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ROOT RESPONSES TO NUTRIENT HETEROGENEITY:
A COMPARISON OF DOMINANT AND SUBORDINATE
SPECIES FROM OLD FIELDS

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ROOT RESPONSES TO NUTRIENT HETEROGENEITY: A COMPARISON OF DOMINANT AND SUBORDINATE SPECIES FROM OLD FIELDS

By

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ABSTRACT

ROOT RESPONSES TO NUTRIENT HETEROGENEITY: A COMPARISON OF DOMINANT AND SUBORDINATE SPECIES FROM OLD FIELDS

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Many potential mechanisms have been proposed to explain coexistence among plant species. Spatial heterogeneity in soil resources at scales smaller than individual plants may promote coexistence by forcing a trade-off between the efficient locating of nutrient patches (scale foraging) and the efficient exploiting of nutrient patches (precision foraging). Evidence from Campbell *et al.* (1991) suggests that dominant species use a scale foraging strategy whereas subordinate species use a precision foraging strategy.

To test whether dominant and subordinate species differed in foraging strategy under heterogeneous soil resource conditions, I conducted a series of greenhouse experiments in which I varied patch size and intensity, using two dominant (*Bromus inermis* and *Solidago canadensis*) and three subordinate (*Achillea millefolium, Rumex acetosella,* and *Silene latifolia*) herbaceous perennial species commonly found in local old fields. One of the dominant species (*Bromus*) consistently had high tissue nitrogen content, but had lower nitrogen contents as patch size and nutrient intensity decreased. This performance pattern was consistent with that expected for a scale forager. The other dominant species (*Solidago*) and the three subordinate species had lower nitrogen contents overall, but more consistent nitrogen contents across patch sizes and nutrient intensities. This performance pattern was consistent with that expected for a precision forager. There appeared to be a trade-off between the

ability to acquire a large amount of nitrogen and the ability to maintain constant acquisition levels in the plant as nutrient patch sizes changed.

I also predicted that precision and scale-foraging species would differ in the mechanisms they used to respond to nutrient heterogeneity. Although I found differences among species with respect to which response mechanisms they used, the differences were not related to precision vs. scale foraging strategies. Two of the four precision-foraging species, *Achillea* and *Solidago*, increased their root branching density in nutrient patches. All species were able to selectively allocate root biomass to nutrient enriched pot quadrants. None of these species changed root system topology in response to nutrient patches. *Rumex* and *Silene*, two of the precision-foraging species, adjusted nitrogen uptake so that root nitrogen concentrations were constant across patch sizes. *Bromus* (a scale forager), *Achillea* and *Solidago* (precision foragers) adjusted nitrogen uptake so that root concentrations in enriched pot quadrants were higher than in background quadrants. These five species did not differ in the patch size or nutrient intensity at which they used a response mechanism.

To fully understand the role that nutrient heterogeneity may play in determining plant community structure, we must also know the size, intensity and frequency of small scale patches in the environment. Spatial sampling and geostatistical analysis techniques are useful tools for quantifying spatial variation of nutrients in the field, but it is unclear what spatial sampling pattern will be most sensitive to multiple scales of heterogeneity. Using computer simulated data and semivariance analysis, I determined that a stratified-nested grid sampling regime would be more sensitive than a random or stratified grid sampling regime when determining the magnitude and scale of environmental heterogeneity across several sites.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1

INTRODUCTION

COMPETITION AND COEXISTENCE

Understanding the mechanisms that allow for species coexistence and determine biodiversity is one of the key questions in ecology (Tilman 1988, Schluter and Ricklefs 1993, Reynolds *et al.* 1997). Plants provide a unique challenge for understanding mechanisms of coexistence, because they are sessile and require the same basic resources. Many potential mechanisms have been proposed to explain coexistence in plant species (e.g. Zobel 1992, Bengtsson *et al.* 1994, Reynolds *et al.* 1997). Reynolds *et al.* (1997) categorize these mechanisms into three types: those that emphasize spatial and temporal resource partitioning; those that emphasize competitive equivalence and thus prolonged times to competitive exclusion; and those that emphasize factors that interrupt or reverse competitive exclusion, like herbivory or disturbance.

THE ROLE OF ENVIRONMENTAL HETEROGENEITY

Considerations of how environmental heterogeneity in plant resources will influence competitive interactions fall into the category of resource partitioning. At scales of resource heterogeneity larger than individual plants, species may have trade-offs in competitive abilities at different resource levels, and thus different species will be favored in different areas of a spatially heterogeneous environment (Tilman 1988, 1994, Tilman and Pacala 1993).

At very small scales of resource heterogeneity (i.e. less than the size of an individual

plant), plants will encounter multiple patches, rather than being entirely within one patch. At this scale, competition between plants will shift from focussing on the exploitation of a single patch to having roots that both find and exploit multiple patches (Hutchings and de Kroon 1994, Casper and Jackson 1997). Species differences in ability to obtain nutrients from a patchy soil environment will translate into differences in growth, performance and competitive ability that will change as the spatial distribution of nutrients changes (Casper and Cahill 1996).

