

Carbohydrate storage in herbs: the forgotten functional dimension of the plant economic spectrum

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- **Background and Aims** Although the plant economic spectrum seeks to explain resource allocation strategies, carbohydrate storage is often omitted. Belowground storage organs are the centre of herb perennation, yet little is known about the role of their turnover, anatomy and carbohydrate storage in relation to the aboveground economic spectrum.
- **Methods** We collected aboveground traits associated with the economic spectrum, storage organ turnover traits, storage organ inner structure traits and storage carbohydrate concentrations for ~80 temperate meadow species.
- **Key Results** The suites of belowground traits were largely independent of one another, but there was significant correlation of the aboveground traits with both inner structure and storage carbohydrates. Anatomical traits diverged according to leaf nitrogen concentration on the one hand and vessel area and dry matter content on the other; carbohydrates separated along gradients of leaf nitrogen concentration and plant height.
- **Conclusions** Contrary to our expectations, aboveground traits and not storage organ turnover were correlated with anatomy and storage carbohydrates. Belowground traits associated with the aboveground economic spectrum also did not fall clearly within the fast–slow economic continuum, thus indicating the presence of a more complicated economic space. Our study implies that the generally overlooked role of storage within the plant economic spectrum represents an important dimension of plant strategy.

Key words: Anatomy, belowground, herbaceous plant, lignin, non-structural carbohydrates, plant economic spectrum, storage, persistence.

INTRODUCTION

The examination of an economic spectrum within plants began with leaves, describing plants towards the fast and slow ends of the continuum favouring an acquisitive strategy and a conservative strategy, respectively (Wright *et al.*, 2004). It was later proposed that an economic spectrum exists within all plant organs and these organs work in tandem (Reich, 2014). For example, plants in a resource-abundant habitat (moist and nutrient-rich) would have a highly acquisitive strategy with short-lived structures and structural investment in organs would be lower, with low leaf dry matter content (LDMC), low wood density, and roots with greater length compared with root mass (Freschet *et al.*, 2010a; Reich, 2014). In contrast, plants in a more stressful habitat would conserve resources by forming long-lived, tougher structures with contrasting characteristics.

Although leaf traits can form a convenient spectrum across different types of plant, there is some dispute regarding the strength of the relationship with other structures (especially root traits; e.g. Valverde-Barrantes *et al.*, 2017), and the characteristics of organs and economic strategy can also vary greatly between woody and herbaceous plants (Klimešová and Herben,

2014). When a plant stem is considered in the plant economic spectrum, it is usually the woody trunk of trees that combines the functions of height growth and competitive ability, vascular connection between roots and leaves, and storage that enables the annual growth of new leaves in temperate zones (Reich, 2014). However, the tree trunk is not completely functionally analogous to the aboveground annual stems of herbaceous perennials (Klimešová *et al.*, 2015). Although both form a connection between belowground organs and leaves, the storage necessary for the regrowth of herbs in seasonal climates is deposited in belowground organs (e.g. rhizomes, bulbs and tubers) (Raunkjær, 1934; Asaeda *et al.*, 2008). This storage represents an important economic allocation of plant resources, yet its role in the plant economic spectrum is largely unknown (Klimešová *et al.*, 2018).

How the inclusion of belowground storage organs would change our perception of the plant economic spectrum could be illustrated by the difference in organ longevity between aboveground stems and belowground storage organs in herbs. Organ longevity is the most important trait in organ economy because plants generally invest less in structures of short-lived organs than in those of long-lived organs; only plants growing

in less stressful conditions may afford to produce new organs instead of investing in the maintenance of old organs (Chapin *et al.*, 1990). Although the aboveground stems of herbs are seasonal structures, belowground storage organs persist from 1 year to several decades (Klimešová and Klimeš, 2008). We can further expect that short-lived storage organs differ in their anatomical structure and storage from long-lived structures (Schweingruber and Poschold, 2005). Thus, we argue that the consideration of storage organ traits (turnover, inner structure and carbohydrate concentration) in herbs will provide a picture of their economy wholly different from that obtained using the traits of aboveground organs.

The turnover of belowground storage organs such as rhizomes (the most common storage organ in the flora of temperate Europe; Klimešová and Klimeš, 2008) was described as falling into two contrasting categories – splitters and integrators – representing the competitive and conservative strategies, respectively (Jónsdóttir and Watson, 1997). Splitters, characterized by low longevity and fast turnover, have a higher lateral spread (horizontal rhizome increment) and multiplication rate (number of ramets) than integrators, which have greater longevity and slower turnover. These strategies correspond to environmental conditions; longevity decreases while lateral spread and multiplication rate increase with greater moisture and nutrient availability (van Groenendael *et al.*, 1996; Klimeš, 2008; Klimešová *et al.*, 2011, 2015; Klimešová and Herben, 2014). The relationship of the morphological characteristics describing the turnover of belowground storage organs to the aboveground traits of the economic spectrum was evaluated for the flora of central Europe; as expected, there was a negative correlation between specific leaf area (SLA) and rhizome persistence (Klimešová *et al.*, 2015).

The persistence and eventual disintegration of a storage organ is a programmed event within plant ontogeny and thus this trait is likely conditioned by organ inner structure, for example by secondary thickening and the abundant presence of lignin (Hay and Kelly, 2008; Watson, 2008). Lignin is an important structural polymer, especially for forming and reinforcing the vascular conduits that connect the roots to the aboveground plant parts (Bazzaz *et al.*, 2000; Klimešová *et al.*, 2018). The content of lignin in storage organs is between that of aboveground stems and leaves (Steen and Larsson, 1986; Freschet *et al.*, 2010a, b; Amougou *et al.*, 2011). Storage organs also contain parenchyma, which is primarily storage tissue (Esau, 1977). It could be expected that the lignin content within storage organs increases with a more conservative strategy (Lens *et al.*, 2016), while the parenchyma content increases with a more acquisitive strategy because greater storage may align with presence in a resource-rich environment. However, greater storage may also be necessary for surviving harsh conditions, and storage organ anatomy has not been studied within the context of the economic spectrum. Additionally, greater vessel diameter has been linked to an increase in hydraulic efficiency but also vulnerability to drought- and frost-induced embolism, and thus it too may be part of the more acquisitive strategy in belowground storage organs (Tyree *et al.*, 1994; Doležal *et al.*, 2019a).

The main function of storage organs is to provide space for the temporary storage of non-structural carbohydrates until later mobilization for use (Chapin *et al.*, 1990). Starch is the most common non-structural carbohydrate in plants but also a large

and immobile polysaccharide; thus, many plants also utilize water-soluble molecules that can function as storage or mediate the response to stressors such as drought and frost (Dias-Tagliacozzo *et al.*, 2004; Hisano *et al.*, 2004; Patton *et al.*, 2007; Janeček *et al.*, 2011). These include poly- and oligosaccharides (including raffinose family oligosaccharides and fructans) and the mono- and disaccharides (and small sugar alcohols, e.g. sorbitol; Lewis, 1984). The smallest carbohydrates (mono- and disaccharides) are always present and serve as transport molecules (Liu *et al.*, 2012; Jensen *et al.*, 2016). Carbohydrate type can be phylogenetically constrained, with the notable cases of raffinose family oligosaccharides in the Lamiales (Lewis, 1984) and fructans within the families Asteraceae, Boraginaceae, Campanulaceae and Amaryllidaceae and the Pooidae subfamily of Poaceae (Hendry, 1987). The composition of carbohydrate storage types is known for numerous plant species (e.g. Hendry, 1987; Gomes de Moraes *et al.*, 2016) and it is expected that osmotically active water-soluble carbohydrates are typical of stressful conditions where the growth of plants is constrained by drought or cold. We can therefore expect that because of the role of these carbohydrates in stress response and avoidance, species storing water-soluble poly- and oligosaccharides as the main carbon storage compound will be aligned to the slow end of the economic spectrum.

