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IDENTIFICATION OF THE MAIN FUNCTIONAL GROUPS IN THE DRY GRASSLANDS OF *FESTUCETALIA VALESIACAE* FROM NORTH-EASTERN ROMANIA

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Abstract: Plant functional traits and plant functional groups are increasingly used to assess the effect of the change in land use on plant species or plant communities, in nature conservation projects, to detect patterns in the expansion some invasive species or to asses the processes of succession or competition in plant communities. In this study, the main objective was to identify the main plant functional groups (based on plant traits) which co-exist in different plant communities of the dry grasslands (*Festucetalia valesiacae*) from North-Eastern Romania using the RLQ analysis (considering the plant traits, environment characteristics and vegetation). As RLQ analysis mainly revealed a soil moisture-soil nutrients gradient along the first axis, a transition from species with traits specific to more dry conditions and less available nutrients to moister and higher nutrients availability sites was observed (from perennial species with short flowering range and traits oriented to resources retention in storage organs to annual species with long flowering range and traits oriented to resource acquisition). Plant functional groups were identified using species scores along the first two RLQ axes via k-means clustering which generated six groups displayed along the above mentioned gradients. The floristic composition of the identified functional groups suggested that, in the context of *Festucetalia valesiacae* vegetation type from North-Eastern Romania, plant communities developed in areas with lower values for soil moisture and nutrients could possibly be richer in autochore and barochore geophyte and hemicryptophyte species with short flowering range and mixed reproduction type. As soil moisture and nutrients increase, in the floristic composition could possibly occur more anthropochore and zoochore therophyte species with long flowering range, mainly reproducing by seeds and, also, some taller endozoochore shrubs species.

Key words: xeric grasslands, functional approach, RLQ, North-Eastern Romania

Introduction

Plant functional traits are morphological, physiological or life-history characteristics influencing the growth, reproduction and even the survival of species and highlights the strategies by which the plant respond to abiotic and biotic environment [NI, 2003; GUBSH & al. 2011; SEEBACHER & al. 2012]. Plant functional groups include non-phylogenetic related species with similar eco-physiological and life-history traits, which respond in a common way to environmental factors and present a similar effect on ecosystem functioning [LAVOREL & GARNIER, 2002; NI, 2003; MOONEN & BARBERI, 2008; FRANKS & al. 2009]. The study of functional groups in different plant communities can be used to assess the effect of the change in land use [ANSQUER & al. 2009; FRANKS & al. 2009] on plant species or plant communities, in nature conservation projects by identification of the best management measures applied to the target species

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[DROBNIK & al. 2011], or to detect patterns in the expansion some invasive species [FARNSWORTH, 2007]. Also, plant functional types can be used to asses the processes of succession or competition in plant communities because certain plant traits are important for the competitive ability of a certain species [DUCKWORTH & al. 2000].

The majority of the xeric grasslands within *Festuco-Brometea* vegetation class are semi-natural communities of herbaceous species, sometimes of an extraordinary diversity, and with a floristic composition including numerous rare or threatened species. As a number of studies concluded that some of the dry grasslands represents the most species rich plant communities at least for small spatial scales [DENGLER & al. 2012; TURTUREANU & al. 2014; WILSON & al. 2012], they present a great conservation value and most of the dry grasslands habitats, as 6240* Sub-pannonic steppe grasslands and 62C0* Ponto-Sarmatic steppes (in Romania) were included in the community interest category [GAFTA & MOUNTFORD, 2008]. One of the possible explanations of the coexistence of a great number of species in relative small areas could be represented by the differences in plant traits which make possible the complementary use of resources. Plant functional traits can be used in order to group the plant species according to their functions, to understand and to predict the assembly and stability of plant communities [GUBSCH & al. 2011]. In this context the plant functional groups could represent also ones of the main determinants of the species composition of the plant communities [MOONEN & BARBERI, 2008].

