RESEARCH PAPER

Shoot: root ratio of seedlings is associated with species niche on soil moisture gradient

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ABSTRACT

- Surviving the seedling phase is crucial for the establishment of plant individuals and populations. In ecosystems with dynamic water availability such as temperate grasslands, seedlings should adjust their growth strategy not only to match the current conditions but also to secure resource acquisition in the future. Here, we explored evolutionary adaptations determining plant early growth strategies in herbaceous species of temperate grasslands differing in their requirements for soil water availability.
- We chose 15 plant genera, within which we selected species differing in their Ellenberg indicator values for moisture. We cultivated the seedlings under standard conditions with sufficient water supply for 4 weeks. Subsequently, we measured length-based and mass-based shoot:root ratio to investigate seedling growth strategy and its association with species ecological niche. Seed size and content of soil-borne nutrients were considered as potential covariates affecting this association.
- Linear mixed-effect models identified the length-based shoot:root ratio of seedlings was positively associated with soil moisture requirements in a congeneric species comparison. Nitrogen and phosphorus seed concentrations had an additional negative effect on the shoot:root ratio. Neither of these trends was found for the mass-based shoot:root ratio.
- We demonstrated for the first time that there might be a general adaptation modifying the seedling shoot:root ratio according to the species niche position on the soil moisture gradient in temperate grassland species across a broad range of angiosperm phylogeny. This adaptation seems to be affected by seed mineral nutrient reserves and may operate in parallel to the well-known phenotypic plasticity.

INTRODUCTION

Understanding plant adaptations to dry conditions is becoming more and more urgent under the current climatic changes. For instance, aridity is expected to increase (Dai 2013), with consequential changes in precipitation (Konapala et al. 2020) and thus changes in the soil moisture regime (Jung et al. 2010). As a result, there is a great interest in studying plant community resilience (Grime et al. 2008) and composition changes under shifting environmental conditions (Shovon et al. 2019). An important determinant of the community assembly is species regeneration niche (Grubb 1977), that is, requirements for establishment of a new plant individual. Every new individual is provided with supplies from the mother plant stored in the seed. The species identity of the mother plant determines the habitat requirements of the new individual. Seedlings thus need to adopt a growth and resource allocation strategy matching the environment that they expect to encounter in their further development. Research on regeneration niche frequently emphasizes the role of the environment and seedling growth plasticity, but little is known about matching the seedling growth strategy and the species ecological niche.

Seedlings first develop roots to obtain water, but their relative investment into leaves *versus* roots changes over time (Gedroc et al. 1996; McConnaughay & Coleman 1999). Asymmetric aboveground competition usually drives investment into shoot development (Bessler et al. 2009), but its significance is much smaller in unfertile or dry environments (Keddy et al. 1997; Dovrat et al. 2019). Conversely, the phenotypic plasticity of plants in response to soil water availability is well known. Usually, under water stress conditions, the root system tends to develop relatively longer roots (Schenk & Jackson 2002), less branching (Yavitt & Wright 2001) and fine roots have a shorter lifespan (Pregitzer et al. 1993). Plants also allocate their biomass into the root part rather than to aboveground parts (Chapin et al. 1993; Kozlowski & Pallardy 2002) to ensure enough water and, concomitantly, soil nutrient acquisition (Lima et al. 2010). The behaviour of seedlings was documented to depend not only on environmental resource availability but also on seed resources. In the first phase of ontogeny, the development of resource-harvesting organs relies on seed resources (Leishman & Westoby 1994; Baraloto et al. 2005; Urbieta et al. 2008). Therefore, seed size limitation may lead to a trade-off between the immediate need for resources against adaptations for future needs (Leishman et al. 2000;

Mašková & Herben 2018). Moreover, this trade-off can be shaped by seed nutrient composition (Milberg & Lamont 1997; Vaughton & Ramsey 2001).

