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Ecological differences between herbs and woody plants, and evolution of the herbaceous habit

Ekologické rozdíly mezi bylinami a dřevinami a evoluce bylinnosti

Doctoral thesis

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“Human society is essentially an herbaceous product.”

Edmund W. Sinnott, Science 1916

Prohlášení

Prohlašuji, že jsem závěrečnou práci vypracoval za přispění kolegů a školitele. Můj podíl na dosažených výsledcích je vymezen na začátku každé studie. Dále prohlašuji, že jsem řádně uvedl všechny použité informační zdroje a literaturu. Tato práce ani žádná její část nebyla využita jako závěrečná práce k získání jiného nebo obdobného druhu vysokoškolské kvalifikace.

Table of contents

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Abstract

Flowering plants (angiosperms), which make up most present-day vegetation, were originally woody. While flowering plants have repeatedly given rise to herbaceous lineages since their first appearance, we lack a clear explanation for these common evolutionary events. Freezing temperatures and drought periods have been proposed as factors which had caused huge success of the younger growth form but the evidence is very limited and not in favour of these hypotheses.

In this thesis, we aimed to build the foundations of research on the evolution of herbs. We outlined new potential drivers of the evolution of herbs, suggested solutions to some methodological challenges and provided evidence about differences between herbs and woody plants relevant to the hypotheses on herb evolution. To this end, we used common garden experiments with young plants of both growth forms and global trait data from public databases which we evaluated using phylogenetic comparative techniques.

Annuality of aboveground biomass and fast life-strategy of herbs are characteristics which differentiate them from woody plants and which in some conditions are expected to be behind their success. Apart from the proposed conditions of freezing and drought, other disturbances damaging aboveground biomass might have played a role in the evolution. We proposed and showed evidence from experiments in favour of unpredictable disturbances like herbivory over predictable winter freezing. Additionally, based on their fast life-strategy, herbs might have an advantage in direct competition with woody plants, which we supported by showing their lower cost of aboveground structures, higher shade tolerance and based on experiment slightly higher growth plasticity. On the other hand, we showed that the two growth forms do not globally differ in fire tolerance and woody plants are more drought-tolerant than herbs.

This entire study is done on species which are phylogenetically related, which has to be, as we have done, taken into account when analysing results of experiments or database data. Also since data are gathered on individuals but conclusions are drawn about populations and their response to studied conditions such as freezing, species' demography must also be considered. We have described a way how demography can be incorporated into analyses.

Our results do not provide a final answer to the question of which ecological driver was behind the evolution of herbs but we have contributed to the evidence, which allows us to mark some of the hypotheses as much more likely than others. Our results show that unpredictable disturbances and fast and plastic growth of herbs are promising areas for further research of evolution of the younger growth form in angiosperms.

Abstrakt

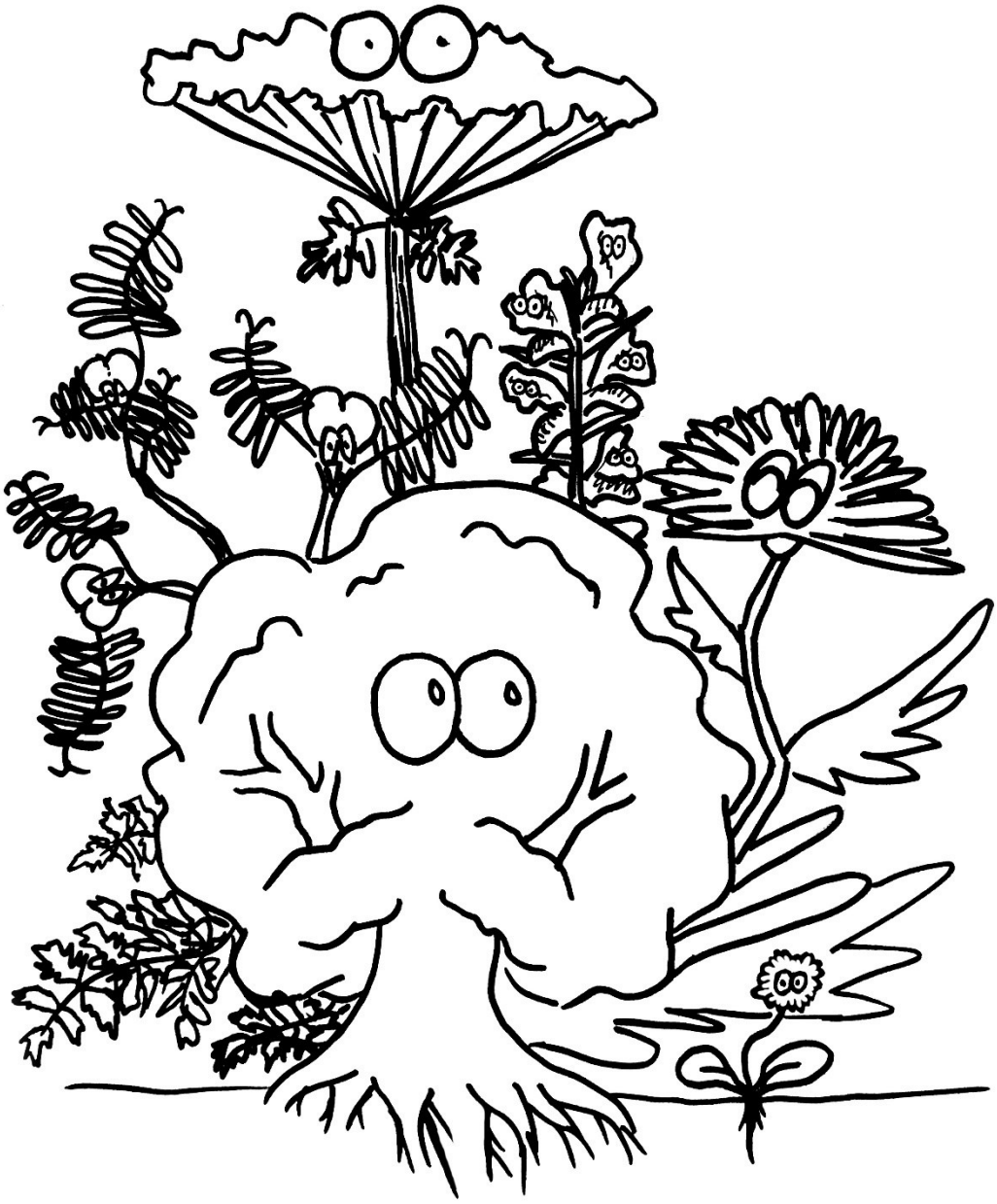
Krytosemenné rostliny (*Magnoliophyta*), představující dnes většinu vegetace na Zemi, byly původně dřevinami. Od jejich vzniku se mezi nimi opakovaně vyvinuly byliny, avšak proč se tak stalo, nevíme. Jako faktory, které mohly stát za úspěchem mladší růstové formy, byly navržené mráz a období sucha. Ovšem oba faktory jsou málo prozkoumané a to, co se ví, nehovoří v jejich prospěch.

V této práci jsme si dali za cíl položit základy pro výzkum evoluce bylinnosti. Navrhli jsme další faktory, které mohly stát za evolucí bylin, přišli jsme s řešením některých metodologických obtíží a představili jsme doklady o rozdílech mezi bylinami a dřevinami, které lze na základě jednotlivých hypotéz o evoluci bylinnosti očekávat. Za tímto účelem jsme provedli několik zahradních pokusů s mladými rostlinami obou růstových forem a analýzu veřejně dostupných databázových dat o vlastnostech rostlin z celého světa za použití fylogenetických komparativních technik.

Byliny odlišuje od dřevin jednoletost jejich nadzemní biomasy a rychlá životní strategie a očekává se, že tyto charakteristiky stojí za jejich úspěchem. Kromě dříve navržených faktorů mrazu a sucha, existují další disturbance, které mohly hrát roli v jejich evoluci. Navrhli jsme a výsledky pokusu podpořili neprediktabilní disturbance jako například herbivorii. Kromě toho na základě rychlé životní strategie bylin lze očekávat, že by mohly mít výhodu v přímé kompetici s dřevinami, což jsme doložili jejich levnější nadzemní biomasou, vyšší tolerancí k zastínění a experimentálně zjištěnou mírně vyšší plasticitou růstu. Na druhou stranu jsme ukázali, že obě růstové formy se globálně neliší v toleranci vůči ohni a že dřeviny jsou v rozporu s dosavadními představami více tolerantní k suchu než byliny.

Biologické druhy, o nichž jsme sbírali data, jsou do různé míry fylogeneticky příbuzné, což je třeba při analýze těchto dat zohlednit. Navíc jsou data sbírána na jedincích a závěry se týkají populací a jejich reakcí na různé podmínky jako například mráz. Proto je třeba vzít v potaz demografii dotčených druhů. Popsali jsme, jak to provést a začlenit demografii do analýzy dat.

Finální odpověď na otázku, který faktor způsobil evoluci bylin, nemáme, ale přispěli jsme výsledky, které nám umožňují některé z možných faktorů označit jako mnohem pravděpodobnější. Ukázali jsme, že neprediktabilní disturbance a rychlý a plastický růst rostlin jsou slibnými oblastmi pro další výzkum evoluce této růstové formy krytosemenných rostlin.



Introduction

Angiosperms (flowering plants) evolved from gymnosperms around 200 million years ago (Soltis et al., 2008; Smith et al., 2010). The first angiosperms were probably shrubby plants with magnolia-like flowers growing in shaded and disturbed habitats (Feild et al., 2004; Sauquet et al., 2017). During the early Cretaceous, they diversified and the first herbs appeared among them (Friis et al., 2003). Angiosperms further prospered and became the dominant plant clade on Earth (Ramírez-Barahona et al., 2020). Herbs have been evolving among them repeatedly up to present day. There have been more than a thousand (see Supplements) such transitions and many in the opposite direction from herbs to woody plants (there are more than 6000 secondary woody species; Dória et al., 2018). There is an old question related to these transitions not yet answered – why does it happen (Sinnott and Bailey, 1915)? What triggers the evolution of herbs? The necessary genetic changes are considered to be quite small since genes that control apical growth are responsible for lignin formation and are present in herbs (Groover, 2005). Consequently, we ask for ecological causes – what conditions have driven the evolution of herbs, what conditions enabled them to proliferate alongside their woody relatives?

In this introduction to the thesis, we summarize what we have contributed to answering this question. Our experiments, analyses and their results are presented. Yet the primary goal of this introduction is to outline the framework in which we have been working, the definitions and assumptions we used, methodological challenges one encounters when studying past evolution and the purpose of all the work. We aim for general conclusions and comments which transcend the topic of research of evolution of herbs and are therefore (to at least some degree) valuable in other parts of ecology.

Evolution of herbs – hypotheses

The question why herbs evolved from woody plants appeared with the first estimations of ancestral growth form of angiosperms more than a hundred years ago (Sinnott and Bailey, 1915). Two hypotheses proposing freezing temperatures and drought periods have been suggested but have not received much attention until recently (Zanne et al., 2014).

The first hypothesis that winter freezing temperatures were the driver of evolution of herbs was proposed by Sinnott and Bailey (1915). They based their suggestion on the global distribution of herbs and woody plants and on freezing events during the Paleogene. Sinnott and Bailey showed that the proportion of herbs is very high in floras of northern countries and much lower near the equator and suggested that it is driven by minimum winter temperature. They explained the advantage of herbs in areas exposed to winter freezing by their ability to survive the unfavourable conditions as seeds or belowground. They add that a fast life-strategy contributed to the success of herbs enabling them to disperse and adapt faster than woody plants.

Nearly a hundred years later, the hypothesis that herbs evolved due to freezing temperatures received further attention. Zanne et al. (2014) had assembled dataset of species occurrences, phylogeny and growth habit. They estimated evolutionary transitions between freezing exposed and unexposed

occurrences and woodiness. In 58% of cases, herbaceous habit evolved prior to exposure to freezing temperatures, which means that plants did not become (in many cases) herbaceous in response to freezing temperatures. Thus, freezing temperatures are an unlikely universal driver of their evolution.

The second hypothesis that drought might be responsible for evolution of herbs was suggested also already by Sinnott and Bailey (1915). They proposed that not all herbs had evolved due to low temperatures but some had been an adaptation to drought periods. Herbs thanks to their fast life-strategy are able to mature and produce seeds during the rainy season and thus avoid dry seasons similarly as winter periods.

Although dry habitats have been associated with transitions to a herbaceous habit in the genus *Echium* (García-Maroto et al., 2009), there is to our knowledge no systematic evaluation of the hypothesis that drought was the driver for the evolution of herbs. Furthermore, during studies of secondary woodiness on islands, a trend was found for woody plants to be more associated with dry habitats than herbs (Dória et al., 2018).

The two proposed hypotheses received only limited attention and the only study explicitly testing the freezing hypothesis dismisses it as a main driver of evolution of herbs (Zanne et al., 2014). There are, however, other ecological factors than drought and freezing that might have been behind the evolution of herbs and were not formulated nor tested so far. The differences between herbs and woody plants that Sinnott and Bailey stated in support of freezing and drought, are likely beneficial in other conditions too. Herbs are able to lose and regrow aboveground biomass and avoid unfavourable conditions belowground or as seeds. This ability can be expected to give them an advantage over woody plants during disturbances that damage primarily aboveground biomass such as fire or herbivory. Indeed, these are known to be important conditions of existence of herbaceous biomes and habitats (Buechner and Dawkins, 1961; Hoffmann et al., 2003; De L. Dantas et al., 2013).

The other difference that Sinnott and Bailey stated is the faster life-strategy of many herbs in comparison with woody plants which allows them to reproduce in very young age (Aarssen, 2008). Apart from the abovementioned disturbances (winter freezing, drought, fire and herbivory), where a faster life-strategy enables herbs to produce seeds between unfavourable conditions, it might be beneficial also in direct competition with woody plants. In early-successional habitats or in the forest understory, where herbs compete for light with young woody plants, fast growth can be expected to be one of the main strategies (Sterck et al., 2006). Furthermore, herbs creating aboveground biomass for a single year probably have more flexibility in their growth than woody plants which have to account for the position of their structures in years to follow and the stability of their (future) heavy aboveground biomass.

In summary, based on two sets of basic differences between herbs and woody plants – annuality vs. perenniality of aboveground biomass and faster vs. slower life-strategy – we have mentioned several hypotheses of evolution of herbs. These were hypotheses suggesting that herbs are more tolerant to freezing, drought, fire or herbivory. Although these forces can work as stress factors when limited, they are disturbances removing aboveground biomass when they are stronger (Bond and Keeley, 2005). Therefore herbs creating only annual aboveground biomass are expected to deal better with them than

woody plants. Further hypothesis suggests that herbs grow faster and have greater growth plasticity, which was the driver of their evolution. Direct evaluation of these hypotheses is mostly missing (apart from the freezing hypothesis) and indirect evidence is quite limited.

Before we present our results, we outline the framework in which our research is positioned – its assumptions, methods and purpose. This can be phrased as a clarification of the question: "What was the driver of evolution of herbs?". The first point of the framework is what is studied. The demarcation between herbs and woody plants is not unequivocal. The second is how it is studied. Study of past evolutionary events has its challenges and we will outline the most important ones. The third point is why we are studying evolution of herbs at all.

Definition of woodiness

Demarcation of herbs and woody plants is the first necessary step for any research on their evolution and although the concept of herbs on the one hand and trees and shrubs on the other is well established in everyday use, when we look closer at some species, their assignment is not unequivocal at all. Any definition of woodiness revolves around the aboveground stem. Stems of both growth forms provide several functions. A stem connects leaves and roots providing a transportation function. It also supports leaves and reproductive structures and mainly in woody plants serve as storage of carbohydrates. Herbs and woody plants differ in their biomass investment into stems, which is usually much higher in woody plants, representing the majority of aboveground biomass, than it is in herbs (Chap. 4). In herbs, the stem encompasses around half of aboveground biomass irrespective of whether it provides full supporting function or not (as is the case in climbers; Chap. 4). The high biomass proportion that woody plants allocate to stems is enabled by two characteristics which also often figure as definitions of woodiness – secondary vascular growth (anatomical definition; Spicer and Groover, 2010) and perenniality of aboveground stems (Beaulieu et al., 2013; Zanne et al., 2014). Both growth forms could be also defined by position of their buds during unfavourable seasons with herbs having buds at or below ground level and woody plants having them on stems aboveground (Raunkiaer, 1934).

However, in nature we can find a continuum of secondary vascular growth from herbs to trees with many intermediate states (Groover, 2005; Spicer and Groover, 2010) and even intraspecific variability (Groover, 2005). Apart from high elevations, most herbs create lignin especially in their perennial belowground parts which also secondary thicken (Büntgen et al., 2014; Crivellaro and Büntgen, 2020). The criterion of perenniality is quite clear only in seasonal climates and even there the rule concerning the position of buds has many exceptions or equivocal cases (epiphytes, subshrubs). Therefore, herbs and woody plants are artificial categories that can by successfully applied to majority of plants but not all of them.

Hypotheses proposed in our studies claim herbs to be the growth form that regrows aboveground biomass anew each year (Chap 1 and 2). This is close to the definition stating woody plants to be those that have perennial aboveground structures. Nevertheless, all species used in our experiments would be classified as herbs or woody plants identically using either this or the definition of woodiness based secondary vascular growth (anatomical definition).

For large (global phylogenetic) studies (as e.g. Chap. 3), the choice of definitions of woodiness can change how some species are classified. But such studies inevitably rely on multiple data sources and metadata are seldom so detailed as to contain information like definition of woodiness. The commonly used approach is then to use consensual information and exclude species which are assigned to different categories by different sources (e.g.: Zanne et al., 2014; Engemann et al., 2016). Furthermore, such studies aim for results which are robust and thus not changed by slightly different definitions (which is necessary since in data from many sources, mistakes and imprecisions are inevitable).

To evaluate the robustness, we repeated the analyses from Chap. 3 giving priority to datasets of woodiness using different definitions (Zanne et al., 2014; Engemann et al., 2016). Results of the analyses employing the anatomical definition of woodiness were very similar to the definition based on perenniality of aboveground structures (see Supplements, Tab. S1).

Methodology

Any study of the evolution of herbs is research of past events and as such it is necessarily indirect. From the present species and their traits, we infer the likely course of nature in the past. Our results are therefore based on assumptions about what traces past evolutionary events left on current species. We specifically assume that current differences between both growth forms mirror past ones and thus any advantage of one of the growth forms in certain conditions makes the growth form an adaptation or exaptation to such conditions.

Although the research seems to be only historic, the evolution of herbs has been repeating, therefore our question can be rephrased from “What was the driver of evolution of herbs?” to “What are the most likely drivers of the recurring events of herb evolution?”. The aim has to be general and not just historic to meet the purpose of the study, which is not only knowledge of past events.

There does not have to be one trigger/driver of all events of evolution of herbs. Herbs could evolve and proliferate thanks to a set of their traits, which could differ in time or across the phylogenetic tree (as pointed out e.g. by Keeley et al., 2011). This means that the question about the driver is a question about the main driver or more than one important driver. Evaluation of a greater number of potential drivers simultaneously can give us information about their relative strength and/or probability (as we have done in Chap. 1 and 3).

A related question concerns the universality of the studied driver(s) – whether they are likely drivers across the phylogenetic tree or only in its part. One suitable approach for answering it is a global study with wide phylogenetic and geographical coverage (Chap. 3), as it enables an evaluation of the hypotheses both in particular clades and across them. This approach has one major disadvantage and that is its data-intensity – only very few traits are available in good quality for a high number of species. Consequently, one often has to analyse indirect proxies for the traits of interest (which adds non-trivial assumptions). A complementary approach is the common garden experiment, in which plants of both growth forms are exposed to specific treatments (Chap. 1 and 2). This allows us to study specific traits directly linked to the

hypothesis of interest but only for very limited number of species. Their combination, we believe, is desirable since global study enables us to outline which hypotheses are worth a detailed experimental study or which trends discovered in garden experiments are generalizable.

Phylogenetic comparative models

When studying differences between the two growth forms (in global comparative studies or in experiments), which might have been behind the evolution of herbs, our observations – datapoints – are individual species. However, sets of species share different portions of evolutionary history and therefore show different degrees of similarity in heritable traits. Close association between two traits (e.g. woodiness and freezing tolerance) of species from one genus does not say as much about the relationship of these traits in a much larger clade (e.g. angiosperms). Such statistical non-independence of species must be controlled for in analyses using phylogenetic comparative models (Felsenstein, 1985).

These models usually assume that observations (species) have residuals corresponding to the variance-covariance matrix derived from their phylogenetic tree. But when we study herbs and woody plants experimentally (e.g.: Chap. 1 and 2), we gather data about individuals rather than species. All individuals from one species then represent estimates of the species value and thus from a statistical perspective the problem is hierarchical.

For analyses of such multispecies data, mixed effect models have been suggested (e.g.: Jamil et al., 2013) and often used with species as a random effect. These models account for the hierarchical structure of the problem; however, they assume species to be independent (a parameter describing the species-specific effect is assumed to be normally distributed). Thus, they are identical to a phylogenetic hierarchical model (as used in Chap. 1 and 2) with a star-like phylogeny (i.e. with Pagel's lambda equal to zero; Pagel, 1999). Therefore, mixed-effect models with species as a random effect are not suitable for phylogenetic data and hierarchical phylogenetic models should be used instead (as we have done in Chap. 1 and 2).

To illustrate why phylogenetic models should be preferred, we compared the type I error of those two models used for phylogenetic data. As has been shown for non-hierarchical models (Revell, 2010), failing to account for phylogeny strongly increases the error rate (see Supplements, Fig. S1).

Demographic correction

When we study differences between growth forms, as in Chap. 1 and 2, we are ultimately interested whether these differences can translate into differences in fitness. Since our research concerns evolutionary process, we are interested in long-term differences in the fitness of populations, not differences between individuals. Translation of observed differences between individuals of both growth forms to differences between populations is not straightforward. Individuals are studied in a particular life-stage and each life-stage can be of a very different importance (for population growth rate) for

different growth forms. Thus, e.g. 50% mortality due to a particular treatment for seedlings of trees and annual herbs is likely to have much stronger effect on a population of herbs than a population of trees. The reason is different demography with different importance of the particular life-stage for population growth rate.

Therefore, one should take the demography into account when making inferences from individual to population level. The adjustment of the data for demography using projection matrix models, which we call demography correction, is presented in Chap. 5. It consists in adjusting impacted transitions in the projection matrix and calculating changes in population growth rates.

As we point out in our study, demographic information is available for a very limited set of species. Although the main drivers of demography have been identified (Adler et al., 2014; Salguero-Gómez, 2017), the relative importance of specific live-stages for herbs and woody plants have yet to be quantified. For young plants, which we used in our experiments (Chap. 1 and 2), we can expect greater relative importance of these life-stages in herbs than in woody plants whose life-cycle is dominated by survival of adult plants (Silvertown et al., 1993). This expectation means that mortality of young woody plants is likely to be relatively less important for the population than mortality of young herbs. Therefore, a demographic correction, as mentioned in the manuscript, to some degree probably reduces the difference observed in the mortality of herbs and woody plants in unpredictable conditions (Chap. 1). On the other hand, it would not influence the relative comparison of predictable and unpredictable conditions as nearly identical sets of species were used. The question of how the demographic correction influences the result of the experiment with green shading (Chap. 2) is less straightforward. Here, responses in terms of changes in direction of growth are measured and compared and these cannot be easily translated into fitness differences. However, if the observed differences in response to shading can lead to advantages of herbaceous individuals in some conditions then this difference has to be greater than some value to retain the advantage after demographic correction. Otherwise, it does not speak in favour of greater growth plasticity of herbs than woody plants to be the driver of their evolution.

Purpose of study

As was already outlined, the driver we are looking for is not of developmental nature – what makes herbs herbs. The question is ecological, namely, what conditions enabled herbs to flourish once the particular mutation or mutations took place. Furthermore, what helps them flourish now and will in the future. Thus the identified differences between both growth forms lead us not only to the understanding of past events but also potential drivers of future events. Differences between woody plants and herbs influence their proportion on a locality and their proportion has enormous impact on what the habitat looks like, what lives in it and what is likely to happen to it in the future.

Results

We have conducted three studies with the aim to contribute to the evidence concerning the evolution of herbs.

In the first study (Chap. 1), we propose that the ability of herbs to lose aboveground biomass, which was expected to be beneficial under winter freezing, is useful also under different disturbances, which damage aboveground biomass. Unlike winter freezing, some of these disturbances are less predictable and thus adaptations of woody plants like deciduousness are not usable. We therefore expected herbs to have an advantage over woody plants when exposed to unpredictable disturbances.

We conducted three common garden experiments with young herbs and woody plants. We exposed them to three treatments of various predictability – the most predictable winter freezing, moderately predictable spring freezing and the most unpredictable herbivory. Freezing was simulated by putting plants overnight into a freezer (for winter freezing we simulated a severe event down to -20°C and for spring freezing much milder conditions down to -5°C) and herbivory by removal of aboveground biomass. We observed no difference between herbs and woody plants under predictable conditions but with increasing unpredictability, the advantage of herbs increased. Thus, the results were in agreement with our expectation.

In the second study (Chap. 2), we propose that biotic interactions among plants should also be considered when studying the evolution of herbs. Herbs build their aboveground structures again each year unlike woody plants which have perennial aboveground biomass. Woody plants therefore have to balance photosynthetic gain in the given year against future years, along with the stability of their aboveground structures. Therefore, herbs can afford to respond in much more plastic fashion to surrounding conditions than woody plants.

To test this hypothesis, we conducted a common garden experiment, where we exposed young herbs and woody plants to directed green shading. We measured their growth direction after two months and compared it between herbs and woody plants. As expected, response of herbs to green shading simulating surrounding vegetation was stronger than response of woody plants. However, the difference was small.

In the third study (Chap. 3), we selected plant traits related to abovementioned hypotheses, assembled them from public databases and assessed differences between herbs and woody plants. We used tolerance to freezing, drought, fire, shade and specific leaf area (SLA) as a proxy for the cost of leaves and clonality as a trait which have been proposed to be generally beneficial under disturbances. We compared both growth forms and found differences between them in freezing, drought and shade tolerances as well as in the SLA. Herbs were more tolerant to freezing and shade and had higher SLA. On the other hand, and contrary to expectations, woody plants had higher drought tolerance. Growth forms did not differ in fire tolerance or clonality.

We studied differences between both growth forms in many aspects which range from investment into stem in their climbing varieties to traits which are proposed to be different under various hypotheses of evolution of herbs. We have shown that climbing habit does not lead to savings in stem biomass in herbs

(Chap. 4), that herbs are likely to have an advantage over woody plants when exposed to unpredictable disturbances (Chap. 1), that they respond more strongly to surrounding green shading (Chap. 2), have greater freezing and shade tolerance and lower drought tolerance (Chap. 3). We also pointed out that different demography of herbs and woody plants might lead to biased results in studies of their differences (Chap. 5).

Conclusion

The question concerning the main drivers of the evolution of herbs remains open. But, based on our work, we are closer to answering it. Primarily, we contributed to the clarification of the question and we outlined possible drivers of the evolution of herbs. Secondly, we provided evidence in favour or against individual hypotheses. This allows us to address the credibility of the hypotheses and focus further research in promising areas.

Since the times of Sinnott and Bailey (1915), many new details about climate and evolution have been described. We now know that angiosperm families diversified in one of the warmest periods on Earth (Zachos et al., 2006; Ramírez-Barahona et al., 2020). Together with evidence of the evolution of the herbaceous habit before encountering freezing (Zanne et al., 2014) and no difference in response of seedlings to winter freezing (Chap. 1), we can conclude that freezing temperatures were not the main driver in the evolution of herbs. Still, herbs do generally deal with freezing temperatures much better than woody plants (Chap. 3) and the main diversification period of angiosperms (100-66 million years ago) is preceded and later succeeded (34 million years ago - Late Cenozoic Ice Age) by glacial periods (the preceeding one might just be a colder period; Veizer et al., 2000). Thus, the herbaceous habit might only be an exaptation for freezing temperatures but we also cannot exclude that it might in some rarer cases be an adaptation to these conditions. Drought, the other driver proposed by Sinnott and Bailey (1915), is also very unlikely the universal driver of the evolution of herbs, primarily because woody plants are generally more tolerant to drought than herbs (Chap. 3; Dória et al., 2018).

Our results suggest that herbs have an advantage over woody plants under less predictable disturbances (Chap. 1). While fire may be a suitable disturbance of limited predictability, the growth forms do not differ in fire tolerance (Chap. 3). Resistance to herbivory is another promising potential driver, unfortunately, data availability is too limited to evaluate global differences in it between growth forms. Finally, we want to draw attention towards direct biotic interactions, where cheap aboveground structures, shade tolerance and probably more plastic growth can give an advantage to herbs (Chap. 2 and 3).

These results outline possible directions for future study of the evolution of herbs. We believe that promising directions include disturbances like fire and herbivory by large mammals, which both currently suffer from limited availability of comparable data across the globe. Apart from disturbances, differences in growth plasticity and growth speed should not be forgotten. The field will also benefit from further development of phylogenetic comparative methods (e.g. hierarchical models) and from combining various approaches (experiments and global trait comparisons) in the study of evolution of herbs.

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Supplements

Estimation of number of transitions into herbaceous habit

We used growth form data from Chap. 3 to estimate number of transitions between growth forms on phylogenetic tree ALLOTB. Growth form data are based on: Engemann et al (2016), Zanne et al (2014) and CLO-PLA database (Klimesova et al., 2017) and the phylogenetic tree is from Smith and Brown (2018). We estimated the number of transition for superasterids (22584 species) and superrosids (23847 species) using stochastic mapping of discrete character with two estimated rates of transition (one for each direction). Stochastic mapping was done using package *phytools* (version 0.7-47; Revell, 2012).

For superasterids, we estimated 955 transitions towards herbaceous habit and 1540 transition in the opposite direction. For superrosids, the estimate was 609 transition towards herbaceous habit and 1077 transition towards woody habit.

Type I error rate in phylogenetic and non-phylogenetic hierarchical model used on phylogenetic data

We simulated phylogenetic data and independent phylogenetic predictor. We tested the effect of the predictor using phylogenetic and non-phylogenetic hierarchical model to evaluate type I error of both models. We calculated 95% credible interval of the parameter of interest (effect of the predictor) and checked whether it encompasses zero (the true value used in simulation of the data). We ran the simulation 100 times and compared error rate of both models. From 100 cases, 95% credible interval of phylogenetic model did not contain true value in 3 cases. In non-phylogenetic model it was in 15 cases (Fig S1).

