

NEW METHODOLOGICAL INSIGHTS FOR THE ASSESSMENT OF TEMPORAL CHANGES IN SEMI-NATURAL DRY GRASSLANDS PLANT SPECIES COMPOSITION BASED ON FIELD DATA FROM THE NORTHERN APENNINES

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Abstract

In Europe, the management of semi-natural grasslands is changing steadily in extent and intensity. These changes represent a serious threat to one of the most species-rich habitats in Europe; therefore, the related shifts in species composition should be assessed by means of sound scientific.

We describe a new methodology aimed at: (i) quantifying the temporal changes in vascular plant species composition of semi-natural grasslands; (ii) identifying the environmental and management variables that drive patterns of change. Our approach consists of a diachronic analysis based on historical phytosociological data associated with detailed vegetation maps. To describe and test our methodology, in 2013 we re-visited 24 historical plots of *Bromus erectus* grasslands that were first sampled in 1982. To designate the new sampling sites, we combined the topographic and typological information available for the historical plots with the spatial information of the associated map. The degree of change in species composition was calculated through ordination techniques; multivariate distances were used in a regression analysis to identify the environmental variables responsible for patterns of compositional change. Our worked example demonstrated that species composition has changed significantly in the last 30 years, with patterns that have been substantially influenced by topography and landscape structure.

Keywords: diagnostic species, Habitats Directive, landscape metrics, orchid species, regression trees, re-visitation study, vegetation dynamics.

Izveleček

Gospodarjenje s pol naravnimi travišči se v Evropi spreminja po obsegu in po intenzivnosti. Spremembe predstavljajo resno grožnjo enim vrstno najbolj bogatih habitatov v Evropi, zato je potrebno ocenjevati spremembe v vrstni sestavi z znanstveno metodo. Opisujemo novo metodologijo, ki stremi h (i) kvantifikaciji časovnih sprememb v vrstni sestavi pol naravnih travišč ter (ii) opredelitvi dejavnikov okolja in gospodarjenja, ki vodijo v spremembe. Naš pristop temelji na diakronični analizi zgodovinskih fitocenoloških podatkov v povezavi z natančnimi vegetacijskimi kartami. Za opis in preverjanje metodologije smo leta 2013 ponovno obiskali 24 starejših ploskev travišč z vrsto *Bromus erectus*, mi smo jih prvič vzorčili leta 1982. Za izbiro novih vzorčnih mest smo kombinirali topografske in tipološke informacije, ki so na voljo za starejše ploskve, s prostorskimi informacijami na povezani karti. Stopnjo sprememb vrstne sestave smo izračunali z ordinacijskimi metodami; uporabili smo multivariatne razdalje v regresijski analizi za opredelitev okoljskih dejavnikov odgovornih za vzorec sprememb vrstne sestave. Z našim primerom smo pokazali, da se je vrstna sestava v zadnjih 30 letih značilno spremenila, na vzorec sprememb pa sta bistveno vplivala topografija in krajinska sestava.

Ključne besede: diagnostične vrste, diagnostic species, Habitatsna direktiva, krajinska metrika, orhideje, regresijska drevesa, študija s ponovnim obiskom, dinamika vegetacije.

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1. INTRODUCTION

Semi-natural habitats represent a noteworthy example of human-environmental interactions, because in these ecosystems human activities, like livestock grazing and haymaking, support a unique set of species originating from a range of natural habitats (Pärtel et al. 2005). Compared with other European vegetation types, semi-natural grasslands host an extremely high plant diversity at multiple levels, from intra-specific genetic variability (Van Treuren et al. 2005) to species richness (Wilson et al. 2012) and landscape diversity (Lindemann-Matthies et al. 2010). Species density is particularly high in calcareous grasslands, which may host as many as 80 vascular plant species per square meter (WallisDeVries et al. 2002). Moreover, semi-natural grasslands host several threatened and rare birds (Calaciura & Spinelli 2008) as well as numerous species of insects (Labadessa et al. 2015). For these reasons, secondary grasslands were classified as habitat type “34. Steppes and dry calcareous grasslands” in PHYSSYS classification of Palearctic Habitats (http://www.kbinirsnb.be/cb/databases/cb_db_physis_eng.htm). Later, several European semi-natural grasslands were included in the Annex I of the European Habitat Directive (92/43/EEC), many of which are considered fully dependent on agricultural management (Halada et al. 2011, Farris et al. 2013).

Nowadays, most of the agricultural practices that maintain these grasslands are no longer economically profitable. Indeed, since many of these habitats occur in areas that are not easily accessible and do not provide favorable local conditions for high productivity livestock farming, the number of farmers and farms has decreased markedly (MacDonald et al. 2000). Changes in socio-economic constraints and the increasing costs of agricultural management in remote areas represent a major threat to grassland ecosystems, especially in mountain areas (Falcucci et al. 2007, Tasser et al. 2007, Niedrist et al. 2009).

The cessation of management in semi-natural grassland systems activates processes of vegetation dynamics that consist of changes in species composition towards mid-successional communities (Vassilev et al. 2011), often leading to the colonization of shrubs and trees (Hansson & Fogelfors 2000, Poschlod et al. 2005, Hegedúšová & Senko 2011). In Italy, these successional processes have been observed above all in the Apennine

chain, where vegetation of mountainous and hilly areas has shifted towards forests, and pasture and grassland cover almost halved from 1960 to 2000 (Falcucci et al. 2007). The assessment of temporal changes in vegetation communities within such a dynamic context would therefore be extremely useful, especially if it leads to the identification of the conditions that determine the patterns of those changes.