Changes in carbohydrate storage are usually studied on an intraspecific level (e.g. seasonal refilling and consumption during resprouting) (Lee and Dunton, 1996; McLaurin *et al.*, 1999; Asaeda *et al.*, 2008), while we have no explanation for interspecific differences because comparative studies of the carbohydrate storage strategies of perennial herbs over numerous species and in relation to specific ecological factors are extremely rare (Palacio *et al.*, 2007; Janeček *et al.*, 2011). The extensive storage of carbohydrates is generally interpreted as an adaptation for surviving stressful conditions and damage (Klimeš *et al.*, 1993; Asaeda *et al.*, 2006; Martínez-Vilalta *et al.*, 2016), thus aligning with a more conservative strategy. However, it is important to note that the carbohydrate pool (the amount of carbohydrates in storage organs per plant) is seldom studied because of methodological difficulties (Klimešová *et al.*, 2019). Carbohydrate concentration (the mass of carbohydrates per unit of storage organ dry mass) is easier to study and its relationship to the plant economic spectrum may or may not be the same as the expected relationship of plant economy to the carbohydrate pool.

In this study, we are interested in whether there is a united economic spectrum that encompasses both the aboveground acquisitive organs and belowground storage organs (morphological, anatomical and storage traits) of perennial herbs (Fig. 1). We hypothesize that plants on the fast end of the economic spectrum [characterized by high SLA, high leaf nitrogen concentration (LNC), low LDMC and greater height] have faster turnover of storage organs (characterized by large lateral spread, low persistence and high multiplication rate) and high percentages of parenchyma, large conduits, and high total non-structural carbohydrate concentration, formed preferentially by starch. In contrast, plants on the slow end of the economic spectrum are characterized by traits of low turnover (e.g. low lateral spread, high persistence), high percentages of lignified tissue, thin conduits, and low carbohydrate concentration formed by water-soluble sugars in the storage organs. We further hypothesized that storage turnover and storage carbohydrate concentration are interconnected

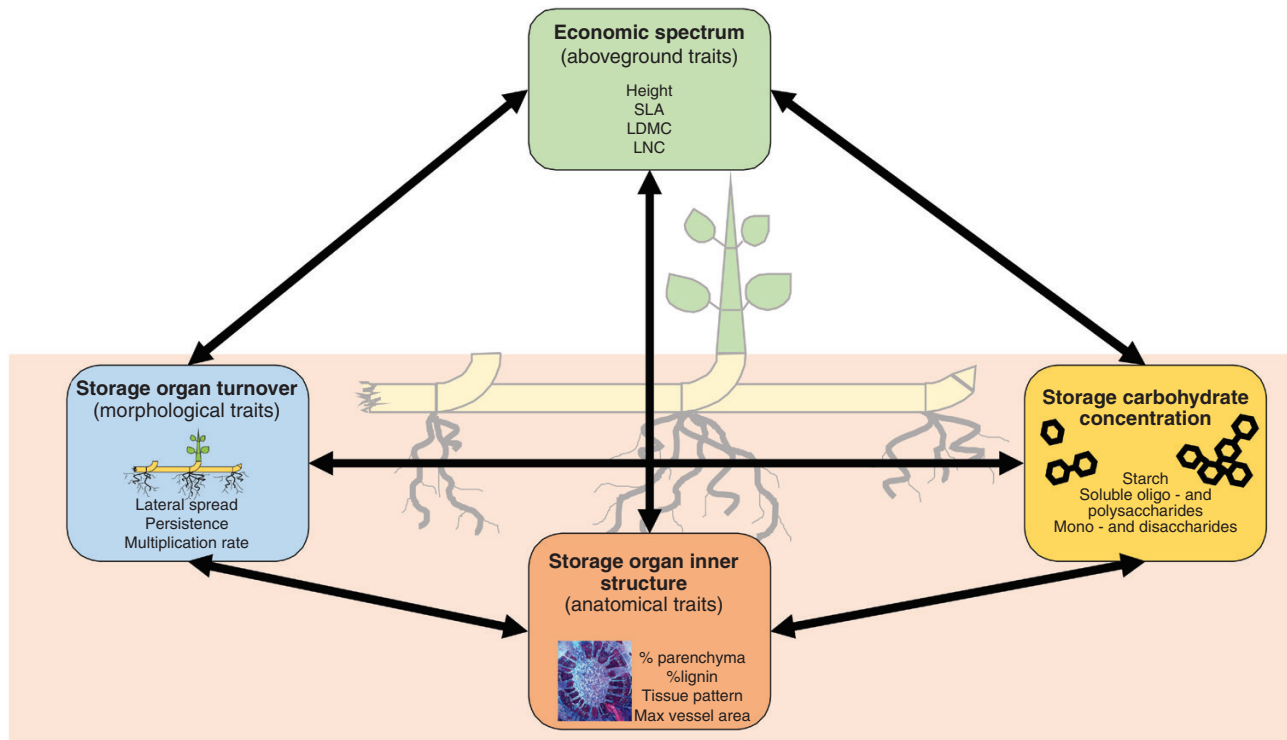


FIG. 1. Conceptual structure of trait groups and the relationships between aboveground traits and belowground storage traits.

through the inner structure of storage organs. Storage organs with high turnover will be characterized by high carbohydrate concentration because of a higher percentage of parenchyma, and storage organs with slow turnover will be characterized by low carbohydrate concentrations and a high percentage of lignified tissue forming a solid cylinder.

MATERIALS AND METHODS

Study site

We sampled plants on two grasslands (meadows mown once annually for hay without grazing or burning) with different moisture levels. The dry meadow [Čertyryje, in the Bílé Karpaty Mountains, south-east Czech Republic (48°54' N, 17°25' E; 440 m a.s.l.)] has a mean annual precipitation around 670 mm, mean annual temperature of 9.1 °C and deep calcium-rich soils that dry out in summer. For a more detailed description see Klimeš (1995) and Doležal *et al.* (2016). The wet meadow [Ohrazení, near the town of České Budějovice (48°57' N, 14°36' E; 500 m a.s.l.)] has a mean annual precipitation around 620 mm, mean annual temperature of 7.8 °C and acidic soil that remains moist throughout the year. For a more detailed description see Lepš (1999) and Doležal *et al.* (2019b).

Plant collection

Overall, we collected 78 perennial herbs (49 dicots and 29 monocots). Our first sampling yielded 22 species in the dry meadow and 19 species in the wet meadow. We sampled plants

in 2006 and 2008 in June (before mowing) and in October (before cold winter temperatures), in five or six replications per species during each sampling. During our second sampling, we collected an additional 36 species in June 2010 in the dry meadow in five replicates. The aboveground biomass was taken into the laboratory and used for measuring aboveground traits (plant height, SLA, LDMC and LNC). The belowground biomass was washed immediately after harvesting, divided into roots, rhizomes and stem bases (if present), and frozen in liquid nitrogen. Because rhizomes and roots of meadow species are intermingled in a dense net, it is virtually impossible to harvest the entire belowground biomass of a plant and thus only fragments of roots and rhizomes were collected. Consequently, only carbohydrate concentrations (and not carbohydrate pool) could be assessed for the harvested plants (see also Klimešová *et al.*, 2017a). Carbohydrates were analysed both in belowground organs of stem origin (stem bases and rhizomes) and in roots; only bulky organs were considered as storage organs for the purpose of this study, even though concentrations in relatively fine roots may be higher than in bulky storage organs (Janeček and Klimešová, 2014).

