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Doktorský studijní program: Botanika

Ph.D. Study program: Botany



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Plant body as a behavioural platform – an ecologist's insight
Role těla v chování rostlin – ekologický pohled

Dissertation / Disertační práce

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Praha, 2015

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V Praze, 10.07.2015

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Summary

Conceptualisation of plant behaviour, or plant phenotypic plasticity, has been proven to be useful both on the ecosystem scale and on the level of individuals, as it allows to predict differentiation of species across ecosystems or results of interactions among individuals. Between these extremes is a vast array of processes that drive community assembly. These processes are difficult to predict, be it at the individual level or based on whether a whole species is plastic or non-plastic. These processes are traditionally investigated at the species level. In this thesis, however, I instead show how species-specific life histories delineate plant behaviour. I hope to convince the reader that it is the plant body, or at least its species-specific properties, not merely its non-specific, theoretical degree of plasticity, that should be used to explain actual cases where plant behaviour underpins species coexistence.

As evidence for my case, I present four studies, each of them dealing with different part of the plant body that underlies different aspects of plant behaviour. In the first study, I show how species' life-history traits are coordinated with their responses to neighbour presence and resource shortage, both of these delivered in the form of changing light quantity and quality. Not only the quantity of the response, but especially its form, is deeply coordinated with life history traits of species. In the second study, I examine the amount of plasticity that plant root systems exhibit in patchy environments and link the extent of root localization plasticity exhibited with the same set of species-specific life history traits as in the first study. Previously, some of these life history traits were linked to plant plasticity in general. It has also been traditionally hypothesized that plasticity is a common property of the whole plant body, both aboveground and belowground. In the third study, I show that seedling root system development is plastic, but that the realized form of the root system is strongly affected by species-specific seed mass and the environment. Seed mass thus sets the boundaries for plasticity in architectural traits of the root system. In the last study, I show how growth forms and different environments set the optima for clonal spread of plants.

I hope that this thesis sheds new light on the interplay between plant behaviour and the plant body. Although all of the presented studies deal with multiple species, I have a feeling that I have only opened the door to research into this topic with a plethora of possibilities and undiscovered links. Even though this puts me further from the goal of linking plant behaviour and coexistence, I believe that this is the way how to shape ideas about plasticity to reflect the physical world, where real living organisms with real bodies struggle for existence.

Keywords: phenotypic plasticity, constraints, plants, behaviour, species traits

Souhrn

Rostliny rostou. Porovnááme-li jedince (rostlin) se stejnou sadou vloh, najdeme rozdíly dvou typů: jedny lze předem předpovědět podle toho, *do jaké velikosti* jedinci narostli, druhý typ rozdílu je dán tím, *jakým způsobem* narostli, a lze jej předpovědět s menší jistotou. Způsob růstu, který rostlina zvolí, je vlastně jejím chováním. Takto nahlížený růst dovolí předvídat výsledky interakcí jedinců či prostředí, kde lze s takovým chováním (zvolenou formou růstu) uspět. Na druhou stranu, prostor pro možné chování omezují vlohy jedinců, a ačkoliv se jedinci vlohami liší obecně, větší rozdíly lze očekávat mezi málo příbuznými jedinci, kteří nemohou dát vzniknout potomkům s kombinacemi svých vloh. Právě možnost soužití takových skupin jedinců, tedy druhů, je předmětem zájmu ekologie společenstev. V této práci se snažím ukázat, jak souvisí chování příslušníků druhu s jejich společnými, druhovými, vlastnostmi. Protože jde o rostliny, zaměřil jsem se na vlastnosti jejich těl, jakožto výsledků a současně platformou růstu. Tím se pokouším poskytnout pravidla (korelativní povahy), která by v důsledku umožnila spojit vlastnosti těl druhů s jejich soužitím, je-li soužití druhů závislé na jejich chování.

Tato práce obsahuje čtyři takové studie a každá z nich se věnuje jinému okruhu vlastností druhů a jejich vlivu na chování jedinců. V první z nich ukazují, jak souvisí vlastnosti těl druhů s jejich chováním v případě nedostatku světla či jeho očekávaného budoucího úbytku v důsledku přítomnosti sousedů. Vlastnosti těl druhů v takových případech určují nejen míru, ale zejména způsob odpovědi. Ve druhé studii se věnují vztahu stejných vlastností těl a preferenčního umístění kořenů do živinami bohatých míst. Tato studie byla inspirována tvrzením, že druhy jsou obecně plastické či neplastické zároveň v nadzemí i podzemí, tedy že stejné vlastnosti těl druhů by mohly souviset s obojím. Na základě svých výsledků se domnívám, že tomu tak do jisté míry je, ale že univerzalita takového tvrzení je spojena s rychlostí růstu, nikoliv se specifickými formami chování. Sama o sobě je ale nízká míra preferenčního umístění kořenů spojena se schopností vegetativního šíření. Třetí studie je věnována vývoji kořenových systémů semenáčů a vztahu průběhu tohoto vývoje ke vzájemně zdánlivě zastupitelným zdrojům – dostupnosti živin v prostředí a velikosti semen, tj. velikosti živinových zásob. Kořenové systémy semenáčů mění svůj vývoj podle podmínek prostředí, ale klíčové parametry tohoto vývoje souvisí s velikostí semen na úrovni druhu a jsou na prostředí nezávislé. Ve čtvrté studii se věnují vztahu klonálního šíření a prostředí. Zatímco vzdálenost šíření stolony lze odhadnout pomocí jednoduchého analytického modelu závislosti této vzdálenosti na podmínkách prostředí, pro šíření pomocí oddenků to neplatí. Z toho vyplývá, že ačkoliv stolony i oddenky mohou sloužit klonálnímu šíření, každá z těchto struktur jej ovlivňuje jinak.

Doufám, že tato práce jako celek přispěje k poznání role rostlinného těla jako platformy chování rostlin. Ačkoliv jsem ve všech studiích pracoval s co největšími počty druhů,

nedomnívám se, že jsem popsal veškerá podstatná pravidla této koordinace. Je zřejmé, že nestejnost rostlinných těl nás posouvá *od* řešení otázky vztahu chování a soužití ve společenstvech rostlin, nicméně vede nás *k* nutnosti zkoumání této koordinace na úrovni, kde je její vznik sjednáván, tedy *k* pokusům různých těl o úspěch v přírodním výběru.

Klíčová slova: fenotypová plasticita, omezení, rostliny, chování, vlastnosti druhů

Poděkování / Acknowledgement

No fun with the research would be possible without my friends and family. If you belong to any of these groups, Thank you.



...

Plant body as a behavioural platform – an ecologist's insight

“Ekologie je etologie” [“Ecology is ethology”] – Z. Neubauer, undated

Body, phenotypic plasticity and behaviour

For an entity/symbol to become an information, the capable interpreter is needed. This simple fact should point a biologist's attention to the crucial role of the body. It is the body that acts in the process of evolution, as it is the body with its traits that acts as the interface through which the inherited gets in touch with its perceived environment (*Umwelt* sensu Uexküll; Kleisner 2008). Bodies are the objects that need to fit their environment in order to survive and reproduce, i.e. subjects of evolution (Dawkins 1982).

The body traits may be static or dynamic. If the trait is dynamic, i.e. changes in time, it may be plastic. If the body trait changes in time, but in a fully predictable way, regardless the environment, the trait is dynamic, but not plastic. Phenotypic plasticity is the ability of a single genotype to become blueprint of several phenotypes (Bradshaw 1965). However, it should be noted that there is no phenotypic plasticity without uncertainty, trait dynamics¹ and the substantial role of environmental stimuli² (Figure 1). Plasticity allows the body to change dynamically, so plasticity yields dynamic body traits. Further, plasticity allows to respond to environmental cues and thus to fit the dynamic environment with dynamic body traits. Such dynamics on both sides of the interactive interface may result in better³ response in plastic trait than a static trait could provide (Bradshaw 1965; Aphalo & Ballaré 1995; Van Kleunen & Fischer 2001; Huber et al. 2011)



Figure 1 – body traits. The toddler and football (soccer) referee. If we assume that the toddler picture is an old photograph of the football referee, we may illustrate several kinds of body traits. During certain period of life, some traits are static – note that he toddler and the referee have the same numbers of body parts. Over the same period of time, some traits are dynamic – note the difference in proportions of body parts between more and less grown state of the individual. Apart of extreme situations, this proportion shifts are inevitable, and allow

an observer to instantly distinguish a child and a man. Some other traits are plastic. At the age of the toddler,

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- 1 Karel Kleisner noted that animals may be phenotypically plastic, but the trait does not *seem* to be dynamic. For example, if pupa of some butterflies is subjected to heat shock, the resulting imago substantially differs from the genetically identical imagos that were not subjected to the shock. The imago stays so, regardless the environment or time. Similar examples may be found e.g. in temperature dependent sex development in reptiles. I argue that even in these cases there is dynamics, if butterfly pupa or reptile egg is considered an individual. Start of the ability to plastically respond to environment signifies the begin of an individual.
 - 2 Phenotypic plasticity may rise even without present environmental stimulus – consider development of immunity cells receptors.
 - 3 In terms of fitness

future football referees, their musculature and lung capacity are difficult (if not impossible) to tell. (Toddler picture: author – Avsar Aras, CC-SA. Football referee (Howard Webb): author – Patrick Telford, CC-SA)

Note that phenotypic plasticity is detectable only through comparison, either in time (the before-after scheme) or by comparison of genetically similar (preferably identical) individuals under different “treatments”. Therefore, phenotypic plasticity cannot be used as a state variable to describe an individual. On the other hand, body traits are measurable, describing the state of an individual (Pigliucci 2005; Violle et al. 2007), and we detect phenotypic plasticity through comparison of their states. This way, phenotypic plasticity is inevitably connected to the body, as heat is observed as a temperature of the object.

As heat may be transferred in several ways (radiation, conduction,...), there are two ways how phenotypic plasticity may happen. First, individuals may differ in their traits because of environmental constraints, e.g. plants may be bigger or smaller according to irrigation or a finger may be lost in an accident. This is known as passive phenotypic plasticity. Second, individuals may actively reflect the environment, so plant may open its flower in the particular part of a day or a cobra may raise its hood when encountering uncomfortable vibrations. This is known as active phenotypic plasticity, or, behaviour⁴. Conceptualisation of behaviour allowed its rigorous study, leading to flourish of ethology as a scientific discipline, with its own methodology and desire for universal laws and solutions (Portmann 2008).

Nature and limits of plant behaviour

In plants, discrimination of passive and active phenotypic plasticity is rather complicated, because most of the changes in plant phenotype are based on growth⁵. At the first glance, difference in size (biomass) may seem like the criterion. In conspicuously different, yet genetically identical individuals, no difference in size means active plasticity, and vice versa. While conceptually true, it is not a useful criterion. Even slight (and hence undetectable) differences in amount of growth may lead to immense differences in phenotype, because of allometry in trait values (Weiner 2004)(Figure 2). Moreover, as active plasticity is usually a response to the certain state of the environment, and the state of the environment that provokes the response is likely to constrain the growth, both types of plasticity may be entangled (Figure 3). Thus, apart from identification of activity of possibly divergent metabolic/regulatory pathways inside the organism, the only useful approach is detection of changes in allometry patterns (Huber & Stuefer 1997; Weiner 2004; Van Kleunen & Fischer 2005).

4 I am not aware of any objective and plausible reason why to differentiate between the terms. Reversibility, perhaps.

5 Tomáš Herben noted that in many languages, Czech included, the word for "plant" comes from the word for growth, indicating that the most salient feature of plants and of their life is growing.

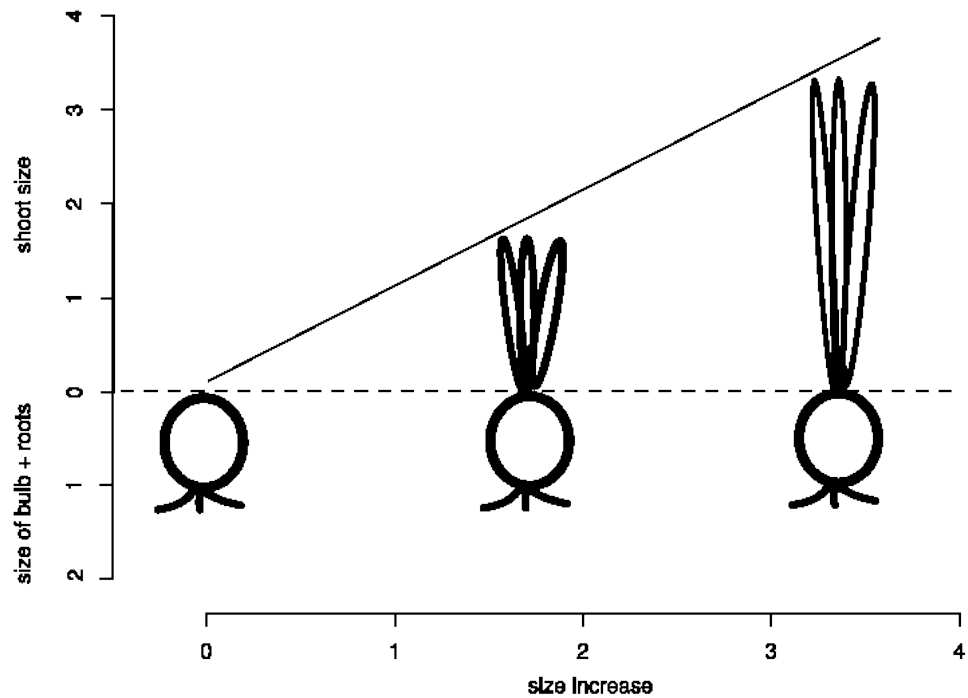


Figure 2 – Simple example of passive plasticity. Increase in size changes the plant body in two ways, yielding phenotype differences: the plants get bigger and leafier. Note that the notion of the difference depends on the selected trait: number of leaves -- number of roots ratio stays constant, also ratio of aboveground and belowground black pixel counts does not follow the same line as “vertical size belowground” - “vertical size aboveground” ratio. For more complicated examples, see e.g. Wright & McConnaughay 2002

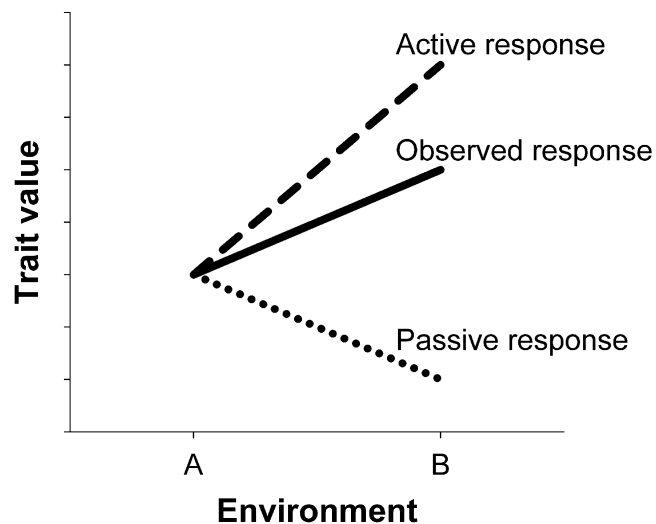


Fig 3 – The interplay of active and passive plasticity. Illustration of how an observed plastic response may be the result of both passive plasticity as a consequence of resource limitation, and active plasticity as a consequence of changes in allocation (from: Van Kleunen & Fischer 2005)

Although difficult, disentangling these two types of plasticity is worth the effort. If understood as behaviour, active plant phenotypic plasticity [or at least its substantial part (Bradshaw 1965)] becomes open to be studied with the methods of ethology⁶. As one of the results, plant behaviour research underwent Cartesian reduction: in order to get universal and general results, it partly abstracted from the role of the plant body as the plasticity bearer⁷. This process happened in several ways and each of these ways brought many fruitful concepts in delimiting the role of plant behaviour in plant ecology. Namely, three general approaches were used, which are reviewed further: i, behaviour of the model plant to model stimulus; ii, interactions among individuals in monocultures, and iii, generalisation of species properties at large scales.

In the simplest case, single model species experiments were used to show behaviour as a response to arbitrary stimulus. Several types of behaviour were demonstrated this way, e.g. ability to cope with temporal trends in nutrients availability (Shemesh et al. 2010), horizontal and vertical escape reactions (Novoplansky et al. 1990; Schmitt 1993), optimal allocation and foraging (Stuefer et al. 1996; Stoll & Schmid 1998), response to herbivory (Karban & Myers 1989), etc. Moreover, this simplest possible experimental setup was used to assess the evolvability of such behavioural traits, both directly (Van Kleunen & Fischer 2001), or indirectly through detecting behavioural differences among genotypes when subjected to various environments (“G×E”) (Skalova et al. 1997; Van Kleunen et al. 2000; Alpert et al. 2003).

The second approach was driven by analysis of feedbacks, i.e. the role of behaviour in interactions between individuals. As such, it used experiments based on the single species systems. Individuals of a single species are likely to have very similar body traits background and therefore just the role of behaviour may be investigated in its purest manifestation. Application of sociobiology with a game theoretic approach as its tool (Maynard Smith & Price 1973) provoked an interest in testing plants to show the predicted behaviour. In this way, tragedy of commons (Gersani et al. 2001; Boonman et al. 2006), recognition of kin or self (Falik et al. 2003; Gruntman & Novoplansky 2004; Dudley & File 2007; Crepy & Casal 2015) or just the immense role of detecting neighbour presence in coexistence (Stoll et al. 2002) were demonstrated.

Research at the level of individual species documented so many forms of behaviour, that it even allowed conceptualisation of plant intelligence (Trewavas 2005; Trewavas 2009). On the other hand, the true adaptive value of these behavioural patterns (i.e. whether they

6 Other views of phenotypic plasticity are of course possible. For example, when the term was coined, phenotypic plasticity was treated namely as a nuisance parameter in genetics and heritability studies, and subsequently as a mechanism that allows accommodation of phenotypic novelties into heritable set of traits. See e.g. (Pigliucci 2005) and references therein.

7 Note that in search for general (aspecific) rules, experiments comparing species reactions are not meaningful, as species identity, with its unique evolutionary history and set of body traits, is a confounding parameter.

improved fitness of their performers) was only seldom determined (but see Novoplansky et al. 1994; Stoll et al. 2002; Boonman et al. 2006; Huber et al. 2011; Keser et al. 2015), although calls for such studies were recurrent (Pigliucci 2005; Van Kleunen & Fischer 2005)⁸.

The third method resigned to experimenting and aimed at the theoretical boundaries of plant behaviour adaptivity. Such scale allows the generalization of species-specific constraints and hence generalisations in the form of the behaviour. Thus, species (or their virtual models) were just classified as more or less plastic, or more or less capable of certain form of behaviour. Again, this type of generalization proved to be useful, as it allowed to treat the role of plasticity in invasions (Richards et al. 2006), link the overall amount plasticity with habitat properties (Grime 1977; Alpert & Simms 2002) or hypothesize the role of certain behaviour in broad environmental context (de Kroon & Schieving 1990; Herben & Novoplansky 2007)

Navigation inside the limits of possible plant behaviour

Result obtained through above-mentioned experiments and generalisations shed light on the boundaries of plant behaviour in terms of possibility and its role in fitness. They revealed a wide palette of behaviours that plants can perform and a large number of settings where it is appropriate for a plant to use behaviour to survive and reproduce, instead to rely on some hard-coded trait. However, the territory inside these limits of possibility is mostly unknown. Specifically, we do not know how is the species diversity reflected in behaviour diversity, and whether there are patterns in body traits and behaviour. What if there is no pattern nor structure within the boundaries?



Figure 4 – “When pigs fly” is an English idiom for impossibility. Note that even for the illustration of impossible event of behaviour, the artist changed the body traits of its agent.

(<https://bulgingbuttons.wordpress.com/2013/11/25/when-pigs-fly/> [HeidiWeinmann]; accessed June 2015)

Although this question would sound ridiculous to any animal ethologist, as no one would expect ritual courtship flights in pigs (Figure 4), plant ecologists may be much more

⁸ Lack of positive evidence for behaviour adaptivity is not very surprising if we take into account that many elaborate structures in the realm of living organisms are not the direct outcome of natural selection, i.e., these structures do not fit the body as an interface for selection (Gould & Lewontin 1979; Portmann 2008). So, until the behavioural pattern does not negatively interfere with fitness of its agent, it may be developed and sustained, potentially becoming a pre-adaptive feature.

uncertain. Most of the plants share common resources and potential niches (Ellenberg 1954), so there is no surprise that neutral models of coexistence (Goldberg & Werner 1983; Hubbell 2005) were inspired by plant communities. To discover the patterns in this realm, I claim the need for a coordinate system, with axes based on similarity of species bodies, i.e. species traits and their syndromes (species blueprints⁹), and forms and extents of behaviours.

Closer look reveals that the set of the body/trait axes is well defined, i.e. plant species differ in their traits and these traits matter in species ecology. There is a good evidence that plant species life history traits determine suitable habitats, therefore predicting passive species co-occurrence (e.g. Grime 1977; Westoby 1998; Silvertown et al. 1999; Cornwell et al. 2014) or even active co-existence (Gaudet & Keddy 1988; Zobel et al. 2010; Wildova et al. 2012; Keser et al. 2015). So, functional differences between plant species exist and there are some principal axes of differentiation between species, therefore differentiation in behaviour may be expected to exist (Portmann 2008).

Homage to species diversity or Why there are so few behavioural data across species

As there is substantial evidence that genotypes of single species differ in their behaviour (Skalova et al. 1997; Van Kleunen et al. 2000), it is parsimonious to expect that species also will differ in their behaviour, too, i.e. that the second set of coordinate axes exists as well. Surprisingly, there is much less evidence than expected. Only a few studies dealt with behavioural patterns across many species (Campbell et al. 1991; Grime et al. 1997; Johnson & Biondini 2001; Lepik et al. 2005, Keser et al. 2015). Moreover, these studies worked with non-responsive stimuli that cannot mimic biotic interactions in community assembly processes. Species coexistence experiments involved only a handful of species (Semchenko et al. 2007; Semchenko et al. 2014), and I am aware only of single truly multispecific study in this context (Lepik et al. 2004).

Of course, the lack of data may simply result from laboriousness of experiments involving large sets of species (Pigliucci 2005), but I see the main reason for rarity of these efforts in incomparability of species¹⁰. For example, mechanism of root foraging for nutrient-rich patches strongly varies between species (Einsmann et al. 1999) and the same holds for upward growth in response to shading (Huber 1996). On the other hand, these differences constitute the species. Therefore, behavioural pattern in groups of species may be only quantified through species covariance in multivariate response space.

