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A comparative analysis of functional traits in semi-natural grasslands under different grazing intensities

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Abstract. The reduction of traditional management practices is a major threat for the conservation of permanent grasslands in many European marginal areas. The ecological importance of grasslands is acknowledged by the European Habitats Directive 92/43/EEC (1992) which includes many natural or semi-natural grassland types, and by the growing attention of society towards functions and services provided by these ecosystems. Nonetheless, the efficiency of conservation policies is questioned also for the lack of local-scale information on trends and state of grasslands hampers the definition of local-tailored schemes. The main objective of this work is to assess the potential of a set of functional traits in discriminating between different management intensities and their capacity to describe the dynamics occurring in semi-natural grasslands. The research was carried out in a hilly area of Tuscany (Italy) on four grassland sites characterized by similar environmental features (soil, climate, topography), and by different management practices for 10 or more years. The survey concerned collection and analysis of different functional traits related to foliar features, litter and botanical composition. The functional traits were able to differentiate the four sites under different management practices, but their effectiveness was different. Results support the possibility to perform a rapid appraisal of grassland successional stages based on leaf functional traits of dominant species and by the assessment of presence of a reduced number of species among those occurring in the community.

Key words: life forms, leaf dry matter content, leaf nitrogen concentration, litter, specific leaf area.

INTRODUCTION

Grasslands are one of the most widespread ecosystems at global level which are represented by many vegetal communities adapted to a range of different environmental conditions (Dixon et al., 2014). The importance of grasslands is not only related to forage production (Conant et al., 2016), but also to the provision of a number of other ecosystem services (Primi et al., 2016) such as protection of vegetal biodiversity (McAllister et al., 2014; Rossetti et al., 2015), conservation of open space for wildlife (Crosby et al., 2015; Cervasio et al., 2016), maintenance of areas for touristic activity (Dossche et al., 2016), and landscape preservation (Argenti et al., 2011; Schmid et al., 2017). Their ecological importance is also acknowledged by the European Habitats Directive 92/43/EEC (1992) which includes many natural or semi-natural grassland types.

In the last decades, complex socio-economic changes have caused the abandonment of traditional management practices such as mowing or grazing in many European marginal areas (Török et al., 2016). That has triggered significant successional dynamics of the herbaceous ecosystems (Dengler et al., 2014). Currently, shrub encroachment and natural reforestation are the main vegetation dynamics which entail negative consequences on biodiversity and landscape aesthetics in marginal grasslands (Argenti et al., 2012; Koch et al., 2015; Braunisch et al., 2016). For these reasons, the Common Agricultural Policy includes measures to encourage the utilization of marginal environments. However, these measures are not always effective for the conservation of grassland biodiversity because they are not able to target efficiently the territorial heterogeneity of mountain areas and they do not strictly focus on the management of grasslands (Burrascano et al., 2016). Consequently, the availability of local-scale information on the grassland conditions is necessary to define conservation strategies able to optimize the efficiency of interventions (Lengyel et al., 2012). Indicators assessing the structure of plant communities in relation to land use and sensitive enough to suggest appropriate grassland management may be used to improve the effectiveness of the agro-environmental programs (Kampmann et al., 2012). However, indicators usually require specific expertise including an advanced knowledge of species taxonomy and local context. Indeed, availability of experts and labour costs are acknowledged among the main hurdles to improve the information base for the policy design (Targetti et al., 2014). The possibility to improve the availability of local-scale information, on the other hand, is necessary to enhance the efficiency and legitimation of agri-environmental schemes (Armsworth et al., 2012; Pe'er et al., 2014).

Functional traits include different plant features, such as morphological, ecophysiological and reproductive ones (Harrington et al., 2010). Some traits concern leaf characteristics of dominant species, whereas others are derived from vegetation composition as growth forms, flowering features or biomass partition (Schweiger et al., 2017). Relationships among these indicators and environmental and management factors are well established and -hence- their employment in ecological modeling is suggested to be potentially useful (Yang et al., 2015). For instance, plant functional traits (PFT) have been used as a key to link plant communities to ecosystem services and are considered able to reveal variations in local environmental conditions (Schweiger et al., 2017). PFT have been also tested in comparative analyses, to underline dynamics related to disturbance regimes, to assess relations among ecosystems services and management and to describe successional stages (Liu & Su, 2017). Nonetheless, constraints concerning data collection have been raised because the collection of functional traits is very often time-consuming and difficult in particular in marginal areas (Musavi et al., 2016).

Main questions arising from previous analysis of the state of the art are those concerning the efficiency of functional traits in explaining vegetation characteristics and dynamics and in the choice of such traits among a large amount of different possible alternatives. Trying to answer to these issues, the objective of the present paper is to assess the potential of a selected set of functional traits to describe the vegetation dynamics in semi-natural grasslands and to test their capacity of discriminating sites with different management history. The research was carried out in a hilly area of Tuscany

(Italy) on semi-natural grasslands characterized by similar environmental features (soil, climate, topography) and presenting different management intensities (animal stocking rate) for 10 or more years.

