogeographic gradients generally favour a conformity of species numbers at large spatial scales, the strong congruence of community similarities between ants and plants was an interesting outcome of the present study. The impact of grazing abandonment on biodiversity has been investigated extensively in different ecosystems worldwide, but the effects on ants and plants have been usually treated separately. In Mediterranean grasslands, past evidence suggests that grazing abandonment harms biodiversity, and this view is mainly based on studies focused on vegetation (Peco et al. 2005, 2006). Mountain grazing abandonment in alpine areas can promote the establishment of vegetation characterised by few species and negatively affect biodiversity (Cislaghi et al. 2019). However, grazing involves different trophic levels (Filazzola et al. 2020), organisms and ecosystems (Maghniaa et al. 2017; Mannu et al. 2018, 2020), and the impact of grazing on biodiversity can be negative or positive. For example, grazing decreased the ant species richness in American and Australian rangelands (Woinarski et al. 2002; Boulton et al. 2005). In temperate rangelands, livestock grazing maintained grassland structure by suppressing woody encroachment and increased ant species richness (Schmidt et al. 2012). In Mediterranean grasslands, grazing abandonment increased the functional and species ant diversity (Azcárate and Peco 2012). In our study, we observed that ant and vascular plant community composition varied in a similar way in response to pastoral land-use abandonment. According to Radnan and Eldridge (2017), grazing in secondary grasslands favoured Hot Climate Specialists. However, the same authors showed that grazing had stronger effects on ants than any increase in shrub cover, as ant and plant communities can respond to pastoral land abandonment in an idiosyncratic way (Német et al. 2016). Although plants are considered poor surrogates of invertebrate biodiversity (Andersen and Majer 2004), our result shows that ants and plants can provide a congruent representation of biodiversity. This finding confirms what has been reported in recent studies involving plant communities, ant communities and other arthropods (Ford et al. 2012; Corcos et al. 2021; Zina et al. 2021). Ant communities might be classified according to structural attributes that parallel those adopted in vegetation science (Andersen 1995), and used as surrogates in biodiversity studies. However, the moderate level of cross-taxon congruence we found at the level of functional groups suggests the need for further investigations to develop new and comprehensive ant metrics suitable for pastoral mountain areas.

Conclusions

Our attempt to compare the community pattern of plants and ants in mountain areas of different biogeographic regions in response to the abandonment of pastoral land use provided encouraging results. Pastoral land use is one of the main driving forces that caused a change of species composition from secondary grasslands to woodlands in the two taxa: the abandonment promoted the woody species colonisation and the conversion of the original grassland ant community. Cross-taxa relationships confirmed this result. Although we did not test congruency within each chronosequence stage or biogeographic region, the analyses of the entire dataset showed that ant communities mirrored the plant species and functional groups at taxonomic and functional levels. The functional groups explain a good proportion of trait variation among species. They allow going beyond the taxonomic barriers and can be a valuable tool in cost-effective ecological surveys. Although the cross-taxon congruence was moderate at the functional group level, the results underline the reliability of ants as bioindicators of land changes and ecosystem functioning. This result is important because it could greatly facilitate biodiversity monitoring and conservation. Due to their role in food webs and ecosystem functioning, ants can provide a more fine-grained and dynamic view of ecosystems than plants. An in-depth study of the main drivers of ant community composition would certainly be a notable improvement in understanding how their structure and function vary in response to land use. However, simplified ant monitoring systems should be developed to meet the needs of land managers. Finally, our findings indicate that the functional group approach and the reduction of the taxonomic complexity could contribute to developing predictive models to detect early environmental changes and biodiversity loss in mountain habitats.

Abbreviations

%SC: Species percentage cover; Grass_G: Grasses Geophytes; Grass_H: Grasses Hemicryptophytes; Grass_T: Grasses Therophyte; Gymno_P: Gymnosperm Phanerophytes; Legume_CH: Legumes Chamaephyte; Legume_H: Legumes Hemicryptophyte; Legume_P: Legumes Phanerophyte; Legume_T: Legumes Therophyte; Oth.dycot_CH: Other Dicotyledons Chamaephyte; Oth.dycot_G: Other Dicotyledons Geophyte; Oth.dycot_H: Other Dicotyledons Hemicryptophyte; Oth.dycot_NP: Other Dicotyledons Nanophanerophyte; Oth.dycot_P: Other Dicotyledons Phanerophyte; Oth.dycot_T: Other Dicotyledons Therophyte; Oth.monocot_G: Other Monocotyledons Geophyte; Oth.monocot_H: Other Monocotyledons Hemicryptophyte; Oth.monocot_T: Other Monocotyledons Therophyte; Pterid_CH: Pteridophytes Chamaephyte; Pterid_G: Pteridophytes Geophyte; Pterid_H: Pteridophytes Hemicryptophyte; Sedge_G: Sedges Geophyte; Sedge_H: Sedges Hemicryptophyte; D: Diurnal; nD: Not strictly Diurnal; FSG: Strictly group foraging strategy; FSC: Strictly collective foraging strategy; FSGC: Both group and collective foraging strategy; FSI: Strictly individual foraging strategy; Mg: Monogyny; MPg: Both monogyny and polygyny; Pg: Polygyny; Md: Monodomous; MPd: Monodomous and polydomous; Pd: Polydomous; Fd: Dependent; Fdi: Both dependent and independent; Fi: Independent; Sub: Subordinate; Dom: Dominant; CS: Colony size; WS: Worker size; WP: Worker polymorphism; Gen: Generalist; LF: Mainly liquid feeder; PLF: Predators/liquid feeder; P: Strictly predator; SH: Seed harvester; Op: Opportunists; Cr: Cryptic species; Cm: Subordinate camponotini; GM: Generalised myrmicinae; CCS: Cold climate specialists; HCS: Hot climate specialists.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-022-00379-9.