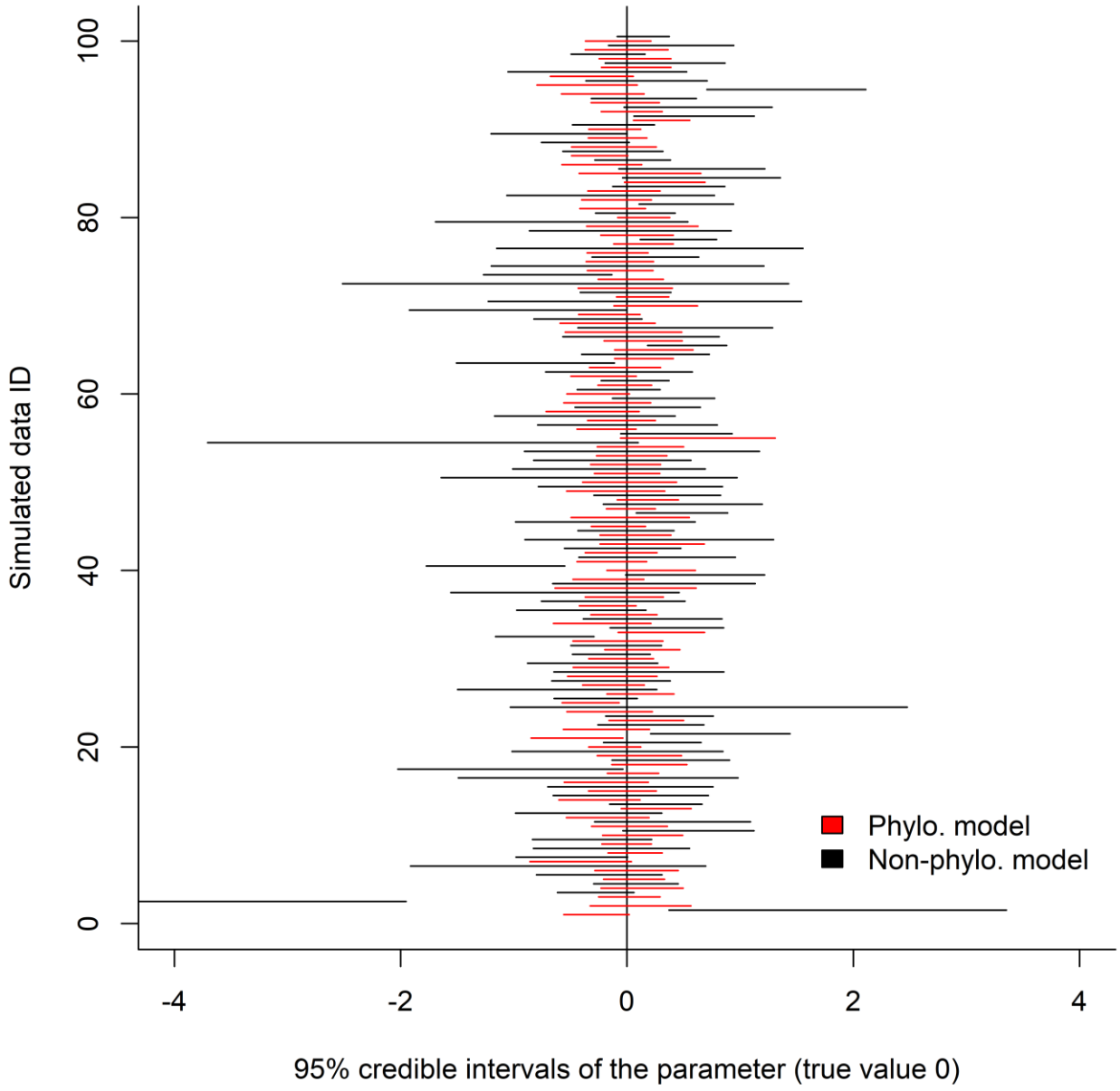

Figure S1: Comparison of credible intervals of phylogenetic and non-phylogenetic hierarchical model applied to phylogenetic data. From 100 simulated datasets and consequent analyses, in 3 cases the 95% credible interval of the phylogenetic model does not encompass true value of the parameter (0) and for non-phylogenetic model it happened 15 times.

Phylogenetic tree for 30 species was simulated by randomly clustering tips (ape::rcoal function). Woodiness was than simulated as binary trait along this tree using symmetric transition Q matrix (via phytools::sim.history function). Species means of the trait were simulated to have arbitrary mean 2 and covariance structure corresponding to Brownian motion model of evolution using the simulated tree (via phytools::fastBM function). We simulated 15 observations per species with normal error with mean 0 and

arbitrary standard deviation 0.5. Woodiness was not used for simulation of the trait (example of the data: Fig S2).

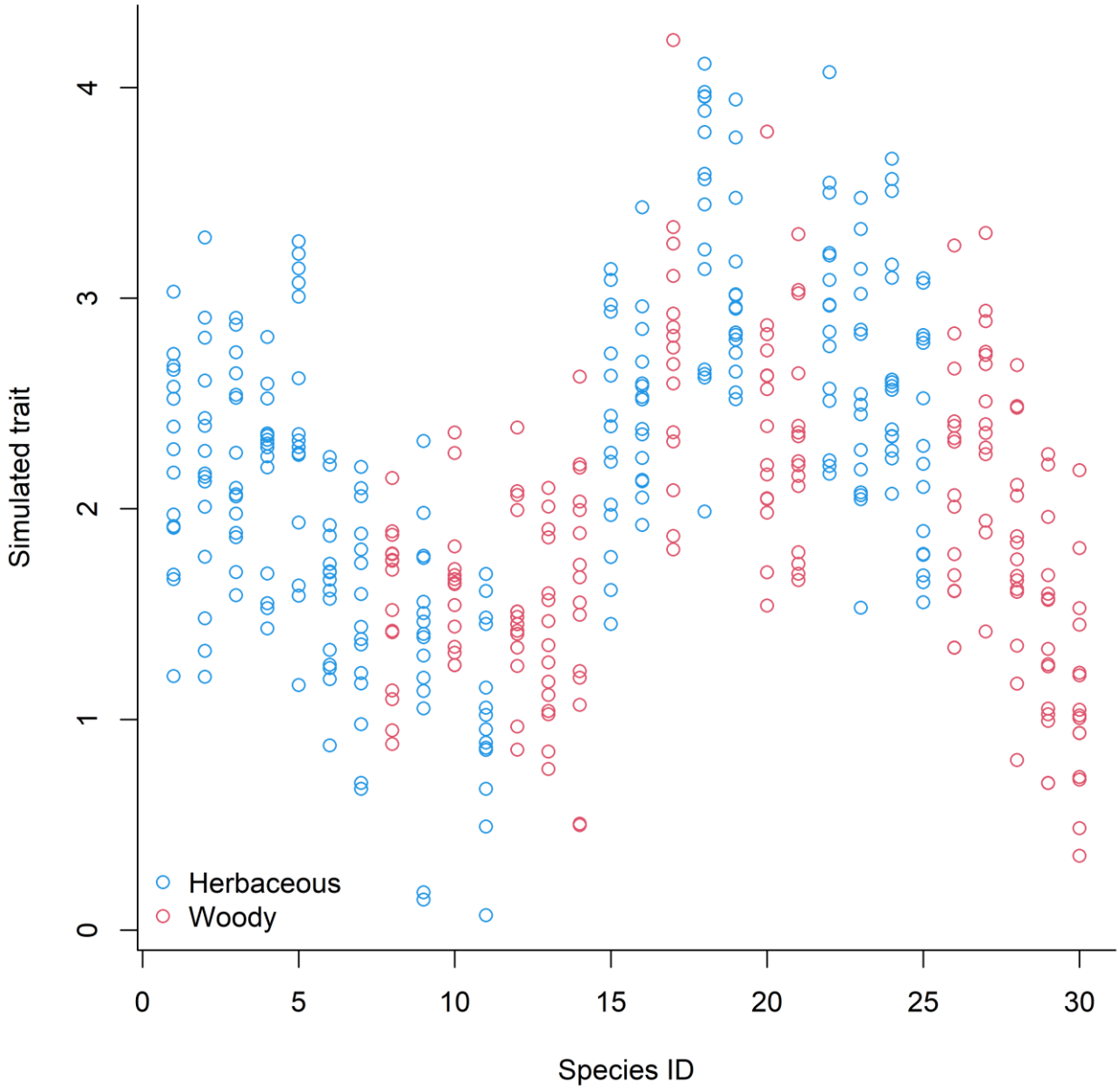

Figure S2: Example of simulated data for comparison of phylogenetic and non-phylogenetic hierarchical models.

Very simple non-phylogenetic and phylogenetic model were used (more complex models are used in Chap 1 and 2). In non-phylogenetic model, the data were modelled:

$$Data \sim \text{Normal}(\alpha + SpecEff, \sigma)$$

$$SpecEff \sim \text{Normal}(\beta * W, \varphi)$$

where α, σ, β, φ were parameters, *SpecEff* was random effect of species, *W* was woodiness and β was the parameter of interest.

The phylogenetic model modelled data:

$$Data \sim \text{Normal}(\alpha + SpecEff, \sigma)$$

$$SpecEff \sim \text{MultivariateNormal}(\beta * W, \varphi * phy)$$

where α, σ, β, φ were parameters, *W* was woodiness, *phy* was variance-covariance matrix calculated from the phylogenetic tree and β was the parameter of interest.

In both models uniform flat priors for all parameters were used. Models were run with 4 chains, 4000 iterations each, half of them as warm-up phrase. Convergence was checked using *R-hat* statistics. Non-centred parametrization (Papaspiliopoulos et al., 2007) was used to avoid Neal's funnel (Neal, 2003) – common sampling inefficiency in hierarchical models. Models were written in *Stan* (Carpenter et al., 2017) using package *rstan* (version 2.19.3; Stan Development Team, 2018). Packages *phytools* (version 0.7-47; Revell, 2012) and *ape* (version 5.3; Paradis et al., 2004) were used for data simulation. All analyses were done in R (version 4.0.0; R Core Team, 2020).

Table S1: Results of the analyses of differences between herbs and woody plants in selected traits (see Chap. 3) using two different definitions of woodiness. Arrows show the direction of the difference (up: higher values of the trait for woody species than for herbs). Absence of the arrow denotes that the direction of the difference depends on the biome. In brackets is the p-value for the difference being zero. In first three columns of the tests (default, GBOTB, annuals), biome was included in the analysis – in case the difference between woody plants and herbs differed among biomes, asterisk is added. Fields showing results in agreement with the hypothesis (and significant on level of 0.05) are in blue, opposite results are in red. Default model is with ALLOTB phylogenetic tree, without annuals and with biomes. Exp. = expectation, GBOTB = phylogenetic tree (Smith and Brown, 2018)*, PLANTS = Plants database* (USDA NRCS, 2017)*, SLA = specific leaf area.*

		Anatomical definition of woodiness							
Trait	Exp.	Default model	GBOTB	Annuals	Without biomes	Temperate mixed broadleaf forest	Tropical, subtropical coniferous woodland	Fabales	Lamiales
Frost free days	↑	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (0.013)	↑ (0.047)	↑ (<0.001)
Minimum temperature	↑	↑ (<0.001)*	↑ (0.001)*	- (0.101)*	↑ (<0.001)	↑ (0.189)	↑ (0.221)	↑ (0.03)	↑ (<0.001)
Freezing exposed	↓	↓ (<0.001)*	↓ (<0.001)*	↓ (<0.001)*	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)
Drought tolerance	↓	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (0.001)	↑ (0.137)	↑ (0.007)
Drought tolerance (PLANTS)	↓	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (<0.001)	↑ (0.069)	↑ (0.058)
Fire tolerance	↓	- (0.244)*	- (0.675)*	- (0.991)*	↓ (0.508)	↑ (0.975)	↓ (0.54)	↓ (0.165)	↓ (0.952)
Shade tolerance	↓	↓ (0.013)*	↓ (0.014)	↓ (0.004)	↓ (<0.001)	↓ (0.16)	↓ (0.022)	↓ (0.578)	↓ (<0.001)
Shade tolerance (PLANTS)	↓	- (0.568)*	- (0.517)	- (0.459)	↓ (0.004)	↑ (0.907)	↓ (0.491)	↑ (0.26)	↓ (0.005)
SLA	↓	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)*	↓ (<0.001)	↓ (<0.001)	↓ (0.031)	↓ (0.407)	↓ (<0.001)
Clonality	↓	- (0.075)	- (0.052)	- (0.007)*	↑ (0.638)	↑ (0.033)	↑ (0.508)	↓ (0.264)	↑ (0.459)
		Perenniality of aboveground definition of woodiness							
Frost free days	↑	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (0.013)	↑ (0.001)	↑ (<0.001)
Minimum temperature	↑	↑ (<0.001)*	↑ (0.001)*	- (0.142)*	↑ (<0.001)	↑ (0.225)	↑ (0.221)	↑ (0.096)	↑ (<0.001)
Freezing exposed	↓	↓ (<0.001)*	↓ (<0.001)*	↓ (<0.001)*	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)
Drought tolerance	↓	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (0.001)	↑ (0.032)	↑ (0.003)
Drought tolerance (PLANTS)	↓	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (<0.001)	↑ (0.01)	↑ (0.018)
Fire tolerance	↓	- (0.617)*	- (0.814)*	- (0.544)*	↓ (0.795)	↑ (0.435)	↓ (0.54)	↓ (0.408)	↑ (0.755)
Shade tolerance	↓	↓ (0.005)	↓ (0.006)	↓ (0.002)	↓ (<0.001)	↓ (0.115)	↓ (0.038)	↓ (0.306)	↓ (<0.001)
Shade tolerance (PLANTS)	↓	- (0.353)*	↓ (0.336)	- (0.277)	↓ (0.006)	↓ (0.842)	↓ (0.491)	↑ (0.556)	↓ (0.002)
SLA	↓	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)*	↓ (<0.001)	↓ (<0.001)	↓ (0.016)	↓ (0.215)	↓ (<0.001)
Clonality	↓	- (0.052)	- (0.04)	- (0.008)*	↑ (0.681)	↑ (0.047)	↑ (0.26)	↓ (0.484)	↓ (0.93)

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Chapter 1

1. Evolution of herbs: Key to the conundrum might be tolerance not avoidance

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TH, AK, MW and TK planned and designed the research, AK, MW and TK performed the fieldwork, AK analysed the data and wrote the manuscript using all other authors' contributions.

Submitted manuscript.

1.1 Abstract

Woody plants represent the ancestral growth form in angiosperms with herbs evolving repeatedly from them. While there are a number of hypotheses about drivers of the evolution of the herbaceous habit, the ability to avoid frost damage in winter by discarding their aboveground biomass has often been invoked as the main force in their evolution. We propose instead that any unpredictable disturbance might have been much more important than the seasonal frost, as herbs easily survive repeated disturbance.

We tested this hypothesis by comparing herbs and woody plants in their ability to deal with three types of simulated disturbances, ranging from the most predictable, i.e. winter freezing, through spring freezing to relatively unpredictable herbivory. Comparison was done in experimental common garden setup with 20 species differing in woodiness. We evaluated effects of these disturbances on mortality and regrowth of plants.

Herbs did not have an advantage over woody plants in survival when exposed to winter freezing. In less predictable conditions of spring freezing herbs survived the treatment better than woody plants and this advantage was even larger in case of the simulated herbivory treatment. In all three treatments, woody plants that survived had tendency to decrease more in size due to treatment than herbs.

Increasing advantage of herbs over woody plants with increasing unpredictability suggests that herbaceous growth form might be an adaptation to unpredictable disturbance, which herbs are able to tolerate thanks to their ability to survive loss of aboveground biomass. Consequently, factors such as mammal herbivory or fire might have been the most likely factors in the transition from woody species to herbs.

Keywords: experiment; herbivory; predictability; spring freezing; winter freezing; woody plants

1.2 Introduction

In Angiosperms, herbs are the younger growth form which evolved repeatedly from the ancestral woody state (Smith et al., 2010). Although being younger, herbs have been very successful and account globally for about half of plant species (Fitzjohn et al., 2014). They are therefore bound to excel under some specific conditions that enabled them to occupy new niches and proliferate along their woody relatives. Since woody plants dominate in long-term competition in most environments, herbaceous habit must provide an advantage under conditions which prevent this from happening, such as temporary stress or physical disturbance.

There are two basic strategies how to deal with such conditions (temporary stress or disturbance) – tolerance and avoidance. Plants tolerate unfavorable conditions by first preparing their life-processes (e.g. by cold acclimation; Wanner and Junttila, 1999) and then enduring. Alternatively, they can prevent effects of such conditions by avoidance. While this strategy is less important in sessile organisms due to their low mobility, they still can avoid temporary unfavorable conditions as seeds or dormant organs hidden below ground. The latter is a characteristic of temperate herbs of seasonal climates which, unlike woody plants, repeatedly lose their aboveground biomass during their life-time. Avoidance was hypothesized to be the strategy which enabled herbs to dominate over woody plants in stressful conditions which occur only intermittently (Sinnott and Bailey, 1915).

Temporary unfavorable conditions with which herbs are expected to cope better than woody plants are freezing temperatures (Sinnott and Bailey, 1915; Zanne et al., 2014). Since winter freezing is largely predictable, avoidance is a possible strategy how to deal with it - that is exactly herb's strategy in temperate climate during winter season. Global distribution of herbs, which exhibits a strong latitudinal gradient with lower proportion in the Tropics and higher towards the poles, speaks in favor of this hypothesis (Sinnott and Bailey, 1915; Moles et al., 2009). However, when Zanne et al. (2014) performed evolutionary reconstruction of transitions into herbaceous habit and freezing exposed environment, evolution of the habit in a slight majority of cases preceded exposure to freezing temperatures.

Predictable winter frost is not the only adversity that plants experience; plants are regularly exposed to a number of other adverse factors which are typically less predictable than winter frost (e.g. late spring frost events, drought, fire or herbivory). All these conditions have one common denominator – they primarily or exclusively damage aboveground biomass. Therefore, even though they are unpredictable and thus not fully avoidable, herbaceous strategy adapted to repeated loss of aboveground biomass might be beneficial when encountering them.

In this paper, we aim to test this hypothesis. Specifically, we aim to test that herbs deal better than woody plants with unpredictable conditions that damage or destroy aboveground biomass. From the large spectrum of possible factors, we selected three which differ in their predictability. First are freezing temperatures in winter. As fully predictable conditions these are avoidable. Second, we selected late spring frost events, which are less predictable, and finally herbivory which is (in many ecosystems (but see: Lennartsson et al., 1997)) even more unpredictable, and plants usually need to tolerate it in order to be able to grow on the given site. Moreover, absence of herbivory on islands has been proposed as a driver of the reverse evolution of woody plants from herbs (insular woodiness; Dória et al., 2018).

All three factors have been extensively studied experimentally (Burke et al., 1976; Belsky, 1986; Junhu et al., 2013), but they have only scarcely been explicitly compared between herbs and woody plants. What is known about freezing resistance is based mainly on alpine plants where both growth forms were reported not to differ in the ability to deal with low temperatures (with exception of graminoids, which

were more resistant than woody plants; Taschler and Neuner, 2004; Sierra-Almeida et al., 2010; Pescador et al., 2016) or shrubs were marked as more resistant than herbs (Venn et al., 2013). Resistance to herbivory by large herbivores has not been, according to our best knowledge, experimentally compared between these two growth forms. Defoliation is typically studied on part of woody plant and on whole herb, resulting in data that cannot be meaningfully compared between these two growth forms (Obeso, 1993; Haukioja and Koricheva, 2000).

While relationships between such conditions and the herbaceous habit can be studied by phylogenetic reconstructions (see Zanne et al. (2014) for freezing temperatures), such approach needs to be complemented by an experimental study which would identify conditions in which herbs have an advantage over woody plants directly. We focus on young plants as this is the life-stage which face similar challenges for both growth forms and its establishment is often crucial for future trajectory of the community. To compare how resistant are both growth forms to winter freezing, spring freezing and herbivory, we conducted three experiments where we exposed young herbs and woody plants to simulations of these conditions. We measured proxies of their fitness (survival and size) and compared the response of herbaceous and woody plants.

1.3 Material and Methods

To explore response of herbaceous and woody plants to treatments simulating freezing and herbivory we selected pairs of closely related herbaceous and woody central European species and obtained their seeds from seed suppliers (Planta naturalis (http://www.plantanaturalis.com), treeseedonline.com and wildflowerseeds.eu). We stratified the woody species according to the information from the suppliers – most plants were treated with 2 months warm and 2 months cold stratification in soil in seed trays, lesser part with 3 months of cold stratification. Species from Fabaceae family were scarified with hot water and only after successful imbibition were sown with the rest. In spring we replanted germinated seedlings from seeder pots into 3L pots with mix of equal portion of sand and universal gardening substrate (Stender B400 (MC510), containing peat, clay, NPK 14-16-18, trace elements, and with pH 5.5-6.0). Only subset of selected species germinated in sufficient numbers which we divided into treatment and control plants. In 2017 plants were exposed to treatments simulating spring freezing and herbivory. In 2018 we repeated the planting of seeds of both growth forms and in the beginning February 2019 we exposed half of them (nearly one year old plants) to simulated extreme winter freezing, the other half remained as control (for number of plants and species in each treatment see Tab. 1; for list of species see Fig. S1).

Winter freezing was simulated by putting plants in the beginning of February for overnight into freezer. We aimed for simulation of extreme winter event, therefore temperature in the freezer reached down to approx. -20°C. As we expected potential freezing damage even to belowground parts of plants, we isolated the pots by polystyrene from sides to limit the frost penetration only from above to simulate natural conditions. At the beginning of May we measured plants' height, largest dimension of plain view and number of leaves and collected their aboveground biomass, dried it and weighted. Biomass of woody plants was separated in biomass produced in the spring and in the previous year.

Spring freezing was simulated by putting plants at the beginning of June for overnight into freezer. Temperature in the freezer was measured and reached down to approx. -5°C. Plants were measured (their height, largest dimension of plain view and number of leaves) before the treatment. After the treatment we returned plants to garden where we measured them and inspected their survival at the beginning of August.

Herbivory was simulated by removal of all aboveground biomass of plants by scissors at the beginning of August. Plants were measured before the treatment (their height and largest dimension of plain view) and again (with inspection of survival) after two months at the beginning of October.

Analyses

To evaluate effect of simulated unfavorable conditions on herbs and woody plants we used set of hierarchical phylogenetic linear models within Bayesian framework. Since they constitute three distinct experiments with slightly different species lists, we evaluated them separately. Within species we assume that survival of plants and their size is affected by treatment and on interspecific level we expect response of species to be affected by their woodiness (thus evaluating difference between both growth forms) and to be similar for phylogenetically closely related species. We estimated the strength of the phylogenetic signal simultaneously with estimation of other parameters of the model. This way we account for phylogenetic relatedness to the appropriate degree (Freckleton et al., 2002; Revell, 2010).

We modeled survival for each treatment as:

$$Surv_i \sim \text{Bernoulli\_logit}(\alpha_i + \beta_i * Treat)$$

$$\alpha \sim \text{MultivariateNormal}(\gamma_\alpha, \vartheta_\alpha * \lambda_\alpha * phy)$$

$$\beta \sim \text{MultivariateNormal}(\gamma_\beta + \delta_\beta * Woody, \vartheta_\beta * \lambda_\beta * phy)$$

where *Surv*, *Treat* and *Woody* are Boolean variables denoting survival of an individual, whether it was subjected to the particular treatment and woodiness of the species respectively, variable *phy* is phylogenetic variance-covariance matrix, and *i* indicates individual species. α, β, γ, δ, ϑ and are model parameters. Parameter ϑ is constrained to positive values and parameter λ multiplies only off-diagonal elements of the matrix and is constrained to interval [0,1] thus it corresponds to Pagel's lambda (Pagel, 1999).

Apart from survival, we analyzed size of plants which survived. We used model similar to the previous one, only we added information about size of the plants before the treatment.

$$Log(FinalSize)_i \sim \text{Normal}(\alpha_i + \beta_i * Treat + \mu_i * Log(StartSize), \sigma_i)$$

$$\alpha \sim \text{MultivariateNormal}(\gamma_\alpha, \vartheta_\alpha * \lambda_\alpha * phy)$$

$$\beta \sim \text{MultivariateNormal}(\gamma_\beta + \delta_\beta * Woody, \vartheta_\beta * \lambda_\beta * phy)$$

$$\mu \sim \text{MultivariateNormal}(\gamma_\mu, \vartheta_\mu * \lambda_\mu * phy)$$

FinalSize is one of the measures of plant size (height, plain view or number of leaves) at the end of the experiment, *StartSize* is the same measure before the execution of the treatment. This model has two extra parameters in comparison with the survival model, namely μ and σ; for description of other parameters and variables see the survival model. We used natural logarithms of size values since growth is a multiplicative process (in three cases where the size value was zero, we substituted it by half of the minimum of the rest values).

In case of winter freezing treatment, we had no information about plant size before the treatment application. Therefore, in this case the model was without *StartSize* and corresponding parameter μ.

Models were implemented in R (version 3.6.1; R Core Team, 2019) package *rstan* (version 2.19.2 Carpenter et al., 2017; Stan Development Team, 2018) and evaluated using Hamiltonian Monte Carlo with No-U-Turn sampler (Hoffman and Gelman, 2014). We used zero centered priors for slope parameters which slightly shrink posteriors towards zero and thus weaken problem with multiple testing (Gelman and Tuerlinckx, 2000). To facilitate parameter estimation and avoid sampling problems due to Neal's funnel (Neal, 2003) common to centered parameterizations of hierarchical models, we used non-centered reparameterization (Papaspiliopoulos et al., 2007). For slopes parameters we used Cauchy distributed priors with zero mean and scale of five. For variance parameters we used half-Cauchy priors with the same parameters. For other parameters we used uniform priors. We ran 4 chains each with 4000 iterations, half of them as warmup phase. Convergence was checked using *R-hat* statistic (smaller than 1.01 in all cases).

1.4 Results

All three treatments caused considerable mortality in both growth forms. In controls, nearly all plants survived (the highest mortality was in herbivory where 4% of controls did not survive). In treated plants, 10% of plants exposed to simulated winter freezing died, 12.1% in case of simulated spring freezing and 38.8% in case of simulated herbivory treatment (Fig. 1, Tab. S1, S2). In all treatments woody plants had higher mortality than herbs. The difference in mortality between growth forms was small in case of simulated winter freezing, considerable in simulated spring freezing and even larger under the herbivory treatment (Tab. 1).

In simulated winter freezing experiment, all species had low mortality in controls (not more than one individual) and some of them had increased mortality under treatment (mortality above 50% was observed for *Cytisus scoparius (W-woody)* and *Verbena officinalis (H-herbaceous)*). In simulated spring freezing experiment, no species had more than one dead plant in controls, whereas the highest mortality for treated plants was 40% in *Atropa belladonna (H)* and several other species had similar mortality (between 30 and 40%: *Colutea arborescens (W)*, *Hippocrepis emerus (W)*, *Cornus sanguinea (W)*, *Ribes uva-crispa (W)*). In simulated herbivory experiment, controls had no more than 2 dead individuals per species and treated plants had mortality up to 100% in the cases of *Coronilla vaginalis (W)* and *Cytisus scoparius (W)*.

Among plants that survived, treated individuals were generally smaller than control ones (Fig. 2, Tab. S1, S2). This decrease was similar for herbs and woody plants. However, in most of the measures woody plants decreased in size slightly more than herbs due to the treatment. The only strong response was observed in height of plants exposed to simulated herbivory – woody plants in this case decreased more in height than herbs (Tab. 1).

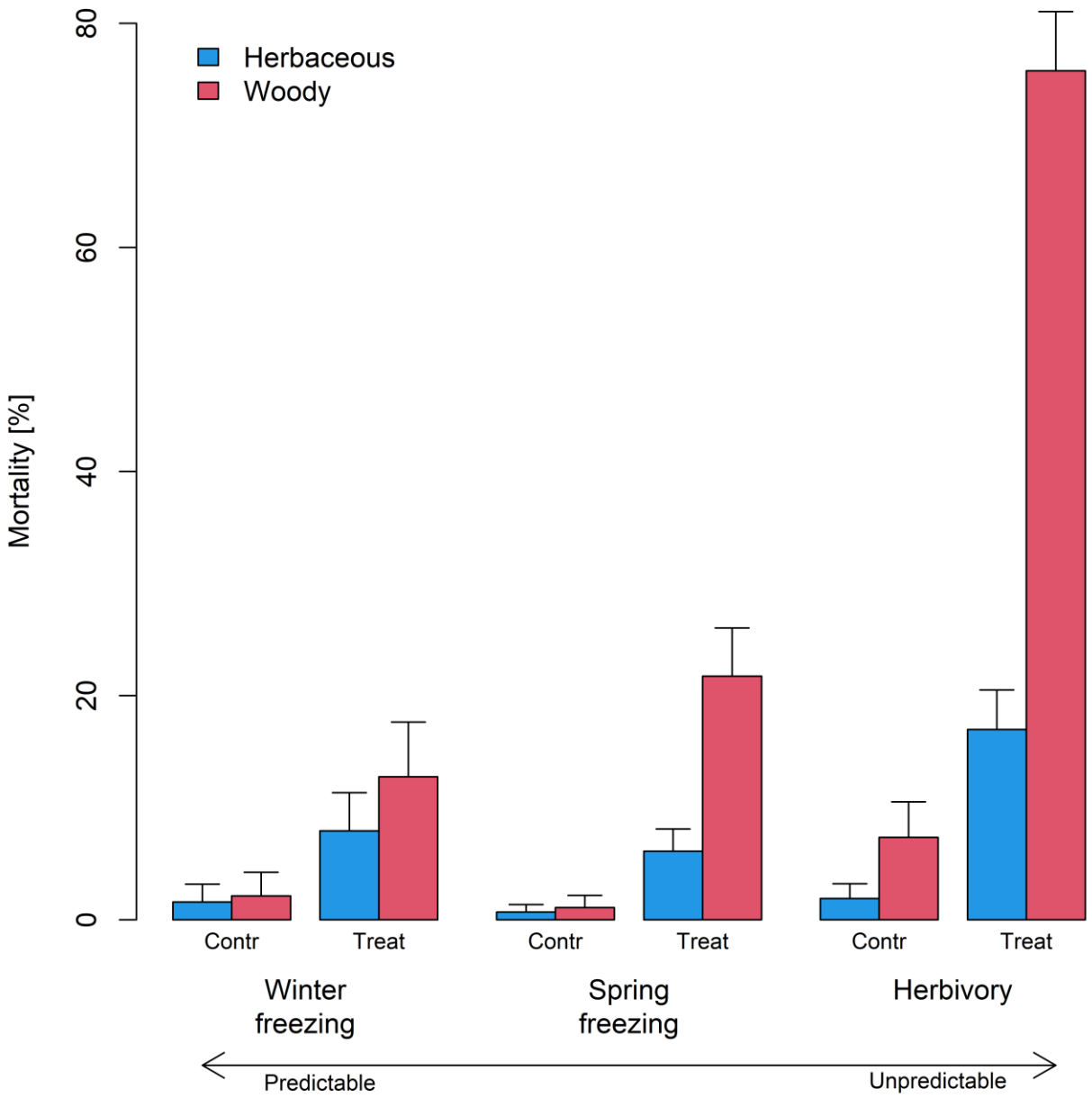

Figure 1: Mortality of herbs and woody plants in control plants and plants exposed to treatments differing in predictability. Treatments are sorted according to predictability from the most predictable - winter freezing to the least predictable - herbivory. „Contr" stands for control plants and „Treat" for plants exposed to a treatment. Error bars are standard errors.

