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# Vegetation Characteristics in Relation to Different Management Regimes of Calcareous Grassland: A Functional Analysis Using Plant Traits

By

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Key words: Functional traits, calcareous grassland, management, multivariate statistics, grazing, C-S-R strategies, species richness.


## Summary

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Designation of management strategies for preservation of calcareous grasslands demands in-depth understanding of vegetation processes. For this purpose the functional approach using plant functional types and traits has been widely promoted. In this study we focused on the analysis of C-S-R established strategies and some simple plant traits to detect general trends in trait responses to abandonment on one side and to eutrophication on the other giving us a basis for future management strategies. Five treatments were applied to calcareous grassland in SW Slovenia representing different combinations of fertilization and grazing regimes. Effects of these two factors along with other environmental variables on species composition were evaluated. Trait composition of original low-intensity grazed vegetation showed importance of stress-tolerance (S component), relatively high abundance of small plants, chamaephytes, phalanx strategy and summer green plants. Abandonment increased abundance of grasses and suppressed forbs and legumes. C component, showing appearance of competitive exclusion, increased, resulting in increased average plant height. Fertilization promoted the abundance of therophytes and persistent green, mesophyllous plant species with guerrilla lateral spread. It also caused significant increase in abundance of species expressing ruderality (R component).


## Introduction

Calcareous grasslands are throughout Europe and widely regarded valuable habitats due to their diversity of flora and fauna (WALLISDEVRIES & al. 2002).

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Since these habitats in Europe are mostly agriculture dependent and are a subject of dereliction and aforestation in modern times, proper management that would take into account biological and ecological factors and social and economical interests of certain area is of vital importance. There have been several experiments on the traditional and alternative management strategies conducted (BAKKER & al. 2002, KAHMEN & al. 2002), which show that sufficient and reliable results might only be judged by long-term observations. Due to time consuming research methods the need for predictive and functional ecology has increased resulting in increasing interest of biological and ecological characteristics (traits) of species, their assembly rules, trade-offs, interactions and function within an ecosystem (PYWELL & al. 2003).

The concept of plant functional types (PFTs) has been designed to predict the impact of management measures on vegetation (KLEYER & al. 2002), moreover, the functional approach is widely used also in theoretical and practical studies of climate change (DIAZ & al. 1999), disturbance effects (LAVOREL & al. 1999), land-use change (VERHEYEN & al. 2003), and biodiversity-ecosystem function relations (LOREAU & al. 2001). PFTs are defined as groups of plants irrespective of their taxonomic status with a similar role in the ecosystem and are thus believed to have certain morphological, physiological, regeneration, life-history, phenological and other traits in common (LAVOREL & al. 1997). The whole set of species of the community is in that way reduced on a much smaller set of PFTs with their known characteristics. The concept of PFTs is quite stretchable. Species might be classified very generally (e.g. Raunkiaer's life-forms (RAUNKIAER 1934), Grime's CSR established strategies (GRIME 1977), Westoby's LSH scheme (WESTOBY 1998) or specifically on response to a certain environmental constraint (e.g. response to sheep grazing, response to lead pollution, etc.) using inductive or deductive approaches (GITAY & NOBLE 1997).

This paper focuses on functional aspects of plant species and communities of a calcareous grassland under different management treatments. Grazing and fertilization effects are evaluated to give some basic theoretical conclusions for future management possibilities. Grazing is regarded as an essential method for nature conservation of low-productivity grasslands (WATKINSON & ORMEROD 2001) although there are also some negative experiences of this management type (FISCHER & WIPF 2002). Nutrient deficiency is the most prominent inhibitor of productivity of calcareous grasslands. Fertilizing generally decreases diversity of plants (RAJANIEMI 2002) but as phosphorus is regarded as the most limiting nutrient of dry grasslands (VIDRIH & LOBNIK 2003), we tested some combinations of N, NP and NPK fertilization to evaluate the effect on community structure. Some facts about vegetation characteristics of early succession stage following abandonment are also presented in this study and compared with other observations showing clear shifts of the communities towards dominance of competitive species (BAZZAZ 1996). The study has the following main objectives: (1) to compare plant species richness and diversity in different grazing and fertilizing regimes, (2) to determine distribution of C-S-R established strategies sensu GRIME 1977 across these regimes and to analyse their relation to environmental factors, plant species richness and diversity, (3) to evaluate individual plant species distribution patterns in relation to management regimes and other environmental factors, (4) to determine the relationship between occurrences of some general plant functional traits and management regime, and (5) to discover shifts of plant function due to eutrophication and dereliction phenomena.

