

Species traits and shoot–root biomass allocation in 20 dry-grassland species

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Abstract

Aims

A plant has a limited amount of resources at any time and it allocates them to different structures. In spite of the large number of previous studies on allocation patterns within single species, knowledge of general patterns in species allocation is still very limited. This is because each study was done in different conditions using different methodology, making generalization difficult. We investigate intraspecific above- versus below-ground biomass allocation among individuals across a spectrum of dry-grassland plant species at two different developmental stages and ask whether allocation is age- and species specific, and whether differences among species can be explained by their life-history traits and phylogeny.

Methods

We collected data on above- and below-ground biomass of seedlings and adult plants of 20 species from a common garden experiment. We analysed data on shoot–root biomass allocation allometrically and studied the relationship between the allometric exponents (slopes on log–log scale), species life-history traits and phylogenetic distances.

Important Findings

We found isometric as well as allometric patterns of biomass allocation in the studied species. Seedlings and adult individuals of more than half of the species differed in their above- versus below-ground biomass allometric exponents. Seedlings and adult individuals of the remaining species differed in their allometric coefficients (intercepts). Annual species generally allocated proportionally more to above- than below-ground biomass as seedlings than as adults, whereas perennial species showed the opposite pattern. Plant life-history traits, such as plant life span, age of first flowering, month in which the species begin flowering and specific leaf area were much more important in explaining differences in shoot–root allometry among species than were phylogenetic relationships. This suggests that allocation patterns vary greatly among closely related species but can be predicted based on species life-history traits.

Keywords: above-ground biomass, below-ground biomass, allometry, isometry, seedling, adult plant, life-history traits, phylogenetic relatedness, root–shoot ratio

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INTRODUCTION

Each plant has a given amount of resources at any time and it allocates them to different functions and structures. Different allocation patterns reflect different strategies that are the results of selection pressures and constraints (Weiner 2004; Weiner *et al.* 2009a). Similarly, changes in allocation during ontogeny reflect the changing priorities of an organism during its development.

Many studies have emphasized the variability in biomass partitioning observed across plants. These studies fall

primarily within the optimal partitioning theory, also called the balanced-growth hypothesis, which suggests that plants should allocate biomass to the organ that acquires the most limiting resource (Bloom *et al.* 1985; McCarthy and Enquist 2007; Shipley and Meziane 2002).

Alternatively, variation in biomass allocation among plants can be driven also by differences in plant size (Coleman *et al.* 1994; McConnaughay and Coleman 1999; Müller *et al.* 2000; Reich 2002; Weiner 2004; Weiner *et al.* 2009a, 2009b). Many morphological and physiological changes, including biomass allocation patterns, occur under the normal course of growth

and development (Coleman *et al.* 1994; McConnaughay and Coleman 1999; Weiner 2004), a phenomenon sometimes referred to as ontogenetic drift (Evans 1972; Gedroc *et al.* 1996). For this reason, it is difficult to distinguish between variation in biomass allocation patterns due to resource limitations in the environment ('true' plasticity) from size-dependent allometric patterns ('apparent' plasticity; McCarthy and Enquist 2007; McConnaughay and Coleman 1999; Weiner 2004).

Another approach to explaining variability in biomass partitioning in relationship to plant size is allometric biomass partitioning theory (Cheng and Niklas 2007; Niklas 2006; Niklas and Enquist 2002). This theory predicts that plants must allocate biomass proportionally among plant parts (such as leaves, stem and roots) and examines how biomass allocation pattern among species changes with plant size according to allometric relationship which can be described by the allometric equation characterized by an allometric (scaling) exponent (slope of log–log relationship between mass of plant parts) and the allometric coefficient (intercept or 'elevation' of the relationship). Under ideal conditions, allometric biomass partitioning theory often predicts that the scaling exponent is equal to one, which means that above- and below-ground biomass scale isometrically, independently of the plant size. Isometric scaling relationships have been documented in numerous studies at various scales—from individual level (Cheng and Niklas 2007; Cheng *et al.* 2015; Niklas 2006; Niklas and Enquist 2002; Zhou *et al.* 2014) to community level (Cheng *et al.* 2015; Enquist and Niklas 2002; Yang *et al.* 2009, 2010). Many other studies, however, revealed scaling exponent different from one, indicating an allometric (i.e. non-isometric) relationship between below- and above-ground biomass—at the individual (Janeček *et al.* 2014; Li *et al.* 2013; Lohier *et al.* 2014; Müller *et al.* 2000; Pan *et al.* 2013; Wang *et al.* 2010; Weiner *et al.* 2009b; Zhou *et al.* 2014) as well as community level (Wang *et al.* 2014a; Wu *et al.* 2013). Because allocation patterns changes with plant size, allocation should be analysed and interpreted allometrically (Weiner 2004; Weiner *et al.* 2009a, 2009b) and not as ratios (e.g. root–shoot ratio) or proportions, which is the traditional and commonly used approach, in which allocation is assumed to be size independent (Mokany *et al.* 2006).

Biomass allocation patterns, including reproductive allocation, were traditionally studied using manipulative experiments studying the effect of two or more resource levels on biomass allocation of one or a few species, usually annual weeds or crops (e.g. Mao *et al.* 2012; Qin *et al.* 2013; Wang *et al.* 2014b; Xie *et al.* 2012) and only rarely for several species (Müller *et al.* 2000; Shipley and Meziane 2002). Most of the data have been collected at one point in time or at the same plant age (McConnaughay and Coleman 1999). Such studies did not usually take plant size into account in interpreting the differences in allocation patterns at different resource levels. More recent studies, however, have considered plant size and demonstrated the importance of ontogenetic drift, sometimes showing no or only small but significant direct effects of resources or other environmental factors on allometric

patterns (Gedroc *et al.* 1996; Janeček *et al.* 2014; Lohier *et al.* 2014; Müller *et al.* 2000).

Biomass allocation patterns could also vary with plant life-history strategy, but studies comparing species of different strategies are rare. For annual plants, it was found that the proportion of allocation to roots declines during growth and development (Coleman *et al.* 1994; Gedroc *et al.* 1996; McConnaughay and Coleman 1999). The direction of preferential allocation to roots or shoots over the course of ontogeny is more complicated and less consistent among perennial plants. The proportion of allocation to roots increased during ontogeny in some perennial species (e.g. *Leontodon hispidus*—Niinemets 2004) whereas it decreased in others (e.g. *Plantago lanceolata*—Janeček *et al.* 2014). Meta-analyses by Poorter *et al.* (2012) compared a large number of species from a wide range of published experiments in the literature (especially focused on the effect of resource levels on allocation of species) and found that most herbaceous species show a trend towards a decreased root allocation and an increased shoot allocation with size, but there was no evidence for differences in allocation patterns between perennials and annuals. Their findings were based on a range of different studies performed with different methods. This variation could mask individual species differences. It was also found that perennials exhibited 'apparent' plasticity in relation to different resource levels and did not change their allocation strategy whereas annuals did, thus exhibiting 'true' plasticity (Mao *et al.* 2012). Similarly, Zhou *et al.* (2014) found isometric allocation or evidence for the optimal partitioning theory in annuals but allometric allocation in ephemeral perennials. These two studies (Mao *et al.* 2012; Zhou *et al.* 2014) included very few (one to three) species within each category. For allocation to reproduction, a review of studies on >50 different species found isometric allocation in many short-lived and clonal plants, but allometric allocation by longer-lived plants (Weiner *et al.* 2009a).