Additional file 1. Appendix S1. Schematic representation of surveys on vascular plants through the vertical point-quadrat method along four transects corresponding to four perpendicular radii of a circular plot. **Appendix S2.** Scheme of ant sampling design: four pitfall traps, 10 m distant from each other, were sunk into the soil and centred on each vegetation plot. **Appendix S3.** Ant dichotomous key reference list. **Appendix S4.** Ant species collected during the survey per each stage. Ant functional groups (FG): Subordinate Camponotini (Cm), Hot-climate Specialists (HCS), Cold-climate Specialists (CCS), Cryptic Species (Cr), Opportunists (Op), Specialised Predators (SP), and Generalised Myrmicinae (GM). Ant traits: Worker size (WS), Worker polymorphism (WP), Colony size (CS), Diet (D), Behavioural dominance (BD), Diurnality (Di), Foraging strategy (FS), Number of nests per colony (nN), Number of queens (nQ), Colony foundation (CF). For ant traits in species for which no data are reported in the literature, ten workers per species were randomly selected and measured for determining the WS; the mean thereof was used as a value for each species. **Appendix S5.** List of plant species found across the study sites associated with their Raunkiaer plant life form (Pignatti 2005), Forage type (Allen et al. 2011), Plant functional group, and the occurrence in the stages of the chronosequence. The latter is the combination of the life form and the forage type. Plant species nomenclature follows Pignatti et al. (2017).

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Author contributions

MV: Unit scientific leader, Conceptualization, Data analysis, Investigation, Writing—Original Draft, Writing—Review & Editing, Funding acquisition, Data Curation, Visualisation. MP: Conceptualization, Data analysis, Investigation, Writing—Original Draft, Writing—Review & Editing, Data Curation, Visualisation. MCC: Investigation, Data Curation, Writing—Review & Editing. GP: Investigation, Data Curation, Writing—Review & Editing. PPR: Unit scientific leader, Investigation, Project administration, Funding acquisition—Review & Editing. GMM: Investigation. SA: Investigation, Data Curation. MLF: Investigation. GL: Project principal investigator, Funding acquisition, Writing—Review & Editing. ML: Conceptualization, Methodology, Writing—Review & Editing. GN: Investigation, Data Curation, Writing—Review & Editing. MS: Unit Scientific leader, Funding acquisition, site management. SB: Unit Scientific leader, Conceptualization, Writing—Original Draft, Writing—Investigation, Review & Editing, Project administration, Funding acquisition. All authors read and approved the final manuscript.

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Data availability statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interests.

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Publisher's Note

CHAPTER 3

SaGRA: a first step towards a thematic database of Sardinian grasslands

Abstract

Databases play a fundamental role in various fields. They are essential for organizing, managing, and efficiently using data in various contexts. Vegetation databases are vital tools for understanding the spatial distribution of species, assessing the conservation status of species and habitats, studying how vegetation changes over time, and guiding nature conservation.

A significant number of georeferenced phytosociological surveys was collected in the last decades in Sardinia. Nonetheless, such surveys have not yet been compiled into a single database. With this work, we moved the first steps towards creating a regional Sardinian Grassland database (SaGRA), organising 1277 field surveys. These data, unevenly distributed on the island, provide an in-depth view of vegetation distribution, environmental conditions, and land use. The database contains information on plant species, vegetation structure, management type grazing livestock (e.g., dairy and cattle sheep, beef cattle, or goats) and environmental variables such as altitude, slope, exposure, vegetation series, geological types, land use providing a comprehensive overview of Sardinian grasslands. The total number of species present is 427, with a strong dominance of Therophytes.

Keywords: Environmental variables, Grassland management, Mediterranean grasslands, Phytosociological surveys, Plant biodiversity, Vegetation plots.