Storage organ samples for anatomy were not collected during sampling in 2006, 2008 and 2010; additional samples for these species were collected in the same meadows in 2016 and 2019, or data were supplemented with existing cross-sections from Schweingruber *et al.* (2020). We cut 2-cm-long fragments from the root collar (the area between the root and stem) in non-clonal forbs, 2-cm-long fragments from the most distal part of the rhizomes in clonal forbs, and 2-cm-long fragments from the culm in graminoids (grasses, sedges) at internodes above the base of the culm (for details see Schweingruber and Poschold, 2005; Klimešová *et al.*, 2019). Fragments were stored in 50 % ethanol

to avoid drying and decomposition of samples until processing in the anatomical laboratory of the Department of Functional Ecology, Institute of Botany, Třeboň, Czech Republic.

Trait assessment

Aboveground economic spectrum (leaf traits and plant height). In the field, we removed one undamaged fully expanded leaf per individual from the stem and weighed it fresh. The maximum height of each plant was measured in the laboratory; this measure is included to indicate plant size because size in herbs can be affected by productivity (Klimešová *et al.*, 2015). For the leafless *Juncus effusus*, part of a young photosynthetic stem (length >4 cm) was considered to be a leaf (Perez-Harguindeguy *et al.*, 2016). In the laboratory, we measured the one-side projected leaf area for each sample with an AM200 leaf area meter (ADC BioScientific, Hoddesdon, UK) or by scanning it and analysing the image. Leaves were dried at 80 °C (minimum 24 h) and weighed. From the leaf measurements we calculated SLA (the ratio of leaf area to dry weight, mm² mg⁻¹) and LDMC (the ratio of leaf dry mass divided by fresh mass, %). Dry leaves were ground to a powder using an MM 200 mixer mill (Retsch, Haan, Germany) and after samples had undergone Kjeldahl digestion the nitrogen concentration was measured using a flow injection analysis system (FIA QC8500, Lachat Instruments, USA) (mg g⁻¹).

Storage organ turnover traits. From the CLO-PLA3 database (the database of clonal traits for the flora of Central Europe; Klimešová *et al.*, 2017b), we assessed three clonal traits: rhizome persistence (longevity of connection between ramets, in years); lateral spread (rhizome increment in horizontal direction grown per year); and multiplication rate (number of ramets per parental ramet per year). Rhizome persistence is difficult to measure because of limitations within both anatomical and morphological techniques (Klimešová *et al.*, 2019), and thus it was included as a categorical variable (less than or more than 4 years, according to the CLO-PLA3 database) in the full dataset. Persistence of non-clonal species was set as more than 4 years. For the analysis of relationships between anatomy and persistence, we used a subset of 59 species with more detailed data available, with a close approximation of the number of years of persistence measured by anatomical or morphological techniques for both clonal and non-clonal species. For non-clonal species, lateral spread and multiplication rate were set to zero.

Storage organ inner structure traits. Plant material was sectioned using a sledge microtome (modified Reichert type; © H. Gartner/F.H. Schweingruber, Birmensdorf, Switzerland). Cross-section thickness was between 15 and 40 µm (Gärtner and Schweingruber, 2013). Cross-sections were double-stained using a 1:1 mixture of blue and red dyes (Astra Blue and safranin, respectively). Double staining provided differentiation of lignified xylem cells (fibres, lignified vessels and lignified cells) and cellulose (parenchyma cells and non-lignified cells). The stained cross-sections were dehydrated with a series of ethanol solutions (75 %, 96 % and absolute), washed with xylene and fixed with Canada balsam. The final slides were

examined using an Olympus BX53 microscope, an Olympus DP73 camera and cellSense Entry 1.9 software.

To determine the proportion of the three basic tissue types with different functions, a single polygon was drawn over each cross-section to contain all tissue types identified by their colour after staining: blue, parenchyma (storage); white, conduits (water conductive/transport); and red, lignin (mechanical/support). Damaged parts were avoided (Crivellaro *et al.*, 2012). Tissue percentages were quantified using the software ImageJ (Schneider *et al.*, 2012): in the selected polygon 100 randomly placed probes were analysed and the average percentage of the tissue types was calculated.

Additionally, cross-sections were divided into four categories based on the anatomical structure and tissue distribution: monocotyledonous herbs with belowground storage stem without hollow pith (Monocot 1) and with hollow pith (Monocot 2); eudicotyledonous herbs with belowground storage stem with parenchyma and rays separating regions of lignified secondary tissue (Dicot 1); and eudicotyledonous herbs with belowground storage stem with solid region of lignified secondary tissue (Dicot 2), (Fig. 2).

Storage carbohydrate concentrations. Frozen samples of storage organs were freeze-dried, weighed and ground in the laboratory. Glucose, fructose and sucrose were extracted in hot ethanol (Klimešová *et al.*, 2019). In *Plantago lanceolata* we analysed the content of sorbitol because it is the common sugar alcohol in this species.

Glucose, fructose, sucrose and sorbitol contents were assessed using high-performance anion exchange chromatography with a pulsed amperometric detector (HPAE-PAD; Dionex ISC-3000). Separation was performed using a CarboPac PA1 analytical column (Dionex, Prague, Czech Republic). The content of raffinose family oligosaccharides was calculated as the difference in ethanol-soluble carbohydrates (galactose, glucose, fructose and sucrose) before and after the addition of α -galactosidase (*Aspergillus niger*; Megazyme, 2020) to the ethanol extract.

Fructan content was analysed by the fructan assay procedure developed by Megazyme International Ireland Ltd (Co. Wicklow, Ireland). This method includes the specific hydrolysis of fructans by fructanase. After hydrolysis, the reducing sugars were measured with the PAHBAH reducing sugar method. The starch content was determined by a total starch assay procedure also developed by Megazyme International. This method uses starch hydrolysis with thermostable α -amylase and amyloglucosidase. The product of these hydrolyses (glucose) is then stained colorimetrically with the glucose-determination reagent GOPOD (glucose oxidase/peroxidase).

All storage carbohydrates were merged to three groups based on size and function (mono- and disaccharides, oligo- and polysaccharides, starch) and expressed as percentages of storage organ dry mass. Mono- and disaccharides (including sorbitol, the small sugar alcohol common in *P. lanceolata*) are small and have additional transport function, whereas our category of oligo- and polysaccharides (including fructans and raffinose family oligosaccharides) contains all other water-soluble carbohydrates, which are typically of an intermediate size between the first category and starch. Total non-structural carbohydrates were obtained by summing all carbohydrate types found in one sample and were also expressed as the percentage of storage organ dry mass.