Differences in biomass allocation patterns of species may also depend on many other life-history traits, such as growth form, Grime's CSR ecological strategy (competitors/stress-tolerators/ruderals), seed size, age of first flowering, clonality, stem height, or leaf traits such as specific leaf area (SLA), leaf thickness or leaf longevity, but almost nothing is known about these effects. An exception is the study based on a global dataset, which showed the importance of growth form for allocation into above-ground organs and positive relationship of leaf mass with leaf life span and negative relationship with SLA and maximum photosynthetic rate (McCarthy *et al.* 2007). Another study (Fortunel *et al.* 2009) found that early successional species allocated proportionally more biomass into reproduction than later successional species, consistent with high colonization abilities of early successional species and high competitive ability of later successional species. Seed size can also be important for seedling biomass allocation as was demonstrated within a single species (Chacon *et al.* 1998). But it is not clear if this relationship applies to other species or across species.

It could be expected that more closely related species will show more similar allocation patterns than distantly related species. Phylogeny was the strongest predictor of biomass allocation pattern in a global dataset at higher taxonomic levels (i.e. between clades and families) and across a wide spectrum of plant sizes (McCarthy *et al.* 2007; McCarthy and Enquist 2007). Nothing is, however, known on the importance of phylogenetic relatedness for biomass allocation patterns at lower taxonomic levels—i.e. between different species from the same habitat and vegetation type.

In spite of the relatively high number of studies carried out on biomass allocation, most of them have focussed on the effect of various resource levels on allocation pattern of one or a few species. The published studies used very different methodologies, making comparisons among different life-history traits and phylogenetic relatedness of different species coming from the same vegetation type difficult. Moreover, there are three fundamentally different kinds of allometric relationships, which address very different questions, but have been conflated throughout much of the literature: (i) broad interspecific, (ii) static intraspecific among individuals within a species and (iii) allometric growth of individuals (Weiner *et al.* 2009a). Mixing of these three types of comparisons further complicates any comparative studies.

The aim of this study was to investigate the pattern of above- versus below-ground biomass allocation across a spectrum of dry-grassland plant species within one community. By comparing data from each species separately and comparing only the resulting coefficients among species, we investigate static intraspecific allometry in our study. We ask the following questions: (i) how does allocation to above- versus below-ground biomass differ (a) between seedlings and adult plants within and (b) among species? (ii) can we explain the between species differences in allocation pattern in terms of species life-history traits? and (iii) do closely related species within the same community show more similar allometric relationships than less related species? We hypothesize that allocation of biomass into different structures changes during ontogeny, that allocation will vary among different life spans/forms and that closely related species will have similar allocation patterns because of their shared evolutionary history.

To answer these questions, we set up a common garden experiment on above- and below-ground biomass of seedlings and adult plants of 20 dry-grassland species from the same community, including species from different families and with different life-history traits. We tested whether the slope of the relationship between allocation into above- and below-ground biomass of each species and each age category is significantly different from one (isometry) and whether the allocation slopes differ between seedlings and adult plants of each species. Then we used the values of allocation slopes and tested them against species life-history traits and phylogenetic distances.

MATERIALS AND METHODS

Study species and data collection

Twenty herbaceous dry-grassland plant species from a wide taxonomic range (20 genera in 11 families) and differing in life-history traits were selected to study the general pattern of biomass allocation above- versus below-ground (Table 1). We collected seeds of all these species in the field from natural dry-grassland plant communities in forest openings in the Protected Landscape Area and Biosphere Reserve Křivoklátsko in the Czech Republic. We sampled 120–200 individuals of each species (for details, see Table 1) occurring at 12–20 localities to obtain a sufficient number of seeds. In autumn of the year when we collected seeds (2009–2011) or in spring of the following year (depending on whether the seeds require cold stratification), we sowed a given number of seeds from each individual into 15 × 15 × 15 cm pots in a substrate consisting of 2 parts garden soil to 1 part sand in an experimental garden. The number of seeds per pot differed among species, reflecting differences in seed size (Table 1).

Two or 3 weeks after most of seeds of each species started to germinate, we counted the number of individuals ('seedlings') and removed them from the soil. We left one randomly chosen individual in each pot to study its later growth. Because all individuals were very small at this time, there was no competition among them. Due to the small size of individuals and their root systems as well as the sandy substrate, it was easy to extract the whole root system of each plant from the soil. All harvested seedlings were washed in water to remove residual soil, divided into above- and below-ground parts, dried to constant weight at 70°C and weighed. We refer to these as 'seedlings'.

The remaining plants were left to grow in the pots in the experimental garden until most of their fruits were mature (4–16 months) and then were harvested. The plants were carefully extracted from the substrate and we were able to extract almost all the root biomass for each individual. The plants did not experience competition as there was only one plant per pot. As with the seedlings, we divided the individuals into above- and below-ground parts, washed the below-ground parts and dried to constant weight at 70°C and weighed them. In the following text, we refer to these as 'adult plants'. All flowers and fruits with seeds were included in the above-ground biomass of adult plants.

Life-history traits and phylogeny of the studied species

To interpret differences among the species in their allocation to above- versus below-ground biomass, we collected data about their life-history traits from several databases as well as making our own measurements using the same methods used in the databases. We measured the seed weight for all individuals of each species that we sampled and calculated the average seed weight per species. We used the data from the experiment to determine if individuals of each species were