FORAGING STRATEGY

Grime and colleagues (Campbell *et al.* 1991, Grime *et al.* 1991, Grime 1994) hypothesized that there would be a fundamental trade-off between a species' ability to explore a large area (or 'scale foraging') and the ability to efficiently exploit resources within that area (or 'precision foraging'). This trade-off may lead to different competitive abilities for species in heterogeneous vs. homogeneous environments, or may promote coexistence of species with different foraging strategies in heterogeneous environments.

Within the scale of a plant's root system, both patch size and nutrient intensity (the magnitude of difference between nutrient concentrations in a patch and the background soil) could vary widely. Some species may be more responsive to patches across a range of sizes or intensities, while other species only respond to the largest and most intense patches. In addition, plants may utilize different mechanisms to respond to nutrient patches such as changing nutrient uptake rates (Caldwell 1994), changing root branching pattern (Fitter 1994), or proliferating roots in patches (Robinson *et al.* 1994). If the trade-off between scale and precision of foraging is important, then there could be differences among species in how well they perform in environments that differ in patch size and nutrient intensity (relative to a "homogeneous" environment). Species with a scale foraging strategy would be expected to be

less sensitive to patches, while precision foraging species would be expected to have the capability of exploiting patches of many sizes and intensities.

Campbell *et al.* (1991) observe that dominant species typically used a scale foraging strategy, while subordinate species adopted a precision foraging strategy. If this is a general pattern, then the trade-off between scale and precision in resource foraging could provide a means by which subordinate species can reduce competitive effects of dominant species in heterogeneous environments and therefore persist. A subordinate species that can more effectively exploit small patches can maintain or improve its performance in patchy environments, relative to the dominant species.

SPECIES DESCRIPTIONS

Mid-successional old fields at the Kellogg Biological Station (KBS) are dominated by herbaceous perennials (Huberty *et al.* 1998). In productive sites, the biomass dominants are *Solidago* species and the perennial grasses *Bromus inermis*, *Phleum pratense*, *Andropogon virginicus* and *Agropyron repens* (Burbank *et al.* 1992, also Goldberg 1987, Goldberg and Gross 1988, Foster 1997). *Centaurea maculosa*, *Hieracium* spp., and *Aster pilosus* are often co-dominants with the above species. Other frequently occurring species are *Achillea millefolium*, *Medicago sativa*, *Euphorbia corollata*, *Daucus carota*, and *Monarda fistulosa* (Burbank *et al.* 1992).

From the suite of perennial herbaceous species found in local mid-successional old-fields, I chose five that represent a range of growth form and root systems: *Achillea millefolium, Bromus inermis, Rumex acetosella, Silene alba* and *Solidago canadensis* (Figure 1.1). These species also differ in their relative abundance in the field. *Solidago* and *Bromus* are generally biomass dominants in these fields, often making up 40 to 80% of the community biomass, whereas the other three species are less common and generally comprise

Figure 1.1 - The five species used in this study: a) *Bromus inermis*, b) *Solidago canadensis*, c) *Achillea millefolium*, d) *Rumex acetosella*, and e) *Silene alba*. Images not drawn to scale. Drawings by K. McMillen.