MATERIALS AND METHODS

The case study area is located at an altitude of 700 m asl in Mugello (North Apennines, Tuscany), a rural region characterized by high presence of semi-natural grasslands. The survey was carried out in semi-natural grasslands assigned to the *Festuco-Brometea* (Br.-bl. et Tuxen) phytosociological class (Ellenberg, 1988). The area is characterized by mean annual temperature of 11.3 °C, with precipitations concentrated during autumn and winter. The mean annual rainfall is about 1,000 mm with an annual potential evapotranspiration of 950 mm. The soil of the study area is classified as silty-loam with a neutral reaction.

Information collected by means of interviews with local farmers allowed to identify the most common management practices and the grassland sites where it was possible to retrieve information on past management of the last ten years. Subsequently, environmental conditions, ecological characteristics and landscape homogeneity were considered for the selection of the grassland sites.

Figure 1. Images from the study site grassland plots.

The selection allowed to identify four sites covering a gradient of grazing intensity (Fig. 1):

- high intensity (site A), mown in spring (usually May) and then grazed with an average stocking rate higher than 0.65 livestock unit (LU) ha^{-1} yr^{-1} in the last ten years;

– medium-intensity (site B), stocking rate between 0.45 and 0.65 LU ha^{-1} yr^{-1} in the last ten years, no mowing performed;

– low-intensity (site C), stocking rate between 0.25 and 0.45 LU ha^{-1} yr^{-1} in the last ten years, no mowing performed;

– abandoned area (site D), where no utilization (grazing or mowing) was performed in the last 25 years and presenting a vegetation mosaic of grasslands and shrub species.

A reference period of 10 years featuring the same or very similar pastoral management practice and stocking rate for each site was considered sufficient to balance floristic composition and functional characteristics in response to livestock grazing regime. All the pasture sites were fenced and grazed with horses as horse breeding is an economic alternative to traditional cattle grazing in the area due to progressive abandonment of dairy and cattle farming over the last decades (Targetti et al., 2013).

At each site, two permanent plots (25 m x 4 m) were randomly selected in order to reflect average environmental conditions and to avoid edge effects. To take into account climatic variability, field sampling covered two years (2006 and 2007) and two growing seasons (May and September) in each year. Vegetation composition was assessed in each plot: a permanent transect line (25 m-long) was established and vascular plant species were recorded every 50 cm according to the point quadrat method (Daget & Poissonet, 1969). Floristic nomenclature of all identified species refers to Pignatti (1982). The percentage contribution of each species (specific contribution, SC) in the sward was quantified according to the formula:

$$SCi = \frac{SF_i}{\sum_{k=i}^{n} SF_i} \times 100 \qquad (1)$$

where SF_i is the specific frequency of a given species, *i.e.* the number of times that a single species is recorded along the transect (Argenti & Lombardi, 2012).

In the present work, we considered three different categories of functional traits:

– leaf functional traits: specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC) (based on field survey);

– vegetation traits: life forms, start and duration of flowering (based on existing database);

– characteristics of litter layer: dry weight, average weight per area, and density (based on field survey).

Leaf functional traits (SLA, LDMC, LNC) were investigated on three different grass species, namely *Brachypodium rupestre, Bromus erectus* and *Festuca* gr. *rubra* (following Pignatti, 1982) which were the most frequent in the plots (average presence between 7 and 24% in the four sites) and in agreement with other researches that emphasized the possibility of functional traits evaluation only on a reduced number of species among those occurring in the grassland community, particularly if the traits are weighted (Ansquer et al., 2005). We considered these species as a useful guide for interpreting the dynamic of the plant communities as response to environmental and management changes (Grime, 2001). SLA measures the light interception per leaf biomass unit, and it is often correlated with the potential growth rate of the species. LDMC is related to the average density of tissue and it is a measure of stress tolerance, as it has important consequences for leaf energy and water balance (Cornelissen et al., 2003). LNC is the total amount of nitrogen per dry leaf mass unit and it is strongly correlated to the concentrations of leaf nitrogen compounds involved in photosynthesis (Garnier et al., 2004).

Vegetation samples for SLA, LDMC, and LNC measurements were collected close to the line transects at 5, 10, 15, 20 and 25 m interval. Specific leaf area and leaf dry matter content were performed at maximum biomass following a standard protocol proposed by Garnier et al. (2001). SLA was calculated as the ratio of leaf area to dry mass and LDMC was calculated as the ratio of leaf dry mass to fresh mass on young and fully expanded leaves. LNC was determined on 240 samples of dry fully expanded leaves of each species. For further details, refer to Targetti et al. (2013).

