Article

Turnover of Plant Species on an Ecological Gradient in Karst Dolines Is Reflected in Plant Traits: Chorotypes, Life Forms, Plant Architecture and Strategies

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Abstract: We analyzed plants and their traits in dolines, which are characteristic enclosed terrain depressions on carbonate (karst) plateaus. These landforms range from a few meters to over 100 m in diameter, their depth generally varying from a few meters to a few tens of meters. A pronounced ecological gradient can be found from the bottom to the top, starting from humid, cool and shaded bottoms to sunny, dry and warm slopes and tops. We sampled dolines of various depths and analyzed the distribution of plant species on the gradient and how this distribution is reflected in plant traits: chorotypes, life forms, plant architecture and strategies. We used the transect method and sampled the floristic composition from the doline bottom to the top. We collected information about plant traits from various literature sources. The results show life forms and plant architecture explain this gradient well and, to a lesser extent, also chorotypes, but functional strategies have a low explanatory power. Life forms and plant architecture are the result of adaptation of species to the environment, and chorotypes are defined as species with an overlapping geographical distribution pattern due to their distribution and environmental histories. Functional strategies, which have evolved to enable plants to succeed in various environments, unexpectedly have a low explanatory power.

Keywords: chorotype; doline; ecology; plant functional strategy; life form; plant architecture; transect

1. Introduction

Enclosed terrain depressions termed dolines (sinkholes in American literature) can be found on carbonate (karst) plateaus representing a characteristic karst surface feature. Environmental factors in dolines deviate markedly from the surroundings, and this reflects also in the appearance of plants species and their communities.

The significance of dolines for local biodiversity has been recognized in the past. Beck-Mannagetta [1] stated that alpine plants in dolines in the Dinaric Alps are remnants of the glacial period. Horvat [2] presented vegetation zonation within various dolines over the whole Dinarides. Bátori and his team [3–10] elaborated on many aspects of dolines. They could be refugia for endangered cool-adapted plant species that have found safe havens here. Besides vascular plants, many other important diagnostic groups of species (e.g., ants) have been found in doline. They used the same transect method as in the present study. Kermavnar and his team [11–14] studied the development of vegetation in the dolines after cutting. They evaluated the microclimatic conditions and initial responses of floor vegetation (plant species and traits) after cutting. All referenced studies identified different ecological conditions in dolines, caused primarily by geomorphology.

Dolines are formed by dissolution of sedimentary carbonate bedrock (limestone, dolomite) and appear in different sizes and shapes. They are commonly sub-circular in plan

and roughly funnel-shaped, but they can also be elliptical. They range from a few meters to over 100 m in diameter, with sides ranging from gently sloping to vertical. Consequently, their depth generally varies from a few meters to a few tens of meters. Ecological and climatic conditions change due to terrain concavity [15–17].

Because of evident topographic deviation, the microclimate in dolines differs significantly from the surrounding (macro)climate of extensive and generally flattened karst plateaus. During clear nights, heavy and cold air flows into these depressions and accumulates there since they are protected from the wind. As the surrounding air warms during the day, it becomes lighter and mixes poorly with the cold air sitting in dolines, causing an extended period of cold. Well-pronounced thermal inversions occur in dolines [2,18].

During rain, due to the steep topography, water erodes soil from the slopes which accumulates in the bottom of dolines, where deep soil layers can be found composed of fine-grained soil with a large proportion of clay and silt. Steep slopes are often covered by bare rocks and cliffs, where water easily sinks into the karst underground, whereas the clay in the bottom has a higher retention ability. These geomorphic features also contribute to different ecological conditions in dolines [15,19,20].

Such rapid changes of ecological conditions in a restricted area offer the opportunity for studying changes in ecological biodiversity [21]. We used vascular plants as a proxy for total biodiversity across environmental gradients, as vascular plants are often used as a proxy in broad taxonomic realms (bryophytes, macrofungi, lichens, plant-galling arthropods, gastropods, spiders, carabid beetles, hoverflies, and genetic richness) [22]. Plants and their communities are also good indicators of environmental site conditions [23], and different environmental conditions are also reflected in chorotypes, functional traits and functional strategies.

Chorotypes are groupings of species with overlapping geographical distribution (e.g., Mediterranean, Central European) [24]. We adjusted the chorotype scheme proposed by Pignatti [25,26]. Species classified within the same chorotype can be of unrelated taxa and do not appear in any specific ecological conditions, or have specific functional traits or strategies. Chorotype is used to describe the distribution pattern of a group of species that appear under the same macroecological conditions. We can thus reach conclusions about the origin and development of vegetation in the area. For instance, it can be hypothesized that mesic forests in dolines (central European chorotype), surrounded by thermophilous forests on karstic plateaus (Mediterranean chorotype), are remnants of cooler and more humid periods in the past [27].

Many examples exist that show that plant functional traits explain community responses to environmental conditions, e.g., disturbances [28], succession [29], climate [30], degradation [31], etc. Special features in the environment can create unique combinations of plant functional traits that can provide important insights about species functioning in different ecological conditions. Čarni and coauthors [32] showed that the distribution patterns of functional traits mirror microclimatic variation in topographically complex karst environments. As functional traits, we applied Raunkier [33] life forms (phanerophyte, chamaephyte, hemicryptophyte, geophyte and therophyte), which were further subdivided by Pignatti [25] according to their architecture.

Plant functional strategies are also crucial in the functioning of communities [34]. A prominent strategy scheme is Grime's scheme [35] (competitor, stress tolerator, ruderal-CSR) and his theory [36] describes the three principal strategies representing viable trait combinations arising under conditions of competition (C), abiotic limitation to growth (S) and periodic biomass destruction (R), respectively [37].

The Kras Plateau areas have been largely deforested and transformed into grasslands, while the bottoms of dolines, where deeper soil was available, have been used as cultivated fields. Since the Second World War, and especially in recent years due to a changed agricultural policy, an intensive process of reforestation has begun [38–40]. During our study, we focused only on areas that have been forested for an extended time (at least 70 years), which are the least disturbed and most natural in the landscape [9].

According to the presented theoretical backgrounds, the following research questions were the focus of the study. (a) Is the ecological gradient from the bottom of a doline to its top the most important for plant species turnover? (b) Do only deeper dolines possess the whole gradient? (c) Can we expect more species with competitor strategy in the bottom of dolines? (d) Are there more species originating from the northern chorotypes in the bottom, whereas more species of southern chorotypes on the top? (e) Can we expect more geophytes in the bottom and more hemicryptophyte on the top? (f) How are ecological conditions reflected in the architecture of plants? (g) Can functional strategies best explain the species turnover?

2. Materials and Methods

2.1. Study Area

The research took place on Kras Plateau, a limestone karstic plateau lying above the Bay of Trieste in the northernmost part of the Adriatic Sea at an altitude of 300–500 m. It is an example of an uplifted and slightly leveled corrosion plain surface in the NW part of the Dinaric Mountains [41].

