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ROOT FORAGING BEHAVIOUR OF PLANTS: NEW THEORY, NEW METHODS AND NEW IDEAS

by

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DEDICATION

To Julia.

ABSTRACT

All organisms, including plants, experience variability in the environment which puts pressure on organisms to evolve flexible responses. The study of these responses by organisms falls into the discipline of behavioural ecology. In this thesis, I am interested in the foraging behaviour of plant roots and I have two goals. First, I will use foraging theory from the animal literature to determine whether plants forage in ways that are similar to animals. Second, I will show how the adoption of foraging theory for plants can lead to a better theoretical understanding of coexistence of plants. I begin with a discussion of the major differences between plants and animals in their foraging behaviour and how this can be incorporated in to a more general predictive framework of plant foraging behaviour. I follow this discussion with two empirical tests of classic foraging models. First, I test a patch use model from the animal literature to determine if it can predict plant foraging behaviour. My results show that plants foraged for patches using the same strategies used by animals. Second, I test a resource choice model from the animal literature. These data indicated that plants select different types of nitrogen using the same resource choice strategies as foraging animals. These two studies reveal some basic foraging abilities of plants, however the experiments were performed in the absence of resource competition, a condition seldom experienced by plants in nature. To overcome difficulties in studying plant roots grown with neighbours I developed a molecular method for the identification of visually indistinguishable plant roots from competition

experiments. Finally, I apply the molecular method to examine whether resource patchiness in soil can increase the intensity of competition experienced by foraging plants, and that the presence of neighbours influences the foraging strategies of plants. Together the results presented in this thesis show that plants use the same basic foraging strategies as animals, and that foraging behaviour can be linked to competition and coexistence of plant species.

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LIST OF SYMBOLS

CHAPTER 2

E – Energy intake during feeding period

T – Total length of feeding period

T_S – Search time

T_H – Handling time

E_{ij} – Energy intake of the *i*th root, on the *j*th ramet

T_{Sij} – Search time of the *i*th root, on the *j*th ramet

T_{Hij} – Handling time of the *i*th root, on the *j*th ramet

$f(r_{ij})$ – any function describing the potential benefits of nutrient capture by plants

$f(c_{ij})$ – any function describing the potential costs of nutrient capture by plants

E_{gain} – Energy gained by the plant through foraging efforts defined by the function $f(r_{ij})$

E_{spent} – Energy spent by the plant through foraging efforts defined by the function $f(c_{ij})$

CHAPTER 4

dI – Isotopic composition of samples

R_{sam} - the ratios of either $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample

R_{sta} - the ratios of either $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the standard

y – the measured $d^{15}N$ value for each plant

a – the proportion of the labeled nitrogen source captured by the plant

b – the known isotopic signature of the labeled nitrogen source

x – the known isotopic signature of the unlabeled nitrogen source

N_i – symbol representing nitrogen i

N_j – symbol representing nitrogen j

R_i – abundance of nitrogen i

N_j – abundance of nitrogen j

log (R_j/R_i) – the log ratio of the abundance of resource j to resource i

CHAPTER 5

T_m – Primer melting temperature

CHAPTER 6

lnRR – Log response ratio, a measure of the intensity of competition

B_{indi} – Biomass of individual plants grown alone

B_{comp} – Biomass of individual plants grown with neighbours

LIST OF ABBREVIATIONS

GENERAL

MVT – Marginal value theorem

GLM – General Linear model

ANOVA – Analysis of variance

GLMM – Generalized linear mixed model

lmer – A GLMM package from the R statistical environment (R v 2.9.2), in the lme4 package.

PROC GLIMMIX – A GLMM package from the Statistical Analysis Software (SAS v2.9)

PROC GLM – A GLM package from the Statistical Analysis Software (SAS v2.9)

PROC ANOVA – An ANOVA package from the Statistical Analysis Software (SAS v2.9)

df – degrees of freedom

DNA – deoxyribonucleic acid

PCR – polymerase chain reaction

(w/v) – weight per volume, to calculate concentrations listed in percent

(v/v) – volume per volume, to calculate concentrations listed in percent

Het – heterogeneous

Hom - homogeneous

CHAPTER 3

GUT – Giving up time

High-L – Soil treatment where plants encounter a high quality patch close to the stem (Figure 3.1)

Low-H – Soil treatment where plants encounter a low quality patch close to the stem (Figure 3.1)

Hom – Soil treatment where plants encounter only homogenous soil, with no patches (Figure 3.1)

CHAPTER 4

RFLP – Restriction Fragment Length Polymorphism

ITS – the internal transcribed spacer region of nuclear ribosomal DNA

Tris – tris(hydroxymethyl)aminomethane, a compound used in DNA buffers

EDTA – Ethylenediaminetetraacetic acid, a compound used in DNA buffers

CTAB - hexadecyltrimethylammonium bromide, a compound used in DNA Extractions

PVP – polyvinylpyrrolidone, a compound used in DNA extractions

PVPP – polyvinylpolypyrrolidone, a compound used in DNA extractions

dATP – Deoxyadenosine triphosphate, a nucleotide precursor used in PCR for DNA amplification.

dCTP – Deoxycytidine triphosphate, a nucleotide precursor used in PCR for DNA amplification.

dGTP – Guanosine triphosphate, a nucleotide precursor used in PCR for DNA

amplification.

dTTP - Thymidine triphosphate, a nucleotide precursor used in PCR for DNA amplification.

LNA – locked nucleic acid

bp – base pairs

Ach – Abbreviated species name, *Achillea millefolium* L. (Asteraceae)

Art – Abbreviated species name, *Artemisia ludoviciana* Nutt. (Asteraceae)

Ast – Abbreviated species name, *Astragalus agrestis* Douglas ex G. Don (Fabaceae)

Bro – Abbreviated species name, *Campanula rotundifolia* L. (Campanulaceae)

Cam – Abbreviated species name, *Bromus inermis* Leyss. (Poaceae)

Fes – Abbreviated species name, *Festuca hallii* (Vasey) Piper (Poaceae)

Koe – Abbreviated species name, *Koeleria macrantha* (Ledeb.) J.A. Schultes (Poaceae)

Poa – Abbreviated species name, *Poa pratensis* L. (Poaceae)

Ros – Abbreviated species name, *Rosa arkansana* Porter var arkansana (Rosaceae)

The – Abbreviated species name, *Thermopsis rhombifolia* (Nutt. Ex Pursh) Nutt. Ex Richardson (Fabaceae)

CHAPTER 5

CTAB method – a DNA extraction protocol using hexadecyltrimethylammonium bromide, described in Chapter 5.

RSPF – Resource selection probability function

AICc – small sample size corrected Akaike Information Criteria score

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1. GENERAL INTRODUCTION

All organisms, including plants, are faced with environments that vary in both time and space. This variability puts pressure on organisms to be flexible in their responses to environmental cues. Furthermore, individuals that can respond adaptively to such variability in environmental stimuli should be favoured by natural selection over individuals that show no response (Krebs and Davies 1987). The idea that plants might exhibit complex responses to their environment is at least as old as Darwin, who studied the movement of plant leaves in response to light, and the movement of plant tendrils in response to touch (Darwin 1865). However the formal study of plant behaviour has only become common in recent years (Satter and Galston 1973; Sutherland and Stillman 1988; Silvertown and Gordon 1989; Kelly 1990; de Kroon and Hutchings 1995; Gleeson and Fry 1997; Schenk *et al.* 1999; Dudley and File 2007). Behaviour has been defined as shifts in growth, movement or physiology by organisms, in the course of their lifetime in response to variable environmental stimuli (*sensu* Silvertown and Gordon 1989), and that is the definition I will use here. This definition is advantageous because it is not taxa specific and can include all forms of life including plants and animals. It should be noted that the above definition of behaviour overlaps with the idea of phenotypic plasticity where plasticity is related to responses to the environment. Behaviour will be considered adaptive if alternate behaviours have a direct impact on components of fitness in differing environments.

A diversity of behavioural topics have been explored in plants, and these include kin selection (Dudley and File 2007; Murphy and Dudley 2009), mate choice (Marshall and Folsom 1991; Lankinen and Kiboi 2007), territoriality (Schenk *et al.* 1999; Cahill *et al.* 2010), communication (Callaway 2002; Falik *et al.* 2003; Gruntman and Novoplansky 2004; Karban 2008), competitive games (O'Brien *et al.* 2007; Falster and Westoby 2003; Gersani *et al.* 2001) and optimal foraging (Kelly 1990; Kelly 1992; Gleeson and Fry 1997). These studies all show that plants exhibit behavioural responses to external stimuli and that plant behaviour may be similar to animal behaviour. This thesis will focus on foraging behaviour in plants, and I seek to develop a predictive framework to understand why plants forage as they do. I also am interested in being able to predict the ecological consequences of plant foraging behaviour.

To develop plant foraging theory, I will draw from existing foraging theory developed to predict animal behaviour and I will address two main questions in my thesis: First, do plants forage following similar foraging rules as animals? Plants and animals are expected to be under similar evolutionary pressure to find and assimilate resources in the most efficient way possible. Thus, there is good reason to think that plants and animals might forage using similar rules. If this is true, it will make the development of a more general theory of plant foraging behaviour relatively simple since plant ecologists will be able to draw from the rich pre-existing literature on animal foraging behaviour. Second, in this thesis I ask, what are the ecological consequences of plant foraging behaviour for competition among species? Foraging can often be helpful to

understand how species compete (Brown *et al.* 1994; Hutchings *et al.* 2003; Stephens *et al.* 2007). The development of predictive theory for plant foraging behaviour should be helpful for understanding how plant foraging behaviour may contribute to plant competition and ultimately plant species coexistence.

In the sections that follow I will briefly introduce some basic concepts from foraging theory, provide a brief overview of how plants forage, and discuss how specific foraging theories may contribute to mechanisms of species coexistence. However, before delving into the realm of foraging theory I must be clear the types of behavioural questions I will address in this thesis.

1.1.1. Questions about behaviour

In the study of behaviour, two kinds of questions are distinguished in the animal literature: proximate and ultimate (Tinbergen 1963; Krebs and Davies 1987). Questions about mechanism and development provide proximate answers about *how* a given behaviour comes about in the lifetime of an organism. For example, it is well known that plants forage by growing organs (leaves, roots or clonals) into spaces which contain resources (Hutchings and de Kroon 1994; Hodge 2004; Hodge 2006). Questions about evolutionary history and functional significance provide ultimate explanations about *why* a given behaviour exists in the repertoire of a species. For example, some have suggested that plants may be motivated to forage because capturing resources more efficiently than a neighbour may enhance competitive ability, and thus fitness (Hodge *et al.* 1999; Robinson *et al.* 1999). Generally, plant behaviourists have not framed their questions this way

(but see Silvertown and Gordon 1989), but doing so might avoid confusion in the development of plant behavioural research programs.

In this thesis I will focus primarily on ultimate questions surrounding plant foraging behaviour. Foraging models in the animal literature are usually constructed by assuming that foragers behave adaptively, and attempt to predict a foraging response from this assumption. I will approach the problem of plant foraging behaviour by testing specific optimal foraging models from the animal literature to determine whether they predict plant behaviour. This approach has several advantages. First, if plants behave like animals then plant behaviour can be integrated into the more general framework of behavioural ecology. Second, the development of plant theory can be significantly enhanced by drawing on the pre-existing literature if it is true that plant behaviour can be explained using the same framework as animal behaviour.

1.1.2. Foraging Behaviour

The essential resources required of all organisms are generally heterogeneously distributed, and thus organisms need behavioural responses that allow them to forage effectively in a heterogeneous world (Macarthur and Pianka 1966; Hutchings and de Kroon 1994; Hodge 2006; Stephens *et al.* 2007; Heineman *et al.* 2008). This heterogeneity can take two forms. First, resources may be aggregated into spatially discrete patches (Macarthur and Pianka 1966; Charnov 1976b; Hutchings and de Kroon 1994; Hodge 2006). When the world is patchy, there is significant selective pressure on organisms to find and use high

quality patches, and a basic prediction is that high value patches should be preferentially used over lower quality areas between patches (Macarthur and Pianka 1966; Charnov 1976b). Second, within patches, resources can be available in multiple substitutable forms which may vary in their abundance and in the net benefits obtained from their consumption (Holling 1959; Macarthur and Pianka 1966; Werner and Hall 1974; Charnov 1976a). Examples of substitutable forms of resources could be different insect prey for a predatory manatid (Charnov 1976a), different bacterial strains for a viral bacteriophage (Heineman *et al.* 2008), or different chemical forms of nitrogen for a plant (Kielland 1994; McKane *et al.* 2002). When there are different types of substitutable resources available, there should also be significant selective pressure for organisms to capture those resources which yield the greatest net benefits to the forager.

Broadly speaking, foraging theory is concerned with understanding the behavioural responses of organisms to these two types of resource heterogeneity (*i.e.* patches and prey). However, there are some clear differences between plants and animals which will alter the applicability of behavioural theory to plants. In Chapter 2, I explore what I perceive to be the major differences between plants and animals which will influence the predictions and applicability of existing foraging theory from the animal literature to plants. I will also suggest how patch use, and prey choice models from the animal literature might be used to make predictions about plant foraging behaviour. These chapters will center on ultimate questions about plant foraging behaviour. But it will be useful to understand a little about how plants forage for either patches or different resources.

1.1.3. How do plants forage for patches?

Research into the foraging ecology of plants has mostly focused on patch use behaviour of plants, either through the placement of interconnected clones into patches (*e.g.* Birch and Hutchings 1994; de Kroon and Hutchings 1995; Wijesinghe and Hutchings 1999) or through the placement of belowground roots into patches (*e.g.* Drew and Saker 1975; Campbell *et al.* 1991; Jackson and Caldwell 1989). In this thesis, I will focus on the root foraging behaviour of plants. For roots, over 100 species have been assayed to determine how they place roots in the soil relative to nutrient distributions (Johnson and Biondini 2001; Hodge 2004; Kembel and Cahill 2005; Hodge 2008). Generally, plants respond to soil nutrient patches by placing more roots into patches than they do in the surrounding soil. The ratio of root mass inside a patch relative to root mass in background soil is often called foraging precision. Throughout this thesis, the difference in root growth inside patches, compared to outside of patches will be the metric I use to determine the magnitude of plant root foraging behaviour in response to patches of different quality, or to compare behaviour among species. However, I do not use the precision ratio that is commonly used because ratios mask differences in magnitude that may be important. For example, 10/1, and 1000/100 yield identical ratios but there may be something very different about the underlying behaviours that yield such ratios, and this magnitude difference may be important.

Despite the large amount of research on the topic of patch use behavior in plants, there has been relatively little theory available to predict the responses of plant roots (but see Grime 2007). Theory is important to develop predictive frameworks, but also to understand the selective pressures which drive the evolution of certain behaviours. In Chapter 3, I test the patch choice model known as the marginal value theorem (*sensu* Charnov 1976b) to explain and predict how plant roots are expected to grow between and within patches in the soil. Using *Achillea millefolium* (Asteraceae, L.) as a model species I show that the marginal value theorem, which is one of the most widely studied animal foraging models, accurately predicts root foraging of plants.

1.1.4. How do plants forage for different resource types?

Foraging by plants for different chemical types of a resource has not been as well studied as foraging for soil patches, though it appears to be a common foraging problem (Lipson and Näsholm 2001; Näsholm *et al.* 2009). Almost all of the research has focused on differences in uptake capacity among plants for different types of nitrogen, such as nitrate, ammonium and different amino acids (Kielland, 1994, McKane *et al.*, 2002, Näsholm *et al.*, 2009). However, it has been suggested that plants may also capture different chemical types of phosphorus (Turner 2008). Näsholm *et al.* (2009) reviewed the literature and claimed that all plants that have been tested, regardless of taxonomic family, or mycorrhizal type, have the capacity to capture at least some forms of intact organic nitrogen types suggesting this is an important problem for a large number

of plant species. In general different plant species capture very different amounts of different types of nitrogen, suggesting that these species may have different preferences (Kielland, 1994, McKane *et al.*, 2002, Forsum *et al.*, 2008, Näsholm *et al.*, 2009).

Ecologists generally agree that differences in nitrogen use (or prefereces) may be linked to species performance, and plant-plant competition; however, like the problem of patch use behaviour there is little theory available to predict or understand why species exhibit preferences. In Chapter 4, I apply some of the ideas from Chapter 2 to test whether plants are capable of adaptive resource choice, a concept analogous to prey choice in animals. Using *Arabidopsis thaliana* (Brassicaceae (L.) Heynh., Ecotype Col-0) as a model species, I show that these plant preferences for different types of nitrogen can be predicted and explained using models of optimal prey choice.

1.1.5. Foraging and Coexistence

There are many mechanisms that potentially explain species coexistence (Tilman 1982; Angert *et al.* 2009; Chesson 2000; Silvertown 2004; Snyder and Chesson 2004) and only some of these might include foraging. Regardless, foraging behaviour can be a mechanism which contributes to the coexistence of multiple species within a community (Macarthur 1958; Brown *et al.* 1994; Hutchings *et al.* 2003; Stephens *et al.* 2007). For example, species that forage for the same resource will compete for that resource and this may limit their ability to coexist (Hardin 1960). Coexisting species often consume different resources and

that minimizes the effects of resource competition among species. These differences in resource use may be the product of fixed morphological traits which limit food consumption such as differences in beak size or shape in birds (*e.g.* Lack 1947), or they may be the product of foraging behaviour (*e.g.* Macarthur 1958; Brown *et al.* 1994). This basic principal has been shown for plants which may specialize on different chemical types of nitrogen (McKane *et al.* 2002; Harrison *et al.* 2007). However, this idea has not been directly linked to foraging theory, and plant ecologists lack general theories to predict or explain the resource choices of plants. In Chapter 4, I present a simple model for how coexistence may be mediated through differences in foraging choices. I show how subtle differences in foraging behaviour of two competing plants can theoretically minimize competition for nitrogen and potentially promote their coexistence.

When species share a resource they may also coexist by spatially segregating into different patches or regions of a habitat. Such spatial segregation also minimizes competition for a resource because species do not come into direct competition when they use different regions of a shared habitat. For example, MacArthur (1958) showed that five species of co-occuring warblers were able to coexist, even though they had similar diets, because they foraged in different parts of a tree. MacArthur (1958) argued that this spatial partitioning promoted coexistence and minimized competition among these competing birds. Similar ideas have been tested in plants. For example, it is thought that plants may avoid direct competition by segregating roots either by depth (Parrish and Bazzaz 1976; Mommer *et al.* 2010), or horizontally in soil (von Felten and Schmid 2008; Cahill

et al. 2010). However, studies of root segregation have been limited by methodological difficulties in identifying roots of co-occurring species since the roots of many species are visually indistinguishable (Mommer *et al.* 2008; Taggart *et al.* 2010). In Chapter 5, I present a molecular method for the identification of co-occurring plant species that makes root identification possible. In Chapter 6, I apply the molecular method developed in Chapter 5 to try to understand how the distribution of roots of 4 co-occurring grassland species shifts as a factor of heterogeneity and the presence of competitors. I develop resource selection probability functions (RSPF) for each species using molecular data (*sensu* Lele and Keim 2006). RSPFs are linear models fit to binomial data which use environmental variables associated with microsites to predict the probability of habitat use of organisms (Lele and Keim 2006).

1.1.6. Summary

Plants exhibit complex responses to nutrient patches (Hutchings and de Kroon 1994; Hodge 2004; Hodge 2008) and different chemical types of nutrients in and among patches (Kielland 1994; Lipson and Näsholm 2001; Näsholm *et al.* 2009). Research has documented a wide range of plant responses, and much is known about the proximate causes and responses of plants (Zhang and Forde 2000; Osmont *et al.* 2007; Chen *et al.* 2008; Hodge 2008; Ho *et al.* 2009; Forde and Walch-Liu 2009; de Kroon *et al.* 2009). However, much less is known about the ultimate causes that underlie plant foraging behaviour and there is little theory available to make predictions about the ecological consequences of plant foraging

behaviour (Robinson 1996; Kembel and Cahill 2005; Kembel *et al.* 2008). Throughout this thesis I will draw from pre-existing foraging theory developed in the animal literature to attempt to fill this gap and address two main questions: First, do plants forage using similar foraging rules as animals? Second, what are the ecological consequences of plant foraging behaviour for competition among species? I expect that answering these questions will advance the study of plant behaviour, and ultimately may serve to move the study of plant behaviour under the broader umbrella of behavioural ecology.

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2. A FRAMEWORK FOR PLANT ROOT FORAGING BEHAVIOUR[1]

2.1.1. Plant Behaviour

Standard definitions of 'behaviour' refer to the action or reaction of an individual to an event or stimulus. Behaviour is typically considered a feature of animals; however, this definition does not exclude the responses of other organisms, including plants (Silvertown and Gordon 1989; Karban 2008). This broad definition of behaviour has encouraged plant ecologists to investigate areas traditionally tackled by animal behaviourists, including territoriality (Schenk *et al.* 1999), kin selection (Dudley and File 2007; Klemens 2008; Dudley and File 2008; Murphy and Dudley 2009), mate choice (Marshall and Folsom 1991; Niesenbaum 1999), sexual conflict (Prasad and Bedhomme 2006; Lankinen and Kiboi 2007), non-random foraging (Hutchings and de Kroon 1994; Hodge 2004; Kembel and Cahill 2005; de Kroon and Mommer 2006; Kembel *et al.* 2008), interspecific communication (Falik *et al.* 2003; Falik *et al.* 2005; Schenk 2006; Hess and de Kroon 2007), and competitive games (Gersani *et al.* 2001; Schenk 2006; O'Brien *et al.* 2007; Hess and de Kroon 2007; O'Brien and Brown 2008). This idea that plants behave in ways that are similar to animals has generated substantial debate, often centered on the term 'behaviour' and whether it can occur without cognition (Gersani *et al.* 2001; Schenk 2006; Hess and de Kroon 2007; Dudley and File

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[1] A version of this chapter has been published. McNickle GG, St. Clair CC and Cahill JF. 2009. *Trends in Ecology and Evolution*. 24:419-426

2007; Klemens 2008; Dudley and File 2008). I do not address this debate here and instead work from the idea that plants do exhibit behaviour, and I seek to integrate plant behaviour within the broader discipline of behavioural ecology. My idea of behaviour assumes that phenotypic plasticity in plant growth is produced by stimuli for which alternative responses would produce differential fitness. In other words I assume that behaviours shift because they are adaptive. These alternative responses are labeled as 'behaviour' under this definition; however, the word used is not critical to the argument. Although plant behavioural ecology is empirically rich, it has lacked a common conceptual foundation to integrate the growing number of mechanistic studies (Kembel *et al.* 2008).

In this chapter, I use the specific case of plant root foraging to show that plant behaviour can be cast in a more general context of behavioural ecology. I argue that the assumption of optimality may serve to generate predictive mathematical models of plant foraging, just as it has done in animal behavioural ecology. I will focus primarily on the ultimate causes of root foraging behaviour (*Sensu* Tinbergen), since the proximate causes of root foraging are reviewed elsewhere (Chrispeels *et al.* 1999; Schiefelbein 2003; Hodge 2004; Hardtke 2006; Hodge 2006; Osmont *et al.* 2007; Chen *et al.* 2008; Hodge 2008). The arguments are centered on general biology of plants and animals, but this does not rule out the potential for atypical taxa to provide critical insights. I begin the discussion of root foraging by contrasting aspects of the basic biology of plants and animals, which will necessarily affect how plants exhibit behaviour.

2.1.2. Expression of plant and animal behaviour

Let us begin with a common metaphor, that plant roots growing in soil are analogous to a foraging animal (Karban 2008; Hodge 2008) (Figure 2.1). At a superficial level, this seems reasonable; both organisms search for, capture and handle resources. But if one looks deeper, this analogy lacks focus. In general, an animal consumes prey in discrete packets that provide energy and essential nutrients while plant roots capture mineral nutrient and water as individual molecules which are used to construct organs, some of which capture energy from the sun. Furthermore, an individual animal is made up of a single foraging unit with one mouth that can perform a limited number of behaviours at once. An individual plant is built from a series of repeating foraging units which can each perform different behaviours simultaneously (de Kroon *et al.* 2005). A moving animal leaves only a trail, but a plant moves by creating or elongating cells behind the advancing root tip, leaving behind semi-permanent tissues which require ongoing maintenance.

These broad differences can be lumped into two main differences that most strongly impact the development of a conceptual foundation for plant foraging ecology; i) modularity – generally plants forage by growing new organs that can occupy many places simultaneously, while animals forage in one place at a time (de Kroon *et al.* 2005); ii) currency – because plants and animals differ in the mechanisms of energy capture, the ‘currencies’ they spend and receive from foraging also differ (Gleeson and Fry 1997). Yet, despite these differences plants should still be expected to behave in ways that enhance fitness, suggesting that the

assumption of optimality might provide an initial foundation for the study of plant behaviour. In the sections that follow, I will expand these ideas and use well established concepts from the animal foraging literature to develop a conceptual model that takes into account these details of plant biology.

2.1.3. The logic of animal foraging models

The foundation of most animal foraging models is strikingly similar. Solitary animals are generally expected to perform behaviours that maximize energy intake per unit time (Holling 1959; Macarthur and Pianka 1966; Werner and Hall 1974; Charnov 1976b; Charnov 1976a; Krebs *et al.* 1977; Stephens and Krebs 1986). This rate can be expressed mathematically (notation follows Charnov 1976a; Krebs *et al.* 1977), where E is the energy intake during a feeding period of length T. Typically, T is subdivided into search and handling times, T_S and T_H respectively. Thus the net rate of energy intake can be expressed as;

$$\frac{E}{T} = \frac{E}{T_S + T_H} \tag{2.1}$$

To accommodate specific questions about animal foraging this basic idea is modified to incorporate parameters such as prey abundance, patch location, or habitat quality (Werner and Hall 1974; Charnov 1976b; Charnov 1976a; Krebs *et al.* 1977; Stephens and Krebs 1986; Stephens *et al.* 2007).

Two seminal questions emerged from this basic model: how should predators choose prey, and how much time and effort should foragers spend in patches? These questions have been addressed with a number of mathematical

models (Krebs 1977; Smith 1978; Pyke 1984; Stephens and Krebs 1986; Krebs and Davies 1987; Parker and Smith 1990; Stephens *et al.* 2007). Prey choice models generally compare the profitability of different prey relative to the costs associated with foraging and solve for the optimum diet (Macarthur and Pianka 1966; Werner and Hall 1974; Charnov 1976a). These models typically demonstrate that the costs of specialization decrease as the relative abundance of favoured prey increases. Patch use models ignore prey quality and recognize that prey are often aggregated into patches of varying quality (Macarthur and Pianka 1966; Charnov 1976b). Patch use models predict that organisms should spend more time in a patch when travel time among patches increases, average resource density within patches increases, or when overall habitat quality is low (Charnov 1976b). I will return to these two basic types of foraging questions after developing a more general conceptual framework for plant root foraging behaviour.

2.1.4. Towards a framework for plant foraging

If heterotrophic animals maximize the rate of energy gain, what should modular, autotrophic foraging plants be expected to maximize? In the sections that follow I will modify the structure and components of Equation (2.1) to enhance its applicability to plant foraging. The equations I generate are simple, not mathematically derived from each other, and do not represent an analytical model for plant foraging behaviour. Instead they are meant to serve as sign posts that crystallize my logic rather than a mathematical proof. I do not claim that this

model will be the end point for theoretical development of plant foraging behavior, but instead hope that these basic ideas will encourage more research in this direction.

2.1.5. The importance of plant modularity

Plant bodies are constructed from a series of repeating units that occur at multiple scales (de Kroon *et al.* 2005). At the larger scale, genetically unique plants (genet) can consist of clonal daughter plants (ramets). Depending upon the species and environmental conditions, ramets can live independently, or can be connected with the potential for resource sharing, division of labour, and communication (de Kroon *et al.* 2005). A second scale of modularity involves repeating organs within the plant body (metameres), each of which includes meristemic tissues (Hodge 2004). Meristems contain undifferentiated cells, allowing the creation of new plant organs in different areas based on local conditions. At a finer level, individual roots contain uptake proteins, and nutrient transport pathways that influence the capture of mineral nutrients (Chrispeels *et al.* 1999; Chen *et al.* 2008). Through plasticity in growth among these foraging units, plants are able to capitalize on opportunities, such as canopy gaps or nutrient rich soil patches (Hutchings and de Kroon 1994; Hodge 2004; Kembel and Cahill 2005). One way to visualize these different levels of organization in plant bodies is to think of plants as having many mouths (roots) spread out over large areas (Figure 2.1). Perhaps even more importantly, these plant mouths

(roots) may act independently, even while selection acts upon the individual as a whole (Houston *et al.* 1988; de Kroon *et al.* 2005).

The issue of modularity poses an immediate challenge to Equation (2.1). Individual animals have one mouth, which means that there is a clear mathematical path from the activity of the mouth to the fitness of the organism. However, in plants, this relationship is more complicated. Like animals, plants can alter the activity of existing foraging units, but unlike solitary animals, plants can also produce new foraging units, and all the foraging units of the plants may be in different envirnoments, and doing different things (de Kroon *et al.* 2005). Any model of plant foraging must account for the semi-separate activities of the collective parts of plants, and it is unclear whether a version of Equation (2.1) should be applied to the whole plant, or to each individual foraging unit. Insight might come from studies of social insect colonies (Figure 2.1).

Individuals of social insect colonies, such as the social hymenoptera, are expected to behave differently than solitary animals because the individuals who forage do not typically also reproduce (Houston *et al.* 1988). In such cases, the behaviour of workers is expected to enhance colony fitness, rather than individual energy gain of workers. This is similar to the situation of foraging in plants, where the foraging behaviour of individual plant parts should be expected to enhance total plant fitness, rather than the rate of foraging of each individual root (Figure 2.1).

