Root Foraging Precision: Do Experimental Conditions Matter?

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Research Article

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Root foraging precision: Do experimental conditions matter?

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Abstract

Purpose: Root foraging precision, i.e., preferential root proliferation in nutrient-rich patches in heterogeneous soil, contributes significantly to plant nutrient acquisition. The ability to forage is usually studied experimentally, but often under different conditions. It remains unclear whether different experimental conditions affect root foraging precision. We studied the effect of experiment duration, pot size and method of root separation (inclusion or exclusion of coarse roots) on root foraging precision and its estimation. This allowed us to comment upon the appropriateness of using root foraging as species-specific values in databases and meta-analyses.

Methods: We cultivated three perennial species in pots with spatially heterogeneous nutrient supplies and manipulated the experiment duration (4 – 10 weeks). We partly replicated the experiment in two consecutive years. In two of the three species, we compared outcomes when root types were separated and unseparated, and for one species, we also manipulated pot size. We assessed the effects of the manipulated factors on foraging precision expressed as the ratio of root biomass in nutrient-rich and poor patches.

Results: Root foraging precision was not affected by experiment duration or pot size. Separating roots to use only the fine ones for root foraging assessment amplified foraging precision values and reduced their intraspecific variation.

Conclusions: Our study investigated various methods of root foraging research and their impact on root foraging precision. Root foraging precision is invariable to the studied factors; therefore, it is suitable as a species-specific trait if other factors (such as nutrient patch characteristics) are considered.
1. Introduction

Root foraging in a heterogeneous soil environment is a hallmark of plant belowground phenotypic plasticity (Grime et al. 1997; Hutchings and de Kroon 1994; Giehl and von Wiren 2014). Because of the nature of the phenomenon, it is usually studied under controlled conditions, i.e., using cultivation experiments. Although the experimental approach allows us to understand species ecology (Lepik et al. 2005), variation in experimental conditions impedes generalization of results and disallows meaningful meta-analytic approaches (e.g., Kembel and Cahill 2005). Considering the methodological diversity in root foraging experiments, it is not surprising that using root foraging in studies such as meta-analysis has been questioned (de Kroon et al. 2012).

In root foraging experiments, plants are usually grown in soil with a heterogeneous nutrient supply, and after some time, the placement of roots (root foraging precision) or root trait variation between nutrient "rich" and "poor" parts is compared (Campbell and Grime 1989). Despite this general framework, several important factors cause possible inconsistencies. These factors include differences in the level of contrast between "rich" and "poor" patches in the heterogeneous environment and also in the spatial scale of this heterogeneity. The challenges that these factors pose to generalization have been well examined (Campbell et al. 1991; Kembel and Cahill 2005; de Kroon and Mommer 2006; Weiser et al. 2016). However, there are other three important factors that have not been well studied: (i) experiment duration, (ii) pot size, and (iii) the way roots of different functions are separated for root foraging precision assessment.

Although these three factors are important when determining the feasibility of an experiment, both the theory and experimental data about their effects on root foraging precision are indirect. Moreover, these effects are likely to be species-specific. Experiment duration and pot size will likely affect all species in the same way but with a different strength of these effects as each species has a different growth rate. While the way of root separation according to their functions or size will likely affect root foraging estimation mainly in species with highly segmented root systems. In turn, these factors vary considerably among experiments. It remains an open question, then, whether suchlike diversity in experimental design allows comparison of root foraging data across experiments, and possibly also across species within an experiment.

The duration of root foraging experiments can vary from days to weeks and even years (Kembel et al. 2008; Reyes and Aguiar 2017; Wang et al. 2018b; among others). Long experiment duration negatively influences root-foraging precision, which is thought to be due to nutrient depletion (Kembel and Cahill 2005). However, although nutrient depletion usually does not occur in root-foraging experiments with constant nutrient supply (standard methodology of Campbell and Grime (1989)), the ontogenetic stage of a plant may affect root foraging precision even under constant nutrient supply. Younger plants observed at the beginning of an experiment may forage more precisely than older ones observed later, because the older plants would have already accumulated enough nutrients, therefore requiring greater contrast with the baseline level of nutrients to initiate foraging (de Kroon et al. 2009). It was also suggested that root foraging precision varies among species because of their different relative growth rates (Aanderud et al. 2003; but see Kembel and Cahill 2005). Fast-growing species are likely to respond by root system growth quickly, while root foraging precision of the slow-growing species in the same period of time may not be developed enough. Thus, the observed interspecific differences in root foraging precision (as well as intraspecific differences found in different studies) may only be an artifact of the experiment duration.
While the experimenter may wish to use the minimal pot size in which the plant can fit, pot size affects plants’ biomass, growth, and morphology (Semchenko et al. 2007; Poorter et al. 2012; Fuellner et al. 2012; Wheeldon et al. 2020) as well as its effectiveness of root nutrient uptake (Kume et al. 2006). Roots reaching the wall and bottom of the pot start to follow their contours, forming a pattern, which may distort root foraging-precision assessment. Therefore, plants in bigger pots may show better root foraging ability, and root foraging precision may be observable for a longer time.