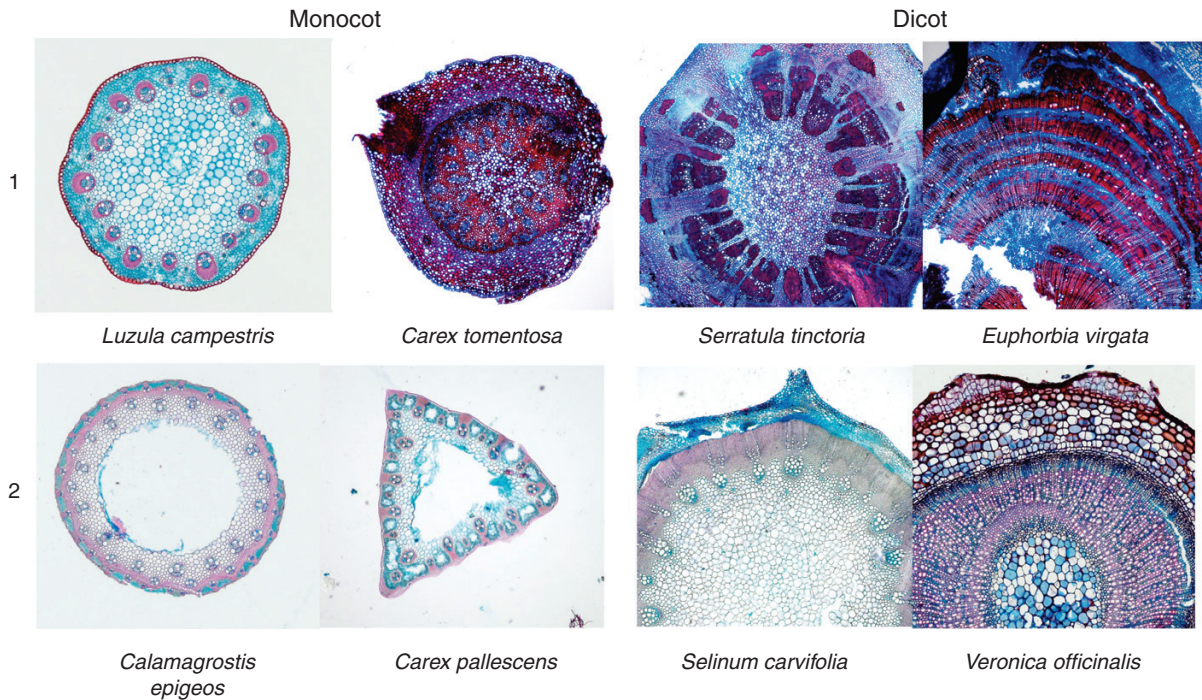


FIG. 2. Examples of the anatomical categories found among the species collected. In monocots, categories are based on the presence or absence of hollow pith, while in dicots they are based on the presence or absence of parenchyma rays separating regions of lignified secondary tissue.

Statistical analysis

We used canonical correlation analysis (Hotelling, 1936) to evaluate the links between groups of plant aboveground and belowground traits for 78 of the collected species (49 dicots and 29 monocots). This technique finds the linear combination of two multivariate datasets that maximizes their correlation and thus is suitable for exploration of symmetrical relationships among groups of traits. We assessed the relationships between aboveground economic spectrum traits, belowground storage organ turnover traits, storage organ inner structure traits and storage carbohydrate concentrations using only the summer storage organ values from 2006, 2008 and 2010 (for traits used see Fig. 1). Trait values for each species are the averages of all individuals assessed. To account for variation in evolutionary relatedness between species (Felsenstein, 1985), we used phylogenetic analysis (for description see Revell and Harrison, 2008) with simultaneous estimation of phylogenetic signal (Pagel's λ ; Pagel, 1999), which accounts for the relatedness to the appropriate degree (Freckleton *et al.*, 2002; Revell, 2010).

Because growth is mainly a multiplicative process, many plant characteristics show highly skewed distributions and multiplicative effects are of greater interest than additive ones. We transformed such traits in our dataset (height, lateral spread, multiplication rate, maximum vessel area and carbohydrate concentrations) prior to analyses using natural logarithms (when traits had zero values, we replaced these values by half of the minimum of the rest of the values). Our traits differed in their units, and thus we standardized them to 0 mean and standard deviation 1 prior to canonical correlation analyses.

For two traits (persistence and total carbohydrate concentration) we had more detailed data for a subset of our species

(for 59 and 38 species, respectively). To provide better insight into their relationship with storage organ inner structure traits (tissue pattern and parenchyma and lignin percentages), we used phylogenetic linear regressions with simultaneous estimation of phylogenetic signal. Specifically, we used the logarithm of persistence (in years) as the response variable and parenchyma and lignin percentages and their interaction as predictors for the first model. To explore the second relationship, we ran models with the logarithm of total carbohydrate concentration as the response variable and parenchyma as the predictor. We used total carbohydrate concentration in summer and compared the results for the same model with total carbohydrate concentration in autumn, about which we had data for a subset of our species. We also explored if tissue patterns differed in parenchyma and lignin percentages and in storage carbohydrate concentrations (for all 78 species) to get better insight into the effects of this anatomical trait.

To visually assess the interplay of traits both among each other and with greater environmental context, we conducted an exploratory principal components analysis (PCA) with the species optima for moisture and nitrogen using Ellenberg indicator values. Ellenberg indicator values represent the environmental preferences of the species of Central Europe, circumscribing their optima along these gradients (Ellenberg *et al.*, 1992; Dieckmann, 2003). Although primarily experimental observations, their effectiveness in ecological studies has been extensively tested (e.g. Schaffers and Sýkora, 2000; Wamelink *et al.*, 2003). The first two axes of the phylogenetic PCA were fitted using the package phytools version 0.7-20 (Revell, 2012). Traits were standardized to 0 mean and standard deviation 1 prior to the analysis. Ellenberg indicator values (Chytrý *et al.*, 2018) and types of anatomy (Monocot 1, 2 and Dicot 1, 2; centroids)

