Behaviour of *Helianthus annuus* L.: an ethogram for sunflower, and the effect of potential competitors on soil nutrient patch use

by

Megan Kathryn Ljubotina

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Ecology

Department of Biological Sciences
University of Alberta

Abstract

Individual organisms mediate their relationship with their environment through behaviour, which can have consequences at higher levels of biological organization such as populations and communities. Plants express diverse behaviours in response to their environments, but many aspects of behaviour remain unexplored in plants. In this thesis, I address behavioural questions about plants using common sunflower, *Helianthus annuus* L.

First, I present an ethogram for *H. annuus*, a catalogue of behaviours observed in the species, here drawn from the literature. The intent of this ethogram was to provide a behavioural perspective on the biology of a widespread and well-known plant, showing that plants in general take in and respond to information about their environments in diverse ways.

Second, using an experimental approach, I test how root foraging behaviour in *H. annuus* is impacted by the location of high nutrient soil patches relative to competitors. For plants, patchiness of soil resources is typical, with high nutrient patches in a matrix of relatively low nutrient soil. Plants commonly respond to high nutrient patches by proliferating roots within them, thereby preferentially increasing investment in and use of these patches. Although patch use is often contingent on social aspects of the environment in other taxa, we understand little about how patch use in plants may be altered by competitors. Here, I measured temporal dynamics of patch use by sunflowers grown alone with sole access to a high quality patch, by sunflowers sharing a high quality patch with an equidistant neighbouring plant, and by sunflowers located in closer proximity to a high quality patch relative to a neighbouring plant. I also compared patch use by sunflowers grown alone with two high quality patches to use by sunflowers grown with two high quality patches and a neighbour, so that one patch was equidistant to a neighbour and the other was in close relative proximity to the focal plant. As expected, sunflowers grown alone preferentially used (grew more roots in) high nutrient patches than lower nutrient background soil. Patch use changed depending on the specifics of the social environment: plants that shared equal access to a single high quality patch no longer preferentially used the patch, but plants in closer proximity to a high quality patch than a neighbour increased use relative to plants grown alone. We suggest that these responses may allow sunflowers to avoid competition in highly contested patches equidistant to neighbours, but to also pre-empt resources in high quality patches that they are closer to and therefore typically have first access to. By contrast, plants with access to two patches decreased use of shared patches but did not increase use of high quality patches they were in closer proximity to. This result is consistent with general predictions about competitive behaviour being less important in less resource-limited environments. These results show that at least for sunflower, root foraging responses are socially contingent, and plants may account for the possibility of monopolizing limited resources in the near future to inform foraging decisions.

Preface

Chapter 2 of this thesis is an invited contribution to a forthcoming book, *The Mind of Plants*, edited by John Ryan, Monica Gagliano, and Patricia Vieira. *The Mind of Plants* will be a collection of essays aimed at a general, non-specialist audience and written in an accessible style with minimal references. I wrote the initial manuscript, and J.F. Cahill Jr. helped with concept development and writing.

Chapter 3 of this thesis has been submitted as "Effects of competitor presence and distance from patch on nutrient patch use by common sunflower", by M.K. Ljubotina and J.F. Cahill Jr., to *Proceedings of the Royal Society B.* I collected and analyzed the data and wrote the initial manuscript. J.F.C. and I conceived of and designed the experiment, and J.F.C. helped write the manuscript.

Acknowledgements

I would like to thank my supervisor James F. Cahill Jr. for his support and generosity during my time as a student in his lab. His dedication to and excellence in his scientific pursuits have inspired me since my second year of university, when I was lucky enough to take an introductory ecology class with him.

I would also like to thank Kimberley J. Mathot for being part of my supervisory committee and providing helpful feedback throughout my program.

Thank you very much to my mentor and friend Tan Bao for his endless help and support throughout my degree.

Thank you to all my wonderful colleagues in the lab, including Gisela Stotz, Greg Pec, Margarete Dettlaff, Charlotte Brown, Amgaa Batbaatar, Jessica Grenke, Karina Salimbayeva, Habba Mahal, Yilei Fan, and Alex Filazzola. Thank you to the undergraduate students I worked directly with during my program: Tianna Barber-Cross and Emily Holden.

Thank you as well to the instructors I had a chance to be a teaching assistant to and learn from: Melissa Roach, Richard Moses, and Gerry Hilchie.

Finally, thank you to my family, including my mom Glenda Macleod, dad Steve Ljubotina, and brothers Sean Ljubotina and Dylan Macleod, as well as family friends Claire and Colin Gallant for their support throughout my program.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

The study of behaviour allows us to understand how organisms respond to their complex and changing environments – what organisms *do*, in the course of their lives (Trewavas 2014). Concepts drawn from the rich literature of behavioral ecology can help us to better understand what plants do in the course of their lives in response to their dynamic environments (Silvertown and Gordon 1989). In this thesis, I address the problem of understanding behaviour in plants using two different broad approaches. Chapter 2 is an essay on sunflower behaviour aimed at a general audience and framed around an ethogram, a classic tool for understanding behaviour. In Chapter 3, I present an experiment testing fundamental questions about the effect of the social environment (presence and location of conspecifics) on high nutrient patch use by plants, using sunflower as a study organism and drawing from theory on patch use in plants and other taxa.

Chapter 2

Plants express choice and respond to their environments in diverse ways that are often best described using concepts drawn from behavioural ecology, such as foraging (Hutchings and de Kroon 1994, Cahill and McNickle 2011), habitat selection (Bazzaz 1991) and mate choice (Lankinen and Karlsson Green 2015). A starting point for behavioural research for a study species is the development of an ethogram, a catalogue of behaviours observed in a given species (Martin and Bateson 2007). These lists of behaviours are important because they can delimit and describe behaviours in a way that makes observation more tractable and coordinate research done by diverse working groups. Ethograms have been produced for a wide variety of taxa, but despite the increasing popularity of behavioural approaches to plant biology, to my knowledge, an ethogram has never been produced for any species of plants. In Chapter 2, I present the first ethogram written for a plant species, *Helianthus annuus* L., common sunflower, in an accessible style, to demonstrate how plants can take in and respond to information about their environments.

Chapter 3

All organisms must acquire resources from their environments. For plants, the belowground resource environment is heterogeneous, often on the scale of a single plant root system, with rich high nutrient patches (e.g. from decomposing animals, or animal waste products) in a matrix of lower nutrient soil (Jackson and Caldwell 1993). Nutrients such as nitrogen can vary by as much as an order of magnitude over the area of the plant root system (Jackson and Caldwell 1993). Plants behaviours to exploit this heterogeneity include changing the morphology of the root system (Hodge 2004) and increasing the rate of nutrient uptake in fertile microsites (Jackson et al. 1990). One tactic for belowground foraging in plants is to increase lateral root proliferation in high nutrient patches, often dramatically (Drew 1975, Jackson and Caldwell 1989, Hodge 2004). This increases the density of roots in areas of the soil with relatively higher nutrient availability.

Plants can use information about other individuals in their environments to make foraging decisions above- and belowground. For example, Gruntman et al. 2017 showed that plants can assess characteristics such as height and density of aboveground competitors and choose between distinct strategies for responding to competition (shade avoidance, shade tolerance, or escape) based on this information. In another experiment, pea plants increased overall root growth in response to the presence of neighbours to pre-empt resources in a tragedy-of-the-commons game

(Gersani et al. 2001). Avoidance of other plants belowground by spatial root segregation is also common (Schenk et al. 1999, Belter and Cahill 2015). Certainly, nutrient depletion by neighbours may result in avoidance belowground due to nutritive cues (i.e. plants place fewer roots near neighbours because there are fewer nutrients near neighbours), but there is also evidence that these responses are sometimes due to cues such as chemical exudates released by plants or physical contact between roots (Mahall and Callaway 1991, 1992). Belowground responses to neighbours by plants has also been shown to be context dependent. For example, some plants are more likely to avoid belowground competition with neighbours that they are closely related to, a response potentially driven by kin selection (Dudley and File 2007), since cooperating with closely related individuals can result in higher inclusive fitness for the cooperator. Additionally, plants can integrate information about both neighbour presence and differing distributions of nutrients (Cahill and McNickle 2011, Mommer et al. 2012), resulting in strategies that do not resemble the response to either differing nutrient distributions or neighbours alone.

How are decisions about investment in high nutrient patches affected by the social environment? Increased root growth in high quality patches can give plants a competitive advantage over other plants because it can allow them to pre-empt resources there (Robinson et al. 1999), but can also be costly because it requires the investment of carbon and energy (Robinson 1996), and patches may be ephemeral. Because plants are sessile, the location of high-resource patches relative to neighbouring plants could impact the dynamics of competition for resources in these patches. If competition in these patches changes in a predictable way based on their proximity to neighbours, the foraging decisions of plants in response to these patches may also change. Certainly, social aspects of the environment and resource distributions can alter resource use in other organisms (Monaghan and Metcalfe 1985, Newman and Caraco 1987).

Therefore, we use an experimental approach to test a series of questions about the use of high quality patches by sunflowers depending on the social environment:

1) How do plants change patch use (amount of root invested) when a high quality patch is shared with a neighbour?
2) Is the impact of a neighbour on patch use contingent on the spatial relationship between patch and neighbour (i.e. plants with a neighbour equidistant to patch vs. plants in closer proximity to patch relative to neighbour)?
3) If plants have access to two patches, one equidistant to a neighbour and one that is closer in proximity to a focal plant, how does it use the two patches?

Literature cited

Bazzaz, F. A. 1991. Habitat selection in plants. The American Naturalist 137:S116–S130.

Belter, P. R., and J. F. Cahill. 2015. Disentangling root system responses to neighbours: identification of novel root behavioural strategies. AoB Plants 7:plv059.

Cahill, J. F., and G. G. McNickle. 2011. The behavioral ecology of nutrient foraging by plants. Annual Review of Ecology, Evolution, and Systematics 42:289–311.

Drew, M. C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. The New Phytologist 75:479–490.

Dudley, S. A., and A. L. File. 2007. Kin recognition in an annual plant. Biology Letters 3:435–438.

Gersani, M., J. S. Brown, E. E. O’Brien, G. M. Maina, and Z. Abramsky. 2001. Tragedy of the commons as a result of root competition. Journal of Ecology 89:660–669.

Gruntman, M., D. Groß, M. Májeková, and K. Tielbörger. 2017. Decision-making in plants under competition. Nature Communications 8:2235.

Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytologist 162:9–24.

Hutchings, M. J., and H. de Kroon. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Pages 159–238 *in* M. B. and A. H. Fitter, editor. Advances in Ecological Research. Academic Press.

Jackson, R. B., and M. M. Caldwell. 1989. The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. Oecologia 81:149–153.

Jackson, R. B., and M. M. Caldwell. 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology 74:612–614.

Jackson, R. B., J. H. Manwaring, and M. M. Caldwell. 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature 344:58.

Lankinen, Å., and K. Karlsson Green. 2015. Using theories of sexual selection and sexual conflict to improve our understanding of plant ecology and evolution. AoB PLANTS 7.

Mahall, B. E., and R. M. Callaway. 1991. Root communication among desert shrubs. Proceedings of the National Academy of Sciences 88:874–876.

Mahall, B. E., and R. M. Callaway. 1992. Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. Ecology 73:2145–2151.

Martin, P., and P. Bateson. 2007. Measuring Behaviour: An Introductory Guide. Third edition. Cambridge University Press, New York.

Mommer, L., J. van Ruijven, C. Jansen, H. M. van de Steeg, and H. de Kroon. 2012. Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? Functional Ecology 26:66–73.

Monaghan, P., and N. B. Metcalfe. 1985. Group foraging in wild brown hares: effects of resource distribution and social status. Animal Behaviour 33:993–999.

Newman, J. A., and T. Caraco. 1987. Foraging, predation hazard and patch use in grey squirrels. Animal Behaviour 35:1804–1813.

Robinson, D. 1996. Resource capture by localized root proliferation: why do plants bother? Annals of Botany 77:179–186.

Robinson, D., A. Hodge, B. S. Griffiths, and A. H. Fitter. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proceedings of the Royal Society B: Biological Sciences 266:431–435.

Schenk, H. J., R. M. Callaway, and B. E. Mahall. 1999. Spatial root segregation: are plants territorial? Pages 145–180 *in* A. H. F. and D. Raffaelli, editor. Advances in Ecological Research. Academic Press.

Silvertown, J., and D. M. Gordon. 1989. A framework for plant behavior. Annual Review of Ecology and Systematics 20:349–366.

Trewavas, A. 2014. Plant Behaviour and Intelligence. Oxford University Press.

Chapter 2: Applying a behavioural approach to understanding plants: an ethogram for common sunflower

Introduction

What is a sunflower? The question may seem strange, since sunflower is one of those plants probably instantly recognizable to much of humankind. But as commonplace as this plant is, there is much that we don't understand about it. In this essay, we argue for the importance of a behavioural approach in addition to more traditional approaches to describing common sunflower, *Helianthus annuus* L. We then present an ethogram (catalogue of behaviours) for common sunflower, in keeping with traditions in behavioural ecology.

Traditionally, our botanical understanding of plant species has been rooted in descriptions of their use to humans, evolutionary relationships with other organisms, physical traits and life histories. For example, one answer to the question of what a sunflower is might describe its significance to human beings. Sunflower is an important crop plant, harvested for its seeds and the oil produced from them (Stefansson 2013) and it is also grown for ornamental purposes. As such, it is an economically important plant. It has a long history of cultivation and domestication by human beings, stretching back to at least 2600 B.C. in Mexico (Lentz et al. 2008). Sunflowers, images of sunflowers, and reproductions of sunflowers are certainly common in art and pop culture, often representing hope, happiness, or nature. Sunflowers are important to humanity, and have left an impression on us for millennia, but can we describe them in terms of their lives, independently of our own?

Another approach to describing what a sunflower is might be to describe its place in the tree of life. Like us, sunflowers are eukaryotes, distinguished from non-eukaryotes like bacteria by the membrane-enclosed nuclei in our cells. They are members of the kingdom Plantae, the plants. Within that kingdom, they are part of a group called angiosperms: plants that produce flowers. And within the angiosperms, they are part of a large family of plants called Asteraceae, which are united by the typical arrangement of many flowers into dense, flower-like heads (other members of this family include dandelions and daisies). They are members of the genus *Helianthus*, and the scientific name that distinguishes them from all other species is *Helianthus annuus* L. This description helps us to understand common sunflower's evolutionary relationships with other organisms and some of the broad characteristics that distinguish it but is lacking some detail for understanding what it is really like, and how it lives its life.

A typical description of the species might read as follows: common sunflower is an annual plant, meaning that it completes its life cycle within a single growing season. It is herbaceous (producing no wood), with an upright stem and coarsely toothed, alternate leaves; belowground it possesses a central tap root and branching lateral roots. Sunflower leaves and stems usually possess trichomes, hair-like structures thought to be important in defense from herbivores. When it reaches reproductive maturity, its flowering head resembles a single flower, but is actually composed of many small flowers, called florets. Many individual flowers called disc florets make up the central "button" of the flower, while each "petal" is an individual flower called a ray floret. Each of the reproductive disc flowers is composed of five fused petals, five fused anthers (the male reproductive parts of the plant), and a single pistil (the female reproductive part of the plant). The individual flowers are hermaphroditic and typically protandrous, meaning that the flower is functionally male first (pollen exposed), and then female (stigmatic surface, where pollen is deposited, exposed) (Free 1993). When the disc florets are pollinated, they produce seeds surrounded by a kind of dry fruit called an achene (what we typically refer to as the shell of the seed). This description provides a fuller idea of what a sunflower is, and yet it is quite static, describing the plant in terms of its anatomy or unchanging aspects of its life history.

All three approaches (of importance to humans, evolutionary relationship to other organisms, and botanical descriptions focused on anatomy and life history) are important components of understanding sunflower, and all plants. Essential as these descriptions are for understanding plants, they are typically somewhat static, providing a snapshot of the organism as it typically looks, a reflection of its evolutionary past, or its relationship with humans. For any living thing, the environment it is constantly changing and full of challenges: scarce resources, competition, and attack by predators or herbivores, among other things. What do sunflowers *do* in the course of their lives: how do they respond to these complex and changing environments? In other words, how do sunflowers behave?

Although biologists do not all agree on what constitutes behaviour or whether plants behave, here we use the definition of behaviour given by Jonathan Silvertown (a plant biologist) and Deborah M. Gordon (an animal biologist) in their paper “A Framework For Plant Behaviour”: “[W]hat a plant or animal does, in the course of an individual's lifetime, in response to some event or change in its environment” (Silvertown and Gordon 1989). This definition is fairly similar to one based on the survey responses of 174 working behavioural biologists: “[T]he internally coordinated responses (actions or inactions) of whole living organisms (individuals or groups) to internal and/or external stimuli, excluding responses more easily understood as developmental changes” (Levitis et al. 2009).

Of course, sunflowers demonstrate a conspicuous and famous behaviour that has been noted by humans for centuries (Kutschera and Briggs 2016): the young flowering heads follow the sun through the day, exhibiting heliotropism. Heliotropism in sunflowers may stand out to humans because it reminds us of how we mediate our relationship with the environment, largely through rapid and conspicuous movements. Modern research has demonstrated that the behaviour also serves an intuitive function, allowing the plant more efficiently gather food in the form of energy from the sun (Atamian et al. 2016). This example notwithstanding, sunflowers and other plants often respond to their environments in ways that are far more difficult for us to perceive or understand. However, the fundamental problems that sunflowers address by responding actively to their environments, like finding food, defending themselves from enemies, and reproducing successfully, can be strikingly similar to the challenges that animals like ourselves must manage in their lives. Therefore, understanding these responses in plants can be fundamental not just to understanding plants but also to understanding commonalities in how living things in general deal with the challenges inherent to their environments.