Table 1: studied species

Studied species	Abbreviation	Life span	No. of sampled individuals and pots in the experiment	No. of sown seeds per pot
<i>Acinos arvensis</i>	Aci arv	Annual	170	40
<i>Arabidopsis thaliana</i>	Ara tha	Annual	200	100
<i>Arenaria serpyllifolia</i> agg.	Are ser	Annual	200	50
<i>Asperula cynanchica</i>	Asp cyn	Perennial	170	30
<i>Dianthus carthusianorum</i> agg.	Dia car	Perennial	138	20
<i>Echium vulgare</i>	Echi vul	Perennial	180	20
<i>Fragaria viridis</i>	Fra vir	Perennial	186	20
<i>Geranium columbinum</i>	Ger col	Annual	200	20
<i>Hieracium pilosella</i>	Hie pil	Perennial	200	20
<i>Inula conyzae</i>	Inu con	Perennial	121	50
<i>Melica transsilvanica</i>	Mel tra	Perennial	200	50
<i>Myosotis ramosissima</i>	Myo rha	Annual	200	20
<i>Origanum vulgare</i>	Ori vul	Perennial	200	40
<i>Phleum phleoides</i>	Phl phl	Perennial	200	50
<i>Potentilla argentea</i>	Pot arg	Perennial	200	50
<i>Scleranthus perennis</i>	Scl per	Perennial	120	50
<i>Teucrium botrys</i>	Teu bot	Annual	141	30
<i>Trifolium arvense</i>	Tri arv	Annual	200	40
<i>Veronica dillenii</i>	Ver dil	Annual	200	40
<i>Vicia hirsuta</i>	Vic hir	Annual	163	10

able to flower in their first season (i.e. if most of individuals of a species flowered in the first year). We collected data on plant life span and SLA from the Leda Trait base (Kleyer *et al.* 2008); plant height and month in which each species usually starts to flower (which corresponded to our observations) from Kubát *et al.* (2002); clonality of each species from CloPla database (Klimešová and de Bello 2009; <http://clopla.butbn.cas.cz>) and CSR strategy and leaf persistence from BiolFlor database (<http://www2.ufz.de/billfold/index.jsp>).

To assess phylogenetic distance between the species, we used the Daphne database (Durka and Michalski 2012; <http://www.esajournals.org/doi/abs/10.1890/12-0743.1>) and calculated matrix of pairwise phylogenetic distances between studied species using R software – Ape package (Paradis *et al.* 2004).

Statistical analysis

Analysis of above- versus below-ground biomass allocation

Since there are no dependent and independent variables in allometric analyses and both variables have errors, the analyses of the above- versus below-ground biomass relationships were performed using the standardized major axis (SMA) regression on log–log-transformed data using SMATR package (version 3.4-3; <http://bio.mq.edu.au/ecology/SMATR/>) in R (version 3.0.2; <https://www.r-project.org/>). SMA is appropriate when the purpose is to estimate the linear relationship between two variables, especially when the slope of the relationship is of primary interest (Warton *et al.* 2012).

To ask whether the allocation patterns of the studied species are species and age specific, we first tested the effect of each factor separately using SMA. Because both factors (species and age) were important for allocation patterns, we used analysis of covariance with type III sum of squares (S-plus 4.6, MathSoft Inc. 1999, Washington, USA) to test the effect of both factors as well as their interactions on allocation patterns (here expressed as log above-ground biomass ~ log below-ground biomass + species + age + species × age) and identify an effect of each particular factor without the effect of all the other factors. We used this analysis because it is not possible to use SMA to test the effect of multiple factors in one model.

We then asked if there is a significant relationship between allocation to above- and below-ground biomass of each species for each age category separately and if the slope of this relationship is significantly different from one (i.e. if the allocation pattern is allometric or isometric; Table 2). Tests of heterogeneity in slopes among seedlings and adult plants of each species were then performed separately (Warton *et al.* 2006). If there was no significant difference in slopes, we tested for difference in intercept (elevation on y-axis; Table 2), which is meaningful only when the allometric slopes are considered to be equal (Li *et al.* 2013; Warton *et al.* 2006, 2012). In addition, we also compared the values of biomass allocation for all seedlings and adult plants together to find out if there is any general allocation pattern for seedlings and adult plants (Table 2).

Table 2: above- versus below-ground allometric relationships for seedlings and adult plants

Species	A. Seedlings: above- and below-ground allometry				B. Adult plants: above- and below-ground allometry				C. Difference in slope of seedlings versus adult plants				D. Difference in intercept			
	Slope	R ²	P value	F statistic	d.f.	Slope	R ²	P value	F statistic	d.f.	P value	LR	Direction of change	P value	Wald	Direction
Act ar	1.058	0.734	0.21	1.59	131	0.851	0.406	0.007	7.421	168	0.004	8.435	S>A			
Ara tha	0.998	0.506	0.977	0.001	77	1.087	0.605	0.2	1.665	95	0.408	0.686	S<A	<0.001	29.809	S<A
Are ser	0.95	0.802	0.137	2.238	170	1	0.753	0.998	0	46	0.531	0.392	S<A	0.07	3.278	
Asp cyn	0.835	0.818	<0.001	21.218	118	0.904	0.396	0.102	2.705	161	0.277	1.183	S<A	0.010	6.717	S<A
Dia car	0.713	0.773	<0.001	44.124	84	1.071	0.644	0.189	1.74	131	<0.001	28.774	S<A			
Echi vul	0.948	0.709	0.41	0.687	71	1.136	0.769	0.01	6.815	96	0.027	4.921	S<A			
Fra vir	0.985	0.845	0.671	0.182	129	0.984	0.567	0.735	0.115	180	0.976	0.001	S=A			
Ger col	1.027	0.797	0.458	0.553	162	0.843	0.387	0.002	9.457	198	0.003	8.79	S>A			
Hie pil	0.89	0.713	0.018	5.75	121	0.751	0.492	<0.001	30.629	184	0.018	5.603	S>A			
Inu con	0.952	0.661	0.534	0.392	54	1.694	0.3	<0.001	44.402	102	<0.001	23.738	S<A			
Mel tra	0.736	0.387	<0.001	24.768	157	0.846	0.627	<0.001	13.856	184	0.071	3.254	S<A	<0.001	12.621	S<A
Myo rha	0.896	0.545	0.143	2.187	82	0.922	0.305	0.179	1.818	191	0.765	0.089	S<A	<0.001	87.419	S<A
Ort vul	0.687	0.393	<0.001	17.013	70	0.97	0.545	0.528	0.399	194	0.001	10.412	S<A			
Phl phl	0.965	0.615	0.546	0.367	112	0.866	0.392	0.013	6.312	183	0.187	1.739	S>A			
Pot arg	0.89	0.574	0.014	6.204	194	1.211	0.651	<0.001	21.12	198	<0.001	23.403	S<A			
Scl per	0.942	0.84	0.165	1.962	88	1.106	0.578	0.093	2.867	118	0.030	4.726	S<A			
Teu bot	1.134	0.854	<0.001	13.644	126	1.389	0.692	<0.001	29.421	81	0.005	8.056	S<A			
Tri arv	1.167	0.848	0.011	7.12	45	1.125	0.925	0.001	12.266	66	0.588	0.294	S>A			
Ver dil	0.772	0.375	<0.001	21.216	194	0.422	0.332	<0.001	93.758	66	<0.001	25.474	S>A			
Vic hir	1.158	0.765	0.02	5.736	62	0.799	0.435	0.002	9.786	108	<0.001	14.706	S>A			
All	0.881	0.77	<0.001	158.89	2285	0.802	0.752	<0.001	557.38	2788	<0.001	47.052	S>A			

