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Relationships among diverse root foraging behaviours: understanding plant behavioural types

by

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ABSTRACT

Behaviours capture the functional response of plants to environmental factors. I explore behaviours for twenty co-occurring grassland species in response to common belowground environmental factors (competition, mycorrhizae, heterogeneous and high nutrients) and their relationship to plant outcomes. First, detailed plant response to neighbours was observed using a specialized apparatus which allowed for visualization of the root system in situ. Species varied in response from strongly aggregating to strongly segregating roots by adjusting overall size and occupation of the soil and/or by altering allocation to roots and the specific placement of roots. In the second study, associations among response to nutrients, mycorrhizae, neighbours, foraging precision, and soil exploration behaviours created behavioural types along two axes: assertiveness and focus. However, neither individual behaviours nor behavioural types influenced plant growth overall. Together, these results suggest the complexity of belowground behaviours and the need to consider associations among behaviours across contexts.

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CHAPTER 1 GENERAL INTRODUCTION

Plants share demand for a common set of resources such as water, mineral nutrients, and light, but species vary greatly in the strategies used to obtain these. Many studies have sought to identify these different strategies (see Table 1.1 for definitions of common terms; Grime 1977; Southwood 1988; Taylor *et al.* 1990; Lavorel *et al.* 1997) in order to link environmental factors to species distribution and community composition. Species traits have commonly been used to construct these strategies (Grime *et al.* 1997), but I argue that most of these measures do not capture the functional aspect of plant responses to environmental conditions. For example, specific root length could be measured under nutrient stress and differences among species used to explain growth under those conditions. What this fails to account for is potential plasticity that can be exhibited in response to the environmental factor of interest (Callaway *et al.* 2003). I propose looking instead at behaviours, what a plant does in response to some change in environment (Silvertown and Gordon 1989), which may provide more complete understanding of the differences in species outcomes. In the above example, species have the potential to change specific root length in response to nutrient stress. Whether or not a species does exhibit a change, and to what degree, however, may be much more informative that the static measure of specific root length at one point in time.

Expanding from the idea of a single behaviour in response to a single environmental factor, what species do across different environmental contexts is likely to be related in some manner. Animal ecologists have recognized that an organism can exhibit consistent variation in behavioural responses across multiple contexts and refer to these correlations as behavioural syndromes (Sih *et al.* 2004; Bell 2007). It is likely that similar correlations exist between plant behaviours. By quantifying these relationships, species behavioural types within a syndrome may be used to explain plant outcomes under various environmental factors. Rather than looking at behaviours in isolation, behavioural types will account for the correlations or constraints imposed by behaviours in different contexts.

In this thesis, I will measure behaviours related to belowground environmental factors such as nutrients, neighbours, and root symbionts for twenty co-occurring grassland plant species. The majority of plant biomass occurs belowground in grassland systems, which means that the majority of plant interactions and behaviours will also occur in the soil (Jackson *et al.* 1996; Cahill 2003). Variation is already known to exist between species in terms of root exploration of the soil (Comas and Eissenstat 2004), nutrient foraging (Kembel and Cahill 2005), ability to capitalize on increased nutrient availability (Chapin 1980), response to neighbour plants (Litav and Harper 1967; Schenk *et al.* 1999; Gersani *et al.* 2001), and mycorrhizal associations (Klironomos 2003). I further explore how variation in behaviours may relate to plant outcome under these environmental factors, and how behaviours in different environmental contexts may be associated with one another.

Thesis Outline

In Chapter 2 I look at detailed root responses to the presence of neighbours through the use of a specialized apparatus which allows for in situ visualization of the root system. I will identify associations between the different ways in which species respond through soil occupancy, root placement, allocation, and overall biomass. This study will also explore the relationships between root placement plasticity and size of the root system, as well as with the amount of competition experienced by a plant. The specific questions I will address are:

1) In what ways do plants respond to neighbours belowground?
2) Is plasticity in root placement associated with root system size?
3) Is plasticity in root placement associated with the amount of competition experienced?

Chapter 3 will combine information about root responses to neighbours with four other belowground behaviours (foraging precision, soil exploration, shifts in root allocation in response to high nutrients and mycorrhizae) to determine if there are suites of root behaviours correlated across contexts. This will identify potential root behavioural types. I will then determine whether plant growth responses to environmental factors are influenced by individual root behaviours, or by root behavioural types. The specific questions I will address are:

1) Are plant growth responses to environmental factors influenced by individual root behaviours?
2) Are there suites of correlated root behaviours which create root behavioural syndromes?
3) Are plant growth responses to environmental factors influenced by root behavioural type?

Chapter 4 will discuss the findings of Chapters 2 and 3.

TABLE 1.1 Definition of terms.

Term	**Definition**
Behaviour	What a plant does in response to some change in its environment.
Behavioural syndrome	Correlations in behavioural responses across multiple contexts.
Behavioural type	The specific level of behaviours expressed by an individual or species within a behavioural syndrome (eg. more assertive versus less assertive behavioural types).
Plant strategy	A combination of adaptations exhibited by a plant which promote success in certain environments.
Plasticity	The capability of an organism to produce different phenotypes in response to different environments.

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CHAPTER 2 BELOWGROUND NEIGHBOURS - PLANT RESPONSE THROUGH SIZE, SOIL OCCUPANCY, AND PLASTICITY IN ROOT PLACEMENT

INTRODUCTION

The close proximity of plants and the fact that they require the same resources for growth mean that competition is a common interaction experienced by plants. Competition often has a major impact on individual fitness and in turn, may affect species distribution and community composition (Schenk 2006). In grassland systems, the majority of plant biomass occurs belowground meaning that most plant interactions will also take place in the soil (Jackson *et al.* 1996; Cahill 2003). Understanding how species respond to belowground competition and the plasticity of these responses will allow researchers to model the outcomes of competitive interactions.

What has been shown in a number of studies is that plants are able to alter root placement in response to neighbours (Cahill and McNickle 2011; Schenk 2006). However, the type of response found is variable. Studies have found segregation of root systems (reviewed in Schenk *et al*. 1999; Baldwin and Tinker 1972; Brisson and Reynolds 1994; Caldwell *et al*. 1996; Holzapfel and Alpert 2003), overproliferation of roots (Gersani *et al*. 2001; Mania *et al*. 2002; Padilla *et al.* 2013), or simply no response (Litav and Harper 1967; Semchenko *et al*. 2007). Furthermore, neighbour identity may also change the type of root behaviour displayed (Mahall and Callaway 1991; Falik *et al*. 2003; Bartelheimer *et al*. 2006; Fang *et al*. 2013).

Studies usually look at the response to competition in terms of overall biomass with or without a neighbour (Gersani *et al.* 2001). Less is known about specific root placement in the soil since the substrate poses a challenge for such studies. In addition, a shortcoming for many studies is the inability to assign root identity. This often results in individual plant root biomass being represented by an average of the root biomass found between competing plants (Gersani *et al.* 2001; Bartelheimer *et al.* 2006). This assumes that both individuals have the same response which, from the variety of root responses observed in various studies, may be a tenuous assumption. In contrast, methods that study roots in-situ allow the placement and extent of a root system to be quantified (Fang *et al*. 2013). With detailed individual measures, plasticity in the responses can also be determined. What is missing from the literature is an evaluation of multiple species responses using a consistent method that gives such detailed measures for not only size and allocation to roots, but also soil occupancy, and placement of roots.

Predictions exist for when a plant should display plasticity in the placement of roots. Campbell *et al*. (1991) predicted that plasticity (specifically root foraging precision) should be related to the scale of foraging, with a negative correlation existing between the two. They suggest large scale foragers will dominate capture of resources through extensive root systems with little flexibility in placement. In contrast, plants of smaller scale have the ability to finely adjust root distribution. These predictions have had some support (eg. Wijesinghe *et al*. 2001), but many studies have found positive correlations instead (Einsmann *et al*. 1999; Rajaniemi and Reynolds 2004). Kembel and Cahill (2005) found no relationship at all when using a large data set compiled from previously published studies and controlling for phylogenetic relatedness. Kembel *et al*. (2008) conducted a meta-analysis of studies testing the predictions of Campbell *et al*. (1991) and found no support for the trade-off. Of those studies that found a correlation, either positive or negative, examined few species, usually less than ten.

Foraging precision, as used by Campbell *et al*. (1991) is essentially a form of plasticity in the root system. In this study I revisit the prediction of a scale-precision trade-off in the context of placement plasticity in response to neighbours using 20 herbaceous prairie species. Beyond resource foraging, if a trade-off does exist between scale and precision, a similar negative correlation should be found when exploring the ability of a plant to respond to a neighbour through plasticity in its root placement.

In response to mixed evidence for a scale-precision trade-off, de Kroon and Mommer (2006) suggest that one of the main questions that remain unanswered is why variation in root proliferation exists. It is conceivable that the ability to alter root responses would be advantageous in competition. Rajaniemi (2007) found that root foraging precision per se was not related to competitive ability, however precision is root placement plasticity specifically related to resource foraging. The amount of competition experienced by a plant may be related to its ability to alter root placement in response to neighbours as a method of avoiding or pursuing interaction with those neighbours.

This study will quantify the root responses of twenty co-occurring grassland species using an in-situ method. Evaluating a large number of species with one detailed methodology will determine if the variation in response to neighbours found across previous studies is representative. Detailed measures of soil occupancy, root placement and plasticity in all measures will be collected in addition to standard biomass measures for size and allocation to roots. Roots will be visualized throughout growth using a unique window box apparatus which will allow roots of individual plants to be identified and separated. The study will use two neighbour treatments (either a monocot or eudicot) to determine if any of the response measures are dependent on neighbour identity.

Using measures of root system size and plasticity in root placement I will also explore the predictions set out by Campbell *et al*. (1999) that suggest large scale root systems will display little plasticity. If the relationship exists it should be linear with smaller root systems having a high degree of plasticity in root placement which decreases as root system size increases. Plasticity will also be compared to the amount of competition experienced by the focal plant. Plants that are most impacted by competition should benefit from a high degree of root placement plasticity to either avoid the neighbour or pre-empt nutrients by aggregating towards the neighbour.

MATERIALS AND METHODS

Study Species

Twenty co-occurring species were chosen across a range of plant families to capture the breadth of root responses: ten Asteraceae; *Achillea millefolium* L., *Artemesia frigid* Willd., *Artemesia ludoviciana* Nutt., *Erigeron glabellus* Nutt., *Gaillardia aristata* Pursh, *Heterotheca villosa* (Pursh) Shinners, *Solidago missouriensis* Nutt., *Symphyotrichum ericoides* (L.) G.L. Nesom, *Symphyotrichum falcatum* (Lindl.) G.L. Nesom, and *Symphyotrichum laeve* (L.) Á. Löve & D. Löve; five Poaceae; *Bouteloua gracilis* (Kunth) Lag. ex Griffiths, *Bromus inermis* Leyss., *Elymus glaucus* Buckley, *Koeleria macrantha* (Ledeb.) Schult., and *Poa pratensis* L; two Rosaceae; *Drymocallis arguta* Pursh, and *Geum triflorum* Pursh; one Brassicaceae; *Descurainia sophia* (L.) Webb ex Prantl; one Fabaceae; *Astragalus agrestis* Douglas ex G. Don; one Polygonaceae; *Rumex crispus* L. Seed for these species were collected from multiple plants in a native rough fescue prairie at the University of Alberta Kinsella Research Ranch located near Kinsella, Alberta, Canada (53°05 N, 111°33 W). Our research group has an extensive history of research in this location and with the species found there (Lamb and Cahill 2008). Using species abundance data, common species were selected for this study.

In order to obtain a generic measure of response to a neighbour plant, the neighbour species were chosen so as to not naturally co-occur with the twenty focal species. One monocot (*Phleum pratense* L., Poaceae) and one eudicot

(*Lactuca sativa* L. cv. Esmeralda M.I., Asteraceae) species were chosen to test if neighbour identity had an influence on response.

Experimental Set Up

Plants were grown in window boxes made of two pieces of Plexiglas (one black, one clear) and side spacers held together with binder clips (Figure 2.1). Polyester batting fibres and a horizontal bamboo skewer were arranged at the bottom of each window box to prevent soil from falling out while still allowing for drainage. This configuration provided approximately 5 mm x 190 mm x 250 mm of soil space for the plant to grow. The window boxes rested at a 40° angle, with the clear Plexiglas facing down to encourage root growth along this window for visualization. To prevent effects of light on the roots, this clear window was covered with a black plastic sheet held in place with elastic bands.

The window boxes were filled with a homogeneous soil composition of 3:1 sand to topsoil mix as well as approximately ~2% manure. The top 1 cm of each window box was filled with peat moss (Sun Gro Horticulture Canada Ltd) to help maintain soil moisture.

Seeds of the focal species were planted into the centre of the window box (9.5cm from each edge). Neighbour seeds were germinated on moist filter paper. When one of the focal seeds had germinated, the other seeds were removed and a germinated neighbour seed was planted. The neighbour was planted halfway between the focal plant and pot edge, to the right of the focal plant (plants were approximately 4.75cm apart).

The experiment was conducted in a growth room at the University of Alberta Biotron under controlled environmental conditions (16:8 hour light:dark cycle and temperature set at 24°C). Replicates were completed across 5 trials from April 2012 – January 2013. The experiment began when the focal plant germinated and pictures of the root system were taken every 3 days (10 picture sessions total). Due to varying germination success, as well as root visibility in the photos, each species-neighbour combination had between 2 and 7 replicates with most having at least 3 replicates.

After 27 days the boxes were opened and plants removed. Roots of each plant were separated and rinsed free of soil. Roots and shoots were separated, oven dried (48 hours at 70°C), and weighed.

All photos were visually inspected and any replicates with roots not visible were removed from further analysis. Using ArcGIS software by Esri (version 10.1) the root pictures were digitized. Roots were traced with lines, and coded as focal or neighbour plant roots. A vertical line was added starting at the base of each plant to indicate the centre and depth intervals were created by placing horizontal lines at every 10mm of depth starting at the plant base.

Ten response measures were compiled for the focal plant species. Four variables correspond to size measures (aboveground biomass, belowground biomass, total biomass, and total root length), two measure the soil occupancy of a plant (total root system area, and maximum width), and the remaining variables measure the allocation and placement of roots (root:shoot ratio, proportion of root length, proportion of area, and depth of maximum width).

Aboveground biomass

Dry mass of all aboveground tissues measured in grams.

Belowground biomass

Dry mass of all belowground tissues measured in grams.

Total biomass

Combined dry mass of aboveground and belowground plant tissues measured in grams.

Root:shoot ratio

Ratio of belowground biomass to aboveground biomass for a given individual.

Total root length

Total length of roots (measured in millimetres) traced using ArcGIS software and attributed to a given individual plant.

Total root system area

A convex hull is created around all of the roots of each individual plant. The area (cm^2) of this convex hull is considered to be the total root system area 'occupied' by the plant.

Maximum width

The vertical soil space was divided into 10mm intervals. For each depth interval the distance between the farthest root points left and right of centre is calculated (mm). The largest of these widths represents maximum width of the root system.

Proportion of root length

Proportion of total root length for a given individual plant that is found to the right of plant centre. When a neighbour is present this measure corresponds to the proportion of total root length placed towards that neighbour.

Proportion of area

Proportion of total root occupation area for a given individual plant that is found to the right of plant centre. When a neighbour is present this measure corresponds to the proportion of total occupation area towards that neighbour.

Depth of maximum width

The 10mm depth interval in which the maximum width is found. The depth measure is the lower end of the interval. Ie. A depth of 10mm would indicate the interval between 0-10mm.

Statistical Analysis

Root response to neighbours

Linear mixed models were used to analyze neighbour effects on the ten response variables. Models incorporated treatment as a fixed factor (plants grown alone, with *Lactuca sativa* neighbour, or with *Phleum pratense* neighbour) and focal species as a random factor. *A priori* contrasts (IBM SPSS Statistics (version 20) TEST subcommand in MIXED) were used to test for a difference between the alone treatment and both neighbours (response to neighbours). Models were run with and without the random factor to determine if focal species improved model fit based on Hurvich and Tsai’s criterion (AICc) which accounts for small sample sizes. In order to have balanced data to perform planned contrasts, these analyses were performed on a subset of the total data which included only species with replicates for both neighbour treatments. Proportion variables (PropLength and PropArea) were arcsine(√) transformed and all other variables were ln transformed to meet assumptions. Analyses were performed using IBM SPSS Statistics (version 20).

For species specific analyses, species log response ratios (*sensu* Hedges *et al.* 1999; Cahill 1999) were calculated as:

$$\mathrm{LRR} = \ln(V_N / V_A)$$

Where V_N is the response value for the focal plant when a neighbour (either *Lactuca sativa* or *Phleum pratense*) was present and V_A is the response value when grown alone. Replicates were paired based on trial number and resting angle of the boxes. Individual replicates of alone plants were not paired more than once within a neighbour treatment. Positive LRR values indicate larger response with neighbours. Negative LRR values indicate a reduced response with neighbours. One-sample t-tests were used to test whether each species response ratio was significantly different from zero (no difference between responses with and without neighbour). Analyses were performed using IBM SPSS Statistics (version 20).

To determine relationships between root response measures Principal Components Analysis (PCA) was used. The PCA was performed using a correlation matrix and equamax rotation method. For each species the mean LRR across both neighbour treatments was used. Only six response variables were used in this analysis to represent the most distinct ways a species could respond within the broader categories of size, soil occupancy, allocation and placement. The variables used were LRR total biomass, LRR total root length, LRR maximum width, LRR root:shoot ratio, LRR proportion of root length, and LRR depth of maximum width. PCA analysis was performed using IBM SPSS Statistics (version 20).

Relationship between placement plasticity and root system size

Two regressions were performed using the absolute value of each species' mean LRR response variable across both neighbour treatments and the mean of Ln belowground biomass of each species when grown alone. The absolute value was used in order to analyze magnitude of plasticity in relation to root system size independent of direction. For exploring horizontal placement plasticity, absolute value of LRR proportion of root length was used as the response variable. For vertical placement plasticity, absolute value of LRR depth of maximum width was used. Analysis was performed using IBM SPSS Statistics (version 20).

Relationship between placement plasticity and competition experienced by focal plant

Two regressions were performed using mean the absolute value of LRR response variable and the mean LRR total biomass across both neighbour treatments. Again, the absolute value was used in order to analyze magnitude of plasticity in relation to competition experience by the focal plant independent of direction. LRR total biomass was used as a measure of competition as it shows the shift in biomass due to the neighbour treatment. For exploring horizontal placement plasticity, LRR proportion of root length was used as the response variable. For vertical placement plasticity, LRR depth of maximum width was used. Analysis was performed using IBM SPSS Statistics (version 20).