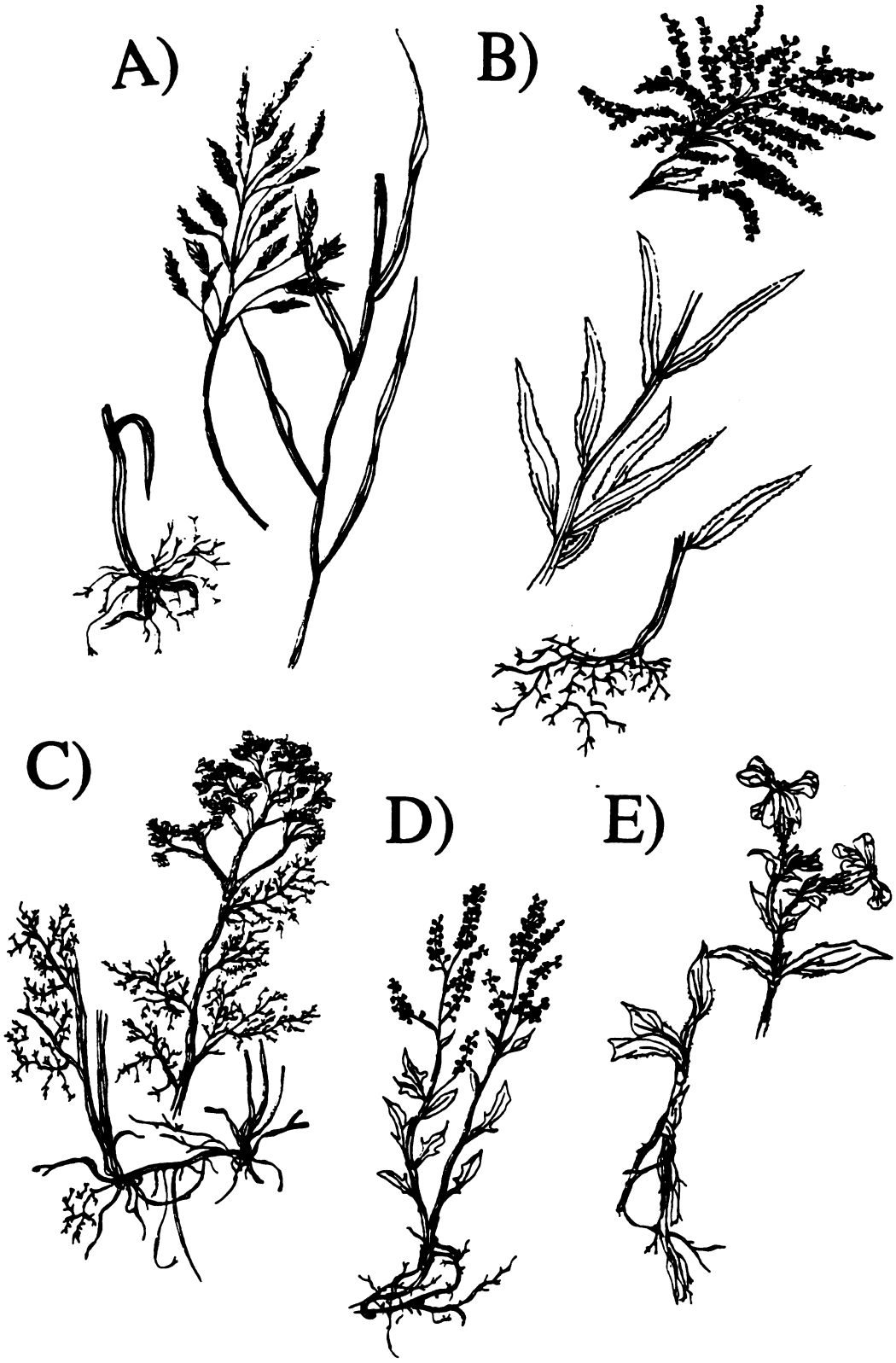


Figure 1.1

less than 10% of the community biomass.

Bromus inermis Leysser (Family: Poaceae) is a perennial cool season grass (C_3) that was introduced to North America from Europe. It grows 50-100 cm tall and can reproduce by seeds, tillers and creeping rhizomes (Stubbendieck *et al.* 1986). Individual leaf blades are 15-40 cm long and 0.4-1.5 cm wide. Inflorescences are 7-20 cm long panicles, with 5-13 flowers per spikelet. *Bromus* has been observed to form mycorrhizal associations (Harley and Harley 1987). This species has been cultivated as a hay and pasture grass, but is also found along roadsides and in waste places (Stubbendieck *et al.* 1986). In southwest Michigan, it can become dominant in successional old fields (Burbank *et al.* 1992, Foster 1997).

Solidago canadensis L. (Family: Asteraceae) is a long-lived perennial forb that occurs in a broad range of habitats having moderately moist to fairly dry soils and moderate to full sun. *Solidago* grows 25-200 cm tall with alternate leaves on the stem and begins to flower in early August. Flowers are yellow, less than 3 mm in size and form terminal inflorescences. *Solidago* has been shown to form mycorrhizal associations and can reproduce vegetatively from underground rhizomes. In the fall, the flowering stems die and plants overwinter as dormant rhizomes. *Solidago* is native to North America, and often becomes the dominant species in the secondary succession of abandoned fields (8-17+ years). It is also found in waste areas, tallgrass prairies and infrequently grazed pastures (Werner *et al.* 1980).

Achillea millefolium L. S.L. (Family: Asteraceae) is a long lived perennial that overwinters as a rosette of dormant leaves. Flowering stems can be 20-100 cm tall, with finely divided alternating leaves and several 2-10 cm composite inflorescences at the top,composed of 2-4 mm flowers. *Achillea* generally flowers in July and August. Clonal reproduction by rhizomes is possible, but *Achillea* generally does not propagate vegetatively (Warwick and Black 1982). *Achillea* has been observed to have mycorrhizal associations (Harley and Harley 1987). It can be found in a wide range of habitats, usually in

the open and it is capable of growing under poor soil or drought conditions. The species is native to Eurasia, but is widely distributed through North America (Warwick and Black 1982).

Rumex acetosella L. (Family: Polygonaceae) is a dioecious perennial that grows 10-40 cm tall from a basal rosette of leaves (Gleason and Cronquist 1991). Flowers are very small in leafless racemes at the top of the stem (Newcomb 1977). *Rumex* can reproduce vegetatively from root buds that are produced at in high numbers (Houssard and Escarré 1995). *Rumex* is considered an amycorrhizal species (Harley and Harley 1987) and its fine roots have abundant root hairs (Corbett, pers. obs.). A native of Eurasia, *Rumex* is found in dry fields, pastures, roadsides and gardens (Newcomb 1977) but also tolerates sandy and acid soils (Gleason and Cronquist 1991). Seedlings will establish in recently disturbed areas, but vegetative propagation dominates in closed communities (Houssard and Escarré 1991).