1. Introduction

Over the past three decades, ecologists have wisely harnessed the potential of “big data” demonstrating a growing interest in their use, mainly in response to global environmental changes (Franklin et al., 2017). "Big data" includes various data types like digital environmental maps, species records, vegetation plots and species details (Franklin et al., 2017). In plant ecology, using “big data” has proven to be a crucial breakthrough that significantly contributed to understanding plant distribution and dynamics (Sabatini et al., 2021; Kapfer et al., 2017). They have also been employed to address emerging issues such as the severe risk of species loss, habitat degradation, and biodiversity decline (Biurrun et al., 2021). Nowadays, with data being generated at an unprecedented rate, there's a challenge in processing this vast information efficiently to gain meaningful insights. This delay in extracting data and producing knowledge is due to the enormous data volume (Song and Zhu, 2016). Datasets and databases that store and manage information efficiently, enabling efficient storage, retrieval, and manipulation of that data are essential to implement “big data”. Creating a database is not the final goal: we need data that is accessible and useful in the long term (Collins et al., 2016). Yet, building a user-friendly database can be quite challenging. So, when selecting the database type, it is vital to consider factors like ease of setup and upkeep, data access, and data clarity (Medlyn et al., 1999).

Given the pivotal structural and functional role of vegetation in terrestrial ecosystems, vegetation databases are essential tools for vegetation classification, the assessment of the conservation status of species and habitats, providing information on nature conservation and policy formulation, and assessing the spread of alien species (Chytrý et al., 2016; Janssen et al., 2023). These abilities are particularly significant for meeting all the goals set by the major international policies for biodiversity conservation.

Vegetation data come in diverse formats due to varying objectives, leading to format differences (Collins et al., 2016). Ensuring data interoperability and standardizing formats or creating a unified structure is essential (Kattge et al., 2011).

Multiple global initiatives collaborate to coordinate vegetation databases (Bruelheide at al., 2019; Sabatini et al., 2021). These include the Global Index of Vegetation-Plot Database (GIVD), the European Vegetation Archive (EVA), and the sPlot Consortium, creating a unified global database of plant species assemblages. They’ve recently released an open-access version (Dengler et al., 2011).

Europe is significantly ahead of other continents regarding the number of existing vegetation plots and plots preserved in electronic format (Chytrý et al., 2016). The profusion of European vegetation plots in the study by Schaminée et al. (2009) underscores their substantial scientific potential and the critical importance of improving their accessibility. The first national plant database projects were launched in the 1980s in France, the Netherlands, and Switzerland (Chytrý et al., 2016). Currently, the five largest vegetation databases in Europe are the Dutch National Vegetation Database (600,000 plots), the French database (SOPHY, 212,244 plots), the Iberian and Macaronesian Vegetation Information System (125,000 plots), the German Reference Database on Vegetation (111,928 plots), and the Czech National Phytosociological Database (99,586 plots) (Landucci et al., 2012).

In Italy up to 2009, Schaminée et al. (2009) documented 150,000 plots, both published and unpublished, with only about 20,000 in electronic format. Thirteen Italian databases are listed in GIVD (Alessi et al., 2022), but some vegetation databases from Italian institutions are not yet included in the Global Index. Furthermore, a relevant step was represented by establishing the national vegetation database called VegItaly (Venanzoni et al., 2010; 2012; 2014; Landucci et al., 2012). VegItaly includes 34,462 vegetation plots, most of which come from published sources. AMS-VegBank has recently contributed 17,505 vegetation-plot data from 1930 to 2021 for the Italian territory (Alessi et al., 2022).

In addition to these broad-scale initiatives, specialised efforts concentrate on specific environments or biogeographical regions. These are known as thematic databases, such as the GrassPlot database, which focuses explicitly on multi-scale plant diversity in Palaearctic grasslands (Alessi et al., 2022; Dengler et al., 2018).

National databases represent a significant step towards more efficient management and sharing of vegetation data. However, we must address significant challenges that require immediate attention. The difficulty in updating the national database and the lack of even regional-level data unification for vegetation are obstacles we cannot underestimate.

To tackle this, creating a regional database structure accessible at the national level could be a solution, improving access and management of vegetation data for future research and environmental conservation.

There are no vegetation plots from Sardinian grasslands in any database, nor in Dengler et al. (2018) within the GrassPlot database. This underscores the potential value of collecting phytosociological data specific to Sardinian pastures and establishing a specialized database tailored to this habitat type.

Mediterranean grasslands on the island are vital for biodiversity conservation and related ecosystem services (Bagella and Caria, 2011). However, their preservation is threatened due to land-use changes like abandonment, which poses a significant risk to local biodiversity.

Our main goal was to create a database of the Sardinian grassland (SaGra), collecting and standardizing existing vegetation data. We georeferenced the plots in a standard coordinate system (EPSG:4326 - WGS 84) and included various environmental variables such as altitude, exposure, slope, land use, geology, and management practices in the survey information. The database will support current research and establish a solid foundation for future botany and environmental management studies. Transitioning from a fine-scale approach to a regional one will broaden our perspective on Sardinian grasslands, helping us understand relationships between different locations, geographical features, and environmental patterns.