Management and environmental factors have a major influence on vascular plant species richness and composition in managed grasslands (Klimek et al. 2007, Pal et al. 2013), as it was also demonstrated in the Apennine region (Blasi et al. 2009, Burrascano et al. 2013). Indeed, species composition is extremely responsive to management type and intensity, especially within regions that have been subjected to traditional livestock management for a long time (Sebastià et al. 2008). Similarly, environmental conditions (e.g. soil properties) may represent relevant constraints to the colonization by woody species (Pärtel & Helm 2007, Wilson 1998).

The knowledge of the processes underlying past changes in species composition is particularly valuable since it would help to predict future trends in species composition within vegetation communities, and in turn to develop adequate land management and conservation strategies (Lomba et al. 2013). Hence, in studies focused on understanding patterns of vegetation changes, information on current and past management, as well as environmental and landscape variables should be collected together with vegetation data (de Bello et al. 2010).

Vegetation data from different points in time can be obtained in three different ways: i) by using permanent plots; ii) from large vegetation databases; and iii) by re-visiting historical plots (Chytrý et al. 2014). Although the first approach is the most reliable, few permanent plots are available and their distribution is frequently biased towards sites where vegetation change was expected to occur (Bekker et al. 2007). The use of existing large vegetation databases is extremely advantageous (Jandt et al. 2011) since it does not require new fieldwork; however, it may yield incorrect results owing to differences in the locations of the sampling plots as well as in the various sampling strategies adopted over time (Chytrý et al. 2014). The main problem linked to the re-visitation of historical plots arises from the approximated, or even undefined, location of the historical plots,

which may result in the assessment of a pseudo-turnover, i.e. change in species composition due to the shift in plot location rather than to a change in time (Fischer & Stöcklin 1997).

However, after analyzing the pros and cons of these three approaches, Chytrý et al. (2014) came to the conclusion that the best strategy to quantify and assess changes that have occurred in the past is the re-visitation of historical plots, especially if they are marked in the field or are associated with fine-scale maps. Indeed, the effectiveness of re-visiting historical plots in different habitat types has been demonstrated by several case studies that relied on the location of the historical plots, which were often hand-marked on topographic maps (Fischer & Stöcklin 1997, von Numers & Korvenpää 2007, Baeten et al. 2010, Jírová et al. 2012, Saar et al. 2012, Catorci et al. 2013, Johnson et al. 2014).

The aim of our study was to develop a full methodological proposal for the assessment of compositional changes in semi-natural dry grasslands based on the re-visitation of historical vegetation plots whose exact location is unknown, a situation that applies to the vast majority of this type of data. In particular, we attempted to over-

come issues related both to the pseudo-turnover and to the lack of ecological data associated with historical plots. To describe and test the methodological novelties of our strategy we provide a worked example based on historical phytosociological data of semi-natural grasslands from central Italy that were resampled after more than 30 years. We provide a step-by-step explanation so that the same strategy may be applied to future analyses of temporal trends in semi-natural dry grasslands. Our underlying hypothesis was that a significant change in species composition occurred in the period between the two vegetation samplings, and that it followed patterns driven by environmental and management conditions.

2. METHODS

In the following paragraphs, we will describe, step-by-step, the methodology we propose for both the assessment of change, and the identification of the drivers of the patterns of change, through the definition of three main methodological steps: 1. Recovery of the historical dataset; 2. Designation of the sampling sites; 3. Diachronic analysis of the data (Figure 1). We will

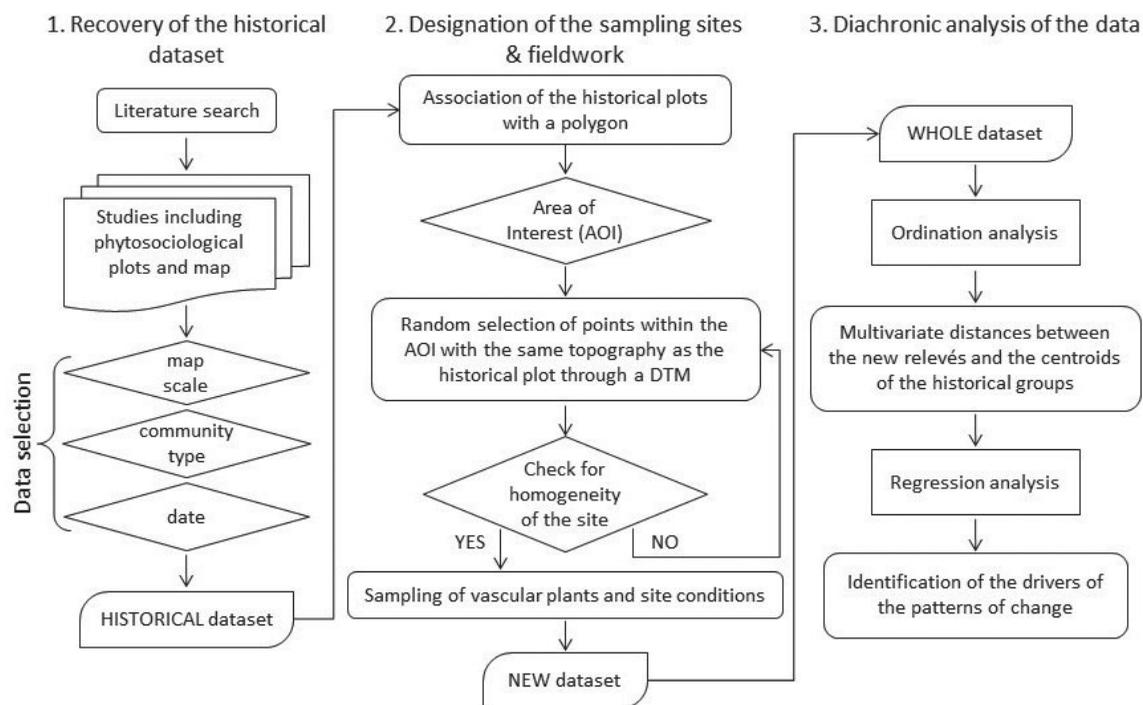


Figure 1: Flow chart summarizing the proposed methodology. The entire procedure is divided in three main phases: i) recovery of the historical dataset; ii) designation of the sampling sites and fieldwork; iii) diachronic analysis of the data.