(A) and (B): the relationship between log above- and log below-ground biomass for seedlings (A) and adult plants (B). Depicted are R² values of the above- versus below-ground relationship (significant in all cases, P < 0.0001) and allocation slopes of these relationships for each species. P values, F statistic and d.f. are parameters of tests whether the allocation slopes are significantly different from 1, i.e. whether the relationship is allometric. Bold values are significantly different from 1. (C) and (D): comparisons of allocation patterns of seedlings and adult plants of each species. (C): test if there is significant difference between allocation slopes of seedlings and adult plants. In case of no significant difference, the results of tests for difference in intercept (D) are depicted, assuming the lines are parallel. For all these tests, P values and statistical value [LR = the Bartlett-corrected likelihood ratio statistic in (C) and Wald statistic in (D)] are shown. Bold values are significant (P < 0.05). Direction of change in (C) means change between slopes of seedlings (S) and adult (A) plants and in (D) shift in intercept between seedlings (S) and adult (A) plants. For abbreviations of plant species, see Table 1. 'All': all species tested together.

The effect of life-history traits on the allocation patterns of species

To identify the effect of life-history traits on allocation patterns of species at seedlings and adult plants, the values of allocation slope of each species were tested against the species life-history traits in one model using stepwise linear regression, as well as separately using analysis of variance or linear regression (S-plus 4.6). For each species we used one value of slope for seedlings and one value for adult plants (from previous analysis) and one average trait value. Thus, we had 20 points (species) in each test.

The effect of phylogeny on allocation pattern

For testing the effect of phylogeny on the allocation pattern of species separately for seedlings and adult plants, we calculated phylogenetic signal using Ape and Picante packages in R (Kembel *et al.* 2010; Paradis *et al.* 2004) and used Blomberg K to express the effect of phylogenetic affinity (as the matrix of pairwise phylogenetic distances between species, expressed according to Daphne database; Durka and Michalski 2012) on the values of allocation slope of seedlings and adult plants of the studied species. We also calculated Pagel's lambda with the 'Crunch' and 'Brunch' function in Caper package in R (Orme *et al.* 2013) to look for a phylogenetic signal in our data. Since the Pagel's lambda as well as the Blomberg K revealed no phylogenetic signal in our data (see below), we did not perform phylogenetically corrected ('pgls') tests.

RESULTS

The effect of species and age

We found strong and significant effects of species and age as well as a species–age interaction on allocation pattern when all the other factors were included as covariates ($F = 98.16$, $P < 0.001$ for species; $F = 1114.09$, $P < 0.001$ for age; $F = 106.29$, $P < 0.001$ for species \times age), indicating that allocation pattern at dry-grassland plants is species and age specific.

Allocation pattern into above- and below-ground biomass at seedlings and adult plants

Ten of the 20 species showed isometric (size independent) biomass allocation to shoots versus roots as seedlings, while the other 10 species showed allometric allocation patterns, i.e. the allometric exponent, estimated from the log above- versus log below-ground biomass, was significantly different from one (Table 2A). Within the latter group, a slope lower than one indicates that larger individuals of these species have relatively more roots than shoots compared to smaller individuals (Table 2A and Fig. 1A and B). Overall, seedlings of all of these species had allometric allocation patterns for above- versus below-ground biomass with a slope significantly lower than one, indicating that larger individuals generally have higher root–shoot ratio than smaller individuals (Table 2A).

For adult plants, 8 of the 20 species' allocation patterns were not significantly different from isometric, while allocation

patterns of the other 12 species were significantly different from isometric (Table 2B). Five of these 12 species had allometric slopes larger than one, which indicates that larger individuals have more above- compared to below-ground biomass than smaller individuals (Table 2B and Fig. 1A and B), whereas 7 species had slopes less than one. Overall, adult individuals of all of these species had an above- versus below-ground allometric allocation slope significantly lower than one, indicating that larger individuals generally have a higher root–shoot ratio than smaller individuals (Table 2B).

Comparison of allocation patterns of seedlings and adult plants

Twelve species' seedling–adult pairs differed significantly in their allocation slopes (Table 2C). Also, overall, seedlings of all species taken together significantly differed from adult individuals in their allocation patterns, with seedlings having higher allometric slopes than adults (Table 2C). There was shift in the value of slope from greater than one to less than one with increasing age in some annual species (Table 2C and Fig. 1A-a). On the other hand, there was shift from slope smaller than one to larger than one in late flowering (i.e. in the second season) perennial species (Table 2C and Fig. 1A-b).

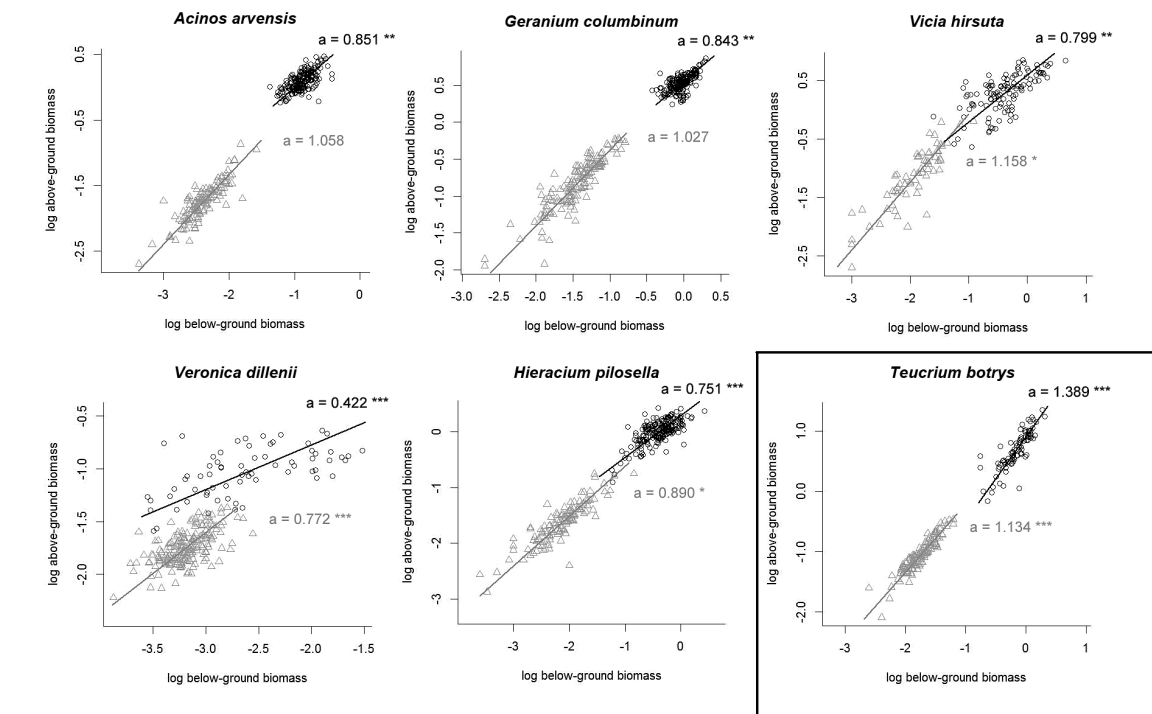
We found a significant decline in the allometric slope between seedlings and adult plants for one annual (*Veronica dillenii*) and one perennial clonal species (*Hieracium pilosella*; Fig. 1A-a) but the slopes were still less than one in all cases. Larger individuals have more roots compared to shoots than smaller individuals, and this tendency is stronger for adults than for seedlings. On the other hand, one perennial clonal species (*Origanum vulgare*) and one annual species (*Teucrium botrys*) significantly increased their slopes from seedlings to adult plants but the slopes remained lower than one and higher than one, respectively (Fig. 1A-b).