Additionally, our phytosociological database will enable us to study how plant species are distributed across the region in response to temperature and precipitation. This information will be crucial for sustainable environmental management, land planning, and biodiversity conservation.

SaGra will contribute to local biodiversity conservation and the future of botanical research.

2. Material and methods

2.1. The territory

Sardinia is located in the middle of the West Mediterranean basin, between 40° 7' 15.15" N and 9° 0' 46.415" E. It has an area of approximately 24,000 km^2 with a coastline of about 1900 km (Fig. 1).

The territory is mainly mountainous; however, the mountains are not very high, with a maximum elevation of 1834 m and an average elevation of 334 m a.s.l. Relieves are represented by massifs divided by plateaux and plains, which permit recognition of large regions with a relevant interest in land use and historical and cultural aspects of the territory (Mori, 1966). From a geological point of view, the island includes four main units: a Variscan basement characterized by Paleozoic magmatic intrusive and metamorphic complexes; a Permian to Oligocene sedimentary succession related to marine transgression; an Upper Oligocene to Upper Miocene volcano-sedimentary succession and the Plio-pleistocenic basaltic lava flows. Moreover, Quaternary deposits of different origins are also present (Carmignani et al., 2016).

Almost 28% of the island's total surface area presents an association made up of outcropping rock and poorly developed soils, the depth of which does not exceed 10-15 cm. Only 18% of the Sardinian territory is occupied by irrigable soil. In the remaining territory, they are mainly Cambisols, Leptosols and Regosols, concerning the different substrates, morphology and the degree and type of vegetation cover used in forestry and agro-pastoral activities (Pietracaprina, 1980).

Due to its geographic position, the climate is typically Mediterranean, with dry and hot summers and relatively rainy and mild winters. Rainfall ranges from 411 to more than 1215 mm in the inner mountainous regions. The measured mean annual temperature ranges from 11.7 °C to 18.1 °C. Two macroclimates were identified: Mediterranean pluviseasonal oceanic, which covers 99.1% of the total area and Temperate oceanic (Canu et al., 2015).

Sardinia is considered a hotspot for plants in the Mediterranean region (Myers et al., 2000). As reported by Bagella et al. (2020) its flora includes 3338 taxa according to Arrigoni (2006-2015) and 2586 taxa according to Bartolucci et al. (2018). The island's heterogeneous climate, morphology and geological substrata determine a wide variety of Potential Natural Vegetation, mainly represented by *Q. ilex* and *Q. suber* woodlands (Bacchetta et al., 2009). Woodlands are replaced mainly by secondary grasslands whose formation has a long history (Malavasi et al., 2023). These grasslands represent a crucial element of agro-silvopastoral systems and are nowadays strongly threatened by abandonment (Farris et al., 2010; Bagella et al., 2020) (Fig. 2).

Fig. 1 - Representation of the study area - Sardinia (Italy). Map created on QGIS.

Fig. 2 – Sardinian grasslands.

2.2. Workflow for Database compilation

2.2.1. Data collection

The vegetation data were derived from ten different Excel datasets created in the framework of ten projects carried out in Sardinia between 2011 and 2021. Each project had specific objectives but shared the common goal of promoting the sustainable use of grassland resources and biodiversity conservation. A separate data file was available for each project, including the species list and the surveys.

In all the projects, vegetation surveys were performed based on Braun-Blanquet (1951) scale in 2x2 m^2 plots (Fig. 3). The plot dimension was calibrated according to the Manual for monitoring species and habitats (Angelini et al., 2016), which suggests grasslands dominated by annual species 0.5 x 0.5 m^2 to 4 x 4 m^2 plots. Three to five random replications were made within homogenous areas, namely fields. The total vegetation cover was assigned to each plot in % value. The surveys were performed in spring and sometimes repeated in different years. Indications concerning vegetation structure were also reported for each survey.

Plant nomenclature was based on different sources. Each species has associated the family, biological form and chorological type.

For each plot, information, not consistently uniform, were associated: the data collector, geographic coordinates, survey date, project and farmer name, field name, replicate number, type of management and livestock grazing.