In the development of the conceptual framework we must now consider the sum of the behaviours of all parts of the plant separately. If there are *n* ramets,

m root meristems, and E_{ij}, T_{Sij} and T_{Hij} are the gains or costs from the foraging activities of root *j* on ramet *i*, then;

$$\frac{E}{T} = \sum_{i=1}^{n} \sum_{j=1}^{m} \frac{E_{ij}}{T_{Sij} + T_{Hij}} \qquad (2.2)$$

In Equation (2.2), if total foraging performance is assumed to be optimal, then the fitness of the plant will include the sum of all foraging roots (*j*), across all the foraging ramets (*i*) within a single genet. For simplicity, this Equation also assumes that there are no interactions between the individual foraging units of a genetic clone, and units of organization smaller than individual roots (*e.g.* uptake proteins) are ignored. This is reasonable since many studies suggest that plant roots within an individual avoid or at least minimize self-competition (Falik *et al.* 2003; Falik *et al.* 2005) and that at least some foraging decisions are made in the root tips (Zhang and Forde 1998; Karban 2008). However, the impact of interactions among foraging units within the individual could be modeled (de Kroon *et al.* 2005). The conceptual framework for plant foraging represented by Equation (2.2) now accounts for plant modularity in a basic way. However, Equation (2.2) represents foraging costs and benefits for plants in units of currency developed for heterotrophs, and must be further modified to account for the differences in currency between plants and animals.

2.1.6. The currency of plant foraging

For foraging animals, the currencies of energy and time make sense when fitness is limited by energy intake and by time (*i.e.* the number of things an animal

can do at once) (Charnov 1976b; Charnov 1976a; Krebs *et al.* 1977; Stephens and Krebs 1986; Stephens *et al.* 2007). However, these currencies are inappropriate to describe the foraging behaviour of modular, autotrophic foraging plants. One possible solution would be to simply replace energy with nutrients in the numerator of Equation (2.2). This solution has been applied in previous attempts to apply optimal foraging models to plants (Kelly 1990; Kelly 1992; Gleeson and Fry 1997). However, the relationship between nutrient uptake and fitness gain is not always positive or linear for plants (Marschner 1997) and thus maximizing capture of a single nutrient would not necessarily maximize plant fitness. There can also be complex interactions among essential resources and plant fitness. For example, a given concentration of nitrogen can be limiting to plants under high phosphorus availability, but not when phosphorus itself is limiting (Tilman 1982; Marschner 1997). Under this common scenario, maximizing the capture of any single nutrient would not necessarily maximize plant fitness.

The implications of non-linear interacting nutrient relationships for Equation (2.2) is that the parameter E_{ij} is too simplistic and should be replaced by some fitness generating function $f(r_{ij})$, which describes the nonlinear benefits and interactions among the resources that limit plant growth (*e.g* Simpson *et al.* 2004). Although there are large numbers of essential resources for plant growth, $f(r_{ij})$ need only focus on a subset depending on the environment, species or system under study, or the question being addressed. If E_{ij} is replaced with $f(r_{ij})$, in Equation (2.2) then;

$$\frac{E}{T} = \sum_{i=1}^{n} \sum_{j=1}^{m} \frac{f(r_{ij})}{T_{Sij} + T_{Hij}} \quad (2.3)$$

In Equation (2.3), $f(r_{ij})$ is different from E in Equations (2.1) and (2.2) because as a function it can account for the multidimensionality of plant mineral nutrition, converting nutrient capture into potential for fitness gain and solving for the optimum (Bloom *et al.* 1985; Simpson *et al.* 2004). I leave $f(r_{ij})$ undefined for this project, as it will likely be species and system specific and will require empirical work to parameterize such a function. Next we must consider the currency of plant foraging costs.

Animal foraging models often differentiate costs as either search or handling time, which are assumed to be mutually exclusive activities (Macarthur and Pianka 1966; Charnov 1976b). At first, a similar distinction appears to apply to plants. Plants have a search cost represented by the ability to locate the nutrient and grow roots nearby (de Kroon and Mommer 2006; Gersani *et al.* 2001). Handling costs may consist of nutrient uptake, processing of nutrients into forms suitable for transport, and transport throughout the plant (Chrispeels *et al.* 1999; Osmont *et al.* 2007; Chen *et al.* 2008).

However, a closer look at plant physiology shows that the costs of search and handling are difficult to separate. For example, when a root is used to both search out and handle a nutrient, how does one score the cost of constructing and maintaining that root? Similarly, transpiration is an energetically expensive process in plants, driving the transport of solutes from roots to shoots in the xylem which could be considered handling costs (Bloom *et al.* 1985; Marschner 1997).

However, transpiration also creates a gradient in soil water potential that influences movement of ions near the roots, which could be considered a search cost. Further complicating this issue, many plants produce exudates or form symbioses with microbes that enhance nutrient availability, soil exploration, and nutrient capture, but at a significant energetic cost to the plant (Godlewski and Adamczyk 2007; Kiers and Denison 2008). Thus I suggest that foraging costs in plants should also be framed by some function, $f(c_{ij})$, which describes the combined sum of costs in units of energy or potential fitness lost through missed opportunities within root *i* on ramet *j*. This sum need not include every biological process, but only those which are thought to be biologically relevant to the species or question at hand. Equation (2.3) now becomes:

$$\frac{E_{Gain}}{E_{Spent}} = \sum_{i=1}^{n}\sum_{j=1}^{m}\frac{f(r_{ij})}{f(c_{ij})} \tag{2.4}$$

The function, $f(c_{ij})$, is different from the term $(T_S + T_H)$ in Equations (2.1-2.3) because it can have more than two terms and the individual costs will be categorized into biological functions rather than as search or handling. A consequence of this adjustment is that Equation (2.4) suggests that foraging should maximize efficiency whereas Equation (2.1) suggested that foraging should maximize a rate. I also leave $f(c_{ij})$ undefined because it will be species and system specific and require significant empirical work to parameterize this equation.

2.1.7. Foraging plants should maximize absolute gains

A problem with foraging gains expressed as a ratio is that very large gains which come at very large costs and very small gains which come at very small costs may be represented by identical ratios (Stephens and Krebs 1986). Since this framework allows the units of benefits and costs of plant foraging to be the same (*i.e.* fitness), the structure of Equation (2.4) can be rethought. I propose that plants should be expected to maximize absolute fitness gains across all foraging roots and ramets (*i.e.* benefits - costs);

$$E_{Gain} = \sum_{i=1}^{n} \sum_{j=1}^{m} \left(f(r_{ij}) - f(c_{ij}) \right) \qquad (2.5)$$

Equation (2.5) is not mathematically derived from the previous Equations. Instead, these 5 Equations serve as sign posts which reveal the logical development of a basic conceptual framework for plant root foraging. First the modular growth form of plants was acknowledged, and incorporated into a basic mathematical idea of foraging behaviour. When modularity is taken into consideration, plants should maximize the sum of all foraging units rather than the behaviour of each unit independently - Equation (2.2). Second, the benefits of plant foraging are complex and must be expressed by a function that describes both the potential benefits and interactions of the limiting nutrients of interest - Equation (2.3). Third, the costs of plant foraging cannot be easily demarcated into search or handling, and are also complex. Instead, the biologically relevant costs of plant foraging must be accounted for individually - Equation (2.4). Finally, it is more appropriate to assume that plants optimize absolute foraging gains than

foraging efficiency - Equation (2.5). With a very general framework for plant foraging in hand, I now return to the two classes of foraging questions that have been asked: patch use and prey choice models, and discuss how they may apply to plant foraging.

2.1.8. Patch use models

When prey are aggregated into a mosaic of patches and patch value declines as an organism depletes it by foraging, how much should an organism invest in that patch? This question has been addressed in animals most often by applying the Marginal Value Theorem (MVT) (Charnov 1976b) and other patch use models (Stephens and Krebs 1986; Stephens *et al.* 2007). Patch use models generally predict that the time spent in individual patches will maximize the rate of energy gain over time (Charnov 1976b). Because there are costs associated with traveling between patches, both the average distance between patches and the profitability of patches influences the optimum patch residency time of foragers (Figure 2.2).

These two ideas concerning the costs and benefits of patch use can be generalized into three basic predictions of patch use models like the MVT. First, as the resource density of the patch increases, it takes longer for resources to be depleted to the average level of other patches. As a result an individual should spend more effort and time in a high quality patch compared to a low quality patch (Figure 2.2). Second, as travel cost between patches increases so too do costs of leaving a patch. As a result, an individual will spend more effort in a

patch before moving on when patches are farther apart (Figure 2.2). Finally, as the overall profitability of the average patch in an environment declines, organisms should spend more time and effort in each patch.

Plants are similarly faced with a patchy distribution of soil resources (Hutchings and de Kroon 1994) and these resources are often depleted with increasing foraging effort (Fransen and de Kroon 2001; Lamb *et al.* 2004). Using Equation (2.5) plants are expected to exert greater costs in the form of increased tissue, and metabolic activity within patches. For example, plants proliferate into patches of varying quality in proportion to their quality (Jackson and Caldwell 1989; Gleeson and Fry 1997). Similarly, foraging effort in patches can be influenced by overall habitat quality (Lamb *et al.* 2004). However, plants should also move between patches in a way that is consistent with MVT. Foraging gains will be maximized when a plant remains inside a patch until the resource supply of the patch drops to the level of the average habitat. Plants may leave patches in two ways; i) through the senescence of roots or; ii) by physically growing through patches and exiting out the other side. Previous studies have linked foraging biomass investment to the marginal value theorem (Kelly 1990; Kelly 1992; Gleeson and Fry 1997; McNickle and Cahill 2009a), but the question of how patch quality influences plant root movement and growth through the soil has not been investigated. Based on Equation (2.5) and the predictions of the marginal value theorem I predict that plant roots should invest in patches until they are depleted, and only once a patch is depleted should the plant move beyond the boundaries of the patch. Thus, plants that encounter high quality patches will

travel shorter distances through the soil than plants that encounter low quality patches. The focus of Chapter 3 will be to test this hypothesis, and the more general predictions of the Marginal Value Theorem using a plant model.

2.1.9. Prey Choice Models

When multiple prey types of varying quality are available to an organism, which prey should be consumed? Animals are assumed to select those prey that maximize energy gain per unit time. This means that prey with the highest energy content, and the lowest search and handling costs should be favoured (Charnov 1976a; Krebs *et al.* 1977; Krebs and Davies 1987). The abundance of prey will also influence prey choice.

The ideas concerning costs and benefits of prey choice can be generalized into two basic predictions of prey choice models which can be expressed both graphically and mathematically (Holling 1959; Macarthur and Pianka 1966; Werner and Hall 1974; Charnov 1976b; Charnov 1976a; Krebs *et al.* 1977). First, when prey can be ranked in order of their profitability, there should be some subset of prey which includes only the highest quality prey and excludes the lowest quality prey. Second, the breadth of the diet will be influenced by the abundance of prey. As the abundance of high quality prey declines, prey become harder to find and this increases search costs. When this happens foragers' preferences may switch and lower quality prey that were originally avoided may be included in the diet of the organism (Figure 2.3).

Plants are faced with choice among multiple chemical forms of many essential nutrients such as nitrogen (Kielland 1994; Näsholm *et al.* 2009) and potentially phosphorus (Turner 2008). As in animals, these different 'prey' choices each can result in different growth rates in plants. For example, nitrogen exists in soil as nitrate, ammonium, and a variety of amino acids. Plant species have maximal growth under different ratios of these different forms of nitrogen (Kielland 1994; McKane *et al.* 2002), and plants can show different growth rates or lifetime fitness when grown on a single nitrogen source (Forsum *et al.* 2008). These findings suggest that plants should be expected to actively select prey in a way that is analogous to prey choice in animals.

Based on these general predictions of prey choice models, and Equation (2.5), plants should be expected to preferentially select the chemical types of nitrogen that maximize net foraging gains. However, plants should also switch to lower quality forms of nitrogen when the highest quality type becomes rare. The focus of Chapter 4 will be to use these very general predictions to predict and understand how plants may select among different types of nitrogen.

2.1.10. Conclusions

In this chapter I have presented a conceptual foundation that places plant root foraging within a larger sub-discipline of behavioural ecology. My goal in this brief essay was to begin to think more quantitatively about how plants might forage if we assume they forage optimality, and not to develop an analytical model of plant foraging. Similar to animal models, Equation (2.5) is a

simplification of reality. The strength of such frameworks comes from the explicit and quantitative predictions they support about the costs and benefits of foraging behaviours. It will take significant theoretical and empirical research to transform $f(r_{ij})$ and $f(c_{ij})$ to fully parameterized models for plant behaviour. Furthermore, the exact form of $f(r_{ij})$ and $f(c_{ij})$ will depend on the foraging questions to be addressed, and the plant system under study. Equation (2.5) is meant to serve as a starting point to begin to think about how to use an optimization framework to explore plant foraging in novel ways. More generally, I would urge plant ecologists to approach the idea of plant behaviour from a more theoretical perspective that accounts for the assumption that behaviour is optimal, and to exert more effort to think at the level of the individual. To achieve this, plant behaviourists should tap into the large volumes of behavioural theory developed for animals, much of which can be applied (with modification) to plants (e.g. Gleeson and Fry 1997; Karban 2008; Kelly 1992; McNickle and Cahill 2009). I believe the assumption of optimality, and the mathematical rigor that it brings to the study of foraging behaviour, has the potential to significantly advance the broader study of both plant behaviour and eventually behavioural ecology as a whole.

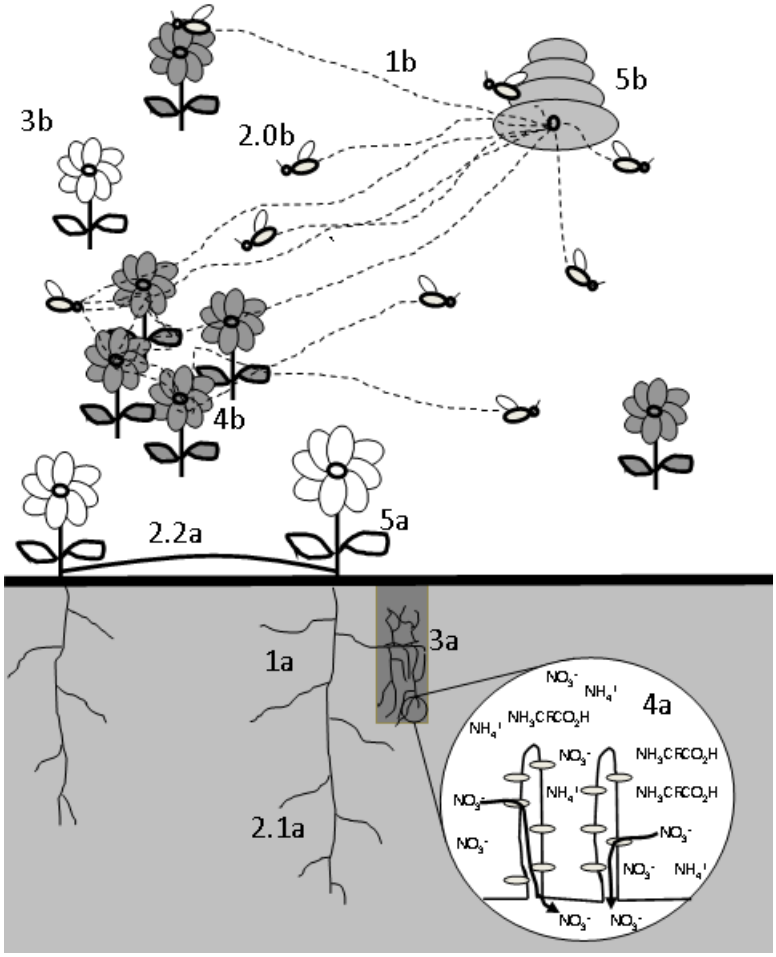

FIGURE 2.1: Cartoon comparison of how plant (a) and social insect foraging (b) can be considered to be analogous. This cartoon comparison is meant to serve only as a simple way of making phenotypic plasticity in plants comparable to the well studied foraging behaviour of animals. However, there are enough significant differences between plants and animals to make this analogy a tenuous one and this figure should therefore not be taken literally, and should be interpreted with extreme caution. Nutrient uptake in plants occurs primarily in the root-tips (2.1a). Root tips move throughout the soil through increased growth and elongation of roots. Roots are attached to daughter ramets (2.2a), which move across the landscape through the growth of new physiologically connected ramets. By comparison, in a social insect colony, resource capture occurs primarily by autonomous workers (2.0b). The individual root tip and the worker have similar genetic composition to the other root tips and workers, but do not reproduce themselves. Thus, the individual foragers (*i.e.* roots or workers) should work to maximize the performance of the plant or the colony as a whole, rather than their own individual performance. When resources are distributed in patches, plants increase the number of root tips in a patch (3a) while social insects will increase the number of visits to a patch (3b). This behaviour is well documented by

FIGURE 2.1 Continued:
empirical studies in both plants and animals. When there are multiple types of a resource with different costs and benefits organisms are expected to select among these resources in a way that maximizes benefits and minimizes costs. For plants (4a), these resources are individual forms of specific nutrients, for example nitrogen is taken up as nitrate (NO_3), ammonium (NH_4) and various amino acids (NH_3CRCO_2H). These are captured through the use of a variety of uptake mechanisms (ovals, 4a), and in this scenario plants preferentially uptake NO_3. For the social insects depicted (4b), these resources are different flowers (gray or white). In this scenario the insects preferentially visit the gray flower species. Captured resources in plants can be transported to the shoot (5a) where they influence energy capture and the production of new foraging units (roots/ramets). For social insects captured resources are transported to the colony (5b) for consumption and eventually can influence the production of new foraging units (workers).

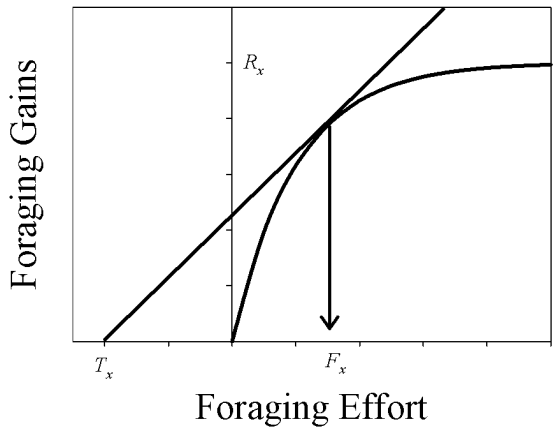

FIGURE 2.2: This figure shows the expected relationship between cumulative resource acquisition (curve), effort spent foraging in a patch and travel cost between patches (Charnov 1976b). As an organism spends more effort foraging in a patch the quality of the patch is depleted and resource acquisition eventually plateaus at some maximum level of resource acquisition (R_x). A tangent line (line) drawn from the travel cost to the gain curve identifies the point where the marginal value of staying declines below the average profitability of all patches and organisms should only invest F_x effort into the patch. Increasing travel cost (T_x) increases the amount of effort spent foraging in the patch (F_x). Increasing patch quality also increases the maximum amount of resources that can be extracted from the patch (R_x) and as a result increases the amount of effort spent in the patch (F_x). Though travel and handling costs are difficult to separate for plants, plants have been shown to expend effort in proportion to patch quality, and to control the timing of patch exit as predicted by patch use models designed for animals.

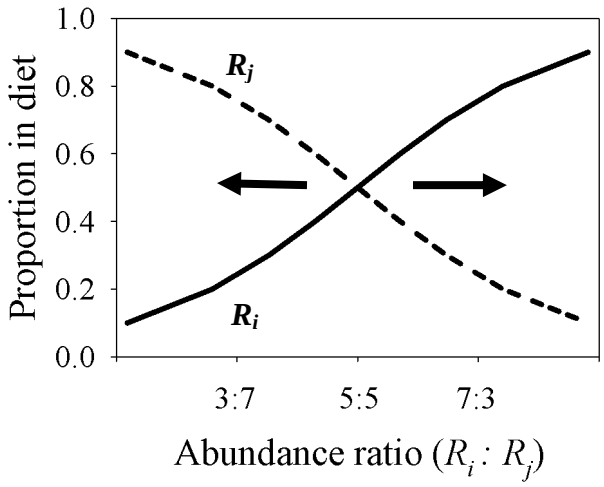

FIGURE 2.3: Change in diet preferences for an organism foraging for two substitutable resources (R_i – solid line and R_j – dashed line). In this example the two resources have equal costs and benefits. When resource *i* is common and resource *j* is rare (left side), the organism prefers the more common resource. However, if the ratio of available resources switches, the diet of the organism switches as well (right side). The point where the two lines cross can move left or right depending on the costs and benefits of resource *i* and *j*. If resource *i* has the higher benefit:cost ratio then the switch point moves to the left, and if resource j has the higher benefit:cost ratio then the switch point moves to the right.

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3. PATCH USE: PLANT ROOT GROWTH AND THE MARGINAL VALUE THEOREM[2]

3.1. INTRODUCTION

All life must find and consume resources to sustain itself, and there exist a diverse array of solutions to this basic problem (Krebs *et al.* 1977; Karban 2008; Hutchings and de Kroon 1994; Hodge 2004; Heineman *et al.* 2008; Charnov 1976a). Though the proximate mechanisms (sensu Tinbergen 1963) of resource collection differ among taxa, they are conceptually linked by a common ultimate cause. Natural selection should favor those individuals who are able to forage more efficiently, within certain lineage specific biological constraints. One approach that has been used to address issues of foraging behaviour has been the application of optimality-based models which formalize the assumption that behaviour is adaptive or optimal (Smith 1978; Stearns and Schmidhempel 1987; Stephens *et al.* 2007). Although natural selection is unlikely to produce perfectly optimal individuals (Gould and Lewontin 1979; Pierce and Ollason 1987), animal behaviourists have been successful at predicting and understanding foraging behaviour through the use of an explicitly quantitative treatment of the assumption of optimality. Such an approach is advantageous because it forces the

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researcher to *a priori* identify the exact costs and benefits which should be associated with different behaviors. It also forces researchers to quantify their assumptions and the biological constraints on behavior. By using an explicitly quantitative approach one gains a precision in the understanding of the system that cannot be achieved with vague references to adaptation. This precision can shed light on both the proximate and ultimate causes of behavior and lead to new research directions and improved understanding of behavior regardless of whether the behavior or the organism is strictly optimal (Smith 1978; Stearns and Schmidhempel 1987; Stephens *et al.* 2007).

Arguably one of the most influential contributions to optimal foraging theory was the Marginal Value Theorem (MVT) (Charnov 1976b; Stephens and Krebs 1986; Stephens *et al.* 2007). MVT uses the assumption of optimality to predict how organisms should allocate foraging effort to patches of differing quality before leaving, and by extension, predicts broad scale patterns of movement across a landscape. Specifically when foraging activities deplete resource abundance in a patch, MVT predicts; *i*) The density of resources which remain in a patch when the organism leaves (*i.e.* the giving up density) should be equal for all patches regardless of initial patch quality; *ii*) as the distance between patches increases the amount of time spent in patches before leaving (*i.e.* the Giving Up Time; GUT) should increase; *iii*) as average habitat quality increases, GUT should decrease and *iv*) within a given habitat, the GUT for higher quality patches should be higher than lower quality patches. It is this fourth prediction, concerning GUT within a habitat that will be the focus of this chapter.

Plants exhibit substantial plasticity in growth in relation to environmental heterogeneity, often preferentially placing their foraging organs in areas of high resource concentration (Hutchings and de Kroon 1994; Hodge 2004; Karban 2008). This phenomenon has been compared to the foraging behavior of animals, and there have been several previous attempts to place plant foraging into the context of MVT (Gleeson and Fry 1997; Gersani *et al.* 1998; Kelly 1990; Kelly 1992). For example, plants increase root growth (Gleeson and Fry 1997; Gersani *et al.* 1998) and the allocation of parasitic stems (Kelly 1992; Kelly 1990) into patches of variable quality in a manner consistent with the predictions of MVT. In these studies, and common to most studies of plant foraging, is a focus on biomass distributions, ignoring the potential movement patterns of plant organs which result in the discovery and eventual exit of patches. This issue of patch leaving behavior is one of the key predictions of MVT, and to my knowledge this issue has not been applied to plants. Here I will focus specifically on plant root foraging behavior, as this area is particular well studied and most obviously similar to patterns of animal movement.

If root growth is taken to be analogous to movement in animals, MVT would predict that actively growing plant roots should not venture outside of a patch until the resource level in that patch had been drawn down to the average resource density in the environment. In other words, those roots which approach the edges of a patch should stop growing until the patch value has been significantly lowered. Thus, the plant could increase its rate of resource capture by focusing all root growth inside the borders of the patch, and only venturing

into poorer quality soil once the patch is depleted. If this were true, and occurred locally among all roots within the patch, it would result in broad differences in the overall breadth of the root system of plants growing in heterogeneous soil. Like animals, plants which encounter the most highly enriched patches would travel the shortest distances, compared to plants which encounter patches of lower enrichment. Furthermore, because plants are constructed from semi-autonomous metamers which form the building blocks of their modular bodies (de Kroon *et al.* 2005; McNickle *et al.* 2009, Chapter 2), I expect this change in distance traveled to be a local response, not a systemic response.

Here I describe an experiment designed to test two predictions of MVT concerning patch use behavior: i) Plant roots should leave low-enriched patches earlier than highly-enriched patches and ii) plants should allocate more foraging effort to high quality patches than low quality patches (Gleeson and Fry 1997; Gersani *et al.* 1998; Kelly 1992; Kelly 1990), as measured by root biomass. I also expect both of these responses to be local rather than systemic because of the modular construction of plants. To test these hypotheses I grew *Achillea millefolium* plants in soil where they would have access to highly-enriched patches, low-enriched patches or no patches at all (Figure 3.1). Patches were placed on one side of the shoot only, which allowed me to measure root growth towards and away from patches to distinguish local responses from systemic responses (Figure 3.1). I tracked root movement through the use of a minirhizotron camera so that I could measure the total distance traveled either towards patches, or away from patches and thus estimate when plants left patches.

Average habitat quality, and average distance among patches was held constant and I varied only patch quality and the presence or absence of patches. A critical aspect of my design was that all treatments received the same total amount of nutrient enrichment, but differed only in the pattern of nutrient enrichment (Figure 3.1). I conclude the study by re-synthesizing some of the existing data in the context of MVT with a goal of assessing the generality of this model for application to plant behaviour.

3.2. METHODS

3.2.1. Study species

Achillea millefolium (Asteraceae, L.) is a herbacious perennial species which is native to much of the northern hemisphere and is thought to have a naturally circumpolar distribution (Purdy and Bayer 1996). Seed was obtained from a local native seed distributor (Bedrock Seedbank**,** Sangudo, Alberta, Canada) who obtains and propagates seed from local populations. I selected *A. millefolium* as the study species for this experiment because the foraging response of *A. millefolium* is well documented, with evidence that it does exhibit a high degree of foraging precision (Johnson and Biondini 2001; Rajaniemi 2007; Rajaniemi and Reynolds 2004). Not all plant species respond to heterogeneity and it was important for this study that I focus on a plant known to exhibit a strong foraging response.

3.2.2. Experimental setup

A. millefolium plants were grown in three soil environments, each of which contained the same total amount of nutrients, but varied in the distribution of those nutrients (Figure 3.1). The three soil treatments were i) High-L, background soil with a highly-enriched patch near one-side of the plant (66% v/v steer manure, Nu-Grow IP inc., Brantford, Ontario, Canada); ii) Low-H, background soil with a low-enriched patch near one side of the plant (25% steer manure), and iii) Hom, a spatially uniform soil environment (4% steer manure spread evenly throughout the soil). The background soil used in the two heterogeneous treatments consisted of a 3:1 ratio of washed sand to commercial top soil (Burnco, Edmonton, Alberta, Canada), and it was this soil that was amended with manure to create nutrient enrichment. I used *A. millefolium* plants in a separate experiment to bioassay each of my soil quality designations (described below).

Individual plants grew in the center of 30x12x30cm (length x width x depth) wooden boxes (Figure 3.1), with 10 replicate boxes per treatment. Patches, when present, were 2cm wide and spanned the width of the pot. Patches were placed 6cm away from the plant on only one side of the stem, which allowed for the differentiation of a localized response (only roots on the side with the patch would vary among treatments), and a systemic response (roots of both sides would respond). Heterogeneous soil treatments received a second patch of opposite quality to the first which was placed 12 cm from stems on the same side of the pot as the first patch (Figure 3.1). This was done to ensure that each pot

received the same total amount of nutrients. However, the experiment was stopped as plants reached the second set of patches. Plants were grown in a growth chamber in the University of Alberta Department of Biological Sciences Biotron facility, with 16:8 light:dark cycle. Plants were watered daily with an automatic mist sprinkler system, preventing any appearance of water stress.

Prior to the experiment, a clear plastic mini-rhizotron tube was inserted lengthwise through each box 5cm below the surface of the soil (Figure 3.1). This tube allowed non-destructive measures of root growth through visual observation with a mini-rhizotron camera (Bartz Technology Co., Carpentaria, CA, USA). It was not possible to measure the exact giving-up-time (GUT) for my plants because fine scale movements of roots turned out to be too difficult to measure as roots would often move in and out of the field of view. Instead I measured the total distance traveled by plant roots over the course of the experiment as a proxy for GUT. Distance traveled was measured as the distance from the base of the shoot to the farthest visible root either towards patches or away from patches (Figure 3.1). Patch and shoot locations were marked on the rhizotron tube before the start of the experiment and patch soil was visually distinguishable from background soil. Root images were captured every 6 days to monitor root progress through the soil volume. Root tracing was performed with Win RhizoTron v2007b (Regent Instruments Inc., Quebec City, Quebec, Canada).

Because small plants have few roots relative to large plants, I expected size-dependent responses to the soil treatments. To estimate plant size I measured the length of the longest leaf on each plant every 6 days, which I knew from

previous studies was a correlate of total plant biomass in *A. millefolium*. To confirm the correlation between leaf length and total biomass I performed a linear regression on leaf length from the day of plant harvest, to the total dry weight of plants from my main experiment. Leaf and biomass data were log transformed and analyzed using linear regression in SAS v 9.1 (SAS institute inc.). There were no differences among treatments, the regression was highly significant ($F_{1, 26}$ = 209, $p < 0.0001$) and leaf length explained 88% of the variation in total plant biomass (Figure 3.2).