In simulated winter freezing, strongest decrease in size due to the treatment was observed in *Cytisus scoparius (W)* (all measures decreased to 12-31% of size of controls). On the other extreme, *Robinia pseudoacacia (W)* increased in all measures (more than two times in all measures except for number of leaves). In simulated spring freezing, the strongest decrease in majority of measures was in *Securigera varia (H)* (48% plain view of controls and 46% of leaves) and strongest increase due to treatment was in *Saxifraga granulata (H)* (24% increase in plain view and 20% increase in height. Largest increase in number of leaves was in *Lonicera xylosteum (W)* (increase 15%). In simulated herbivory, all treated plants were in

average smaller in plain view and height than controls with exception of increase of plain view by 1% in *Urtica dioica (H)* and by 5% in *Securigera varia (H)*. The decrease was strongest in *Malva sylvestris (H)* in plain view (33% of size of controls) and in *Hippocrepis emerus (W)* in terms of height (15% of size of controls).

Table 1: Differences between response of herbs and woody plants to treatments differing in predictability. Posterior means and 95% credible intervals are reported for the parameter δ_θ which denotes difference between herbs and woody plants; negative values indicate larger decrease of the response variable due to treatment in woody species. Survival of plants as the key response is highlighted in bold.

Treatment	Response variable	Posterior mean	Lower 95% CI	Upper 95% CI	Number of species	Number of plants
Winter freezing						
	Survival	**-0.4974**	**-3.6007**	**2.5083**	**17**	**220**
	Plain view [cm]	-0.3142	-0.6909	0.0549	17	207
	Height [cm]	-0.1859	-0.4794	0.0892	17	207
	Number of leaves	-0.1282	-0.7818	0.4825	17	207
	Total biomass [g]	-0.0462	-0.6813	0.5363	17	207
	New biomass [g]	-0.1562	-0.8921	0.5236	17	207
Spring freezing						
	Survival	**-2.0224**	**-4.0016**	**-0.2339**	**20**	**478**
	Plain view [cm]	0.0000	-0.2298	0.2402	20	408
	Height [cm]	-0.0120	-0.3283	0.2812	20	400
	Number of leaves	0.0153	-0.2897	0.3289	20	408
Herbivory						
	Survival	**-2.9990**	**-5.0126**	**-1.1113**	**17**	**352**
	Plain view [cm]	-0.2341	-0.7803	0.3167	17	275
	Height [cm]	-0.8415	-1.4188	-0.2785	17	275

1.5 Discussion

We exposed woody and herbaceous plants to simulated unfavorable conditions with different predictability and thus avoidable to different degree. Under stressful but predictable conditions (simulated winter freezing) herbs did not have an advantage over woody plants; however, with increasing unpredictability herbs had increasingly higher survival rate than woody plants. Woody plants that survived had also slight tendency to be smaller, relative to controls, than surviving herbs.

Our results suggest that herbs are likely to have an advantage over woody plants in unpredictable conditions which we represented by simulated herbivory. We believe that the ability of herbs to lose and regenerate aboveground biomass is behind this advantage. Woody plants are more limited in their ability to regenerate from belowground organs since their carbohydrate storages and buds are often aboveground (Palacio et al., 2018) and they need to invest more into structural tissues, thus they suffer more by removal of their aboveground biomass in conditions like our simulated herbivory.

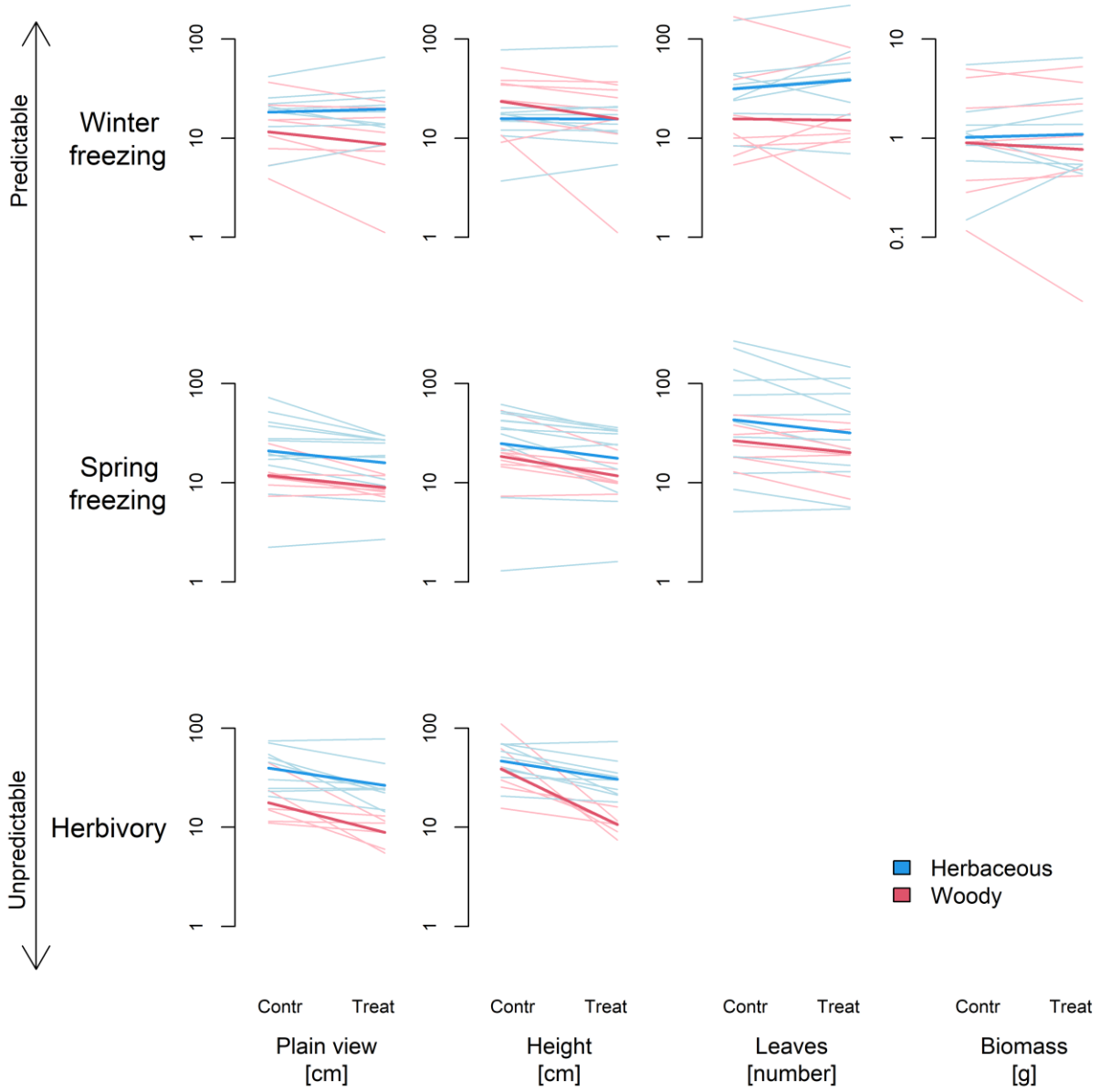

Figure 2: Size of herbs and woody plants in control plants and plants exposed to the experimental treatments differing in predictability. Treatments are sorted according to predictability from the most predictable - winter freezing to the least predictable - herbivory. Light lines connect mean values per species and bold lines connect interspecific means. Biomass and number of leaves are not available for all treatments. „Contr" stands for control plants and „Treat" for plants exposed to a treatment.

This supports our hypothesis that evolution of herbs was driven primarily by unpredictable damage of aboveground plant parts, which herbs can survive (and regenerate from it) much more easily than woody species. Herbivory by large herbivores that are able to consume or destroy whole aboveground biomass is a good candidate for such unpredictable agent. This is also supported by the reversal to woodiness in insular floras (Böhle et al., 1996; Dória et al., 2018) as oceanic islands, where this phenomenon is most prominent, typically lack large mammalian herbivores. Apart from herbivory, plants are exposed to many

other unpredictable disturbances which also remove or damage aboveground biomass. Specifically, fire is the disturbance which calls for future studies in this context as it is known to be an important factor preserving open landscape rich in herbaceous species in many environments (Buechner and Dawkins, 1961; Hoffmann et al., 2003; De L. Dantas et al., 2013).

We would like to point out that the experiment is based on comparison of young plants of both growth forms, and does not address response to disturbance in their older life stages. We see this as an advantage for two reasons. (1) Young plants are an important part of plant's life cycle. In open landscape establishment of young plants after disturbance of any kind is crucial for future state of the biotope leading to different succession trajectories depending whether herbs or woody plants prevail (Pausas and Bond, 2020). (2) Both growth forms are similar in this life-stage facing similar challenges and thus comparable. However, they represent only short part of plant's life and might have different importance for survival of populations of respective species (Chap. 5). This importance is likely to be higher for herbs which depend more on reproduction and survival of seedlings than for woody plants whose lifecycle is dominated by survival of adult individuals (Silvertown et al., 1993).

It remains to be answered which of the potential multitude of disturbance causes, herbivory, fire or other type of unpredictable conditions was the driver of evolution of herbs, and how common it was in individual plant lineages, as well as how these causes contribute to the maintenance or loss of the herbaceous habit in the present. To answer this question, common garden experiments like the one presented might be used to further narrow the range of potential candidate conditions, namely if linked with detailed phylogenetic reconstructions. Such combination of approaches will permit us to see whether herbaceous habit is truly an adaptation to them or whether it arose in response to entirely other factors and it only happened to be beneficial under conditions of unpredictable disturbance.

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1.7 Supplements

Table S1: Response of herbs to treatments differing in predictability. Posterior means and 95% credible intervals are reported for parameter γβ. Survival of plants as the key response is highlighted in bold.

Treatment	Response variable	Posterior mean	Lower 95% CI	Upper 95% CI	Number of species	Number of plants
Winter freezing						
	Survival	**-0.5397**	**-3.0470**	**2.2877**	**17**	**220**
	Plain view [cm]	0.1010	-0.1813	0.3960	17	207
	Height [cm]	-0.0113	-0.2129	0.2095	17	207
	Number of leaves	0.2287	-0.2551	0.7525	17	207
	Total biomass [g]	-0.0061	-0.4861	0.4895	17	207
	New biomass [g]	0.0485	-0.5258	0.6623	17	207
Spring freezing						
	Survival	**-0.7719**	**-2.4270**	**1.1217**	**20**	**478**
	Plain view [cm]	-0.1719	-0.3554	-0.0157	20	408
	Height [cm]	-0.2569	-0.5001	-0.0389	20	400
	Number of leaves	-0.1312	-0.3706	0.0782	20	408
Herbivory						
	Survival	**-1.2719**	**-2.9274**	**0.5303**	**17**	**352**
	Plain view [cm]	-0.3767	-0.8409	-0.0054	17	275
	Height [cm]	-0.4057	-0.8648	-0.0365	17	275

Table S2: Response of woody plants to treatments differing in predictability. Posterior means and 95% credible intervals are reported for parameter $\gamma_\theta + \delta_\theta$. Survival of plants as the key response is highlighted in bold.

Treatment	Response variable	Posterior mean	Lower 95% CI	Upper 95% CI	Number of species	Number of plants
Winter freezing						
	Survival	**-1.0370**	**-3.7381**	**1.8900**	**17**	**220**
	Plain view [cm]	-0.2132	-0.5396	0.1019	17	207
	Height [cm]	-0.1972	-0.4627	0.0520	17	207
	Number of leaves	0.1005	-0.5042	0.6272	17	207
	Total biomass [g]	-0.0523	-0.5931	0.4700	17	207
	New biomass [g]	-0.1077	-0.7839	0.5196	17	207
Spring freezing						
	Survival	**-2.7944**	**-4.6413**	**-1.0596**	**20**	**478**
	Plain view [cm]	-0.1719	-0.3919	0.0387	20	408
	Height [cm]	-0.2689	-0.5710	0.0085	20	400
	Number of leaves	-0.1159	-0.4145	0.1655	20	408
Herbivory						
	Survival	**-4.2710**	**-6.1474**	**-2.3923**	**17**	**352**
	Plain view [cm]	-0.6109	-1.1608	-0.1233	17	275
	Height [cm]	-1.2472	-1.8247	-0.7044	17	275

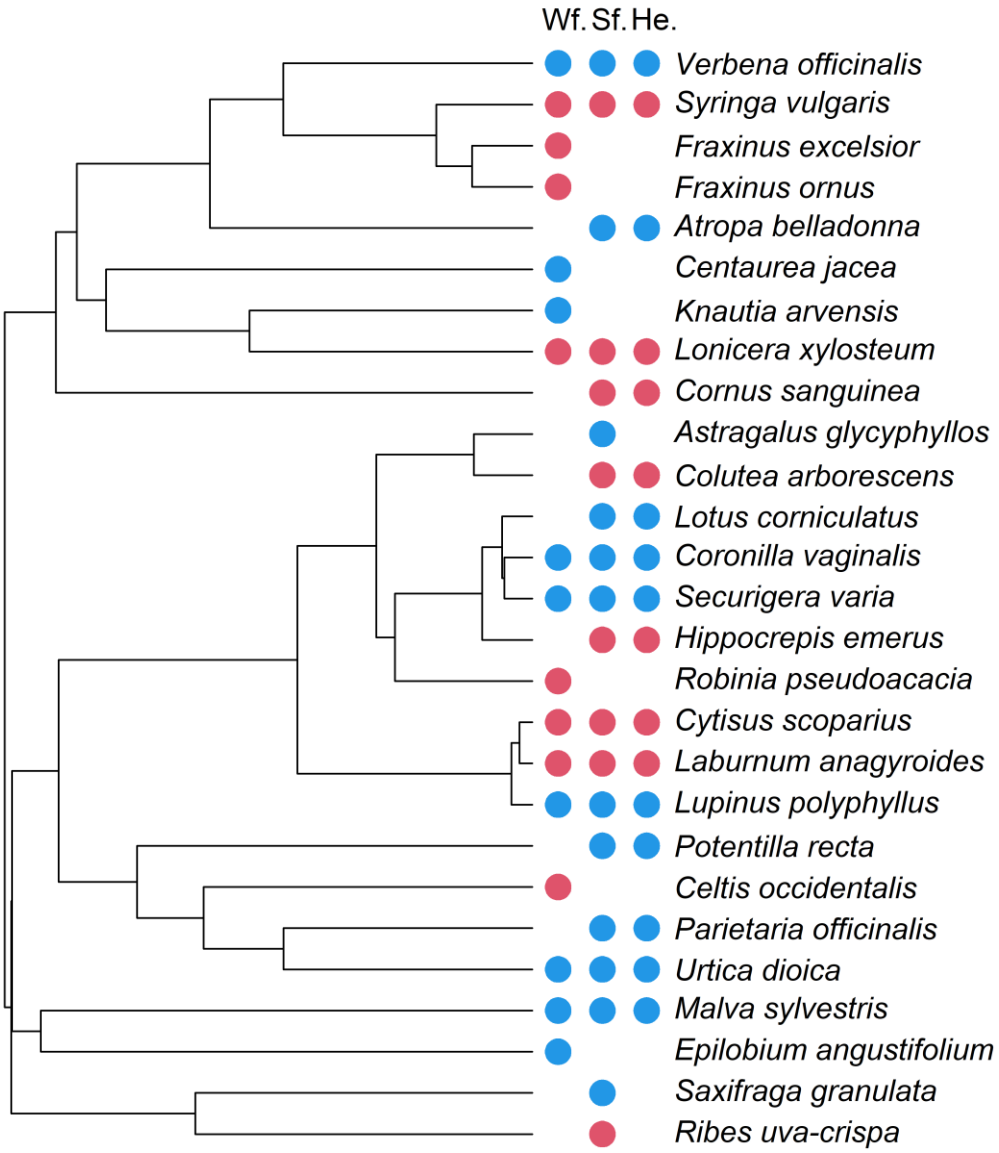

Figure S1: Phylogenetic tree of plants used in experiments. Points mark which species were used in each experiment. Shortcuts were used for the experiments: Wf. = winter freezing, Sf. = spring freezing, He. = herbivory. Red points were used for woody plants, blue points for herbs.

Chapter 2

2. Growth plasticity in response to shading as a potential key to the evolution of herbs

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Submitted manuscript.

2.1 Abstract

In angiosperms herbs evolved from ancestral woody growth form. A number of hypotheses on what was the driver of their evolution have been proposed, but none of them has received clear support so far. We are putting forward a new hypothesis that an important advantage of the herbaceous growth form lies in its greater capacity for plastic response to neighbour shading. Since most herbs form aboveground structures only for one year, they can respond to light heterogeneity more plastically than woody plants with long-living structures which need to pursue long-term goals such as stability and upward growth.

To test the hypothesis, we carried out an experiment comparing plastic response to directional green shading of 21 species of young herbaceous and woody plants. We measured change of their tilt and length and compared it between herbs and woody plants using phylogenetic techniques.

Both herbs and woody plants in our experiment responded to directed green shading by growing away from the shading plastic film. Overall response of herbs was, in agreement with the hypothesis, slightly stronger than response of woody plants, although there was high interspecific variation.

The data indicate that herbs indeed have greater plasticity of stem growth in response to neighbour shading than woody plants, although the overall difference is not very strong. This capacity for plastic response might have played a role as one of the drivers of the evolution of the herbaceous growth form.

Keywords: evolution of herbs; growth plasticity; light heterogeneity; multispecies experiment; shading; tilt

2.2 Introduction

There are two main life strategies in the plant kingdom – herbs and woody plants. Their comparable global abundance in terms of number of species marks them both as very successful (Fitzjohn et al., 2014). Among flowering plants (angiosperms) woody growth form was the first one on the Earth surface (Feild et al., 2004). Herbs evolved repeatedly from it with the first occurrence shortly after the woody ones (Stebbins, 1965; Smith et al., 2010). Although both growth forms differ in many ecological aspects (Díaz et al., 2016; Šímová et al., 2018) and it is likely that success of the herbaceous growth form was due to the whole syndrome of these differences, we still do not know which of them were important, let alone relative importance of each of them. The key difference may stem from the fact that construction of aboveground body in woody plants takes a long time (many years), while in herbs it is a short-term process often repeated many times during the individual's lifetime.

Several hypotheses concerning the ecological drivers of evolution of herbs have been proposed. Stress factors such as freezing temperatures and drought, assuming physiological differences between both growth forms, have been suggested (Sinnott and Bailey, 1915; Zanne et al., 2014), but a global study (Zanne et al., 2014) concluded that freezing temperatures probably were not responsible for the evolution of herbs. Drought was only studied in relation to secondary evolution of woodiness; again, it seems that there are no differences in tolerance to drought between both growth forms (Lens et al., 2016). Some studies even showed that woody plants are more tolerant (Neupane et al., 2017; Dória et al., 2018), which contradicts the hypothesis about drought being the driver of evolution of herbs. Thus, the stress factor hypotheses are based on inconclusive evidence and can hardly explain success of the herbaceous growth form.

Apart from these physiological differences, there are profound morphological and architectural differences between both growth forms which might represent other potential drivers of the evolution of herbs. Strategy of herbs forming, at least in the temperate regions, their aboveground structures for one year and rebuilding them anew each spring strongly contrasts with perennial aboveground biomass of woody plants. This obvious difference might have been responsible for the evolution of herbs in several ways. First, woody plants invest much more to aboveground biomass than herbs (Poorter, Niklas, et al., 2012). Thus, loss of aboveground structures due to various kinds of disturbance is more severe for them than for herbs. It is known that disturbances such as fire, grazing and browsing, and trampling contribute to maintenance of the open landscape in which herbs typically prevail (Bond and Keeley, 2005; Charles-dominique et al., 2016; Dantas et al., 2016), although direct evidence from comparison of herbs and woody plants is scarce (Vesk, 2006).

Second, woody plants, thanks to their perennial aboveground structures, should be more constrained in their response to environmental heterogeneity than herbs. Herbs might show more plastic response to temporal cues than the woody species do, because herbs form aboveground structures for only one year and thus may develop them according to the short-term (i.e. one-year) context of surrounding vegetation. In contrast, woody plants develop stem as a long-term project and thus should be more bound to the long-term goals, hence trading-off the advantage of exploiting short-term heterogeneity, e.g. in light gaps distribution. As a consequence, herbs might be expected to position their stem in a way to optimize the photosynthetic gain in the given year, while woody plants should optimize growth not only for that particular year but also for future years. Further, with often large and long living aboveground structures woody plants also must maintain mechanical stability. If their stem is highly bent in the younger age, they would face danger of fall in higher age which is expected to further limit their plasticity (Alméras and Fournier, 2009). Consequently, herbs should be able to afford to respond more to shading by vegetation in a particular year (via bending their stem) than woody plants. It may even be speculated that since first

angiosperms probably evolved in understory of gymnosperm vegetation light have been limiting factor for them. Newly evolved herbaceous habit among the first angiosperms might represent adaptation to this selective pressure.

A lot is known about response of plants to shading, although herbs and woody plants have as a rule been studied separately. Both growth forms are known to decrease biomass (Ammer, 2003; Macek and Lepš, 2003; Weijschedé et al., 2006), increase SLA (Loach, 1970; van Hees, 1997; Gong et al., 2015) and investment into growing upward (Kasperbauer, 1971; Aphalo and Lehto, 1997; Anten et al., 2009) when shaded. Apart of minimizing the resource itself, shading by vegetation has an important informational aspect for plants of both growth forms as a signal of future competition. Light passing through vegetation can be recognized by plants from other shading because of decreased ratio of red to far red light and plants can adjust their growth in response to it (Novoplansky et al., 1990; Aphalo et al., 1999). Heterogenous light conditions were studied on herbs and might lead to directed growth of an individual (Gruntman et al., 2017) or directed ramet placement in case of clonal plants (Xie et al., 2014).

Despite the striking differences in the later life, when the woody species grow upon the stems accumulated in the previous years, both growth habits have very similar starting conditions in their early life. Yet because of the perennial nature of the stems build by the woody species, directional growth decisions taken at the early stage have long-lasting consequences. Thus, the first-year plants are the optimal phase to study the plasticity, since at the stage, the two guilds differ just by the stem growth expectations.

Difference in response to shading between these two growth forms has never been directly compared. To fill this gap in our knowledge, we conducted an experiment testing the hypothesis that young herbs respond more strongly to shading by vegetation than young woody plants. Specifically, we tested response to green directional shading that mimics shading by vegetation. It involves both resource (photosynthetically active radiation - PAR) and information (red/far red) components of the light signal. We exposed individuals of woody and herbaceous species selected to be phylogenetically as related as possible to experimental shading. The shading was spectral (green) and was directed from south. After 2 months we measured growth of the experimental plants and its direction to assess the responses of their stems and evaluated differences between both growth forms.

2.3 Materials and Methods

We selected pairs of phylogenetically related Central European herbaceous and woody species (for the list of species see Fig. 1; for phylogenetic tree see Fig. S1). All selected species had low or medium shade tolerance (Ellenberg indicator values for light > 3 (Ellenberg et al., 1991)).

Seeds of most of the trees were bought from e-shop treeseedonline.com, except for *Lonicera xylosteum* bought from wildflowerseeds.eu. Seeds of all herbs were bought from seed supplier Planta naturalis (http://www.plantanaturalis.com). Seeds of woody plants were stratified according to information from the suppliers: most plants were treated with 2 months warm and 2 months cold stratification, lesser part only with 3 months of cold stratification. Woody species from Fabaceae family were scarified with hot water and sown directly together with species that needed no stratification. In spring, 11 herbaceous and 10 woody species germinated in sufficient numbers. Individuals of these species were transplanted in 3L pots (each plant in separate pot) with mix 1:1 of sand and universal gardening substrate (Stender B400 (MC510), containing peat, clay, NPK 14-16-18, trace elements, and with pH 5.5-6.0). In beginning of July, 296 plants were positioned into the experimental garden. All plants were shaded from south with 1.2m

high plastic film (positioned approx. 15cm from the pots). In case when young plants were slightly bent (there were no highly bent plants), we rotated them to be bent towards the plastic film. The control set of plants was shaded with transparent plastic film (http://www.leefilters.com/; Clear, no. 130) and the other half of pots (treatment) with a green one (Moss green, no. 089; for number of individuals per species and treatment see Tab. S1). The experiment was carried out in Prague (50°N) in mid-summer 2018, thus in afternoon Sun was shining over the plastic film on plants and they had therefore information where to grow to avoid the shading. Plants were drip irrigated by tap water two times per day to avoid any drought.

At the beginning of the experiment we measured plants' stretched height and number of leaves in case of rosettes. After two months we measured stretched height and position of all apical meristems in 3D space, number of leaves pointing towards and away from the plastic film in case of rosettes and we dried (at 65°C) and weighed their aboveground biomass.

Analyses

Direction of growing of each plant at the end of experiment was evaluated as mean of position of its apical meristems in 3D space weighted by height of its apical meristems (since main apical meristems are higher (than minor ones) and determine direction of growth). While this multivariate information may be summarized in a number of different ways, we used a single measure that in our opinion best expresses their stem response to the directional shading, which we call tilt. The tilt was assessed as follows: First, we computed coordinates of intersection of ray from rooting point towards the course of growing and sphere with radius one. Second, the tilt was horizontal distance of this intersection and rooting point (Fig. S2). In case of rosette plants, which produce stems only when flowering, their aboveground biomass being otherwise formed by leaf rosettes only, "the tilt" was determined as proportion of leaves in the half of pot towards the plastic film. From this proportion we subtracted 0.5 and then multiplied it by 2 to get values in the same range as in case of non-rosette plants. Thus, the tilt variable of zero means growing upward; one means growing directly towards the plastic film and minus one means directly away from the film.

The tilt, computed in this way, represents summary measure of direction of growth of stems. It captures responses to the treatment which we believe are of relevant scale – i.e. not only positioning of leaves in case of non-rosette plants. The tilt measure ignores changes in directions which are not towards or away from the plastic film.

There were large differences in the final size attained by individual species at the end of the experiment. Because higher growth means greater potential response to shading, we used relative growth (RG) as a predictor of variability of the tilt (see below). RG was computed as proportional increase in height of stretched plants at the end of the experiment in relation to the beginning. In case of rosette plants, we used proportional increase of number of leaves instead of height. RG was standardized per species (divided by maximum value per species).

Apart from changes in tilt, plants can adjust growth of their stems to reach the light by lengthening. Woody plants could compensate their lower tilt by this upward growth. Therefore, we also compared changes of RG due to shading in herbs and woody plants (this was done only for non-rosette plants).

The tilt of plants was analysed using hierarchical phylogenetic model in Bayesian framework. We modelled the tilt of plants for each species separately. Estimated coefficients denoting tilt of control plants and shaded plants (difference from controls). At the species level, we then modelled these coefficients and

explored if the second one differs between herbs and woody plants. At this (species) level, we also expect response of plants to be more similar in closely related species. Thus, we accounted for phylogeny. We modelled the tilt (*Y*) for each species as:

$$Y_i \sim Normal(\alpha_i + \beta_i * T, \sigma_i + \gamma * G)$$

where *T* is Boolean variable standing for treatment, *G* is relative growth, i is index of species, γ is parameter of the model and $\alpha_i, \beta_i, \sigma_i$ are species specific parameters of the model. Species specific means of control plants (α_i) and response to treatment (β_i), which is of our primary interest, were modelled as:

$$\alpha \sim MultivariateNormal(\mu, \theta_\alpha * \lambda_\alpha * phy)$$

$$\beta \sim MultivariateNormal\big(\delta + \vartheta * W, \theta_\beta * \lambda_\beta * phy\big)$$

where *phy* is phylogenetic variance-covariance matrix calculated from phylogeny from Zanne et al. (2014), *W* is Boolean variable standing for woodiness and $\mu, \lambda, \delta, \vartheta, \theta$ are parameters of the model. Parameter λ multiplies only off-diagonal elements of the variance-covariance phylogenetic matrix and is constrained to interval [0,1]; thus, it corresponds to Pagel's lambda (Pagel, 1999). Parameter δ denotes mean effect of treatment for herbaceous species and sum of parameters δ and ϑ mean effect of treatment for woody plants.

Wide Cauchy distributed priors were used (with 0 mean and scale 5) for all parameters apart from α, μ, λ where uniform flat priors were used on R and interval [0, 1] for λ respectively.

Model was written in Stan (Carpenter et al., 2017) using *rstan* package (version 2.17.3; Stan Development Team, 2018) in R program (R Core Team, 2018). It was evaluated using Hamiltonian Monte Carlo with No-U-Turn sampler (Hoffman and Gelman, 2014). We ran 4 chains each with 10000 iterations, half of them as warmup phase. In addition, we also fitted analogical models to examine whether rosette species differ in their tilt from non-rosette species and whether inclusion of RG or exclusion of *Lotus corniculatus*, a herb with the far strongest response, influenced our results (Supporting information 1). The same model was used for the comparison of RG.

To assess whether there is a cost of shading in terms of plants fitness, we looked at plant's aboveground biomass (as proxy of fitness) and effect of treatment and woodiness on it. We used difference between treatments in mean biomass for each species as response variable and woodiness as explanatory variable. We ran phylogenetic generalized least squares model with lambda parameter simultaneously estimated using maximum likelihood. We used the same phylogenetic tree as in the previous model (from Zanne et al. (2014)). For this model, we used R package *Caper* (version 1.0.1; Orme et al., 2013).

2.4 Results

Mean tilt of species ranged from -0.106 to 0.187 for controls and from -0.607 to 0.098 for shaded plants (negative values denote growing away from the shading film). Only *Celtis occidentalis* responded to shading by growing more towards the shading film (mean change of the tilt 0.141). However, it was also the most variable species and with long and flexible stem likely to be bent by e.g. wind. Strongest response to shading was observed in *Lotus corniculatus* (mean change of the tilt -0.786; exclusion of this species did not change the results – see Supplements).