Slika 1: Diagram poteka, ki prikazuje predlagano metodologijo. Celoten potek je razdeljen v tri faze: i) pridobitev starejšega niza podatkov; ii) določitev vzorčnih mest in terenskega dela; iii) diakronična analiza podatkov.

only marginally provide insights on the field sampling since it has to closely fit both the features of the communities investigated and the strategy adopted in the historical sampling. Lastly, we will describe a practical example for the proposed methodology.

2.1 RECOVERY OF THE HISTORICAL DATASET

2.1.1 Literature search

We decided to base our approach on phytosociological data since: i) they represent the vast majority of the historical vegetation data in Europe (Schaminée et al. 2009, Dengler et al. 2011), ii) the phytosociological classification has often been used in detailed vegetation maps, iii) the habitats listed in Annex I of the Habitat Directive are defined on the basis of the phytosociological classification.

The literature search can largely be conducted using vegetation databases, several of which are available online, such as the database of phytosociological and syntaxonomic references of Italy (LISY; <http://www.scienzadellavegetazione.it/siv/lisy/index.jsp>) and the “Sistema de Informacion de la Vegetacion Iberica y Macaronesica” (SIVIM; <http://www.sivim.info/sivi/>). Additional hints on existing publications may be provided by local scientists, to whom the process of data selection for each specific study should be fully explained (see following paragraph).

2.1.2 Data selection by date, map scale and community type

Since the aim of the re-visitation approach is to assess changes in plant communities, the historical plots have to date back far enough for any changes to be detected. This time span may differ widely depending on both the vegetation type and the topographic and microclimatic conditions. For instance, in Sweden it has been shown that 15 years may be long enough for a closed deciduous forest to replace semi-natural grassland communities (Hansson & Fogelfors 2000), whereas in central Italy, it may take 23 years for a secondary succession to take place (Bracchetti et al. 2012); and slower successional dynamics were observed in Bulgaria, where 20 years of abandonment did not result in an increase in tree

and shrub species, but merely in a shift in herb species composition (Vassilev et al. 2011). In our research, we were interested in any changes in vegetation, either in herb species composition or in major successional changes, and, based on the results obtained in southern Europe (Vassilev et al. 2011, Bracchetti et al. 2012), we only considered historical data collected at least 20 years ago.

In order to reduce the possibility of inaccuracy in the designation of the sampling sites, we only used historical plots associated with fine-scale vegetation maps according to Chytrý et al. (2014) and Wesche et al. (2012). Indeed, these maps provide important spatial information (e.g. the map polygons referred to plant associations) that should be used in conjunction with the topographic information in the phytosociological tables to designate the new sampling sites. Deciding to use a trade-off between the availability of fine-scale vegetation maps associated with the historical vegetation plots and the accuracy of spatial information included in such maps, we considered only those datasets made up of vegetation associated with maps with a scale of up to 1:25,000.

To standardize the general environmental conditions, it is important to focus on a specific community type (Vanderwalle et al. 2014), for which a single sampling protocol may be adopted. We chose to apply the re-visitation of historical plots approach on semi-natural grasslands listed in the Habitat Directive. In particular, we focused only on vegetation communities that could be referred to Habitat 6210(*) “Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (*important orchid sites)”, following the national interpretation manual of Annex I Habitats (Biondi et al. 2012). In order to do so, we searched for grassland communities that have *Bromus erectus* as dominant or co-dominant species, and that are included in the endemic alliance of the Apennines *Phleo ambigu-Bromion erecti* (Biondi et al. 2005). These communities are generally not mowed, but only subjected to extensive grazing.

2.2 DESIGNATION OF THE SAMPLING SITES

The spatial information included in the vegetation map, together with the geographic and topographic data reported in the phytosociological table, substantially reduces the likelihood of

spatial inaccuracy during the designation of the new sites (Chytrý et al. 2014). Indeed, thanks to the information provided on the vegetation unit (*syntaxon*), and on the locality and topography of the historical plots, it is possible to confidently associate each historical plot with an individual map polygon, or with part of a polygon, of the same vegetation unit thereby identifying an Area of Interest (AOI) where the new sampling sites will be designated.

Once this association is established, we randomly identified a set of possible target coordinates within the AOI where the new sampling was to be carried out. We used a stratified random approach that was recently applied to the diachronic analysis of floodplain grasslands (Wesche et al. 2012). Stratification is particularly important in hilly and mountain areas owing to the morphological variability and the resulting floristic differentiation. For this reason, we stratified grassland polygons on the basis of the topographic information (i.e. altitude, slope and aspect) by means of a Digital Terrain Model with a very fine grid (20 m) and considered only those sites with the same values of the historical plots.