To determine potential short-term biomass effects of the treatments, plants were harvested when the mean foraging distance of roots from at least one treatment approached the second patch (day 48, Figure 3.3). This was done to allow growth benefits from root foraging activities in the first patch to accrue without allowing potential confounding effects associated with accessing the second patch. Shoots were cut at the soil surface, and dried at 60°C until they reached a constant mass. Two 2.5 x 5cm (diameter x depth) cylindrical root cores were taken from the 'towards' side of the pot inside the first patch (or equivalent location in treatment Hom) and pooled, and two root cores were taken from the same location on the 'away' side of the plant. Roots were stored at -20°C and washed in a 1mm sieve, dried at 60°C and weighed.

3.2.3. Statistical analyses

The general pattern of growth rate among treatments was similar at each time period (Figure 3.3), and analyses of repeated measures data did not converge

because of high overdispersion in the data. Since the primary goal was to compare patch leaving behaviour among treatments, distance traveled data from the time period when plants left the patches were analyzed. Thus, data from after 36 days only, were analyzed using the PROC GLIMMIX procedure for Generalized Linear Mixed Models (GLMMs) in SAS (v 9.1, SAS Institute Inc.). The model included direction of growth (towards, away) and soil treatment (High-L, Low-H, Hom) as main effects, length of longest leaf (leaf size) as a covariate and box as a random factor nested in soil treatment to control for pseudoreplication. The data generally followed a Poisson distribution, but exhibited significant over dispersion. Thus a negative binomial error distribution was used to account for the over dispersion, with a convergence criteria of $1x10^{-6}$ (pconv = 1EXP-6). The fit of this model was 1.00 (generalized Chi-square / df).

Root mass data were analyzed using the PROC GLIMMIX procedure for Generalized Linear Mixed Models (GLMMs) in SAS. The model included direction of growth (towards, away) and soil treatment (High-L, Low-H, Hom) as main effects and box as a random factor nested in soil treatment to control for pseudoreplication. The data were again fit to a negative binomial distribution, with a convergence criteria of $1x10^{-6}$ (pconv = 1EXP-6). The fit of the model was 0.77 (generalized Chi-square / df). Means were compared using the nlsmeans post hoc analysis in SAS.

Total plant biomass data were log transformed for normality and analyzed using the PROC ANOVA procedure in SAS with soil treatment as the main factor. Means were compared using a post hoc Tukey's test in SAS.

3.2.4. Soil bioassay

In a separate experiment I performed a bioassay of soil quality using *A. millefollium* plants on pure batches of my 4 different soil types to validate my assertion that high quality soil > low > poor = background soil for plant growth. I did not expect that poor quality soil would differ from background soil. Plants were grown in 15cm diameter cylindrical pots on pure high (66% v/v steer manure), low (25% v/v steer manure), poor (4% v/v steer manure) and background soil (0% v/v steer manure). After 6 weeks the experiment was harvested. Shoots were collected and dried at 60°C, and weighed. Shoot mass was log transformed for normality and analyzed using the PROC ANOVA procedure in SAS with soil type as the main factor. Means were compared using a post hoc Tukey's test in SAS. Plant biomass was largest in High quality soil, intermediate in low quality soil, and plant size in homogeneous and background soil were smallest but did not differ from each other ($F_{3, 29} = 20.16$, $p < 0.0001$). This shows that my ranking and designation of soil quality (*i.e.* High > Low > Background = Hom) for *A. millefolium* growth was consistent with plant growth (Figure 3.4).

3.3. RESULTS

3.3.1. Patch leaving behaviour

When grown in heterogeneous soil, plant roots grew beyond low-enriched patches earlier than they grew beyond highly-enriched patches, resulting in

differences in the overall distance traveled by roots among these soil treatments (Figure 3.5, 3.6). This finding is indicated by a significant three way interaction between body size, direction of growth and heterogeneity on the total distance traveled by plants over a 36 day period (Table 3.1, Figure 3.5, 3.6). This complex interaction indicates that plant root growth depends simultaneously on *i*) whether the plant is grown in heterogeneous or homogeneous soil - roots travel less in homogeneously poor soil, *ii*) whether the roots encounter a patch or not – they travel farther when they encounter a patch (*i.e.* this is a local response and not a systemic response) and; *iii*) the size of the plant - bigger plants have more roots, and therefore travel farther than smaller plants. The most important conclusion of this result is that, as predicted by MVT, plant roots grow more slowly through more highly-enriched patches than in low-enriched patches before moving (Figure 3.5, 3.6).

3.3.2. Foraging effort within patches

Plants allocated more foraging effort per unit soil volume (root biomass) to highly-enriched patches than to low-enriched patches (Figure 3.7). The amount of foraging effort was the same in homogeneous soil as it was in the background soil in the heterogeneous soil treatment (Figure 3.7). This is evidenced as a two-way interaction between soil heterogeneity, and the direction of growth on total foraging effort (Table 3.2, Figure 3.7), indicating that the amount of root growth in a particular soil location depends upon the quality of the soil at that location. Plants allocate more root growth in areas of better quality soil. Because plant root

allocation differed as a function of the side of the plant in which roots were measured (side with the patch versus side without the patch), I can infer that plant root responses are local and not systemic (Figure 3.7).

3.3.3. Short term benefits

Total plant biomass was larger ($F_{2, 24} = 5.31$, $p = 0.012$) when plants encountered high quality patches than in the other treatments (Figure 3.8). Because the bioassay showed that my background soil limited plant growth, this result suggests that the foraging efforts of plants in high quality patches led to increased nutrient capture in the short term. There was no difference between the low-enriched patches and homogeneous soil for total plant growth (Figure 3.8). Biomass in this context only reflects differences in short term nutrient capture, and may or may not correlate to long-term fitness.

3.4. DISCUSSION

The results from my experiment are consistent with both of the hypotheses generated by MVT, with plants spending both more time (Figure 3.5, 3.6) and effort (Figure 3.7) in highly-enriched patches than in low-enriched patches. This study creates a picture of plant root movement in relation to patches that mirrors patterns of movement among patches in animals. Both plants and animals that encounter high quality patches will remain in those patches longer (Stephens *et al.* 2007; Stephens and Krebs 1986), and as a result travel shorter distances in search

of resources compared to conspecifics which encounter low quality patches (Figure 3.5, 3.6). To my knowledge this is the first time that plant root movement patterns have been linked to the idea of patch leaving as envisioned by the MVT. However, this is not the first study to conceptually link patterns of biomass allocation in relation to resource heterogeneity to MVT (Gersani *et al.* 1998; Gleeson and Fry 1997; Kelly 1992; Kelly 1990). Furthermore, increased biomass allocation to patches by plants seems to be a ubiquitous ability of plants and many studies have shown this with no links to behavioural theory (*e.g.* Campbell *et al.* 1991; Hutchings and de Kroon 1994; Johnson and Biondini 2001; Hodge 2004; Kembel and Cahill 2005). In the following section I will briefly discuss past findings concerning plant root foraging behaviour in relation to MVT.

3.4.1. Links to the Marginal Value Thereom

One of the predictions of MVT is that organisms should invest foraging effort in proportion to patch quality. This has been demonstrated for the allocation of foraging effort of roots (Gersani *et al.* 1998) and parasitic stems (Kelly 1992; Kelly 1990) which occur in proportion to patch quality as predicted by MVT. Patterns of fine root biomass allocation in response to soil nutrient heterogeneity are well studied and there are studies in the literature which have similarly documented this relationship between patch quality and foraging effort, even if the original authors did not cast them in the context of MVT. Not all studies of root foraging measure patches of different quality, but when they do almost every species studied expends foraging effort in proportion to patch quality (*e.g.* Lamb

et al. 2004; Pregitzer *et al.* 1993; Jackson and Caldwell 1989; Drew 1975; Fransen and de Kroon 2001). In other words, the assumption that plants that focus root growth primarily in nutrient rich soil to maximize the benefits of foraging and minimize the costs of root growth and nutrient uptake seems like it is valid.

Another prediction of MVT is that organisms should spend less effort in patches as total habitat quality increases. Less data is available to test this prediction, though available data suggests this hypothesis may be supported. Lamb *et al.* (2004) found fewer total roots were produced by plants as total habitat quality increased and when the total number of patches remained constant. However, there was only a non-significant trend towards reduced effort in patches as habitat quality increased (Lamb *et al.* 2004). Similar results have been found for competing plants which avoid areas of high competition in favor of root free soil, where resource uptake is presumably higher (Gersani *et al.* 1998). Many plants seem to favor soil with low competition over soil with large numbers of competitors (Schenk *et al.* 1999; Cahill *et al.* 2010), though this is not always the case (O'Brien and Brown 2008). Few studies have included experimental designs that vary total habitat fertility; this preliminary evidence suggests that some plants behave in a way that is predicted by MVT, but more studies are needed.

A third prediction of MVT is that the density of resources which remain in a patch when the organism leaves (the 'giving up density') should be equal among all patches regardless of initial quality (Charnov 1976b; Brown 1988). I am aware of no studies that test this hypothesis. Measuring giving up density for plants will

require precise and repeated measures of both root movement and available soil nutrients and will likely prove difficult.

3.4.2. Similarity to daughter ramet placement

Similar to the foraging response of fine roots, the vegetative spread of clonal plants through the environment tends to track heterogeneity in resource distribution, and has also been described as foraging (de Kroon and Hutchings 1995; Birch and Hutchings 1994; Wijesinghe and Hutchings 1999). Generally, clonal plants increase the density of daughter ramets inside high quality patches compared to the background environment which increases the level of resource capture (de Kroon and Hutchings 1995; Birch and Hutchings 1994; Wijesinghe and Hutchings 1999). This allocation of ramet biomass in relation to heterogeneity also matches the predictions of MVT, though again most authors do not link their results to an optimality framework. Clonal plants also may decrease the spacer length between daughter ramets in high quality patches compared to other areas of the environment (de Kroon and Hutchings 1995; Birch and Hutchings 1994; Wijesinghe and Hutchings 1999). This change in spacer length is traditionally thought to serve only as a mechanism for increasing foraging effort (de Kroon and Hutchings 1995; Birch and Hutchings 1994; Wijesinghe and Hutchings 1999). However, the reduction of spacer length in high quality patches means that plants will spend more time in those patches compared to background soil. This behaviour in stem plasticity is similar to the behaviour of the fine roots of *A. millefoloum* described in this study (Figure 3.6, 3.7). This suggests that there

is likely a similar underlying ultimate cause behind these behaviours. Specifically, I argue that those plant species who were capable of devoting more time and effort to highly enriched soil gathered more resources had higher fitness and left more descendants than those that were incapable of this behaviour.

3.4.3. Using optimality theory to move forward

Although most species respond to nutrient patches through precise placement of biomass into patches, there are some that do not, and this has puzzled some authors (Hutchings and de Kroon 1994; Hodge 2004; Kembel and Cahill 2005). I suggest that quantitative models like MVT that are based on the assumption of optimality may provide clues to the range of responses among species, and among contexts. Optimal foraging does not mean that an organism must always respond to a resource stimulus as has been expected in many plant studies and as many plant ecologists have expected. Instead optimality implies that organisms should maximize benefits, and minimize costs subject to certain constraints (Smith 1978; Stearns and Schmidhempel 1987; Stephens *et al.* 2007). When the potential costs exceed the benefits the organism should not respond to the resource. By taking a comparative approach to measuring costs and benefits of foraging and quantifying ones assumptions as well as the biological constraints, those species with low foraging ability may turn out to be the most important for assessing the applicability of any specific foraging theory.

Despite the fact that few authors have linked their results to MVT, the data from this study and others suggests much of the patch use behaviour of plants is at

least qualitatively consistent with a framework of optimality described by MVT. Based on this congruency I suggest that plant ecologists should begin to develop more explicitly quantitative frameworks based on the assumption of optimal foraging in plants. As shown in this chapter MVT can make surprisingly good qualitative predictions about plants, despite the fact that it was developed with animal foraging in mind. However, the development of plant specific models of optimal foraging will likely lead to more precise predictions about plant behavior and ultimately a better understanding of plant foraging. These models should take into account issues such as modular growth, which is one of the biggest differences between plants and (most) animals (de Kroon *et al.* 2005). But why does this matter and how does the synthesis above differ from a series of nice stories about adaptation?

Plant ecologists have admittedly gone down some blind alleys in the study of plant foraging (de Kroon and Mommer 2006). For example, the study of root foraging for several decades has often focused on possible trade-off in the scale and precision of root foraging (Campbell *et al.* 1991; Grime 2007; Kembel *et al.* 2008; de Kroon and Mommer 2006; Kembel and Cahill 2005). The idea behind the scale-precision trade-off was that species with large-scale root systems would be imprecise foragers, and species with small-scale root systems should be precise foragers (Campbell *et al.* 1991; Grime 2007). In other words, this theory implicitly assumed that the ultimate evolutionary drivers of behavior should be reversed depending on the size of the plant, or at least that the proximate abilities of closely related species differed as a function of their size. After decades of

research on over one hundred species, two recent meta-analyses of this literature do not result in support for a tradeoff in scale and precision (Kembel *et al.* 2008; Kembel and Cahill 2005). With the doubt cast on this dominant paradigm, plant ecologists are in need of new directions for foraging theory (Kembel *et al.* 2008; de Kroon and Mommer 2006; Kembel and Cahill 2005 but see Grime 2007).

Given the incredible success of the assumption of optimality for the investigation of animal foraging (Stephens *et al.* 2007; Stearns and Schmidhempel 1987), and the fact that much of the relevant plant foraging behavior described above is consistent with this assumption, I suggest that plant ecologists should work towards an explicitly quantitative development of optimal foraging theory for plants. This will involve a change of focus in this research program to measuring potential fitness losses through missed opportunities, the potential benefits of resource capture for fitness gain and how these benefits and costs interact to shape the total response of foraging plants. It will also require plant ecologists to quantify their assumptions and the constraints on behaviour. Such an explicitly quantitative approach takes the vague notion of a behavior being adaptive which has always been assumed and brings it into sharper focus by precisely quantifying what is meant by the word adaptive. This shift in thinking about plant behaviour can be simplified by gaining insight from the lessons learned throughout the history of the animal foraging literature (McNickle *et al.* 2009) as I have shown with a combination of experimentation and literature review.

3.4.4. Conclusions

My data and much of the existing data in the literature show that patterns of plant root growth through soil qualitatively mirror movement strategies of animals as predicted by the marginal value theorem. My predictions were born out of a theory of animal movement, but they are consistent with much of the empirical evidence concerning plant responses to soil patches. I have argued that a more explicit treatment of the assumption of optimality will bring a level of quantitative precision to the study of plant behavior which is sorely needed. I have discussed one such optimal foraging model from the animal literature and shown how it can provide novel insights into plant ecology. However, ultimately I believe that plant ecologists will need to forge their own mathematical models which account for the unique biology of plants.

TABLE 3.1: Results of GLM on raw distance traveled by plants. Soil is the soil nutrient heterogeneity treatment (Hom, High-L or Low-H), direction is the direction of growth (towards or away) and leaf is the covariate length of longest leaf as an estimate of plant size.

Factor	**df**	**F**	**P**
Leaf	1	45.36	<0.0001
Soil	2	2.46	0.1074
direction	2	1.63	0.2174
leaf*soil	1	0.91	0.3489
leaf*direction	1	2.86	0.2044
treat*direction	2	7.89	0.0025
leaf*soil*direction	2	5.36	0.0123
Residuals	23		

TABLE 3.2: Results of GLM on patch exploration by plants. Soil is the soil nutrient heterogeneity treatment (Hom, High-L or Low-H), direction is the direction of growth (towards or away).

Factor	**df**	**F**	**p**
soil	2	1.69	0.2051
direction	1	17.94	0.0003
soil*direction	2	5.78	0.0084
Residuals	26		

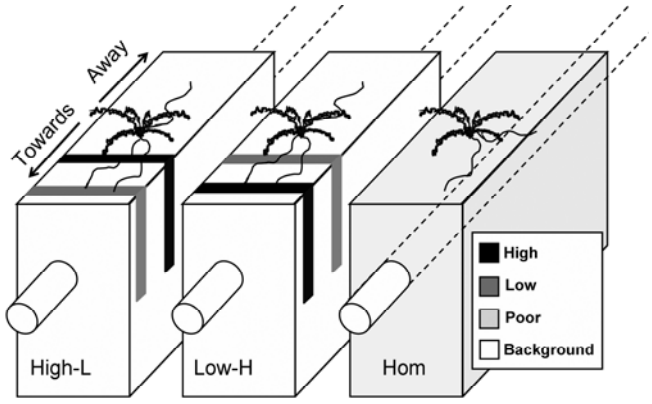

FIGURE 3.1: Schematic representation of experimental design. Regions of higher soil quality are indicated by regions of darker shading. High quality soil contained 66% manure mixed with background soil (v/v), low contained 25%, poor 4% and background soil 0%. Transparent plastic tubes spanned the length of each box so that roots traveling away from the shoot could be visualized through the use of a minirhizotron camera inserted into the tubes. Mini-rhizotron tubes below the soil in the schematic are indicated by dashed lines and are shown for the Hom treatment only. Distance traveled by roots searching for nutrients in the soil was measured as the distance from the base of the shoot to the farthest visible root either towards patches, or away from patches. Schematic is not to scale.

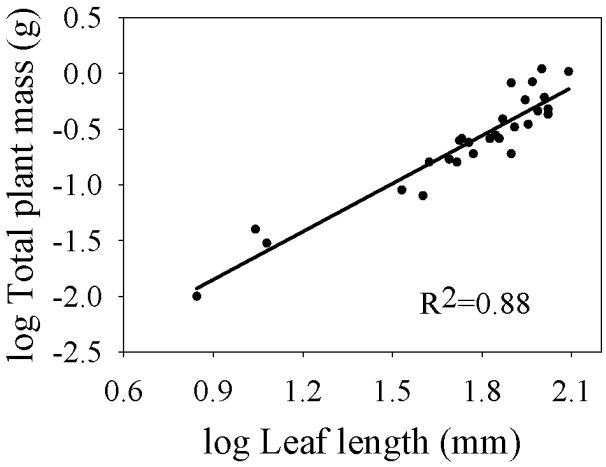

FIGURE 3.2: Relationship between log leaf length and log total plant mass. Though there appear to be several outliers, removal of these data points did not alter the relationship depicted here. Thus, these data points were retained.

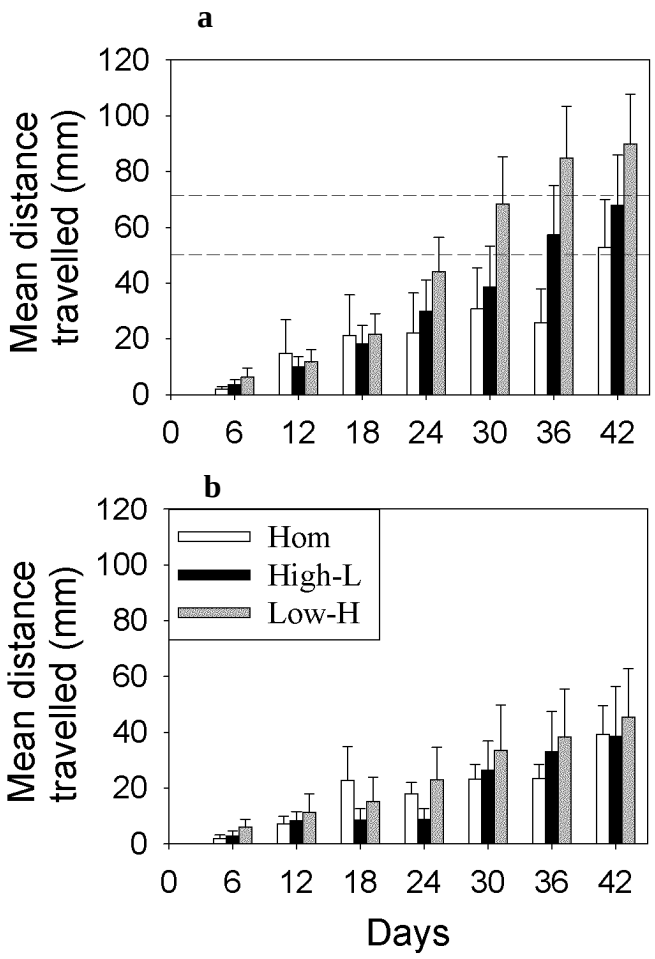

FIGURE 3.3: Mean raw distance traveled by plant roots through the soil in 6 day intervals across all soil treatments either towards patches (panel a) or away from patches (panel b). The location of the first patch in panel a, is denoted by dashed horizontal lines. Large variability, and presence of large numbers of zeros at early time periods made repeated measures analysis impossible, and thus these data are not analyzed. However, patterns in root growth among soil treatments are similar at each time period once plants began to reach the borders of the patch (~day 24-30). Error bars are 1SE.

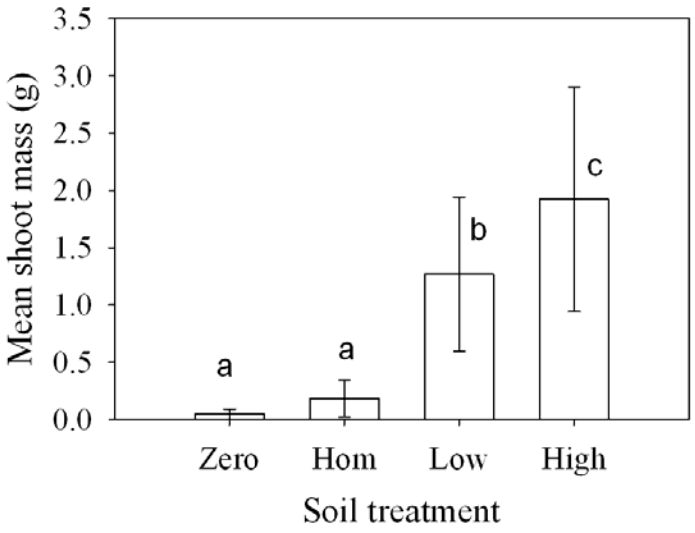

FIGURE 3.4: Mean shoot mass in the bioassay experiment. Letters above the means indicate statistical differences detected by a Tukey's test. Error bars are 1 SD.

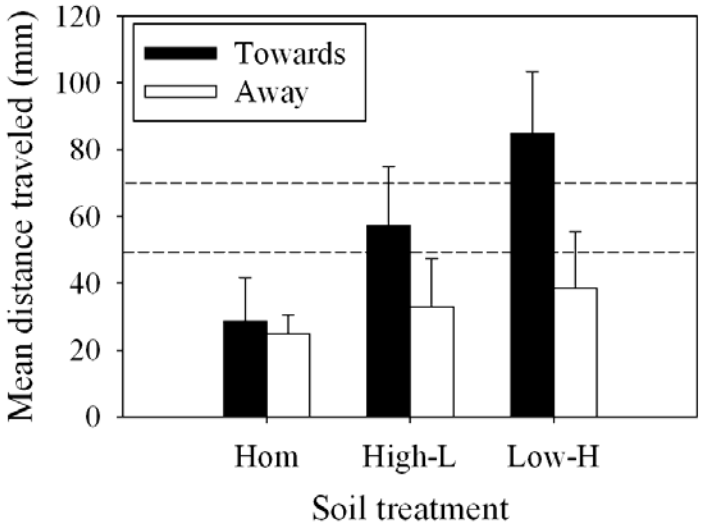

FIGURE 3.5: Mean raw distance traveled by plant roots through the soil either towards patches, or away from patches across all treatments without accounting for shoot size, after 36 days of growth. This is a subset of the data from Figure 3.7. The location of the first patch is denoted by dashed horizontal lines. Error bars are 1SE.

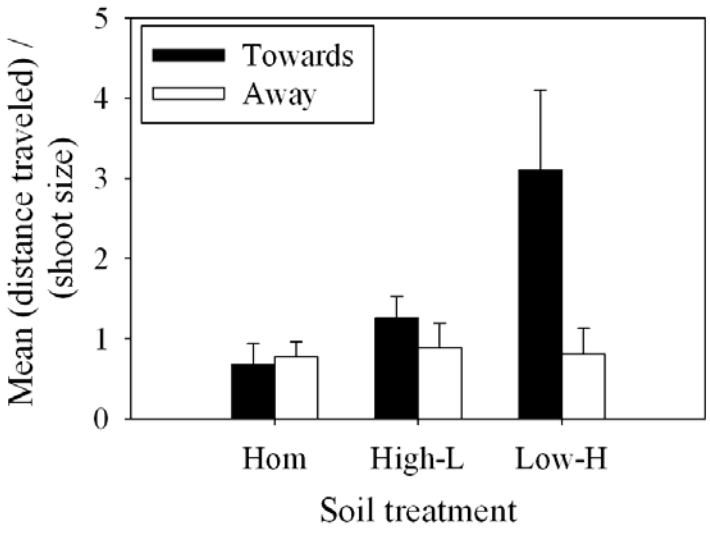

FIGURE 3.6: Mean distance traveled by roots either towards patches or away from patches standardized by plant size for all treatments after 36 days of growth. This accounts for differences in distance traveled by roots which are related only to plant size. Error bars are 1SE.

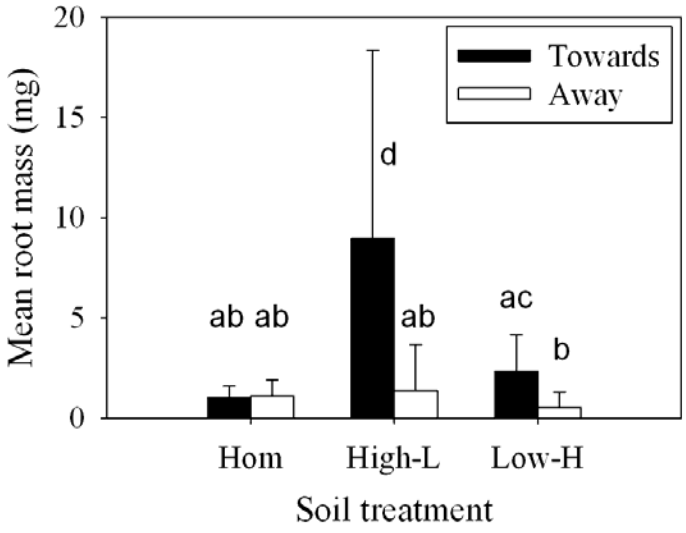

FIGURE 3.7: Mean soil exploration measured as biomass of roots inside the boundaries of the first patch only (Towards) as well as the equivalent location on the opposite side of the plant (Away) for each treatment. Letters above bars represent the differences in mean proliferation designated by the least square means post hoc comparison in SAS. Data is after 48 days of growth. Error bars are 1 SD.

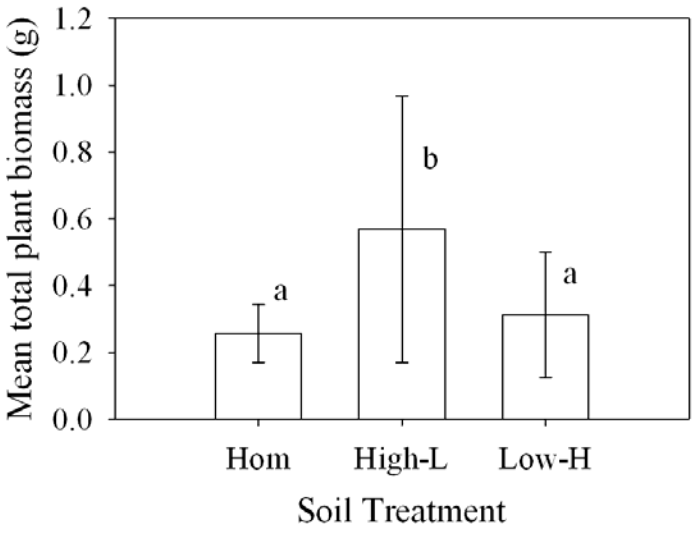

FIGURE 3.8: Mean total biomass of plants grown in each soil treatment after 48 days of growth. Changes in biomass reflect differences in nutrient uptake among plants. Letters above the means indicate statistical differences detected by a Tukey's test. Error bars are 1 SD.

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4. RESOURCE CHOICE: PLANT NITROGEN PREFERENCES AND ADAPTIVE FORAGING

4.1. INTRODUCTION

Food is often organized into patches and organisms must choose among resource patches of differing quality (Chapter 2; Chapter 3; Macarthur and Pianka 1966; Charnov 1976b; McNickle and Cahill 2009). However, even within a patch, foragers face choices. Resources may come in different forms, and foragers must choose between different types of substitutable resources (Macarthur and Pianka 1966; Werner and Hall 1974; Charnov 1976a; Krebs *et al.* 1977; Abrams 2010b). For example, a predator may be capable of capturing and consuming many types of prey, but may also be limited in its ability to handle many prey at once. What factors influence the composition of that predator's diet? Each prey species may have different benefits (*e.g.* nutrient or energy content), costs (*e.g.* metabolic breakdown or defences) and relative abundance. Not surprisingly, foragers have been shown to integrate the benefits, costs and abundance of different prey to make tradeoffs that shape their foraging choices (Macarthur and Pianka 1966; Werner and Hall 1974; Charnov 1976a; Krebs *et al.* 1977; Abrams 2010b). For example, all else being equal, adaptive foragers should generally prefer the prey with the highest net benefits. However, the adaptive choice of foragers can shift towards prey with lower net benefits if higher quality prey become rare (Charnov 1976a).

Animals are not the only organisms faced with resource choice. Plants often show preferences for different chemical types of nitrogen (Kielland 1994; McKane *et al.* 2002; Näsholm *et al.* 2009), and possibly phosphorus (Turner 2008). Logically, plants face the same selection pressures as animals to choose those resources that maximize individual fitness (Chapter 2; McNickle *et al.* 2009). Though plants have been shown to forage for patches using similar foraging rules as animals (Kelly 1990; Gleeson and Fry 1997; McNickle and Cahill 2009; Chapter 3), and to integrate information about nutrients and neighbour distributions in the soil (Cahill *et al.* 2010), resource choice has not been demonstrated in plants. Instead plant ecologists have defined preferences of plants in the absence of choice among multiple types of resources. Preference is often defined as the amount of a type of nitrogen captured (*e.g.* Kielland 1994; McKane *et al.* 2002). However, an alternative prediction would be that preference varies as a function of resource quality and resource abundance when multiple resource types are available simultaneously.