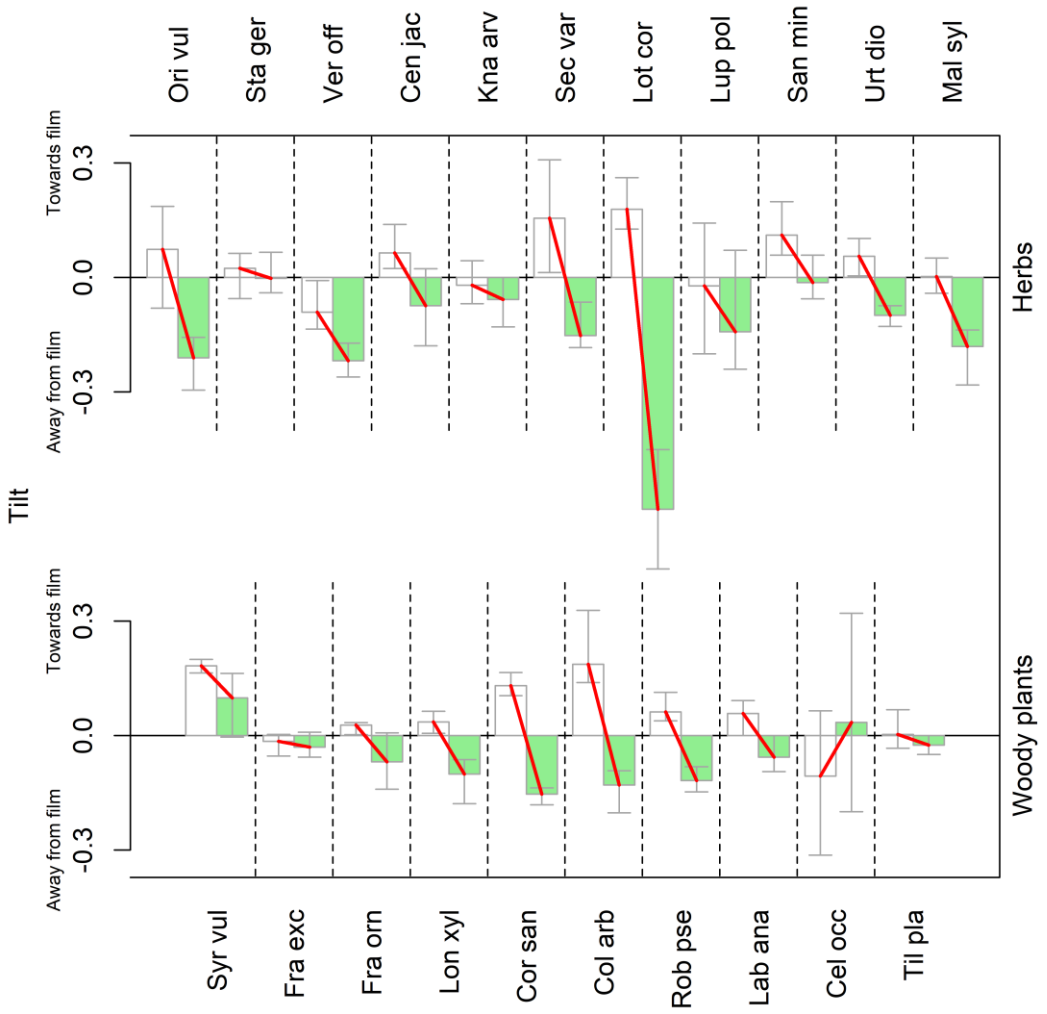

Figure 1: The tilt of all herbaceous and woody species. Left (transparent) bar of each pair corresponds to control plants and right (green) bar to plants exposed to green shading. Height of bars denotes mean and error lines show first and third quartile. Red lines connect medians of control and treatment plants. Positive values of the tilt show growth towards the plastic film, negative away from it. Shortcuts are used instead of full names: Ori vul: Origanum vulgare, Sta ger: Stachys germanica, Ver off: Verbena officinalis, Cen jac: Centaurea jacea, Kna arv: Knautia arvensis, Sec var: Securigera varia Lot cor: Lotus corniculatus, Lup pol: Lupinus polyphyllus, San min: Sanguisorba minor, Urt dio: Urtica dioica, Mal syl: Malva sylvestris, Syr vul: Syringa vulgaris, Fra exc: Fraxinus excelsior, Fra orn: Fraxinus ornus, Lon xyl: Lonicera xylosteum, Cor san: Cornus sanguinea, Col arb: Colutea arborescens, Rob pse: Robinia pseudoacacia, Lab ana: Laburnum anagyroides, Cel occ: Celtis occidentalis, Til pla: Tilia platyphyllos. Species within each growth form are ordered according to the phylogeny.

Species responded to the shading treatment and directed their growth away from the shading film in comparison with control plants. This holds for both growth forms. Response of herbs was stronger than response of woody plants as hypothesized (taking into account phylogenetic relatedness of the species). However, this difference was assessed on the background of fairly large interspecific variation (Fig. 1, 2, Tab. 1). Results were not changed when we distinguished rosettes from other herbs nor by exclusion of the effect of RG on variability of the tilt (see Supplements).

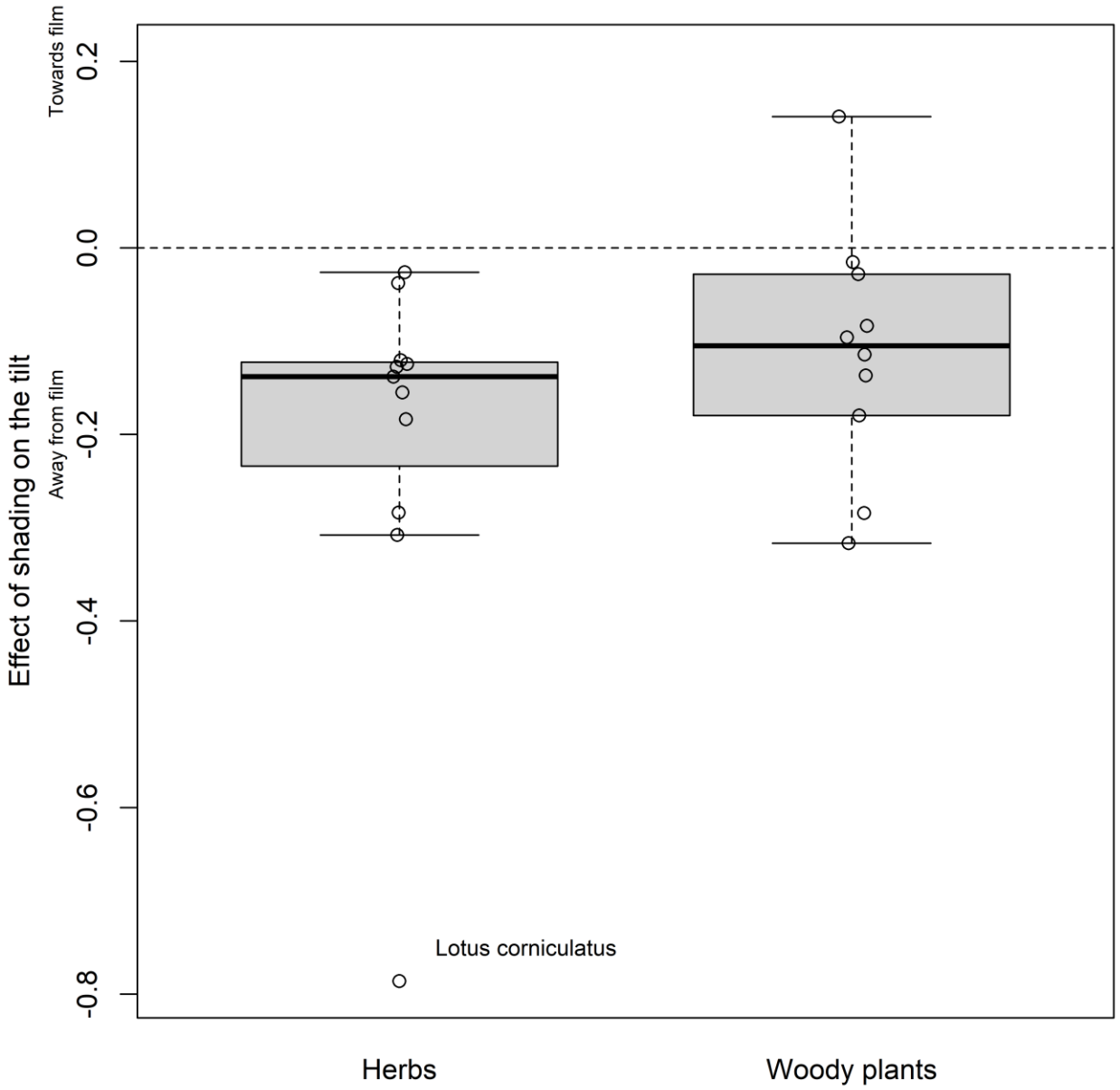

Figure 2: Effect of shading on herbs and woody plants. Effect of shading is the tilt of shaded minus the tilt of control plants (mean values per species). Positive values denote growth towards the plastic film as a response to shading, negative values growth away from the film. Response of Lotus corniculatus is much stronger than response of other species. However, exclusion of it does not change the results (see Supplements). Boxes range from first to third quartile, whiskers up to 3/2 of interquartile range from the box and thick line denotes median.

Relative growth (RG) was higher for both growth forms in shaded plants than in controls. In herbs the effect of shading was considerable (apart from *Origanum vulgare*), in woody plants it was weaker (but still present apart from *Syringa vulgaris* and *Cornus sanguinea*). The difference between strength of the response for herbs and for woody plants was weak (Tab. 1, Fig. S3).

Table 1: Results of model of the tilt and relative growth (RG): Means and 95% credible intervals (in brackets) of posterior distributions of parameters (corresponding variables) of interest.

Parameter	Interpretation	Tilt	RG
δ	*Effect of shading on herbs*	-0.1789 [-0.2805, -0.0852]	0.2029 [0.0435, 0.3698]
$\delta + \vartheta$	*Effect of shading on woody plants*	-0.1113 [-0.2047, -0.0097]	0.1213 [-0.0095, 0.2530]
ϑ	*Difference in effect of shading between woody plants and herbs*	0.0676 [-0.0354, 0.1840]	-0.0816 [-0.2565, 0.0911]

Biomass of plants ranged from 0.035 to 12.098 g with largest plants being *Lotus corniculatus* (mean biomass 6.024 g) and smallest *Tilia platyphyllos* (mean biomass 0.341 g). Biomass of shaded plants did not differ from controls and the effect of treatment on biomass was similar for both growth forms (Tab. 2). There was considerable variability among species with *Stachys germanica* having only 58% (mean) of biomass of controls when shaded and *Robinia pseudoacacia* having 223% (mean).

Table 2: Results of model of biomass: We report estimates, their standard errors and p-values (for null hypotheses of the given parameter equal to zero). Mean values per species were used; response variable was biomass of control plants minus biomass of treatment; predictor was woodiness (i.e. intercept denotes estimate for herbaceous plants).

Parameter	Estimate	Std. Error	P-value
Intercept	-0.2007	0.3344	0.5556
Woodiness	-0.1425	0.4595	0.7598

2.5 Discussion

We examined the hypothesis that herbs show larger plastic response of stem to light heterogeneity than woody plants with an implication that such difference might have contributed as a potential driver to the evolution of the herbaceous growth form. Our results from the experimental shading provide some support for this hypothesis. Herbs responded more to directed green shading than woody plants and grew more strongly away from the shading film. Nevertheless, woody plants also responded, only their response was weaker (Fig. 2). Woody plants did not compensate their small plasticity in tilt by growing more upwards than herbs. Aboveground biomass of both growth forms was not affected by the shading treatment.

Our results represent the first, albeit weak, corroboration of the hypothesis that herbs have larger growth plasticity of stem than woody plants. This finding supports the notion that the requirement of the long-term development of the aboveground shoot in woody plants can represent a constraint for plastic response to shading. Woody plants could respond less to short-term conditions since they pursue long-term goals such as stability and ongoing vertical growth in following years (Alméras and Fournier, 2009). In contrast, the faster turnover of stems allowed herbs to exploit light patches and gaps in vegetation more efficiently by plastic responses to short-term signals. In this respect, herbs might have made an additional step in the direction that was started by the first woody angiosperms and appeared to be advantageous in gymnosperm understory. Woody angiosperms have already less constrained architecture than gymnosperms and therefore larger potential to respond to light heterogeneity, due to weaker apical dominance in angiosperms than in gymnosperms which allows them to position branches

more efficiently into light gaps (Poorter, Lianes, et al., 2012; Pretzsch, 2014). Herbs probably took this trait further, which allowed them to potentially occupy small unused light patches.

However, it should be pointed out that even if herbs respond stronger to directional shading than woody plants (for which this experiment provides some evidence), it does not necessarily mean that this was the driver of their evolution. Primarily, it would be necessary to understand how plants respond in natural conditions where they are not isolated and the light heterogeneity is determined by neighbouring plants with potentially changing positions in time. Design of an experimental approach to get insight into such system under controlled conditions will be challenging, but necessary to support or falsify our expectation that herbs might have proliferated alongside woody plants thanks to their growth plasticity. However, this expectation is indirectly supported by the findings that plasticity in response to light favours coexistence among herbs in grassland communities (Lepik et al., 2005; Lepik and Zobel, 2015).

Another further step is to link mechanical responses to shading (the tilt) with fitness. As both growth forms differ highly in their life-span, fitness evaluation is not straightforward. In the presented experiment, mechanical response was of our primary interest however we also measured aboveground biomass of plants as a proxy of fitness. Based on presented results, no link between mechanical responses and fitness can be established as we did not observe any systematic differences between growth forms in effect of shading on aboveground biomass (Tab. 2).

We would also like to point out that there are important limitations inherent in any experimental comparison of plasticity between woody plants and herbs. First, we had to study response of young plants. However, we are convinced that this is the only meaningful comparison between these two growth forms, as growth plasticity of woody plants is very likely to differ in older woody plants with lignified stems and no meaningful counterpart in herbs. Young plants are likely to represent the key life-stage and their establishment in favourable conditions thus determines future success of individuals, although they represent only a small part of the plant's life cycle, both in herbaceous perennials and in woody plants.

Second, there is an additional limitation due to the fact that many herbaceous plants are clonal and can spread over considerable distances (Klimesova et al., 2017). This enables them to develop new ramets in high-light patches and share resources with shaded ramets (Stuefer et al., 1996). This is true especially for stoloniferous plants (Xie et al., 2014). Therefore, clonal herbs do not need to alter growth direction of their apical meristem to get into the light patches (see Weiser et al., 2016 for a similar effect of clonality on root foraging). Again, this strategy limits experimental approaches for comparison with woody plants as clonality is much less common there and is typically confined to root sprouting which is functionally very different from stem-based clonality (Klimesova et al., 2017; Herben and Klimešová, 2020). Nevertheless, both these effects are likely to affect the results in the same direction, namely reducing the difference between both growth forms and thus suggest even larger difference in plasticity of growth between herbs and woody plants.

The present results imply that our new hypothesis based on the difference in lifespan of their aboveground parts between herbs and woody plants deserves further attention. Larger growth plasticity of herbaceous stem might have enabled herbs to proliferate alongside their woody relatives, both in understory and in open habitats where they had to compete with other non-woody species. Young plants, on which we tested the hypothesis, are the critical life-stage that can be expected to face similar challenges, namely competition for light, in both growth forms. We believe that experimental approaches (as the one presented in this paper) with sets of species are a necessary complement to observational and phylogenetic studies. These approaches can specifically address functional differences which cannot be easily captured by differences in measurable traits which are amenable to phylogenetic studies. In

particular, experimental studies can bring proper understanding of the plastic response to signals and thus may bring further insights on its role for species growth forms, their distribution, habitat requirements and abundance (Abakumova et al., 2016; Dostál et al., 2016).

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2.7 Supplements

Table S1: Number of individuals used in the experiment per species.

Species	Control	Treatment
Celtis occidentalis	8	7
Centaurea jacea	7	8
Colutea arborescens	4	4
Cornus sanguinea	8	7
Fraxinus excelsior	7	8
Fraxinus ornus	7	7
Knautia arvensis	6	8
Laburnum anagyroides	7	8
Lonicera xylosteum	8	7
Lotus corniculatus	7	7
Lupinus polyphyllus	8	7
Malva sylvestris	7	8
Origanum vulgare	7	8
Robinia pseudoacacia	8	7
Sanguisorba minor	8	7
Securigera varia	7	8
Stachys germanica	7	7
Syringa vulgaris	6	8
Tilia platyphyllos	4	4
Urtica dioica	8	7
Verbena officinalis	8	7

Supplementary analyses

Because effect of relative growth (*RG*) on variability of the tilt on level of individuals was very low (mean posterior distribution of parameter γ was 0.0163 with 95% credible interval [0.0006, 0.0467]), we ran also variation of the model without *RG*. Results are very similar (Tab. S2).

Table S2: Comparison of results of the model of tilt with and without relative growth. We report means and 95% credible intervals (in brackets) of posterior distributions. Model with RG is the model described in the Methods section and its results are in Table 1.

Parameter	Interpretation	With RG	Without RG
δ	*Effect of shading on herbs*	-0.1789 [-0.2805, -0.0852]	-0.1775 [-0.2780, -0.0844]
$\delta + \vartheta$	*Effect of shading on woody plants*	-0.1113 [-0.2047, -0.0097]	-0.1126 [-0.2064, -0.0100]
ϑ	*Difference in effect of shading between woody plants and herbs*	0.0676 [-0.0354, 0.1840]	0.0648 [-0.0387, 0.1788]

Because we have two types of herbs – rosettes and non-rosettes – and for each group tilt was calculated differently (see Methods), we explored whether rosettes differ in their response to shading from other herbs. We ran the model with added parameter for rosettes at species level in case of mean of control plants (α) and mean response to the treatment (β). Thus, model equations for this alternative were:

$$\alpha \sim \text{MultivariateNormal}(\mu + \tau * R, \theta_\alpha * \lambda_\alpha * phy)$$

$$\beta \sim \text{MultivariateNormal}(\delta + \vartheta * W + \kappa * R, \theta_\beta * \lambda_\beta * phy)$$

where all parameters and variables are the same as in the main variant described in Methods section apart from *R* which stands Boolean variable denoting that species is rosette plant and new parameters τ and κ.

The tilt of rosette control plants was very similar to other control plants. On the other hand, rosettes responded slightly less to the shading treatment than other herbs. Nevertheless, accounting for rosettes have not changed main results of the experiment (Tab. S3).

Because response of *Lotus corniculatus* was by far the strongest among herbs and thus arouses suspicion that difference between both growth forms is driven by this species, we fitted the model without it. Results are very similar (Tab. S4).

Table S3: Comparison of results of the model of tilt with and without parameters for rosettes. We report means and 95% credible intervals (in brackets) of posterior distributions. Model without parameters for rosettes is the model described in the Methods section and its results are in Table 1.

Parameter	Interpretation	Without parameters for rosettes	With parameters for rosettes
δ	*Effect of shading on herbs*	-0.1789 [-0.2805, -0.0852]	-0.2070 [-0.3229, -0.0883]
$\delta + \vartheta$	*Effect of shading on woody plants*	-0.1113 [-0.2047, -0.0097]	-0.1151 [-0.2069, -0.0166]
ϑ	*Difference in effect of shading between woody plants and herbs*	0.0676 [-0.0354, 0.1840]	0.0919 [-0.0322, 0.2177]
T	*Effect of being a rosette on control plants*	-	-0.0179 [-0.1045, 0.0653]
K	*Difference in effect of shading between rosettes and non-rosettes*	-	0.0677 [-0.1041, 0.2144]

Table S4: Comparison of results of the model with and without Lotus corniculatus (Lot_cor). We report means and 95% credible intervals (in brackets) of posterior distributions. Model with Lotus corniculatus is the model described in the Methods section and its results are in Table 1.

Parameter	Interpretation	With Lot_cor	Without Lot_cor
δ	*Effect of shading on herbs*	-0.1789 [-0.2805, -0.0852]	-0.1553 [-0.2379, -0.0773]
$\delta + \vartheta$	*Effect of shading on woody plants*	-0.1113 [-0.2047, -0.0097]	-0.1199 [-0.1981, -0.0386]
ϑ	*Difference in effect of shading between woody plants and herbs*	0.0676 [-0.0354, 0.1840]	0.0354 [-0.0533, 0.1265]

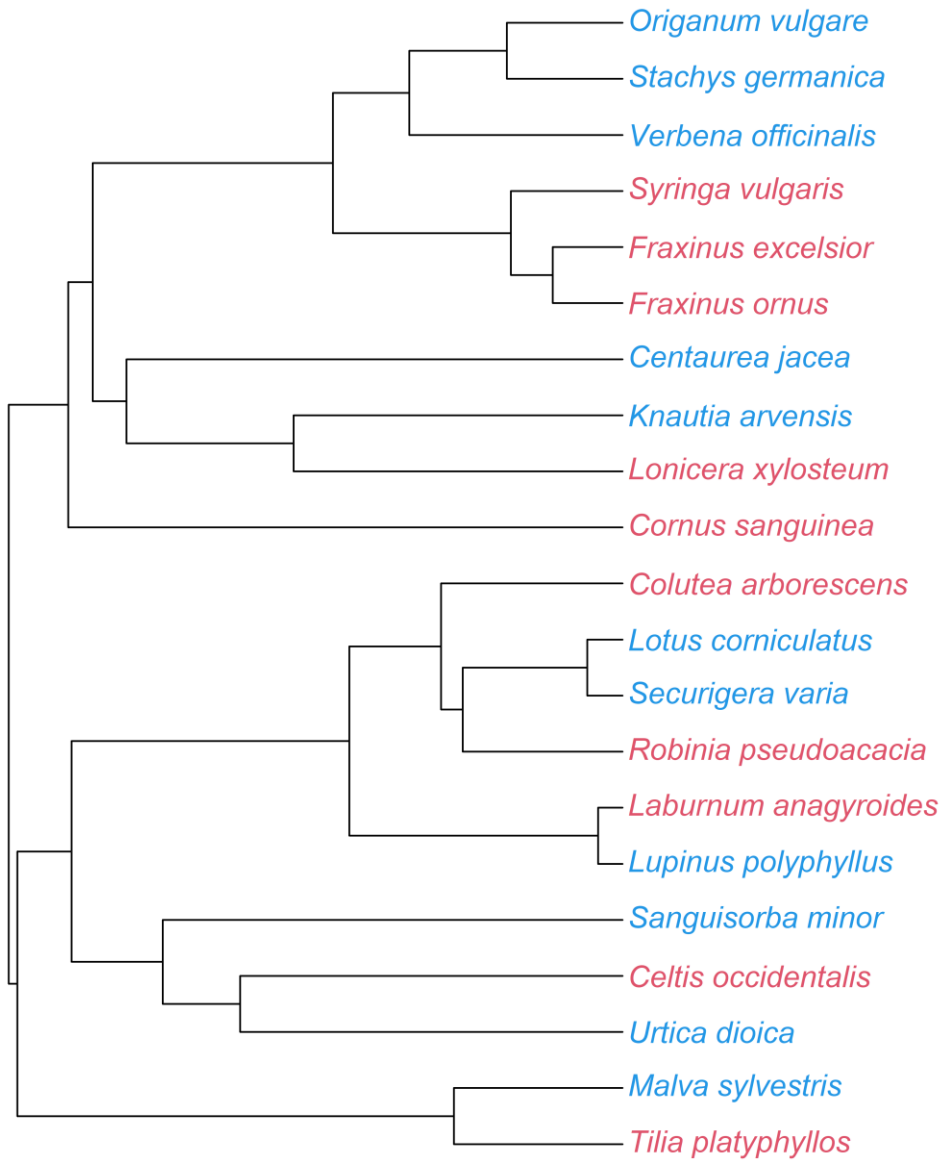


Figure S1: Phylogenetic tree of species used in the experiment. Names of woody species are in red, herbs are in blue.

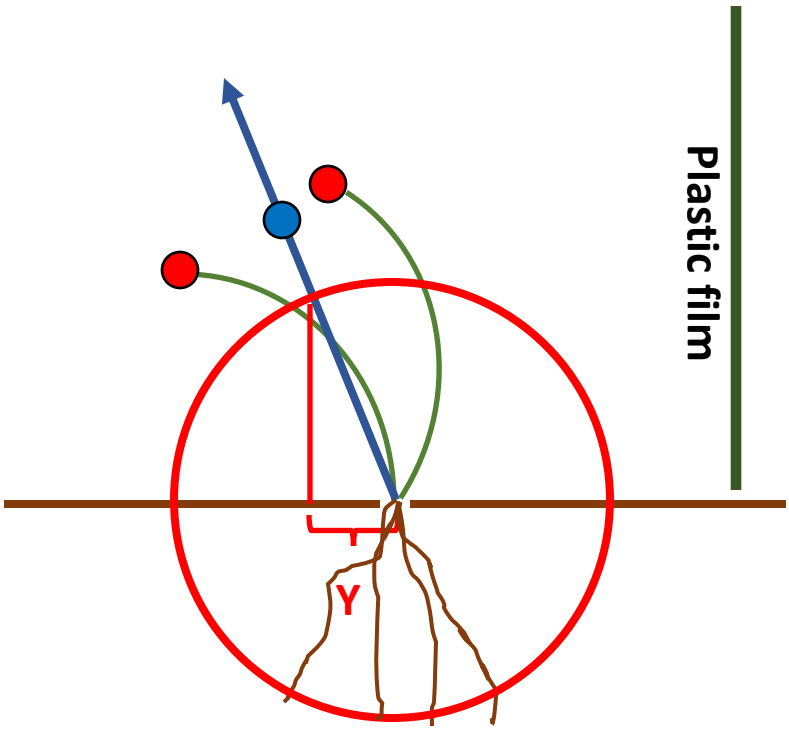


Figure S2: Diagram showing determination of the tilt – sideview of experimental plant. Red points are apical meristems, blue dot is their weighted mean (weighted by their height). Blue arrow is ray from rooting point towards the mean apical meristem. Red circle is unit circle and Y is the tilt.

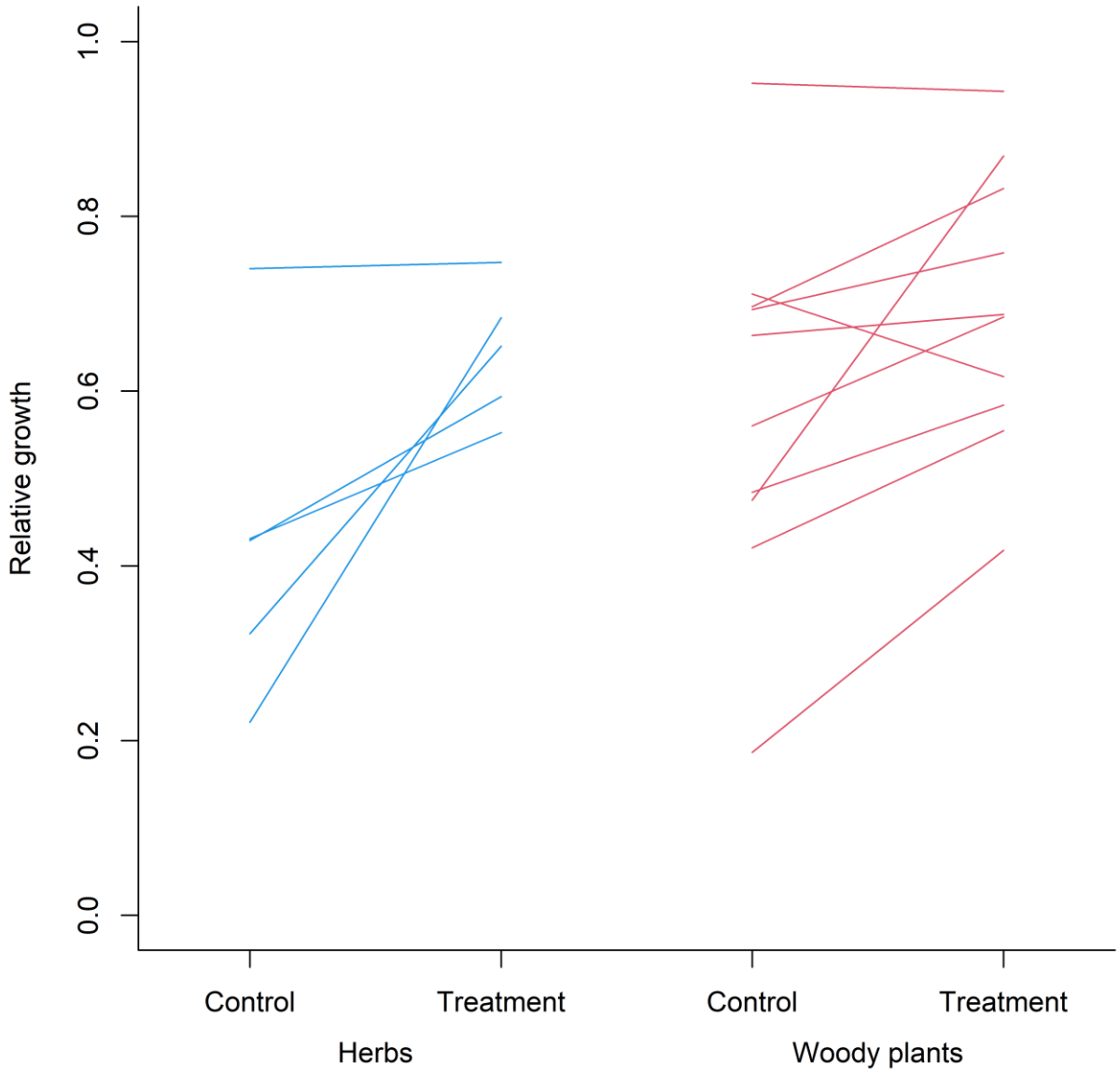
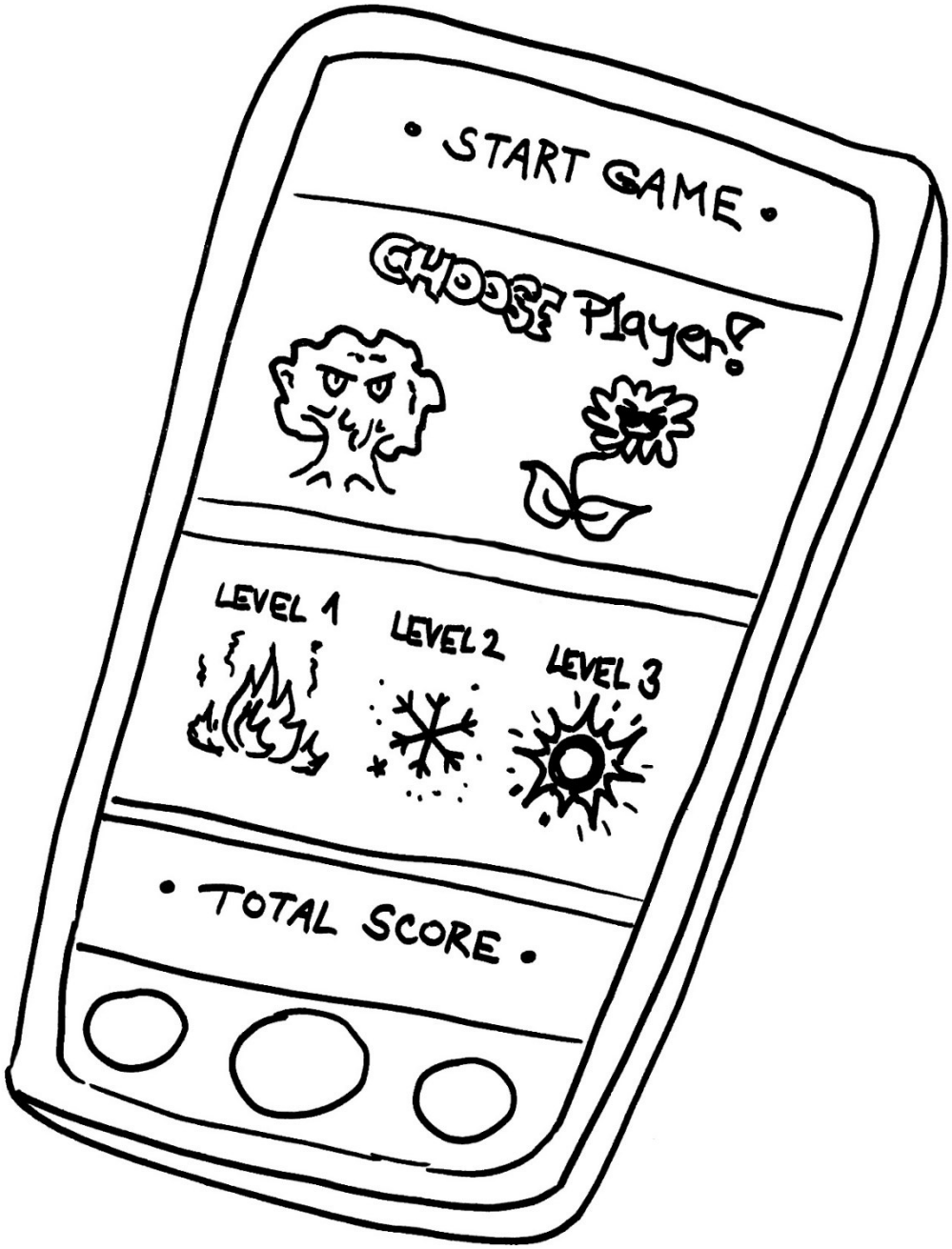


Figure S3: Relative growth (RG) of control and shaded non-rosette herbs and woody plants. Lines connect species means. The herb with similar RG in control and treatment conditions is Origanum vulgare; woody plants with mean negative effect of shading on their RG are Syringa vulgaris (small decrease) and Cornus sanguinea (larger decrease).