Common garden experiments have demonstrated the capacity of seedlings of species from nutrient-rich habitats to adjust their morphology in terms of specific leaf area and specific root length and, consequently, exploit abundant soil nutrients better than species from nutrient-poor habitats (Fichter & Schulze 1992; Elberse & Berendse 1993). These findings point to evolutionary adaptations in seedling morphology at the species level. However, such allocation strategies are poorly studied in relation to water deficiency (e.g. Dyer et al. 2001). Intuitively, species of dry habitats may be expected to invest relatively more into root growth to ensure water supply. In contrast, species of moist habitats should invest more into shoot growth to escape competition for light. In ecosystems with high water availability dynamics, such as temperate grasslands, seedlings should adjust their resource allocation based not only on the current conditions but also to secure resource acquisition in the future. For instance, seedlings of species of dry habitats may experience plentiful water at the beginning of growth. Conversely, seedlings of plants occurring in productive grasslands with unlimited belowground resources but strongly limited by competition for light typically germinate in periods of temporary high light availability at ground level. Following a fixed growth strategy suitable for the whole season may thus more advantageous than the phenotypic plasticity. Therefore, we hypothesize that there should be differences in the early growth strategies of plant species, and that this will be related to their habitat requirements. To the best of our knowledge, this hypothesis was only tested by Dyer et al. (2001) with plants originating from a coastal Mediterranean grassland and adjacent desert, but the results were inconclusive. However, such results may be caused by considering only annual species and focusing on the dry end of the soil moisture gradient.

This study explores allocation strategies of plants in their early growth phase through an experimental test with seedlings of 33 temperate grassland species differing in habitat requirements across the whole gradient of soil water availability. We also consider the effect of seed traits as potential covariates affecting such strategies. Namely, we hypothesize that (i) seedlings of species from dry environments adjust their strategy to capture more belowground resources, while species of moist ecosystems prefer aboveground vertical growth to escape competition for light; and (ii) this association between early plant growth and species ecological niche will be affected by seed size and seed content of soil-borne nutrients (i.e. nitrogen and phosphorus).

MATERIAL AND METHODS

Species selection and cultivation

Species for the study were selected from the species pool of temperate grassland habitats where similar processes affecting seedling survival are to be expected. To cover a broad evolutionary range of Central Europe, we chose 15 genera from nine families for the study. Two, three or four species from each genus were selected that differed in requirement for moisture as described by the Ellenberg indicator value (EIV) for moisture (Ellenberg et al. 1992; Chytrý et al. 2018). Although EIV

are semiquantitative and not based on direct measurement, they are known to provide reliable information on species niche along principal environmental gradients (Schaffers & Sýkora 2000; Diekmann 2003). While the Ellenberg values are ordinal, they show a reasonable monotonic relationship with measurable variables in test datasets (Schaffers & Sýkora 2000, Wamelink et al. 2002) and can be safely used as predictors in regression analyses. In each genus, we intended to cover a moisture gradient that was as wide as possible. All selected species displayed the same Raunkiær life form – hemicryptophytes (Raunkiaer 1934; Kaplan et al. 2019; Chytrý et al. 2021) and perennial growth strategy (Chytrý et al. 2021) – to minimize the potential impact of growth form on biomass partitioning. Based on these criteria and seed germination or cultivation limitations, we obtained data for 33 species from 15 genera (see Table S1 for details).

We obtained all seeds from a local commercial supplier (Planta Naturalis; www.plantanaturalis.com; 50.42°N, 15.19°E) which had grown all the species in the same place for more than 20 years. Thus, possible impacts of different environmental conditions of the mother plant environment arising from maternal effects (Galloway 2005) were minimized. Seeds of Carex and Iris species were surface sterilized before germination (5% NaClO for 15 min with subsequent rinsing in water); cold stratification (constant temperature 5°C for 2 months) and then mechanical scarification (part of the integument removed with sandpaper to break the physical dormancy) were applied (Royal Botanic Gardens Kew 2017). Due to space limitations, seed germination and plant cultivation were conducted in two rounds with each species cultivated in a single round. The growing conditions were identical between the two rounds, but the germination conditions differed moderately. All seeds in both rounds were germinated in Petri dishes on filter paper moistened with deionized water. The Petri dishes were kept in a growth chamber (first round: light intensity 160 µmol m^{-2}s^{-1} with 12-h light/12-h dark at 18°C/12°C; second round: 200 µmol m^{-2} s^{-1} 14-h light/10-h dark, 18°C/15°C).

Immediately after germination, seeds were moved to plastic pots (21.5 × 10.5 × 10.5 cm) filled with vermiculite:sand (9:1), moistened with deionized water, placed in a greenhouse (light/dark regime: 17°C for 11 h/12°C for 13 h, mean soil temperature was 14.7°C, mean relative air humidity was 78%) and arranged in a completely randomized design. In addition, locations of individual pots were re-arranged on a weekly basis to prevent possible effects of environmental heterogeneity. Ten and eight individuals per species were grown in the first and second rounds, respectively. The plants were watered sufficiently to keep the substrate surface moist and thus prevent any water limitation. Commercial fertilizer, Ferty 3 (N:P:K = 3:2:3) was added continuously to each pot through irrigation (each plant received 0.02 g N over 4 weeks).