Third, besides adequate duration and pot size, experiments vary in which root system parts are considered when estimating root foraging precision (Keser et al. 2015; Weiser et al. 2016; Wang et al. 2018a). Whether to measure root traits on fine or coarse roots may differ according to the research question (Freschet and Roumet 2017). Since only fine roots take up nutrients, these are the most relevant to root foraging assessment (Pregitzer et al. 1993). On the other hand, the position of coarse roots reflects past responses to heterogeneity, as even the coarse roots were once fine ones. For this reason, coarse roots could also be included when assessing root-foraging precision.

Another reason for discarding the coarse roots is that they may negatively affect the root foraging precision assessment. The central part of the root system with thick roots usually grows at the interface of rich and poor patches. During harvest, it could end up in either of these parts randomly, thus increasing the variance of the root foraging precision estimate. However, separating fine roots from coarse roots and taproots is quite demanding.

To address these issues, we tested the effects of different experimental conditions on root foraging precision and its values, using three species differing in their (previously estimated) root foraging precision and growth rates. We followed their root foraging precision development for ten weeks. Also, we experimented using two pot sizes with the fast-growing species and separated the fine, coarse, and taproots. Specifically, we asked whether: (i) the root foraging precision changes with the duration of the experiment; (ii) the root foraging precision and its dynamics change with the size of the pot; and (iii) whether separating roots by type affects the root foraging precision values and their intra-specific variability. Because we partly replicated the study in a second year, we were also able to address the inter-annual variability of the root foraging precision. Answering these questions allowed us to assess the usability of root foraging precision in meta-analyses.

2. Materials and Methods

2.1 Species selection and experimental setup

To answer our questions, we carried out two types of experiments. In the first type, we focused on root foraging precision development through time and its comparison between species. In the second one, we aimed to elucidate the effects of pot size on root foraging precision development in a fast-growing species. We used material from both to estimate the effect of root type separation on root foraging precision. The experiments were run during 2018 and 2019 in the greenhouse in the experimental garden of the Faculty of Science, Charles University (50.069N, 14.425E).

In both types of experiments, we employed pots that differed from each other in their soil environments: heterogeneous pots and control pots. In the pots with the heterogeneous soil
environment, the halves of the individual pots differed from each other in the amounts of a fertilizer administered. In the control pots, the fertilizer was distributed evenly among the pot halves, but with the same total fertilizer amount per pot as in the heterogeneous pots. We used a modified version of a nutrient dripping method of Campbell and Grime (1989) since the method allowed us to create separate nutrient-rich and nutrient-poor patches without any physical barrier. This method uses surface drip irrigation on a non-retentive substrate, so that the main flow of the water and fertilizer is vertical with only limited mixing, creating heterogeneities in the horizontal plane. We used standard round pots with holes in the bottoms (Fig. 1 in Supplementary Material). We filled the pots with washed river sand (washed in a concrete mixer with tap water until the water was clear) and placed them on water-leveled perforated tables to allow excess water from the drip irrigation to flow freely out of the pots. Each pot was drip irrigated from two opposite sides, and the drippers were set to deliver a constant amount of the irrigation solution at the same time. The pots were irrigated so that the sand was kept wet at field capacity (irrigated two times per day). The fertilizer was added proportionally to the water, using flow-powered proportional dosing pumps (Dosatron, D25RE2).