A necessary condition for the type of resource choice described above is a menu of substitutable resources. Here I focus on nitrogen, which plants can capture as at least 20 different chemical types, and which is widely studied in plants (Kielland 1994; Lipson and Näsholm 2001; McKane *et al.* 2002; Forsum *et al.* 2008; Forde and Walch-Liu 2009; Näsholm *et al.* 2009). For example, a review of the literature on plant amino acid uptake claims that all tested plant species possess the capacity to capture intact amino acids (Näsholm *et al.* 2009; Lipson and Näsholm 2001). For example, plants from every family tested in

systems as diverse as arctic and alpine ecosystems (Kielland 1994; McKane *et al.* 2002; Xu *et al.* 2006), temperate ecosystems (Falkengren-Grerup *et al.* 2000; Weigelt *et al.* 2005; Harrison *et al.* 2007), and tropical ecosystems (Schmidt and Stewart 1999; Wanek and Portl 2008) have all been shown to have the capacity for amino acid uptake. Furthermore, plants that possess the ability to capture amino acids may be either non-mycorrhizal or any mycorrhizal types (Lipson and Näsholm 2001; Hodge 2003; Clemmensen *et al.* 2008; Näsholm *et al.* 2009).

Plant preferences for different types of nitrogen have been shown to influence coexistence of species in intact plant communities. For example, in an arctic tundra system where organic nitrogen is the dominant form of nitrogen in the soil, there was a clear relationship between species preferences and species abundance. Specifically, the most common plant species preferred the most common types of nitrogen, and rare species preferred rare types of nitrogen (McKane *et al.* 2002). However, in more temperate soils organic nitrogen is rarer (Lipson and Näsholm 2001; Näsholm *et al.* 2009), and plant preferences are more subtle. For example, in a temperate grassland the variability in nitrogen preferences among species was small, and there was no obvious relationship between nitrogen preference and species abundance (Harrison *et al.* 2007). Given that plants seem to be generalists for many types of nitrogen (Lipson and Näsholm 2001; Näsholm *et al.* 2009), and that coexisting species may exhibit only slight differences in nitrogen preference (Falkengren-Grerup *et al.* 2000; McKane *et al.* 2002; Harrison *et al.* 2007), theory and data are needed to understand how subtle

differences in nitrogen preferences may or may not influence competition and species coexistence.

In this chapter I have two goals. First, I show that plant nutrient preferences can be viewed as a problem of adaptive resource choice. This finding will be important because, it will provide a theoretical framework to understand why plants may have evolved preferences for certain types of nitrogen. Furthermore, this theoretical framework can also be used to make predictions concerning competition among foraging plants. To test this idea, I provided plants with pairwise choice between three types of nitrogen, and test the hypothesis that, like animals, plants should integrate information about the net benefits of each nitrogen type, as well as the abundance of that nitrogen type. If plants exhibit adaptive resource choice they should generally prefer the highest quality nitrogen types, but diet preferences may switch to lower quality nitrogen when the preferred type is rare. My second goal in this chapter is to show how adaptive resource choice theory can be used to predict plant competition for different types of nitrogen. Using this framework, plant competition for nitrogen can be viewed in a food web context, with plants as consumers of different types of nitrogen resources. I present a graphical model of such a plant-nitrogen food web, and show how very slight differences in preference among two generalist plant foragers can minimize competition and promote plant coexistence.

4.2. METHODS

4.2.1. Study species

Arabidopsis thaliana (Brassicaceae (L.) Heynh., Ecotype Col-0) was selected because the physiology, genes and molecular mechanisms behind nitrogen uptake are relatively well understood for this species, and it is known to take up a variety of amino acids (Zhang *et al.* 1999; Forsum *et al.* 2008; Forde and Walch-Liu 2009). *A. thaliana* grows well in sterile media, which was important because the experiment needed to be performed in a microbe free environment so that nitrogen types would not be consumed and transformed by microbes. *A. thaliana* is also non-mycorrhizal which means that nitrogen uptake can be attributed solely to plant root uptake. Finally, *A. thaliana* is a relatively short lived annual which allowed for the estimation of lifetime reproductive effort and a link between foraging and fitness.

4.2.2. General growth conditions

In all experiments described below, individual *A. thaliana* plants were grown inside sterile 50 mL culture tubes (Eppendorf), in a growth chamber (16:8 light:dark, 20°C, 180 µmol/m^2/sec, 22 °C, 18% relative humidity) for 4 weeks (Figure 4.1a). The growth media was 15mL of a sterile agar based nutrient media in the bottom of the tube. I used a modified 1x Hoagland's solution recipe that contained no nitrogen: 5mM K_2SO_4, 2mM $MgSO_4$, 0.5mM KH_2PO_4, 4.5mM $CaSO_4$, 46.3µM H_3BO_3, 0.76µM $ZnSO_4$, 0.32µM $CuSO_4$, 0.0025% (w/v) Iron

Chelate (Plant Products Co. Ltd.), 0.66µM $NaMoO_4$ (Hoagland 1920). The nutrient media plants were grown in contained 0.75% (w/v) phytablend (Caisson Laboratories, Inc.) and 15mL of 0.1X nitrogen free Hoagland's solution. These nutrient concentrations were selected based on the lower range of the high affinity uptake system of *A. thaliana* (Marschner 1997), and because they represent ecologically meaningful concentrations of nitrogen based on measurements of field soils (Näsholm *et al.* 2009). These concentrations were also selected because toxicity may occur at higher concentrations of certain nutrients (Marschner 1997).

Nitrogen sources used were nitrate, asparagine, or glutamine. These were chosen based on results that showed that of all the amino acids that *A. thaliana* can capture, *A. thaliana* grew best on nitrate (supplied as $Ca(NO_3)_2$), second on glutamine, and third on asparagine (Forsum *et al.* 2008). When it was necessary to measure precise amounts of nitrogen captured, I used dual stable isotope labeled ($^{13}C/^{15}N$) amino acids mixed with unlabeled nitrogen sources so that I could precisely measure nitrogen uptake from each nitrogen source. When nitrogen was labeled, ^{15}N concentrations were elevated to approximately double atmospheric ^{15}N concentrations of nitrogen(~74‰), while unenriched sources of nitrogen remained at atmospheric ^{15}N concentrations (~37‰).

4.2.3. Trait-Fitness correlations

Fresh tissue was required for stable isotope analysis, and thus it was impossible to allow plants from the main experiments to grow to senescence and measure lifetime reproductive effort. However, it was desirable to be able to relate

measurements of plant size to plant fitness. Thus, to determine which plant traits, at 4 weeks of age, could be used as a predictor of plant fitness (measured as seed production) I performed an experiment where plants were grown in Hoagland's solution with only calcium nitrate as a source of nitrogen (1X Hoagland's as above + 20mM $CaNO_3$). To maximize the range of plant sizes to make fitness-trait correlations I varied the concentration of the whole Hoagland's mixture at 0.001X, 0.034X, 0.067X, 0.101X or 0.135X. Each concentration was replicated 25 times for a total of 125 plants. After 4 weeks of growth, approximately half of the plants had begun to bolt. At this point, I photographed each plant individually through the transparent wall of the tube, and measured rosette diameter, stem height, leaf number, presence of nutrient stress, and flower number using ImageJ (v1.43, http://rsbweb.nih.gov/ij/). Measurements were calibrated for distance and angle by photographing a tube that contained a paper disc at the same location that a plant would be. The disc was marked with a series of ruled lines of known length for calibration so that distances measured on images could be calculated in millimeters. When plants had fully senesced they were harvested, and seeds of each plant were collected into transparent trays. The seed crop of each plant was scanned individually and I used the particle counter in ImageJ to count seeds for each plant. Survival of parent plants was measured, but did not differ among treatments (mean 74% survival, Table 8.1), and thus survival was not considered further as a relevant aspect of fitness for the main experiments.

4.2.4. Single nitrogen nutrition

To make predictions about adaptive choice in plants it was necessary to estimate the net benefits of each source of nitrogen individually. A single estimate of the net-benefits of each nitrogen type was needed. Here I use the slope of plant size vs nitrogen concentration as an estimate of net benefits of each nitrogen type. To estimate this slope, I performed a second experiment where I grew plants on 9 different concentrations of each nitrogen source individually for 4 weeks as described above. The concentrations of nitrogen ranged from 0.1mM – 0.9mM in 0.1mM increments. All other nutrient concentrations were held constant with 0.1x N-free Hoagland's solution. This allowed me to measure the changes in growth that were caused solely by changing nitrogen abundance, and concentration. Each concentration and nitrogen type was replicated 8 times for a total of 216 plants. Changes in pH that might be caused by different types of nitrogen were considered to be an intrinsic cost or benefit associated with that type of nitrogen. Such pH differences would not be controlled in a natural setting and might influence plant resource choice and thus I decided not to control for pH. However, pH was relatively constant among treatments (Table 4.1). Amino acids were dual labeled with $^{13}C/^{15}N$ as described above, as a check to ensure plants were capturing intact amino acids. At the end of 4 weeks of growth plants were harvested, and I measured rosette diameter only as a predictor of lifetime reproductive effort (Figure 4.2). Each plant was rinsed in deionized water to remove any isotopes that may have been on the surface of the leaves, and shoots were dried at 60 °C. It was too difficult to extract roots from the agar medium

without significant amounts of agar and nutrient media clinging to the roots, and thus root measurements were not taken. Three plants from every treatment were randomly selected for stable isotope analysis. The entire shoot biomass was ground and carbon and nitrogen isotopic composition ($^{13}C/^{15}N$) was determined using an Elemental Combustion System (Costech ECS 4010, Costech Analytical Technologies Inc.) which was coupled to a continuous flow isotope ratio mass spectrometer (Finnigan Delta Plus Avantage, Thermo Finnigan, Bremen, Germany).

The isotopic composition of the samples (*dI*) was calculated from the following equation:

$$dI = 1000\left(\frac{R_{sam}}{R_{sta}} - 1\right) \tag{4.1}$$

where R_{sam} and R_{sta} are the ratios of either $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample and standard. For carbon, National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) standards of NBS 19, NBS 22 and LSVEC were used to calibrate internal standards of BMO, Corn Stover, Pea Grain Protein, and Red Clover which were used as the working standards. The internal standards had carbon isotope composition of –23.91‰, –12.5‰, -24.68‰, –27.32‰, and –27.42‰ relative to international standards (Pee Dee Belemnite), respectively. For nitrogen, NIST standards of IAEA-N1, IAEA-N2 and IAEA-N3 were used to calibrate the same internal standards as above. Standards had a nitrogen isotope

composition of 8.20‰, 9.10‰, 2.42‰, 6.16‰, and -0.56‰ relative to international standards, respectively.

4.2.5. Choice - Mixed nitrogen nutrition

To determine whether plants could choose among different nitrogen sources I performed a third experiment where plants were offered all possible pairs of the three nitrogen sources. Nitrogen was supplied using the same concentrations as above, but two sources were mixed so that the overall abundance of nitrogen always summed to 1mM. This created nine different ratios of nitrogen from 0.1mM:0.9mM – 0.9mM:0.1mM of each possible pair (Figure 4.1b). For graphical presentation ratios are expressed as log (R_i/R_j) so that each ratio was evenly spaced along an x-axis (Figure 4.1c). Each ratio was replicated 8 times for a total of 216 plants. Only one form of nitrogen in each pair was labeled with C^{13}/N^{15} as above. Labeling only one form of nitrogen allowed for the precise measurement of the amount of each nitrogen type that was captured by a plant. Glutamine was labeled in the nitrate/glutamine choice experiment, and asparagine was labeled in the nitrate/asparagine and glutamine/asparagine experiments. For the same reasons described above, pH was not controlled but was relatively constant among treatments (Table 4.1). Many plants in the glutamine/asparagine choice experiment were too small to obtain enough tissue for stable isotope analysis, and thus only incomplete isotope data is available for this choice experiment. Also, all tubes in the nitrate/glutamine treatment at a ratio of

0.4mM:0.6mM became contaminated with bacteria and all the plants died in this treatment.

After 4 weeks of growth all shoot biomass was harvested, and rosette diameter of all plants was measured. Shoots were rinsed in deionized water, and dried at 60°C. Survival was similar among all treatments, and similar to the experiments described above (mean 69% survival). Again, three plants from every treatment were randomly selected for stable isotope analysis. The entire shoot biomass was ground and carbon and nitrogen isotopic composition (d13C/d15N) was determined as described previously.

To calculate the amount of each type of nitrogen captured by plants in the mixed nutrition experiment, I took advantage of the fact that each nitrogen source had a unique isotopic signature. One source was elevated to ~74‰ while the second nitrogen was not elevated and was at the atmospheric concentration of ~37‰. Since there were only two sources of nitrogen available, there was only one possible combination of each nitrogen source that could yield the ^{15}N isotopic signature I measured in each plant which could be found by solving:

$$y = ax + b(1 - x) \tag{4.2}$$

Where y was the measured ^{15}N‰ value for each plant, x was the proportion of the labeled nitrogen source captured by the plant, (1-x) was the proportion of the unlabeled nitrogen source captured by the plant, a was the known isotopic signature of the labeled nitrogen source and b is the known isotopic signature of the unlabeled nitrogen source. For each nitrogen type I analyzed a sample of the raw nitrogen used in the experiment and thus, a and b were 0.74 and 0.37,

respectively, in each case. For each data point I solved for *x*, and thus could estimate the proportion of each nitrogen source captured by the plant.

Finally, I also investigated how changing the nitrogen types offered to plants and shifting nitrogen abundance ratios affected foraging benefits received by the plants. As above, benefits were estimated based on rosette diameter at 4 weeks of age, which is highly correlated with total lifetime seed production in the annual *A. thaliana* (Figure 4.2).

4.2.6. Statistical Analyses

For plant fitness trait correlations, I used stepwise multiple regressions to determine which plant trait or combination of traits from plants at 4 weeks of age would be the most efficient predictor of lifetime reproductive effort at senescence (R Statistical Environment, R Development Core Team 2009, v2.9.2).

To compare plant growth on each type of nitrogen in the no-choice experiment, I analyzed log transformed rosette diameter using GLMs with a Gaussian distribution, nitrogen type as a fixed factor and nitrogen abundance as a continuous variable, and a type III sum of squares (R Statistical Environment, v2.9.2; R Development Core Team 2009; Fox and Weisberg 2010). I was interested only whether the slope of plant size versus nitrogen concentration varied among nitrogen types. If slopes vary among each type of nitrogen then there would be a significant interaction between nitrogen type, and the nitrogen abundance covariate.

Nitrogen capture data from the choice experiment was arcsine square root transformed for normality and continuity. I compared the transformed nitrogen capture (percent captured) values calculated above to a null expectation that uptake would simply track nitrogen availability using a GLM with a Gaussian distribution, nitrogen concentration as a covariate, observed/expected as a fixed effect, and a type III sum of squares (R Statistical Environment, v2.9.2). For this analysis I was simply interested in whether the slope of observed nitrogen diet deviated significantly from the slope of the expected nitrogen diet which would indicate that plants deviate from the null 1:1 expectation.

Finally, for plant size in the choice experiment, natural log transformed rosette diameter for each choice experiment was analyzed using a GLM with a Gaussian distribution, nitrogen choice experiments as a fixed effect, log nitrogen abundance ratio as a covariate and a type III sum of squares.

4.3. RESULTS

4.3.1. Trait fitness correlations

The stepwise regression indicated that a model that included only rosette diameter was the most efficient model ($F_{1,91}$=254.9, $p<0.0001$), and rosette diameter explained 73% of the variance in lifetime reproductive output for seed production (Figure 4.2). Other variables were such poor predictors of seed set that they were not considered further (Appendix 1, Table 8.2, Figures 8.1-8.4). Thus,

all subsequent experiments were run for 4 weeks, and analyses used rosette diameter at 4 weeks of age to estimate the lifetime benefits of nitrogen nutrition.

4.3.2. Single nitrogen nutrition

Not surprisingly, plants grew significantly bigger when more nitrogen was provided regardless of type (*i.e.* positive slopes). However, fitness varied as a function of the nitrogen type offered to plants (Table 4.2, Figure 4.3). Plants always grew significantly larger on nitrate compared to either amino acid. At low concentrations of nitrogen glutamine and asparagine produced plants of similar size, but at higher concentrations plants grew significantly larger on glutamine compared to asparagine resulting in different slopes. Very low concentrations of nitrogen were expected to produce very small plants regardless of nitrogen type. Since toxicity was not expected within the range of concentrations used, the slope of plant size versus nitrogen concentration is the simplest estimate of the potential benefits of each nitrogen source over a range of concentrations, and thus this was used as an estimate of net-benefits for each type of nitrogen. Based on the comparison of these slopes, the general value of each nitrogen source for plant growth was ranked nitrate > glutamine > asparagine (Figure 4.3).

4.3.3. Mixed nitrogen nutrition

If plants exhibit significant preferences for certain types of nitrogen then the amount of each type of nitrogen captured should significantly deviate from the null prediction of no active choice, and this deviation should be in favour of the

most beneficial type of nitrogen. In support of the adaptive foraging model I found that in every choice experiment, there was a significant interaction indicating that plant preferences significantly deviated from the null model (Table 4.3, Figure 4.4 a-c). Plants consistently preferred the nitrogen type with the highest potential for plant growth in each choice experiment. Specifically, nitrate was preferred over glutamine (Figure 4.4a) and asparagine (Figure 4.4b), and glutamine was preferred over asparagine (Figure 4.4c). The strength of these preferences also depended on the difference in nitrogen quality, with nitrate being preferred more strongly over asparagine compared to glutamine (Figure 4.4a vs 4.4b) which is consistent with the ranking of net benefits for each type of nitrogen as nitrate > glutamine > asparagine. Plants also switched their preferences to lower quality types of nitrogen when their preferred type became very rare (Figure 4.4 a,b).

For plant size, there was a significant interaction between the types of nitrogen provided, and the ratio of available nitrogen concentrations (Table 4.4). For all combinations of nitrogen in the choice experiment, there was a slight decline in plant size as the amount of nitrate available declined (Figure 4.5 a-b). Plants were smallest at the lowest abundances of nitrate even though total nitrogen availability remained constant. Plants were the smallest in the glutamine/asparagine experiment indicating that amino acids are generally a poor source of nitrogen for plant growth compared to nitrate. Plants were similar in size for both the nitrate/glutamine and nitrate/asparagine choice experiment.

4.4. DISCUSSION

In this study I tested whether plant preferences for different types of nitrogen followed the basic qualitative predictions of adaptive resource choice models. As predicted, plants showed significant preferences for the most highly ranked form of nitrogen provided when given a choice between two types of nitrogen (Figure 4.4, Table 4.3). However, plant preferences were not static and plants often switched to lower ranked types of nitrogen when higher quality types of nitrogen were rare. Adaptive foraging theory sheds light on the reasons why plants may show preferences for different types of nitrogen. Specifically, plants should favour those nitrogen types which maximize net benefits, while incorporating nitrogen abundance into their choice (Werner and Hall 1974; Krebs *et al.* 1977; Abrams 2010b; Charnov 1976a).

There are several sources of potential bias in the isotope data gathered in this experiment. First, I analyzed only shoot material rather than whole plants. It is possible that there is preferential allocation of nitrogen types to either shoots or roots which could influence my estimate of nitrogen capture by plants. Shoot material was analyzed because roots could only be removed from agar by melting it, and the concern for contamination of heat damaged roots by isotopes was greater than the concern that shoots might provide a biased estimate of nitrogen capture. Second, biological reactions often discriminate against stable isotopes, resulting in biases in isotopic signatures in tissue known as fractionation. Fractionation occurs because ^{15}N contains one more neutron than ^{14}N and this makes it slightly heavier. The slight difference in molecular weight means that

^{15}N requires a slightly higher activation energy to be used in any enzymatic reactions or biological pathways compared to ^{14}N (Handley and Raven 1992). This leads to slightly lower isotopic signatures than would otherwise occur in the absence of fractionation. For example, fractionation for ammonium and nitrate uptake has been estimated to range between -2‰ and -20‰ depending on species and environmental conditions (Handley and Raven 1992). However, if the fractionation of nitrate, glutamine and asparagine were equal than this effect cancels out in equation 4.2. Furthermore, even with a fractionation correction of more than -50‰ on my data there would be no change in the conclusions drawn above indicating that fractionation effects are not driving the conclusion that plants selectively forage for different types of nitrogen. Thus, I am confident that the conclusions drawn are valid even with no correction for fractionation.

In the following sections I will briefly discuss potential links between previous research and adaptive foraging theory.

4.4.1. Links to adaptive foraging theory

There are few studies available that allow for any broad review that links plant nitrogen nutrition to adaptive foraging predictions. To fully assess whether plant nitrogen nutrition is really a problem of adaptive foraging additional research is necessary. For example, many plant species show preferences for different types of nitrogen (Kielland 1994; McKane *et al.* 2002; Lipson and Näsholm 2001; Näsholm *et al.* 2009). For these preferences to be adaptive species preferences should be correlated with species growth potential on each form of

nitrogen as I have shown here with *A. thaliana*. This would be valuable data to collect, but I am unaware of any studies that make this link.

Also, experiments that offer plants choice between several types of nitrogen simultaneously over a range of ratios (*e.g.* Krebs *et al.* 1977) are lacking. If plant nitrogen nutrition is a problem of adaptive resource choice, then species should show preferences for different resources that are related to the net benefits of those resources (*e.g.* Figure 4.4). Finally, theoretical work is needed to develop plant specific models of adaptive resource choice. The predictions made in this study were simple qualitative predictions. However, it should be possible to develop more comprehensive mathematical models of adaptive resource choice by plants (McNickle *et al.* 2009). In the following section I develop a simple graphical model of resource choice by plants, and demonstrate how species specific differences in nitrogen preferences may minimize competition between plants.

4.4.2. Using foraging theory to move forward

Based on the data presented in this chapter, and my survey of the literature that suggests plant preferences for nitrogen are common and vary among species, I argue that plant preferences for different types of nitrogen can be understood using a framework of adaptive resource choice. Specifically, plants should prefer those types of nitrogen that maximize fitness; however, plants should also integrate the abundance of each nitrogen type in their foraging decisions causing them to switch to lower quality types of nitrogen when preferred types are rare.

Plants commonly show preferences for different types of nitrogen, and these preferences have been empirically linked to plant coexistence and plant community structure (McKane *et al.* 2002; Harrison *et al.* 2007). However, plant ecologists have lacked a rich conceptual framework to understand why plants show these preferences, or how plant preferences can actually lead to differences in competition or coexistence. My second goal in this chapter is to show how adopting foraging theory into plant ecology can be used to generate specific predictions about competition and coexistence among plant species by integrating resource choice with food web models.

Here I present a simple graphical model (Figure 4.6) to demonstrate how competition could shift as a function of foraging behaviour for the simplest case of a system containing two generalist plant foragers with different resource preferences foraging for two perfectly substitutable resources, *i* and *j*. This system is identical to the one described above (Figure 4.1) except there are now two competing plant species with only slightly different resource preferences. Plant preferences are small in this example, because though most plant species vary in their nitrogen preferences, the differences in preference are typically small (McKane *et al.* 2002; Weigelt *et al.* 2005; Harrison *et al.* 2007). As above, the abundance of resource *i*, R_i, is equal to 1-R_j. I use a graphical model to illustrate my point; however analytical models are well developed elsewhere that produce these graphs and predictions (Stephens *et al.* 2007; Abrams 2010a; Abrams 2010b). Competition theory suggests that species that share a resource will experience higher competition than those that do not (Hutchinson 1957; Hardin

1960; Chesson 2000; Silvertown 2004). Thus, in my theoretical system competition for nitrogen is expected to be high when plants prefer the same type of nitrogen, and low when plants have different nitrogen preferences. Based on these ideas, I measure the potential for competition as the inverse of the Cartesian distance between nitrogen diet composition among the two species (Figure 4.6a).

In this simple model, at either end of the extremes along the resource abundance gradient both competing plants use the same resources (Figure 4.6a, b). This is because one type is very common, and the other very rare which limits the ability of the plants to select one type of nitrogen over another. At these extremes, the diets are the most similar, and thus the intensity of competition is strong (Figure 4.6c). However, at intermediate resource ratios, there is more potential for nitrogen usage to differ among plants via differences in preference. If the foraging plants exhibit different resource preferences (Figure 4.6a, b) then this foraging behaviour can lead to a decline in the intensity of competition (Figure 4.6c). Resource partitioning in this simple system is not caused by static specialization, but rather is dynamic and depends on the foraging preferences of generalist competitors, and the abundance of each resource. The scenario depicted in Figure 4.6 could represent two individuals foraging over time as resources are drawn down, or many individuals that compete at distinct points along a resource gradient. A key prediction of this general model is the ‘U’ shaped relationship (Fig. 3c) between the intensity of competition along a gradient of shifting resource ratios in a two plant, two resource system where each species has a different

foraging response to resources *i* and *j* if it is assumed that similar resource use begets competition for that resource (Fig. 3b).

4.4.3. Conclusions

Plant preferences for different types of nitrogen are common, but there has been little theory available to understand why plants show different preferences, or predict meaningful ecological outcomes from the preferences of plants. Using evidence from the model plant *A. thaliana* I argue that plant nitrogen preferences can be conceptualized as a problem of adaptive foraging, and that plants integrate information about the net-benefits, and abundance of each type of nitrogen and make choices that are adaptive. Based on this finding, I present a simple graphical model to show how foraging theory can contribute general predictions about plant-plant competition and coexistence. Much theoretical and empirical work is still needed to develop a rich theory of adaptive foraging for plants (McNickle *et al.* 2009). However, like others, I believe that adopting a framework of adaptive foraging for plants will allow ecologists to gain a better understanding of plant nitrogen preferences, and the ecological consequences of these preferences (McNickle *et al.* 2009). Here I have shown how such a framework can be used to predict nitrogen preferences, and further I have suggested how these preferences may predict competition based on small differences in diet among competing plants.

TABLE 4.1: Initial pH of each nutrient solution used in the no-choice experiment (top) and the choice experiment (bottom) for each type or combination of nitrogen, and each abundance or ratio of nitrogen.

Concentration (mM)	Nitrate	Glutamine	Asparagine
0.1	7.02	7.31	7.35
0.2	7.00	7.63	7.33
0.3	7.02	7.67	7.30
0.4	6.98	7.61	7.30
0.5	7.00	7.68	7.28
0.6	6.91	7.67	7.24
0.7	7.04	7.63	7.22
0.8	6.96	7.59	7.20
0.9	7.07	7.59	7.19

Ratio	Nitrate + glutamine	Nitrate + asparagine	Glutamine + asparagine
1:9	6.98	7.13	7.22
2:8	6.95	7.02	7.19
3:7	6.89	6.91	7.19
4:6	6.85	6.85	7.15
5:5	6.83	6.82	7.13
6:4	6.80	6.80	7.11
7:3	6.82	6.78	7.11
8:2	6.82	6.80	7.11
9:1	6.67	6.80	7.13

TABLE 4.2: Results of GLM on plant size for nitrogen type (nitrate, glutamine or asparagine), and nitrogen abundance in the no choice experiment.

Factor	Df	F	p
Nitrogen	2	11.7	0.0001
abundance	1	37.7	<0.0001
Nitrogen* abundance	2	5.3	0.0058
Residuals	154		

TABLE 4.3: GLM results for observed vs expected nitrogen capture for the nitrate/glutamine, nitrate/asparagine and glutamine/asparagine experiment. Since the amount of each type of nitrogen captured always sums to 1, then analyzing for the amount of either type of nitrogen in each choice experiment yields identical results. The factor 'Model' is either observed or expected nitrogen diet values, abundance represents the nitrogen abundance (mM). Different numbers of residual Df represent differences in the number of individuals available for analysis in each choice experiment based on growth and survival.

	nitrate/glutamine			nitrate/asparagine			glutamine/asparagine		
Factor	Df	F	P	Df	F	p	Df	F	p
Model	1	4.5	0.0387	1	8.182	0.006	1	208.9	<0.0001
abundance	1	639.7	<0.0001	1	638.5	<0.0001	1	2.7	0.109
Model* abundance	1	41.1	<0.0001	1	112.2	<0.0001	1	33.2	<0.0001
Residuals	44			50			32		

TABLE 4.4: Results of GLM on plant size for nitrogen choice experiment, and nitrogen abundance ratio in the choice experiment.

Factor	**Df**	**F**	**p**
Choice	1	33.2	<0.0001
Ratio	2	116.2	<0.0001
Choice*Ratio	2	4.3	0.0151
Residuals	164		

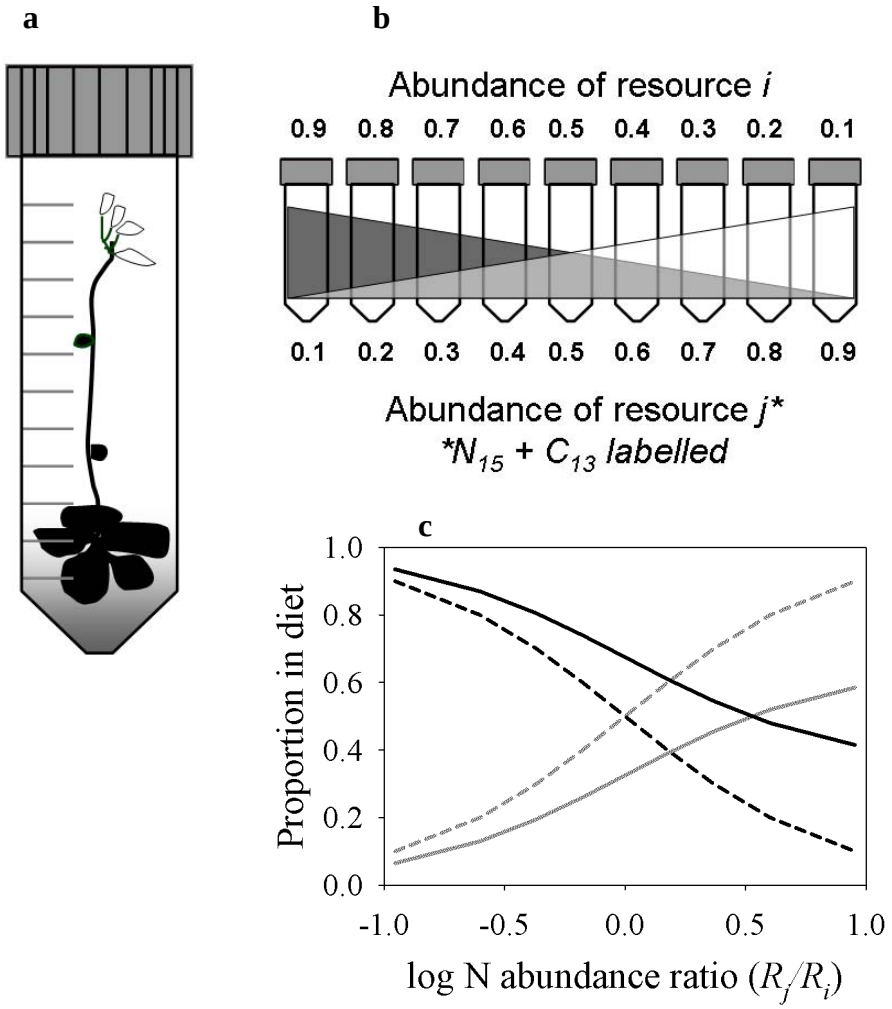

FIGURE 4.1: Schematic representation of the experimental design (a,b) and model predictions (c). a, The system is the simplest choice system with one foraging plant and two substitutable resources represented here by N_i (Dark shading; all panels) and N_j (light shading; all panels). b, Each choice experiment consisted of choice between two types of nitrogen at nine different ratios, where the relative concentration of each nitrogen type varied from common to rare, but the total concentration of nitrogen was constant. In my experimental system R_i=1-R_j. One nitrogen type (resource *j*) was labeled with ^{13}C and ^{15}N. **c,** Hypothetical data demonstrating preferences predicted by adaptive resource choice. The *x*-axis is the log ratio (R_j/R_i) from each of the nine mixtures depicted in panel b. Dotted lines depict a situation where neither resource *i* or *j* are preferred, and represent a null expectation. Solid lines represent an example where N_i (black) provides the higher per unit growth benefits than N_j (gray) and thus is consumed at a rate greater than its proportional abundance.