Apart of insufficient data for behavioural differences between species, the answer to the question of non-random patterns in species trait – behaviour combinations is further

9 EVO-DEVO: archetypes

10 My naive intent when I started my thesis research was exactly to perform such studies. In particular, I was interested in quantifying “competitive behavioural response” across many species, and linking this quantity (“strategy”) with species coexistence. While I am still interested, I see that it is the species diversity itself that in the same time allows and obscures such “strategies”.

obscured by above-mentioned interaction of passive plasticity and active plasticity (behaviour). Multiplicative nature of plant growth allows false positives in plant behaviour identification. Even small and therefore possibly random behavioural shifts in blueprint may be multiplied to large extents, making it conspicuous to the researcher but not necessarily so to natural selection. Apart of overall growth rate (e.g. Campbell et al. 1991; Grime & Mackey 2002), this pitfall may even involve discrete structures: modular nature of plants makes meristems also depletable resource, so shortage in their count may also hinder the growth, leading to allometric shifts, whose source is difficult to be identified correctly (Watson 1984).

Looking for the interplay of behaviour and its agent

Because of the difficulties, it is not surprising that although prevalent in the life of plants, documented indubitable cases of plant body traits in interplay with behaviour (*active* plasticity) are so rare (e.g. Huber 1996; Pecháčková 1999). I hope that the four studies I hereby present as my thesis would elucidate the plant body traits – behaviour interplay further.

In the first study, which I performed together with Tomáš Koubek and Tomáš Herben, I aim to address all the issues mentioned above. My main intent is to show how species life-history traits are coordinated with plant behaviour. In the same time, I show how multivariate is such behaviour. I document it using growth experiment with 40 species. Using a common garden setup, individuals of these species were treated with light of manipulated quantity and spectral composition. These manipulations allowed us to discern the effects of behaviour and passive plasticity. Therefore, we were able to identify species life history traits that underlie the amount of active and passive plasticity of shoot-expressed behaviour. However, the result I value most is that we clearly identified links between species life history traits and specific forms of the behaviour. Precision in trait definition allowed us to show that *seemingly* similar species traits (lateral spread and potential for vegetative reproduction) underlie non-overlapping behavioural patterns.

In the second study, which was performed by the same team, I examined the amount of plasticity that plant root systems exhibit in the patchy environments, and link the exhibited amount of root foraging plasticity with the same set of species specific life history traits as in the first study. Again, we arranged it as common-garden experiment with 37 species. Previously, some of the life history traits we used were linked to plant plasticity in general and it was hypothesized that plasticity is a common quantity for the whole plant body, both aboveground and belowground. Here we show that this conclusion may stem more from the multiplicative plant growth than from the active behavioural coordination of body traits development. Moreover, we discovered an unexpected link between root system behaviour and plant clonality: clonal species seem to perform much less root foraging than the non-clonals.

The third study was performed by Tereza Mašková, while I participated in its design, method development, data analysis and results reporting. We aimed to show how seed mass constrains root system development and whether these constraints could be relaxed by environmental supply of nutrients. For four weeks, we followed root development of seven species from the Fabaceae family in artificial substrate with manipulated levels of available nutrients. We show that seedling root system development is plastic, but the realized form of the root system is strongly affected by species specific seed size and the environment, thus that the seed size sets the boundaries for plasticity in root system architectural traits.

The last study is aimed to clarify the interpretation of clonal plant behaviour. I teamed up with Jan Smyčka to show that simple environmental constraints may substantially shape growth patterns in clonal plants. We modelled initially homogeneous belowground environment, where ramet presence was the only source of heterogeneity. In this environment, we described optimal distance between connected ramets as a function of spacer cost and belowground resource availability. In this way, we obtained a model for lateral spread of ramets according to productivity of the environment, i.e. a null model to test whether certain observed pattern of clonal lateral spread may be derived just from this modelled principle or whether there are other factors. Using database of species lateral spread traits, we showed that stolon-based clonal spread follows the predicted patterns to the great extent, while the rhizome-based spread does not. So, we demonstrated how lateral spread of clonal plants is constrained by the nature of body features used to spread.

Coda

Plant modularity predisposes them to clonality, and switch between these two forms is rather common (Klimeš et al. 1997). So, it is surprising that behavioural patterns of these two groups are so distinct and that the distinction holds even across the root/shoot boundary. Bearing this in mind, I wonder how “occasionally clonal” plants perform their roles.

Switch from the unitary organism, as a seed and early seedling is, to the highly modular one, as a plant is, may be even more dramatic change (Grime et al. 1997). However, commonness of this abrupt shift in body organization pushes it out of research limelights. Therefore, how this shift actually happens in the context of an environment is an open question.

I am happy that my friends, past and current students and collaborators seem to be fascinated with these questions, too. Together, we watch great performances in the plant diversity circus. As far as I can tell, costumes and tricks are amazing.

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Active and passive plastic response to light: are there any relationships to functional traits

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Introduction

Coexistence within a community challenges its members with the constant pressure to respond to the actions of others. One form this takes, competition for resources, is a major driving force in community assembly, so we can expect that its varying severity in space and time leads to evolution of adaptive behaviour. For example, potential competitors may use cues indicating possible conflict and take specific measures that allow them to respond with proper timing and magnitude. In this context, two extreme strategies may be described (Maynard Smith & Price 1973): strong “lions” which approach the resource regardless the presence of competitors, and highly mobile “vultures” which approach the resource only if no cues of “lion” presence exist (Novoplansky 2009). Although sessile, plants do possess abilities that are necessary for these behavioural patterns: they are able to respond to signals of competitor proximity (Novoplansky et al. 1990; Falik et al. 2003) and recognize competitors (Dudley & File 2007; Semchenko et al. 2014). However, for an individual, its fitness effects from following a particular behavioural approach to competition depend not only on the identities of potential competitors, but also on the abilities of the individual itself. Lions need to be strong and vultures need to be good flyers if lion-like or vulture-like behaviour, respectively, should bring them benefit.

Plant reactions (plasticity), like those of animals, include those on the physiological level, but it is plants' growth responses that correspond to animal behaviour (Bradshaw 1965). Growth-based plasticity necessarily yields changes in size and shape, and both size and shape are likely to change when plants compete for light (Schmitt 1993; Stuefer & Huber 1998; Valladares & Niinemets 2007). There are two principle possible causes of this. First, photosynthetically active radiation (PAR) is a resource needed for growth, and thus its abundance, by being a limiting factor for growth, can directly yield plasticity. Additionally, however, spectral composition of the light may serve as a cue of neighbour presence, *per se* (e.g. red/far red ratio, R/FR (Aphalo & Ballare 1995)), and this attribute of light may be used by plants as information to determine whether and how to grow in order to reduce the competitive effects of neighbour presence (Van Kleunen & Fischer 2001; Stoll et al. 2002). Light changes therefore cause both passive effects of changed resource (photosynthate) availability and active responses (active plasticity) (Weiner 2004; Van Kleunen & Fischer 2005). Importantly, capacity for the active response to light cues is constrained by the amount of light available as a resource to the plant.

However, in addition to the limitation imposed by its passive response to light resources, plastic response to environmental signals is further constrained by the individual plant body,

in terms of its state and form and life-history traits (Valladares & Niinemets 2007). These constraints differ among plant species, corresponding to their difference in key life-history traits, such as growth rate, height, and longevity. Species-specific sets of traits form the blueprint of the plant body (such as erect or creeping growth form, branching pattern, and module size) and are likely to constrain the range of options available to individual plants for plastic responses to environmental signals. Interestingly, although there are extensive data on interspecific and intraspecific trait variation, their role in constraining plastic responses to environmental stimuli is virtually unknown.

Insufficient knowledge of plasticity constraints contrasts with our better-developed understanding of the roles of plant life-history traits, namely size and height, in competition and coexistence. These traits are good predictors of the outcomes of forced plant competition (Gaudet & Keddy 1988; Goldberg 1996; Tracey & Aarssen 2011; Wildova et al. 2012), because it is the asymmetry of competing individuals either in size or in resource-economy that links traits and competition outcomes (Titman[Tilman] 1976; Schwinning & Weiner 1998). Although such direct competitive situations may involve plastic responses of plants to their neighbours, plasticity is also, perhaps more importantly, involved in response to cues of neighbour presence before actual competition takes place (Novoplansky et al. 1990; Aphalo & Ballare 1995). In terms of the lions/vultures metaphor, if a subject is forced to play the lion's role, its strength matters, although its other traits may be more appropriate to cope with the situation if allowed to do so (e.g. flight abilities if flying away to an unoccupied patch were possible). In a plant's world, low ability to outcompete neighbours may be more than balanced by the ability to avoid such situations in space and time (Grime 1977). However, experiments that involve forced competition necessarily reduce the amount of observed plasticity and are likely to falsely identify single relevant traits as the only crucial ones, although in fact many more traits, forming the phenotypic or behavioural niche, may play roles.

This raises the issue of recognizing the life-history traits that do in fact plant behaviour. As noted above, surprisingly, the trait-plasticity interplay has seldom been studied, so there are no direct cues to the identities of these traits. Only a few model species and a handful of structural, life-history traits have been examined so far (e.g. Huber et al. 1999; Benot et al. 2010, but see Grime et al. 1997). Therefore, a broad scan of life-history traits is appropriate. However, since some traits are known to correlate with species coexistence, namely those that describe module and shoot dynamics, spatial extent and reproduction rate (Westoby 1998; Herben et al. 2013), and plasticity is also correlated with species coexistence (Lepik et al. 2005), we can narrow the set of traits worth investigation to these, relevant traits.

In the present study, we aimed to investigate the plasticity-life-history trait relationship in competition for light. Because of the dual role that light plays in plant competition, we examine the links between plant traits and plant responses to both resource shortage and

neighbour presence cues. We used neutral and R/FR-changed shading of plants in a factorial design and compared their morphology to unshaded individuals grown in a common garden experiment. From the available life-history traits, we selected those that are known to both describe architectural blueprints and growth dynamics and to be correlated with coexistence of individuals. Diverse trait combinations and phylogenies were represented by using 40 herbaceous species, which we examined with respect to how the capacity of plastic response relates to life-history traits. Patterns in the plant response-plant traits matrix allowed us to discern whether plastic responses to light are associated with plant life-history traits and to identify the traits that are good predictors of magnitude of plant response to light, and to identify trait effect direction. Because of the complex nature of responses, we applied multivariate techniques that allowed us to pool the variability we observed in various measured response components. Separate analyses of neutral and R/FR-changed shading were used to identify sets of traits that constrain/determine responses to light signals.

Methods

Species selection

We selected 40 herbaceous species from six families of the Czech Republic flora using the following criteria: (i) perennial hemicryptophyte growth form; (ii) occurring in mesic unshaded or moderately shaded habitats (Ellenberg indicator value (EIV; Ellenberg 1992) for moisture <9 and EIV for Light >4). We also avoided species known for taxonomic complexity. Within each family, we selected species in a stratified manner according to species abundance in the Czech National Phytosociological Database (Chytrý & Rafajová 2003) and species height at maturity (taken from Kubát et al. 2002).

Experimental setup

The species were sown in the second half of April 2012 from seeds obtained from a commercial supplier (Planta Naturalis, <http://www.plantanaturalis.com>) in seedling trays in greenhouse. All plants germinated within one month and were transferred to 3-litre pots by the end of May. Both the seeding trays and the pots were filled with a 1:1 mixture of washed sand and potting substrate (Stender B400 (MC510), containing peat, clay, NPK 14-16-18, trace elements, and with pH 5.5-6.0) to simulate field conditions of low to medium productivity. Pots were arranged in 24 blocks with one pot of each species in each block, resulting in 960 pots altogether. Plants were drip irrigated twice a day for 10 minutes (approx. 330 ml per pot per day). During the week after the transfer, plants were surveyed daily and replaced if needed due to transfer shock.

During the second week after the transfer, we subjected all plants to one of six treatments (160 plants in each) that manipulated the total amount of photosynthetically active

radiation (PAR) and/or the red/far-red ratio (R/FR). These treatments were arranged in 24 shading blocks (four per treatment), spatially arranged in a systematic fashion. Each shading block was covered with a triangular “tent”, five meters long, 0.5 meter wide and 1 meter high with vents to avoid overheating. The tents were located one meter apart from each other with their long sides were facing south.

Three of these treatments just consisted of different levels of PAR: control, light grey and dark grey. The other three treatments consisted of combinations of PAR levels with R/FR shifts, to produce – light green, dark green and dark grey-green (see Table 1). Shading was achieved by using plastic colour film with known spectral characteristics (Lee filters) and shading cloths (Juta Adamov). Transparent film (Clear, Lee Filters no. 130) was used for control treatments and both grey treatments. Light green (Fern green, Lee Filters no. 122) was used for the light green and dark grey-green treatments, and dark green film (Moss green, Lee Filters no. 89) was used for the dark green treatment. Shading cloths were added to grey treatments to manipulate PAR in two steps with only a slight effect on R/FR. The exact spectral characteristics were measured with an Avantes spectroradiometer (AvaSpec-2048, VA 300, reading wavelengths from 326 to 1100 nm (see Table 1) at noon on a homogeneously cloudy day during the experiment (close to its end). PAR was characterised in terms of the ratio of irradiance (1-minute irradiance between 400–700 nm in $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$) in the shading block to the mean of the irradiances measured just outside the shading block 30 seconds before and after the measurement in the treated block. The R/FR ratio was calculated as the ratio of irradiance between 650–670 nm and 730–750 nm measured simultaneously with PAR.

Data collection

Images of the plants were recorded on the 16th and 17th of July 2012. Each plant was placed against a neutral grey background and photographed from both the top and the side. While being photographed, each plant was arranged so that the amount of leaf overlap was minimised in the side picture. For each plant, we used one camera to take photographs from above and another to capture images from the side. Both cameras were Canon DSLRs with APS-C chips and rectilinear lenses with negligible distortions and aberrations. The camera used for the top views would be placed a 2.5 meters above the plant and had a 17mm Sigma lens, while the one used for side views was placed 3.5 meters from the plant and had a 50mm Canon lens. Both cameras were attached to fixed tripods. Images were stored as high-resolution JPG image files.

Image analysis

Photographs were retouched to remove any images representing pieces of moss, and lightness was normalised using Adobe Photoshop CS6 to increase the dynamic range when needed. The photographs taken from above were loaded to FIJI (free ImageJ 1.48 frontend; (Schindelin et al. 2012)) and using the software, horizontal positions of plants rooting points were manually pointed at in the photographs, and their position recorded and stored in separate files. Similarly, we marked the vertical positions of the pot rims in the photographs taken from the side. All photographs were transformed to black and white images with white representing the green pixels and all remaining colours changed to black. So that no living aboveground parts of the plant were excluded by this process, green pixels were operationally defined for each batch of photos separately, such that they included not only all actual green parts, but also living tissue of other dominant colours (e. g. reddish); dead tissue (brown or yellow) was not included. Side- and top-view images were then denoised using different approaches for each of these views. Side-view images were denoised using 2 rounds of 9-cell kernel median filtering. Top-view images were denoised by removing particles smaller than 2 % of the biggest green part. All images were visually examined for concordance between their raw and cleared versions. The image analysis was done in Octave 3.6.1 (Eaton 2002).

The images were further processed to yield several variables representing both vertical and horizontal components of each plant's size and shape. Because the plant body could not be expected to grow homogeneously in space, we used non-parametric measures. From the side images we estimated **Median Height** (i.e. height above which 50 % of pixels occurred). As a second moment, we used an interquartile range of pixel heights, i.e. the distance between the height of the top of the 1st and bottom of the 3rd quartile pixels ("middle half of the pixels", further denoted **Vertical Range**). As a third moment, we used Bowley nonparametric skewness (Groeneveld & Meeden 1984), i.e. the normalized ratio of the spans of the 2nd and 3rd quartile (further denoted **Vertical Skewness**). As an overall measure of plant size and a proxy for the leaf area index, we used the **Total Area** of the green parts shown in the side view. For all side-view variables, only plant parts above the pot rim were used.

Similar measurements were taken from the top-view images, using distance from the rooting point instead of height (**Median Distance** of green pixels from the centre, **Distance Range** as the width of the central 50% of green pixels and **Distance Skewness** calculated as above). In addition, we measured the distance between each rooting point and the plant centroid (gravity centre in the flat image) (**Asymmetry**). This measurement was used to detect directional horizontal growth or change in the growth form from upright to prostrate.

Species life-history traits data

The following species life-history traits (hereinafter referred to as “traits”) were selected from various databases to represent size and growth dynamics of the species involved: plant height at maturity, mean area of leaf, specific leaf area (SLA), shoot lifespan (cyclicality, see Klimešová & de Bello 2009), capacity for vegetative and sexual reproduction assessed by long-term observation in a botanical garden (Herben et al. 2012), clonality (i.e. capacity to form new ramets by clonal growth, a binary trait), and lateral spread (in meters. J. Klimešová, unpublished data, Klimešová & de Bello 2009). Values of these traits were taken from the LEDA traitbase (Kleyer et al. 2008), CLOPLA database ver. 3.2 (Klimešová & de Bello 2009) and Kubát et al. (2002). Mean leaf area was log-transformed before analysis.

Treatment response data – estimation of the reaction norm magnitude

The measured variables express the sizes and shapes of individual plants and their responses to shading. As these responses potentially involve all the measured variables, for the comparison between shaded plants and unshaded controls, we used redundancy analysis – a multivariate analogue of multiple regression, implemented in the *rda* procedure from the *vegan* package (ver. 2.0-0, (Oksanen et al. 2011) in the R statistical environment (ver. 2.15.1, R Core Team 2012). We treated the measured variables as dependent variables and values of red/far red ratio (RFR) and/or amount of photosynthetically active radiation (PAR) as independent variables. Each species was analysed separately. Plain numeric values of both R/FR and PAR (Table 1) were used in the analyses because initial checks showed that lack-of-fit due to non-linearity of the response was low (Figure S1 in the Appendix).

We first determined the overall magnitude of response of each species to the light signal and contributions of its components, i.e. gross and net contributions of R/FR, gross and net contributions of PAR, and their overlap using adjusted R² (this is the only unbiased method; see Peres-Neto et al. 2006). For variance partitioning, we used the *varpart* procedure from the *vegan* package. The adjusted R² values express whether and how much the given species responded to the light treatment, whether it responded more to R/FR or PAR, and whether the responses to PAR and R/FR are independent of each other (i.e. show no interaction). We call these values response magnitude (or magnitude of the response).

We assessed the correlation of magnitudes of response with species traits. Pearson correlation coefficients were used for numeric variables; Spearman correlation coefficients were used for binary and ordinal traits (function *rcorr* from *Hmisc* package ver. 3.9-3, Harrell 2012). Their values are reported with uncorrected significance values; overall significance of the relationships between the R² matrix and trait matrix was tested using a Mantel test employing Euclidean distances of standardized variables; species with non-complete traits

data were omitted.

Linking components of species reaction norms to species traits

To examine how individual measured variables (i.e. shape parameters) contributed to species reaction norms, we determined raw scores of individual measured variables along the first canonical axis of the respective ordination.. In cases where light availability axis direction was not concordant with canonical axis direction, the raw scores were multiplied by -1 to unify the results. We call these scores components of species response. For each species, we ran four redundancy analyses, to determine these scores of the measured variables as related to each of the following four effects: (i) effect of PAR; (ii) effect of R/FR; (iii) net effect of R/FR, with PAR as a covariate; and (iv) net effect of PAR, with R/FR as a covariate.

Next, we assessed the relationships of these species response components (scores) with the same species traits whose correlations with reaction norm magnitudes we had already determined. For each ordered set of scores, we estimated the position of the neutral reaction, i.e. the position of the (hypothetical) species with score equal to zero. If it was in the middle third of the set, i.e. between the 33% and 66% quantile, we concluded that the reaction occurred in both of the other thirds of the set. Likewise, position of the neutral reaction in the lower or upper third of the ordered set of scores indicated that reaction increased or decreased towards the other respective end of the score set.

Results

Interspecific variation in reaction norms

Effects of treatments varied substantially among species. The total effect of the light treatment explained between zero (*Hypochaeris radicata*, *Geum urbanum* or *Silene nutans*) and 40% (adjusted R^2) of the total variance in size and shape (in *Bothriochloa ischaemum* and *Gypsophila paniculata*). Individual species also differed strongly in the specificity of their responses to RFR and PAR. Some species showed high overlap (low specificity) in their responses, making active and passive components of the plasticity merely indistinguishable (e.g. *Filipendula ulmaria*, *Avenula pratensis*, *Cerastium arvense*), whereas others responded with active plasticity to RFR, independently of their response to PAR (e.g. *Fragaria vesca*, *Prunella vulgaris*, *Geum rivale*, *Filipendula vulgaris*), (Figure 1). Similarly, mean reactions of measured variables differed strongly in response to treatment type (Appendix S3).

Species traits and reaction norm magnitude

The magnitude of species responses was significantly related to species traits, namely traits that represent vegetative reproduction (Mantel test, Mantel $r = 0.162$, $P = 0.039$, 999 permutations; $r = 0.179$, $P = 0.032$ if gross effects of R/FR and PAR are excluded). Species

with high capacity for vegetative reproduction and/or morphological adaptations for lateral spread showed strong specific responses to R/FR (even stronger when the effect of PAR was partialled out; Table 2). In contrast, species that are able to attain greater height at maturity responded more strongly to PAR, and showed a tendency to not respond to RFR. Plants with bigger and softer leaves (high SLA) tended to not respond to PAR.

Species traits and reaction norm components

Among species traits, generative reproduction rate, clonality *per se* and height at maturity were not correlated with any particular treatment-based difference in measured variables (“shading syndrome”), although some of these traits were correlated with the magnitude of the reaction (see above). Likewise, the ability to manipulate vertical skewness (i.e. to manipulate degree of asymmetry in distribution of photosynthetic organs around the median height), was not significantly linked to any species trait (Figure 2, Appendix S2).