The climate is transitional between Mediterranean and continental (i.e., sub-Mediterranean), with rainy, cool winters and hot summers. The precipitation amount is around 1400 mm and the average annual temperature around 11 °C. Kras Plateau consists of karstified Mesozoic limestone covered predominately by Rendzinas and Cambisols. The main forest tree species in the area are hop hornbeam (*Ostrya carpinifolia*) and pubescent oak (*Quercus pubescens*) [39,42,43]. Kras Plateau is on the border between continental and Mediterranean biogeographic regions [44]. Since disturbance has a negative impact on vegetation and could impact the natural gradient, we sampled floristic composition only in forested dolines, which are the least disturbed habitats in the landscape, with high conservation values [9].

The many dolines (over 14,000) on Kras Plateau are quite diverse, having various depths, diameters, shapes, and soil depths (on average 3 m, while on slopes, the soil depth is related to the type of doline) [45]. Oak-hornbeam, or even beech forests can be found at the bottom of dolines. On slopes, there are ravine forests dominated by sycamore and Norway maple (*Acer pseudoplatanus, A. plantanoides*), little-leaved and large-leaved limes (*Tilia cordata, T. platyphyllos*), wych elm (*Ulmus glabra*), and common ash (*Fraxinus excelsior*). On slopes exposed to the south that are warmer due to environmental conditions, forests dominated by Turkey oak (*Quercus cerris*) and hop hornbeam (*Ostrya carpinifolia*) appear [26,46–48].

2.2. Vegetation Sampling

We sampled vegetation (floristic composition) by transects. Each transect consisted of adjoining 2 m × 2 m large plots, positioned from south-north, from one edge of the doline through the bottom to the other edge [32,49,50]. The transects were of different lengths depending on the size of the doline (Table 1, Figure 1). We thus recorded the greater part of (vascular plant) biodiversity. Distance from the edge of doline was measured and depth of individual sample plot was determined from Lidar data. All ground floor vascular plants were identified, and we estimated the cover of individual plants visually in each plot by the 7-degree scale proposed by Braun-Blanquet [51]. We also visually estimated the proportion of bare rock on the surface in each plot. The plots were stored in the TURBOVEG database program [52] and entered in the JUICE program for analysis [53].We obtained a matrix of 286 plots × 124 plant species that were used in the analysis.

Detrended correspondence analysis (DCA) was performed on the plot matrix (Table S1). The original cover values of plants (Br.-Bl. values) were transformed to percentages and subjected to square root transformation. We extracted the first two axes of the analysis (Axis 1 and Axis 2) and used them in further analyses. The first axis represents the main floristic gradient. The calculations were done using the mass module in the vegan program package [54] run R program environment [https://cran.r-project.org, accessed 20 December 2021].

Table 1. Selected dolines of different depth. The length of the transect from center to the northern and southern edge of doline is presented.

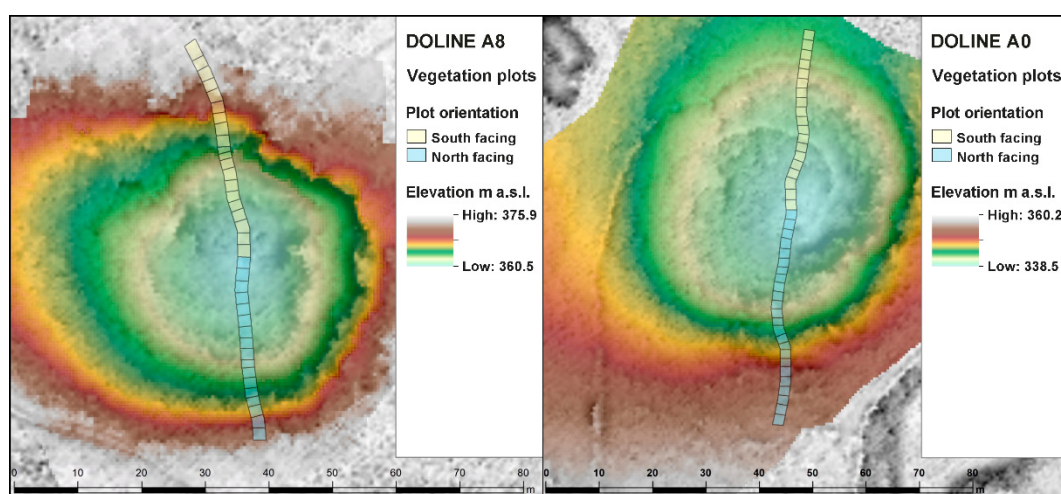| Doline	Depth (m)	Transect Length (m) North	Transect Length (m) South
A1	2.41	10	22
A2	5.98	14	20
A3	7.51	14	24
A4	8.17	18	34
A5	8.71	22	18
A6	13.38	32	36
A7	13.55	30	30
A8	15.4	40	34
A9	18.84	34	54
A0	21.74	38	48

Figure 1. Sampling design with transect method by which the plant composition was sampled from the doline bottom to the top. Doline A8 and A0 are presented as examples of bigger dolines to visualize the ratio between doline depth and length of transects.

We calculated unweighted bioindicator (Ellenberg) values (EIV) [55,56] for each plot using the JUICE program [53] and passively projected them onto an ordination plane.

2.3. Analyzing Plant Composition

We calculated the chorotypes, plant functional traits and strategies of the studied vegetation plots by community-weighted means (CWM) of each trait. This can be considered to be the average trait value in the vegetation plot reflecting the relative abundances of species. As abundance of the species, we took the estimated cover value transformed into percentage [51]. We used unweighted indicator values. The results were visualized by their projection onto the ordination plane.

Chorotypes were calculated based on classification of geoelements proposed by Pignatti [57], but we amalgamated some categories to broader ones, such as Eurasian, Mediterranean (incl. sub-Mediterranean), Central European (incl. Mediterranean-montane), Balkan, SE European, Eurocaucasian and Northern (incl. here Boreal, Eurosibirian, Holoarctic) chorotypes.

We calculated plant functional traits according to the morphological features of plants [57], based on Raunkier's life forms [33], as nanophanerophytes, hemicryptophytes, therophytes and geophytes, and architecture of growth defined by Pignatti [25] as caespitose, rosulate, suffrutescens, liane, crawling and rhizomatous, to mention only the most significant ones.

We also calculated functional strategies, for which we used Grime's model of CSR [35], as competitors, stress tolerators and ruderals. This model and determination of functional

strategies are the result of long-term research conducted in field surveys, laboratories, monitoring of permanent plots and manipulative experiments. There also exist methods for rapid determination of strategy based on canopy height, dry matter content, flowering period, flowering start, lateral spread, leaf dry weight and specific leaf area [58]. The position of each plot can be determined in the CSR triangle. The community thus obtains a functional signature [59]. The data were provided by the Biolflor database [60] and are shown in Table S1.

We tested the effect of the explanatory power of variables on floristic composition using CCA ordination analysis in CANOCO using the Monte Carlo permutation test with 9999 permutations. Since the results of variation partitioning overlapped, they were displayed in a diagram using Venn circles [61,62].