## Material and Methods

The study was conducted on the mountain Vremščica area (SW Slovenia), on the pasture belonging to the Centre for Sustainable Recultivation, where small ruminant grazing was reintroduced in 1992 after the area had been abandoned in the previous decades. Before abandonment, transhumant grazing was present on this area. Due to high altitude (830 m.a.s.l.) and frequent drought in summer the vegetation period is short and consequently grazing is limited between June and September. The climate is transitional between mediterranean and continental with mean annual rainfall of 1800 and 2000 mm with a noticeable dry period in August. The mean annual temperatures are between 6.0 and 7.0 °C with mean minimum temperature of -5.0 °C in January and mean maximum temperature of 21.5 °C in July. Severe NW wind (burja) is also typical of this area. The soil is classified as rendzina, with quite unstable soil depth (frequent pockets of deeper soil in between shallower areas) and is lying on a limestone substrate. Grassland vegetation of the study site belongs to Festuco-Brometea Br.-Bl. et Tx. 43 class. Depending on the nutrient availability and land use type it passes from Mesobromion erecti Br.-Bl. et Moor 36 alliance with higher degree of mesophyllous plant species to more thermophyllous and oligotrophic Satureion subspicatae Ht. 62 alliance. Grasslands in the vicinity, where the soil is even shallower and rocky, are classified as Carici humilis-Centaureetum rupestris Ht. 31.

Vegetation and environmental data sampling were carried out in 2004 taking into consideration all the activities from the past 11 years since the area had been recultivated. Five treatments were included in the research to evaluate the effects of two factors: nutrient availability (especially phosphorus) and grazing/no grazing regime. Since all the combinations of these two factors could not be carried out, we chose the following: treatment A: grazing + fertilization with 30 kg N/ha and 90 kg P/ha, treatment B: grazing + fertilization with 90 kg P/ha, treatment C: grazing + fertilization 30 kg N/ha, 90 kg P/ha and 90 kg K/ha, treatment D: grazing + no fertilization, and treatment E: no grazing + no fertilization. Five plots representing five treatments were split into twelve 3 x 5 m large rectangles where vegetation sampling was conducted over three terms (beginning of June, end of July, middle of September) investigating four random rectangles at each term. Plant species abundance was estimated using the Braun-Blanquet method. In the spring term, soil samples were collected in four replicates per each treatment. Laboratory analysis of these samples included total N, total C and organic matter, available phosphate, and soil reaction. Since available N was not measured we estimated it using Ellenberg N values (ELLENBERG & al. 1992). Other Ellenberg values were also calculated as abundance-weighted means. Nomenclature of species follows taxonomic key Mala flora Slovenije (MARTINČIČ & al. 1999).