We created heterogeneous (2:0) and homogeneous (1:1) nutrient treatments by changing the proportions of fertilizer in the drippers, but both treatments received the same amount of fertilizer. The homogeneous treatment received water with fertilizer from all the drippers in the pot, whereas the heterogeneous treatment received water with fertilizer from all drippers on just one side of the pot while the other side was supplied with water to which fertilizer had not been added. The distinct separation of the nutrient-rich and poor parts in the heterogeneous pots was made visible by algae growing on the surface (Fig. 1 in Supplementary Material). We supplied pots with the recommended amount of fertilizer for adult plants (0.1% v/v solution; Wuxal Super, NPK 8:8:6 + micro-nutrients, Aglukon; for full contents of fertilizer see Table 1 in Supplementary Material) by mixing water and fertilizer (10:1) in the storage barrel and then diluting it further to achieve 2 % (heterogeneous treatment) or 1 % (homogeneous treatment) by drip irrigation.

As the studied plants we selected three herbaceous species - Achillea millefolium (L.), Lythrum salicaria (L.), and Verbena officinalis (L.). Because the study aims to observe changes in root-foraging precision, we selected species with some degree of root-foraging precision shown previously (Weiser et al. 2016, Stiblíková et al. [in press]), and their different levels of the root-foraging precision forming a gradient. Thus, L. salicaria has shown a high level of root foraging precision, and A. millefolium and V. officinalis were found to have low levels. We obtained the seeds from a commercial supplier (Planta Naturalis Ltd., Markvartice, Czech Republic, www.plantanaturalis.com). Before we started the experiments, we sterilized the seeds with a solution of sodium hypochlorite (5% w/w) and water in a ratio of 1:10 v/v for ten minutes and washed them in demineralized water afterward. We sowed the seeds into trays (TEKU Pöppelman, JP3050/104) with washed river sand and placed them in the greenhouse. We transferred the seedlings to the experimental pots when they had their first true leaves.

2.2 Experiment 1 – Effects of experiment duration

To investigate changes in root foraging precision over time, in 2018 we planted the three species in pots with heterogeneous and homogeneous nutrient treatments. First, we sowed the seeds into the trays at the end of June 2018. On July 31, seedlings were big enough for transplantation to the experimental pots. We transplanted the seedlings into the middle of round 3-L pots (TEKU
Pöppelman, MCI 19) filled with washed river sand (Fig. 1-a in Supplementary Material). After the 4th, 5th, 6th, 8th, and 10th weeks from transplantation we harvested the plants from 42 pots each time, representing the combination of the three species under two nutrient treatments (homogeneous and heterogeneous) in seven replicates. Altogether, we harvested the plants from 210 pots. However, the final number of pots used in analyses was 174 due to some plants dying, the discarding of samples with whole root system weight < 0.01 g, and losses during processing of samples.

At harvest, we would cut the aboveground part of the plant, dry it (72 hours, 65°C) and weigh it. We precisely divided each pot into two halves, so that the plane of the cut was vertical and perpendicular to the line connecting the positions of the drippers (thus perpendicular to the nutrients gradient in heterogeneous pots), and it included the rooting point of the plant. We did the cuts using a sharpened metal plate. Next, we washed both halves on the fine sieve and gathered all the roots. We carefully washed sand out of the roots and dried them on Petri dishes (72 hours, 65°C), and weighed them.

We followed the same protocol in 2019, using just *A. millefolium* and *L. salicaria*, but with 15 replicates per treatment, species, and harvest, resulting in 300 pots; however, the final number of pots in analyses was 294 (deaths, losses during processing, discarding of samples with whole root system weight < 0.01 g).

### 2.3 Experiment 2 – Pot size effects

Among the species we used, *L. salicaria* was the fastest growing one. Therefore, we used it in the experiment estimating the effects of pot size on root foraging limitation. In this experiment, we used two sizes of pots: 3L (regular, as above) and 10L (TEKU Pöppelman, MCI 29; Fig. 1 in Supplementary Material). Whereas the regular pots had one dripper on each side of the pot, the big pots had three drippers on each side, thus tripling the input of water and fertilizer into the big pots compared to the regular ones. Aside from this, we followed the same protocol as in Experiment 1.

The experiment took place in 2018, with the seedlings transplanted on July 30. Harvesting took place after the 4th, 5th, 6th, 8th, and 10th weeks from the transplantation. On each occasion, 28 pots were harvested (1 species × 2 nutrient treatments × 2 pot sizes × 7 replicates), yielding 140 pots in the experiment altogether. The final number of pots included in analyses was 121 (due to pots eliminated because of deaths, losses during processing, or discarding of samples with whole root system weight < 0.01 g).