The other eight seedling–adult species pairs did not differ in their above- versus below-ground allometric slopes but there was a shift in elevation (i.e. y-intercept; Table 2D and Fig. 1B) or no difference between allocation patterns of seedlings versus adults (*Arenaria serpyllifolia*; Fig. 1C).

The effect of life-history traits

Allometric patterns varied significantly with life-history traits (Table 3). Seedlings of annual species allocated proportionally more to above- compared to below-ground biomass with increasing size (Fig. 2). On the other hand, seedlings of perennials (Fig. 2) allocated proportionally more into below- compared to above-ground biomass with increasing size. We also found a marginally significant positive effect of seed weight on seedlings allocation preferences, indicating that species with heavier seeds allocated proportionally more in above- and less in below-ground biomass with increasing size than species with lighter seeds. There was a marginally significant effect of leaf persistence on the allocation pattern of seedlings. Plants with persistent green or overwintering green leaves tended to allocate proportionally more into above- compared

A) Difference in slopes between seedlings and adult plants
 A-a) The value of slope is larger at seedlings than at adult plants.



A-b) The value of slope is smaller at seedlings than at adult plants.

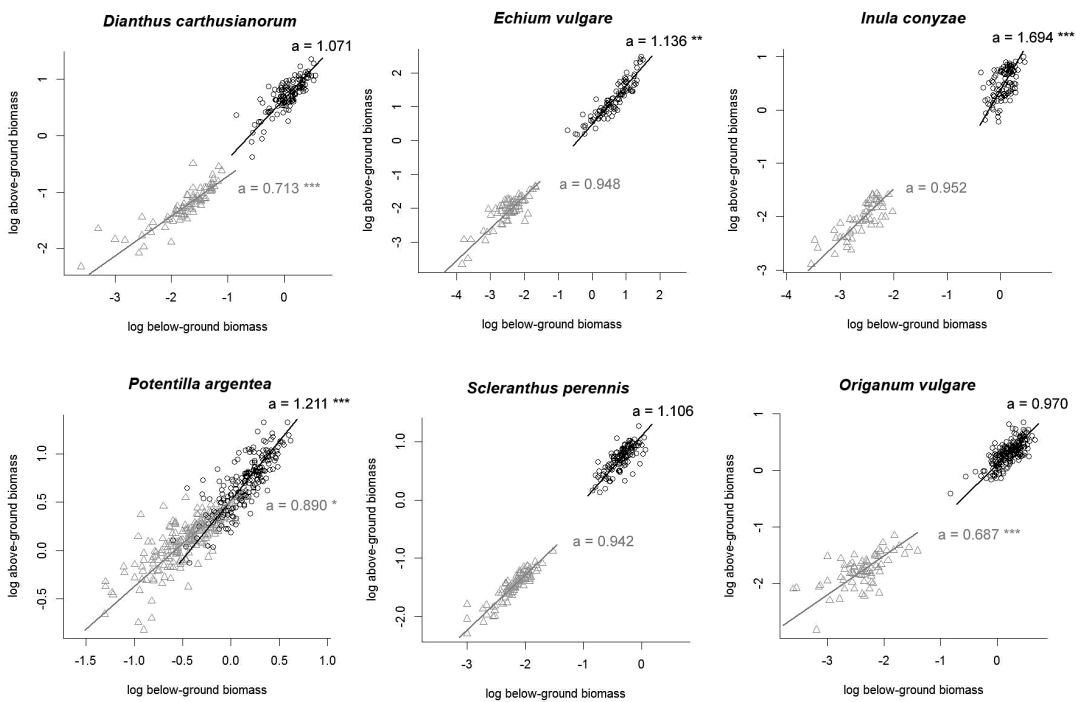
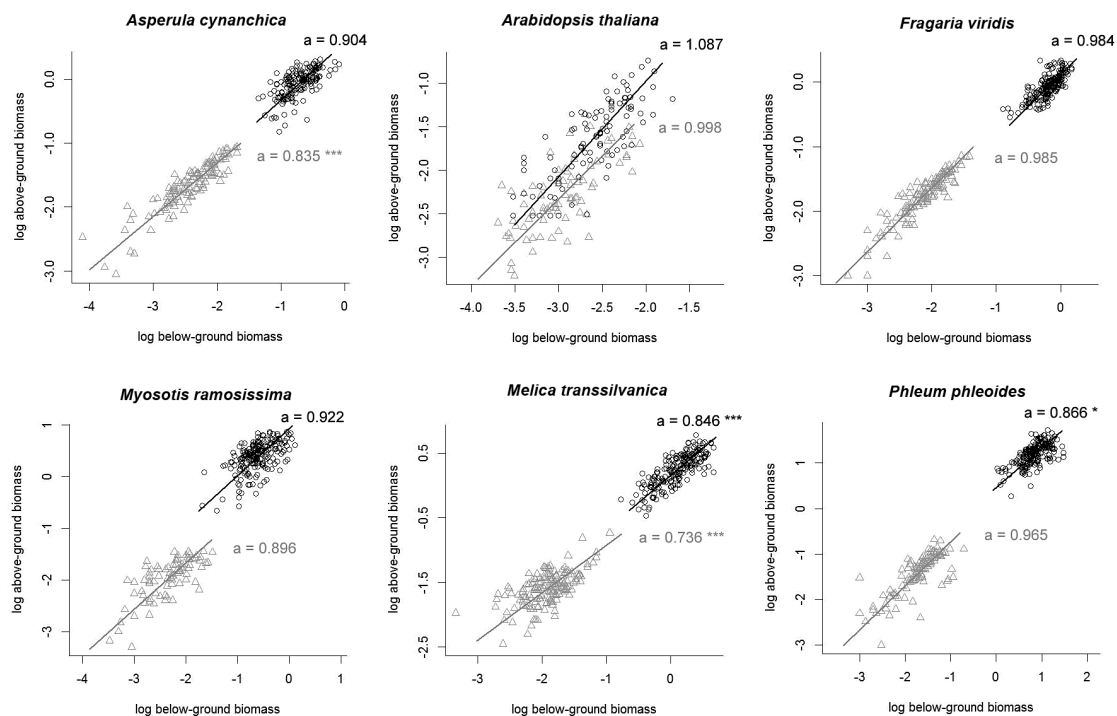