RESULTS

Root response to neighbours

Across species, and independent of neighbour identity (*A priori* contrast of alone, versus *Lactuca sativa*, and *Phleum prantense*), the presence of a neighbour had no significant effect on any of the ten response variables (Table 2.1: aboveground biomass, belowground biomass, total biomass, total root length, total root system area, maximum width, root:shoot ratio, proportion of root length, proportion of area, and depth of maximum width). Neighbour identity also had no significant effect on any of the response variables (Table 2.2).

When focal plant identity is included as a random factor in the general linear mixed model, there is an increase in model fit for the majority of response variables as shown by change in AICc values (Table 2.3). In general, change in AICc values less than 2 are considered to increase model fit. Eight of the ten response variables (aboveground biomass, belowground biomass, total biomass, total root length, total root system area, maximum width, root:shoot ratio, and depth of maximum width) are largely negative values which indicate focal species should be included in the model (Table 2.3). For those variables, the focal species factor was able to explain 20 – 80% of the remaining variation in the model

(Table 2.4) with most explaining over 60%. For proportion of root length and proportion of area variables, the change in AICc value is 2.054 indicating that the focal species term does not greatly increase the model fit. With the term included, only 0.06% of remaining variance in proportion of area was explained and for the proportion of root length variable the focal species factor did not explain any (Table 2.4).

When considering each species independently, there is a range in LRR for each response variable with some species increasing, decreasing, or having no effect with presence of a neighbour and there are no clear trends in response type across plant groups (eudicot versus monocot) or families (Figure 2.2). A t-test for each species and response variable combination shows those increases, or decreases in response with a neighbour that are significant (see Tables 2.5 through 2.14 for test values). The individual species responses appear to be independent of neighbour identity as well. In some cases the magnitude, but rarely the direction (segregation with one neighbour and aggregation with the other), of response varied with neighbour identity.

Six mean response variables (total biomass, root:shoot ratio, total root length, proportion of root length, maximum width, and depth of maximum width) were used in principal components analysis representing the variety of response variables measured within the broad categories of size, soil occupancy, allocation and placement. The variables were reduced to two main component axes (Figure 2.3). Component 1 accounts for 39% of the variation with the measures LRR total root length, LRR total biomass, and LRR maximum width contributing the most to this axis, all in the same direction. Component 2 accounts for another 29% of the variation with the measures LRR root:shoot ratio, and LRR proportion of root length positively associated with the axis and LRR depth of maximum width associated negatively. Monocot and eudicot species are scattered throughout the axis loadings indicating that the variation is not explained by plant group (Table 2.15).

Relationship between placement plasticity and root system size

Placement plasticity was measured by two variables: absolute value of LRR proportion of root length which represents the horizontal dimension, and absolute value of LRR depth of maximum width which represents the vertical dimension. There was no significant linear relationship between absolute value of LRR proportion of root length and root biomass when grown alone (Figure 2.4; R^2=0.074, $F_{1,18}$=1.436, p=0.246). There was also a non-significant decreasing linear relationship between absolute value of LRR depth of maximum width and root biomass when grown alone (Figure 2.5; R^2=0.150, $F_{1,18}$=3.166, p=0.092). Non-linear regression models were also tested however AIC values failed to indicate a better fit than linear regression.

Relationship between placement plasticity and competition experienced by focal plant

Once again, placement plasticity was measured by two variables: absolute value of LRR proportion of root length and absolute value of LRR depth of maximum width. The amount of competition experienced by the focal plant was categorized as the LRR of total biomass as this represents the difference in size when grown with a neighbour. There was no significant linear relationship between absolute value of LRR proportion of root length and LRR total biomass (Figure 2.6; $R^2=0.017$, $F_{1,18}=0.303$, $p=0.589$). There was also no significant linear relationship between absolute value of LRR depth of maximum width and LRR total biomass (Figure 2.7; $R^2=0.017$, $F_{1,18}=0.309$, $p=0.585$). Non-linear regression models were also tested however AIC values failed to indicate a better fit than linear regression.

DISCUSSION

Root response to neighbours

Root response to neighbours has varied across studies from no response (eg. Litav and Harper 1967; Semchenko *et al.* 2007), to segregation (eg. Baldwin and Tinker 1972; Brisson and Reynolds 1994; Caldwell *et al*. 1996; Schenk *et al*. 1999) or over-proliferation (eg. Gersani *et al*. 2001; Mania *et al*. 2002; Padilla *et al.* 2013). My findings support the idea that this variation is the norm rather than one set response type. In the general linear mixed model, no general response was found for any of the ten root responses measured. Rather, the inclusion of species explained much of the variation in responses. Individually, species responses ranged from strongly proliferating, segregating or having no response to the presence of a neighbour. Unsurprisingly, when these varying response types were averaged across all species, no general response could be identified.

One of the unique features of this study is the number of response variables measured for a large group of species. Where other studies have focused on overall size measures (Gersani *et al.* 2001) for response, I have obtained detailed soil occupancy measures and placement of roots in addition to size and allocation metrics. Species have different responses to the presence of a neighbour but there are trends in the ways in which they respond. Using PCA analysis of six variables within the three broad measurement categories of size, soil occupancy, allocation and root placement, 68% of the variation in responses was accounted for along two main component axes. Most of the variation (39%) in species response to neighbours can be explained by size and soil occupancy. The component separates species who display higher total biomass, total root length and larger maximum width of the root system when neighbours are present from those with the opposite set of traits. Allocation to roots and their placement explain the next largest portion of variation species responses (29%). This second component of variation separates species which display a higher root:shoot ratio, shallower placement of the widest part of the root system, and place a greater proportion of root length towards the neighbour when a competitor is present in contrast to those species with the opposite set of traits.

The partitioning of the variation in response separates species that aggregate with neighbours from those that avoid or segregate their roots. The results show plants obtain this aggregation or segregation by two means (1) by adjusting overall size and occupation of the soil, and/or (2) by altering allocation to roots and the specific placement of roots. By increasing overall size and soil occupancy the plant increases its presence in space shared with the neighbour. The plant may further enhance aggregation with the neighbour by also altering how much of the increased biomass is allocated to root structures and where those structures are placed. This was the case for *Bouteloua gracilis* which increased in size and soil occupancy and also selectively placed more biomass into roots when a neighbour was present. In addition, a greater proportion of the root system was placed towards the neighbour with the widest part of the root system occurring at a shallower depth. In contrast, a species such as *Elymus glaucus* also displayed an aggregation response; however this was mostly achieved by increasing overall size and soil occupancy with placement of and allocation to roots playing a lesser role. The opposite scenario was also found, where no increase in size or soil occupancy occurred but aggregation with the neighbour was displayed through allocation to roots and their placement towards the neighbour with the greatest width at a shallower depth. This pattern occurred for *Achillea millefolium* which actually had a slight decrease in size and soil occupancy while still strongly aggregating with the neighbour through allocation and root placement.

What these findings highlight is the need to recognize multiple methods of response to neighbours. By measuring only size and soil occupancy, some species that also display aggregation with neighbours may be overlooked as they may only do so through allocation to roots and their selective placement. Depth of the widest part of the root system has not previously been explored in studies of root responses to neighbours. Here we find that it is a characteristic related to how plants are able to aggregate or segregate from a neighbour. By altering the depth of placement, a plant accesses another method by which to concentrate roots with its competitor or to avoid competition by placing the widest part of soil exploration deeper than the neighbour's root system.

What is also interesting to note about the variation explained by the two main components discussed here, is that neither component resulted in a separation of monocot and eudicot species. Kembel and Cahill (2005) found broad differences in the plasticity of monocot and eudicot species in response to nutrient heterogeneity. Similarly, we might expect to see clustering of these two groups in terms of plasticity in root placement described by the second component. Instead monocot and eudicot species are scattered across both components in no discernible pattern. A meta-analysis by Kiær *et al.* (2013) showed different competitive effects across plant groups. Combined with the current findings of responses to neighbours, this highlights that there is no one strategy of response that confers greater competitive effects on the neighbour.

At no point in the analyses of species responses to neighbours did identity of neighbour (monocot versus eudicot) matter. Although previous studies have not always included neighbour identity as a variable for investigation, when they have the comparison is usually between inter- and intra-specific competition (Mahall and Callaway 1991; Bartelheimer *et al.* 2006) or genotypes of the same species (Callaway and Mahall 2007; Dudley and File 2007; Murphy and Dudley 2009; Fang *et al.* 2013). Evidence shows that plants are able to identify their neighbours at the root level (Chen *et al.* 2012), and they alter their root responses according to that identity (Mahall and Callaway 1991; Bartelheimer *et al.* 2006; Callaway and

Mahall 2007; Dudley and File 2007; Murphy and Dudley 2009; Fang *et al.* 2013). In contrast, this study found that the response of a plant was not dependent on neighbour identity. The neighbours used, however, were specifically chosen to not naturally occur with any of the focal species being observed. Because of this, there would be no evolutionary history of interaction between species to trigger differential responses. Finding no difference between the monocot and eudicot neighbour does indicate that these plant groups alone are not enough to produce significant variation in the response type employed.

Relationship between placement plasticity and root system size

In this study I revisited the predictions of Campbell *et al*. (1991) for a trade-off in size and plasticity. Originally plasticity was presented as foraging precision; however there has been little support for a relationship between precision and size (Kembel and Cahill 2005; Kembel *et al.* 2008). Rather than foraging precision I used measures of placement plasticity in response to neighbours to determine if any evidence exists for a trade off in root system size and plasticity of the root system.

Limited support was found for a trade-off between placement plasticity and root system size. Specifically a very weak relationship was found between root system size and plasticity in the depth of placement of the widest part of the root system (R^2=0.150, $F_{1,18}$=3.166, p=0.092). This trend indicated that plants with larger root systems likely do not respond to neighbours by means of shifting the depth of their widest part; their extensive root system means they will in all likelihood interact with the neighbour regardless of a shift in depth of maximum width. In contrast, plants with smaller root systems could benefit from plasticity in where they place the widest part of the root system. This plasticity would allow segregation from a neighbour by placing the widest part of the root system in an area not used by the neighbour plant. Our second measure of placement plasticity was the proportion of roots placed towards the neighbour. No significant relationship was found for this measure of plasticity and root system size (R^2=0.074, $F_{1,18}$=1.436, p=0.246).

This study used a larger number of species than most that have previously tested relationships between plasticity and size (Kembel *et al.* 2008). Despite this, support for a trade-off is weak, again suggesting that plasticity may not be linked to root system size. In order to progress our understanding of why such variation in plasticity exists, it must be considered within a broader context of costs and benefits (de Kroon and Mommer 2006).

Relationship between placement plasticity and competition experienced by focal plant

Plasticity which allows for aggregation or segregation of root systems is thought to confer a competitive advantage over neighbours (Craine *et al.* 2005). By having the flexibility to adjust where roots are placed in the soil in relation to a neighbour, a plant would possess the ability to respond optimally under competition. For both of the measures of placement plasticity in this study, proportion of root length placed towards the neighbour and depth of point of maximum width, there was no significant correlation to the amount of competition experienced as measured by change in total biomass with neighbours (R^2=0.017, $F_{1,18}$=0.303, p=0.589 and R^2=0.017, $F_{1,18}$=0.309, p=0.585 respectively). Wang *et al.* (2010) suggested that competitive response may be the product of many plant traits when no correlation could be found with any of their measured traits ranging from size, relative growth rate, and root:shoot ratio. These findings show that plasticity in root placement is also not the key to competitive response, and the role it may have likely is in concert with many other traits.

Conclusions

In this study, I explored response to neighbours through size, soil occupancy, allocation and detailed placement of roots. The findings highlight the need to consider species individually when determining their response to neighbours. There is no overarching tactic of segregation, avoidance, or for that matter, no response at all in any of the response categories. Instead, each of these root responses is possible and it would be dependent on the species being observed. The variation in method by which species respond to neighbours can be partitioned into two main sources. The first separates species based on their size, and soil occupancy and the second based on allocation to roots and their placement. By only using measures of size species that respond solely through specific root placement may be overlooked. This study found that the response of a plant was not dependent on neighbour identity; however this may be due to the purposeful selection of neighbours not previously associated with any of the observed species.

There was no clear support for a size-plasticity trade-off. Despite having a large sample size, only a weak relationship was found for plasticity in the depth of maximum root system width and no relationship with plasticity in placement of roots towards the neighbour. Nor was there any support for a relationship between the amount of competition experienced and placement plasticity. To move our understanding of root plasticity forward we need to go beyond simple correlations as these are continually shown to be incomplete.

TABLE 2.1 A priori contrast of response to neighbours (alone (1) versus *Lactuca sativa* (-0.5), or *Phleum pratense* (-0.5) neighbour) in a general linear mixed model analysis with neighbour treatment (as a fixed factor and focal species a random factor. Only species with replicates for all neighbour treatments are included in this analysis.

	Contrast Alone versus *L. sativa* & *P. pratense* neighbours			
Response variable	Estimate	df	*t*	*P*
Aboveground biomass	0.046169	136.169	0.486	0.627
Belowground biomass	-0.022935	136.115	-0.225	0.822
Total biomass	0.024208	136.126	0.266	0.791
Total root length	-0.008342	136.290	-0.095	0.924
Total root system area	-0.048216	136.318	-0.367	0.715
Maximum width	-0.069496	136.349	-0.798	0.426
Root:shoot ratio	-0.066268	136.527	-0.945	0.346
Proportion of root length	-0.021461	151.000	-0.436	0.664
Proportion of area	-0.023802	141.616	-0.471	0.638
Depth of maximum width	0.173350	137.860	1.586	0.115

*All response variables were ln transformed except proportion of root length and proportion of area which were arcsine(√) transformed.

TABLE 2.2 General linear mixed model analysis of the fixed factor neighbour treatment (alone, *Lactuca sativa*, or *Phleum pratense*) on 10 response variables with focal species included as a random factor. Only species with replicates for all neighbour treatments are included in this analysis.

	Neighbour treatment (fixed factor)		
Response variable	Df	*F*	*p*
Aboveground biomass	2, 136.112	0.472	0.625
Belowground biomass	2, 136.078	0.571	0.567
Total biomass	2, 136.084	0.604	0.548
Total root length	2, 136.209	0.052	0.949
Total root system area	2, 136.227	0.153	0.858
Maximum width	2, 136.241	0.752	0.473
Root:shoot ratio	2, 136.392	0.519	0.596
Proportion of root length	2, 151.000	0.327	0.721
Proportion of area	2, 140.322	0.381	0.684
Depth of maximum width	2, 137.442	1.864	0.159

*All response variables were ln transformed except proportion of root length and proportion of area which were arcsine(√) transformed.

TABLE 2.3 Change in AICc value for 10 response variables when focal species is included as a random factor in the general linear mixed model analysis with neighbour treatment (alone, *Lactuca sativa*, or *Phleum pratense*) as a fixed factor. Negative values indicate that the random factor increased fit of the model. Only species with replicates for all neighbour treatments are included in this analysis.

Response variable	Change in AICc
Aboveground biomass	-129.172
Belowground biomass	-174.233
Total biomass	-160.145
Total root length	-100.500
Total root system area	-90.532
Maximum width	-77.003
Root:shoot ratio	-63.170
Proportion of root length	2.054
Proportion of area	2.054
Depth of maximum width	-11.796

*All response variables were ln transformed except proportion of root length and proportion of area which were arcsine(√) transformed.

TABLE 2.4 Percent of remaining variation explained by focal species (random factor) in the general linear mixed model analysis with neighbour treatment (alone, *Lactuca sativa*, or *Phleum pratense*) as a fixed factor. Only species with replicates for all neighbour treatments are included in this analysis.

Response variable	% of variance explained by focal species
Aboveground biomass	71.84
Belowground biomass	80.06
Total biomass	77.88
Total root length	63.84
Total root system area	60.86
Maximum width	56.63
Root:shoot ratio	50.34
Proportion of root length	Redundant parameter
Proportion of area	0.06
Depth of maximum width	19.64

*All response variables were ln transformed except proportion of root length and proportion of area which were arcsine(√) transformed.