Measurements

Plants were harvested after 4 weeks of cultivation, with the substrate carefully removed from the roots. Subsequently, the stem length and longest root were measured. Then root and shoot were separated, dried to constant weight and dry biomass of shoot and root determined. Based on these data, we calculated the following seedling growth parameters: length- and mass-

based shoot:root ratio, specific shoot height (stem length:dry shoot mass) and specific root length (longest root length:dry root mass). Information on species-specific seed mass was obtained by counting 100 seeds, allowing them to dry in the air and weighting them (Kleyer *et al.* 2008).

Seed nutrient reserves were measured as the content of nitrogen (N), phosphorus (P) and non-structural carbon (C). C content was calculated as the sum of fructans, starch and oils, corrected to C content. N content was measured by flow injection analysis after Kjeldahl mineralization. P content was measured by flow injection analysis after perchloric acid mineralization. Starch and fructans were measured with the enzymatic procedure Megazyme (McCleary *et al.* 1994). Oil content of seeds was measured by Soxhlet (International Organization for Standartization 2016). See Maškova & Herben (2021) for more details.

Data analysis

We first explored the correlation structure among seed traits, seedling growth parameters and species moisture EIV using a partial principal components analysis (PCA). Mean values for each species were used as independent observations in the analysis. Genus identity was used as a covariate to focus analysis on the variability within genera. Seedling growth parameters based on biomass or length measurements were log-transformed, and all parameters centred and standardized by dividing them by their standard deviation. In the data analysis exploratory phase, we identified a strong positive correlation between N and P content in seed biomass ($r = 0.82$, $P < 0.001$); therefore, we combined N and P content into a single variable by computing another PCA (standardization by variables) with just these two parameters. Subsequently, we used the first PC (explained 91.25% of variability) of this analysis as the 'seed N & P concentration' parameter in further analyses. This parameter was scaled on a relative scale, ranging from 0 to 1 in seeds, with the lowest and highest nutrient concentration, respectively.

Linear mixed-effect models were used to test the associations between seedling growth parameters (length- and mass-based shoot:root, specific shoot height and root length) on the one side and species moisture EIV and seed traits on the other. The random term of the model included species identity nested within genus. This implies individual species are considered as independent observations and the focus of the analysis is comparisons within the genera. We tested both simple effects of individual predictors and also built minimum adequate models by a stepwise forward selection of significant predictors based on likelihood ratio tests ($P < 0.05$).

All analyses were conducted in R version 4.02 (R Core Team 2020). Packages vegan (Oksanen *et al.* 2019) and nlme (Pinheiro *et al.* 2018) were used for PCA and mixed effect models, respectively.

RESULTS

The partial PCA identified 79.1% of the variability in the whole dataset as being associated with differences among genera. As a result, 20.9% of the variability was left for analyses within genera, which was the focus of the study. The partial PCA identified a notable correlation between seed traits, seedling growth parameters and species moisture EIV (Fig. 1). The EIV,
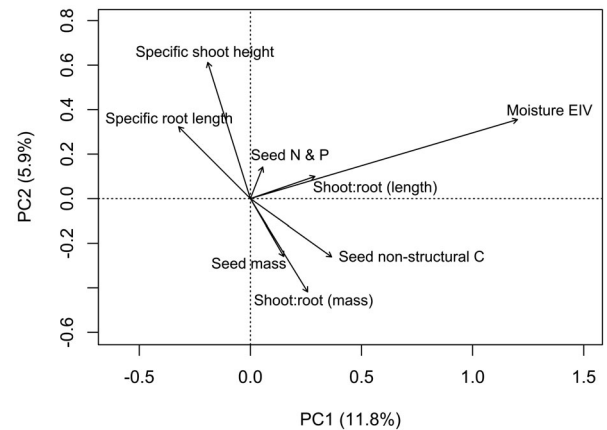

Fig. 1. Partial principal components (PC) ordination plot displaying correlations among seed traits, seedling growth parameters and species moisture Ellenberg indicator value (EIV). The first two PC account for 17.6% of total variability, which corresponds to 67.9% of variability within the genera.

however, seemed only moderately positively correlated with the length-based shoot:root ratio. There also appeared to be a slight correlation with seed N & P concentration. Most other traits seemed orthogonal to the main gradient defined by the EIV.