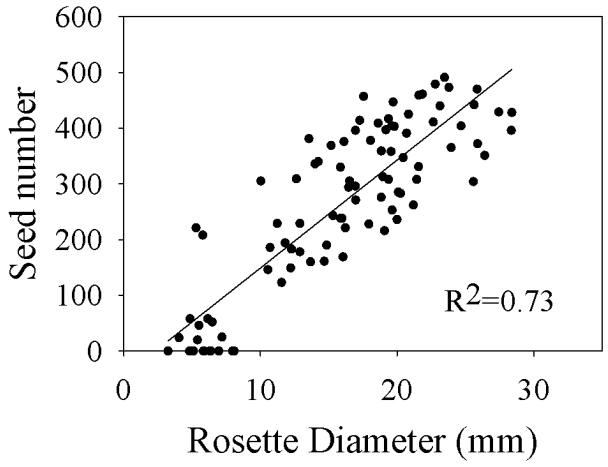

FIGURE 4.2: Relationship between seed production at senescence (~10 weeks) and rosette diameter at 4 weeks of age.

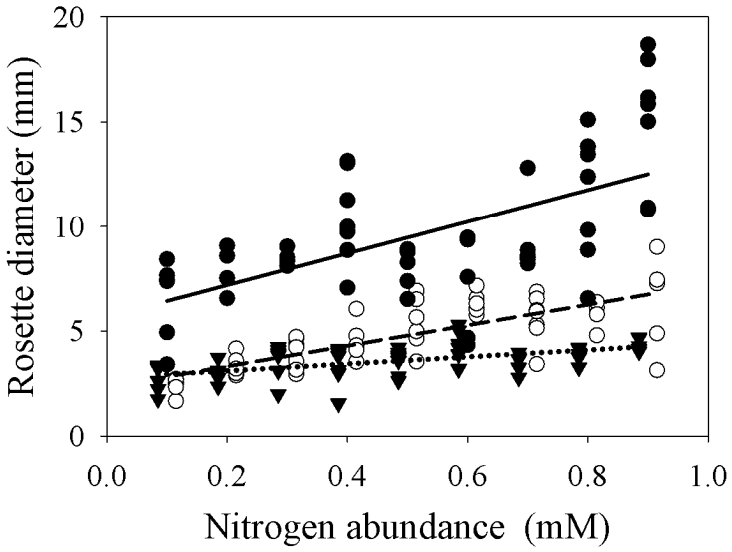

FIGURE 4.3: Scatter plots and linear fits of rosette diameter of plants grown on only one type of nitrogen in the no-choice experiment for each type of nitrogen, and each concentration of nitrogen. Nitrate (Filled circles, solid line), glutamine (Empty circles, dashed line) and asparagine (triangles, dotted line).

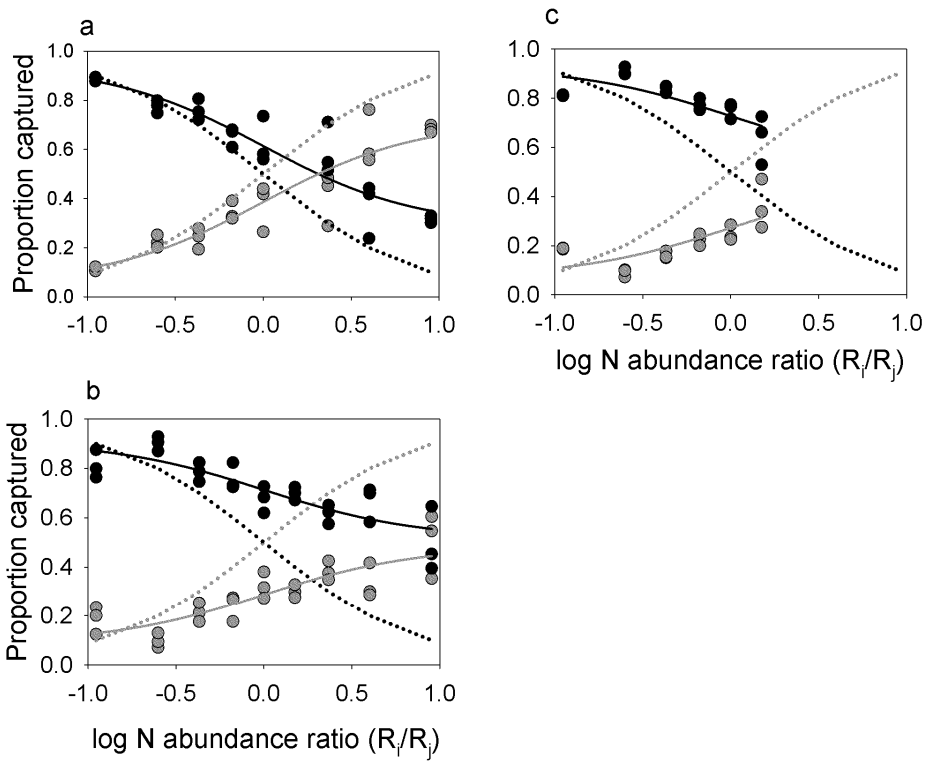

FIGURE 4.4: Summary nitrogen capture data from choice experiment. **a**, nitrate (N_i; black) versus glutamine choice (N_j; gray); **b**, nitrate (N_i; black) versus asparagine choice (N_j; gray); **c**, glutamine (N_i; black) versus asparagine choice (N_j; gray). Observed nitrogen preferences (circles), with fitted linear models (solid lines) compared to null no choice expectations (dotted lines). Lines appear non-linear because of the log scale of the *x*-axis.

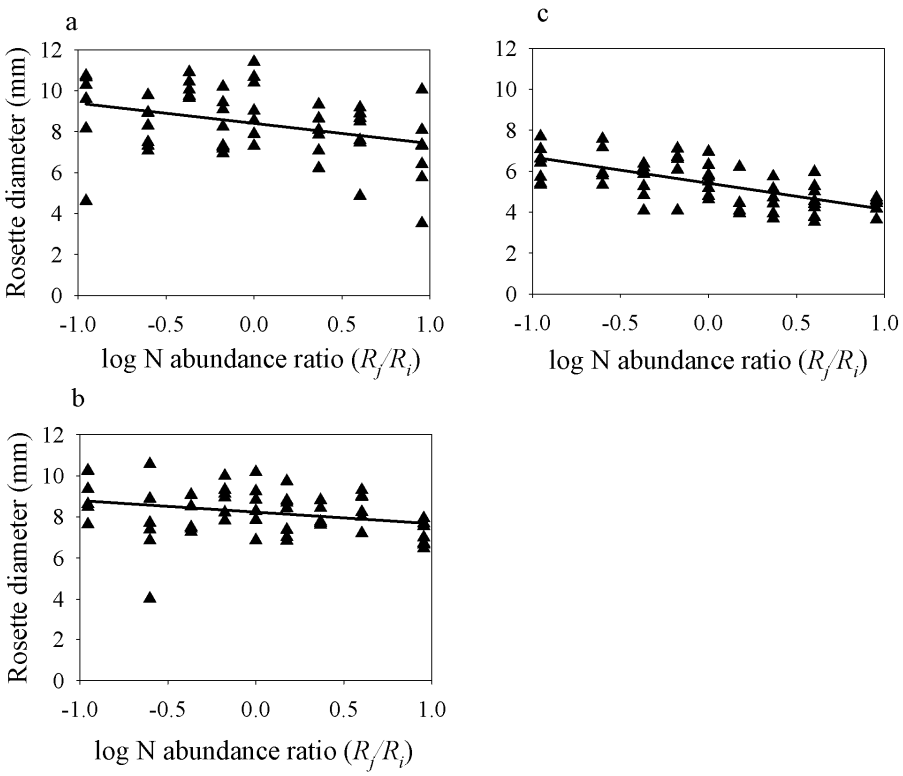

FIGURE 4.5: Relationship between plant size, and the log nitrogen abundance ratio (log R_j/R_i) from the choice experiment. log R_j/R_i is calculated based on the abundance of each type of nitrogen (R_i or R_j), see Fig 1. a, nitrate (R_i) versus glutamine (R_j). b, nitrate (R_i) versus asparagine (R_j). c, glutamine (R_i) versus asparagine (R_j).

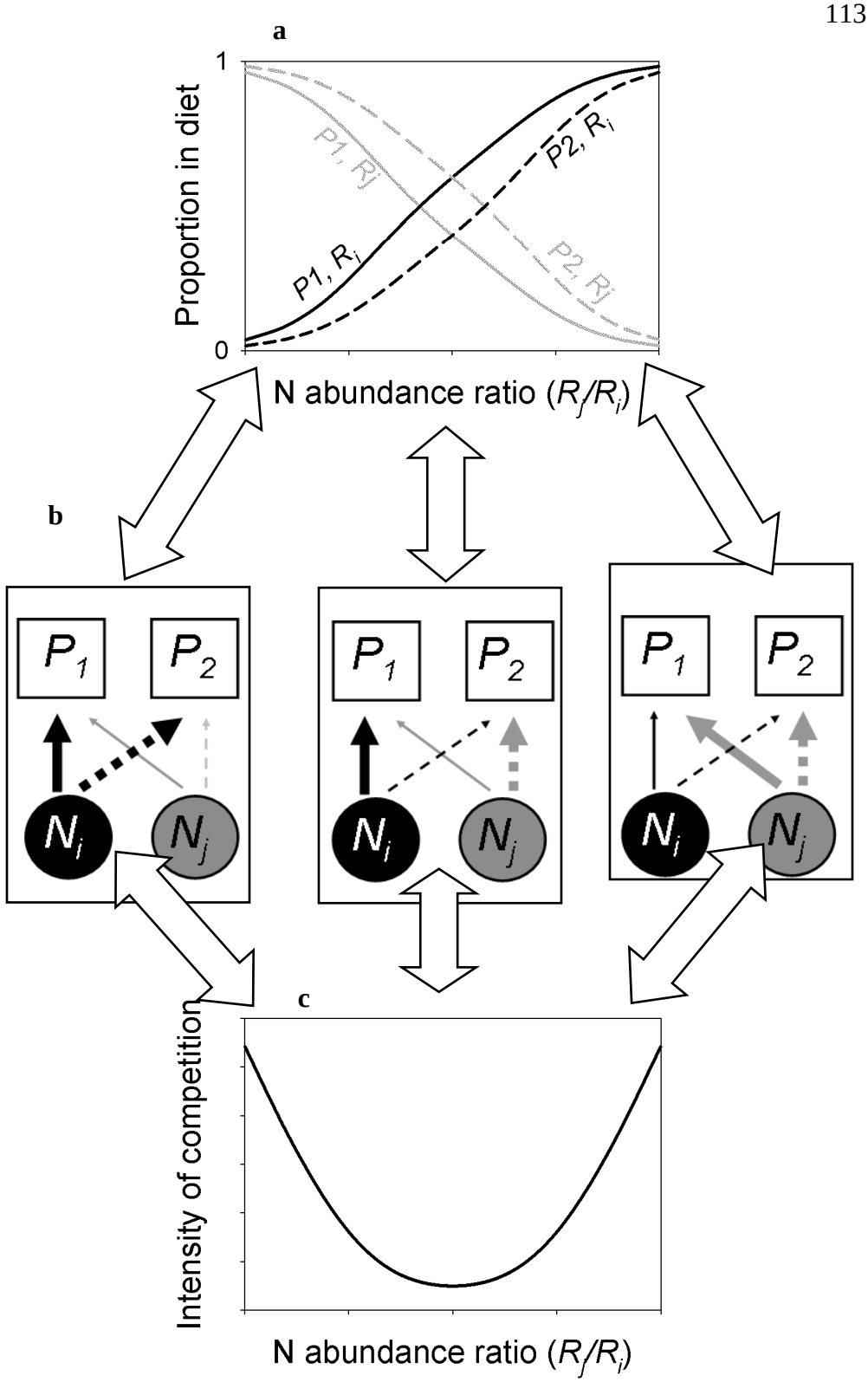
a
1
0
Proportion in diet
P1, Ri
P2, Ri
P1, Rj
P2, Rj
N abundance ratio (Rj/Ri)
b
P1
P2
Ni
Nj
c
Intensity of competition
N abundance ratio (Rj/Ri)

FIGURE 4.6

FIGURE 4.6 continued: Graphical food web theory to explain plant coexistence via adaptive resource choice for the simplest system of two hypothetical plants (*P1* and *P2*), foraging for 2 perfectly substitutable types of a resource N_i (black) and N_j (gray). **a**, Graphical depiction of the foraging preferences of P1 (solid lines) and P2 (dashed lines) for resource *i* (black) and resource *j* (gray). As abundance ratios shift the preferences of each plant shift yielding differences in resource preference. Each curve must necessarily pass through the points (0,0) and (1,1). **b**, Cartoon food web representation of the foraging choices of P1 (solid lines) and P2 (dashed lines) for resource *i* (black) and resource *j* (gray) represented graphically in panel a. Thicker lines represent stronger preferences. Block arrows link each of the foraging situations depicted in panel b to the appropriate point along the resource gradient in panels b and c. **c**, Intensity of competition between P1 and P2 along the resource gradient. More dissimilar diets are assumed to result in less intense competition and this is calculated as the inverse of the diet similarity (Cartesian distance between preference curves) of each plant in panel a.

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5. A PCR BASED METHOD FOR THE IDENTIFICATION OF THE ROOTS OF 10 CO-OCCURING GRASSLAND SPECIES IN MESOCOSM EXPERIMENTS[3]

5.1. INTRODUCTION

Factors that affect the productivity and nutrient flux within ecosystems have great potential to affect ecosystem structure and function. Most plant species have the ability to non-randomly place their roots in regions of high soil fertility (Campbell *et al.* 1991; Hutchings and de Kroon 1994; Kembel and Cahill 2005; McNickle and Cahill 2009, Chapter 3). This ability leads to higher nutrient capture, and thus greater productivity of individual plants than if roots were randomly arranged in the soil. An understanding of these basic patterns of root distribution should be intimately linked to an understanding of plant coexistence and community structure. However, little is known about the importance of root responses in natural systems (Hutchings *et al.* 2003; Hodge 2004; Kembel and Cahill 2005). A major limitation to linking patterns of small scale root distributions to larger scale ecological processes has been a lack of robust methods for identifying roots of co-occurring species (Bobowski *et al.* 1999; Linder *et al.* 2000; Brunner *et al.* 2001; Ridgway *et al.* 2003; Moore and Field 2005).

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[3] A version of this chapter has been published. McNickle GG, Cahill JF and Deyholos MK. 2008. *Botany*. 86: 485-490.

Essentially all of the evidence concerning root placement in soil comes from mesocosm experiments. Mesocosms are useful tools for addressing questions about root distributions, because in a field setting it is impossible to know the distribution of plant roots before the start of an experiment. Furthermore it has been necessary for studies concerning root distributions to focus on individual plants grown in isolation (*e.g.* Campbell *et al.* 1991; Lamb *et al.* 2004), or on single species grown in monoculture (*e.g.* Casper and Cahill 1996; Casper and Cahill 1998; Fransen *et al.* 2001). This is because the fine roots of different species are not visually distinguishable and species specific differences cannot typically be measured when more than one species co-occur. However, the results of single species mesocosm experiments are of limited relevance to natural systems, where plants rarely occur in the absence of interspecific competition (Robinson *et al.* 1999; Fransen *et al.* 2001; Bliss *et al.* 2002; O'Brien *et al.* 2005; Rajaniemi 2007).

As a response to these technical challenges, several methods have been developed that use DNA based methods to identify individual root fragments, one at a time (Bobowski *et al.* 1999; Linder *et al.* 2000; Brunner *et al.* 2001; Ridgway *et al.* 2003). These methods are all based on either DNA sequencing or RFLP keys which work best on homogeneous single species samples. While such methods represent a significant technological advance, it should be clear that even a modest sized mesocosm experiment would contain an impractically large number of root fragments, each of which would have to be isolated and analyzed individually. Moore and Field (2005) recognized this fact and took root

identification one step further. They showed that samples containing mixtures of up to four species could be distinguished with their RFLP keys. However, this method is not easily scalable, in part because RFLP patterns have an increasing chance to blend together and overlap as more species co-occur in a sample.

Each of these previously described root identification methods begins with PCR, and proceeds to use various downstream reactions to characterize the PCR products and assign taxonomic identity to each specimen. It would be much more cost and time effective if PCR were the first and last step in the process. Furthermore, since questions about root distributions are almost always confined to mesocosms, the high taxonomic resolution of more expensive techniques such as DNA sequencing or RFLP keys are not required. My goal was to develop a new method for root identification which was; 1) applicable to multi-species samples of any number of species, and; 2) was more cost and time efficient than previously described methods. In this chapter I describe a set of species specific PCR primers designed for this purpose. I show that these primers are robust and species specific and can lead to species identifications using simple agarose gel electrophoresis.

5.2. METHODS

5.2.1. Species and Tissue collection

I selected 10 naturally co-occurring grassland species to develop this method. Species selection was influenced by my primary goal of addressing central questions in plant ecology. I included species from a wide range of natural

abundances (common to rare), members of all grassland functional groups (shrubs, grasses, forbs) and species which have been extensively studied in previous mesocosm studies (*e.g. Achillea millefolium* and *Poa pratensis*) (Table 5.1).

Leaf tissue and seed were field collected from spatially separated individuals by haphazardly sampling along an 11 km transect every two weeks in the summer of 2005 at the University of Alberta Kinsella Ranch (N 53º00.950', W 111º32.403'). Leaf tissue from each species was always sampled from flowering individuals to guard against misidentification. Leaf tissue was immediately dried using silica gel and stored at -20°C (Chase and Hills 1991). Seeds were stored at 4°C. Reference specimens were collected at the same time from the field site and deposited in the University of Alberta Vascular Plant Herbarium (ALTA) (Table 5.1).

5.2.2. Species Specific Primer Design

I selected the Internal Transcribed Spacer (ITS) region of ribosomal DNA as the site for primer design. This was done for three reasons. First, the ITS region has been successfully applied to the problem of root identification (Linder *et al.* 2000; Moore and Field 2005). Second, sequence variability in the ITS region has been shown to be among the most useful regions for phylogenetic inference (White *et al.* 1990; Baldwin *et al.* 1995; Alvarez and Wendel 2003). Third, the ITS region is one of the most widely available DNA sequences in GenBank. Any

available DNA sequence could conceivably be used to design species specific primers, and in all cases, specificity must be demonstrated empirically.

Based on the general topology of the ITS region (Baldwin *et al.* 1995; Alvarez and Wendel 2003), and the fact that I did not require high taxonomic resolution for mesocosm experiments, I reasoned that I did not need to design two unique primers for each species. Instead, I designed a single species-specific primer which could be paired with the universal ITS5m primer (Saar *et al.* 2001) in all PCR reactions. By doing this I reduced the amount of unique sequence necessary for positive species identification to about 20 base pairs (bp). ITS sequence data for each species were downloaded from Genbank (Table 5.1). Primers were designed using the Primer3 program (Rozen and Skaletsky 2001). The PCR product size was designed to be approximately 500bp for half of my focal species, and approximately 200bp for the other half (Table 5.1). This was done to add additional landmarks for the visualization and quantification of PCR products, and to potentially permit multiplexing of PCR reactions. The assignment of species to each PCR product size class was based on the best location for a primer, as predicted by the Primer3 software.

5.2.3. DNA Extractions

All DNA extractions were done using a method for DNA extraction of roots described by Brunner et al. (2001), and further modified for this study. Briefly, 10mg of dry plant tissue was ground to a fine powder in liquid nitrogen, mixed with 650ul of extraction buffer [100mM Tris-HCl pH8.0, 25mM

ethylenediaminetetraacetic acid (EDTA), 2M NaCl, 2% (w/v) hexadecyltrimethylammonium bromide (CTAB), 500mg/L spermidine, 2% (w/v) polyvinylpyrrolidone K30 (PVP), 5% (w/v) polyvinylpolypyrrolidone (PVPP) and 2% (v/v) 2-mercaptoethanol], and incubated for 30 minutes at 65°C. Different from Brunner et al. (2001), I found that the best results were obtained by removing cell debris by centrifugation for 10 minutes at 12,000 rpm with a table top centrifuge (Hettich Zentrifugen Mikro20), prior to chloroform extraction. The supernatant was collected and two sequential chloroform extractions were performed using 700uL of chloroform, followed by a 10 minute centrifugation at 12, 000rpm. The supernatant was collected, 1.5 vol of chilled isopropanol was added and the sample was incubated at -20°C for at least 1 hour. The sample was then centrifuged at 13000 rpm, and the supernatant was poured off. I also found that the addition of a wash with chilled 70% ethanol increased the success of downstream enzymatic reactions. Finally the pellet was air dried for 30 minutes and then re-suspended in Tris-EDTA buffer (100mM Tris, 10mM EDTA, pH 7.4).

5.2.4. Specificity using homogeneous templates

To test the species specificity of each primer, I conducted PCR with genomic DNA of two individuals of each of the 10 species, in a full factorial design of each combination of a unique primer and genomic DNA from an individual species. Reactions were performed in a total volume of 15 µL containing 1X PCR buffer (New England Biolabs), 0.2mM each of dATP, dCTP, dGTP, and dTTP (Invitrogen), 0.25 units of Taq polymerase (New England

Biolabs) and 2.5ng of template DNA. Reaction temperature conditions were: 1 minute initial denaturation at 94°C, and 25 cycles of 30 seconds denaturation at 94°C, 30 seconds annealing, 30 seconds extension at 72°C, followed by a final extension of 5 min at 72°C. The optimal annealing temperature differed among primers (Table 5.1). Seven µL of each reaction was run on a 1% (w/v) agarose gel and visualized using ethidium bromide to confirm successful amplification.

Primers that failed to be species specific were redesigned as necessary. If re-designed primers were also non-specific, I used Locked Nucleic Acid (LNA, Exiqon) modifications to increase primer melting temperature and thus primer specificity (Letertre *et al.* 2003).

5.2.5. Multiplex PCR

To minimize the number of PCR reactions, combinations of primers were tested in multiplex PCR reactions until five multiplex mixtures of two primers each (plus the universal primer) were identified (Table 5.1). To compensate for potential differences in efficiency related to target sequence length, I added 0.2 µM of the species specific primer that corresponded to the ~200bp target sequence, and 0.4 µM of all other primers in all multiplex PCR reactions. Although further multiplexing could potentially reduce the cost of the assay, I did not attempt to optimize my method for a larger number of primers in a single reaction. Increasing the number of primer pairs in a PCR reaction increases the potential for undesirable interactions among components of the reactions. Moreover, because PCR efficiency is related to the length of amplified product,

the large range of product sizes that would be required for electrophoretic resolution of bands from ten multiplexed primers could differentially affect the sensitivity of detection for individual species. This is especially true in my case since different products competed not only for typical components of the reaction but also for access to the ITS5m primer.

5.2.6. Assay of DNA extracted from a mixture of roots

I grew monocultures of eight of my ten focal species in a greenhouse. Root tissue was collected and dried in silica gel (Chase and Hills 1991). Eight different combinations of four randomly selected species were made by mixing equal biomass of root tissue of four species (Table 5.2). Combinations of species were limited to only eight of my ten focal species because the field-collected seed from *Rosa arkansana* and *Astragalus agrestis* did not germinate. The DNA was extracted from each root mixture, and the species present were identified using multiplex PCR reactions per sample as described above.

5.2.7. Assays of heterogeneous templates

To further test the primers, I mixed genomic DNA samples that had been extracted from individual species (rather than mixing the tissue and then extracting the DNA). This was done so that I could include DNA from *R. arkansana* and *A. agrestis* in my validation of my method. A total of 16 different mixtures of genomic DNA were made that included five mixtures each of four, six and eight randomly selected focal species, and one mixture of all ten species

(Table 5.3). DNA stocks of each species were diluted to a concentration of 10ng/µL and mixtures were created by adding equal volumes of the DNA of each species to a constant final volume.

5.2.8. Assays of sensitivity

To test the limits of detection I performed PCR reactions on a dilution series of three samples containing two selected species each. In these reactions the DNA of one species was not diluted and remained common, while the second target species was increasingly diluted making it increasingly rare. The abundances of the target species in these reactions were; 10, 8, 6, 4, 2, 1 and 0.5% of 2.5ng total DNA, and the common species made up the remainder of the DNA in these samples. The species were randomly selected and included *Campanula rotundifolia* (136bp), *Festuca hallii* (558bp), and *Bromus inermus* (224bp) as target species and *A. millefolium* as the common species in all three cases.

5.3. RESULTS

5.3.1. Specificity using homogeneous templates

After reaction conditions were optimized, all primers produced species-specific PCR products in a full factorial design of each combination of a unique primer and genomic DNA from an individual species. In the case of *P. pratensis,* species specificity was achieved only after introducing LNA modifications to the primer (Table 5.1). It was also necessary to introduce LNA modifications into the *Thermopsis rhombifolia* primer and ITS5m primer for use in multiplex group B

(Table 5.1). This was not necessary to achieve species specificity for the *T. rhombifolia* primer, but only to ensure proper annealing at the increased annealing temperature required for the *P.pratensis* primer in a multiplex reaction.

5.3.2. Assays of DNA extracted from a mixture of roots

In the multi-species root mixtures, I always detected all species known to be present in a mixture (true positives), and never detected anything known to be absent (false positives) (Table 5.2). This was true regardless of the combination of species in a mixture.

5.3.3. Assays of heterogeneous DNA templates

Similarly, in the multi-species DNA mixtures I never observed any false positives or false negatives (Table 5.3). That is, my DNA based species identification method worked perfectly in 100% of my assays. The accuracy of the method was not influenced by the number, identity or even the abundance of species in an unknown sample.

5.3.4. Assays of sensitivity

When 2.5g of mixed DNA from more than one species was used as a template in my assays, I was able to detect the presence of as little as 0.05ng (2%) of DNA from within this mixture. To put this measurement of sensitivity in perspective, my standard DNA extraction procedure yields an average of 3000ng of DNA from a 5mg root sample, and thus, 0.05ng is theoretically the amount of

DNA that could be extracted from 83ng of root tissue. However, due to practical limitations of isolating roots from soil, the actual amount of root mass that can be detected in practice is expected to be somewhat higher.

5.4. DISCUSSION

My objective was to demonstrate that identification of soil grown roots from a mixture of species is possible using PCR and simple gel electrophoresis. The species specific primers I report here provided reproducible positive species identifications in 100% of my validation assays. As with any application of PCR, the method is extremely sensitive. I was able to detect extremely low absolute amounts of template DNA using PCR. This PCR based method is more cost and time efficient than previous methods designed for root identification because it relies exclusively on PCR and does not require any downstream reactions. Furthermore, a PCR based method is more robust than previous methods because it can be applied directly to mixed species samples of any number.

Because my technique is PCR-based, it may be more easily adapted to certain applications than previously described methods. For example, future modifications of my method may allow for quantification of relative species abundance, using quantitative real-time PCR with fluorescent probes or dyes such as SYBR green (*e.g.* Mommer *et al.* 2008). Resolution and throughput of species detection could also be increased using multiple fluorescent dyes in automated DNA sequencers (*e.g.* Taggart *et al.* 2010).

I have shown that, when present, very small amounts of template DNA present in a multi-species sample can be detected using PCR. However, in practice the type of soil and how thoroughly roots are washed prior to DNA extraction can influence the yield and quality of extracted DNA and may influence sensitivity (Brunner *et al.* 2001, GGM personal observation). Thus, instead of attempting to validate every possible set of extraction and growing conditions, I have shown that the basic behaviour of my species-specific primers in PCR reactions was robust. Thus, researchers should calibrate the sensitivity of the method on actual roots for their particular set of growing conditions prior to application of the method.

The method I have presented here should not be confused with DNA barcoding. Though DNA barcoding methods also seek to make species identifications, the overarching goal of DNA barcoding initiatives is the development of methods for species identification which are universal across all taxonomic groups (Hebert *et al.* 2003; Savolainen *et al.* 2005). My method stands in contrast: I was interested in distinguishing between a small set of specific, ecologically interesting species in mesocosms. Because the identities of all potential members of a mesocosm experiment can be known from the outset of the experiment, I did not require the universally high taxonomic resolution of standard DNA barcoding methods. Instead I developed a simple, cost-effective technique with sufficient resolution for identification of each species within a mesocosm. I have shown only that my method works consistently in mixed species samples from mesocosms in which only those 10 species occur.

