The impact of defoliation on the root foraging behaviour of sunflower

(*Heliathus annuus* L.)

by

Tianna Barber-Cross

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ABSTRACT

Plants have a remarkable ability to proliferate roots and increase nutrient uptake within nutrient patches in the soil. This behaviour, known as root foraging, describes this ability and what factors may influence or modify this response. It has been observed that plants integrate multiple environmental cues to inform their root foraging behaviour, resulting in non-additive responses to novel combinations of stimuli such as nutrient distribution and the presence and identity of neighbouring plants. These responses are highly species-specific and context-dependent. While the effect of nutrient distribution and competitors on foraging behaviour has been studied quite comprehensively in both plants and animals, the impact of injury on the foraging behaviour of plants remains largely unexplored.

In plants, defoliation can be considered a form of injury. Although both plants and animals need to forage for nutrients, it is unclear if they respond to injury in a similar manner. The many physiological and ecological differences between plants and animals, as well as their distinct interactions with the environment, cannot be disregarded. Therefore, it is essential to experimentally test the assumption that they may behave in the same way.

To experimentally evaluate the impact of injury and its severity on root foraging behaviour over time, we conducted a study using the model organism, common sunflower (*Helianthus annuus* L.). Individual sunflower seeds were planted in experimental arenas that allowed us to observe root growth over time. These arenas contained soil with low nutrient levels and a nutrient-rich patch, enabling us to measure root foraging responses to heterogenous nutrient distribution in the soil. Defoliation treatments were applied at two different severities: half clipped and fully clipped, alongside a control treatment in which no defoliation occurred.

Defoliation was found to suppress the overall root length grown, but had no effect on root foraging precision. Over time, root foraging precision decreased from an initially high proliferation response to nutrients to an agnostic response to nutrient patch by the fourth week of growth. Other measures of root morphology, namely average root diameter and average root length, exhibited no changes over time or in response to defoliation. The average root diameter was consistently larger within the nutrient patch, while average root length remained equivalent in both the patch and background soil throughout the study.

These findings generally align with assumptions based on optimal foraging and optimal defence theory. However, they also highlight the influence the ecological and physiological differences between plants and animals can have on the applications of optimality theories. Additionally, this study emphasizes the importance of considering the various different measures of root foraging behaviour and the timing of data collection. Different measures and their timing yielded distinct results, and only when all measures were compared could a comprehensive understanding be obtained. Ultimately, comparing the possible effects of timing and types of measures on a study's findings and experimentally testing the applicability of established theories to novel organisms enhances our understanding of how past and future studies can be effectively compared. This includes considering the chosen measures and timing of data collection, as well as the theories themselves and their applicability in future research.

PREFACE

This thesis is an original, collaborative work between myself, Dr. James Cahill, and Stasha Lysyk at the University of Alberta. My responsibilities included methods development, data collection and processing, statistical analysis and interpretation, and manuscript composition. Dr. James Cahill was involved in concept and manuscript development. Stasha Lysyk was involved in methods development and data collection and processing. No part of this thesis has been previously published.

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made this an incredible and rewarding experience that I will remember fondly for the rest of my days. I send you my best wishes for the future!

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1. INTRODUCTION

The study of plant behavioural ecology strives to apply behavioural ecological theory, once only applied to animals, to plants in order to better understand how plants interact with other organisms and their environment. Studying plant behaviour also allows researchers the opportunity to test assumptions of well-known behavioural ecological theories in a novel study organism. Over the years, the study of plant behaviour has steadily gained traction within ecology, and it is now widely accepted that plants exhibit responses consistent with expectations posed by many behavioural ecological theories, such as the marginal value theorem (McNickle & Cahill, 2009), game theory (McNickle & Dybzinski, 2013), and energetic trade-offs (Bao et al., 2018; Jensen et al., 2011). Consequently, the literature surrounding the study of plant behavioural ecology is now moving away from "do plants behave?" and towards "what informs or changes these behavioural responses?" as studies strive to examine the impact of other factors, like injury, on established behavioural responses. This thesis aligns with this evolving trend in the literature.

Plants demonstrate a tendency to proliferate roots and increase nutrient uptake within nutrient-rich patches compared to less nutrient-dense areas in the soil (Robinson, 1994). Root foraging behaviour describes this tendency of plants to proliferate more roots within nutrient patches and explores what factors may impact or change this response (Cahill & McNickle, 2011). Root foraging precision describes how strong this response is in a species or individual, with higher root foraging precision involving placing a greater amount of roots within a nutrient patch in comparison to a less nutrient-dense area. However, research indicates that plants integrate multiple environmental cues to inform their root foraging behaviour, resulting in non-additive responses to novel combinations of stimuli such as nutrient distribution and the presence

and identity of neighbouring plants that is species- specific and context- dependent (Cahill et al., 2010; Garlick et al., 2021; Kembel & Cahill, 2005; Ljubotina & Cahill, 2019; McNickle & Brown, 2014; Sattler & Bartelheimer, 2018; P. Wang et al., 2020).

Several previous studies have displayed that plants adjust their root foraging behaviour based on a hierarchal set of decision rules related to nutrient distribution and neighbour presence. In comparison to plants grown alone, common sunflowers (*Helianthus annuus* L.) with a nutrient patch equidistant between themselves and a neighbour would decrease root proliferation within the patch (Ljubotina & Cahill, 2019). However, these focal plants would increase their root proliferation within the nutrient patch if it was closer to themselves. Conversely, the addition of a second nutrient patch resulted in decreased root growth in the patch equidistant to its neighbour, but no significant increase in the patch closest to the focal plant. In another study by Cahill et al. (2010) *Abutlon theophrasti* exhibited a broad root foraging strategy when grown alone, irrespective of nutrient placement and integration into the soil. The presence of a neighbouring plant induced a change in the foraging strategy of the focal plant, with the effect also dependent on the placement and integration of the nutrient patch into the soil. In the presence of a neighbour, the focal plant adopted a much narrower root foraging strategy across all treatments compared to when it was grown alone, resulting in complete separation of the root space between the plants (Cahill et al., 2010). However, the focal plant would proliferate more into the nutrient patch if it was placed in between the focal plant and its neighbour, so that the root spaces of the two plants overlapped. When the nutrient patch was placed on the edge closest to the focal plant and far away from the neighbour, the response was intermediate between the two other neighbour treatments (Cahill et al., 2010).

Root foraging behaviour, including the impact of factors like nutrient distributions and neighbour presence, is also species-specific. The tendency to place more roots within nutrient patches is not an all-encompassing truth across every plant species studied. In fact, species responses to nutrient patches within the soil vary not only in the strength of their response, but even the presence or directionality of the proliferation response to nutrients at all. Some species do not display a significant proliferation response within nutrient patches, indicating an agnostic response, while others place more roots to an equivalent area of lower nutrients in comparison to the nutrient patch, indicating an avoidance response (Cahill & McNickle, 2011). Studies that have investigated whether there is a phylogenetic pattern in the root foraging behaviour of plants have found that, generally, eudicots forage more precisely than monocots (Grime & Mackey, 2002; Kembel & Cahill, 2005). However, beyond this broad phylogenetic signal there has been no support for phylogenetic patterns within multiple genera of species (McNickle & Brown, 2014).

While the influence of nutrient distribution and competitors on foraging behaviour has been studied quite comprehensively in both plants and animals (Charnov, 1976; Grand & Dill, 1999; Milinski, 1982; Nash et al., 2012; Pyke et al., 1977), the impact of injury on foraging behaviour has received greater attention in animals (Rennolds & Bely, 2023). In animals, injury to feeding organs, such as mouthparts, automatically reduces foraging via feeding efficiency and overall food intake, as they become unable to forage and feed (Rennolds & Bely, 2023). The trade-off associated with healing from injury likely involves the redirection of energy allocated to growth and reproduction towards recovery. Additionally, studies have shown that injury to organs not immediately involved in foraging can also affect the foraging behaviour of animals, altering their feeding patterns and frequency (Lindsay & Woodin, 1992; Werner et al., 2006).

The impact of injury on where an animal feeds can result in either an increase or decrease in risk-taking, likely due to other factors such as the animals internal energetic state and the severity of injury (Rennolds & Bely, 2023). When risk-taking is reduced, leading to reduced exploration or feeding events, the benefits associated with successful feeding or potential reward from risky behaviour may be outweighed by the possible costs to further injury or predation. Conversely, increased risk-taking, manifested as more frequent feeding events in potentially risky habitats, may be necessary when the costs of starvation and injury repair and benefit of a successful risk taken outweigh the potential for further injury and predation. Therefore, it is plausible that changes in the foraging behaviour of plants may involve and increase in root growth at lower injury severities and a decrease in root growth if the injury is severe enough. Although the impact of injury on plant foraging behavior is less explored compared to animals, considering the analogous responses observed in the animal kingdom, it is reasonable to expect similar adaptations in plants.

In plants, defoliation can be equated to injury, wherein the location and extent of the defoliation represent changes in severity of the damage. Leaves, like roots, are a foraging organ that are essential to a plant's acquisition of food. Signaling between shoots and roots has been well documented in literature pertaining to the root-shoot axis, particularly in regards to induced defenses in response to stress (Bezemer et al., 2004; Erb et al., 2009; Soler et al., 2013; G. Wang et al., 2019). In accordance with optimal defense theory and energetic trade-offs between recovery and defense, the impact of injury to the shoot on root behaviour may depend on its severity. A degree of tolerance to damage may be seen in injury to less critical tissues like old leaves. In the cases of moderate injury, this response may increase to a systematic communication leading to the release of defense hormones. Alternatively, if the injury is severe

and foraging is disrupted, the plant may completely divert their energy from defense to recovery and re-growth of the injured organ (Van Dam & Bezemer, 2006).

Preliminary work on the impact of defoliation of root foraging behaviour has yielded mixed results. Defoliation in leaf veins, but not leaf mesophyll, was shown to suppress root foraging behaviour in *Plantago asiatica* and *Prunus jamasakura* (Yamawo et al., 2019). However, other studies show that defoliation can both suppress or induce root proliferation responses depending on the soil conditions. For instance, in a cadmium hyperaccumulating plant, increased root foraging for and nutrient uptake of cadmium was induced by defoliation in plants taken from a non-metalliferous region (Mohiley et al., 2021). Conversely, plants taken from a metalliferous region displayed greater cadmium sharing between ramets and did not show alterations in root foraging (Mohiley et al., 2021). These findings suggest that both suppression and induction of precise root foraging behaviour can occur.

It is important to note, however, that the type and extent of defoliation varied between these studies. Yamawo et al. (2019) used solely mechanical defoliation of either the mesophyll or veins of the leaves, whereas Mohiley et al. simulated herbivory by puncturing small holes in the leaves and applying jasmonic acid (2021). Consequently, the disparities observed in these findings may be due to differences in the extent of mechanical defoliation and the simulation of herbivory via applied plant hormones. It is evident that further investigations are necessary to unravel the intricate relationship between defoliation, root foraging behavior, and the varying factors that influence plant responses.

In summary, both plants and animals must forage for nutrients, but it is unclear if they respond to injury similarly. If this behaviour is fundamentally based on a cost-benefit analysis of the situation, as it is in animals and as it is understood to be by current literature. Then, similar

responses and adjustments to foraging behaviour should be seen in both plants and animals. However, we cannot ignore the many physiological and ecological differences between plants and animals and how they interact with the environment. Thus, we cannot just assume that they will behave the same, this assumption must be tested experimentally.

In order to help evaluate the impact of injury and its severity on root foraging behaviour over time we used the model organism, common sunflower (*Helianthus annuus* L.). Common sunflower has been used in other studies on root foraging behaviour and evaluated to be a precise forager, where they reliably proliferate significantly more roots within nutrient patches in comparison with nutrient lacking background soil (Ljubotina & Cahill, 2019). Individual sunflower seeds were then planted in experimental arenas that allowed for viewing of root growth over time. These arenas had low nutrient background soil and a higher nutrient patch to allow for root foraging precision measures to be taken in response to heterogenous nutrient distribution within the soil. Defoliation was applied at two different severities, half clipped and fully clipped, alongside a control treatment in which no defoliation occurred.

Therefore, to examine the effect of defoliation and its severity on the temporality of root foraging behaviour we asked a series of questions:

1. How does the presence and severity of a defoliation event affect patterns of resource patch use?
2. How do patterns of patch use vary as a function of the temporal scale of measures?
3. What can different measures of root morphology and patch use tell us about the impact of defoliation and root foraging behaviour in general?

2. METHODS

Study Species

The study species selected for this study was the common sunflower (*Helianthus annuus* L.). This choice was based on previous studies that have shown this species to exhibit precise root foraging behaviour, reliably proliferating its roots within nutrient patches (Ljubotina & Cahill, 2019). Furthermore, this species has been grown in the experimental arenas used in this study, ensuring reliable growth and enabling us to make direct comparisons with previous literature (Ljubotina & Cahill, 2019). To obtain the seeds for this species, black oil sunflower seeds were purchased from an Edmonton area seed supplier (Apache Seeds Lte.).

Experimental Arenas

The experimental arenas used in this study consisted of two Plexiglass sheets (27.9 cm tall × 21.5 cm wide × 0.6 cm thick) with spacers, enclosing a thin layer of soil. A clear window was included on one side of the arena, through which the researcher could observe roots growth (Image 1). The arena construction and experimental processes were adapted from Ljubotina and Cahill (2019). The resulting arena had a soil volume of 25.9 cm tall × 17.8 cm wide × 0.6 cm deep. A soil mix consisting of 3:1 sand to soil was used as the background soil within these arenas. To create the nutrient patches, the background soil was mixed with composted manure in a ratio of 2:1, resulting in patches that were the same depth and length as the arena and 1.5 cm wide. Nutrient patches were randomly assigned to either the left or right side of the arena, located 3 cm from the center where the plant was seeded. To prevent root exposure to light, a laminated black sheet of construction paper was attached to the window side of the arena with a rubber band. This sheet was removed only when scanning the plants and was immediately replaced.

Growth Conditions

The experiment was conducted in a controlled growth chamber located in the University of Alberta Biotron Facility under the same conditions of Ljubotina and Cahill (2019). The growth chamber maintained a 16:8 hour light-dark cycle at a temperature of 24°C with ambient humidity. Plants were grown at an approximately a 45° angle with the window side of the window box facing downwards to promote greater contact of roots with the window section, which aids in the visualization of root growth (Image 2).

Sunflower seeds were planted directly into the center of the window boxes and allowed to grow for one week prior to initiating scans. Each plant was placed alone in its own window box. Window boxes with unsuccessful germination were re-seeded and put into a second round of measurements. Consequently, individuals numbered one through five of each treatment belonged to the first run, a result of an approximately 50% germination rate of the 30 window boxes. As well, individuals numbered six through nine were from the second run, a result of the approximately 80% germination rate of the re-seeded arenas. Throughout the four-week duration of the experiment, plants were watered daily *ad libitum* until soil saturation.

Experimental Treatments

To investigate the impact of defoliation and its severity on the root foraging behaviour of sunflower three defoliation treatments were applied, including two different severities of defoliation and one control treatment. The treatment application occurred after two weeks of growth and one week after the scanning began. Treatment assignment was also completely randomized at time of treatment application.

The control treatment served as the baseline and involved no defoliation. In contrast, the "half-clipped" treatment consisted of removing half of the true leaves at the time of defoliation,

while the “fully-clipped” treatment involved the complete removal of all true leaves at the time of defoliation. At the point of defoliation, every plant had a minimum of two true leaves and two cotyledons. Consequently, one leaf was removed in the half-clipped treatment, and both leaves were removed in the fully-clipped treatment. Leaves were cut as close to the base of the petiole as possible to ensure complete removal.

Harvest Measures

Following four weeks of growth, the experiment was harvested to determine plant biomass. The plant was initially divided into leaf, stem, and cotyledon sections, which were then dried at 70°C for 48 hours and weighed to determine their respective biomasses. To determine root biomass, the entire root system was carefully stored in a plastic bag and refrigerated until root washing could be performed. Subsequently, the roots were washed over a 1 mm sieve to remove excess soil and dried for 72 hours at 70°C before being weighed. To limit the degradation of the fine roots in storage, all root sections were washed and weighed within a week. The scale utilized had a d-value of 0.005 mg/0.01 mg.

Root Scanning and Tracing

Root scanning and tracing were conducted using the following approach. After seeding, a one week growth period was allowed before the commencement of scanning. This initial period was not scanned because the plant roots would not be sufficiently developed for meaningful measurements, and could potentially disrupt small, underdeveloped seedlings. The first week of daily scanning which took place during the second week of growth, served to determine the plants’ baseline root foraging behaviour prior to defoliation. The treatments were applied on the seventh day of scanning (14^{th} day of growth), immediately following the daily scan.

Subsequently, the plants were grown for an additional two weeks: one week post-defoliation (third week of growth) and the second-week post-defoliation (fourth week of growth).

The decision to grow the plants for a total of four weeks, with two weeks post-defoliation, was based on observations from a pilot study conducted as a precursor to this thesis and personal observations of plant health. Previous pilot study findings indicated that root foraging behaviour typically recovered over a span of approximately two weeks after defoliation (JC personal communication 2021), while my own observations suggested that plants began to exhibit signs of senescence around four weeks of growth (TBC personal observation 2022).