The average weighted values for Leaf Functional Traits (LFT) on the three target species were calculated as follows:

$$wLFT_{i,s} = \left(\frac{p_{i,s}}{\sum_{i} p_{i,s}}\right) \times LFTi \tag{2}$$

where $wLFT_{i,s}$ is the weighted leaf functional trait of species i in transect s; $p_{i,s}$ is the relative abundance of species i in transect s; LFT_i is the average leaf functional trait of species i.

Based upon existing database (Pignatti, 1982), functional traits including life forms (*sensu* Raunkiaer, 1934), and start and duration of flowering were also assessed. These traits were selected with the aim to evaluate the performance of easy to measure traits which were available for a wide range of species and, therefore, transferable to sites with different species composition (Kahmen & Poschlod, 2008). Raunkiaer's life forms is an integrative trait comprising plant species strategies to survive to adverse seasons. We considered 6 categories of life form: therophytes, geophytes, hemicryptophytes, chamaephytes, nano-phanerophytes and phanerophytes. Concerning the start and duration of flowering period, three categories for the trait 'start of flowering period' (March or early, April or May, and June or later) and three categories for the trait 'duration of flowering period' (1 month or less, between 2 and 3 months and more than 4 months) were considered. The contribution of these traits was assessed for the complete set of species for each transect. Since these traits are categorical, the contribution of each trait category was calculated as the sum of the relative contribution of each species within that category.

The parameters referring to the litter layer were adopted since grazing pressure may affect plant characteristics primarily via biomass and litter removal (Mapfumo et al., 2002). Five litter layer heights (cm) were randomly measured in a sampling quadrat (0.25 m x 0.25 m) and repeated three times for each plot and sampling period. A sample of the standing phytomass was collected in the same quadrat. Each sample was transported and dried in laboratory at 60 °C for 48 hours until a steady weight was reached. Then the samples were weighted to determine dry weight (g), average litter weight per area (g m^{-2}), and density (g m^{-3}).

Linear discriminant analysis (LDA) was performed after standardization of data to assess the effectiveness of the measured functional traits in discriminating the four studied pasture sites. LDA is a multivariate statistical technique which classifies a set of variables in terms of their capacity to 'discriminate' different groups of observations. The technique is usually used for dimensionality reduction in data mining and for the interpretation of the importance of a given set of variables (R Development Core Team, 2012). The general condition of normality of data for the discriminant analysis was checked using the Shapiro-Wilk test (Shapiro & Wilk, 1965).

RESULTS AND DISCUSSION

The three grass species selected for the measurement of the leaf functional traits (*Brachypodium rupestre, Bromus erectus,* and *Festuca* gr. *rubra*) were by far the most frequent in the four plots, except in site B where *Arrhenatherum elatius* was more frequent than *Festuca* gr. *rubra*. In Fig. 2 the relative frequency, expressed as specific contribution, and variability of the three selected species in the four different sites is presented.

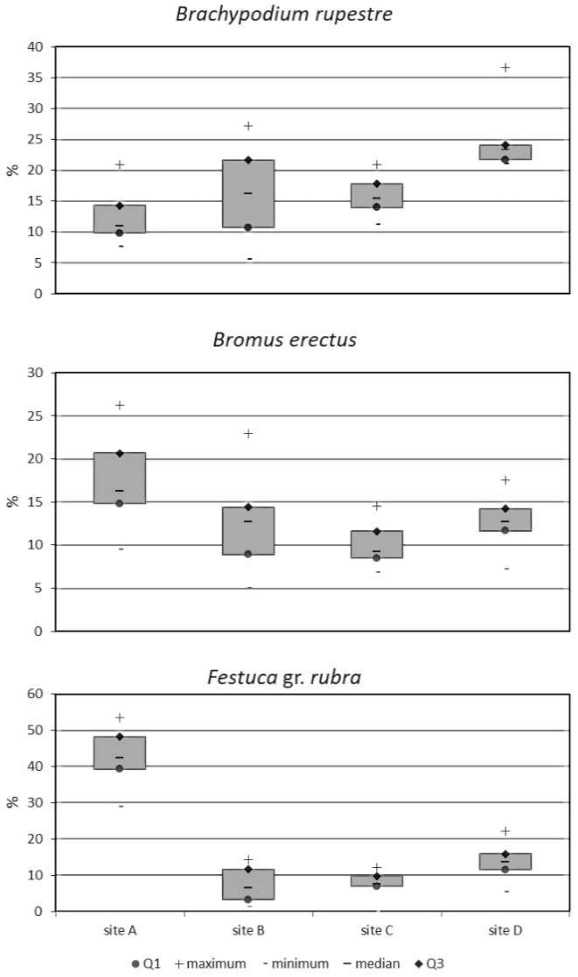

Figure 2. Boxplot charts concerning variability of specific contribution of the three most common species in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Brachypodium rupestre tended to increase its abundance as grazing management decreased; on the contrary, *Bromus erectus* displayed an opposite tendency from site A to C. *Festuca* gr. *rubra* showed a more controversial trend between sites with an increase of its presence under high grazing pressure (site A) and in abandoned plots (site D).

