



# Re-visiting historical semi-natural grasslands in the Apennines to assess patterns of changes in species composition and functional traits

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## Keywords

*Bromus erectus*; Calcareous grasslands; Drivers of changes; Habitat 6210(\*); Habitats Directive; Leaf–height–seed scheme; Multi-Temporal analysis; Raunkjær life forms; Vegetation dynamics

## Nomenclature

Conti et al. (2005)

## Abbreviations

AC group(s) = group(s) of plots identified by the study area and the original plant community  
CWM shift(s) = changes in community-level weighted means

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## Abstract

**Questions:** To what extent have semi-natural grassland plant species composition and functional traits changed during the past five decades? Which are the main directions of change? Which environmental, management and landscape factors have determined these changes?

**Location:** Central and southern Apennines of Italy.

**Methods:** In 2013–2014, we used a stratified random sampling design to revisit 132 semi-natural grasslands originally sampled between 1966 and 1992. The exact locations of the historical plots were not available but they were associated with detailed vegetation maps. Plots were divided in 17 groups based on study area and original community type. To assess changes in species composition we ran PERMANOVA and RDA. The extent of change was estimated by comparing each new plot with the quantitative species pool of the corresponding historical group; species composition changes were calculated using Bray-Curtis coefficient, and shifts in functional traits were quantified as differences in community-weighted means. These measures of change were used as response variables in mixed effects models including climate, topography, management and landscape metrics as explanatory variables.

**Results:** The largest changes were related to successional dynamics, which occurred mainly in grasslands developing at low altitude and in the flattest sites where the soil organic horizon was relatively thick. Colonization by shrubs and trees was facilitated by high soil pH and amount of adjacent forests. Also, management factors affected the patterns of shifts in species composition and functional traits: grazing intensity was negatively related to the degree of compositional change as well as to the abundance of woody species, whereas the increase of species associated with frequent grazing disturbance was found at sites with higher values of soil salinity.

**Conclusions:** Apennine semi-natural grasslands have undergone substantial changes in species composition and functional traits during recent decades. Compositional changes mainly indicated successional dynamics, likely following a decrease in grazing intensity, whereas some more accessible grasslands experienced an increase in disturbance-tolerant species. The combination of the re-visit approach with functional traits allowed us to clarify different directions of changes in species composition, adding information on the ecological processes related to these changes.

## Introduction

The European landscape is characterized by the abundance of semi-natural grasslands, i.e. anthropogenic grasslands at sites where forests represent the natural potential vegetation. The expansion and maintenance of these grasslands has been supported by centuries or millennia of low-intensity land use, represented by pastoralism and regular mowing (Hejcman et al. 2013). Worryingly, these ecosystems are being lost or degraded at very high rates. Indeed, the semi-natural grasslands of the European Union experienced a reduction in extent of more than 5 million hectares between 1990 and 2013 (faostat.org). This trend is the result of both the land-use intensification and the abandonment of agricultural land that is taking place, especially in economically marginal regions (Burrascano et al. 2016). Land-use intensification includes artificial fertilization, increased cutting frequency or stocking rate and frequent re-sowing with agronomically valuable species (Hejcman et al. 2013; Dengler et al. 2014). On the other hand, the cessation of land management activates processes of vegetation dynamics that result in the progressive succession toward forests (Assini et al. 2015).

European semi-natural dry grasslands are among the most species-rich ecosystems globally (Chytrý et al. 2015) and host about 18% of the European endemic vascular plant species (Hobohm & Brunchmann 2009). Moreover, numerous plant and animal species of Community interest restricted to grassland ecosystems are included in the threatened categories of the International Union for Conservation of Nature, e.g. 15% of the species of vascular plants, 28% of amphibian and 23% of bird species (EEA 2010).

Due to their vulnerability to land-use changes and their importance for biodiversity, it is crucial to understand the vegetation dynamics that occur in semi-natural ecosystems. The assessment of changes in species composition can be achieved through different strategies: permanent plots, re-visitation of historical plots, use of large databases, each of which has specific pros and cons (Chytrý et al. 2014). We chose the approach based on the re-visitation of historical plots associated with fine-scale maps, basing our choice on a trade-off between the availability of historical data and the reliability of their relocation. Indeed, several recent studies effectively applied this approach to semi-natural grasslands, and were able to show a general tendency towards species-poor communities dominated by competitive species at the expense of the stress-tolerant species (e.g. Bennie et al. 2006; Wesche et al. 2012; Stevens et al. 2016). Nevertheless, to the best of our knowledge, few attempts have been made to investigate temporal shifts in functional traits through

re-visitation data (Saar et al. 2012; Wesche et al. 2012; Lewis et al. 2014), although their analysis can give relevant insights on the ecological processes at the base of changes in species diversity. Indeed, the use of functional traits of organisms rather than or in addition to taxonomic categories is now accepted as an excellent means to understand community dynamics in response to environmental gradients (Kleyer et al. 2012), disturbance regime (Burrascano et al. 2015) and management practices (Sabatini et al. 2014). In semi-natural grasslands, a complex interplay of environmental and management variables was found to determine the species composition and diversity (Burrascano et al. 2013; Catorci et al. 2013), and it is likely to also affect species functional traits within these ecosystems.