Chapter 3



3. Differences between herbs and woody plants as clues about the evolution of herbaceous habit

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AK, TH and IS planned and designed the research, AK assembled the data with the help of IS, AZ and AA, AK analysed the data and wrote the manuscript with contributions of all other authors.

Manuscript in preparation.

3.1 Abstract

Herbaceous habit is the evolutionary younger growth form of angiosperms, which evolved repeatedly from ancestral woody state. Why herbs evolved and which ecological conditions enabled their success is not known. Several hypotheses suggesting biotic and abiotic drivers of the evolution have been proposed but their evaluation was limited and support equivocal.

We evaluated these hypotheses by testing the differences between woody plants and herbs in a set of traits that have been hypothesized to underlie their functional differences. We gathered data from public databases about freezing, drought, fire, shade tolerances, about clonality and specific leaf area. We compared these two growth forms both globally and within selected biomes and clades using phylogenetic comparative analyses.

In agreement with the hypotheses, herbs were more frost and shade tolerant than woody plants and had higher specific leaf area. Both growth forms, however, did not differ in their fire tolerance and clonality. Also, contrary to the expectation, woody plants were more drought tolerant than herbs.

Our results suggest that both biotic and abiotic factors, namely shade and frost, may have played a role in the evolution of herbs. Success of herbs has been likely enabled by their ability to tolerate low temperatures and shade and by their fast life strategy. Nevertheless, across phylogenetic tree, none of the factors had universal effect suggesting different drivers of transitions into herbaceous habit.

Keywords: clonality; drought; fire; freezing; SLA; tolerance

3.2 Introduction

Although flowering plants (angiosperms) are associated, at least for modern search engines, with flowering herbs, half of them are woody (Fitzjohn et al., 2014). Furthermore, their common ancestor was woody (Smith et al., 2010) but the underlying factors responsible for the evolution of herbaceous habit are unknown. Herbaceous species have been appearing throughout evolutionary history independently in many plant lineages and became very successful representing the majority of species in many habitats. As genetical change to herbaceous growth form is probably simple (Groover, 2005), there must have been an ecological driver or drivers which enabled herbs to proliferate alongside their woody relatives. Uncovering this driver would help us to understand the two main growth forms and conditions in which they flourish.

We define an ecological driver of evolution of herbs as a condition under which differences between the two growth forms led to an advantage of herbs over woody plants. Herbs and woody plants differ in many ways (Díaz et al., 2016) ranging from position on r-K spectrum with herbs being the faster strategy and woody plants the slower surviving one (Silvertown et al., 1993) to stem construction which is perennial for woody plants and short-lived or annual for herbs.

The ecological drivers of evolution of herbs could be biotic or abiotic. Abiotic condition is likely to be some kind of disturbance or stress since woody plants usually dominate in long term competition in unstressed and undisturbed conditions; stress or disturbance thus can prevent their dominance. Based on the ability of herbs to repeatedly lose and regrow their aboveground biomass, freezing temperatures have been proposed to be the driver of their evolution (Sinnott and Bailey, 1915). The proportion of herbs to woody plants follows a strong latitudinal trend with more herbs growing in higher latitudes exposed to freezing temperatures (Moles et al., 2009). However, phylogenetic reconstruction showed that in about half of the transitions from ancestral woody to herbaceous habit, evolution of herbaceous habit preceded exposure to freezing temperatures. This suggests that freezing temperature is unlikely to be a universal driver of evolution of herbs (Zanne et al., 2014). Also, experimental evidence shows no advantage of young herbaceous plants over woody ones when exposed to severe winter freezing (Chap. 1).

The ability of herbs to discard their aboveground biomass is likely to be beneficial also under other conditions than is the exposure to freezing temperatures. Fire primarily damages aboveground biomass and is known to maintain open landscape dominated by herbs (Bond et al., 2005). Another potential driver of evolution of herbs are periods of drought that herbs can avoid belowground or in form of seeds (Sinnott, 1916). This was confirmed for *Echium* where evolution of herbaceous growth form have been found to be associated with dry habitats (García-Maroto et al., 2009).

Besides the abiotic drivers, the advantage of herbaceous habit might be in biotic interaction. Herbs actively compete with young woody plants of comparable stature both in understory and in open landscape and results of this competition can strongly influence future appearance of the habitat in which the competition takes place (Pausas and Bond, 2020). Tolerance to shaded conditions and high growth rate of cheap structures are traits which can be expected in herbs to achieve aboveground competitive advantage (Aarssen, 2008). Indeed, relative growth rate of herbs was found to be higher than in trees (linked to differences in lignin formation; Poorter, 1990). Also demography of herbs is mostly dominated by growth in contrast to woody plants for which it is survival (Silvertown et al., 1993).

We explored differences between herbs and woody plants in several traits that have been hypothesized to underlie evolution of herbaceous habit. These hypotheses suggest frost, drought, fire and shade tolerances as drivers of the evolution. We obtained trait data representing species environmental

tolerances from public databases (TRY, BIEN). As proxies of frost tolerance we used (1) frost free days, (2) minimum temperature and (3) freezing exposure within species distributional range. We further included tolerances to (4) drought, (5) fire and (6) shading. To evaluate cost and turnover of leaves (Reich et al., 1992), we compared both growth forms in (7) specific leaf area (SLA). Finally, we compared herbs and woody plants in (8) clonality, a trait that is proposed to be beneficial under disturbances (Bellingham and Sparrow, 2000; Klimešová and Klimeš, 2003).

We compared the difference in trait values between both growth forms while accounting for phylogenetic dependence among species. As global trait data suffer from uneven sampling intensity, we performed the analyses for global dataset as well as for well sampled biomes and clades to explore potential differences and robustness of our results.

3.3 Materials and Methods

To evaluate if woody plants and herbs differ in traits presumably related to the hypotheses of evolution of herbs, we assembled data from large public databases. We gathered plant tolerances to abiotic conditions (tolerance to frost, drought and fire), two traits which are related to biotic interactions (specific leaf area and shade tolerance) and clonality as a trait generally beneficial under disturbances.

Tolerance to frost consisted of three traits: 1) “Frost free days” which is a trait from PLANTS database (USDA NRCS, 2017) defined as the minimum average number of frost-free days within the plant’s known geographical range; 2) “minimum temperature”, again from PLANTS database, defined as the lowest temperature recorded in the plant’s historical range; 3) “freezing exposed” which is a binary trait denoting if species encounters freezing temperatures in its area of distribution – from Zanne et al 2014 (2014). Drought tolerance was compiled from PLANTS database and The Ecological Flora Database (Fitter and Peat, 1994). Fire tolerance was from PLANTS database and shade tolerance was compiled from PLANTS database, Wirth and Lichstein (2009) and The Ecological Flora Database. SLA was taken from BIEN database (Enquist et al., 2009). Clonality as binary trait was compiled from multiple sources (see supplementary methods).

Plant tolerances are often classified into ordered categories (e.g.: low, medium, high); which we substituted by integer values. As these categories might differ among individual datasets, we combined datasets for traits with multiple source datasets (drought and shading tolerances) by using phylogenetic linear models with data on overlapping species (for details see supplementary methods). The model was used to predict values on scale of PLANTS database for species missing in this dataset and present in other datasets. In further analyses, we used not only the combined dataset but also the largest original dataset (which was from PLANTS database in both cases). For clonality, we considered a species to be clonal if at least one source marked it as clonal.

Since annual herbs represent different strategy than perennials, we assembled information about plant lifespan to excluded annual plants from further analyses. Lifespan data were obtained from multiple sources (see supplementary methods) and a species was marked as annual if at least one source marked it as annual (or belonging to category which could contain annual species – e.g. “annual and biennial”). Therefore, our lifespan data was conservative – considering as annuals all annuals and probably also some perennials. Consequently, we ran analyses with and without exclusion of annuals.

Woodiness data were compiled from Engemann et al. (2016), Zanne et al. (2014) and CLO-PLA database (Klimesova et al., 2017). To account for phylogenetic relatedness, we used the largest up-to-date

phylogenies ALLOTB and GBOTB (Smith and Brown, 2018) which combine genetic data from GenBank with phylogenetic data and taxa from Open Tree of Life project. GBOTB phylogenetic tree contains only species for which genetic data were available.

Global trait data suffer from uneven sampling intensity (Kattge et al., 2020a). Stratification of global data into smaller units which would be more evenly sampled and would not differ so much in proportion of herbaceous species would reduce potential biases. Therefore, we stratified trait data according to biomes. Consequently, we used biome as a covariate and also explored separately biomes with highest number of species (temperate mixed broadleaf forest biome – further temperate forest; tropical, subtropical coniferous woodland biome – further tropical woodland). We assigned species to one of the world's biomes (Olson et al., 2001) based on the information of species distribution obtained from Global Biodiversity Information Facility (GBIF) database (see supplementary methods; GBIF, 2017). Exploration of differences within biomes has an advantage of more even geographical completeness of observations but it is on expense of a lower and non-random cover of phylogeny.

Assembled dataset does not make possible combined analysis of all traits due to low overlap of species for individual traits. Therefore, to uncover differences between herbs and woody plants in individual traits, we ran phylogenetic linear model for each trait separately. To explore the difference between herbs and woody plants, we tested for an effect of biome, woodiness and their interaction on each trait.

We ran the default model where we excluded annuals, used ALLOTB phylogenetic tree and used biomes as predictor. Then to explore whether our results are sensitive to these choices, we ran three models which differed from the default model in one of the choices. First model was with GBOTB phylogenetic tree instead of ALLOTB, the second one was without exclusion of annuals and the third one was without biomes.

In all models, all response variables were standardized to 0 mean and standard deviation 1. Strength of the phylogenetic signal (Pagel's lambda (Pagel, 1999)) was estimated to account for phylogenetic relatedness to the appropriate degree (Freckleton et al., 2002; Revell, 2010). For binary traits (clonality and exposure to freezing temperatures) we used the same model since it was shown to be more accurate than phylogenetic logistic regression (as suggested by Ives and Garland, 2014), however this model still exhibited moderately inflated type I error (Klimes unpublished simulations). Phylogenetic analysis of relationship between categorical variables thus represent a problem which had not been yet satisfactory solved (Maddison and Fitzjohn, 2015).

In the large scale phylogenetic models, trait evolution is likely to differ in different branches of the tree and estimation of a single phylogenetic signal might not be appropriate. To check the robustness of our results to the assumption of one phylogenetic signal, we ran the model for clades Fabales and Lamiales for which we had the best trait coverage (for number of species see Tab. S1). In this case, biomes were not included due to low number of observations.

Analyses were done in R (version 4.0.0; R Core Team, 2020) using packages *nlme* (version 3.1-148; Pinheiro et al., 2019) and package *phylolm* (version 2.6.2; Ho and Ané, 2014) for phylogenetic linear models, package *speciesgeocodeR* (Töpel et al., 2017) to assign species to biomes, package *msm* to calculate confidence intervals for visualization based on delta method (version 1.6.8; Oehlert, 1992; Jackson, 2011) and package *BIEN* (version 1.2.4; Maitner, 2017) to access the database of the same name. Trait values apart from traits from BIEN and CLO-PLA databases were accessed via TRY database (Kattge et al., 2020b).

3.4 Results

We explored differences between herbs and woody plants in traits related to hypotheses of evolution of herbaceous habit. For the frost tolerance traits, woody plants had higher number of frost free days and higher minimum temperature within species distributional range and lower proportion of freezing exposed plants, all in agreement with our expectation. Only in case of the minimum temperature, some of the models showed small (non-significant) differences - for species categorized into temperate forest and tropical woodland biomes and also with annual species included herbs and woody plants did not differ in values of this trait (Tab. 1, Fig. 1).

Table 1: Differences between herbs and woody plants in selected traits related to hypotheses of the evolution of herbs. Arrows show the direction of the difference (up: higher values of the trait for woody species than for herbs). Absence of the arrow denotes that the direction of the difference was biome-dependent. The number in the brackets is p-value for the difference being zero. The first three columns represent the tests when biome was included in the analysis as an explanatory variable. The significant difference between woody plants and herbs among biomes is represented by asterisk (*). Significant results that are in agreement with our expectations are highlighted in blue. Significant results that contrast our expectations are highlighted in red. Default model means the model based on ALLOTB phylogenetic tree, without annuals and with biomes as an explanatory variable. Exp. = expectation, GBOTB = phylogenetic tree (Smith and Brown, 2018)*, PLANTS = database* (USDA NRCS, 2017)*, SLA = specific leaf area.*

Trait	Exp.	Default model	GBOTB	With annuals	Without biomes	Temperate forest	Tropical woodland	Fabales	Lamiales
Frost free days	↑	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (0.013)	↑ (0.002)	↑ (<0.001)
Minimum temperature	↑	↑ (<0.001)*	↑ (0.001)*	- (0.102)*	↑ (<0.001)	↑ (0.194)	↑ (0.221)	↑ (0.05)	↑ (<0.001)
Freezing exposed	↓	↓ (<0.001)*	↓ (<0.001)*	↓ (<0.001)*	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)
Drought tolerance	↓	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (0.001)	↑ (0.05)	↑ (0.004)
Drought tolerance (PLANTS)	↓	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)*	↑ (<0.001)	↑ (<0.001)	↑ (<0.001)	↑ (0.018)	↑ (0.022)
Fire tolerance	↓	- (0.402)*	- (0.954)*	- (0.768)*	↓ (0.806)	↑ (0.668)	↓ (0.54)	↓ (0.361)	↑ (0.851)
Shade tolerance	↓	↓ (0.007)	↓ (0.008)	↓ (0.002)	↓ (<0.001)	↓ (0.129)	↓ (0.04)	↓ (0.431)	↓ (<0.001)
Shade tolerance (PLANTS)	↓	- (0.399)*	↓ (0.361)	- (0.308)	↓ (0.003)	↓ (0.91)	↓ (0.491)	↑ (0.37)	↓ (0.003)
SLA	↓	↓ (<0.001)	↓ (<0.001)	↓ (<0.001)*	↓ (<0.001)	↓ (<0.001)	↓ (0.021)	↓ (0.275)	↓ (<0.001)
Clonality	↓	- (0.052)	- (0.034)	- (0.006)*	↑ (0.612)	↑ (0.025)	↑ (0.442)	↓ (0.395)	↑ (0.959)

For the drought tolerance trait, the difference between herbs and woody plants was unequivocal for both combined dataset and subset from PLANTS database. All analyses showed higher tolerance of woody plants to drought stress than of herbs, that contrasts our expectation. Only for Fabales, the tolerance of both growth forms was similar (Tab. 1).

For the fire tolerance, our expectation that herbs would be more tolerant than woody species was not confirmed. Both growth form groups showed similar degree of fire tolerance in all analyses. There was even no consistent sign of the estimated difference between herbs and woody plants (Tab. 1).

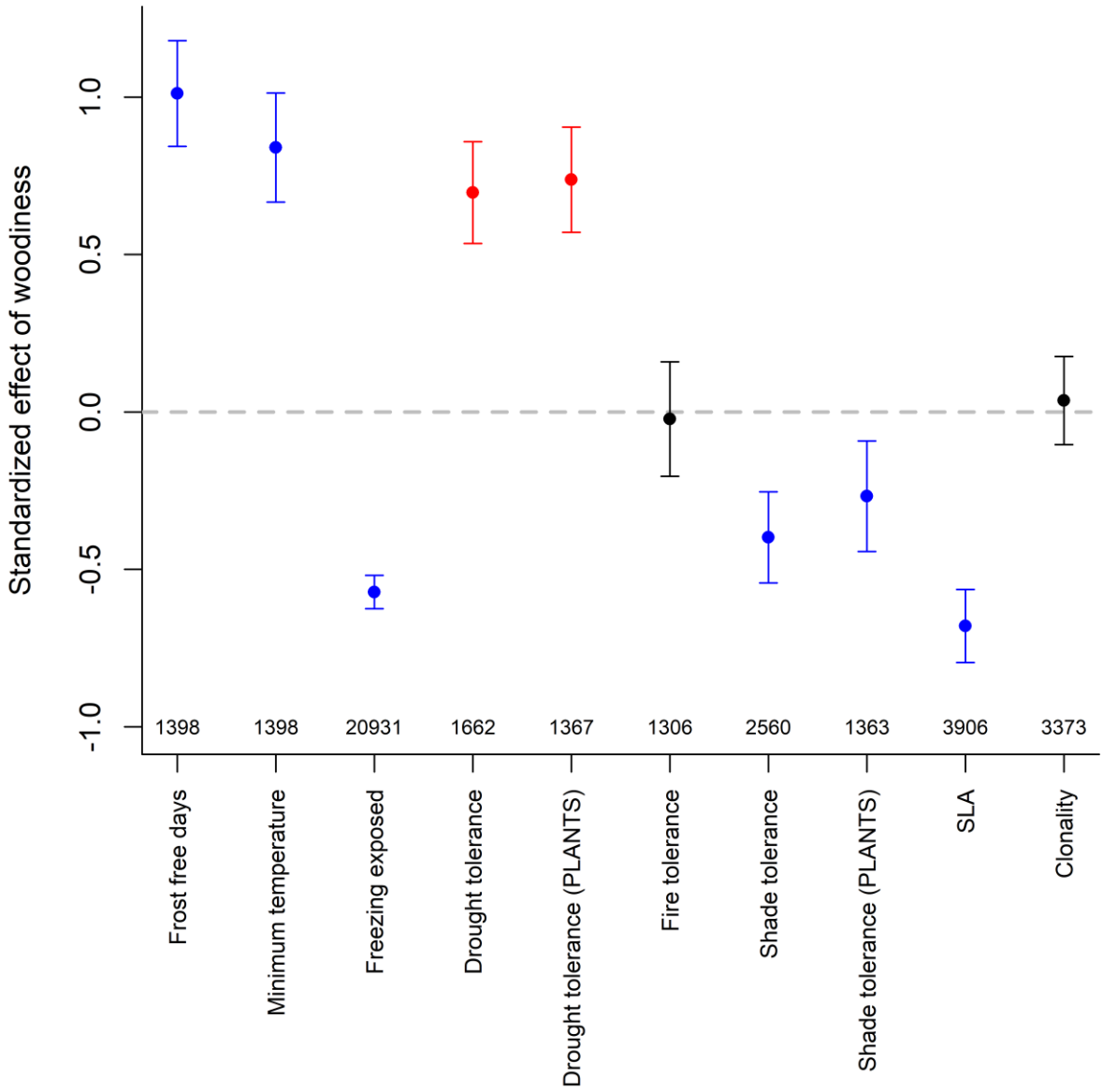

Figure 1: Standardized effects of woodiness for selected traits related to hypotheses of the evolution of herbs. Estimates and 95% confidence intervals are visualized for the model without biomes (without annuals and with ALLOTB phylogenetic tree). Blue colour denotes the difference in agreement with the hypothesis, red colour denotes disagreement and black colour is for no difference between herbs and woody plants. Numbers at the bottom show the number of species used in each analysis. SLA stands for specific leaf area and PLANTS marks traits from PLANTS database.

For the shade tolerance and SLA, our expectation of herbs being more tolerant than woody species was confirmed in most of the analyses. The two exceptions were Fabales where the difference between the

growth forms was not significant and temperate forest biome where our expectation was confirmed for SLA only. Also when analysing the PLANTS data subset, our expectation was confirmed for the model without biomes only and, when focusing on different clades, for Lamiales only (Tab. 1).

For the clonality, the difference between both growth form groups was negligible in all cases, contrasting our expectation of herbs being more clonal than woody species. Moreover, within temperate forest biome woody species were significantly more clonal than herbs, which is opposite of what we expected (Tab. 1).

3.5 Discussion

We proposed several hypotheses explaining the evolution of herbaceous habit. Then we tested whether differences between herbs and woody plants correspond to the expectations based on these hypotheses. Our results of analyses of global trait data show that herbs are more tolerant to frost than woody plants. On the other hand, they were less tolerant to drought and we found no difference in clonality, trait which is proposed to be beneficial under disturbances. Further, herbs were expected to have greater shade tolerance which was confirmed although the difference was not very strong in some groups. Herbs had also higher specific leaf area than woody plants.

Frost tolerance as the driver of evolution of herbaceous habit was rejected by previous study which showed that many plant lineages evolved herbaceous habit before they encountered freezing temperatures (Zanne et al., 2014). Also young plants of both growth forms have been shown to deal similarly well when exposed to freezing temperatures (Chap. 1). Our results show that herbs have consistently higher frost tolerance (based on their distribution) than woody plants. This is unsurprisingly in agreement with global distribution of herbs – their proportion increases towards temperate region and decreases towards tropics (Moles et al., 2009). However, this could be explained by dispersal limitation - high proportion of herbs in temperate region is likely enhanced by stronger exclusion of woody plants in glacial ages. Slower dispersal of woody plants facilitated further radiation of herbaceous plants in temperate regions (Willson et al., 1990; Svenning and Skov, 2007; Murphy et al., 2016).

Drought tolerance have been hypothesized as the driver due its impact on aboveground biomass which is easier renewable in herbs than in woody plants and due to its association with evolution of herbs in genus *Echium* (García-Maroto et al., 2009). However, our results represent strong evidence of woody plants to be more drought tolerant than herbs. Woody plants have longer thicker roots than herbs (Valverde-Barrantes et al., 2020) which enables them to reach belowground water when soil moisture is deficient and herbs ability to avoid short-term drought belowground seems not to balance out this advantage. This is in agreement with the observation of secondary woody species (woody species which evolved from herbs) to be associated with dry localities (Dória et al., 2018) and with vulnerability to drought embolism being negatively correlated with plant woodiness (Dória et al., 2019). Therefore, drought might be more likely a driver of reverse evolution of woody plants from herbaceous habit than of herbs.

Fire was suggested as another disturbance which damages mainly aboveground biomass and thus herbs can be expected to deal better with it than woody plants. However, fire tolerance of both growth forms did neither differ for global dataset nor when subsetting the data for particular clades and biomes. This results suggests that fire is unlikely the driver of evolution of herbs. The problem could be that the fire tolerance trait we used is defined as an ability of plants to resprout or regrow after fire and as such is likely studied mainly on plants encountering fire. Nevertheless, this would probably lead to underrepresentation of woody plants (in case they would be less fire tolerant) in the data which was not the case (Fig. S1). Due to efficient adaptations in resprouting or, in case of woody plants, in bark traits,

both growth forms are able to survive conditions with frequent fire (Keeley et al., 2011). It is also possible that differences among woody and herbaceous plants are more subtle and depends on fire frequency or its intensity (Robertson and Hmielowski, 2014), that was not captured by our data.

We expected greater shade tolerance and cheaper leaves with faster turnover (traits related to SLA; Reich et al., 1992) in herbs than in woody plants as traits beneficial in competition of young plants. Indeed, our results confirmed herbs to be more shade tolerant and to have higher specific leaf area. Notable exception were Fabales were we found none of the differences being significant. Also the difference in shade tolerance was generally weaker than the difference observed in frost tolerance (Fig. 1, S2). Smaller stature and faster growth of herbs in comparison with woody plants might enable them to occupy early successional habitats and in combination with greater shade tolerance to effectively compete with young trees and shrubs in understory.

Clonality was included in our analyses as a trait expected to be beneficial under disturbances (Bellingham and Sparrow, 2000; Klimešová and Klimeš, 2003). Consequently, we hypothesized herbs to be more clonal than woody plants which was not confirmed. Previous studies showed that the relationship between clonality and disturbance is rather complex, however, as clonality is probably not beneficial when the disturbance is severe or when the plants affected by disturbance are young (Herben et al., 2018; Martínková et al., 2020). Furthermore, our dataset contained much higher proportion of clonal species (in some biomes over 80%) than it is expected to be in the nature (Fig. S3; 60% of plants are clonal in central Europe where clonality is well studied; Klimesova et al., 2017). Also clonality in woody plants is studied mainly in relation to resprouting after disturbance (see supplementary methods) and thus does not have to be in all cases active clonality (i.e. some plants marked as clonal do not clonally reproduce when not disturbed). Although the dataset might not fully represent plant clonality, our analyses are based on the best data we currently have (but see: Aarssen, 2008). In most biomes proportion clonal plants is even slightly higher for woody plants than in herbs which is opposite to our expectation. Therefore, we consider our results as an evidence against the proposed difference in clonality.

The study of differences between woody plants and herbs in tolerances is limited by data availability and quality which is of concern in some traits – e.g. clonality or herbivory tolerance which we have not compared for this reason, thus awaits further study before conclusive conclusions can be drawn. On the other hand, other traits (e.g.: frost and shade tolerances) are explored for many species and corresponding hypotheses can be, as we have done, evaluated. Our results outline hypotheses promising for further experimental research which can provide direct evidence of competitive advantage of herbs in given conditions over woody plants.

In conclusion, we found that both growth forms differ in frost, drought and shade tolerance. Unexpectedly, drought is likely a candidate to be the driver of the reverse evolution of woody plants from herbs. Frost and shade tolerances might be drivers of evolution of herbs. Nevertheless, they both have limitations. Freezing hypothesis is limited by the fact that many lineages became herbaceous before encountering freezing temperatures (Zanne et al., 2014). Shade hypothesis is limited by small differences between herbs and woody plants in shade tolerance (Fig. S2). Therefore, frost and shade tolerances might have played a role as drivers of evolution of herbs in part of the transitions towards herbaceous habit but not in all of the cases.

Acknowledgements

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3.7 Supplements

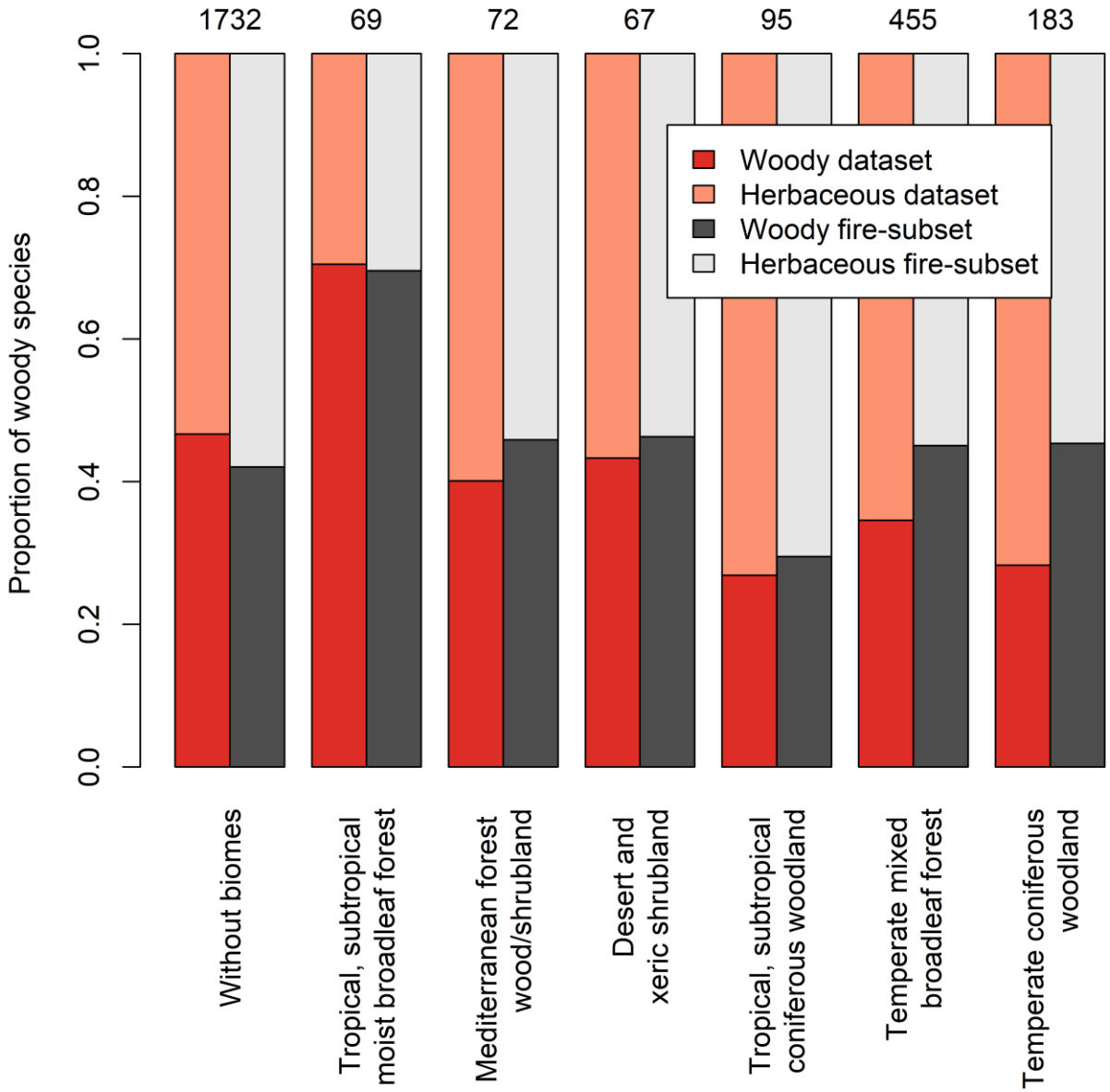

Figure S1: Proportion of woody species in whole dataset and in fire tolerance data. Proportion is visualized for all the data (without accounting for biomes) and for selected biomes. Biomes were selected in the same way as in the analyses – only such biomes where were data about at least 20 woody and 20 herbaceous plants. Numbers on top denote number of species with fire tolerance information (grey columns). Proportion of woody plant is very similar for the dataset and for subset with information about fire tolerance.