In the present study, the main objective was to identify the main plant functional groups (based on plant traits) that co-exist in different plant communities of the dry grasslands (*Festucetalia valesiacae*) from North-Eastern Romania using a vegetation dataset (including 45 releves) realized in 2014 and a RLQ analysis in which the plant traits, environment characteristics and vegetation are considered.

Material and methods

The study area (Fig. 1: 46°30’ – 47°40’ N and 26°40’ – 28°00’ E), situated in the Moldavian Plateau, is characterized by a fragmented relief and a mainly agricultural landscape, with mean altitudes of 150-250 m. The general climate is temperate continental, with mean annual temperatures of 9-10 °C and mean annual precipitations of 400-600 mm/m^2. The main soil types are the mollisols and chernozems. The region is situated at the intersection of two floristic regions: the Euro-Siberian region and the Irano-Turanian region, each presenting their particular floristic elements [CHIFU & al. 2006]. From another perspective almost all territory is included in the continental biogeographical region and only the southern, smaller, part is included in the steppic biogeographical region [HABITATS DIRECTIVE, 1992].

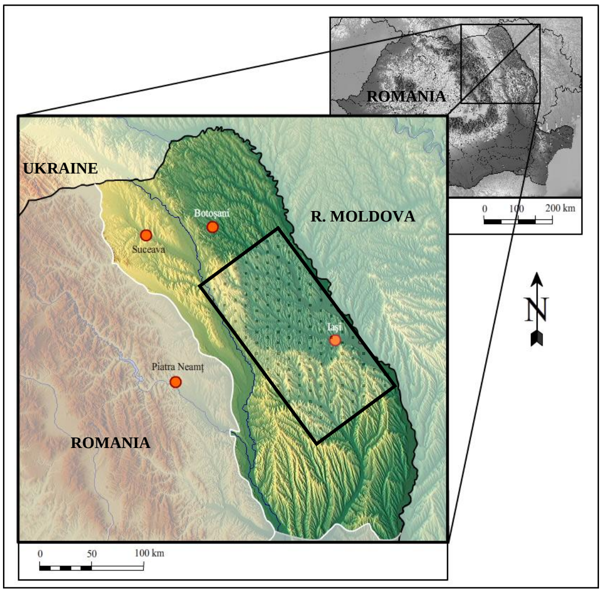

Fig. 1. Geographical location of the study area within Romanian territory

In order to identify the main functional groups in the dry grasslands from North-Eastern Romania, 45 relevés (including 273 species) were used. The relevés covered the main plant communities within *Festucetalia valesiacae* in the region (communities of *Stipa capillata*, *Stipa lessingiana*, *Festuca valesiaca*, *Dichanthium ischaemum*, *Chrysopogon gryllus* etc.), and were realized using the method elaborated by the Central European School for vegetation study adapted for Romanian vegetation [BORZA & BOŞCAIU, 1965]. Plant species cover was visually estimated using a 6 level scale: + (<5%); 1 (5–10%); 2 (10–25%); 3 (25–50%); 4 (50–75%); 5 (>75%). All relevés have 100 m^2 in size. Species present only in one relevé were removed (final dataset included 45 relevés x 208 species). Each plant species was characterized by the following seven traits (factors in the RLQ analysis):