2.3 DIACHRONIC ANALYSIS OF THE DATA

Since the designation of the new sampling sites is inevitably affected by a certain degree of spatial inaccuracy, this inaccuracy should also be taken into account during the data analysis. In particular, any comparison between the historical and new plots in pairs should be avoided, i.e. the historical plot should not be compared with a new plot whose location is based on the former.

To overcome this problem, we first performed an ordination of the historical and new plots, and then summarized the compositional information of the historical plots in their centroid. The ordination should be based on non-Euclidean measures of dissimilarity, since metric measures are not compatible with ordinal scores such as phytosociological cover scores (Podani 2006). We then used the distances between each individual new plot and the centroid of the historical plots as a measure of the amount of compositional change that had occurred.

This is particularly effective if the centroids of each individual phytosociological association are used. Indeed, plots included in the same association always display a very high degree of homo-

geneity and their centroids can be used as single starting points from which any change may be measured.

Another issue that needs to be taken into account is the scarcity of environmental and management data associated with the historical plots that could be used as explanatory variables in the analysis of change. Indeed, only a very limited number of topographic characteristics are reported for the historical plots (usually altitude, slope and aspect) that are by assumption equal to those of the newly sampled sites. However, past data on management and landscape metrics may be retrieved, respectively, from the records of local agencies (townships and other public authorities) and from historical vegetation maps.

Since understanding the relative importance of environmental and management variables is of primary relevance when studying vegetation change (Wilson et al. 2011, Catorci et al. 2014), the new sampling should include the recording of management and environmental variables, and of landscape metrics (de Bello et al. 2010).

Our approach to the data analysis allows the whole set of the available explanatory variables (i.e. past and current management and environmental features, and landscape metrics) to be associated exclusively with the new plots. These variables may be used in a regression analysis in which the distances between the new plots and the centroids of the historical groups of plots are used as the response variable.

2.4 WORKED EXAMPLE

In order to test the feasibility of our methodology, we applied it to the semi-natural grasslands of Mount Catria (central Italy).

In the LISY on-line database, we found historical data (both phytosociological plots and map) published by Biondi & Ballelli (1982) for the Mount Catria massif. The semi-natural grasslands were sampled between 1976 and 1981 and were associated with a fine-scale map (scale of 1:15000, year of publication 1982) covering an area of about 3920 ha, ranging between 300 and 1701 m a.s.l. Within this area, we considered the only semi-natural dry communities reported in the paper of Biondi & Ballelli (1982), which were referred to three different associations of the endemic *Phleo ambigu-Bromion erecti* alliance of the Apennines: *Asperulo purpureae-Brometum erecti*,