Silene latifolia Poiret (Family: Caryophyllaceae) (formerly known as *Silene alba* (Miller) E. H. L. Krause, *Lychnis alba* Miller and *Melandrium album* (Miller) Gracke) is a short lived perennial or biennial that requires habitats of well drained soil and high light levels. *Silene* is dioecious and produces flower shoots 30-100 cm tall from basal rosettes of leaves from June through October (McNeill 1977). Flowers are white, 2.5-3.0 cm wide and sepals are fused into a finely-veined bladder-shaped calyx (Newcomb 1977). Plants have a prominent tap root and no special structures for vegetative reproduction (McNeill 1977). *Silene* is an amycorrhizal species (Harley and Harley 1987) and has long dense root hairs on the fine roots (Corbett, pers. obs.). Introduced from Eurasia, it is found in waste places, roadsides and field edges, and usually does not persist in closed communities (McNeill 1977). These five species are all commonly found in successional old fields in southwest Michigan, but differ in their abundances (Table 1.1). *Bromus* and *Solidago* dominate in terms of biomass when they occur ($>40\%$ and $>20\%$, respectively). *Achillea* and *Rumex* occur

frequently, but generally at lower biomass abundance. *Silene* occurs at only a few sites, and when it is found it is always in lower biomass abundance.

THESIS OVERVIEW

I used a series of greenhouse experiments to investigate whether these species differ in the magnitude and/or mechanism of response to nutrient heterogeneity as patch size and patch intensity vary. I then assessed if there were similarities in the magnitude and mechanism of response to nutrient heterogeneity among species based on relative dominance, foraging strategy, mycorrhizal status or how closely related the species are. Chapter 2 summarizes the magnitude of response to nutrient heterogeneity. I compared the growth performance of these species in response to different patch sizes and intensities to test the hypothesis that more precise foragers will have more consistent performance as patch size and nutrient intensity change. A companion paper in chapter 3 investigates whether the mechanisms used by plants to adjust their root systems to patchy nutrient conditions differ across patches of different size and intensity. The greater emphasis on effective patch exploitation by precision-foraging species leads to several expectations. Precision-foraging species are expected to: 1) use more response mechanisms than scale foraging species; 2) respond to a wider range of patch sizes and nutrient intensities; and 3) employ those mechanisms with finer control than scale-foraging species, such that roots in enriched nutrient patches will be more different from roots in unenriched areas.

To understand how species responses to spatial heterogeneity in resources relates to their competitive ability or the pattern of diversity we see in the field, we need to know not only the potential of different species to respond to small nutrient patches, but also what the patterns of nutrient heterogeneity are in the field. Only a few studies have documented small scale nutrient heterogeneity in the field (Robertson *et al.* 1988, Jackson and Caldwell 1993,

Table 1.1 - Biomass abundance of study species in several old fields at the W.K. Kellogg Biological Station. Total biomass adjusted to amount per square meter. Amount per species is expressed as a percentage of total biomass. Values are averages for multiple samplings at a given site on a given date. All data collected in 1996 and unpublished unless otherwise specified.

Location (Source)	Total Biomass g/m^2	Bromus inermis g/m^2 (%)	Solidago canadensis g/m^2 (%)	Achillea millefolium g/m^2 (%)	Rumex acetosella g/m^2 (%)	Silene latifolia g/m^2 (%)
Upper Louden (H. Reynolds unpubl.)	445	271.3 (61.0)	40.3 (9.1)	9.9 (2.0)	0.5 (0.1)	0.0 (0.0)
Lux Arbor (L. Broughton unpubl.)	254	0.0 (0.0)	104.6 (30.9)	13.0 (3.8)	2.0 (0.6)	0.0 (0.0)
Cantlon (K. Gross *et al.* unpubl.)	698	0.0 (0.0)	171.4 (24.6)	26.2 (3.8)	0.6 (0.1)	2.4 (0.3)
LTER Treatment 7 (K. Gross *et al.* unpubl.)	367	0.0 (0.0)	131.5 (35.8)	2.6 (0.7)	1.6 (0.4)	1.5 (0.4)
Upper Pond Lab (K. Gross & G. Mittelbach unpubl.)	217	95.8 (44.0)	9.0 (4.1)	4.7 (2.2)	1.5 (0.7)	0.0 (0.0)
Lower Pond Lab (K. Gross & G. Mittelbach unpubl.)	302	213.2 (70.7)	15.0 (5.0)	6.6 (2.2)	0.4 (0.1)	0.0 (0.0)
McKay (B.L. Foster 1996)	403	200.3 (49.7)	0.0 (0.0)	31.7 (7.9)	1.0 (0.3)	0.0 (0.0)

Gross *et al.* 1995), but we still know little about the size, intensity and frequency of small scale patches under most field conditions and how heterogeneity might vary across different communities. Spatial sampling and analysis using geostatistical techniques is a useful way to quantify nutrient variation under field conditions. Chapter 4 addresses the question of how to choose a spatial sampling scheme for geostatistical analysis that is sensitive to several scales of heterogeneity when sampling several sites to test an ecological hypothesis.

Chapter 2

WHOLE-PLANT RESPONSES TO NUTRIENT HETEROGENEITY: A COMPARISON OF DOMINANT AND SUBORDINATE SPECIES.

INTRODUCTION

Heterogeneity of soil resources in space and time can influence community structure and the coexistence of plant species (Bengtsson *et al.* 1994, Tilman 1994). Differences among species in their ability to respond to nutrient heterogeneity can explain variation in their relative competitive abilities and community structure (Casper and Cahill 1996, Campbell and Grime 1989a, Grime 1994).