We applied a re-visitation study on semi-natural grasslands dominated or co-dominated by *Bromus erectus* that, according to the Habitats Directive (92/43/EEC), belong to the habitat 6210(\*) 'Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*)(\*important orchid sites)'. This habitat is one of the most widespread among the European semi-natural habitats, and is considered as fully dependent on low-intensity management (Halada et al. 2011). Our aims were to: (1) analyse changes in vascular plant species composition and functional traits that occurred during recent decades; (2) identify the main directions of these changes; and (3) assess the factors that have influenced both compositional and functional changes. We expected the changes in species composition that occurred in the last 50 yrs to be reflected in shifts in functional traits, and that these changes show different patterns in relation to shifts in climatic parameters, topographic and soil conditions, grazing intensity and landscape metrics. In particular, we expected an increase in the abundance of tall and woody species in those areas less subjected to environmental constraints and with a low grazing intensity (de Bello et al. 2005; Stevens et al. 2016); in contrast, we expected an increase in grazing-tolerant species (e.g. small stature and short life span) in the most accessible sites (i.e. near roads and drinking points) where animals usually gather, rest and feed (Catorci et al. 2012).

## Methods

### Study areas and sampling design

We performed our study in the central and southern part of the Apennine mountain chain along the Italian peninsula, which reaches 2912 m a.s.l. with the peak of Corno Grande, and hosts a huge portion of the Italian plant species diversity (Blasi et al. 2011). We selected seven areas (Fig. 1) by searching for published and unpublished

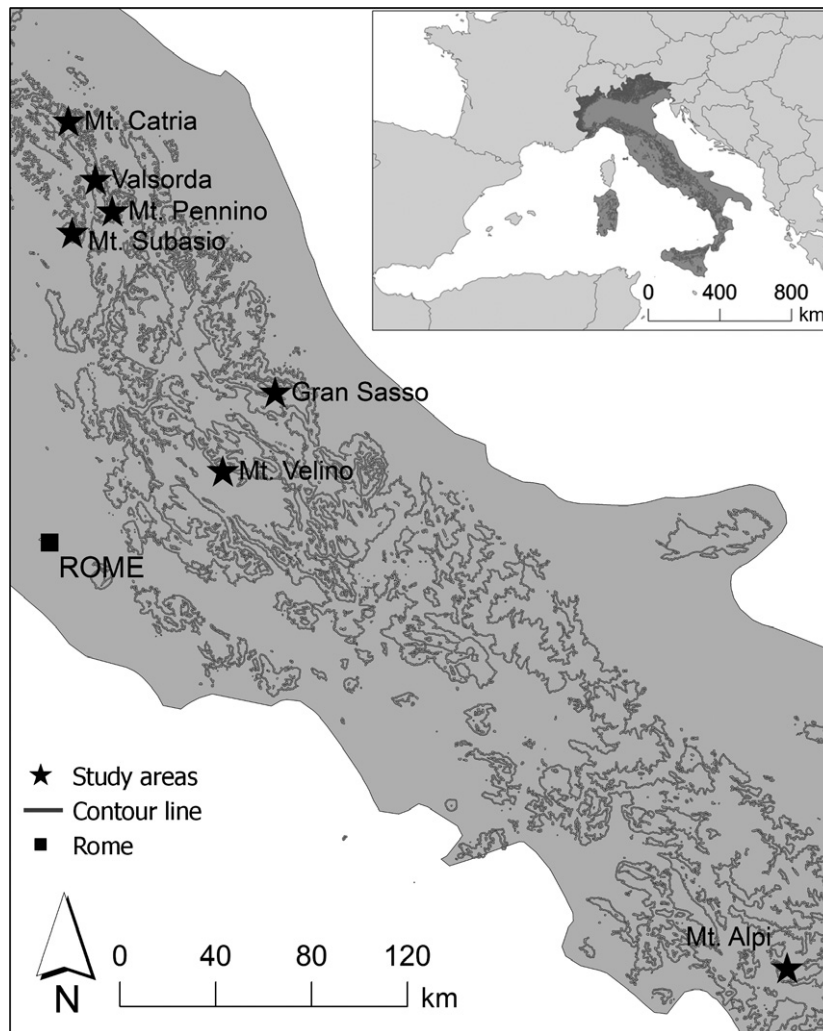


Fig. 1. Distribution of the seven study areas along the Apennine chain in Italy.

studies that include vegetation plots associated with fine-scale vegetation maps. To be selected, the historical studies should be composed of: (1) vegetation plots dated at least 20 yrs ago and sampled on calcareous substrates; (2) map with a scale range from 1:25000 to 1:10000 and with phytosociological legend at the association level; and (3) community types referred to the Apennine endemic alliance *Phleo ambiguus-Bromion erecti* of the habitat 6210(\*) (Biondi et al. 2005).