We chose some simple plant attributes that are generally considered as being surrogates for so-called hard traits. The latter are difficult to measure but are directly related to specific function (WEIHER & al. 1999). Special emphasis was given on traits that are related to grazing and nutrient availability (see LANDSBERG & al. 1999, LAVOREL & al. 1998, MCINTYRE & LAVOREL 2001 for lists of traits). The trait data for species recorded was obtained from BiolFlor database (KLOTZ & al. 2002) and other sources (GRIME & al. 1988, HUNT & al. 2004). The following set of simple morphological descriptions of plant species was compiled: 'life form' sensu RAUNKIAER 1934, 'guild (group) of grassland species' (grass, sedge, forb, legume, woody plant), 'life span' (annual, perennial, biennial), 'height' (three classes - H1 (<10 cm), H2 (10-25 cm), H3 (>25 cm)), 'lateral spread type' (guerrilla, phalanx, compact, therophyte), 'canopy structure' (erosulate, hemirosette, rosette plant), 'leaf persistence' (summer green, persistent green, over wintering green, spring green), 'leaf anatomy' (hygromorph, mesomorph, skleromorph), and 'reproduction type' (seed,

seed/vegetatively, mostly vegetatively). Additionally, 'grazing tolerance', 'trampling tolerance' and 'forage values' were collected from BiolFlor database (9 class scale) as we regarded these as ecologically relevant descriptors. For six species (all of them low abundant) out of 166 recorded, trait data was not available and these species were excluded from the analysis. Secondly, species were classified with respect to established strategies (functional types) sensu GRIME 1977; to each species one of 19 functional types (HODGSON & al. 1999) was assigned and that classification was used to derive 'functional signature' for the whole vegetation sample. Using the methodology of HUNT & al. 2004, average values of C, S and R dimensions were calculated for each vegetation sample.

The data was compiled into three matrices: floristic data (160 species × 60 samples), environmental data (7 environmental factors × 60 samples) and plant trait data (160 species × 9 plant traits). Two canonical correspondence analyses (CCAs) were conducted using CANOCO for Windows 4.5 (TER BRAAK & ŠMILAUER 2002): one on floristic matrix with environmental factors as explanatory variables and one on transposed floristic matrix and trait data as explanatory variables (trait attributes transformed as dummy variables). Environmental factors were management treatment (grazing, fertilization), soil parameters and Ellenberg values (F, N, K). These factors showed sufficient variability among samples and were not highly correlated. Forward selection of variables within CANOCO and Monte Carlo permutation tests (499 permutations) were used for the selection of significant explanatory variables at $p < 0.05$. Variations in relative abundance of individual plant attributes across treatments were analysed using general linear model (GLM) within Statistica 6.0 software. Due to simple experiment design, results of both CCAs and GLMs were directly interpretable; hence, there was no need for numerical analysis of plant trait-environment relationship.

## Results

Before continuing with the analysis we evaluated the influence of the term of sampling using some additional statistical testing (correspondence analysis, GLM). As the term of sampling showed no significant differences regarding vegetation composition and plant trait response, we treated the whole set of 12 samples of each treatment as being one term. That shows the characteristic of grasslands of the study area - growth burst in late spring and negligible growth later on.

### Plant species richness and diversity

In total, 166 plant species were recorded in the study. As expected, plant richness was the lowest on fertilized plots. There were no significant differences between treatments A, B and C (median 36 species per sample). Species richness was highest on treatment D (50 species) but did not differ significantly from treatment E (46 species). Fertilization showed to be a more influential factor than grazing, considering plant species richness. On the contrary, grazing appeared important for the diversity of vegetation samples. Shannon index of diversity, showing distribution of species and evenness of vegetation stand, was the lowest in treatment E (mean 1.32) due to the dominance of *Brachypodium rupestre* and low abundance of other species. Diversity was highest on treatments B (mean 2.17) and C (2.5) and intermediate in A (mean 1.80) and D (1.87).

### C-S-R functional signature of vegetation samples

General linear model of C, S and R components of vegetation samples showed highly significant differences between treatments ($F_{8,11} = 39.9$, $p = 0.000$) (see Fig. 1b for ratios of components). Ordination of samples is presented in standard C-S-R ternary diagram (Fig. 1a) showing relative importance of R component (ruderality) in treatments A, B and C. Variability of fertilization regimes displayed no significant differences. The absence of fertilization resulted in significant increase of S component (stress tolerance), which is especially evident in samples of treatment D. R and S components were highly correlated ($r = -0.89$, $p = 0.000$). In samples of nongrazed plot (treatment E) component C (competition) was significant, showing dereliction and competitive exclusion effects. In short, compared to grazing, nutrient availability appeared as a more fundamental factor of the relative proportions of C-S-R functional types.