Soil resource heterogeneity can occur over a range of spatial scales (Robertson and Gross 1994, Ehrenfeld *et al.* 1997). At large scales of heterogeneity, soil resource patches are larger than individual plants, and plant responses to heterogeneity may be expressed in terms of the relative abundance of species in different types of patches, the ability to colonize patches, or disturbances (Goldberg and Werner 1983, Tilman 1988, 1990, Casper and Jackson 1997). Field studies have documented that soil nutrients can be heterogeneous at small scales (i.e. less than 1 m, Schlesinger *et al.* 1996, Jackson and Caldwell 1993, Gross *et al.* 1995). At this scale, nutrient patches are often smaller than the size of individual plant root systems. The ability of the plant root system to make plastic adjustments to compensate for spatial heterogeneity of nutrients could be important in determining how much resource the plants can obtain from the soil (Campbell *et al.* 1991). The spatial arrangement of the nutrients could be as vital to a plant's survival as the total amount of nutrients.

Grime and colleagues (Campbell *et al.* 1991, Grime 1994) have proposed that plants in environments with small-scale nutrient heterogeneity experience a trade-off between the ability to have roots foraging over a large volume of soil ("scale" foragers) and the ability to forage intensively in a smaller area ("precision" foragers). Many studies have documented that plant species can selectively proliferate fine roots or adjust uptake in nutrient-enriched soil patches, but that not all species exhibit this plasticity (see review by Robinson 1994).

These previous studies have usually investigated plant responses to only one size of nutrient patch, or compared a single heterogeneous environment to a homogeneous environment. Yet, both patch size and nutrient intensity (i.e. the magnitude of difference between nutrient concentrations in a patch and in the background soil) can vary widely, while still remaining within the scale of a plant's root system. If there is a trade-off between scale and precision of foraging, as suggested by Grime and colleagues (Campbell *et al.* 1991, Grime 1994) then one can expect that there will be differences among species in their performance at different patch sizes and nutrient intensities, based on which foraging strategy they use. Precision foragers would be expected to take advantage of patches of smaller sizes or intensities and thus perform equally well under heterogeneous and homogeneous nutrient conditions. If the pattern of their performance is measured across a range of patch sizes, they would exhibit either a "responsive" (the same nutrient acquisition whether the same total amount of nutrients is patchy or is spread uniformly through the soil) or "extra-responsive" (obtain more nutrients under patchy conditions than uniform conditions; e.g. Borkert and Barber 1985, Anghinoni and Barber 1980) pattern (Figure 2.1). Scale foragers, on the other hand, would be less efficient at exploiting nutrients in patches, and their performance would be expected to be poorer under heterogeneous nutrient conditions than under homogeneous conditions. The pattern of response across a range of patch sizes would be "partially responsive": they respond to fertilization but obtain fewer nutrients when fertilizer is patchy

Figure 2.1 - Possible patterns of response to heterogeneity by plants of different species, showing hypothetical performances of plants as enriched patch size varies from very small to as large as the whole root system. The performance of an unfertilized control (no patch) is included for reference.

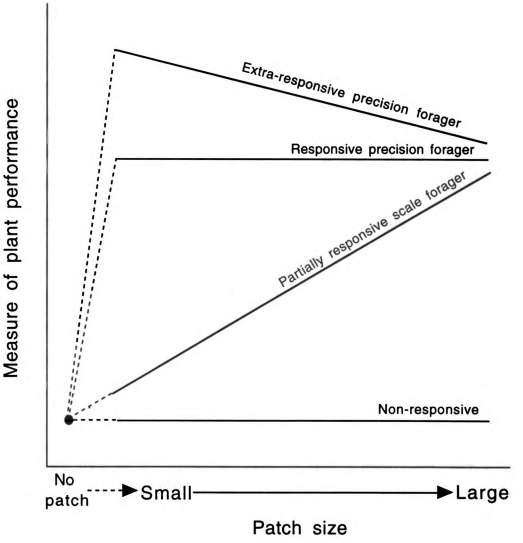

Extra-responsive precision forager
Responsive precision forager
Partially responsive scale forager
Non-responsive
Measure of plant performance
No patch
Small
Large
Patch size

Figure 2.1

than when it is uniform (see Figure 2.1). If a plant obtained no nutrients from fertilization, it would exhibit a "non-responsive" pattern.

Precision foragers will be either "responsive" or "extra-responsive" to a range of patch sizes because the ability to be plastic at small scales allows the detection and exploitation of nutrients even when patches are small. Scale foragers will be "partially responsive" to changes in patch size, because the trade-off between scale and precision prohibits efficient exploitation of small patches.

Similarly, as the intensity (or magnitude) of a patch decreases, greater precision will be needed by a plant to detect an enriched patch, relative to background areas. Thus, scale foragers are expected to be less able to respond to nutrient patches (vs. uniform enrichment) as nutrient intensity decreases, while precision foragers remain either "responsive" or "extra-responsive" to nutrient patches.