The annual mean temperature averaged across the seven study areas is 12.3 °C (with minimum and maximum values of 8.4 °C for Mt. Velino and 15.1 °C for Mt. Subasio, respectively); the annual mean rainfall is 943 mm, with strong differences between minimum and maximum values (Mt. Alpi 675.5 mm and Mt. Catria 1405.3 mm, respectively), during June–August the mean rainfall is 157.4 mm (minimum for Mt. Velino

107.1 mm, maximum for Mt. Pennino 203 mm). All the study areas occur on Jurassic and Cretaceous limestones, with some incursions of marly limestone and calcareous marls.

Since the historical vegetation plots did not have an exact geographic reference, we used locality and map legend to identify a site for each historical plot. Then we applied a stratified random approach using altitude, slope and aspect of each historical plot, to identify the points where the new sampling should be performed (Giarrizzo et al. 2015). Our approach allowed the new plots to be surveyed in areas originally occupied by the target community type, and in the same topographic conditions. However, since this process does not ensure a perfect overlay between the historical and the new plots, we did not compare them directly in pairs (details on the methods applied are described below).

### Vegetation data and functional traits

During 2013 and 2014, we revisited a total of 132 plots originally sampled between 1966 and 1992 and at that time dominated or co-dominated by *Bromus erectus* (Appendix S1). The current vegetation was sampled during the peak of vegetation biomass, in the same period of the year and with the same method (Braun-Blanquet approach) adopted by the previous authors (Muller-Dombois & Ellenberg 1974). Special attention was paid to taxonomic standardization between the historical and new data sets. Besides assuring that synonyms were correctly recognized as a single species, for the taxonomically complicated groups as well as in those cases with a substantial risk of misidentification, we aggregated the species. It is worth noting that closely related species usually have negligible differences in functional traits (Penone et al. 2014).

To analyse the shifts in plant functional traits, we focused on three Raunkiær life forms that give crucial indication for the analysis of vegetation dynamics in semi-natural grasslands and three quantitative traits chosen according to the leaf–height–seed scheme (Westoby 1998; see Table 1). Since the ecosystem functions are widely determined by trait values of the dominant plant species (Grime 1998), we directly measured the three quantitative traits for those species with a value of 3 or more on the Braun-Blanquet scale in at least one new plot, following an international handbook (Pérez-Harguindeguy et al. 2013). For the trait values of the remaining species and the Raunkiær life form of all the species, we mainly referred to the LEDA database (Kleyer et al. 2008) and to Pignatti (1982), respectively. In case of missing species in LEDA,

we integrated the trait values from scientific literature and other databases (Table 1).

### Environmental, management and landscape data

To understand which factors have played a key role in influencing the changes in species composition and functional traits, we considered a wide range of explanatory variables collected either at the area or at the plot scale (Table 2). At the study area scale, we considered climate and livestock. Climatic data were derived from the meteorological stations closest to the sampling plots and were included in the analyses as the differences between the two sampling periods in: (1) minimum mean temperature of the coldest month of the year and (2) rainfall of June, July and August (Blasi et al. 1999). For both historical and new plots, we averaged these parameters considering the 15 yrs before the sampling of each plot. Data on livestock (number of individuals for species per hectare) were provided by the public bodies responsible for the land management. Since the time span and the temporal resolution of these data differed in relation to the study area, we decided to take into account only recent and current management (approximately since 2009).

Landscape variables were calculated based on the historical maps. In particular, for each grassland polygon (i.e. a continuous portion of land mapped as an individual legend category) including one or more re-visited plots we calculated an index of shape complexity that indicates how jagged is the edge of the polygon (McGarigal et al. 2012), and the percentage of perimeter shared with forest and shrubland polygon(s) (Smiraglia et al. 2007).

**Table 1.** Functional traits considered in the analyses, with the data source and the number of species for which we have the trait value. Phanerophytes and nano-phanerophytes were grouped together in one category. We directly measured quantitative traits for those species that reached a value of 3 or more on the Braun-Blanquet scale of abundance (cover > 25%) in at least one new plot.

| Trait              | u.m.                              | Range  | Source |                      |   | No. of Species |
|--------------------|-----------------------------------|--|--------|----------------------|---|----------------|
|                    |                                   |  | Field  | Databases            | Scientific Literature   |                |
| Life Form          | –                                 | Hemipterophytes;<br>Therophytes;<br>(Nano)Phanaerophytes | –      | –                    | Pignatti (1982)   | 580            |
| Specific Leaf Area | mm <sup>2</sup> ·mg <sup>-1</sup> | 22.3 (3.1–74.3)  | Yes    | LEDA, TRY            | Huovinen-Hufschmid & Körner (1998); Caccianiga et al. (2006); Navas et al. (2010); Dujardin et al. (2011); Raavel et al. (2012); Pierce et al. (2013); Nunes et al. (2014); Burrascano et al. (2015); Puglielli et al. (2015) | 337            |
| Canopy Height      | m                                 | 0.69 (0.029–20)  | Yes    | LEDA, TRY            | Pignatti (1982); Caccianiga et al. (2006); Navas et al. (2010)  | 410            |
| Seed Mass          | mg                                | 33.37 (0–4263)   | Yes    | BROT, Kew, LEDA, TRY | Navas et al. (2010); Raavel et al. (2012)   | 340            |