Daily root scans were performed using an Epson Perfection V850 Pro scanner, capturing images at a resolution of 900 dpi and saving them as TIFF (*.tif) files. Root tracing was accomplished using WhinRHIZO Tron (WinRhizo Tron 2021a, Regent Instruments, GC, Canada) to determine root measurements Tracing was limited to a designated "patch" and "non-patch" area, each measuring 1.5 cm x 22 cm (Image 3). The patch box was positioned over the nutrient patch (patch), with the non-patch box was placed on the opposite side of the plant in an equivalent area without added nutrients (non-patch). Adobe Photoshop 2023 was used to add these boxes during the tracing process.

Metrics

Daily values of root length (cm), average diameter (mm), and average length (cm) within a nutrient patch (P) and equivalent non-nutrient areas (NP) were determined by either summing (i.e. root length) or averaging (i.e. average diameter and average length) the root values extracted from the raw dataset generated by WhinRHIZO Tron (WinRhizo Tron 2021a, Regent Instruments, GC, Canada). Linear Mixed Models (LMMs) were employed, and p-values were estimated using Kenward-Roger degrees of freedom calculations based on type three ANOVAs

with Wald F-tests. It should be noted that there is ongoing debate regarding the inclusion of degrees of freedom and p-value estimates in analysis using Restricted Maximum Likelihood (REML), and while the creators of the *lme4* package discourage their use, we decided to include them for the sake of interpretation and discussion.

The average root length was used in this study as a proxy for root verticality. This assumption that longer roots were more vertical as a function of the rectangular sample area for P and NP was tested by performing a linear regression that modelled the angle of the root as a function of root length. This regression confirmed that the angle of roots was significantly impacted by their length ($df = 1,31.699$, $F = 47.934$, $p < 0.0001$) with longer roots more likely to be closer to 90° (Supplemental Figure 1). This test was performed on data derived from a small subset of root scans, specifically the last image scan from every control individual. The angle of the roots were manually calculated using a protractor and printed out root scans. A line was drawn connecting the entry and exit point of the root, and an angle of that line was taken. Roots that entered and exited on the same plane were removed from analysis.

Weekly root length within P and NP were calculated by taking the difference of the total root length value in the area on day 7 and day 1 of scanning (Week 1), day 14 and day 8 of scanning (Week 2), and day 22 and day 15 of scanning (Week 3) (Image 4). The weekly average diameter and length within P and NP were determined by averaging the daily values within each weekly period (Image 4). For example, Week 1's average was calculated from the values from days 1, 2, 3, 4, 5, 6, and 7 of scanning. This process was then repeated each week for both measures. The final values of root length, average diameter, and average length in P and NP were obtained from the calculated daily values for day 22 (Image 4).

Root foraging precision was calculated by taking the weekly total root length values in P and dividing them by the total combined root length grown in that week (P and NP) for each individual. Final values were obtained via the same method, but instead of using the root length grown in that week only the last values of the root length in P and NP on day 22 of scanning were used.

Statistical Analyses

All graphing and statistical analyses were performed using RStudio v.1.3.959, employing the packages *lme4*, *car*, *ggplot2*, *MuMIn*, *tidyverse*, and *dplyr*. Many different types of analyses were performed because we wanted to consider various different root foraging variables (precision, total length, average diameter, and average length) over different temporalities (weekly and final measures) in a comprehensive manner. To simplify our analyses further would be to ignore the testing of assumptions and implementation of treatments or remove the comparison of different measures and different time frames.

To examine the influence of plant size on different foraging behavioural variables, linear models were employed to analyze various plant size-related variables and their relationship to root foraging precision. Final root foraging precision was modelled as a function of total biomass. The total combined root length grown, including P and NP values, was modelled as a function of final height (measured on the last day of the experiment). Additionally, the total combined root length was modelled as a function of final total plant biomass. To test the assumption of similar plant sizes pre-defoliation, plant height immediately before defoliation (day 14 of growth, day 7 of data collection) was modelled as a function of treatment. Furthermore, to ensure the effectiveness of the defoliation treatments, the final total plant biomass was modelled as a function of treatment.

To assess root foraging precision in response to defoliation and time, LMMs were performed. Root foraging precision was modelled as a function of the fixed effects of treatment and week, and the random effect of individual. This model was also tested with an additional fixed effect of initial height (first measurement, one week after planting) to examine if plant size influenced root foraging precision. Akaike Information Criteria, corrected for a small sample size (AICc), were then used to determine the best-fitting model. Considering concerns that the half-clipped treatment was not displaying root foraging precision values pre-defoliation that matched the other treatments and past studies, the analyses were conducted both including and excluding the half-clipped treatment.

Separate LMMs were run for each week on the response variables of total root length to investigate the effects of defoliation over time. Total root length was evaluated as a function of the fixed effects of treatment and patch, and the random effect of individual. Similar to the root foraging precision models, the weekly models were performed with and without the half-clipped treatment.

To assess the final measures of root foraging behaviour, a LM and LMM were conducted on root foraging precision and total root length, respectively. Firstly, final root foraging precision was modelled as a function of the fixed effect of treatment. Secondly, the final total root length was modelled as a function of the fixed effects of treatment and patch and the random effect of individual. As well, one-sample t-tests were performed separately for each treatment group, comparing them to an agnostic value of 0.5 to determine if the treatment groups displayed significant root foraging precision in their final measures. The value 0.5 was chosen as it represents an exactly equal proportion of root length grown within P and NP areas.

Other measures of patch use and root morphology, average root diameter and average root length, were measured utilizing similar methods to total root length. Separate LMMs were run for each week on both the response variables of average root length and average root diameter to investigate the effects of defoliation over time. The response variables were evaluated as a function of the fixed effects of treatment and patch, and the random effect of individual. These weekly models were also performed with and without the half-clipped treatment and applied to the second week of growth (first week of data collection, immediately pre-defoliation) and third week of growth (immediately post-defoliation) for the control and fully-clipped treatments. Final measures of average root diameter and average root length were modelled as a function of the fixed effects of treatment and patch and the random effect of individual in similar fashion to the final measures of total root length.

Artificial Intelligence

The artificial intelligence software ChatGPT was utilized in editing of the final version of this thesis. While there are no stated standards for citation or use of artificial intelligence software, I have a responsibility to acknowledge how I used ChatGPT in order to increase transparency and also aid future students who may interested in incorporating the software into their research. ChatGPT was used at no point in the experimental or concept development of this thesis, including the methods, data collection, and statistical analysis. Drafts of this thesis were created by TBC and then feedback was received from JC. Afterwards, the initial feedback was incorporated and the final draft sections of the thesis were then input into ChatGPT with a prompt similar to “The text included is part of the introduction of a biological science master’s student's thesis. Please make the writing better. Thanks! The text is …”. The adjustments made

by ChatGPT were then vetted by TBC, and only those deemed an improvement by TBC were actually incorporated into the final thesis.

3. RESULTS

In regards to the general effects of plant size and testing of the adequacy of treatment application, we found that final root foraging precision measures was not significantly affected by total biomass independent of whether the half-clipped treatment was included (Supplemental Figure 2, df = 1,25, F = 0.1646, p = 0.69) or excluded (Supplemental Figure 3, df =1,16, F = 0.3604, p = 0.56). The final total combined root length grown increased significantly with increased total biomass both when the half-clipped was included (Supplemental Figure 4, df =1,25, F = 9.3542, p = 0.0052) and excluded (Supplemental Figure 5, df = 1,16, F = 14.4460, p = 0.0016). In accordance with our assumptions, plant height before defoliation did not significantly differ between treatments (Supplemental Figure 6, df = 2,24, F = 0.2495, p = 0.78), but total biomass did significantly differ at the end of the experiment (Supplemental Figure 7, df =2,24, F = 6.8981, p = 0.0043) with total biomass decreasing with increased defoliation severity.

How does the presence and severity of a defoliation event affect patterns of resource patch use?

Our first LMM (Table 1) illustrated that weekly root foraging precision was significantly affected by week (df = 1,50.767, F = 4.5250, p = 0.038). It appears that root foraging precision in the weeks immediately pre- and post- defoliation (weeks two and three of growth) show precise foraging behaviour (Figure 1). These values approximately match values depicted in other studies of sunflower root foraging (Ljubotina & Cahill, 2019). Whereas, the second week post-defoliation (week four of growth) has values closer to 0.5, representing no proportional preference for the nutrient patch or equivalent non-nutrient area. Inclusion or exclusion of the half clipped treatment did not change the results, only strengthening the significant effect of

week when excluded (Table 2, Figure 2, df = 1, 33.758, F = 5.5350, p = 0.025). The same LMM investigating root foraging precision was also ran on all treatments with initial height as a factor and compared using AICc to the LMM without initial height as a factor. Initial height was shown to not have a significant effect on root foraging precision whether the half-clipped treatment was included (Supplemental Table 1) or excluded (Supplemental Table 2). As well, the AICc output supported the model excluding initial height to be the best fit with or without the half clipped treatment (Supplemental Table 3).

The half clipped treatment did not visually display high root foraging precision throughout the entire experiment, which is not in concordance with other studies in sunflower root foraging behaviour and our findings in the control and fully-clipped treatments. Thus, the half clipped treatment was then removed from the rest of the main analysis due to the lack of significant root foraging precision, with analyses performed including and excluding the half-clipped treatment to better investigate the changes, or lack thereof, that was caused by the treatments inclusion.

When looking at the root length grown within a patch or non-patch area by week we see changes in the significance of different factors and interactions over time (Figure 3). In week two of growth (pre-defoliation) there was a significant effect of patch (df = 1, 15.723, F = 5.5840, p = 0.031), but no effect of treatment or interaction of treatment and patch (Table 3). Plants placed more roots within the nutrient patch. In week three of growth (immediately post-defoliation) there was a significant effect of patch (df = 1,16, F = 16.0419, p = 0.0010) and treatment (df = 1,16, F = 4.6390, p = 0.047). Plants placed more roots within a nutrient patch, independent of treatment, and the fully clipped treatment grew significantly less roots than the control treatment that was not defoliated. Furthermore, there was a non-significant trend towards an interaction

between treatment and patch (df = 1,16, F = 3.1845, p = 0.093). In the fourth week of growth (two weeks post-defoliation) there were no significant main effects of patch or treatment or a significant interaction between patch and treatment (Table 3). In this final week the root length grown, overall and within a patch vs non-patch, is equivalent between the treatments.

How do patterns of patch use vary as a function of the temporal scale of measures?

Final root foraging precision and final root length grown in a patch vs non-patch values show that overall the control and fully clipped treatments foraged precisely as they placed more roots, proportionally (Figure 4, df = 17, t = 6.1158, p < 0.0001) and totally (Figure 5, df = 1,16, F = 24.7070, p = 0.00014), within a patch versus a non-patch (Table 4, Table 5). Even though final total root length was determined to be significantly greater in the nutrient patch than in the non-nutrient added equivalent area, there was no significant difference between treatments (df = 1,16, F = 1.8815, p = 0.19). Root foraging precision also did not significantly vary between treatments (Table 6, df = 1,16, F = 0.2061, p = 0.66). Furthermore, despite both the control and fully clipped treatments displayed significant root foraging precision in comparison to an agnostic baseline of 0.5, the half clipped treatment did not display foraging precision in final values (Table 4) which supports its removal from our main analyses.

When only taking into account the final measures significant root foraging precision and patch response were found, alongside a lack of treatment effects. This juxtaposes our weekly findings discussed above, wherein weekly root foraging precision decreased to an agnostic level in the fourth week of growth (Figure 2) and equivalent total root lengths were grown within P and NP areas in the same week (Figure 3). As well, a significant effect of patch and treatment on the weekly root length grown were displayed in the week immediately following defoliation as

defoliation decreased the total amount of root length grown in the fully clipped treatment (Figure 3).

What can different measures of root morphology and patch use tell us about the impact of defoliation and root foraging behaviour in general?

The average root diameter was consistently larger within P than NP (Figure 6, Table 7). When excluding the half-clipped treatment, the second week of growth (pre-defoliation) displays a trend towards a larger root diameter within the nutrient patch. However, it should be noted that this was not significant (df = 1,16, F = 3.4538, p = 0.082). In both other weeks, week three (df = 1,16, F = 4.9813, p = 0.040) and four (df = 1,16, F =8.0001, p = 0.012) of growth, the average root diameter is significantly larger within nutrient patches. This also coincides with the final measures of average root diameter taken on the last day of scanning (Table 8), as the average root diameter was shown to be significantly larger within nutrient patches as well (df = 1,16, F = 11.1251, p = 0.0042). Conversely, the addition of the half-clipped treatment does not change these findings, except for small changes in the significance or strength of the effect of patch on average root diameter (Table 9). This is especially apparent in the first week where the non-significant trend towards a larger average root diameter within nutrient patches becomes significant once the half-clipped group is added (df = 1,24, F = 5.2751, p = 0.031). However, I believe that this is due to an increase in statistical power by the inclusion of the samples from the half-clipped treatment group and not a difference in the treatment group or findings themselves. At no time point or in final measures were treatment or the interaction between patch and treatment significant (Table 7, Table 8).

The average root length was consistently not significantly different between P and NP areas at any time point (Figure 7) , including any week (Table 10) or final measures (Table 11).

As well, at no time point or in final measures were treatment or the interaction between patch and treatment significant (Table 10, Table 11).

4. DISCUSSION

The objective of this thesis was to investigate the effects of defoliation on the root foraging behaviour of common sunflower, focusing on the temporality of its foraging response and the potential variations in interpretation resulting from different measures of behaviour and root morphology. Past studies have demonstrated that both suppression and induction of root foraging behaviour in plants is possible (Mohiley et al., 2021; Yamawo et al., 2019). In this study, it was observed that severe defoliation led to a reduction in overall root length grown in the week following the event. However, defoliation did not affect root foraging precision, average root diameter, or average root length (Figure 2, Figure 6, Figure 7). Interestingly, root foraging precision, as calculated using weekly new growth, decreased gradually over the experimental time period, ultimately reaching agnostic values in the final week of measures. This finding aligns with the timelines of nutrient patch persistence found in field conditions (Březina et al., 2019; Lamb et al., 2004).

Moreover, this study highlighted the importance of considering different foraging and root morphological variables, as well as the timing of their assessment, when comparing past studies and designing future experiments. Trait-based ecology has long recognized that different measures of leaves and roots can elucidate distinct aspects of plant function and performance (Klimešová et al., 2018; Pérez-Harguindeguy et al., 2013). While this notion may seem intuitive, this thesis represents the first empirical demonstration of how such considerations can impact the outcomes of plant behavioral studies. By examining the effects of defoliation on root foraging

behavior in common sunflower and taking into account various measures of behavior and root morphology, this study provides valuable insights into the dynamics of plant responses to injury.

Although plants exhibited similar responses to animals in response to injury by reducing overall feeding, there are ecological and physiological differences that resulted in a slightly different outcome. Exploration was not decreased due to injury, a finding which contradicts assumptions based on findings in animal behavioural ecology (Lindsay & Woodin, 1992; Rennolds & Bely, 2023; Werner et al., 2006). However, when considering a plants immobility and their inability to move to safer habitats, it can be thought that reducing exploration via changes in root foraging precision would not decrease the likelihood of further injury. Instead, it would only decrease possible nutrient gain that could be used for repair and regrowth (Van Dam & Bezemer, 2006). Therefore, this study aligns with the movement in plant behavioural ecology that strives to expand on the literature pertaining to established behavioural responses in plants by studying the impact of novel factors, like injury. It highlights the need to study the assumptions and applicability of animal behavioural theory within the context of plant behaviour, promoting a more comprehensive understanding of plant responses.

How does the presence and severity of a defoliation event affect patterns of resource patch use?

In this study, we considered new root growth as an indicator of feeding occurrence in plants. However, root foraging precision is not as easily equated to patch or prey selection. Instead, higher root foraging precision could be equated to a reduction in exploration and investment within a patch or prey animal. This comparison admittedly still does not completely align between animals and plants. While proportionally increased growth into a patch does indicate a reduction in the relative exploration of the soil, it cannot be ignored that the plant is still exploring to some extent if root foraging precision is anything less than 100% within the

nutrient patch, which is typically the case. This is in direct juxtaposition to a fundamental assumption of optimal foraging theory that exploration and feeding are mutually exclusive behaviours (Charnov, 1976; Pyke et al., 1977; Samu, 1991). I raise this discordance not to invalidate the connection I am drawing between animal habitat exploration and root foraging precision, but rather to highlight the differences in physiology and ecology of plants and animals that need to be taken into account. It is crucial to consider and acknowledge these differences in order to fully understand the similarities and differences between them and how we can effectively apply behavioural theory.

Animal foraging studies investigating the impact of injury on foraging behaviour are typically framed in the context of the sublethal or indirect effects of predation (Lindsay & Woodin, 1992; Rennolds & Bely, 2023; Werner et al., 2006; Wirsing et al., 2008). Thus, these studies generally reveal a reduction in feeding instances and a shift in food selection towards safer but potentially less rewarding patches, reflecting a decrease in exploration behavior (Lindsay & Woodin, 1992; Rennolds & Bely, 2023; Werner et al., 2006; Wirsing et al., 2008). In our study, the common sunflower responded to injury by reducing overall new growth, indicating a decrease in feeding. However, there was no change in their level of exploration, or where they were feeding, as root foraging precision values did not change in response to defoliation. Furthermore, this suppression of growth was recovered from quickly, over one week. While these findings align with animal foraging behavioural theory, they are also consistent with assumptions of optimal defence theory in plants and previous research on the effect of defoliation on overall root growth.