If we are interested in describing sunflower behaviour, how might it be done? One approach that has been utilized by behavioural biologists for decades is the ethogram. An ethogram is simply a dictionary or catalogue of behaviours observed in a given species. It can provide an important starting point for behaviourists attempting to quantify and record behaviour in a species (e.g. Greenberg 1977), or it can be a synthesis drawn from prior research on an important species (e.g. Kalueff et al. 2013). Although behavioural approaches to understanding plants are increasingly prevalent, to our knowledge nobody has ever produced an ethogram for a plant species. By presenting an ethogram for common sunflower, we are interested in describing what sunflower does in response to its environment, and how these responses help the plant to deal with the environment, and so our descriptions focus on these aspects of behaviour.

Ethograms in animals are often created by a single observer recording behaviours of individuals of a species. Of course, many animal behaviours can easily be observed by humans watching the animal as it moves. Plants are often less showy in their behaviours: when they move, they move more slowly; they do many things microscopically; and half the plant is hidden belowground in the soil. Therefore, we draw many examples from the literature, focusing our search on examples of behaviour observed in plants in general.

Ethogram of *Helianthus annuus* L.

Attract parasites of herbivores: Following attack by herbivore, produce compounds that attract parasites of herbivores to plant, therefore defending self against herbivore (Dias et al. 2016).

Control stomata: Close stomata (which mediate gas and water exchange in the leaves) in response to soil dryness i.e. anticipated drought; sunflowers can close stomata even when leaf water status is kept constant, suggesting a signal from roots to stomata regulates the response (Gollan et al. 1986).

Curve stigmatic lobes down: Move stigmatic lobes (the receptive female part of the flower) downward so that they touch the style, where the lobes can pick up pollen from the same floret that has been deposited there. Seen in florets where pollination by another individual has not yet occurred and may be a "last resort" strategy when a suitable non-self mate has not been encountered (Free 1993).

Decrease reproductive allocation: Decrease relative allocation to reproduction (offspring), and number of seeds produced (analogous to clutch size in animals) under lower resource or higher density conditions (Kawano and Nagai 1986).

Defend, chemically: Respond to perceived herbivory by increasing or initiating production or secretion of chemical defenses that deter further attack (Roseland and Grosz 1997). This is referred to as “induced defense”, which means that the sunflower does not always express this defensive trait. This allows sunflowers to avoid spending excessive energy on defense when it is not necessary, but to quickly and reversibly respond to attack when it occurs.

Emerge: Germinate and transition from seed to seedling; in sunflowers, the timing of emergence is controlled by genetic and environmental factors, and can influence fitness (Mercer et al. 2011). Dormancy is an alternate state where seeds stay alive but do not germinate, often due to unsuitable growing conditions. Sunflowers can “choose” when and where they live as plants to some extent by remaining dormant.

Explore soil: Extend roots into new area of the soil, vertically or horizontally. Can be contingent on the social environment: sunflowers more quickly explore deeper layers of the soil when grown in higher densities (Sadras et al. 1989).

Heliotropic behaviour: Bend shoot toward the sun throughout the day, a behaviour mediated by both external and internal signals. (Schaffner 1898). Observed in young sunflowers, but not reproductively mature individuals. As well, young sunflowers reorient shoots toward the east at dawn, anticipatory behaviour that allows them to face the direction of the sun when it rises. This behaviour allows plants to be more efficient in harvesting light energy from the sun (Atamian et al. 2016). In concert with the movement of the stem, plants may also bend individual leaves to increase surface area exposed to sun (Kutschera and Briggs 2016).

Incline stems: Incline stem in the opposite direction of nearby neighbour to avoid light competition. This behaviour only occurs at high densities; populations of sunflowers can self-organize by inclining stems in the opposite direction of their nearby neighbours, thereby increasing population-wide and average individual fitness (Pereira et al. 2017). Population-level organization (alternating angles of inclination of stems) can therefore arise from local interactions between individuals.

Increase allelopathy: Increase the production of chemical compounds that inhibit germination of other plants (allelochemicals; the effect of allelochemicals is called allelopathy) when stressed for nutrients (Hall et al. 1982). This has been suggested to be the increased expression of a territorial behaviour under more limited resource conditions (Schenk et al. 1999).

Increase anchorage locally: Produce locally thicker and stiffer roots in response to unidirectional stem flexure (like that stimulated by wind). This results in greater mechanical support in the direction of the wind (Goodman and Ennos 1998), and better anchorage when pulled over.

Iron foraging: Produce specialized transfer cells at tips of roots that increase uptake of iron (Kramer et al. 1980) while also releasing protons that enable greater uptake rates of iron (Romheld et al. 1984). A response to iron deficiency; both responses are localized at the root tips, likely to increase efficiency of responses acting together.

Mating attempt by male: Initiate formation of pollen tube and growth into style (Gotelli et al. 2010); stimulated by landing on stigma.

Nyctinasty: Vertically reorient leaves downward at night (Schaffner 1898); function of this behaviour is unclear (Minorsky 2019), but common and conspicuous across plant species. Proposed functional explanations include ridding the leaves of excess water, avoiding frost damage, appearing less conspicuous to herbivores, or even exposing herbivores to predation from above (Minorsky 2019).

Root forage precisely: Increase frequency of lateral root proliferation behaviour after encountering high nutrient patch in soil (Grime and Mackey 2002); grow more roots where more nutrients are available. This allows the plant to expend more energy and carbon where there are more nutrients available to the root system.

Secrete stigmatic exudate: Upon deposition of pollen, release extracellular exudate from stigmatic surface which functions to hydrate the pollen grain (Elleman et al. 1992).

Shade avoidance: Elongate stems and leaf petioles in response to nearby plants blocking light (Shirley 1929); a strategy used to overtop other plants in competitive interactions by positioning leaves above neighbour interference.

Conclusion

In this ethogram, we have described some of sunflower's behavioural repertoire: how it actively responds to its environment and makes choices based on the information it perceives. Sunflower, like many other plants, demonstrates a variety of behavioural responses to its environment. The known behaviours of this species also represent only a fraction of what a sunflower does in a continuous stream of behaviours throughout its life; there is still much to learn by carefully observing what individual sunflowers do. The reason why it is useful, perhaps even essential, to consider these responses to be behaviour is because the sessile plant often expresses choice through these changes: choice in where it lives, in how it finds food, in how it mates and cares for its offspring. We hope to have demonstrated that approaches to botany that view plants as insensitive or unresponsive cannot allow us to fully understand a ubiquitous species like common sunflower, or any other plant.

Literature cited

Atamian, H. S., N. M. Creux, E. A. Brown, A. G. Garner, B. K. Blackman, and S. L. Harmer. 2016. Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits. Science 353:587–590.

Dias, A. M., M. Pareja, M. Laia, M. C. Blassioli-Moraes, M. Borges, and R. A. Laumann. 2016. Attraction of *Telenomus podisi* to volatiles induced by *Euschistus heros* in three different plant species. Arthropod-Plant Interactions 10:419–428.

Elleman, C. J., V. Franklin-Tong, and H. G. Dickinson. 1992. Pollination in species with dry stigmas: the nature of the early stigmatic response and the pathway taken by pollen tubes. New Phytologist 121:413–424.

Free, J. B. 1993. Insect pollination of crops. 2nd Revised edition. Academic Press, London.

Gollan, T., J. B. Passioura, and R. Munns. 1986. Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. Australian Journal of Plant Physiology 13:459–464.

Goodman, A., and A. R. Ennos. 1998. Responses of the root systems of sunflower and maize to unidirectional stem flexure. Annals of Botany 82:347–357.

Gotelli, M. M., B. G. Galati, and D. Medan. 2010. Structure of the stigma and style in sunflower (*Helianthus annuus* L.). Biocell 34:133–138.

Greenberg, N. 1977. An ethogram of the blue spiny lizard, *Sceloporus cyanogenys* (Reptilia, Lacertilia, Iguanidae). Journal of Herpetology 11:177.

Grime, J. P., and J. M. L. Mackey. 2002. The role of plasticity in resource capture by plants. Evolutionary Ecology 16:299–307.

Hall, A. B., U. Blum, and R. C. Fites. 1982. Stress modification of allelopathy of *Helianthus annuus* L. debris on seed germination. American Journal of Botany 69:776–783.

Kalueff, A. V., M. Gebhardt, A. M. Stewart, J. M. Cachat, M. Brimmer, J. S. Chawla, C. Craddock, E. J. Kyzar, A. Roth, S. Landsman, S. Gaikwad, K. Robinson, E. Baatrup, K. Tierney, A. Shamchuk, W. Norton, N. Miller, T. Nicolson, O. Braubach, C. P. Gilman, J. Pittman, D. B. Rosemberg, R. Gerlai, D. Echevarria, E. Lamb, S. C. F. Neuhauss, W. Weng, L. Bally-Cuif, and H. Schneider. 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish 10:70–86.

Kawano, S., and Y. Nagai. 1986. Regulatory mechanisms of reproductive effort in plants 1. Plasticity in reproductive energy allocation and propagule output of *Helianthus annuus* L. (Compositae) cultivated at varying densities and nitrogen levels. Plant Species Biology 1:1–18.

Kramer, D., V. Römheld, E. Landsberg, and H. Marschner. 1980. Induction of transfer-cell formation by iron deficiency in the root epidermis of *Helianthus annuus* L. Planta 147:335–339.

Kutschera, U., and W. R. Briggs. 2016. Phototropic solar tracking in sunflower plants: an integrative perspective. Annals of Botany 117:1–8.

Lentz, D. L., M. D. Pohl, J. L. Alvarado, S. Tarighat, and R. Bye. 2008. Sunflower (*Helianthus annuus* L.) as a pre-Columbian domesticate in Mexico. Proceedings of the National Academy of Sciences 105:6232–6237.

Levitis, D. A., W. Z. Lidicker, and G. Freund. 2009. Behavioural biologists do not agree on what constitutes behaviour. Animal Behaviour 78:103–110.

Mercer, K. L., H. M. Alexander, and A. A. Snow. 2011. Selection on seedling emergence timing and size in an annual plant, *Helianthus annuus* (common sunflower, Asteraceae). American Journal of Botany 98:975–985.

Minorsky, P. V. 2019. The functions of foliar nyctinasty: a review and hypothesis. Biological Reviews 94:216–229.

Pereira, M. L., V. O. Sadras, W. Batista, J. J. Casal, and A. J. Hall. 2017. Light-mediated self-organization of sunflower stands increases oil yield in the field. Proceedings of the National Academy of Sciences 114:7975–7980.

Romheld, V., C. Muller, and H. Marschner. 1984. Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiology 76:603–606.

Roseland, C. R., and T. J. Grosz. 1997. Induced responses of common annual sunflower *Helianthus annuus* L. from geographically diverse populations and deterrence to feeding by sunflower beetle. Journal of Chemical Ecology 23:517–542.

Sadras, V. O., A. J. Hall, N. Trapani, and F. Vilella. 1989. Dynamics of rooting and root-length: leaf-area relationships as affected by plant population in sunflower crops. Field Crops Research 22:45–57.

Schaffner, J. H. 1898. Observations on the nutation of *Helianthus annuus*. Botanical Gazette 25:395–403.

Schenk, H. J., R. M. Callaway, and B. E. Mahall. 1999. Spatial root segregation: are plants territorial? Pages 145–180 *in* A. H. F. and D. Raffaelli, editor. Advances in Ecological Research. Academic Press.

Shirley, H. L. 1929. The influence of light intensity and light quality upon the growth of plants. American Journal of Botany 16:354–390.

Silvertown, J., and D. M. Gordon. 1989. A framework for plant behavior. Annual Review of Ecology and Systematics 20:349–366.

Stefansson, B. R. 2013, May 4. Oilseed crops. Historica Canada, Toronto.

Chapter 3: Effects of competitor presence and distance from patch on nutrient patch use by common sunflower

Introduction

In their pursuit of resources, plants face environmental challenges traditionally central to foraging theory: resources required by plants such as light and mineral nutrients are often scarce, patchily distributed, and highly contested by other individuals in the environment (Jackson and Caldwell 1993, Gruntman et al. 2017). Plants move through their environments as they grow and discard body parts (Harper 1985), encountering patches of higher and lower resource supply, and they are often very plastic in their growth and uptake kinetics in response (Hutchings and de Kroon 1994, Silvertown 2004, de Kroon et al. 2005, Metlen et al. 2009). This plasticity allows them to actively forage for the resources they need.

Plants forage for soil nutrients via several different mechanisms, including changes in root growth (Hodge 2004, McNickle and Cahill 2009) and physiology (Jackson et al. 1990, Robinson 2002, Metlen et al. 2009). Most seed plants can produce lateral roots at any point along the root axis depending on environmental conditions (Motte and Beeckman 2019). Because natural soils are often patchy in nutrient content (Jackson and Caldwell 1993), one tactic for belowground foraging in many plant species is to produce more roots in high nutrient soil patches than in low nutrient soil (Drew 1975, Jackson and Caldwell 1989, Hodge 2004, Cahill and McNickle 2011). Increasing use of high quality patches by growing more roots there can improve the ability of plants to efficiently capture nutrients from patchy soil (Fransen 1999, Robinson 2002). Biologists have meaningfully applied a behavioural ecological approach to root foraging in plants to better understand both the generality of foraging theory and plant biology. For example, the marginal value theorem is a classic model in foraging theory predicting that organisms should spend longer in high quality patches than low quality patches (Charnov 1976a), which has been used to explain patterns of plant root growth (McNickle and Cahill 2009).

The simplest models of foraging focus on responses to differences in resource supply (Charnov 1976a) or resource type (Charnov 1976b). However, aspects of the environment not directly related to resource supply or quality can also strongly influence foraging decisions. For example, there are inherent trade-offs between avoiding predation risk and foraging efficiently: more energy-starved individuals are likelier to accept more risk of predation while foraging than well-fed ones (Lima et al. 1985, Lima and Dill 1990, Jensen et al. 2011). Aggressive behaviour of conspecifics can also change foraging strategies (Fretwell and Lucas 1969, Schoener 1983, Lima et al. 1985), as in territoriality (Gill and Wolf 1975, Schenk et al. 1999); even the presence of non-aggressive conspecifics can decrease the value assigned to a habitat of a given resource supply by individuals (Fretwell and Lucas 1969). Because the behaviour of other individuals can be so influential, game theoretical models of behaviour that account for the contingencies of behaviours on those of other individuals have become increasingly prominent in behavioural ecology (Sirot 2000, Křivan et al. 2008). Many of these broad ideas in behavioural ecology apply to plants (e.g. foraging efficiency-predation risk trade-offs (Jensen et al. 2011); territoriality (Schenk et al. 1999); the ideal free distribution (Gersani et al. 1998, McNickle and Brown 2014); game theory (Gersani et al. 2001, Dudley and File 2007, O'Brien and Brown 2008)). Like many other organisms, plants can detect neighbouring plants through a variety of cues or signals (Aphalo and Ballare 1995, Karban 2008), and respond in complex ways, but many areas of social dynamics in foraging in plants remain unexplored.

How plant roots respond to high quality soil patches may be especially important in competition for nutrients with other plants, because increased root growth in high quality patches can allow plants to pre-empt resources from neighbours (Robinson et al. 1999, Hodge et al. 1999, Fransen et al. 2001). When it has been investigated, there is evidence that plants alter patch use in response to the social environment. For example, *Abutilon theophrasti* increased exploration of the soil space in response to a high quality patch when grown with another plant (Cahill et al. 2010), but not when grown alone. In studies involving more species, there is evidence that some plants increase their use of low-quality soil in the presence of neighbours, thereby reducing competition in higher quality areas of the soil that are highly contested (Mommer et al. 2012, McNickle et al. 2016). Combined, there is evidence of socially-mediated patch use in plants which may help plants respond to changing competitive dynamics for resources. However, experiments and studies on high nutrient patch use by plants have in large part examined the response in plants grown alone (Grime and Mackey 2002), and very few studies have measured foraging behaviour of individual plants in different social environments. Consequently, many aspects of dynamics of patch use remain unexplored.

The arrangement of resources in the environment can alter the dynamics of competition for those resources, and organisms often alter foraging strategies accordingly. For example, individuals may be more likely to be aggressive toward others when food is clumped in a patch rather than spread out (Monaghan and Metcalfe 1985, Grant and Guha 1993), because it becomes possible to monopolize clumped resources. Because plants are sessile and encounter resources by growing from a central point, closer initial proximity of the plant's body to high quality patches can give plants an advantage in competition (Casper and Cahill 1996, Day et al. 2003), but it not known whether this is mediated by changes in foraging behaviour. Therefore, we tested the effect of proximity to patches relative to potential competitors on plant behaviour by altering the location of high nutrient patches relative to neighbouring plants. If competition in these patches changes in a predictable way based on their spatial relation to neighbours, the foraging behaviours of plants in response to these patches may also change.