TABLE 2.5 One-sample t-tests for difference between mean log response ratio (LRR) for aboveground biomass (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR aboveground biomass									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	-0.3657	0.60415	2	-0.605	0.606	-0.5769	0.40166	2	-1.436	0.287
Asteraceae	*Artemesia frigida*	-	-	-	-	-	0.0609	0.85588	2	0.071	0.950
Asteraceae	*Artemesia ludoviciana*	-0.0017	1.19267	1	-0.001	0.999	-0.1642	0.63890	2	-0.257	0.821
Asteraceae	*Erigeron glabellus*	-0.0125	0.40841	2	-0.031	0.978	0.6188	1.34970	1	0.458	0.726
Asteraceae	*Gaillardia aristata*	0.3802	0.24253	3	1.568	0.215	0.2631	0.17020	2	1.546	0.262
Asteraceae	*Heterotheca villosa*	0.4283	0.66173	2	0.647	0.584	-0.1473	0.52374	2	-0.281	0.805
Asteraceae	*Solidago missouriensis*	-0.5595	0.40628	2	-1.377	0.302	-0.4755	0.11312	2	-4.203	**0.052**
Asteraceae	*Symphyotrichum ericoides*	-0.1465	0.05989	2	-2.447	0.134	-0.1305	0.31414	2	-0.416	0.718
Asteraceae	*Symphyotrichum falcatum*	-0.0076	0.67417	2	-0.011	0.992	0.0843	0.52970	2	0.159	0.888
Asteraceae	*Symphyotrichum laeve*	-0.1389	0.69398	1	-0.200	0.874	-0.1014	0.06601	1	-1.536	0.367
Polygonaceae	*Rumex crispus*	-0.6136	0.65160	1	-0.942	0.519	-0.6548	0.19435	2	-3.369	**0.078**
Rosaceae	*Drymocallis arguta*	0.1627	0.19074	3	0.853	0.456	0.4199	0.84565	1	0.497	0.707
Rosaceae	*Geum triflorum*	0.1080	0.12331	2	0.876	0.474	0.1401	0.34641	2	0.404	0.725
Fabaceae	*Astragalus agrestis*	-0.4066	0.26634	1	-1.527	0.369	-0.4087	0.47064	2	-0.868	0.477
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.7401	0.63505	1	1.165	0.451
Poaceae	*Bouteloua gracilis*	0.2731	0.26820	2	1.018	0.416	-0.1724	0.70178	2	-0.246	0.829
Poaceae	*Bromus inermis*	-0.1776	0.11401	2	-1.558	0.260	-0.2084	0.03144	1	-6.631	**0.095**
Poaceae	*Elymus glaucus*	0.3353	0.41834	2	0.802	0.507	0.3763	0.33389	2	1.127	0.377
Poaceae	*Koeleria macrantha*	-	-	-	-	-	-0.3446	0.16479	2	-2.091	0.172
Poaceae	*Poa pratensis*	-	-	-	-	-	0.0735	0.27923	2	0.263	0.817

TABLE 2.6 One-sample t-tests for difference between mean log response ratio (LRR) for belowground biomass (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR belowground biomass									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.1628	0.48306	2	0.337	0.768	-0.1678	0.33674	2	-0.498	0.668
Asteraceae	*Artemesia frigida*	-	-	-	-	-	0.0007	0.90084	2	0.001	0.999
Asteraceae	*Artemesia ludoviciana*	0.4124	1.20441	1	0.342	0.790	0.1192	0.42096	2	0.283	0.804
Asteraceae	*Erigeron glabellus*	0.0469	0.11393	2	0.412	0.721	-0.0579	0.81560	1	-0.071	0.955
Asteraceae	*Gaillardia aristata*	-0.1051	0.32046	3	-0.328	0.765	0.1855	0.12612	2	1.471	0.279
Asteraceae	*Heterotheca villosa*	0.3517	0.60117	2	0.585	0.618	-0.7236	0.46636	2	-1.552	0.261
Asteraceae	*Solidago missouriensis*	-0.8365	0.66704	2	-1.254	0.337	-0.8069	0.39060	2	-2.066	0.175
Asteraceae	*Symphyotrichum ericoides*	0.0649	0.40791	2	0.159	0.888	-0.4638	0.63678	2	-0.728	0.542
Asteraceae	*Symphyotrichum falcatum*	-0.1408	0.57484	2	-0.245	0.829	-0.0377	0.38835	2	-0.097	0.932
Asteraceae	*Symphyotrichum laeve*	0.2222	0.47998	1	0.463	0.724	0.1388	0.20454	1	0.679	0.620
Polygonaceae	*Rumex crispus*	-0.5079	0.51395	1	-0.988	0.504	-0.5600	0.31171	2	-1.797	0.214
Rosaceae	*Drymocallis arguta*	0.6041	0.18847	3	3.205	**0.049**	0.7921	0.73580	1	1.077	0.477
Rosaceae	*Geum triflorum*	0.0801	0.17082	2	0.469	0.685	0.2619	0.38246	2	0.685	0.564
Fabaceae	*Astragalus agrestis*	-0.7489	0.10942	1	-6.844	**0.092**	-0.1247	0.75455	2	-0.165	0.884
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.6118	0.36116	1	1.694	0.340
Poaceae	*Bouteloua gracilis*	0.7699	0.04749	2	16.211	**0.004**	0.7145	0.74750	2	0.956	0.440
Poaceae	*Bromus inermis*	-0.4033	0.07311	2	-5.516	**0.031**	-0.0436	0.14362	1	-0.303	0.812
Poaceae	*Elymus glaucus*	0.5277	0.33598	2	1.571	0.257	0.1304	0.05206	2	2.505	0.129
Poaceae	*Koeleria macrantha*	-	-	-	-	-	-0.0202	0.31598	2	-0.064	0.955
Poaceae	*Poa pratensis*	-	-	-	-	-	-0.1508	0.29229	2	-0.516	0.657

TABLE 2.7 One-sample t-tests for difference between mean log response ratio (LRR) for total biomass (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate $p < 0.10$.

		LRR total biomass									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	-0.2700	0.58012	2	-0.465	0.687	-0.5064	0.38719	2	-1.308	0.321
Asteraceae	*Artemesia frigida*	-	-	-	-	-	0.0388	0.85540	2	0.045	0.968
Asteraceae	*Artemesia ludoviciana*	0.1014	1.21062	1	0.084	0.947	-0.1074	0.59869	2	-0.179	0.874
Asteraceae	*Erigeron glabellus*	0.0089	0.20788	2	0.043	0.970	0.3509	1.13484	1	0.309	0.809
Asteraceae	*Gaillardia aristata*	0.2758	0.16660	3	1.655	0.196	0.2346	0.15433	2	1.520	0.268
Asteraceae	*Heterotheca villosa*	0.4153	0.61880	2	0.671	0.571	-0.3001	0.47741	2	-0.629	0.594
Asteraceae	*Solidago missouriensis*	-0.6085	0.48014	2	-1.267	0.333	-0.5355	0.20108	2	-2.663	0.117
Asteraceae	*Symphyotrichum ericoides*	-0.0560	0.18110	2	-0.309	0.786	-0.2207	0.41050	2	-0.538	0.645
Asteraceae	*Symphyotrichum falcatum*	-0.0502	0.62122	2	-0.081	0.943	0.0514	0.47140	2	0.109	0.923
Asteraceae	*Symphyotrichum laeve*	-0.0081	0.61332	1	-0.013	0.992	-0.0158	0.11974	1	-0.132	0.917
Polygonaceae	*Rumex crispus*	-0.5454	0.56294	1	-0.969	0.510	-0.5706	0.24913	2	-2.290	0.149
Rosaceae	*Drymocallis arguta*	0.2831	0.17544	3	1.614	0.205	0.5310	0.80873	1	0.657	0.630
Rosaceae	*Geum triflorum*	0.1051	0.12953	2	0.812	0.502	0.1721	0.35361	2	0.487	0.675
Fabaceae	*Astragalus agrestis*	-0.4831	0.18225	1	-2.651	0.230	-0.3315	0.52390	2	-0.633	0.592
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.7298	0.57306	1	1.274	0.424
Poaceae	*Bouteloua gracilis*	0.3977	0.19499	2	2.039	0.178	0.0447	0.70703	2	0.063	0.955
Poaceae	*Bromus inermis*	-0.2619	0.08909	2	-2.940	**0.099**	-0.1374	0.04329	1	-3.174	0.194
Poaceae	*Elymus glaucus*	0.4098	0.38909	2	1.053	0.403	0.2882	0.22235	2	1.296	0.324
Poaceae	*Koeleria macrantha*	-	-	-	-	-	-0.2183	0.11189	2	-1.951	0.190
Poaceae	*Poa pratensis*	-	-	-	-	-	-0.0043	0.28052	2	-0.015	0.989

TABLE 2.8 One-sample t-tests for difference between mean log response ratio (LRR) for total root length (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR total root length									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.0835	0.34587	2	0.241	0.832	-0.1763	0.51807	2	-0.340	0.766
Asteraceae	*Artemesia frigida*	-	-	-	-	-	-0.1370	0.62413	2	-0.219	0.847
Asteraceae	*Artemesia ludoviciana*	0.4246	1.41746	1	0.300	0.815	0.4547	0.27499	2	1.653	0.240
Asteraceae	*Erigeron glabellus*	-0.1024	0.60094	2	-0.170	0.880	0.3346	1.22438	1	0.273	0.830
Asteraceae	*Gaillardia aristata*	-0.4643	0.35560	3	-1.306	0.283	0.1419	0.09903	2	1.433	0.288
Asteraceae	*Heterotheca villosa*	0.6384	0.56829	2	1.123	0.378	0.0324	0.50093	2	0.065	0.954
Asteraceae	*Solidago missouriensis*	-0.2944	0.39417	2	-0.747	0.533	-0.3230	0.16106	2	-2.005	0.183
Asteraceae	*Symphyotrichum ericoides*	-0.0018	0.22744	2	-0.008	0.995	-0.3353	0.42277	2	-0.793	0.511
Asteraceae	*Symphyotrichum falcatum*	0.0035	0.57998	2	0.006	0.996	0.1170	0.42483	2	0.275	0.809
Asteraceae	*Symphyotrichum laeve*	0.2217	0.04631	1	4.786	0.131	0.4293	0.22406	1	1.916	0.306
Polygonaceae	*Rumex crispus*	-0.5185	0.16858	1	-3.076	0.200	-0.5976	0.20263	2	-2.949	**0.098**
Rosaceae	*Drymocallis arguta*	0.2231	0.25039	3	0.891	0.439	0.1022	0.38769	1	0.264	0.836
Rosaceae	*Geum triflorum*	0.1232	0.28188	2	0.437	0.705	0.5225	0.13922	2	3.753	**0.064**
Fabaceae	*Astragalus agrestis*	-1.0978	0.35427	1	-3.099	0.199	-0.6523	0.60141	2	-1.085	0.391
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.7682	0.51384	1	1.495	0.375
Poaceae	*Bouteloua gracilis*	0.5169	0.23093	2	2.239	0.155	-0.2142	0.68152	2	-0.314	0.783
Poaceae	*Bromus inermis*	-0.0890	0.24447	2	-0.364	0.751	-0.0124	0.26892	1	-0.046	0.971
Poaceae	*Elymus glaucus*	0.3520	0.22446	2	1.568	0.257	0.1461	0.18030	2	0.810	0.503
Poaceae	*Koeleria macrantha*	-	-	-	-	-	0.0096	0.14489	2	0.066	0.953
Poaceae	*Poa pratensis*	-	-	-	-	-	-0.2139	0.65190	2	-0.328	0.774

TABLE 2.9 One-sample t-tests for difference between mean log response ratio (LRR) for root system area (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR total root system area									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.0037	0.73909	2	0.005	0.996	-0.8461	0.65433	2	-1.293	0.325
Asteraceae	*Artemesia frigida*	-	-	-	-	-	0.0819	1.08828	2	0.075	0.947
Asteraceae	*Artemesia ludoviciana*	0.1440	1.12617	1	0.128	0.919	0.1505	0.13758	2	1.094	0.388
Asteraceae	*Erigeron glabellus*	-0.0853	0.69860	2	-0.122	0.914	0.4826	1.60205	1	0.301	0.814
Asteraceae	*Gaillardia aristata*	-0.2787	0.50107	3	-0.556	0.617	0.0360	0.43501	2	0.083	0.942
Asteraceae	*Heterotheca villosa*	1.1770	0.84293	2	1.396	0.297	0.4579	0.61466	2	0.745	0.534
Asteraceae	*Solidago missouriensis*	-0.2333	0.57076	2	-0.409	0.722	-0.3441	0.30149	2	-1.141	0.372
Asteraceae	*Symphyotrichum ericoides*	-0.2593	0.20592	2	-1.259	0.335	-0.6314	0.30817	2	-2.049	0.177
Asteraceae	*Symphyotrichum falcatum*	0.2033	0.48982	2	0.415	0.718	0.6523	0.70297	2	0.928	0.451
Asteraceae	*Symphyotrichum laeve*	0.4719	0.40268	1	1.172	0.450	0.5158	0.45409	1	1.136	0.460
Polygonaceae	*Rumex crispus*	-0.2369	0.09672	1	-2.449	0.247	-0.4939	0.27159	2	-1.819	0.211
Rosaceae	*Drymocallis arguta*	0.9020	0.53352	3	1.691	0.189	0.5284	1.09952	1	0.481	0.715
Rosaceae	*Geum triflorum*	0.2787	0.33204	2	0.839	0.490	1.0013	0.11740	2	8.529	**0.013**
Fabaceae	*Astragalus agrestis*	-2.0718	0.10868	1	-19.064	**0.033**	-1.3082	1.37989	2	-0.948	0.443
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.4468	0.49617	1	0.900	0.533
Poaceae	*Bouteloua gracilis*	0.8751	0.53400	2	1.639	0.243	-0.1999	1.03539	2	-0.193	0.865
Poaceae	*Bromus inermis*	0.0567	0.24138	2	0.235	0.836	-0.0965	0.33856	1	-0.285	0.823
Poaceae	*Elymus glaucus*	0.4137	0.12828	2	3.225	**0.084**	0.3478	0.22844	2	1.522	0.267
Poaceae	*Koeleria macrantha*	-	-	-	-	-	0.0328	0.42276	2	0.078	0.945
Poaceae	*Poa pratensis*	-	-	-	-	-	-0.1205	1.42810	2	-0.084	0.940

TABLE 2.10 One-sample t-tests for difference between mean log response ratio (LRR) for maximum width of root system (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR maximum width of root system									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.0472	0.65808	2	0.072	0.949	-0.3479	0.44915	2	-0.775	0.520
Asteraceae	*Artemesia frigida*	-	-	-	-	-	0.4993	0.87355	2	0.572	0.625
Asteraceae	*Artemesia ludoviciana*	0.3550	0.73533	1	0.483	0.714	-0.0993	0.05479	2	-1.813	0.212
Asteraceae	*Erigeron glabellus*	-0.1178	0.37092	2	-0.318	0.781	0.7762	0.67088	1	1.157	0.454
Asteraceae	*Gaillardia aristata*	-0.2751	0.38051	3	-0.723	0.522	-0.0849	0.21127	2	-0.402	0.727
Asteraceae	*Heterotheca villosa*	0.8032	0.49426	2	1.625	0.246	0.3693	0.54299	2	0.680	0.567
Asteraceae	*Solidago missouriensis*	-0.3180	0.67031	2	-0.474	0.682	-0.2254	0.07063	2	-3.191	**0.086**
Asteraceae	*Symphyotrichum ericoides*	-0.4006	0.26869	2	-1.491	0.274	-0.4311	0.06404	2	-6.733	**0.021**
Asteraceae	*Symphyotrichum falcatum*	-0.2893	0.22462	2	-1.288	0.327	0.4035	0.37833	2	1.066	0.398
Asteraceae	*Symphyotrichum laeve*	0.0172	0.21037	1	0.082	0.948	0.4540	0.03041	1	14.929	**0.043**
Polygonaceae	*Rumex crispus*	-0.2590	0.12411	1	-2.087	0.285	-0.3285	0.18406	2	-1.784	0.216
Rosaceae	*Drymocallis arguta*	0.8085	0.56277	3	1.437	0.246	0.7898	0.78606	1	1.005	0.498
Rosaceae	*Geum triflorum*	-0.1601	0.19316	2	-0.829	0.494	0.4466	0.05512	2	8.102	**0.015**
Fabaceae	*Astragalus agrestis*	-0.7879	0.21870	1	-3.603	0.172	-0.2898	0.59226	2	-0.489	0.673
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	-0.0498	0.09163	1	-0.544	0.683
Poaceae	*Bouteloua gracilis*	0.4082	0.19818	2	2.060	0.176	-0.0564	0.34007	2	-0.166	0.884
Poaceae	*Bromus inermis*	0.1598	0.15054	2	1.062	0.400	0.0681	0.39736	1	0.171	0.892
Poaceae	*Elymus glaucus*	0.2386	0.12618	2	1.891	0.199	0.5110	0.36574	2	1.397	0.297
Poaceae	*Koeleria macrantha*	-	-	-	-	-	0.1352	0.42549	2	0.318	0.781
Poaceae	*Poa pratensis*	-	-	-	-	-	0.0431	0.86404	2	0.050	0.965

TABLE 2.11 One-sample t-tests for difference between mean log response ratio (LRR) for root:shoot ratio (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate $p < 0.10$.

		LRR root:shoot ratio									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.5285	0.1260	2	4.196	**0.052**	0.4090	0.0660	2	6.202	**0.025**
Asteraceae	*Artemesia frigida*	-	-	-	-	-	-0.0601	0.1930	2	-0.311	0.785
Asteraceae	*Artemesia ludoviciana*	0.4141	0.0117	1	35.257	**0.018**	0.2834	0.2395	2	1.183	0.358
Asteraceae	*Erigeron glabellus*	0.0594	0.5220	2	0.114	0.920	-0.6767	0.5341	1	-1.267	0.425
Asteraceae	*Gaillardia aristata*	-0.4853	0.4838	3	-1.003	0.390	-0.0775	0.044/1	2	-1.727	0.226
Asteraceae	*Heterotheca villosa*	-0.0766	0.3680	2	-0.208	0.854	-0.5762	0.4101	2	-1.405	0.295
Asteraceae	*Solidago missouriensis*	-0.2770	0.2615	2	-1.059	0.400	-0.3314	0.2898	2	-1.144	0.371
Asteraceae	*Symphyotrichum ericoides*	0.2114	0.3524	2	0.600	0.610	-0.3333	0.3269	2	-1.020	0.415
Asteraceae	*Symphyotrichum falcatum*	-0.1332	0.2602	2	-0.512	0.660	-0.1220	0.2589	2	-0.471	0.684
Asteraceae	*Symphyotrichum laeve*	0.3611	0.2140	1	1.687	0.341	0.2402	0.1385	1	1.734	0.333
Polygonaceae	*Rumex crispus*	0.1057	0.1377	1	0.768	0.583	0.0948	0.2112	2	0.449	0.697
Rosaceae	*Drymocallis arguta*	0.4414	0.1492	3	2.959	**0.060**	0.3723	0.1099	1	3.389	0.183
Rosaceae	*Geum triflorum*	-0.0279	0.1081	2	-0.258	0.820	0.1218	0.1273	2	0.957	0.440
Fabaceae	*Astragalus agrestis*	-0.3423	0.3758	1	-0.911	0.530	0.2840	0.4066	2	0.699	0.557
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	-0.1284	0.2739	1	-0.469	0.721
Poaceae	*Bouteloua gracilis*	0.4968	0.2261	2	2.197	0.159	0.8869	0.1823	2	4.865	**0.040**
Poaceae	*Bromus inermis*	-0.2257	0.1305	2	-1.730	0.226	0.1649	0.1751	1	0.942	0.519
Poaceae	*Elymus glaucus*	0.1924	0.0964	2	1.997	0.184	-0.2460	0.3169	2	-0.776	0.519
Poaceae	*Koeleria macrantha*	-	-	-	-	-	0.3244	0.4040	2	0.803	0.506
Poaceae	*Poa pratensis*	-	-	-	-	-	-0.2244	0.0971	2	-2.311	0.147

TABLE 2.12 One-sample t-tests for difference between mean log response ratio (LRR) for proportion of root length towards neighbour (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR proportion of root length towards neighbour									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.17060	0.97452	2	0.175	0.877	0.45120	0.27607	2	1.634	0.244
Asteraceae	*Artemesia frigida*	-	-	-	-	-	-6.25770	2.96956	2	-2.107	0.170
Asteraceae	*Artemesia ludoviciana*	2.07300	0.23329	1	8.886	**0.071**	0.84350	0.88096	2	0.958	0.439
Asteraceae	*Erigeron glabellus*	-0.72400	1.01736	2	-0.712	0.551	0.81880	0.47424	1	1.727	0.334
Asteraceae	*Gaillardia aristata*	-0.32740	0.40368	3	-0.811	0.477	-0.37960	0.55150	2	-0.688	0.562
Asteraceae	*Heterotheca villosa*	0.72620	0.85179	2	0.853	0.484	-0.04690	1.00459	2	-0.047	0.967
Asteraceae	*Solidago missouriensis*	-2.12560	1.04688	2	-2.030	0.179	-1.25660	0.95521	2	-1.316	0.319
Asteraceae	*Symphyotrichum ericoides*	0.53890	0.92603	2	0.582	0.619	0.66650	0.20877	2	3.192	**0.086**
Asteraceae	*Symphyotrichum falcatum*	0.45790	0.77484	2	0.591	0.614	1.02280	0.74848	2	1.367	0.305
Asteraceae	*Symphyotrichum laeve*	1.02820	0.95035	1	1.082	0.475	-1.94120	3.09150	1	-0.628	0.643
Polygonaceae	*Rumex crispus*	-0.10050	0.27564	1	-0.365	0.777	-0.27810	0.06846	2	-4.063	**0.056**
Rosaceae	*Drymocallis arguta*	1.00630	1.03305	3	0.974	0.402	-4.92000	6.59290	1	-0.746	0.592
Rosaceae	*Geum triflorum*	-1.20340	1.22341	2	-0.984	0.429	-1.15400	0.17008	2	-6.785	**0.021**
Fabaceae	*Astragalus agrestis*	-5.85440	7.26799	1	-0.806	0.568	-4.24170	4.67723	2	-0.907	0.460
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.56640	0.22161	1	2.556	0.237
Poaceae	*Bouteloua gracilis*	0.43500	0.32069	2	1.356	0.308	0.95120	0.83855	2	1.134	0.374
Poaceae	*Bromus inermis*	-0.30730	0.31150	2	-0.986	0.428	-0.22300	0.16660	1	-1.339	0.408
Poaceae	*Elymus glaucus*	0.60310	0.59050	2	1.021	0.415	1.09520	0.48857	2	2.242	0.154
Poaceae	*Koeleria macrantha*	-	-	-	-	-	-0.26690	0.22577	2	-1.182	0.359
Poaceae	*Poa pratensis*	-	-	-	-	-	-3.83950	3.83351	2	-1.002	0.422

TABLE 2.13 One-sample t-tests for difference between mean log response ratio (LRR) for proportion of area towards neighbour (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate p < 0.10.