Application to other systems where any additional species outside my 10 focal species might occur (*e.g.* the field, or in pots with additional species) would require empirical testing of the species specificity of my primers against the DNA of those additional species.

The historic understanding of the factors influencing root distributions has been overwhelmingly dependent on results from mesocosm experiments with single plants grown in isolation (Robinson *et al.* 1999; Fransen *et al.* 2001; Bliss *et al.* 2002; O'Brien *et al.* 2005; Rajaniemi 2007). Despite a proliferation of these types of experiments in the literature there has been very little success in drawing connections between conclusions based on single plant pot experiments and patterns of plant communities. This is partially because there is little data available on the interaction between interspecific competition and root foraging ability. With a robust method for identification of roots to the level of species, previous single plant experiments can now be replicated to include multi-species competition treatments which more closely approximate the competitive environment a plant experiences in the natural world.

TABLE 5.1: Species specific primer sequences and characteristics for all focal species. ITS5m, LNA bases are indicated by lowercase letters.

Species (Family)	**Species specific primer sequence**	**Tm**	**Multiplex grouping**	**PCR Product Size (bp)**	**Genbank accession number**	**Herberium Accession number**
Rosa arkansana Porter var arkansana (Rosaceae)	TCA CGC CGG TGT TCA GTA	62	C	186	AJ631862.1	116082
Festuca hallii (Vasey) Piper (Poaceae)	CGG ATG CAC TGC GTT TAG T	58	D	558	AF532952.1	116079
Achillea millefolium L. (Asteraceae)	CGT CAA TGA CAC ATT CAC CAG	62	E	113	AY603186.1	116074
Poa pratensis L. (Poaceae)	GGT CcT TAA GGc CAT CAC	67	B	588	AF171183.1	116081
Koeleria macrantha (Ledeb.) J.A. Schultes (Poaceae)	GGG TCT TTA GAG GCC ATC G	62	C	532	Z96910.1	116080
Artemisia ludoviciana Nutt. (Asteraceae)	AAA GCG TCG AAA GGA TCA AA	62	E	497	AF514349.1& AF514350.1	116075
Thermopsis rhombifolia (Nutt. Ex Pursh) Nutt. Ex Richardson (Fabaceae)	GGG AcG CAc TAG ACA ATC T	67	B	208	AF007468.1	116083

TABLE 5.1: Continued

Species (Family)	Species specific primer sequence	Tm	Multiplex grouping	PCR Product Size (bp)	Genbank accession number	Herberium Accession number
Astragalus agrestis Douglas ex G. Don (Fabaceae)	GCA TGC ACA TGA TCG GTA TC	60	A	404	L10758.1& L10759.1	116076
Campanula rotundifolia L. (Campanulaceae)	GAC AAG GAA GGG GTC AAA TG	58	D	136	DQ304615.1	116078
Bromus inermis Leyss. (Poaceae)	CAA CAC AAG AGA TGA CCA GCA	60	A	224	AY367915.1	116077
ITS5m	GAA GGa GAA GTC GTA AcA AGG					

TABLE 5.2: Summary of species combinations used in root mixtures, species names are abbreviated to the first three letters of their genus, + indicates that a species was both present and detected. Combinations of species were randomly chosen.

Replicate	Ach	Art	Bro	Cam	Fes	Koe	Poa	The
1	+	+				+	+	
2		+	+				+	+
3	+				+	+	+	
4	+	+	+					+
5			+		+		+	+
6	+		+	+		+		
7			+			+	+	+
8	+	+					+	+

TABLE 5.3: Summary of species combinations used in genomic DNA mixtures, species names are abbreviated to the first three letters of their genus, 'Rep' indicates replicate number, + indicates that a species was both present and detected.

Number of Species	Rep	Ach	Art	Ast	Bro	Cam	Fes	Koe	Poa	Ros	The
4	1			+		+		+		+	
4	2				+		+		+	+	
4	3		+			+		+			+
4	4			+	+	+	+				
4	5	+	+	+					+		
6	1	+		+		+	+	+			+
6	2	+	+		+	+			+		+
6	3		+	+	+		+	+	+		
6	4		+		+	+		+	+		+
6	5	+		+	+	+	+		+		
8	1	+	+		+	+		+	+	+	+
8	2	+	+	+		+	+		+	+	+
8	3	+	+	+			+	+	+	+	+
8	4	+	+	+	+	+			+	+	+
8	5	+	+	+	+		+	+		+	+
10	1	+	+	+	+	+	+	+	+	+	+

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6. ROOT FORAGING BEHAVIOUR OF FOUR CO-OCCURING GRASSLAND SPECIES ALONE AND WITH NEIGHBOURS

6.1. INTRODUCTION

Heterogeneity in the distribution of soil nutrients (Jackson and Caldwell 1993; Hutchings and de Kroon 1994; Hodge 2004) and belowground competition for those nutrients (Casper and Jackson 1997; Schenk 2006) are ubiquitous pressures experienced by plants in the natural world. Hundreds of plant species have been assayed for their response to either heterogeneity (Hutchings and de Kroon 1994; Johnson and Biondini 2001; Hodge 2004; Kembel and Cahill 2005; Hodge 2006) or root competition (Keddy *et al.* 1994; Casper and Jackson 1997; Schenk 2006) independently. Yet only a handful of plant species have been assayed to investigate how plants respond to both of these pressures simultaneously (Casper and Cahill 1996; Cahill and Casper 1999; Fransen *et al.* 2001; Bliss *et al.* 2002; Day *et al.* 2003; Rajaniemi 2007; Cahill *et al.* 2010; Mommer *et al.* 2010). Furthermore, these studies do not typically measure the root foraging behaviour of plants because of the difficulty in identifying the roots of co-occuring species (McNickle *et al.* 2008; Mommer *et al.* 2008). Thus, little is known about how root foraging behaviour may shift when plants are grown with neighbours.

It has been hypothesized that soil heterogeneity should increase the intensity of competition among competing plants (Schwinning and Weiner 1998; Day *et al.* 2003; Schenk 2006). Increased competition in heterogeneous soils was

predicted because when plants are grown alone in heterogeneous soils, nearly all plants studied will place a large amount of root biomass within high quality soil patches (Hutchings and de Kroon 1994; Hodge 2004; Kembel and Cahill 2005; Hodge 2006). Therefore, it follows that if competing plants use the same foraging strategy with neighbours as they do when grown alone, then the aggregation of roots within patches should intensify competition in heterogeneous relative to homogeneous soils (Schwinning and Weiner 1998; Day *et al.* 2003; Schenk 2006). Some studies find support for this idea (Fransen *et al.* 2001; Day *et al.* 2003), while others find no support (Casper and Cahill 1996; Cahill and Casper 1999; Bliss *et al.* 2002). However, most studies do not measure root behaviour and are forced to assume root behaviour of individuals grown with neighbours is the same as when grown alone (*e.g*. Casper and Cahill 1996; Cahill and Casper 1999; Hodge *et al.* 1999; Robinson *et al.* 1999; Fransen *et al.* 2001; Bliss *et al.* 2002; Day *et al.* 2003; Rajaniemi 2007). Yet, from the few studies that have been able to separate the roots of individuals, there is good reason to think that plants alter their root foraging behaviour in the presence of neighbours, compared to when grown alone (*e.g.* Cahill *et al.* 2010; Mommer *et al.* 2010).

In response to both soil nutrients and neighbour distributions, plant root growth is plastic and plants alter the growth and movement of roots to influence occupancy of fine scale locations in the soil (Gleeson and Fry 1997; de Kroon *et al.* 2009; McNickle *et al.* 2009; Mommer *et al.* 2010). For example, plants have the ability to preferentially place roots into regions of elevated soil nutrients (Hutchings and de Kroon 1994; Hodge 2004; Kembel and Cahill 2005; de Kroon

et al. 2009; McNickle and Cahill 2009), to preferentially allocate roots towards (Gersani *et al.* 2001; O'Brien *et al.* 2005) or away from neighbours (Gersani *et al.* 1998; Schenk *et al.* 1999; Schenk 2006; Dudley and File 2007), and plants can integrate information about both neighbours and nutrients to alter occupancy of locations in the soil (Cahill *et al.* 2010; Mommer *et al.* 2010). Relatively few studies have been able to precisely track the root placement behaviour of competing plants due to the fact that the roots of most plant species are visually indistinguishable. However, current evidence suggests that plants significantly alter their root placement strategies in the presence of neighbours (*e.g.* Dudley and File 2007; Cahill *et al.* 2010; Mommer *et al.* 2010). Some authors have argued that increased root overlap with neighbours will be the best way for plants to win competitive encounters (Hodge *et al.* 1999; Robinson *et al.* 1999; Gersani *et al.* 2001; O'Brien and Brown 2008), while other authors have argued that plants should avoid the roots of neighbours to maximize resource capture (Parrish and Bazzaz 1976; Schenk *et al.* 1999; Cahill *et al.* 2010; Mommer *et al.* 2010). Ultimately, more work is needed to gain a better understanding of the factors that control root placement by plants, and the strategies employed by plants for root competition.

The goal of this study was to gain a better understanding of how root competition influenced the root foraging behaviour of four co-occuring grassland species. I conducted a mesocosm experiment where plants were grown with or without soil heterogeneity and with or without neighbours sharing the soil. I used molecular techniques described in Chapter 5 to measure the presence or absence

of roots throughout the soil in the presence of neighbours (McNickle *et al.* 2008). I tested the following 4 questions; (1) Do plants experience more intense competition in heterogeneous compared to homogeneous soils? (2) What factors determine the presence of roots of each species in the soil, and specifically do neighbours influence root presence? (3) How many species co-occur in each soil location, and do species aggregate into patches as previously expected? (4) Do root biomass distributions in mixtures shift as a function of either soil heterogeneity or the presence of neighbours.

6.2. METHODS

6.2.1. Study species

Four naturally co-occurring species were selected for this study: *Achillea millefolium* (Asteraceae, L.), *Artemisia ludoviciana* (Asteraceae, Nutt.), *Koeleria macrantha* (Poaceae, Ledeb. (Schult.)), and *Poa pratensis* (Poaceae, L.). These four species were selected based on several criteria. First, broad differences have been observed between monocots and eudicots in their overall foraging ability with monocots showing lower foraging precision than eudicots (Kembel *et al.* 2008; Kembel and Cahill 2005). I wanted to include a mixture of monocots and eudicots in order to get a gradient of foraging precision among species. Generally, monocots show lower foraging precision compared to eudicots (Kembel and Cahill 2005; Kembel *et al.* 2008). Further, the species included in this study have been studied previously and show a broad range of foraging responses (Robinson *et al.* 1999; Johnson and Biondini 2001; Kembel and Cahill 2005; Rajaniemi

2007; McNickle and Cahill 2009). Finally, molecular methods were available to allow me to map belowground root distributions of these four species (Chapter 5; McNickle *et al.*; 2008).

Seeds of *P. pratensis* and *K. macrantha* were collected from native rough fescue prairie at the University of Alberta Kinsella Ranch (+53°05, -111°33). Seeds of *A. ludiviciana* and *A. millefolium* were obtained from a local supplier of native seeds (Bedrock Seed Bank**,** Sangudo, Alberta, Canada).

6.2.2. Experimental Design

I used two levels of analysis in this experiment: analysis at the pot level and analysis at the level of soil locations within a pot. At the pot level, the experiment consisted of a fully factorial randomized block design with two levels of heterogeneity (heterogeneous or homogenous), and two levels of a neighbour treatment (alone or with neighbours) (Figure 6.1). Thus at the pot level the experiment consisted of heterogeneity by neighbours in a fully factorial randomized block design. In total there were 12 replicate blocks, which resulted in a total of 120 pots for each combination of four species grown alone in heterogeneous or homogeneous soil (8 pots/block) and one species mixture grown in heterogeneous or homogeneous soil (2pots/block).

Within pots, at the level of soil locations, there were two levels of soil type (patch or background), and four root cores were taken from each pot. Thus at the level of soil locations there was a fully factorial design of heterogeneity by soil type by neighbours (Figure 6.1). This yielded 480 root cores across the whole

experiment. There was also potentially an effect of distance from the stem (close or far), in the occupancy of soil locations by plant roots (Figure 6.1). Because of the limits of sample size, distance was included in statistical models as an additive effect, and the inclusion of distance in the model was judged using information theoretic criteria (details below). Also, in each analysis, appropriate random effects which include pot number were employed to control for the spatial autocorrelation among cores taken from the same pot (described in detail below).

The experiment was grown outside of the University Alberta Phytotron facility (+53°31, -113°31) in ambient weather conditions from June 2007-September 2007. Ambient rainfall was supplemented by an automatic mist sprinkler system for approximately 20mins each day to minimize any water stress on the plants. I used large pots (20cm diameter by 30cm deep) to allow substantial volume of soil for root growth and movement. The background soil used consisted of a 3:1 mixture of sand to commercial topsoil (Burnco, Edmonton, Alberta, Canada).

Soil heterogeneity was constructed by adding steer manure (Nu-Grow IP inc., Brantford, Ontario, Canada) to the soil mixture in different spatial configurations. First, the heterogeneous treatment received 120 mL of steer manure divided equally between two cylindrical patches that were 2.5cm in diameter and 30cm deep and made from a 1:1 ratio of steer manure and the sand:soil mix and were placed on diagonally opposite sides of the pot (Figure 6.1). Second, homogenous treatments also received 120 mL of steer manure, but the manure was spread evenly through the soil (Figure 6.1). The two locations in

homogeneous treatment which were spatially equivalent to the patch locations were called patch for the purposes of comparison between soil treatment and for statistical analysis. Using this labeling system, a plant response to high quality patch soil within heterogeneous soil would be indicated by a significant interaction between soil type and heterogeneity in this statistical design.

Neighbour treatments were constructed by (a) growing plants alone, or (b) in species mixtures that contained four plants (one individual of each of the four species) in a ring around the pot (Figure 6.1). In mixtures species were 6cm from each other and from the edge of the pot. The planting arrangement of species grown in mixtures was randomized among blocks (*i.e.* different among blocks), but was identical among pots within a block (*i.e.* identical within blocks). This was done to control for potential differences in planting arrangement within a block, but this design did not allow me to test for any differences in root occupancy that were due to planting arrangement or neighbour identity.

6.2.3. Soil Bioassay

In a separate experiment I performed a bioassay of soil quality by growing each of the four focal species on pure batches of the three different soil types used in this study to validate my expectation that in a plant growth assay, patch soil > homogeneous soil = background soil. For this assay, plants were grown in 6cm diameter, 10cm deep cylindrical pots, with 10 replicates per species on three soil types which resulted in an additional 120 pots in total. Soil types consisted of 50% volume/volume (v/v) steer manure (equivalent to pure patch soil), 1.5% v/v steer

manure (equivalent to homogeneous soil) and 0% v/v steer manure (equivalent to background soil). After 6 weeks this experiment was harvested. Shoots were collected and dried at 60°C, and weighed. Shoot mass in mg was analyzed using a general linear model (R Development Core Team, 2009, v2.9.2) with soil type and species as fixed effects, a quasipoisson distribution of errors and a type III sum of squares (using the Anova command from the car package; Fox and Weisberg 2010).

Each species responded similarly to the addition of manure to soil in this bioassay. Only the fertility of the soil had a significant effect on shoot mass for each species (Table 6.1). The shoot mass of each species was largest in the soil containing 50% manure (equal to patch soil), while plants were significantly smaller in 1.5% manure and 0% manure (Table 6.1, Figure 6.2). This shows that my ranking and designation of soil quality (*i.e.* Patch > Background = Homogeneous) for the growth of each species was consistent with growth (Figure 6.2).

6.2.4. Harvest and Measurements

From the main experiment (*i.e.* neighbours by heterogeneity), four spatially referenced 5cm diameter root cores were taken from each pot for a total of 480 root cores. Cores were centered on each of the two patches, or equivalent control locations in all pot treatments (Figure 6.1). Roots were removed from the cores by washing over a 2mm sieve within 24 hours of coring. Roots were then dried in silica gel at room temperature, weighed and stored dry at -20°C for

molecular analysis (Chase and Hills 1991; McNickle *et al.* 2008). Shoot mass was also harvested, dried at 60°C, and weighed. To measure the effect of heterogeneity on competitive intensity, I calculated log response ratios (*sensu* Hedges *et al.* 1999) for each of the four species. The log response ratio was calculated as:

$$\ln RR = \ln\left(B_{indi} / B_{Comp}\right) \quad (6.1)$$

Where B_{indi} is the mass of individual plants grown alone, and B_{comp} is the mass of individual plants grown with neighbours. lnRR values equal to zero indicate that the plants were not affected by the presence of neighbours, a positive lnRR value indicates plants were negatively influenced by the presence of neighbours, while a negative lnRR value indicates plants were positively influenced by the presence of neighbours (*sensu* Hedges *et al.* 1999).

6.2.5. Molecular root identification

I used the methods of McNickle et al (2008) which are described in Chapter 5 to determine the identity of species in a given root core. In brief the presence or absence of the roots of each species was detected by extracting DNA from bulk root cores. Briefly, all root tissue was ground to a fine powder using a bead beater mill (MM301 Ball Mill, Retsch inc., Germany) and DNA was extracted from roots using a modified CTAB method developed specifically for roots, and described elsewhere (Chapter 5; Brunner *et al.* 2001; McNickle *et al.* 2008). One PCR reaction was performed for each species on each root core using previously reported species specific primers. Primer sequences, and PCR reaction

conditions are described elsewhere (Chapter 5; McNickle *et al.*; 2008). These primers have been previously reported to be 100% accurate in making species identifications, perfectly detecting species presences with no false positives and are capable of detecting as little as 0.05ng of DNA (Chapter 5; McNickle *et al.* 2008).

6.2.6. Modeling occupancy of soil by roots

The major goal of this study was to examine shifts in foraging strategy of plants in the presence of neighbours and soil heterogeneity. The most popular methods for quantifying resource use of species from occupancy data are resource selection models, which are used extensively in animal systems (MacKenzie *et al.* 2002; Manly *et al.* 2002; Lele and Keim 2006; McLoughlin *et al.* 2010). By fitting linear models to binomial occupancy data, resource selection models give the probability that a resource condition will be used by an organism (Manly *et al.* 2002; Lele and Keim 2006; McLoughlin *et al.* 2010). To generate resource selection models one must measure ecologically important attributes of microsites, which can be categorical or continuous, and measure the presence of the target species within each microsite. Where true absence and true use of the resource by the target species is known, ecologists typically fit a special case of the resource selection model known as the resource selection probability function (RSPF). The RSPF is fit using a binomial generalized linear mixed effects model with a logit link function, presence as the response variable and microsite attributes as the predictors (Manly *et al.* 2002; Lele and Keim 2006; McLoughlin

et al. 2010). Here I fit RSPFs to occupancy data generated by molecular identification of roots at the whole core level (present or absent in the core).

6.2.7. Multimodel inference

The factors that influence root placement by competing plant species are not well understood. Thus, it was desirable to determine, based on the weight of evidence in the data, which factors were important for determining occupancy of soil locations by roots rather than simply base inferences on the factors included in the model. The important factors may or may not include those that were included in the experimental design. Therefore, to generate the most parsimonious resource selection model (i.e. the RSPF) I used information theoretic criteria to rank candidate models based on the relative importance of the variables in candidate models (Burnham and Anderson 2002; Anderson 2008). This approach has two advantages. First, it focuses inference on the weight of evidence in the data for different models and for the importance of each factor. Second, this approach allows for the estimation of the size and direction of effects by generating parameter estimates of the effect of each factor.

To determine the RSPF of each plant, I developed eight candidate models based on the factors included in the experimental design, and the current literature on plant responses to either nutrients or neighbours in the soil. The placement of roots in soil by plants has been shown to respond to nutrients only (Hutchings and de Kroon 1994; Gleeson and Fry 1997; Kembel and Cahill 2005; Kembel et al. 2008; McNickle and Cahill 2009), neighbours only (Gersani et al. 1998; Gersani

et al. 2001; Dudley and File 2007) and interactions between nutrients and neighbours have also been shown (Cahill et al. 2010; Mommer et al. 2010). Thus, candidate models were developed which would test for shifts in root occupancy which were related to (a) only nutrients, (b) only neighbours and (c) both nutrients and neighbours. Distance from the stem was also tested because I expected roots would be more commonly found close to the stem of plants (Casper and Cahill 1996; Hutchings et al. 2003). The individual factors used in model construction are described in Table 6.2, and each candidate model is described in detail in Table 6.3. The models are described briefly as follows. (1) Only pot as a random effect – i.e. none of the experimental factors explained root occupancy. (2) Only distance from the stem predicted soil occupancy. (3) Resource distributions (heterogeneity x soil quality) were the only factors that predict soil occupancy. (4) The presence or absence of neighbours was the only factor that explains root occupancy. (5) Resources and neighbours (heterogeneity x soil quality x neighbours) potentially interact to explain root occupancy. The final three models, (6)-(8) were the same as models (3)-(5), but distance is added as an additive effect (Table 6.2).

Models were ranked based on Akaike information criterion scores, corrected for small sample size (AICc) (Burnham and Anderson 2002; Anderson 2008). Rather than select one model as the most parsimonious, I used a model averaging approach which weights the parameter estimates of each model based on the difference in AICc scores. Model averaging is especially advantageous, when several models have similar AICc scores and no clear model emerges as the

highest ranked (as in this study; Table 6.5). Thus the parameter estimates of all eight candidate models, as well as their standard errors are averaged by their AICc weight, and summed to create the full averaged model (Burnham and Anderson 2002; Anderson 2008).

6.2.8. Statistical Analysis

All analyses were conducted using the R statistical environment (v2.9.2, R Development Core Team 2009). The intensity of competition (lnRR) was analyzed using generalized linear mixed effects modeling (GLMM) with lmer in the lme4 package (Bates 2007a; Bates 2007b) with heterogeneity and species as fixed effects, pot nested in heterogeneity as a random effect, and a Gaussian distribution. Markov chain Monte Carlo resampling was used to generate estimates of F, df and p values from the lmer output (languageR package; Baayen 2010).

To develop the RSPF, root presence/absence data were analyzed using lmer with a binomial distribution and a logit link function. For statistical modeling, soil type (patch or background), neighbours (alone or with neighbours) and heterogeneity of soil (heterogeneous or homogeneous) were included as potentially orthogonal fixed effects (Table 6.3). Distance from stem (close or far from stem) was included as a fixed effect but was not able to interact with the other fixed effects in the model (Table 6.3). To control for the non-independence of multiple root cores taken from within pots (Figure 6.1), random effects included pot identity nested inside either heterogeneity or neighbours or both

depending on the model tested (Table 6.3). Eight candidate models were fit with different combinations of factors as described above, and in Table 6.3.

To determine how many species co-occurred at any soil location, I analyzed species richness at each soil location pooled by soil type (Patch or background). To generate a null expectation of the distribution of species richness I summed the presence of each individual plant when grown alone for each location. If species aggregate in the soil then species richness at any location in the species mixtures should be higher than expected based on root placement of individual plants grown alone. Thus the presence of neighbours should be significant as either a main effect or in an interaction term if species aggregate when grown in mixture. To test this hypothesis, a generalized linear mixed effects model (lmer) with a Gaussian distribution of errors was used to examine how species richness varied as a function of soil type (patch or background), heterogeneity (heterogeneous or homogenous) or neighbours (null expected from alone or actual with neighbours). Pot was included as a random effect to control for repeated measures within a pot. Significance of main effects is judged via Wald's z-test employed by lmer, which provides a more unbiased test of fixed effects than Wald's F-test when quasilikelihood methods are employed and sample size is low (Bolker *et al.* 2009; Bates 2010a; Bates 2010b).

The distribution of biomass in the soil was examined by pooling biomass data by soil type (patch or background). A null expectation of root mass distribution was created by summing the response of each individual plant when grown alone. This would be the null expectation of root mass distribution in the

absence of a response to neighbours. However, if species respond to neighbours by altering the distribution of root mass then a significant neighbour by heterogeneity or a significant neighbour by heterogeneity by soil interaction would be expected. A generalized linear mixed effects model (lmer) with a Poisson distribution of errors was used to examine how root mass varied as a function of soil type (patch or background), heterogeneity (heterogeneous or homogenous) or neighbours (null expected from alone or actual from with neighbours). Pot was included as a random effect to control for repeated measures within a pot. Biomass data was restricted to the response of the whole community. This is because it was not possible to measure species level root biomass responses in the with neighbours treatments, since the PCR method could only detect presence or absence of species. Significance of main effects is judged via Wald's z-test employed by lmer, which provides a more unbiased test of fixed effects than Wald's F-test when quasilikelihood methods are employed and sample size is low (Bolker *et al.* 2009; Bates 2010a; Bates 2010b).

6.3. RESULTS

6.3.1. General

Mortality was low across the experiment. *A. millefolium* had the highest mortality rate with 92% survival, *A. ludiviciana* had 96% survival and *P. pratensis* and *K. macrantha* had 100% survival. Plants had not become pot bound in any treatment by the time of harvest (GGM personal observation). Furthermore,

the shoots of all plants remained well spaced and there was no evidence of shading, which suggested that any competition that occurred was belowground.

6.3.2. Intensity of competition

The intensity of competition was influenced by a significant species by heterogeneity interaction (Table 6.4, Figure 6.3). There was a general trend towards more intense competition in heterogeneous compared to homogenous soils for *A. millefolium*, *K. macrantha* and *A. ludoviciana* and this was strongest for *K. macrantha* (Figure 6.3).

6.3.3. Resource selection probability functions

Investigation of the averaged model parameter estimates for each species revealed the factors that influenced root occupancy for each species. Parameter estimates close to zero, and parameter estimates with standard errors larger than 1.96 x the value of the estimate (*i.e.* with confidence intervals that span 0, Anderson, 2008) will have a very small influence on model predictions and are therefore deemed unimportant. For all four species there was a strong negative influence of distance from the stem on the probability of finding a root (Table 6.6, Figures 6.4-6.7). Generally, the root occupancy of *A. millefolium*, and *K. macrantha* was relatively unaffected by any of the factors included in this study other than distance (Table 6.6 Figures 6.4, 6.6). For *P. pratensis*, the probability of finding a root was negatively influenced by neighbours and distance (Table 6.6, Figure 6.7). *A. ludoviciana* was strongly affected by interactions between

heterogeneity, soil quality and neighbours as well as distance from the stem (Table 6.6, Figure 6.5). This three way interaction can be explained as follows. When grown alone, *A. ludoviciana* is most commonly found in patch soil within heterogeneous pots (Figure 6.5). However, in the presence of neighbours, the probability of finding the roots of *A. ludoviciana* outside of a patch increased, while neighbours had no effect on the probability of finding roots inside a patch for this species (Figure 6.5). The probability of finding a root of A. ludoviciana also increased in homogeneous soil in the presence of neighbours (Figure 6.5).

Significance of parameters is not judged in this information theoretic framework (Burnham and Anderson 2002; Anderson 2008; Bolker *et al.* 2009). However, the individual results, including tests of significance, for each of the eight individual models fit can be found in Tables 9.1-9.8 in Appendix 2.

6.3.4. Species richness belowground

I also investigated the number of species found at each soil location. If species tended to aggregate in species mixtures then there should be fewer species found at each location in mixture than would be expected based on the distribution of species roots in the alone treatments. The analysis revealed that there were no treatment effects on species richness (Table 6.7, Figure 6.8). The species richness found at any soil location in the species mixtures was 2.36 ± 0.93 while the expected richness based on plant root distributions from the alone treatments was 2.40 ± 0.61 (Figure 6.8). There was a slight trend towards fewer

species than expected inside patches in heterogeneous soil for species mixtures (Figure 6.8), however this was not significant (Table 6.7).

6.3.5. Root biomass distributions

For root biomass there was a significant 3-way interaction between heterogeneity, soil type and neighbours (Table 6.8, Figure 6.9). In general, there were more roots in heterogeneous compared to homogeneous pots, but within a pot at the patch level, the amount of root mass present depended on both the soil type within heterogeneous pots and presence of neighbours. There were more roots in background soil, and less in patch soil than expected based on the behaviour of plants grown alone which generated the 3-way interaction (Figure 6.9).

6.4. DISCUSSION

The first goal of this study was to gain a better understanding of how heterogeneity altered competitive interactions among four co-occuring species. There was a trend towards increased competition in heterogeneous compared to homogeneous soils for *A. millefolium*, *A. ludoviciana* and *K. macrantha* but this was strongest for *K. macrantha* (Table 6.4, Figure 6.3). This result fits with the current literature in that soil heterogeneity seems to intensify competitive interactions for some species but not all (Bliss *et al.* 2002; Day *et al.* 2003; Schenk, 2006).

The second goal of this study was to determine how neighbours interacted with heterogeneity to alter root foraging behaviour of plants. Some authors have predicted that plants should increase root overlap with neighbours to win competitive encounters (Hodge *et al.* 1999; Robinson *et al.* 1999; Gersani *et al.* 2001; O'Brien and Brown 2008), while other authors have suggested that plants should avoid the roots of neighbours (Parrish and Bazzaz 1976; Schenk *et al.* 1999; Cahill *et al.* 2010; Mommer *et al.* 2010). Rather than one single strategy for dealing with the presence of neighbours, I found evidence for three distinct strategies for selecting soil locations in the presence of neighbours as revealed by the RSPFs developed for each species (Table 6.6). First, the probability of detecting roots of *P. pratensis* declined in the presence of neighbours suggesting this species may avoid neighbours in the soil (Figure 6.7). Second, the probability of detecting roots of *A. ludoviciana* generally increased in the presence of neighbours suggesting that this species may actually aggressively grow roots towards neighbours (Figure 6.5). Third, *A. millefolium* and *K. macrantha* appeared to be indifferent to the presence of neighbours, as the neighbour treatment had no effect on the probability of detecting a root for either species (Figures 6.4, 6.6). Furthermore, the biomass data suggest that species may allocate biomass away from competitors, though my methods did not allow us to determine which species were involved in this effect. Finally, the treatments in this study had little influence on the species richness found at each location (Table 6.7, Figure 6.8). In sum, my results suggest that species do alter root placement strategies, and root biomass allocation strategies in the presence of neighbours

compared to when they grow alone. However, this does not seem to influence the co-occurrence of roots in space, and similarly heterogeneity had variable effects on the intensity of competition experienced by three of the species included in this study (Table 6.4, Figure 6.3).