a) grasses (gr), non leguminous forbs (fo) and legumes (le) – a priori classification of plant species in the above functional categories [WRIGHT & al. 2006]. In adition to this classification, some species as *Rosa gallica*, *Rosa canina*, *Prunus spinosa* were treated as shrubs (sh);
b) life form – six categories: therophyte (t), hemitherophyte (he), hemicryptophyte (h), geophyte (g), chamaephyte (c) and phanerophyte (ph) [SÂRBU & al. 2013];
c) reproduction type - two categories: reproduction by seeds (s) and mixed (m) - by seeds and vegetative [KOVÁCS, 1979];
d) dispersal mode – nine categories: anemochory (wind dispersal – an), endozoochory (seeds passed through digestive system of various animals – en), anthropochory (dispersal by humans – at), myrmecochory (ant dispersal – my), mixed mode of dispersal (anemochory / anthropochory – mi), epizoochory (dispersal by animals – ep), autochory (self dispersal – au), barochory (unassisted dispersal – ba) and hydrochory (water dispersal – hy) [KOVÁCS, 1979];
e) pollinating mode – three categories: wind (w), insects (i) and mixed (m) [KOVÁCS, 1979];
f) plant height [SÂRBU & al. 2013] – three categories: low (< 0.3 m), medium (0.3 – 0.7 cm - med) and high (> 0.7 m);
g) flowering range [SÂRBU & al. 2013]: six categories (from one to six months).

Next, a RLQ analysis [DOLÉDEC & al. 1996; DROBNIK & al. 2011] was conducted using three matrices previously constructed: a sites x species matrix (the L matrix), a sites x environment matrix (the R matrix), including the non weighted average values of the Ellenberg indicator values [ELLENBERG & al. 1992] for light (EIV L), temperature (EIV T), continentality (EIV C), soil moisture (EIV F), soil pH (EIV R), soil nutrients (EIV N), altitude and heat load (derived from aspect and slope [OLLSON & al., 2009]) and a species x traits matrix (the Q matrix). A correspondence analysis was firstly performed on the L matrix, on the arcsin-square root transformed values of the plant species cover percentages. After that, Hill-Smith ordinations were conducted on the R matrix (using the row species scores from the correspondence analysis as canonical factor) and on the Q matrix (using the column species scores from the correspondence analysis as canonical factor). The RLQ analysis link the vegetation data, species traits and environmental characteristics [BORCHARDT & al. 2013] finding two sets of coefficients (representing linear combination of traits and vegetation data and linear combination of environment variables and vegetation data) between which the covariance is maximized and equal to the square root of the corresponding eigenvalue [MINDEN & al. 2012]. Correspondence analysis, Hill-Smith ordinations and the RLQ analysis were made in the 'ade4' package [DRAY & al. 2015] from R software [R Development Core Team, 2004]. In order to identify the main functional groups, species scores from the RLQ analysis (only the first two axes) were further subjected to a k-means clustering procedure, trying partitions with 2 to 10 clusters. Optimal number of clusters was assessed using the average *silhouette* width and *Calinski-Harabasz* criteria. K-means clustering was conducted in the fpc package [HENNING, 2015] from the R software [R Development Core Team, 2004].

Results and discussion

Analysis of vegetation plots – environmental variables relationship – In the separate analysis of vegetation plots – environmental variables (Fig. 2), the first ordination axis (the most important one, presenting the highest eigenvalue) explained almost half of the total variance (47.14%) in species composition and was correlated with plant species preferences for temperature, soil moisture and soil nutrients. The second ordination axis explained only 15.40% of the variation and was correlated with the heat load and soil reaction. The first axis distinguished the dry sites, with plant species tolerating high temperatures growing on soils with less available nitrogen (left side) from the more moist sites with higher amounts of available nitrogen and species preferring lower temperatures. The second axis represented a gradient of heat load and soil reaction separating the vegetation plots from steep, south and south-western slopes and lower values of soil pH (upper part of the ordinogram) from the plots from north and north-eastern slopes, with higher values of soil ph (the lower part of the ordinogram).