In contrast, shoot lifespan, vegetative reproduction potential and leaf size seem to be the most constraining traits, because they strongly underlie several particular reaction norms. Plants with high potential for generative reproduction place their biomass higher when subjected to the high R/FR treatment, while plants with low potential for vegetative reproduction respond in the opposite way. Shoot lifespan is deeply connected to the reaction to PAR shortage. With increasing PAR, plants with long-lived shoots showed increase symmetry around their rooting points, and decreasing distance ranges. Plants with short-lived shoots mostly had the same degree of asymmetry regardless of PAR and increased the distance ranges of their horizontal biomass distribution with increasing PAR. Reaction to R/FR, i.e. a signal of competition, was strongly correlated with leaf size. Plants with big leaves were shorter (i.e. concentrated their biomass closer to the ground) and were smaller (in terms of side-view area) when the red part of light spectrum was not depleted, whereas plants with small leaves reacted in the opposite way. Specific leaf area correlated with ability to react to R/FR changes: plants with cheap unit leaves (high SLA) decreased their aboveground cover when there was no sign of competing neighbours, whereas plants with expensive (and small, see above) leaves had greater aboveground cover when there was no sign of competing neighbours.

Vegetative reproduction potential was associated with two distinct patterns of responses to light. In the first, involving responses to PAR, plants with high vegetative reproduction potential filled the vertical space more fully and more evenly with increasing PAR supply. In contrast, plants with low vegetative reproduction potential responded to increasing PAR, by concentrating the leaves vertically, but did not change the overall amount of aboveground live material. In the second, involving responses to R/FR, if the red part of the light spectrum is not depleted, plants that are likely to reproduce vegetatively tend to be more symmetric around the rooting point. Also, if there is no sign of neighbour presence (i.e. if the R/FR is

high), plants with high potential for vegetative growth arrange their photosynthetically active biomass further from the rooting point, while plants that have low vegetative reproduction potential concentrate their light harvesting tissues closer to the rooting point. Although lateral spread is usually viewed as tightly correlated to vegetative reproduction potential, our results show that it is connected to a PAR-induced reaction and does not constrain reaction to the neighbour presence signal (R/FR). With full PAR supply, plants with short- distance lateral spread tend to be symmetric, while far-spreading ones stay the same as those treated with low levels of PAR.

Discussion

Plant life-history traits constrained plant behaviour at two distinct levels. Either they constrained the magnitude of phenotypic plasticity exercised in the experiment, or they formed the basis for particular reactions, i.e. pronounced distinct components of the reaction norms. Furthermore, these roles did not always overlap. Thus, while vegetative reproduction potential and ability to spread laterally constrained behaviour in both ways, plant height at maturity was related to the magnitude of PAR-related reaction norm but did not correlate with any specific component of this reaction. In contrast, leaf economy did not limit either PAR- or R/FR-related reaction magnitude, but strongly affected the form of the latter.

With respect to the reaction norm magnitude limitation, plants that reproduce vegetatively and spread far laterally displayed strong responses to R/FR, i.e. neighbour presence signal, with only slight traces of PAR limitations. This suggests the actively plastic nature of the response to R/FR, already documented for some creeping plants (Novoplansky et al. 1990; Stuefer & Huber 1998; Vermeulen et al. 2008; Skálová 2010). This earlier evidence, however, was only anecdotal, not allowing for assessment of the relative role of this set of life-history traits. Here, on the other hand, we show its crucial importance in the ability to react to neighbour presence.

In contrast, tall plants are likely affected by overall light supply, as the magnitude of their reaction norms are highly correlated with available PAR, and with relatively high overlap between PAR and R/FR effects. Therefore, their reaction norm magnitudes probably mirror just the degree of darkness, not the spectrum of the remaining light. Lack of specificity in their reaction norms may mirror passive species-specific size-related shifts in allometric space (Wright & McConnaughay 2002; Weiner 2004). This would occur because as tall species are likely to be fast-growing in absolute terms, and therefore more prone to passive effects of resource shortage than are small, slowly growing species. However, this allometric shift also may be either active or adaptive. Nevertheless, it is the potential magnitude of the reaction that yields reduction in above-ground competition asymmetry, and thus the presence of more individuals, and, in turn, coexistence of more species (Stoll et al. 2002; Lepik et al. 2005).

Our examination of relationships between life-history traits and reaction norm components to both PAR and R/FR treatments revealed specific components of reaction norms related mainly to the following groups of traits (or trait groups): leaf characteristics, shoot lifespan and vegetative reproduction potential. Thus, the set of trait correlated with the most pronounced components of the reaction norms only partially overlaps with the set of traits correlated with reaction norm magnitude *per se*.

Among the traits correlated with expression of reaction norm components, only the leaf economy traits have been unequivocally reported as correlated with the reaction that we observed (Lepik et al. 2004; Boonman et al. 2006). Broad-leaved, high-SLA species were likely to form dense and high “umbrellas” of leaves when encountering the neighbour presence signal, presumably using leaf biomass surplus to outcompete their neighbours (e.g. compare *Lespedeza* to *Miscanthus* in (Anten & Hirose 1999)). Inability of small-leaved species to perform such a reaction forces them to respond by avoiding competition in vertical growth and cover density (Givnish 1982; Falster & Westoby 2003) and allows them to allocate the resources to the structures that are more likely to be profitable (Novoplansky 2009).

Several reaction norm patterns have been reported for clonal plants capable of lateral growth: shading inducing spacer elongation (e.g. Slade & Hutchings 1987; Dong & de Kroon 1994) or not (e.g. Caradus & Chapman 1991) and shading increasing the proportion of far-reaching structures (Skálová 2005) or not (Huber & Stuefer 1997; Skálová 2005). The settings of the experiments that reported these results varied from field experiments to spectrally controlled shading in greenhouses. We found substantial differences in the effects of PAR shortage and decreased R/FR, which may partially underlie the discrepancies in the results reported in these earlier studies. In response to increasing R/FR, i.e. to decreasing strength of the neighbour presence signal, plants with high vegetative reproduction potential decreased growth asymmetry and increased relative amount of far-reaching structures. This reaction syndrome suggests reallocation of resources to a unidirectional, phalanx-like crawl in the presence of neighbours. Plants with low potential for vegetative reproduction responded by decreasing the amount of far-reaching parts, which may be achieved by shortening the clonal spacers or petioles and branches.

Life-history traits that form the clonal plant archetype may cause further confusion. The reaction norms to R/FR that we observed seem to be connected solely with the rate of vegetative reproduction, not with either plant clonality or distance of lateral spread. Furthermore, there is only partial overlap among PAR-related reaction norm components constrained by seemingly similar “clonal” traits. High vegetative reproduction potential allowed plants to respond to increasing PAR by filling the aboveground space more fully, probably due to higher numbers of developed ramets or stems. In contrast, in plants that do not spread laterally very much, increased PAR supply led to decreased asymmetry, while far-

spreading ones stayed the same. Thus, responses to neighbour presence and neutral shading partially overlap, but are mainly based on seemingly similar, but functionally different life-history traits.

Moreover, reaction to decreased PAR may arise because of an allometric shift (Weiner 2004). This shift may underlie the apparent connection of PAR, symmetry, shoot longevity and lateral spread, as the response may reflect side-leaning of plants with feeble, single-season stems that do not spread laterally. Whether this is an adaptive reaction, enabling lateral movement to better lighted spots or instead is just an incidental outcome of weak stems remains an open question, but some evidence suggests that, as a part of the plant-shading syndrome, it is in fact adaptive (Huber et al. 2011).

Plant body modularity allows several modes of reaction, viz. changing the number of modules, changing the size and shape of the modules and combinations of these processes over all modules or just subsets of them. Clearly, such diversity of possible response norms is difficult to comprehend. We transformed this diversity into spatial characteristics, with a few cardinal moments each, but the number of dimensions still remained high, prompting our usage of multidimensional methods. This allowed us to summarize the responses in a single quantity, the response norm magnitude. Because we were able to detect the response norms of substantial magnitude, we worked backwards to shed light on underlying mechanisms, describing the links between life-history traits and response reaction components. The need for the analysis of life-history trait- response component structure is twofold. First, when assessing the correlations of only the magnitudes of the reaction norms, which are directionless, with species traits, cases of strong reactions in species on the opposite side of the trait scale are disregarded. This is less likely to be an issue in passive reactions, but may hinder detection of actively plastic reactions. Second, correlations with response components demonstrate proximate mechanisms of the reaction and suggest its general life-history constraints.

Although we believe that our results bring important insights, two caveats should be kept in mind. First, intraspecific variation in phenotypic plasticity may be tremendously important (e.g. (Schmitt 1993; Skálová et al. 1997; Hinsberg & Tienderen 1997)), so attempts to generalize from results should always be treated with caution. Second, apart from intraspecific variability and local adaptation, the experimental setup we used may be inappropriate for some species and their reaction modes. Namely, some species are known to adjust their reactions according to the spatial homogeneity in shading (Semchenko & Zobel 2007; Vermeulen et al. 2008; Bittebiere et al. 2012). However, even unexpected and seemingly extreme combinations of life-history traits and plant behaviour have been demonstrated to have positive fitness effects (Huber et al. 2011), so in a setting that is at least marginally realistic, it may be expected for traits and reaction norms to be in accord.

Conclusion

Based on the obviously non-random patterns displayed by the data, we believe that reported life-history traits effects substantially shape plant plasticity niches and, consequently, affect community assembly in situations where phenotypic plasticity is a major driving force (Grime & Mackey 2002; Lepik et al. 2005). However, we show that contrasting effects of seemingly similar life history traits, viz. lateral spread distance and vegetative reproduction potential, may lead to improper generalizations, as apparent similarity in traits from an experimenter's point of view may be only a weak indicator of trait similarity from their bearer's point of view.

Author contributions

Designed the experiment: MW, TH, TK; Performed the experiment: MW, TK; Analysed the data: MW, with discussions with TK and TH; Written the paper: MW, with contributions of TK and TH.

Acknowledgement

This study was supported by Czech Science Foundation grant no. P505-12-1007.

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Tables and Figures

Table 1 – Spectral characteristics of the treatments

Treatment	clear	light green	dark green	dark grey-green	dark grey	light grey
			moss			
Lee filter	clear (130)	fern green (122)	green (89)	fern green (122)	clear (130)	clear (130)
shading cloths	none	none	none	2 × loose	2 × dense	loose + dense
mean background PAR ($\mu\text{M}\cdot\text{s}^{-1}\cdot\text{m}^2$)	334.66	328.13	290.52	258.33	270.08	272.43
mean PAR in shading block ($\mu\text{M}\cdot\text{s}^{-1}\cdot\text{m}^2$)	321.10	129.14	70.17	45.71	30.58	55.13
PAR ratio (%)	95.95	39.36	24.15	17.69	11.32	20.24
background R/FR ratio	1.076	1.077	1.080	1.087	1.084	1.082
R/FR ratio in the shading block	1.077	0.304	0.173	0.220	0.799	0.892

Table 2 - Correlations between species traits and species responses to treatment factors. Values in the table are Pearson correlation coefficients except in the cases of Seed Reproduction, Vegetative Reproduction, Shoot Lifespan (cyclicality) and Clonality, for which Spearman rank correlation values are used. + - $P < 0.1$, * - $P < 0.05$, ** - $P < 0.01$ (uncorrected significance levels). The overall relationship between traits and variance explained is significant (Mantel test, Mantel $r = 0.162$, $P = 0.039$; $r = 0.179$, $P = 0.032$ if gross effects of R/FR and PAR are excluded).

Trait	Variance explained by					
	RFR and PAR	RFR	RFR partial	PAR	PAR partial	Overlap
Leaf area (log)	-0.213	-0.017	-0.034	-0.223	-0.24	0.012
SLA	-0.133	-0.041	0.001	-0.159	-0.127	-0.053
Height at maturity	0.172	0.006	-0.185	0.337*	0.231	0.233
Seed reproduction	0.262	0.208	0.044	0.168	0.087	0.175
Vegetative reproduction	0.334*	0.305+	0.496**	0.146	0.184	0.019
Shoot lifespan (cyclicality)	-0.062	-0.09	-0.002	-0.086	0.012	-0.136
Clonality	0.054	-0.068	0.25	-0.032	0.118	-0.2
Distance of lateral spread	0.158	0.107	0.447**	-0.139	0.11	-0.389*

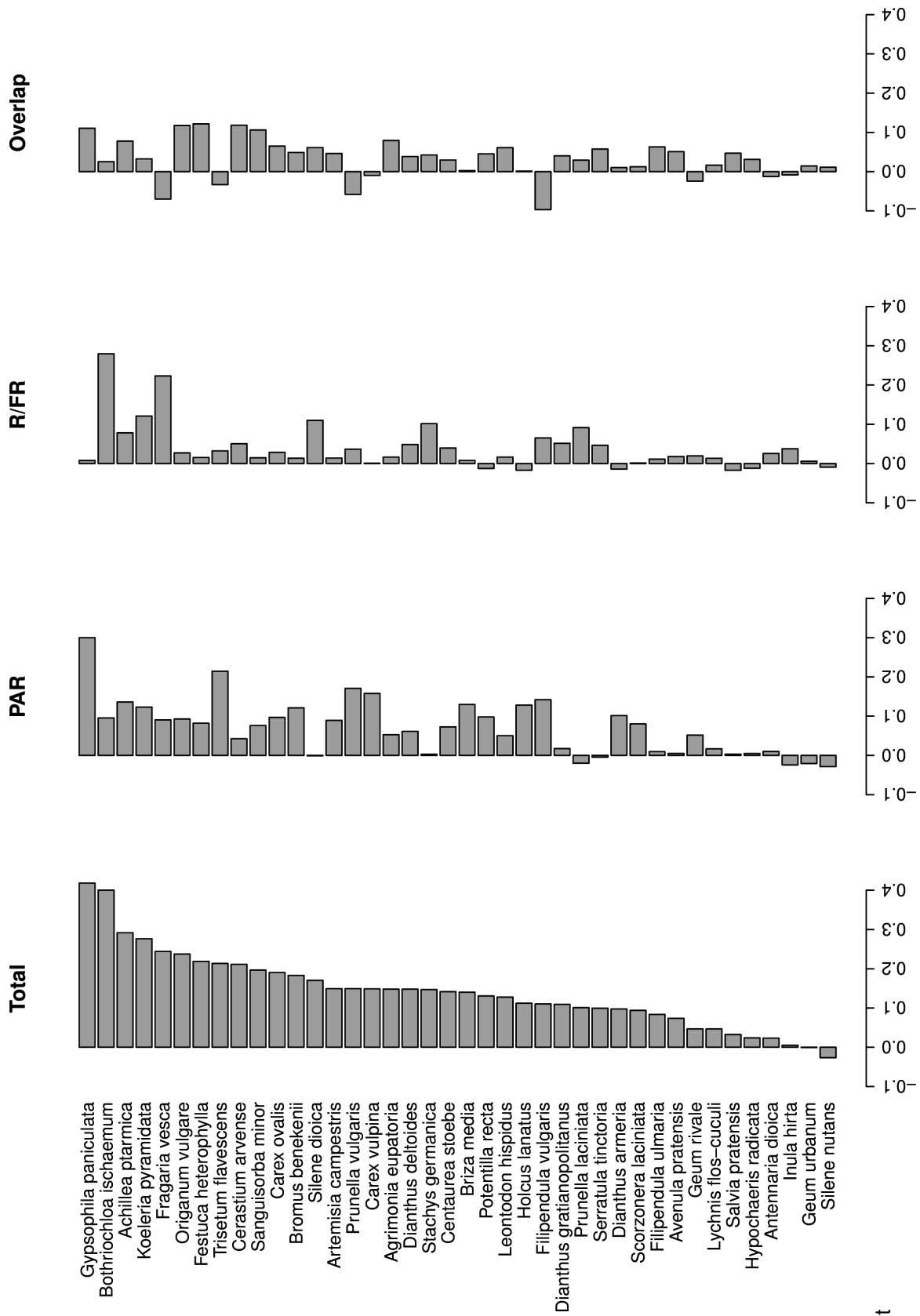


Figure1 - Species response norms and their partitioning. Depicted are adjusted R^2 for measured variables affected by both linear effects of PAR and RFR (Total), just PAR, R/FR. Overlap is the amount of variability in measured variables that can be attributed both to the effect of PAR and R/FR

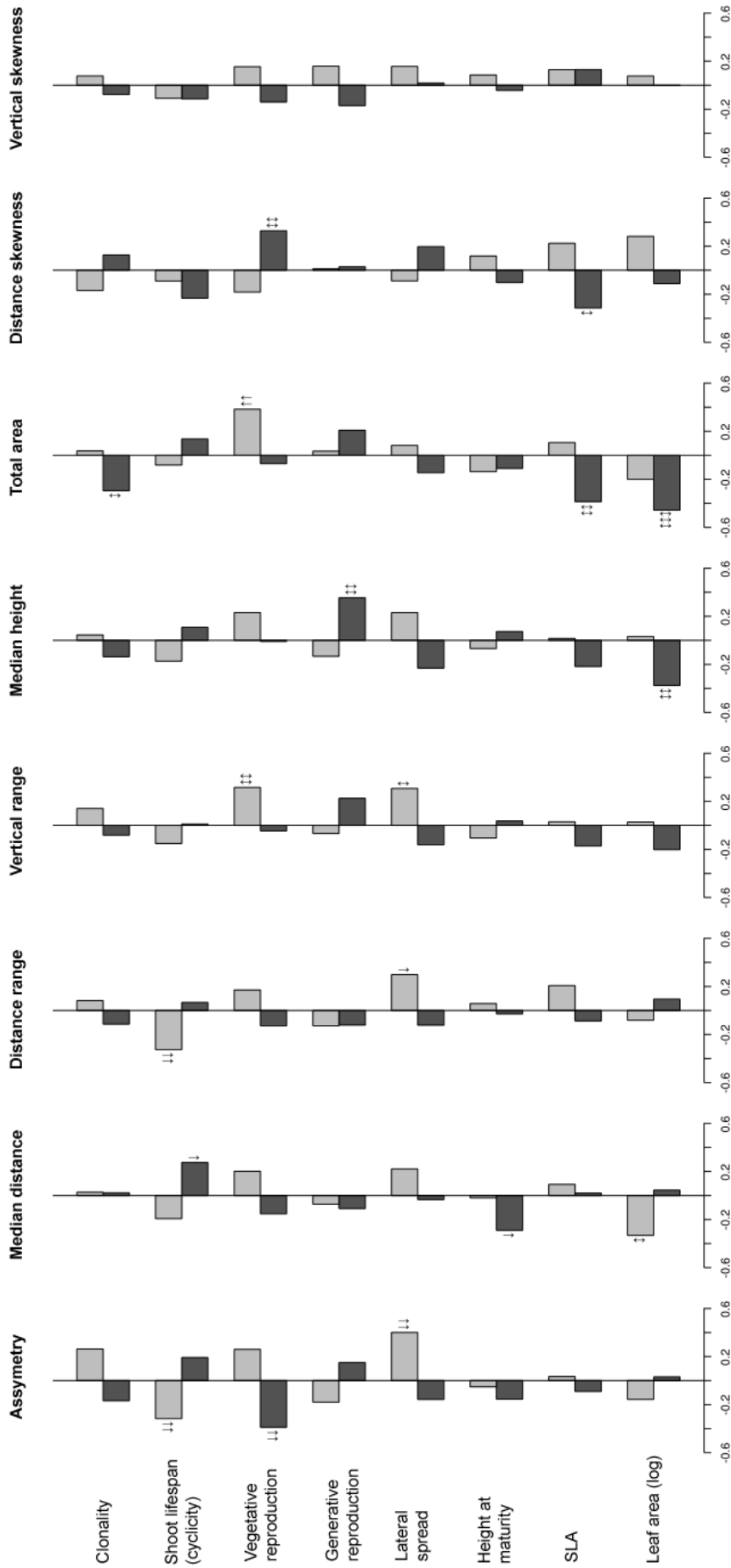


Figure 2 – Life-history traits affect reaction modes to change in PAR and RFR. grey bars – reaction to increasing PAR, green bars – reaction to increasing R/FR, i.e. diminishing competitive signal. Negative values: measured variable score decreases with species trait value. Positive values: measured variable score increases with species trait value.

↑ - at least 2/3 of the measured variable scores are above 0, i.e. in the case of positive bars, with increasing species trait value, the reaction of measured variable to increasing light (increasing light or decreasing competitive signal) increases from less pronounced to more pronounced; in the case of negative bars, the reaction changes from strongly positive to neutral,

↕ - measured variable scores are dispersed around 0, i.e. in the case of positive bars, the reaction of the measured variable to increasing light changes from negative to positive with increasing values of the species trait, and in the case of negative bars, reaction of the measured variable to increasing light changes from positive to negative with increasing values of the species trait

↓ - at least 2/3 of the measured variable scores are below 0, i.e. in the case of positive bars, the reaction changes from strongly negative to neutral, and in the case of negative bars, the reaction changes from neutral to strongly negative

↑, ↑↑, ↑↑↑ - significance levels ($p < 0.1$, $p <= 0.05$, $p <= 0.01$)

Prediction of root foraging ability by life history traits

Authors: Martin Weiser, Tomáš Koubek, Tomáš Herben

Introduction

For plants, soil is the source of various essential resources with contrasting repletion and depletion dynamics and spatial patterns (Craine & Dybzinski 2013). In contrast, the light supply above ground is continuous, with instant repletion and a general pattern of the gradient. Therefore, as the soil heterogeneity pattern is the sum of the heterogeneities of the individual resources, the below-ground environment is likely to be more heterogeneous and spatially less predictable than the above-ground environment. Plant roots need to develop in a way that allows efficient extraction of the resources from the highly heterogeneous environment that soil is. Phenotypic plasticity in root growth, architecture, and spatial placement may be an answer to this problem (Bradshaw 1965).

Ample evidence of a plastic response in root growth and placement has been obtained from experimental systems illustrating root searching patterns in response to gradients of water and nutrients (Drew 1975; Hodge et al. 1999). Indeed, roots are the plant organ for which foraging for resources has been most convincingly demonstrated (Hutchings & de Kroon 1994). However, the degree of such root plasticity strongly differs among species (e.g., Campbell et al. 1991; Kembel & Cahill 2005; Keser et al. 2015), indicating the existence of a factor that constrains this potential. We see two possible sources of such constraint: (i) differences in growth rate and resulting overall root system size, and (ii) differences in the processes that determine the size-independent component of root system shape.

Differences among individuals in degree of growth and growth rate constitute their passive plasticity. Passive plasticity arises when different degrees of growth and the resulting disparity in size produce different phenotypes in genetically identical individuals. These dissimilarities may be even more pronounced if the growth multiplies initial differences in body form. In contrast, differences in body form that change allometric patterns among individuals constitute active plasticity, that is, the size-independent changes in body shape that typically occur as a result of responsive behaviour to an environmental signal. A common example of this is root foraging, that is, changes in spatial root system allocation in response to a nutrient or water gradient (Hutchings & de Kroon 1994; McNickle & Brown 2014b). Although both types of response are based on growth, passive plasticity and active plasticity are in principle independent of each other. Only passive plasticity is the direct outcome of overall amount of growth (Weiner 2004; Van Kleunen & Fischer 2005). However, it may constrain options available for active plasticity to take place.