Van Dam and Bezemer posit that if one of a plant's resource capturing organs, namely the roots or shoots, is severely damaged to the extent that it loses the capacity to acquire its

specific resource, a signal should be triggered for reallocation of resources for growth and repair that would involve an immediate decrease in growth of the opposite organ as resources are utilized in rebuilding the injured part of the plant (2006). Additionally, there would be a temporary but significant decrease in access to resources for new growth, as one of the main resource capturing organs is unusable until it is repaired or regrown. The findings from this thesis directly coincide with this prediction, providing support for optimal defence theory in plants (McCall & Fordyce, 2010; Van Dam & Bezemer, 2006; Zangerl & Rutledge, 1996). Furthermore, these findings are consistent with previous studies that demonstrated a decrease in overall root growth following defoliation (Chapin & Slack, 1979; Oswalt et al., 1959; Volesky et al., 2011). The connections made in this study, linking optimal foraging behavior and optimal defense theory, reinforce the associations between different optimality theories across various organisms.

How do patterns of patch use vary as a function of the temporal scale of measures?

Our findings indicate that root foraging precision and the length of root growth within a patch and equivalent area without added nutrients become more agnostic, or equivalent, over time. This can be interpreted as a plant "leaving" a patch as it is depleted. The timeline presented in this study matches with other papers that have found that nutrient patches are ephemeral and randomly placed within an environment, with nutrient spikes lasting only a couple weeks (Březina et al., 2019; Lamb et al., 2004). The ubiquity of this response across all individuals studied, regardless of defoliation treatment, is in line with findings of the marginal value theorem (Charnov, 1976; McNickle & Cahill, 2009; Menezes, 2022; Pyke et al., 1977). Accordingly, given the same amount of nutrients within a nutrient patch, organisms should leave the patch at a standardized or similar rate.

An important distinction should be made between interim and final measures of root foraging precision. The timing of these measures, whether taken on the cumulative root growth at the end of a period of growth or on new growth over time, can greatly impact the findings of a study. While plants may display strong foraging precision at the end of a defined time period, the actual dynamics of patch utilization over time can change rapidly, as demonstrated in this study. Our findings may also help provide insights into a prominent question in root foraging behaviour literature: why do we find such strong root proliferation into nutrient patches in certain species if those nutrients will be quickly depleted (Aanderud et al., 2003; Lamb et al., 2004)? While this phenomenon has been attributed to the persistence of plant roots once they are grown, no studies have shown decisively that plant growth within the patch is limited growth and the observed proliferation researchers are seeing is the result of past growth.

Studies of animal habitat use and movement ecology regularly employ measures of both movement over time and cumulative values of total movement within a given space (Katzner & Arlettaz, 2020; Li et al., 2021; Rubenstein & Hobson, 2004). These studies have been primarily utilized in conservation biology to help mitigate human-animal conflicts, assess seasonal changes in habitat use, and evaluate the potential impacts of habitat alterations, such as road construction, on wildlife populations (Katzner & Arlettaz, 2020; Li et al., 2021; Rubenstein & Hobson, 2004). Although the application of these types of measures in plants may differ, they have underscored the importance of incorporating both interim and final measures of habitat use. Solely relying on endpoint measures, or measures of taken after a longer time span, can obscure critical insights into movement dynamics and patch utilization (Postlethwaite & Dennis, 2013).

What can different measures of root morphology and patch use tell us about the impact of defoliation and root foraging behaviour in general?

Measures of average root diameter and average root length exhibit greater stability and less susceptible to change over time or in response to injury compared to other measures of patch use, such as root foraging precision. We observed a consistent pattern of significantly larger average root diameter within a nutrient patch compared to an equivalent non-nutrient added area, and this pattern remained unchanged over time or in response to injury. These findings are consistent with other studies that have found average root diameter to increase in response to certain nutrients (Oswalt et al., 1959; Zobel et al., 2007). Additionally, although average root length served as a reliable proxy for assessing root verticality, there was no observed changes in verticality in response to nutrient distributions or over time.

This study illustrates the importance of recognizing the variability in measures of root foraging behavior and their differential responses to injury and time, as some measures are more responsive to injury and time. Specifically, root length measures of patch use that compare length within a nutrient patch and non-nutrient added area showed change in response to defoliation and time. Root foraging precision displayed change in response to time, but not defoliation. Finally, average root diameter and average root length did not change in response to defoliation or time, with average root length showing no response even to heterogenous nutrient distribution within the soil.

This study reveals that average root length, and root verticality by association, may not serve as reliable measures of root foraging behaviour and may offer limited utility in future studies on this subject. On the other hand, average root diameter could be a useful addition in root foraging studies focusing on final measures, rather than changes over time. Despite its limited responsiveness to time or injury, average root diameter remains a meaningful functional trait. It can provide insights into root longevity, nutrient uptake, and can be utilized in various

functional measures such as specific root length (Klimešová et al., 2018; Pérez-Harguindeguy et al., 2013; Zobel et al., 2007).

5. FUTURE DIRECTIONS

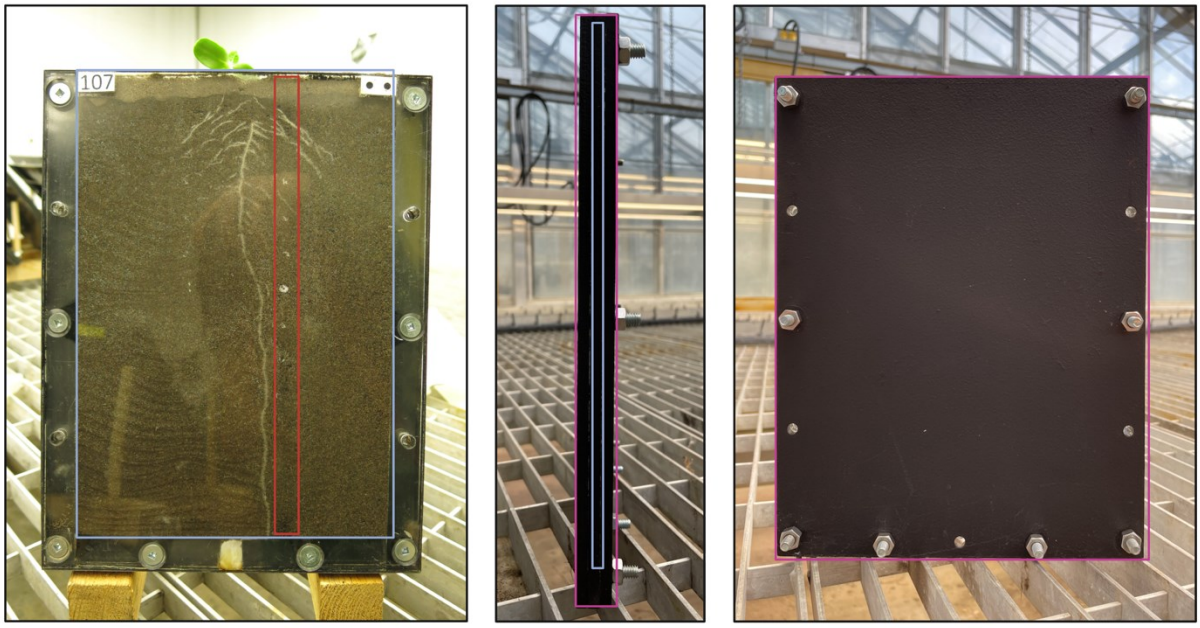
The study of the effect of novel factors on establish plant behaviours requires further exploration in order to expand our understanding of these processes. It is crucial to examine the different dependencies of various behaviours and what they tell us about the plants, behaviour, or the applied theory itself. Specifically, I believe that future studies can build upon this research by considering how the timing and type of measurements can impact plant behavioural findings during study creation and comparison. This study demonstrates that these factors can drastically change interpretation, and only if they are all taken together can a clear picture be generated. However, despite the many different measures analyzed in this experiment it still did not include every root foraging behavioural measurement taken or calculated in the field. Therefore, further study in the field should aim to either empirically compare new measures with well-established ones or standardize the protocols for measures of plant foraging and calculations.

Currently, the literature pertaining to the effect of defoliation on root foraging behaviour is preliminary. There are few studies looking at a few species, all with very different methods of defoliation (Mohiley et al., 2021; Yamawo et al., 2019). Future studies should strive to incorporate more species in diverse contexts. By incorporating more species, we can begin to expand our exploration of the potential phylogenetic patterns of plant behaviour. Current studies into phylogenetic patterns of root foraging focus on root foraging precision, in which eudicots are generally more precise foragers than monocots (Grime & Mackey, 2002; Kembel & Cahill, 2005). However, by incorporating more species into studies going beyond precision measures we could then explore if there are patterns associated with injury or other novel factors. For

example, are certain phylogenetic groupings of species more responsive to defoliation in terms of changes to their root foraging behaviour? Conversely, future studies could also examine other factors that could influence a plants response to defoliation, such as changes to nutrient distribution or the presence of neighbouring plants. These factors have been shown to change the root foraging behaviour of species in non-additive ways, but the inclusion of defoliation has not been studied yet. Thus, the field of root foraging behavior is continuously evolving, and experiments are becoming more complex as novel factors and contexts are integrated into established understandings.

6. IMAGES, TABLES, AND FIGURES

Image 1. Constructed window box visual aid. The pink areas represent the dimensions of the entire window box (27.9 cm × 21.5 cm × 0.6 cm), the blue areas represent the available soil space (25.9 cm × 17.8 cm × 0.6 cm), and the red area represents the nutrient patch (25.9 cm × 1.5 cm × 0.6 cm). The red markers are excluded from the side image, as they would be the same length and depth as the blue area.



Front Side Back

Image 2. Visual aid of the window box positioning within the plant stands.

Image 3. Example root scan. Scans were taken at 900 dpi on the Epson Perfection V850 Pro scanner. The red boxes (1.5 cm x 22 cm) were added after the scans were taken using Adobe Photoshop 2023. In this particular arena the left area is centered on the nutrient patch (P), with the right area representing the equivalent non-nutrient added area (NP).

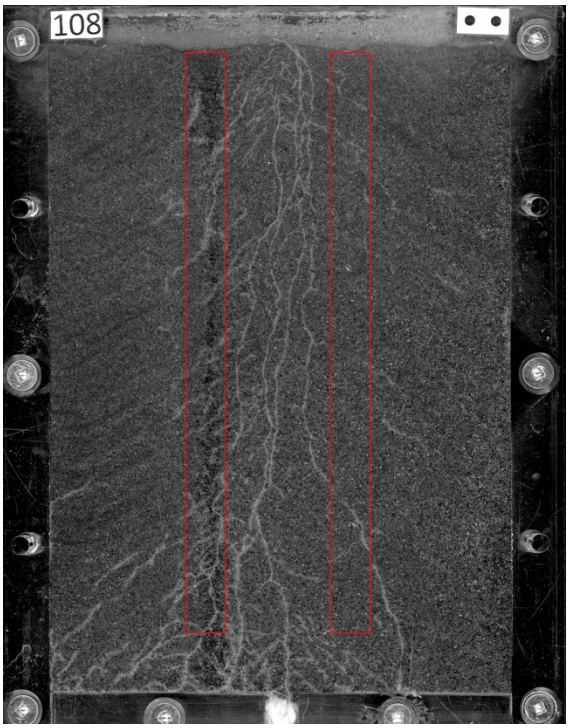


Image 4. Experimental timeline and example measures. Each measure was done for both patch (P) and non-patch (NP) area and each week.

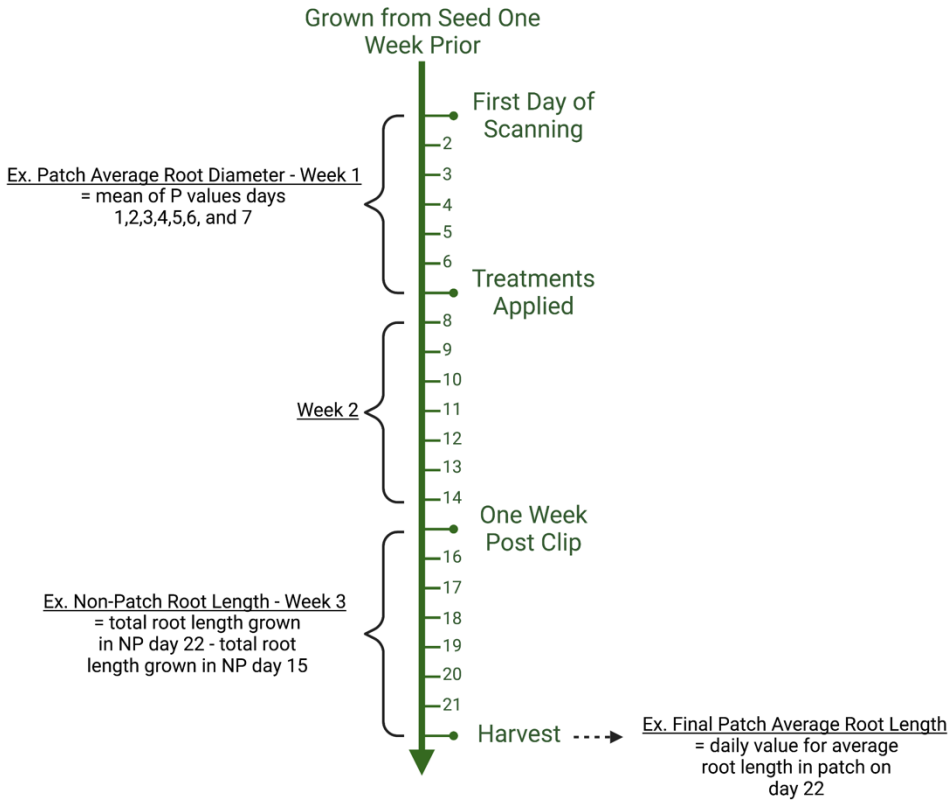


Table 1. A linear mixed model testing the effect of week and treatment on weekly root foraging precision in sunflower, *Helianthus annuus* L. Treatments included: no clipping, half clipping, and fully clipped defoliation severities. Fixed effects included week, treatment, and their interaction. Individual was included as a random effect (v = 0, sd = 0).

	root foraging precision		
source	df	F-value	p-value
week	1,50.77	4.53	**0.038**
treatment	2,65.06	0.92	0.41
week x treatment	2,50.75	0.36	0.70

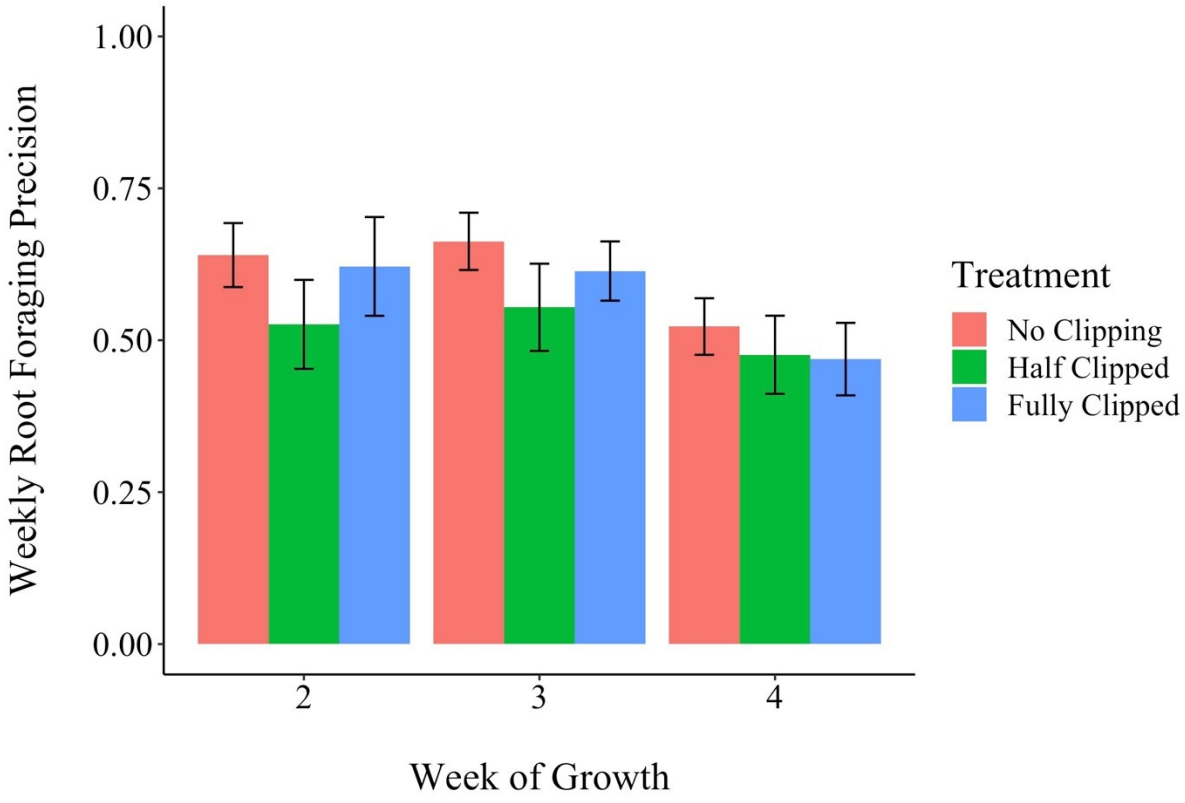


Figure 1. Weekly root foraging precision in each week of growth, separated by defoliation treatment (mean ± se). Calculations were performed on the new root growth within each week, where the root grown within a nutrient patch was divided by the total combined root length grown within a nutrient patch and equivalent non-nutrient added area. The no clipping defoliation treatment was subject to no leaf removal, the half-clipped defoliation treatment had half of the true leaves removed, and the fully-clipped defoliation treatment had all true leaves removed.