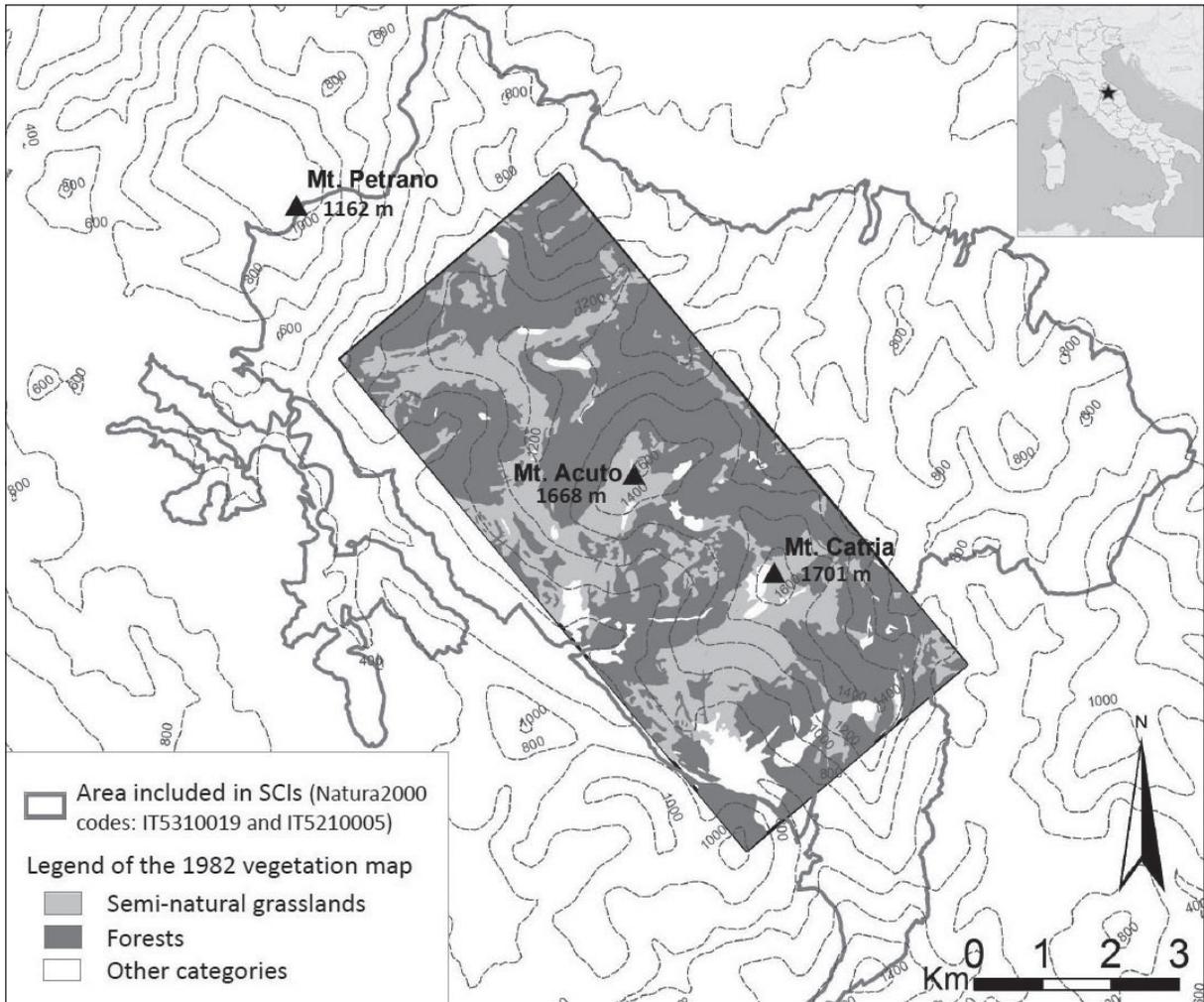


Figure 2: Location of the study area in Italy, boundaries of the Sites of Community Importance (SCIs) and distribution of *Bromus erectus* semi-natural grasslands within the area covered by the 1982 map.

Figure 2: Raziskovano območje v Italiji, meje posebnih ohranitvenih območij (SCI) in razširjenost pol naravnih travišč z vrsto *Bromus erectus* znotraj območja, prikazanega na karti iz leta 1982.

Briza mediae-*Brometum erecti* and *Sesleria nitidae*-*Brometum erecti*. These *Bromus erectus* dominated grasslands show a high frequency of hemicryptophytes like *Hieracium pilosella*, *Koeleria lobata*, and *Knautia arvensis*, which occur in at least 80% of the historical plots.

The area covered by the historical data includes part of two Sites of Community Importance (Natura 2000 codes: IT5310019 and IT5210005) (Figure 2). The substrate is mainly calcareous, and the climate is sub-Mediterranean, with a limited summer drought and mean annual rainfall ranging from 1100 to 1700 mm.

In accordance with the season in which the historical plots were sampled, we carried out the new fieldwork in June and July 2013. We desig-

nated 24 new sampling sites based on the information of the historical plots. In particular, for each historical sampling site, three pairs of coordinates with the same topographical features were randomly selected within the same AOI. Once in the field, if the target site that was selected firstly did not meet the requirement of microtopographic and physiognomic homogeneity, we moved on to next pair of target coordinates (Figure 1).

For each new plot, we identified several potential drivers of vascular plant species composition (Table 1). In particular, we recorded in the field environmental characteristics such as topographic features (i.e. altitude, slope, and aspect); percentage cover of outcropping rocks and stones,

Table 1: Explanatory variables used in the regression analysis as the response variable for the distances of each new vegetation plot from the centroid of the historical group of plots. Numbers in superscript identify the explanatory variables that were used to produce the grazing ⁽¹⁾ and the soil skeleton ⁽²⁾ indices.

Table 1: Neodvisne spremenljivke v regresijski analizi kot odvisne spremenljivke za razdalje za vsako novo vegetacijsko ploskev od centroida starejše skupine ploskev. Nadpisane številke prikazujejo neodvisne spremenljivke, ki smo jih uporabili za izračun indeksov za pašo ⁽¹⁾ in skeletnost tal ⁽²⁾.

Type of drivers	Variable	Unit	Mean	Minimum	Maximum
Environmental	Altitude	m a.s.l.	1127	858	1502
	Slope	°	21	0	42
	Aspect	°	183	0	320
	Rocks	%	0	0	1
	Stones	%	10	0	50
	Bare soil	%	0	0	2
	Litter	%	2	0	15
Landscape metrics	Polygon area	ha	65	9	130
	Polygon Shape Index	-	3	2	4
	Perimeter shared with forest polygons	%	59	21	87
	Distance from road ¹	m	286	8	624
Management	Droppings ¹	n	1	0	10
	Cattle-horses density ¹	n / ha	1	0	1
	Sheep density ¹	n / ha	0	0	1
Soil	Depth of horizon O	cm	0	0	5
	Boulders ²	%	19	0	90
	Cobbles ²	%	13	0	60
	Coarse gravel ²	%	14	0	60
	Medium gravel ²	%	4	0	60
	Large roots	n / dm ²	0	0	2
	Fine roots	n / dm ²	49	11	125
CaCO ₃	%	5	0	10	

bare soil, and litter. We also recorded the number and type of droppings within each sampling unit, and we noted several soil variables based on a 25 cm deep mini-pit (i.e. depth of horizon O, percentage of boulders and cobbles, percentage of coarse and medium gravel, number of large and fine roots per dm², and percentage of CaCO₃).

Data on livestock structure (number and types of animal per municipality) from 1968 to 2012 were provided by the local agency “Azienda Speciale Consorziale del Catria” (Figure 3).

We also considered the landscape metrics calculated from the 1982 map, which was first digitalized and georeferenced within a GIS environment (ArcGis 10). In particular, for each polygon associated with the re-visited sites we took into account: the polygon area, the Shape Index as proposed by Comber et al. (2003), and the percentage of perimeter shared with forest polygon(s) (Smiraglia et al. 2007).

In addition, we calculated the distance from each new sampling site to the nearest road, and

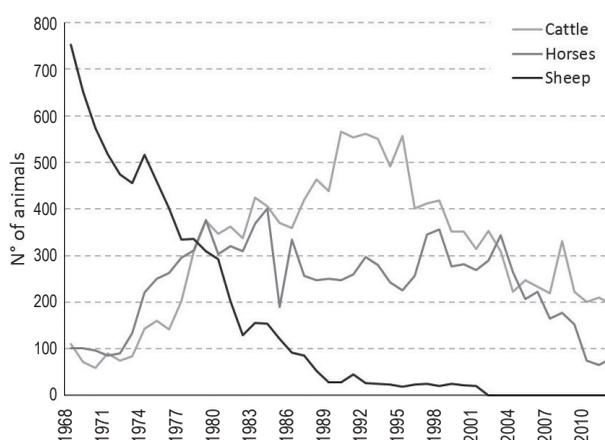


Figure 3: Trends in livestock structure from 1968 to 2012.