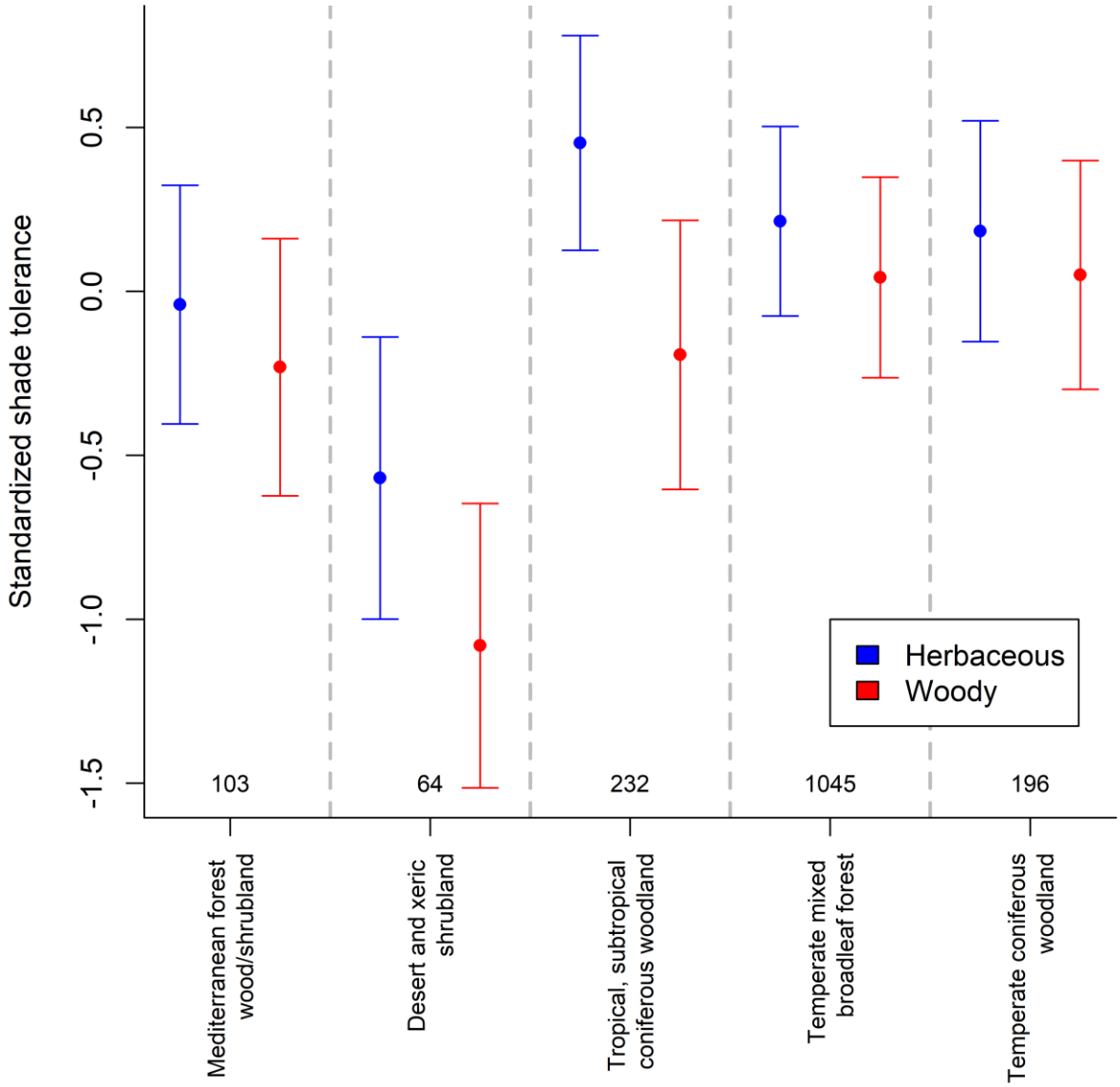

Figure S2: Standardized shade tolerance for herbaceous and woody plants from five biomes. Estimates and approximate 95% confidence intervals (computed using the delta method via package *msm* version 1.6.8; Oehlert, 1992; Jackson, 2011) *are visualized from the default model of shade tolerance. Numbers at the bottom denote number of species used in the analysis for each biome.*

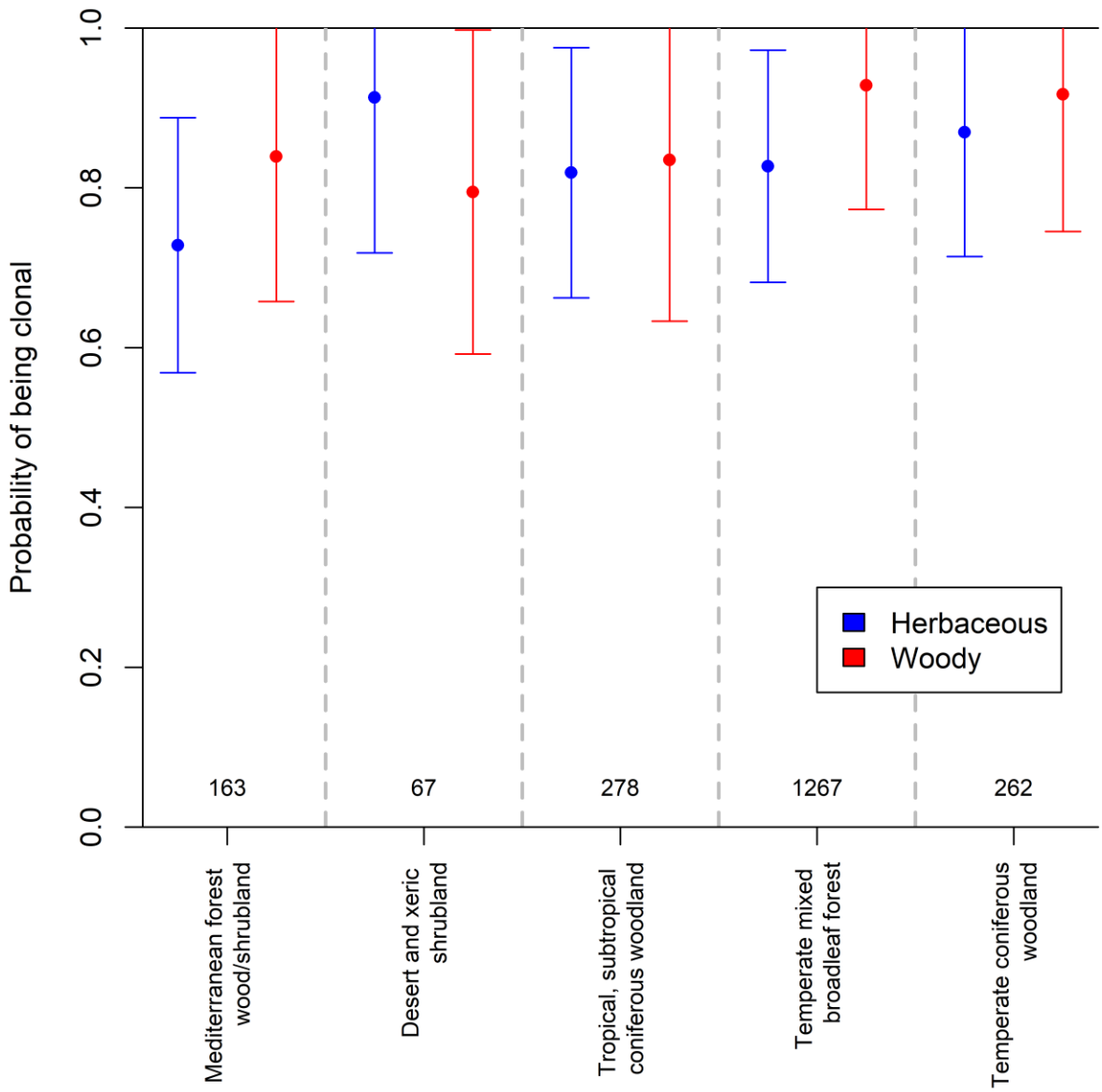

Figure S3: Probability of being clonal for herbaceous and woody plants from five biomes. Estimates and approximate 95% confidence intervals (computed using the delta method via package *msm* version 1.6.8; Oehlert, 1992; Jackson, 2011) *are visualized from the default model of clonality. Numbers at the bottom denote number of species used in the analysis for each biome. The model is not logistic regression (see methods) therefore confidence intervals reach outside of interval [0,1].*

Table S1: Number of species used in each analysis and sources for each trait. Used shortcuts: BIEN – Botanical information and Ecology Network, EFD – The Ecological Flora database (Fitter and Peat 1994), GBIF – Global Biodiversity Information Facility, GBOTB – phylogenetic tree from Smith and Brown 2018, SLA – specific leaf area, PLANTS – database under United States Department of Agriculture.

Trait	Default model	GBOTB	With annuals	Without biomes	Temperate mixed broadleaf forest	Tropical, subtropical coniferous woodland	Fabales	Lamiales	Sources
Frost free days	792	646	938	1398	425	84	103	70	PLANTS
Minimum temperature	793	648	940	1398	426	85	103	70	PLANTS
Freezing exposed	10216	8435	10692	20931	2647	482	1592	970	Zanne et al 2014 (based on GBIF)
Drought tolerance	991	814	1135	1662	571	111	114	78	PLANTS, EFD
Drought tolerance (PLANTS)	789	644	918	1367	422	85	100	67	PLANTS
Fire tolerance	753	612	814	1306	398	82	97	64	PLANTS
Shade tolerance	1640	1472	1891	2560	1045	232	144	157	PLANTS, Wirth and Lichstein 2009, EFD
Shade tolerance (PLANTS)	788	644	922	1363	423	84	97	68	PLANTS
SLA	1667	1530	1834	3906	1032	217	351	213	BIEN
Clonality	2037	1799	2371	3373	1267	278	204	248	TRY (see supplements)

Supplementary Methods - Data preparation

All datasets were processed in the following manner: For categorical trait data, species with equivocal values were excluded and then categories were substituted by integers from the lowest tolerance with value 1 up to the highest tolerance (following approach of Fitter and Peat, 1994). For continuous variables, mean values per species were computed.

Shade tolerance

Shade tolerance information was obtained from PLANTS database (USDA NRCS, 2017), Wirth and Lichstein (2009) and The Ecological Flora Database (Fitter and Peat, 1994) accessed via TRY database (Kattge et al., 2020). For species which were not present in PLANTS dataset, we predicted tolerance values on PLANTS scale using phylogenetic linear model with estimated Pagel's lambda (Pagel, 1999) based on species overlap between the datasets. Results from the dataset containing only PLANTS values (i.e. without predictions) are presented as well. Phylogenetic information was taken from Zanne et al. (2014) and the model was fitted using package nlme (version 3.1-148; Pinheiro et al., 2019) in R (version 4.0.0; R Core Team, 2020).

Drought tolerance

Drought tolerance information was from PLANTS database and The Ecological Flora database. For species present only in The Ecological Flora database, we predicted their values on PLANTS scale as was done in the case of shade tolerance. Again, results using only PLANTS values are presented as well.

Freezing tolerance

Freezing tolerance is represented by three traits: Freezing exposed/unexposed plants from Zanne et al. (2014) based on GBIF occurrences (GBIF, 2017), minimum temperature and number of frost free days in the area of species distribution from PLANTS database. We used each of the traits separately since two PLANTS traits are for nearly identical set of species and the other dataset is much larger with binary values thus not suitable for predictions such as were done for shade and drought tolerances.

Fire tolerance

Fire tolerance values were from PLANTS database.

Clonality

We combined information from CLO-PLA database with TRY database traits "Plant vegetative regeneration capacity", "Plant vegetative reproduction: clonality of ramets" and "Plant resprouting capacity" (only for woody plants). Species marked by at least one source as clonal were considered clonal.

Lifespan

We wanted to exclude annual herbs, so we took lifespan values from TRY database. All categories which can be expected to contain annuals were marked as annuals. Since some of these plants are probably perennials, we ran models with and without them and report both sets of results.

Biome

Each species was assigned to one biome. The assignment was done based on occurrences of the species which we obtained from the Global Biodiversity Information Facility (GBIF, 2017) and biome definition from Olson et al. (2001) using R package *specisegeocodeR* (Töpel et al., 2017). Biome with most unique occurrences for each species was selected. We have downloaded up to 200 000 occurrences per species from GBIF using *rgbif* (Chamberlain et al., 2020) package. Species selection for download was done using "Species matching" tool from www.gbif.org to which we put all angiosperm species from phylogeny of

Zanne et al (2014). From the match only matches which were not classified as “higherrank” were used. We than downloaded occurrences excluding occurrences without coordinates and with “geospatial issue”. Occurrences were rounded and only unique values after rounding were used to exclude pseudo-replication created by repeated observation. Rounding was to 0.001 degree of latitude (approximately corresponding to 110 meters) and 0.001 degree of longitude on equator (110 meters; and corresponding degree on higher latitudes to get 110 meters grid). Corresponding degree on higher latitudes was computed as 0.001 multiplied by inversed proportion of distance (to 110 meters) on nearest whole degree of latitude corresponding to change of 0.001 longitude. Distance was calculated using haversine formula.

Species' names

For species which were not present in the ALLOTB phylogenetic tree (Smith and Brown, 2018), we looked for their synonyms in The Plant List (The Plant List, 2013) using package Taxonstand (version 2.2; Cayuela et al., 2019) and used these if they were not present in the dataset.

Woodiness

We used datasets of Engemann et al. (2016), Zanne et al. (2014) and information from Kubát et al. (2002) accessed via CLO-PLA (Klimesova et al., 2017) as sources of growth form information. Categories *vine*, *non-woody epiphyte* and *herb* from Engemann's dataset were classified on basis of their description as herbs and *liana*, *shrub*, *tree* and *woody epiphyte* as woody species. From Zanne's dataset we excluded species classified as “variable“. We merged first two datasets excluding all species where there was disagreement among datasets. Then we included growth form information from CLO-PLA for missing species.

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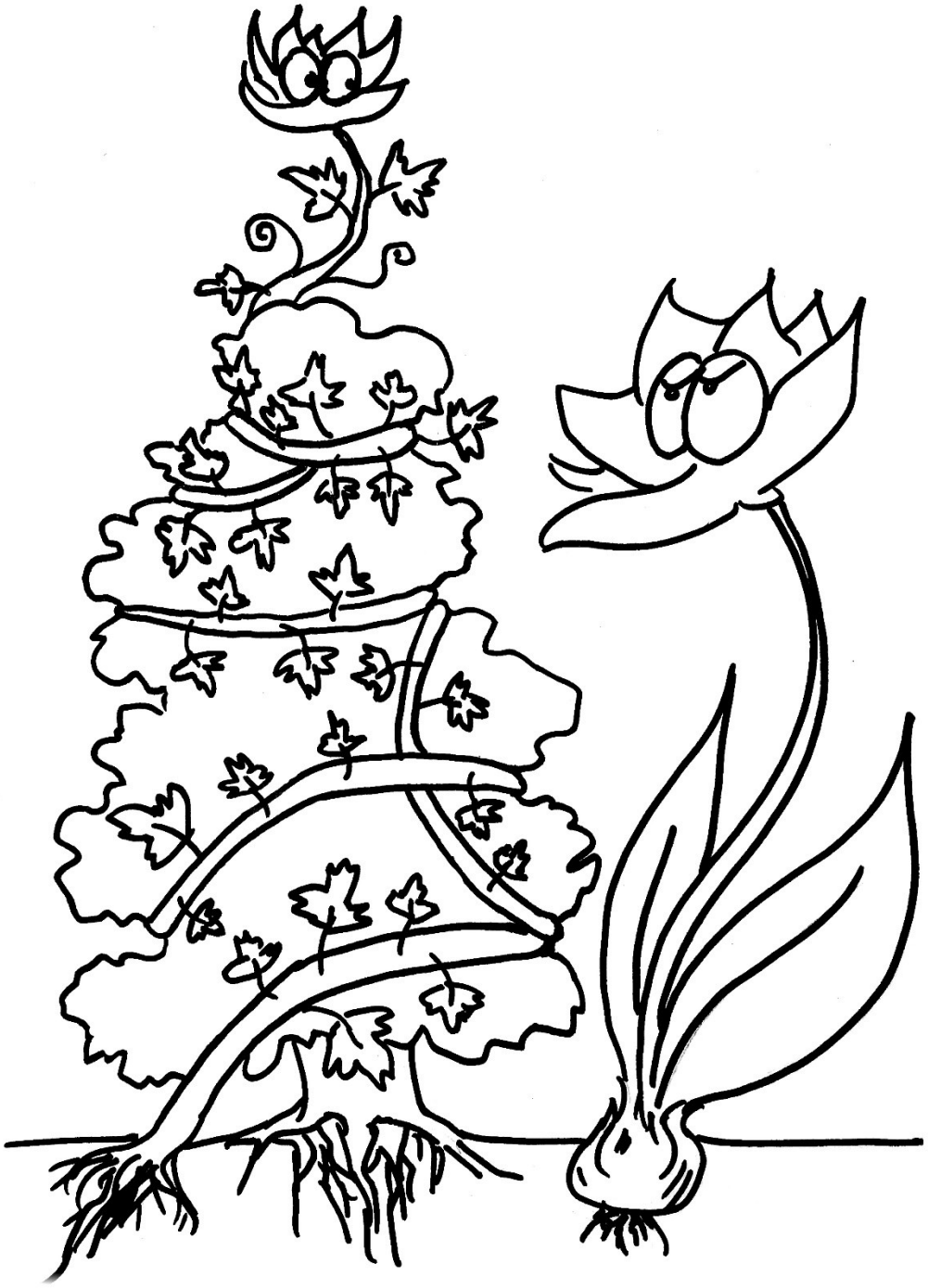
Chapter 4



4. Climbing strategy in herbs does not necessarily lead to lower investments into stem biomass

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JK planned and designed the research. LK did fieldwork with assistance of other authors. AK analysed the data and wrote the manuscript with contributions of all other authors.

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4.1 Abstract

Herbaceous climbers (vines) represent a growth strategy in which the stem lacks most of its supporting function. This has led to the hypothesis that herbaceous climbers are structural parasites that invest less into stems than self-supporting plants. So far, the support for this idea has been ambiguous, as woody and herbaceous plants have been discussed jointly and evidence is often based on young plants in pot experiments.

We collected in wild fully grown temperate herbaceous climbers and self-supporting herbs to examine the idea. We made a phylogenetically informed comparison of biomass allocation into stems and leaves of 16 climber species and 74 self-supporting herbs. Furthermore, we compared our results with those published for woody climbers to gain insight into different biomass allocation between herbaceous and woody growth forms.

We found that herbaceous climbers and self-supporting herbs do not differ in their proportion of stem biomass to leaf biomass. Herbaceous climbers reach much higher in the canopy thanks to their climbing habit and in average more than 7 times longer stems, but contrary to the expectation and unlike their woody counterparts, they do not save on investment into the stem. Herbaceous climbers and self-supporting herbs represent a study system which provides insight into biomass scaling with versus without supporting function where both stems as well as leaves are seasonal.

Keywords: biomass allocation; herbaceous climbers; structural parasitism; supporting function; vines; woody

4.2 Introduction

The biomass of plants is partitioned among different organs of the plant body and this partitioning follows certain rules resulting from the fact that each plant is a functional unit. The plant functions are ensured by conduit connection from roots through stems to leaves, by mechanical safety and longevity of organs (Enquist and Niklas, 2002; Poorter et al., 2015). The ratios of organ biomass following from these rules differ between growth forms and taxonomical groups according to their morphological and anatomical constraints (Poorter et al., 2015). Insight into these differences may provide evidence about relative contribution of individual factors governing biomass allocation. However, despite the availability of large amounts of data, certain growth forms remain underexplored.

One such underexplored growth strategies is climbing strategy represented by woody lianas and herbaceous vines. Climbers rely on the support of other plants and are expected to invest less into the supporting function of stems and more into leaves. The stem of climbers does not need to provide a full supporting function, but it still contains phloem and xylem, which connect leaves and roots and provide transport functions. In the light of this fact, Darwin stated: "[Climbers reach the light] with wonderfully little expenditure of organized matter, in comparison with trees, which have to support a load of heavy branches by a massive trunk" (Darwin, 1882 p. 189). An assessment of how much climbers save on supporting biomass thanks to their habit would provide insight into the stem's support function. Variations of Darwin's hypothesis have been tested but no general consensus has been reached so far (review: Wyka et al., 2013).

Available data on biomass allocation in climbers has some limitations. They are often based on early ontogenetic stages (den Dubbelden and Verburg, 1996; Cai et al., 2007; Toledo-Aceves and Swaine, 2008) and distinction between woody and herbaceous climbers is rarely examined. However, general allocation trends in plants suggest that biomass investments to stems depends on the ontogenetic stage, the size of the plant and whether it is herbaceous or woody (Poorter et al., 2012). Biomass allocation of young climbers might differ from later stages due to allometry (Poorter et al., 2015) and might be influenced by self-supporting growth in young climbers (Cai et al., 2007; Selaya and Anten, 2008). The stem of woody plants is perennial and usually thickens secondarily and accumulates biomass over years, whereas the stem of herbaceous species is annual and is formed anew each year. Therefore, biomass allocation to stems of woody plants can be expected to differ from allocation of herbs. To assess the cost of the supporting function, woody climbers should thus be compared with woody self-supporting plants and herbaceous climbers with self-supporting herbs.

There is considerable evidence regarding Darwin's hypothesis for woody climbers and trees. This evidence is not decisive – some field studies have shown that woody climbers invest less into stems than trees of the same leaf biomass (Ichihashi & Tateno, 2015; Wyka et al., 2013) others report no difference (Kaneko and Homma, 2006; Cai et al., 2007; Selaya and Anten, 2008; Wyka et al., 2019). In the case of herbaceous climbers, experiment of den Dubbelden and Verburg (1996) suggests no difference or even higher stem biomass per leaf biomass for climbers than for self-supporting herbs. However, data about fully-grown wild growing herbaceous climbers from natural communities which are necessary for generalization are missing. To provide the missing evidence, we compared herbaceous climbers with self-supporting herbs. Our data on 16 species of temperate herbaceous climbers and 74 self-supporting herbs (50 grassland herbs and 24 so-called megaherbs to cover the full range of heights attained by herbaceous climbers) allowed us to compare these two growth strategies. Specifically, we addressed the question whether herbaceous climbers allocate less biomass to stems than self-supporting herbs at the same leaf biomass. We also compared our results with those published for woody climbers and trees to gain insight into differences in biomass allocation between the two growth forms. Up until today, most of the studies on

climbers have focused either on allometric relationships within species (e.g. Niklas, 1994; Toledo-Aceves and Swaine, 2008) or compared few species from one genus (Kaneko and Homma, 2006; Cai et al., 2007). Here, we provide an interspecific comparison of climbers and self-supporting species while accounting for their phylogenetic relatedness.

4.3 Materials and Methods

Data collection

We restricted our sampling to the temperate zone, where herbaceous climbers, similarly to other herbs, produce their aboveground biomass each year anew from seed or from belowground perennating organs. The climber growth strategy is rather rare in the temperate zone and is concentrated in a few families, namely the Convolvulaceae and Fabaceae; less often it can be found also in the Asteraceae and Cucurbitaceae. Most climbers are of small stature, occur in herbaceous vegetation and climb on other herbs. The largest representatives, however, occur on forest edges and climb on trees. Some species inhabit anthropogenic habitats and climb on human constructions like poles and fences. The method of climbing varies among taxa; climbers use twining, tendrils or hooks.

We gathered individuals of 16 herbaceous climber species and 50 self-supporting grassland herbs. Climbers were collected in the Czech Republic and in Maryland (USA; see Tab. S1 for localities)), self-supporting grassland herbs were from Bílé Karpaty Mts in the Czech Republic (48°53’21.7”N, 17°38’18.8”E; Tab. S2). For each species we chose several (3-12 in case of climbers and 10 in case of grassland herbs) healthy-looking (without signs of herbivory or pathogens) individuals which we collected late in the vegetation season when their extension growth was already finished and plants were flowering or fruiting (grassland herbs were collected in June; climbers in June-October – see Tab. S1). In climbers, species were selected with aim to cover evenly their size range. As the small herbaceous climbers are much more common than large ones, the sampling of small climbers was random. Concerning large stature climbers, we sampled nearly all available species with help of local floras and experts (e.g. Kubát et al., 2002, Dennis Whigham personal communication). In large stature climbers we aimed for at least three replicates; the acquisition of additional replicates was logistically constrained because fully grown climber specimens were often entangled among trees, shrubs, artificial constructions, or other climbing species. In self-supporting grassland herbs, we sampled semirosette and erosulate shoot architectures and avoided lodging plants as they are not fully self-supporting, and rosette plants as they produce stems that bear no leaves but only flowers. In collected plants we measured stretched length (from rooting point to the furthest apical meristem) and separated stems, leaves and reproductive structures which were dried prior to the determination of dry biomass.

Stem length of our climber species was much greater (species mean 308 cm) than the length of our grassland species (species mean 39 cm). Therefore, to account for possible allometric relationships between stem and leaf biomass dependent on plant length, we added data from temperate herbs which can reach a length comparable to our climber species occurring on the Kamchatka Peninsula and the island of Sakhalin. From Morozov and Belaya (1988) we extracted data on 24 species with a mean length of 247 cm (further megaherbs; information on length available for only 6 species; see Tab. S3 for list of species; for data summary see Tab. S4).

We extracted phylogenetic information from Zanne et al. (2014) to encompass evolutionary relationships between our species. Since some of our species are missing in this dataset, we estimated their position on the phylogenetic tree. For species which were not present in the phylogenetic tree, we randomly

assigned them position of one of their congeneric species present in the dataset. For 19 species, congeneric species represented monophyletic cluster, thus the random assignment did not influence the final phylogenetic tree. This was not the case for 17 species, therefore we generated 100 random phylogenetic trees and ran the analysis with all of them. Results were nearly identical, so we report mean values across 100 model runs.

Analyses

For the purposes of our analyses, we computed the mean of each variable for each species. As temperate herbaceous climbers are restricted to a few higher taxa, which amplifies statistical non-independence of their characteristics (Felsenstein, 1985), we used a phylogenetic least squares model (Freckleton et al., 2002) to model stem biomass (response variable). As explanatory variables we used leaf biomass, growth form (herbaceous climbers/megaherbs/grassland herbs) and their interaction. The lambda parameter scaling the phylogenetic tree (Pagel, 1999) was simultaneously estimated by the model (based on maximum likelihood) to account for appropriate degree of phylogenetic signal (Freckleton et al., 2002; Revell, 2010). We ran the model with all 100 phylogenetic trees. The data were analysed in R (R Core Team, 2018) using the packages 'caper' (Orme et al., 2013) and 'ape' (Paradis et al., 2004). Since growth is a multiplicative process (West et al., 1920), we used (natural) logarithmic transformations of all our variables (stem and leaf biomass) in the model. The logarithm of leaf biomass was centred to a zero mean to facilitate parameter estimation (results are visualized and reported in non-centred form).

Reproductive biomass in some species represents a non-negligible fraction of the total biomass. Since we were interested in the supporting function of the stem, this might have influenced our results. Therefore, we evaluated the model also with reproductive biomass summed with leaf biomass (for herbaceous climbers and self-supporting grassland herbs; we did not have information on the reproductive biomass of megaherbs). We also used the same model structure to test relationship between stretched length (as a response variable) and aboveground biomass to get better insight into stature of growth forms.

For comparison with woody species, we used published data and equations. The relationship between leaf and stem biomass of tropical trees and woody climbers was estimated from equations given by Gerwing and Fabias (2000) and Higuchi et al. (1998) (for details see Supplements). We also included data about temperate climbers and trees from Ichihashi and Tateno (2015). They studied ontogenetic relationships, so their dataset is comprised of small to large individuals. We focus on interspecific relationship among fully grown individuals, therefore we selected largest individuals per species (9 woody climbers and 46 trees) from their dataset (Fig. S1 which shows that our results are not sensitive to the selected size percentile).

We provide only a visual comparison for herbs vs. woody plants. A rigorous test would require a larger dataset of especially woody climbers and a greater overlap of leaf biomass between herbaceous and woody growth forms.

4.4 Results

Herbaceous climbers and self-supporting herbs

Stem biomass was larger in herbaceous climbers than in grassland herbs for mean leaf biomass across our dataset and smaller than in megaherbs. The slopes of the relationships did not differ among the three

growth forms, although the largest value was estimated for herbaceous climbers and the smallest for megaherbs (Fig. 1; Tab. 1).

When we modelled the relationship between stem biomass and the remaining aboveground biomass (reproductive biomass plus leaf biomass), the difference in stem biomass between herbaceous climbers and grassland herbs diminished. The slopes did not change considerably – remaining similar for all three growth forms (Fig. 2; Tab. 1).

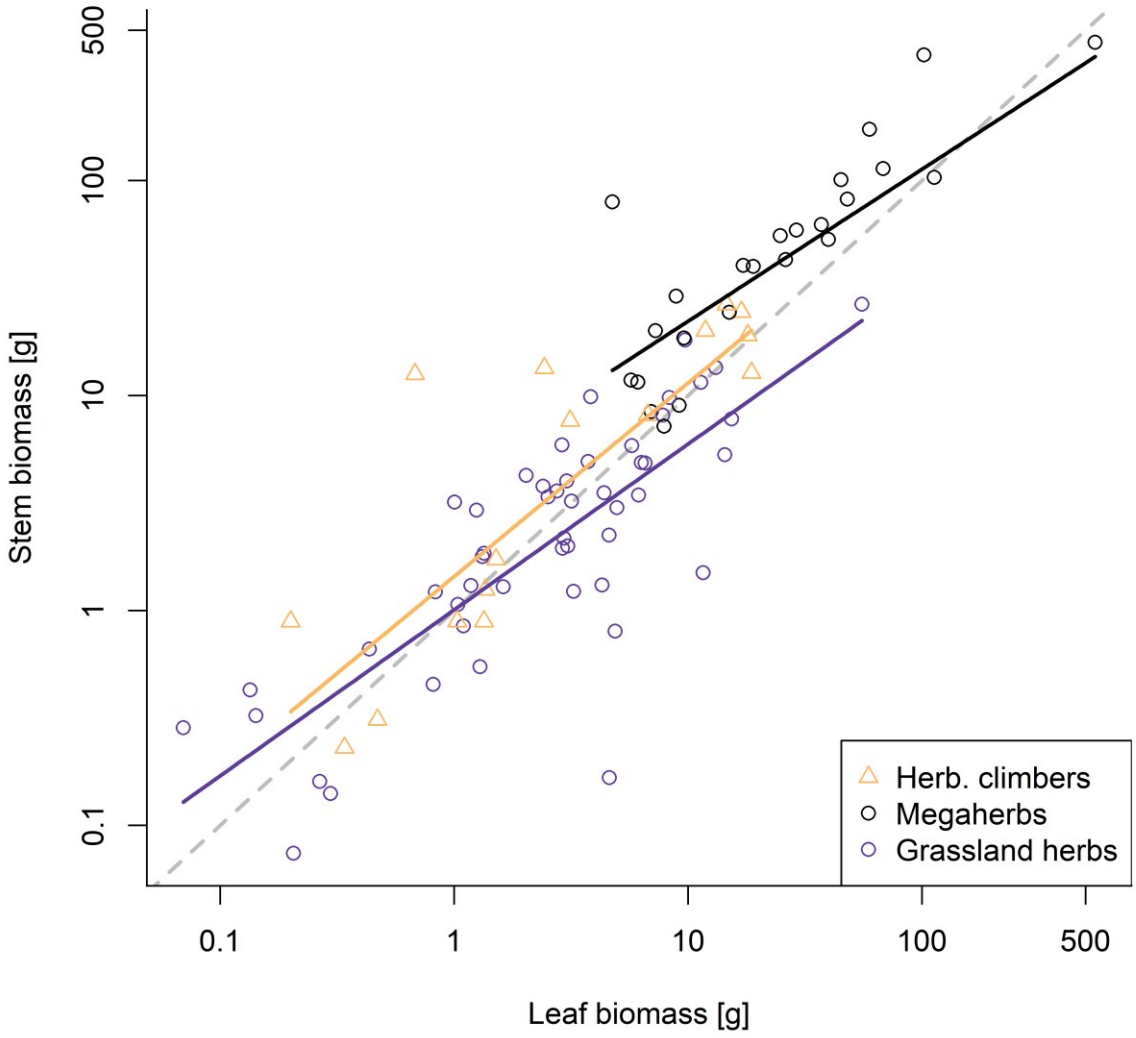

Figure 1: Relationship between leaf biomass (log) and stem biomass (log) of herbaceous climbers and self-supporting herbs. Lines are modelled using a phylogenetic generalized least squares model. The slope of the relationship between the growth forms is not significantly different. The grey dashed line marks slope 1 corresponding to the same amount of leaf and stem biomass.