3. Results

3.1. Ordination

Detrended correspondence analysis showed that the main floristic gradient along the slope from the bottom of dolines to their top is reflected on the first axis (Axis 1), while the second axis (Axis 2) represents the proportion of bare rock on the surface (Figures 2 and 3). The highest species richness was found on the bottom, while the most moderate was on steep and stony slopes (Figure 2). Humid and nutrient-rich sites can be found in the doline bottoms, whereas the tops are warm, dry, and sunny. Rocky sites can be found on some doline slopes (Figures 2 and 3).
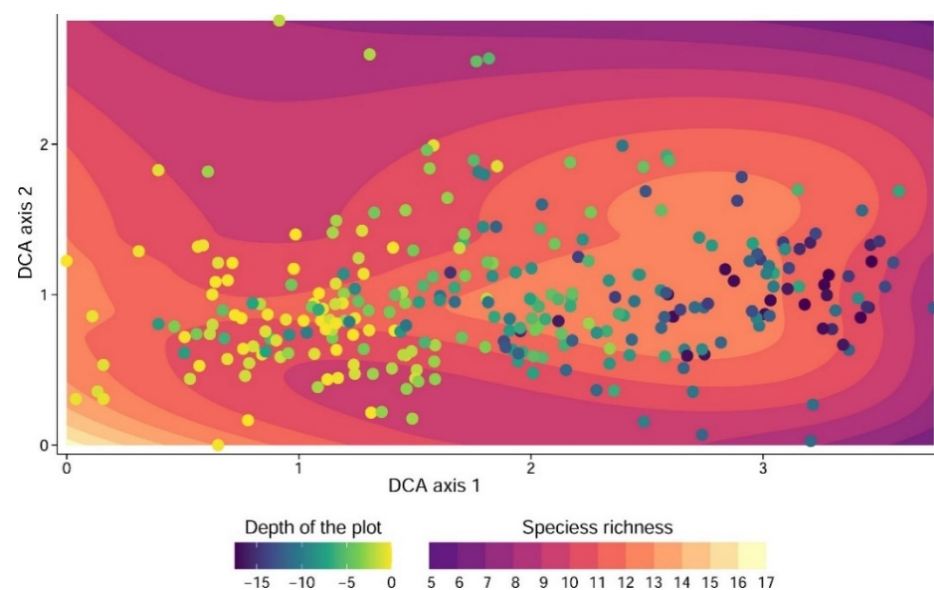

Figure 2. Detrended correspondence analysis (DCA) diagram of vegetation plots. The first two axes are presented (eigenvalues are 0.49 and 0.26). At the same time, the number of species in the plots is interpolated into the background of the diagram plane. Each plot is colored according to its depth.

It can be observed that only plots from the deepest dolines are positioned along the whole gradient, while all plots from shallow dolines are positioned only in the left-hand part of the gradient (Figure 4).
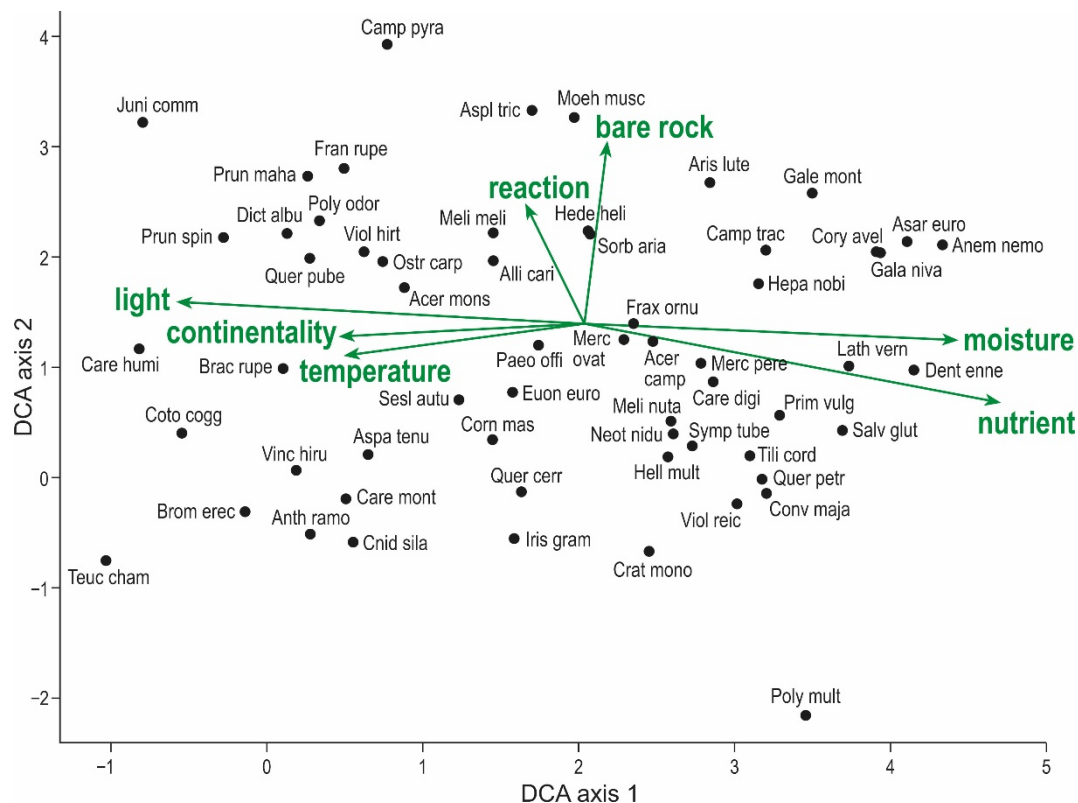
Figure 3. Detrended correspondence analysis (DCA) diagram of species with passively projected bioinicator values. Only half of the species are presented, i.e., which had the best fit to the first DCA axis. Names of species are indicated by four letters indicating genus and four letters for species name. Legend: Acer camp–*Acer campestre*, Acer mons–*Acer monspessulanum*, Ajug rept–*Ajuga reptans*, Alli cari–*Allium carinatum* ssp. *pulchellum*, Anem nemo–*Anemone nemorosa*, Anth ramo–*Anthericum ramosum*, Aris lute–*Aristolochia lutea*, Asar euro–*Asarum europaeum*, Aspa tenu–*Asparagus tenuifolius*, Aspl tric–*Asplenium trichomanes*, Brom erec–*Bromopsis erecta*, Camp pyra–*Campanula pyramidalis*, Camp trac–*Campanula trachelium*, Care digi–*Carex digitata*, Care humi–*Carex humilis*, Care mont–*Carex montana*, Cnid sila–*Cnidium silaifolium*, Conv maja–*Convallaria majalis*, Corn mas–*Cornus mas*, Cory avel–*Corylus avellana*, Coti cogg–*Cotinus coggygria*, Crat mono–*Crataegus monogyna*, Dent enne–*Dentaria enneaphyllos*, Dict albu–*Dictamnus albus*, Euon euro–*Euonymus europaeus*, Fran rupe–*Frangula rupestris*, Frax ornu–*Fraxinus ornus*, Gala niva–*Galanthus nivalis*, Gale mont–*Galeobdolon montanum*, Hede heli–*Hedera helix*, Hell mult–*Helleborus multifidus* ssp. *istriacus*, Hepa nobi–*Hepatica nobilis*, Iris gram–*Iris graminea*, Juni comm–*Juniperus communis*, Lath vern–*Lathyrus vernus*, Meli nuta–*Melica nutans*, Meli meli–*Melittis melissophyllum*, Merc ovat–*Mercurialis ovata*, Merc pere–*Mercurialis perennis*, Moeh musc–*Moehringia muscosa*, Neot nidu–*Neottia nidus–avis*, Ostr carp–*Ostrya carpinifolia*, Paeo offi–*Paeonia officinalis*, Poly odor–*Polygonatum odoratum*, Prim vulg–*Primula vulgaris*, Prun maha–*Prunus mahaleb*, Prun spin–*Prunus spinosa*, Quer cerr–*Quercus cerris*, Quer petr–*Quercus petraea*, Quer pube–*Quercus pubescens*, Salv glut–*Salvia glutinosa*, Sesl autu–*Sesleria autumnalis*, Sorb aria–*Sorbus aria*, Symp tube–*Symphytum tuberosum*, Teuc cham–*Teucrium chamaedrys*, Tili cord–*Tilia cordata*, Vinc hiru–*Vincetoxicum hirundinaria*, Viol hirt–*Viola hirta*, Viol reic–*Viola reichenbachiana*.
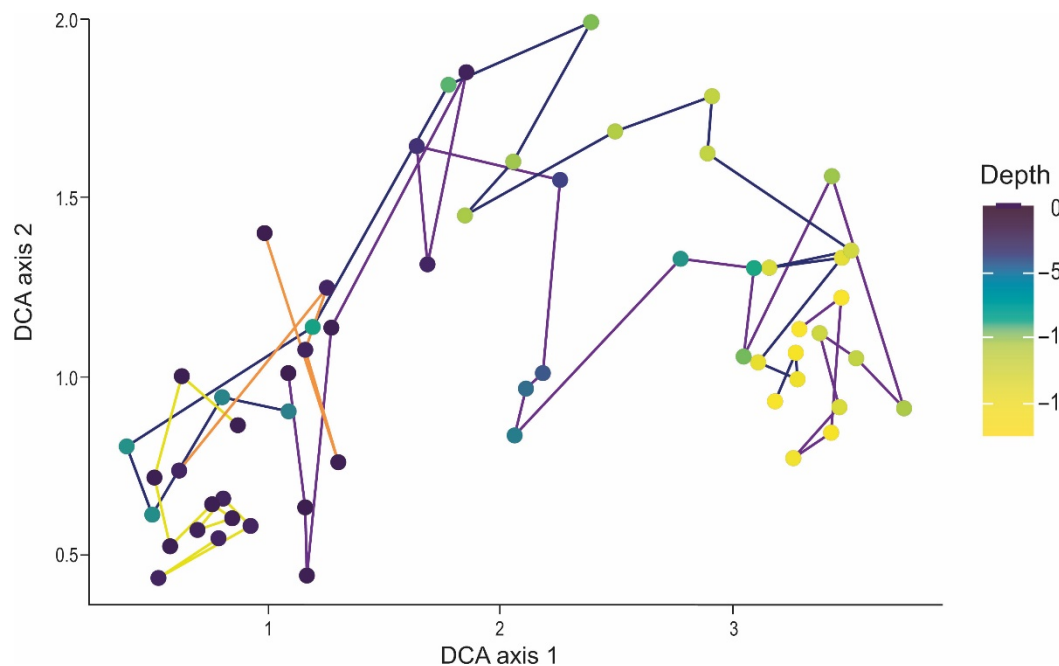
Figure 4. DCA diagram of vegetation plots. Only plots belonging to A4 and A8 are presented for clarity (A4 lines in yellow and orange shades, A8 lines in blue and purple shades). Plots belonging to the same transect are connected. Each doline has two transects, from the center to the north and from the center to the south. The difference between shallow (A4) and deep (A8) dolines is evident.