Slika 3: Trend sestave živine med letoma 1968 in 2012.

we used this information jointly with other proxies of grazing intensity (i.e. number of droppings and density of grazing animals) in a Principal Component Analysis. The sample scores on the first axis of the ordination were used as the val-

ues of an index of grazing. The same procedure was adopted to obtain an index of the soil skeleton from the variables boulders, cobbles, coarse gravel, and medium gravel.

In order to test for significant differences between the two vegetation samplings (historical and new as a whole), we compared the vascular plant species' composition through permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) on the basis of a plot-to-plot dissimilarity matrix calculated by means of the Bray-Curtis coefficient on species abundances. The analysis was conducted via the function *adonis*, in the *vegan* package of the program R (Oksanen et al. 2013, R Core Team 2014).

In order to visualize the differences in the species composition between the historical and new plots, we performed the Principal Coordinate Analysis (PCoA) using the same dissimilarity matrix as that used for the PERMANOVA (function *cmdscale* in *stats* base package, R).

To quantify the changes in vascular plant species composition, we computed the distance of each new plot from the centroid of the corresponding historical group of plots (i.e. the centroids of the historical group of plots on which we based the designation of the new sampling sites) within the ordination space (functions *betadis* and *dist* from packages *vegan* and *stats*, respectively, R).

Since our analysis is aimed at assessing changes in the species composition of plant communities referred to Habitat 6210(*), we tested whether the frequencies of the diagnostic species of the Habitat changed over time by comparing them in the historical plots (expected frequencies) and in the new plots (observed frequencies) using the χ^2 test. We also compared the number of diagnostic species and the abundance of each diagnostic species in the two vegetation samplings (historical and new) using the non-parametric Wilcoxon rank-sum test.

To identify the drivers of patterns of change in vascular plant species composition, we performed a regression tree analysis using the variables listed in Table 1 as explanatory variables and, as response variable, the distances of each new plot from the centroid of the corresponding historical group of plots. The regression analysis splits the data in clusters of plots through binary recursive partitioning, and associates each split with a threshold for one explanatory variable; the split which maximizes the reduction in impurity

of the clusters is chosen, after splitting the dataset the process is repeated. For this analysis, we used the function *tree* in the *tree* package (Ripley 2014) for the R software that sets six as the minimum number of objects (i.e. plots) in each terminal node. In our case study, due to the relatively low number of plots considered in our case study (24) the original tree had only five nodes (one root node, one non-terminal node and three terminal nodes), therefore no pruning was applied.

3. RESULTS

The PERMANOVA highlighted significant differences between the historical and the new groups of plots (pseudo- $F = 4.79$, $p = 0.001$). In fact, in the PCoA biplot, the historical plots are clearly separated in three dense clusters that correspond to the three original grassland associations, whereas the 24 new plots are widely scattered throughout the ordination space (Figure 4). The majority of the new plots are spread along the first axis, some of them are located far from the corresponding historical groups (e.g. *Asperulo purpurae-Brometum erecti* and *Seslerio nitidae-Brometum erecti*), whereas other new plots are definitely close, such as those sampled within the polygon mapped as *Brizo mediae-Brometum erecti*.

The historical dataset included 19 diagnostic species for Habitat 6210(*) (Interpretation Manual of European Union Habitats -EU28), whereas only 15 diagnostic species were found in the new

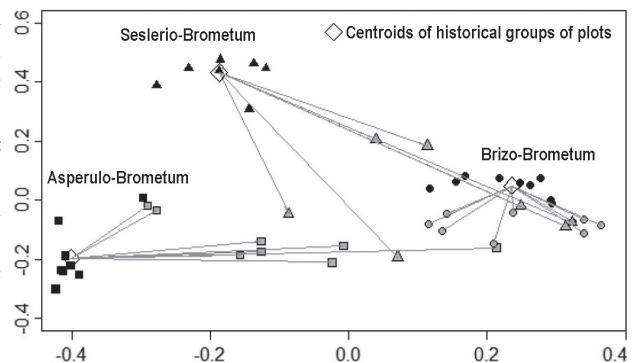


Figure 4: Ordination diagram of the PCoA based on Bray-Curtis dissimilarity coefficients calculated from species abundance values. Black and gray symbols indicate historical and new plots, respectively.

Slika 4: Ordinacijski diagram PCoA s koeficientom različnosti (Bray-Curtis), izračunanim iz vrednosti abundanc posameznih vrst. Črni in sivi simboli prikazujejo starejše in nove vzorčne ploskve.