		LRR proportion of area towards neighbour									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	0.62430	0.57269	2	1.090	0.390	0.52990	0.29261	2	1.811	0.212
Asteraceae	*Artemesia frigida*	-	-	-	-	-	-7.80690	2.37208	2	-3.291	**0.081**
Asteraceae	*Artemesia ludoviciana*	1.51570	0.47359	1	3.200	0.193	0.65690	0.76367	2	0.860	0.480
Asteraceae	*Erigeron glabellus*	-0.08030	1.01914	2	-0.079	0.944	0.52610	0.08056	1	6.530	**0.097**
Asteraceae	*Gaillardia aristata*	-0.58860	0.59817	3	-0.984	0.398	-0.11790	0.40547	2	-0.291	0.799
Asteraceae	*Heterotheca villosa*	0.28440	0.65471	2	0.434	0.706	-0.81200	1.19216	2	-0.681	0.566
Asteraceae	*Solidago missouriensis*	-1.81780	1.65771	2	-1.097	0.387	-1.52700	1.64849	2	-0.926	0.452
Asteraceae	*Symphyotrichum ericoides*	0.52950	0.66517	2	0.796	0.509	0.30310	0.17802	2	1.703	0.231
Asteraceae	*Symphyotrichum falcatum*	1.42350	1.04947	2	1.356	0.308	1.20630	0.99018	2	1.218	0.347
Asteraceae	*Symphyotrichum laeve*	-0.06380	0.95265	1	-0.067	0.957	-3.98800	4.92964	1	-0.809	0.567
Polygonaceae	*Rumex crispus*	-0.31870	0.17168	1	-1.856	0.315	-0.23160	0.09521	2	-2.432	0.135
Rosaceae	*Drymocallis arguta*	1.68430	1.79637	3	0.938	0.418	-3.57780	6.24878	1	-0.573	0.669
Rosaceae	*Geum triflorum*	-1.91120	1.94715	2	-0.982	0.430	-0.76840	0.10487	2	-7.327	**0.018**
Fabaceae	*Astragalus agrestis*	-5.65380	7.46861	1	-0.757	0.587	-5.39040	4.73113	2	-1.139	0.373
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	-0.13130	0.03673	1	-3.576	0.174
Poaceae	*Bouteloua gracilis*	0.82640	0.34882	2	2.369	0.141	1.56020	0.20853	2	7.482	**0.017**
Poaceae	*Bromus inermis*	-0.34910	0.38235	2	-0.913	0.458	-0.12320	0.03673	1	-3.353	0.185
Poaceae	*Elymus glaucus*	0.57390	0.69891	2	0.821	0.498	1.07780	0.64094	2	1.682	0.235
Poaceae	*Koeleria macrantha*	-	-	-	-	-	0.36260	0.36003	2	1.007	0.420
Poaceae	*Poa pratensis*	-	-	-	-	-	-3.75440	2.39058	2	-1.570	0.257

TABLE 2.14 One-sample t-tests for difference between mean log response ratio (LRR) for depth of maximum width (when grown with neighbour) and zero (indicating no response to neighbour). Each species and treatment combination was analyzed separately. Bold values indicate $p < 0.10$.

		LRR depth of maximum width									
		Lactuca sativa neighbour					*Phleum pratense* neighbour				
Family	Focal species	Estimate	S.E.	df	*t*	*p*	Estimate	S.E.	df	*t*	*p*
Asteraceae	*Achillea millefolium*	-0.46900	0.20260	2	-2.315	0.147	-0.82160	0.26589	2	-3.090	**0.091**
Asteraceae	*Artemesia frigida*	-	-	-	-	-	1.02400	0.09393	2	10.902	**0.008**
Asteraceae	*Artemesia ludoviciana*	0.52470	0.41352	1	1.269	0.425	0.29730	0.35501	2	0.837	0.491
Asteraceae	*Erigeron glabellus*	0.70060	0.31129	2	2.251	0.153	>-0.0001	1.09862	1	>0.001	1.000
Asteraceae	*Gaillardia aristata*	-0.53410	0.59719	3	-0.894	0.437	-0.13720	0.11410	2	-1.203	0.352
Asteraceae	*Heterotheca villosa*	0.55020	0.16898	2	3.256	**0.083**	0.43470	0.66412	2	0.655	0.580
Asteraceae	*Solidago missouriensis*	1.30670	0.79228	2	1.649	0.241	0.64860	0.37028	2	1.752	0.222
Asteraceae	*Symphyotrichum ericoides*	-0.18650	0.91029	2	-0.205	0.857	-0.18650	0.34066	2	-0.548	0.639
Asteraceae	*Symphyotrichum falcatum*	-0.91890	0.42605	2	-2.157	0.164	-1.18510	0.27875	2	-4.252	**0.051**
Asteraceae	*Symphyotrichum laeve*	-0.02700	0.02703	1	-1.000	0.500	-0.38480	1.17329	1	-0.328	0.798
Polygonaceae	*Rumex crispus*	0.13410	0.39196	1	0.342	0.790	-0.52800	0.38602	2	-1.368	0.305
Rosaceae	*Drymocallis arguta*	-0.24360	0.44187	3	-0.551	0.620	-0.62090	0.98858	1	-0.628	0.643
Rosaceae	*Geum triflorum*	-0.34470	0.06077	2	-5.672	**0.030**	>0.0001	0.10526	2	>0.001	1.000
Fabaceae	*Astragalus agrestis*	-1.06850	1.37384	1	-0.778	0.579	0.01550	0.28770	2	0.054	0.962
Brassicaceae	*Descurainia sophia*	-	-	-	-	-	0.31980	0.16568	1	1.930	0.304
Poaceae	*Bouteloua gracilis*	-1.08920	0.35705	2	-3.051	**0.093**	-0.68790	1.29266	2	-0.532	0.648
Poaceae	*Bromus inermis*	0.30060	0.05245	2	5.732	**0.029**	-0.07710	0.07708	1	-1.000	0.500
Poaceae	*Elymus glaucus*	-0.15140	0.09857	2	-1.536	0.264	-0.14730	0.08312	2	-1.772	0.218
Poaceae	*Koeleria macrantha*	-	-	-	-	-	0.11220	0.11216	2	1.000	0.423
Poaceae	*Poa pratensis*	-	-	-	-	-	0.03180	0.36399	2	0.087	0.938

TABLE 2.15 Species axis loading scores for the two extracted PCA components.

Family	Identification number	Species	Component 1	Component 2
Asteraceae	1	*Achillea millefolium*	-0.860	1.476
Asteraceae	2	*Artemesia frigida*	0.262	-2.297
Asteraceae	3	*Artemesia ludoviciana*	0.643	0.474
Asteraceae	4	*Erigeron glabellus*	0.574	-0.780
Asteraceae	5	*Gaillardia aristata*	-0.245	-0.103
Asteraceae	6	*Heterotheca villosa*	1.169	-0.977
Asteraceae	7	*Solidago missouriensis*	-1.122	-1.530
Asteraceae	8	*Symphyotrichum ericoides*	-0.805	0.374
Asteraceae	9	*Symphyotrichum falcatum*	-0.111	0.973
Asteraceae	10	*Symphyotrichum laeve*	0.440	0.585
Polygonaceae	11	*Rumex crispus*	-1.670	0.461
Rosaceae	12	*Drymocallis arguta*	1.215	0.584
Rosaceae	13	*Geum triflorum*	0.500	0.071
Fabaceae	14	*Astragalus agrestis*	-2.270	-0.313
Brassicaceae	15	*Descurainia sophia*	1.686	-0.233
Poaceae	16	*Bouteloua gracilis*	0.317	1.988
Poaceae	17	*Bromus inermis*	-0.181	-0.272
Poaceae	18	*Elymus glaucus*	1.030	0.201
Poaceae	19	*Koeleria macrantha*	-0.149	0.354
Poaceae	20	*Poa pratensis*	-0.424	-1.037

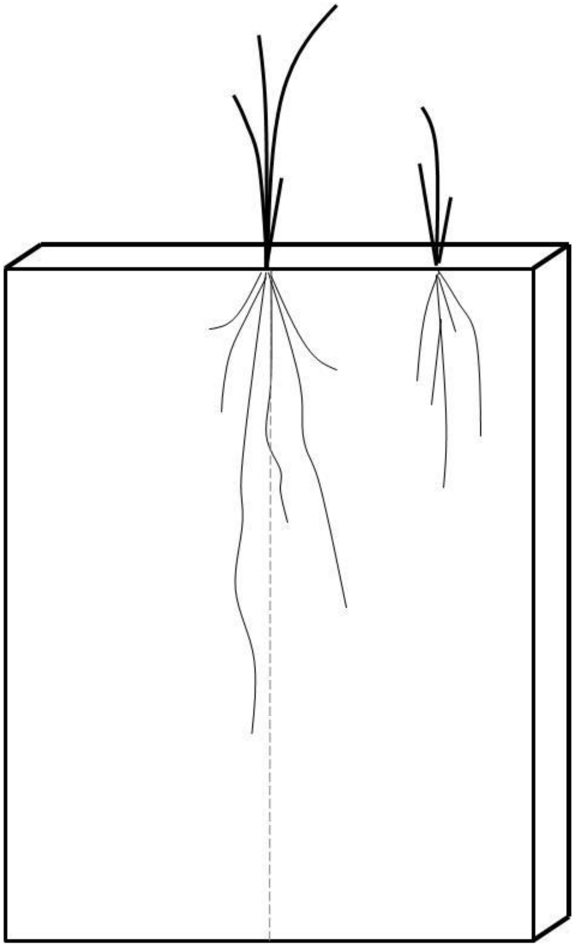

FIGURE 2.1 Schematic of experimental window boxes. Soil space available to the plants is approximately 5 x 190 x 250mm. For competition treatments center plant is the focal species with neighbour planted to the right, halfway between focal plant and box edge. No neighbour plant would be present in the control alone treatment. Dashed vertical grey line delineates the right and left side of focal plant for measures of proportion of root length placed towards a neighbour (to the right).

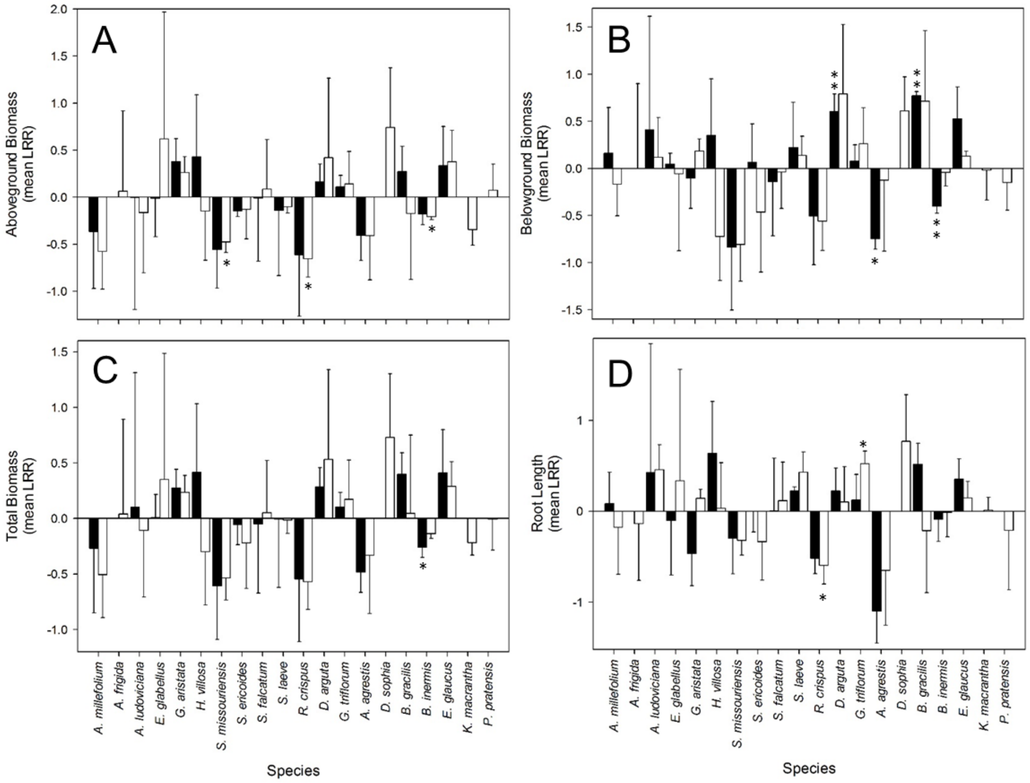

FIGURE 2.2 Mean response (+ 1 S.E.) of twenty species to neighbour treatment (LRR). Graphs show LRR response measures A) aboveground biomass B) belowground biomass C) total biomass D) total root length E) total root system area F) maximum width of root system G) root:shoot ratio H) depth of point of maximum width I) proportion of root length towards neighbor J) proportion of area towards neighbour. Closed bars are species mean with *Lactuca sativa* neighbour, open bars are species mean with *Phleum pratense* neighbour. Asterisks indicate results of one-sample t-tests for a difference from zero (no difference between responses with and without neighbour). Single asterisks indicate p<0.10 and double asterisks indicate p<0.05.

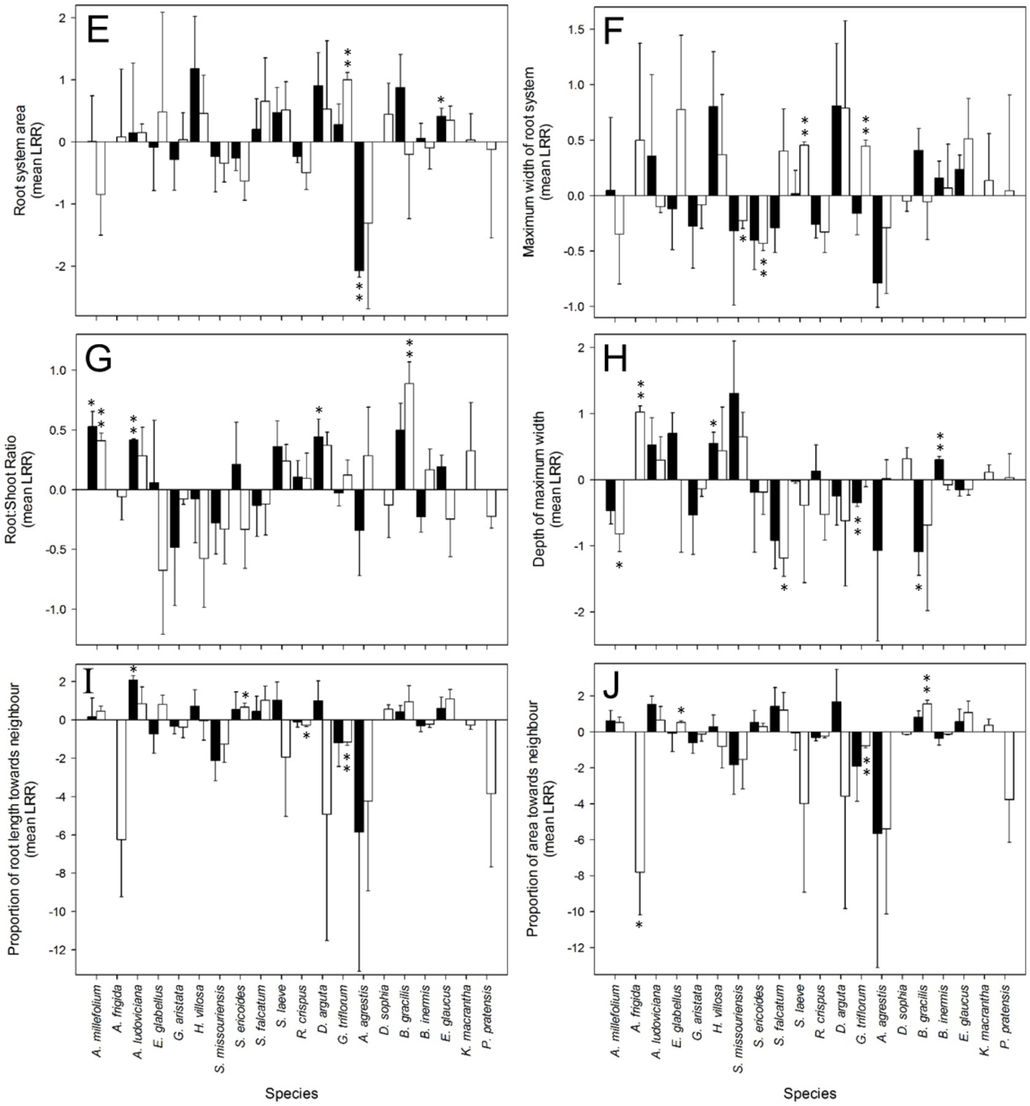

FIGURE 2.2 Extended.