Linear mixed-effect models identified a significant association with moisture EIV only for the length-based shoot:root ratio. This parameter was significantly associated not only with the EIV (Likelihood ratio = 6.64, df = 1, $P = 0.010$) but also with seed N & P concentration (Likelihood ratio = 6.27, df = 1, $P = 0.012$; Fig. 2). The minimum adequate model included moisture EIV as a positive predictor ($t_{16} = 3.12$, $P = 0.007$) and seed N & P concentration as a negative predictor ($t_{16} = -3.17$, $P = 0.006$) of the shoot:root ratio. Of the other seedling growth parameters, mass-based shoot:root ratio was only positively associated with seed non-structural C concentration ($t_{17} = 2.34$, $P = 0.032$ Figure S1) and the relationship between specific root length and seed mass was marginally non-significant ($t_{17} = -2.02$, $P = 0.06$). Other associations between growth parameters and seed traits and moisture EIV were not significant (Table S2).

DISCUSSION

Our experiment demonstrated a systematic trend in the length-based shoot:root ratio of closely related species associated with their ecological niche on the soil moisture gradient. Observation of such pattern despite sufficient water supply suggests that seedling growth is driven by adaptation to water conditions expected to be encountered in later life stages, as is typical for temperate dry grasslands (Fischer *et al.* 2020). Exactly as expected, species from dry habitats had deeper roots, important for securing water supply (Schenk & Jackson 2002; Holdo & Timberlake 2008), the availability of which typically increases with soil depth (Grieu *et al.* 2001). In contrast, seedlings of moist habitat species grew relatively taller, which could be crucial to escape competition for light (Kobayashi & Kamitani 2000; Deng *et al.* 2006). This pattern may even be slightly stronger than observed here. We sourced seeds from a company where the maternal culture is in a common garden. This minimizes the potential influence of epigenetic effects (*e.g.* maternal effect; Galloway 2005), but may have led to selection of genotypes adapted to these particular conditions, which may
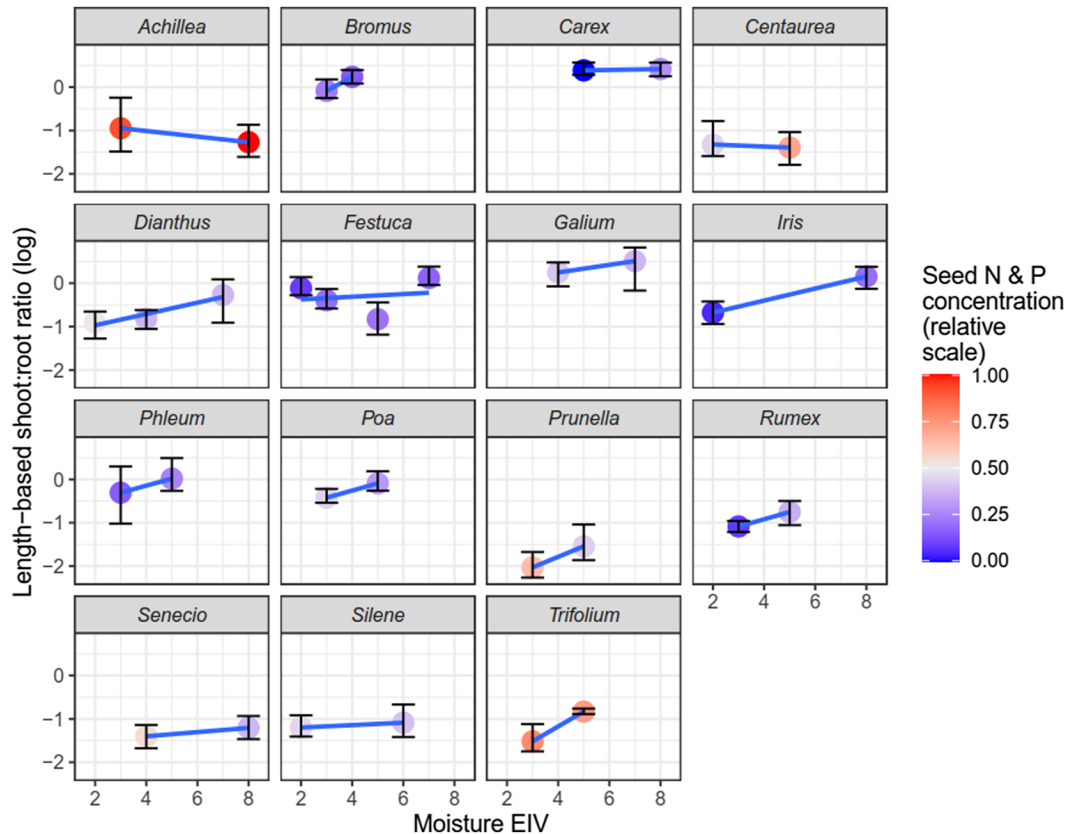
Fig. 2. Association between length-based shoot:root ratio and moisture Ellenberg indicator value (EIV), with additional seed N & P concentration, illustrated using a colour scale. Coloured dots and error bars indicate mean and range of shoot:root ratio values, respectively. Blue lines show genus-specific linear fits.