**Table 2.** Factors potentially affecting the change in species composition and functional traits included in the analyses as explanatory variables. We report the scale at which the variable was collected and the range of values for each variable. Grazing resulted from the first axis of a PCA (37.3% of variance explained) and summarized information collected at both study area and plot scale (number of individuals for species per hectare, number of droppings and distance of the new plots from roads and drinking points).

|                   | Variable                                      | u.m.                | Scale of Analysis | Mean (min–max)       |
|-------------------|---|---------------------|-------------------|----------------------|
| Climatic Shifts   | Minimum mean temperature of the coldest month | °C                  | Area              | −0.08 (−5.18–3.79)   |
|                   | Mean rainfall of the three summer months      | mm                  | Area              | −3.10 (−42.07–53.14) |
| Topography        | Altitude                                      | m a.s.l.            | Plot              | 1263 (579–1863)      |
|                   | Slope   | °                   | Plot              | 21.64 (0–50)         |
|                   | Stones  | %                   | Plot              | 13.64 (0–95)         |
|                   | Bare soil                                     | %                   | Plot              | 4.23 (0–40)          |
| Soil              | Thickness of soil organic horizon             | cm                  | Plot              | 0.86 (0–5)           |
|                   | C/N   | –                   | Plot              | 15.28 (5.08–29.19)   |
|                   | pH  | –                   | Plot              | 7.28 (5–8.4)         |
|                   | Salinity                                      | mg·kg <sup>−1</sup> | Plot              | 0.8 (0.15–5.46)      |
| Management        | Grazing                                       | –                   | Area/Plot         | 0 (−0.43–0.78)       |
| Landscape Metrics | Polygon shape index                           | –                   | Plot              | 2.74 (1.2–5.92)      |
|                   | Perimeter shared with forest polygons         | %                   | Plot              | 33.2 (0–100)         |

During the fieldwork we noted in each plot topographic features and the percentage of bare soil and outcropping stones; we recorded the thickness of the organic material laying on the soil (i.e. soil organic horizon) and we collected, at the centre of each plot, a soil sample from the top 25 cm of mineral soil. After air-drying and sieving at 2-mm, the soil samples were analysed in laboratory for pH and salinity, according to the national ‘Guidelines for soil description’ (2000). The nitrogen (N) and organic carbon (C) concentrations were measured by dry combustion (ThermoFinnigan Flash EA112 CHN), after neutralizing the inorganic C using HCl.

For each new plot, we also recorded three proxies of grazing pressure by counting the number of droppings within the plot, and measuring the distance from roads and drinking points within a GIS environment (ArcGIS 10). These distances are indicative on how accessible the site is for the animals. Based on livestock data and these proxies, we calculated an index of grazing pressure (hereafter ‘grazing’) using the first axis (37.3% of variance explained) of a PCA (function *rda* in the *vegan* R package; R Foundation for Statistical Computing, Vienna, AT).

### Changes in species composition and functional traits

Based on the study areas and the original plant community type we divided the total data set in 17 groups (hereafter ‘AC groups’), each of them having the same number of historical and new plots (from five to 12 for group). Before running the analyses, species abundance scores were converted to the mean values of each Braun-Blanquet class considering ‘r’ and ‘+’ as the same (i.e.  $r/+ = 0.5$ ; 1 = 3; 2 = 15; 3 = 37.5; 4 = 62.5; 4 = 87.5).

We tested for significant changes in vascular plant species composition between the two sampling periods with a

PERMANOVA (Anderson 2001) using the sampling period as predictor, and the 17 AC groups as permutation strata, i.e. permutations occur only within each group (function *adonis*, *vegan* R package). We also ran PERMANOVA within each AC group.

We identified the species that increased or decreased in abundance through a RDA (function *rda*, *vegan* R package), on the species’ abundance scores in each historical and new plot, after applying Hellinger’s transformation on these. We set the sampling period as constraining variable (historical sampling = 0, new = 1) and the AC group as covariable.

We obtained 17 quantitative species pools averaging the cover values of the species that occurred within the historical plots belonging to each AC group. Afterwards, to quantify the change in species composition, we measured Bray-Curtis dissimilarity between each new plot and the corresponding historical species pool, and considered these dissimilarities as a measure of the occurred changes. Although the AC groups contain different numbers of plots in each one, we tested the effect of this difference on our measure of change and it was not relevant. This approach was preferred to that applied in Giarrizzo et al. (2015; i.e. distance between each new plot and the corresponding historical centroid in a two-dimensional space) because using Bray-Curtis dissimilarities allowed us to take into account all the variation between the two sampling periods.

Quantitative traits were log-transformed and normalized. For each trait and each life-form category considered, we computed CWMs (function *functcomp*, *FD* R package – Laliberté et al. 2014) for each species pool and for each new plot. To quantify the shifts in functional traits that occurred over time (hereafter ‘CWM shifts’) we subtracted from the CWMs of the new plots the CWMs of the

corresponding historical species pools, for a total of six CWM shifts for each plot.