3.2. Explanatory Power of Chorotypes, Functional Traits and Strategies

Chorotypes (Figure 5b) reflect the origin of vegetation, and most species can be found with Eurocaucasian and Northern chorotypes (e.g., *Corylus avellana*, *Galanthus nivalis*, *Galeobdolon montanum*) in the humid, nutrient rich and shaded bottom of the dolines. The central European and Mediterranean chorotype appear on stony slopes (*Campanula pyramidalis*, *Frangula rupestris*, *Moehringia muscosa*). On less stony slopes with deeper soils (reflecting lower pH), many Balkan species (*Cnidium silaifolium*, *Iris graminea*, *Symphytum tuberosum)* appear. Eurasian, SE European and Mediterranean chorotypes appear on top plots that are dry and warm with high light availability (*Bromopsis erecta*, *Carex humilis*, *Vincetoxicum hirundinaria*).

Functional traits (Figure 5c,d) reflect ecological conditions. Geophytes (*Dentaria enneaphyllos*, *Lathyrus vernus*, *Galanthus nivalis*) can be found in humid and nutrient rich soil in the bottom. Therophytes (*Geranium purpureum*, *G. robertianum*) can be found on warm and stony sites on slopes. Top sites are dominated by hemicryptophytes (various graminoid species: *Bromopsis erecta*, *Carex humilis*) and nanophanerophytes (various shrubs: *Cotinus coggygria*, *Juniperus communis*). Crawling (*Asarum europaeum*) and rhizomatous (*Anemone nemorosa*, *Dentaria enneaphyllos*, *Lathyrus vernus*) species are found in the bottom of dolines. On active stony slopes, where difficult conditions prevail, rosulate *(Asplenium trichomanes*, *Campanula pyramidalis)* species can be found. Plots on the top are dominated by caespitose (*Sesleria autumanis*, *Brachypodium rupestre*, *Carex humilis*) and suffrutescent (*Teucrium chamaedrys*) plants.