Separating size-dependent and size-independent components of root foraging is also necessary for a careful analysis of the commonly discussed concepts of foraging scale and

precision (Campbell et al. 1991). Both of these concepts are intimately linked to growth rate and resulting differences in size, and analysis of their potential relationship must consider these two components separately. Indeed, Kembel & Cahill (2005) reported that the size-independent (because of the log transformation they used) component of foraging is only weakly related to growth rate.

Although growth and the resulting size patterns constitute one of the most fundamental ecological differences (Gaudet & Keddy 1988; Cornwell et al. 2014; Aarssen 2015), differences in size have a special bearing on response to environmental heterogeneity as encountered in the soil. With an increasing rate of root growth, root-perceived spatial heterogeneity naturally decreases. The faster the root tip moves through the soil matrix, temporal heterogeneity it processes decreases in grain size. Root plasticity cannot reflect soil heterogeneity if heterogeneity occurs on too small a scale (Alpert & Simms 2002), but scale size is determined by traits of the individual, namely, size and growth rate. This concerns both intra- and interspecific differences in size. As plant species differ in their growth rate even within a single environment (Grime & Hunt 1975), differences between fast and slow species may open the field for root systems specialized in harvesting small-scale patches, that is, patches at the level of heterogeneity overlooked by fast-growing species.

Passive plasticity, based purely on growth rate, however, is very unlikely to be the only factor underlying interspecific differences in root system plasticity, namely, root foraging. If root system plasticity is more pronounced in fast-growing species simply because of multiplicative growth effects (Aanderud et al. 2003; Kembel & Cahill 2005), it would result in a paradox: slow species should forage better, that is, should be more plastic, to respond to rich patch presence at the small scale, but cannot, whereas fast-growing species could be more plastic, but need not to forage, as they average the outcome of resource acquisition across the patches and grow through the rich patches (Alpert & Simms 2002). The solution to this paradox involves an actively plastic element of behaviour that is (or at least can be) more pronounced in slow species.

Further evidence that root foraging is the behaviour beyond passive plasticity comes from the similarity between root foraging and root competition, which is another root system plasticity phenomenon. In root competition, it is active plasticity that obviously plays a major role (e.g., Gersani et al. 2001). Because of the symmetric nature of plant interactions below ground (Schwinning & Weiner 1998) and their high frequency (Goldberg & Barton 1992; Craine & Dybzinski 2013), it is probable that the root systems meet the heterogeneity generated by another individual, at a scale that allows a plastic response (Craine et al. 2005; Craine 2006). Various species-specific strategies have been reported to be expressed in this context [e.g., nutrient pre-emption (Padilla et al. 2013), root overproliferation (Gersani et al. 2001), patch avoidance (McNickle & Brown 2014a)], which suggests that a universal solution does not exist. These responses to neighbour presence were found to be context specific

(Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Dudley & File 2007; Semchenko et al. 2007). Diversity in these responses to neighbour presence implies that apart from the growth rate, there are several life-history traits (body constraints) that constrain the plastic response of root systems to soil heterogeneity.

We, however, possess almost no information explaining the existing large interspecific differences in root system plasticity and, in particular, active plasticity. Although plant plasticity has seldom been studied in interspecific comparisons (Lepik et al. 2004; Lepik et al. 2005), and therefore its interspecific patterns are little known, the constraints shaping the plasticity of plant shoots are better known. For example, growth form and clonality constrain the plasticity of plant shoots (Dong & de Kroon 1994; Huber 1996; Huber et al. 1999, Weiser et al., this volume). Because root and plasticity have been found to be correlated (Grime et al. 1997), we may expect that the same or similar life-history traits could be used equally well to predict root plasticity. Moreover, the existing knowledge of ecological functions of life-history traits (Westoby 1998; Cornwell et al. 2014) may help to identify potential constraints/predictors of passive and active plasticity. Some traits are likely to determine interspecific differences in size and growth (i.e., components of passive plasticity). Such traits include, for example, maximum height or specific leaf area as a proxy for growth rate. Constraints on active plasticity are more elusive, but should be searched for among traits that constrain size-independent differences in plant bodies. A great advantage of all these life-history traits is that their values are known for large sets of species. Either these traits are already catalogued in species descriptions (e.g., species height at maturity, clonality, leaf size) or may be easily obtained from species cultivation, namely, collections in botanical gardens. Therefore, they can potentially be used as proxies for the ability of species to respond to below-ground heterogeneity, which is much more difficult to measure. In the work described in this article, we therefore determined differences in root system plasticity in a large set of herbaceous species and examined potential constraints and predictors of these differences. To obtain root system plasticity estimates comparable to those in previous studies (e.g., Campbell & Grime 1989; Campbell et al. 1991), we essentially replicated the approach based on root foraging, that is, allocation of roots in a patchy environment. However, we used data analysis techniques that allowed us to demonstrate plasticity regardless of the body size of its bearer, that is, to identify the component of active plasticity. We linked these estimates with life-history species traits that presumably predict either growth rate and size or size-independent differences in plant bodies. In addition, we also used realized vegetative and seed reproduction rates as estimates of the functional outcome of these traits (Herben et al. 2012). We compared the predictive power of these life-history traits with that of specific leaf area (SLA), the prominent trait reported to determine root system plasticity earlier (Grime et al. 1997).

Methods

Species selection

We selected 43 herbaceous species from 14 families of the flora of the Czech Republic using the following criteria: (i) perennial hemicryptophyte growth form and (ii) occurrence in mesic unshaded or moderately shaded habitats [Ellenberg indicator value (EIV) for moisture <9 and EIV for light >5 (Ellenberg 1992)]. We avoided species known for their taxonomic complexity. To represent the taxonomic composition of the flora, we used several species from four widespread families (Asteraceae, Caryophyllaceae, Poaceae, and Rosaceae), together with a few species from less diverse families. Ten species were tested in the year 2013; another 33 were tested in 2014. For the final analysis, we excluded 6 species because they had very small roots and, therefore, were vulnerable to errors in root biomass processing. See the full species list with additional information in Supplementary1 (S1).

Species life-history trait data

The following species life-history traits (hereinafter referred to as “traits”) were selected from several databases to represent the size and growth dynamics of the species involved: plant height at maturity, mean area of leaf, specific leaf area, shoot life span [cyclicality, see Klimešová & de Bello (2009)], clonality (i.e., capacity to form new ramets by clonal growth, a binary trait), and lateral spread (in metres) (J. Klimešová, unpublished data; Klimešová & de Bello 2009). Values of these traits were taken from the LEDA trait base (Kleyer et al. 2008), CLOPLA database Version 3.3 (Klimešová & de Bello 2009), and Kubát et al. (2002). Further, we used capacity for vegetative and sexual reproduction assessed by long-term observation in a botanical garden (Herben et al. 2012) as additional information on species reproductive strategy. Mean leaf area was log-transformed before analysis. Some trait values were defined only for subsets of species, for example, lateral spread data were defined only for clonal species, whereas other trait values were simply unavailable in the databases we used. Missing values of both types were not included in the calculation of species trait-foraging ability correlations (see below). The species trait correlation matrix did not show any unexpected collinearity (S2).

Experimental setup

The species were obtained as seeds from a commercial supplier (Planta Naturalis, <http://plantanaturalis.com>). Seeds were sown into seeding trays with clean sand in a greenhouse in June 2013 and the end of May 2014. All plants germinated within 1 month from sowing and were planted in August 2013 (July 2014) by species in a time sequence that spanned 2 weeks. We did this for two reasons: (i) to start with each species at approximately the same size and (ii) to spread the harvest period. The plants were planted into 3-L pots

with washed sand. The sand was washed with tap water in small batches in a concrete mixer until the water was clear. We took extra care to place the plant in the middle of the pot. Pots were placed on water-levelled perforated plates to avoid uneven mixing and leaks into other pots. Each pot was drip irrigated from two sides, and all pots received the same amount of fertilizer in the water. The treatments were created by changing the proportion of fertilizer in the drippers. There were three treatments: (i) control (no contrast, 2:2), (ii) low contrast (3:1), and (iii) high contrast (4:0). The precise dosage was dispensed by a mechanical dosing system (Dosatron, D25RE2). The commercial fertilizer was Wuxal Super (NPK 8:8:6 + micronutrients, Aglukon). We used the recommended dilution for adult plants (0.2%) as the maximum by diluting 10% stock fertilizer to 2%; the other concentrations were mixed similarly by diluting to 1.5, 1, and 0.5% of the stock. So we achieved final concentrations at the levels of 0.2%, 0.15%, 0.1%, and 0.05% of the original fertilizer concentration.

The plants were harvested after 5 weeks in the same sequence as they were planted. Each pot was divided into two halves in the middle of the plant's rooting point by a sharpened iron sheet. Both halves of the pot were washed in water on a fine sieve, and all roots were extracted. The roots were dried at 65°C and weighed.

Data analysis

For each pot, we calculated the natural logarithm of root weight in each half of the pot and expressed root placement pattern as $\log(\text{root quantity in nutrient-rich half}/\text{root quantity in nutrient-poor half})$. Logarithmic transformation effectively removes any effect of plant size; that is, the values obtained are likely to express effects that are independent of it (active plasticity). For control pots with no contrast, instead of nutrient-poor and nutrient-rich halves, we used (arbitrarily chosen) the left and right halves of the pot. Hereafter we call this parameter "balance."

Even in control pots, the balance data per species per treatment exhibited substantial skewness, as measured with the robust *medcouple* method [package *robustbase*, Version 0.8-1-1, (Rousseeuw et al. 2012)]. Therefore, we used medians to represent species by treatment response and used non-parametric methods in species response estimation.

To assess whether the treatments used were effective in eliciting a root allocation response, we compared balance data for the control with data for low-contrast treatment and data for high-contrast treatment. Comparisons were done pairwise according to species identity, using the Wilcoxon test as implemented in *wilcox.test* procedure. Control data were used twice; therefore, we report the Bonferroni-corrected (multiplied by 2) *p*-values of these tests.

Species-specific treatment effects— response as a shift in balance— were quantified using Mann–Whitney test statistics, divided by the product of the numbers of individuals subjected to each treatment, $U/(m \times n)$, where *U* is the Mann–Whitney test score for

difference in balance across contrasts, reported by *wilcox.test*; m is the number of control pots of the species; and n is the number of pots of the species subjected to low- or high-contrast treatment (Newcombe 2006). These species-specific responses were centred to zero by subtracting 0.5. In this way, we obtained two response parameters per species: one for balance difference between control and low contrast and the other for balance difference between control and high contrast.

The reliability of the response (i.e., stability of the difference between individuals from the control and contrast groups) was estimated by 1,000 bootstrap iterations on the data. In each iteration, both control and contrast balance values were bootstrapped. Because we did not assume any probability distribution for the balance differences, we used the ordinary bootstrap method, as implemented in the *boot* procedure [package *boot*, Version 1.3-5; (Davison & Hinkley 1997; Canty & Ripley 2015)]. These stability measures were compared across contrasts with the Wilcoxon paired test, for which we used the difference between the 0.25 and 0.75 quantiles (i.e., “middle half”) of the bootstrapped values for each response estimate (high values mean low stability).

The species response estimates in low and high contrast correlated substantially; therefore we used only the high-contrast species response estimates for correlation with species traits. To calculate these correlations, we always used the untransformed form of the parameter and its absolute value. The first approach allowed us to take rich patch avoidance response into account; the second one is an estimate of the directionless amount of plasticity.

We correlated high-contrast response estimates in both forms with species traits using procedure *rcorr* (*Hmisc* package, Version 3.9-3; Harrell 2012). For binary and ordinary traits (i.e., plant clonality, vegetative reproduction potential, generative reproduction potential, shoot longevity), we used Spearman’s correlation coefficient (ρ). We used Pearson’s correlation coefficient (r) for the remaining data. All analyses were performed in the R statistical environment, Version 2.15.1 (R Core Team 2012).

Species-specific treatment effects – response as a shift in balance – were quantified using Mann-Whitney test statistics, divided by product of number of individuals subjected to each treatment ($U/(m \times n)$), where U is Mann-Whitney test score for difference in balance across contrasts, reported by *wilcox.test*; m – number of control pots of the species; n – number of pots of the species subjected to low or high contrast treatment; (Newcombe 2006). These species-specific responses were centred to zero by subtracting 0.5. In this way, we obtained two response parameters per species: one for balance difference between control and low contrast and the other one for balance difference between control and high contrast.

Results

In general, high contrast elicited a substantial response in root allocation, whereas low contrast did not (Figure 1) (control vs. low contrast $V = 299$, $p = 0.87$; control vs. high contrast $V = 120$, $p < 0.001$).

More species exerted a strong foraging response in high-contrast treatment than in low contrast treatment, but the responses under both treatments were substantially correlated to each other (Spearman's $\rho = 0.46$, $p = 0.004$) (Figure 2, S3). Rather surprisingly, few species (*Bromus benekenii*, *Hypericum perforatum*, *Thalictrum lucidum*) avoided nutrient-rich patches in both contrast levels; their responses seemed to be quite stable. Response to the high-contrast treatment was significantly more stable (i.e., using bootstrap, we obtained a narrower set of the response estimate) than response to low-contrast treatment ($V = 170.5$, $p = 0.018$), but the stability of the responses within species across treatments did not correlate substantially (Spearman's $\rho = -0.24$, $p = 0.158$).

Root foraging ability substantially negatively correlated with vegetative reproduction potential (Figure 3) and, less convincingly, with plant clonality (binary trait) (Table 1). The correlation was negative; that is, clonal plants had lower estimated reaction norms (Figure 4). Results were approximately the same regardless of the form of reaction (directionless or directed) used. The only exception was correlation of root allocation with lateral spread. Correlation of plant lateral spread with the directed response (level of foraging, i.e., including rich patch avoidance as a negative response) was low, whereas it was much higher with the directionless measure of plasticity. Plants with low lateral spread tend to respond to soil heterogeneity level much more than plants with high lateral spread.

Discussion

We demonstrated that plants strongly differ in root foraging ability. Although the foraging response is expected to be related to steepness of the gradient between rich and poor patches, root foraging is apparent regardless of the size of an individual. We therefore interpret it as an outcome of active phenotypic plasticity, that is, plant behaviour. We illustrated that this behaviour is much less pronounced in plant species capable of rapid vegetative reproduction, that is, clonal plants. Quite surprisingly, we found no relationship between species traits used as proxies of growth rate (i.e., leaf economy traits) and root foraging when the latter was expressed using a size-invariant measure. Therefore, we assume that such relationships, reported in a previous study (Keser et al. 2014), are passive plasticity effects caused by simple differences in size (Aanderud et al. 2003).

The difference between clonal and nonclonal plants is the only strong pattern in active (i.e., size-independent) root plasticity found in the experiment. This is, to our knowledge, the first report of such a difference between clonal and nonclonal plants. Although we have no

mechanistic understanding of the phenomenon, it is likely that the low root foraging ability of clonal plants may derive from their ability to form stands of several ramets. Low root foraging of individual ramets may thus be compensated at the level among ramets (de Kroons & Hutchings 1995). In such a scenario, instead of proliferation and elongation of costly roots, which are effective in harvest but less so in transport (Alpert & Mooney 1986), an entirely new semi-autonomous harvesting unit (ramet) may be deployed at the resource-rich patch, with a stem-derived spacer capable of high-capacity transport. Such transport is much more efficient than the transport efficiency of roots, so it allows specialisation among ramets, that is, division of labour (Stuefer et al. 1996). On the other hand, we did not limit ramet development (by any other means than by the length of the experiment), so either we see demonstration of root placement according to future plans for ramet placement (de Kroon & Schieving 1990; Huber et al. 1999; Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Herben & Novoplansky 2007) or a lack of root foraging ability for an unknown reason. However, clonal plants are known for their root system plasticity (Hutchings & de Kroon 1994; Holzapfel & Alpert 2003; Gruntman & Novoplansky 2004; Semchenko et al. 2007), so we are more likely to expect purpose than inability of the behaviour.

Lack of root preference for nutrient-rich patches may also result from the lower nutrient optima of clonal plants (Groenendaal et al. 1996). We do not expect this to be the mechanism underlying the observed root foraging pattern. If it were so, we should have observed the differences between directionless and directed response, which was not the case.

In any ecological setting, the effects of the species traits are modified by growth, and the modification becomes stronger with greater growth differences. We therefore believe that differences in growth rates among species are the basis of previously reported strong correlations between root foraging ability and growth rate or leaf economy spectrum (Grime et al. 1997; Aanderud et al. 2003). These differences may also underlie the tight correlation between above-ground and below-ground plasticity (Campbell et al. 1991). Also, although we contend that active plasticity *detection* must be done after effects of absolute size and growth rate are removed, active plasticity *exhibition* is an outcome of the detected active plasticity patterns and growth. Growth modifies active plasticity effects, either weakening or enhancing them. Furthermore, because of allometry in growth (Weiner 2004), some species traits that we did not find to be correlated with root foraging ability may become related to root foraging in certain environments, provided these environments constrain or favour growth rate (Grime 1977).

Regardless of the relative significance of active or passive plasticity in the root foraging process, it may be useful to quickly identify species capable and not capable of root foraging, for example, in community assembly research. However, traits (e.g., relative growth rate, shoot plasticity, leaf economy) that have been previously linked to root plasticity (Grime et al.

1997) are rather derived, with strong links to plant physiology and its potential niches. Such traits, even though linked to proximate mechanisms in the life of the individual, are difficult to obtain without the necessary burden of direct estimation. For large-scale studies, this burden may render these traits unusable. Herein lies the strength of our study: Albeit in a correlative way, we identified easy-to-obtain species traits that fit such purpose. Moreover, if species coexistence is interaction driven, traits that describe the interactive interface of the individuals may be more important than the inner, nonmanifested traits.

Conclusions

Simple life-history species traits (namely, potential for vegetative reproduction, clonality) seem to be good correlates of root foraging as a form of active behaviour. The unexpected effect of clonality on root foraging may shed new light on our understanding of clonal species growth patterns in response to resource availability and spatial heterogeneity (de Kroon & Schieving 1990; Oborny et al. 2012). This is likely to be another example of deep functional differences between clonal and nonclonal species (Klimešová et al. in revision). Moreover, at the same time, the correlation is likely to be related to the fast species–slow species continuum (Kembel et al. 2008). If this is the case, it will be necessary to replace the single axis of fast–slow continuum to a fast–slow × clonality plane.

Further, it has been reported that active plasticity in root placement may contribute to the ecological success of species (Keser et al. 2015)). A similar finding has been reported for plastic response to light, where species with better plastic response are likely to occur in more species-rich communities (Lepik et al. 2005). This implies that the ability for plastic response is involved in community-level processes, such as patterns of species abundance and coexistence. These processes have hitherto been analysed mainly in terms of species (soft) traits, both because of the conceptual simplicity of such analysis and because of the relative ease with which trait data can be obtained (Cornwell et al. 2014). We propose that the ability of species to respond to their neighbours, both above ground and below ground, may be an important missing component in such analyses. Here we show that root placement is, to some extent, predictable by easily obtainable traits, but the generality of this finding remains to be determined.

Author contributions

Designed the experiment: MW, TH, TK; Performed the experiment: MW, TK; Analysed the data: MW, with discussions with TK and TH; Written the paper: MW, with contributions of TK and TH.

Acknowledgement

This study was supported by Czech Science Foundation grant no.P505-12-1007.