This study is the first to develop an RSPF for plants in the context of habitat use by the roots of individuals. RSPFs are common tools used in wildlife management to predict the use of sites within a habitat by wildlife species of interest (Manly *et al.* 2002; Lele and Keim 2006; McLoughlin *et al.* 2010). I have used similar statistical methods to develop a linear model that quantifies the use of soil locations within a mesocosm for four co-occuring plant species based on binary presence/absence data. Given that molecular tools for root identification are increasing in availability it should be increasingly possible to generate the presence/absence data which will be required to develop RSPFs for individual plants. This could be an important tool for plant ecologists because RSPFs may reveal hidden plant strategies. For example, the RSPF analysis revealed that the root placement strategy of *A. ludoviciana* was complex and involved a 3-way interaction between the presence of neighbours, the presence of soil heterogeneity, and the quality of soil at locations within a pot (Table 6.6, Figure 6.5). RSPFs may also be useful because it has been suggested that the RSPF can be envisioned as an estimate of the realized niche in the animal literature (McLoughlin *et al.* 2010).

Several potentially important factors that may be related to the resource selection by plants were excluded from this study. For example, this study was

limited to root locations along a horizontal plane as roots grow out and away from the stem of the plant. However, other studies have found that depth can be important, and that species may select different depths as part of their foraging strategy (Parrish and Bazzaz 1976; von Felten and Schmid 2008). Second, the rooting location of each plant was also destructively measured at a single time period. It is possible that these species have a strategy for temporal root segregation that was not measured in this study (*e.g.* Chesson 2000; Angert *et al.* 2009). Finally, the soil used in this study was relatively simple and consisted of either poor quality background/homogeneous soil, or high quality patch soil crossed with the presence or absence of neighbours (Figure 6.1). In field soil there can be significant heterogeneity not only in the total amount of nutrients available, but in the ratios and abundances of multiple mineral nutrients (Jackson and Caldwell 1993). Other factors such as pH, soil texture, and moisture gradients, were not manipulated in this study but might potentially control the placement of roots of these and other species (Hutchings and de Kroon 1994; Hodge 2004; Hodge 2006). Similarly, microbes (Hodge 2001; Hodge 2003), root herbivores (Stevens and Jones 2006; Stevens *et al.* 2007) as well as the identity of nearest neighbours (Schenk *et al.* 1999; Robinson *et al.* 1999) may potentially control root occupancy in soils, and these factors were also ignored.

While data from experimental mesocosms may be important to gain an initial insight into the root foraging behaviour and resource selection strategies of plants (Table 6.5; Mommer *et al.* 2010), ultimately field data will be the most valuable. Methods for mapping root distributions in the field are also becoming

available (*e.g.* Brunner *et al.* 2001; Taggart *et al.* 2010) and these will facilitate the development of RSPFs for species from field data. Field studies should include as much information as possible about the soil characteristics (*e.g.* pH, texture, nutrient availability) and neighbours (*e.g.* stem or root identity/proximity, local community richness). The distance from its stem should also be taken into account since distance has been shown to be important in previous studies (Casper and Cahill 1996; Hutchings *et al.* 2003), and since distance was important for all species included in this study (Table 6.5). Manipulative field experiments would be valuable, but simple observational studies are likely a good start. Developing such RSPFs for a multitude of species in a variety of communities has the potential to significantly enhance our understanding of plant niches, plant foraging behaviour and the mechanisms that structure plant communities. Given that plants have historically presented difficulties for the generality of concepts surrounding the ecological niche, and species coexistence (Silvertown 2004), I expect that the development of RSPFs for plants has great potential in the future of plant ecology.

6.4.1. Conclusions

Relatively little is known about how the presence of soil heterogeneity influences the intensity of competition experienced by plants, or how the presence of neighbours influences the root foraging behaviour of plants. Here I have shown that the presence of heterogeneity increased the intensity of competition for some, but not all species. Similarly, the presence of neighbours can influence the

foraging strategy of some but not all plant species. I identified three distinct strategies for dealing with the presence of neighbours. In the presence of neighbours, one species increased root occupancy, one species decreased root occupancy, and two other species did not alter root occupancy. There was also evidence that species reorganized the way that they allocated biomass to soil patches when grown in mixture, but it was not possible to measure this at the species level. More work is needed to gain a better understanding of the root foraging behaviour of individual plants and the factors that influence root placement in the soil. In general, the results of this study fit with the broader literature that suggest foraging behaviour is highly complex and highly species specific (Kembel and Cahill 2005; Kembel *et al.* 2008), and that plants do not exhibit one strategy (*e.g.* Gersani *et al.* 2001; O'Brien and Brown 2008), or even two strategies(Campbell *et al.* 1991) as has been previously suggested. I suggest that the development of RSPFs for a wide variety of plant taxa will be a valuable tool for understanding plant root foraging behaviour, for potentially understanding plant niches, and for understanding the potential breadth of possible plant strategies.

TABLE 6.1: Results of GLM on shoot size in the soil bioassay. The factor 'Fertility' represents the amount of manure added to the soil.

	df	**F**	**P**
Species	3	0.83	0.477
Fertility	2	29.27	<0.001
Species x Fertility	6	1.88	0.094
Residuals	84		

TABLE 6.2: Description of factors included in soil occupancy models. Heterogeneity, Soil and Neighbours were fully crossed, and interaction terms among factors are also included in models when appropriate. Distance was only ever included as an additive effect. See Figure 1 for a schematic description of these factors.

Factors	**Effect**	**Data**
Pot	Random effect	Pot ID
Heterogeneity	Fixed effect	Heterogeneous / Homogeneous
Soil	Fixed effect	Patch / Background
Distance	Fixed effect	Close / Far
Neighbours	Fixed effect	Alone / Neighbours

TABLE 6.3: Description of the models included in multi-model inference. See Table 6.1 and Figure 6.1 for more details on experimental design and factor descriptions. Random effects are enclosed in parentheses, * denotes a fully factorial design among factors while : denotes just the single interaction term among factors. Pot is included (with appropriate nesting within treatments as required) in all models as a random effect to control for repeated measures taken within pots.

Model	**Factors**	k_i	**Description**
Null	(Pot)	1	Null model - no measured variables explain occupancy by roots
Distance	Distance+(Pot)	2	Only distance from the stem in the soil volume explains root occupancy. Plants are expected to be found more commonly near stems than far from stems.
Resources	Heterogeneity*Soil+ (Hetero:Pot)	4	Resource model - Only resource based variables explain root occupancy.
Neighbours	Neighbours+ (Neighb:Pot)	2	Neighbours model - Only the presence of neighbours explain root occupancy.
Resources x Neighbours	Neighbours* Heterogeneity*Soil +(Neighb:Hetero:Pot)	8	Fully factorial model - Neighbours and resources can interact to explain root occupancy.
Resources + Distance	Heterogeneity*Soil+ Distance +(Hetero:Pot)	5	Resource model - Only resource based variables and distance from the stem explain root occupancy.
Neighbours + Distance	Neighbours+ Distance+ (Neighb:Pot)	3	Neighbours model - Only the presence of neighbours and distance from the stem explain root occupancy.
Resources x Neighbours +Distance	Neighbours* Heterogeneity*Soil+ Distance +(Neighb:Hetero:Pot)	9	Fully factorial model - Neighbours and resources can interact to explain root occupancy. Distance from the stem is also included.

TABLE 6.4: F table results of GLMM (lmer, R v2.9.2) on intensity of competition among species.

	df	**F**	**P**
Species	3	4.33	0.007
Heterogeneity	1	6.07	0.016
Species x Heterogeneity	3	2.90	0.04
Residuals	83		

TABLE 6.5: AICc scores and model weights (w_i) for all soil occupancy models tested, for all four species. Models are described in detail in Table 6.2

	Achillea		**Artemisia**		**Koeleria**		**Poa**	
Model	**AICc**	w_i	**AICc**	w_i	**AICc**	w_i	**AICc**	w_i
Null	108.3	0.01	168.2	0.00	257.0	0.00	247.0	0.00
Distance	100.6	0.56	163.5	0.00	206.4	0.54	213.4	0.06
Resources	108.8	0.01	159.1	0.02	258.0	0.00	242.2	0.00
Neighbours	110.6	0.00	170.1	0.00	261.5	0.00	248.6	0.00
Resources x Neighbours	110.3	0.00	157.8	0.04	267.5	0.00	247.8	0.00
Resources +Distance	101.7	0.31	154.0	0.29	207.0	0.38	208.3	0.79
Neighbours + Distance	104.1	0.09	165.4	0.00	210.3	0.07	213.7	0.05
Resources x Neighbours +Distance	108.5	0.01	152.4	0.64	215.6	0.01	212.6	0.09

TABLE 6.6: Full averaged soil occupancy model for all four species averaged across all tested models. Individual models are described in Table 6.3, Factors are described in Table 6.1and AICc scores with model weights (w_i) can be found in Table 6.5. The full output from each model tested can be found in Appendix 2.

	Achillea		**Artemisia**		**Koeleria**		**Poa**	
Factor	**Estimate**	**(SE)**	**Estimate**	**(SE)**	**Estimate**	**(SE)**	**Estimate**	**(SE)**
Intercept	8.04	(2.84)	1.53	(0.73)	0.56	(0.49)	0.63	(0.55)
Distance	-2.37	(1.82)	-1.25	(0.56)	-2.42	(0.38)	-2.15	(0.37)
Neighbours	0.77	(1962)	3.02	(1.47)	-0.17	(0.23)	-0.94	(0.35)
Heterogeneity	-0.31	(0.78)	0.25	(0.74)	0.00	(0.15)	-0.12	(0.13)
Soil	0	(0.52)	2.28	(1.41)	-0.05	(0.15)	-0.12	(0.14)
Neighbours x Heterogeneity	-0.23	(1962)	-0.36	(1.75)	0	(0.07)	0.06	(0)
Neighbours x soil	0	(2560)	-3.32	(1.65)	0	(0.07)	-0.10	(0)
Heterogeneity x Soil	0.04	(0.60)	-2.41	(1.47)	0.05	(0.21)	0.22	(0.18)
Neighbours x heterogeneity x soil	-0.02	(2560)	3.45	(2.28)	0	(0.10)	0.08	(0)

TABLE 6.7: lmer mixed effects model of null vs neighbours belowground species richness in soil microsites. Presence of species grown alone were summed as a null model to compare the richness expected when species were grown together.

Factor	Estimate	SE	z value	P
intercept	0.92	0.13	7.10	<0.001
Heterogeneity	-0.14	0.19	-0.70	0.482
Soil	0.05	0.18	0.27	0.787
Neighbours	-0.04	0.19	-0.21	0.833
Heterogeneity x Soil	0.01	0.27	0.04	0.965
Heterogeneity x Neighbours	0.16	0.28	0.57	0.567
Soil x Neighbours	-0.21	0.28	-0.74	0.459
Heterogeneity x Soil x Neighbours	0.18	0.39	0.46	0.643

TABLE 6.8: lmer mixed effects model of null vs neighbours root biomass in soil microsites. Root mass of species grown alone were summed as a null model to compare the sum of roots when species were grown together.

Factor	Estimate	SE	z value	p
intercept	1.51	0.18	8.29	<0.001
Heterogeneity	-1.08	0.32	-3.40	<0.001
Soil	0.56	0.16	3.51	<0.001
Neighbours	-0.88	0.3	-2.92	0.004
Heterogeneity x Soil	-0.38	0.35	-1.10	0.273
Heterogeneity x Neighbours	0.26	0.52	0.50	0.615
Soil x Neighbours	1.22	0.27	4.47	<0.001
Heterogeneity x Soil x Neighbours	-1.32	0.59	-2.22	0.027

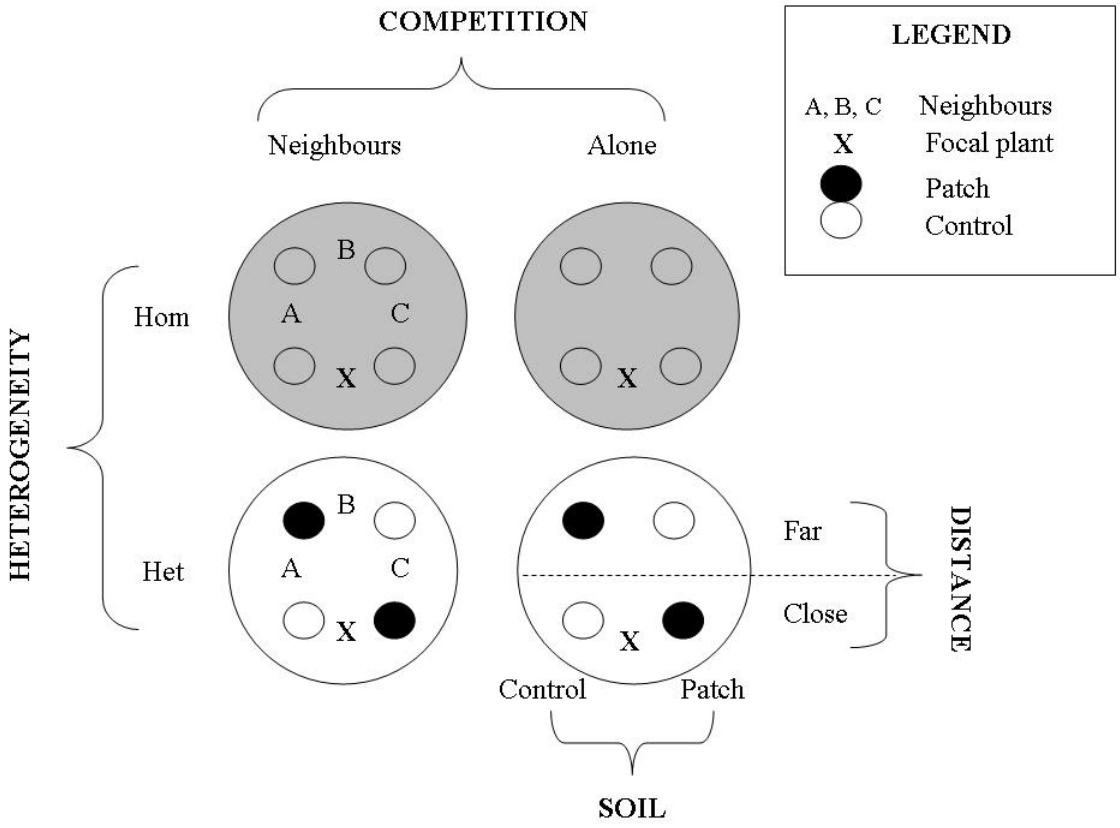

FIGURE 6.1: Schematic of experimental design. Homogenous (Hom) soil is represented by gray shading, and Heterogeneous soil (Het) is represented by white background soil with small patches (black circles). Four root cores were taken from each pot in the locations represented by circles within each pot. The factors Heterogeneity x Neighbours x Soil are fully orthogonal.

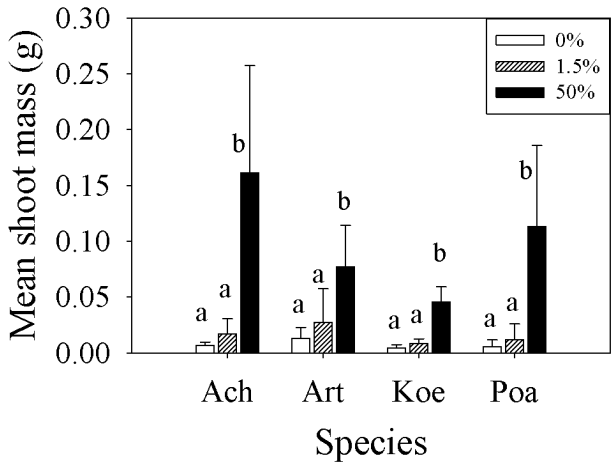

FIGURE 6.2: Results from soil bioassay. Species names are abbreviated to the first three letters of their genus name. Soil types were made from taking the background soil mix and amending with manure concentrations that matched the soil types used in the main experiment, and included either: 0% manure (background soil), 1.5% manure (homogeneous soil), or 50% manure (patch soil). Error bars are +/- 1 standard deviation, letters represent differences among means from a post-hoc Tukey's test.

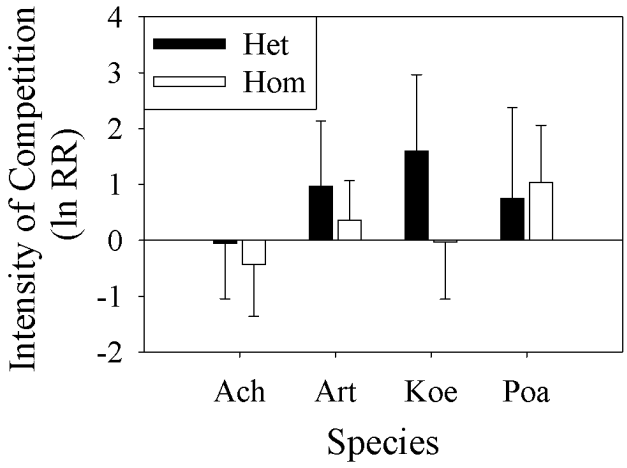

FIGURE 6.3: The intensity of competition (lnRR) for each species, in heterogeneous (Het) or homogeneous (Hom) soils. Species names are abbreviated to the first three letters of their genus name, error bars are 1 SD.

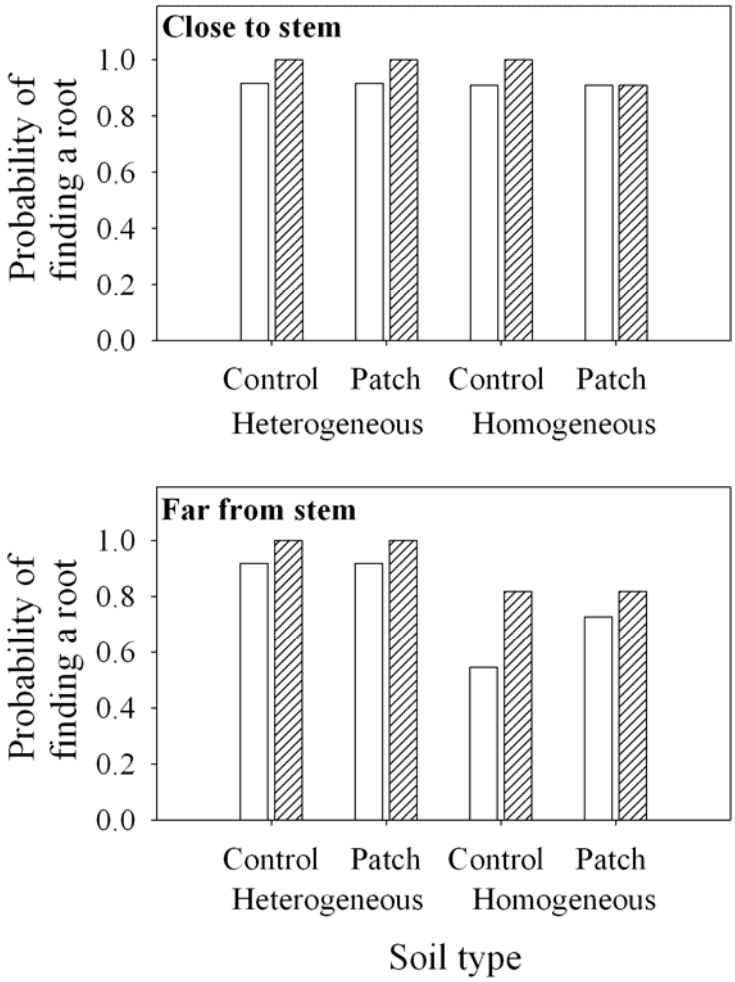

FIGURE 6.4: Root occupancy data for *A. millefolium* close to the stem or far from the stem for plants grown alone (open bars) or plants grown with neighbours (hatched bars) in heterogeneous or homogeneous soil. Patches or background soil (Control) are denoted. Note that homogeneous soil did not actually contain patches, but equivalent locations in homogenous soil are simply labeled as patch for purposes of comparison to actual patches in heterogeneous soil.

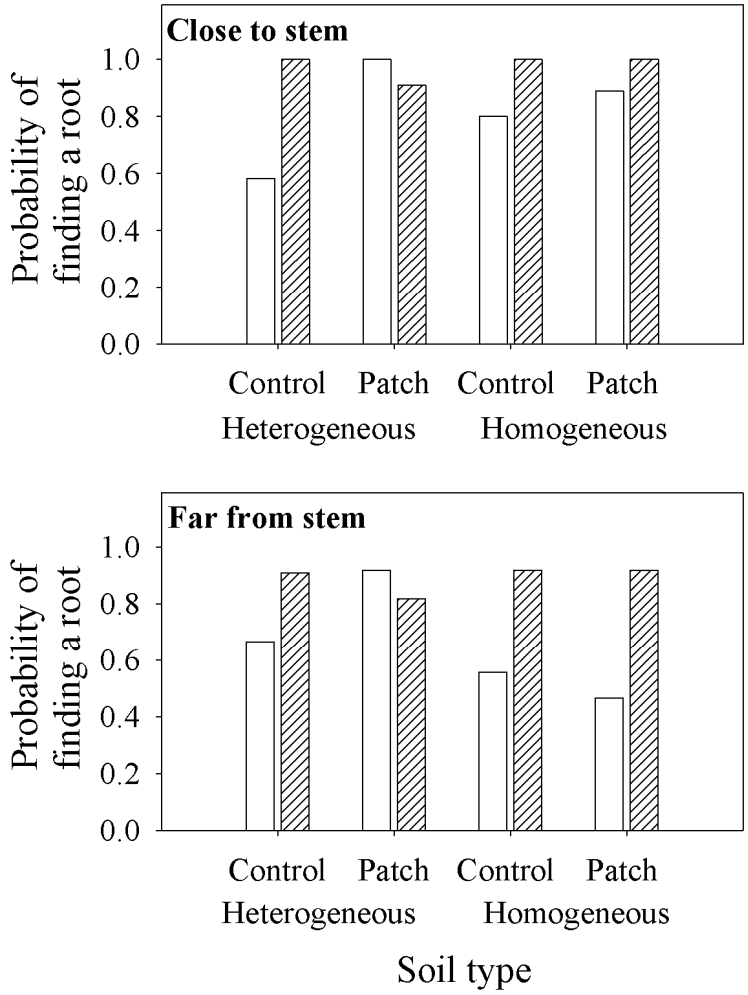

FIGURE 6.5: Root occupancy data for *A. ludoviciana* close to the stem or far from the stem for plants grown alone (open bars) or plants grown with neighbours (hatched bars) in heterogeneous or homogeneous soil. Patches or background soil (Control) are denoted. Note that homogeneous soil did not actually contain patches, but equivalent locations in homogenous soil are simply labeled as patch for purposes of comparison to actual patches in heterogeneous soil.

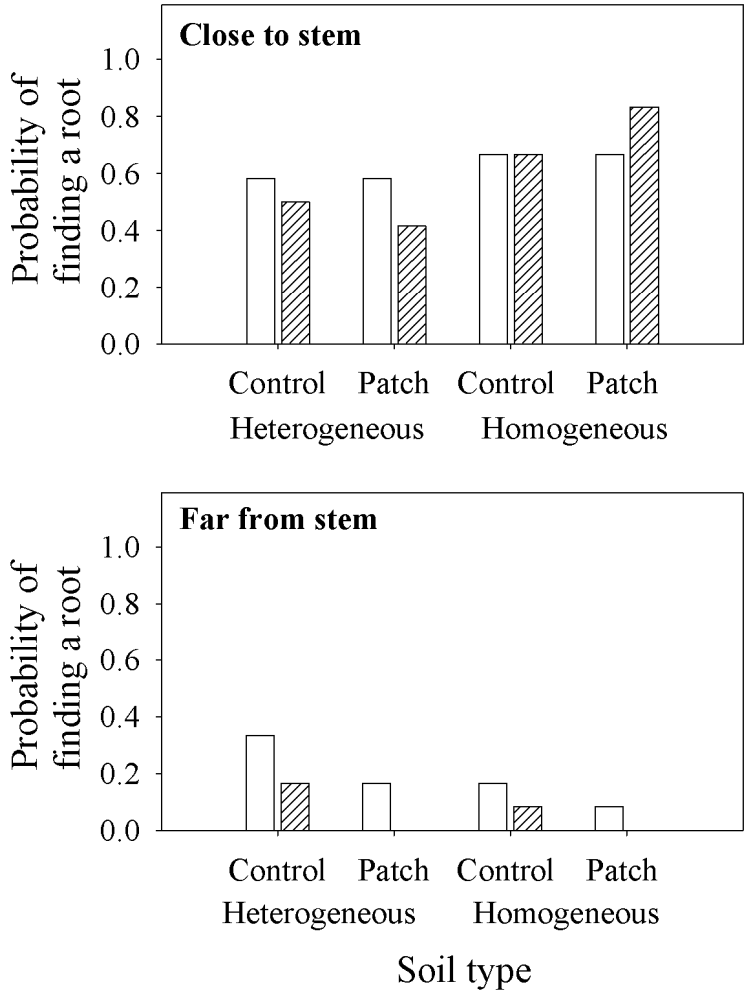

FIGURE 6.6: Root occupancy data for *K. macrantha* close to the stem or far from the stem for plants grown alone (open bars) or plants grown with neighbours (hatched bars) in heterogeneous or homogeneous soil. Patches or background soil (Control) are denoted. Note that homogeneous soil did not actually contain patches, but equivalent locations in homogenous soil are simply labeled as patch for purposes of comparison to actual patches in heterogeneous soil.

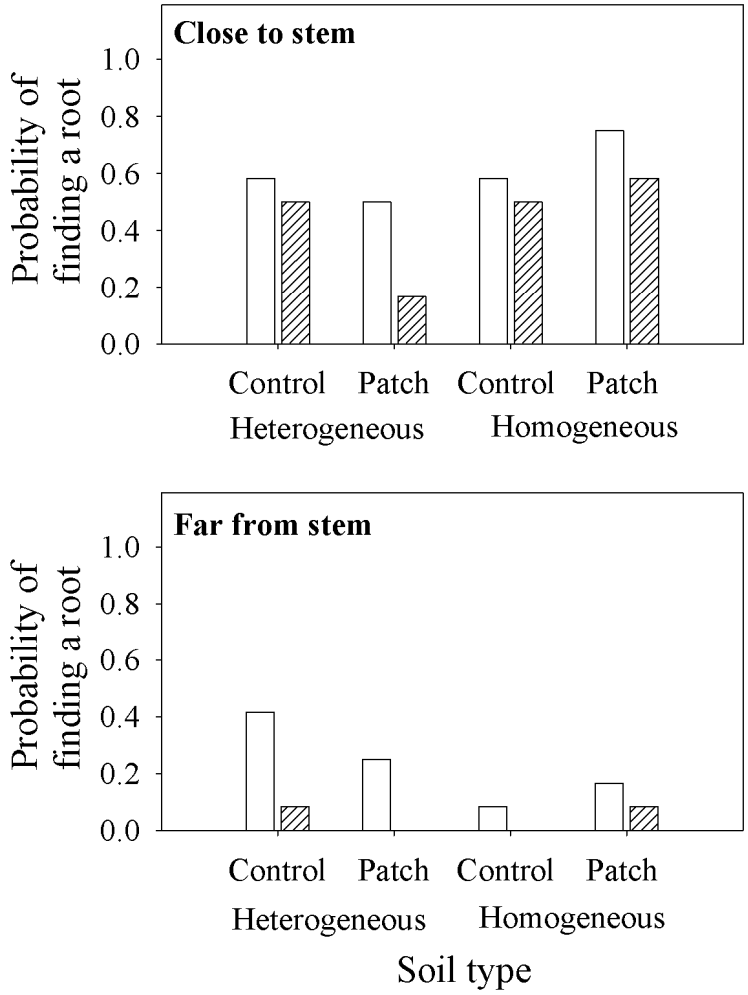

FIGURE 6.7: Root occupancy data for *P. pratensis* close to the stem or far from the stem (panel B) for plants grown alone or plants grown with neighbours (hatched bars) in heterogeneous or homogeneous soil. Patches or background soil (Control) are denoted. Note that homogeneous soil did not actually contain patches, but equivalent locations in homogenous soil are simply labeled as patch for purposes of comparison to actual patches in heterogeneous soil.

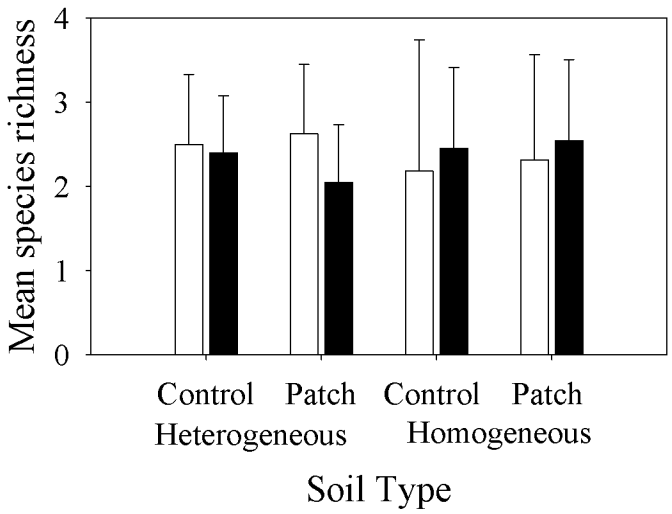

FIGURE 6.8: Mean observed species richness (black bars) when plants were grown with neighbours, compared to expected species richness (open bars) derived from summing data from when species were grown alone. Error bars are 1 SD.