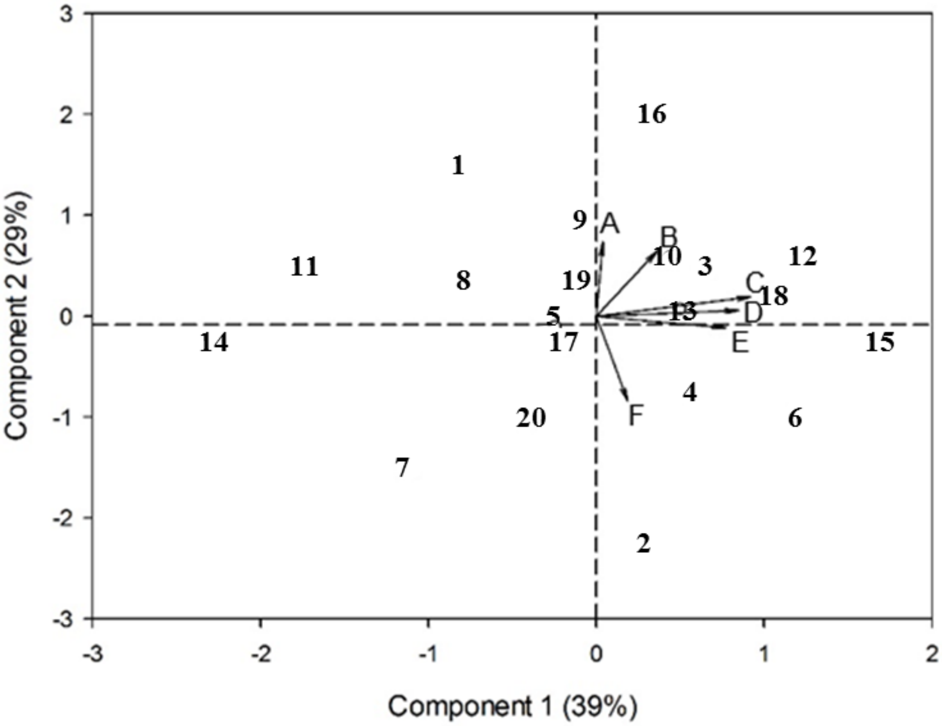

FIGURE 2.3 Rotated components plot for the principal components analysis of six mean response variables of twenty species to neighbour treatment (LRR). Response variables are: A) root:shoot ratio B) proportion of root length towards neighbor C) root length D) total biomass E) maximum width of root system F) depth of maximum width. Component 1 explains 39% of the variance and component 2 explains 29%. Numbers identify species from Table 2.15.

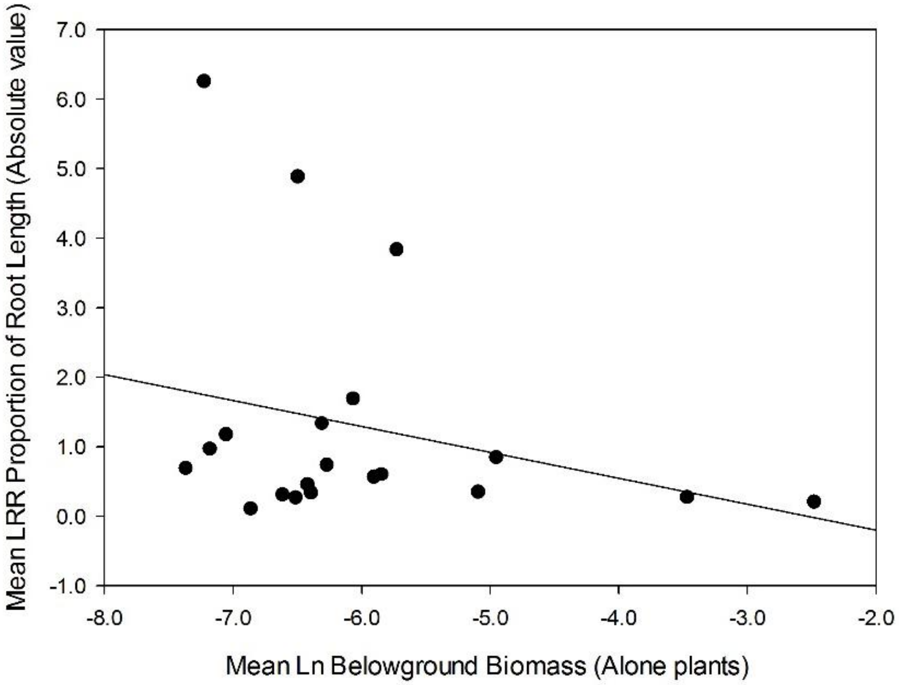

FIGURE 2.4 Mean plasticity in root placement response (Absolute value of LRR proportion of root length placed towards neighbour) of twenty species under the neighbour treatment in relation to mean root system size (as represented by belowground biomass) of species grown alone. R^2=0.074, $F_{1,18}$=1.436, p=0.246.

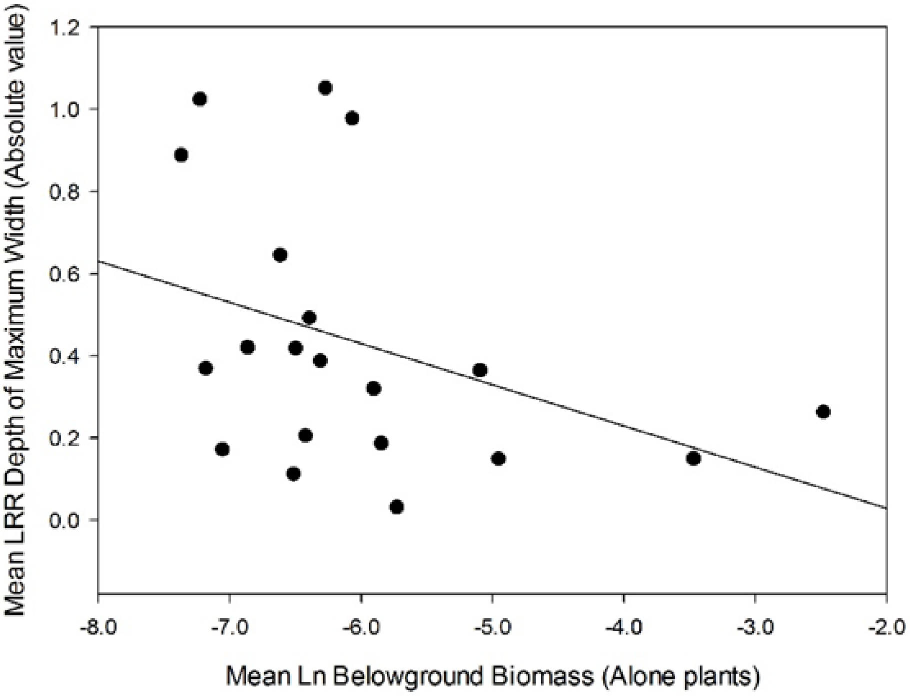

FIGURE 2.5 Mean plasticity in depth of widest point of root system (Absolute value of LRR depth of maximum width) of twenty species under the neighbour treatment in relation to mean root system size (as represented by belowground biomass) of species grown alone. R^2=0.150, $F_{1,18}$=3.166, p=0.092.

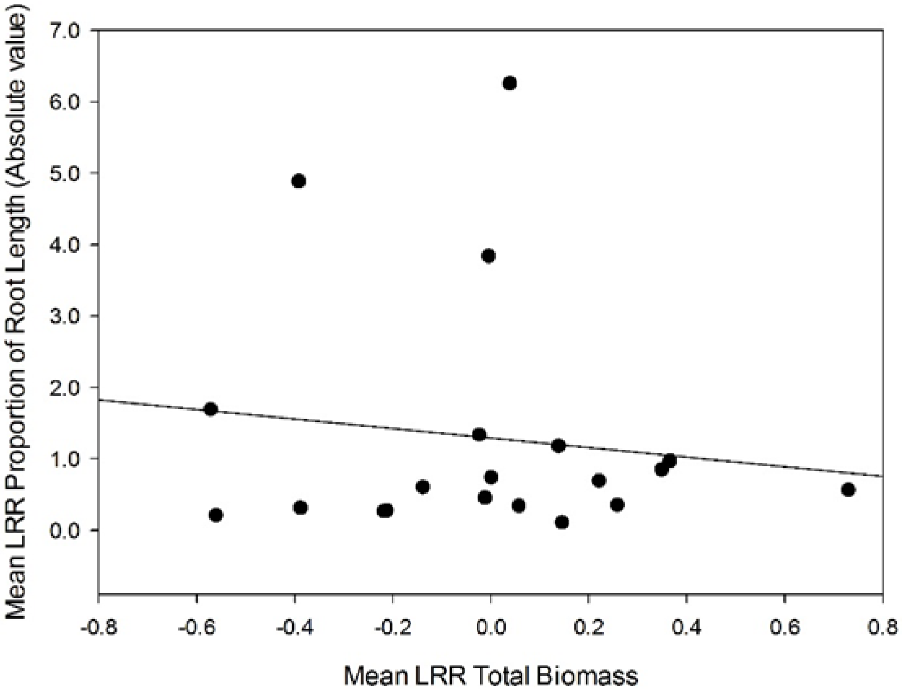

FIGURE 2.6 Mean plasticity in root placement response (Absolute value of LRR proportion of root length placed towards neighbour) of twenty species under the neighbour treatment in relation to mean competition experienced (LRR total biomass). R^2=0.017, $F_{1,18}$=0.303, p=0.589.

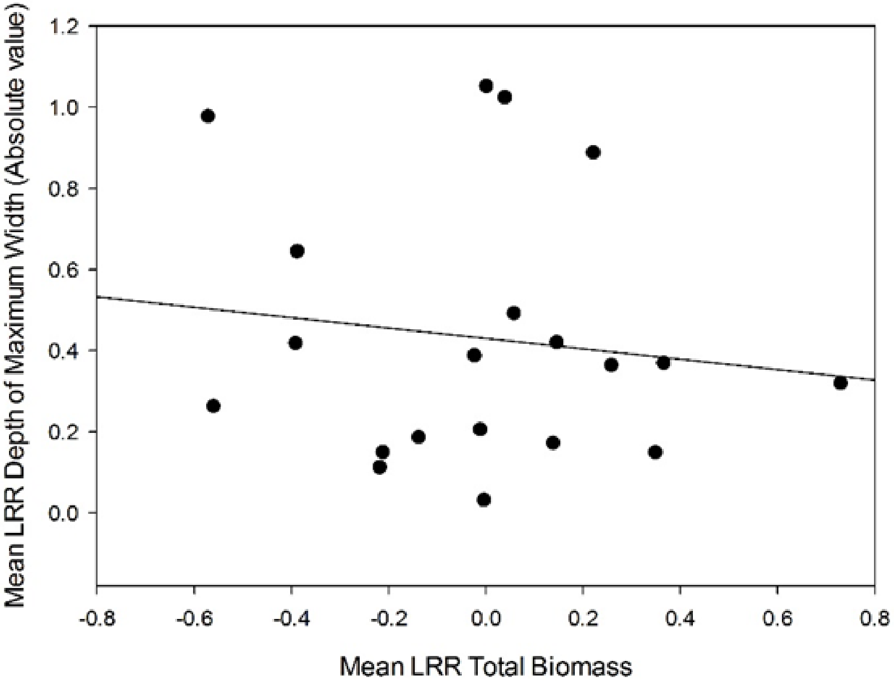

FIGURE 2.7 Mean plasticity in depth of widest point of root system (Absolute value of LRR depth of maximum width) of twenty species under the neighbour treatment in relation to mean competition experienced (LRR total biomass). R^2=0.017, $F_{1,18}$=0.309, p=0.585.

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CHAPTER 3 IDENTIFYING BELOWGROUND BEHAVIOURAL TYPES – CAN WE BETTER PREDICT PLANT OUTCOMES?

INTRODUCTION

All plants require a common set of resources, however, the diversity of plant life reflects the numerous strategies employed by plants to acquire these resources in varying environments. Strategies describe different combinations of adaptations that may allow a plant to be successful in these different environments. (Grime 1977; Southwood 1988; Taylor *et al.* 1990). By identifying strategies, plant ecologists attempt to develop general rules that link environmental factors to species composition (Lavorel and Garnier 2002). When environmental factors change, knowing how species with different strategies respond can help to predict how community composition might also change.

Many steps and processes will influence the plant outcome that ultimately arises from an environmental factor (Figure 3.1). For example, high nutrients in the soil could elicit root branching, or shift allocation of biomass to other organs such as shoots or reproductive parts, all of which might have an impact on plant size, survival, or fitness. At each of these steps, variation in response has the potential to result in different outcomes for an individual plant. To determine differences in individual plant outcomes, most studies have used plant traits to group species and attempt to predict ecological outcomes (Grime *et al*. 1997; Craine *et al.* 2001; Westoby *et al.* 2002; Lavorel and Garnier 2002; Diaz *et al*.

2004). One of the most extensive of these groupings is Grime's CSR (competitive, stress-tolerant, and ruderal) triangle which identifies strategies emerging from varying intensity of stress and disturbance (Grime 1974; Grime 1977; Grime *et al.* 1997). This classification system has been largely based on relationships between plant traits (Grime *et al.* 1997). While some of the traits used are more functional in nature, for example measures of root and shoot foraging, others such as mineral content of tissues or seed size represent plant measures at a snapshot of time.

In contrast to non-plastic traits, functional responses to environmental factors are likely those which will be most informative about plant outcomes. These functional responses are also known as behaviour and are increasingly the focus of plant studies (Karban 2008; McNickle *et al.* 2009; Cahill and McNickle 2011). As defined by Silvertown and Gordon (1989), behaviour is explicitly what a plant does in response to some change in its environment. For example, in response to heterogeneous soil nutrients, a plant is able to selectively proliferate roots within the nutrient rich patches (McNickle *et al*. 2009). Traits may play a role in constraining the types of behaviour that can be exhibited by a given plant (Figure 3.1), but measuring a trait will not necessarily inform what behaviour is actually expressed. From this, I suggest that it will be differences in species behaviours that will determine individual plant outcomes (Figure 3.1).

Plants will express different behaviours in different environmental contexts and it is conceivable that these behaviours may be related in some manner. Foraging behaviour for example, may be related to competition responses. Studies of animal behaviour have recognized that groups of behaviours exist that are consistent across different contexts (Sih *et al.* 2004a; Bell 2007). These suites of correlated behaviours are described as behavioural syndromes, with individuals expressing a specific behavioural type within the syndrome (Bell 2007). Variation in plant behaviours may also be correlated and the concept of behavioural syndromes may assist in synthesizing this information.

Animal behavioural syndromes have been used to explain species abundance, interactions and responses to environmental change (Sih *et al.* 2012). It would follow that this framework could also benefit the study of plant outcomes to environmental factors. Rather than considering a single behavioural response to environmental factors in isolation, the correlations and constraints imposed on that behaviour by other behaviours may play a larger role in informing any given individual plant outcome. By incorporating information about a plant's behavioural type, predictive power for the outcome should increase.

For natural systems such as grasslands, the majority of plant interactions occur belowground (Cahill 2003). I will use this system to examine plant outcomes in response to common belowground environmental factors (nutrient distribution, nutrient level, competition, and arbuscular mycorrhizal associations) and the behaviours expressed in these contexts (Table 3.1). Some of these behaviours, foraging precision (Kembel and Cahill 2005; Lamb *et al.* 2004) and responses to neighbours (Cahill *et al.* 2010; Schenk *et al*. 1999), have been extensively studied in plants already. Other behaviours, shifts in root allocation: nutrients (Klironomos 2003; Egger and Hibbett 2004), shifts in root allocation:

mycorrhizae (Chapin 1980), and soil exploration (Comas and Eissenstat 2004), build on variation that has been observed in plant species and, in the case of soil exploration, draws parallels to behaviours explored in animal behaviour studies.

First, I investigate the relationship among root behaviours exhibited in response to belowground environmental factors and the resulting plant outcomes. I will then explore interactions among behaviours from these different contexts to identify suites of correlated behaviours (with each species displaying a behavioural type). This will be used to determine if understanding a species' root behavioural type increases the understanding of plant outcome. Specifically I address the following questions:

1) Are plant growth responses to environmental factors influenced by individual root behaviours?
2) Are there suites of correlated root behaviours which create root behavioural syndromes?
3) Are plant growth responses to environmental factors influenced by root behavioural type?

MATERIALS AND METHODS

Study Species

Twenty co-occurring species were chosen across a range of plant families to capture a diversity of root behaviours that may occur among potentially naturally co-occurring species: ten Asteraceae; *Achillea millefolium* L., *Artemesia frigid* Willd., *Artemesia ludoviciana* Nutt., *Erigeron glabellus* Nutt., *Gaillardia* *aristata* Pursh, *Heterotheca villosa* (Pursh) Shinners, *Solidago missouriensis* Nutt., *Symphyotrichum ericoides* (L.) G.L. Nesom, *Symphyotrichum falcatum* (Lindl.) G.L. Nesom, and *Symphyotrichum laeve* (L.) Á. Löve & D. Löve; five Poaceae; *Bouteloua gracilis* (Kunth) Lag. ex Griffiths, *Bromus inermis* Leyss., *Elymus glaucus* Buckley, *Koeleria macrantha* (Ledeb.) Schult., and *Poa pratensis* L; two Rosaceae; *Drymocallis arguta* Pursh, and *Geum triflorum* Pursh; one Brassicaceae; *Descurainia sophia* (L.) Webb ex Prantl; one Fabaceae; *Astragalus agrestis* Douglas ex G. Don; one Polygonaceae; *Rumex crispus* L. Seed for these species were collected from multiple plants in a native rough fescue prairie at the University of Alberta Kinsella Research Ranch located near Kinsella, Alberta, Canada (53°05 N, 111°33 W). Our research group has an extensive history of research in this location and with the species found there (Lamb and Cahill 2008). Using species abundance data, common species were selected for this study.