### Mixed effect models

To understand which factors have influenced the changes in species composition and functional traits, we applied several linear mixed effects models considering either the Bray-Curtis dissimilarities or the CWM shifts as response variables. AC groups were included in the random part, while climatic, topographic, soil, management, and landscape variables were in the fixed part. We did not include the time lag between the historical and the new samplings among the explanatory variables because, in our case, it does not convey any information on the occurred changes in management, which would be the key factor influencing the changes in semi-natural grasslands. Moreover, this information is substantially embedded in the AC groups, and when we tested the effect of this time lag on the occurred changes, we found no significant results (data not shown).

To obtain differences in climatic conditions avoiding pair-wise comparisons between historical and new plots, we averaged the minimum temperature of the coldest month and the rainfall of the three summer months for each historical group, and subtracted these values from those associated with each new plot belonging to the same AC group.

We standardized all the explanatory variables, scaling them to zero mean and unit variance. We ran mixed models for each response variable, choosing the appropriate random part through Restricted Maximum Likelihood and AIC criterion (Zuur et al. 2009), and finally selecting the best-fixed part through a backward elimination of the least relevant terms (function *lmer*, *lme4* R package – Bates et al. 2015). Since for some traits CWM shifts were close to zero (canopy height and seed mass) or zero (phanerophytes) in the majority of the plots and reached very high values for a small portion of plots, for these traits we excluded those plots that showed a CWM shift within the range of  $-0.01$  and  $0.01$  before applying mixed effects models. All the analyses were performed in R software.

## Results

### Changes in species composition and functional traits

The final data set is composed of 264 sampling units (132 historical vs 132 new) and 580 vascular plant species. Through PERMANOVA, we observed significant differences in species composition between the two sampling periods (pseudo-F = 9.74,  $R^2 = 0.02$ ,  $P = 0.001$ ), both across the whole data set and within all the individual AC groups but one (Appendix S2).

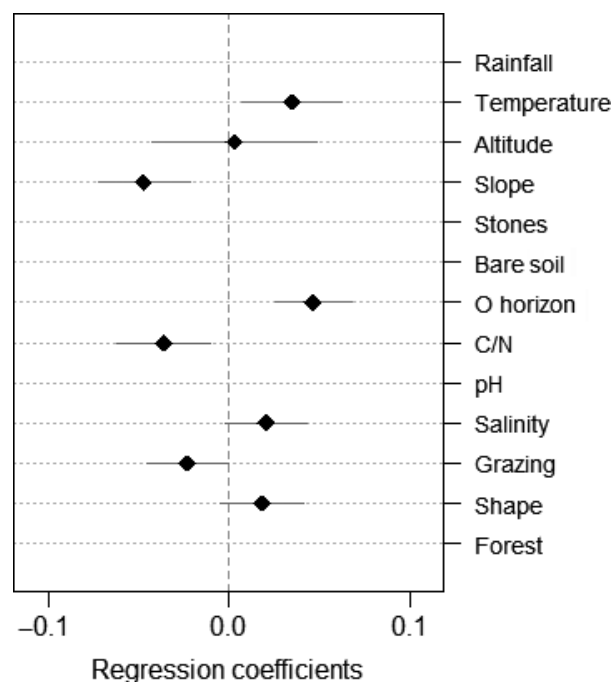
The proportion of the total variance explained by the sampling period in the RDA is extremely low (1.7%; adjusted  $R^2 = 0.015$ ) due to the high variability of the data set, especially that among the new plots, which cannot be explained by the time variable. However, it is interesting to analyse those plants that have particularly high or low scores along this temporal gradient (Appendix S3). In particular, several of the species with RDA scores greater than zero (i.e. species that colonized or increased in abundance in the new plots) indicate successional changes. This is the case for numerous shrub and tree species (see life form and canopy height values in Appendix S3), which were found exclusively during the new sampling, such as *Fraxinus ornus* and *Prunus spinosa* and *Juniperus communis* that showed an increase in abundance. In addition to woody species, many others with high RDA scores are characterized by high values of SLA. These high SLA species can be associated either with successional changes for their affiliation to fringe communities (e.g. *Hypericum perforatum*), with frequent disturbance to which they are adapted through clonality (e.g. *Cynodon dactylon*) or annual life cycle (e.g. *Veronica arvensis*). Contrarily, the species that decreased in abundance over time (with negative RDA scores) are mainly short herbs included among the habitat 6210(\*) diagnostic species (e.g. *Sesleria nitida*, *Satureja montana*, *Helianthemum apenninum*).

The extent of change in species composition (quantified through Bray-Curtis dissimilarities) varied across the AC groups, ranging from a minimum mean value of  $0.57 \pm 0.16$  ( $\pm$ SD) to a maximum of  $0.83 \pm 0.03$ . CWM shifts did not show a clear increase or decrease over time since some traits showed ranges of CWM shifts evenly distributed around zero, e.g. hemicryptophytes that ranged from  $-0.78$  to  $0.57$ , SLA from  $-0.25$  to  $0.21$ . In some cases CWM shift was almost negligible since only a few plots displayed very high values. This was the case of phanerophytes, which attained CWM shift  $> 0.5$  in more than ten plots (those with a pronounced tree encroachment), and shifts between  $-0.05$  and  $0.004$  in 75% of the plots.