Campbell *et al.* (1991) observed that fast-growing, competitively dominant plants were able to acquire more nutrients in total and thus grow larger (scale foraging). In contrast, the smaller slow-growing subordinates were more flexible in allocating root biomass to nutrient patches (precision foraging). Based on this previous work, I hypothesized that, across a range of patch sizes and intensities, the dominant species in old fields would be scale foragers whereas the subordinate species in these communities would be precision foragers.

To examine these predictions, I compared five perennial species that are common in mid-successional old fields in southwest Michigan, but differ in relative abundance. I classified these species as either dominant or subordinate based on their proportional biomass abundance in local old fields (see Chapter 1). In a series of greenhouse experiments I measured the ability of these species to respond to nutrient patches of three different sizes and two different intensities. I measured their response in terms of total plant biomass, shoot nitrogen concentration and plant nitrogen content.

METHODS

Study species

The five species I selected for these experiments represent a range of growth forms and rooting characteristics typical of herbaceous perennials. *Bromus* and *Solidago* are generally dominant species in relative biomass abundance in old fields (Table 1.1 and Burbank *et al.* 1992). *Achillea, Rumex* and *Silene* occur in many mid- to late-successional old fields, but in much lower abundance, usually less than 10% of total community biomass and so I classified them as subordinates. *Bromus inermis* Leysser (Poaceae) is a C_3 perennial grass that reproduces clonally by rhizomes and forms mycorrhizal associations (Gleason and Cronquist 1991, Harley and Harley 1987). *Solidago canadensis* L. (Asteraceae) is a long-lived clonal perennial and is the only one of my species native to North America. It forms mycorrhizal associations and produces rhizomes (Werner *et al.* 1980). *Achillea millefolium* L. S.L. (Asteraceae) is a rosette-forming short-lived perennial or biennial that can reproduce vegetatively by rhizomes and forms mycorrhizal associations (Warwick and Black 1982, Harley and Harley 1987). *Rumex acetosella* L. (Polygonaceae), a dioecious perennial that can reproduce vegetatively, is common in dry, sandy and acidic soils (Gleason and Cronquist 1991). It has not been observed to form mycorrhizal associations (Harley and Harley 1987). *Silene latifolia* Poiret (Caryophyllaceae - formerly *Silene alba* (Miller) E. H. L. Krause, *Lychnis alba* Miller and *Melandrium album* (Miller) Gracke) is a short-lived dioecious perennial that has no vegetative reproductive structures (McNeill 1977). It is also amycorrhizal (Harley and Harley 1987).

Pre-experiment conditions

I collected seeds of all five study species from field populations at the W.K. Kellogg Biological Station (KBS) of Michigan State University in southwest Michigan. Seeds were

collected during the summer of 1995 at the time of seed maturation for each species and stored dry. I germinated seeds of all species on wet sand in petri dishes placed in growth chambers under 16 hours of light and at a constant temperature of 25°C. *Rumex* seeds, which required pre-treatment to enhance germination (Anderson 1968), were soaked for 10 minutes in 10 ml 1:1 concentrated sulfuric acid: distilled water and rinsed in distilled water before being placed in the petri dishes.

Two weeks after germination, I transferred individual seedlings of each species to 5 cm diameter containers filled with silica sand, with a total of 10 seedlings transplanted per species. I grew the seedlings in the greenhouse at temperatures of 20-23°C, under metal halide lights set to a 14 hour day (mean light intensity of 388 μmol m^{-2} sec^{-1}, measured under the lights, mid-morning on a cloudless sunny day). Temperatures were maintained by greenhouse heaters in winter and evaporative coolers in summer. I watered plants daily and fertilized them once a week with 10 ml of a 1 g/L Peter's 20-10-20 Peat-lite Special Fertilizer solution with micronutrients (7.77% ammonium nitrogen, 12.23% nitrate nitrogen, 10% available phosphoric acid, 20% soluble potash, 0.15% soluble magnesium, 0.02% boron, 0.01% chelated copper, 0.1% chelated iron, 0.056% chelated manganese, 0.01% molybdate, and 0.0162% zinc).

After 2-3 weeks, I re-potted single individuals of each species into 20 cm pots filled 10 cm deep with silica sand (6 pots per species). The plants were grown under the same light and temperature conditions as above, and I continued to water the plants daily and give them the weekly fertilizer treatments for an additional 3-4 weeks. This allowed the root systems to spread throughout the volume of the pot, without becoming pot-bound. I then selected four plants of each species of similar size for experimentation.

The experimental apparatus

I created different nutrient treatments, using a continuous drip irrigation system modelled after that used by Campbell *et al.* (Campbell and Grime 1989b, Campbell *et al.* 1991, Hendry and Grime 1993). Each pot had four equally spaced Tygon tubes held in place by a PVC frame, dividing the pot into four equal sized quadrants (see Figure 2.2). Two peristaltic pumps pumped fertilizer solutions through the tubing at a rate of 20 ml per hour. As long as the solution was flowing through all four tubes in a pot at the same rate, there was no diffusion between quadrants. Therefore, by varying the nutrient concentrations flowing through each tube I could create a variety of patchy environments (Campbell and Grime 1989b). To verify that the experimental apparatus was generating distinct patches, I ran two day and week-long trials with potassium iodide (an analog for NO_3^-) as a marker in the irrigation system before starting the experiments (see van Ommen *et al.* 1988). I removed the soil in 2 cm layers, and traced the location of potassium iodide in pots that had either one or two enriched quadrants. I observed that patches retained their integrity to the bottom of the pot (i.e. there was no horizontal spread).