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Tables and Figures

Table 1 – Correlation of foraging response and species traits. Foraging response either included the direction of the response (FORAGING) or just its (directionless) magnitude (PLASTICITY). Type of the correlation coefficient used is indicated columnwise, r – Pearson's correlation coefficient, ρ – Spearman's correlation coefficient (rho). n – number of cases (species), p – p-value

FORAGING								
	SLA [m ² /g] (r)	log Leaf area [m ²] (r)	Height at maturity [m] (r)	Lateral spread [m](r)	Generative reproduction potential [1..5] (ρ)	Vegetative reproduction potential [1..5] (ρ)	Shoot lifespan [years; 1/2](ρ)	Clonality [0/1] (ρ)
r/ρ	-0.03	0.24	-0.25	-0.1	0.17	-0.41	0.19	-0.34
n	33	31	37	22	35	37	37	37
p	0.862	0.191	0.133	0.648	0.331	0.012	0.256	0.038
PLASTICITY								
	SLA [m ² /g] (r)	log Leaf area [m ²] (r)	Height at maturity [m] (r)	Lateral spread [m](r)	Generative reproduction potential [1..5] (ρ)	Vegetative reproduction potential [1..5] (ρ)	Shoot cyclicality [1/2](ρ)	Clonality [0/1] (ρ)
r/ρ	-0.03	0.2	-0.23	-0.34	0.17	-0.42	0.08	-0.31
n	33	31	37	22	35	37	37	37
p	0.889	0.28	0.177	0.118	0.334	0.01	0.62	0.066

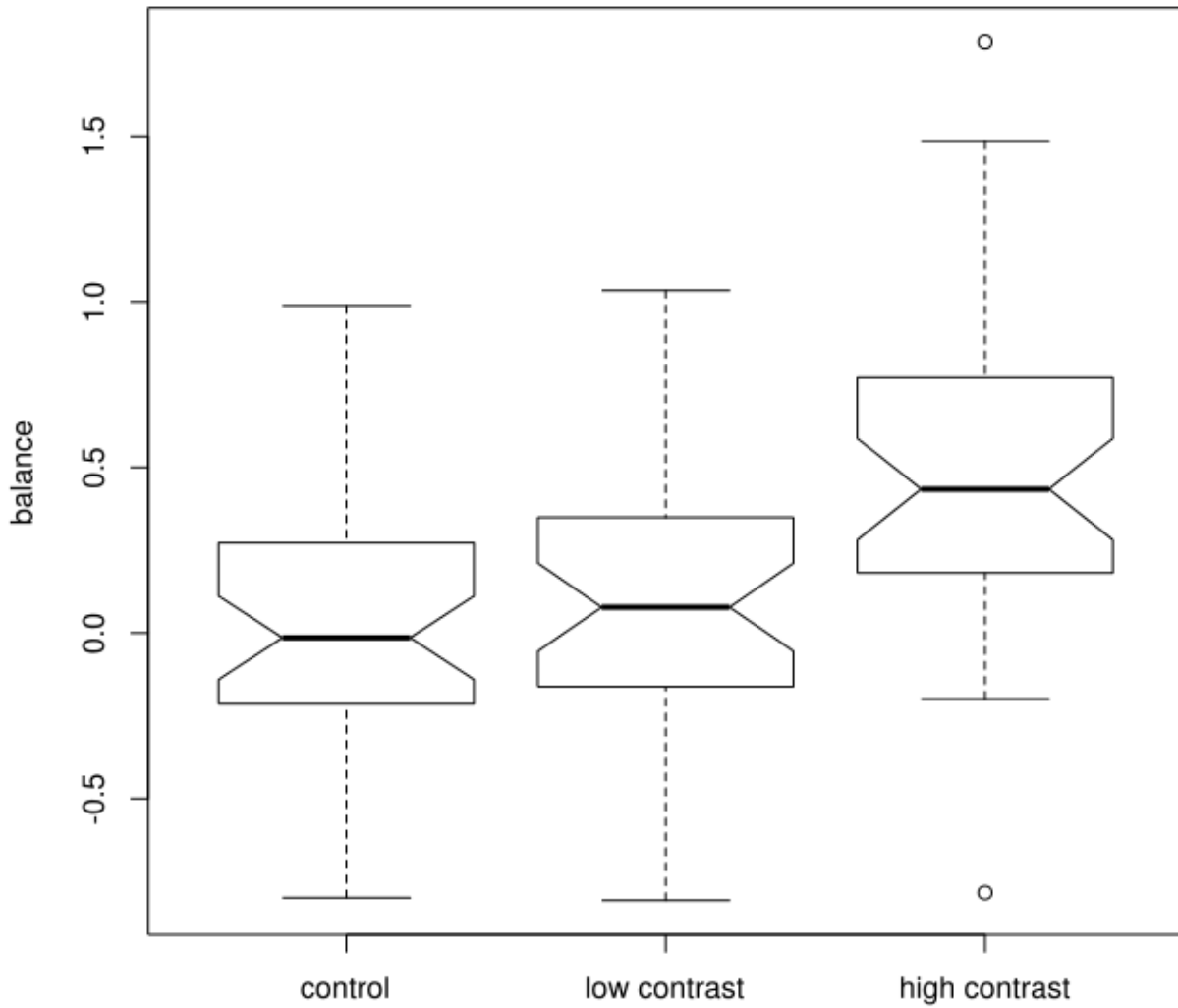
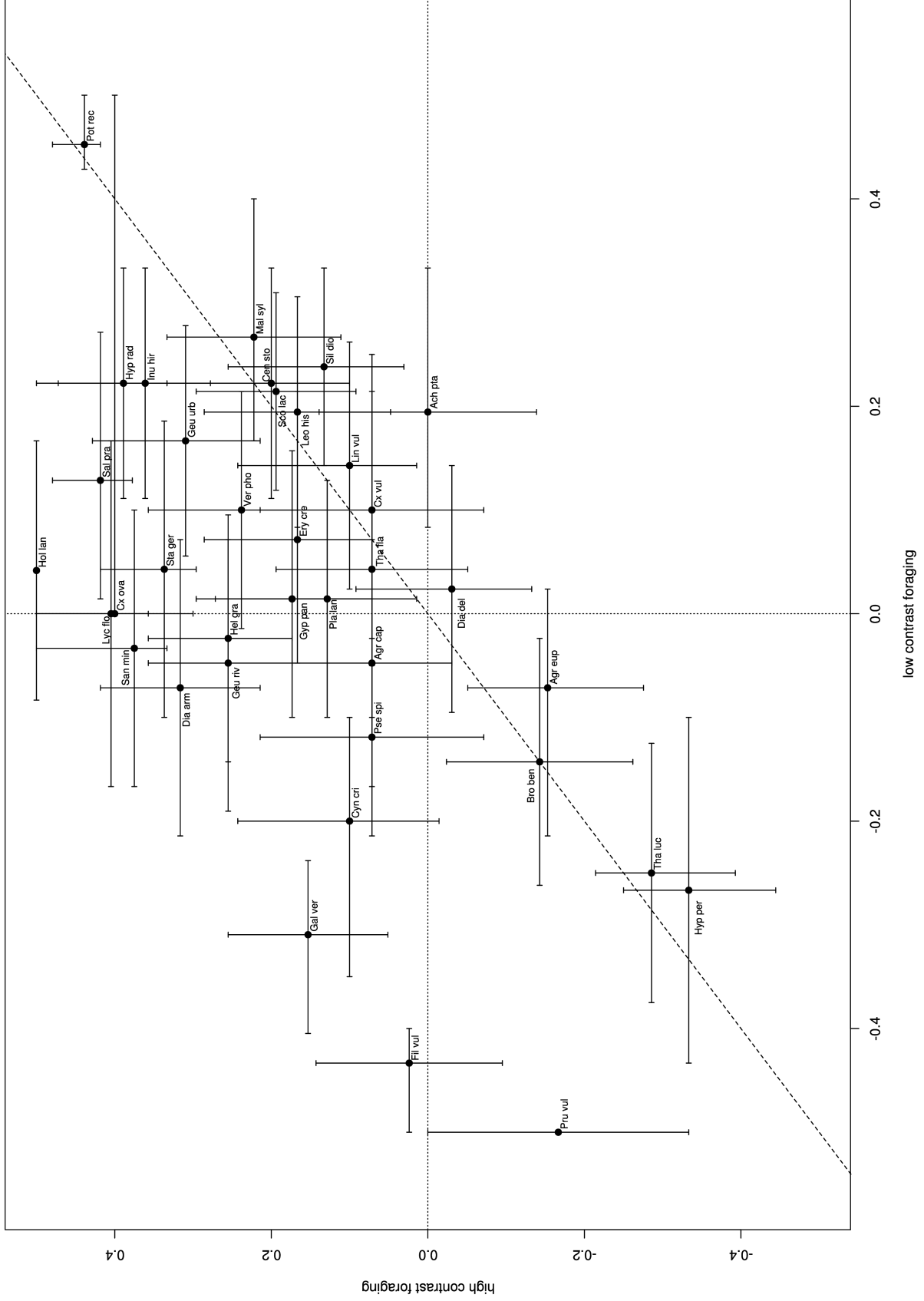


Figure 1 - Root placement under the treatments. Zero value means equal amount of roots in both halves of the pot. Positive values mean more roots placed into the nutrient rich patch.

Figure 2 – Species response to different levels of contrast. Low contrast foraging – difference between low contrast and control treatment, high contrast foraging – high contrast – control treatment difference. Error bars indicate middle half of the bootstrapped value for species full names.



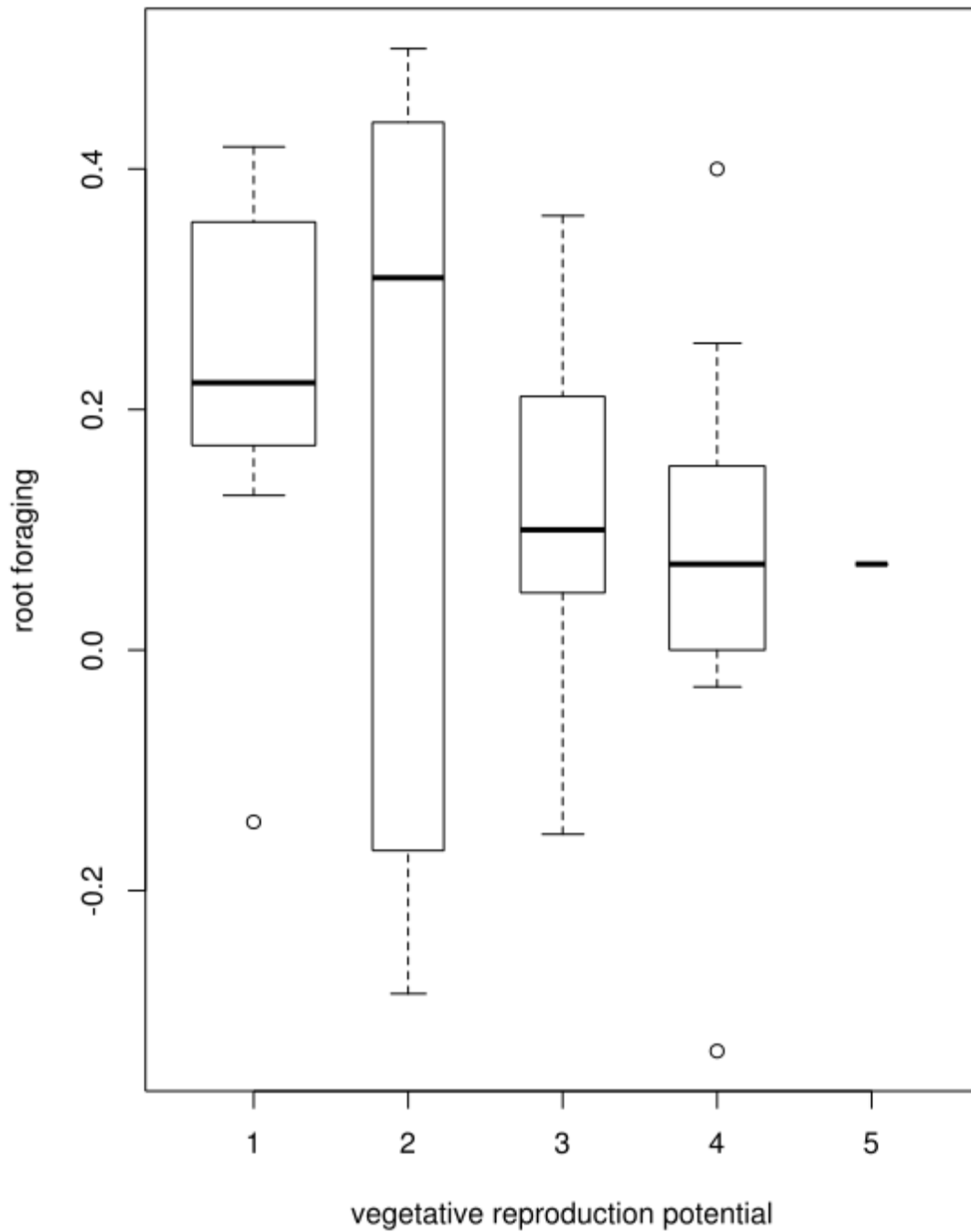


Figure 3 – Species-wise root foraging estimates and vegetative reproduction potential. Widths of the boxes are proportional to number of cases (species)

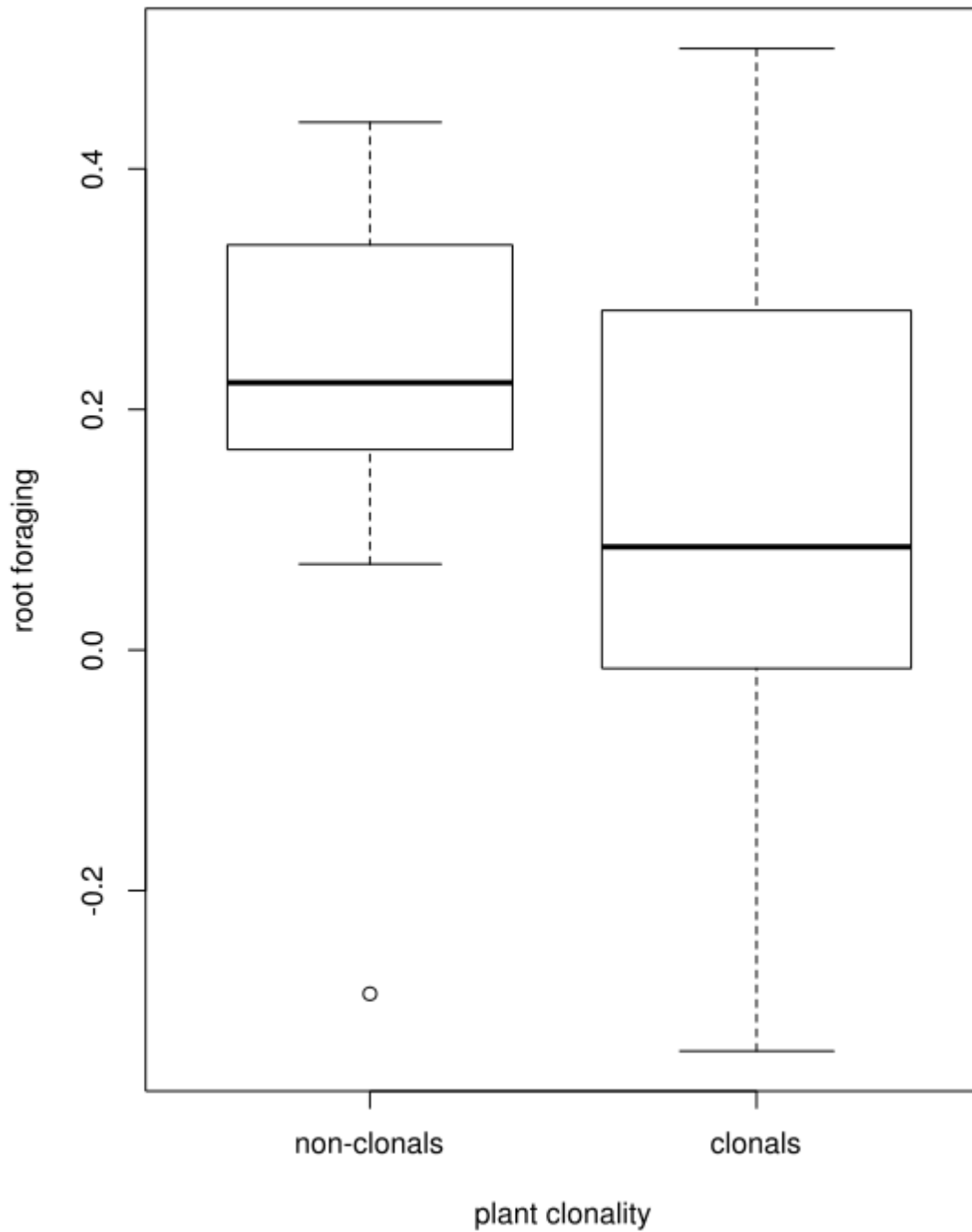


Figure 4 – Foraging estimate in clonal vs. non-clonal species. Widths of the boxes are proportional to number of cases (species).

The role of seed mass and resource availability in root system development

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Introduction

Establishment of seedlings is a critical part of the life cycle of plants (Jakobsson & Eriksson 2000). In the seedling stage, plant individuals are small, easily destroyed and do not possess well developed resource-harvesting organs. On the other hand, they do possess a special pool of nutrients in seed tissues from which they germinated (e.g. cotyledons in the case of legumes, the endosperm in grasses, etc.). These seed-stored nutrients are not subjected to competition over resources with neighbours, what makes this pool particularly reliable. Depletion of this pool is driven solely by the seedling itself, allowing better management of its use over time.

Due to the limited amount of nutrients stored in the seed, the seedling is forced to use its internal reserves to build its own belowground and aboveground resource-harvesting body parts. After exhausting these reserves, the seedling itself must be able to gain all nutrients, water and light from the surrounding environment. Similarly, the capacity for correcting errors in this growth phase is also limited by the pool of stored resources. Lack of resources for corrections needed due to wrongly predicted gradients in the environment enhances the criticality of developmental decisions even further.

Interspecific variation in the amount of nutrients stored is enormous, exceeding corresponding intraspecific variation by several orders of magnitude (Harper et al. 1970, Ellison 2001). This underlies differences in starting conditions among different species and represents species-specific solutions of various trade-offs in seed mass (Westoby et al. 1996; Coomes & Grubb 2003). Seedlings of species with small seeds are more resource-limited than seedlings of species with larger seeds. The development constraints outlined above are pronounced to different extents in different species. Particularly in species with small seeds, seed size limitation may therefore lead to a trade-off between the future need of a well developed root system and the immediate need for underground resources, as both targets are likely to be attained by different root system morphology. Finding at least some resources may be a good short-term goal, while good conductivity and efficiency may be a good strategy in the long run (Fitter 1987).

As regards seed size, it has been hypothesized that larger seed size enhances seedling survival in competitive environments (Leishman et al. 2000). Differences in seed mass act as one of the key traits in species differentiation (Westoby 1998; Moles et al. 2006). The trade-off between seed size and number favours small but numerous seeds in highly heterogeneous or unpredictable environments, while fewer and larger seeds benefit

reproduction in stable but competitive environments (Harper et al. 1970). However, little is known about the direct relationship between seed size and hard traits responsible for competitive traits of seedlings. It is obvious that not seed size *per se*, but the way how seed resources are translated into the development of competitive organs, play the crucial role in seedling competitive ability.

Both these effects of seed size (i.e. the economy of buildup of resource-acquiring organs) and competition with neighbours are likely to be strongly affected by the nutrient status of the environment in which the seed has germinated. In nutrient rich environments, even small and young, short-rooted seedlings are likely to meet their nutrients needs easily; on the other hand, severe competition for light is likely to occur in such habitats (Grime 1977). Compared to the internal seedling resource pool, resources present in the environment are not available to a single individual only, so they may be subject to direct competition. Moreover, because of spatial and temporal heterogeneity, which may be either intrinsic, or resulting from neighbour presence, future supply of these resources is only vaguely predictable from limited contact zone between the seedling and the soil (Tielbörger & Prasse 2009).

For adult plants, the dichotomy between species-specific and environmentally plastic root system traits has already been documented (e.g. Lynch 1995). Root architecture, the number of lateral roots and their position in the whole root system is very important not just for the occupied soil volume, but also for the functioning of whole root system (Fitter 1987). Dichotomous and herringbone root systems differ in their efficiency of resource acquirement and transport (Fitter 1987). This feature also predetermines potential competitive ability in various environments. Although the basic architecture of root system of each species is fixed genetically (Fitter et al. 1991), its connection to seed mass is only little known (Gross et al. 1992). On the other hand, the highly plastic morphology of the plant root system with regard to the soil environment is well documented (Audus 1975, Jackson & Barlow 1981, Vartapetian & Jackson 1997, Day et al. 2003), but unfortunately not for seedlings.

Here we investigate the roles that environmental nutrient richness and the amount of stored nutrients play in seedling root system development. We followed seedling root system development of seven species from the Fabaceae family under four levels of nutrients over a period of four weeks. During the experiment, we tracked changes in root system structure, size and shape. Because environmental and seed resource pools are not predictable to the same extent, we hypothesized that the amount of resources in these two pools may affect seedling root system development in different ways. As small-seeded species seedlings possess less internal resources, we hypothesize that these seedlings are more sensitive to nutrient levels in the environment.

Materials and methods

Plants cultivation

We worked with seven species from the Fabaceae family, selected to represent a substantial range of seed masses (see Table 1). All seeds were bought from a commercial provider (Planta naturalis, Markvartice u Sobotky). Nutrients were added in the form of a universal fertilizer solution (Wuxal Super; manufactured by AGLUKON Specialdünger GmbH & Co.KG, Düsseldorf; N:P:K = 8:8:6) diluted by water to four concentrations: 0.025%, 0.05%, 0.1% and 0.0% (pure deionized water). The highest administered concentration was in the lower half of the range recommended by the fertilizer manufacturer. As a substrate matrix, we used expanded perlite (expanded amorphous volcanic glass). We chose perlite because it provides good aeration and leaks practically no substantial nutrients, so we were able to fully control the amount of available nutrients by watering and fertilization.

Seeds germinated individually in Petri dishes on filter paper wetted with 3 ml of the respective fertilizer solution. Deionized water was added throughout the germination as needed. The Petri dishes were kept in a growth chamber (Adaptis A 1000 with TC kit, Conviron, Canada; light intensity 225 $\mu\text{mol}/\text{cm}^2/\text{s}$ in the distance 12.5 cm from the light source) under the following diurnal temperature regime: 20°C for 12 hours during the day and 10°C for 12 hours during the night. Each seed was transferred into its own experimental container on the day the radicle had torn its testum.

The experimental containers (inner dimensions height \times width \times depth: 9.5 \times 15 \times 1.5 cm) consisted of PVC boards sealed with silicone putty, one of the larger sides being transparent. During the cultivation, the transparent front side was covered with a non-transparent panel, so roots developed in darkness. The containers were sloped at an angle of 40°, with the transparent (but covered) side facing downwards. This caused the roots to grow on the transparent surface.

Each container was filled with perlite and 200 ml of fertilizer solution. During the cultivation, the plants were watered with deionized water as needed. Each germinated seed was transferred into one container with the same fertilizer concentration as used during germination. Cultivation of plants took place in the same growth chamber under the same settings as the one used for the germination. Relative air humidity was set to 50 % during the day and 70 % during the night. The plants were cultivated for four weeks after germination. Initially, we aimed for at least 10 replicates per species per nutrient level, leading to 40 containers per species and 280 containers in total, but due to technical reasons, we did not achieve the full set. The actual number of replicates per species per nutrients level was from 6 to 14. Root system development was checked weekly for four weeks, totalling 1116 observations.

After each week, we drew the root system of each seedling on the front side of the container. We used different colours for the main root and lateral roots in each week to make the growth of both root types in different weeks distinguishable in the final picture. During the experiment, only a negligible amount of tertiary roots was observed (in addition, most of its at *Trifolium pratense*), both in terms of count and length. We therefore excluded them from further analyses. After four weeks of growth (four drawings), we scanned the colour picture of each root system from the front side of the container using an office table-top document scanner.

Measurements

Raw scanned images were not suitable for direct skeletonization, so we redrew them in GIMP (version 2.6.12 (Anon n.d.)) using the scanned originals as templates and skeletonized the redrawn images. For skeletonization, we used the built-in algorithm in ImageJ (version 1.45, Schneider et al. 2012). From each of the skeletonized images, we extracted parameter values in three groups: root system structure, size and shape (table 2.).

Structure of the root system was represented by the length of the main root, total length of lateral roots and the number of lateral roots in each week, plus mean length of lateral roots in each week. The length of the roots was measured as the number of square pixels of corresponding colour in the skeletonized image minus one (1 cm corresponded to 118 pixels in a straight line). Two pixels with a common side were counted as 1; two pixels with a common vertex only were counted as $\sqrt{2}$. Pixels and lengths were counted using a Python script. With only a few exceptions, root systems branched to the second order roots only (main root and non-branched lateral roots), so classification into dichotomous vs herringbone roots became meaningless, while the number of lateral roots and their length became the principal parameter.

To describe both the size and shape of the whole root system, we marked its topmost (denoted A), leftmost (C), rightmost (D) and bottommost (B) points in each week (see Fig. 1), yielding 16 (four per week) points per scanned image, and noted the coordinates of these points. Size parameters depict the area occupied by the root system. We used three measures that were described using distances between A-D points as follows. Length of the root system was defined as the vertical distance between points A and B of the image, width of the root system as the horizontal distance between points C and D, and the area of the root system as the area of the ACBD tetragon for each of the weekly measurements.