### 2.4 Root type separation effects

We tested the effect of root separation on evaluation of root foraging precision of *L. salicaria* and *V. officinalis* because these species had root systems differentiated into fine and coarse roots (root system of *A. millefolium* consisted mostly of fine roots, i.e., roots smaller than 2 mm in diameter). We used the roots of *V. officinalis* from Experiment 1 and the roots of *L. salicaria* from both Experiment 1 (only year 2018) and Experiment 2 (both small and big pots). We only used samples from the pots in which plants had differentiated root systems (coarse roots distinct from fine ones). Because we dried all the roots without separation immediately after the harvest of each experiment, we needed to let them soak in water and then we sorted them. We put the dry roots into beakers with water and let them soak the water up for 12 hours. Then we separated
roots by hand into three categories using a ruler – the taproot, coarse roots (diameter > 2 mm), and fine roots (< 2 mm). We dried all the separated parts of the roots on Petri dishes (72 hours, 65°C, reaching constant weight) and weighed them. The final number of separated pots was 144, but for analyses we used only those in the heterogeneous treatment, thus a total of 69 pots, comprising 55 of *L. salicaria* and 14 of *V. officinalis*).

In all experiments, we calculated root foraging precision in each pot as the decadic logarithm of \( N/W \), where \( N \) was the biomass of dried roots from the nutrient-rich half of a given pot and \( W \) was the biomass of dried roots from the nutrient-poor half of the pot. Thus, values above zero mean that plants created more roots in the nutrient-rich half of the pot, and values below zero mean that plants created more roots in the nutrient-poor half of the pot; for homogeneous pots, we assigned \( N \) or \( W \) to their halves randomly. For separated roots from the experiment on root type separation, we calculated root foraging precision in the same way as for the heterogeneous pots in the other experiments but used only the biomass of the fine roots.

Unless otherwise stated, in analyses we used the root foraging data calculated from the root samples not separated by type, as the type-separated data were available only for a part of the dataset. Moreover, using separated or non-separated roots did not affect the outcome of any statistical analyses.

### 2.5 Data analysis

The primary assumption of our analyses was that root foraging precision in heterogeneous treatment is different from the one in homogeneous treatment. We tested it using linear regression with nutrient treatment as a predictor of root foraging precision. After verifying this assumption, we could use only root foraging precision in heterogeneous treatment in the following analyses without including nutrient treatment variable into the models.

In estimating the roles of species identity, and experiment duration on root foraging precision, we used these factors as predictors in linear models of root foraging precision with experiment duration as a continuous predictor (number of days since the beginning of the experiment). First, we analyzed data from Experiment 1 in 2018, as all three species together (*A. millefolium, L. salicaria, and V. officinalis*) were present only this year. Then we tested the effect of the same predictors and interannual variability on root foraging precision using data from Experiment 1 from 2018 and 2019 but only for species *A. millefolium* and *L. salicaria* (*V. officinalis* was not grown in 2019). We re-ran the above analyses with experiment duration as a categorical predictor expressed as harvest week identity (using the same data as described).

Pot size and experiment duration effects upon root foraging precision were estimated using linear models. We fitted linear regressions with these factors as predictors (experiment duration as a continuous variable – number of days since the beginning of the experiment) and foraging precision as a dependent variable. For this analysis, we used data from Experiment 2. Further, we re-ran the above analyses with experiment duration as a categorical predictor expressed as harvest week identity (using the same data as described).

To test whether root foraging precision calculated from separated fine roots differs from foraging precision assessed from non-separated roots, we used Model II Regression (method MA) with permutation number 999 using lmodel2 package (Legendre 2018; version 1.7-3). We also compared the root foraging precision calculated from separated and non-separated roots for
homogeneity of variances using an F-ratio test. Further, we re-ran the analyses of the results from

the Experiment 1 (duration) and Experiment 2 (pot size) using root foraging precision calculated from separated thin roots.

We performed analyses with R (R Core Team 2020; version 3.6.3) in Rstudio (RStudio Team 2020; version 1.3.1056). All images were done using the ggplot2 package (Wickham 2016; version 3.3.0).

3. Results

Root foraging precision of all species in heterogeneous treatment significantly differed from the root foraging precision in homogeneous treatment in all experiments (higher in heterogeneous than homogeneous treatment; $F_{1,569} = 120.9, p < 0.001$; Fig 2 in Supplementary Material). Root foraging precision was also higher in heterogeneous than homogeneous treatment when compared values calculated only from separated fine roots ($F_{1,137} = 90.4, p < 0.001$). Thus, we could perform all subsequent analyses of experiment duration and pot size only with data of root foraging precision in heterogeneous nutrient treatment.