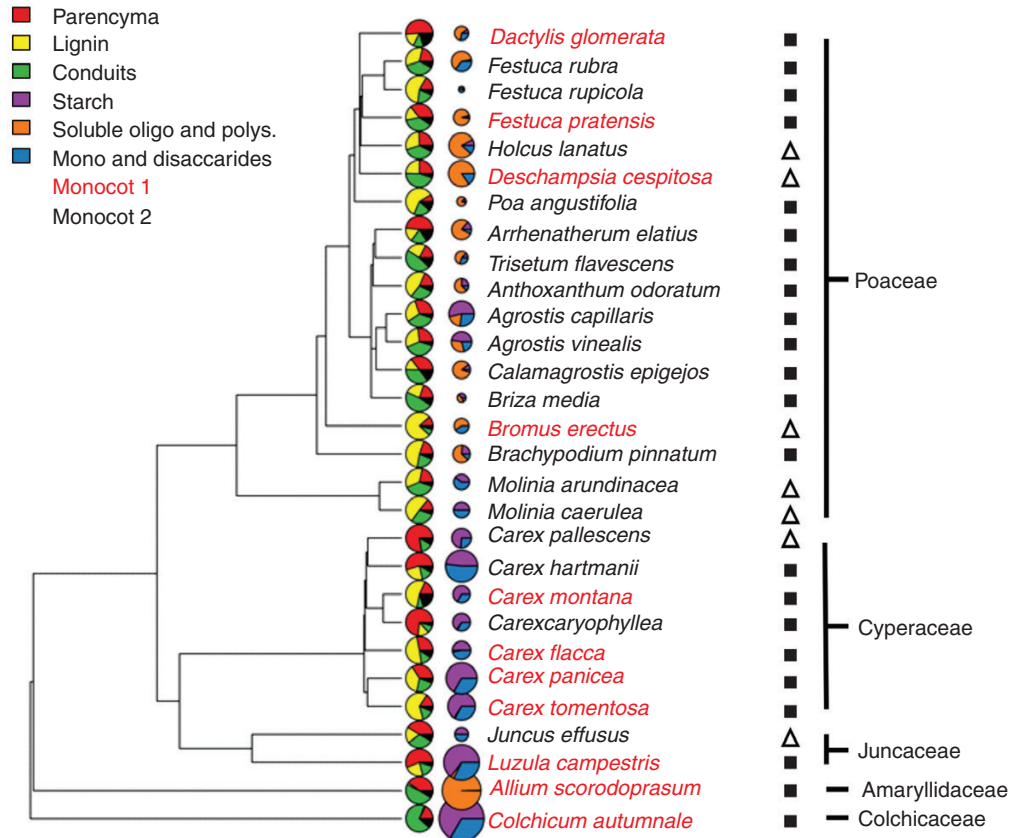


FIG. 3. Phylogenetic tree of studied monocots with visualization of anatomy and storage proportions, anatomical type and family. The size of the pie charts for carbohydrate concentrations corresponds to the logarithm of total storage carbohydrate concentration and species name colour denotes anatomical category. Storage organ type is denoted by a black square for rhizomes and a white triangle for stem bases.

were passively projected using the function `envfit` from package `vegan` (version 2.5-6; Oksanen *et al.*, 2018).

Analyses were done in R (version 4.0.0; R Core Team, 2020) using the package `phytools` for canonical correlation analysis (version 0.7-47; Revell, 2012) and package `caper` for phylogenetic regression (version 1.0.1; Orme *et al.*, 2013). Phylogenetic information about our species was taken from Durka and Michalski (2012).

RESULTS

Within our dataset, storage organ lignin percentage varied from <1 % in *Vicia sepium* to over 76 % in *Bromus erectus* and parenchyma was from 7 % in *Leucanthemum vulgare* to 63 % in *Selinum carvifolia* (Figs 3 and 4). Starch and oligo- and polysaccharides ranged from near total absence in many species to almost 100 % of dry mass of storage organs in *Allium scorodoprasum* (oligo- and polysaccharides) and *Angelica sylvestris* (starch). Mono- and disaccharides were similarly variable but never composed >56 % of dry mass of storage organs (*Primula veris*).

We evaluated links between the four groups of traits and resource acquisition and storage (economic spectrum traits, storage organ turnover traits, storage organ inner structure traits

and storage carbohydrate concentrations). There were no significant links between the three belowground storage organ trait groups. However, we found strong links between economic spectrum traits and two of the belowground groups – storage organ inner structure and storage carbohydrate concentrations (Figs 5 and 6). Relationships among aboveground economic traits and storage organ inner structure (Fig. 6B) were driven mainly by high correlations of anatomical types and vessel area with economic traits, whereas percentages of parenchyma and lignin were independent of other compared traits. The relationship between aboveground economic traits and carbohydrate storage concentration was mainly a result of the correlation of plant height and, to a lesser degree, leaf nitrogen with carbohydrate types, while SLA and LDMC were mostly independent (Fig. 6C).

In the analyses of more detailed data for subsets of our species, parenchyma and lignin were negatively correlated (Pearson correlation coefficient, -0.728), and thus their effects on persistence were not fully separable. They had a weak positive effect (P -value of the model, 0.070, $\lambda = 0.42$) on persistence, explaining 7.12 % of the variability (adjusted R^2).

In our ordination combining all traits with Ellenberg indicator values, the first axis of the PCA explained 21.3 % and the second 15.95 % of the variability (Fig. 7). The estimated Pagel's λ was 0.40. The main traits representing the first axis were SLA

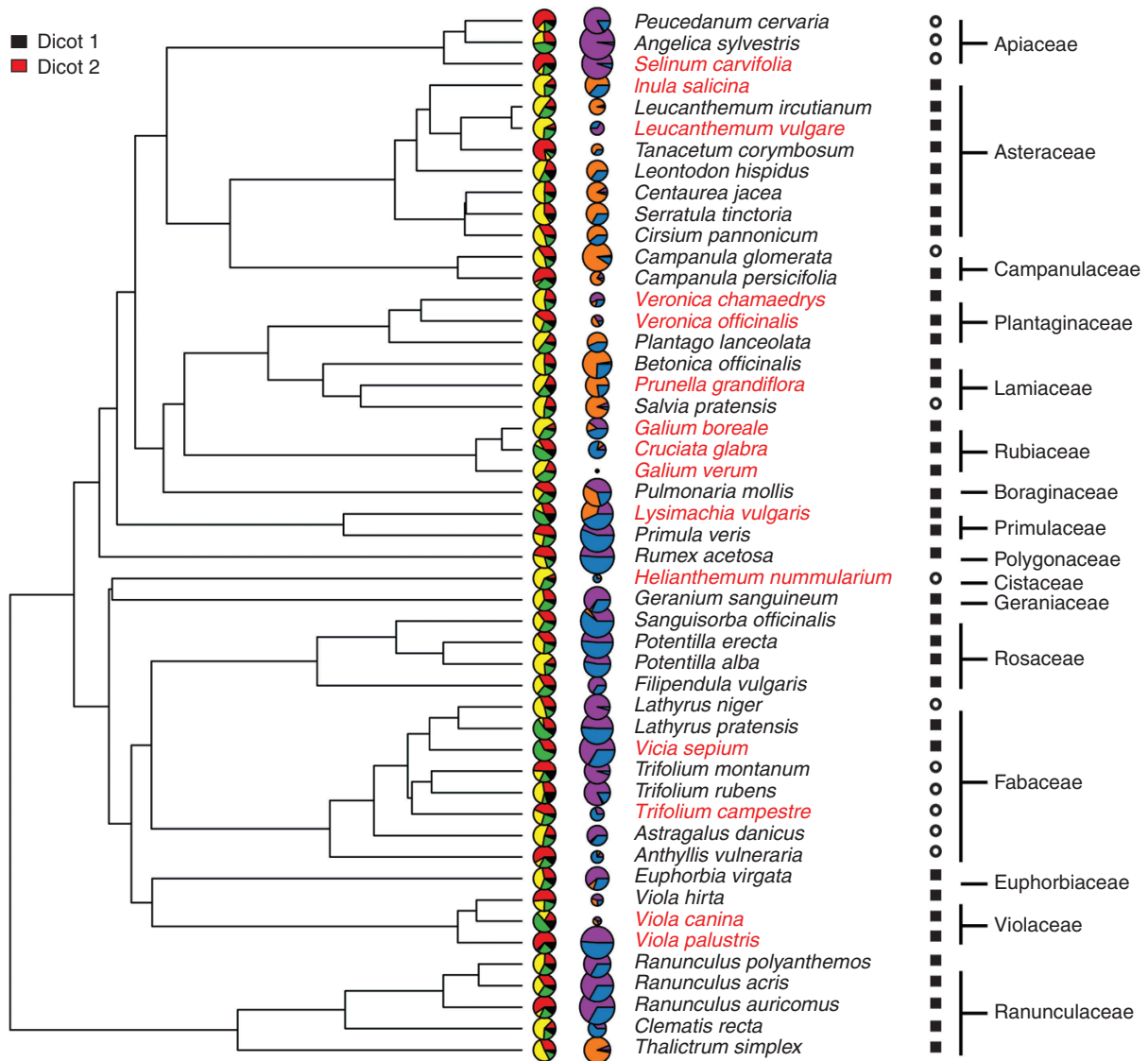


FIG. 4. Phylogenetic tree of studied dicots with visualization of anatomy and storage proportions, anatomical type and family. The size of pie charts for carbohydrates corresponds to the logarithm of total storage (for key see Fig. 3) and species name colour denotes anatomical category. Storage organ type is denoted by a black square for rhizomes and a white circle for roots.

and persistence of the storage organ, while the second axis was best represented by plant height, lateral spread and multiplication rate. Passively projected environmental preferences of species were correlated with the first axis.