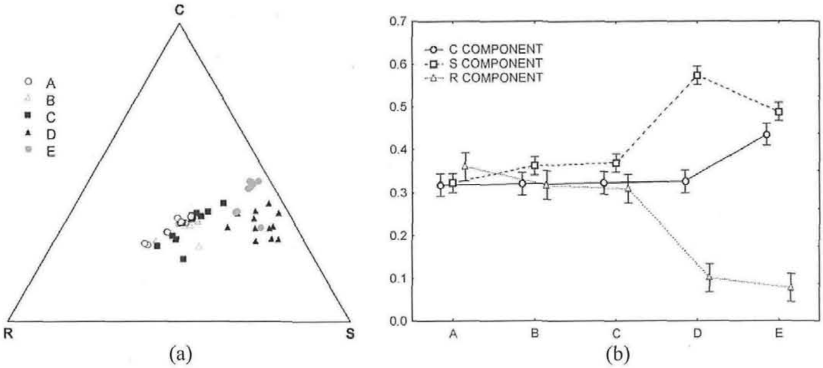


Fig. 1. (a) C-S-R ordination of vegetation samples. (b) Ratios between C-S-R components of vegetation samples across treatments (vertical bars indicate 95 % confidence interval).

Relating vegetation composition to treatments

Six environmental factors out of seven (Fig. 2a) were significant for floristic gradient (measured pH was excluded and is displayed as supplementary variable on Fig. 2a). Eigenvalues of the first 4 canonical axes were 0.32, 0.11, 0.07 and 0.06. These axes explained cumulatively 15.0, 20.1, 23.3 and 26.0 % of species data and 51.2, 68.5, 79.6 and 88.9 % of species-environment relationship. First canonical axis is negatively correlated with fertilization regime, phosphorus availability and Ellenberg N value and positively with Ellenberg K. Second axis is well correlated with the presence of grazing and Ellenberg F value. Samples are ordinated in three clusters (Fig. 2a). Since the fertilization regime was reduced on presence/absence of fertilizing, differences between A, B and C treatments were not expected. The bulk of plant species are ordinated around axis 1 showing to be present in the majority of samples. Species associated with treatment D are the species, typical of low-intensity managed grasslands of the investigated area (*Carex humilis, Potentilla australis, Coronilla vaginalis, Thalictrum minus, Polygala nicaeensis, Leucanthemum liburnicum*, etc.). In treatment E some species of forest edge, scrubland and abandoned grasslands were present, namely *Thalictrum aqui-*

*legiifolium, Rubus idaeus, Daphne mezereum, Hypericum perforatum, Astrantia major*, etc.

Plant trait analysis

Canonical correspondence analysis of floristic data with respect to plant traits as explanatory variables was conducted primarily to visualize the effects of plant trait attributes on ordination of vegetation samples. As it can be seen from Fig. 2b, three clusters are formed, proving the existence of gradient in plant functional traits. Eigenvalues of this CCA are 0.51, 0.17, 0.12 and 0.09. Canonical axes explain cumulatively 42.4, 56.0, 66.1 and 73.1 % of variance of species - plant trait relation. Trait attributes, which well separate samples of treatments A, B, and C from group of samples of treatments D and E, are correlated with correspondence axis 1. These are the attributes of two leaf traits: 'leaf anatomy' and 'leaf persistence'. Axis 2 is well correlated with some attributes of the following traits: 'lateral spread', 'guild (group) of grassland species', 'plant height', 'canopy structure', and 'reproduction type'.