3.1 Effect of time

There was no general change in the root foraging precision of *A. millefolium*, *L. salicaria*, and *V. officinalis* over time in 2018 ($F_{1,74} = 0.106, p = 0.745$) or *A. millefolium* and *L. salicaria* in 2018 and 2019 together ($F_{1,191} = 1.314, p = 0.253$; Table 1, Fig. 1). Root foraging precision of *A. millefolium* and *L. salicaria* also did not change between years ($F_{1,191} = 2.516, p = 0.114$), although we found a slight effect of the interaction of species identity, experiment duration, and year ($F_{1,191} = 3.705, p = 0.056$). Root foraging precision slightly differed between *A. millefolium* and *L. salicaria* if data from both years were taken into account ($F_{1,191} = 3.141, p = 0.078$). When experiment duration was coded as a categorial variable, the results did not change except the interaction of species, experiment duration, and year being non-significant ($p = 0.289$) (Table 2 in Supplementary Material).

3.2 Effect of pot size

After eight weeks, we observed that roots in small pots reached the wall and the bottom and started to grow along them. In contrast, roots in big pots did not reach the pot edge until the tenth week. Soil volume did not affect the root foraging precision dynamics of *L. salicaria* ($F_{1,53} = 0.465, p = 0.498$) and this relationship did not change over time ($F_{1,53} = 0.151, p = 0.7$; Fig. 2; Table 2). The results did not change when experiment duration was coded as a categorial variable (Table 3 in Supplementary Material).

3.3 Root type separation effects

The root separation method significantly affected root foraging precision values ($p < 0.001$, Table 3). When the root foraging precision of non-separated roots was approximately 0.8 or less, its value was underestimated compared to the foraging precision of separated roots (Fig. 3). The root foraging precision calculated from the separated roots did not change the results of the analyses of the effects of time and pot size. We observed high variation in root foraging precision in all experiments, which declined when root foraging precision was estimated using only fine roots ($F_{68,68} = 2.06, p = 0.003$; Fig. 2 in Supplementary Material).
4. Discussion

Our study aimed to assess the influence of experimental conditions, in particular the duration of the experiment, pot size, and root separation, on root foraging precision. Different experimental conditions could affect the results of root foraging experiments limiting the ability to generalize from them or use them in meta-analyses. We found that the duration of the experiment and pot size did not affect the root foraging precision in the three studied species. However, the root separation significantly affected the root foraging precision assessment.

The root foraging precision of *L. salicaria*, *V. officinalis*, and *A. millefolium* did not change between the fourth and tenth week, in contrast with the results of a meta-analysis (Kembel and Cahill 2005) and theoretical predictions based on the plant's ontogenesis (de Kroon et al. 2009). Both studies suggested that the root foraging precision declines with time. The meta-analysis identifies nutrient depletion as the cause, while predictions based on the ontogenesis are independent of nutrient depletion.

The contrasting results from those of the meta-analysis by Kembel and Cahill (2005) are not surprising, as that study suggested that root foraging precision declined over time due to nutrient depletion (Falik et al. 2005). However, only 5 out of the 12 studies included in the meta-analysis used methodology that incorporated nutrient depletion. The contrasting results between the meta-analysis and our experiment may arise from the fact that the long-term studies used in the meta-analysis were those with nutrient depletion (e.g., a two-year study by Fransen and de Kroon (2001)). In our experiments, we used a modified methodology of Campbell and Grime (1989) with continuous nutrient supply. This method does not exactly simulate natural conditions, but it is a suitable way to determine species' root foraging ability. The continuous nutrient supply allows us to observe the root foraging precision at any time without the confounding effect of nutrient depletion. Moreover, each study used in the meta-analysis (Kembel and Cahill 2005) experimented with different species, which could also affect the results because species differ in their root foraging precision (Campbell et al. 1991). In our experiments, we examined the development of root foraging precision of the same species.