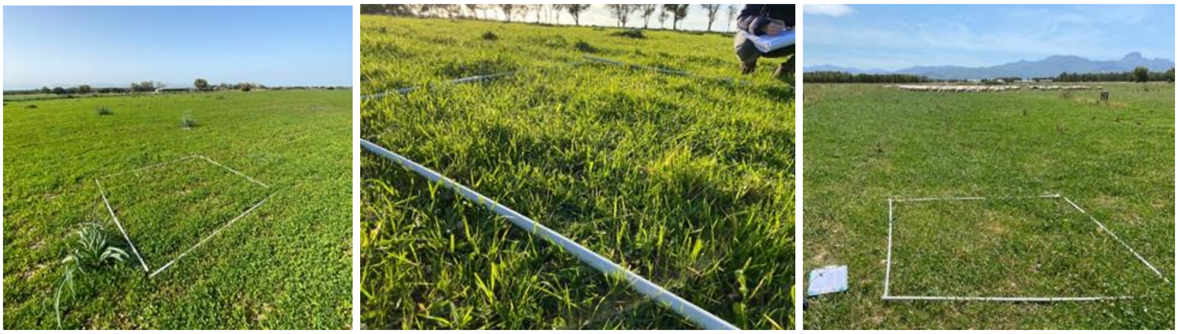
Fig. 3 - The 2 x 2 m^2 plot used for the field surveys.

2.2.2. Data validation and harmonization

To achieve consistency among the ten datasets, we ensured that all Excel files from each project had the same structure, contained the same information, and maintained the same names and row arrangements. Moreover, new fields were added to improve the administrative information, namely country, province and municipality.

Information about vegetation structure was uniform considering the following types: open grasslands, bushed grasslands, wooded grasslands, forbs and clearings. For wooded grasslands, the additional information "position" was added to indicate if the survey was performed below or outside the canopy of trees.

Management information includes the management units and grazing animals. The management units were categorized into annual forage crops, grasslands, meadows and meadow-grasslands. For some of them, the following specific subunits were added. Subunit1: annual forage crops (temporary-alternate); grasslands and meadow-grasslands (temporary-permanent). Subunit2: annual forage crops (grazed-mown grazed); grasslands (grazed-mown grazed). Livestock included the four types: beef cattle, dairy cattle, dairy sheep, goats and mixed.

Plant names were standardized according to the Euro+Med PlantBase nomenclature. Data from different projects were merged into a single database using Turboveg v. 2.135b (Hennekens and Schaminée, 2001).

2.2.3. Data entry

The plots were digitized and georeferenced using QGIS (QGIS 3.26.2 – Geographic Information System software). Georeferencing the quadrats was a crucial phase that required careful attention and time. Most geographic coordinates were originally entered in Excel files in different formats and spatial references, which were later cleaned up and converted into vector files (Esri shapefile – SHP). While some surveys had exact coordinates, only the indication of its spatial location within the area was available for others. Consequently, it was necessary to accurately estimate the precise positions of the quadrats within the fields in collaboration with those who conducted the initial surveys. All coordinates were then transformed into a standard coordinate system: World Geodetic System 1984 (WGS 84 - EPSG:4326).

Once the coordinates for all surveys were organized for each project, separate SHP files were generated, which were later merged into a single SHP file. Subsequently, the variables were processed, and all obtained information was loaded into QGIS.

Standardizing the attribute tables in QGIS was also crucial to ensure they contained all the information in the Excel files, providing consistent correspondence between them. Each plot was identified and indexed by an alphanumeric code comprising the project abbreviation, a progressive number, and all derived variables.

2.2.4. Environmental context

The datasets were integrated by adding information derived from the intersection of open-source or specific maps with the SHP file of vegetation plots.

Altitude, slope and aspects were derived from open-source data Copernicus images Sardinia (Copernicus Land Monitoring Service – EU-DEM v1.1- https://land.copernicus.eu/imagery-in-situ/eu-dem/eu-dem-v1.1?tab=metadata). Specifically, the Digital Elevation Model (DTM) of Sardinia with a resolution of 25 m and vertical accuracy of +/- 7 m RMSE was used.

Additional information was obtained overlapping the SHP file the map of the vegetation series of Sardinia (Bacchetta et al., 2009), the bioclimatic map of Sardinia (Canu et al., 2015), the geological map of Sardinia and the land use map of Sardinia up to 5 levels (Sardegna Geoportale https://www.sardegnageoportale.it/).

3. Results: the SaGra database

3.1. Plot number and distribution

A single georeferenced regional database with 685 vegetation plots, mainly located in the central-western and northeastern parts of the island, was obtained. A total of 1277 vegetation surveys have been compiled (Fig. 4).

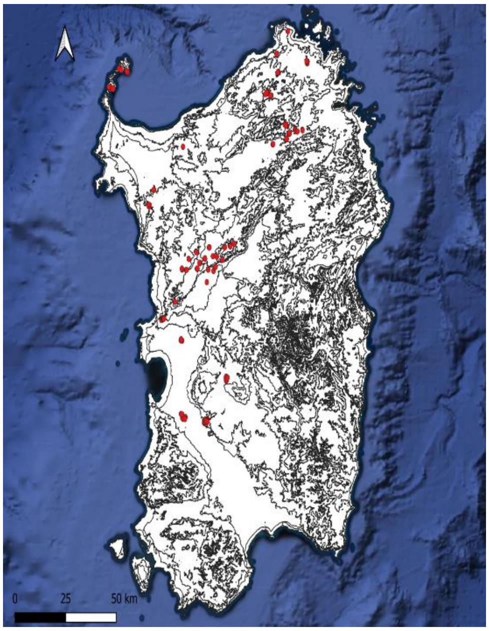

Fig. 4 - Map of Sardinia with the 685 georeferenced plots (red dots).