Interestingly, the relationships between the change in species composition and the CWM shifts indicate successional dynamics as the main trend, since we found strong positive correlations between Bray-Curtis dissimilarities and increase in phanerophytes, canopy height and seed mass (0.49, 0.47 and 0.47 respectively; see Appendix S4).

### Factors influencing compositional and functional changes

Only two out of the 13 variables potentially affecting the shifts in species composition and functional traits did not exhibit any significant effect in any of the models (summer rainfall and percentage of outcropping stones; Figs 2, 3).



**Fig 2.** Regression coefficients (diamonds) and confidence intervals (gray lines) of the variables included in the final mixed effects model (random intercept and slope) on the Bray-Curtis dissimilarities of the new plots versus the corresponding historical species pool. It modelled the change in species composition (i.e. Bray-Curtis dissimilarities) in response to climatic shifts (mean rainfall of the three summer months and minimum mean temperature of the coldest month), topographic conditions (altitude, slope, percentage of stones and of bare soil), soil variables (thickness of soil organic horizon, C/N ratio, pH and soil salinity), grazing, and landscape metrics calculated from the historical maps (polygon shape index and percentage of adjacent forests). Even if not significant, altitude was retained in the figure because it was included in the random part (intercept and slope model). Confidence intervals were calculated as the average  $\pm 1.96$  SE (0.975 quantile of the normal distribution).

Conversely, the variables that attained a significant effect in all the models are the thickness of the soil organic horizon and altitude, both of them primarily influencing the occurrence of successional dynamics. The former was positively related to the degree of compositional change, as well as to the abundance of woody species, and to positive shifts in SLA, canopy height and seed mass, whereas the opposite relationships were observed for altitude. Also slope, grazing and pH played a key role for successional change. The first two showed a negative relationship with the changes in species composition and with the shift in phanerophytes and SLA. Soil pH had no significant effect on Bray-Curtis dissimilarities but contrasting effects on three quantitative traits, i.e. a negative relation with SLA, and a positive relation with canopy height and seed mass.

Among the other variables, the shift in the temperature of the coldest month and soil salinity significantly affected

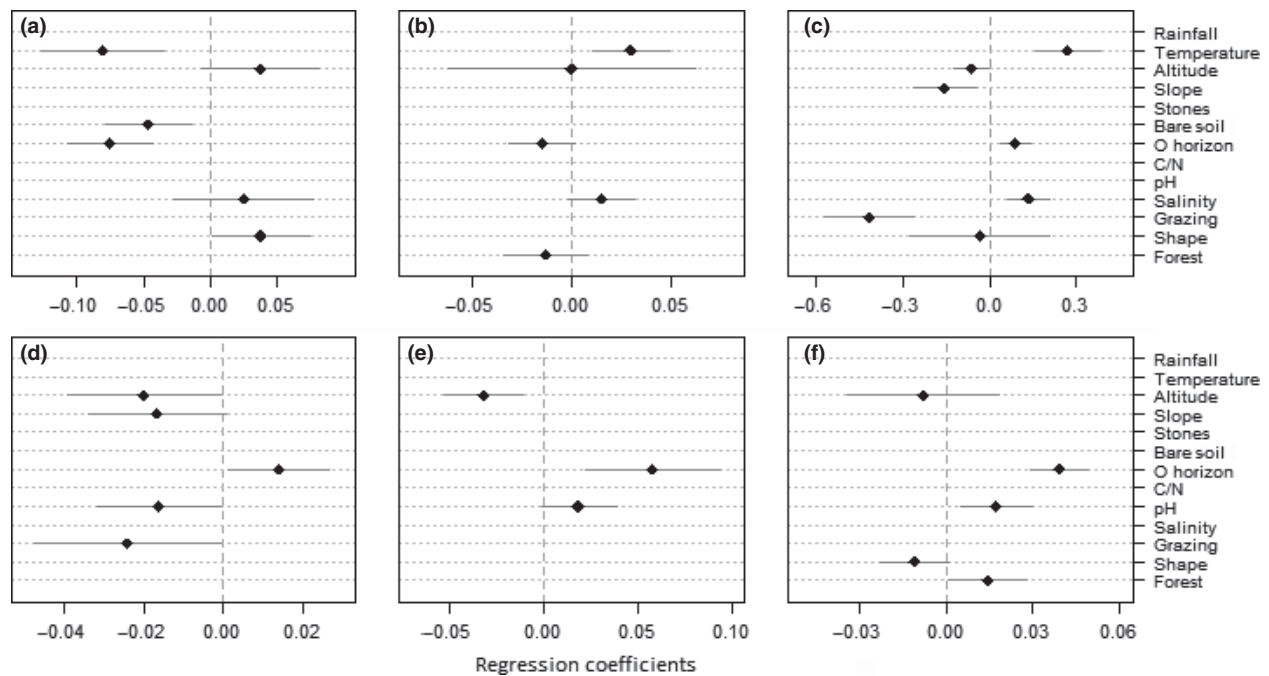
both the changes in the three life-form categories as well as species composition across the study areas. Landscape metrics were relevant especially for seed mass, while bare soil and the ratio C/N were significantly related only to one response variable: shift in hemicryptophytes and compositional changes, respectively.