Based on the theoretical model, root foraging precision should decline with the age of the plant (de Kroon et al. 2009). The model suggests that plants are formed from individual parts ('modules'), which respond to the local environment, but modules also receive signals from the whole-plant level, which may influence their response. This interplay between local and systemic signals can lead to the situation in which the foraging response of roots in a nutrient-rich environment, which would otherwise be stimulated to proliferate, is suppressed by signals from the whole-plant level if the overall nutrient status of the plant is high. Thus, plants with better nutrient status, i.e., older plants that have accumulated nutrients during their lives, would forage less. Our results did not support this hypothesis, as our species showed the same foraging ability in the fourth as well as in the tenth week. Our experiments lasted from four to ten weeks, which are all very common lengths of root foraging experiments (e.g., Johnson and Biondini 2001; Day et al. 2003; Lamb et al. 2004; Jansen et al. 2006; Keser et al. 2015). However, ten weeks could be too short to accumulate enough nutrients to result in decline in root foraging precision. This possibility suggests the need for further investigation. Importantly, plants started to forage during the first four weeks after transplantation to the pots. This shows that the root foraging precision can occur even in very young plants, indicating that root foraging precision during the first weeks of plants' life is also under-examined.
The root foraging precision and dynamics of *L. salicaria* and *A. millefolium* that we observed tended to change between years although the magnitude of the effect does not exclude random events. Although this trend was only marginal, it should be kept in mind when comparing data from experiments conducted in different years.

Root foraging precision was not affected by the size of the pot. Despite this, the growth pattern of the roots of *L. salicaria* strongly differed between small and big pots. At the eighth week, roots in small pots had already reached the wall and the bottom of the pot and started to follow their contours, but in big pots, roots did not reach the pot edge until the tenth week. These results are to our best knowledge the first of their kind, as no one has previously explored the effect of pot size on root foraging precision.

Plant roots produce various exudates which alter plant growth. Three types of the exudates may affect root foraging precision. First, roots produce allelopathic exudates in the vicinity of obstacles (i.e., a pot wall; Falik et al. (2005)), causing root growth suppression. Second, in the early stage of plant growth, plants produce 'substrate volume-sensing signals' (SVS) (Wheeldon et al. 2020). The SVS are used to detect the substrate volume so that the plant adapts its future growth accordingly. The third type of substances are 'root density-sensing signals' (RDS) (Semchenko et al. 2007; Wheeldon et al. 2020). The RDS affect the plant in the later stage and suppress its growth according to the density of roots. The RDS affect the root system size and thus indirectly also the shoot growth, while the SVS are thought to affect only shoot growth (Wheeldon et al. 2020; but see Semchenko et al. 2007).

We assume that the conflict between non-affected root foraging precision and root growth in the small pots stems from the method we used. In our experiments we used surface drip irrigation of a non-retentive substrate, thus the water trickled vertically through the pots, possibly removing accumulated substances. This removal could cause the similarity of results between small and big pots, even if roots in small pots grow along the walls and bottom from the eighth week onwards. It means that this method can help observe root foraging precision without needing to use unduly large pots to eliminate the potentially confusing effect of inhibitory substances. But the RDS are not very mobile throughout the substrate (Wheeldon et al. 2020), thus their removal by trickling water was not very likely. There are several possible explanations why we did not detect any RDS effect upon root foraging of the examined plants. First, even though the RDS are not very mobile our water flow was intensive enough to remove them. Second, the concentration of roots in our experiment was not high enough to suppress their growth by RDS. Third, the root response to RDS was suppressed by another set of cues urging the plants to proliferate into the nutrient-rich zone. And fourth, the response to RDS might take place at the level of the whole root system, thus averaging out patches with high and low root density. These hypotheses need further investigation, nevertheless, our results show that experimenters should choose pot sizes based mainly on the size of the plant used, since in root foraging experiments, the size of the pot is probably not very important.

Experiment duration and pot size should theoretically have interacting effects. Bigger the pot, the longer the experiment can last before the roots fill it. After a longer time, the nutrient-poor half could get more roots once the nutrient-rich half reached the maximum root length density, consequently decreasing the observed root foraging precision. This did not occur, even though in the small pots after ten weeks, the nutrient-rich halves of the small pots were overgrown by roots (judged by visual observation). We cannot rule out some subtle effects, but we conclude that
there is no need to set the harvest time precisely to the point when roots touch the pot wall, and a few more weeks is acceptable.