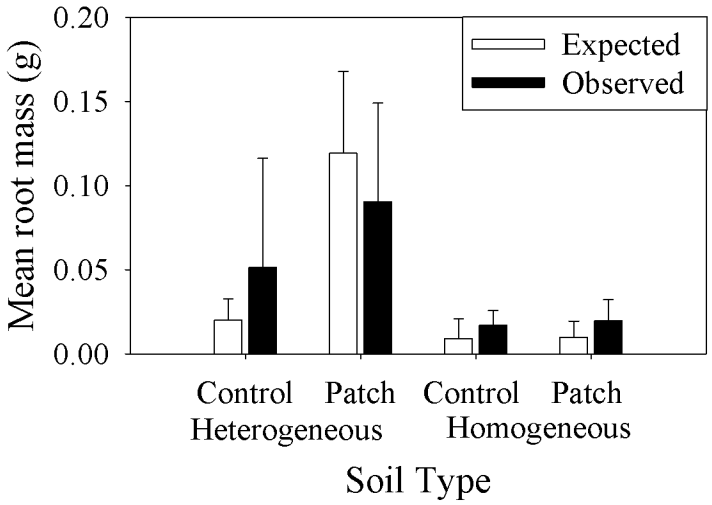

FIGURE 6.9: Mean root mass observed for plants grown with neighbours (black bars) compared to expected root mass derived from when plants grew alone (white bars) in all soil types. Error bars are 1 SD.

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7. GENERAL DISCUSSION

Since many environmental factors seem to vary in time and space, it is expected that organisms should evolve plastic responses that allow them to cope with variable environments. In the animal literature the study of these plastic responses constitutes the study of behavioural ecology. Increasingly it has been recognized that plants exhibit plasticity which is analogous to animal behaviour (*e.g.* Silvertown and Gordon 1989; Kelly 1990; Gleeson and Fry 1997; Schenk *et al.* 1999; Gersani *et al.* 2001; Dudley and File 2007; O'Brien and Brown 2008; de Kroon *et al.* 2009; Murphy and Dudley 2009). Within the broader context of behavioural ecology, foraging behaviour studies the responses of organisms to variability in resource based stimuli. In this thesis, I was particularly interested in the root foraging behaviour of some plant species. I had two goals in this thesis: First I tested whether foraging theory which had been developed for animals might be useful to predict plant foraging behaviour (Chapters 2-4). Second, I wanted to show how adopting a more rigorous foraging theory, and development of new methods may help generate a greater understanding of plant competition (Chapters 4-6).

For my first goal to determine whether plant foraging behaviour could be predicted from animal foraging theory, all of my data support the idea that plants foraged using similar rules as animals. This suggests that models of animal foraging behaviour can provide a good starting point to develop plant foraging theory. This finding is important because plant ecologists have lacked a general

predictive framework to understand plant foraging behaviour (Kembel and Cahill 2005; de Kroon and Mommer 2006; Kembel *et al.* 2008). My work helps to bring the study of plant foraging behaviour into the more general realm of behavioural ecology. Furthermore, my work shows that plant ecologists can draw from a decades old research paradigm on foraging behaviour (*e.g.* Holling 1959; Macarthur and Pianka 1966; Schoener 1971; Werner and Hall 1974; Charnov 1976b; Charnov 1976a) instead of developing plant foraging models from first principals. My second goal was to show how foraging theory can provide some new mechanisms to understand plant competition, and potentially plant coexistence. Plant ecologists have widely studied plant foraging responses and discussed the potential for foraging to influence competition among species (Campbell *et al.* 1991; Hutchings and de Kroon 1994; Johnson and Biondini 2001; Hodge 2004; Kembel and Cahill 2005; Hodge 2006), but have been relatively unsuccessful at developing predictive models that link foraging to competition (Kembel *et al.* 2008; de Kroon and Mommer 2006; Kembel and Cahill 2005, but see; Grime 2007). This is in contrast to the animal literature, where foraging theory is well developed and well linked to concepts of competition and coexistence among competing species (Brown 1988; Brown *et al.* 1994; Stephens *et al.* 2007; Abrams 2010a; Abrams 2010b). The introduction of predictive animal foraging theory to the plant literature may facilitate the development of models that make clear predictions about how plant foraging behaviour can influence competition among plants. However, there are some

differences between plants and animals that require existing animal foraging models to be adjusted if these models are to be applied to plants.

Though animals and plants differ in the mechanisms employed for foraging (*e.g.* growth versus movement), all organisms should be expected to forage in ways that maximize benefits but minimize costs, within taxonomic constraints. In Chapter 2, I showed how two major differences between plants and animals can be taken into account when adapting animal foraging theory to plant systems. Specifically, I discussed how modularity of plant growth means that plants have many mouths which can be in many places at once. In this way the individual foraging tissues of modular plants may be more like a colony of animals than a single solitary animal. I also discussed how plants forage for many individual nutrients simultaneously, and potentially independently. This means that the costs and benefits of plant foraging are likely multivariate while for animals a single variable might explain choice. Based on these two major differences I also discussed how two classic foraging models for patch use and prey choice could be adapted to shed light on plant foraging behaviour. In Chapters 3 and 4, I tested these two foraging models individually.

In Chapter 3, I tested the applicability of a classic patch use model, the marginal value theorem (*sensu* Charnov 1976b), to predict patch use by plants. Like animals, the plants in this study spent more time and effort in the highest quality patches compared to lower quality patches (Figures 3.6, 3.7). This behaviour enhanced plant size (Figure 3.8) and also altered spatial use of the soil volume. Since plants remained in higher quality patches for the longest amount of

time, plants that encountered higher quality patches had root systems that explored the smallest volume of space (Figure 3.6). This showed that plants are capable of altering their movement through the soil in ways that maximize foraging gains and shows how insights may be obtained by borrowing theory from the animal literature. Such patterns of movement between patches were a novel behaviour that had not been previously described in plants.

Chapter 4 examined the applicability of a classic model of resource choice (Charnov, 1976a, Abrams, 2010) to predict nitrogen preferences of plants. Similar to the way that animals may prefer certain prey, the plants in this study preferred types of nitrogen with the highest net benefits in terms of potential plant growth (Figure 4.4). I also presented a simple graphical model showing how intensity of competition might change as a function of resource choice among two competing species if competition is assumed to correlate with similarity in resource use (Figure 4.6). Many plants exhibit preferences for different types of nitrogen (Kielland 1994; McKane *et al.* 2002; Harrison *et al.* 2007); however, there has been no clear predictive framework to understand why plants exhibit these preferences, or how they might lead to coexistence among species. This work provides one such possible framework.

The studies described in Chapters 3 and 4 investigated plant behaviour in the absence of competition. This is because the roots of many plant species are visually indistinguishable making the study of behaviour difficult in mixed species communities. This has meant that it was historically difficult to measure the roots of competing individuals. However, competition for soil resources is an

inescapable pressure faced by natural populations of plants (Casper and Jackson 1997; Schenk 2006), and it will be important to measure root foraging behaviour of competing plants. In Chapter 5, I presented a simple PCR based method for the identification of plant roots of 10 naturally co-occurring species in Mesocosms. This method accurately identified species present in all tested samples 100% of the time, and was sensitive to very small fragments of roots. Such molecular methods are becoming ubiquitous and have the potential to significantly enhance the understanding of plant foraging in mesocosms (Mommer *et al.* 2008), and in natural systems (Brunner *et al.* 2001; Taggart *et al.* 2010).

In Chapter 6, I applied the molecular method described in Chapter 5 to investigate the factors that influence soil microsite selection by four naturally co-occurring plant species. To do this, I developed a resource selection function for each species in the study. I investigated the role of microsite fertility, pot level heterogeneity, the presence of neighbours and distance of the soil microsite from the stem. I found that species exhibited three basic resource selection strategies based on occupancy data (Table 6.6., Figure 6.4-6.7). Specifically the probability of detecting a root in the presence of neighbours increased for one species, decreased for one species and was unaltered for two species. Furthermore, biomass distributions in species mixtures were more evenly distributed throughout the soil in mixtures than expected based on individually grown plants (Figure 6.9). However, species richness at each soil location was not influenced by any factor in the experiment (Figure 6.8). This is the first resource selection function developed for plant roots, and this statistical approach has great promise to shed

light on the realized niches of naturally occurring plant species if applied in natural systems.

Together these 5 chapters address the two main goals I set out to address in my thesis. Chapters 2-4 are primarily about the basic behavioural ecology of plants. These chapters discuss, and demonstrate some of the basic behaviours that plants possess. I also present methods and ideas about competition and coexistence of plant species that may arise simply through foraging behaviour in Chapters 4-6. In the sections that follow I will discuss the implications of my results for the behavioural ecology of plants and plant coexistence. I will also discuss some limitations of my studies in each of these sections.

7.1.1. Behavioural ecology of plants

Empirically, the data I present in this thesis are not so different from previous studies. I show that plants respond to patches, and that plants capture different types of nitrogen. Both of these facts have been known for 50 years or more (Hutchings and de Kroon 1994). However, the theoretical framework I have developed to understand plant foraging is novel, and theory is important to understand a system fully. Only a few plant ecologists have attempted to develop optimal foraging models specific to plant foraging behaviour in the past (Sutherland and Stillman 1988; Kelly 1990; Kelly 1992; Gleeson and Fry 1997). However, plant ecologists have not typically based their research in any theoretical context (*e.g.* Hutchings and de Kroon 1994; Casper and Cahill 1996; Fransen *et al.* 1998; Casper and Cahill 1998; Hodge *et al.* 1999; Robinson *et al.*

1999; Fransen *et al.* 2001; Bliss *et al.* 2002; Day *et al.* 2003; Hodge 2004; Lamb *et al.* 2004; Hodge 2006; Rajaniemi and Reynolds 2004; Rajaniemi 2007; de Kroon *et al.* 2009). A great advantage of using optimality models is that one is forced to quantify assumptions, and specifically, one is forced to quantify what is meant by adaptive. For example, in Chapter 4 I assumed that a nitrogen type which enhanced growth and seed output more than another nitrogen type would be preferred. I then quantified adaptive based on the potential for growth and seed production. In this way, I was able to reveal that plants in fact forage for different types of nitrogen in a way that appears to be adaptive. Another advantage of developing quantitative models and assuming optimality is that one can make and test specific quantitative predictions. For example, in Chapter 4 I showed that a quantitative theory may emerge that not only predicts plant preferences, but can predict more complex ecological phenomena such as competition or coexistence of species. This is something that plant ecologists have only occasionally done for plant foraging behaviour (*E.g* Sutherland and Stillman 1988; Gleeson and Fry 1997). I believe the study of plant foraging will be greatly advanced by the development of plant foraging models and theory that make specific quantitative predictions. Finally, my data suggest that the process of developing plant foraging theory can be simplified by drawing from pre-existing foraging theory in the animal literature.

In Chapters 2-4, I have taken the approach of qualitatively testing some previous models instead of developing new plant models that make specific quantitative predictions. This was done because if experiments can show that

animal foraging models predict plant foraging behaviour this will allow plant ecologists to draw from a rich pre-existing literature on foraging behaviour. My qualitative tests showed that plants generally behave using similar foraging rules as animals (Chapters 3 and 4) and provide a first step in the process of developing a more general framework for understanding plant foraging behaviour. However, ultimately plant specific models will likely need to be developed in order to make specific quantitative predictions about plant foraging behaviour and the consequences of that behaviour. Similarly, I believe that more data is needed to test the generality of my findings across taxa and ecosystems. For example, the experiments in Chapters 3 and 4 were limited to 1 plant species each (*Achillea millefolium* in Chapter 3, and *Arabidopsis thaliana* in Chapter 4). When possible I have tried to supplement my own data by reviewing the literature and data from other studies is generally consistent with my own results. However, more research is needed on a diversity of plant taxa to determine if the behaviours I have identified are exhibited among many plant species, or if these are just interesting case studies. Lack of data is currently the biggest limitation to the development of foraging theory for plants. Finally, it should be pointed out that the experiments in Chapters 3 were conducted in pots in a greenhouse, and the experiments in Chapter 4 were done in sterile agar media. Although greenhouse studies are useful in shedding light on the basic abilities of plants, to truly understand the ecological consequences of plant foraging behaviour studies in more natural field conditions will be necessary.

7.1.2. Foraging behaviour and competition

Foraging behaviour is one of many possible mechanisms that may contribute to patterns of competition among species (Brown *et al.* 1994; Stephens *et al.* 2007). At some level, all resource competition is related to resource consumption and therefore to foraging behaviour. For example, species that prefer different prey or resources due to behavioural choices will compete less than species with identical preferences. Similarly, species that select different patches within the landscape may not encounter one another, and similarly may not compete for resources. In these basic ways, foraging behaviour may be linked to competition in natural systems. However, the role of foraging in plant coexistence and competition has not been fully explored for plant communities. In this thesis I have also tried to make links between foraging theory and its potential to make predictions about plant competition and coexistence. For example, in Chapter 5 I presented a molecular method for measuring the root foraging behaviour of plants grown with neighbours, and in Chapters 4 and 6 I have shown how foraging may be linked to competition and ultimately species coexistence.

Because of the logical link between foraging and competition, I have discussed competition and coexistence in relation to foraging theory throughout this thesis. However, it should be noted that these discussions are limited to theory as I have little data on coexistence in this thesis. For example, in Chapter 4 I present a general model of plant competition for two species with slightly different foraging behaviour. This model is a mathematical consequence of the resource choice behaviour (*e.g.* Abrams 2010a; Abrams 2010b); however I have

not tested this model empirically. Similarly, in Chapter 6 I show how the intensity of competition may increase for some species in heterogeneous soil and that foraging behaviour may shift in the presence of neighbours. However, competition and foraging behaviour are not independent in this study and the direction of causality between these two results is not clear. Further experiments are needed to gain a better understanding of the link between plant foraging behaviour and plant competition and coexistence. Similar to above, the lack of species diversity, and the lack of field studies limit my understanding of how plant foraging may link to ideas of plant coexistence. Again, the biggest limitation to the development of theory that links foraging and coexistence is data from a diversity of species and systems. Despite these limitations, I have provided some new theoretical ideas (Chapter 4, Chapter 6), and some new methodological advances (Chapter 5) which will contribute to the development of future research in this field.

7.1.3. General conclusions

The work contained within this thesis is just a small part of the increasing trend towards incorporating behavioural theory in to the study of plant ecology (*e.g.* Silvertown and Gordon 1989; Kelly 1990; Gleeson and Fry 1997; Schenk *et al.* 1999; Gersani *et al.* 2001; Dudley and File 2007; O'Brien and Brown 2008; de Kroon *et al.* 2009; Murphy and Dudley 2009). My goal in this thesis was to provide some new data and novel ideas to move the study of plant ecology forward and I believe I have achieved this. I have shown that plants forage in

ways that are analogous to animal foraging, and that animal foraging theory can provide a useful scaffold from which to develop plant-specific behavioural theory. I have also discussed how a more quantitative theoretical approach to plant foraging has the potential to add new ideas to the understanding of some mechanisms which regulate plant competition and competition. These two findings have great potential to advance our understanding of plant foraging and plant species coexistence. Specifically, like animals, plants forage for resources through relatively complex responses to environmental heterogeneity and a quantitative theory that can predict these responses has the potential to contribute to the understanding of plant competition in natural systems.

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8. APPENDIX 1

Results of fitness-trait regressions for *Arabidopsis thaliana* from Chapter 4. Details of the experimental statistical analysis, experimental design and plant traits can be found in Chapter 4.

TABLE 8.1: Percent survival at the end of the experiment for the no-choice experiment (top) and the choice experiment (bottom) for each type or combination of nitrogen, and each abundance or ratio of nitrogen. There were no obvious trends in survival.

Concentration (mM)	**Nitrate**	**Glutamine**	**Asparagine**
0.1	0.625	0.625	0.75
0.2	0.625	0.5	0.5
0.3	0.625	0.875	0.5
0.4	1	0.75	0.875
0.5	0.875	0.75	0.625
0.6	0.875	0.75	0.625
0.7	0.75	0.875	0.75
0.8	0.75	0.75	0.875
0.9	0.75	0.875	0.875

Ratio	**Glutamine + Asparagine**	**Nitrate + Gsparagine**	**Nitrate + Glutamine**
1:9	0.75	0.875	0.875
2:8	1	0.75	1
3:7	0.75	0.625	0.75
4:6	0.5	0.875	
5:5	1	0.875	0.875
6:4	0.75	0.75	1
7:3	0.875	0.875	0.625
8:2	0.875	0.75	0.875
9:1	0.875	0.75	0.75

TABLE 8.2: Parameter estimates, standard errors (SE), t and p values for the stepwise regression on Arabidopsis traits and lifetime seed production. Exl

Parameter	**Estimate**	**SE**	**t**	**p**
Intercept	-44.8	20.8	-2.15	0.034
Diameter	19.4	1.2	15.97	<0.001
Excluded variables				
Bolting stem height			0.67	0.503
Leaf number			-0.014	0.989
Flower number			0.82	0.413
Stress score			-1.98	0.051

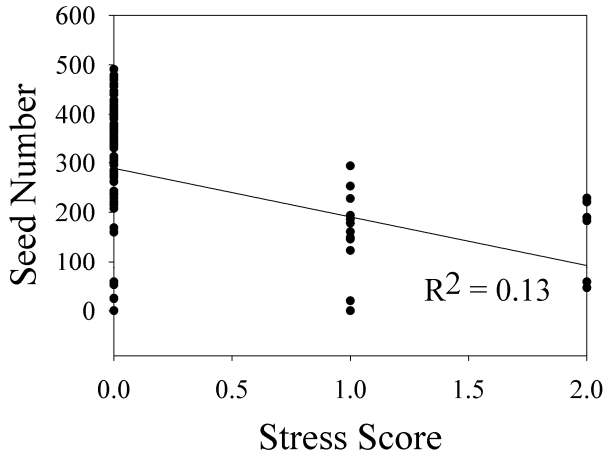

FIGURE 8.1: Relationship between seed production at senescence (~10 weeks) and stress score given at 4 weeks of age. The stress score was 0 for green healthy plants, 1 moderate nutrient stress (one or more leaf was discoloured) and 2 for severe stress (all leaves were severely discoloured).

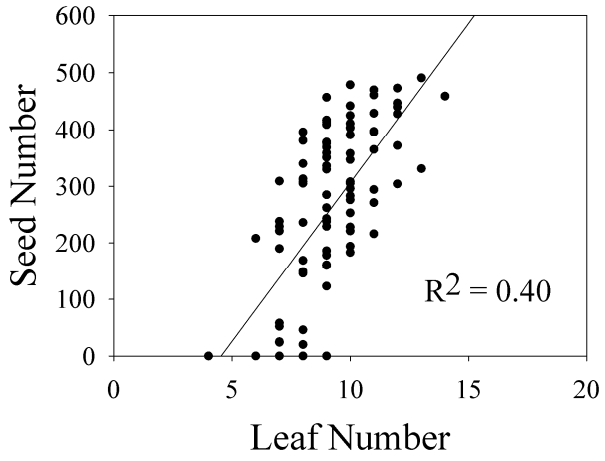

FIGURE 8.2: Relationship between seed production at senescence (~10 weeks) and number of leaves at 4 weeks of age.

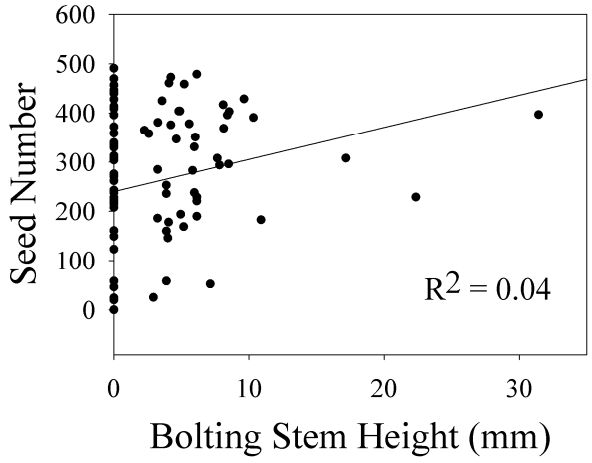

FIGURE 8.3: Relationship between seed production at senescence (~10 weeks) and height of the bolting stem at 4 weeks of age. A value of 0 indicates the plant had not begun to bolt at 4 weeks of age.

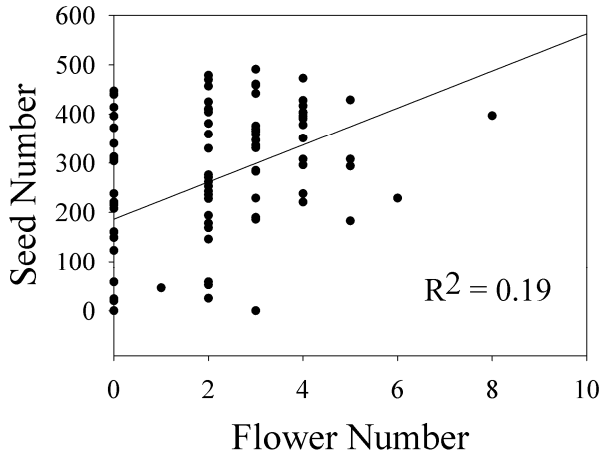

FIGURE 8.4: Relationship between seed production at senescence (~10 weeks) and number of flowers or flower buds at 4 weeks of age.

9. APPENDIX 2

Results of each individual generalized linear mixed effects model from the root occupancy analysis in Chapter 6 which were used to generate the averaged model for the resource selection probability function. Details of the parameters included in the model can be found in Table 6.2, and a description of each model can be found in Table 6.3. The parameter estimates in Tables 9.1-9.8 can be combined with the model weights in Table 6.5 to derive the averaged model presented in Table 6.6 following accepted information theoretic methods (Burnham and Anderson 2002; Anderson 2008).

TABLE 9.1: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the null model containing no fixed effects (Chapter 6).

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	5.27	0.97	5.43	<0.001
Artemsia	Intercept	2.06	0.29	7.06	<0.001
Koelaria	Intercept	-0.53	0.15	-3.57	<0.001
Poa	Intercept	-0.73	0.16	-4.60	<0.001

TABLE 9.2: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the distance model containing only distance from the stem as a fixed effect (Chapter 6).

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	8.72	1.75	4.99	<0.001
	Distance	-2.51	0.89	-2.83	0.005
Artemesia	Intercept	2.81	0.45	6.30	<0.001
	Distance	-1.18	0.47	-2.49	0.0129
Koelaria	Intercept	0.47	0.21	2.23	0.026
	Distance	-2.41	0.37	-6.47	<0.001
Poa	Intercept	0.10	0.23	0.42	0.678
	Distance	-2.12	0.38	-5.63	<0.001

TABLE 9.3: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the neighbours model containing only the presence of neighbours as a fixed effect (Chapter 6).

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	3.58	0.84	4.27	<0.001
	Neighbours	1.60	1.54	1.03	0.301
Artemesia	Intercept	1.20	0.32	3.71	<0.001
	Neighbours	1.95	0.63	3.07	0.002
Koelaria	Intercept	-0.38	0.21	-1.83	0.068
	Neighbours	-0.31	0.30	-1.05	0.296
Poa	Intercept	-0.34	0.21	-1.63	0.104
	Neighbours	-0.82	0.32	-2.59	0.010

TABLE 9.4: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the resources model containing heterogeneity and soil type, as a fixed effects (Chapter 6). Factor labels are abbreviated to the first letter only for interaction terms.

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	5.29	1.49	3.54	<0.001
	Heterogeneity	-2.41	1.70	-1.42	0.156
	Soil	0.00	1.37	0.00	1.000
	H x S	0.25	1.55	0.16	0.870
Artemesia	Intercept	1.72	0.49	3.50	<0.001
	Heterogeneity	0.42	0.72	0.59	0.559
	Soil	1.29	0.72	1.78	0.075
	H x S	-1.63	0.93	-1.75	0.080
Koelaria	Intercept	-0.42	0.30	-1.43	0.152
	Heterogeneity	0.00	0.42	0.00	1.000
	Soil	-0.46	0.43	-1.07	0.284
	H x S	0.46	0.60	0.77	0.440
Poa	Intercept	-0.43	0.30	-1.43	0.152
	Heterogeneity	-0.47	0.44	-1.07	0.285
	Soil	-0.80	0.45	-1.77	0.08
	H x S	1.28	0.63	2.03	0.042

TABLE 9.5: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the resources x neighbours model containing heterogeneity, soil type, and the presence of neighbours as a fixed effects (Chapter 6). Factor labels are abbreviated to the first letter only for interaction terms.

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	3.96	1.29	3.07	0.002
	Neighbours	17.55	10460.00	0.00	0.999
	Heterogeneity	-2.33	1.57	-1.49	0.137
	Soil	0.00	1.31	0.00	1.000
	N x H	-15.46	10460.00	0.00	0.999
	N x S	-0.22	14040.00	0.00	1.000
	H x S	0.82	1.60	0.51	0.608
	N x H x H	-1.20	14040.00	0.00	1.000
Artemesia	Intercept	0.65	0.56	1.17	0.243
	Neighbours	3.11	1.39	2.24	0.025
	Heterogeneity	0.47	0.81	0.58	0.56
	Soil	3.03	1.27	2.39	0.017
	N x H	-0.56	1.98	-0.28	0.776
	N x S	-4.42	1.89	-2.34	0.019
	H x S	-3.50	1.43	-2.45	0.014
	N x H x H	4.89	2.64	1.85	0.064
Koelaria	Intercept	-0.17	0.41	-0.41	0.683
	Neighbours	-0.53	0.60	-0.88	0.377
	Heterogeneity	-0.17	0.58	-0.29	0.771
	Soil	-0.34	0.59	-0.58	0.559
	N x H	0.35	0.84	0.42	0.675
	N x S	-0.30	0.89	-0.34	0.737
	H x S	0.17	0.83	0.20	0.839
	N x H x H	0.65	1.22	0.53	0.595
Poa	Intercept	0.00	0.41	0.00	1.00
	Neighbours	-0.89	0.61	-1.46	0.14
	Heterogeneity	-0.69	0.60	-1.16	0.24
	Soil	-0.51	0.59	-0.87	0.38
	N x H	0.48	0.88	0.55	0.59
	N x S	-1.00	1.05	-0.96	0.34
	H x S	1.04	0.84	1.24	0.22
	N x H x H	0.88	1.36	0.65	0.52

TABLE 9.6: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the neighbours + distance model containing the presence of neighbours and distance from the stem as a fixed effect (Chapter 6).

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	7.25	1.69	4.28	<0.001
	Distance	-2.40	0.87	-2.76	0.596
	Nieghbours	1.58	2.99	0.53	0.006
Artemesia	Intercept	1.92	0.46	4.15	<0.001
	Distance	-1.22	0.48	-2.57	0.010
	Nieghbours	2.10	0.68	3.11	0.002
Koealaria	Intercept	0.68	0.28	2.45	0.015
	Distance	-2.43	0.38	-6.47	<0.001
	Nieghbours	-0.42	0.35	-1.21	0.226
Poa	Intercept	0.62	0.30	2.07	0.039
	Distance	-2.14	0.38	-5.57	<0.001
	Nieghbours	-1.05	0.39	-2.70	0.007

TABLE 9.7: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the resources + distance model containing heterogeneity, soil type, and distance from the stem as a fixed effect (Chapter 6). Factor labels are abbreviated to the first letter only for interaction terms.

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	7.88	2.22	3.54	0.000
	Distance	-2.16	0.82	-2.64	0.247
	Heterogeneity	-2.77	2.40	-1.16	1.000
	Soil	0.00	1.58	0.00	0.008
	H x S	0.31	1.78	0.18	0.861
Artemesia	Intercept	2.47	0.61	4.05	<0.001
	Distance	-1.21	0.49	-2.50	0.012
	Heterogeneity	0.37	0.76	0.48	0.630
	Soil	1.39	0.75	1.85	0.06
	H x S	-1.58	0.96	-1.64	0.101
Koelaria	Intercept	0.63	0.37	1.69	0.090
	Distance	-2.44	0.38	-6.48	<0.001
	Heterogeneity	0.00	0.49	0.00	1.000
	Soil	-0.62	0.50	-1.23	0.218
	H x S	0.62	0.70	0.88	0.379
Poa	Intercept	0.53	0.40	1.30	0.193
	Distance	-2.24	0.39	-5.73	<0.001
	Heterogeneity	-0.63	0.55	-1.16	0.247
	Soil	-1.07	0.52	-2.07	0.04
	H x S	1.71	0.72	2.37	0.018

TABLE 9.8: Parameter estimates, standard errors (SE), z and p values estimated by the lmer procedure in the resources x neighbours + distance model containing heterogeneity, soil type, presence of neighbours and distance from the stem as fixed effects (Chapter 6). Factor labels are abbreviated to the first letter only for interaction terms.

Species	Parameter	Estimate	SE	z	p
Achillea	Intercept	6.24	1.83	3.41	0.001
	Distance	-2.08	0.79	-2.62	0.999
	Neighbours	17.84	17880.00	0.00	0.137
	Heterogeneity	-3.08	2.07	-1.49	1.000
	Soil	0.00	1.51	0.00	0.009
	N x H	-15.06	17880.00	0.00	0.999
	N x S	-0.37	23230.00	0.00	1.000
	H x S	1.04	1.82	0.57	0.567
	N x H x H	-1.40	23230.00	0.00	1.000
Artemesia	Intercept	1.42	0.69	2.07	0.038
	Distance	-1.40	0.52	-2.71	0.007
	Neighbours	3.48	1.50	2.32	0.021
	Heterogeneity	0.36	0.89	0.40	0.69
	Soil	3.34	1.34	2.50	0.013
	N x H	-0.52	2.12	-0.25	0.806
	N x S	-4.86	1.99	-2.44	0.015
	H x S	-3.51	1.50	-2.34	0.019
	N x H x H	5.04	2.76	1.83	0.068
Koelaria	Intercept	1.00	0.52	1.94	0.052
	Distance	-2.49	0.38	-6.51	<0.001
	Neighbours	-0.73	0.70	-1.04	0.299
	Heterogeneity	-0.24	0.69	-0.35	0.729
	Soil	-0.48	0.70	-0.69	0.488
	N x H	0.49	0.99	0.49	0.62
	N x S	-0.32	1.02	-0.32	0.751
	H x S	0.24	0.99	0.24	0.807
	N x H x H	0.81	1.42	0.57	0.569
Poa	Intercept	1.12	0.54	2.08	0.038
	Distance	-2.24	0.40	-5.68	<0.001
	Neighbours	-1.20	0.74	-1.63	0.104
	Heterogeneity	-0.94	0.73	-1.30	0.195
	Soil	-0.70	0.68	-1.03	0.305
	N x H	0.67	1.05	0.64	0.524
	N x S	-1.09	1.16	-0.95	0.345
	H x S	1.41	0.97	1.45	0.146
	N x H x H	0.91	1.52	0.60	0.552

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