The weighted leaf functional traits did not show a linear relation to management intensity (Fig. 3). The variables wLDMC and wLNC reflected the gradient of management intensity from site A to site C. All the three PFT highlighted noticeable differences between sites C and D.

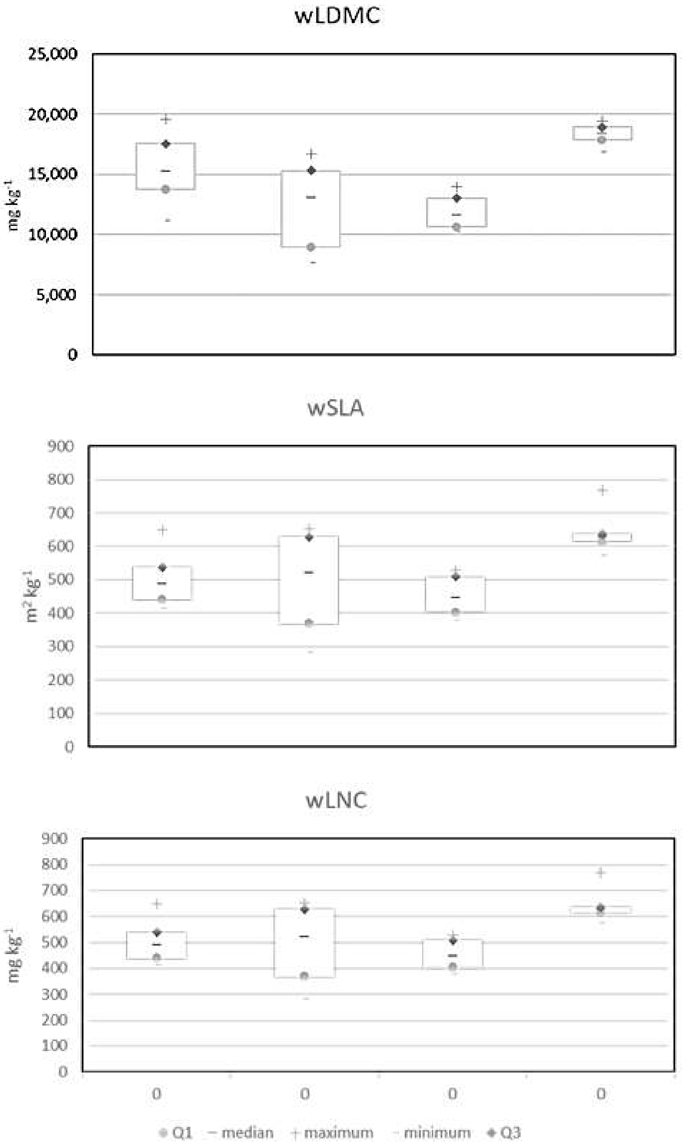

Figure 3. Boxplot charts concerning variability of the weighted leaf functional traits (wLDMC, wSLA, wLNC) for the three species in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Results show different performances of the considered variables as proxies for management intensity, and in some cases are consistent with previous studies (*e.g.* Martin et al., 2009). In particular, some functional traits seem to have a linear relation with intensity of management, whereas others highlight a gradient that does not link clearly to management. Interestingly, variables that could be assessed with a rapid botanic survey such as the wLDMC and the frequency of *Brachypodium rupestre* seem to be effective indicators of management intensity. The use of wLDMC would however be feasible only if LDMC values for the most frequent species are available as the contextual collection and measurement of that parameter is not affordable in a day-by-day assessment. The increase of *Brachypodium rupestre* and the reduction of *Bromus erectus* from site A to site D was the main cause of the highlighted trend of wLDMC. The behaviour of *Festuca* gr. *rubra* was less linear because it reached higher frequencies at the two management extremes (sites A and D) and a low frequency in sites B and C. That species has a wide ecological spectrum (Gaucherand et al., 2006) and probably takes advantage of the heterogeneous ecological conditions in site D. Despite this fact, wLDMC calculated on these three species was the parameter recording the highest coefficient in the linear discriminant analysis (see hereafter) and outlines a relevant potential as indicator related to management intensity.

The effectiveness of wSLA in differentiating the sites would likely be more evident when comparing high intensive grasslands with low-input and abandoned grasslands as reported by Kahmen & Poschlod (2008). Indeed, the relation between disturbance and higher SLA is more evident in other studies including a wider range of species and management intensities (Garnier et al., 2007). The oligotrophic character of the four sites induced to discard species typical of more fertile environments like *Lolium multiflorum* or *Dactylis glomerata*, which are characterized by high values of SLA (Ansquer et al., 2009). The high SLA value of *Brachypodium rupestre* has probably had a masking effect on the wSLA in our plots. Similar results have been presented by Saar et al. (2012) and Timmermann et al. (2015) where high SLA values in abandoned sites were related to litter accumulation and the creation of mesic conditions which facilitates tall and high SLA species, as pointed out also by Giarrizzo et al. (2017).