In root system shape estimation, we again used the coordinates of the ACBD vertices. Using these points, we selected shape parameters to represent size-independent measures. First, we calculated the complementary variable called “depth of the root system” as the vertical distance between point A and the point of intersection of the ACBD tetragon

diagonals, i.e. the position of the ACBD centroid. Relative depth of the root system was thus defined as the depth:length ratio, and relative width of the root system was defined as the width:length ratio. We calculated these parameters for each of the four weeks.

Data analysis

Each aspect of root system development (structure, size, shape) consisted of several parameters. Because of this multivariate nature of the data, we analysed them using redundancy analysis (RDA), a multivariate analogue to multiple regression, using the *vegan* package version 2.1-8 (Oksanen et al. 2011) in R version 2.14.1 (R Development Core Team 2011). Prior to the analysis, each response variable (i.e. describing the root system) was scaled to zero mean and unit variance. In each of the analyses, we used the same set of predictors (constraining variables): seed mass, nutrient level, time (the number of weeks since the beginning of the experiment) and interactions of time with seed mass and time with nutrient level. These interaction terms describe root system development over time in terms of seed mass and nutrient levels. Further, we included the three-way interaction of time, seed mass and nutrient level, reflecting the hypothesis that root system development is driven both by the nutrient level and seed mass in a non-additive way. Seedling identity was used as a covariate to account for temporal pseudoreplication, as each seedling was measured four times throughout the experiment. It should be noted that, as we describe development over a time period which necessarily starts at a common point for all experimental units, not the additive effects of internal or external nutrient pools, but their interaction with time, is the most important parameter. Statistical significance was estimated using permutation-based analysis of variance under a reduced model (function *anova* in the *vegan* package, 499 permutations). Marginal effects of predictors and their interactions are reported in terms of adjusted R^2 (Peres-Neto et al. 2006), using the *varpart* procedure of the *vegan* package.

First, all measured variables were used in the analysis, and the relative effect of the model was estimated as the fraction of variability explained after fitting covariates (seedling identity). The three-way interaction explained less than a random amount of variability (negative adjusted R^2), so it was not used further, and models without this interaction are hereafter referred to as full. Measured variables pertaining to structure, size and shape were analysed separately in order to separate their effects. The same variance partitioning procedure as described above was used.

Results

Root system development was strongly determined by the predictors (unadjusted R^2 = 19.65% of the full variability, i.e. 36.7% after fitting covariables, 36.4% after adjusting for the number of predictors; $p=0.001$, 499 permutations). Note that we observed only additive

effects of environmental nutrient levels and seed mass. Although the largest part of the root system change is connected to its growth in time, it is modified more by seed mass than by the nutrient level (Table 3, Fig. 2).

As regards root system structure, plants grown under higher nutrient levels developed more lateral roots and started to produce them earlier than plants grown under low nutrient levels (incl. the treatment with distilled water). This effect was more pronounced in species with low seed mass, as these started to produce lateral roots earlier and more abundantly than large-seeded species (Fig. 2).

The speed of development in root system size was negatively linked to seed mass, but plants grown under higher nutrients levels developed denser root systems (see Fig. 2 and Fig. 3). On the other hand, root system shape was totally independent of the amount of nutrients, but its development was connected with seed mass (see Fig. 4). Seedlings from smaller seeds (in all species except *Trifolium medium*) stopped the initial narrowing earlier and even started to widen their root system again. Seedlings from smaller seeds branched more in the upper part of the root system whereas seedlings from larger seeds branched at greater relative depths. This was probably caused by an earlier onset of lateral root production in smaller seeds.

Discussion

Our results show that both observed parameters (the amount of available nutrients in the substrate and seed mass) play important roles in root system development in the early phase of plant ontogeny, but that their effects do not interact. Seed mass determines the size and shape of the root system and development of this shape over time. The amount of nutrients available in the substrate underlies the number of lateral roots, which adjust to the basic shape of the root system determined by seed mass.

Seedling root systems branch more in environments with higher nutrient levels. This is also frequently the case of root systems of fully grown plants (Drew 1975; Hodge et al. 1999). Structural similarities across developmental stages, i.e. early seedlings and full-grown plants, probably reflect a response to a common challenge, although some other, as yet undetected mechanisms may also play a role. We therefore argue that proliferation of seedling lateral roots that we observed in nutrient-rich environments has the same cause and purpose as lateral root proliferation in developed plants and that internal seed reserves do not play a role. On the other hand, as we do not compare root systems of seedlings and full grown plants directly, this statement is only qualitative, not quantitative, i.e. root proliferation reflects environmental nutrient levels, but the exact representation may differ between seedlings and adults. Lateral roots in root systems of developed plants are known to proliferate in resource-rich environments (Robinson 1994), although the response differs depending on nutrient

stoichiometry and mobility in the substrate matrix (Craine & Dybzinski 2013). The proliferation rate of lateral roots, i.e. the number of active absorption zones in the nutrient patch, is the factor that underlies the absorption rate (Hodge et al. 1999; Robinson et al. 1999).

While root system density corresponded to the environment, the development of root system shape was strongly affected by seed mass. Compared to branching intensity, overall root system shape is seldom studied (but see Gross et al. 1992), so any interpretation of our results by means of analogy between root system shape and function in seedlings and adult plants has to be more cautious. One of the possible explanations invokes the trade-off between root systems that are effective at harvesting resources and those that are effective at transporting them (Fitter 1987), so larger-seeded species simply start with building structures that are going to be fully used later in the life of the plant whereas small-seeded species need to start with structures that are ready to use even though they will have to be reconstituted later. On the other hand, the shape of the root system in adult plants is strongly related to the environment, namely water dynamics (Schenk & Jackson 2002). Therefore, another possible interpretation of the pattern we observed is that larger internal seed reserves allow seedlings to place the main branching zone deeper into the soil, where the risk of drying out is lower. Because of small internal reserves, small seeds are forced to branch their roots as soon as possible to reach nutrients in the environment. Early and thus shallow branching of small seeded seedlings puts their root system at a higher risk of drying out, as a higher portion of the root system is in the more dangerous shallow zone, even though deeper levels may be reached in the meantime.

Seed size correlates with the productivity of the environment, and hence with the amount of aboveground biomass (Westoby 1998). Plants with larger seeds dominate in dense stands (Moles et al. 2006). On the other hand, seed size seems to be more related to the development of the overall shape of the root system than to the proliferation of roots. But it is the amount of roots that plants change in competitive situations (Gersani et al. 2001; Craine et al. 2005; Craine 2006), and the outcomes of such investments are more predictable (Schwinning & Weiner 1998). However, in competition for light, an early start could bring life-long profit (Schwinning & Weiner 1998; Olsen et al. 2005; Verdú & Traveset 2005) so ready-to-use seed-stored nutrients are likely to enhance survival.

Further research is needed to prove whether our results and their interpretations are general enough. Studies across phylogenetic clades would be especially beneficial, as it is possible that the reported phenomena are tightly linked to legumes. Furthermore, regardless of phylogeny, legumes are known for their symbiosis with nitrogen-fixing bacteria and high seed protein content, and these ecological traits might be somehow linked to root system development. Again, this may be investigated using another set of species with different

nutrient consumption rates and seed stoichiometry. More species would also allow to test not only the generality of the observed patterns and their proximate mechanisms, but also our interpretation of the observed patterns regarding their ultimate roles in species ecology. Apart from the correlative evidence, the relationship of seed mass with root system development and species ecology should be tested directly in the field to provide the ultimate answer.

On the other hand, if the patterns we observed prove to be general, the increased resistance to unexpected periods of drought as a feature of large seeds may be one of the mechanisms that restrict the solutions to the trade-off in seed size and numbers in habitats where unexpected dry periods may occur (Amir & Cohen 1990; Wong & Ackerly 2005). In turn, this claim can be extrapolated to link this feature of seed mass to the broad applicability of the LHS scheme (Westoby 1998) for species differentiation across large scales. On the other hand, root system traits linked to nutrient availability, namely rooting density, size and branching pattern, may play a major role in the coexistence of individuals in cases where root competition changes available nutrient levels (Craine 2006; Craine & Dybzinski 2013).

Conclusion

Both observed parameters, seed mass and nutrient availability in the substrate, play important roles in the development of the root system at the seedling stage. However, each of these parameters has a different impact. While seed mass determined the relative shape of the root system, environmental nutrient availability was still important for the length and number of lateral roots. We therefore propose the following hypothesis: Seed size sets the field, but does not win the game if root system interactions drive local coexistence. Further, as plants with larger seeds dominate highly competitive sites, it is probable that the main role of the seed-stored pool of nutrients is to enhance survival during competition for light.

Acknowledgement

Tomáš Herben and Sylvie Pecháčková provided us with useful comments throughout the paper development.

Author contributions

Designed the experiment: TM, MW; Performed the experiment: TM; Analysed the data: TM, MW; Written the paper: TM, MW.

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Tables and Figures

Table 1: Seed mass of used species.

Species	Mass of 100 seeds (g)
<i>Lotus corniculatus</i>	0.1104
<i>Trifolium pratense</i>	0.1487
<i>Trifolium medium</i>	0.1948
<i>Securigera varia</i>	0.3511
<i>Cytisus nigricans</i>	0.5718
<i>Lathyrus pratensis</i>	0.9371
<i>Lathyrus vernus</i>	1.4642

Table 2.: Three groups of observed parameters.

Structure	Size	Shape
length of the main root	length of root system	relative depth of root system
total length of lateral roots	width of root system	relative width of root system
number of lateral roots	area of root system	
mean length of lateral roots		

Table 3: Results of RDA models. Explained variance was adjusted to the number of model parameters. Seedling identity was partialled out.

RDA model of:	Full model - explained variability (%)	Full model - p value	time x nutrients - explained variability (%)	time x nutrients - p value	time x seed mass - explained variability (%)	time x seed mass - p value	seed mass - explained variability(%)	nutrients level alone variability explained (%)
all traits	35.4	0.002	0.2	0.014	4	0.002	4	0.1
structure	34.1	0.002	0.4	0.002	5.3	0.002	5.2	0.4
size	58.7	0.002	<0.1	0.108	4.8	0.002	4.8	<0.1
shape	2	0.002	<0.1	0.884	0.4	0.016	0.3	<0.1



Figure 1: Image of the root system with marked sixteen points which were used for calculating size and shape parameters of the root system in each of four weeks. Point A is the topmost, point B is the bottommost, point C is the leftmost, and point D is the rightmost. The numbers correspond to the measurement week. Each colour represents a different entity, i.e. the order of the root (primary vs lateral) and its length gain in each week.

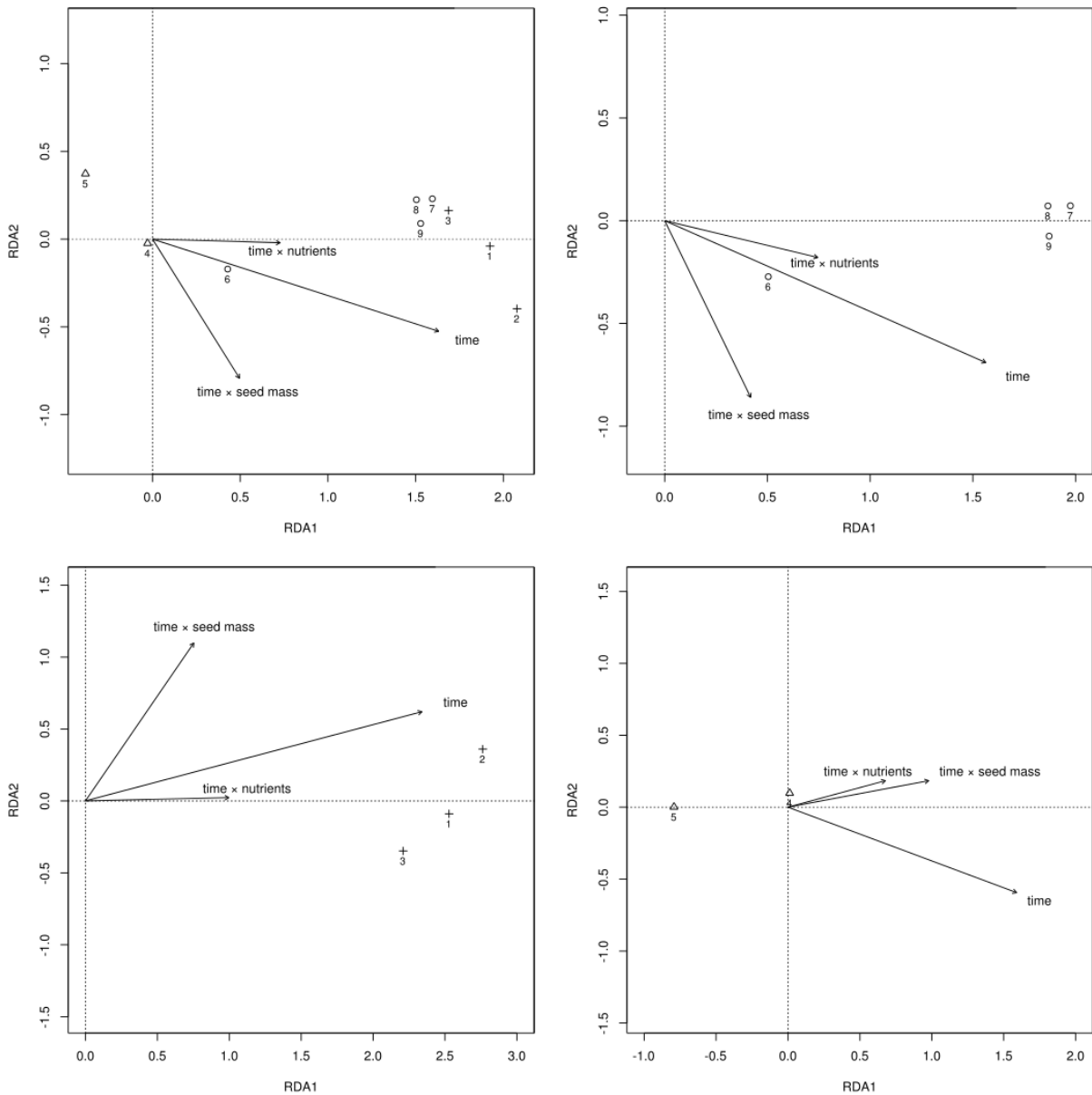


Figure 2: Root system development. The symbols indicate root traits positions in a constrained ordination space (RDA). The subplots depict ordination of: the overall trait set (RDA axis 1: 34.7%, RDA axis 2: 0.9% of overall variability after fitting covariables), root system structure (34%, 0.3%), root system size (58%, 0.8%) and root system shape (2.4%, 0.04%). Circles – structure-related traits, Crosses – size-related traits, Triangles – shape-related traits. The numbers refer to different root system traits: 1 – width, 2 – length, 3 – area, 4 – relative depth, 5 – relative width, 6 – main root length, 7 – length of the lateral roots, 8 – number of lateral roots, 9 – mean lateral root length.

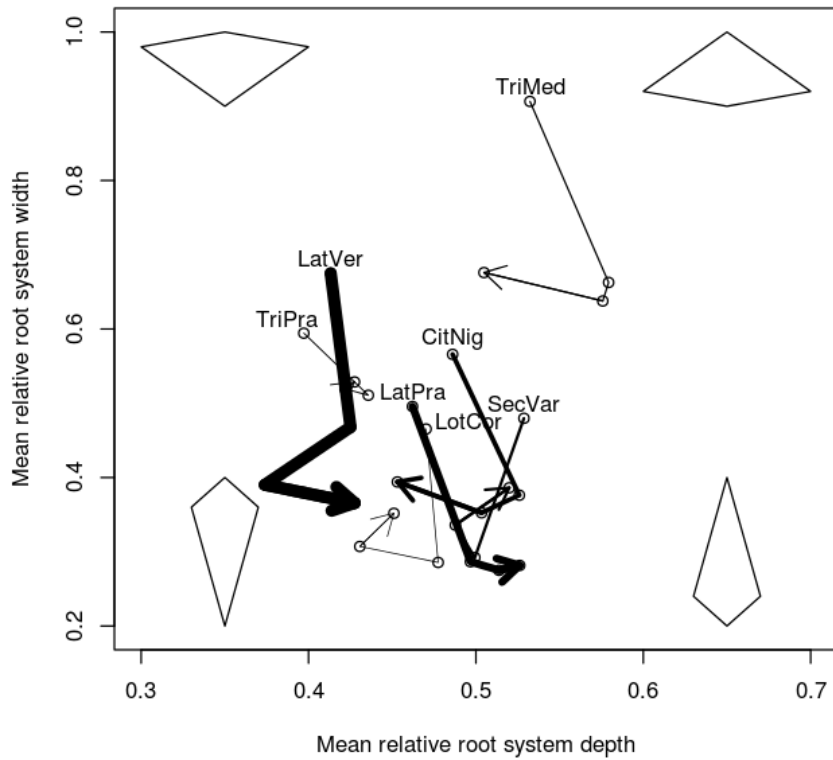


Figure 4: Development of root system relative shape depends on the species. Line thickness is proportional to seed mass; the deltoids depict idealized root system shape at the respective margins; temporal development is indicated by the arrows. Regardless of seed size, the centre of each root system more or less stays at the same relative depth (e.g. at the half the total depth). Compared to large-seeded species, small-seeded species tend to stop narrowing their root systems earlier and even widen them. Seedlings from larger seeds branch relatively deeper than those from small seeds.

A simple model for the influence of habitat resource availability on lateral clonal spread

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Fully featured version of the article is available from Proceedings of the Royal Society B:
<http://rspb.royalsocietypublishing.org/content/282/1806/20150327>

DOI: 10.1098/rspb.2015.0327

Introduction

Clonal plants are frequently the dominant guild in terms of both biomass and number of species. In temperate areas, their relative abundance with respect to non-clonal plants is greater in less productive habitats [1], which may indicate a possible relationship between habitat productivity and selection for clonality. Among clonal plants, the majority of species spread by stem-derived spacers, located either belowground in the form of rhizomes or aboveground as stolons, reaching distances that span over an order of magnitude [2]. Selection seems to have influenced the spreading distance, as some general trends link higher environmental productivity with farther lateral spread [3,4], although this relationship bears strong signals of phylogenetic and morphological constraints [3,5]. However, the mechanism underlying the relationship between productivity and spread distance is still not known.

Spread by spacers allows plants to integrate resources across environmental heterogeneities [6,7]. This integration may improve the fitness of the new ramet as an eventual independent individual, or it may increase the level of resources available to the clone as a whole. However, it incurs a cost in the resources needed to make and maintain conductive spacers. This is especially true for stoloniferous plants, as their roots are situated only at the nodes bearing new ramets, with the spacers not self-sustaining. Thus, spread by spacers is an effective strategy in terms of resource acquisition only if the new ramet can be placed such that the net resource income is non-negative [8,9].

The question of ramet placement in intrinsically heterogeneous environments has been the subject of considerable research including experimental studies [10–12] and construction of *in silico* models (reviewed by Oborny *et al.* [13]). Surprisingly, much less attention has been paid to understanding ramet placement in intrinsically homogeneous environments, i.e. those in which heterogeneity occurs not as the driver, but as the result, of the presence of plants at particular locations (yielding self-generated heterogeneity) [14,15].

Here we develop a model of spacer length between two directly connected ramets as a basis for overall lateral spread extent. The model seeks to explain spacer lengths simply in terms of overall soil resource availability and spacer production cost rather than relying upon external environmental heterogeneity. As such, the model may be used as a basis for

quantification of plant spreading strategies, that frequently deviate from simple maximization of resources in heterogeneous environments [16,17].

Further, we compare the model's predictions to those yielded by an alternative one based solely on the assumption of overall plant size, including its spreading structures, increasing with soil resource productivity in a unified manner (hereafter referred as isometric model). In this isometric model both overall plant size and the distance between ramets have the same relationship with environmental productivity, i.e. plants in higher resource environments have greater spreading distances simply as a linear function of greater size of spacers. To differentiate between these two models, we compare clonal plants with two different types of spacers, namely stolons and rhizomes. While both spacer types serve for clonal growth, they differ in their additional functions, with rhizomes being much more often involved in storage and soil resource acquisition in addition to clonal growth[18]. As our model does not take into account these processes, it is likely to hold for stoloniferous plants only; in contrast, if response to increasing soil resource availability is based on simple isometry, it is likely to affect both stolons and rhizomes in the same way.

Plausibility of these qualitative predictions of these two models is then compared to the relationships between database values of spatial clonal growth and species optimum along the gradient of belowground resource availability, assessed by Ellenberg indicator values [19].

The model

The underlying idea of the model is that spacer length is dictated by the need of the entire interconnected clonal fragment to maximize resource net gain, and for ramets to therefore be located accordingly. While maximum spacer length would then be limited by the cost of the spacer itself, minimal spread distance would derive from the need to avoid potential future competition between mother and daughter ramets. We represented the cost of distance in a “common currency” as resource gain, enabling us to perform additive operations to optimize overall plant performance.

Our model assumes symmetric competition between mother and daughter ramets for belowground resources [20]. We did not include aboveground competition because of the strong asymmetry that characterises competition for light, which would result in daughter ramets, necessarily smaller than the mother ramet, being strongly suppressed by shading in the close vicinity of the mother. For the sake of simplicity, we assumed permanently connected mother and daughter ramets of the same final size.

We assumed that the cost of a spacer (S) is linearly determined by its length (spreading distance, D , in meters) and unit spacer cost paid for both establishment and maintenance of the spacer during its lifetime (u , in resource/meter):

$$S=D \cdot u \tag{2.1}$$

In a given environment, the amount of resource acquired would be determined by the rhizosphere size, with the length of each root optimized for the resource availability: as the root incurs a cost per unit of its length, maximal root length would be limited to that at which it acquires just more than it costs [21–23]. Thus, a more productive environment would lead to bigger plants with bigger rhizospheres.

To keep the model simple, we assumed that the cost per unit root is constant. This would lead to a hemispherical rhizosphere. However, as environments differ in their depths of maximal resource content, and natural selection should act towards maximizing acquisition capacity by the roots at that depth, we decided to instead use a half spheroid (i.e., half an ellipsoid of revolution), with its width-to-depth ratio determined by the depth that contains the maximal amount of the resource. This representation allowed us to include rhizosphere shapes from nearly cylindrical to nearly flat.