Table 2. A linear mixed model testing the effect of week and treatment on weekly root foraging precision in sunflower, *Helianthus annuus* L. Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included week, treatment, and their interaction. Individual was included as a random effect (v = 0, sd = 0).

	root foraging precision		
source	df	F-value	p-value
week	1,33.76	5.54	**0.025**
treatment	1,43.22	0.0060	0.94
week x treatment	1,33.76	0.074	0.79

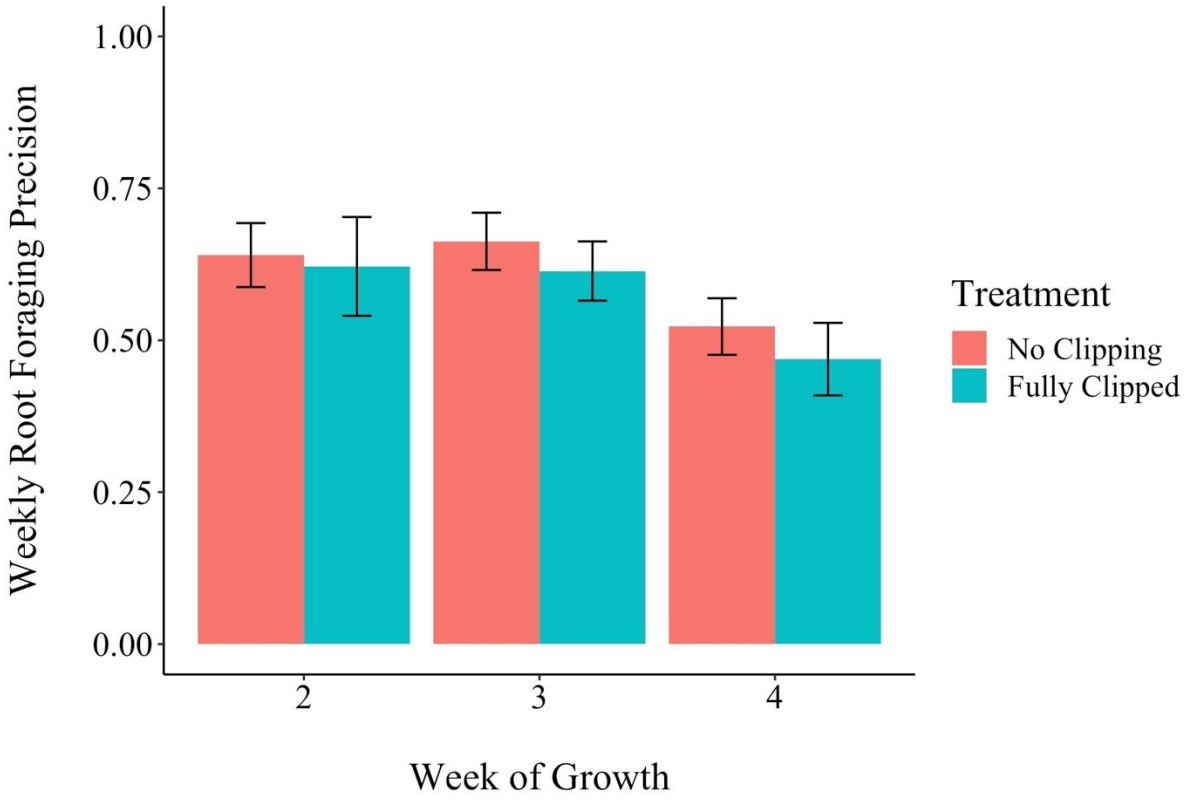


Figure 2. Weekly root foraging precision in each week of growth, separated by defoliation treatment (mean ± se). Calculations were performed on the new root growth within each week, where the root grown within a nutrient patch was divided by the total combined root length grown within a nutrient patch and equivalent non-nutrient added area. The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed.

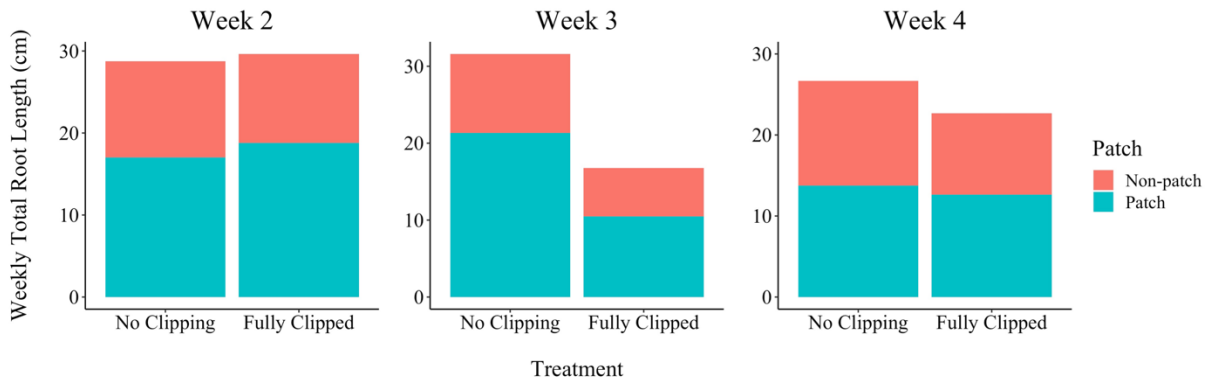


Figure 3. Weekly total root length grown in each week, separated by defoliation treatment (mean). Calculations were performed on the new root growth within each week in a nutrient patch (patch) and non-nutrient added equivalent area (non-patch). The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true

Table 3. Three linear mixed models testing the effect of nutrient patch and treatment on the weekly root length grown in sunflower, *Helianthus annuus* L. One model was performed on each week of data (the second, third, and fourth week of growth). Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random factor for the second week of growth (v = 0, sd = 0), third week of growth (v = 37.08, sd = 6.09), and fourth week of growth (v = 30.39, sd = 5.51).

		patch use: total root length		
week of growth	source	df	F-value	p-value
Second	patch	1,15.72	5.58	**0.031**
	treatment	1,15.72	0.026	0.87
	patch x treatment	1,15.72	0.22	0.65
Third	patch	1,16	16.04	**0.0010**
	treatment	1,16	4.64	**0.047**
	patch x treatment	1,16	3.18	0.093*
Fourth	patch	1,16	0.90	0.36
	treatment	1,16	0.40	0.54
	patch x treatment	1,16	0.24	0.63

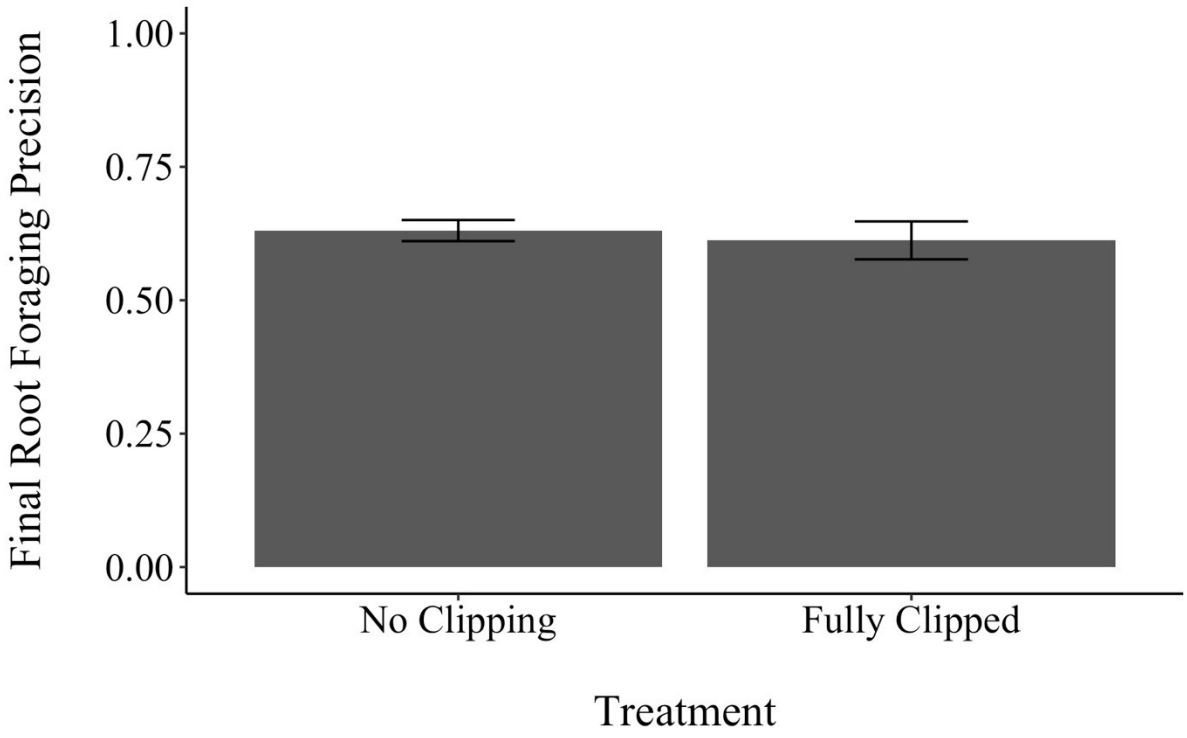


Figure 4. Final root foraging precision separated by defoliation treatment (mean ± se). Calculations were performed on the final recorded root growth values, where the total root length within a nutrient patch was divided by the total combined root length within a nutrient patch and equivalent non-nutrient added area. The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed.

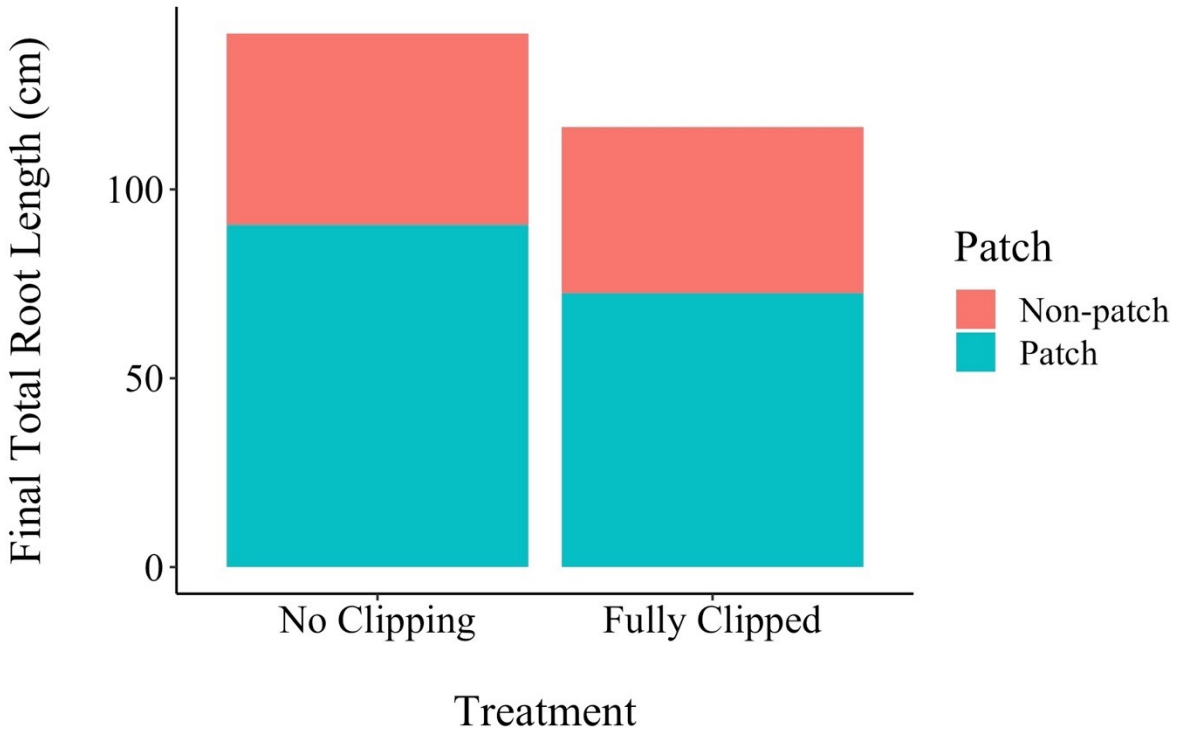


Figure 5. Final total root length grown in each week, separated by defoliation treatment (mean). Calculations were performed on the final recorded root growth values in a nutrient patch (patch) and non-nutrient added equivalent area (non-patch). The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed.

Table 4. Five one-sample t-tests comparing final root foraging precision to an agnostic value of 0.5 in sunflower, *Helianthus annuus* L. Treatments subsets include: all treatments, excluding the half clipped, only the no clipping treatment, only the half clipped treatment, and only the fully clipped treatment.

		final root foraging precision		
treatment	expected mean value	df	t-value	p-value
all inclusive	0.5	26	4.72	**7.03e-05**
no half clipped	0.5	17	6.12	**1.14e-05**
no clipping	0.5	8	6.60	**0.00017**
half clipped	0.5	8	1.03	0.33
fully clipped	0.5	8	3.16	**0.013**

Table 5. A linear mixed model testing the effect of patch and treatment on the final total root length grown in sunflower, *Helianthus annuus* L. Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random effect (v = 154.90, sd = 12.44).

	final patch use: total root length		
source	df	F-value	p-value
patch	1,16	24.71	**0.00014**
treatment	1,16	1.88	0.19
patch x treatment	1,16	0.68	0.42

Table 6. A linear model testing the effect of treatment on the final root foraging precision in sunflower, *Helianthus annuus* L. Treatments included the no clipping and fully clipped defoliation severities. Fixed effect of treatment.

	final root foraging precision		
source	df	F-value	p-value
treatment	1,16	0.21	0.66

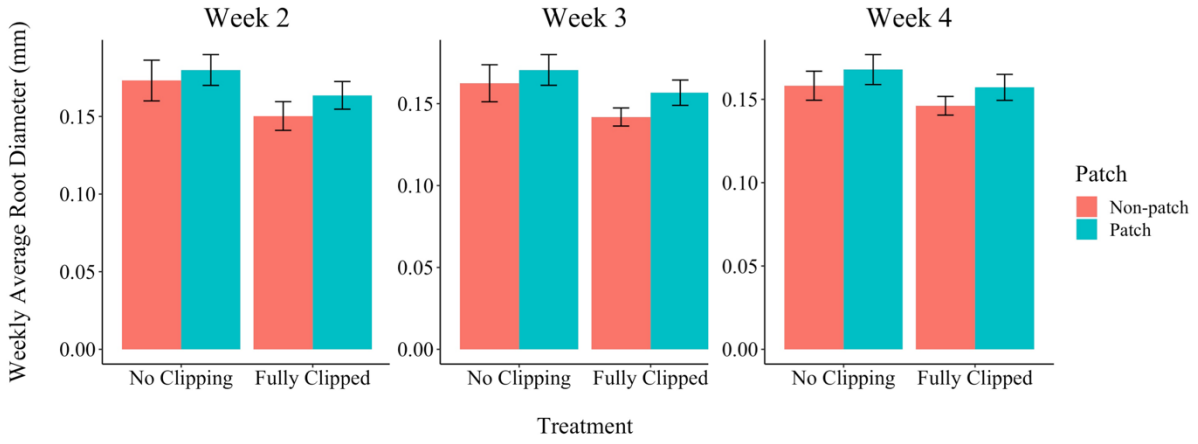


Figure 6. Weekly average root diameter (mm) grown in each week, separated by defoliation treatment (mean ± se). Calculations were performed on the new root growth within each week in a nutrient patch (patch) and non-nutrient added equivalent area (non-patch). The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed.