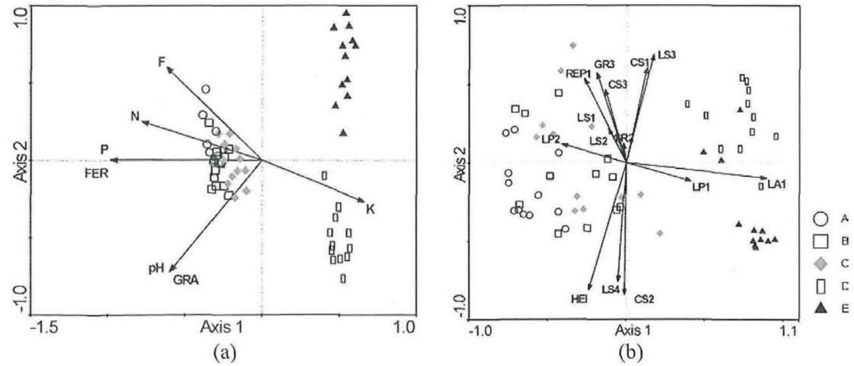


Fig. 2. Ordination results of two CCAs. (a) Effects of environmental variables (F- Ellenberg F value, N Ellenberg N value, P - phosphorus availability, pH - soil reaction, GRA - presence/absence of grazing, FER - presence/absence of fertilization, K - Ellenberg K value) on ordination of vegetation samples. (b) Ordination of vegetation samples and plant attributes as explanatory variables (for abbreviations see table 1; some attributes were combined due to similarity or low occurrence).

Samples of fertilized plots appear very diverse, but no clear pattern is noticeable. Once again, the separation of samples within axis 1 is primarily due to fertilization and not due to grazing. Results of GLMs for each plant trait and attributes within traits are presented in Table 1. Except for 'reproduction type' trait the differences of relative abundances of trait attributes between treatments were predominantly significant. Nonsignificant attributes (e.g. geophytes, rosette plants, hygromorphous leaves) had very sparse occurrence among all the vegetation samples or the occurrence among samples (abundance) was stable (compact plants, herbaceous legume guild).

## Discussion

As there were no differences in majority of floristic, biological and ecological parameters within fertilized treatments A, B and C, the whole work is generally the analysis of three management regimes: fertilized/grazed, not fertilized/grazed and not fertilized/not grazed. We might regard the stand of treatment D as original for this area (low intensity sheep grazing on nutrient poor, shallow soil) and the other two regimes as two risks for the biodiversity of calcareous grasslands of the area: eutrophication on one side and dereliction on the other. The highest species richness and the most valuable species composition are associated with the nongrazing/nonfertilization regime (treatment D). Comparatively to other treatments, plants of treatment D are in average small. There is relatively high abundance of chamaephytes (e.g. *Thymus praecox*) and phalanx lateral spread type (low, dense patches of *Thymus praecox, Hippocrepis comosa, Teucrium montanum*, etc.). Forage value is low and, quite surprisingly, grazing and trampling tolerances are also low. The latter might indicate that the proper management demands very low stocking rates and short grazing periods. Despite the presence of both environmental constraints sensu GRIME 2001, namely stress and disturbance, on plot D stress tolerance is of greater importance (Fig. 1). In both unfertilized treatments D and E plants with scleromorph leaf anatomy prevailed, indicating low nutrient availability and low soil moisture. Plants that dominated in unfertilised plots are typical summer green species, giving a typical brown appearance of these grasslands in winter.