Total carbohydrate concentrations in summer and autumn were highly correlated (Pearson correlation coefficient after logarithmic transformation, 0.819). Parenchyma had no effect on summer concentration (adjusted $R^2 = 1.94\%$, $P = 0.116$, $\lambda = 0.40$) or on autumn concentration (adjusted $R^2 = 3.28\%$, $P = 0.142$, $\lambda = 0.06$). However, imperfect stain absorption in some species (possibly *Carex pallescens* and *Tanacetum corymbosum*) could have weakened the relationship.

Anatomical types defined by tissue patterns in storage organ cross-sections did not differ in parenchyma (adjusted $R^2 = -1.66\%$, $P = 0.630$, $\lambda = 0.00$) or lignin percentage (adjusted $R^2 = -2.87\%$, $P = 0.836$, $\lambda = 0.00$). Tissue patterns differed in the logarithm of total storage carbohydrate

concentration (adjusted $R^2 = 14.81\%$, $P = 0.001$, $\lambda = 0.00$), with higher values for Monocot 1 and Dicot 1 and lower values for Monocot 2 and Dicot 2. On the other hand, tissue patterns did not differ in the concentration of any type of storage carbohydrate (starch, adjusted $R^2 = -1.51\%$, $P = 0.605$; soluble oligo- and polysaccharides, adjusted $R^2 = 2.88\%$, $P = 0.162$; mono- and disaccharides, adjusted $R^2 = -2.75\%$, $P = 0.815$). Estimated phylogenetic signal (λ) was high for models with the individual types of storage carbohydrates (starch, 0.958; soluble oligo- and polysaccharides, 0.878; mono- and disaccharides, 0.390).

DISCUSSION

The traits of belowground storage organs for 78 species from temperate grasslands were, contrary to expectations, largely

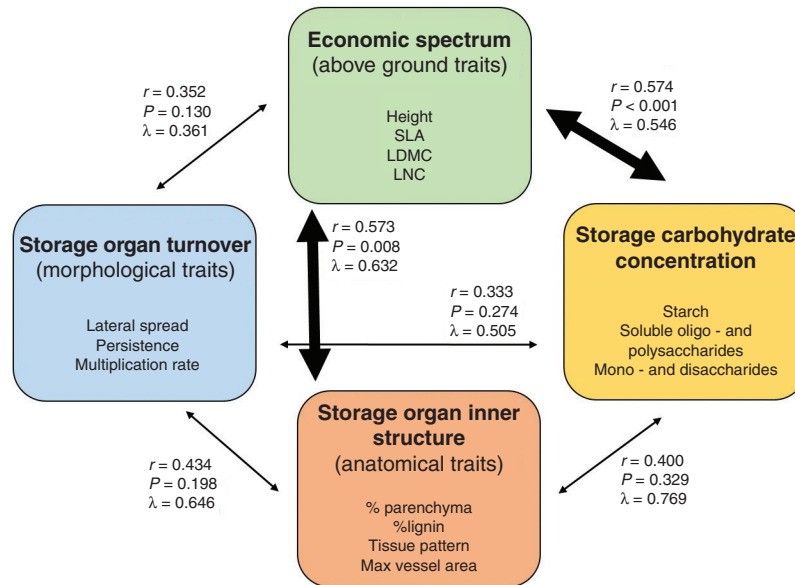


FIG. 5. Relationships between groups of traits. For each pair the figure shows the correlation among first canonical axes (r), the P -value of the first and all other correlations being zero, and λ (phylogenetic signal). All groups contained 78 species except for storage organ turnover, with only 76 species. Wide arrows denote significant relationships. Details of multivariate comparisons are depicted in Fig. 6.

independent of one another. However, there were correlations of aboveground economic traits with storage organ inner structure, maximum vessel size, and the concentrations of individual types of storage carbohydrate. Although different organ inner structure arrangements did not vary significantly in the concentrations of individual carbohydrate types, they differed in total carbohydrate concentration, indicating an effect of anatomy on carbohydrate storage.

Storage organs and anatomical constraints of their function

Temperate grasslands are a rich mixture of different taxonomical lineages, with 21 families represented in our dataset (five monocotyledonous and 16 eudicotyledonous). This taxonomic diversity enhances richness in anatomical and carbohydrate types (Esau, 1977; Lewis, 1984; Hendry, 1987). The monocots studied are incapable of secondary thickening and have scattered vascular bundles in stem cross-sections; additionally, some species have specialized storage organs like tubers and bulbs. The eudicots have or do not have secondary thickening and have a variety of different patterns of vascular tissue in their stems. In this study, we primarily distinguished between the presence of regions of parenchyma separating those of secondarily thickened lignified tissues (Fig. 2).

In the correlation of aboveground economic traits with organ inner structure, monocot anatomical types formed divergent strategies. Monocot 1 correlated positively with LNC and Monocot 2 with LDMC. Maximum vessel area was also correlated with LDMC and plant height, showing a pattern similar to that found for graminoids in the dry environment of the Western Himalayas, where water-stressed low-altitude plants were characterized by high stature and large vessels (Doležal et al., 2019a) in comparison with plants from wetter and colder

high altitudes. Large vessels are usually reported as a component of a faster and more competitive strategy in trees that are not subjected to drought (Reich, 2014; Olson et al., 2018), but their relationship to drought in herbs seems not to mirror that of trees (Dória et al., 2019; Doležal et al., 2019a).

We found that the percentage of storage organ cross-section consisting of parenchyma was largely independent of the concentration of individual types of non-structural carbohydrates. This might be because we used only a single anatomical cross-section for storage organ characterization and thus did not describe possible variability along the length of the organ. On the other hand, we found a relationship between inner structure (rough anatomical types) of the storage organs and total non-structural carbohydrates (i.e. when all carbohydrate types were summed). Their concentrations were higher in species containing additional regions of parenchyma (the pith in Monocot 1 or rays and other regions between areas of secondary thickening in Dicot 1), indicating that anatomical structure is an important clue to interspecific differences in the amount of carbohydrate storage. In trees, the ray parenchyma fraction from species across a broad elevation gradient is correlated with environmental factors, but varies considerably across different clades (Plavcová et al., 2016; Godfrey et al., 2020). Increased parenchyma, and hence storage carbohydrate concentration, in tree species is linked to cold or dry conditions and interpreted as a means of ensuring greater capacity to rescue vascular tissue from hydraulic embolism caused by freezing and drought stress (Morris et al., 2016; Trifilò et al., 2019). The quantity and arrangement of parenchyma may have similar advantages against stress for herbs.