Plant functional strategies (Figure 5a) reflect the functioning of communities. Most species with a ruderal strategy can be found, coping with disturbances (e.g., wild boars, past land usage) in the bottom of dolines. A stress tolerator strategy is somewhat more pronounced on stony slopes, where site conditions are the hardest. A competitor strategy is slightly more pronounced on top, where disturbances and hard conditions are less pronounced. The CSR signature (Figure 6) shows that there is not much variation in functional strategies, and this also explains the low explanatory power of this trait.
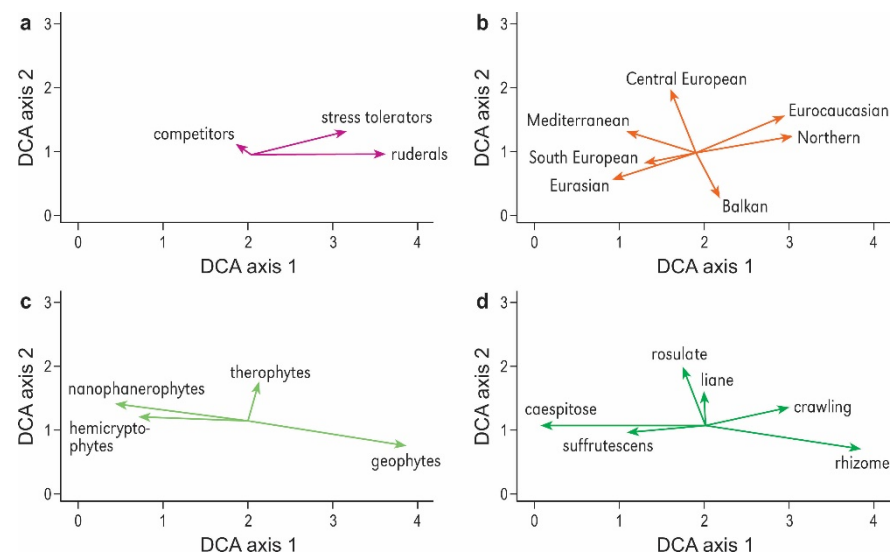
Figure 5. DCA diagram plane. For clarity only passive projection of functional strategies (**a**), chorotypes (**b**), life forms (**c**) and architecture of plants (**d**) is shown.
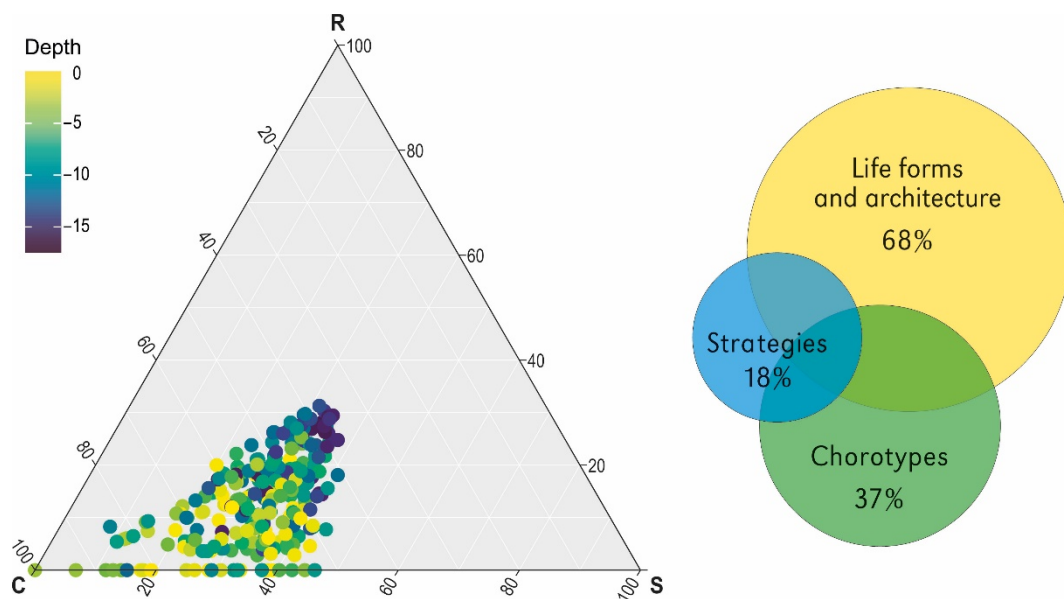

Figure 6. The CSR signature of plots (**left**) presents the position of plots in the CSR triangle. The Venn diagram (**right**) presents the explanatory power of explanatory variables. The explanatory powers overlap. Strategies alone explain only 8% of the explained variation, while plant functional traits (life forms and architecture) alone explain 50%, and chorotypes 23%.

Functional traits, chorotypes and functional strategies explain, together, 61% of the whole variance in floristic composition. The Venn diagram (Figure 6) shows that the most powerful are functional traits (life forms and architecture of plants), which explain 68% of explained variance, whereas functional strategies have low explanatory power, 18%.

4. Discussion

4.1. High Species Richness in Dolines

Our research confirmed the results of previous studies, that dolines possess different ecological conditions than surrounding vegetation. They offer favorable climatic conditions for cool-adapted plant species and are often treated as safe havens in the event of foreseen climatic changes [49], although it should be taken into consideration that probably some

species (e.g., *Sesleria autumnalis* or *Paeonia officinalis*) might not be capable of retreating from increasing heat and drought to the bottom of dolines due to other factors, such as the lack of light and soil conditions at such sites. Dolines already offer space for various biota [8,63] that would otherwise be excluded from, or be much rarer, in the local species pool (e.g., *Dentaria enneaphylos*, which needs moist and cool conditions and *Campanula pyramidalis*, which needs rocky crevices). It is important to note that the species richness at the bottoms of the deep dolines is consistently higher compared to shallow dolines and tops on the plateau. This is especially significant when accounting for the much smaller area covered by doline bottoms.

4.2. Plant Changes along the Ecological Gradient in Dolines

We established that moist and nutrient-rich habitats appear in the bottom of dolines, dominated by oak-hornbeam forests (*Quercus petraea-Carpinus betulus*) [64]. They are characterized by a high proportion of geophytes [65]. Geophytes are early spring perennial plants that are common in broad-leaved deciduous forests, where they appear before spring leafing takes place [66]. There are also harsh conditions, with thermal inversion and snow accumulation [2], and plants invest carbohydrates into below-ground organs of perennation instead of above-ground biomass. This increases protection from freezing, and plants are additionally protected by plant litter and snow cover [67]. Many wild boars appear in the region, and they are active in rooting and looking for food [68], which supports the ruderal functional strategy.

Since our results show an increased tendency for stress-tolerant plants in the bottom, compared to the plateau area, it may be that the main stress factor for the herb layer over all is not drought or heat, which are the expected stressors on the plateau, but the lack of light and activity of wild animals, both more prominent at the bottom of dolines [9,68]. However, since the strategies alone explained very little of the total variance, further studies are needed to discover the significance of the pattern of strategies distribution.

On slopes, ravine forests can be found, dominated by sycamore (*Acer pseudoplatanus),* little-leaved lime *(Tilia cordata),* and large-leaved lime *(Tilia platyphyllos)* [48]. These forests are adapted to stony slopes with moving rocks on the surface. These habitats are characterized by scapose phanerophytes, woody species that are adapted to moving bedrock [69]. Many of these scapose phanerophytes (e.g., *Fraxinus ornus*) are of SE European origin. In cases in which wood species cannot survive these hard conditions, chasmophytic vegetation composed of rosette, biennial and scapose hemicryptophytes appears [70], with which the dominant functional strategy is stress tolerance [71,72].

Plots on top can be classified within sub-Mediterranean hop hornbeam-pubescent oak (*Ostrya carpinifolia-Quercus pubescens*) forests [73] dominated by caespitose phanerophytes that often build coppiced forests [74]. Due to the open canopy, a well-developed herb layer can be found dominated by caespitose hemicryptophytes (graminoids). Since many graminoids belong to the Eurasian chorotype, originating from non-forested areas situated in the eastern part of Europe and further in Asia [75], this chorotype is well represented in the region. It seems that the competitor functional strategy is not characteristic in these plots, but it is only diagnostic, since the stress tolerance strategy is more pronounced on rocky slopes and ruderal in deep humid plots in the bottom of dolines.

4.3. High Explanatory Power of Functional Traits (Life Forms, Plant Architecture) in Relation to Functional Strategies

This is the first study to have compared the explanatory power of chorotypes, functional traits and strategies in relation to ecological changes in karst dolines. The results show that functional traits best reflect ecological changes from the bottom to the top of dolines. Functional traits (life forms and architecture of plants) explain 68% of explained variance.

Chorotypes are defined as species with overlapping distribution patterns [24] and they cannot, therefore, fully reflect ecological differentiation along the gradient. Some differences can be found, since it can be hypothesized that species from a certain chorotype

appear in specific site conditions (e.g., Mediterranean species appear in warm and xeric site conditions), but it is not a basis for a definition of chorotype.