In our study, use of a thorough root separation method affected the evaluation of root foraging precision in *L. salicaria* and *V. officinalis*. This is probably because usually only fine roots take up the nutrients and therefore are relevant to root foraging (Pregitzer et al. 1993). When we did not perform this separation, the estimated root foraging precision values were lower in most cases. Root foraging precision values were greater for unseparated roots versus separated roots only in cases in which the root foraging precision values were particularly high. The comparison of the root foraging precision of 123 species (Stiblíková et al. [in press]) with the distribution of the root foraging values of our species revealed, that most species-specific root foraging precision values are concentrated in the lower part of the range. It means that an experimenter who does not employ a careful separation method will typically underestimate the value of root foraging precision. Thus, non-separation estimates of the root foraging may therefore be viewed as conservative but affected with an unnecessary amount of variance.

We observed high intraspecific variability in root foraging precision in all of our experiments. This variability decreased when we used only fine roots for calculation of root foraging precision. It can likely be explained by the central part of the root system, with thicker roots, ending up by chance in one of the halves of the pot during harvest. The change of root foraging values and variability when using separated roots was not systematic, as it did not affect the results of all the analyses performed regarding time and pot size. We recommend separating the roots and using only the fine ones for root foraging precision assessment, as the variability of such estimates is lower, and we consider these estimates to be more accurate with respect to the functions of the individual parts of the root system (Pregitzer et al. 1993).

Another option would be to use smaller nutrient patches located further from the rooting point (Belter 2014). This would reduce the influence of random deviation of thicker, primary roots towards one of the pot halves. On the other hand, this solution raises issues with the appropriate distance of the patches, because if the patches are located far from the rooting point, small species will have a lower chance to reach them, regardless of the precision of their foraging. With decreasing relative size of the rich patches (and/or increasing average distance between the initial rooting point and a rich patch), the result is increasingly affected by the plant’s ability to find the patch, compared to the current design, where the plant has full information about the substrate heterogeneity from the beginning. Aside from the studied factors, the results of the root foraging precision experiments may be affected by the technical aspects of nutrient patch creation. In particular, the level of the contrast between the patch and the background is likely to affect root foraging precision (Blouin and Puga-Freitas 2011; Weiser et al. 2016). If the contrast between the patch and the background soil is too small, roots will not proliferate into the patch. Also, the nutrient composition of the patches may influence root foraging precision, as the particular nutrients and micronutrients included, and whether they are organic versus inorganic may affect root foraging differently (Drew 1975; Hodge 2006; Cheng et al. 2016; Ferrieri et al. 2017). All these effects should be considered if the data are to be pooled across studies.

In general, meta-analysis and comparison of results from different root foraging studies are valid approaches if the experiments meet several conditions. First, creating nutrient patches by using water flow and continuous nutrient supply will ensure that experiment duration and pot size will not affect the root foraging precision, although we cannot claim that other methodologies of
creating patches would act differentially, as we did not test other approaches of creating nutrient heterogeneities. Second, we recommend separating the fine roots and using only these for root foraging precision assessment, as the lack of separating them generally decreases assessed root foraging values and also increases their intraspecific variability. Third, we recommend being aware of the potential for interannual variability in root foraging precision of species, as we found subtle differences between values obtained from two different years.
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Tables:

Table 1 The effects of species identity, experiment duration, and year on root foraging precision. Experiment duration was coded as a continuous predictor (number of days since the beginning of the experiment). Data are from Experiment 1 and pots with heterogeneous fertilization treatment. We analyzed all three species in the analysis of 2018; in the analysis of 2018 + 2019, only *A. millefolium* and *L. salicaria* were analyzed, as *V. officinalis* was not grown in 2019.

|                     | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---------------------|----|--------|---------|---------|--------|
| 2018                |    |        |         |         |        |
| Species             | 2  | 0.034  | 0.017   | 0.102   | 0.903  |
| Experiment duration | 1  | 0.018  | 0.018   | 0.106   | 0.745  |
| Species * Experiment duration | 2  | 0.307  | 0.154   | 0.933   | 0.398  |
| Residuals           | 74 | 12.179 | 0.165   |         |        |
| 2018 + 2019         |    |        |         |         |        |
| Species             | 1  | 0.419  | 0.419   | 3.141   | 0.078  |
| Experiment duration | 1  | 0.175  | 0.175   | 1.314   | 0.253  |
| Year                | 1  | 0.336  | 0.336   | 2.516   | 0.114  |
| Species * Experiment duration | 1  | 0.359  | 0.359   | 2.692   | 0.103  |
| Species * Year      | 1  | 0.292  | 0.292   | 2.187   | 0.141  |
| Experiment duration * Year | 1  | 0.005  | 0.005   | 0.037   | 0.848  |
| Species * Exp. duration * Year | 1  | 0.495  | 0.495   | 3.705   | 0.056  |
| Residuals           | 191| 25.502 | 0.134   |         |        |