Contrary to our expectation, lignin percentage was not correlated with the persistence of storage organs and therefore lignification is probably not a major cause of organ persistence, even though it does play a role in decomposition after the life of the organ (Freschet et al., 2012). Similarly, the secondary

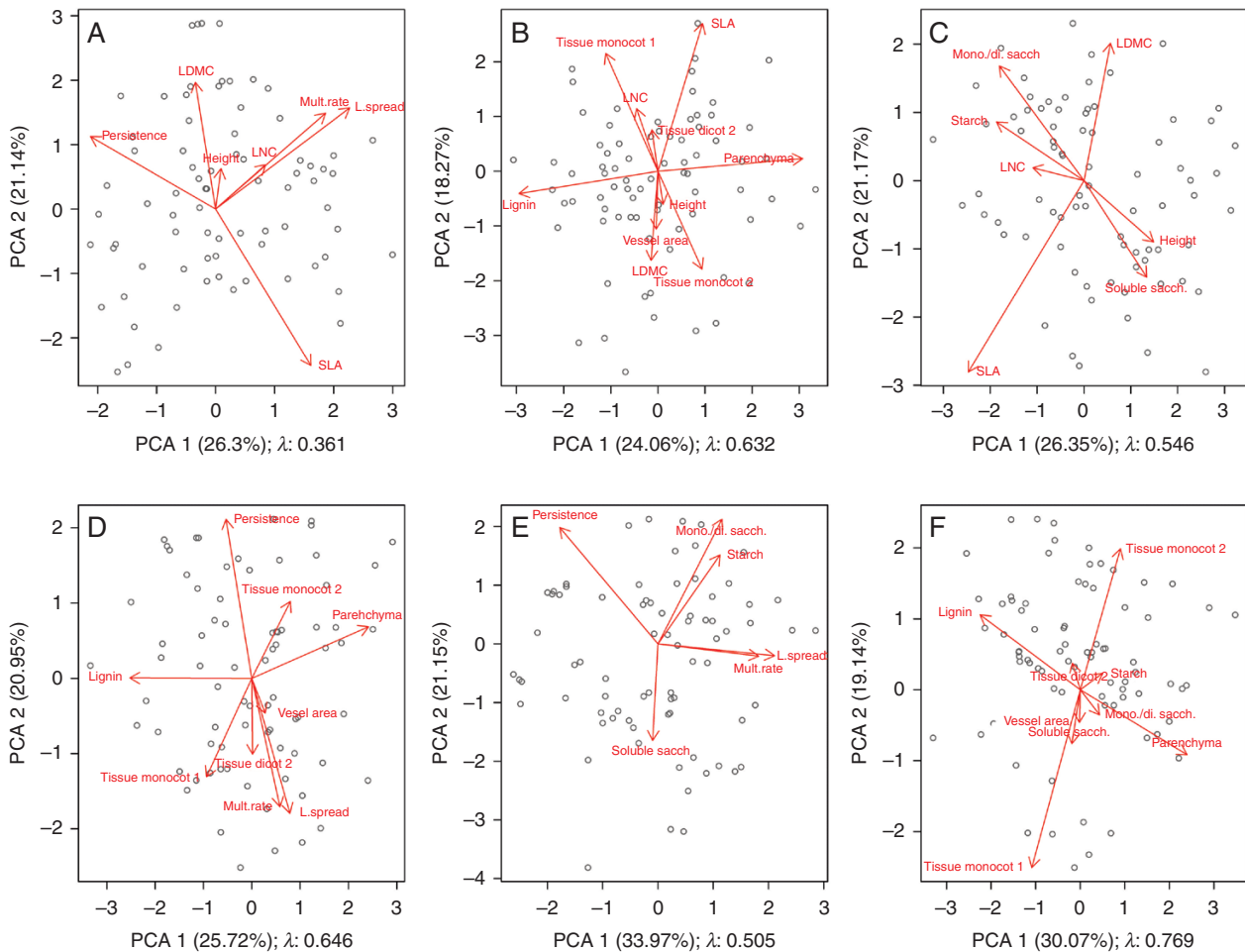


FIG. 6. Ordinations of paired groups of traits for the economic spectrum traits and storage organ (SO): turnover, inner structure and storage carbohydrates. (A) Economic spectrum and SO turnover. (B) Economic spectrum and SO inner structure. (C) Economic spectrum and storage carbohydrates. (D) SO turnover and SO inner structure. (E) SO turnover and storage carbohydrates. (F) SO inner structure and storage carbohydrates. Visualized are the first two axes of the phylogenetic PCAs with estimated strength of phylogenetic signal. In (B) and (C) there is significant correlation of canonical axes between groups. Mult. rate, multiplication rate; L. spread, lateral spread; sacch., saccharides

thickening that is generally considered responsible for the persistence of belowground storage organs (Hay and Kelly, 2008; Watson, 2008) did not show the expected pattern. Although the categories of anatomical type did not differ in morphological traits or the concentrations of different storage carbohydrates, they were correlated with traits of the aboveground economic spectrum. Storage organ anatomy deserves further study using experiments and longer environmental gradients. Additionally, more studies should include characterization of more cross-sections along storage organs and with larger regions of the cross-section to better describe a large diversity of anatomical types.

Storage carbohydrates

Although the main storage carbohydrate type can be constrained within certain taxonomical groups, there is still variability, as illustrated by the high concentration of water-soluble oligo- and polysaccharides in *Thalictrum simplex* in the otherwise starch-rich family of Ranunculaceae (Figs 3 and

4). The identified correlation of carbohydrate type concentration with traits of the aboveground economic spectrum is not easy to explain because of a scarcity of comparative data. The aboveground economic traits and storage carbohydrate concentrations appear to diverge into two strategies, but with different traits within the fast end of the economic spectrum. Small soluble molecules (mono- and disaccharides) and starch aligned with the fast-strategy trait of LNC, while the medium-sized molecules aligned with greater plant height. The main leaf traits, SLA and LDMC, however, remained independent of carbohydrate storage traits. This relationship does not follow the fast–slow dichotomy and hints at an economic space with greater variation and complexity.

The smallest storage molecules are found in all plants (Lewis, 1984), and their use increases when plants that primarily invest in starch are under stress (Alves Vieira et al., 2017). Greater water-soluble sugar concentration is linked to recovery from drought-triggered embolism in trees (Savi et al., 2016) and protection against drought (Küchenmeister et al., 2013; Du et al., 2020), frost (Hisano et al., 2004; Patton et al., 2007) and fire (Gomes de Moraes et al., 2016) in herbaceous plants. However,