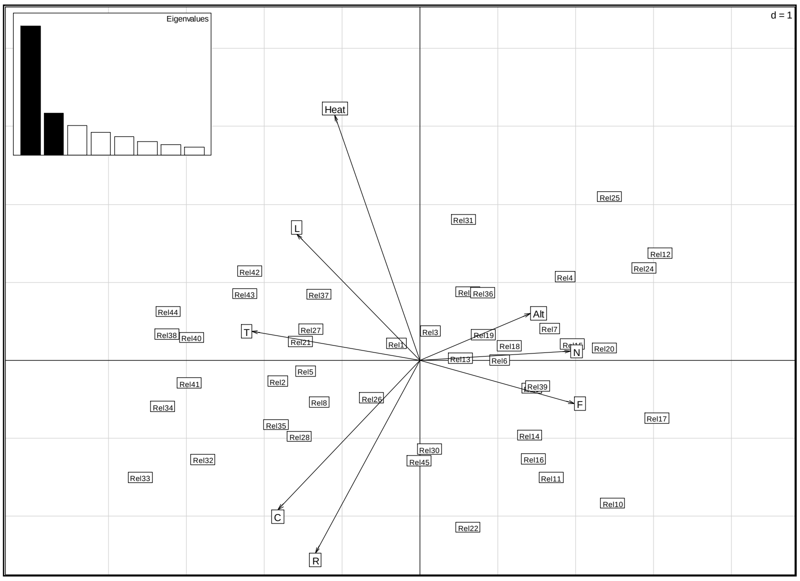

Fig. 2. Hill-Smith ordination of sites and environmental variables (R table: sites x environment) using the row weights from the site scores of the CA on L table as canonical factors.
Eigenvalues: Axis 1: 3.771, Axis 2: 1.233. Variance explained (%): Axis 1: 47.143, Axis 2: 15.406. Squared correlation coefficients: Axis 1: T (0.7577), F (0.6411), N (0.6099), C (0.5391), L (0.4065), Alt (0.3291), R (0.2933), Heat load (0.1943); Axis 2: Heat load (0.5263), R (0.3266), C (0.1965), L (0.1392), Alt (0.0191), F (0.0164), T (0.0072), N (0.0006).

The soil moisture-soil nutrients gradient along the first ordination axis separated the *Chrysopogon gryllus* phytocoenoses from those edified by *Stipa lessingiana*, *Dichanthium ischaemum* and *Festuca valesiaca* while the soil reaction and heat load gradients along the second axis differentiated the *Stipa capillata* community from *Festuca valesiaca community* (Fig. 2).

Hill - Smith ordination of the species traits – Relationship among species traits revealed by the Hill-Smith ordination of the traits table (Q), also emphasized the first axis as the most important one, accounting for 11.28% of total variance, compared to axis two which explained only 7.65%. As all of the plant traits were qualitative, the correlation ratios suggested that plant classification into grasses, forbs and legumes (0.83), pollination mode (0.81) and reproduction type (0.52) are the main traits correlated with the first axis while life form (0.61) and the dispersal mode (0.4) were correlated with the second axis. The first axis distinguished the grass species pollinated by wind and mixed reproduction type (left side of the ordinogram) from the forbs having a mixed pollinating mode and reproduction (mainly) by seeds (the right side of the ordinogram). The second axis opposed the therophyte and hemitherophyte species with long flowering range and mixed (anemochory/anthropochory) dispersal mode to endozoochore geophyte and phanerophyte (shrubs) species presenting a short flowering range.