Raunkiaer life forms highlighted a trend following the management intensity with a gradual reduction of hemycryptophytes and terophytes from site A to site D, and with a parallel increase of phanerophytes from site A to site D (Fig. 4). The other life forms classes evidenced a non-linear relation with the different management intensities. Geophytes frequency was between 2% in site B and 0.1% in site C, whereas no geophytes was recorded in site A. Chamaephytes were present in all sites, except site B. The highest concentration of chamaephytes was recorded in site C and D (8.9 and 5.6% respectively). The differences of frequency of nano-phanerophytes across the four sites was not relevant.

As expected, management rate reduction was related with frequency of chamaephytes (shrub-species) in site C and phanerophytes (woody-species) in site D. Higher stocking rates and soil disturbance in sites A and B had a clear inhibitive effect on these life forms due to two main effects: the mechanical effect of large herbivores trampling and the annual mowing in site A, and the exceptional low grazing selectivity which is a typical feature of horses in comparison to cattle (Menard et al., 2002). Effects of grazing intensity on different vegetal functional group found in our study are consistent with previous research conducted in Mediterranean environment (Papanikolaou et al., 2011).

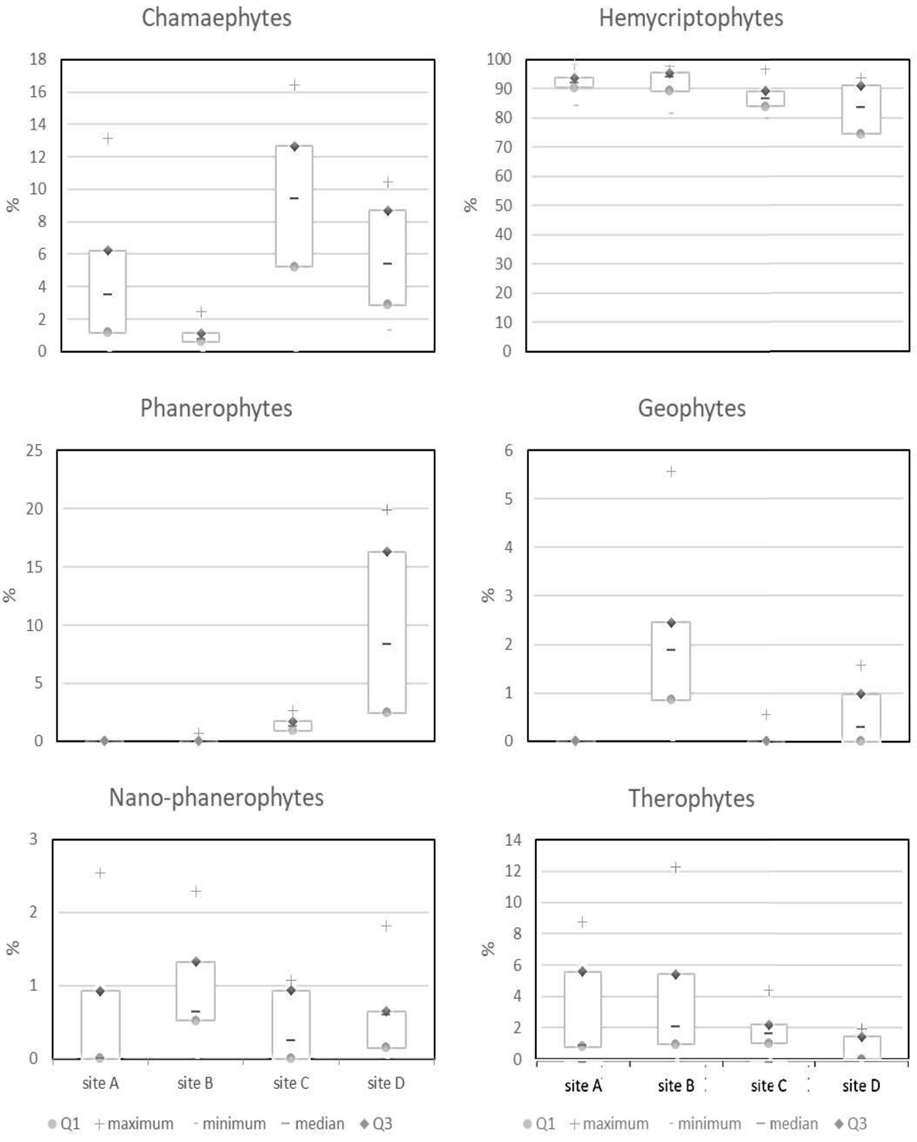

Figure 4. Relative frequency of the Raunkiaer biological forms (according to Pignatti, 1982) in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Species flowering in April and May were by far the most present in the four sites (Fig. 5).