3.2. Floristic composition and vegetation structure

Overall, 427 species were listed in the database. Fabaceae represented the most significant family, with 76 species, followed by Poaceae and Asteraceae (Fig. 5).

Therophytes were much more abundant than perennial species (Fig. 6). Stenomediterranean species (130) were the most abundant in the chorotypes, followed by Eurimediterranean (127). Four endemic species were recorded: *Glechoma sardoa*, *Pancratium illyricum*, *Romulea requienii* and *Saxifraga corsica* (Fig. 7).

Open grasslands represented the dominant vegetation structure, followed by wooded grasslands. The other types were represented by very few plots (Fig. 8).

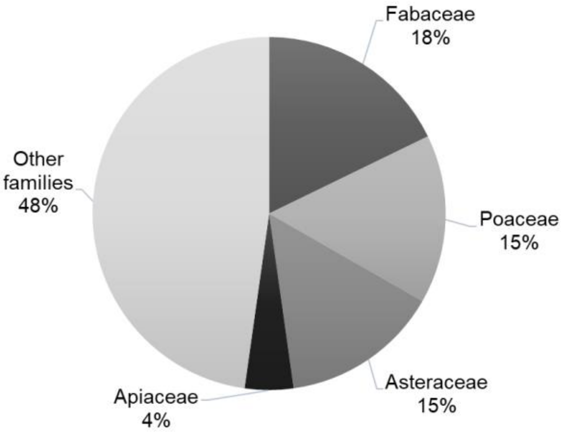

Fig. 5 - Family spectrum

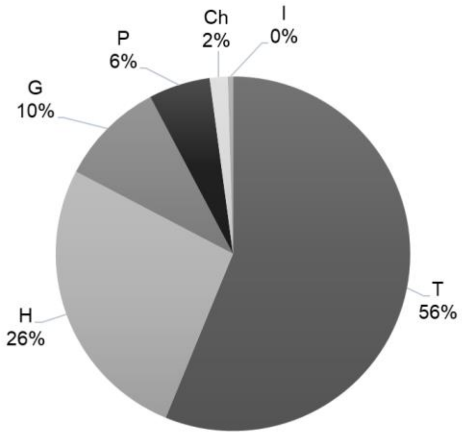

Fig. 6 - Biological spectrum

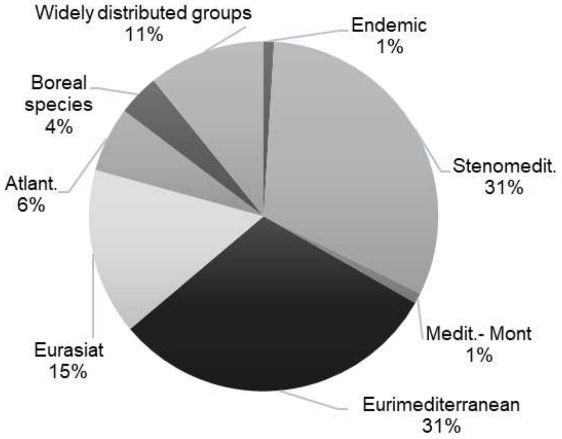

Fig. 7 – Chorological spectrum

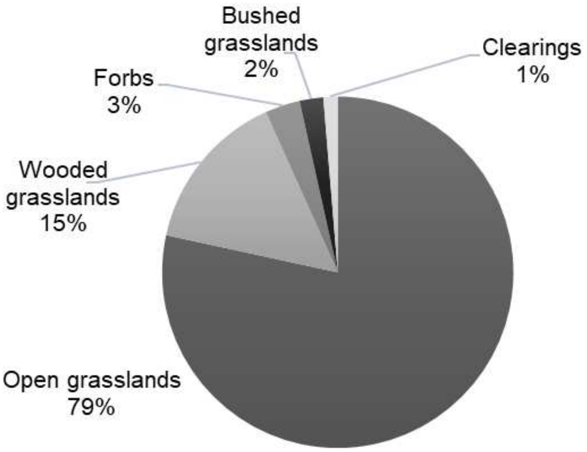

Fig. 8 – Vegetation structures

3.3. Management types

The predominant management units were grasslands followed by annual forage crops (Fig. 9). As for livestock, beef cattle and dairy sheep were nearly equally represented (Fig. 10).