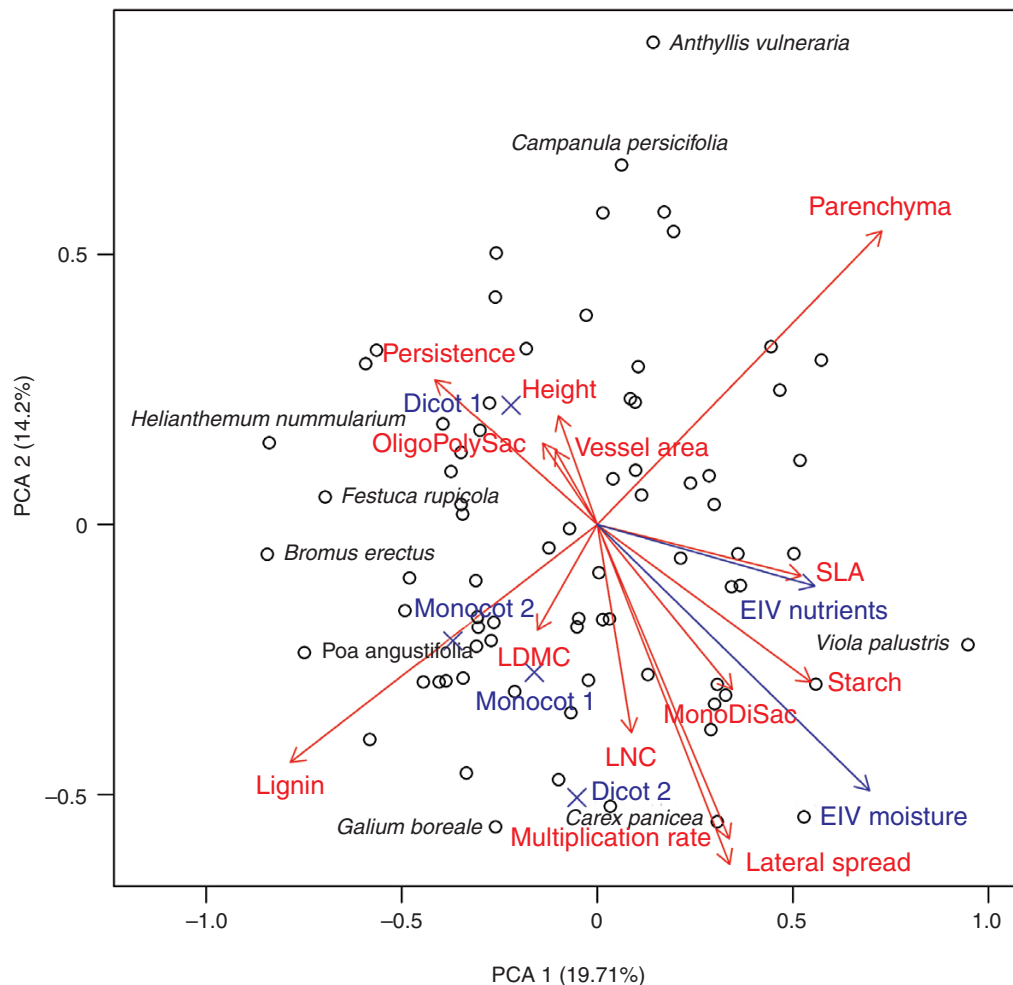


FIG. 7. Ordination of traits (aboveground economic spectrum traits, morphological traits, anatomical traits and storage carbohydrates). The first two axes of the phylogenetic PCA (fitted using package phytools version 0.7-20; Revell, 2012) are visualized. Names of species with high scores on the first or second axis are displayed. Shortcuts were used for some variables: SLA, LDMC, LNC, Ellenberg indicator value (EIV), mono- and disaccharides (MonoDiSac) and oligo- and polysaccharides (OligoPolySac). Traits were standardized to mean 0 and standard deviation 1 prior to the analysis. Estimated Pagel's λ was 0.44. Percentages denote how much variability is captured by each axis. EIVs (Chytrý et al., 2018) and types of anatomy (Monocot 1, 2 and Dicot 1, 2; centroids denoted by an X) are passively projected using the envfit function from the vegan package (version 2.5-6; Oksanen et al., 2018). The relative length of red to blue arrows is arbitrary.

comparative studies testing these relationships using representative sets of species are rare, and further comparative studies are necessary.

It has also been observed that many species rich in fructans (a common group of oligosaccharides) have fast early-spring growth (Brocklebank and Hendry, 1989), but the cited study compared only 20 species. Additionally, some of the fructan-bearing taxa are incredibly speciose (e.g. Asteraceae, Amaryllidaceae, Pooidae subfamily of Poaceae), feature numerous strategies, and include many species with comparatively late phenology or long growing seasons. The tall meadow plants in our dataset that store oligo- and polysaccharides are usually of late phenology and may be subjected to summer drought. In contrast, many starch-storing small plants in meadows are usually early-flowering species that might be subjected to early-spring frosts and then shading from dominants later in the season. The relationship of storage carbohydrate

type to economic strategy in terms of stress response and mobilization needs to be further studied.

Contrary to the lack of relationship of the inner anatomical structure and concentrations of carbohydrate types, we found differences in total non-structural carbohydrate concentration among storage organ anatomical types. This implies that we are on the right track to understanding the relationship between carbohydrate concentrations and carbohydrate pools. However, we must remember that we need to know the storage organ biomass in order to calculate the carbohydrate pool from data about concentration. This parameter is difficult to study, especially in rhizomatous plants (Klimešová et al., 2017a). We can expect that ecosystems other than temperate grasslands, where plants are more compact belowground and not so intermingled with each other (e.g. tropical grasslands), would probably be more suitable for addressing questions regarding the relationship between carbohydrate pool and the aboveground economic spectrum.

Environment

The meadow species we collected on two grasslands represent plants specialized to a gradient of nutrient and water availability, albeit not to its extreme values. In our canonical correlation analysis, we did not confirm the trend found in a previous study surveying clonal and bud bank traits in Central Europe, wherein aboveground traits and storage organ turnover traits were correlated (Klimešová *et al.*, 2015), specifically with a positive relationship between height and lateral spread and a negative relationship between SLA and persistence. This lack of confirmation is probably a result of the shorter environmental gradient and lower number of species than in the previous study. Nevertheless, we do still recognize two main gradients of trait specialization: resource-demanding species (according to preferences for water and nutrients evaluated using Ellenberg indicator values) with high SLA, starch, and mono- and disaccharide concentrations separate from species with high lignin percentages and water-soluble oligo- and polysaccharides, and long persistence of storage organs. In the second gradient, tall plants separate from those with extensive lateral spread and a high multiplication rate. While the first axis combines two well-known relationships within the leaf economic spectrum (Wright *et al.*, 2004) and the splitter–integrator continuum (van Groenendael *et al.*, 1996; Jónsdóttir and Watson, 1997; Klimeš, 2008; Klimešová *et al.*, 2011, 2015), the second seems to reflect the gradient of demographic strategies from tall species with low lateral spread and multiplication rate to small species that quickly spread using short-lived rhizomes (van der Maarel and Sykes, 1993; Herben *et al.*, 2019). The demographic specialization was at least partly connected with the moisture gradient, indicating that stationary persistence is the primary strategy for species adapted to dry and less dense meadows, while mobile individuals (by clonal growth in the horizontal direction) are adapted to the competitive environment of wet meadows. Although the trait groups may have few connections to one another, there may be alignments of these traits at different levels.

To accumulate this dataset of a broad range of traits for a large number of species, we have pooled data collected in different years and from a variety of different specimens. Although the strength of our study would be greater if all measurements had been conducted on all the same individuals and consistently studied the same way and repeated during multiple years, this would have been a massive undertaking. Although there is great need for careful and precise study of the variability and response of these traits under specific conditions, it is also our hope that the belowground traits studied here will be a valuable tool in studying plant ecology across broader scales or when it is not possible to acquire values for all the destructive measures we have used here.

Conclusions

This study is an unprecedented assessment of the relationships between plant traits of the leaf economic spectrum and storage organs of perennial herbs. Although the associations between belowground trait groups within this study are generally weak, we have identified previously unknown links between the aboveground economic spectrum traits and those of

storage organ inner structure and storage carbohydrate concentration. Because our study is the first attempt to broaden the understanding of the plant economic spectrum with the inclusion of storage traits, we hope that it opens our vision to new axes of plant specialization and points out that the economic strategies of plants are not a simple spectrum but rather a complicated economic space that should be further explored in its full complexity. Future studies should examine these traits on broader environmental gradients, in different community types and in floras with other evolutionary histories.

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