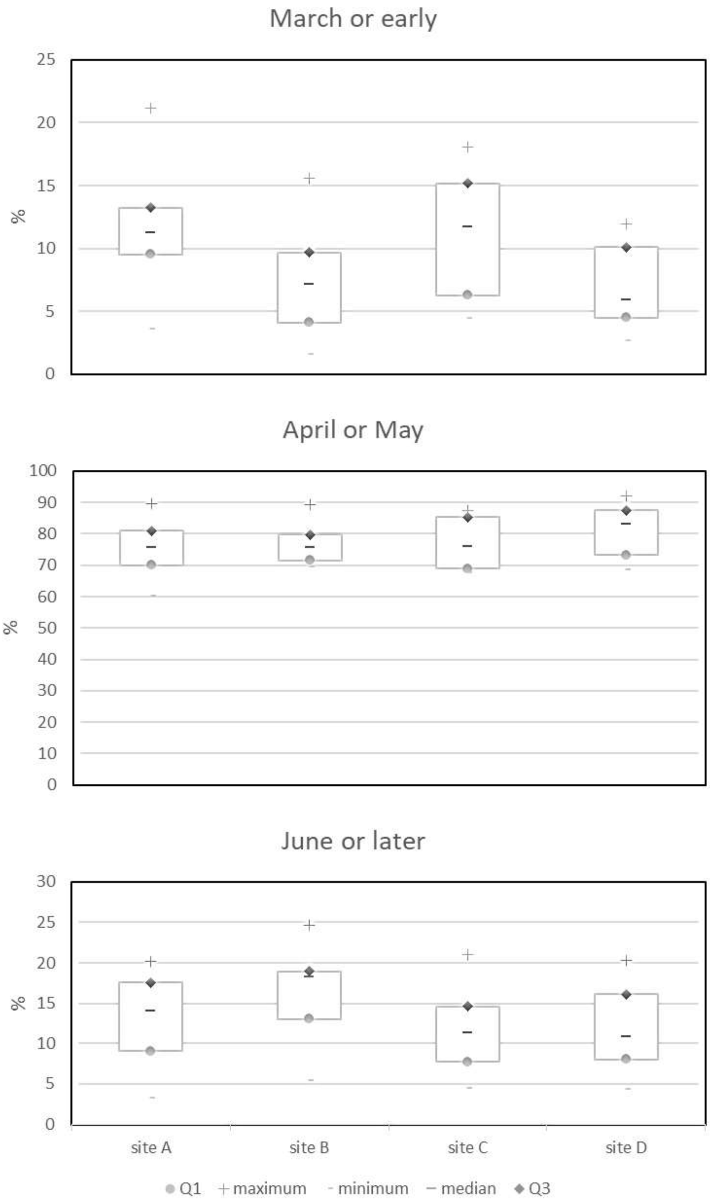

Figure 5. Relative frequency of the start flowering class (according to Pignatti, 1982) in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Even though a slightly higher frequency of early flowering species was found in sites A and C, the functional trait related to the starting of flowering period did not show clear differences between the four surveyed sites and did not seem to be linked with the management intensity. The duration of flowering seemed to be more useful as an indicator to differentiate the management intensity (Fig. 6). The frequency of species with short duration of flowering period decreased from more intensive to extensive and abandoned sites. On the contrary, the frequency of species with medium duration of flowering showed the opposite behaviour increasing from the intensive to the abandoned site even though the relation was less clear.