Using an experimental approach, we addressed the following questions, with multiple working hypotheses:

1) How do plants change patch use when a high quality patch is equidistant to a neighbour? Since different plant species vary in their behavioural responses to neighbours (Belter and Cahill 2015), we had multiple working hypotheses: sunflowers may increase use of high quality patches to pre-empt resources (Gersani et al. 2001), or they may decrease use of patches to avoid competition (Mommer et al. 2012) or in response to resource depletion (Maina et al. 2002).
2) Is the impact of a neighbour on patch use contingent on the spatial relationship between patch and neighbour? In other words, if a plant is in closer proximity to a high quality patch than another plant, how does its use of the patch compare to those plants grown alone, or with a patch equidistant to a neighbour (patch between plants)? As above, we had multiple working hypotheses: plants may alter their use in a similar fashion regardless of spatial relationship between patch and neighbour; plants may not increase investment as rapidly as plants grown with a patch equidistant to neighbour, since the resources are less fiercely contested (Gersani et al. 2001); or they may increase investment in patches that they are in closer proximity to relative to neighbours since they are more likely to be able to monopolize those resources (Monaghan and Metcalfe 1985).

3) If a plant has access to two patches, one equidistant to a neighbour and one that is closer in proximity to the focal plant, how does it invest in the two patches? We predicted that the dynamics in the two patches should reflect behaviour seen in the above behavioural assays, but that plants may be less competitive when resources are more abundant (Sirot 2000, Grant et al. 2002)
4) Do plants in closer proximity to a high quality patch relative to a neighbouring plant have an advantage reflected in increased biomass or nutrient acquisition when compared to plants that share a patch with an equidistant neighbour? Based on previous experimental work, we predicted that individuals in closer proximity to a high quality patch would have an advantage (Casper and Cahill 1996).

We tested these questions by growing focal plants in experimental arenas that differed in the arrangement of high quality patches of soil and neighbour plants, potential competitors for those resources. To answer our first two questions, we used treatments differing in proximity of high quality patches to focal plants relative to neighbouring plants (sole access/no neighbours, equal proximity, or focal plants in closer proximity). To answer our third question, we used a separate set of treatments where plants had access to two high quality patches and were either grown alone or with one patch in equal proximity to a neighbour and the other in closer proximity to the focal plant. To test whether plants with first access to a patch had an advantage, we measured biomass and nutrient concentration in the tissues of plants at the end of the experiment.

Methods

(a) Species selection

We used common sunflower, *Helianthus annuus* L. for this experiment because prior work indicates that it demonstrates increased use of patches with higher nutrient availability (Grime and Mackey 2002, Kembel and Cahill Jr. 2005) and is amenable to the experimental conditions used here (Bao et al. 2018). Seed was obtained from a local supplier (Apache Seeds Ltd.).

(b) Experimental design

Treatments used in this experiment are illustrated in Figure 3-1. In the first set of treatments we tested the response of plants to a single high quality patch in the soil that differed in terms of its spatial relationship to neighbours. We grew focal plants either alone with sole access to the high quality patch (1P-Sole), with another individual (neighbour) planted so that it was equidistant to the high quality patch (1P-Equal), or with another individual (neighbour) present but further away from the high quality patch so that the focal plant was in closer proximity to it (1P-Closer). These three treatments were used to test our first two questions about how patch use is altered by neighbours depending on the spatial relationship between patch and neighbour.

We also grew plants in experimental arenas with two high quality patches, either alone (2P-Sole) or with another plant (2P-Shared) so that there was one patch another plant had equal access to, and one that the focal plant was in closer proximity to. These treatments were used to test our third question about how plants make choices about investment in patches when two are available that differ in terms of spatial relationship to neighbour. Because plants in treatments with two patches were grown in more resource rich environments, their behaviour was not directly compared to that of plants grown with one patch.

Fifteen replicates of each treatment were planted, but due to mortality of seedlings and technical issues, between 11 - 14 replicates of each treatment were used in the final analysis.

(c) Experimental conditions and procedures

Plants were grown and observed for 15 days in experimental arenas. Experimental arenas were constructed from two plexiglass sheets (21.5 cm wide, 27.9 cm tall, 0.6 cm thick) held apart by plexiglass spacers, which created a soil volume of 24.9 cm tall x 17.8 cm wide x 0.6 cm deep (Figure 3-1). Experimental arenas were filled with a matrix of relatively low nutrient background soil (a 3:1 mixture of washed sand : topsoil, BURNCO, Edmonton, AB) with the one or two high resource patches composed of manure-enriched soil (a 1 : 1 mixture of background soil : composted manure (0.5-0.5-0.5 minimum analysis, Premier Tech Home & Garden, Mississauga, ON)). High nutrient patch soil contained threefold greater available nitrate and ammonium than background soil (see Appendix, Table 5-2). The thin design of the arenas forces root foraging dynamics into (nearly) two dimensions, allowing visualization of roots as they contact the plexiglass (Figure 3-2). We recognize this design is a simplification of the complex three dimensional foraging space available to plants, though it was necessary to allow for non-destructive observation of foraging decisions made by individual plants. Experimental arenas were held in mounts that angled the front piece of plexiglass 45 degrees toward the ground (Figure 3-2), encouraging gravitropic root growth toward the front plexiglass sheet and further increasing root visibility. The back/upper piece of plexiglass was painted black to block light from the roots. We used black card to block light from the front/lower plexiglass sheet so that it could be removed during imaging of roots. Black card was also used to separate the aboveground parts of plants in separate experimental arenas (Ballaré et al. 1990, Gundel et al. 2014).

Prior to the experiment, seeds were germinated on damp paper towel, with each seed in a separate container so that there were interactions among individuals before the experiment began. Two days after germination began, healthy-looking seedlings with radicles 2-3 mm long were planted in the arenas with the radicle centred and pointing down in the planting location. The planting location was always in the center of the arena for focal plants, and to one side for neighbour plants. This was so that the focal plant was centred in the box and did not encounter one edge before the other (Semchenko et al. 2008).

Experimental arenas were located in a controlled growth room (16 : 8 h light : dark cycle at 24 °C) at the University of Alberta Biotron Facility. Water was provided *ad libitum.* Due to space constraints in the growing space, the experiment was performed in 3 runs, each consisting of 5 replicates of each treatment. To account for spatial variation within the growth chamber, experimental arenas were randomly assigned initial position in a growth chamber and position in growth chamber was re-randomized every other day.

(d) Behavioural assays

In all treatments, we measured individual plant root length in consistent soil locations corresponding to the dimensions and location of nutrient patches when they were present (Figure 3-1). Locations were equidistant from the focal plant's stem, controlling for the effect of distance from planting location on root measures. To capture images of root growth, we scanned the lower sheet of plexiglass of the experimental arenas using a flatbed scanner (Epson Perfection V700). For the first 9 days of root growth, boxes were scanned every second day (on day 1, day 3, … day 9). After this (days 9 - 15), boxes were scanned every day as root growth became more extensive and complex.

A challenge in root behavioural studies is assigning root segments to individual plants (Semchenko et al. 2007). This is simple when attachment points are visualized, but more difficult when they are not. The latter is common as roots are not always in contact with the plexiglass, and thus periodically are outside the field of view, hidden behind opaque soil. To facilitate behavioural assessment, we digitized roots by tracing them using the image analysis software WinRHIZO Tron (WinRHIZO Tron 2018a, Regent Instruments, QC, Canada). Traced root segments were assigned identity (focal or neighbour) based on direct connection to the tap root or based on contextual cues. Contextual cues were formalized into a ruleset (see Appendix, Table 5-1) and applied consistently to all images and all treatments. In general, rules were based on observed patterns of root growth in plants grown alone. Root length measured this way was highly correlated with roots carefully followed back to the taproot and measured at the endpoint of the experiment to confirm their identity (see Appendix, Figure 5-2).

(e) Endpoint measures

Plants were harvested after 15 days of growth in the experimental arenas. Shoots were cut at the surface of the soil, dried at 65℃ for 48 hours, and weighed. The individual root systems (focal and neighbour, if present) were carefully washed and separated, dried at 65℃ for 48 hours, and weighed.

To assess nutrient acquisition by individual plants, we measured % N in the two most recently expanded leaves of both focal and neighbour plants. We chose the most recently expanded leaves because of the aboveground parts of the plants, they were produced most recently and thus should most fully reflect any treatment differences. Dried leaves were ground into a homogenous powder using a bead mill (TissueLyser II; Qiagen, Holden, Germany). Approximately 5 mg of the resulting powder was weighed out and analyzed using an elemental analyser (FLASH 2000 HT Elemental Analyzer; Thermo Fisher Scientific, Milan, Italy) to determine % N.

(f) Statistical analyses

All statistical analyses were performed using R v. 3.4.1, using the packages lme4 and emmeans. Figures were produced using IBM SPSS Statistics 20. We used a suite of general linear mixed models to assess patch use (root length) in different areas of the soil, as well as final biomass and % N of leaf tissue. There was minimal root growth in the sampled areas of the soil during the first seven days of the experiment, since many plants had not yet reached those areas of the soil with their roots. Consequently, our analyses of root foraging decisions are restricted to days 9-15. We did not use run as a factor in our statistical analysis because there was no significant difference in metrics such as weight and root length between plants among the three experimental runs, and because environmental conditions were unchanging in the growth chambers where plants were grown. In all models, significance of fixed effects was determined using two-tailed Satterthwaite's F-tests.

Behavioural root length data was initially analyzed using two separate models, one that included all treatments with a single patch, and one that included both treatments with two patches. Plant identity was included as a random effect in all models discussed in this paragraph, to account for multiple measures of root length taken on each individual plant over time and across space. We first used general linear mixed models with treatment, soil area (high nutrient patch or background soil for the first model, and equal proximity or closer proximity patch for the second model), day of experiment (9-15), and all interaction terms were fixed effects. There were significant three-way interactions between treatment, soil area, and day in both models, unsurprisingly indicating that the effect of the social environment was not independent of the soil area and day of experiment (see Appendix, Table 5-3, Table 5-4). We then used a two-step approach to analyze the data and disentangle this interaction. First, we ran separate general linear mixed models on data separated by treatment, with soil area, day, and their interaction as fixed effects, to determine how plants within each treatment used different parts of the soil over time. Next, we ran separate general linear mixed models on data separated by soil area, with treatment, day and their interaction as fixed factors, to determine the effect of social environment in each area over time. After performing the general linear mixed models, we used Tukey-adjusted post-hoc comparisons within days for all models to determine the timing of differences and tease apart significant interactions between day and soil area, or treatment.

In treatments with two high nutrient patches, the two soil areas in each treatment were identical in terms of nutrient composition (composed of high nutrient patch soil), and the key difference was whether the focal plant shared equal proximity to the patch as a neighbour or was in closer proximity relative to a neighbour. To compare the treatments with neighbours to those grown alone, the two sides of the soil for individuals grown alone (2P-Sole treatment) were randomly assigned to either soil area (equal proximity or closer proximity) for comparison. There were no significant differences between the two sides assigned this way for plants grown alone in the 2P-Sole treatment (Table 3-3).

We compared biomass and % nitrogen of most recently expanded leaves between focal plants in different treatments and between neighbour plants in different treatments using general linear mixed models with treatment as a fixed effect. To compare focal vs. neighbour plants within treatments, we used general linear models with focal vs. neighbour identity as a fixed effect and experimental arena as a random effect to account for the fact that the focal and neighbour plants were paired within experimental arenas.

Results

(a) How do plants change patch use when a high quality patch is shared with a neighbour?

As expected, plants grown alone preferentially grew roots in a high nutrient patch relative to background soil, producing nearly twice as much root length on average in high nutrient patches (Figure 3-3a; Table 3-1; df = 1,169, F = 122.33, p <0.0001). By contrast, plants grown with a neighbour equidistant to the high quality patch did not preferentially use the high quality patch: there was no difference in root length between high nutrient soil and background soil over the growing period (Figure 3-3b; df = 1,156, F = 0.89, p = 0.3457).

(b) Is the impact of a neighbour on patch use contingent on the spatial relationship between patch and neighbour?

In contrast, when a focal plant was in relatively closer proximity to the patch, and the neighbour plant was located farther away from it, focal plants again preferentially used the high quality patch (Figure 3-3c; df = 1,143, F = 466.45, p <0.0001). There was also a significant interaction between day and treatment in the 1P-Closer model (df = 1,143, F = 11.65, p < 0.0001), indicating that the difference in root length between the high nutrient hotspot and the background soil became more exaggerated over time (Figure 3-3c). Separate tests of the impact of treatment and day within the high nutrient patch (Table 3-2) further supported differences in patch use depending on the social environment, with root length in high nutrient patches significantly affected by treatment (Figure 3-3, green lines indicated root length in patch soil; df = 2,36, F = 16.33, p < 0.0001). Although plants in closer proximity to the high quality patch initially responded similarly to the patch as plants grown alone, by the end of the experiment they had on average twice as much root length in the high quality patch as plants grown alone, as indicated by the significant interaction between treatment and day (df = 12,216, F = 22.44, p <0.0001). By contrast, there was no significant effect of treatment on root length in background soil, indicating no response to neighbour presence or access relative to a neighbour in the background soil (Table 3-2); changes in soil use by focal plants were only observed in the high quality patch.

(c) **If plants have access to two patches, one shared with a neighbour and one that is closer in proximity to a focal plant, how does it invest in the two patches?**

For plants grown alone in arenas with two equidistant patches, individuals invested equally in the two patches (Figure 3-4a; Table 3-3; df = 1,143, F = 2.11, p = 0.1487). When plants were grown with one patch equidistant to a neighbour and one patch that they were in closer proximity to, they ultimately invested almost 50% less root length in the patch that a neighbour shared equal access to, compared to the patch that they had first access to (Figure 3-4b; Table 3-3; df = 1,130, F = 87.22, p < 0.0001). By comparing root length to plants grown with sole access to the two patches in the 2P-Sole treatment by the end of the growing period, we determined that this was driven by a decrease in growth in shared patches that occurred later in the growing period (Figure 3-4; Table 3-4; significant interaction effect; df = 6,126, F = 11.01, p < 0.0001), rather than an increase in patches that individuals had first access to (Figure 3-4; Table 3-4; df = 1,21, F = 0.34, p = 0.5651).

(d) **Do plants in closer proximity to a high quality patch relative to a neighbouring plant have a competitive advantage?**

Metrics of aboveground biomass, belowground biomass, and total biomass did not differ among treatments compared here (see Appendix, Table 5-11; aboveground and belowground biomass not shown). Three comparisons of % N (see Appendix, Table 5-12) in most recently expanded leaves indicated significant differences: focal plants in the three treatments with a single patch varied significantly in % N of leaf tissues, with plants grown alone (1P-Sole) having the highest concentration of nitrogen in those tissues (3.04 ± 0.08 % N), focal plants grown with neighbours in the 1P-Equal treatment having the lowest (2.68 ± 0.05 % N), and focal plants in the 1P-Closer treatment with an intermediate concentration (2.84 ± 0.08 % N). In the comparison of neighbour plants in the 1P-Equal and 1P-Closer treatments, neighbours grown in the 1P-Closer treatment also had significantly higher leaf nitrogen concentrations (3.03 ± 0.10 % N) than neighbours grown in the 1P-Equal treatment (2.74 ± 0.09 % N). By contrast, there was no difference in % N between focal plants in the 2P-Sole and 2P-Shared treatments.

Discussion

Our results show that sunflowers alter their use of high quality patches when nearby neighbours are present, and that the specific spatial social context matters, as does the number of patches/resource level in the environment. As expected, plants grown alone with a single high quality patch preferentially grew more root in that patch (Figure 3-3a). When a high nutrient patch was equidistant to a neighbour, focal plants showed decreased use of the high quality patch relative to plants grown alone (Figure 3-3b). However, when a focal plant was in closer proximity to the high quality patch relative to a nearby neighbour, the opposite trend was observed: focal plants increased their use of the patch compared to plants grown alone (Figure 3-3c). We found that plants grown with two high nutrient patches, one which they had first access to, and one which they shared equal access to, ultimately decreased foraging effort in the patch a neighbour shared access to but did not alter their use of the patch they were more proximate to relative to plants grown alone with two patches (Figure 3-4a,b). Finally, we found limited evidence for an advantage for plants located closer to high nutrient patches in terms of nutrient acquisition, but no differences in biomass over the course of this experiment despite large changes in observed behaviour.

Here, sunflowers integrated spatially disparate cues (a valuable patch of soil on one side, and a neighbour on the other side) to inform foraging decisions early on in their growth, before neighbour roots had yet reached the valuable patch. Therefore, our results lend further support to previous studies that show that plants can use social information in their foraging decisions (Cahill et al. 2010, Mommer et al. 2012, McNickle et al. 2016). They also suggest that plants are capable of anticipatory behaviour in their foraging, by altering their behaviour such that they remove mineral nutrients from the soil before neighbours can reach a high quality patch. This kind of anticipatory response in plant root foraging may be common. In one experiment, pea plants allocated more roots to areas of the soil where nutrient levels were increasing, even over areas where nutrients are maintained at a higher constant concentration (Shemesh et al. 2010). In response to the presence of neighbours, plants sometimes increase total root growth (Gersani et al. 2001); plants can even respond to the local application of root exudates from another plant in a similar way (Semchenko et al. 2014). However, to our knowledge this is the first example of a plant increasing its use specifically of a relatively high quality patch in response to a neighbour.

Increased root proliferation in high nutrient patches that individuals have first access to might allow plants to more quickly exploit high nutrient patches before they are accessed by a competitor, but why don't plants grown alone respond as strongly? As in animals (Stephens 1987), there can be a trade-off between exploration of the environment and exploitation of resources by plants (Peng et al. 2012). Although this trade-off hasn't been tested directly in plant root foraging, some plants do stop exploring the soil after encountering a high nutrient patch and proliferating roots within it (McNickle and Cahill 2009), which may be in part due to trade-offs between exploitation and further exploration of the soil. Certainly, root proliferation can be costly in terms of carbon invested (Robinson 1996, 2002). Plants grown alone may not proliferate as much in high-nutrient patches because they are allocating more energy toward exploring more areas of the soil, while plants grown with a neighbour may be able to detect the obstruction to exploring further areas of the soil (Semchenko et al. 2008) and therefore invest more energy in pre-empting resources within the high nutrient patch they do have access to.