Experimental procedure

The experiment was set up in a nested randomized block design. Each experimental block consisted of all five species exposed to four different patch size treatments with one plant of each species per treatment. The number of channels on the pumps limited the number of plants that could be used in an experimental block, so replication was generated by repeating blocks through time. There were four patch size treatments applied to the plants (Figure 2.3): an unfertilized control with background fertilizer solution to all four quadrants, and small, medium or large patch sizes with one, two or all four quadrants enriched with fertilizer. In the fertilized treatments, the remaining unenriched quadrants received

Figure 2.2 - a) Side view of a plant in pot, showing how experimental apparatus was positioned and b) Top view of pot showing the four quadrants that the apparatus creates in the pot (based on Grime 1994). Numbers indicate placement of drip tubes.

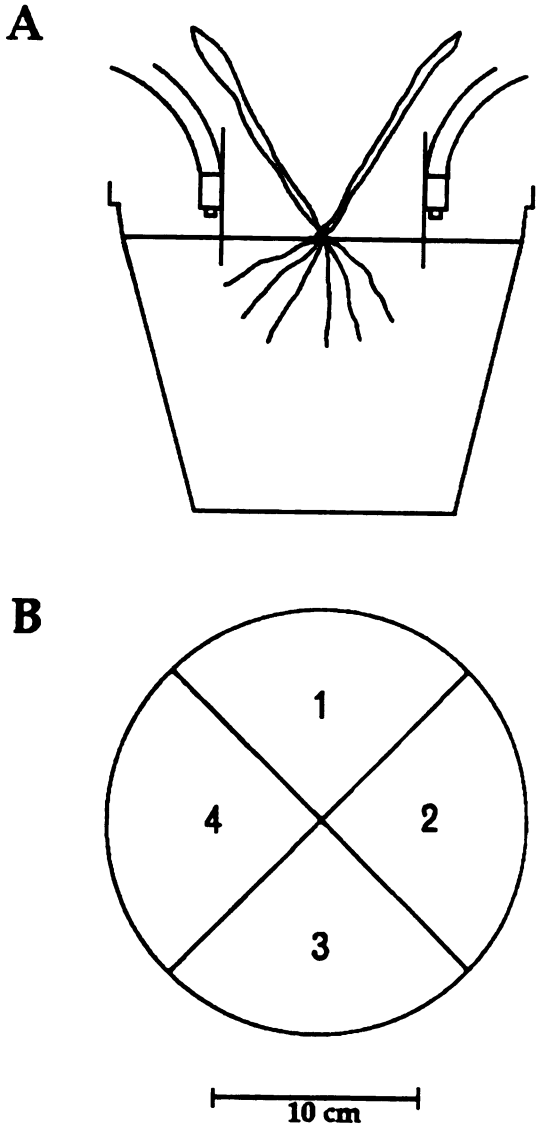


Figure 2.2

Figure 2.3 - The four patch-size treatments applied during the experimental period.

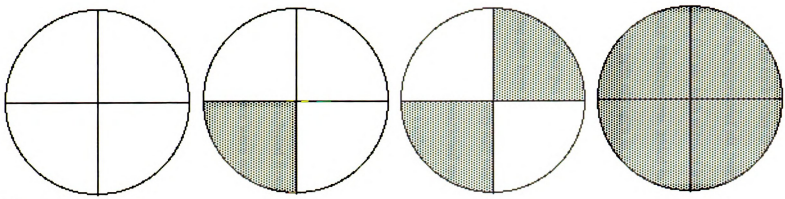

Patch size:
Control
Small
Medium
Large
(# of quadrants fertilized):
0
1
2
4
Plant's "view" of environment:
Homogeneous
Heterogeneous
Heterogeneous
Homogeneous

Figure 2.3

background solution. In the medium-sized patch treatment, the two quadrants that were enriched were opposite each other (Figure 2.3).

I repeated the experiment six times at two nutrient intensity levels between March and December, 1996. For the high nutrient intensity treatment, a total of 7.5 mg of Peters 20-10-20 Peat-lite Special fertilizer was added in solution to each pot per hour. I calculated that this was equivalent to 20 ppm nitrogen in a pot, an amount chosen to approximate the high end of available nitrogen in soil patches in local old fields (Gross *et al.* 1995). The total amount of fertilizer enrichment per pot was kept constant, delivered as either 7.5 mg per hour to one quadrant, 3.75 mg per hour to 2 quadrants or 1.875 mg per hour to four quadrants. The lower nutrient intensity level was half that of the high level (*i.e.* 3.75 mg of fertilizer added in solution per pot per hour). Unenriched quadrants in all pots (including all four quadrants in the control treatment) received a solution of 0.0375 mg fertilizer per pot per hour, or 0.4 ppm nitrogen. Consequently, enriched patches were 50-200 times more concentrated in fertilizer content than background patches (depending on patch size and intensity levels). The solutions kept the moisture level of the sand saturated, so no further watering was required. *Bromus* and *Silene* had to be excluded from the last replicate of the experiment because of an equipment malfunction, so there were only 5 replicates for these two species at the lower nutrient intensity treatment.