On the contrary, fertilized plots were green most of the year. Early succession of original grasslands (transition from treatment D to E) leads to competitive exclusion effects (BAZZAZ 1996) and the dominance of competitive perennial grass *Brachypodium rupestre*. This grass species is capable of forming almost monospecific patches due to its long-distance lateral spread capability. Forbs and legumes were greatly suppressed, some woody species emerged, indicating the beginning of invasion of shrubs (*Corylus avellana, Juniperus communis*). The absence of biomass removal (grazing) favours the species, which invest the energy and nutrients in above ground organs (GRIME 2001) resulting in increase of average plant height. Wide C/N ratio prevents the litter decomposition, which results in increase of O-soil layer depth and lower pH value. Therefore some acidophyllous species appeared (*Potentilla erecta, Calluna vulgaris*) in treatment E. Fertilization (eutrophication) of original grasslands (transition from D to group of treatments A, B, C) led to decrease of species richness and increase of Shannon diversity index. In respect of CSR functional types, transition revealed greater importance of ruderality. Mutual effects of nutrient availability and higher stocking rates promote species with higher regeneration ability, which is correlated with R component (HODGSON & al. 1999, GRIME 2001).

Table 1. General linear modelling results shows changes in relative abundance of trait attributes in relation to treatments (A-E). Linear regressions were conducted separately for every trait; significance of differences between treatments is indicated as p value.

| Trait | Attribute | Abrr. | Mean relative abundance (%) | | | | | Multiple R | F | p |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | A | B | C | D | E | | | |
| Life form | Phanerophyte | LF1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.3 | 0.40 | 2.54 | 0.0497 |
| | Geophyte | LF2 | 1.9 | 5.0 | 0.3 | 4.4 | 1.5 | 0.32 | 1.57 | 0.1963 |
| | Hemichriptophyte | LF3 | 97.1 | 90.8 | 94.3 | 76.0 | 95.9 | 0.70 | 13.46 | 0.0000 |
| | Therophyte | LF4 | 0.4 | 1.5 | 0.6 | 0.2 | 0.1 | 0.54 | 5.57 | 0.0008 |
| | Hameaphyte | LF5 | 0.6 | 2.8 | 4.8 | 19.4 | 2.3 | 0.77 | 20.03 | 0.0000 |
| Life span | Perennial | LN1 | 99.5 | 95.6 | 95.1 | 99.7 | 99.9 | 0.55 | 5.99 | 0.0004 |
| | Biennial | LN2 | 0.0 | 2.0 | 3.8 | 0.1 | 0.1 | 0.45 | 3.56 | 0.0119 |
| | Annual | LN3 | 0.4 | 2.4 | 1.1 | 0.2 | 0.1 | 0.53 | 5.40 | 0.0010 |
| Grassland guild | Sedge | GR4 | 0.0 | 0.1 | 0.0 | 1.5 | 0.9 | 0.52 | 5.18 | 0.0013 |
| | Forb | GR3 | 41.1 | 41.6 | 40.9 | 40.6 | 16.3 | 0.63 | 8.95 | 0.0000 |
| | Herbaceous legume | GR2 | 5.9 | 9.3 | 12.6 | 8.4 | 6.9 | 0.31 | 1.42 | 0.2389 |
| | Woody plant | GR1 | 0.0 | 0.0 | 0.0 | 0.1 | 0.4 | 0.46 | 3.69 | 0.0098 |
| | Grass | GR4 | 53.0 | 49.1 | 46.4 | 49.3 | 75.6 | 0.61 | 8.35 | 0.0000 |
| Canopy structure | Erosulate plant | CS1 | 9.2 | 14.6 | 18.6 | 28.7 | 13.3 | 0.55 | 5.96 | 0.0005 |
| | Hemirosette plant | CS2 | 76.7 | 72.4 | 68.6 | 58.5 | 80.8 | 0.45 | 3.45 | 0.0139 |
| | Rosette plant | CS3 | 14.1 | 12.9 | 12.8 | 12.8 | 5.9 | 0.29 | 1.23 | 0.3075 |
| Plant height Class | H1 (0 - 10 cm) | | 1.8 | 3.1 | 5.6 | 23.3 | 3.0 | 0.83 | 29.54 | 0.0000 |