We saw no increase in patch use by plants grown with neighbours compared to plants grown alone when there were two patches available (Figure 3-4). Although we predicted that plants may respond similarly to patches they are in closer proximity to when two patches are available, in an environment with more resources (two high quality patches vs. one), pre-emption of resources in high quality patches could be less important. Consistent with this interpretation, plants grown with a neighbour and two patches did not have significantly lower %N in their leaf tissue when compared with plants grown without a neighbour and two patches. This may be analogous to lower levels of aggression observed in animals when more resources are available (Sirot 2000, Grant et al. 2002), since proliferation in high quality patches can similarly allow plants to monopolize resources (Hodge et al. 1999). This finding emphasizes the great deal of contingency in plant root foraging responses.

In the context of this experiment, it is not certain what cues sunflowers used to assess neighbour presence and location and alter foraging behaviour accordingly. Certainly, ample evidence exists for the importance of chemical cues produced by plants in the soil on neighbour detection and root behaviour (Mahall and Callaway 1991, Falik et al. 2005, Biedrzycki et al.

2010, Semchenko et al. 2014), and a chemical cue or signal could be at play here. It is also important to note that the cue or signal may be nutritive (O'Brien and Brown 2008), with plants using information about nutrient distributions across the root system (i.e. a decrease in nutrients near neighbours) as a cue to increase root growth in certain areas of the soil (Gersani and Sachs 1992). This is plausible because root proliferation responses depend not just on absolute but relative values of high nutrient areas, and plants respond differently to a given nutrient level depending on whether it is localized or applied to the entire root system (Drew 1975, Gersani and Sachs 1992). This proposed proximate mechanism is supported by the lack of increased investment when plants were given access to two patches, potentially due to the overall higher level of nutrients in that treatment. Regardless of the mechanism, the chosen tactic of plants in response to social context (decreasing or increasing foraging effort in a patch) occurred very early in growth, indicating that plants can use relatively small initial cues in order to change their behaviour early in their lives.

Plants that shared equal access to a patch with another plant had significantly lower % N than plants with sole access (grown alone). However, plants in closer proximity to the patch had an intermediate leaf nitrogen concentration that did not differ significantly from plants with sole access to patches. This suggests that closer proximity to a patch benefitted plants that were subject to competition, consistent with previous experiments (Casper and Cahill 1996, Day et al. 2003). Curiously, neighbour plants (which were never in closer proximity to patches) also had higher concentrations of leaf nitrogen in the treatment where focal plants were closer to a high quality patch than in the treatment where focal plants shared equal access to a patch. This result potentially reflects a benefit to *both* plants when one plant has first access to a patch rather than when access is shared, perhaps because plants that share access are more subject to over-proliferation resulting in a tragedy of the commons (Gersani et al. 2001) (see Appendix, Figure 5-4), although ultimately the mechanism for this remains unclear based on this experiment. Although we saw limited effects on metrics such as biomass and nutrient acquisition in this relatively short experiment, small differences in the timing of access to resources certainly can have cascading consequences in the lives of plants (Ross and Harper 1972, Day et al. 2003).

The finding that plants use information about the specific spatial social context to inform their responses to high nutrient patches is part of a growing body of literature showing that plants use information about their social environment to modify root foraging behaviour (Mahall and Callaway 1991, Schenk et al. 1999, Cahill et al. 2010, Cahill and McNickle 2011, Belter and Cahill 2015). More broadly, plants, like many other organisms, use cues or signals from other organisms in their environments to make decisions about foraging (Karban 2008). If such contingencies in foraging behaviour are common in plants, competitive outcomes between individuals and potentially community composition and structure could be impacted (Kembel and Cahill Jr. 2005). More work is needed to disentangle the proximate mechanisms at play in responses such as these (for example, whether cues used by the plants are based on changes in nutrient levels or another cue from neighbours) and whether these contingent responses have impacts on plant performance throughout the natural lifetime of a plant. Although understanding qualitative changes in behaviour are key to beginning to understand plant behavioural ecology, further work is also needed to place these responses in a quantitative, optimality framework (McNickle et al. 2009).

Literature cited

Aphalo, P. J., and C. L. Ballare. 1995. On the importance of information-acquiring systems in plant-plant interactions. Functional Ecology 9:5–14.

Ballaré, C. L., A. L. Scopel, and R. A. Sánchez. 1990. Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. Science 247:329–332.

Bao, T., G. W. Melenka, M. K. Ljubotina, J. P. Carey, and J. F. Cahill. 2018. A new method for the rapid characterization of root growth and distribution using digital image correlation. New Phytologist 218:835–846.

Belter, P. R., and J. F. Cahill. 2015. Disentangling root system responses to neighbours: identification of novel root behavioural strategies. AoB Plants 7:plv059.

Biedrzycki, M. L., T. A. Jilany, S. A. Dudley, and H. P. Bais. 2010. Root exudates mediate kin recognition in plants. Communicative & Integrative Biology 3:28–35.

Cahill, J. F., and G. G. McNickle. 2011. The behavioral ecology of nutrient foraging by plants. Annual Review of Ecology, Evolution, and Systematics 42:289–311.

Cahill, J. F., G. G. McNickle, J. J. Haag, E. G. Lamb, S. M. Nyanumba, and C. C. St Clair. 2010. Plants integrate information about nutrients and neighbors. Science 328:1657–1657.

Casper, B. B., and J. F. Cahill. 1996. Limited effects of soil nutrient heterogeneity on populations of *Abutilon theophrasti* (Malvaceae). American Journal of Botany 83:333–341.

Charnov, E. L. 1976a. Optimal foraging, the marginal value theorem. Theoretical population biology 9:129–136.

Charnov, E. L. 1976b. Optimal foraging: attack strategy of a mantid. The American Naturalist 110:141–151.

Day, K. J., M. J. Hutchings, and E. A. John. 2003. The effects of spatial pattern of nutrient supply on the early stages of growth in plant populations. Journal of Ecology 91:305–315.

Drew, M. C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. The New Phytologist 75:479–490.

Dudley, S. A., and A. L. File. 2007. Kin recognition in an annual plant. Biology Letters 3:435–438.

Falik, O., P. Reides, M. Gersani, and A. Novoplansky. 2005. Root navigation by self inhibition. Plant, Cell & Environment 28:562–569.

Fransen, B. 1999. Disentangling the effects of root foraging and inherent growth rate on plant biomass accumulation in heterogeneous environments: a modelling study. Annals of Botany 84:305–311.

Fransen, B., H. de Kroon, and F. Berendse. 2001. Soil nutrient heterogeneity alters competition between two perennial grass species. Ecology 82:2534–2546.

Fretwell, S. D., and H. L. Lucas. 1969. On territorial behavior and other factors influencing habitat distribution in birds. Acta biotheoretica 19:16–36.

Gersani, M., Z. Abramsky, and O. Falik. 1998. Density-dependent habitat selection in plants. Evolutionary Ecology 12:223–234.

Gersani, M., J. S. Brown, E. E. O'Brien, G. M. Maina, and Z. Abramsky. 2001. Tragedy of the commons as a result of root competition. Journal of Ecology 89:660–669.

Gersani, M., and T. Sachs. 1992. Development correlations between roots in heterogeneous environments. Plant, Cell & Environment 15:463–469.

Gill, F. B., and L. L. Wolf. 1975. Economics of feeding territoriality in the golden-winged sunbird. Ecology 56:333–345.

Grant, J. W. A., I. L. Girard, C. Breau, and L. K. Weir. 2002. Influence of food abundance on competitive aggression in juvenile convict cichlids. Animal Behaviour 63:323–330.

Grant, J. W. A., and R. T. Guha. 1993. Spatial clumping of food increases its monopolization and defense by convict cichlids, *Cichlasoma nigrofasciatum*. Behavioral Ecology 4:293–296.

Grime, J. P., and J. M. L. Mackey. 2002. The role of plasticity in resource capture by plants. Evolutionary Ecology 16:299–307.

Gruntman, M., D. Groß, M. Májeková, and K. Tielbörger. 2017. Decision-making in plants under competition. Nature Communications 8:2235.

Gundel, P., R. Pierik, L. Mommer, and C. Ballaré. 2014. Competing neighbors: light perception and root function. Oecologia 176:1–10.

Harper, J. L. 1985. Modules, branches, and the capture of resources. Pages 1–33 Population biology and evolution of clonal organisms. Yale University Press, New Haven.

Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytologist 162:9–24.

Hodge, A., D. Robinson, B. S. Griffiths, and A. H. Fitter. 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. Plant, Cell & Environment 22:811–820.

Hutchings, M. J., and H. de Kroon. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Pages 159–238 *in* M. B. and A. H. Fitter, editor. Advances in Ecological Research. Academic Press.

Jackson, R. B., and M. M. Caldwell. 1989. The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. Oecologia 81:149–153.

Jackson, R. B., and M. M. Caldwell. 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology 74:612–614.

Jackson, R. B., J. H. Manwaring, and M. M. Caldwell. 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature 344:58.

Jensen, E. L., L. M. Dill, and J. F. Cahill. 2011. Applying behavioral-ecological theory to plant defense: light-dependent movement in *Mimosa pudica* suggests a trade-off between predation risk and energetic Reward. The American Naturalist 177:377–381.

Karban, R. 2008. Plant behaviour and communication. Ecology Letters 11:727–739.

Kembel, S. W., and J. F. Cahill Jr. 2005. Plant phenotypic plasticity belowground: a phylogenetic perspective on root foraging trade-offs. The American Naturalist 166:216–230.

Křivan, V., R. Cressman, and C. Schneider. 2008. The ideal free distribution: A review and synthesis of the game-theoretic perspective. Theoretical Population Biology 73:403–425.

de Kroon, H., H. Huber, J. F. Stuefer, and J. M. Van Groenendael. 2005. A modular concept of phenotypic plasticity in plants. New Phytologist 166:73–82.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619–640.

Lima, S. L., T. J. Valone, and T. Caraco. 1985. Foraging-efficiency-predation-risk trade-off in the grey squirrel. Animal Behaviour 33:155–165.

Mahall, B. E., and R. M. Callaway. 1991. Root communication among desert shrubs. Proceedings of the National Academy of Sciences 88:874–876.

Maina, G. G., J. S. Brown, and M. Gersani. 2002. Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons. Plant Ecology 160:235–247.

McNickle, G. G., and J. S. Brown. 2014. An ideal free distribution explains the root production of plants that do not engage in a tragedy of the commons game. Journal of Ecology 102:963–971.

McNickle, G. G., and J. F. Cahill. 2009. Plant root growth and the marginal value theorem. Proceedings of the National Academy of Sciences 106:4747–4751.

McNickle, G. G., M. K. Deyholos, and J. F. Cahill. 2016. Nutrient foraging behaviour of four co-occurring perennial grassland plant species alone does not predict behaviour with neighbours. Functional Ecology 30:420–430.

McNickle, G. G., C. C. St Clair, and J. F. Cahill. 2009. Focusing the metaphor: plant root foraging behaviour. Trends in Ecology & Evolution 24:419–426.

Metlen, K. L., E. T. Aschehoug, and R. M. Callaway. 2009. Plant behavioural ecology: dynamic plasticity in secondary metabolites. Plant, Cell & Environment 32:641–653.

Mommer, L., J. van Ruijven, C. Jansen, H. M. van de Steeg, and H. de Kroon. 2012. Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? Functional Ecology 26:66–73.

Monaghan, P., and N. B. Metcalfe. 1985. Group foraging in wild brown hares: effects of resource distribution and social status. Animal Behaviour 33:993–999.

Motte, H., and T. Beeckman. 2019. The evolution of root branching: increasing the level of plasticity. Journal of Experimental Botany 70:785–793.

O'Brien, E. E., and J. S. Brown. 2008. Games roots play: effects of soil volume and nutrients. Journal of Ecology 96:438–446.

Peng, Y., K. J. Niklas, and S. Sun. 2012. Do plants explore habitats before exploiting them? An explicit test using two stoloniferous herbs. Chinese Science Bulletin 57:2425–2432.

Robinson, D. 1996. Resource capture by localized root proliferation: why do plants bother? Annals of Botany 77:179–186.

Robinson, D. 2002. Root proliferation, nitrate inflow and their carbon costs during nitrogen capture by competing plants in patchy soil. Pages 41–50 *in* D. S. Powlson, G. L. Bateman, K. G. Davies, J. L. Gaunt, and P. R. Hirsch, editors. Interactions in the Root Environment: An Integrated Approach. Springer Netherlands, Dordrecht.

Robinson, D., A. Hodge, B. S. Griffiths, and A. H. Fitter. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proceedings of the Royal Society B: Biological Sciences 266:431–435.

Ross, M. A., and J. L. Harper. 1972. Occupation of biological space during seedling establishment. The Journal of Ecology 60:77.

Schenk, H. J., R. M. Callaway, and B. E. Mahall. 1999. Spatial root segregation: are plants territorial? Pages 145–180 *in* A. H. F. and D. Raffaelli, editor. Advances in Ecological Research. Academic Press.

Schoener, T. W. 1983. Simple models of optimal feeding-territory size: a reconciliation. The American Naturalist 121:608–629.

Semchenko, M., E. A. John, and M. J. Hutchings. 2007. Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. New Phytologist 176:644–654.

Semchenko, M., S. Saar, and A. Lepik. 2014. Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. New Phytologist 204:631–637.

Semchenko, M., K. Zobel, A. Heinemeyer, and M. J. Hutchings. 2008. Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. New Phytologist 179:1162–1170.

Shemesh, H., A. Arbiv, M. Gersani, O. Ovadia, and A. Novoplansky. 2010. The effects of nutrient dynamics on root patch choice. PLoS ONE 5:e10824.

Silvertown, J. 2004. Plant coexistence and the niche. Trends in Ecology & Evolution 19:605–611.

Sirot, E. 2000. An evolutionarily stable strategy for aggressiveness in feeding groups. Behavioral Ecology 11:351–356.

Stephens, D. W. 1987. On economically tracking a variable environment. Theoretical Population Biology 32:15–25.

Table 3-1. Results of three separate linear mixed models comparing relative use of high nutrient patches to equidistant low nutrient soil, within three different social environments (treatments). Soil area (high nutrient patch or background soil equidistant to the plant's stem), day (9-15), and their interaction were fixed factors, plant identity was included as a random factor, and root length was the response variable (Figure 3-3). Models were run within each treatment to assess responses of plants to patches within treatments.

	1P-Sole			**1P-Closer**			**1P-Equal**		
Source	**df**	**F-value**	**p-value**	**df**	**F-value**	**p-value**	**df**	**F-value**	**p-value**
Soil area	1,169	122.33	<0.0001	1, 143	466.45	<0.0001	1,156	0.89	0.3457
Day	6,169	20.18	<0.0001	6, 143	32.66	<0.0001	6,156	9.37	<0.0001
Soil area * Day	6,169	1.35	0.2367	6, 143	11.65	<0.0001	6,156	0.22	0.9715

Table 3-2. Results of two separate linear mixed models testing the response of plants to social environment (grown alone, with another plant equidistant to a high nutrient patch, or in closer proximity to a high nutrient patch than a nearby neighbouring plant) within soil types (high nutrient patch vs. equidistant low nutrient background soil). Treatment (1P-Sole, 1P-Equal, or 1P-Closer; Figure 3-1), day (9-15), and their interaction were fixed effects, plant identity was a random effect and root length was the response variable (Figure 3-3). Models were run within soil types to test the response of plants to social context within soil types.

		Background soil model		**High nutrient patch model**	
Source	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Treatment	2,36	1.14	0.3326	16.33	<0.0001
Day	6,216	69.28	<0.0001	192.46	<0.0001
Treatment*Day	12,216	1.28	0.2288	22.44	<0.0001

Table 3-3. Results of two separate linear mixed models testing patch use of plants in two equidistant high nutrient patches, with soil area (equidistant to neighbour or further from neighbour/closer to focal plant), day (9-15), and their interaction as fixed effects, plant identity as a random effect, and root length as the response variable (Figure 3-4). Models were run within treatments to test whether plants grown alone (2P-Sole) invested equal amounts of roots on both sides, as expected, and whether plants changed investment contingent on distance of patch to neighbour within the 2P-Shared treatment.

	2P-Sole			**2P-Shared**		
Source	**df**	**F-value**	**p-value**	**df**	**F-value**	**p-value**
Soil area	1,143	2.11	0.1487	1,130	87.22	<0.0001
Day	6,143	62.13	<0.0001	6,130	36.74	<0.0001
Soil area * day	6,143	0.12	0.9936	6,130	6.48	<0.0001

Table 3-4. Results of two separate linear mixed models testing effect of social environment (neighbour plant present or absent) within soil types. Treatment (2P-Sole or 2P-Shared; Figure 3-1), day (9-15), and their interaction were fixed effects, plant identity was included as a random effect, and root length was the response variable (Figure 3-4). Models were run within soil types to compare use of patches by plants grown with neighbours to use by plants grown alone.