Table 1: Result of models of stem biomass. Both models have as response variable logarithm of stem biomass. First model predicts it using logarithm of leaf biomass, growth form and its interaction. The second model uses sum of reproductive biomass and leaf biomass instead of only leaf biomass. Herbaceous climbers is the reference growth form (i.e. intercept denotes estimate for herbaceous climbers and estimates for megaherbs and grassland herbs show difference between given growth form and herbaceous climbers). Model was fitted with centred predictors, here are reported recalculated values for original predictors. P-values show test results for particular parameter to be equal to zero on centred scale.

Model without reproductive biomass (Multiple R^2: 0.772)			
	Estimate	Std. Error	p-value
Intercept	0.364	0.426	<0.001
Log(leaf b.)	0.900	0.130	<0.001
Megaherbs	1.106	0.374	0.031
Grassland herbs	-0.358	0.259	0.037
Log(leaf b.):Megaherbs	-0.193	0.198	0.331
Log(leaf b.):G. herbs	-0.129	0.148	0.389

Model with reproductive biomass (Multiple R^2: 0.875)			
	Estimate	Std. Error	p-value
Intercept	-0.268	0.170	<0.001
Log(leaf b.+reprod. b.)	0.979	0.114	<0.001
Megaherbs	1.471	0.276	<0.001
Grassland herbs	0.013	0.201	0.414
Log(leaf b.+re. b.):Megaherbs	-0.152	0.167	0.366
Log(leaf b.+re. b.):G. herbs	-0.102	0.138	0.462

All 100 runs of the model using random phylogenetic trees had nearly identical results with the estimated strength of the phylogenetic signal (Pagel's lambda) ranging from 0.498 to 0.528 for the model without reproductive biomass and 0 for the model with reproductive biomass.

Herbaceous climbers had higher stretched length than grassland herbs of the same aboveground biomass (p-value < 0.001; Fig. 3).

Comparison with woody species

We compared relationships estimated by our model for herbaceous growth forms with published equations for tropical woody climbers and trees. Due to a small number of temperate woody species with available data we only visualized the data in Fig. 2 and did not analyse them. Based on visual comparison: Plants belonging to all herbaceous growth forms had a similar amount of stem and leaf biomass. In contrast, trees and woody climbers had a larger stem biomass than leaf biomass. Among herbs, climbers were usually species with the highest proportion of stem biomass. In case of woody plants, the trend is opposite – climbers were those with a lower proportion of stem biomass (Fig. 2).

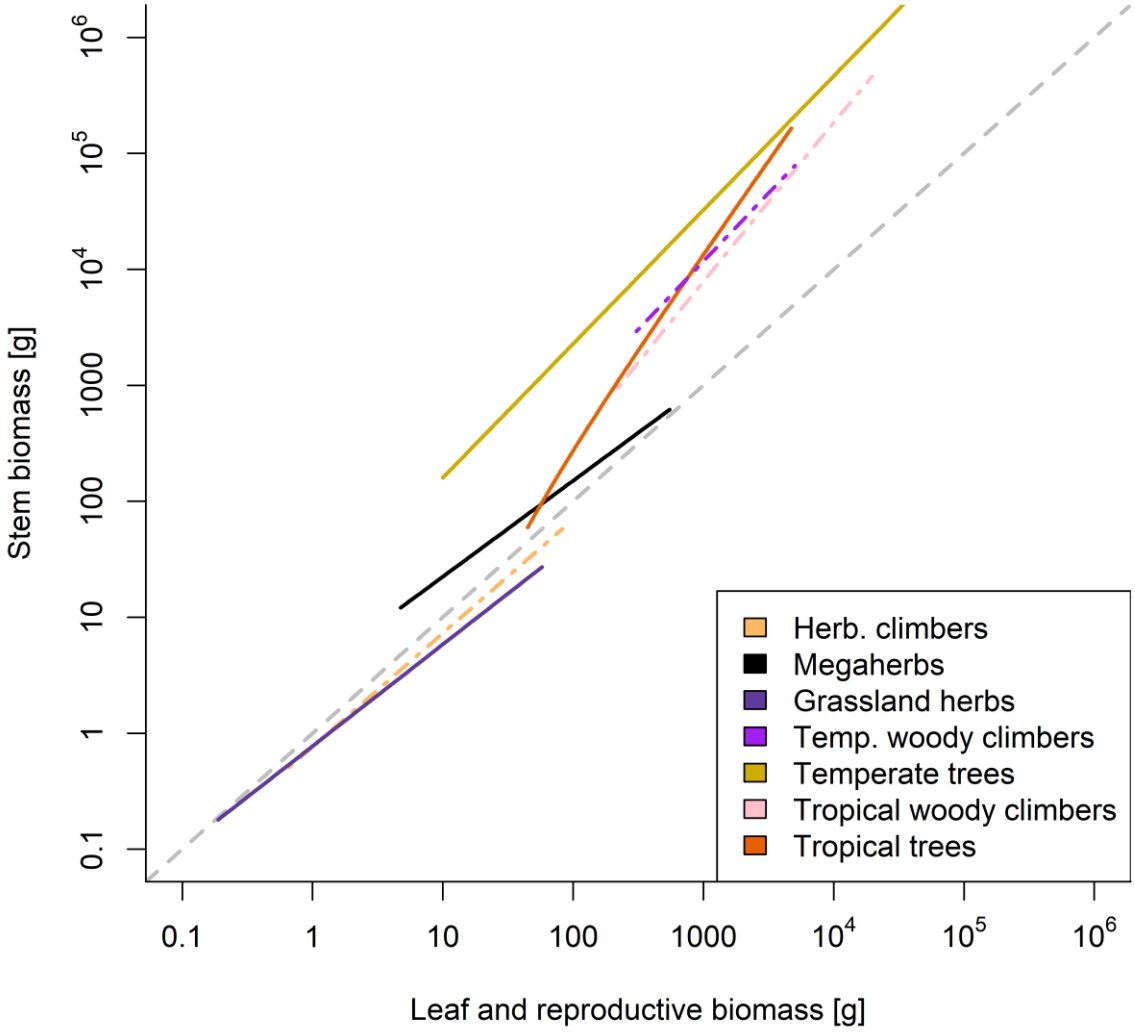

Figure 2: Relationship between stem biomass (log) and remaining aboveground biomass (log) of climbers and self-supporting plants. Lines for herbaceous climbers, megaherbs and grassland herbs are modelled using a phylogenetic generalized least squares model. For megaherbs, data on reproductive biomass is not available, thus only stem biomass is included (identical to Fig. 1). In case of temperate woody plants, we visualized fitted lines from linear regression (for visualized data see Fig. S3). Lines for climbers are dash-and-dotted. The grey dotted line marks slope 1.

The data on temperate woody climbers were in line with the relationship for tropical ones (Fig. 2). Also, temperate tree data do not differ much from relationship for tropical trees, even when proportion of stem biomass may be higher than that of tropical trees (Fig. S3).

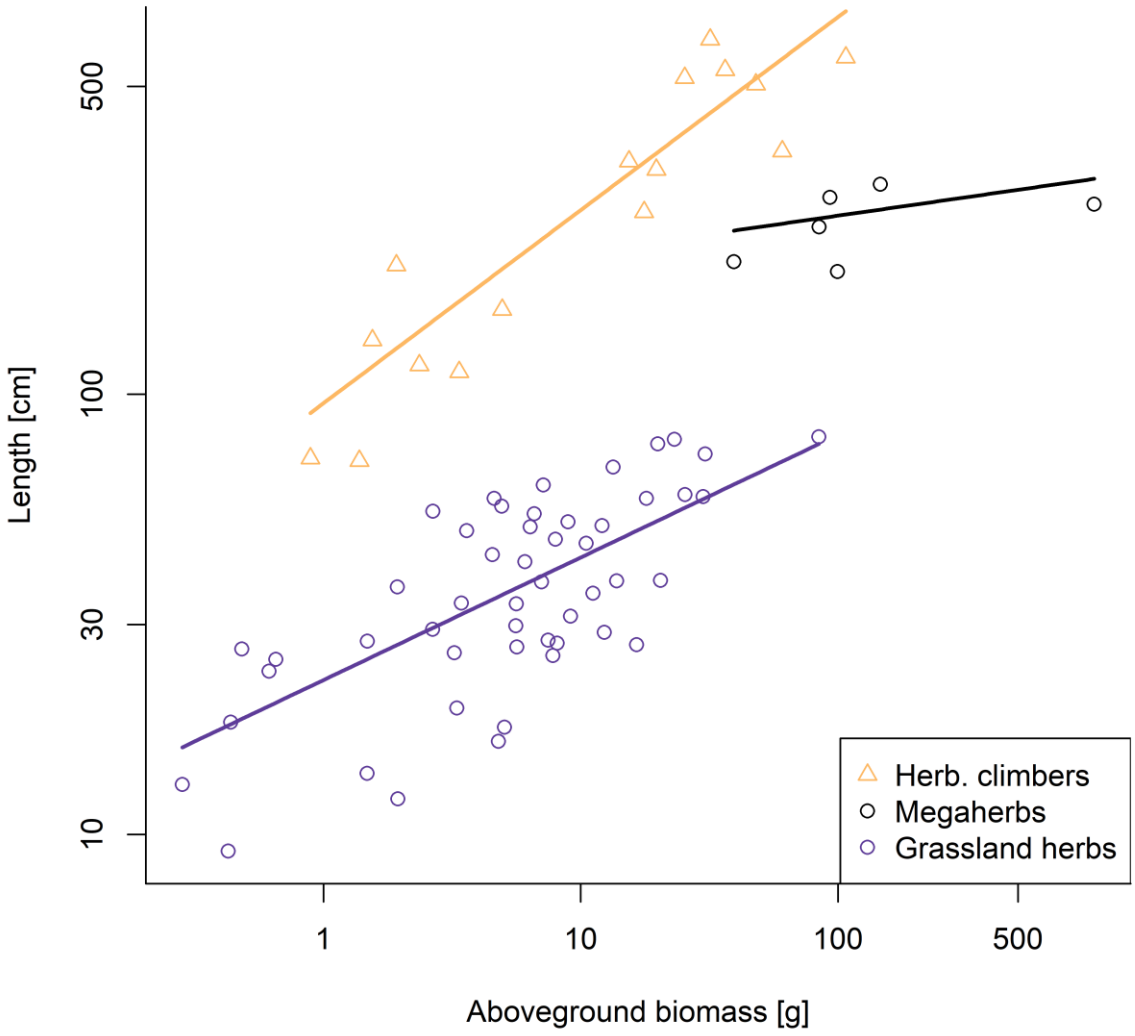

Figure 3: Relationship between aboveground biomass (log) and stretched length (log) of herbaceous climbers and self-supporting herbs. Lines are modelled using phylogenetic generalized least squares model.

4.5 Discussion

In the present study we found that (i) for a given amount of leaf biomass, herbaceous climbers allocated the same amount or more biomass into stems than self-supporting herbs. (ii) When reproductive biomass was added to the leaf biomass, the difference between herbaceous climbers and grassland herbs diminished. (iii) The slope of the relationship between leaf and stem biomass was similar for climbers and self-supporting herbs.

We interpret the high investment of climbers into stem biomass as a result of their strategy. In the early stage of their life, climbers need to reach for support, therefore they strongly invest into vertical growth.

This leads to a higher proportion of stem biomass found in seedling stages in comparison to self-supporting plants (den Dubbelden and Verburg, 1996). Later, herbaceous climbers find their support (den Dubbelden and Oosterbeek, 1995) which is often a tree or shrub at a forest edge and reach their canopy. Therefore, they can reach much higher or wider when they are in grassland than their self-supporting relatives and their stem is much longer (more than 7 times in our dataset; Fig. 3; den Dubbelden and Verburg, 1996; Bitomský et al., 2019). This strong vertical growth leads to higher proportion of stem to leaf biomass for climbers than for self-supporting herbs which we found in our study (Fig. 1).

Plant stems support not only leaves but also reproductive organs, which, in the case of climbers, represent often a large part of aboveground biomass (especially for annuals; Hancock and Pritts, 1987; Ploschuk et al., 2005). The proportion of biomass devoted to reproductive organs was 37.2% for annual climbers in our dataset. This is in range of values reported for annual self-supporting herbs (Bell et al., 1979). When we merge the biomass of reproductive organs with leaves, the proportion of stem biomass in climbers is the same as in self-supporting grassland herbs.

It is costly in terms of investment into supporting function to reach in the same height as climbers. Herbs of similar height (megaherbs from our dataset) have the same or greater amount of stem biomass for given leaf biomass (Fig. 1, 2). This corresponds to general trend of increasing stem biomass proportion with size (Poorter et al., 2012) although within our herbaceous growth forms this trend is not detectable (estimated slope is lower than 1 for all of them but for woody ones it seems to hold; Fig. 1). Unfortunately, we do not have the data about reproductive organs of megaherbs which might represent non-negligible amount of biomass and accounting for it might shift proportion of their stem biomass to be similar to the other two herbaceous growth forms of our dataset.

Our dataset has a modest number of herbaceous climbers. Therefore, we are not able to compare allocation in annuals and perennials and between different climbing techniques (no trends visible in our dataset; Fig. S2). Another important limitation of our dataset is the absence of root biomass which limits comparability with experimental data (e.g. den Dubbelden and Verburg, 1996). Root biomass is usually not obtainable in the field (but see Kaneko and Homma, 2006). It has been shown to differ between woody climbers and self-supporting shrubs (review Wyka et al., 2013, 2019) however available evidence in herbs points to no difference in controlled conditions between self-supporting ones and climbers (den Dubbelden and Verburg, 1996).

Unlike for woody plants where field evidence in most cases supported Darwin's hypothesis (Wyka et al., 2013; Ichihashi and Tateno, 2015), in case of herbaceous plants we reported no difference between self-supporting herbs and climbers in stem biomass for the same amount of leaf biomass. Also, unlike the woody plants (Cai et al., 2007; Wyka et al., 2019), these results are supported by available, nevertheless modest, experimental evidence (den Dubbelden and Verburg, 1996). The climbing strategy for herbs does not represent savings in total investment into stem but enables them to reach high in the canopy.

4.6 Conclusion

We compared biomass allocation between temperate herbaceous climbers and self-supporting herbs and showed differences from woody climbers and trees. We cannot confirm Darwin's hypothesis for herbaceous plants since their investment into stem biomass is not (for the same leaf biomass) lower than that of self-supporting herbs. Thus, herbaceous climbers do generally not save on investment into the stem thanks to their climbing strategy. On the other hand, herbaceous climbers have much longer stems and are able to reach much higher and wider in a canopy (climbing on trees) than self-supporting herbs

and this is in contrast with woody climbers that cannot grow higher than their woody self-supporting counterparts (trees) along which they are climbing. Thus, from the perspective of biomass allocation into plant organs, herbaceous climbers are similar to grassland herbs only in that their stem is in many cases much longer which enables them to climb woody plants or wide areas of upper layers of grasslands. The fact that they occupy this special niche makes some of them troublesome weeds and invaders (Hough-Goldstein et al., 2012; Grašič et al., 2019) but their occurrence, contrary to woody climbers, is not dependent on disturbance (Bitomský et al., 2019).

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4.8 Supplements

To compare allocation of herbaceous climbers and self-supporting herbs with their woody counterparts we used published equations about tropical woody climbers and trees. These are:

1) $AGB_{climbers}=10^{0.07+2.17*\log(D)}$

2) $AGB_{trees}=0.603*e^{-1.754+2.665*\ln(D)}$

3) $LM_{climbers}=10^{-0.57+0.81*\log(BA)}$

4) $LM_{trees}=10^{-1.26+0.84*\log(BA)}$

where *AGB* stands for aboveground biomass, *D* for stem diameter, *LM* for leaf biomass and *BA* for basal area ($BA=\pi*(D/2)^2$), log being the decimal logarithm and ln being the natural logarithm with base *e*. Equations 1, 3 and 4 are from Gerwing and Fabias (2000); equation 2 is from Higuchi et al. (1998).

From these equations we estimated relationship between leaf and stem biomass (rest of aboveground biomass – reproductive organs included) separately for trees and woody climbers. This was done by simulation of stem diameter values in reported range of 1 to 16 cm. We simulated 1501 of such values and computed leaf and stem biomass from each of them using abovementioned equations. Intercept and slope were estimated using linear model with this computed stem biomass (as response) and leaf biomass (as predictor). Computed leaf and stem biomass values represented a line (explained variability of the linear model: $R^2_{climbers}=1$, $R^2_{trees}=0.9996$).

Resulting relationships (Fig. 2):

5) $\ln(SM_{climbers}) = -0.497 + 1.370 * \ln(LM_{climbers})$

6) $\ln(SM_{trees}) = -1.991 + 1.659 * \ln(LM_{climbers})$

where *SM* stands for stem biomass, *LM* for leaf biomass (plus reproductive organs) and Ln for natural logarithm with base *e*.

Table S1: Collected species of herbaceous climbers, their localities and characteristics. T = twining; S = spines or hooks; E = tendrils. Mean annual precipitation and temperature: Straznice – 627 mm, 9.3°C, Klet – 848 mm, 7.2°C, Trebon – 713 mm, 8.0°C, Edgewater - 1071 mm, 13.3°C

Species	Collection site	GPS coordinates	Date of collection	Collected shoots	Climbing mechanism	Perennial/ annual
Bryonia alba L.	Strážnice	48°53'57.9"N, 17°18'56.0"E	18 Sep 2014	4	E	perennial
Fallopia dumetorum (L.) Holub			18 Sep 2014	4	T	annual
Lathyrus pratensis L.	Kleť	48°51'54.9"N, 14°17'01.7"E	21 Jun 2014	3	E	perennial
Calystegia sepium (L.) R. Br.	Třeboň	49°00'16.1"N, 14°46'16.5"E	19 Jun 2011	12	T	perennial
Echinocystis lobata (Michx.) Torr. & A.Gray			22 Sep 2011	7	E	annual
Galium aparine L.			28 Jun 2011	12	S	annual
Humulus lupulus L.			27 Sep 2013	3	T	perennial
Vicia cracca L.			18 Sep 2011	11	E	perennial
Vicia grandiflora Scop.			21 Jun 2014	3	T	annual
Vicia hirsuta (L.) Gray			25 Sep 2011	9	E	annual
Vicia tetrasperma (L.) Schreb.			28 Jun 2011	10	E	annual
Fallopia convolvulus (L.) Á. Löve	Edgewater	38°53'15.0"N, 76°33'14.0"W	4 Sep 2012	4	T	annual
Mikania scandens (L.) Willd.			15 Oct 2012	4	T	perennial
Persicaria perfoliata (L.) H. Gross			7 Sep 2012	4	S	annual
Persicaria sagittata (L.) H. Gross			17 Oct 2012	3	S	annual
Strophostyles helvula (L.) Elliott			17 Sep 2012	4	T	annual

Table S2: List of grassland herbs. Mean annual precipitation and temperature: Straznice – 627 mm, 9.3°C.

Grassland herbs	
Achillea collina (Becker ex Rchb.f.) Heimerl	*Leontodon hispidus* L.
Asperula cynanchica L.	*Leucanthemum vulgare* (Vaill.) Lam.
Asperula tinctoria L.	*Linum catharticum* L.
Astragalus danicus Retz.	*Melampyrum cristatum* Hablitz ex Steud.
Campanula glomerata L.	*Peucedanum cervaria* (L.) Cusson ex Lapeyr.
Campanula persicifolia L.	*Polygala major* Jacq.
Centaurea jacea L.	*Potentilla alba* L.
Centaurea scabiosa L.	*Potentilla erecta* (L.) Raeusch.
Centaurium erythraea Rafn	*Prunella grandiflora* (L.) Scholler
Chamaecytisus virescens (Kováts ex Neilr.) Dostál	*Pulmonaria angustifolia* L.
Cirsium pannonicum (L.f.) Link	*Ranunculus acris* L.
Clematis recta L.	*Salvia pratensis* L.
Dorycnium pentaphyllum Scop.	*Scorzonera hispanica* L.
Euphorbia cyparissias L.	*Senecio umbrosus* Waldst. & Kit.
Euphorbia esula L.	*Serratula tinctoria* L.
Filipendula vulgaris Moench	*Stachys officinalis* (L.) Trevis.
Galium boreale L.	*Symphytum tuberosum* L.
Galium verum L.	*Tanacetum corymbosum* (L.) Sch.Bip.
Geranium sanguineum L.	*Teucrium chamaedrys* L.
Hypochoeris argentina Cabrera	*Thesium linophyllon* L.
Inula ensifolia L.	*Trifolium montanum* L.
Inula hirta L.	*Trifolium ochroleucon* Huds.
Inula salicina L.	*Trifolium pratense* L.
Knautia kitaibelii (Schult.) Borbás	*Veronica austriaca* L.
Lathyrus niger (L.) Bernh.	*Vincetoxicum hirundinaria* Medik.

Table S3: List of megaherbs from Kamchatka Peninsula and the island of Sakhalin. Data on these species were used in the analysis and taken from Morozov and Belaya (1988).

Megaherbs
Aconitum fischeri Rchb.
Aconitum maximum Pall. ex DC.
Angelica genuflexa Nutt.
Angelica sachalinensis Maxim.
Angelica ursina (Rupr.) Maxim.
Anthriscus sylvestris (L.) Hoffm.
Aralia cordata Thunb.
Aruncus dioicus (Walter) Fernald
Parasenecio hastatus (L.) H. Koyama
Parasenecio auriculatus (DC.) J. R. Grant
Cardiocrinum cordatum (Thunb.) Makino
Actaea simplex (DC.) Wormsk. ex Prantl
Cirsium kamtschaticum Ledeb. ex DC.
Cirsium weyrichii Maxim.
Filipendula camschatica f. glabra Koidz.
Filipendula camschatica f. typica Koidz.
Heracleum lanatum Michx.
Ligularia fischeri (Ledeb.) Turcz.
Pleurospermum uralense Hoffm.
Polygonum weyrichii F. Schmidt
Jacobaea cannabifolia (Less.) E.Wiebe
Urtica platyphylla Wedd.
Veratrum grandiflorum (Maxim. ex Miq.) O. Loes.
Veratrum oxysepalum Turcz.

Table S4: Means and standard deviations (SD) of length and biomass measures for climbers and self-supporting herbs. In brackets are species numbers for each growth form. Length of megaherbs was available only for 6 species.

	Annual climbers (10)		Perennial climbers (6)		Megaherbs (24)		Grassland herbs (50)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Length (cm)	302.052	196.801	318.047	217.609	246.667	44.572	39.353	18.608
Biomass of leaves (g)	5.536	7.163	7.312	7.629	52.504	110.014	5.045	8.216
Biomass of stems (g)	9.990	9.222	8.488	10.054	82.029	109.753	4.019	4.950
Biomass of reproductive organs (g)	12.032	20.233	1.335	2.685	-	-	0.801	0.941

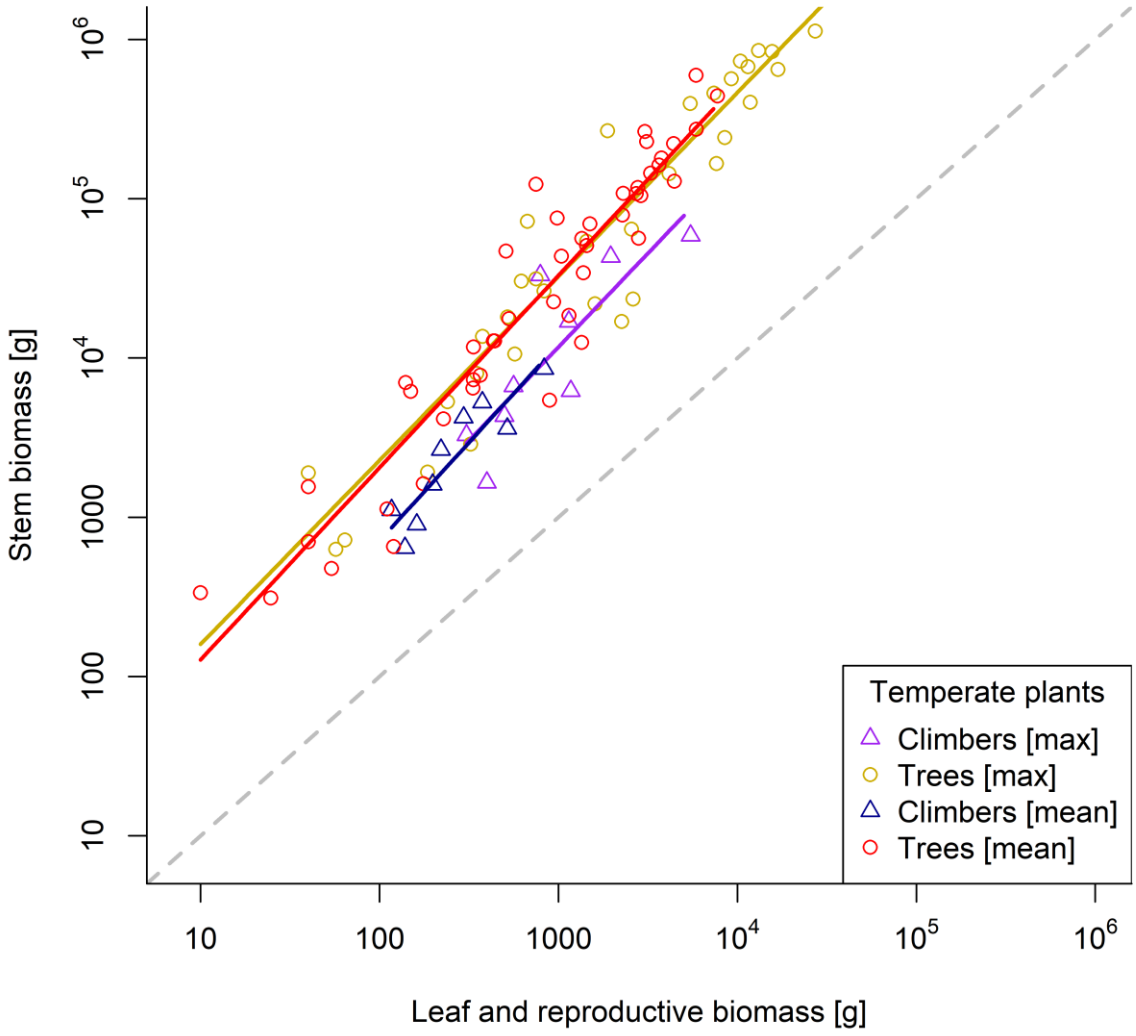


Figure S1: Relationship of leaf and reproductive biomass (log) and stem biomass (log) for temperate woody climbers and trees. Relationships fitted using linear model are visualized for mean and max values per species showing nearly identical slopes. Data are from Ichihashi and Tateno (2015).

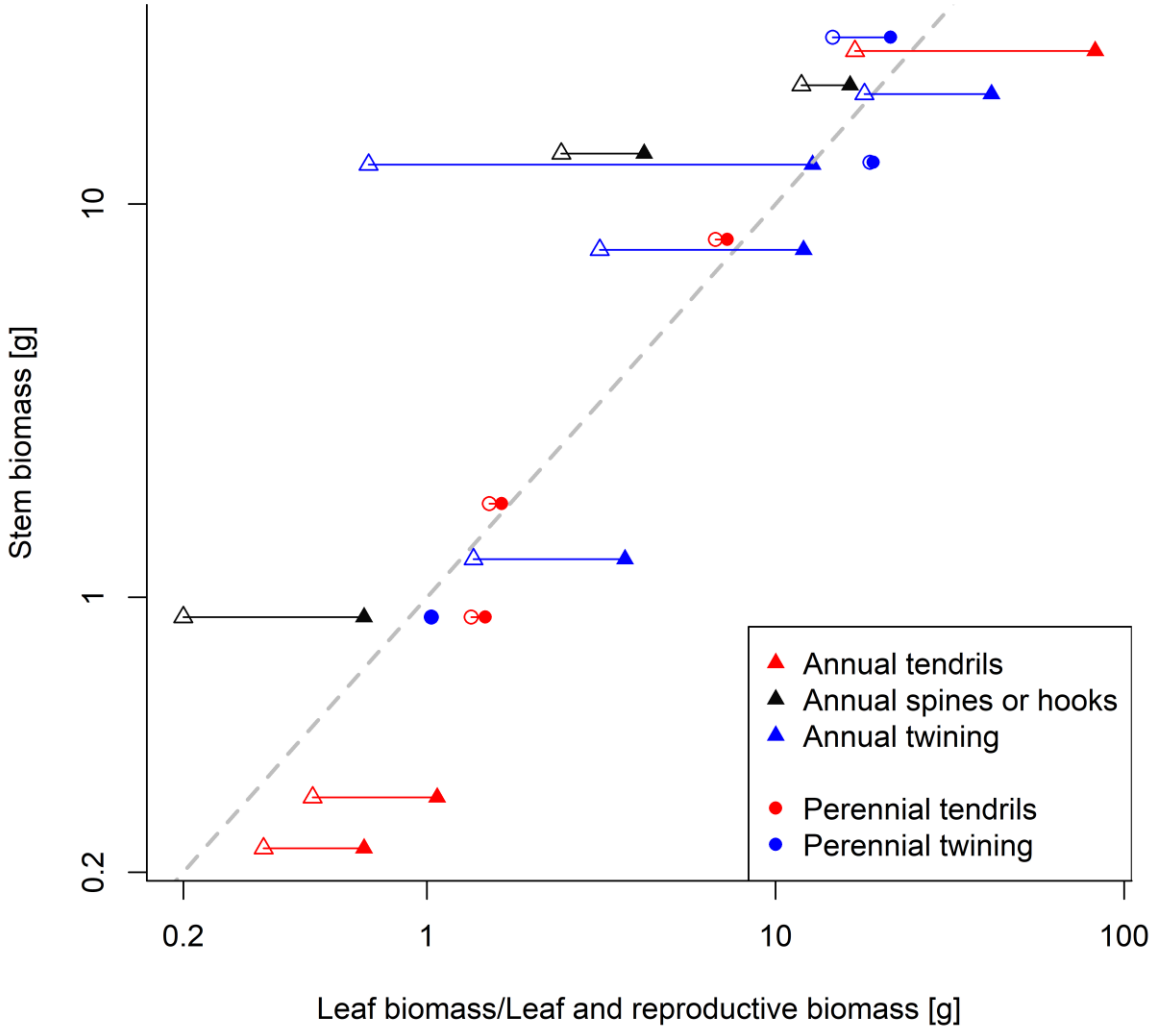


Figure S2: Stem, leaf and reproductive biomass of herbaceous climbers. Empty symbols denote leaf biomass; filled symbols denote leaf plus reproductive biomass. The same species with and without reproductive biomass is connected by black line. Grey dotted line marks slope 1.