RLQ analysis (relationship species traits – environmental variables) – The first two axes of the RLQ analysis explained 70.01% and 12.87% of the total inertia of the plant traits – environmental variables relationship and for this reason we further took into consideration these two axes, as they accounted for 82.88% of the variance in the analysis. Compared to the separate analysis of the sites x environmental variables (R table) the RLQ analysis (three tables ordination) accounted for 95.04 for the first axis and 88.69% for the first two axes of the separate analysis. Also, compared to the separate analysis of the species x traits (Q table) and sites x species (L table) the RLQ analysis accounted for 39.14 (the first axis) and 54.73% for the first two axes of the separate analysis (Fig. 3.). The climatic variables (continentality and temperature) showed negative correlations with the first RLQ axis while soil properties (represented by moisture and nutrients content) were positively correlated with the same axis. The second axis was more strongly positively correlated to light and altitude (Fig. 3). Thus, a soil moisture-soil nutrients gradient along RLQ axis one showed a transition from more dry conditions, with less available nutrients to more moist and higher nutrients availability sites, and, also, a climate continentality-temperature gradient along the same axis showed a transition from the sites characterized by a more continental climate, exposed to higher temperatures to sites with species preferring lower values of temperatures and climate's continentality. In this way, the plant traits aggregated at the negative end of the first RLQ axis separated the geophyte and chamaephyte species with a short flowering range, able to self disperse (autochory) or unassisted dispersal (barochory) from the therophyte species, characterized by a long flowering range and anthropochory (e.g. separating *Chrysopogon gryllus*, *Iris aphylla*, *Muscari comosum*, *Muscari tenuiflorum*, *Elymus hispidus* from *Trifolium arvensis*, *Anagallis arvensis*, *Acinos arvensis*, *Arenaria serpyllifolia*, *Centaurium erythraea*, *Sideritis montana*) and, also, from the few shrub species with a medium flowering range and mixed dispersal mode (*Rosa gallica*, *Rosa canina*, *Prunus spinosa*). Another transition along the first axis is that from grasses to forbs and legumes (*Stipa capillata*, *Stipa lessingiana*, *Stipa tirsa*, *Ajuga laxmannii*, *Adonis vernalis*, *Allium rotundum*, *Crambe tataria* to *Cytisus austriacus*, *Medicago falcata*, *Trifolium pratense*, *Trifolium campestre*, *Trifolium repens*). This could be interpreted as a transition from perennial species with short flowering range and traits oriented to resources retention in storage organs to annual species with long flowering range and traits oriented to resource acquisition.

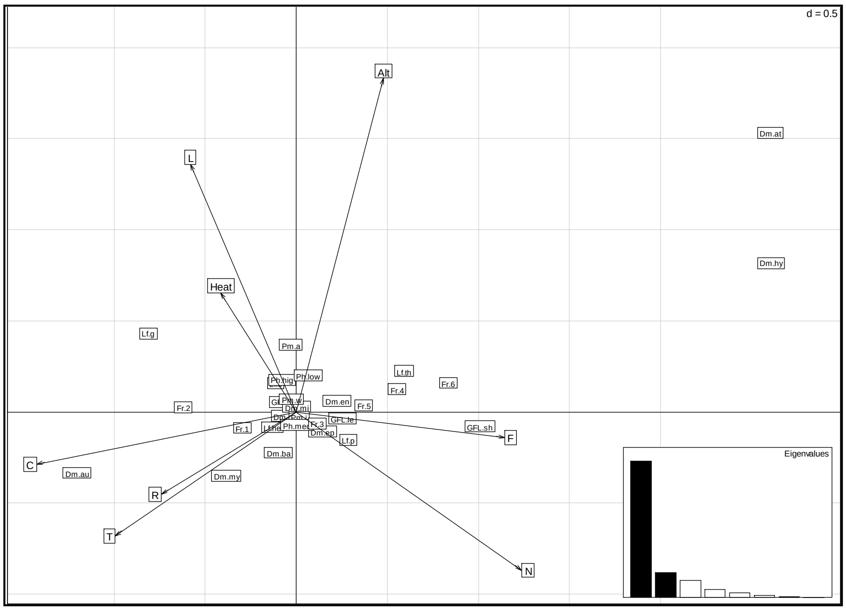

Fig. 3. The RLQ-analysis (relationship plant traits - environmental variables): Eigenvalues: Axis 1: 0.299, Axis 2: 0.055. Variance explained (%): Axis 1: 70.01, Axis 2: 12.87. Covariance: Axis 1: 0.546, Axis 2: 0.234. **Inertia & coinertia R:** Table R (separate ordination) - Axis 1: 3.584, Axis 1+2: 4.438; Table R (RLQ) - Axis 1: 3.771, Axis 1+2: 5.003; % RLQ - Axis 1: 95.04, Axis 1+2: 88.69. **Inertia & coinertia Q:** Table Q (separate ordination) - Axis 1: 1.192, Axis 1+2: 2.799; Table Q (RLQ) - Axis 1: 3.047, Axis 1+2: 5.114; % RLQ - Axis 1: 39.14, Axis 1+2: 54.73. **Correlation L:** Table L (separate ordination) - Axis 1: 0.264; RLQ - 0.409.