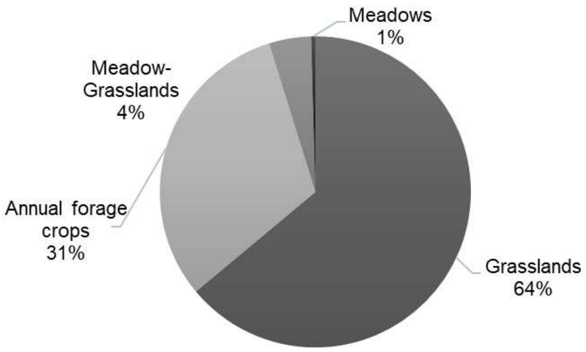

Fig. 9 – Management units

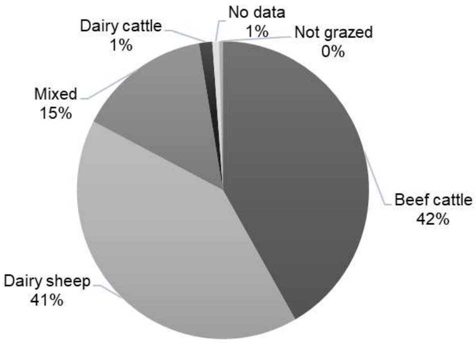

Fig. 10 – Grazing livestock

3.4. Environmental context

The plots were distributed across a wide range of altitudes, ranging from 3 to 995 m a.s.l. Most were located in the hilly plane on flat or gently sloping terrain with an inclination of less than 10°. In terms of exposure, the northwest-oriented (NW) quadrant prevailed.

Regarding isobioclimates, the plots felt into 12 different types. The most numerous were those that fell in the Lower Mesomediterranean, mainly Lower Mesomediterranean, lower subhumid, weak euoceanic (Table 1).

Type of isobioclimate	***Number of plots***
Lower Mesomediterranean, Lower Subhumid, Weak Euoceanic	**244**
Upper Thermomediterranean, Upper Dry, Weak Euoceanic	**126**
Lower Mesomediterranean, Upper Dry, Weak Euoceanic	104
Upper Mesomediterranean, Upper Subhumid, Weak Euoceanic	71
Upper Thermomediterranean, Lower Dry, Weak Euoceanic	51
Upper Thermomediterranean, Upper Dry, Strong Euoceanic	31
Upper Mesomediterranean, Lower Subhumid, Weak Semicontinental	16
Upper Mesomediterranean, Upper Subhumid, Weak Semicontinental	16
Upper Mesomediterranean, Lower Humid, Weak Semicontinental	16
Other categories	10

Tab. 1- Distribution of the plots with respect to the isobioclimatic types.

Geologically, the plots are located mainly on granitic (Gallura granitoid and Goceano-Bittese Granitoid complex) and secondarily volcanic substrates (Table 2).

Geology	***Number of plots***
Gallura Granitoid Complex	**138**
Goceano-Bittese Granitoid Complex	**90**
Plateau Basalts	74
Monte Arci Volcanic Complex	68
Alluvial Sediments	67
Gravitationally Bound Sediments	56
Capo Marargiu Volcanic District	35
Oligo-Miocene Sedimentary Succession of Campidano-Sulcis	30
Bonorva Volcanic District	25
Plio-Pleistocene Sedimentary Succession	21
Pleistocene Deposits of the Continental Area	17
Other categories	64

Tab. 2 - Distribution of the plots in respect to the geological complexes.

The plots were distributed within thirteen different vegetation series, mainly in the 'Sardinian, calcifuge, thermo-mesomediterranean cork oak series (*Galio scabri*-*Quercetum suberis*)' and in the 'Sardinian, calcifuge, mesomediterranean cork oak series (*Violo dehnhardtii-Quercetum suberis*)' (Table 3).

Vegetation series	***Number of plots***
Sardinian, calcifuge, thermo-mesomediterranean cork oak series (*Galio scabri-Quercetum suberis*)	**224**
Sardinian, calcifuge, mesomediterranean cork oak series (*Violo dehnhardtii-Quercetum suberis*)	**132**
Sardinian, thermomediterranean olive tree series (*Asparagus albi-Oleetum sylvestris*)	73
Sardinian, thermo-mesomediterranean holm oak series (*Prasio majoris-Quercetum ilicis quercetosum ilicis* and *phillyreetosum angustifoliae*)	66
Sardinian-Corsican, calcifuge meso-supramediterranean holm oak series (*Galio scabri-Quercetum ilicis*)	39
Sardinian, calcicolous thermo-mesomediterranean Virgil's oak series (*Lonicero implexae-Quercetum virgilianae*)	30
Sardinian, neutral- acidophilous, mesomediterranean of the Sardinian oak series (*Ornithogalo pyrenaici-Quercetum ichnusae*)	30
Sardinian, calcifuge, meso-supratemperate in submediterranean variation of the holm oak series(*Saniculo europaeae-Quercetum ilicis*)	22
Sardinian, neutral- acidophilous, meso-supratemperate in submediterranean variation of the twisted oak series (*Glechomo sardoae-Quercetum congestae*)	20
Sardinian, thermomediterranean holm oak series (*Pyro amygdaliformis-Quercetum ilicis*)	19
Western Mediterranean geosigmet, edaphohygrophilous and/or planizial, eutrophic (*Populenion albae* , *Fraxino angustifoliae -Ulmenion minoris* , *Salicion albae*)	18
Other categories	12

Tab. 3- Distribution of the plots with respect to the vegetation series.