Table 7. Three linear mixed models testing the effect of nutrient patch and treatment on the weekly average root diameter in sunflower, *Helianthus annuus* L. One model was performed on each week of data (the second, third, and fourth week of growth). Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random factor for the second week of growth (v = 0.00072, sd = 0.027), third week of growth (v = 0.00045, sd = 0.021), and fourth week of growth (v = 0.00044, sd = 0.021).

		patch use: average root diameter		
week of growth	source	df	F-value	p-value
Second	patch	1,16	3.45	0.082*
	treatment	1,16	2.033	0.17
	patch x treatment	1,16	0.36	0.55
Third	patch	1,16	4.98	**0.040**
	treatment	1,16	2.35	0.14
	patch x treatment	1,16	0.42	0.52
Fourth	patch	1,16	8.00010	**0.012**
	treatment	1,16	1.16	0.30
	patch x treatment	1,16	0.035	0.85

Table 8. A linear mixed model testing the effect of patch and treatment on the final average root diameter in sunflower, *Helianthus annuus* L. Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random effect (v = 0.00042, sd = 0.020).

	final patch use: average root diameter		
source	df	F-value	p-value
patch	1,16	11.13	**0.0042**
treatment	1,16	0.90	0.36
patch x treatment	1,16	0.0061	0.94

Table 9. Three linear mixed models testing the effect of nutrient patch and treatment on the weekly average root diameter in sunflower, *Helianthus annuus* L. One model was performed on each week of data (the second, third, and fourth week of growth). Treatments included: no clipping, half clipping, and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random factor for the second week of growth (v = 0.00061, sd = 0.025), third week of growth (v = 0.00041, sd = 0.020), and fourth week of growth (v = 4.091e-04, sd = 0.020).

		patch use: average root diameter		
week of growth	source	df	F-value	p-value
Second	patch	1,24	5.28	**0.031**
	treatment	2,24	1.37	0.27
	patch x treatment	2,24	0.30	0.74
Third	patch	1,24	6.71	**0.016**
	treatment	2,24	1.38	0.27
	patch x treatment	2,24	0.77	0.47
Fourth	patch	1,24	10.38	**0.0036**
	treatment	2,24	0.64	0.54
	patch x treatment	2,24	0.73	0.49

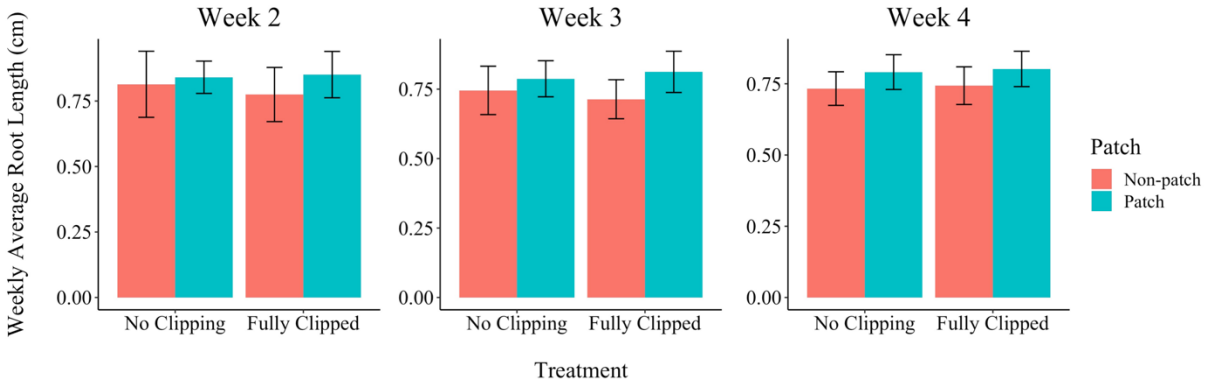


Figure 7. Weekly average root length (cm) grown in each week, separated by defoliation treatment (mean ± se). Calculations were performed on the new root growth within each week in a nutrient patch (patch) and non-nutrient added equivalent area (non-patch). The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed.

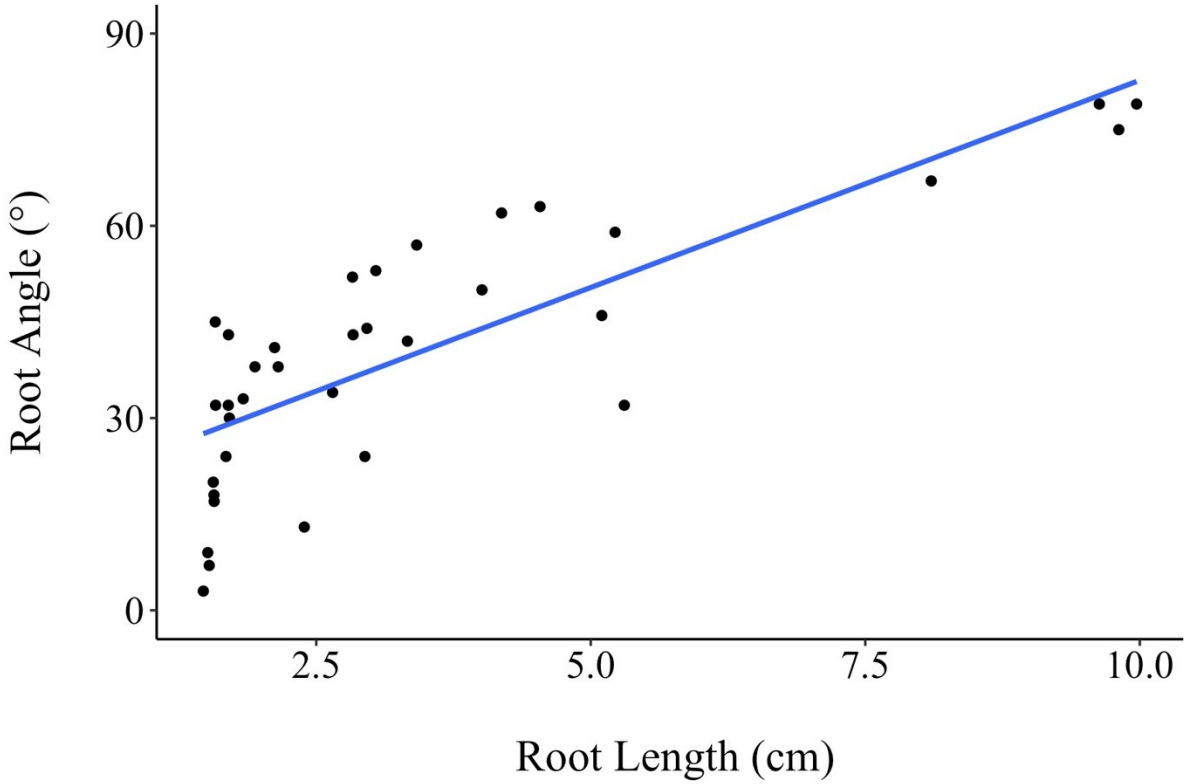
Table 10. Three linear mixed models testing the effect of nutrient patch and treatment on the weekly average root length in sunflower, *Helianthus annuus* L. One model was performed on each week of data (the second, third, and fourth week of growth). Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random factor for the second week of growth (v = 0.022, sd = 0.15), third week of growth (v = 0.0073, sd = 0.085), and fourth week of growth (v = 0.0046, sd = 0.068).

		patch use: average root length		
week of growth	source	df	F-value	p-value
Second	patch	1,16	0.37	0.55
	treatment	1,16	0.017	0.90
	patch x treatment	1,16	0.086	0.77
Third	patch	1,16	1.037	0.32
	treatment	1,16	0.0019	0.97
	patch x treatment	1,16	0.17	0.69
Fourth	patch	1,16	1.015	0.33
	treatment	1,16	0.026	0.87
	patch x treatment	1,16	0.0000	0.995

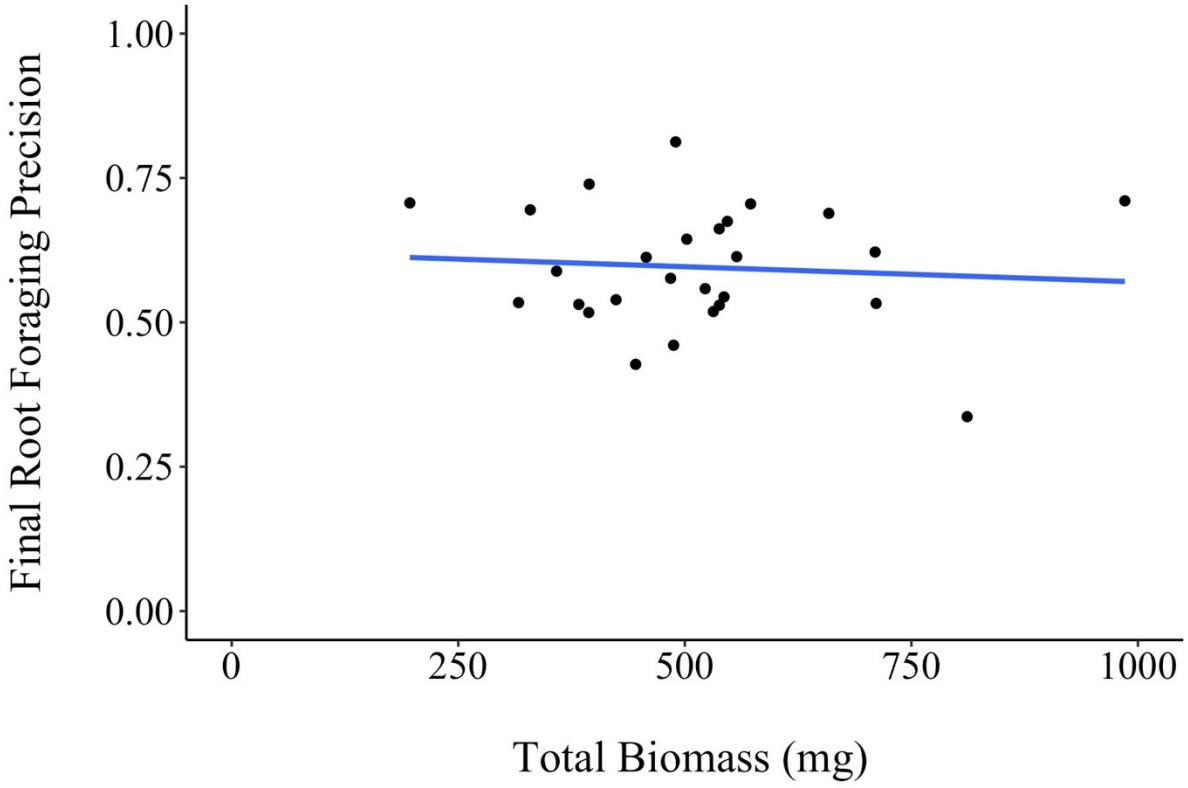
Table 11. A linear mixed model testing the effect of patch and treatment on the final average root length in sunflower, *Helianthus annuus* L. Treatments included the no clipping and fully clipped defoliation severities. Fixed effects included patch, treatment, and their interaction. Individual was included as a random effect (v = 0.0053, sd = 0.073).

	final patch use: average root length		
source	df	F-value	p-value
patch	1,16	1.29	0.27
treatment	1,16	0.068	0.80
patch x treatment	1,16	0.051	0.82

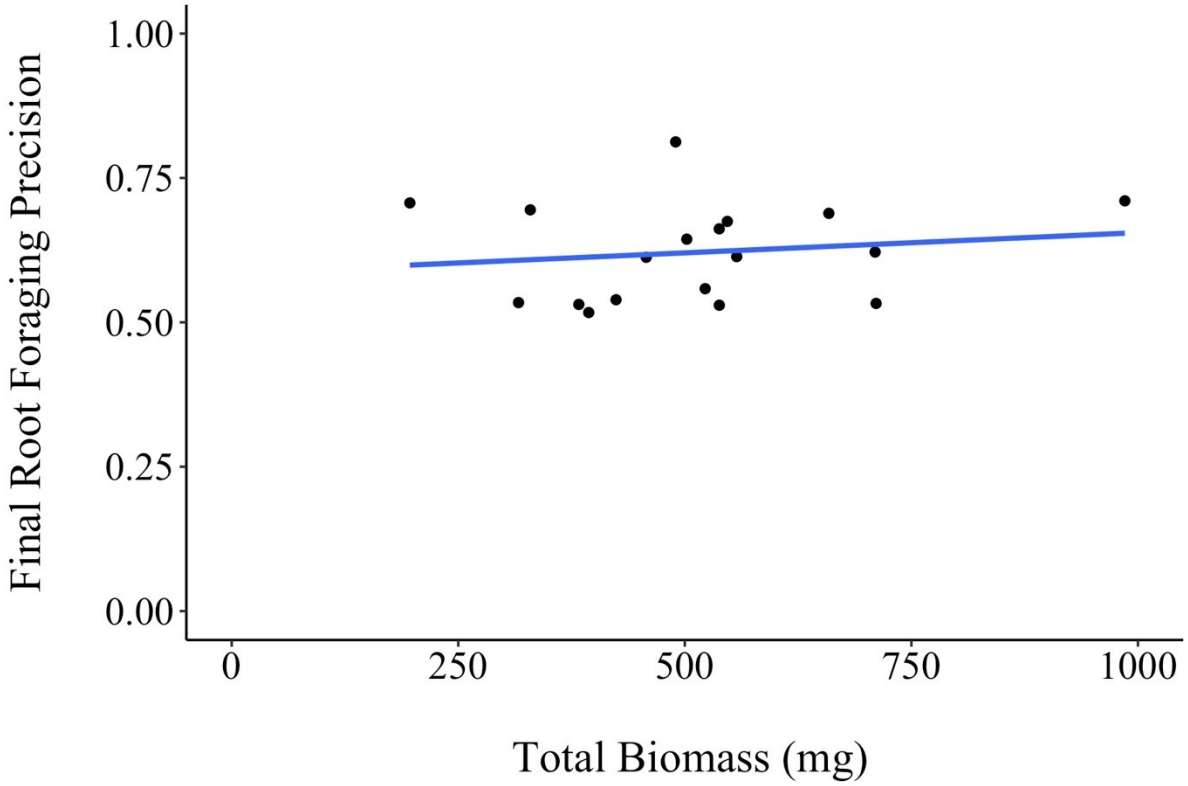
7. SUPPLEMENTARY MATERIAL



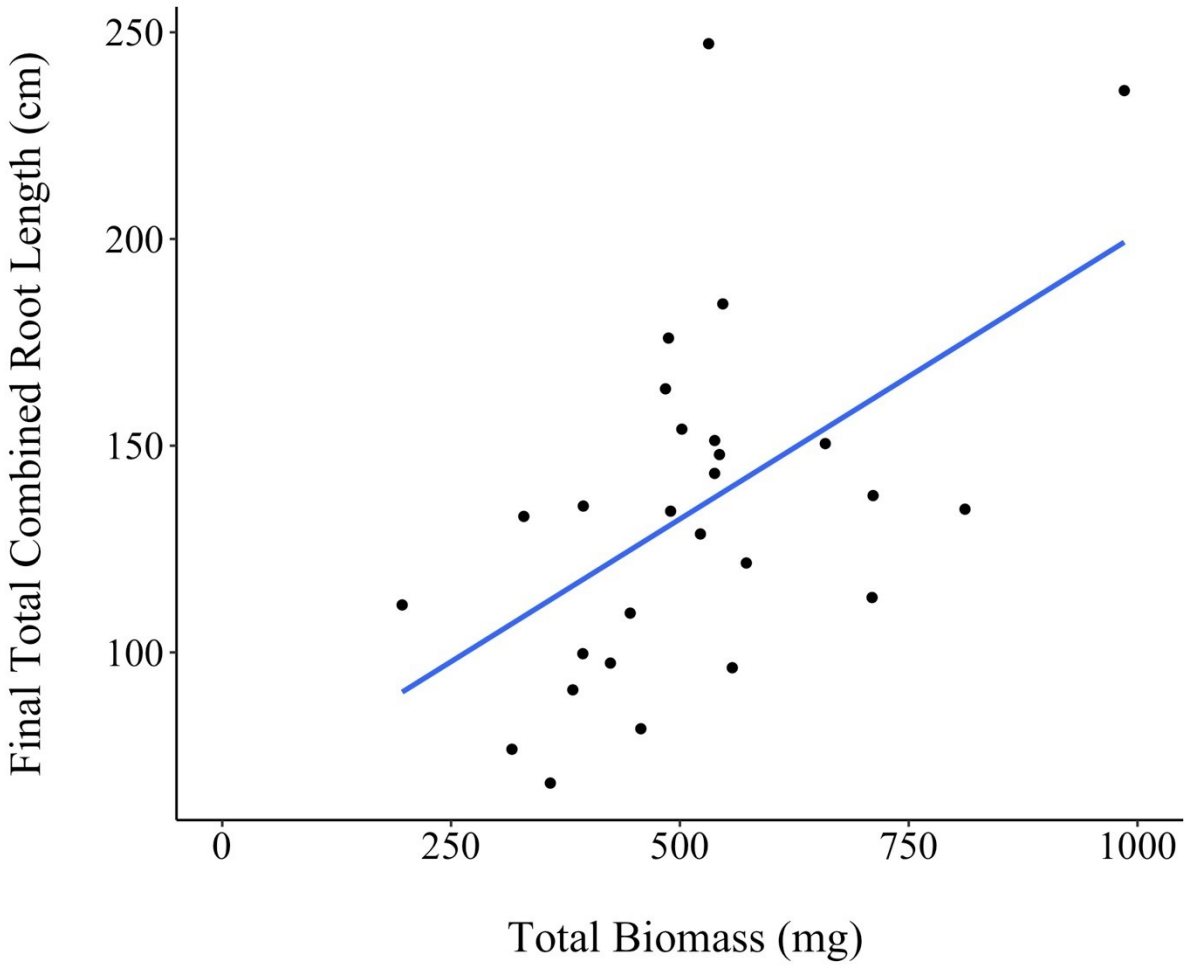
Supplemental Figure 1. Root angle (0-90°) as related to root length (cm). The angle of the root was calculated manually using printed out images of the root scans. A lines was drawn from the entry to exit point and taking the angle of that line. Roots entering and exiting on the same plane were removed.