		Soil equidistant to neighbour		**Soil farther from neighbour**	
Source	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Treatment	1,21	2.86	0.1055	0.34	0.5651
Day	6,126	73.77	<0.0001	148.52	<0.0001
Treatment * Day	6,126	11.01	<0.0001	0.72	0.6331

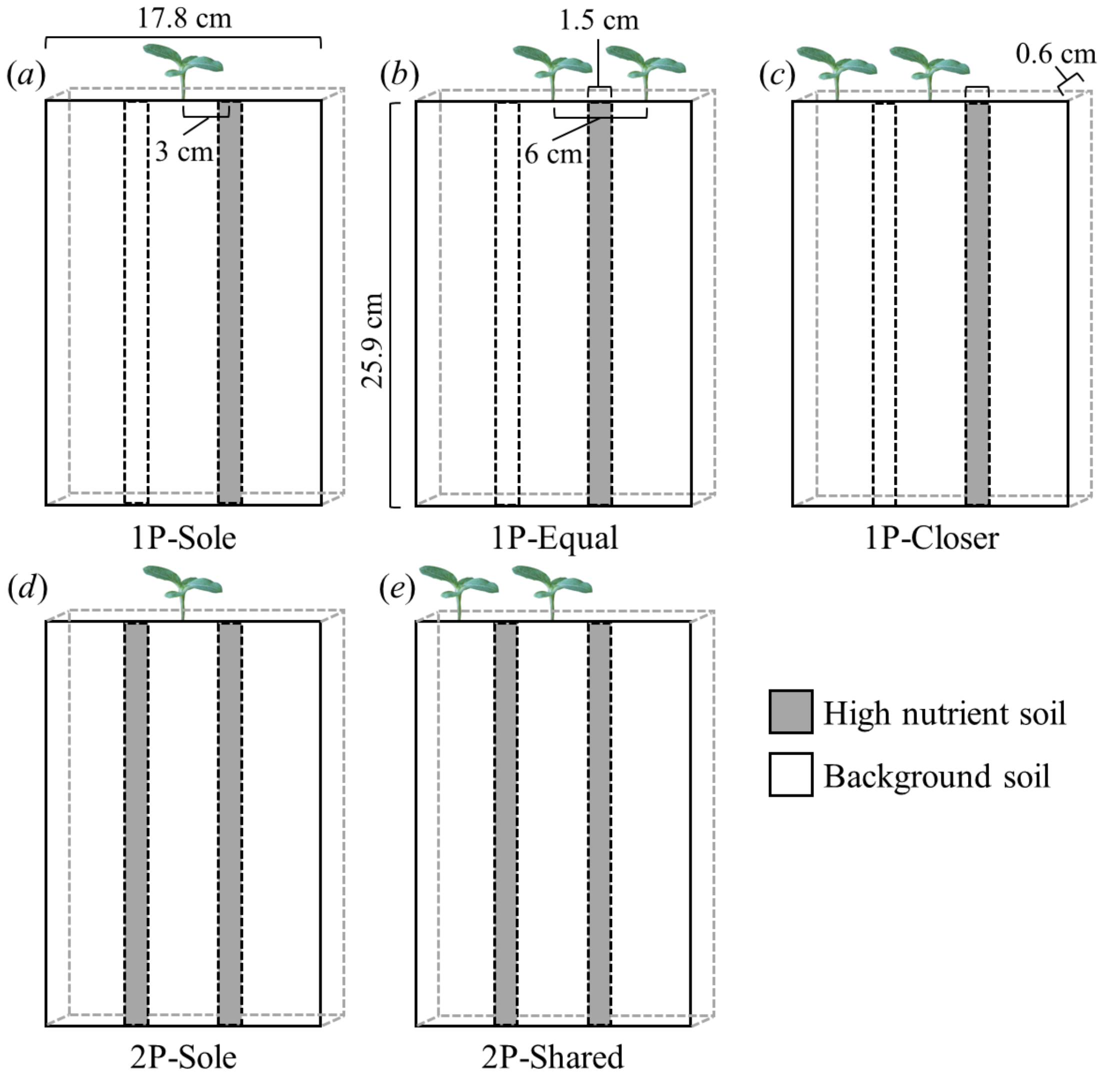


Figure 3-1. Schematic of treatments and growing dimensions of the experimental arenas. Treatment names are below each diagram. Focal plants were always placed in the center of arenas so that focal plant root growth was not affected by encountering one edge before the other. Neighbour plants when present were always placed 6 cm from the focal plant and 3 cm from the edge of the experimental arena. Shading represents the relative nutrient content of the soils used. Root length was measured through the front, transparent plane of the boxes, and the areas of the soil where root length was measured are highlighted with dashed lines. These correspond to the area of the nutrient hotspots where present.

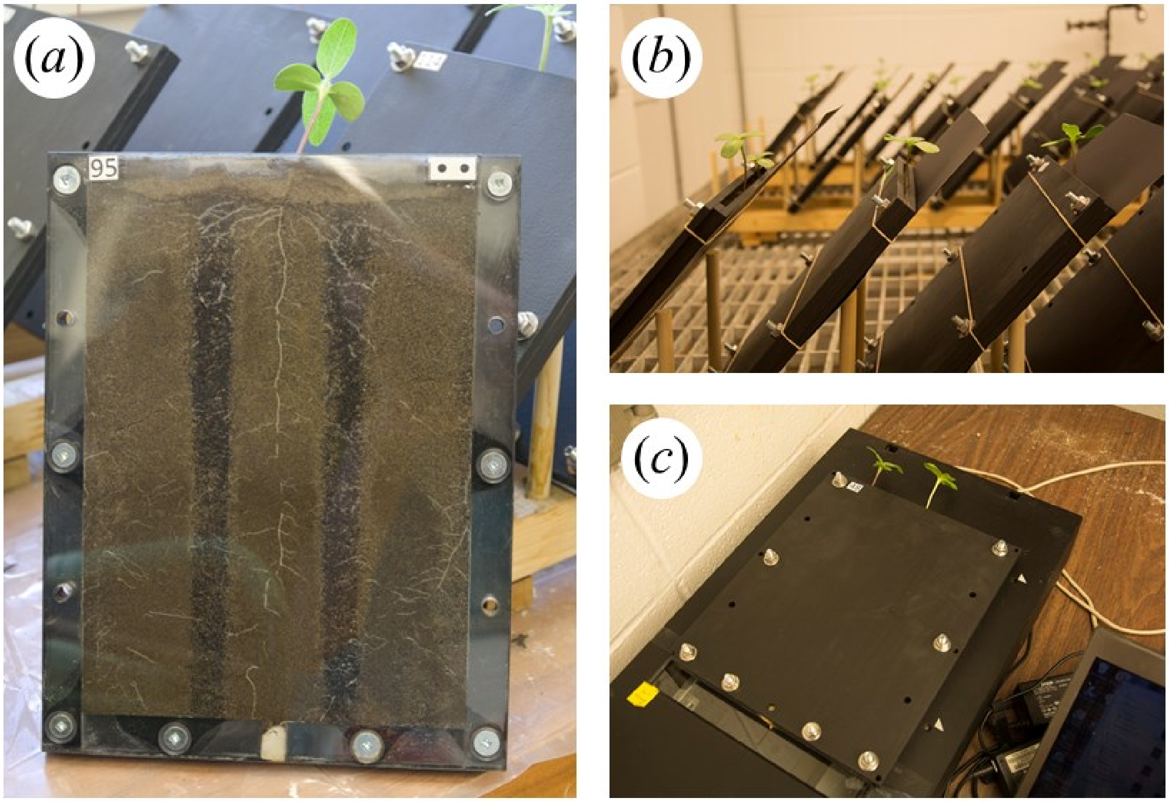


Figure 3-2. Experimental arenas and growing conditions. (*a*) Experimental arena showing the lower plexiglass sheet through which roots were imaged. (*b*) Experimental arenas held in wooden mount. Black pieces of card were attached to the lower piece of plexiglass to block light from the roots and to separate the aboveground parts of plants in adjacent arenas. (*c*) Experimental arena being scanned on flatbed scanner for imaging and subsequent root digitization.

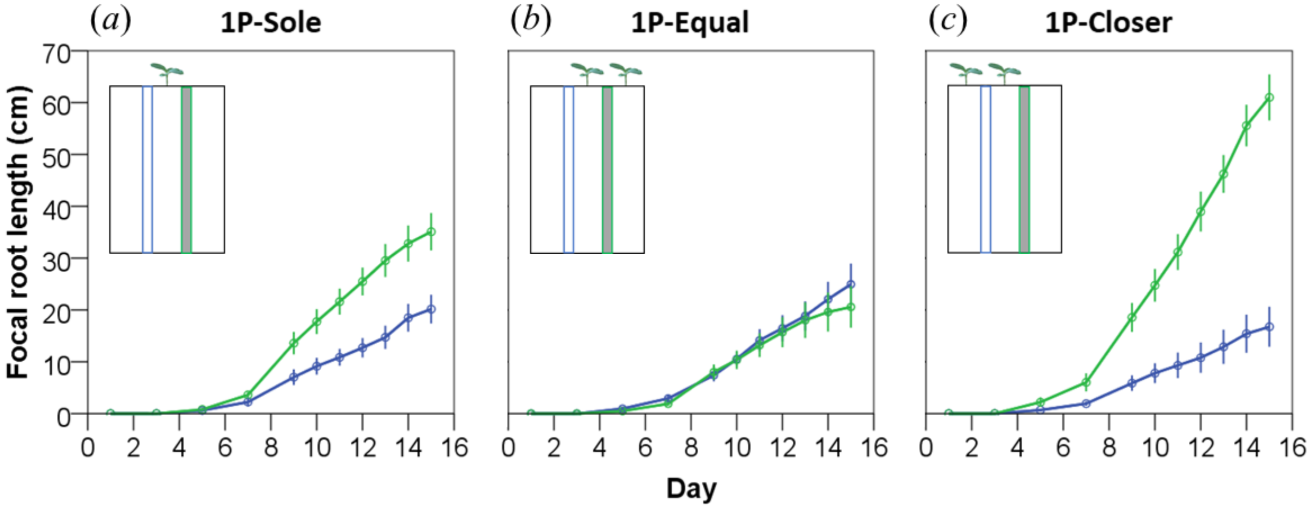


Figure 3-3. Mean traced focal plant root length (± SEM) in centimeters in two areas of the soil measured over fifteen days for the three treatments containing a single high nutrient patch. Green represents focal plant root length in the high nutrient patch, and blue represents root length in the low nutrient soil on the other side of the plant. The measured areas are shown in the inlaid schematics for each treatment. Only focal plant root length is shown here (neighbour plant root length not shown; see Appendix, Figure 5-4).

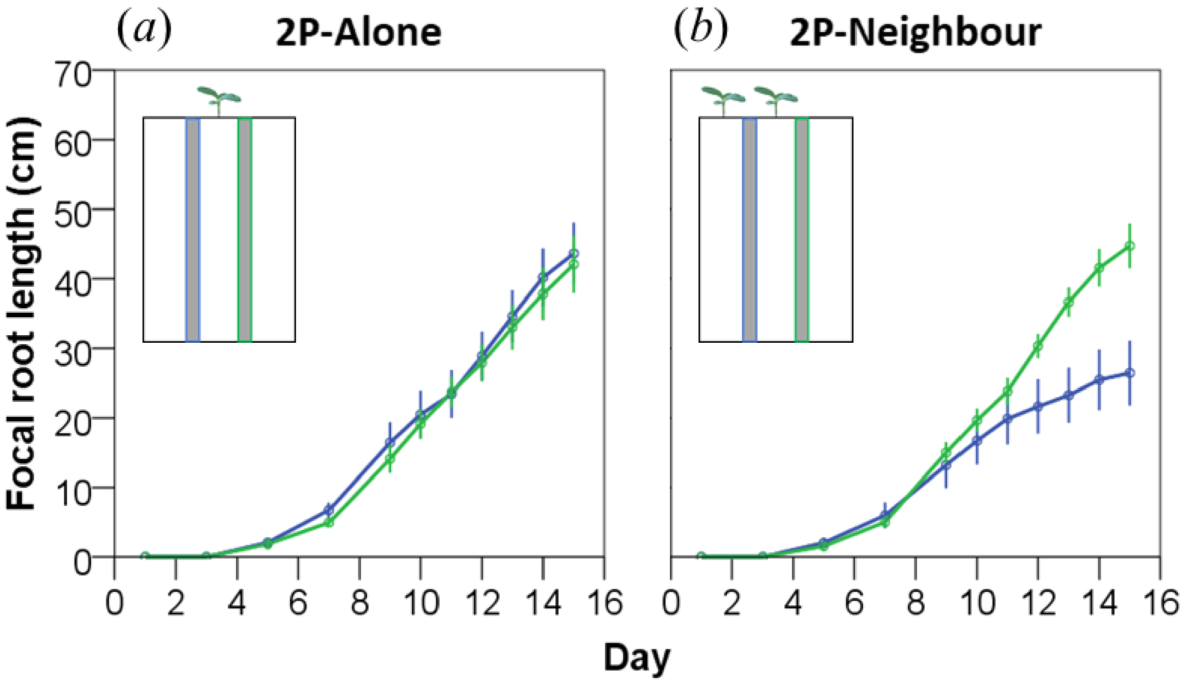


Figure 3-4. Mean traced focal plant root length (± SEM) in centimeters in two areas of the soil measured over fifteen days of growth for the two treatments containing two high nutrient patches. The measured areas are shown in the inlaid schematics for each treatment. Only focal plant root length is shown here; neighbour plant root length not shown (see Appendix, Figure 5-5).

Chapter 4: Conclusion

In this thesis, I have applied behavioural approaches to the study of plants in two chapters, using common sunflower (*Helianthus annuus L.*) as a study organism.

In Chapter 2, I presented an ethogram written for common sunflower, a widespread and well-known species of plant. By listing behaviours drawn from the literature in *Helianthus annuus* L., we illustrated how plants in general respond actively to their environments. Although descriptions of plants based on static traits are incredibly important for understanding botany, biology, and ecology, understanding the behaviours of plants is a key component of understanding as well. Our ethogram was written for a general audience, but ethograms may prove useful in the study of plant behaviour, especially for workers interested in comparing multiple behavioural traits across species (Schleidt et al. 1984).

In Chapter 3, I presented the results of an experiment showing that nutrient patch use by *H. annus* is contingent on the social environment. We found that:

1) Compared to plants grown alone, focal individuals equidistant to a single high quality patch as a neighbour decreased their use of the patch, but focal individuals in closer proximity to a high quality patch relative to a neighbour increased patch use. We suggest that this may represent a strategy that allows plants to avoid competition in highly contested patches (when a valuable patch is equidistant to neighbour), but also to increase pre-emption of resources in patches they have first access to (when in closer proximity to a valuable patch relative to a neighbour).
2) Plants did not increase their use of high quality patches in the same way when grown with more abundant resources (two high quality patches). If increasing root investment in patches is a competitive strategy to pre-empt resources, this result is

consistent with general predictions stemming from game theory and optimal foraging that organisms should alter the frequency of competitive behaviours (in animals, often aggression) in response to resource abundance (Sirot 2000, Grant et al. 2002).

3) There were no differences in biomass of focal plants as a function of competitor presence/location, although there were some differences in nutrient acquisition depending on the social environment.

This work joins a handful of studies that have shown that root foraging behaviour in plants changes depending on the social environment (Cahill et al. 2010, Mommer et al. 2012, McNickle et al. 2016). Since root proliferation in high quality patches can play a role in the pre-emption of resources in those patches and therefore competitive ability (Hodge et al. 1999), then perhaps it is unsurprising that plants change these responses depending on the social environment. More research is needed to understand how common such behavioural shifts are in different species of plant, and the diversity of strategies that may exist among different species, populations, and individuals. Because the observed behavioural shifts occurred early in growth and in areas of the soil where neighbours had not yet reached, this work also suggests that plants make anticipatory changes in root foraging behaviour based on information about competitors (Shemesh et al. 2010).

A major limitation of the experiment was the length of the experiment, which was only fifteen days. The short growing period here was necessary to capture the early responses of plants to the different treatments and their immediate consequences, but limited our ability to assess long-term consequences of competition throughout the plants' lives. Ideally, experiments assessing the effect of competition on plants would do so repeatedly throughout a longer growing period, although historically this approach has been unusual because it likely necessitates repeated harvests of different plants throughout the growing period (Trinder et al. 2012). However, in competitive interactions between plants, early access to resources can certainly play a large part in the dynamics of competition for the remainder of individuals' interactions. For example, plants may germinate sooner in the presence of neighbouring seeds than when grown alone (Dyer et al. 2000), which can give them a long-term competitive advantage due to the initial conquest of resources (Ross and Harper 1972). Therefore, these early changes may have cascading consequences in the lives of plants and in the communities in which they live. More work is needed to understand the diversity and consequences of behavioural responses of plants to each other and their environments.

Literature cited

Cahill, J. F., G. G. McNickle, J. J. Haag, E. G. Lamb, S. M. Nyanumba, and C. C. St Clair. 2010. Plants integrate information about nutrients and neighbors. Science 328:1657–1657.

Dyer, A. R., A. Fenech, and K. J. Rice. 2000. Accelerated seedling emergence in interspecific competitive neighbourhoods. Ecology Letters 3:523–529.

Grant, J. W. A., I. L. Girard, C. Breau, and L. K. Weir. 2002. Influence of food abundance on competitive aggression in juvenile convict cichlids. Animal Behaviour 63:323–330.

Hodge, A., D. Robinson, B. S. Griffiths, and A. H. Fitter. 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. Plant, Cell & Environment 22:811–820.

McNickle, G. G., M. K. Deyholos, and J. F. Cahill. 2016. Nutrient foraging behaviour of four co-occurring perennial grassland plant species alone does not predict behaviour with neighbours. Functional Ecology 30:420–430.

Mommer, L., J. van Ruijven, C. Jansen, H. M. van de Steeg, and H. de Kroon. 2012. Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? Functional Ecology 26:66–73.