In the context of the Corine land use classification with five levels of detail, the most commonly observed land use type was represented by 'Non-irrigated arable land', followed by 'Artificial meadows' (Table 4).

Type of land use	*Number of plots*
Non-Irrigated Arable Land	**187**
Artificial Meadows	**160**
Temporary Crops Associated with Permanent Crops	63
Garigue	60
Cork Oak Groves	49
Simple Arable Land and Open-Field Horticultural Crops	38
Natural Grazing Areas	30
Agroforestry Areas	22
Shrublands and Bushlands	22
Mediterranean Scrub	16
Other categories	38

Tab. 4 - Distribution of the plots with respect to categories of land use types.

4. Discussions

In this study, we have initiated establishing a regional Grassland Database for Sardinia (SaGRA). Our methodological approach involved collecting vegetation data from ten distinct projects between 2011 and 2021. The compilation of this database was the outcome of rigorous data collection efforts and a meticulous process of data organization, digitalization and structuring. As a result, we compile a georeferenced regional database that includes 685 plots, predominantly located in the central-western and northeastern parts of the island, along with 1277 vegetation surveys.

The floristic list is representative of the grasslands of Sardinia, with a clear dominance of annual species and the Mediterranean chorological element. The prevalence of therophytes reflects the adaptation of these species to seasonal precipitation patterns and their tolerance to disturbances, indicating the type of secondary grassland present in the Mediterranean basin (Tárrega et al., 2009; Fernández-Moya et al., 2011).

Although the plots are not evenly distributed throughout the territory, as they are derived from projects that took place in specific areas of Sardinia, they are located in areas representative of different environmental situations and characterized by different types of management. Environmental variables such as altitude, exposure, and slope will be useful to explore plant distribution and abundance along environmental gradients. Additionally, vegetational series, geological types, bioclimate and land use will contribute to relating plant biodiversity with ecological diversity. On the other hand, punctual knowledge of the type of turf management and grazing animals will be useful in looking for relationships between floristic composition and use patterns. Replicating surveys in the some plots throughout the years should be useful to monitor the interannual floristic variation.

Critical issues should be arise by the disparity in the number of plots sampled in different areas, the absence of surveys conducted in the southern part of the island, and the data collection carried out without following a random sampling approach but rather opportunistically based on project areas. Nevertheless, the availability of this georeferenced database represents a fundamental step for promoting scientific research and the sustainable management of Sardinian grasslands. The data provide an overview of the vegetation, ecology, and environmental variables that characterize this habitat, providing the foundation for further investigations. This tool will not only aid in identifying areas of particular conservation interest but will also enable dynamic monitoring of environmental changes over time. Furthermore, the collected data will be processed to define spatial models of grassland diversity in Sardinia and made available through vegetation databases for broader-scale analyses. We hope this work can serve as a starting point for further research and significantly contribute to understanding and preserving Sardinia's plant biodiversity.

The future steps for the database involve creating a regional structure accessible at the national level to enhance data management and accessibility. The database will be operational for users, hosted on a web platform, providing documentation and support. This initiative opens up new opportunities for developing a database dedicated to Sardinian grasslands, which can be integrated into operational contexts like the VegItaly database.

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General conclusion

The PhD thesis aimed to contribute to increasing knowledge on Mediterranean grasslands in Sardinia, Italy. It examines the environmental aspects, management, and biotic interactions within these habitats in a historical and cultural context where they play a significant role.

Our research has contributed to the expansion of knowledge concerning these grasslands, by examining their responses to environmental changes and human activities, while also providing tools for their management and conservation.

We conducted research on the inter-annual variability of floristic composition with a focus on precipitation using a five-year dataset. It would be highly advantageous to expand our data collection over a longer time period to conduct more in-depth analyses. Extended time intervals are of crucial importance in the context of assessing potential scenarios related to climate change.

Cross-taxon research, such as that conducted on vascular plants and ants, has proven to be of great value as it can lead to the assessment of synthetic indices. These indices (e.g., pastoral value, ecological value) are valuable tools for assessing different habitats as a whole.

Finally, the SaGRA project provided an overview of Sardinian grasslands. We hope that the project will evolve into a dynamic and collaborative database, which will continue to integrate suitable future datasets to promote scientific research, environmental conservation, and knowledge dissemination.