## Discussion

### Successional dynamics and adaptation to overgrazing

Our study highlighted that during the last five decades substantial changes in species composition and functional traits occurred in *Bromus erectus* semi-natural grasslands along the Apennines. The main shift was represented by successional dynamics. In fact, a large compositional change occurred together with an increase in the abundance of phanerophytes, in canopy height and seed mass, which can be associated with the colonization by woody and fringe community species. The increase in woody species is a common process in semi-natural grasslands and it is usually associated with cessation of management (Bennie et al. 2006; Jírová et al. 2012; Timmermann et al. 2015; Stevens et al. 2016). For instance, in Danish grasslands trees and shrubs significantly increased their cover after the reduction in management intensity (Timmermann et al. 2015), and similar results were found in English calcareous grasslands (Stevens et al. 2016). In our study, we also found an increase in species that, although non-woody, indicate successional dynamics, such as *Hypericum perforatum* or *Knautia arvensis*, whose increase was already found in relation to the cessation of management (Bennie et al. 2006; Jírová et al. 2012). Indeed, the absence of biomass removal by grazing animals allows for litter accumulation that creates relatively mesic conditions and facilitates tall and high SLA species, which effectively compete for light with small and light-demanding species typical of calcareous dry grasslands (Saar et al. 2012; Timmermann et al. 2015).

However, some species that increased over time and have a relatively high canopy and SLA, are toxic to ruminants (Marsh & Clawson 1930) and could spread under high grazing intensity (*Asphodelus albus* pers obs). Nevertheless, most of the species that indicate high grazing intensity invest few resources in height growth and per unit leaf area (i.e. short and high SLA plants) so that they have less accessible leaves growing near the soil surface and are able to rapidly regenerate their leaf structures (Moog et al. 2005). In our study, several species associated with the new plots have these characteristics and some of them, such as *Cynodon dactylon*, *Cynosurus echinatus* or *Veronica arvensis*, are also characterized by other adaptations to frequent disturbance, i.e. clonality or annual life form (Pakeman 2004; Pérez-Harguindeguy et al. 2013).



**Fig 3.** Regression coefficients (diamonds) and confidence intervals (gray lines) of the variables included in the final mixed effects model (random intercept and slope) on the CWM-shifts of the three life form categories and the three quantitative traits. (a) hemicytrophytes, (b) therophytes, (c) phanaerophytes, (d) SLA, (e) canopy height and (f) seed mass. Even if not significant, explanatory variables were retained in the figure if they were used also in the random part (intercept and slope models). Confidence intervals were calculated as the average  $\pm$  1.96 SE (0.975 quantile of the normal distribution).

Altogether our results suggest that both the abandonment of some areas and the intensification of grazing in some others, are affecting the changes in species composition over time. This regional pattern is in accordance with our hypotheses and it is highly representative of what is happening throughout Europe, i.e. the abandonment of the most remote pastures and the intensification of the use of the most accessible and productive ones (EEA 2010; Halada et al. 2011). In addition to this general trend, what we observed (i.e. the increase in species indicating either successional dynamics or intense grazing disturbance) is also due to a trend that is occurring at a more local scale. In particular, across the Apennines the traditional grazing by sheep has been largely replaced by grazing of freely ranging cattle and horses that tend to over-exploit small areas rather than to feed over wide areas (Catorci et al. 2012). This change in management tends to magnify the effect of the reduction in the overall number of grazing animals, leaving wider areas to abandonment and producing over-grazing in those areas that are still managed.

By analysing the species that increased or decreased over time in a community perspective, it is clear that many of these are diagnostic of the habitat 6210(\*) or of the associations that are included therein; but while dominant or co-dominant generalist species (e.g. *Festuca gr. ovina*, *Phleum hirsutum*) have tended towards a stronger

dominance, those usually occurring with low abundance values have faced a decrease (e.g. *Satureja montana*, *Armeria canescens*, *Helianthemum apenninum*). This confirms the results found by several authors (Bennie et al. 2006; Diekmann et al. 2014; Timmermann et al. 2015) and it is likely to result in a decreasing evenness at the expenses of the  $\alpha$ - and  $\beta$ -diversity of the habitat (Stevens et al. 2016). Indeed, the spread of tall grass dominant species, which is probably linked to their competitive ability, leads to a decrease of those species with poor seedling establishment and survival under competitive conditions (Saar et al. 2012).