The functional strategies do not, in practice, reflect ecological differences and explain very little variation. Plots are positioned mainly along the CS gradient that is characteristic for forest communities [29]. We expected a stress tolerance strategy to prevail on slopes and the top; however, the current results show this strategy is more prevalent on slopes and at the bottom. It is not yet clear whether this is an artefact due to the low explanatory power of the strategies or a real result. If it is not an artefact, one possible explanation for the prevalence of stress-tolerant plants at the bottom of dolines is that factors not yet fully considered should be taken into account. Bottoms and north facing slopes of dolines receive less light, which could be a contributing factor, as well as possibly less favorable, clayey soil conditions at the bottom and the presence of rocks and moving slopes there. Although the trees appear to show that heat and water stress are the main stress factors for the absence of otherwise big and dominant trees such as *Fagus sylvatica, Carpinus betulus* from the plateau, which are replaced by *Quercus pubescens* and *Ostrya carpinifolia,* and by decreased sizes of the trees, the herb layer looks as if it may have a different response to the same macroclimatic conditions.

5. Conclusions

Our research found that functional traits best reflect the ecological gradient from the bottom to the top of dolines. We confirmed that the main floristic gradient is from the bottom of a doline to its top, and reflect the change of ecological conditions and only the transects of deeper dolines extend over the whole gradient. We found that species from the bottom originate from cooler regions and species on the top from warmer ones. We found more geophytes in the bottom and hemicryptophytes on the top. Concerning architecture, we found rhizomatous and crawling species in the bottom, liane, and rosulate on rocky slopes, whereas the tops are dominated by caespitose and suffrutescens plants. We could not confirm the hypothesis that competitor strategy dominates in the bottom, and that strategies are among the traits that best explain the species turnover. However, this pattern should be verified in other regions with different macroclimatic conditions.

The research contributed to the identification of dolines with a high conservation value, as only deeper dolines can provide shelter for rhizomatous species originating from cooler regions. On rocky slopes, we found therophytic and rosulate species that also contribute to the biodiversity of the karstic landscapes.

References

1. Beck-Mannagetta, G. *Die Vegetationsverhältnisse der Illyrischen Länder, Begreifend Südkroatien, die Quarnero-Inseln, Dalmatien, Bosnien und die Hercegovina, Montenegro, Nordalbanien, den Sandžak Novipazar und Serbien*; Verlag von Wilhelm Engelmann: Leipzig, Germany, 1901.
2. Horvat, I. Vegetacija ponikava. *Geogr. Glas.* **1953**, *14*, 1–15.
3. Bátori, Z.; Erdős, L.; Morschhauser, T.; Török, P.; Körmöczi, L. Vegetation of the dolines in Mecsek mountains (South Hungary) in relation to the local plant communities. *Acta Carsologica* **2009**, *38*, 237–252. [CrossRef]
4. Bátori, Z.; Gallé, R.; Erdos, L.; Körmöczi, L. Ecological conditions, flora and vegetation of a large doline in the Mecsek mountains (South Hungary). *Acta Bot. Croat.* **2011**, *70*, 147–155. [CrossRef]
5. Bátori, Z.; Lengyel, A.; Maróti, M.; Körmöczi, L.; Tölgyesi, C.; Bíró, A.; Tóth, M.; Kincses, Z.; Cseh, V.; Erdős, L. Microclimate-vegetation relationships in natural habitat islands: Species preservation and conservation perspectives. *Időjárás* **2014**, *118*, 257–281.
6. Bátori, Z.; Vojtkó, A.; Farkas, T.; Szabó, A.; Havadtői, K.; Vojtkó, A.E.; Tölgyesi, C.; Cseh, V.; Erdős, L.; Maák, I.E.; et al. Large- and small-scale environmental factors drive distributions of cool-adapted plants in karstic microrefugia. *Ann. Bot.* **2017**, *119*, 301–309. [CrossRef]
7. Bátori, Z.; Vojtkó, A.; Maák, I.E.; Lőrinczi, G.; Farkas, T.; Kántor, N.; Tanács, E.; Kiss, P.J.; Juhász, O.; Módra, G.; et al. Karst dolines provide diverse microhabitats for different functional groups in multiple phyla. *Sci. Rep.* **2019**, *9*, 7176. [CrossRef]
8. Bátori, Z.; Lőrinczi, G.; Tölgyesi, C.; Módra, G.; Juhász, O.; Aguilon, D.J.; Vojtkó, A.; Valkó, O.; Deák, B.; Erdős, L.; et al. Karstic microrefugia host functionally specific ant assemblages. *Front. Ecol. Evol.* **2020**, *8*, 613738. [CrossRef]
9. Aguilon, D.J.; Vojtkó, A.; Tölgyesi, C.; Erdős, L.; Kiss, P.J.; Lőrinczi, G.; Juhász, O.; Frei, K.; Bátori, Z. Karst environments and disturbance: Evaluation of the effects of human activity on grassland and forest naturalness in dolines. *Biologia* **2020**, *75*, 1529–1535. [CrossRef]
10. Kiss, P.J.; Tölgyesi, C.; Bóni, I.; Erdős, L.; Vojtkó, A.; Maák, I.E.; Bátori, Z. The effects on intensive logging on the capacity of karst dolines to provide potential microrefugia for cool-adapted plants. *Acta Geogr. Slov.* **2020**, *60*, 37–48. [CrossRef]
11. Kermavnar, J.; Eler, K.; Marinšek, A.; Kutnar, L. Initial understory vegetation responses following different forest management intensities in Illyrian beech forests. *Appl. Veg. Sci.* **2019**, *22*, 48–60. [CrossRef]
12. Kermavnar, J.; Marinšek, A.; Eler, K.; Kutnar, L. Evaluating short-term impacts of forest management and microsite conditions on understory vegetation in temperate fir-beech forests: Floristic, ecological, and trait-based perspective. *Forests* **2019**, *10*, 909. [CrossRef]
13. Kermavnar, J.; Ferlan, M.; Marinšek, A.; Eler, K.; Kobler, A.; Kutnar, L. Effects of various cutting treatments and topographic factors on microclimatic conditions in Dinaric fir-beech forests. *Agric. For. Meteorol.* **2020**, *295*, 108186. [CrossRef]
14. Kermavnar, J.; Eler, K.; Marinšek, A.; Kutnar, L. Post-harvest forest herb layer demography: General patterns are driven by pre-disturbance conditions. *For. Ecol. Manag.* **2021**, *491*, 119121. [CrossRef]
15. Ford, D.C.; Williams, P. *Karst Hydrogeology and Geomorphology*; John Wiley & Sons: Chichester, UK, 2007.
16. Kranjc, A. Classification of closed depressions in carbonate Karst. *Treatise Geomorphol.* **2013**, *6*, 104–114. [CrossRef]
17. Cernatič Gregorič, A. Typical doline and surface landforms of Kras (Slovenia): Karst landscape features and possibilities for their conservation. *Geoheritage* **2021**, *13*, 26. [CrossRef]
18. Vilović, T.; Buzjak, S.; Buzjak, N. Floristic and microclimatic features of the Sovljak doline (Mt. Velika Kapela, Croatia). *Šumarski List* **2019**, *143*, 35–43. [CrossRef]
19. Roa García, C.; Brown, S.; Krzic, M.; Lavkulich, L.; Roa-García, M.C. Relationship of soil water retention characteristics and soil properties: A case study from the Colombian Andes. *Can. J. Soil Sci.* **2021**, *101*, 147–156. [CrossRef]
20. Li, X.Y.; Contreras, S.; Solé-Benet, A. Unsaturated hydraulic conductivity in limestone dolines: Influence of vegetation and rock fragments. *Geoderma* **2008**, *145*, 288–294. [CrossRef]
21. Dala-Corte, R.B.; Melo, A.S.; Siqueira, T.; Bini, L.M.; Martins, R.T.; Cunico, A.M.; Pes, A.M.; Magalhães, A.L.B.; Godoy, B.S.; Leal, C.G.; et al. Thresholds of freshwater biodiversity in response to riparian vegetation loss in the Neotropical region. *J. Appl. Ecol.* **2020**, *57*, 1391–1402. [CrossRef]
22. Brunbjerg, A.K.; Bruun, H.H.; Dalby, L.; Fløjgaard, C.; Frøslev, T.G.; Høye, T.T.; Goldberg, I.; Læssøe, T.; Hansen, M.D.D.; Brøndum, L.; et al. Vascular plant species richness and bioindication predict multi-taxon species richness. *Methods Ecol. Evol.* **2018**, *9*, 2372–2382. [CrossRef]
23. Breg Valjavec, M.; Zorn, M.; Čarni, A. Bioindication of human-induced soil degradation in enclosed karst depressions (dolines) using Ellenberg indicator values (Classical Karst, Slovenia). *Sci. Total Environ.* **2018**, *640–641*, 117–126. [CrossRef] [PubMed]
24. Fattorini, S. On the concept of chorotype. *J. Biogeogr.* **2015**, *42*, 2246–2251. [CrossRef]
25. Pignatti, S. *Flora d'Italia*; Edagricole: Bologna, Italy, 1982; Volume 1–3.
26. Poldini, L. *La Vegetazione del Carso Isontino e Triestino*; Edizioni Lint: Trieste, Italy, 1989.
27. Šercelj, A. *Začetki in razvoj gozdov v Sloveniji*; Slovenska akademija znanosti in umetnosti: Ljubljana, Slovenia, 1996; Volume 50.
28. Herben, T.; Klimešová, J.; Chytrý, M. Effects of disturbance frequency and severity on plant traits: An assessment across a temperate flora. *Funct. Ecol.* **2018**, *32*, 799–808. [CrossRef]
29. Stupar, V.; Čarni, A. Ecological, floristic and functional analysis of zonal forest vegetation in Bosnia and Herzegovina. *Acta Bot. Croat.* **2017**, *76*, 15–26. [CrossRef]