To assess the five behaviours, data was collected from two experiments. The first, a mesocosm experiment, defined behavioural measures related to shifts in root allocation as well as foraging precision under varied soil nutrient treatments and arbuscular mycorrhizal fungi (AMF) spore addition. Because of the difficulties in visualizing roots in situ, a specialized apparatus was required to obtain detailed measures of soil exploration and root responses to neighbours. A second study, growth chamber experiment, was used to asses these behaviours.

Mesocosm Experiment

Experimental Set Up

Seeds of all eudicot species (except *D. sophia* which had excellent germination success with no stratification) were cold stratified for two weeks. *Astragalus agrestis* seeds were passed through a sieve to scarify prior to stratification. All seeds were germinated in seedling trays (sterilized with 10% bleach solution) filled with a 1:1 mixture of play sand and topsoil (Canar, Edmonton, Alberta, Canada). Topsoil was passed through a 6.3mm sieve to remove large debris and both substrates were autoclaved twice for 60 minutes at 121°C and 50psi. Seedlings were watered daily.

The five treatments consisted of four soil compositions (Figure 3.2) A) Heterogeneous, background soil (3:1 ratio of sand and topsoil) with a 2.5 cm diameter high nutrient patch (50% v/v composted cow manure, Sure-Gro Inc., Ste-Thérèse, Quebec, Canada) placed to one side approximately 3 cm from the pot centre; B) Homogeneous, the volume of manure from the heterogeneous nutrient patch mixed evenly throughout background soil; C) High nutrient, manufacturer recommended manure dose mixed evenly with background soil (33% v/v manure); D) Sterilized homogeneous, the same soil mix and nutrient content as the homogeneous soil but sterilized by autoclaving twice for 60 minutes at 121°C and 50psi. Pots were 1.67L and 15cm in diameter. Filter paper was placed at the bottom of all pots before filling to prevent soil loss through drainage holes and pots for the sterilized homogenous environments were sterilized using 10% bleach solution.

Homogeneous soil composition was used as a control with heterogeneous composition in order to measure foraging precision (Figure 3.2). The same homogeneous plants were used as the low nutrient treatment along with high nutrient configuration to measure shifts in root allocation (nutrients). The sterilized homogeneous soil was used for AMF treatments, one where AMF spores were added and one with no AMF spores (Figure 3.2). AMF spores were extracted from *Sorghum* trap cultures (soil originally collected from Kinsella, Alberta, Canada) using a sucrose gradient; filtrate from these extractions was saved.

Seedlings were bare root transplanted into the heterogeneous, homogeneous and high nutrient configurations on May 24 and 25, 2011. For the AMF treatments, seedlings were bare root transplanted into sterilized homogeneous soil on June 2 and 3, 2011. For the AMF treatment (1AMF) 1ml of filtrate from the spore extraction was applied to the bare roots followed by 1ml of spore solution (approximately 500-700 spores) before covering with soil. For the no AMF treatment (0AMF) only 1ml of filtrate was applied to roots before covering with soil. Each species-treatment combination was replicated five times and individuals that died within two weeks of transplant were replaced.

Pots were placed on the roof of the University of Alberta Biotron (Edmonton, Alberta, Canada) in five randomized blocks with each species-treatment combination represented once in each block. Plants were watered *ad libitum* throughout the experiment.

Harvest and Sampling

All plant shoots were clipped on September 5, 2011 after 15 weeks of growth. Tissues were dried at 70°C for a minimum of 72 hours and weighed. For the heterogeneous, homogeneous and high nutrient treatments two cores, 2.2cm in diameter and 11cm in depth, were taken opposite each other approximately 2.5cm from the pot edge. For heterogeneous treatments, one of these cores was taken in the high nutrient patch and the other in background soil. Remaining roots and the entire root systems of both AMF treatments were dry sieved (2mm opening) to remove excess soil and later washed, dried at 70°C for a minimum of 72 hours and weighed.

Response ratios (*sensu* Hedges *et al.* 1999; Cahill 1999) for behaviour and growth response variables were calculated as the natural logarithm of the ratio between treatment and control values:

$$\mathrm{LRR} = \ln(V_T / V_C)$$

Where V_T is the response value under the treatment condition (high nutrients, AMF spore addition, or heterogeneous high nutrient patch) and V_C is the response value under baseline control conditions. Response ratios were calculated per species by block. Positive LRR values indicate larger response under treatment conditions. Negative LRR values indicate reduced response under treatment conditions. For a listing of behaviour and growth response calculations see Table 3.2. Plant outcomes were measured as growth responses under treatment conditions since no measure of fitness in terms of reproductive output was measured.

Growth Chamber Experiment*

*For convenience, methods from Chapter 2 have been repeated here.

Experimental Set Up

Plants were grown in window boxes made of two pieces of Plexiglas (one black, one clear) and side spacers held together with binder clips (Figure 3.3). Polyester batting fibres and a horizontal bamboo skewer were arranged at the bottom of each window box to prevent soil from falling out while still allowing for drainage. This configuration provided approximately 5 mm x 190 mm x 250 mm of soil space for the plant to grow. The window boxes rested at a 40° angle, with the clear Plexiglas facing down to encourage root growth along this window for visualization. To prevent effects of light on the roots, this clear window was covered with a black plastic sheet held in place with elastic bands.

The window boxes were filled with a homogeneous soil composition of 3:1 sand to topsoil mix as well as approximately ~2% manure. The top 1 cm of each window box was filled with peat moss (Sun Gro Horticulture Canada Ltd) to help maintain soil moisture.

Seeds of the focal species were planted into the centre of the window box (9.5cm from each edge). For neighbour treatments, seeds of the neighbour were germinated on moist filter paper. When one of the focal seeds had germinated, the other seeds were removed and a germinated neighbour seed was planted. The neighbour was planted halfway between the focal plant and pot edge, to the right of the focal plant (plants were approximately 4.75cm apart) (Figure 3.3). In order to obtain a generic measure of response to a neighbour plant, the neighbour species were chosen so as to not naturally co-occur with the twenty focal species. One monocot (*Phleum pratense* L., Poaceae) and one eudicot (*Lactuca sativa* L. cv. Esmeralda M.I., Asteraceae) species were used. This resulted in three treatment configurations: alone plants with no neighbour, plants with *Phleum pretense* neighbour, and plants with *Lactuca sativa* neighbour.

The experiment was conducted in a growth room at the University of Alberta Biotron under controlled environmental conditions (16:8 hour light:dark cycle and temperature set at 24°C). Replicates were completed across 5 trials from April 2012 – January 2013. The experiment began when the focal plant germinated and pictures of the root system were taken every 3 days (10 picture sessions total). Due to varying germination success, as well as root visibility in the photos, each treatment combination had between 2 and 7 replicates with most having at least 3 replicates.

Harvest and Sampling

After 27 days the boxes were opened and plants removed. Roots of each plant were separated and rinsed free of soil. Roots and shoots were separated, oven dried (48 hours at 70°C), and weighed.

All photos were visually inspected and any replicates with roots not visible were removed from further analysis. Using ArcGIS software by Esri (version 10.1) the root pictures were digitized. Roots were traced with lines, and coded as focal or neighbour plant roots. A vertical line was added starting at the base of each plant to indicate the centre (Figure 3.3) and depth intervals were created by placing horizontal lines at every 10mm of depth starting at the plant base.

The following response measures were compiled for the focal plant at the final picture session (harvest):

BELOWGROUND BIOMASS

Dry mass of all belowground tissues measured in grams.

TOTAL BIOMASS

Combined dry mass of aboveground and belowground plant tissues measured in grams.

TOTAL ROOT SYSTEM AREA

A convex hull is created around all of the roots of each individual plant (Figure 3.3). The area (cm^2) of this convex hull is considered to be the total root system area ‘occupied’ by the plant.

PROPORTION OF ROOT LENGTH

Proportion of total root length (measured in millimetres) traced using ArcGIS software for a given individual plant that is found to the right of plant centre. When a neighbour is present this measure corresponds to the proportion of total root length placed towards that neighbour.

DEPTH OF MAXIMUM WIDTH

The 10mm depth interval with the maximum width of the root system. The depth measure is the lower end of the interval. Ie. A depth of 10mm would indicate the interval between 0-10mm. To measure width at each interval, the distance between the farthest root points left and right of centre is calculated. The largest of these widths represents maximum width of the root system.

When calculating LRR values (as described for the mesocosm experiment), replicates were paired based on trial number and resting angle of the boxes. Individual replicates of alone plants were not paired more than once with a neighbour treatment. Positive LRR values indicate larger response with neighbours. Negative LRR values indicate a reduced response with neighbours. For a listing of the behaviour and plant response calculations see Table 3.2. Plant outcome was measured as growth response under the neighbour treatment since no measure of fitness in terms of reproductive output was measured. No plant outcome was calculated in relation to soil exploration since the measure of growth in alone treatments could not be standardized.

Data Analysis

Although a breadth of plant families are represented by the twenty species used in the analysis, no phylogenetic analysis was performed. Therefore, the analyses do not account for any variation that is shared between species due to close evolutionary relatedness.

1) Are plant growth responses to environmental factors influenced by individual root behaviours?

To test whether behaviours are associated with plant outcomes linear regression analysis was performed. The mean behaviour measure for each species was entered as the dependent variable with the corresponding mean LRR plant outcome as the response variable. Because two responses to neighbour measurements were obtained, horizontal as well as vertical root placement, there were two analyses performed on the growth response: competition outcome. Plant outcomes were all measures of growth (change in total biomass) under environmental treatment conditions. Analysis was performed using IBM SPSS Statistics (version 20).

2) Are there suites of correlated root behaviours which create root behavioural syndromes?

To determine the associations among the six root behaviour measures (foraging precision, shifts in root allocation: nutrients, shifts in root allocation: mycorrhizae, soil exploration, horizontal response to neighbours, and vertical response to neighbours) Principal Components Analysis (PCA) was used. PCA is a data reduction method that reduces the number of variables by creating uncorrelated components that account for as much of the variation in the original data as possible (Gotelli and Ellison 2004). It assumes a linear relationship between variables. The PCA was performed using a correlation matrix and equamax rotation method. A fixed number of two factors was extracted. PCA analysis was performed using IBM SPSS Statistics (version 20).

3) Are plant growth responses to environmental factors influenced by root behavioural type?

To test whether behavioural types are associated with plant outcomes linear regressions were performed between the species axis loadings determined by the PCA conducted in analysis 2 and mean LRR plant outcome (change in biomass) as the response variable. Each PCA axis was analyzed with the LRR plant outcome separately resulting in a total of eight analyses. Analysis was performed using IBM SPSS Statistics (version 20).

RESULTS

1) Are plant growth responses to environmental factors influenced by individual root behaviours?

For most of the environmental factors considered here, behaviours and plant outcomes (as measured by growth effects) were uncorrelated (Table 3.3). A significant linear relationship was found between shifts in root allocation under high nutrients and the growth response in these conditions (Table 3.3; R^2=0.305, $F_{1,18}$=7.893, p=0.012). No significantly linear relationships were found for any of the other four behaviours (foraging precision, shifts in root allocation: mycorrhizae, horizontal response to neighbour, and vertical response to neighbour) and their corresponding growth response outcomes (Table 3.3).

2) Are there suites of correlated root behaviours which create root behavioural syndromes?

The six behavioural measures (foraging precision, shifts in root allocation: nutrients, shifts in root allocation: mycorrhizae, soil exploration, horizontal response to neighbours, and vertical response to neighbours) were reduced to two components in the principal components analysis, explaining 51% of the observed variation among species (Figure 3.4). Component 1 accounts for 29% of the variation with the measures shifts in root allocation: mycorrhizae, vertical response to neighbours, and shifts in root allocation: nutrients positively associated with the axis and horizontal response to neighbours and soil exploration associated negatively. Component 2 accounts for another 22% of the variation with measured foraging precision, and horizontal response to neighbours positively contributing to the axis and soil exploration negatively. Monocot species were spread evenly throughout the first component axis but tended to be slightly more clustered around zero in the second component (Figure 3.4 and Table 3.4).

3) Are plant growth responses to environmental factors influenced by root behavioural type?

No significant linear relationships were found between either of the PCA components and any of the four plant outcomes (Table 3.5). PCA component 2 explained more of the variation in growth response to heterogeneous nutrients than the behaviour of foraging precision alone however, neither correlation was significant (Table 3.3 and Table 3.5; R^2=0.015 and R^2=<0.001 respectively). None of the other regressions with PCA components explained more of the variation in plant outcomes than the corresponding behaviours alone.

DISCUSSION

1) Are plant growth responses to environmental factors influenced by individual root behaviours?

Shifts in root allocation under high nutrients was the only behavioural measure that was significantly correlated with its corresponding plant outcome (growth response: nutrients) (R^2=0.305, $F_{1,18}$=7.893, p=0.012). While all plants benefited from an increase in nutrients, those that expressed an increased allocation to roots garnered the largest benefit. This is opposed to findings of increased root allocation under low nutrient conditions which suggest that biomass should be distributed towards the limiting resource (Tilman and Wedin 1991; McConnaughay and Coleman 1999; Hermans *et al.* 2006). Nitrogen uptake however, has been linked to increasing fine root biomass density (Craine *et al.* 2002). If the additional roots produced by species which shifted their root allocation were similar fine roots, this may explain how they capitalized on an increased pool of resources for growth benefits.

The remaining behavioural measures in this study were not able to explain individual plant outcomes. In contrast to shifts in root allocation to nutrients, these behaviours did not have a clear optimal expression level. This result was not surprising in the case of foraging precision. No relationship between precision and the growth response to heterogeneous nutrients has been found in other studies (Kembel and Cahill 2005). This suggests that precise foraging behaviour is perhaps a derivative of some other advantageous process (de Kroon and Mommer

2006). For example, foraging precision has been beneficial in the presence of competition (Robinson *et al*. 1999).

The association between mycorrhizal fungi and plant roots is known to range from mutualistic to parasitic (Johnson *et al.* 1997; Klironomos 2003; Egger and Hibbett 2004) and this was reflected in the growth responses across species. Behavioural shifts in root allocation however, were unable to predict this growth response with mycorrhizal fungi (R^2=0.029, $F_{1,18}$=0.540, p=0.472). There were species across all behavioural levels that experienced negative as well as positive growth with mycorrhizal fungi. Hetrick *et al.* 1991 found that mycorrhizal dependent plants were able to alter root architecture in response to colonization and suggested that this conserves energy that might otherwise be allocated to roots. If this were the case, positive growth responses would be associated with lower allocation to roots in the presence of AMF. In contrast, my findings do not support this hypothesis and instead indicate that there is no shift in root allocation optimal for overall growth.

Root competition can result in large reductions in plant biomass and factors such as plant size may have an impact on the size of these effects (Kiær *et al.* 2013). Studies looking specifically at the fitness consequences of root responses to neighbours have found diminished reproductive yield associated with an over-proliferation of roots (Gersani *et al.* 2001; O'Brien *et al.* 2005). In the opposite scenario, segregation of roots can result in larger plants (Schenk *et al.* 1999). For the detailed root behaviours measured in this study, neither horizontal, nor vertical response to neighbour were able to explain growth response under competition (R^2=0.049, $F_{1,18}$=0.936, p=0.346 and R^2=0.003, $F_{1,18}$=0.053, p=0.820, respectively). Plasticity in root placement at these scales appear to simply be one behavioural response possible, which alone does not impact the individual plant outcome as measured by growth.

Most of the measured behaviours were unable to predict plant growth responses to environmental factors. Although these behaviours do not show a benefit for plants in terms of size, this does not exclude the possibility that the behaviours are beneficial in other ways (e.g. for reproduction or survival). Root placement response to neighbours, for example, may result in overall fitness benefits when the neighbour is kin (Dudley and File 2007; Murphy and Dudley 2009).

2) Are there suites of correlated root behaviours which create root behavioural syndromes?

Across the environmental treatments applied in this study, two main relationships among belowground behaviours were identified: assertiveness and focus. Together, the two extracted components account for 51% of the total variability in the six behaviours. The main component of variation (29%) separates species who exhibit low measures of soil exploration (less area of soil explored per gram of root tissue), horizontal as well as vertical avoidance of neighbours, and a shift to root allocation under high nutrients and AMF from those species with the opposite set of behaviours. Assertive species score low on the axis overall, with a tendency to venture further in the soil, aggregate with competitors, and shift to shoot allocation under high nutrient conditions or with the addition of AMF spores (which has the potential for a species to compete assertively aboveground).

The primary axis of variation for the trait-based plant strategies of Grime *et al.* (1997) separates species with acquisitive versus retentive traits. Aspects of the assertiveness axis identified here, such as aggregation with neighbours, high soil exploration and shifts to shoot biomass, may illustrate behaviours associated with high rates of resource acquisition. This study, however, did not test the resource capture of species and any congruence with the axis described by Grime *et al*. (1997) should be treated cautiously.

Additionally, the axis of assertiveness mirrors behavioural syndromes commonly explored by animal ecologists which involve boldness, exploratory behaviour, and aggression (Sih *et al.* 2004b). In this case, the behaviours of shifts to shoot allocation, soil exploration and aggregation with neighbours could be viewed as the plant equivalents that contribute to the correlation of these axes.

The second component accounted for an additional 22% of the variation in belowground behaviours. This component further separated species with high foraging precision, horizontal aggregation with neighbours and low soil exploration from species with the opposite suite of behaviours. The suite depicted by this axis is one of focus and localized intensity of roots. Species that exhibited high focus responded to the treatments through concentration of roots in specified areas such as in a high nutrient patch, or towards a competitor. Unsurprisingly, these species also do not explore the soil volume to a large extent which again displays the overall tendency to localize the intensity of root growth. This suite of behaviours reflects current behavioural research for plants that recognizes the variability in species abilities to selectively place roots in the soil (Hodge 2009; Cahill and McNickle 2011). It also reflects a trade-off between scale and precision. Campbell *et al.* 1991 originally proposed a trade-off between the scale and precision of foraging with scale represented by size of the root system. The focus component revealed by this analysis suggests that the trade-off exists between ability to localize the intensity of root growth and scale as measured by soil exploration (area occupied per gram of root tissue). This measure of scale is different than that of Campbell *et al.* 1991 in that it explicitly incorporates dimension and extent. A large root system (in terms of biomass) could have either high or low soil exploration values depending on its density.