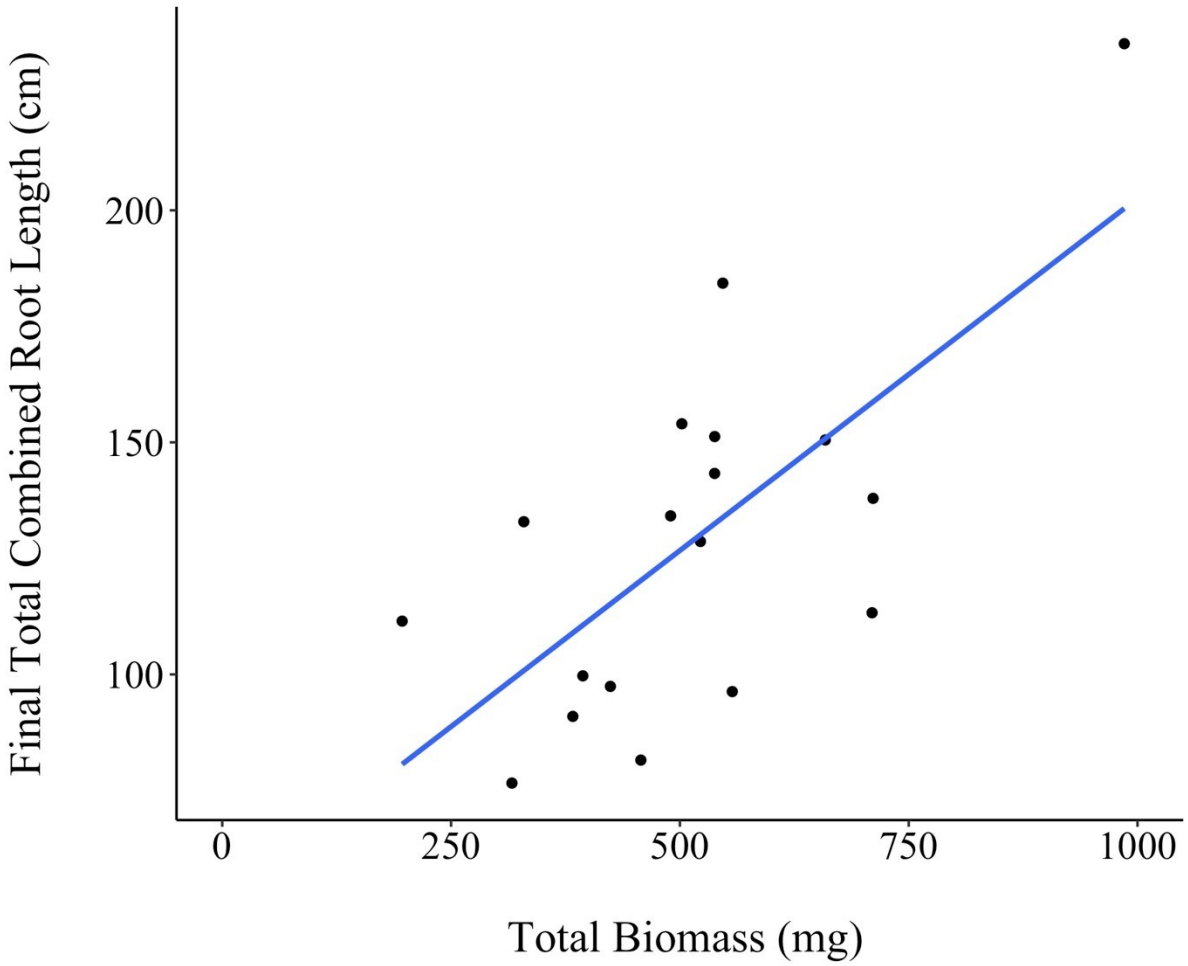
Supplemental Figure 2. Final root foraging precision in relation to the final total dried biomass (mg). Calculations were performed on the final recorded root growth values, where the total root length within a nutrient patch was divided by the total combined root length within a nutrient patch and equivalent non-nutrient added area to derive root foraging precision. The no clipping defoliation treatment was subject to no leaf removal, the half-clipped defoliation treatment had half of the true leaves removed, and the fully-clipped defoliation treatment had all true leaves removed. All individuals of all treatments were pooled for this visualization.



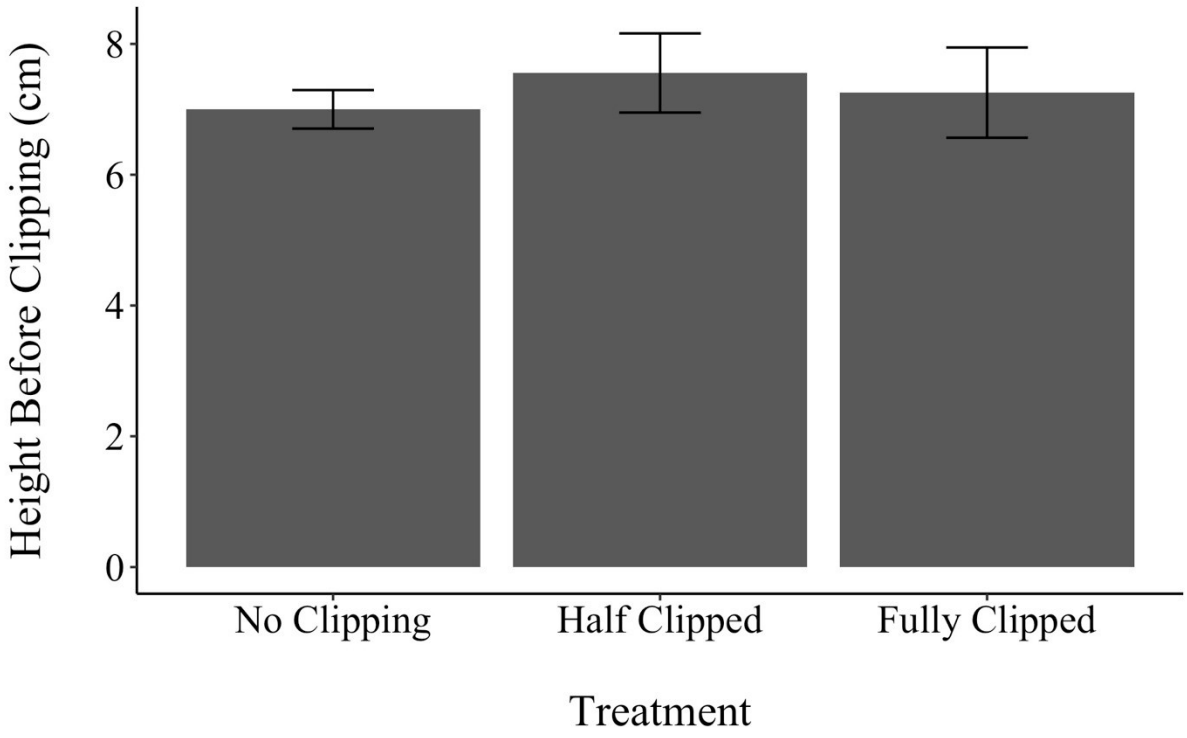
Supplemental Figure 3. Final root foraging precision in relation to the final total dried biomass (mg). Calculations were performed on the final recorded root growth values, where the total root length within a nutrient patch was divided by the total combined root length within a nutrient patch and equivalent non-nutrient added area to derive root foraging precision. The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed. All individuals of the no clipping and fully clipped treatments were pooled for this visualization. The half-clipped treatment was excluded.



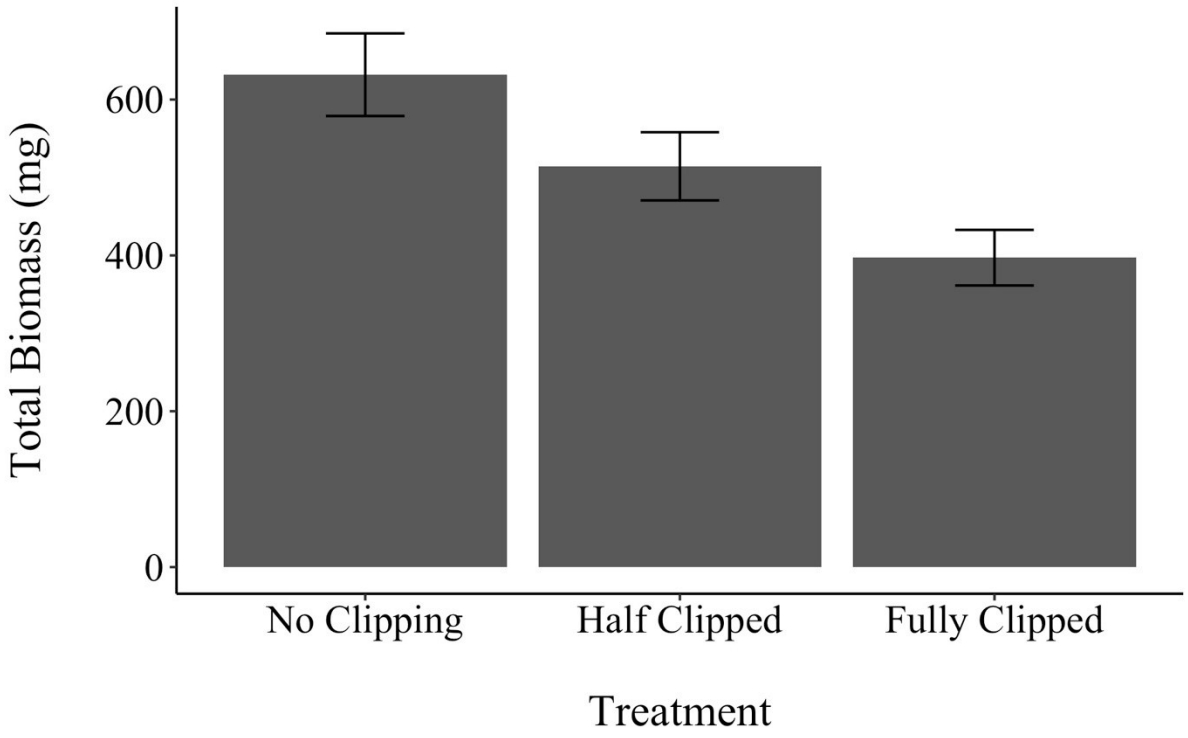
Supplemental Figure 4. Final total combined root length (cm) in relation to the final total dried biomass (mg). Calculations were performed on the final recorded root growth values, where the total root length within a nutrient patch added to the total root length within a nutrient equivalent non-nutrient added area to derive the total value. The no clipping defoliation treatment was subject to no leaf removal, the half-clipped defoliation treatment had half of the true leaves removed, and the fully-clipped defoliation treatment had all true leaves removed. All individuals of all treatments were pooled for this visualization.



Supplemental Figure 5. Final total combined root length (cm) in relation to the final total dried biomass (mg). Calculations were performed on the final recorded root growth values, where the total root length within a nutrient patch added to the total root length within a nutrient equivalent non-nutrient added area to derive the total value. The no clipping defoliation treatment was subject to no leaf removal and the fully-clipped defoliation treatment had all true leaves removed. All individuals of the no clipping and fully clipped treatments were pooled for this visualization. The half-clipped treatment was excluded.



Supplemental Figure 6. Plant height recorded immediately before clipping (cm) separated by treatment (mean ± se). The no clipping defoliation treatment was subject to no leaf removal, the half-clipped defoliation treatment had half of the true leaves removed, and the fully-clipped defoliation treatment had all true leaves removed.



Supplemental Figure 7. Plant final total dried biomass (mg) separated by treatment (mean ± se). The no clipping defoliation treatment was subject to no leaf removal, the half-clipped defoliation treatment had half of the true leaves removed, and the fully-clipped defoliation treatment had all true leaves removed.

Supplemental Table 1. A linear mixed model testing the effect of initial height, week, and treatment on weekly root foraging precision in sunflower, *Helianthus annuus* L. Treatments included: no clipping, half clipping, and fully clipped defoliation severities. Fixed effects included initial height, week, treatment, and their interaction. Individual was included as a random effect (v = 0, sd = 0).

	root foraging precision		
source	df	F-value	p-value
initial height	1,61.94	1.01	0.32
week	1,49.36	2.16	0.15
treatment	2,61.64	0.32	0.73
initial height x week	1,48.88	0.90	0.35
initial height x treatment	2,61.42	0.14	0.87
week x treatment	2,48.95	0.089	0.92
initial height x week x treatment	2,48.57	0.12	0.89

Supplemental Table 2. A linear mixed model testing the effect of initial height, week, and treatment on weekly root foraging precision in sunflower, *Helianthus annuus* L. Treatments included: no clipping and fully clipped defoliation severities. Fixed effects included initial height, week, treatment, and their interaction. Individual was included as a random effect (v = 0, sd = 0).

	root foraging precision		
source	df	F-value	p-value
initial height	1,41.16	1.32	0.26
week	1,33.40	2.057	0.16
treatment	1,41.43	0.25	0.62
initial height x week	1,32.92	0.70	0.41
initial height x treatment	1,41.16	0.22	0.64
week x treatment	1,33.40	0.19	0.66
initial height x week x treatment	1,32.92	0.26	0.61

Supplemental Table 3. AICc output as calculated using the *MuMIn* r package.

		AICc output	
treatments	model	df	AICc
All	week*treatment	8	0.59
	initial height*week*treatment	14	49.61
Half-clipped excluded	week*treatment	6	-4.83
	initial height*week*treatment	10	28.66

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APPENDIX 1: An exploration of the effect of species-relatedness on root foraging behaviour in a subset of the *Linum* genus

Introduction

There is evidence that root foraging behaviour in plants may be based on species-relatedness, with more closely related species exhibiting more similar behaviour. However, the existing evidence is very broad in scope, primarily highlighting the contrasting responses of eudicots and monocots, with eudicots displaying more significant root proliferation responses than monocots (Grime & Mackey, 2002; Kembel & Cahill, 2005). Studies investigating multiple genera of species have found no phylogenetic signal for root foraging behaviour in plants, potentially due to the great variation in the methods of measuring root foraging behaviour (McNickle & Brown, 2014). Therefore, it is essential to clarify if root foraging behaviour is based on species-relatedness, and if so, what levels of relatedness and types of behavioural measures matter. Comparisons of root length- and root biomass-based root proliferation calculations, two of the most common methods of measuring this behaviour, within a single study can facilitate comparison between the two methods. Additionally, further study in the area may want to focus on simple exploration of behavioural similarity within a single species or genus first before expanding to broader assessments, as many studies have already examined the relationships of root foraging behaviour and species relatedness at higher taxonomic levels.

The *Linum* genus is particularly well suited to the study on the impact of species-relatedness due to the agricultural background of *Linum usitatissimum.* Given this background, the genus is already prominent in genetic and phylogenetic studies, and includes a combination of agricultural and wild species that allow for comparisons not only between closely related

species but also species with distinct evolutionary histories of human selection (Bolsheva et al., 2019; Jhala et al., 2008; Saha et al., 2019; Sveinsson et al., 2014; Wang et al., 2012).

To investigate whether closely related flax species exhibit similar root foraging behaviour and if this similarity is a function of the varying relatedness within those species, we conducted an experiment involving the cultivation of young individuals from the *Linum* genus in pots with added nutrient patches. After four weeks of growth, the root systems were excavated and separated into sections to evaluate root growth within the patch and equivalent non-nutrient added area. We measured root length and obtained dried root biomass values in order to explore the possible differences between these two popular methods of root foraging precision measures.

This study was ultimately created to address two key questions regarding the phylogenetic patterns of root foraging behaviour and the impact of measurement methods on root foraging study outcomes. These questions included: 1) Is the root foraging behaviour in closely related flax species a function of the varying relatedness within those species? 2) Do our findings of root foraging behaviour, and the possible similarities/differences between related species of flax, depend on the type of precision measure used?

Methods

Species

Five *Linum* spp. were used in this experiment; *Linum usitatissimum* (cultivar "Bethune"), *Linum lewisii*, *Linum bienne*, *Linum grandiflorum*, and *Linum perenne*. These species were utilized because a phylogeny for them already exists (Sveinsson et al., 2014). *Linum usitatissimum* seed was acquired from Dr. Michael Deyholos of the University of British Columbia, *Linum lewisii* and *Linum bienne* seed were acquired from the U.S. National Plant Germplasm System, and *Linum grandiflorum* and *Linum perenne* seed were acquired from

commercial distributers. In line with findings from Sveinsson et al. (2014) we grouped these species into two broader clades named: "Phylogenetic Group A", which included *Linum usitatissimum, Linum grandiflorum,* and *Linum bienne*, and "Phylogenetic Group B", which included *Linum perenne* and *Linum lewisii*.

Experimental Set Up

All species had seeds germinated and were grown individually in potting soil-filled plant trays prior to transplant. Plants were allowed to grow for three weeks and then were bare-root transplanted into experimental pots. Ten individuals for each species (for a total of 50 individuals) were bare root transplanted, each individual in their own pot. Experimental pots were square, 10 cm (4 inches) in diameter and 9 cm deep, filled with a background soil mix of 3:1 sand:soil. Within every pot we created a cylindrical 2:1 manure:soil mix nutrient patch that was 2 cm in diameter and the same depth of the pot. The nutrient patch was placed to either the left or the right side of the individual, equidistant from the edge of the pot and the plant. The plants were then allowed to grow for an additional four weeks until harvest.