| | H2 (10 - 25 cm) | HEI | 8.2 | 18.3 | 22.9 | 19.9 | 12.4 | 0.49 | 4.25 | 0.0045 |
| | H3 (>25 cm) | | 90.1 | 78.5 | 71.6 | 56.8 | 84.6 | 0.70 | 13.31 | 0.0000 |
| Reproduction type | Seed | REP1 | 16.1 | 16.1 | 17.3 | 9.4 | 3.7 | 0.42 | 3.00 | 0.0261 |
| | Mostly seed, rarely vegetatively | REP1 | 3.9 | 4.3 | 3.9 | 7.6 | 6.8 | 0.29 | 1.30 | 0.2824 |
| | Seed/vegetatively | REP2 | 79.9 | 79.3 | 78.7 | 82.9 | 89.3 | 0.30 | 1.40 | 0.2464 |
| | Mostly vegetatively, rarely seed | REP3 | 0.1 | 0.3 | 0.1 | 0.1 | 0.2 | 0.23 | 0.74 | 0.5701 |
| Lateral spread | Therophyte | LS1 | 0.4 | 3.4 | 4.4 | 0.2 | 0.1 | 0.50 | 4.64 | 0.0027 |
| | Compact | LS2 | 20.0 | 21.7 | 17.8 | 17.8 | 18.6 | 0.12 | 0.22 | 0.9285 |
| | Phalanx | LS3 | 8.6 | 14.2 | 20.9 | 36.0 | 8.5 | 0.73 | 16.03 | 0.0000 |
| | Guerilla | LS3 | 70.9 | 60.7 | 56.9 | 46.0 | 72.9 | 0.54 | 5.57 | 0.0008 |
| Leaf anatomy | Hygromorph | LA1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.1 | 0.31 | 1.43 | 0.2351 |
| | Mesomorph | LA2 | 92.2 | 78.1 | 76.3 | 24.4 | 18.7 | 0.92 | 76.25 | 0.0000 |
| | Skleromorph | LA3 | 7.8 | 21.9 | 23.7 | 75.6 | 81.2 | 0.92 | 75.82 | 0.0000 |
| Leaf persistence | Overwintering green | LP1 | 0.4 | 3.4 | 4.3 | 0.1 | 0.0 | 0.50 | 4.65 | 0.0026 |
| | Persistent green | LP1 | 57.1 | 51.5 | 50.2 | 31.7 | 14.0 | 0.84 | 33.30 | 0.0000 |
| | Spring/summer green | LP2 | 42.6 | 45.1 | 45.5 | 68.1 | 86.0 | 0.85 | 36.95 | 0.0000 |
| Grazing tolerance | / | GT | 6.11 | 5.84 | 5.75 | 5.02 | 5.40 | 0.72 | 14.62 | 0.0000 |
| Trampling tolerance | / | TT | 5.77 | 5.71 | 5.63 | 5.04 | 5.43 | 0.57 | 6.71 | 0.0002 |
| Forage value | / | FV | 6.38 | 5.96 | 5.74 | 3.77 | 3.82 | 0.89 | 53.79 | 0.0000 |

In subsequent analysis we wanted to identify plant functional types regarding traits we had evaluated. Since this study is an example of short floristic, environmental and plant trait gradient, rather than replacements of trait attributes, changes in relative abundance of these traits were observed. For the identification of PFTs, as many traits as possible should be included (PILLAR & SOSINSKI 2003) but the more traits we include the less clear the differences between PFTs are. Across short gradients, so called fuzzy PFTs of lower predictive quality are almost always present. In that respect PFT analysis and identification is more useful on the landscape, regional and global level, where some generalizations might be possible. On the basis of this analysis it is difficult to propose the optimal management regime. Traditional low intensity grazing with no fertilization applied seems to be the most appropriate in view of floristic composition and species richness but future work on preservation of these grasslands will demand testing the effects of combinations of grazing animals, stocking rates and duration of grazing. Among alternative methods there are few choices. Due to rocky and steep slopes mechanical removing of biomass (mowing, mulching) is generally not possible. Burning is not appropriate method for this windy landscape as well and is not in line with the efforts of the world to decrease global warming.

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