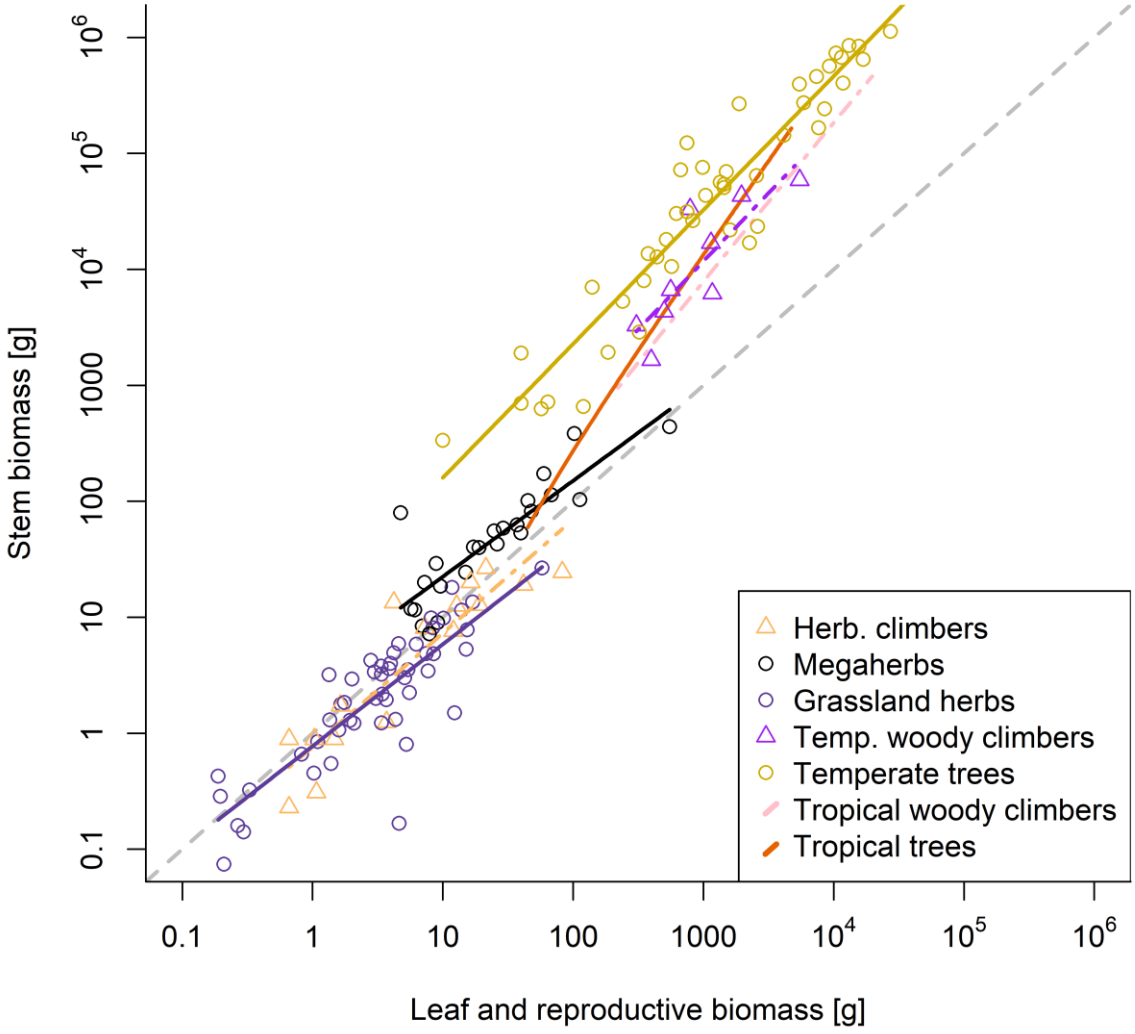
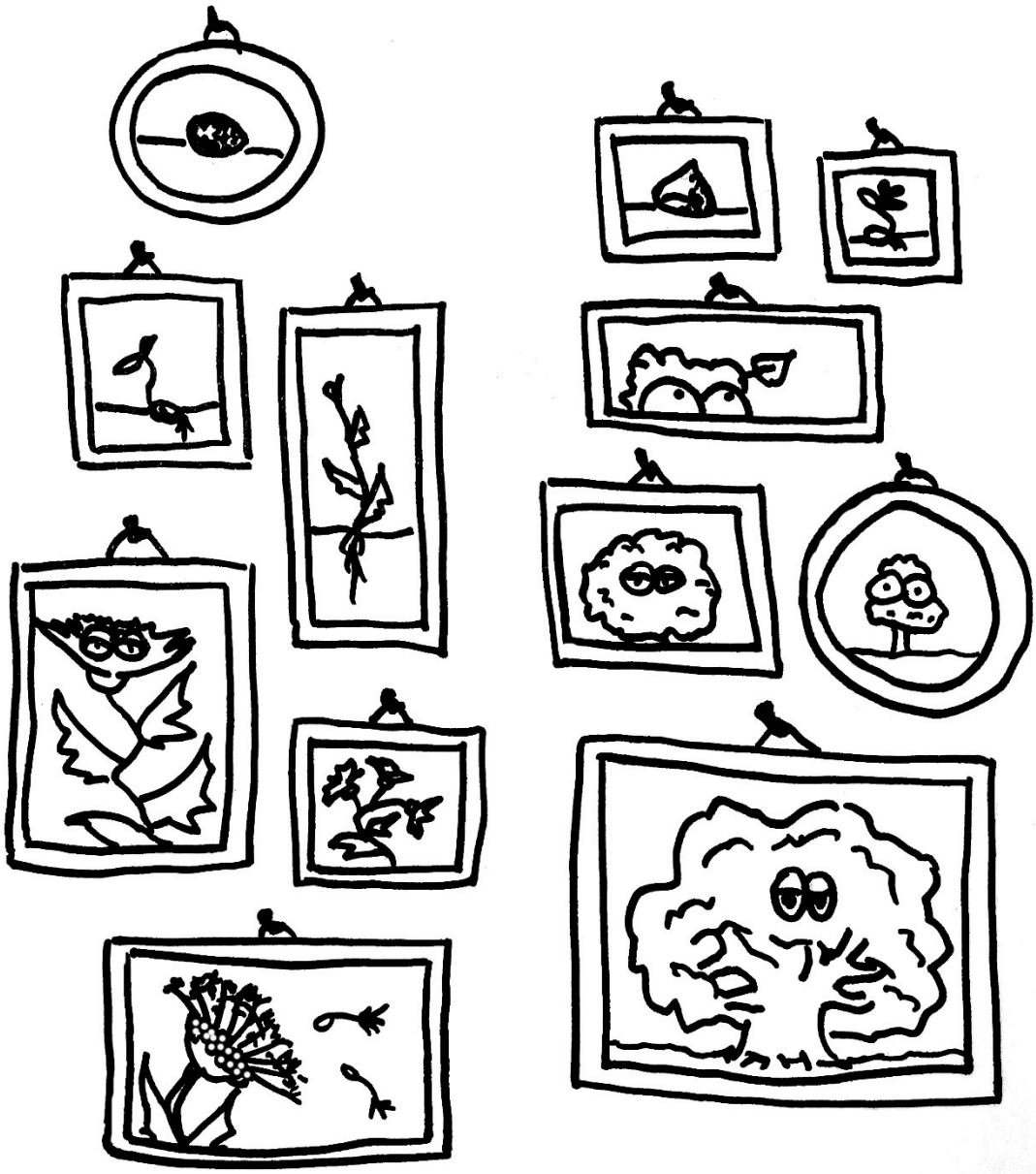


Figure S3: Relationship between stem biomass (log) and remaining aboveground biomass (log) of climbers and self-supporting plants. Lines for herbaceous climbers, megaherbs and grassland herbs are modelled using a phylogenetic generalized least squares model. For megaherbs, data on reproductive biomass is not available, thus only stem biomass is included (identical to Fig. 1). In case of temperate woody plants, we visualized fitted lines from linear regression. For tropical woody plants datapoints are not available. Lines for climbers are dash-and dotted. The grey dotted line marks slope 1.

Chapter 5



5. Demographic correction – a tool for correct inference from individuals to populations

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AK, JK and ZJ conceived the research, AK designed the analysis and wrote the manuscript with contributions of all other authors.

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5.1 Abstract

Response of populations of species to their environment, and comparison of such responses across sets of species, is of primary interest to ecological science. However, the most common approach to address this task is based on comparison of such responses of individuals of one life stage. Typically, they are exposed to contrasting environments and, based on the observed differences in their performance, conclusions are drawn about the effect of such environmental change on interspecific differences in population dynamics. The tricky part is that interspecific fitness differences at one single life stage are not directly comparable. Only the total life cycle of the population determines the value of individuals in one life stage for survival/growth of their populations. Omitting demographic information can thus strongly bias conclusions based on comparison of individuals of one life stage, both in experimental studies with a few species, and in large comparative studies.

We illustrate this effect using a fictitious example showing that under simple assumptions, demographic differences between two species can completely revert conclusion reached by naive comparison that does not take demography into account. We suggest a correction, namely to translate observed effects into changes of demographical models. These changes are done by adjustment of transitions in projection matrix models. While solution is limited by the availability of demographical information, we believe that existing data (and data likely to be collected in the near future) permit at least approximate handling of this problem.

Keywords: vital rates; loop analysis; population growth rate; life history strategy; projection matrices; comparative demography

5.2 Introduction

As ecologists we often ask how populations respond to environmental factors. However, it is not always easy to measure their effect on species' long-term population dynamics directly. Instead, we typically collect data on the effect of changing environment on individuals in one life stage; this is indeed one of the most common designs of all ecological studies. Interpretation of such data is based on the assumption that these differences are informative about the prospect of the whole population. This makes sense intraspecifically: fitness differences of two comparable individuals of a species reflect their relative contributions to species' population dynamics. However, could we infer the same when comparing two or more species?

Interspecific comparisons necessarily face the problem that fitness differences of two similar individuals are not directly comparable in their effects on population dynamics. Namely, population-level effects of differences in vital rates of individuals strongly depend on species' demography (but see e.g. Ehrlén et al., 2005). As a result, reproduction and survival of individuals may have different relative importance in different species (Silvertown et al., 1993). Annual plants and rodents rely more on reproduction, trees and large mammals more on survival (Harper et al., 1996). Therefore, death of a young annual plant would impact its population less than death of a sapling would impact the population of a tree. The impact of changes in the vital rate (mortality, growth, reproduction) highly depends on the life stage which it affects and on the importance of the impacted life-stage for population dynamics of that species.

Owing to recent syntheses of plant's demographical information across many plant taxa (Adler et al., 2014; Salguero-Gómez, 2017) we now know much better how plants differ in importance of individual life stages. These effects of demography are obvious, but are too often neglected in studies when single life stages are compared across species. The extent of the possible differences between individual and population performance can be illustrated by a rare explicit treatment of this problem in two species of genus *Cirsium*, one rare and one common (Münzbergová, 2005). Collected data showed that the individuals of the rare species suffered less from seed predation than the individuals of the common one. Naive interpretation of this finding would imply that rarity/commonness of these species cannot be attributed to the levels of their seed predation. However, when demography of both species were taken into account, the conclusion was opposite, the life cycle of the rare species relied much more on seed production and thus its population growth rate suffered more from seed predation in spite of its overall lower levels (Münzbergová, 2005).

In studies with a low number of species, the error due to omission of species' demography is often avoidable by careful selection of studied species, e.g. by focusing on species with similar life-history strategy. However, the need to correct for different demography of species is much more urgent in large comparative studies promoted by the ever increasing demand for production of general conclusions (Enquist et al., 2009; Kattge et al., 2020). Such large studies frequently compare plants of different growth forms which are bound to differ strongly in their life-history strategies (Salguero-Gómez, 2017), and consequently, in the population-level effects of differences in vital rates. Thus, omission of demographical information might introduce unpredictable biases. This is especially tricky in studies focusing on a specific life-stage in groups of organisms which highly differ in their life-history. For example, many experiments in plant ecology compare seedling recruitment among species, or compare annual and perennial herbs or herbs and trees (Vesk et al., 2004; Whitman and Aarssen, 2010) or clonal and nonclonal herbs (e.g. Klimešová et al., 2013; Vítová et al., 2017; Barak et al., 2018; Albert et al., 2019), without paying attention to the fact that the same life stage transition may have tremendously different effects on the population growth rate and dynamics in each of these life strategies.

In this paper, we illustrate the problem of ignoring species' demography when extrapolating from individuals to population performance using a fictitious example. We are showing that this problem can indeed have major effect on interpretation of data collected on individuals of one life stage. As a remedy, we propose to use information on the whole life cycle (e.g. from population projection matrices) and to incorporate it into the inference. We call this procedure "demographic correction" (Fig. 1) and discuss its utility and limitations.

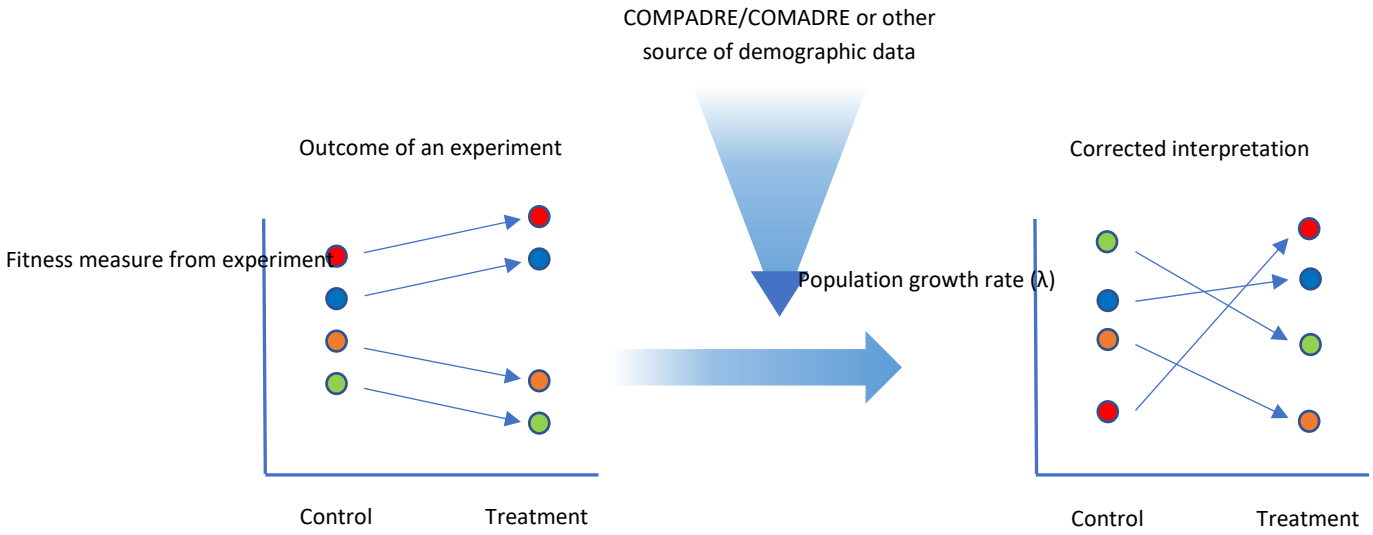

Figure 1: Diagram of demographic correction. Coloured points denote species. Outcome of an experiment shows increase of fitness for two species and decrease for the other two species due to the treatment. After demographic correction population growth rate is obtained which likely to show different effect of the treatment. Omitting the demography and assuming that the right (population level) is the same as the left one (individual level) might lead to biased conclusions.

5.3 Effect of demography – a fictitious example

Demography of species is typically recorded as transition probabilities of individuals from the one life-stage into another. These transitions constitute so called projection matrix (Caswell, 2006). From viewpoint of population fate, the most used value computed from such projection matrices is the asymptotic population growth rate (λ; dominant eigenvalue of the projection matrix) which tells how much the population will grow or decline in one time-step if the population has a stable stage distribution (Caswell, 1989). Thus, we will use this value to interpret the fate of populations in the following fictitious example.

Let's imagine that we are interested in the response of two species to grazing and we conduct a comparative (*sensu* Sanford et al., 2002) greenhouse experiment to investigate that. We obtain seedlings of both species, plant each seedling into one pot and let them grow into maturity. We divide adult plants randomly into treatment and control group and simulate grazing on plants assigned to the treatment group. After the treatment we check plants and note their survival. We analyse (for both species) differences between control and treatment group. Let's say that treatment led (on average) to mortality of 30% for species A and 50% for species B (and this difference was statistically significant; Fig. 2). We would typically conclude that species B will according to our results suffer much more when exposed to grazing then species A.

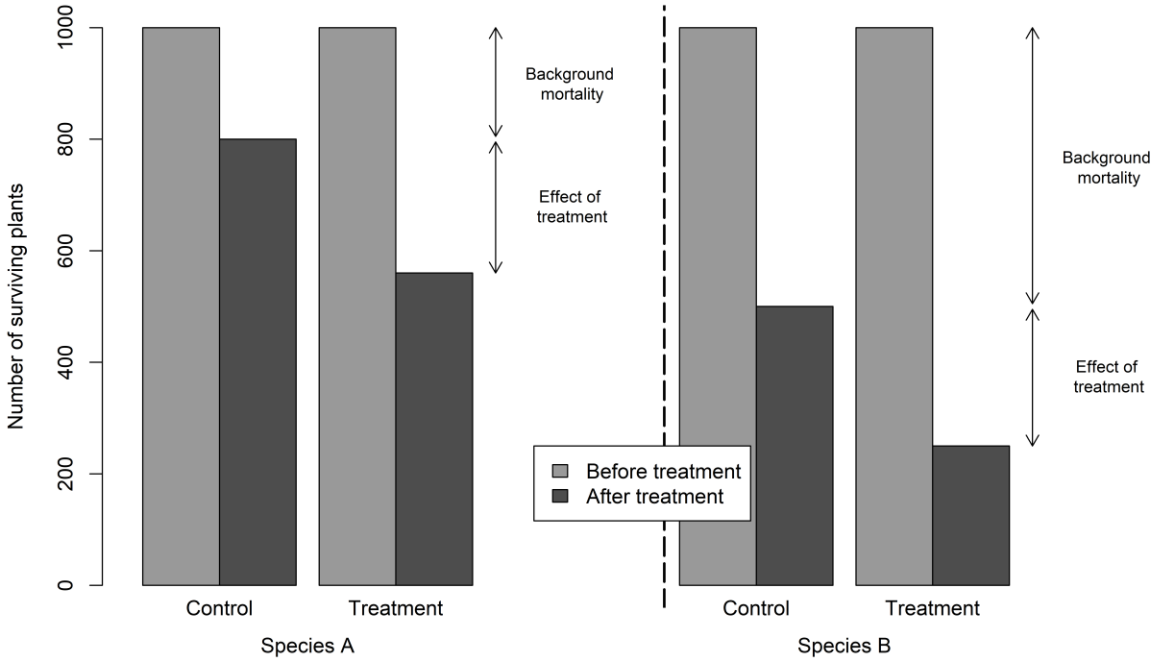

Figure 2: Mortality of species A and B in the fictitious example. Mortality due to treatment was 30% of plants for species A and 50% for species B. Background mortalities correspond to mortalities of "Young" life-stages in projection matrix models (Fig. 3).

Let us further assume that species A and B differ in their demography. We have projection matrix models for both (Fig. 3). Both species in this example have a very simple and similar life-history – there is no stasis and no retrogression, only growth and reproduction of young and adult plants. Populations of both species are stable – asymptotic growth rate equals one.

Our grazing treatment impacted adult plants shortly before reproduction. We can correct for demography by adjusting particular transition in the projection matrices (highlighted in Fig. 3) and inspect predicted population growth rate. Adult plants do not survive therefore the only impacted transition is their reproduction. In species A that transition was 4 and expected mortality due to treatment is according to the experiment 30% resulting in new transition of 2.8 (4*(1-0.3)=2.8). For species B 50% mortality changes the transition into 1 (2*(1-0.5)=1). New asymptotic population growth rate is 0.9293 for species A and 0.9584 for species B. Thus, when we took demography of our species into account, in contrast to the first conclusion, the species A suffers more when grazed then species B.

Figure 3: Population transition matrices for two fictitious species, their adjusted version and population growth rates. They serve as an example of the impact of demography on inference from individuals to populations.

We can see that conclusions drawn from our data can dramatically differ if we use the transition matrix to correct for demography of species or not. What seemed to be without demography a clear advantage of one species under the treatment conditions, might turn to disadvantage when demography is accounted for.

Strong effect of demography in the example is caused by majority of reproduction of species B realized at "Young" life-stage. Thus, when only reproduction of "Adult" life-stage is impacted by the treatment – population of species B suffers much less than would correspond to observed data. On the other hand, species A has mainly reproducing "Adult" life-stage. Therefore, treatment impacts most of its reproduction. This can be nicely described using loop analysis (van Groenendael et al., 1994): Both species have two loop – one goes through reproduction of "Young" life-stage the other though "Adult". Species A and B differ in relative importance of loops in their lifecycle. The main loop of Species A was impacted by the treatment, for species B only the loop with lower importance for population growth rate was impacted by the treatment (Fig. S1).

5.4 Discussion

The huge effect of demography in our fictitious example might seem unlikely since the difference between species in early reproduction is so large, but the same effect (only of different strength) is an inevitable outcome of the demographic consequences of any individual-based data (Fig. S2). We can expect an analogous effect to this example generally in all cases when studied species differ in the importance of impacted transitions for the asymptotic population growth rate (i.e.: when they differ in elasticity of these transitions; but see Carslake et al. (2008)).

Consequently, we are proposing here that such ***demographic correction*** is a necessary step for reliable assessment of any fitness difference measured on individuals (Fig. 1). Interpretation of any individual-derived data requires demographic correction that uses population projection matrix to translate the observed effects into changes in meaningful population characteristics (such as λ) in order to make a valid conclusion about the population response to the studied treatment.

As we already pointed out, in experiments with a few species, demography has often been considered when designing the experiment. In some cases, researchers even choose to gather data about demography and correct for it as we have done in the fictitious example (e.g. Münzbergová, 2005; Struckman et al., 2019). However, this demographic correction has by far not become a standard tool in interpretation of individual-based data. Still we believe that using demographic information in this way is necessary and cannot be avoided, not only in experimental studies with a small number of species, but also in large comparative studies based on plant traits.

Consideration of demography in studies (especially with many species) has one major difficulty. Demography is known only for a very limited subset of existing species (but it is increasing, see: Salguero-Gómez et al., 2015, 2016) and its collection is laborious and time-demanding. Rarity of demographical information is thus surely a limitation. However, the ever-increasing amount of demographic data available has already permitted comparative analyses based solely on demography (Adler et al., 2014), demonstrating that such data becomes available at a comparative basis.

We would also like to point out that phylogenetic information was also quite rare when Felsenstein described its importance in analyses (Felsenstein, 1985). As with phylogenetic information, one solution is to gather demography for species selected for the study. If there is no available demography and we cannot obtain it ourselves, we can still estimate it based on similar species (if we have independent information for the estimation; Salguero-Gómez, 2017; but see: Che-Castaldo et al., 2018). Thus, we can end up with more or less accurate estimation of demography of populations about which we want to make inference.

Further, it is important to note that life-history of species is known to be dependent on conditions in which the species lives (Ballinger, 1979). Importance of life-stages also differ in growing and declining populations (Oostermeijer et al., 1996). Therefore, available demographical information will in many cases be only approximate. Still we can safely say that demographical correction with such information is better than none as it is the case with incomplete phylogenetic information in phylogenetic comparative analysis (Freckleton et al., 2002).

5.5 Conclusion

We have illustrated the potentially large effect of omitting demography in studies with multiple species and showed that it can lead to erroneous conclusions about environmental factors on populations of these species. We are proposing here the demographic correction as a concept and as tool to deal with this problem. Adjustment of transition(s) of population projection matrices according to change expected from an experiment is a way to make justifiable inference from individuals to populations. We see this approach as a way how the increasing amount of demographic information may shed light on one of the most common data type in ecology, namely data sets comparing fitness differences among species in one life stage.

Acknowledgements

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5.7 Supplements

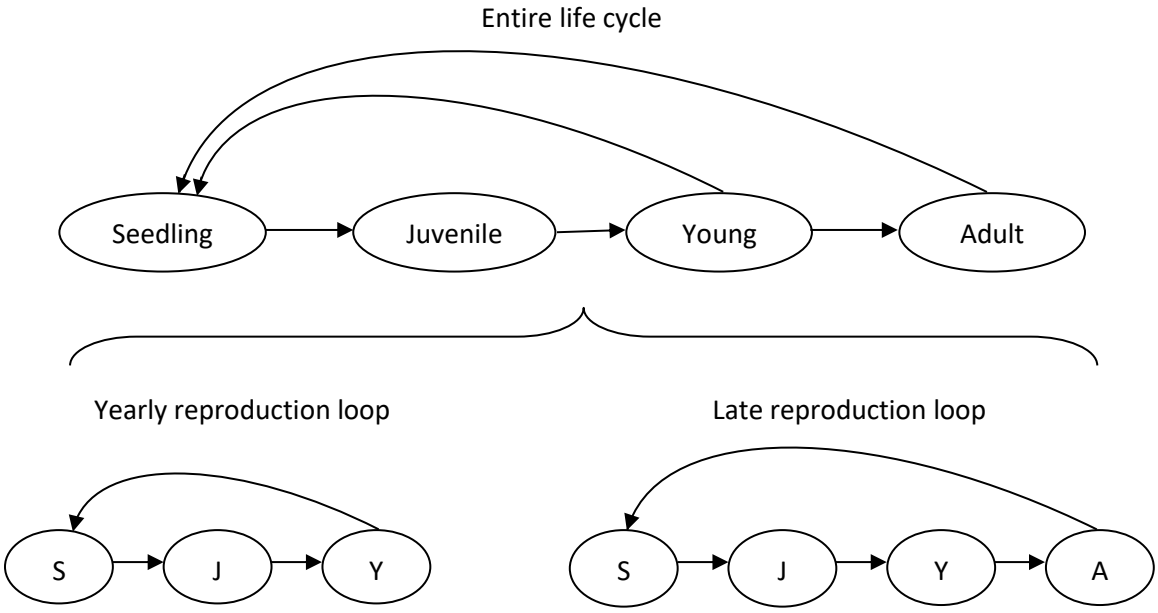

Figure S1: Loops in projection matrix models of species A and B. For species A "Late reproduction loop" is more important (sum of elasticities: 0.84); for species B "Yearly reproduction loop" is more important (sum of elasticities: 0.69).

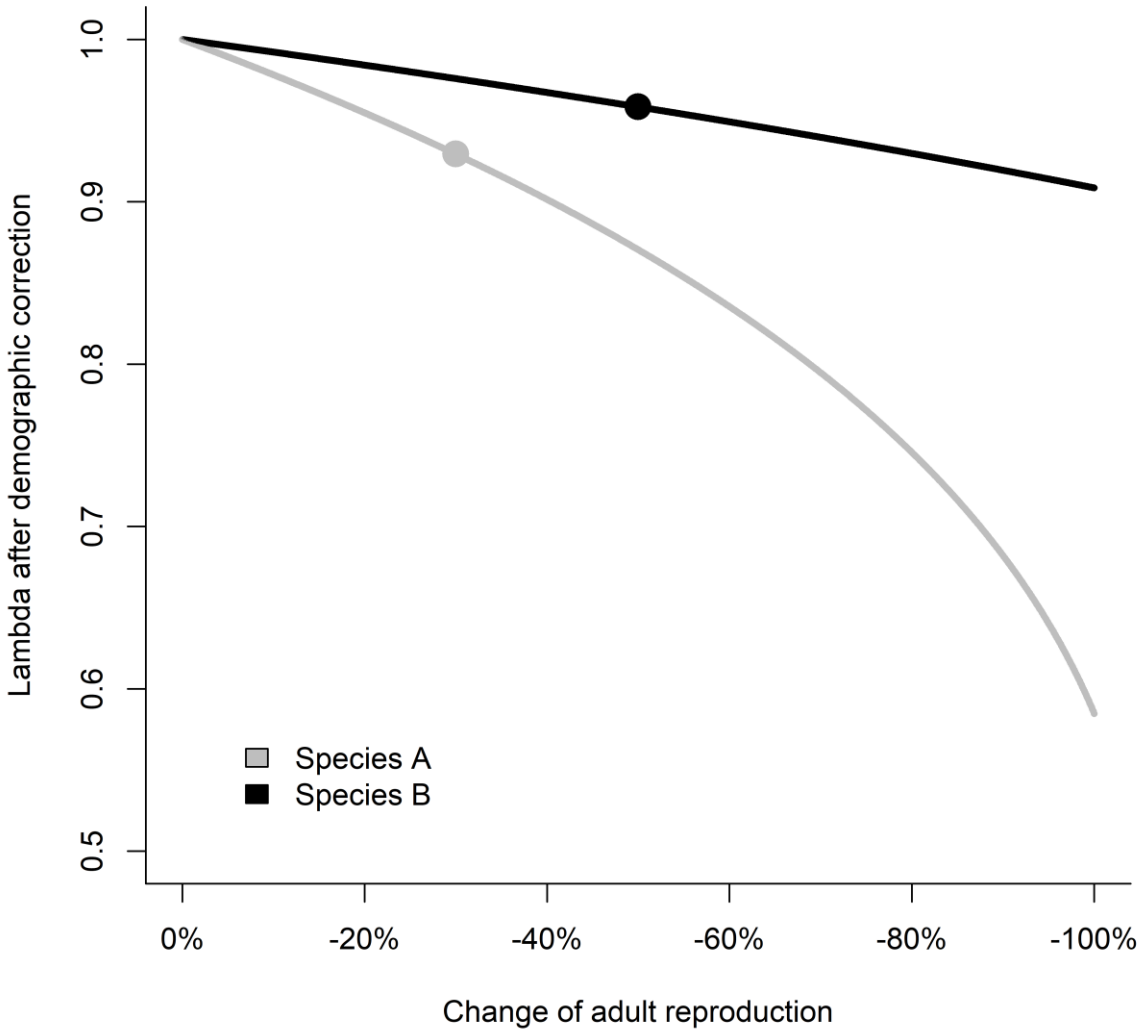

Figure S2: Effect of change of adult reproduction (due to the treatment) on population growth rate (λ) for species A and B from the fictitious example. Observed changes are highlighted by points: Although adult reproduction of species B is highly reduced, the effect on population growth rate is weaker than for species A where adult reproduction is preserved to higher degree.