Plant pots were rotated weekly to ensure no confounding variable of patch side. Pot placement was also randomized every week to ensure no confounding variable of pot location. Both the plant trays and plant pots were bottom watered daily *ad libitum* until soil saturation.

Harvest

At harvest, the shoot was cut off at the base of the plant and dried and weighed for shoot biomass. There were no significant reproductive structures present, so reproductive biomass was not separated. After shoot removal the pots were turned over upside down, so that the top was on the bottom and the bottom was facing the top. The pot was then removed and a mound of dirt and roots was left in the shape of the pot. This mound of dirt was cut into three equivalent

sections, resulting in three sections of roots. These sections were labelled as the middle, patch side (P), and equivalent non-patch side (NP). Root sections were washed over a 1 mm sieve to remove excess soil and stored in water within 50 mL conical centrifuge tubes until they could be scanned. No sample was stored for more than a week within the water to ensure degradation of the fine roots was limited. All washed root sections were scanned via WhinRHIZO (WinRHIZO 2021a, Regent Instruments, QC, Canada) to determine middle, patch side (P), and equivalent non-patch side (NP) root length. Afterwards, the washed and scanned root segments were dried for at least 48 hours at 70C before weighing.

Statistical Analysis

All graphing and statistical analyses were performed using RStudio v.1.3.959, employing the packages *lme4*, *car*, *ggplot2*, *tidyverse*, and *dplyr*. Root foraging precision values based on biomass (mg) were calculated by dividing the mass of the patch (P) section over the combined mass of the patch and non-patch (NP) section (P/(P+NP)). Root foraging precision values based on root length (cm) were calculated the same way.

To test if root foraging precision differed between species we performed a linear model (LM) that examined root foraging precision as a function of species. This LM was performed using the biomass-based and root length-based precision values. To test if root foraging precision differed between phylogenetic groups a linear mixed model (LMM) was performed that modelled root foraging precision as a function of the fixed effect of phylogenetic group and the random effect of species. This LMM was used to evaluate both the biomass-based and root length-based precision values. Comparison between the findings based on the precision values calculated via biomass or root length was completed visually in reference to the statistical outputs and graphs derived from the data. The p-values for the LMM's were estimated using

Kenward-Roger degrees of freedom calculations based on type three ANOVAs with Wald F-tests.

Results

Root foraging precision values derived from dried biomass did not display significantly different precision values from the 0.5 baseline representing an agnostic response to nutrient distribution (Appendix Table 1, Appendix Figure 1). However, there was a trend in *Linum perenne* towards avoidance of the nutrient patch ($df = 9$, $t = 1.9485$, $p = 0.083$). Root foraging precision values derived from root length also largely did not display significantly different precision values from the 0.5 baseline (Appendix Table 2, Appendix Figure 2). However, the trend depicted in *Linum perenne* towards avoidance became significant ($df = 9$, $t = -2.3929$, $p = 0.040$). Species did differ significantly in final total biomass ($df = 4,43$, $F = 27.123$, $p < 0.0001$, Appendix Figure 3), but the two phylogenetic groups did not differ significantly in final total biomass ($df = 1,3.0114$, $F = 3.7084$, $p = 0.15$, Appendix Figure 4). From visual examination it appears that *Linum usitatissimum* was the largest, *Linum bienne* and *Linum grandiflorum* were intermediate in size, and *Linum lewisii* and *Linum perenne* were the smallest (Appendix Figure 3). This aligns with the fact that *Linum usitatissimum* is an agricultural crop, and has likely been bred for such size.

Root foraging precision values derived from dried biomass did not show a significant difference between the behaviour of different species (Appendix Figure 1) or clades (Appendix Figure 5). As well, root foraging precision values derived from total root length also did not show a significant difference between the behaviour of different species (Appendix Figure 2) or clades (Appendix Figure 6). Thus, root foraging behaviour was similar across all related *Linum* spp. studied (Appendix Table 3, Appendix Table 4).

The findings of the effect of species relatedness on root foraging precision of these five Linum spp. did not change with the change in the methods used to derive precision values. Both LMMs derived from either dried biomass or total root length displayed non-significant differences among the studied species and clades. Furthermore, the findings are incredibly similar with visual comparison (Appendix Figure 1, Appendix Figure 2, Appendix Figure 5, Appendix Figure 6).

Future Directions

This preliminary exploration of the root foraging behaviour of members of the *Linum* genus highlights some important considerations for future work. I have ordered them in reference to what I perceive as the relative importance for potential studies, with the most important being discussed first.

First and foremost, this study provides supports for the comparability of root foraging precision values derived from biomass or root length. The variation in methods employed across different studies has been acknowledged as a potential issue in the ability to compare findings across various root foraging behavioural research (McNickle & Brown, 2014). However, it should be noted that this study does not account for differences in growth conditions, including the effects of field versus greenhouse studies or the use of different nutrients. Nevertheless, given the same, or very similar, experimental conditions the type of precision measure calculated should give analogous results. Root length-based foraging precision measures can be incredibly time consuming to collect, and the washed root segments are usually dried afterwards and weighed anyways for additional data and sample storage. If there are time or cost restraints to using root scanning programs, like WhinRHIZO (WinRHIZO 2021a, Regent Instruments, QC, Canada), using a purely biomass-based precision measure may be adequate. This could

potentially enable more studies to be conducted in a shorter timeframe at a reduced cost, facilitating the production of larger-scale investigations into root foraging behaviour.

Secondly, this study provides evidence to support that phylogenetic patterns in root foraging behaviour are observed primarily at broader taxonomic levels and should not be regarded as universal rules. From my personal observations of the data, it appeared that as the taxonomic groupings became broader, from species to clade, the differences in root foraging behaviour grew more pronounced. Although the results of this particular study were not determined to be statistically significant, the pattern suggests that phylogenetic signals for root foraging behaviour exist at broader levels of species relatedness beyond the species or genus level. Furthermore, studies have found that eudicots generally have higher foraging precision than monocots, but that this is not an all-encompassing rule (Grime & Mackey, 2002; Kembel & Cahill, 2005). This study confirms findings in Grime and Mackay (2002) and Kembell and Cahill (2005) that not all eudicot species display high root foraging precision. However, there is evidence that some species that initially display an agnostic response to nutrients when grown alone can adjust this pattern in response to other stimuli, such as the presence of neighbouring plants (Cahill et al., 2010). This in no way means that the broader phylogenetic pattern is incorrect. Instead, it supports the need for preliminary testing of a species root foraging precision before use in experiments regarding root foraging behaviour. Researchers cannot simply use a eudicot species and assume it will forage precisely.

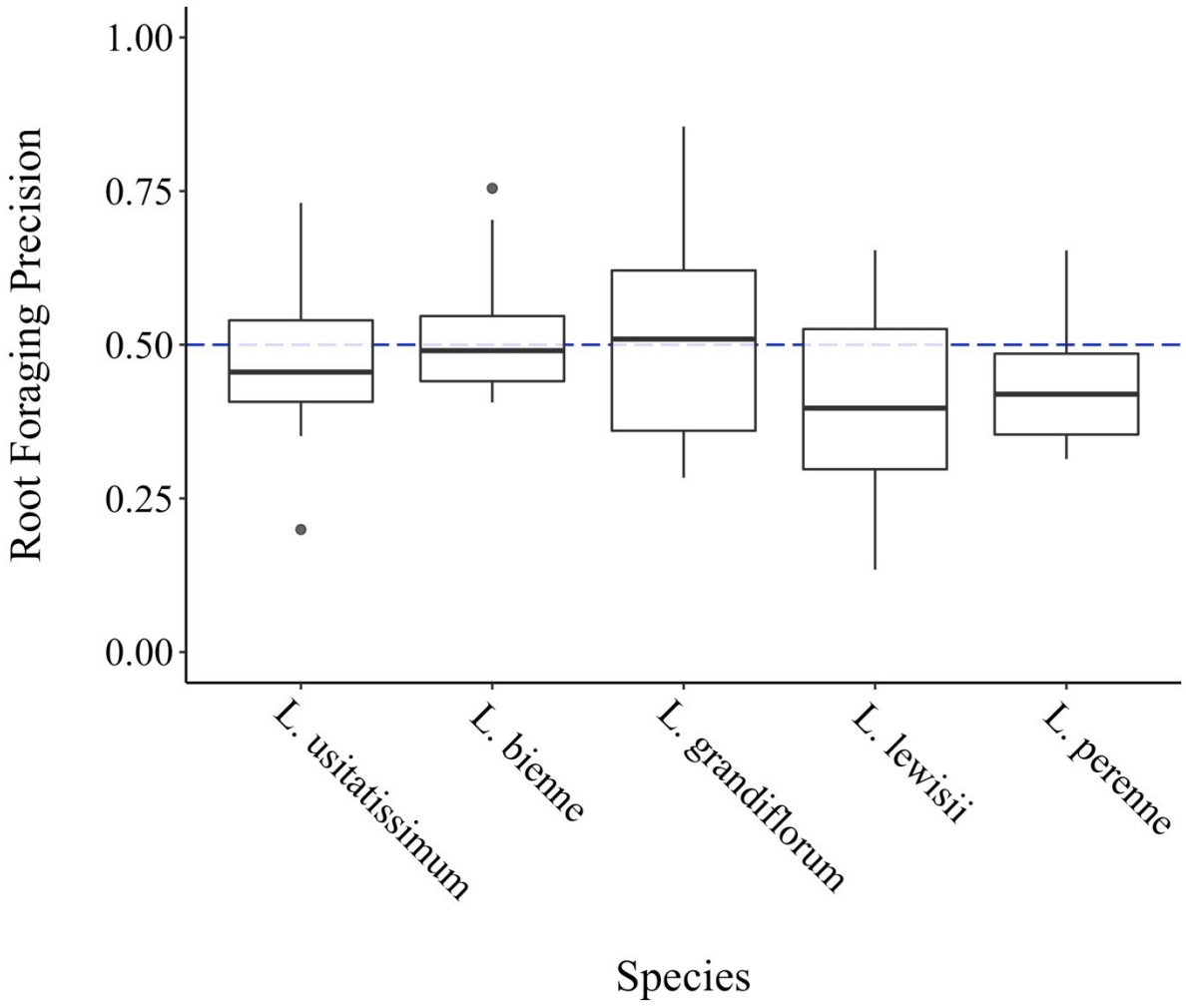
Lastly, the *Linum* genus may not be well-suited to plant behavioural study into root foraging behaviour due to its agnostic response to nutrient patches within the soil. While an agnostic response does not necessarily mean a lack of behaviour, as it can be and is a behavioural type associated with root foraging in plants (Cahill et al., 2010; Cahill & McNickle, 2011). It

poses challenges when evaluating changes or variations in root foraging precision between species or in response to other stimuli. If an agnostic response remains unchanged despite the introduction of a particular stimuli, is it because the agnostic response is still the optimal strategy from the plant's perspective, or is it because the plant is unable to behave anyway otherwise? In other studies investigating root foraging behaviour, study organisms that demonstrate root foraging precision are typically used because they can be used as a baseline to determine if the plants are growing correctly and treatments have been adequately applied (Karst et al., 2012; Ljubotina & Cahill, 2019). An immediate example would be this present thesis, in which common sunflower (*Helianthus annuus* L.) was utilized precisely for that purpose.

APPENDIX 1: Figures and Tables

Appendix Table 1. Five one-sample t-tests comparing biomass-based root foraging precision values of five different flax species to an agnostic value of 0.5. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*.

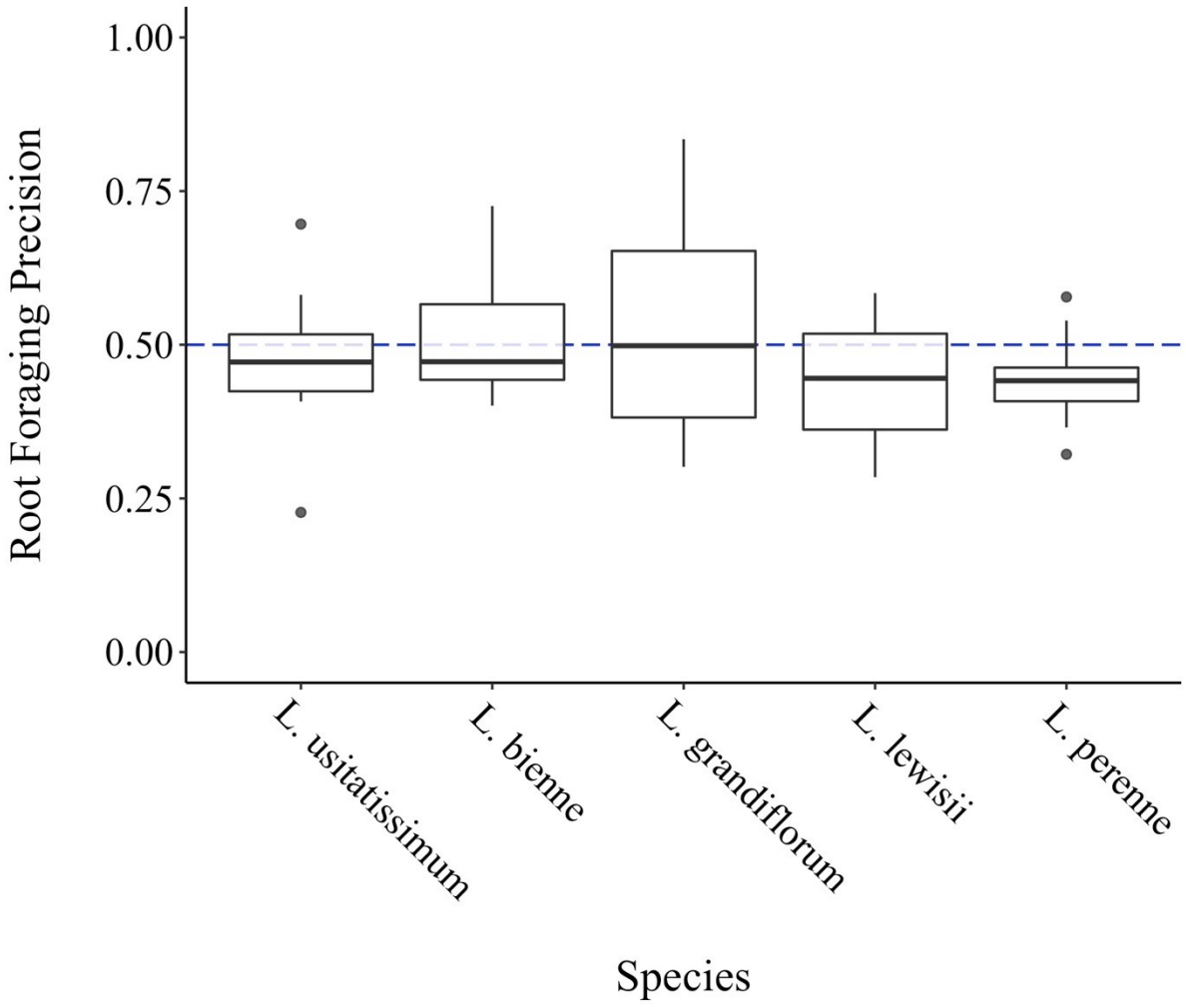
		root foraging precision: biomass		
species	expected mean value	df	t-value	p-value
Linum usitatissimum	0.5	9	-0.51	0.62
Linum bienne	0.5	9	0.66	0.53
Linum grandiforum	0.5	9	0.19	0.86
Linum lewisii	0.5	7	-1.42	0.20
Linum perenne	0.5	9	1.95	0.083*



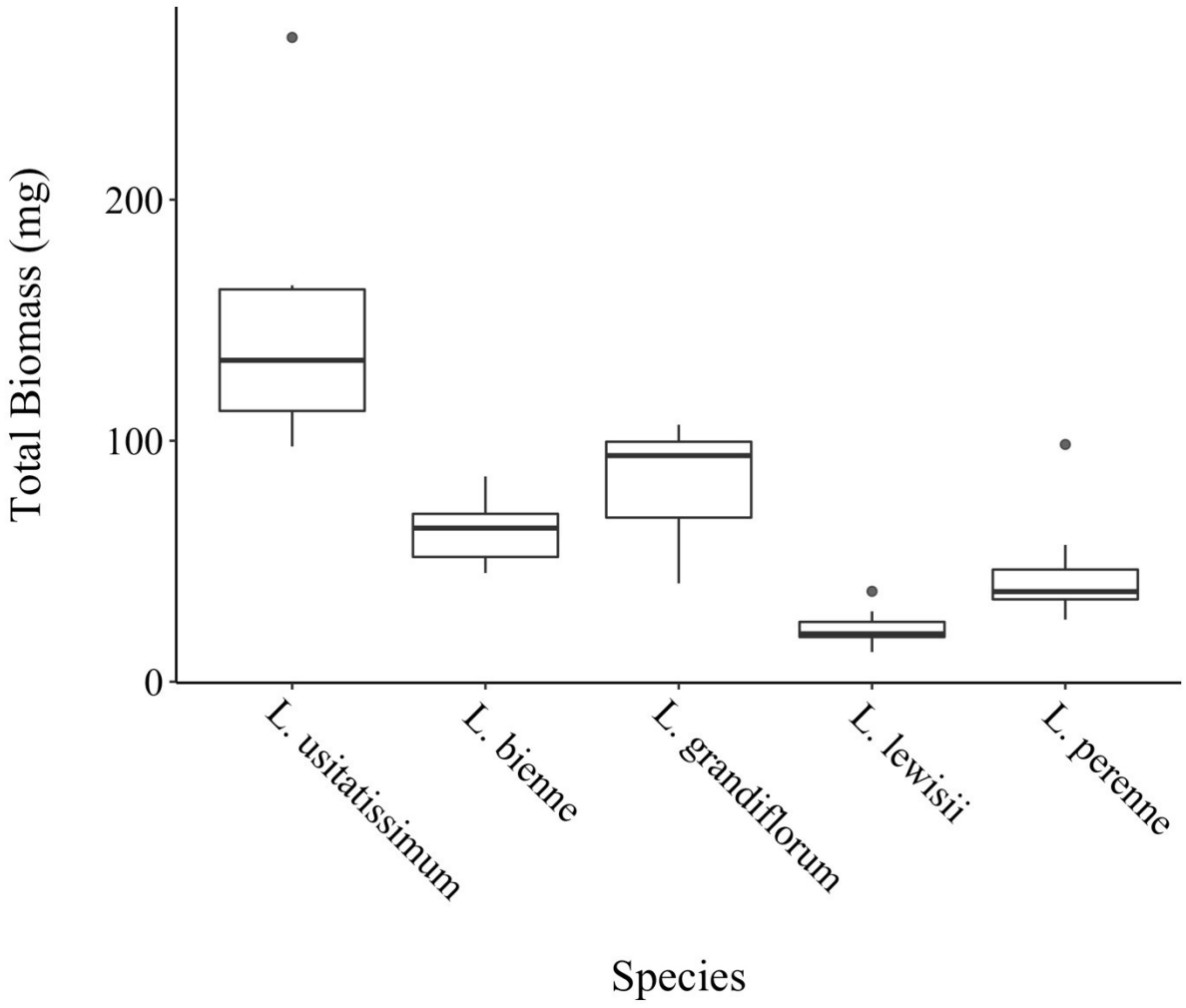
Appendix Figure 1. Root foraging precision by flax species, calculated using biomass. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*.