Ross, M. A., and J. L. Harper. 1972. Occupation of biological space during seedling establishment. The Journal of Ecology 60:77.

Schleidt, W. M., G. Yakalis, M. Donnelly, and J. McGarry. 1984. A proposal for a standard ethogram, exemplified by an ethogram of the bluebreasted quail (*Coturnix chinensis*). Zeitschrift für Tierpsychologie 64:193–220.

Shemesh, H., A. Arbiv, M. Gersani, O. Ovadia, and A. Novoplansky. 2010. The effects of nutrient dynamics on root patch choice. PLoS ONE 5:e10824.

Sirot, E. 2000. An evolutionarily stable strategy for aggressiveness in feeding groups. Behavioral Ecology 11:351–356.

Trinder, C., R. Brooker, H. Davidson, and D. Robinson. 2012. Dynamic trajectories of growth and nitrogen capture by competing plants. New Phytologist 193:948–958.

Bibliography

Aphalo, P. J., and C. L. Ballare. 1995. On the importance of information-acquiring systems in plant-plant interactions. Functional Ecology 9:5–14.

Atamian, H. S., N. M. Creux, E. A. Brown, A. G. Garner, B. K. Blackman, and S. L. Harmer. 2016. Circadian regulation of sunflower heliotropism, floral orientation, and pollinator visits. Science 353:587–590.

Ballaré, C. L., A. L. Scopel, and R. A. Sánchez. 1990. Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. Science 247:329–332.

Bao, T., G. W. Melenka, M. K. Ljubotina, J. P. Carey, and J. F. Cahill. 2018. A new method for the rapid characterization of root growth and distribution using digital image correlation. New Phytologist 218:835–846.

Bazzaz, F. A. 1991. Habitat selection in plants. The American Naturalist 137:S116–S130.

Belter, P. R., and J. F. Cahill. 2015. Disentangling root system responses to neighbours: identification of novel root behavioural strategies. AoB Plants 7:plv059.

Biedrzycki, M. L., T. A. Jilany, S. A. Dudley, and H. P. Bais. 2010. Root exudates mediate kin recognition in plants. Communicative & Integrative Biology 3:28–35.

Cahill, J. F., and G. G. McNickle. 2011. The behavioral ecology of nutrient foraging by plants. Annual Review of Ecology, Evolution, and Systematics 42:289–311.

Cahill, J. F., G. G. McNickle, J. J. Haag, E. G. Lamb, S. M. Nyanumba, and C. C. St Clair. 2010. Plants integrate information about nutrients and neighbors. Science 328:1657–1657.

Casper, B. B., and J. F. Cahill. 1996. Limited effects of soil nutrient heterogeneity on populations of *Abutilon theophrasti* (Malvaceae). American Journal of Botany 83:333–341.

Charnov, E. L. 1976a. Optimal foraging, the marginal value theorem. Theoretical population biology 9:129–136.

Charnov, E. L. 1976b. Optimal foraging: attack strategy of a mantid. The American Naturalist 110:141–151.

Day, K. J., M. J. Hutchings, and E. A. John. 2003. The effects of spatial pattern of nutrient supply on the early stages of growth in plant populations. Journal of Ecology 91:305–315.

Dias, A. M., M. Pareja, M. Laia, M. C. Blassioli-Moraes, M. Borges, and R. A. Laumann. 2016. Attraction of *Telenomus podisi* to volatiles induced by *Euschistus heros* in three different plant species. Arthropod-Plant Interactions 10:419–428.

Drew, M. C. 1975. Comparison of the effects of a localized supply of phosphate, nitrate, ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. The New Phytologist 75:479–490.

Dudley, S. A., and A. L. File. 2007. Kin recognition in an annual plant. Biology Letters 3:435–438.

Dyer, A. R., A. Fenech, and K. J. Rice. 2000. Accelerated seedling emergence in interspecific competitive neighbourhoods. Ecology Letters 3:523–529.

Elleman, C. J., V. Franklin-Tong, and H. G. Dickinson. 1992. Pollination in species with dry stigmas: the nature of the early stigmatic response and the pathway taken by pollen tubes. New Phytologist 121:413–424.

Falik, O., P. Reides, M. Gersani, and A. Novoplansky. 2005. Root navigation by self inhibition. Plant, Cell & Environment 28:562–569.

Fransen, B. 1999. Disentangling the effects of root foraging and inherent growth rate on plant biomass accumulation in heterogeneous environments: a modelling study. Annals of Botany 84:305–311.

Fransen, B., H. de Kroon, and F. Berendse. 2001. Soil nutrient heterogeneity alters competition between two perennial grass species. Ecology 82:2534–2546.

Free, J. B. 1993. Insect pollination of crops. 2nd Revised edition. Academic Press, London.

Fretwell, S. D., and H. L. Lucas. 1969. On territorial behavior and other factors influencing habitat distribution in birds. Acta biotheoretica 19:16–36.

Gersani, M., Z. Abramsky, and O. Falik. 1998. Density-dependent habitat selection in plants. Evolutionary Ecology 12:223–234.

Gersani, M., J. S. Brown, E. E. O'Brien, G. M. Maina, and Z. Abramsky. 2001. Tragedy of the commons as a result of root competition. Journal of Ecology 89:660–669.

Gersani, M., and T. Sachs. 1992. Development correlations between roots in heterogeneous environments. Plant, Cell & Environment 15:463–469.

Gill, F. B., and L. L. Wolf. 1975. Economics of feeding territoriality in the golden-winged sunbird. Ecology 56:333–345.

Gollan, T., J. B. Passioura, and R. Munns. 1986. Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. Australian Journal of Plant Physiology 13:459–464.

Goodman, A., and A. R. Ennos. 1998. Responses of the root systems of sunflower and maize to unidirectional stem flexure. Annals of Botany 82:347–357.

Gotelli, M. M., B. G. Galati, and D. Medan. 2010. Structure of the stigma and style in sunflower (*Helianthus annuus* L.). Biocell 34:133–138.

Grant, J. W. A., I. L. Girard, C. Breau, and L. K. Weir. 2002. Influence of food abundance on competitive aggression in juvenile convict cichlids. Animal Behaviour 63:323–330.

Grant, J. W. A., and R. T. Guha. 1993. Spatial clumping of food increases its monopolization and defense by convict cichlids, *Cichlasoma nigrofasciatum*. Behavioral Ecology 4:293–296.

Greenberg, N. 1977. An ethogram of the blue spiny lizard, *Sceloporus cyanogenys* (Reptilia, Lacertilia, Iguanidae). Journal of Herpetology 11:177.

Grime, J. P., and J. M. L. Mackey. 2002. The role of plasticity in resource capture by plants. Evolutionary Ecology 16:299–307.

Gruntman, M., D. Groß, M. Májeková, and K. Tielbörger. 2017. Decision-making in plants under competition. Nature Communications 8:2235.

Gundel, P., R. Pierik, L. Mommer, and C. Ballaré. 2014. Competing neighbors: light perception and root function. Oecologia 176:1–10.

Hall, A. B., U. Blum, and R. C. Fites. 1982. Stress modification of allelopathy of *Helianthus annuus* L. debris on seed germination. American Journal of Botany 69:776–783.

Harper, J. L. 1985. Modules, branches, and the capture of resources. Pages 1–33 Population biology and evolution of clonal organisms. Yale University Press, New Haven.

Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytologist 162:9–24.

Hodge, A., D. Robinson, B. S. Griffiths, and A. H. Fitter. 1999. Why plants bother: root proliferation results in increased nitrogen capture from an organic patch when two grasses compete. Plant, Cell & Environment 22:811–820.

Hutchings, M. J., and H. de Kroon. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Pages 159–238 *in* M. B. and A. H. Fitter, editor. Advances in Ecological Research. Academic Press.

Jackson, R. B., and M. M. Caldwell. 1989. The timing and degree of root proliferation in fertile-soil microsites for three cold-desert perennials. Oecologia 81:149–153.

Jackson, R. B., and M. M. Caldwell. 1993. The scale of nutrient heterogeneity around individual plants and its quantification with geostatistics. Ecology 74:612–614.

Jackson, R. B., J. H. Manwaring, and M. M. Caldwell. 1990. Rapid physiological adjustment of roots to localized soil enrichment. Nature 344:58.

Jensen, E. L., L. M. Dill, and J. F. Cahill. 2011. Applying behavioral-ecological theory to plant defense: light-dependent movement in *Mimosa pudica* suggests a trade-off between predation risk and energetic Reward. The American Naturalist 177:377–381.

Kalueff, A. V., M. Gebhardt, A. M. Stewart, J. M. Cachat, M. Brimmer, J. S. Chawla, C. Craddock, E. J. Kyzar, A. Roth, S. Landsman, S. Gaikwad, K. Robinson, E. Baatrup, K. Tierney, A. Shamchuk, W. Norton, N. Miller, T. Nicolson, O. Braubach, C. P. Gilman, J. Pittman, D. B. Rosemberg, R. Gerlai, D. Echevarria, E. Lamb, S. C. F. Neuhauss, W. Weng, L. Bally-Cuif, and H. Schneider. 2013. Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. Zebrafish 10:70–86.

Karban, R. 2008. Plant behaviour and communication. Ecology Letters 11:727–739.

Kawano, S., and Y. Nagai. 1986. Regulatory mechanisms of reproductive effort in plants 1. Plasticity in reproductive energy allocation and propagule output of *Helianthus annuus* L. (Compositae) cultivated at varying densities and nitrogen levels. Plant Species Biology 1:1–18.

Kembel, S. W., and J. F. Cahill Jr. 2005. Plant phenotypic plasticity belowground: a phylogenetic perspective on root foraging trade-offs. The American Naturalist 166:216–230.

Kramer, D., V. Römheld, E. Landsberg, and H. Marschner. 1980. Induction of transfer-cell formation by iron deficiency in the root epidermis of *Helianthus annuus* L. Planta 147:335–339.

Křivan, V., R. Cressman, and C. Schneider. 2008. The ideal free distribution: A review and synthesis of the game-theoretic perspective. Theoretical Population Biology 73:403–425.

de Kroon, H., H. Huber, J. F. Stuefer, and J. M. Van Groenendael. 2005. A modular concept of phenotypic plasticity in plants. New Phytologist 166:73–82.

Kutschera, U., and W. R. Briggs. 2016. Phototropic solar tracking in sunflower plants: an integrative perspective. Annals of Botany 117:1–8.

Lankinen, Å., and K. Karlsson Green. 2015. Using theories of sexual selection and sexual conflict to improve our understanding of plant ecology and evolution. AoB PLANTS 7.

Lentz, D. L., M. D. Pohl, J. L. Alvarado, S. Tarighat, and R. Bye. 2008. Sunflower (*Helianthus annuus* L.) as a pre-Columbian domesticate in Mexico. Proceedings of the National Academy of Sciences 105:6232–6237.

Levitis, D. A., W. Z. Lidicker, and G. Freund. 2009. Behavioural biologists do not agree on what constitutes behaviour. Animal Behaviour 78:103–110.

Lima, S. L., and L. M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68:619–640.

Lima, S. L., T. J. Valone, and T. Caraco. 1985. Foraging-efficiency-predation-risk trade-off in the grey squirrel. Animal Behaviour 33:155–165.

Mahall, B. E., and R. M. Callaway. 1991. Root communication among desert shrubs. Proceedings of the National Academy of Sciences 88:874–876.

Mahall, B. E., and R. M. Callaway. 1992. Root communication mechanisms and intracommunity distributions of two Mojave Desert shrubs. Ecology 73:2145–2151.

Maina, G. G., J. S. Brown, and M. Gersani. 2002. Intra-plant versus inter-plant root competition in beans: avoidance, resource matching or tragedy of the commons. Plant Ecology 160:235–247.

Martin, P., and P. Bateson. 2007. Measuring Behaviour: An Introductory Guide. Third edition. Cambridge University Press, New York.

McNickle, G. G., and J. S. Brown. 2014. An ideal free distribution explains the root production of plants that do not engage in a tragedy of the commons game. Journal of Ecology 102:963–971.

McNickle, G. G., and J. F. Cahill. 2009. Plant root growth and the marginal value theorem. Proceedings of the National Academy of Sciences 106:4747–4751.

McNickle, G. G., M. K. Deyholos, and J. F. Cahill. 2016. Nutrient foraging behaviour of four co-occurring perennial grassland plant species alone does not predict behaviour with neighbours. Functional Ecology 30:420–430.

McNickle, G. G., C. C. St Clair, and J. F. Cahill. 2009. Focusing the metaphor: plant root foraging behaviour. Trends in Ecology & Evolution 24:419–426.

Mercer, K. L., H. M. Alexander, and A. A. Snow. 2011. Selection on seedling emergence timing and size in an annual plant, *Helianthus annuus* (common sunflower, Asteraceae). American Journal of Botany 98:975–985.

Metlen, K. L., E. T. Aschehoug, and R. M. Callaway. 2009. Plant behavioural ecology: dynamic plasticity in secondary metabolites. Plant, Cell & Environment 32:641–653.

Minorsky, P. V. 2019. The functions of foliar nyctinasty: a review and hypothesis. Biological Reviews 94:216–229.

Mommer, L., J. van Ruijven, C. Jansen, H. M. van de Steeg, and H. de Kroon. 2012. Interactive effects of nutrient heterogeneity and competition: implications for root foraging theory? Functional Ecology 26:66–73.

Monaghan, P., and N. B. Metcalfe. 1985. Group foraging in wild brown hares: effects of resource distribution and social status. Animal Behaviour 33:993–999.

Motte, H., and T. Beeckman. 2019. The evolution of root branching: increasing the level of plasticity. Journal of Experimental Botany 70:785–793.

Newman, J. A., and T. Caraco. 1987. Foraging, predation hazard and patch use in grey squirrels. Animal Behaviour 35:1804–1813.

O'Brien, E. E., and J. S. Brown. 2008. Games roots play: effects of soil volume and nutrients. Journal of Ecology 96:438–446.

Peng, Y., K. J. Niklas, and S. Sun. 2012. Do plants explore habitats before exploiting them? An explicit test using two stoloniferous herbs. Chinese Science Bulletin 57:2425–2432.

Pereira, M. L., V. O. Sadras, W. Batista, J. J. Casal, and A. J. Hall. 2017. Light-mediated self-organization of sunflower stands increases oil yield in the field. Proceedings of the National Academy of Sciences 114:7975–7980.

Robinson, D. 1996. Resource capture by localized root proliferation: why do plants bother? Annals of Botany 77:179–186.

Robinson, D. 2002. Root proliferation, nitrate inflow and their carbon costs during nitrogen capture by competing plants in patchy soil. Pages 41–50 *in* D. S. Powlson, G. L. Bateman, K. G. Davies, J. L. Gaunt, and P. R. Hirsch, editors. Interactions in the Root Environment: An Integrated Approach. Springer Netherlands, Dordrecht.

Robinson, D., A. Hodge, B. S. Griffiths, and A. H. Fitter. 1999. Plant root proliferation in nitrogen-rich patches confers competitive advantage. Proceedings of the Royal Society B: Biological Sciences 266:431–435.

Romheld, V., C. Muller, and H. Marschner. 1984. Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiology 76:603–606.

Roseland, C. R., and T. J. Grosz. 1997. Induced responses of common annual sunflower *Helianthus annuus* L. from geographically diverse populations and deterrence to feeding by sunflower beetle. Journal of Chemical Ecology 23:517–542.

Ross, M. A., and J. L. Harper. 1972. Occupation of biological space during seedling establishment. The Journal of Ecology 60:77.

Sadras, V. O., A. J. Hall, N. Trapani, and F. Vilella. 1989. Dynamics of rooting and root-length: leaf-area relationships as affected by plant population in sunflower crops. Field Crops Research 22:45–57.

Schaffner, J. H. 1898. Observations on the nutation of *Helianthus annuus*. Botanical Gazette 25:395–403.

Schenk, H. J., R. M. Callaway, and B. E. Mahall. 1999. Spatial root segregation: are plants territorial? Pages 145–180 *in* A. H. F. and D. Raffaelli, editor. Advances in Ecological Research. Academic Press.

Schleidt, W. M., G. Yakalis, M. Donnelly, and J. McGarry. 1984. A proposal for a standard ethogram, exemplified by an ethogram of the bluebreasted quail (*Coturnix chinensis*). Zeitschrift für Tierpsychologie 64:193–220.

Schoener, T. W. 1983. Simple models of optimal feeding-territory size: a reconciliation. The American Naturalist 121:608–629.

Semchenko, M., E. A. John, and M. J. Hutchings. 2007. Effects of physical connection and genetic identity of neighbouring ramets on root-placement patterns in two clonal species. New Phytologist 176:644–654.

Semchenko, M., S. Saar, and A. Lepik. 2014. Plant root exudates mediate neighbour recognition and trigger complex behavioural changes. New Phytologist 204:631–637.

Semchenko, M., K. Zobel, A. Heinemeyer, and M. J. Hutchings. 2008. Foraging for space and avoidance of physical obstructions by plant roots: a comparative study of grasses from contrasting habitats. New Phytologist 179:1162–1170.

Shemesh, H., A. Arbiv, M. Gersani, O. Ovadia, and A. Novoplansky. 2010. The effects of nutrient dynamics on root patch choice. PLoS ONE 5:e10824.

Shirley, H. L. 1929. The influence of light intensity and light quality upon the growth of plants. American Journal of Botany 16:354–390.

Silvertown, J. 2004. Plant coexistence and the niche. Trends in Ecology & Evolution 19:605–611.