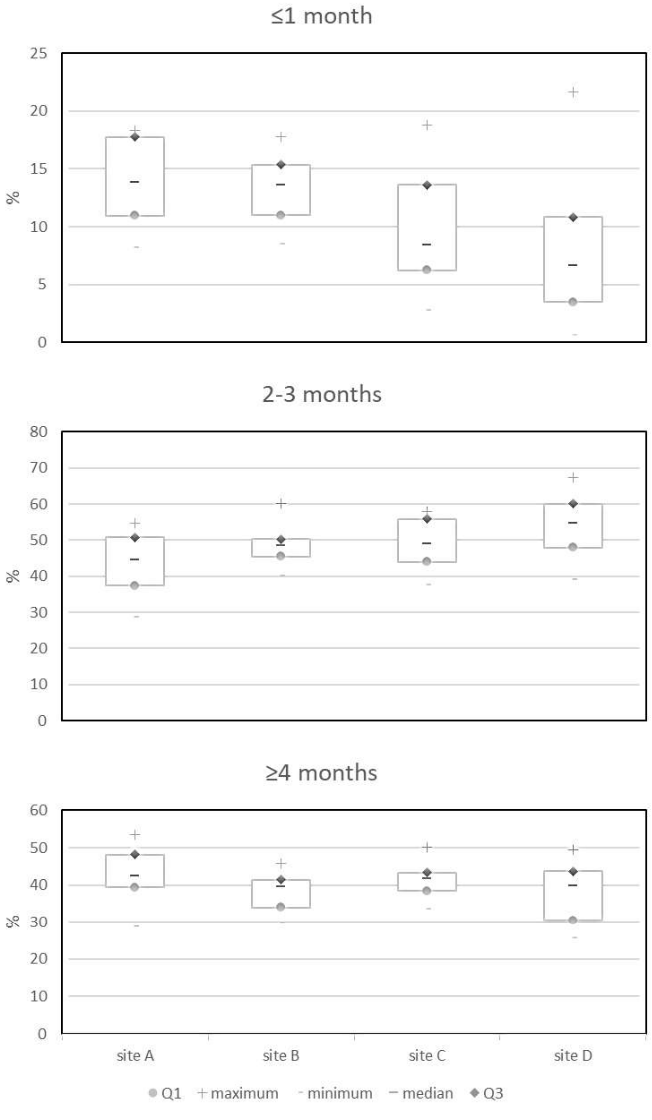

Figure 6. Relative frequency of the duration of flowering class (according to Pignatti, 1982) in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

In our study, functional traits linked with species phenology (start and duration of flowering) showed a marginal importance in relation with management and this is consistent with other studies (Farnsworth 2007; Kahmen & Poschlod, 2008; Storkey et al., 2013). Nevertheless, duration of flowering revealed an increasing trend of short flowering duration species from abandoned to high-disturbed sites. Similarly to findings of Köhler (2001), this suggested an advantage for species able to concentrate the reproductive period in areas under more severe utilization. On the contrary, delay and anticipation of flowering did not give any specific trend across the study sites.

The sites A and B recorded the highest values of litter density whereas the dry weight and thickness was higher in the site D (Fig. 7). Both the dry weight and the thickness highlighted a clear gradient from the more intensive plots to the abandoned ones.

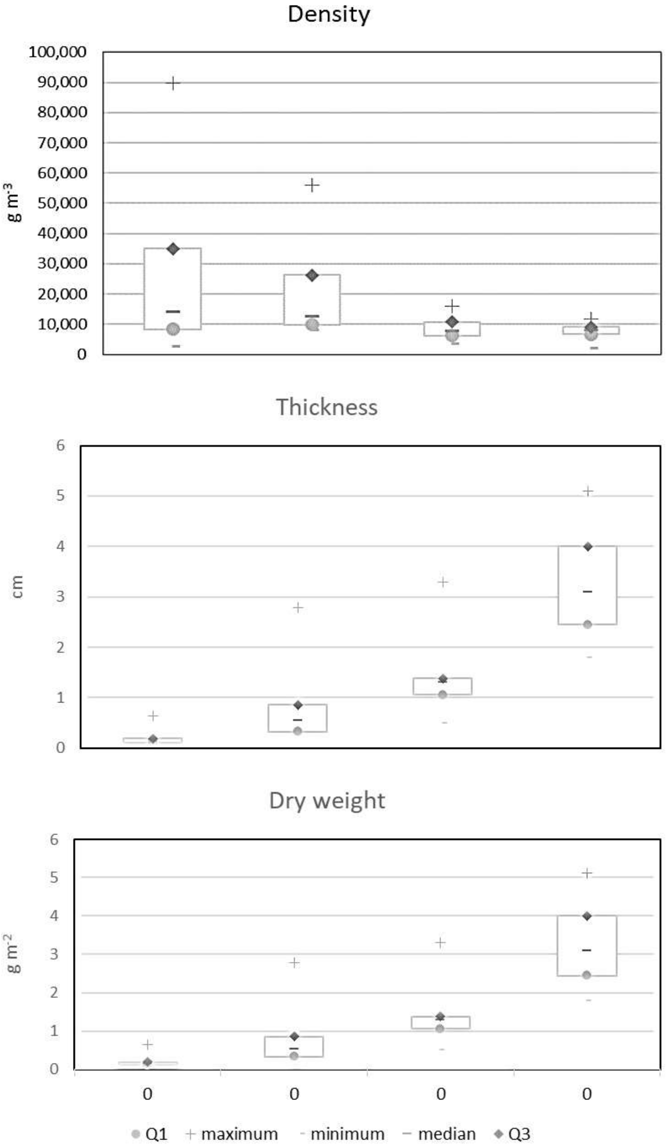

Figure 7. Thickness, dry weight, and density of litter layer in the four different sites. Q1: 1st interquartile; Q3: 3rd interquartile.

Similarly to other findings (Catorci et al., 2014), land abandonment favoured oligotrophic species such as *Brachypodium rupestre.* The litter accumulation in the abandoned site seems therefore related to the combined effect of the reduced biomass removal by grazing animals and the higher presence of grass species producing large amount of litter (Bonanomi et al., 2013).