Appendix Table 2. Five one-sample t-tests comparing root length-based root foraging precision values of five different flax species to an agnostic value of 0.5. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*.

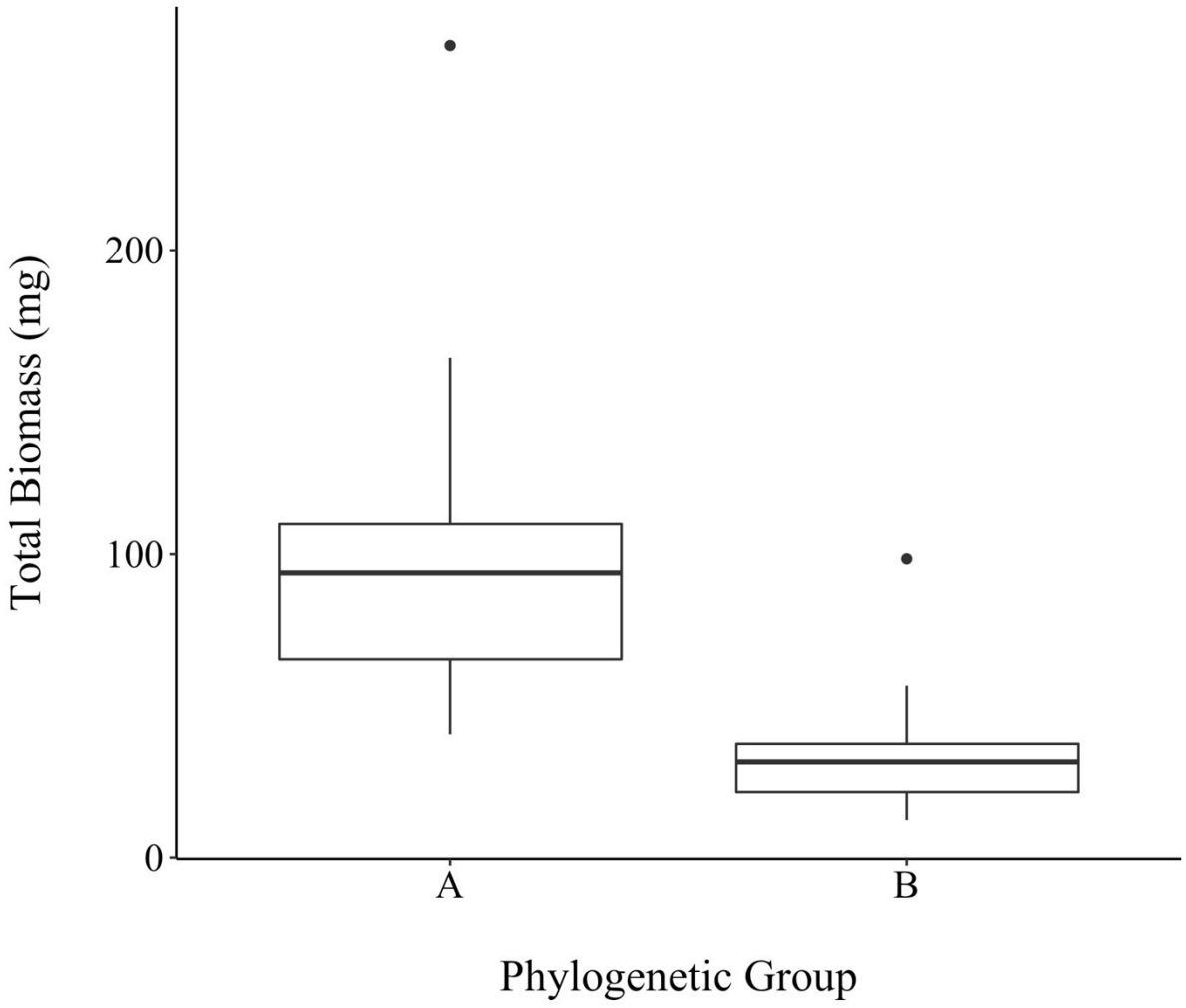
		root foraging precision: root length		
species	expected mean value	df	t-value	p-value
Linum usitatissimum	0.5	9	-0.69	0.51
Linum bienne	0.5	9	0.57	0.58
Linum grandiforum	0.5	8	0.30	0.77
Linum lewisii	0.5	6	-1.49	0.19
Linum perenne	0.5	9	-2.39	**0.040**



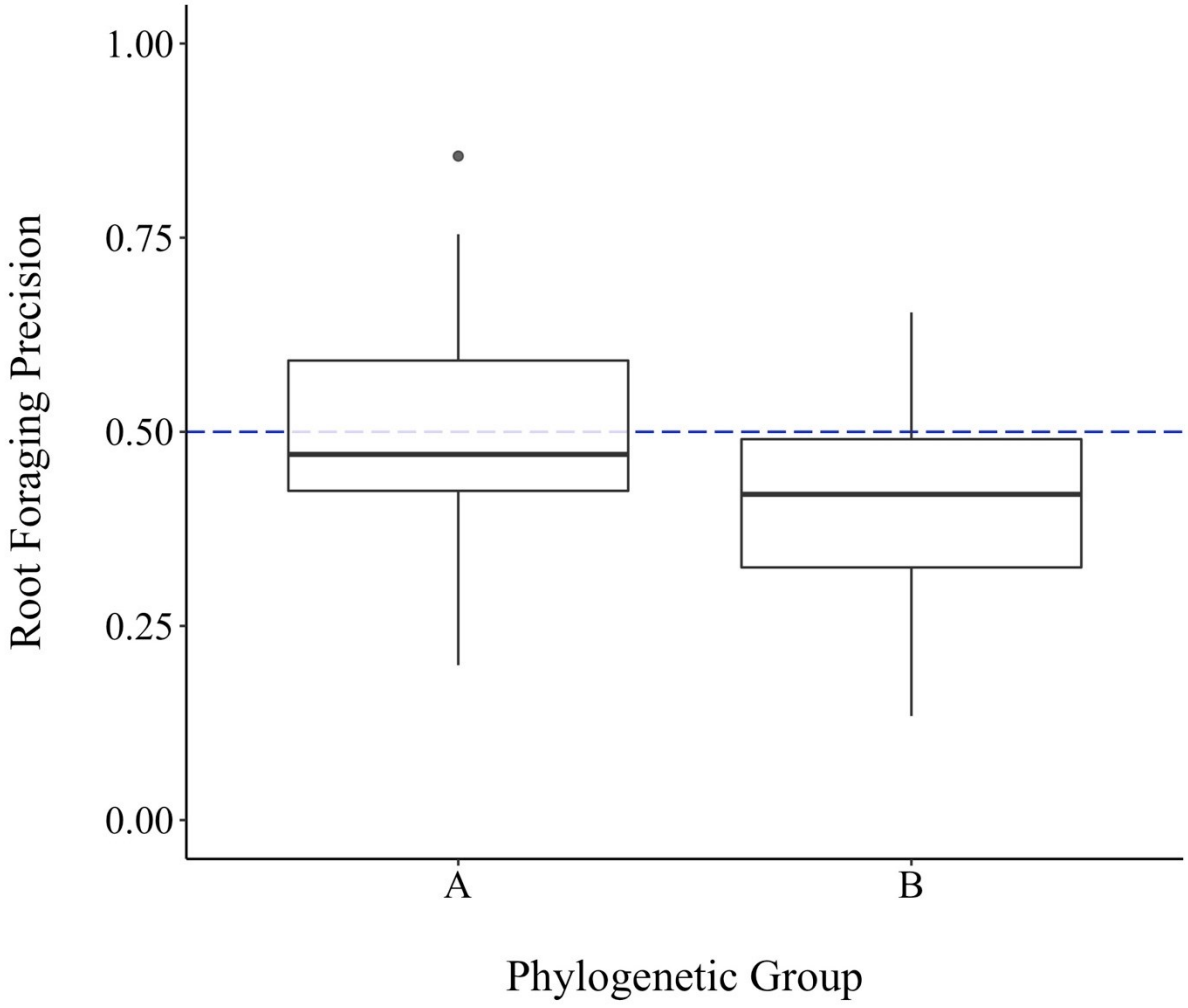
Appendix Figure 2. Root foraging precision by flax species, calculated using root length. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*. The box portion represents the 25th and 75th quartile, with the median represented by the middle black line. The whiskers represent 1.5 times the inter-quartile range either above the 75th quartile (top whisker), or below the 25th quartile (bottom whisker).



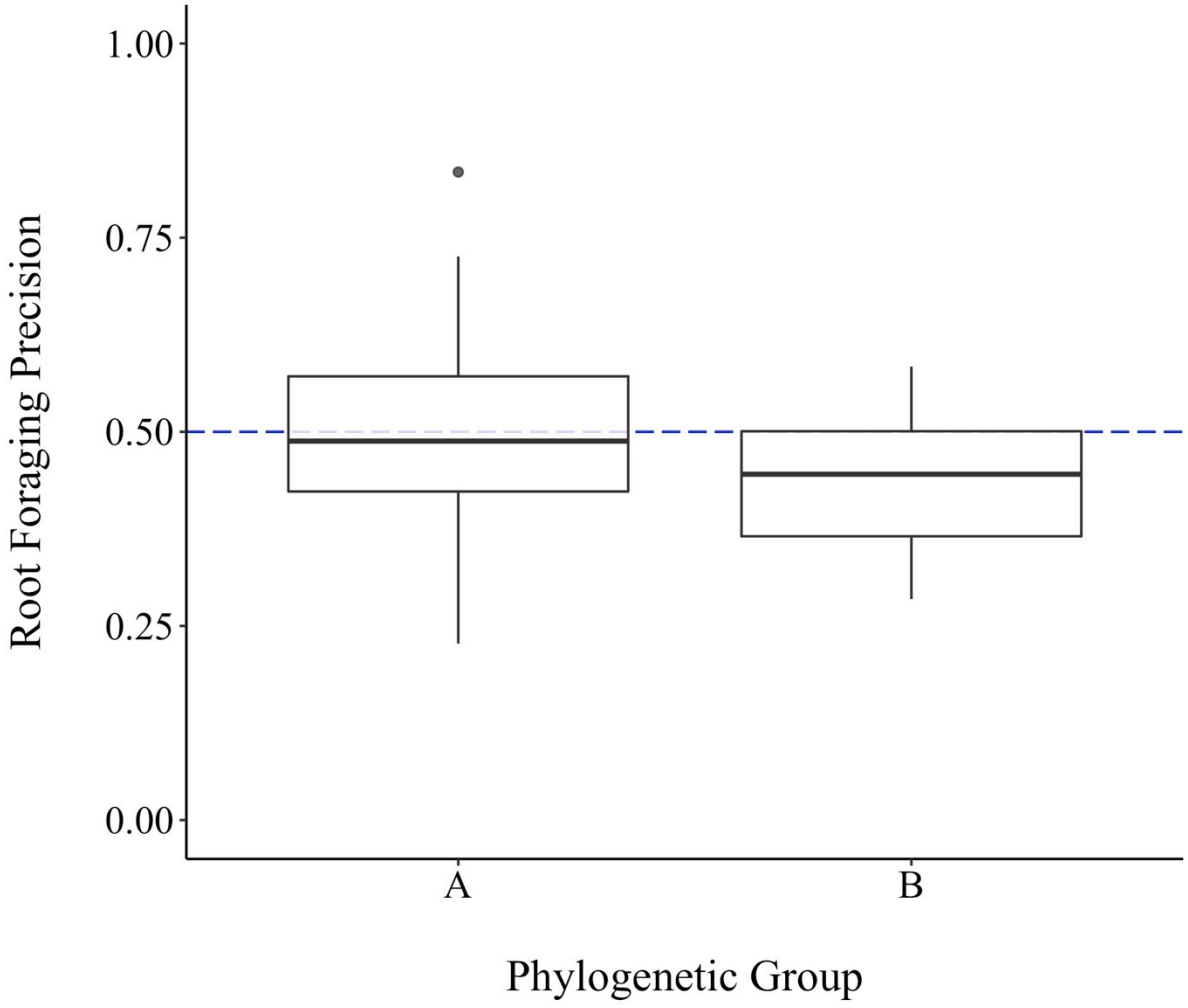
Appendix Figure 3. Total dried biomass (mg) by flax species. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*. The box portion represents the 25th and 75th quartile, with the median represented by the middle black line. The whiskers represent 1.5 times the inter-quartile range either above the 75th quartile (top whisker), or below the 25th quartile (bottom whisker).



Appendix Figure 4. Total dried biomass (mg) by flax phylogenetic group. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*. "Phylogenetic Group A" included *Linum usitatissimum*, *Linum grandiflorum*, and *Linum bienne*. "Phylogenetic Group B" included *Linum perenne* and *Linum lewisii*. The box portion represents the 25th and 75th quartile, with the median represented by the middle black line. The whiskers represent 1.5 times the inter-quartile range either above the 75th quartile (top whisker), or below the 25th quartile (bottom whisker).



Appendix Figure 5. Root foraging precision by flax phylogenetic group, calculated using biomass. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*. "Phylogenetic Group A" included *Linum usitatissimum*, *Linum grandiflorum*, and *Linum bienne*. "Phylogenetic Group B" included *Linum perenne* and *Linum lewisii*. The box portion represents the 25th and 75th quartile, with the median represented by the middle black line. The whiskers represent 1.5 times the inter-quartile range either above the 75th quartile (top whisker), or below the 25th quartile (bottom whisker).



Appendix Figure 6. Root foraging precision by flax phylogenetic group, calculated using root length. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*. "Phylogenetic Group A" included *Linum usitatissimum*, *Linum grandiflorum*, and *Linum bienne*. "Phylogenetic Group B" included *Linum perenne* and *Linum lewisii*. The box portion represents the 25th and 75th quartile, with the median represented by the middle black line. The whiskers represent 1.5 times the inter-quartile range either above the 75th quartile (top whisker), or below the 25th quartile (bottom whisker).

Appendix Table 3. Two linear models analyzing root foraging precision as a function of flax species, using either biomass- or root length-based precision measures. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*.

		root foraging precision: species level		
measure	source	df	F-value	p-value
biomass	species	4,43	0.99	0.42
root length	species	4,41	0.91	0.46

Appendix Table 4. Two linear mixed models analyzing root foraging precision as a function of flax phylogenetic group, using either biomass- or root length-based precision measures. Species was included as a random factor. Species utilized include *Linum usitatissimum* (cultivar "Bethune"), *Linum bienne*, *Linum grandiflorum*, *Linum lewisii*, and *Linum perenne*. "Phylogenetic Group A" included *Linum usitatissimum*, *Linum grandiflorum*, and *Linum bienne*. "Phylogenetic Group B" included *Linum perenne* and *Linum lewisii*.

		root foraging precision: clade level		
measure	source	df	F-value	p-value
biomass	phylogenetic group	1,3.13	3.35	0.16
root length	phylogenetic group	1,3.06	2.80	0.19

APPENDIX 1: Bibliography

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