Plant functional groups

The k-means clustering algorithm generated six groups according to the position of species along the first RLQ axes (according to average *silhouette* width and *Calinski-Harabasz* criteria) based on their particular traits and environmental variables. The groups are mainly displayed along gradients of soil moisture and nutrients.

The first group comprised a mix of low and some taller species, predominantly grasses and few forbs (16 species, with no legume species in this cluster), most of them geophytes, pollinated by wind, having an short (only two months) flowering range with mixed (anemochory/anthropochory) dispersal mode and reproduction type (seeds/vegetative); *Arrhenatherum elatius*, *Chrysopogon gryllus*, *Asparagus officinalis*, *Dactylis glomerata*, *Elymus hispidus* etc. were some of the component species of the group.

The second group included preponderantly hemicryptophyte forbs and (a lower proportion of) grasses (but no legumes) with short flowering range (two months), medium to low height and mixed dispersal, pollination and reproduction types. Among the 36 species within this group there were: *Artemisia austriaca*, *Bromus squarrosus*, *Carthamus lanatus*, *Cerinthe minor*, *Phlomis tuberosa*, *Stipa tirsa*, *Teucrium chamaedrys* etc.

The third group contained predominantly low and medium height perrenial forbs and legumes, characterized by a mixed (anemochory/anthropochory) dispersal mode and a three-four months flowering range, most of them pollinated by insects. Among the 75 species within this group there were: *Achillea setacea*, *Anthemis tinctoria*, *Astragalus onobrychis*, *Ferulago campestris*, *Hypericum perforatum*, *Medicago falcata*, *Silene otites*, *Trifolium montanum*, *Veronica spicata* etc.

Tab. 1. Characteristics of the five clusters (groups) resulted from k-means clustering of species scores in the RLQ analysis

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
No. of species	16	36	75	63	15	3
EIV L	7.80±1.20	8.20±1.14	7.59±1.05	7.76±1.16	7.60±1.80	7.50±0.55
EIV T	6.58±1.16	6.86±1.19	6.24±1.17	6.61±1.01	6.16±0.83	5.75±0.75
EIVC	5.07±0.70	6.02±1.16	5.55±1.21	5.90±1.04	5.07±0.75	5.25±0.50
EIV U	3.84±1.21	3.00±1.13	3.57±1.19	3.36±1.08	4.07±1.20	4.33±0.57
EIV R	5.75±1.48	6.68±1.71	7.00±1.82	6.80±1.38	6.00±1.30	7.50±0.50
EIV N	3.30±2.28	3.56±1.45	3.40±1.76	3.15±1.54	3.63±1.96	5.50±2.25
Functional category	gr (68.7%); fo(31.3%)	gr(33.3%); le (66.7%)	fo (65.5%); le (24.3%)	fo(98.5%)	fo (93.3%)	sh (100%)
Life form type	g (75%)	t (27.7%); h (52.7%)	h (65.5%)	h (79.4%)	t (86.6%)	ph (100%)
Reproduction type	m (87.5%)	s (41.6%); m (58.4%)	s (50%); m (50%)	s (73%); m (27%)	s (93.3%)	m (100%)
Dispersal mode	mi (75%)	an (25%); mi (63.9%)	mi (66.6%)	an (22.2%); mi (47.6%)	mi (73.3%)	en (100%)
Pollination mode	w (62.5%)	w (33.3%); m (44.4%)	i (57.7%); m (24.3%)	i (57%); m (43%)	i (33.3%); m (66.7%)	i (100%)
Plant height category	low (43.7%); high (56.3%)	low (25%); med (54.7%)	low (59%); med (21.8%)	med (93.6%)	low (80%)	med (25%) high (75%)
Flowering range	2 (62.5%); 3 (37.5%)	2 (88.8%)	3 (46.2%); 4 (34.7%)	2 (31.7%); 3 (68.3%)	4 (60%); 5 (20%)	2 (100%)