30. Bruelheide, H.; Dengler, J.; Purschke, O.; Lenoir, J.; Jiménez-Alfaro, B.; Hennekens, S.M.; Botta-Dukát, Z.; Chytrý, M.; Field, R.; Jansen, F.; et al. Global trait–environment relationships of plant communities. *Nat. Ecol. Evol.* **2018**, *2*, 1906–1917. [CrossRef]

31. Breg Valjavec, M.; Ribeiro, D.; Čarni, A. Vegetation as the bioindicator of human-induced degradation in karst landscape: Case study of waste-filled dolines. *Acta Carsologica* **2017**, *46*, 95–110. [CrossRef]

32. Čarni, A.; Čonč, Š.; Breg Valjavec, M. Landform-vegetation units in karstic depressions (dolines) evaluated by indicator plant species and Ellenberg indicator values. *Ecol. Indic.* **2022**, *135*, 108572. [CrossRef]

33. Raunkiaer, C. *The Life Forms of Plants and Statistical Plant Geography, Being the Collected Papers of C. Raunkiaer*; Oxford University Press: Oxford, UK, 1934.

34. Paušič, A.; Čarni, A. Functional response traits and plant community strategy indicate the stage of secondary succession. *Hacquetia* **2012**, *11*, 209–225. [CrossRef]

35. Grime, J.P. *Plant Strategies, Vegetation Processes, and Ecosystem Properties*, 2nd ed.; Wiley: New York, NY, USA, 2001.

36. Pierce, S.; Negreiros, D.; Cerabolini, B.E.L.; Kattge, J.; Iaz, S.D.; Kleyer, M.; Shipley, B.; Wright, S.J.; Soudzilovskaia, N.A.; Onipchenko, V.G.; et al. A global method for calculating plant CSR ecological strategies applied across biomes world-wide. *Funct. Ecol.* **2017**, *31*, 444–457. [CrossRef]

37. Ravnjak, B.; Bavcon, J.; Čarni, A. Plant species turnover on forest gaps after natural disturbances in the Dinaric fir beech forests. *Diversity* **2022**, *14*, 209. [CrossRef]

38. Breg Valjavec, M.; Zorn, M.; Ribeiro, D. Mapping war geoheritage: Recognising geomorphological traces of war. *Open Geosci.* **2018**, *10*, 385–394. [CrossRef]

39. Kaligarič, M.; Ivanjšič, D. Vanishing landscape of the "classic" Karst: Changed landscape identity and projections for the future. *Landsc. Urban Plan.* **2014**, *132*, 148–158. [CrossRef]

40. Kaligarič, M.; Čuš, J.; Škornik, S.; Ivajnšič, D. The failure of agri-environment measures to promote and conserve grassland biodiversity in Slovenia. *Land Use Policy* **2019**, *80*, 127–134. [CrossRef]

41. Mihevc, A.; Prelovšek, M.; Zupan Hajna, N. Introduction to the Dinaric Karst. *J. Chem. Inf. Model.* **2010**, *53*, 71.

42. Ogrin, D. *Podnebje Slovenske Istre (The Climate of Slovenian Istria)*; Zgodovinsko društvo za Južno Primorsko: Znanstveno-raziskovalno središče Republike Slovenije: Koper, Slovenia, 1995.

43. Vrščaj, B.; Repe, B.; Simončič, P. *The Soils of Slovenia*; Springer Netherlands: Dordrecht, The Netherlands, 2017.

44. Cervellini, M.; Zannini, P.; Di Musciano, M.; Fattorini, S.; Jiménez-Alfaro, B.; Rocchini, D.; Field, R.; Vetaas, O.R.; Irl, S.D.H.; Beierkuhnlein, C.; et al. A grid-based map for the biogeographical regions of Europe. *Biodivers. Data J.* **2020**, *8*, e53720. [CrossRef]

45. Breg Valjavec, M.; Zorn, M.; Čarni, A. Human-induced land degradation and biodiversity of Classical Karst landscape: On the example of enclosed karst depressions (dolines). *Land Degrad. Dev.* **2018**, *29*, 3823–3835. [CrossRef]

46. Dakskobler, I. Prispevek k poznavanju gozdne vegetacije Krasa (jugozahodna Slovenija). *Ann.-Ser. Hist. Nat.* **2006**, *16*, 57–76.

47. Dakskobler, I.; Sadar, Z.; Čarni, A. Phytosociological analysis of Quercus cerris woods in the sub-mediterranen phytogeographical region of Slovenia. *Folia Biol. Geol.* **2017**, *58*, 5–34.