### Topography and soil parameters affected the changes more than other factors

The largest changes in species composition and functional traits occurred at the sites with low slope values and thick soil organic horizon. The strong positive relationship of soil organic horizon with the compositional changes, the shifts in phanerophytes and the three quantitative traits clearly indicates the high relevance of this soil parameter in facilitating successional dynamics. However, due to the tight relationship that exists between this soil layer and the vegetation, the thickness of the soil organic horizon should be considered as both a factor influencing successional change and an outcome of this process (Loydi et al. 2014). Indeed,



succession implies litter accumulation and a fast decay, which in turn enhance the development of a thicker soil organic horizon; at the same time, more biomass on the ground surface favours species typically occurring in intermediate stages of succession (Jírová et al. 2012).

By analysing this variable together with topographical conditions and management intensity it is possible to achieve a better interpretation of which are the factors that affected the successional changes in vegetation. Indeed, also altitude, slope and grazing were shown to significantly affect both compositional and functional changes, although with different degrees and with an opposite sign when compared to soil organic horizon. The joint analysis of these factor points to a more rapid colonization of the lowest and flattest areas by species usually occurring in intermediate stages of succession or by shrubs and trees. In the flattest areas more mesic conditions associated with deeper soil enhanced the development of tall-growing competitive species and the invasion of woody species (Pärtel & Helm 2007). Where grazing pressure is scarce, these plants spread successfully at the expense of less competitive herbs. On the contrary, steeper slopes are buffered to some extent against the colonization by competitive species. Indeed, these steeper areas are often characterized by higher abundance of rocks and stones (see correlation between slope and stone in Appendix S4), which are assumed to be more suitable to dry grassland species (Bennie et al. 2006).

The colonization of grassland patches by woody species may also derive from the combination of the degree to which seed sources were available in the past (Bagaria et al. 2015) and of the proximity to source populations (Dovciak et al. 2015). The positive relation between seed mass and percentage of adjacent forest indicates that even if heavy-seeded species may be dispersed through long distances by birds and rodents, e.g. *Corylus avellana*, *Prunus spinosa* or *Quercus pubescens* (Kollmann & Schill 1996), the abundance of mother shrubs and trees was a key driver of woody encroachment. Moreover, most of the plots that faced successional development were close to forest patches (mean distance 108 m) with higher chances to be colonized.

Other relevant variables that influenced compositional change were soil pH and salinity, which showed heterogeneous relationships with several functional traits. The importance of pH can be explained by the strong influence of limestone bedrock on shallow soils that characterized our study areas (see also Jírová et al. 2012). In fact, 40% of the new plots have shallow soils (<24-cm deep) and 88% have pH values > 6. On these soils, the succession is likely to act through the colonization by sclerophyllous shrubs with low SLA but heavy seeds and a canopy height above that of most herb species (e.g. *Arctostaphylos uva-ursi* and

*Juniperus communis*). Therefore, soil pH influences the pathway of woody encroachment by mediating the environmental conditions suitable for the colonization by either sclerophyllous shrubs or woody species adapted to more mesic conditions.

The concentration of salts in the topsoil is generally associated with the continued presence of animals (Di Bella et al. 2014) as was confirmed by the slight positive correlation of this variable with grazing (Appendix S4). This association helps to explain the relationship between soil salinity and the abundance of therophytes, which were often related to frequent grazing intensity (Pakeman 2004). Nevertheless, contrary to our expectations, salinity has a positive relationship also with the abundance of hemicryptophytes and woody species.

Among the climatic variables only the increase in the minimum temperature positively affected compositional change and the occurrence of both annual and woody species, and this is not surprisingly since this parameter is likely to favour both the successional changes at mid to high altitudes and annual species at low to mid altitudes.

## Conclusion

Our approach combined the re-visitation of historical plots associated with detailed vegetation maps and stratified random sampling based on environmental variables. By using this sampling strategy and the historical species pools as a reference we were able to quantify the communities' compositional and functional changes, avoiding paired comparison of historical and new plots and thus reducing the probability of pseudo-turnover. The combination with the analysis of functional traits clarified the ecological processes that influenced these changes, as well as their effects on species composition and habitat structure.

We were able to show substantial changes in species composition and functional traits of *Bromus erectus* semi-natural grasslands along the central and southern Apennines during the last five decades. The shifts in functional traits allowed us to differentiate two different directions of change in relation to the interplay of management and environmental factors. Lowest and flattest areas with low grazing intensity showed a clear successional path with a substantial increase of woody and competitive species. In contrast, a smaller number of sites faced an increase in herb species associated with frequent grazing disturbance.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Summary of the historical data set.

**Appendix S2.** Results of PERMANOVA within each of the 17 AC groups.

**Appendix S3.** Results of RDA on species abundance.

**Appendix S4.** Tables of correlations between Bray-Curtis dissimilarities and CWM shifts, and all the explanatory variables included in the mixed effects models.

## Graphical Abstract

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Re-visitation of historical plots is a precious tool to assess vegetation changes. By using stratified random sampling and historical species pools as a reference, we quantified changes in species composition of Apennines semi-natural grasslands. We identified the ecological processes behind these changes through the analysis of functional traits, and found two main trends: successional dynamics and grazing disturbance.