The linear discriminant analysis (LDA) outlined clearly the difference between the four plots (Fig. 8).

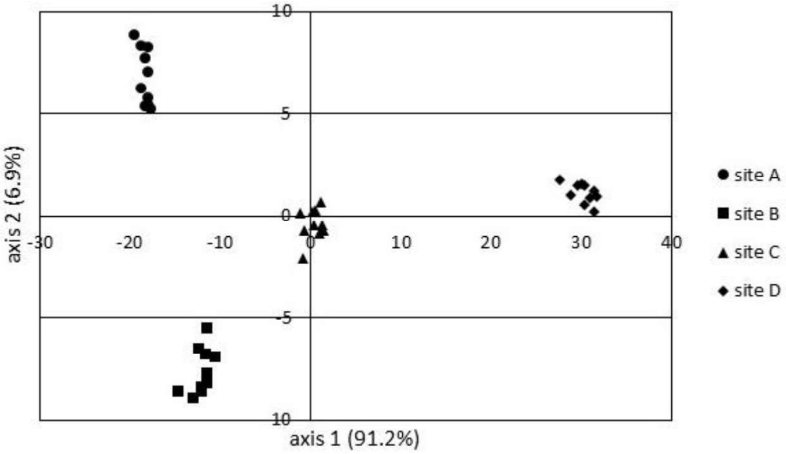

Figure 8. Linear discriminant analysis (LDA): position of observations on the first two axes (in brackets the proportion of variability explained by each axis).

The first axis accounted for more than 90% of variability and outlined the difference between site A and D. The second axis accounted for a reduced amount of explained variability (about 7%) but it seemed useful to differentiate within the managed sites (A, B, and C). By far, the first axis coefficient of *w*LDMC, *w*SLA, and the frequency of *Brachypodium rupestre* were the highest (Table 1). This result highlights the relevance of these variables in discriminating the four plots in comparison to the other considered variables. The coefficients of *Brachypodium rupestre* and *w*LDMC were high also in the second axis, where also the frequency of *Festuca* gr. *rubra* and *Bromus erectus* recorded the highest coefficients. The coefficients of the other functional trait groups were clearly lower along all the three axes.

Table 1. Linear discriminant analysis: variables scores on the first three axes

Leaf functional trait/vegetation trait/characteristics of litter layer’	Axis 1	Axis 2	Axis 3
	Coefficients of linear discriminants		
SC of *Brachypodium rupestre*	30.43	-81.76	72.09
SC of *Bromus erectus*	12.89	-47.59	33.02
SC of *Festuca* gr. *rubra*	1.48	-47.60	26.08
wLDMC	40.79	74.66	-33.96
wSLA	-38.24	24.31	-49.24
wLNC	-23.96	4.97	9.52
Chamaephytes	-1.52	-5.34	-6.58

Table 1 (continued)

Hemicryptophytes	-2.11	-9.04	-8.56
Phanerophytes	-1.29	-7.07	-6.82
Geophytes	0.06	-2.20	-0.43
Nano-phanerophytes	0.80	-0.45	-1.32
Therophytes	-0.88	-3.98	-3.25
Flowering Season A	0.48	-0.04	-0.62
Flowering Season B	-0.19	-0.17	0.04
Flowering Season C	-0.07	0.42	0.29
Flowering Duration A	-0.20	-0.39	0.49
Flowering Duration B	0.06	0.30	-0.13
Flowering Duration C	0.07	-0.07	-0.18
Litter thickness	-0.93	2.08	0.53
Litter dry weight	0.32	0.78	1.05
Litter density	1.08	-1.49	0.25

The discriminant analysis supported the interpretation of the results and showed the effectiveness of the considered parameters in discriminating the four study sites. In particular, sites B and C were clearly separated from the ‘extreme’ sites (A and D) on the first axis. This evidence suggested a good attitude of functional traits in the discrimination of the effect of abandonment in site D from the effect of the grazing/mowing management in site A. On the contrary, the studied traits were less effective in the characterization of the different grazing rates between sites B and C. Our results confirmed the potential of functional traits for the characterization of semi-natural grasslands as reported in other studies (Cruz et al., 2010).

CONCLUSIONS

In our study, functional traits were able to differentiate the four sites related with different management practices but they showed different effectiveness. In particular, results support the possibility to base the assessment of management intensity on a rapid appraisal consisting on the estimation of the frequency of a reduced set of abundant species together with the calculation of wLDMC. That involves the need of further studies to assess the feasibility and validity of such approach to different areas and at dissimilar scales. In particular, results concerning wLDMC encourage further developments for the implementation of the existing database (*e.g.* TRY database) to be used in combination with a rapid botanic assessment. This is an important knowledge-based support for land managers and local decision makers to define areas where concentrate the available resources for conservation. Nevertheless, the inclusion of other sites with different management options like inorganic inputs is particularly required for understanding the potentialities of the method as an operational tool.

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