Silvertown, J., and D. M. Gordon. 1989. A framework for plant behavior. Annual Review of Ecology and Systematics 20:349–366.

Sirot, E. 2000. An evolutionarily stable strategy for aggressiveness in feeding groups. Behavioral Ecology 11:351–356.

Stefansson, B. R. 2013, May 4. Oilseed crops. Historica Canada, Toronto.

Stephens, D. W. 1987. On economically tracking a variable environment. Theoretical Population Biology 32:15–25.

Trewavas, A. 2014. Plant Behaviour and Intelligence. Oxford University Press.

Trinder, C., R. Brooker, H. Davidson, and D. Robinson. 2012. Dynamic trajectories of growth and nitrogen capture by competing plants. New Phytologist 193:948–958.

Appendix

Supplemental material to Chapter 3

(a) Additional details of experimental arenas

The total volume of soil used in the arenas was 275 mL. High nutrient patches were always composed of 25 mL of the manure-enriched high nutrient patch soil, which was a 1 : 1 mixture of background soil : composted manure (0.5-0.5-0.5 minimum analysis, Premier Tech Home & Garden, Mississauga, ON). Patches spanned the height and depth of the boxes, and 1.5 cm of the arena horizontally (see Chapter 3, Figure 3-1). The rest of the box was filled with the background soil, composed of a 3:1 mixture of washed sand : topsoil, BURNCO, Edmonton, AB, 250 mL for treatments with one patch, and 225 mL for treatments with two patches.

Relative to the focal plant as seen from the front of the experimental arena, patch and neighbour location were assigned within treatments and runs such that half of the patches and half of the neighbours were located on the right, and the other half were located on the left. The effect of relative location (left/right) on root length was tested using paired t-tests on otherwise symmetrical treatments and found to be statistically insignificant in those treatments (Figure 5-3) and so were excluded from further analysis.

(b) Ruleset for root tracing

Rules were based on observed patterns of root growth (Table 5-1). Plants produced a tap root, then lateral roots extending diagonally downward from the top part of the tap root, then lateral roots continued to be produced along the tap root and lateral roots. Especially in the early stages of growth, lateral roots were typically angled diagonally downward from the tap root of plants, so this was used to identify roots. Later in the growing period (after day 10) root growth became more complex so roots were assigned identity primarily based on visible or inferred attachment to previously identified roots. For roots that could not be assigned identity (no attachment to other roots or clear contextual cues), the resulting root length was attributed to focal or neighbour plants based on the percentage of focal vs. neighbour roots at the timepoint and in the area of the soil that they grew in. We validated this method of allocating bulk root length to individuals by comparing digitized, traced root length assigned to focal plant at harvest (day 15) to the root length of roots that were carefully identified and excavated during the harvest (day 15), therefore confirming their identity (Figure 5-2; see **Excavation at harvest**, below).

(c) Excavation at harvest

The front pieces of plexiglass of the experimental arenas were removed. We removed sections of root that were within the areas we measured throughout the experiment, that belonged to the focal plant, and that were visible through the front of the experimental arena. Because much of the root system was obscured by soil, every piece of root removed during this process was followed back to the taproot to confirm its identity, gently removing soil from the roots as necessary. We carefully cut the roots, removed with tweezers, washed, scanned in water, and assessed for root length using a root measurement software (WinRHIZO 2015a, Regent Instruments Inc., QC, Canada). Root pieces were then dried at 65℃ for 48 hours and weighed to be included in total biomass measures. We only measured focal root length this way because cutting the neighbour root system would have made separating and identifying the remaining root systems impossible (neighbour root system needed to remain intact to successfully separate root systems). Root length measured this way was highly correlated with traced root length at the endpoint of the experiment (Figure 5-2). The strong correlation between the two methods shows that the method of assigning roots to individuals in the measured areas (Table 5-1) is accurate, despite not always being able to visually trace roots back to individuals during digitization of the roots via tracing.

(d) Growth in homogenous soils without patches

Homogeneous treatments were used to establish responses of focal plants to neighbour presence and neighbour distance from soil when grown in homogeneous soils with no patches, although they were not used in our final analysis. Because the treatments with two high nutrient patches had higher pot-level nutrients than those with only one high nutrient patch, we used two different types of soil: one with the equivalent of one patch mixed homogeneously into the soil ("LowHom-", comparable nutrient levels to one patch treatments), and one with the equivalent of two patches mixed homogeneously into the soil ("HiHom-", comparable nutrient levels to two patch treatments). Focal plants were either grown alone ("LowHom-Alone", n=15; "HiHom-Alone", n = 12) or with a neighbour the same distance from the focal plant as in the other treatments ("LowHom-Neighbour", n = 14; "HiHom-Neighbour", n = 12) (Figure 5-1). Experimental conditions, behavioural assays, and endpoint measures were as outlined in Chapter 3.

To assess the effect of neighbour presence and location on root length in homogenous soil we used similar general linear mixed models (separate for high homogenous and low homogenous soil) as were used to assess the treatments with two patches, with treatment (alone or with neighbour in homogenous soils), soil area (near vs. opposite neighbours relative to focal plant), days since planting, and all interaction terms as fixed effects. As in treatments with two patches, the two soil areas in each treatment were identical in terms of nutrient composition, and the key difference was whether they were equidistant to neighbours or farther away from neighbours/closer to focal plants. To compare the treatments with neighbours to those grown alone, the two sides of the soil for individuals grown alone were randomly assigned to either soil area for comparison. As expected, there were no significant differences between the two sides arbitrarily assigned this way. We then separated our data first by treatment, then by soil area, and ran separate general linear mixed models.

Responses to neighbour location in homogenous soil were not the same as for plants grown in patchy soil (Table 5-5; Table 5-8), indicating support for the integration by plants of information about both high nutrient patches and neighbours in plant root foraging decisions. In the low homogenous treatment with comparable nutrient levels as the treatments with one high quality patch, the effect of treatment (neighbour presence/absence in homogenous soil) never impacted root foraging decisions in soil that focal plants were in closer proximity to (df = 1,27, F = 2.06, p = 0.1929; Table 5-7; Figure 5-6), indicating that plants do not increase use of soil that they are in closer proximity to when soil is lower in nutrients. This contrasts with the significant increase in use of a high quality patch that plants were in closer proximity to relative to a competitor (see Chapter 3, Figure 3-3).

Table 5-1. Ruleset for root tracing with rules and justification for rule if necessary.

Rule	**Justification**
Tap root	
Root that are visibly attached to the tap root	
Root not visibly attached to an identified root, but aligned such that: 1) There is a clear, linear path to an identified root. 2) There is no clear, linear path to a root of another identity.	
Roots within 1 cm of planting location and <2.5 cm from the top of pot	Because of gravitropic root growth, unlikely to belong to other plant
By day 10, root angled diagonally down from location of planting toward other plant	Because of gravitropic root growth, unlikely to belong to other plant
By day 5 of growth, root on the opposite side of a neighbour	Unlikely that a plant has reached this area of the soil by day 5 of growth
By day 10, < 3 cm from point of planting, <3.5 cm from top of box, diagonally down from point of planting	Because of gravitropic root growth, unlikely to belong to other plant
Connection obscured, but root resembles in angle, size, direction of growth other nearby roots that are assigned an identity/connection not obscured.	

Table 5-2. Total N (µg/g soil), calculated using KCl extraction and colorimetric analysis for nitrate and ammonium, of the four different soil types used. Soil was sampled during the third run of the experiment and dried at room temperature before analysis.

Soil type	Technical replicates (n)	Mean total N (µg/g soil)	SEM total N (µg/g soil)
Background	4	19.91	0.71
Patch	5	71.04	0.96
HiHom	5	23.85	0.68
LowHom	5	21.68	1.01

Table 5-3. Results of linear mixed model testing the effects of treatment (1P-Sole, 1P-Equal, or 1P-Closer; see Chapter 3, Figure 3-1), soil area (high nutrient patch or background soil), day (9-15), and their interactions on root length. Plant identity was included as a random effect. To tease apart the 3-way interactions, we ran separate models to assess the effect of soil type within treatments and the effect of treatments within soil type (see Chapter 3, Table 3-1, Table 3-2).

Source	**df**	**F-value**	**p-value**
Treatment	2,36	6.98	0.0027
Day	6,468	58.11	<0.0001
Soil area	1,468	325.06	<0.0001
Treatment * Day	12,468	1.99	0.0238
Treatment * Soil area	2,468	136.26	<0.0001
Day * Soil area	6,468	5.64	<0.0001
Treatment * Soil area * Day	12,468	4.08	<0.0001

Table 5-4. Results of linear mixed model testing effect of treatment (2P-Sole and 2P-Shared), soil area (equidistant to or farther from neighbour/closer to focal plant), day (9-15) and their interaction. Plant identity was included as a random effect. To tease apart the 3-way interactions, we ran separate models to assess the effect of soil area within treatments and the effect of treatments within soil area (see Chapter 3, Table 3-3, Table 3-4).

Source	df	F-value	p-value
Treatment	1,21	0.73	0.4012
Day	6,273	96.56	<0.0001
Soil area	1,273	32.03	<0.0001
Treatment * Day	6,273	1.54	0.1643
Treatment * Soil area	1,273	59.09	<0.0001
Day * Soil area	6,273	3.09	0.0061
Treatment * Soil area * Day	6,273	3.65	0.0017

Table 5-5. Results of linear mixed model testing effect of neighbour presence and distance of soil to neighbour on root length in low homogenous soil. Treatment (LowHom-Alone vs. LowHom-Neighbour), soil area (equidistant to or farther from neighbour/closer to focal plant), day (9-15) and their interaction were included as fixed effects, and plant identity was included as a random effect. We ran separate models to assess the effect of soil area within treatments and the effect of treatments within soil area.

Source	**df**	**F-value**	**p-value**
Treatment	1,27	0.53	0.4735
Day	6,351	56.63	0.0000
Soil area	1,351	111.32	0.0000
Treatment * Day	6,351	0.04	0.9997
Treatment * Soil area	1,351	140.27	0.0000
Day * Soil area	6,351	4.37	0.0003
Treatment * Soil area * Day	6,351	1.91	0.0784

Table 5-6. Results of two separate linear mixed models testing soil use by plants in low homogenous soil with soil area (equidistant to neighbour or further from neighbour/closer to focal plant), day (9-15), and their interaction as fixed effects, plant identity as a random effect, and root length as the response variable. Models were run within treatments to test whether plants grown alone (LowHom-Alone) invested equal amounts of roots on both sides, as expected, and whether plants changed investment contingent on distance of soil to neighbour within the LowHom-Neighbour treatment.

	LowHom-Alone			**LowHom-Neighbour**		
Source	**df**	**F-value**	**p-value**	**df**	**F-value**	**p-value**
Soil area	1,182	0.88	0.3504	1,169	239.07	<0.0001
Day	6,182	30.45	<0.0001	6,169	26.36	<0.0001
Soil area * day	6,182	0.29	0.9390	6,169	5.72	<0.0001

Table 5-7. Results of two separate linear mixed models testing effect of social environment (neighbour plant present or absent) within soil types. Treatment (LowHom-Alone or LowHom-Neighbour), day (9-15), and their interaction were fixed effects, plant identity was included as a random effect, and root length was the response variable. Models were run within soil types to compare use of soil by plants grown with neighbours to use by plants grown alone.

		Soil same side as neighbour		**Soil opposite neighbour**	
Source	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Treatment	1,27	5.59	0.0255	2.06	0.1629
Day	6,162	32.37	<0.0001	115.33	<0.0001
Treatment * Day	6,162	2.36	0.0328	2.18	0.0472

Table 5-8. Results of linear mixed model testing effect of neighbour presence and distance of soil to neighbour on root length in high homogenous soil. Treatment (HiHom-Alone and HiHom-Neighbour), soil area (equidistant to or farther from neighbour/closer to focal plant), day (9-15) and their interaction were included as fixed effects, and plant identity was included as a random effect. We ran separate models to assess the effect of soil area within treatments and the effect of treatments within soil area.

Source	**df**	**F-value**	**p-value**
Treatment	1,22	0.16	0.6959
Day	6,286	28.42	<0.0001
Soil area	1,286	20.89	<0.0001
Treatment * Day	6,286	0.31	0.9328
Treatment * Soil area	1,286	43.84	<0.0001
Day * Soil area	6,286	1.10	0.3611
Treatment * Soil area * Day	6,286	0.29	0.9434

Table 5-9. Results of two separate linear mixed models testing soil use by plants in high homogenous soil with soil area (equidistant to neighbour or further from neighbour/closer to focal plant), day (9-15), and their interaction as fixed effects, plant identity as a random effect, and root length as the response variable. Models were run within treatments to test whether plants grown alone (HiHom-Alone) invested equal amounts of roots on both sides, as expected, and whether plants changed investment contingent on distance of soil to neighbour within the HiHom-Neighbour treatment.

	HiHom-Alone			**HiHom-Neighbour**		
Source	**df**	**F-value**	**p-value**	**df**	**F-value**	**p-value**
Soil area	1,143	3.09	0.0810	1,143	47.47	<0.0001
Day	6,143	16.98	<0.0001	6,143	13.02	<0.0001
Soil area * Day	6,143	0.22	0.9692	6,143	0.94	0.4700

Table 5-10. Results of two separate linear mixed models testing effect of social environment (neighbour plant present or absent) within soil types. Treatment (HiHom-Alone or HiHom-Neighbour), day (9-15), and their interaction were fixed effects, plant identity was included as a random effect, and root length was the response variable. Models were run within soil types to compare use of soil by plants grown with neighbours to use by plants grown alone.

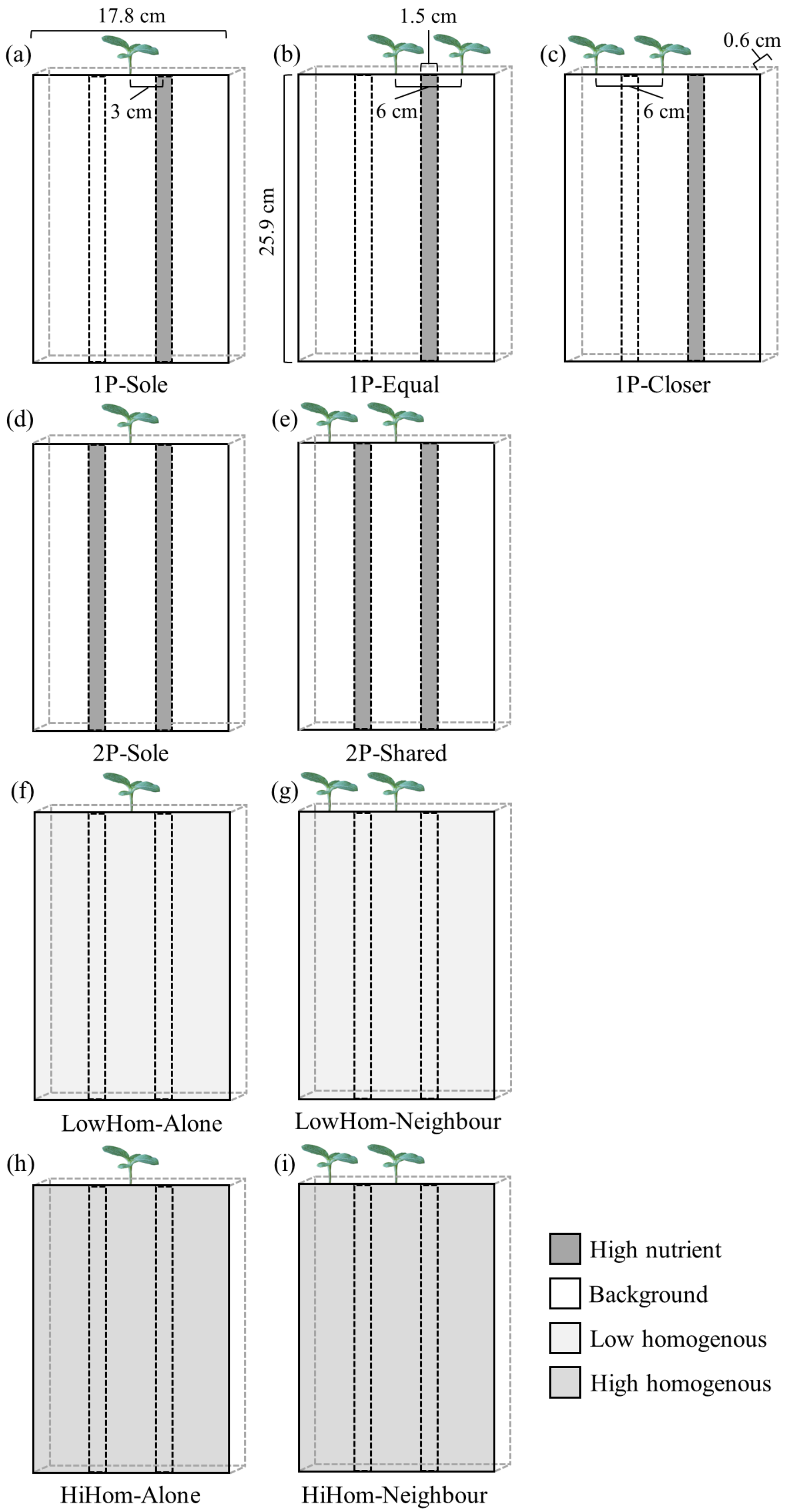
		Soil equidistant to neighbour		**Soil farther from neighbour**	
Source	**df**	**F-value**	**p-value**	**F-value**	**p-value**
Treatment	1,22	0.76	0.3934	2.56	0.1240
Day	6,132	34.36	<0.0001	80.63	<0.0001
Treatment * Day	6,132	0.03	0.9999	2.35	0.0342

Table 5-11. Total biomass (mg) of individual focal and neighbour plants in different treatments. *A priori* comparisons were performed on plants adjacent in a row or column and shaded in the same way (e.g. focal plants in 1P-Sole, 1P-Closer and 1P-Equal treatments; focal and neighbour plants in each of the treatments). No significant differences between any comparisons were found.