In the fourth group there were mostly hemicrytophyte forbs (63 species, but no grass species included) as *Adonis vernalis*, *Aster amellus*, *Campanula sibirica*, *Dianthus membranaceus*, *Echium maculatum*, *Falcaria vulgaris*, *Inula germanica*, *Oxytropis pilosa*, *Salvia nemorosa*, *Verbascum blattaria* etc. Most of the component species were pollinated by insects, of medium height and medium flowering range (three months). The predominant reproduction type was by seeds and the predominant dispersal mode was the mixed one (but a significant proportion was represented by anthropochore species).

Almost all species of the fifth group (15 species) were low height therophyte forbs (no grass species included), reproducing by seeds, with a long flowering range, mixed (anemochory/anthropochory) dispersal mode and mixed pollination mode (wind/insects). As representatives for this group there were: *Anagallis arvensis*, *Centaurium erythraea*, *Sideritis montana*, *Trifolium campestre*, *Viola arvensis* etc.

The last group comprised the shrubs species (as *Rosa gallica*, *Rosa canina*, *Prunus spinosa*) with short flowering range (two months), medium to increased height and endozoochory as dispersal mode, pollinated by insects and mixed reproduction type.

The floristic composition of the six groups generally suggest a shift from low to medium height geophyte species (not necessarily *Liliaceae*) with short flowering range, pollinated by wind adapted to grow in soils with water and nutrients deficit (short time of optimum conditions for plant development) to: a) low height therophyte with long flowering range, reproduction by seeds and mixed dispersal and pollination modes (anthropic influence) and b) to increased height phanerophytes (shrubs) pollinated by insects and endozoochore dispersal mode developed on soils with higher availability of water and nutrients. From another perspective the increase of the mean height of species in grasslands with N availability was observed and highlighted in other studies [SCHELLBERG & PONTES, 2012].

As a final remark, in the context of this particular vegetation type (*Festucetalia valesiacae*) and this particular region (North-Eastern Romania), plant communities developed in areas characterized by higher climate continentality, higher values of temperatures and soil reaction and lower values for soil moisture and nutrients will possibly be richer in autochore and barochore geophyte and hemicryptophyte species (grasses and forbs) with short flowering range and mixed (seeds and vegetative) reproduction type. As soil moisture and soil nutrients increase, in the floristic composition could possibly occur more legume species and, in the richest in nutrients areas the floristic composition could possibly be infiltrated one by more anthropochore and zoochore therophyte species with long flowering range, mainly reproducing by seeds and, second, by some taller endozoochore shrubs species occur with short flowering range also reproducing by seeds.

Conclusion

Six main plant functional groups in which species with similar ecological requirements and life-history traits co-exist were identified in the dry grasslands (*Festucetalia valesiacae*) from North-Eastern Romania via RLQ analysis and k-means clustering. The groups are mainly displayed along gradients of soil moisture and nutrients. Their floristic composition generally suggest a shift from low to medium height geophyte species with short flowering range, pollinated by wind adapted to grow in soils with water and nutrients deficit to low height therophyte with long flowering range, reproduction by seeds and mixed dispersal and pollination modes and to increased height phanerophytes (shrubs) pollinated by insects and endozoochore dispersal mode developed on soils with higher availability of water and nutrients. The groups' species composition suggests transition from perennial species with short flowering range and traits oriented to resources retention in underground organs to annual species with long flowering range and traits oriented to resource acquisition.

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