also involve adjustment of the shoot:root ratio. Therefore, the association between moisture EIV and shoot:root ratio may have been underestimated in our experiment. Remarkably, we observed a relationship between shoot:root ratio and moisture EIV in species from multiple genera, spanning a wide range of angiosperm phylogeny, which suggests the potential universality of this pattern. Our experiment is thus the first to demonstrate the role of morphological evolutionary adaptations, which act together with the well-known phenotypic plasticity (Pregitzer *et al.* 1993; Yavitt & Wright 2001; Schenk & Jackson 2002) to secure species persistence in a given environment throughout the soil moisture gradient.

The association with species-specific moisture habitat requirements was only present in the length-based shoot:root ratio, which directly reflects resource acquisition ability. On the other hand, the relationship between moisture EIV and mass-based shoot:root ratio was much weaker and not significant (albeit present in some genera; Figure S1). This agrees with a previous investigation of evolutionary adaptations of plant growth to habitat conditions (Dyer *et al.* 2001) where no consistent pattern was found for the mass-based ratio. The mass-based ratio better reflects the differential investment to above- and belowground growth. Thus, it seems that there may be alternative strategies that allow species to partially decouple resource acquisition ability (length-based ratios) from investments (mass-based ratios).

We identified seed concentration of mineral nutrients as a significant covariate to the trend between moisture EIV and

seedling length-base shoot:root ratio. This points to the role of mineral nutrients, another soil-borne resource, in the seedling resource economy. Seeds with a higher concentration of mineral nutrients tended to produce seedlings with relatively deeper roots. Such an outcome can be expected because species from nutrient-poor environments are known to have higher seed N concentrations (Mašková & Herben 2021). This suggests that the relationship between environmental moisture gradient and seedling growth pattern is not straightforward. It may also be shaped by different soil nutrient requirements of individual species, which was the case for *Achillea* and *Centaurea* genera in our experiment – the two genera with the weakest observed trend.

Our study is the first to suggest that there may be a general adaptive role of seedling shoot:root ratio in relation to the wide range of soil moisture gradients in temperate grassland species. There are nevertheless many outstanding questions. We know that these evolutionary adaptations operate in parallel with extensive phenotypic plasticity. Relative contributions of these effects to seedling growth morphology, as well as plasticity in root system architecture, are obvious questions raised by our research. The effects of mineral nutrients also call for explicit testing to reveal possible conditional or interactive effects of these soil-borne resources. Such experiments may not be easy to conduct due to the high factor-level combinations required. However, they may substantially advance in developing the regeneration niche theory, relating seedling growth strategies to plant species ecological requirements.

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AUTHOR CONTRIBUTIONS

J.T. designed the study, J.M. performed the experiment, J.T. analysed the data, J.T. and T.M. interpreted results, T.M. wrote the text with contributions from J.T. All authors approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

FUNDING INFORMATION

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available in Table S1.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Association between mass-based shoot to root ratio and moisture EIV (non-significant), with additional display of seed non-structural C concentration illustrated by color scale. Color dots and error bars indicate mean and range of shoot:root ratio values, respectively. The blue lines show genus-specific linear fits

Table S1. Species used in the experiment, their moisture EIV, seed mass, seed nutrient content and shoot and root biomass and length.

Table S2. Results of linear mixed-effect models between seedling growth parameters (length- and mass-based shoot: root, specific shoot height and root length) and species moisture EIV and seed traits.

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