Treatment	**n**	**Mean focal biomass (mg)**	**SEM focal biomass (mg)**	**Mean neighbour biomass (mg)**	**SEM neighbour plant biomass (mg)**
1P-Sole	14	110.4	7.8		
1P-Closer	12	124.9	8.2	117.6	11.0
1P-Equal	13	108.2	5.5	123.4	9.0
2P-Sole	12	133.9	8.2		
2P-Shared	11	111.5	8.2	111.1	8.5
LowHom-A	15	104.2	5.4		
LowHom-N	14	102.6	9.4	111.0	7.6
HiHom-A	12	136.7	7.9		
HiHom-N	12	136.2	9.1	117.1	8.0

Table 5-12. Per cent nitrogen (% N) in two most recently expanded leaves of individual focal and neighbour plants in different treatments. *A priori* comparisons were performed on plants i) shaded in the same way and ii) adjacent in a single row or column. Significant differences among these groups are indicated by letters in brackets.

Treatment	**n**	**Mean focal leaf % N**	**SEM focal leaf % N**	**Mean neighbour leaf % N**	**SEM % neighbour leaf % N**
1P-Sole	14	3.04 (a)	0.08		
1P-Closer	12	2.84 (ab)	0.08	3.025011 (z)	0.102349
1P-Equal	13	2.68 (b)	0.05	2.741042 (x)	0.086138
2P-Sole	12	3.10	0.06		
2P-Shared	11	2.97 (q)	0.11	2.601808 (p)	0.075135
LowHom-A	15	2.86	0.08		
LowHom-N	14	2.76	0.09	2.596961	0.050744
HiHom-A	12	2.84	0.10		
HiHom-N	12	2.72	0.07	2.596073	0.059985



(Caption on next page)

(Figure on previous page)

Figure 5-1. Schematic of treatments and growing dimensions of the experimental arenas, including treatments with homogenous soil. Treatment names are below each diagram. Focal plants were always placed in the center of the arena so that focal plant root growth was not affected by encountering one edge before the other. Neighbour plants when present were always placed 6 cm from the focal plant and 3 cm from the edge of the experimental arena. Shading represents the relative nutrient content of the soils used. Low homogeneous soil had the equivalent of one patch mixed into background soil and high homogenous soil had the equivalent of two patches mixed into background soil. Root length was measured through the front, transparent panel of the boxes, and the areas of the soil where root length was measured are highlighted with black dashed lines. These correspond to the area of the nutrient hotspots where present.

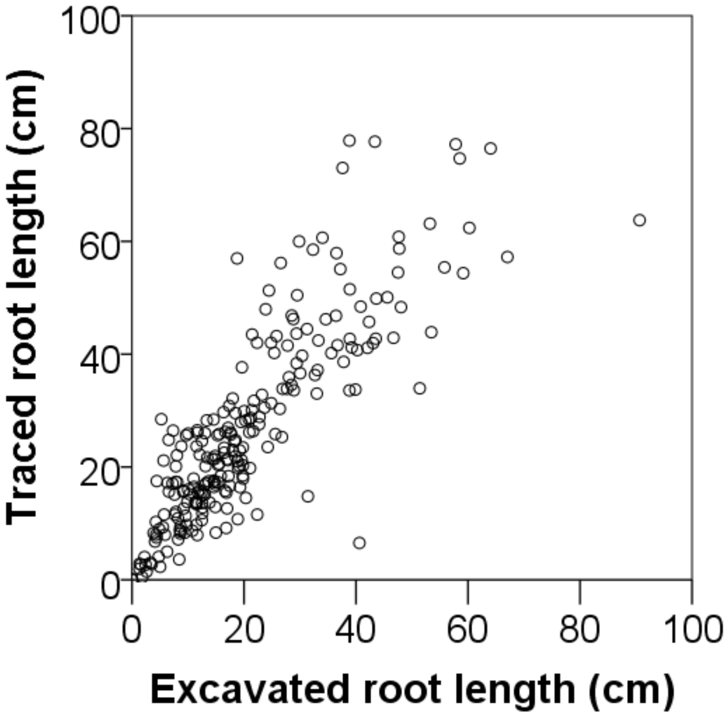


Figure 5-2. Root length (cm) of focal plants in the measured areas of the soil at harvest (day 15), measured in centimeters using two different methods. “Excavated” roots were removed at harvest after being carefully followed back to the taproot of the focal plant to confirm their identity, scanned, and measured, and “traced” roots are those that were digitized and identified from images of the roots scanned through the front of the experimental arenas taken throughout the growing period.

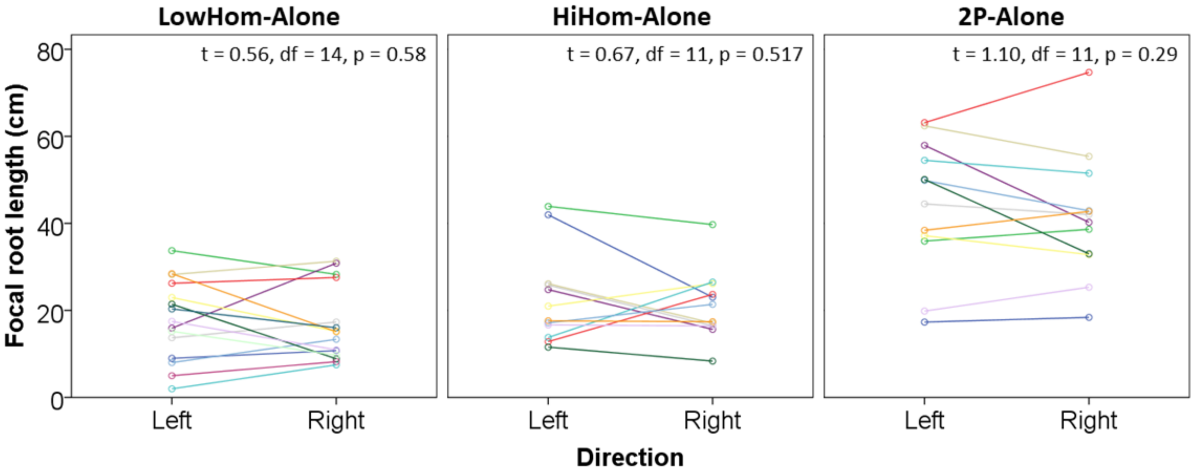


Figure 5-3. Right vs. left focal root length in centimeters of treatments with symmetrical soil distributions and no neighbours at harvest time (day 15). Each colour represents a single individual plant in a single box. Focal root length did not differ significantly between the left and right sides of these treatments (results of paired t-tests for each treatment shown in figure), so direction (left/right) was not included as a factor in the model.

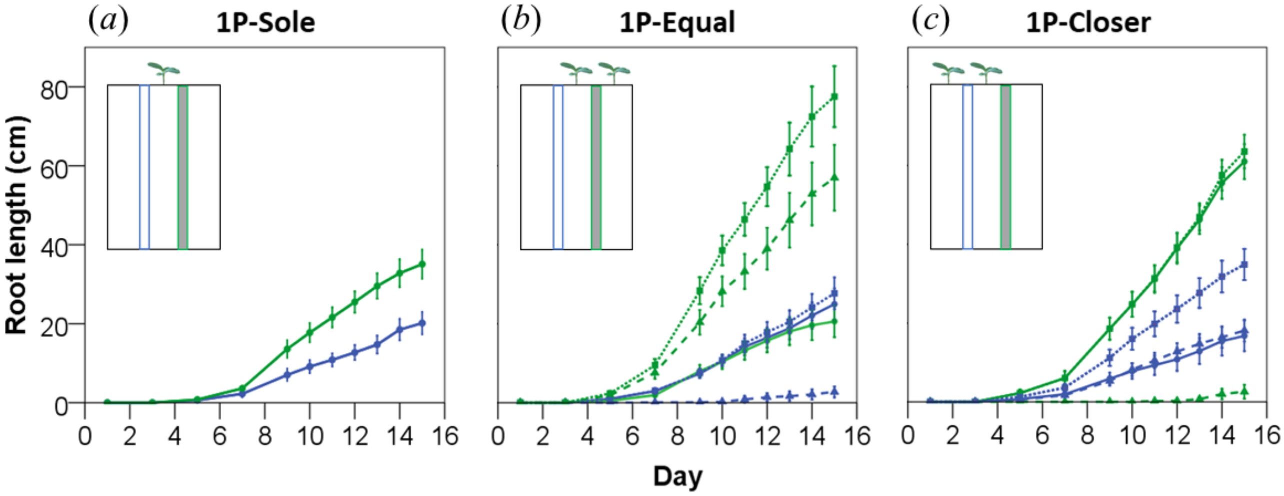


Figure 5-4. Mean traced focal, neighbour, and total plant root length (± SEM) in centimeters in two areas of the soil over fifteen days for the three treatments containing a single high nutrient patch. Green represents focal plant root length in the high nutrient patch, and blue represents root length in the low nutrient soil on the other side of the plant. Focal plant root length is represented by circles and solid lines, neighbour plant root length with triangles and dashed lines; and total root length (focal + neighbour root length) with squares and dotted lines. The measured areas are shown in the inlaid schematics for each treatment.

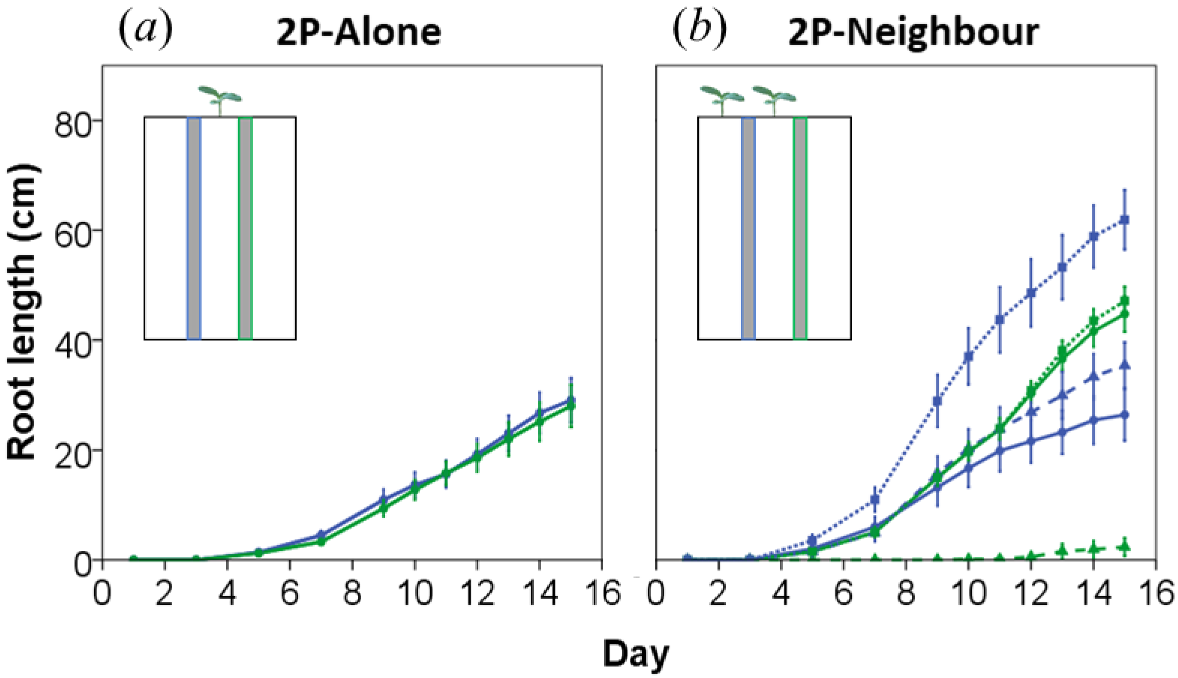


Figure 5-5. Mean traced focal, neighbour, and total plant root length (± SEM) in centimeters in two areas of the soil over fifteen days of growth for the two treatments containing two high nutrient patches. Focal plant root length is represented by circles and solid lines; neighbour plant root length by triangles and dashed lines; and total root length (focal + neighbour root length) by squares and dotted lines. The measured areas are shown in the inlaid schematics for each treatment.

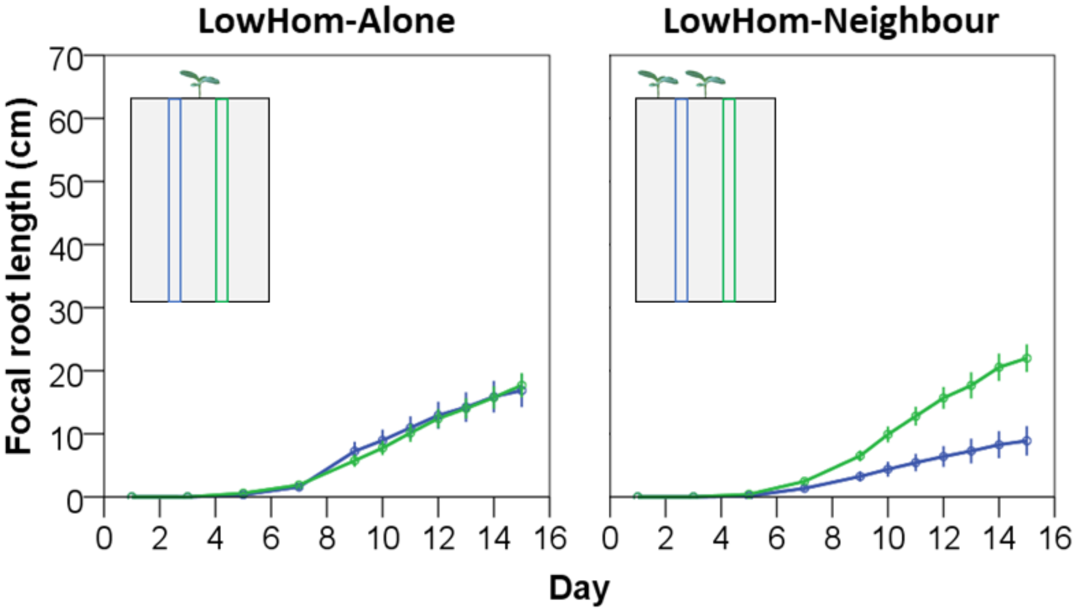


Figure 5-6. Mean traced focal plant root length (± SEM) in centimeters in two areas of the soil over fifteen days of growth for two treatments with plants grown in low homogeneous soil, with the equivalent of one patch mixed into background soil. The measured areas are colour coded and shown in the inlaid schematics for each treatment. Only focal plant root length is shown here.

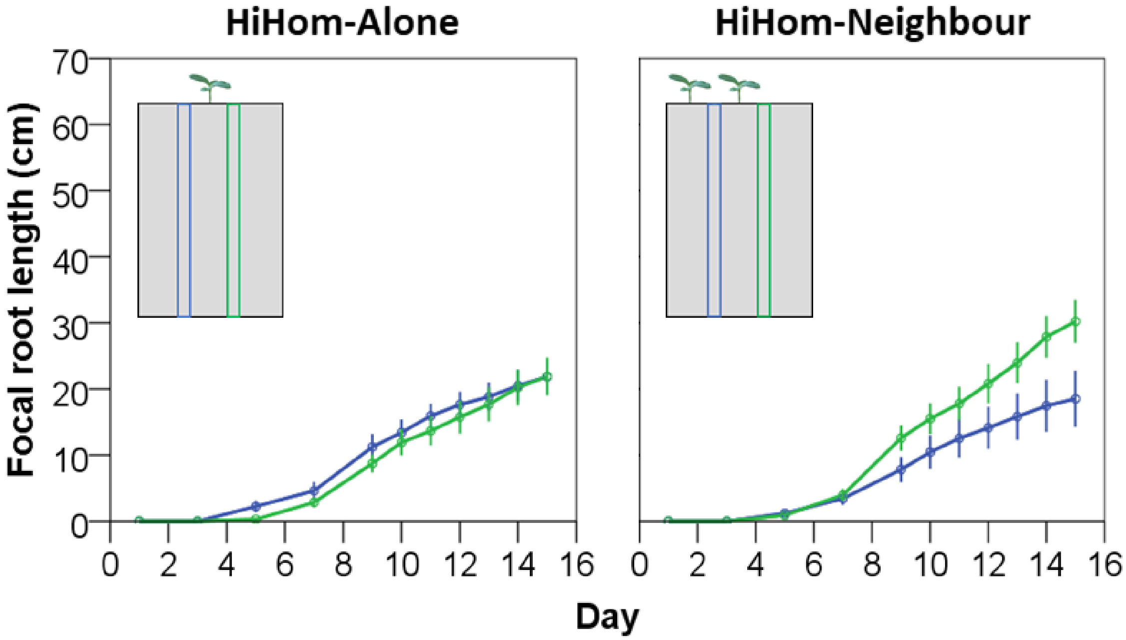


Figure 5-7. Mean traced focal plant root length (± SEM) in centimeters in two areas of the soil over fifteen days of growth for two treatments with plants grown in high homogeneous soil, with the equivalent of two patches mixed into background soil. The measured areas are colour coded and shown in the inlaid schematics for each treatment. Only focal plant root length is shown here.