Although distinguishing between monocot and eudicot species was not the original focus of this study, it was interesting to note that while the primary component of variation did not separate out these two groups, the second component did to some extent. This component clusters monocot species closer to zero values of focus and localized intensity of root growth. This finding is intriguing, as it has previously been observed that monocots display lower proliferation responses relative to eudicots (Kembel and Cahill 2005) which corresponds to a measure of focus.

3) Are plant growth responses to environmental factors influenced by root behavioural type?

Studies of correlations in animal behaviours have demonstrated fitness consequences for the boldness, exploration and aggression axes using measures of reproductive success or survival (Smith and Blumstein 2008). In contrast, the two PCA components identified here (assertiveness and focus) showed no significant linear relationships with any of the plant outcomes. Understanding behavioural types of the twenty species did not account for any more of the variation in outcome than single behaviours alone – rather they fit worse in general. Behavioural type appears to have no influence on fitness, as measured by growth response, under these environmental factors. Overall, this suggests that these plant outcomes may be governed by aspects not accounted for in these two axes.

One exception was the relationship between PCA component 2 (focus) and growth response in heterogeneous nutrients. While the relationship was not significant, it was able to explain more of the variation than the behaviour of foraging precision alone did ($R^2=0.015$ compared to $R^2<0.001$). Precision may provide an advantage under competition (Robinson *et al.* 1999) and the focus axis incorporates both foraging precision and response to neighbour behavioural measures. This supports the previous assertion that precise foraging behaviour may be a by-product of some other process (de Kroon and Mommer 2006). By understanding the interplay among behaviours in different contexts, we are able to expand our knowledge of how these relationships exhibit the plant outcomes we observe.

Future directions

This study initiated the exploration of plant outcomes through behaviours both singly, and in combinations of correlated suites. Root behavioural types did not influence growth responses to the environmental factors however, it would be ideal to test the validity of these behavioural strategies against an independent data set of species responses to changes in environmental factors. The benefits of behavioural types may be discovered when multiple environmental factors exist simultaneously. For example, species high on the assertive axis may perform better when competition, high nutrients and mycorrhizae are all applied. The outcomes that these behavioural types influence may emerge to be more complex than simply growth response. Discovering whether behavioural types can be used to predict species composition at the community level will determine their importance for plant ecology.

TABLE 3.1 Description of the five belowground behaviours investigated.

Behaviour	Description
Foraging precision	The ability to selectively place roots into patches of high nutrients.
Response to neighbours	Distribution and selective placement of roots when grown with a neighbour resulting in either avoidance, aggregation, or no response.
Shifts in root allocation: nutrients	The potential of a species to capitalize on increased nutrients through shifts in root:shoot ratio.
Shifts in root allocation: mycorrhizae	Shifts in root:shoot ratio due to association with arbuscular mycorrhizal fungi (AMF).
Soil exploration	The extent of soil breadth actively explored.

TABLE 3.2 Calculations for behaviour and plant outcomes in the two experiments.

	Behaviour		Plant Outcome	
Experiment	Behaviour	Calculation	Growth Response	Calculation
Mesocosm	Shifts in root allocation: nutrients	Ln $\frac{(\text{Root:Shoot}_{\text{High nutrients}})}{(\text{Root:Shoot}_{\text{Low (homogeneous) nutrients}})}$	Nutrients	Ln $\frac{(\text{Total Biomass}_{\text{High nutrients}})}{(\text{Total Biomass}_{\text{Low nutrients}})}$
	Shifts in root allocation: mycorrhizae	Ln $\frac{(\text{Root:Shoot}_{\text{AMF spore addition}})}{(\text{Root:Shoot}_{\text{No AMF}})}$	Mycorrhizae	Ln $\frac{(\text{Total Biomass}_{\text{AMF spore addition}})}{(\text{Total Biomass}_{\text{No AMF}})}$
	Foraging precision	Ln $\frac{(\text{Root Biomass}_{\text{High nutrient patch core}})}{(\text{Root biomass}_{\text{Background soil core}})}$	Heterogeneous Nutrients	Ln $\frac{(\text{Total Biomass}_{\text{Heterogeneous nutrients}})}{(\text{Total Biomass}_{\text{Homogeneous nutrients}})}$
Growth Chamber	Soil exploration	$\frac{(\text{Total root system area})}{(\text{Root biomass})}$		
	Response to neighbours: Horizontal root placement	Ln $\frac{(\text{Root Length Proportion}_{\text{Towards neighbour}})}{(\text{Root Length Proportion}_{\text{Towards right - no neighbour}})}$	Competition	Ln $\frac{(\text{Total Biomass}_{\text{With neighbour}})}{(\text{Total Biomass}_{\text{Alone}})}$
	Vertical root placement	Ln $\frac{(\text{Depth of maximum width}_{\text{Neighbour}})}{(\text{Depth of maximum width}_{\text{Alone}})}$		

TABLE 3.3 Results of linear regression analysis between behaviour and plant outcomes. Bold values are significant at $p<0.05$.

		Linear Regression			
Plant Outcome	Behaviour	R^2	df	F-value	P-value
Growth response: mycorrhizae	Shifts in root allocation: mycorrhizae	0.029	1, 18	0.540	0.472
Growth response: nutrients	Shifts in root allocation: nutrients	0.305	1, 18	7.893	**0.012**
Growth response: heterogeneous nutrients	Foraging precision	<0.001	1, 18	<0.001	0.994
Growth response: competition	Response to neighbour (horizontal)	0.049	1, 18	0.936	0.346
Growth response: competition	Response to neighbour (vertical)	0.003	1, 18	0.053	0.820

TABLE 3.4 Species axis loading scores for the two extracted PCA components.

Family	Identification number	Species	Component 1	Component 2
Asteraceae	1	*Achillea millefolium*	-1.191	-1.005
Asteraceae	2	*Artemesia frigida*	1.293	-0.786
Asteraceae	3	*Artemesia ludoviciana*	0.321	0.591
Asteraceae	4	*Erigeron glabellus*	0.439	-0.342
Asteraceae	5	*Gaillardia aristata*	-0.078	-1.019
Asteraceae	6	*Heterotheca villosa*	0.037	0.608
Asteraceae	7	*Solidago missouriensis*	1.630	1.080
Asteraceae	8	*Symphyotrichum ericoides*	-1.641	0.942
Asteraceae	9	*Symphyotrichum falcatum*	-0.758	0.141
Asteraceae	10	*Symphyotrichum laeve*	0.468	0.956
Polygonaceae	11	*Rumex crispus*	-0.559	1.161
Rosaceae	12	*Drymocallis arguta*	0.301	-0.023
Rosaceae	13	*Geum triflorum*	0.350	0.275
Fabaceae	14	*Astragalus agrestis*	-0.428	-3.194
Brassicaceae	15	*Descurainia sophia*	0.403	-0.323
Poaceae	16	*Bouteloua gracilis*	-1.777	0.172
Poaceae	17	*Bromus inermis*	0.067	0.479
Poaceae	18	*Elymus glaucus*	-0.981	0.291
Poaceae	19	*Koeleria macrantha*	-0.011	0.465
Poaceae	20	*Poa pratensis*	2.114	-0.468

TABLE 3.5 Results of linear regression analysis between the two extracted PCA components and plant outcomes.

	PCA Component 1				PCA Component 2			
Plant Outcome	R^2	df	F-value	P-value	R^2	df	F-value	P-value
Growth response: mycorrhizae	0.017	1, 18	0.305	0.587	0.007	1, 18	0.130	0.722
Growth response: nutrients	0.015	1, 18	0.272	0.608	0.001	1, 18	0.015	0.904
Growth response: heterogeneous nutrients	0.001	1, 18	0.024	0.878	0.015	1, 18	0.282	0.602
Growth response: competition	<0.001	1, 18	<0.001	0.991	0.003	1, 18	0.061	0.808

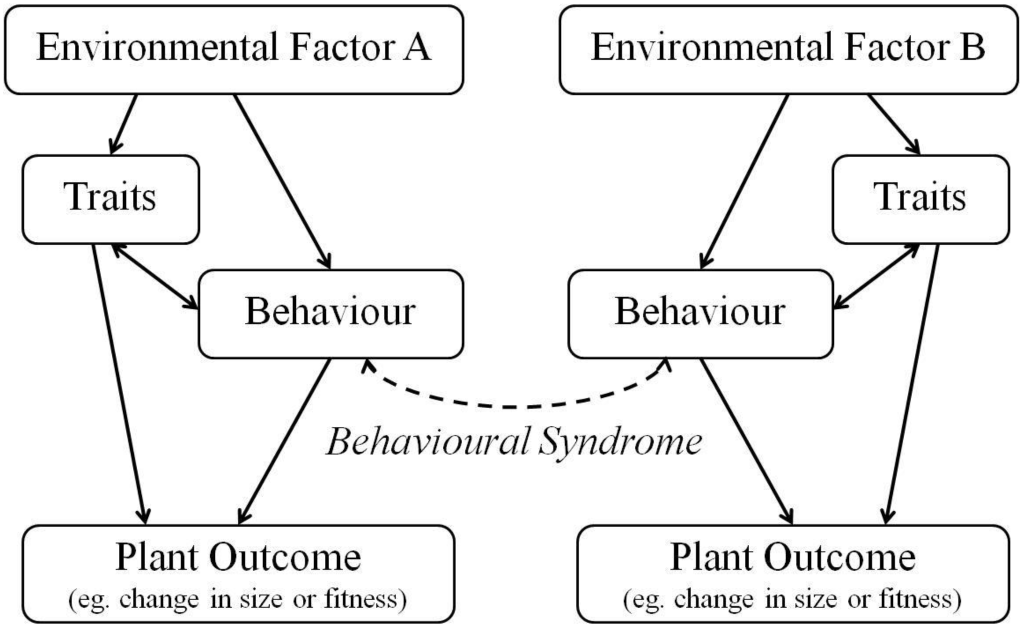

FIGURE 3.1 Conceptual diagram of putative relationships among two environmental factors and associated plant outcomes. Here, each environmental factor elicits both changes to specific traits, as well as behavioural responses. Combined, these determine the plant outcome in response to environmental factors. Traits may influence which behaviours can be expressed, but behaviour will also determine which traits are observed at a given point in time. Behaviours expressed in different contexts may be correlated akin to behavioural syndromes. Behavioural syndromes have the potential for behaviour in one context to constrain the type of behaviour exhibited in another.

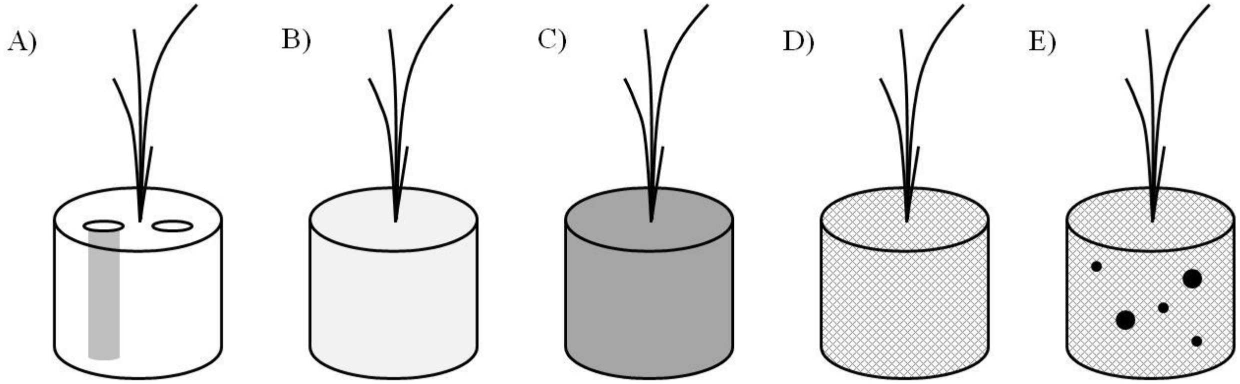

FIGURE 3.2 Six soil treatments used in the mesocosm experiment. A) Heterogeneous nutrients. Grey cylinder indicates high nutrient patch (50% v/v manure) and circles indicate the location of soil cores. Background soil (white) is a 3:1 sand to topsoil mix. B) Homogeneous nutrients. The volume of manure from the high nutrient patch in heterogeneous treatments mixed evenly throughout background soil. C) High nutrients. Manufacturer recommended manure dose mixed evenly with background soil (33% v/v manure) D) Sterilized soil with filtrate added (no arbuscular mycorrhizal fungi (AMF) spores). Nutrient quality is the same as homogeneous. E) Sterilized soil with AMF spores added. Nutrient quality is the same as homogeneous.

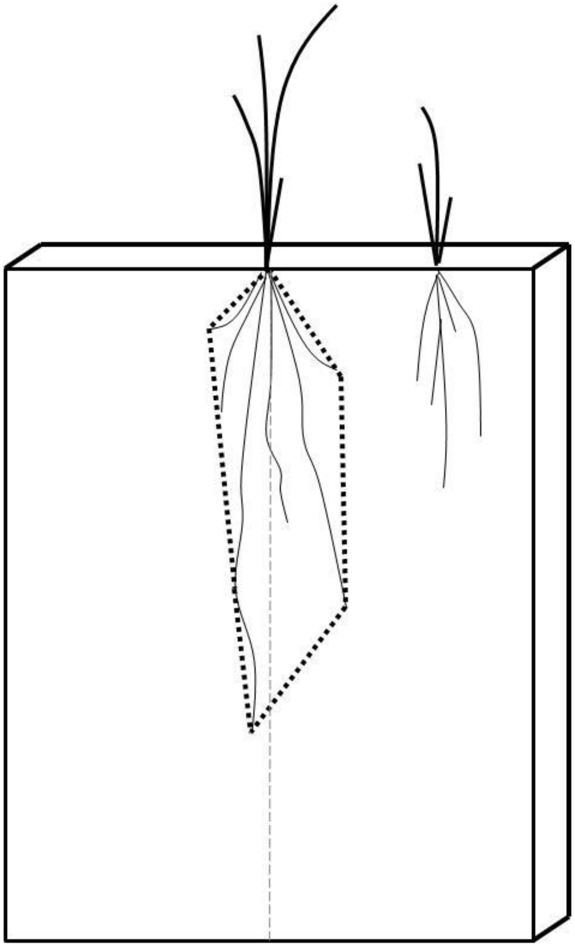

FIGURE 3.3 Schematic of the window box used in growth chamber experiment. For competition treatments center plant is the focal species with neighbour planted to the right, halfway between focal plant and box edge. No neighbour plant would be present in control (alone) treatment. Dashed vertical grey line delineates the right and left side of focal plant for measures of proportion of root length placed towards a neighbour (to the right). Dotted line represents the convex hull for calculating the area occupied by the focal plant.

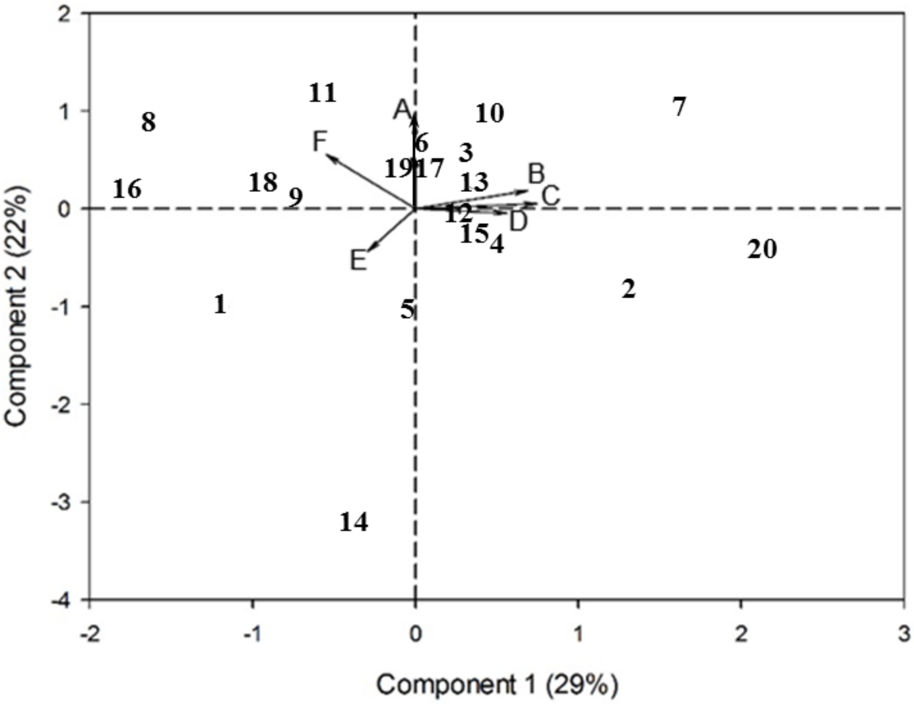

FIGURE 3.4 Rotated components plot for the principal components analysis of six mean behaviour variables for twenty species. Component 1 explains 29% of the variance and component 2 explains 22%. Response variables are: A) foraging precision B) vertical response to neighbours C) shift in root allocation: nutrients D) shift in root allocation: mycorrhizae E) soil exploration F) horizontal response to neighbours. Numbers identify species from Table 3.4.

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CHAPTER 4 GENERAL DISCUSSION

This thesis set out to describe plant behaviours related to belowground environmental factors of nutrient distribution and level, neighbours, and arbuscular mycorrhizal fungi (AMF). By measuring behaviours, I intended to capture the dynamic activities of plants belowground. Specifically, I investigated the following questions:

Chapter 2

1) In what ways do plants respond to neighbours belowground?
2) Is plasticity in root placement associated with root system size?
3) Is plasticity in root placement associated with the amount of competition experienced?

Chapter 3

1) Are plant growth responses to environmental factors influenced by individual root behaviours?
2) Are there suites of correlated root behaviours which create root behavioural syndromes?
3) Are plant growth responses to environmental factors influenced by root behavioural type?

Chapter 2 Conclusions

In what ways do plants respond to neighbours belowground?

Root responses to neighbours varied widely across species with both aggregation and segregation with neighbours observed. This supports the mixed findings for root response to neighbours in many other studies (Litav and Harper

1967; Baldwin and Tinker 1972; Brisson and Reynolds 1994; Schenk *et al.* 1999; Gersani *et al.* 2001; Semchenko *et al.* 2007). The two main means by which species aggregate or segregate with neighbours was via overall size and soil occupancy, and/or by altering allocation to roots and their placement.

Is plasticity in root placement associated with root system size?