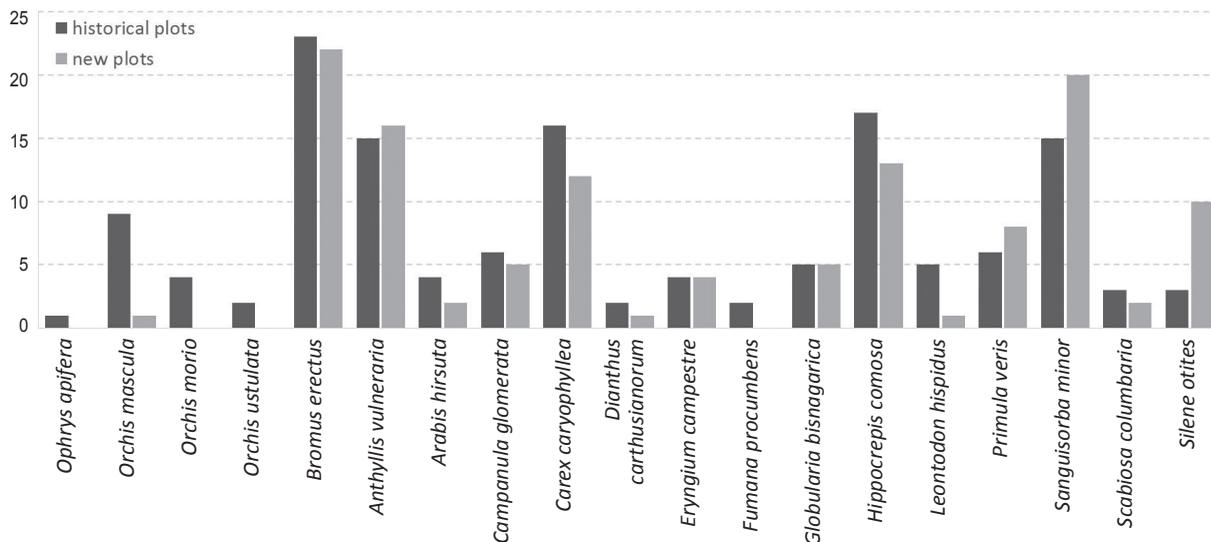
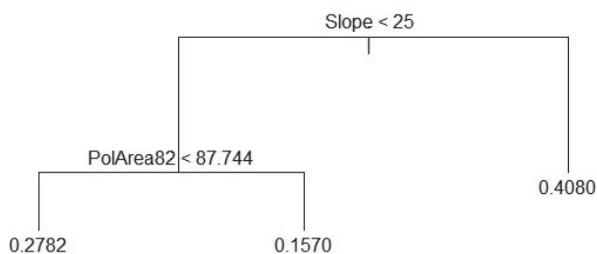


Figure 5: Frequencies of the 19 diagnostic species of Habitat 6210(*) in the historical plots and the new vegetation plots. Orchid species are clustered on the left side of the graph. Based on the χ^2 test, none of the diagnostic species showed significantly different frequencies between 1982 and 2013.

Slika 5: Frekvence 19 diagnostičnih vrst habitata 6210(*) na starejših in novih vegetacijskih ploskvah. Orhideje so prikazane na levem delu grafa. Nobena frekvenca diagnostične vrste se na osnovi χ^2 testa med letoma 1982 in 2013 statistično značilno ne razlikuje.

plots (Figure 5). The Wilcoxon sum-rank test on the number of diagnostic species and the chi-squared test did not detect significant differences in the number and frequencies of the diagnostic species between the historical and new vegetation samplings ($W = 202.5$, $p = 0.075$). However, significant differences were found in the abundance of the two orchids species *Orchis mascula* and *Orchis morio* ($W = 187.5$, $p = 0.003$ and $W = 240$, $p = 0.041$, respectively), which showed a decrease in their abundance over time, and of *Sanguisorba minor* ($W = 370.5$, $p = 0.041$), which conversely increased in abundance.

The regression tree highlighted the strong influence of slope and polygon area on the degree of change in species composition (Figure 6). The steepest sites (right end of the tree) displayed the highest degree of change (mean distance value from the corresponding historical centroids = 0.41). The polygon area is the factor that influences the most the variation in species composition in the flatter areas (i.e. all the sampling sites with a slope < 25°). Indeed, the plots that occurred in smaller polygons (area < 88 ha) were subjected to an intermediate degree of compositional change (mean distance value = 0.2782). The plots displaying the lowest degree of change (mean distance value = 0.157) occurred in polygons with an area greater than 88 ha and with slope values lower than 25°.



4. DISCUSSION

4.1 STRONG SHIFTS IN SPECIES COMPOSITION DERIVE FROM THE COMPLEX INTERPLAY OF MULTIPLE DRIVERS

The diachronic analysis of the Mount Catria dataset pointed to strong differences in vascular plant species composition between the historical and the new plots, as is often observed in re-visitation studies (Fischer & Stöcklin 1997, Baeten et al. 2010, Saar et al. 2012, Wesche et al. 2012).

Indeed, only a small number of new plots remained similar in plant species composition to the corresponding historical groups. This was especially true for few plots within the polygons mapped as *Asperulo purpureae-Brometum erecti* and *Seslerio nitidae-Brometum erecti*, in which the extremely xeric conditions of sampling sites due to high cover of rocks (>50%) substantially slowed down successional dynamics (Vassilev et al. 2011).

The majority of sites sampled within the historical areas of *Brizo mediae-Brometum erecti* also did not show substantial changes in species composition. These communities that develop at low to medium slope (<25°) are still dominated by *Bromus erectus* and host many of the diagnostic species of the Habitat 6210(*), although all four orchid species are missing. The stability of these communities may relate to the fact that they occur in the largest polygons (>88 ha), as previous reports indicate the high probability of grassland species occurrence in larger patches (Cousinset al. 2007; Saar et al. 2012).

Another reason for the stability of the communities originally ascribed to *Brizo mediae-Brometum erecti* is that they are generally subjected to horse and cattle grazing, which has not substantially varied in the whole area since 1968 (Figure 3) and can filter plant species able to avoid or resist a prolonged pressure by these type of animals (Catorci et al. 2012). Indeed, cattle and horses have substantially similar grazing behaviors, strongly preferring moist and productive areas where they spend the majority of their time (Putfarken et al. 2008).