We assumed that within the rhizosphere the roots homogeneously extract the resource and that rhizospheres are discrete in space, with sharp boundaries (or very steep gradients) between fully exploited and non-exploited areas. The amount of the resource acquired (A) would thus be proportional to the radius (a) and depth (b) of the spheroid [Eqn. 2.2], with scaling constant (c) in resource/m³, converting rhizosphere volume to plant extractable nutrients. As a is linearly dependent on b , then b can be interpreted as a scale (size) parameter of the rhizosphere, and $k=a/b$ as its shape parameter.

$$A = \frac{2\pi}{3} c \cdot a^2 b \quad (2.2)$$

If ramets are too close to each other (i.e. the distance between their centres is less than the diameter of the rhizosphere), their potential rhizospheres overlap. With overlapping rhizospheres, future clonal fragments would acquire less of the resource than two ramets farther apart from each other (Figure 1). We denote the difference in resource acquisition due to rhizosphere overlap as the competition cost (C), i.e. it is the sum of the resources that would not be acquired because of ramets proximity during their life. It is proportional to the volume of the overlap of the rhizospheres, which can be calculated as an ellipsoidal side cap volume of height $a - D/2$ as follows:

$$C \approx \iint_{x > D/2} \frac{b}{a} \sqrt{a^2 + x^2 + y^2} dx dy = 2a^2 b \frac{1}{48} \left(\frac{D}{b} - 2 \right)^2 \left(\frac{D}{b} + 4 \right) \pi \text{ for } 0 < D < 2a \quad (2.3)$$

The overall loss (T) in the capacity to gain the resource is then the sum of both costs:

$$T=cC+S \quad (2.4)$$

and it relates to the spreading distance:

$$T=\frac{ca^2 b\pi}{24}\left(\frac{D^3}{b^3}-12\frac{D}{b}+16\right)+Du \quad (2.5)$$

The optimal spreading distance would have the minimal value of T

$$D_{T_{min}}=2b\sqrt{1-\frac{2u}{ca^2\pi}}=2b\sqrt{1-\frac{2u}{ck^2b^2\pi}} \quad (2.6)$$

which would occur only if unit spacer cost is low relative to the size of the rhizosphere

$$u\leq\frac{ca^2\pi}{2} \quad (2.7)$$

Predictions yielded by the model

Our model yields several qualitative predictions that can be tested by examining relationships between clonal plant lateral spread and resource availability. First, at low-resource sites, among clonal plants, species that spread laterally through use of spacers (stolons, rhizomes) should be rare in comparison to root-splitters (i.e. plants in which the senescing taproot can fragment to yield new ramets), because the costs of growing and maintaining minimal usable spacers could exceed the possible gain at that site (Equation 2.7, Figure 2).

Second, because increased resource concentration yields increased maximum root length, and in turn a larger rhizosphere, it would drive an increase in optimal spreading distance. This is because in richer environments, the additional cost of growing a longer

spacer would now be exceeded by the competition cost incurred if a daughter ramet were placed at the same distance as in the poorer environment. However, in contrast with simple isometric growth, our model would produce non-linearity between productivity of the environment and spreading distance. Nevertheless, if both rhizosphere width (a) and depth (b) have the same allometric dependence on the resource concentration – i.e. the rhizosphere shape (k) is constant along the resource gradient – the relationship between the rhizosphere size reflecting environmental productivity and the optimal spreading distance approaches linearity (Figure 3).

Furthermore, higher unit spacer cost will yield more pronounced curvature of this relationship (Figure 3). Different kinds of spacers vary in form and function [24], and therefore also vary likely in the unit cost. This would lead to spacer type-specific relationships between lateral spread and environmental productivity.

Additionally, if the distribution of belowground resources differs between environments (e.g. persistently wet soils vs. arenaceous soils with water available only in upper horizons after rain), this would affect the shape of the rhizosphere. Plants with different rhizosphere geometry will differ in optimal spreading distance, with the relationship between rhizosphere shape and this distance not necessarily monotonic (Figure 4).

Testing the model

Data

As a test of our model, we analysed the relationship between resource richness and spacer distance as shown by growth data from a plant clonal trait database [25] and Ellenberg indicator values (EIV, [19]) for soil moisture and nutrient availability. From the database, we selected terrestrial (i.e., EIV for moisture <10) species that produce overlapping, physically connected generations using stolons, hypogeogenous rhizomes or epigeogenous rhizomes. Hypogeogenous rhizomes are produced below ground, whereas epigeogenous rhizomes originate above ground, but with the distal portion typically situated beneath soil or litter [25]. The unit stolon cost is sometimes considered lower than the cost of rhizomes [26], also rhizomes are more often built to last longer than stolons and serve functions other than spread (namely storage). We considered all of these spacer categories both together and separately. If a given species was listed as using more than one of these different spacer structures, we would input the relevant data for the species and test the model for each of these spacer categories. Lateral spread distance was coded as an ordinal factor having three possible levels, as presented in the database: <1 cm; 1-25 cm; and >25cm. Availability of EIV as values characterizing the most common habitat for particular species limited the testing species pool to Central European species.

Methods

Because both the EIVs and lateral spread values are on ordinal scales, we used Kendall's τ

rank correlation coefficient as a measure of the association between the resource levels and the lateral spread values. Calculations were done using the *cor.test* procedure available in the *stats* package of the R statistical environment (version 2.11.1,[27]). We estimated probability levels of null hypotheses using one-sided tests, because, according to our model, lateral spread would be greater in richer environments.

Results

When considering the whole dataset, positive relationships between lateral spread and resource availability were apparent, as predicted. While the relationship between spread and water availability was highly significant ($p < 0.01$), it was less so in the case of the spread-nutrient availability relationship ($p < 0.1$). In both cases, the associations were rather weak (Table 1).

When the dataset was analysed separately by spacer type, substantial, significant positive associations between both water ($p = 0.014$) and nutrients ($p < 0.001$) and lateral spread were found for stolon-based spread. There were no significant positive associations between rhizome length and belowground resource availability (Table 1, Figure 5).

Discussion

We predicted that plants that spread by spacers would occur in relatively resource-rich environments, compared to root splitters. This was based on our inference that in extremely poor environments, even the small cost of the minimal usable spacer would need to be avoided, with other strategies to avoid local competition preferable. A study of the relationship between clonal plant spacer types and various habitats, using largely the same data to those we employed, revealed this prediction to be well supported (see Van Groenendael *et al.* [4] for additional support of our hypothesis).

In our study, when all the plants were considered together, the distance of lateral spread increased with the availability of belowground resources in the environment, as predicted by our model, although the effect size was rather small. However, this qualitative outcome would also be predicted by simple isometry. The crucial distinction between the outcomes generated by the two models is that the former would yield a non-linear relationship, and the latter, a linear one. While the existing data do not allow direct assessment of linearity to distinguish the form of the response, additional features of the model can help separating these two potential mechanisms.

In simple isometry, lateral spread distance would not be influenced by unit spacer cost, thereby yielding the same relationship between lateral spread and belowground resource availability for all spacer types. In contrast, our cost-based model would produce different outcomes according to spacer types and their unit costs. The data showed that lateral spread based on different spacer types differed in their relationships to belowground resource availability, thus providing support for our model, while indicating that lateral spread distance

was not governed by simple isometry. In particular, the overall relationship we found between water availability and lateral spread was mainly driven by stolon-bearing plants, and these plants showed a positive relationship not just between water and lateral spread, but also between nutrients and lateral spread. Rhizomatous plants, which possess more costly spacers [26], did not manifest these relationships. This could be due to the predicted strong curvature in the relationship between resource availability and lateral spread for species that employ costly spacers.

On the other hand, the lack of a relationship between resource availability and lateral spread in the two groups of plants that employ rhizomes (epigeogenous and hypogeogenous) is likely to be due to the different nature of rhizomes. Unlike stolons, rhizomes frequently serve both as structures to achieve spreading and as storage organs [24,28] and are built to last [5]. The roles of rhizomes other than spreading may pose functional and geometrical constraints that limit their spreading capacity, but may be vital in environments and life histories in which storage capacity is important [18], subjecting rhizomes to other evolutionary pressures [18,29]. This limitation could make our model inadequate for consideration of spread by rhizomatous plants, but should not occur under the isometric model. Stolons are essentially only for clonal spread and support of young ramets [24,18](but see [30,31]), therefore stolon-based spread fits perfectly into our modelling framework. Dong and de Kroon [26] performed an experimental study on this issue, and their results are in accord with our prediction and argumentation: in their experiment, plants generated shorter stolons in low nutrient treatment compared to high nutrient treatment, whereas rhizome length was unaffected. We suggest that our hypotheses can be tested by further experimental studies, preferably on stoloniferous species. Directly manipulated resource availability as well as spacer cost estimation would allow us to discriminate between our model and isometry in lateral spread of clonal plants.

An additional prediction of our model shows that even in a horizontally homogeneous environment, rhizosphere shape may have a profound effect on the optimal spreading distance. Moreover, rhizosphere shape is likely to vary in accord with the vertical heterogeneity of the environment [22,32], and vertical heterogeneity of the environment is likely to differ across the broad scale we used for testing the model. Furthermore, innate differences in rhizosphere shape between species from various phylogenetic clades [33] could also influence the outcome of a study, such as ours, that examines multiple species across different habitats. To account for such variation, it would be necessary to collect new and compile already available rhizosphere shape data.

Limitations of the model

Our simplifying assumptions fell into two categories. The first category comprised conceptual assumptions essential to building an intentionally simple model, in which the only environmental heterogeneity was self-generated, horizontal, and belowground environment.

Our model used the idea of Sachs *et al.* [34], viewing the plant both as a single body and as a population of competing organs at the same time. The model's simplicity allows its usage as a null model in testing hypotheses concerning the effects of phenomena such as external heterogeneities and competition with other individuals on ramet placement and vegetation composition. So it allows detection of ramet placement strategies that occur in such environments, e.g. foraging [16]

The second group of simplifications were done to ease model construction and decrease the number of parameters needed. Among them, we see the following four as those needing specific attention: (i) rhizospheres rigid in shape, i.e. not adjusting according to the presence of roots of sibling ramets; (ii) initial asymmetry in size between ramets; (iii) rhizospheres discrete in space, with boundaries (or very steep gradients) between fully exploited and non-exploited areas; and (iv) stolons only transporting input, with some constant amount lost per unit length. We discuss each of these limitations below.

First, regarding rhizosphere shape rigidity, in nature, this assumption is not met, as plants can adjust rhizosphere shape in response to the presence and identity of neighbours [35,36], e.g. to minimize overlap with siblings [37]. However, this shape adjustment incurs a cost for the plant, as isolated ramets develop non-adjusted rhizosphere shapes. In other words, the shape of rhizospheres is adjusted to neighbour ramets when needed because it is suboptimal for individual ramets. Incorporation of rhizospheres with a plastic shape response to competition into our model would thus require parametrisation of the cost and would necessarily lead to less general models, moreover using a parameter of unknown properties [38]. Importantly, this additional parameter would only modify the cost of competition and would not alter the model's logic.

The second problem is closely related to the first one, as it partly deals with the rhizosphere shape. It could be assumed that at the moment of rooting, the younger ramet from a pair has no rhizosphere and therefore can be placed at the margin of the depletion zone of the older ramet without incurring competition cost. Growth of the younger ramet's rhizosphere is then possible by two means. Either the younger ramet develops a distorted rhizosphere and pays the above-mentioned cost, even propagating this cost to the next generation, or it has to pay a high cost for competing with its parent in the future. Shorter spacer (lower spacer cost) needs to be balanced with the sum of competition costs increasing over time. While this can be accommodated in the model, more parameters would be needed. This would make the model more complicated and possibly less useful as a simple tool for exploring strategic and behavioural patterns of ramet placement.

Third, the assumption of discrete rhizospheres that are exploited uniformly is unlikely to be strictly satisfied in nature, too. Indeed, the intensity of exploitation is likely to be uneven within the rhizosphere due to belowground resource diffusion and branching allometries [21,38]. In particular, real rhizospheres may be exploited more in their centres, and depletion zones for individual resources can vary. Moreover, as resources differ in their migration rate

through the soil matrix, these depletion zones may be resource-specific in size, internal spatial structure, stability and time to reach the replacement-depletion equilibrium. However, incorporating these more exact representations would not lead to qualitatively different results and would merely provide scaling variables for different models of the rhizosphere. According to Liebig's law, one particular soil resource is appropriate for any given situation, and resource-specific functions and constants may be involved in the model at the expense of its simplicity and general applicability.

Finally, in choosing a linear relationship between spacer length and cost, we also considered the possibility that the cost could be proportional to the transported amount, and the relationship therefore exponential. Spacer cost estimation, which would be needed to address this issue, has been done only rarely, with inconclusive results (e.g. [15,39–41]). We chose the linear relationship because it has the same main attributes and a simpler form than the other option. An exponential relationship would increase the rate of growth of the spacer cost, which would limit the maximal spread more strongly and change the shape of the placement function. However, its general properties (a single minimum of total cost) would not be altered. An exponential relationship of spacer length and its cost would probably increase the curvature in the optimal spreading distance–environment productivity relationship.

Conclusions and implications

Limitations in the test of our model are due mostly to the nature of the available data. Experiments directly manipulating resource levels, performed both within and across species, would be welcome, as the first type would allow control for phylogenetic signal and the second enable assessment of effects of innate differences in rhizosphere shape and unit spacer cost. Phylogenetic signal is strongly pronounced in variability in unit spacer cost [3]. Experiments across species could help to quantify this cost, and particularly to evaluate for rhizomes, the most common spreading structures [25], the influence of roles other than spreading. Additionally, we believe that compiling and analysing case studies on root distributions in various environments both within and across taxa [22,33,42] can yield insights into environmental effects on clonal plant spacing, as mediated by rhizosphere shape.

Although our model could best be tested through manipulative experiments, its simplicity makes it more broadly useful as a null model against which to test hypotheses based on system attributes, such as intrinsic environmental heterogeneity, that it explicitly excludes. Therefore, it may be used in testing the presence of clonal growth strategies such as foraging or clumping [16]. Far spreading fast moving plants are beyond the capacity of the model, as they leave the patch before stable rhizospheres develop [16]. In those cases, the model could only estimate an upper bound of the spread.

We believe that the main ideas underlying our model (modularity of plant body resulting

in possible self-competition and resulting selection to diminish total costs) are general enough to be applied to many more systems dealing with plant growth, including positioning of aboveground parts. Such systems are numerous; with clonal spread being especially subject to recurrent investigation in plant ecology [13,43,44]. The particular example that we examined lies at the intersection of two areas of interest in plant ecology: the role of ramet positioning in small-scale vegetation composition [45–48] and ramet placement plasticity and its effect on plant behaviour [10,49,50]. Our model can serve as a suitable null model of ramet positioning to test hypotheses regarding environmental effects, enabling further inquiry into both of these topics.

Authors' contributions

MW formulated the idea and drafted the model, compiled the data for testing and tested the model, drafted the manuscript. JS formalized the model, thoroughly and critically reviewed the draft in several rounds. Both authors gave final approval for publication.

Funding statement

MW was funded by Czech Science Foundation project P505/12/1007.

Data accessibility

Data for model testing are publicly available [19,25].

Acknowledgement

We wish to thank Tomáš Herben for valuable discussions of both ideas and the text. Jonathan Rosenthal provided us with editorial service in the terms of text clarification and language quality. M.W. is personally indebted to Ariel Novoplansky for his support and personal introduction to amazing works of Dan Cohen and Tsvi Sachs during M.W.'s stay at The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev under Drylands Research Specific Support Action. Further, Jitka Klimešová and Ariel Novoplansky substantially commented early versions of the manuscript.

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Tables and Figures

Table 1. Associations of belowground resource and spacer lengths of clonal species categorized by spacer type. (Kendall's correlation coefficients (τ), probability levels of corresponding null hypotheses (p), number of available species data per category (n) are shown both for moisture and nutrients, as indicated by Ellenberg indicator values.)

type of spacer	moisture			nutrients		
	n	τ	p	n	τ	p
all	1014	0.07	0.005	1003	0.036	0.092
stoloniferous	115	0.174	0.014	106	0.395	<0.001
epigeogenous rhizome	663	0.032	0.175	657	0.033	0.170
hypogeogenous rhizome	236	-0.095	0.956	240	0.046	0.203

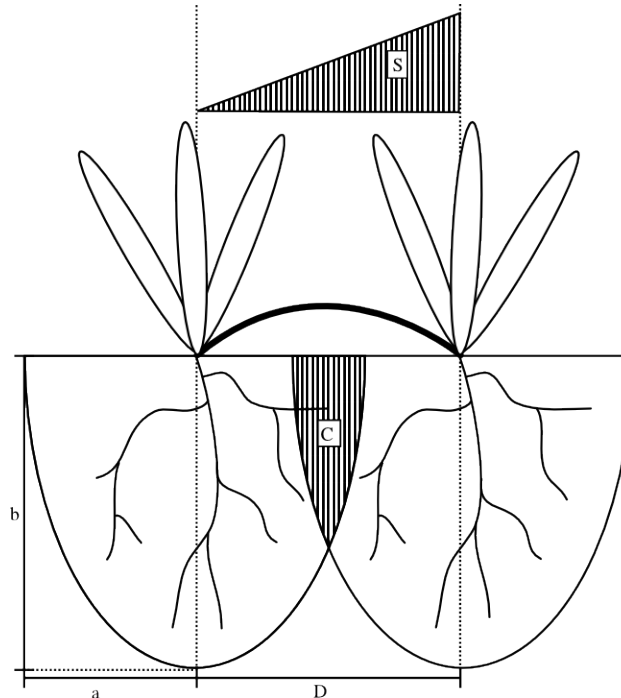


Figure 1. Two interconnected ramets, growing at distance (D) apart. If, as in the case shown, their rhizospheres (with depth b and radius a) overlap, they would incur a competition cost (C). Growing the spacer also incurs a cost (S), which the model assumes will be at the expense of soil resource acquired. Thus, in any case, two interconnected ramets would not acquire twice the amount of the belowground resource captured by a single ramet with the same size rhizosphere.

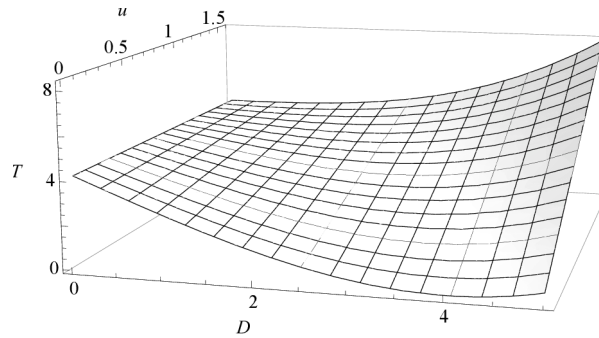


Figure 2. Total cost for spacer-based spread (T) in response to unit spacer cost (u) and spreading distance (D). Single minimum of total cost of spread, and hence the optimal spreading distance, exists for each value of unit spacer cost in each environment. If we assume that there is some minimal unit spacer cost for usable spacer, and this cost is relatively high regarding to the resource availability in the particular environment, optimal spreading distance may decrease to zero, meaning that spread could be reached by other modes (e.g. root-splitting) more effectively. As an example, the figure is plotted for a species and an environment where nutrients level is reflected in rhizosphere depth (b) = 2 and radius (a) = 1; scaling constant (c) = 1.

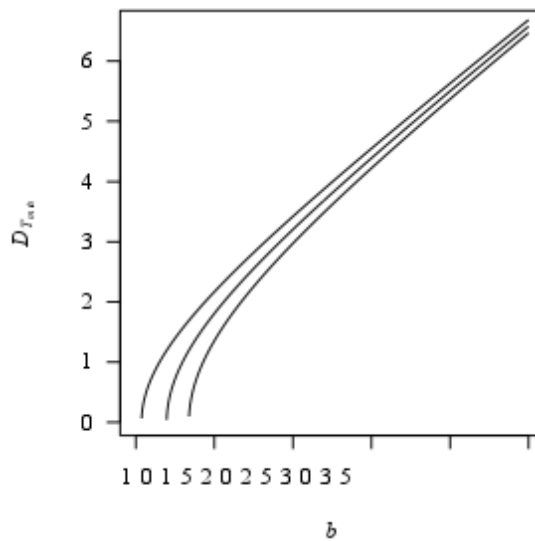


Figure 3. Optimal spreading distance (D_{Tmin}) in environments with different resource availability, as reflected by rooting depth (b). Rhizosphere shape held constant ($k = 2/3$). Under low resource availability conditions, the relationship is strongly non-linear. Moreover, the curvature increases with higher unit spacer cost (u). From top to bottom, lines drawn for $u = 0.75, 1, 1.25$.

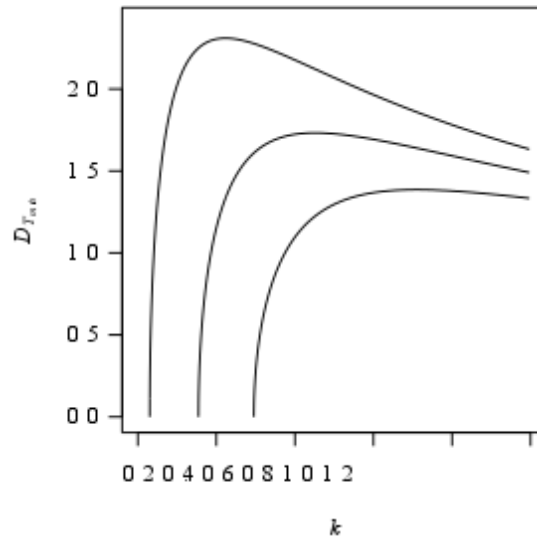


Figure 4. Rhizosphere shape (k , $k = a/b$) determining optimal spreading distance (D_{Tmin}) at the certain level of resource availability ($A = 3$, $c = 1$). Relationship is not monotonic – when root layer is thinner than certain threshold, rhizospheres seem to overlap more when seen from above. Curvature increases with higher unit spacer cost (u). From top to bottom, lines drawn for $u = 0.75, 1, 1.25$.