Table 2 The effects of experiment duration and size of the pot on root foraging precision. Experiment duration was coded as a continuous predictor (number of days since the beginning of the experiment). Data are from Experiment 2 and pots with heterogeneous fertilization treatment.

|                     | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---------------------|----|--------|---------|---------|--------|
| Experiment duration | 1  | 0.387  | 0.387   | 1.697   | 0.198  |
| Pot size            | 1  | 0.106  | 0.106   | 0.465   | 0.498  |
| Exp. duration * Pot size | 1  | 0.034  | 0.034   | 0.151   | 0.700  |
| Residuals           | 53 | 12.079 | 0.228   |         |        |

Table 3 The effect of root type separation on root foraging precision values. Data for species *L. salicaria* (Experiment 1 – 2018, Experiment 2) and *V. officinalis* (Experiment 1). Root foraging precision was calculated as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part); for calculation of foraging precision of separated roots only the fine ones were used.

|                     | MA regression | 2.5% confidence interval | 97.5% confidence interval |
|---------------------|---------------|--------------------------|---------------------------|
| Intercept           | 0.26          | 0.22                     | 0.29                      |
| Slope               | 0.63          | 0.52                     | 0.76                      |
Figure captions:

**Figure 1** Root foraging precision of *A. millefolium* (circle), *L. salicaria* (triangle), and *V. officinalis* (square) over time. Points indicate mean ±SE. Plants were harvested at five time points. Root foraging precision was calculated as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part) using non-separated roots. Values above zero indicate root foraging for nutrients, values below zero indicate avoiding nutrient patches. Grey line indicates root foraging in homogeneous treatment; black line indicates root foraging precision in heterogeneous treatment. Data are from Experiment 1 (both years).

**Figure 2** Effect of soil volume on root foraging precision dynamics of *L. salicaria*. Points indicate means ±SE. Plants grew in two sizes of pot – big (10 l) and small (3 l) and were harvested at five time points. Root foraging precision was calculated as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part) using non-separated roots. Values above zero indicate root foraging for nutrients, values below zero indicate avoidance of nutrient patches. Grey line indicates root foraging in homogeneous treatment; black line indicates root foraging precision in heterogeneous treatment. Data are from Experiment 2.

**Figure 3** The effect of root-type separation on root foraging precision values. We plot the results of MA regression (black line) with 95% confidence intervals (grey line) and 1:1 line to compare (black dashed line). Data for species *L. salicaria* (Experiment 1 – year 2018, Experiment 2) and *V. officinalis* (Experiment 1). The point size reflects the logarithm of the total biomass of the plant. Root foraging precision was counted as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part).
Figure 1

Root foraging precision of *A. millefolium* (circle), *L. salicaria* (triangle), and *V. officinalis* (square) over time. Points indicate mean ± SE. Plants were harvested at five time points. Root foraging precision was calculated as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part) using non-separated roots. Values above zero indicate root foraging for nutrients, values below zero indicate avoiding nutrient patches. Grey line indicates root foraging in homogeneous treatment; black line indicates root foraging precision in heterogeneous treatment. Data are from Experiment 1 (both years).
Effect of soil volume on root foraging precision dynamics of *L. salicaria*. Points indicate means ± SE. Plants grew in two sizes of pot – big (10 l) and small (3 l) and were harvested at five time points. Root foraging precision was calculated as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part) using non-separated roots. Values above zero indicate root foraging for nutrients, values below zero indicate avoidance of nutrient patches. Grey line indicates root foraging in homogeneous treatment; black line indicates root foraging precision in heterogeneous treatment. Data are from Experiment 2.
**Figure 3**

The effect of root-type separation on root foraging precision values. We plot the results of MA regression (black line) with 95% confidence intervals (grey line) and 1:1 line to compare (black dashed line). Data for species *L. salicaria* (Experiment 1 – year 2018, Experiment 2) and *V. officinalis* (Experiment 1). The point size reflects the logarithm of the total biomass of the plant. Root foraging precision was counted as log (biomass of roots in nutrient-rich part/biomass of roots in nutrient-poor part).

**Supplementary Files**

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- SupplementaryMaterial.pdf