Figure 1: comparison of seedlings and adult plants allocation patterns. **(A)** Shoot-root allometric slopes differ between seedlings and adult plants. **(A-a)** The value of slope is significantly larger at seedlings than at adult plants of these species. **(A-b)** The value of slope is significantly smaller at seedlings than at adult plants of these species. **(B,C)** Shoot-root allocation slopes do not differ between seedlings and adult plants, but there are **(B)** shifts in elevation (intercepts) as well as shifts along common slope or **(C)** only shift along common slope. Grey triangles and lines are for seedlings, black circles and lines are for adult plants. Depicted are the values of allocation slopes (a), which were significant at probability levels of $P < 0.0001$ in all cases. *, ** and *** indicate whether the allocation slopes are significantly different from 1, i.e. whether the relationship is allometric, at probability levels of 0.05, 0.01 and 0.001, respectively.

B) No difference in slope, but shift in elevation (intercepts) as well as shift along common slope



C) No difference in slope and no shift in elevation but only shift along common slope

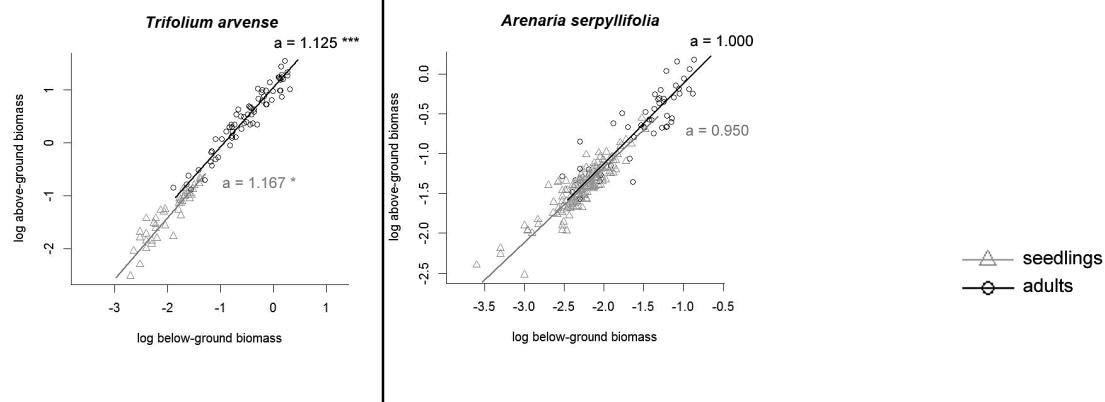


Figure 1: Continued

to below-ground biomass with increasing size than did plants with deciduous green leaves. SLA was selected by stepwise regression as the second most important trait related to differences in allocation for seedlings after life span.

We did not find a significant difference in allocation pattern between annuals versus perennials at adult plants. But species with higher allometric slopes in the adult stage (i.e. allocated proportionally more to above- compared to below-ground biomass with increasing size) usually did not flower in the first year of life (Fig. 3) and/or started flowering later in the growth season (usually from June and July; Fig. 4). Adult plants with lower SLA (Fig. 5) allocated proportionally more to above- compared to below-ground biomass with increasing size.

The effect of phylogeny

We found no evidence for the effect of phylogeny on seedling or adult plants allocation patterns (for seedlings: Blomberg $K = 0.434$, $P = 0.659$; for adult plants: Blomberg $K = 0.384$, $P = 0.850$). Species that were closely related did not have similar allometric allocation patterns.

DISCUSSION

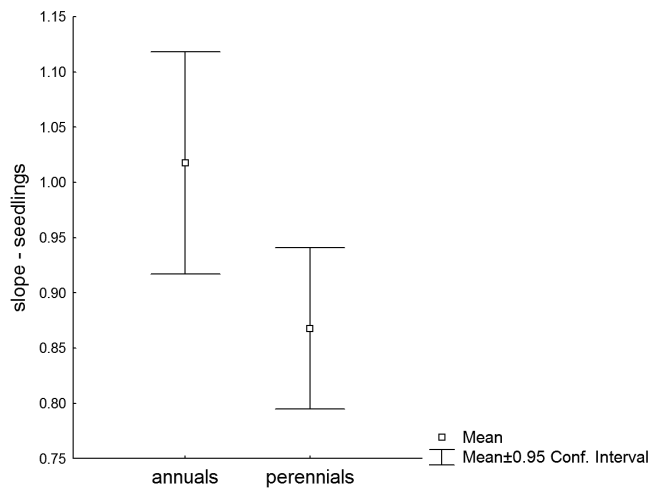
Overall allocation pattern of all species together

Generally, the results showed that allocation to above- versus below-ground organs differed between seedlings and adult plants when analysed across all the 20 dry-grassland species.

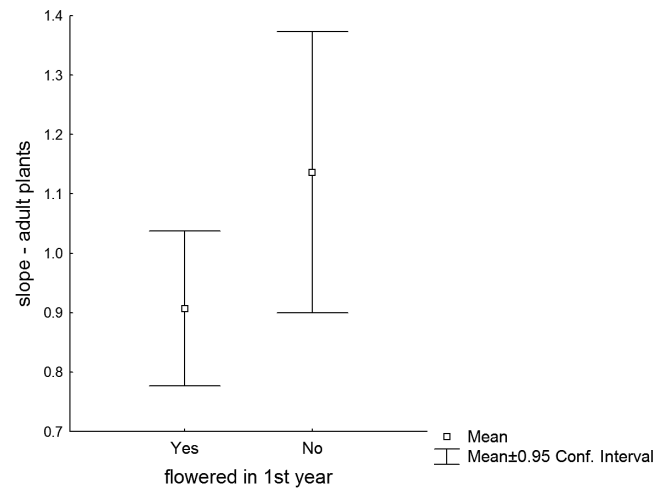
Table 3: the effect of species traits on the average value of shoot–root allometric slope of each species