Support for a trade-off between root placement plasticity and size was limited. Vertical root placement plasticity had a weak linear relationship to root system size, but horizontal plasticity did not. Originally, Campbell *et al.* (1991) proposed a trade-off with foraging precision as the measure of plasticity. My findings add to the growing number of studies that suggest any relationship between plasticity and size is weak at best (Kembel and Cahill 2005; Kembel *et al.* 2008).

Is plasticity in root placement associated with the amount of competition experienced?

The ability to alter root placement, either to avoid or aggregate with neighbours, was thought to provide a means of lowering the cost of competition. Neither vertical, nor horizontal root placement plasticity, however, were associated with the amount of competition experienced by a focal plant as measured by a change in total biomass with neighbours. What determines competitive outcome has proven to be much more complex than the single factor of placement plasticity.

Chapter 3 Conclusions

Are plant growth responses to environmental factors influenced by individual root behaviours?

Of the five behaviours explored, only one (shifts in root allocation under high nutrients) was able to explain the corresponding growth response. Plants that shifted more of their biomass to roots had the largest increase in biomass under high nutrient soils. Previous studies have focused on shifts to root biomass under low nutrient conditions and suggested that plants should shift allocation towards the limiting resource (Tilman and Wedin 1991; McConnaughay and Coleman 1999; Hermans *et al.* 2006). The relationship found here indicates that a shift in allocation to roots is also beneficial under high nutrients in order to capture more of the abundant resource.

Are there suites of correlated root behaviours which create root behavioural syndromes?

The six belowground behaviours studied (foraging precision, soil exploration, shifts in root allocation under high nutrients, with mycorrhizae, and horizontal and vertical responses to neighbours), were correlated along axes of assertiveness and focus. Species with both a high assertiveness and focus type would exhibit shifts to shoot allocation under high nutrient conditions, and with mycorrhizae, aggregate with competitors, and forage precisely.

Are plant growth responses to environmental factors influenced by root behavioural type?

Assertiveness and focus behavioural types were not associated with plant growth outcomes under the applied environmental factors. Overall, these types were unable to account for any more of the variation in outcomes than single behaviours alone. One exception to this, although not significant, was the relationship between focus behavioural type and growth response in heterogeneous nutrients. In this instance, behavioural type was able to explain more of the variation in growth than foraging precision did alone.

Synthesis and Future Directions

The results of this thesis indicate that correlations exist among suites of root response behaviours. Over half of the variation observed among species could be explained by two axes in both the detailed study of responses to neighbours (Chapter 2) and of behaviours from different environmental contexts (Chapter 3). At both levels (species response to one environmental factor, and correlation in responses across multiple factors), variation in the specific placement of roots was represented by the second axis and accounted for over a fifth of the observed variation. This emphasizes the importance of root placement plasticity for plant responses. Given that many studies have paid attention to the variation in root placement plasticity in terms of foraging precision (de Kroon and Hutchings 1995; Einsmann *et al*. 1999; Kembel and Cahill 2005), this is not an entirely surprising result. In contrast, the CSR triangle developed on the basis of many static traits does not account for this axis of plant variation (Grime 1974; Grime 1977; Grime *et al*. 1997), despite the inclusion of some measures of foraging precision.

Despite finding correlations across belowground behaviours in Chapter 3, the behavioural types were not associated with any growth responses to environmental factors. Suites of animal behaviours resulting in dimensions of boldness, exploration, and aggression have all shown some impact on fitness, either through survival or reproductive success (Smith and Blumstein 2008). These dimensions of animal behaviour share parallels with the assertiveness dimension found in Chapter 3 and press for the need to explore other measures of fitness before discounting the usefulness of root behavioural types. In addition, the role of these behavioural types may only become fully apparent when environmental factors are applied in combination.

One plant outcome, growth response under heterogeneous soil conditions, hints at the potential ability of behavioural types in understanding plant responses. Similar to previous studies (Kembel and Cahill 2005), growth response had no relationship to foraging precision behaviour alone and suggested that this behaviour may be the result of some other advantageous process (de Kroon and Mommer 2006). Root behavioural types identified by the focus dimension in Chapter 3 were able to increase, although not completely, the understanding of the variation in this plant outcome. Since this dimension also incorporated behaviour associated with competition, this could explain situations where foraging precision has been beneficial in contexts that include competition (Robinson *et al.* 1999).

By comparison, root placement plasticity was unable to explain competitive response in Chapter 2, which suggested that more behaviours might influence this outcome. The behavioural types of Chapter 3, however, were unable to increase the understanding of this growth response. Others have also concluded that competitive response must therefore incorporate multiple plant functions (Wang *et al.* 2010). Expanding knowledge of plant behaviours and behavioural types in such scenarios may lead to the explanation of this multifaceted plant outcome.

The findings of this thesis suggest that syndromes exist among belowground root behaviours. The benefit of correlated suites of behaviours, if any, is still unclear however, as growth response was not influenced by these behavioural types. Future studies are needed to further explore the potential benefits associated with specific behavioural types. In particular, direct measures of fitness, such as viable seed production, should be incorporated.

Another avenue of research will be to determine if behavioural syndromes are associated with species abundance in natural communities. Certain behavioural types may be best suited for specific combinations of environmental factors. Natural species abundance levels may reflect an optimal mix of behavioural types present in a community. In addition, the behavioural types of species could determine their potential for coexistence. For example, species with high assertiveness types may compete heavily for resources and exclude species exhibiting avoidance behaviour associated with low assertiveness types. By incorporating multifaceted information about species response to multiple environmental factors, behavioural syndromes may explain patterns of coexistence.

Behavioural syndromes in animals have been used to provide novel insights into species distribution, abundance, invasiveness, and response to environmental change (Sih *et al.* 2004; Sih *et al.* 2012). With a deeper understanding of plant behavioural types and the role they play in terms of plant fitness, species abundance, and coexistence, the field of plant ecology has great potential to benefit from the use of this animal behaviour concept.

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APPENDIX

EXPERIMENTAL DATA

Included in this thesis are electronic copies of datasets from the two experiments conducted. The datasets can be accessed through ERA: Education and Research Archive at the University of Alberta by using the following link: http://hdl.handle.net/10402/era.38312. Below are the experimental methods used to collect the data and tables with descriptions of variables.

PRB MSc Data – Mesocosm Experiment – May to September 2011

Seedlings were started in the University of Alberta Biological Sciences Biotron greenhouse (Edmonton, Alberta, Canada). Seeds of all eudicot species, except *Descurainia sophia*, were cold stratified for two weeks. Seeds of *Astragalus agrestis* were scarified prior to stratification. Seeds of all species were germinated in seedling trays (sterilized with 10% bleach solution) filled with a 1:1 mixture of sand and topsoil.

Five treatments consisted of four soil compositions: 1) Heterogeneous, background soil (3:1 sand to topsoil) with a 2.5 cm diameter high nutrient patch (50% v/v composted cow manure) placed to one side approximately 3 cm from pot centre; 2) Homogeneous, the volume of manure from the heterogeneous nutrient patch mixed evenly throughout the background soil, 3) High nutrient, manufacturer manure dose mixed evenly with background soil (33% v/v manure), 4) Sterilized homogeneous soil, the same soil mix as homogeneous but autoclaved twice for 60 minutes at 121°C and 50psi. All pots were 1.67L and 15cm in diameter with filter paper placed at the bottom to prevent soil loss. Pots for the sterilized homogeneous soil treatment were sterilized using 10% bleach solution.

Plants were bare root transplanted into 1.67L round pots; heterogeneous, high, and homogeneous treatments on May 24 and 25, 2011, and both AMF treatments on June 2 and 3, 2011. Two AMF treatments were created by adding to the bare roots 1mL of AMF spore solution (approximately 500-700 spores) extracted from *Sorghum* trap cultures for the positive AMF treatment, and 1mL of spore extraction filtrate to the no AMF treatment. Each species-treatment combination was replicated five times and pots were placed outside on the University of Alberta Biological Sciences Biotron rooftop (Edmonton, Alberta, Canada) in five randomized blocks. On May 31 and June 7 (14 days since transplant) dead seedlings in heterogeneous, high & homogeneous treatments were replanted. On June 20 (18 days since transplant) one dead plant in the AMF spore addition treatment was replanted.

On Sept 5, 2011 all plants were clipped at their base and shoots were separated into 3 categories: leaves/photosynthetic organs, reproductive (buds, flowers, and seeds), and support/structural/stem. Heterogeneous, homogeneous, and high treatments had two cores taken opposite one another, approximately 1" from pot edge (~2 1/4" apart) and 7/8"in diameter, 11cm in depth. One of these cores corresponded to the high nutrient patch in heterogeneous treatments. All cores were stored in freezer until they were washed, roots dried and weighed. Remaining roots of all treatments were dry sieved over 2mm sieve to remove excess soil, stored in cold room until being washed, dried and weighed. For the two AMF treatments, approximately 10 1.5cm fine root segments were clipped randomly throughout the root system and preserved in 75% EtOH for AMF staining. All plant tissues were dried at 70C for at least 72 hours and then weighed. To see a description of the variables included in the dataset please see Table 5.1.

PRB MSc Data – Growth Chamber Window Box Experiment – April 2012 to January 2013

This experiment was conducted from April 2012 to January 2013 with 5 trials taking place. The experiment took place in the University of Alberta (Edmonton, Alberta, Canada) biotron growth chamber with a 16:8 hour light:dark cycle and temperature set at 24°C. Plants were grown in window boxes made of two pieces of Plexiglas and side spacers held together with binder clips (Figure 5.1). Polyester batting prevented soil from falling out of the bottom. Soil composition: 3:1 sand to topsoil and approximately 2% steer manure mixed throughout, top 1cm of window box was filled with peat moss to maintain moisture. This configuration provided approximately 5mm x 190mm x 250mm of soil space. Window boxes rested at a 40° angle and clear window was covered with a black plastic sheet.

Focal plants were planted in the centre of the window box (approx. 9.5cm from each edge). In neighbour treatments, a neighbour was planted when the focal plant germinated. It was planted to the right, halfway between focal plant and pot edge (plants were approx. 4.75cm apart).

The experiment began (day zero) when the focal plant germinated and ended after 27 days. Pictures were taken every three days (10 picture sessions total). At the end of the experiment, roots of each plant were carefully separated from their neighbour (if present) and rinsed of soil. Roots and shoots were separated and oven dried for 48 hours at 70°C then weighed. All photos were visually inspected and replicates with no visible roots were removed from analysis. Root pictures were digitized using ArcGIS software by Esri (version 10.1) with roots traced and coded as focal or neighbour plant roots. A vertical line starting at the base of each plant was added to indicate the centre for left and right measurements. Depth intervals were created by horizontal lines every 10mm of depth starting at the plant base. To see a description of the variables included in the dataset please see Table 5.2.

TABLE 5.1 Description of variables included in the mesocosm data set (PRB MSc Data – Mesocosm Experiment – May to September 2011).

Variable	Level of Measure	Definition
Block	N/A	Designates to which of the five blocks this replicate belongs
Species	N/A	Species name
Treatment	N/A	0AMF = sterile soil with no AMF spores added; 1AMF = AMF spores added to sterile soil; Het = Heterogeneous; Hom = Homogeneous ;High nutrient treatments
Leaves	Plant	Dried biomass of leaf tissue in grams
Stem	Plant	Dried biomass of supportive stem tissue in grams
Reproductive	Plant	Dried biomass of reproductive structures in grams
TOTAL AG	Plant	Total dried biomass of all aboveground parts (leaf, stem & reproductive) in grams
Other BG	Plant	Dried root biomass not found in cores in grams (for AMF treatments this will be all of the root biomass)
Patch	Plant	Dried root biomass in high nutrient patch core (for Hom or High treatment the soil conditions of this core and Core2 are the same) in grams
Core2	Plant	Dried root biomass in opposite core (background soil for Het treatment) in grams
TOTAL BG	Plant	Total dried biomass of all belowground parts (Patch, core2 & other belowground) in grams
TOTAL BIOMASS	Plant	Total dried biomass of the plant (Aboveground & Belowground parts) in grams

TABLE 5.1 Extended.

Variable	Level of Measure	Definition
RSRatio	Plant	Root:Shoot ratio calculated as (BG Biomass/AG Biomass)
Fprec	Heterogeneous plant	Foraging precision calculated as (Patch/Core2) in the Het treatment
MycBen	Species measure for block	Mycorrhizal benefit calculated as (Total biomass in 1AMF/Total biomass in 0AMF)
NutExpAb	Species measure for block	Nutrient exploitation ability calculated as (Total biomass in High/Total biomass in Homogeneous)
BenHet	Species measure for block	Benefit from heterogeneous soil calculated as (Total biomass in Heterogeneous/Total biomass in Homogeneous)
Nut_RSRatioChange	Species measure for block	Change in Root:Shoot ratio with high nutrients (R:S Ratio in High/R:S Ratio in Homogeneous)
Myc_RSRatioChange	Species measure for block	Change in Root:Shoot ratio with mycorrhizae (R:S Ratio in 1AMF/R:S Ratio in 0AMF)

TABLE 5.2 Description of variables included in the growth chamber data set (PRB MSc Data – Growth Chamber Window Box Experiment – April 2012 to January 2013).

Variable	**Definition**
TrialNum	Trial number (1 = April 22 to June 3, 2012; 2 = June 3 to July 2, 2012; 3 = August 21 - September 29, 2012; 4 = October 9 - November 17, 2012; 5 = December 3, 2012 - January 14, 2013)
Focal	Focal species abbreviation
Neighbour	Neighbour species originally planted (may not have germinated, however)
Group	Indication of whether focal plant is a grass or forb [Note: in the case of NL or NP, this column will indicate grouping for the remaining neighbour plant eg. NP = Grass]
Treatment	P= Phl pra L= Lac sat A= Alone, no neighbour NL= Lac sat neighbour, no focal NP= Phl pra neighbour, no focal
Replicate	Replicate number of this species-neighbour combination
Plant	1= Focal plant 2= Neighbour plant
Angle	Potential slant of window box
Session	Image session # 1-10 for this replicate [note that only sessions 1, 2, 4, 6, 8 & 10 were traced from most replicates]
DateInfo	Actual date image was taken
TrialName	Full image file name that tracing measures were extracted from

TABLE 5.2 Extended.

Variable	**Definition**
FocalAGBio	Biomass of aboveground parts of focal plant (g)
NeighAGBio	Biomass of aboveground parts of neighbour plant (if present) (g)
FocalBGBio	Biomass of belowground parts of focal plant (g)
NeighBGBio	Biomass of belowground parts of neighbour plant (if present) (g)
FocalTotalBio	Total biomass of focal plant (g)
FocalRSRatio	Root to shoot ratio of focal plant
NeighbourTotalBio	Total biomass of neighbour plant (g)
NeighbourRSRatio	Root to shoot ratio of neighbour plant
NumRoots	Number of roots traced
RootLength	Length of roots traced (mm)
Length_L	Length of roots traced to the left of plant centre (mm)
Length_R	Length of roots traced to the right of plant centre (mm)
LengthRatioRL	Ratio of root length on either side of plant centre
PropLength	Proportion of roots towards right side
MaxDepth_L	Maximum depth of roots on left side (mm)
MaxDepth_R	Maximum depth of roots on right side (mm)
MaxDepth	Maximum depth of entire root system (mm)
Width_L	Maximum root distance to the left from vertical centre line (mm)

TABLE 5.2 Extended.

Variable	**Definition**
Width_R	Maximum root distance to the right from vertical centre line (mm)
MaxWidth	Maximum distance across entire root system at any given 10mm depth interval (mm)
DepthMW	Depth interval in which maximum width of root system is found
DMW_L	Depth interval in which maximum width of root system to the left is found
DMW_R	Depth interval in which maximum width of root system to the right is found
Area	Total area occupied by the root system (convex hull) (mm^2)
Area_L	Area of convex hull to the left of vertical centre line (mm^2)
Area_R	Area of convex hull to the right of vertical centre line (mm^2)
AreaRatioRL	Ratio of area towards (right) and away (left) from neighbour
PropArea	Proportion of area towards right side/neighbour
NullArea	Area of convex hull created by null model using total width of each depth interval centred on the vertical centre line (mm^2)
SNOverlap	Total area of overlap between the species area and its null area (mm^2)
SSOverlap	Total area of overlap between the focal species area and neighbour area
PropOverlap	Proportion of Focal species area overlapped with Neighbour
SSExclude	Total area of exclusion between the focal and neighbour species
NNOverlap	Total area of overlap between the focal and neighbour species null areas
NNExclude	Total area of exclusion between the focal and neighbour species null areas

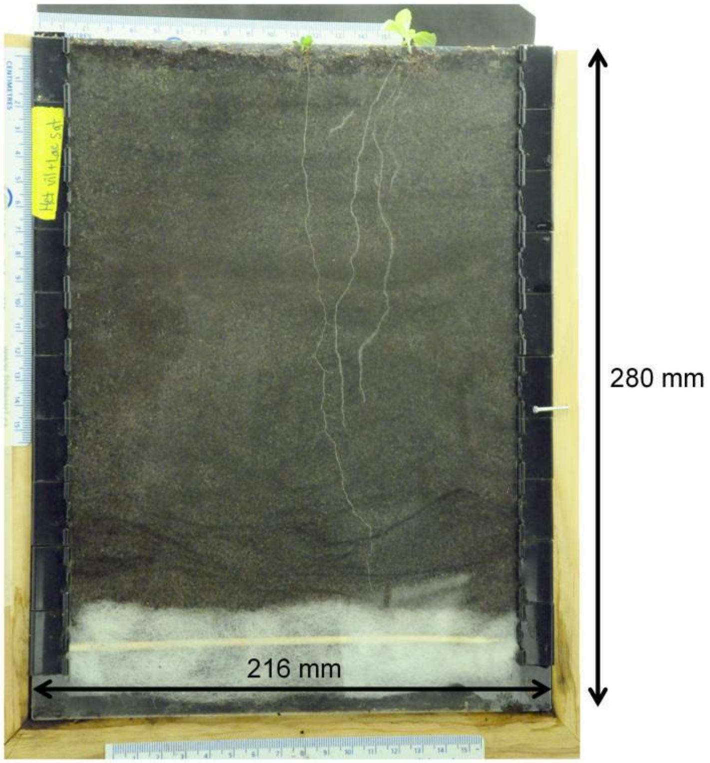

FIGURE 5.1 Window box apparatus used to visualize roots in situ. Two Plexiglas sheets with side spacers are held together with binder clips to create the box. Polyester batting at the bottom of the frame was used to prevent soil from falling out.