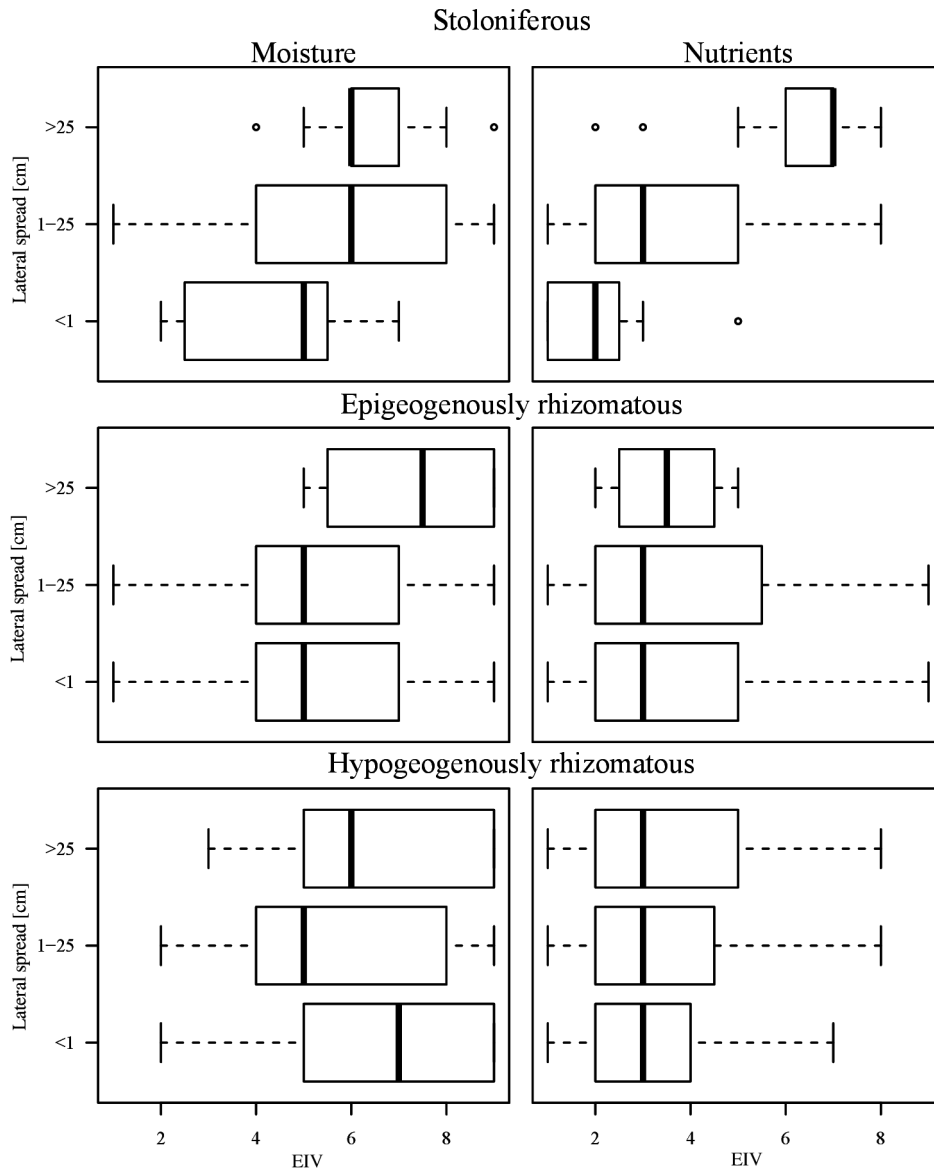


Figure 5. Relationships of clonal spread distances to environmental gradients, grouped by spacer type. Spread distances are categorized as <1cm; 1-25cm; >25cm. Left panels indicate relationships to moisture, right panels indicate relationships to nutrient availability.

Supplementary material

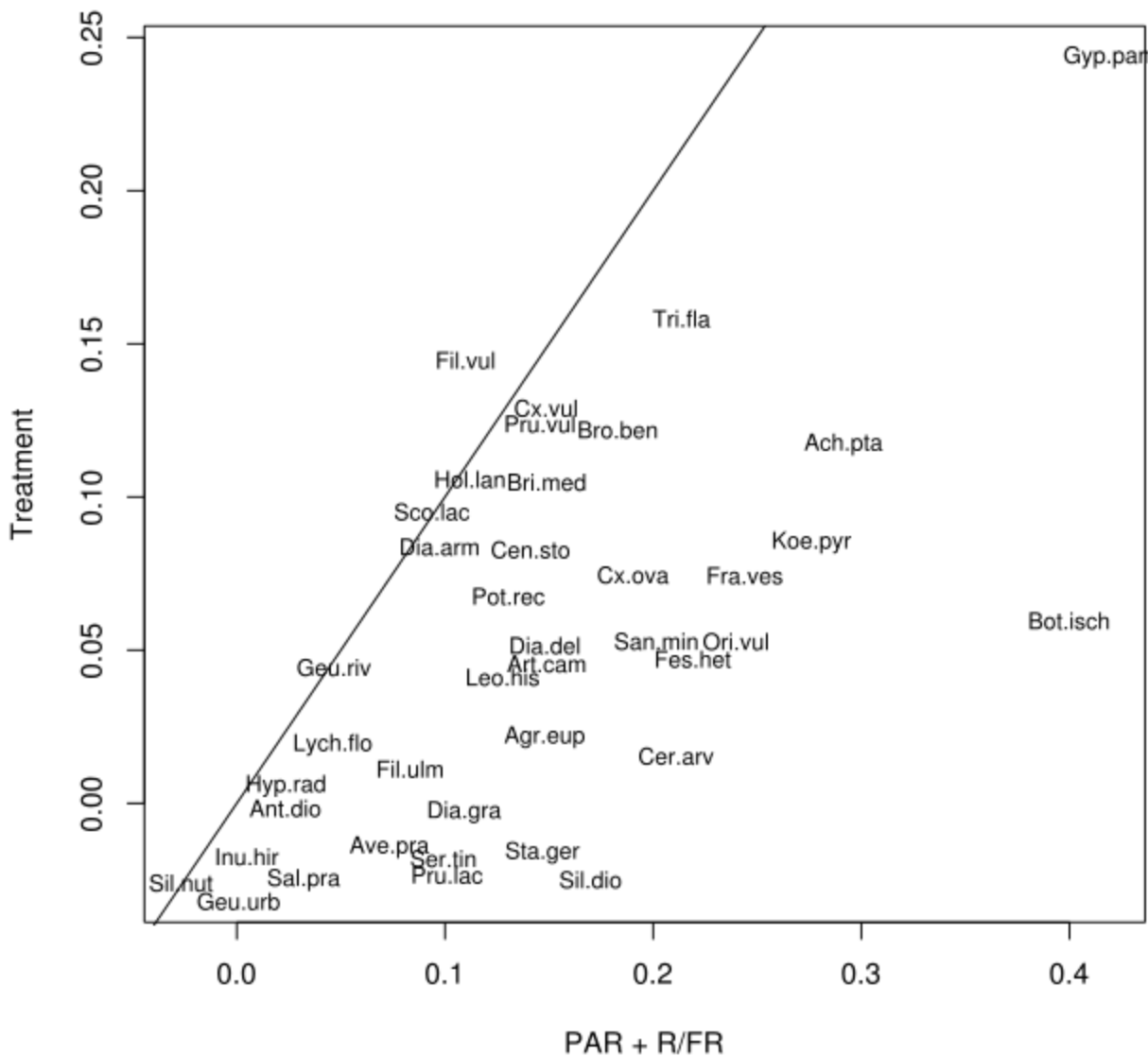
Active and passive plastic response to light: are there any relationships to functional traits

Supplementary material for this study

S1: Fig LACK OF FIT

S2: Table SCORES

S3: Figure MESVAR_SCORES



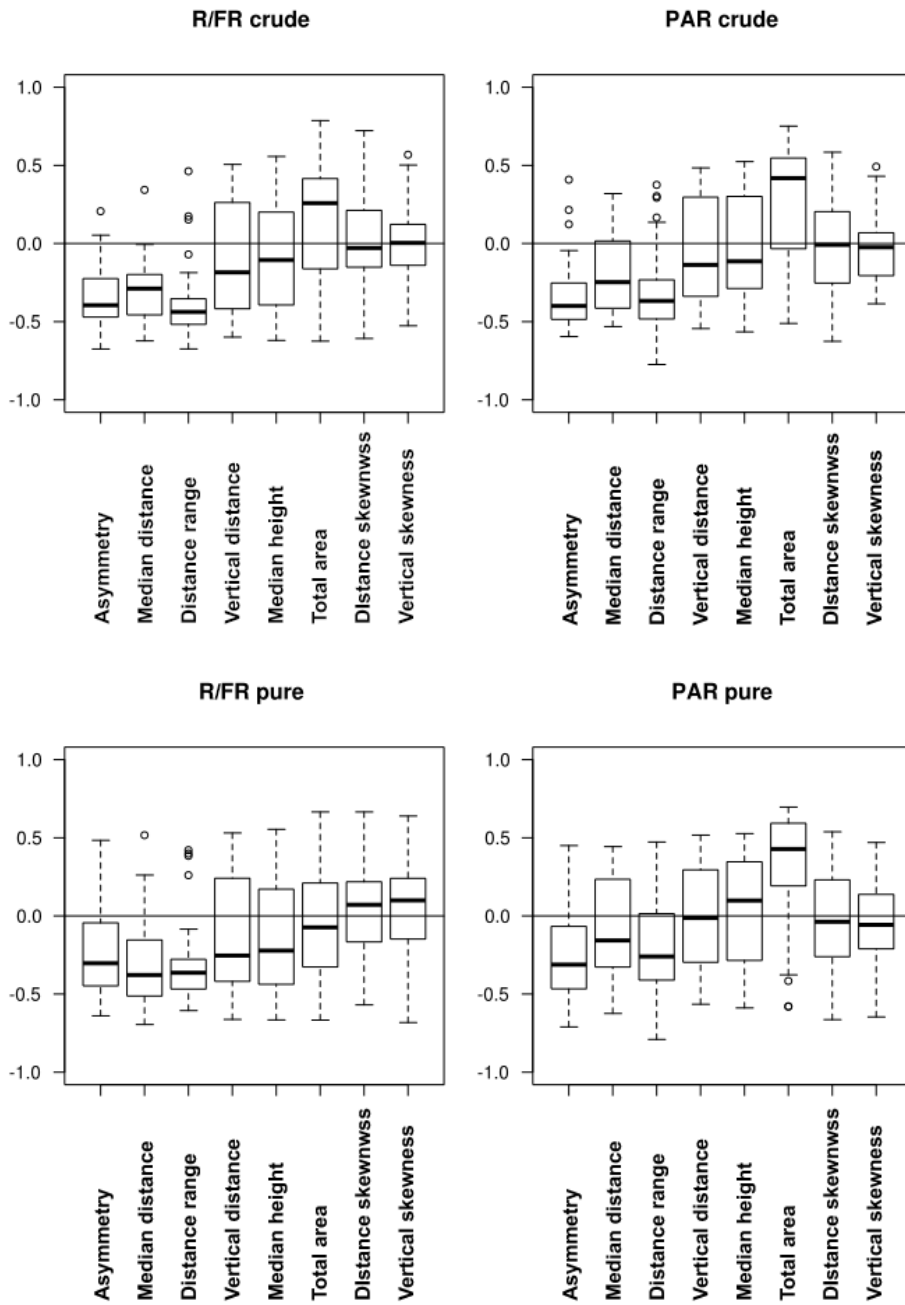
S1: Figure LACK OF FIT. Adjusted R² achieved by linear additive combination of PAR and R/FR levels (i.e. "sum of two lines") vs. Adjusted R² for six discrete treatments. Response of species below the line is better predicted by combination of two linear predictors rather than 6 discrete levels of treatment.

S2: Table SCORES. Contributions of individual measured variables to the plant's response to the light environment. Values in the table are correlation coefficients of scores of measured variables on the first canonical axis constrained by either R/FR (first two panels) or PAR (last two panels). In the first and third panels, the effect of the other light variable was removed by treating it as a covariate. + - $p < 0.1$, * - $p < 0.05$, ** - $p < 0.01$

RFR_pure								
	Assymetry	Median Disatance	Distance Range	Vertical Range	Median Height	Total Area	Distance Skewness	Vertical Skewness
Leaf area (log)	0.031	0.045	0.095	-0.201	-0.375*	-0.457**	-0.111	-0.001
SLA	-0.091	0.02	-0.087	-0.171	-0.218	-0.386*	-0.314+	0.131
Height at maturity	-0.155	-0.29+	-0.028	0.037	0.073	-0.11	-0.103	-0.044
Distance of lateral spread	-0.157	-0.034	-0.122	-0.161	-0.231	-0.145	0.196	0.018
Seed reproduction	0.15	-0.108	-0.121	0.226	0.354*	0.208	0.029	-0.17
Vegetative reproduction	-0.388*	-0.153	-0.127	-0.046	-0.009	-0.07	0.328*	-0.14
Shoot lifespan (cyclicality)	0.192	0.275+	0.067	0.012	0.109	0.136	-0.234	-0.113
Clonality	-0.168	0.023	-0.114	-0.082	-0.136	-0.295+	0.127	-0.077
RFR_crude								
	Assymetry	Median Disatance	Distance Range	Vertical Range	Median Height	Total Area	Distance Skewness	Vertical Skewness
Leaf area (log)	0.061	-0.105	0.168	-0.145	-0.31+	-0.476**	-0.042	-0.038
SLA	-0.151	0.117	-0.049	-0.095	-0.161	-0.219	-0.29+	0.173
Height at maturity	-0.137	-0.154	0.164	0.052	0.108	-0.068	-0.003	-0.096
Distance of lateral spread	-0.079	-0.111	-0.108	-0.014	-0.142	-0.073	0.129	0.176
Seed reproduction	0.046	-0.097	-0.18	0.108	0.239	0.096	0.002	-0.099
Vegetative reproduction	-0.35*	-0.168	0.2	0.048	0	-0.017	0.231	-0.009

Shoot lifespan (cyclicality)	0.03	0.123	-0.238	-0.067	0.025	0.118	-0.294+	-0.243
Clonality	-0.177	-0.036	0	0.041	-0.082	-0.186	-0.027	0.091
PAR_pure								
	Assymetry	Median Disatance	Distance Range	Vertical Range	Median Height	Total Area	Distance Skewness	Vertical Skewness
Leaf area (log)	-0.07	-0.275+	-0.039	0.016	0.001	-0.234	0.205	0.082
SLA	0.114	0.103	0.186	0.086	0.056	0.087	0.16	0.056
Height at maturity	-0.004	0.062	0.114	-0.023	0.001	-0.027	0.18	-0.015
Distance of lateral spread	0.336*	0.226	0.273+	0.304+	0.231	0.139	-0.071	0.122
Seed reproduction	-0.2	-0.027	-0.166	-0.124	-0.149	0.059	-0.013	0.196
Vegetative reproduction	0.201	0.171	0.13	0.295*	0.214	0.287*	-0.131	0.149
Shoot lifespan (cyclicality)	-0.326*	-0.183	-0.372*	-0.173	-0.206	-0.067	-0.21	-0.03
Clonality	0.195	0.05	0.091	0.136	0.054	0.032	-0.204	0.086
PAR_crude								
	Assymetry	Median Disatance	Distance Range	Vertical Range	Median Height	Total Area	Distance Skewness	Vertical Skewness
Leaf area (log)	-0.094	-0.261	0.043	-0.034	-0.118	-0.3+	0.121	0.116
SLA	0.024	0.175	0.211	-0.005	-0.059	-0.007	0.037	0.217
Height at maturity	-0.016	0	0.149	-0.041	0.004	-0.113	0.039	-0.026
Distance of lateral spread	0.286+	0.204	0.24	0.255	0.136	0.127	0.06	0.254
Seed reproduction	-0.126	-0.079	-0.084	-0.062	-0.079	0.039	0.042	0.136
Vegetative reproduction	0.119	0.114	0.13	0.272+	0.186	0.217	-0.003	0.208

Shoot lifespan (cyclicality)	-0.247	-0.09	-0.307+	-0.164	-0.15	0.016	-0.146	-0.275+
Clonality	0.173	-0.073	-0.032	0.173	0.068	-0.059	-0.086	0.154



S3 Figure: Overall values of measured variables in response to treatments

Prediction of root foraging ability by life history traits

Supplementary material for this study:

S1: List of species used, abbreviations of species names

S2: Correlation matrix of species traits

S3: Species response table

S1: Species list, pots per treatment

species	abbreviation	family	year	control	low	high
Agrimonia eupatoria	Agr.eup	Rosaceae	2014	7	6	7
Agrostis capillaris	Agr.cap	Poaceae	2014	7	6	7
Achillea ptarmica	Ach.pta	Asteraceae	2014	6	6	6
Bromus benekenii	Bro.ben	Poaceae	2013	7	6	6
Carex ovalis	Cx.ova	Cyperaceae	2014	2	3	5
Carex vulpina	Cx.vul	Cyperaceae	2014	5	4	7
Centaurea stoebe	Cen.sto	Asteraceae	2013	6	6	5
Cynosurus cristatus	Cyn.cri	Poaceae	2014	5	4	7
Dianthus armeria	Dia.arm	Caryophyllaceae	2014	7	6	7
Dianthus deltoides	Dia.del	Caryophyllaceae	2014	7	6	7
Erysimum crepidifolium	Ery.cre	Brassicaceae	2014	7	6	6
Filipendula vulgaris	Fil.vul	Rosaceae	2014	6	5	7
Galium verum	Gal.ver	Rubiaceae	2014	7	6	7
Geum rivale	Geu.riv	Rosaceae	2014	7	6	7
Geum urbanum	Geu.urb	Rosaceae	2013	6	6	7
Gypsophila paniculata	Gyp.pan	Caryophyllaceae	2013	7	5	7
Helianthemum grandiflorum	Hel.gra	Cistaceae	2014	7	6	7
Holcus lanatus	Hol.lan	Poaceae	2013	4	6	6
Hypericum perforatum	Hyp.per	Hypericaceae	2014	6	5	6
Hypochaeris radicata	Hyp.rad	Asteraceae	2013	6	6	6
Inula hirta	Inu.hir	Asteraceae	2014	6	6	6
Leontodon hispidus	Leo.his	Asteraceae	2013	6	6	7
Linaria vulgaris	Lin.vul	Plantaginaceae	2014	7	6	5
Lychnis flos-cuculi	Lych.flo	Caryophyllaceae	2014	3	6	7
Malva sylvestris	Mal.syl	Malvaceae	2014	6	5	6
Plantago lanceolata	Pla.lan	Plantaginaceae	2014	7	5	5
Potentilla recta	Pot.rec	Asteraceae	2014	7	6	7

Prunella vulgaris	Pru.vul	Lamiaceae	2014	3	3	4
(S1 cont.) Species	abbreviation	family	year	control	low	high
Pseudolysimachion spicatum	Pse.spi	Plantaginaceae	2014	7	6	5
Salvia pratensis	Sal.pra	Lamiaceae	2013	7	5	7
Sanguisorba minor	San.min	Rosaceae	2013	6	5	4
Scorzonera laciniata	Sco.lac	Asteraceae	2013	7	6	7
Silene dioica	Sil.dio	Caryophyllaceae	2014	7	6	7
Stachys germanica	Sta.ger	Lamiaceae	2013	7	5	7
Thalictrum flavum	Tha.flu	Ranunculaceae	2014	7	5	7
Thalictrum lucidum	Tha.luc	Ranunculaceae	2014	4	4	7
Verbascum phoeniceum	Ver.pho	Scrophulariaceae	2014	7	5	6

S2: Correlation matrix of species traits. Spearman's ρ

	SLA	Height at maturity	Seed reproduction	Vegetative reproduction	Shoot lifespan (cyclicality)	Clonality	Distance of lateral spread	Leaf area (log)
SLA	1	0.05	-0.01	0.04	0.05	0.18	-0.04	0.27
Height at maturity	0.05	1	0.06	-0.18	-0.15	-0.26	-0.16	0.04
Seed reproduction	-0.01	0.06	1	-0.51	0.37	-0.1	-0.55	0.07
Vegetative reproduction	0.04	-0.18	-0.51	1	-0.35	0.68	0.57	-0.33
Shoot lifespan (cyclicality)	0.05	-0.15	0.37	-0.35	1	-0.04	-0.38	-0.07
Clonality	0.18	-0.26	-0.1	0.68	-0.04	1	0.04	-0.26
Distance of lateral spread	-0.04	-0.16	-0.55	0.57	-0.38	0.04	1	-0.31
Leaf area (log)	0.27	0.04	0.07	-0.33	-0.07	-0.26	-0.31	1

S3: Species foraging estimates for low and high contrast. Reliability gives width of the interval in which 50% (“middle half”) of bootstrapped estimates fit in.

Species	Response Low contrast	Reliability Low contrast	Response High contrast	Reliability High contrast
Bro ben	-0.143	0.238	-0.143	0.238
Cen sto	0.222	0.222	0.2	0.222
Geu urb	0.167	0.222	0.31	0.222
Gyp pan	0.014	0.257	0.173	0.257
Hol lan	0.042	0.25	0.5	0.25
Hyp rad	0.222	0.222	0.389	0.222
Leo his	0.194	0.222	0.167	0.222
Sal pra	0.129	0.257	0.418	0.257
San min	-0.033	0.267	0.375	0.267
Sco lac	0.214	0.19	0.194	0.19
Sta ger	0.043	0.286	0.337	0.286
Agr cap	-0.048	0.238	0.071	0.238
Agr eup	-0.071	0.238	-0.153	0.238
Ach pta	0.194	0.25	0	0.25
Cx ova	0	0.5	0.4	0.5
Cx vul	0.1	0.3	0.071	0.3
Cyn cri	-0.2	0.25	0.1	0.25
Dia arm	-0.071	0.286	0.316	0.286
Dia del	0.024	0.238	-0.031	0.238
Ery cre	0.071	0.262	0.167	0.262
Fil vul	-0.433	0.1	0.024	0.1
Gal ver	-0.31	0.167	0.153	0.167
Geu riv	-0.048	0.262	0.255	0.262
Hel gra	-0.024	0.238	0.255	0.238
Hyp per	-0.267	0.333	-0.333	0.333
Inu hir	0.222	0.222	0.361	0.222
Lin vul	0.143	0.238	0.1	0.238

Lyc flo	0	0.333	0.405	0.333
(S3 cont.) Species	Response Low contrast	Reliabilty Low contrast	Response High contrast	Reliabilty High contrast
Mal syl	0.267	0.233	0.222	0.233
Pla lan	0.014	0.229	0.129	0.229
Pot rec	0.452	0.071	0.439	0.071
Pru vul	-0.5	0	-0.167	0
Pse spi	-0.119	0.19	0.071	0.19
Sil dio	0.238	0.19	0.133	0.19
Tha fla	0.043	0.314	0.071	0.314
Tha luc	-0.25	0.25	-0.286	0.25
Ver pho	0.1	0.229	0.238	0.229