	Shoot–root allometric slope for seedlings				Shoot–root allometric slope for adult plants			
	Selected in stepwise regression	<i>P</i>	<i>R</i> ²	Effect	Selected in stepwise regression	<i>P</i>	<i>R</i> ²	Effect
Log seed weight	*	0.091	0.151	+		0.850	—	
Life span	*	0.012	0.304	A>P		0.353	—	
CSR strategy		0.501	—			0.619	—	
Flowering in first year		0.737	—			0.049	0.198	No > yes
Flowering from (month)		0.893	—		*	0.004	0.375	Later flowering
Clonal		0.171	—			0.833	—	
Plant height		0.538	—			0.288	—	
SLA	*	0.774	—			0.044	0.207	—
Leaf persistence		0.081	0.256	w>p>s		0.376	—	

Shoot–root allometric slope for seedlings and for adult plants. Average values of species life-history traits: log seed weight (mg), life span (A = annuals, P = perennials), CSR strategy, flowering in first year of species life (yes, no), flowering from which month, clonal growth (yes, no), average plant height (m), SLA (mm²/mg), leaf persistence (p = persistent green leaves, w = overwintering green leaves, s = summer green leaves). Results of stepwise regression (* indicates selected trait) and linear regression or analysis of variance with the *R*² and *P* values and the direction of the effect (+/–) or the particular value are presented. Bold values are significant (*P* < 0.05).

**Figure 2:** shoot–root allometric slope of seedlings. *P* = 0.012, *R*² = 0.304.

This is in agreement with our hypothesis and several previous studies (Coleman *et al.* 1994; McConnaughay and Coleman 1999; Weiner 2004) suggesting that ontogenetic drift changes allocation (Evans 1972; Gedroc *et al.* 1996). Consistent with other studies (Janeček *et al.* 2014; Li *et al.* 2013; Lohier *et al.* 2014; Müller *et al.* 2000; Pan *et al.* 2013; Wang *et al.* 2010; Weiner *et al.* 2009b; Zhou *et al.* 2014), we also found that root–shoot ratio changes with plant size for both seedlings and adult plants. In contrast to some previous studies, however, we generally found increasing root–shoot ratio with increasing plant size in the analyses across species and this pattern was stronger for adult plants than for seedlings. A similar pattern was observed by Shipley and Meziane (2002), who found a preferential allocation to roots during plant ontogeny in a dataset of 22 herbaceous plant species. In contrast to our

**Figure 3:** shoot–root allometric slope of species that flowered in first year of their lives and those that did not. All adult plants of each species were harvested at the time of fruiting and maturing of most of their fruits. *P* = 0.049, *R*² = 0.198.

study, their experiment was very short: only 35 days post-germination, and thus their plants were very young even at time of the last harvest. As Shipley and Meziane (2002) state, a possible explanation for the observed allocation patterns is that the rate of nutrient uptake decreases as roots became older and larger. Moreover, during the growing season or over the plant's life, the amount of resources available, especially in pots, becomes depleted, which may result in higher demands on roots. An alternative explanation could be that studied species are typical dry-grassland species, originating from dry, rocky localities where water and nutrients are limiting factors. For this reason, large plants need to have proportionally much more roots to acquire sufficient amount of water and

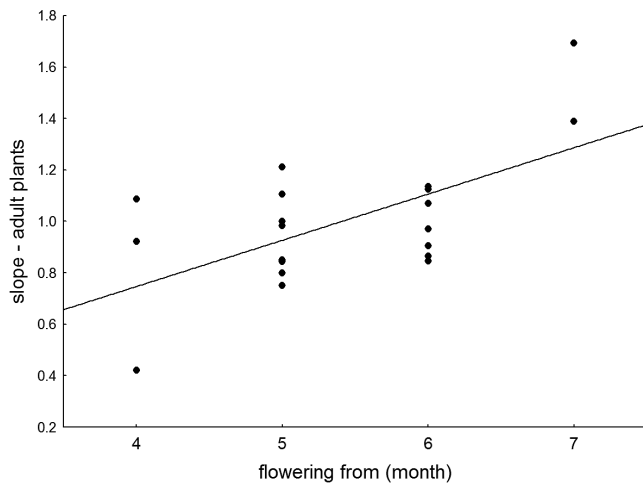


Figure 4: relationship between shoot–root allometric slope and month in which plants of each species start flowering within the season (4 = April, 5 = May, 6 = June, 7 = July). $P = 0.004$, $R^2 = 0.375$.

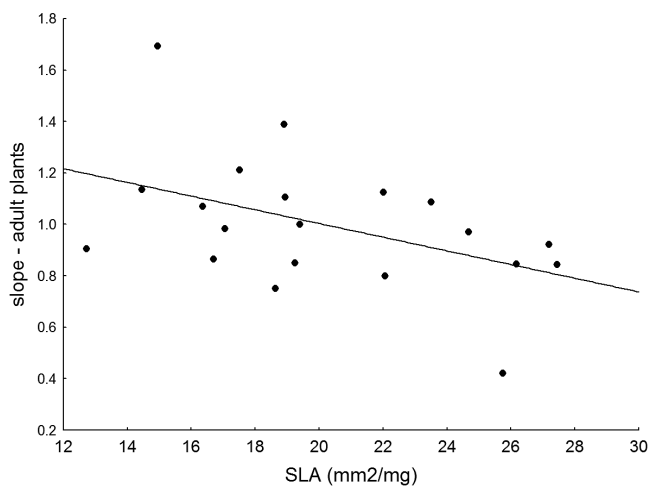


Figure 5: relationship between shoot–root allometric slope of adult plants and SLA. $P = 0.044$, $R^2 = 0.207$.

nutrients than small plants. The maternal environment could affect root allocation in the plants in our experiment in spite of the fact that the studied plants were regularly watered and grew in nutrient rich soil (Weiner *et al.* 1997). This is consistent with a previous study in which plants transplanted from a low-nutrient to high-nutrient environment did not change their shoot versus root allometries (Gedroc *et al.* 1996).

Allocation patterns of seedlings and adult plants of individual species

We found that patterns of biomass allocation differed among species and between developmental stages when analysing data on each species and stage separately. Our results are in contrast to the expectation of isometric allocation at annuals and allometric allocation of perennials (Mao *et al.* 2012; Zhou *et al.* 2014). We found allometric as well as isometric

patterns of allocation in both groups of plants. Specifically, seedlings of annual species with short life cycles and ruderal strategies had allometric slopes greater than one, indicating that larger individuals have relatively more shoots compared to roots than smaller individual, whereas young perennials usually had slopes lower than one. For adults, allometric slopes greater than one were observed in perennials, possibly because of higher allocation to flowers and fruits by larger individuals. On the other hand, adult annuals, one clonal perennial and grasses had slopes lower than one, indicating that larger individuals had more roots compared to shoots than did smaller individuals. For the one clonal perennial (*H. pilosella*) with an allometric slope less than one, the slope could be a consequence of its growth form: larger individuals consist of a main rosette plus newly originated secondary rosettes, each of which has its own roots. For grasses, the preferential allocation into roots than to shoots with increasing size could be related to their mode of persistence and life form. A higher root–shoot ratio for grassland vegetation than for other vegetation types (e.g. understory plants or canopy trees in subtropical forests) has been reported in previous studies (Cheng *et al.* 2015; Mokany *et al.* 2006).