48. Dakskobler, I.; Poldini, L. Phytosociological analysis of noble hardwood forests (Ostryo-Tilienion platyphylli) in the Karst and its neighbouring regions (SW Slovenia). *Hacquetia* **2021**, *20*, 327–372. [CrossRef]

49. Bátori, Z.; Erdős, L.; Gajdács, M.; Barta, K.; Tobak, Z.; Frei, K.; Tölgyesi, C. Managing climate change microrefugia for vascular plants in forested karst landscapes. *For. Ecol. Manag.* **2021**, *496*, 119446. [CrossRef]

50. Kermavnar, J.; Marinšek, A.; Kuntnar, L. Variation in floristic and trait composition along environmental gradients in the herb layer of temperate forests in the transition zone between Central and SE Europe. *Plant Ecol.* **2022**, *223*, 229–242. [CrossRef]

51. Braun-Blanquet, J. *Pflanzensoziologie, Grundzüge der Vegetationskunde*, 3rd ed.; Springer: Wien, Austria, 1964.

52. Hennekens, S.; Schaminée, J. TURBOVEG, a comprehensive data base management system for vegetation data. *J. Veg. Sci.* **2001**, *12*, 589–591. [CrossRef]

53. Tichý, L. JUICE, software for vegetation classification. *J. Veg. Sci.* **2002**, *13*, 451–453. [CrossRef]

54. Oksanen, J.; Blanchet, F.G.; Friendly, M.; Kindt, R.; Legendre, P.; McGlinn, D.; Minchin, P.R.; O'Hara, R.B.; Simpson, G.L.; Solymos, P.; et al. CRAN Vegan 2.5-5, community ecology package. Available online: http://packages.renjin.org/package/org.renjin.cran/vegan (accessed on 15 December 2021).

55. Ellenberg, H.; Weber, H.E.; Düll, R.; Wirth, V.; Werner, W.; Paulißen, D. Zeigerwerte von Pflanzen in Mitteleuropa. *Scr. Geobot.* **1991**, *18*, 1–248.

56. Tölgyesi, C.; Bátori, Z.; Erdos, L. Using statistical tests on relative ecological indicator values to compare vegetation units—Different approaches and weighting methods. *Ecol. Indic.* **2014**, *36*, 441–446. [CrossRef]

57. Pignatti, S.; Menegoni, P.; Pietrosanti, S. Valori di bioindicazione delle piante vascolari della flora d'Italia. *Braun-Blanquetia* **2005**, *39*, 1–97.

58. Hodgson, J.G.; Wilson, P.J.; Hunt, R.; Grime, J.P.; Thompson, K. Allocating C-S-R plant functional type: A soft approach to hard problem. *Oikos* **1999**, *85*, 282–294. [CrossRef]

59. Hunt, R.; Hodgson, J.; Thompson, K.; Bungener, P.; Dunnett, N.; Askwe, A. A new practical tool for deriving a functional signature for herbaceous vegetation. *Appl. Veg. Sci.* **2004**, *7*, 163–170. [CrossRef]

60. Klotz, S.; Kühn, I.; Durka, W. *BIOLFLOR—Eine Datenbank zu Biologisch-Ökologischen Merkmalen der Gefäßpflanzen in Deutschland*; Schriftenreihe für Vegetationskunde 38 Bundesamt für Naturschutz (BfN): Bonn, Germany, 2002.

61. Ter Braak, C.J.F.; Šmilauer, P. *CANOCO Reference Manual and User's Guide: Software for Ordination (Version 5.0)*; Microcomputer Power: Ithaca, NY, USA, 2012.

62. Šmilauer, P.; Lepš, J. *Multivariate Analysis of Ecological Data Using CANOCO 5*; Cambridge University Press: Cambridge, UK, 2014; ISBN 9781139627061.

63. Čonč, Š.; Oliviera, T.; Portas, R.; Černe, R.; Breg Valjavec, M.; Krofel, M. Dolines and cats: Remote detection of karst depressions and their application to study wild felid ecology. *Remote Sens.* **2022**, *14*, 656. [CrossRef]

64. Poldini, L. L'Asaro-Carpinetum betuli Lausi 64 del Carso nordadriatico. *Stud. Geobot.* **1985**, *5*, 31–38.

65. Stajić, S.; Čokeša, V.; Miletić, Z.; Eremija, S.; Ćirković-Mitrović, T.; Marković, M.; Jovanović, F. Phytosociological characteristics of sessile oak and hornbeam forests (Querco petraeae-Carpinetum betuli Rudski 1949. s.l.) in the area of Kosmaj. *Sustain. For.* **2020**, *81–82*, 41–51.

66. Koljanin, D.; Milanović, Đ.; Stupar, V. New data on the distribution and threat status of three rare spring geophytes from Bosnia and Herzegovina. *Phytol. Balc.* **2021**, *27*, 107–114.

67. Lubbe, F.C.; Klimešová, J.; Henry, H.A. Winter belowground: Changing winters and the perennating organs of herbaceous plants. *Funct. Ecol.* **2021**, *35*, 1627–1639. [CrossRef]

68. Mori, E.; Lazzeri, L. Does wild boar rooting affect spatial distribution of active burrows of meadow-dwelling voles? *Biologia* **2021**, *76*, 981–986. [CrossRef]

69. Košir, P.; Čarni, A.; Di Pietro, R. Classification and phytogeographical differentiation of broad-leaved ravine forests in southeastern Europe. *J. Veg. Sci.* **2008**, *19*, 331–342. [CrossRef]

70. Sekulić, D.; Karadžić, B.; Kuzmanović, N.; Jarić, S.; Mitrović, M.; Pavlović, P. Diversity of Ostrya carpinifolia forests in ravine habitats of Serbia (S-E Europe). *Diversity* **2021**, *13*, 59. [CrossRef]

71. Kontopanou, A.; Panitsa, M. Habitat islands on the Aegean Islands (Greece): Elevational gradient of chasmophytic diversity, endemism, phytogeographical patterns and need for monitoring and conservation. *Diversity* **2020**, *12*, 33. [CrossRef]

72. Panitsa, M.; Kokkoris, I.P.; Kougioumoutzis, K.; Kontopanou, A.; Bazos, I.; Strid, A.; Dimopoulos, P. Linking taxonomic, phylogenetic and functional plant diversity with ecosystem services of cliffs and screes in Greece. *Plants* **2021**, *10*, 992. [CrossRef]

73. Stupar, V.; Brujić, J.; Škvorc, Ž.; Čarni, A. Vegetation types of thermophilous deciduous forests (Quercetea pubescentis) in the Western Balkans. *Phytocoenologia* **2016**, *46*, 49–68. [CrossRef]

74. Trajkov, P.; Dubravac, T.; Tanovski, V.; Nestorovski, L.; Sotirovski, K.; Trajanov, Z. Coppice forest management planning and the regeneration potential of pure and mixed oak coppice forests in North Macedonia. *South-East Eur. For.* **2019**, *10*, 165–172. [CrossRef]

75. Török, P.; Neuffer, B.; Heilmeier, H.; Bernhardt, K.G.; Wesche, K. Climate, landscape history and management drive Eurasian steppe biodiversity. *Flora* **2020**, *271*, 151685. [CrossRef]