Other plots accumulated great differences from the corresponding historical groups especially from *Asperulo purpureae-Brometum erecti* and *Seslerio nitidae-Brometum erecti*. High degrees of changes (mean distance value from the corresponding historical centroids = 0.41) are mainly related to the colonization of woody species in

former grassland sites, which included both the young individuals of tree species (e.g. *Quercus pubescens*, *Ostrya carpinifolia*, *Crataegus monogyna* and *Juniperus communis*), and chamaephytes attaining high abundance values (equal or greater than 3 on the Braun-Blanquet scale). The sites that were subjected to this process were those located on the steepest slopes, as it was previously found (Pellissier et al. 2013), furthest from the roads (Vassilev et al. 2011), and within historical polygons that shared a high percentage (about 60%) of their perimeter with forest patches in 1982. These remote and less productive areas have faced a drastic reduction in grazing intensity, mainly due to the fact that sheep, which usually feed in dry and nutrient-poor habitats and cover relatively long distances to reach feeding areas (Evangelou et al. 2014), were widely replaced by cattle and horses (Figure 3). Indeed, in 1968 more than 700 sheep actively grazed the study area, whereas since 2002 sheep had been completely replaced by cattle and horses, whose numbers in 2012 were about 200 and 100, respectively. The variation in livestock structure has affected the spatial distribution of grazing pressure in the Mount Catria area, leading to an abandonment of grazing in the most remote sites and an increase in some of the more accessible areas.

Intermediate degrees of change (mean distance value = 0.2782) were observed in flatter sites within grassland polygons that were smaller than 88 ha. In these conditions, we observed two different directions of change: on the one hand, we noticed the already described encroachment by woody species, on the other hand the occurrence of species favored by over-grazing, such as *Malva sylvestris* and *Lolium perenne*, especially in those plots that lay close to roads and where grazing pressure was high.

The general shift in the type of animals that are being farmed within the study area may also be the reason of the substantial decrease in both frequency and abundance we observed for the orchid species diagnostic of the Habitat 6210(*) in the whole dataset (Figure 5). The reduction of orchids and the increase in frequency and abundance of sub-cosmopolitan species like *Sanguisorba minor* point to a decrease in the conservation value of the habitat in general, which is by definition related to orchid species, whether it be to their overall richness or to the occurrence and abundance of rare and uncommon species. The underlying mechanism behind such a worry-

ing decrease is related especially to the different behavior of cattle and horses when compared to sheep. Indeed, the sedentary behavior of these animals, which graze from early spring to late summer in the Mount Catria area, do not allow orchids to accomplish their life cycle, even if this is relatively brief. It was already demonstrated that early flowering geophytes, such as the orchid species like *Orchis morio*, are strongly affected by grazing in springtime (Catorci et al. 2013).

4.2 PROS AND CONS OF THE PROPOSED METHODOLOGY

Here we propose a new methodology based on the re-visitation of historical vegetation plots for which the exact location is not available. All the spatial information achievable in both historical plots and maps was used to designate the new sampling sites while reducing the probability of pseudo-turnover as much as possible. This method allows the vegetation change to be assessed by means of an approach that combines spatial and typological information. Although our approach does not ensure the exact overlay between historical and new plots, it does ensure that the new plots are surveyed: i) as closely as possible to the historical plots, ii) and in the same topographic conditions of the historical plots. Moreover, the coupling of the historical plots with the map polygons of the same vegetation unit guarantees that the new sampling plots are in sites where the same vegetation unit of the historical plots occurred.

The second important novel aspect of this approach is the use of the distances between each new plot and the centroid of the historical plots as a measure of the amount of compositional change. This strategy avoids paired comparisons of historical and new plots, which should only be performed if the two plots were located in exactly the same site. This approach proved to be particularly effective when the centroids of each individual historical association were used.

The distance of each new plot from the centroid of the corresponding historical group of plots can also be used as the response variable when identifying the drivers of patterns of change. It is thus possible to relate these distances to the environmental and management variables associated with the new plots. This is particularly useful given the lack of environmental data available for the historical data.

We are aware that other problems related to the historical vegetation plots may arise from the use of different taxonomic and syntaxonomic schemes by different authors (Jansen & Dengler 2010, Dengler et al. 2013). Indeed, while we performed our analysis and we went through the species list, we identified some differences clearly ascribable to different taxonomic interpretations and classifications. We tackled this problem by using two taxa (one in the historical dataset, the other in the new dataset) as a single row in the final dataset. We believe that the new and the historical species lists always need to be analyzed and homogenized when re-visitation studies are carried out. With regards to the syntaxonomic scheme, we used a broad syntaxon (alliance) in order to select the studies that were most relevant to ours. We suggest to avoid the analysis of changes in syntaxonomic classification of the communities since it would require an accurate comparison between historical and current syntaxonomic schemes.

5. CONCLUSIONS

Diachronic analyses of vegetation always require a greater effort and are affected by more uncertainties than synchronic analyses. However, the knowledge gained on the temporal and spatial patterns of variation in plant communities clearly justifies the effort made to retrieve past data and to overcome these uncertainties.

Our study proposes a feasible means of highlighting both the degree and the patterns of the changes that have occurred in semi-natural grasslands over recent decades. We hope that our findings may encourage other vegetation scientists to re-visit sites that were sampled in the past in view of setting up a network of monitoring sites for which data from the past are available.

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