Comparison of allocation patterns of seedlings versus adults

Intraspecific comparisons of allocation patterns of seedlings and adult plants of each species indicated that nearly all species had different allometric patterns at the two developmental stages. Seedlings and adults of more than half of the species differed significantly in their allometric slopes, the remaining species differed only in their proportion of above- and below-ground biomass with size, but in a constant way.

Comparing allocation patterns at these two stages among species, we found that annual species generally decreased their allocation slopes from seedlings to adult individuals whereas perennial species flowering in the second year or later usually increased in their allocation slopes with the age. There were, however, few species that deviated strongly from this pattern.

Our results are partly in accordance with previous studies (Coleman *et al.* 1994; Gedroc *et al.* 1996; Janeček *et al.* 2014; McConnaughay and Coleman 1999; Müller *et al.* 2000; Poorter *et al.* 2012; Weiner 2004), which found that most herbaceous species show a trend towards decreased root allocation and an increased shoot allocation with size during development. Some of our findings, however, are not consistent with these previous studies, especially those for annuals, where we found changes in allocation pattern between seedlings and adult plants opposite to most previous studies (Coleman *et al.* 1994; Gedroc *et al.* 1996; McConnaughay and Coleman 1999). These differences could be due to the short life cycle of some annuals that grew rapidly when they were young, but later, during flowering and maturing fruits and seeds, needed more water and nutrients to sustain such large number of fruits and seeds, so large adults allocated proportionally more to roots than shoots than did small adults.

The differences in our results from previous studies could be also due to the different ages and condition of the harvested plants. All our seedlings were harvested 2 or 3 weeks after most of the seeds of that species started to germinate. Many previous studies investigated species allocation earlier, so our seedlings could be older than those in some previous studies (e.g. [Gedroc *et al.* 1996](#); [McConnaughay and Coleman 1999](#); [Shipley and Meziane 2002](#); [Weiner 2004](#)). Also, we considered all seedlings to be at the same developmental stage, but they may not have been. Seedlings of some annuals may have already lived a quarter of their lifespan, whereas some perennials would be just establishing in this time. Adults of each species were harvested at the time of maturation of most of their fruits and seeds, so it is reasonable to assume that they were all at a similar developmental stage, although the length of their growth periods varied among the species. At maturation, annuals and perennials may look similar but they behaved differently: annuals finish their life cycle after fruiting and seeding, while perennials store resources for the next season. This could be why our results are different from those of [Niinemets \(2004\)](#), who studied allocation patterns over the whole life cycle of one perennial species and found increasing allocation to roots during ontogeny.

The effect of life-history traits and phylogeny on intraspecific allocation patterns

Intra- and interspecific biomass allocation pattern can be very different, so it is important not to conflate them ([Weiner *et al.* 2009a](#)). To study interspecific differences in allocation patterns, we used values of intraspecific allocation slopes for each species and we tested how they depend on various species life-history traits and phylogeny.

In our study, seedlings of annuals allocated proportionally more to above- than below-ground biomass with increasing size compared to seedlings of perennials. [McCarthy *et al.* \(2007\)](#) also found important effects of growth form (evergreen trees, deciduous trees, shrubs, forbs and graminoids) for allocation to above-ground organs, but allocation to roots was not influenced by growth form. Contrary to our hypothesis, there was no effect of life span on shoot–root allometry of adult plants. The most important factors affecting adult allocation pattern were the age of first flowering and the month in which the species start to flower. Species that did not flower in the first year of life or started flowering late in the growing season, allocated proportionally more to above- compared to below-ground biomass with increasing size and thus had a higher root–shoot allometric slope as adults. This could be due to the rate of growth and development of the species within the growing season.

Our results also indicate that species with lower SLA allocated proportionally more to above- compared to below-ground biomass with increasing size as adults. Also, species with higher allometric slopes and thus higher allocation to above-ground biomass as seedlings tended to have marginally significantly more persistent leaves than species with

higher allocation to roots. These results are consistent with those of [McCarthy *et al.* \(2007\)](#) who also showed higher allocation to shoots in species with longer-lived leaves and smaller SLA. There was a marginally significant positive effect of seed weight on preferential shoot allocation of seedlings, as observed by [Chacon *et al.* \(1998\)](#). Our results show this over a wide spectrum of species differing in seed weight. We also tested the effect of other species life-history traits on allocation slopes: CSR strategy, plant height and clonality, but none of these were significant. Species habitat requirements may play an important role in biomass allocation, but all our studied species come from the same habitat type, so there was very little variation in habitat requirements. Other factors, such as mycorrhiza, root or shoot herbivory, can have important effects on biomass allocation but they are outside of the scope of our study.

Despite the importance of species life-history traits for allocation patterns and in contrast to our hypothesis, we did not find any effect of phylogenetic relatedness of species on the allocation patterns. These results are not consistent with previous studies ([McCarthy *et al.* 2007](#); [McCarthy and Enquist 2007](#)) that found phylogeny to be the strongest predictor of biomass allocation. These studies were, however, carried out within a global dataset at higher taxonomic levels and across a wide spectrum of plant sizes. Our results thus demonstrate that phylogenetic relatedness of species at a lower taxonomic level—i.e. between different species from the same habitat and vegetation type, may not be important for explaining the variation in biomass allocation patterns, and that other factors, such as life-history traits, could thus play much more important role. This could be because biomass allocation patterns are evolutionarily labile and can evolve quickly ([Weiner 2004](#)).

CONCLUSION

Our results demonstrate the importance of changes in biomass allocation to above- versus below-ground organs between seedlings and adult plants at both intraspecific and interspecific level. The results are consistent with allometric biomass partitioning theory, as we found isometric as well as allometric patterns of biomass allocation in dry-grassland species. In general, seedlings as well as adult plants of the studied dry-grassland species showed allometric allocation patterns with root–shoot ratio increasing with increasing size, and this tendency was stronger for adult plants than for seedlings. Annual species usually decreased their shoot–root allometric slopes from young to adult individuals, whereas perennial species flowering in the second year or later usually increased their shoot–root slopes from seedlings to adults. Differences in species' life span had the strongest effect on intraspecific biomass allocation patterns in seedlings. For adult plants, the most important traits affecting intraspecific allocation patterns were the age of first flowering and month in which the species usually start to flower. Leaf traits, such as SLA, also had

important effects on biomass allocation. Our results confirm that biomass allocation pattern is species- and age specific and that plant life-history traits are much more important in explaining allocation pattern among species than is phylogenetic relatedness among species within a habitat.

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