I placed 10 plants around each of the two pumps and then randomly assigned two treatments to each pump. The plants were exposed to the nutrient treatments for two weeks. Light and temperature conditions in the greenhouse were maintained at the same levels used during the pre-experimental growth period.

At the end of two weeks, I harvested plants by first severing the shoots at the surface of the sand and then used two perpendicular metal sheets as cutting edges to separate the sand and roots into the four quadrants created by the drip irrigation apparatus. I rinsed the sand

from the roots in each quadrant over a 2 mm sieve. The shoots and roots were dried at 45°C to minimize loss of volatile nitrogen compounds. I weighed the dried biomass of shoots and roots on a Mettler AE260 Analytical Balance (Mettler-Toledo Instruments, 1991, Hightstown, NJ) to four decimal places, and then ground the dried plant material for elemental analysis. Nitrogen content of the shoot and root tissues was determined using an elemental analyzer (Nitrogen Analyser 1500 Series 2, 1990, Carlo-Erba Instruments, Milan, Italy).

To assess plant performance, I evaluated total plant biomass, shoot nitrogen concentration and total plant nitrogen content. Total plant N content was estimated by summing the nitrogen in shoot (%N in shoot / 100 * shoot biomass) and root tissues [(%N of roots in enriched quadrant(s) / 100 * root biomass in enriched quadrant(s)) + (%N of roots in unenriched quadrants / 100 * root biomass in unenriched quadrants)]. Plant N content can be used as an index of total N acquired by the plant during the experiment if we assume that all plants of a given species in a given block had approximately the same initial N content (i.e. were the same initial size and N concentration). This assumption is reasonable, because all plants were grown for the same length of time under standard conditions.

Statistical Analyses

All analyses were performed using Systat 5.0 for Windows (Systat Inc. 1992), using a 4-way mixed model nested ANOVA. Nutrient intensity, patch size and species were all fixed effects, while block was random. Block effects were nested within nutrient intensity level in the ANOVA model because blocks were replicated through time and the blocks at high nutrient intensity were run at different times than the blocks at low nutrient intensity. The model was left unbalanced where data was missing in one block for *Bromus* and *Silene*. Total biomass measures were log transformed to better fit assumptions of normality and homoscedasticity in the analyses. The log transformation removes inherent size effects and

makes the tests within the model proportionate, because log (a) - log(b) = log (a/b).

In these analyses, significant interaction terms indicate that species differed in their ability to detect and respond to patches of different sizes and intensities. If there are no significant interaction terms, then the species all exhibited the same pattern of response, even if there were absolute differences in performance. A significant patch size x species interaction indicates differences among species in performance as patch size changes. A significant nutrient intensity x species interaction indicates differences among species in performance as nutrient intensity changes. A three-way interaction (nutrient intensity x patch size x species) indicates species differences in pattern of response across both patch size and nutrient intensity, where some species responses across patch size are more influenced by nutrient intensity than other species.

RESULTS

Total Biomass

The ANOVA of $\log_{10}$ total biomass gave significant block, species and patch size main effects, but no significant interactions (Table 2.1). There was sufficient variation in the size of plants among the experimental replicates within nutrient intensity levels to produce a significant block effect. The significant species effect was due to differences in plant size which were consistent across all treatments. Seedlings of *Bromus* and *Silene* were the largest, *Solidago* were the smallest, and *Achillea* and *Rumex* were intermediate in size (Figure 2.4). Although there were differences in size among species, there were no differences among species in pattern of biomass response to nutrient heterogeneity. Plants grown in treatments with fertilized patches had much greater biomass than those in the unfertilized control treatment, but there were no differences in plant size among the three fertilized treatments (1, 2 and 4 quadrants fertilized; Scheffe post-hoc pairwise comparisons, $P<0.05$; Figure 2.4).

Table 2.1 - F-values from three-way analysis of variance on total biomass, shoot percent nitrogen concentration and total plant N content comparing effects of nutrient intensity, species, patch size and the interactions between nutrient intensity, species, and patch size.

		Variate		
Source	df	Log_{10} (Total Biomass)	Shoot N Concentration	Plant N Content
Nutrient intensity	1	0.916	0.004	8.052 **
Block (Nutrient intensity)	11,8†	7.619 ***	6.345 ***	1.867
Species	4	57.977 ***	4.653 **	40.579 ***
Patch size	3	26.015 ***	211.128 ***	85.089 ***
Nutrient intensity x Species	4	2.154	2.839 *	4.159 **
Nutrient intensity x Patch Size	3	0.774	2.280	0.946
Species x Patch Size	12	0.555	1.653	4.920 ***
Nutrient intensity x Species x Patch Size	12	0.425	2.849 **	1.596

*** $P<0.001$; ** $P<0.01$; * $P<0.05$.

† Only 9 of the 12 blocks were analyzed for N content, thus block df are 11 for biomass and 8 for N concentration and N content.

Figure 2.4 - Log_{10} total biomass $\pm$ S.E. for two dominant (solid line) and three subordinate (dashed line) species at a) low and b) high nutrient intensity levels.

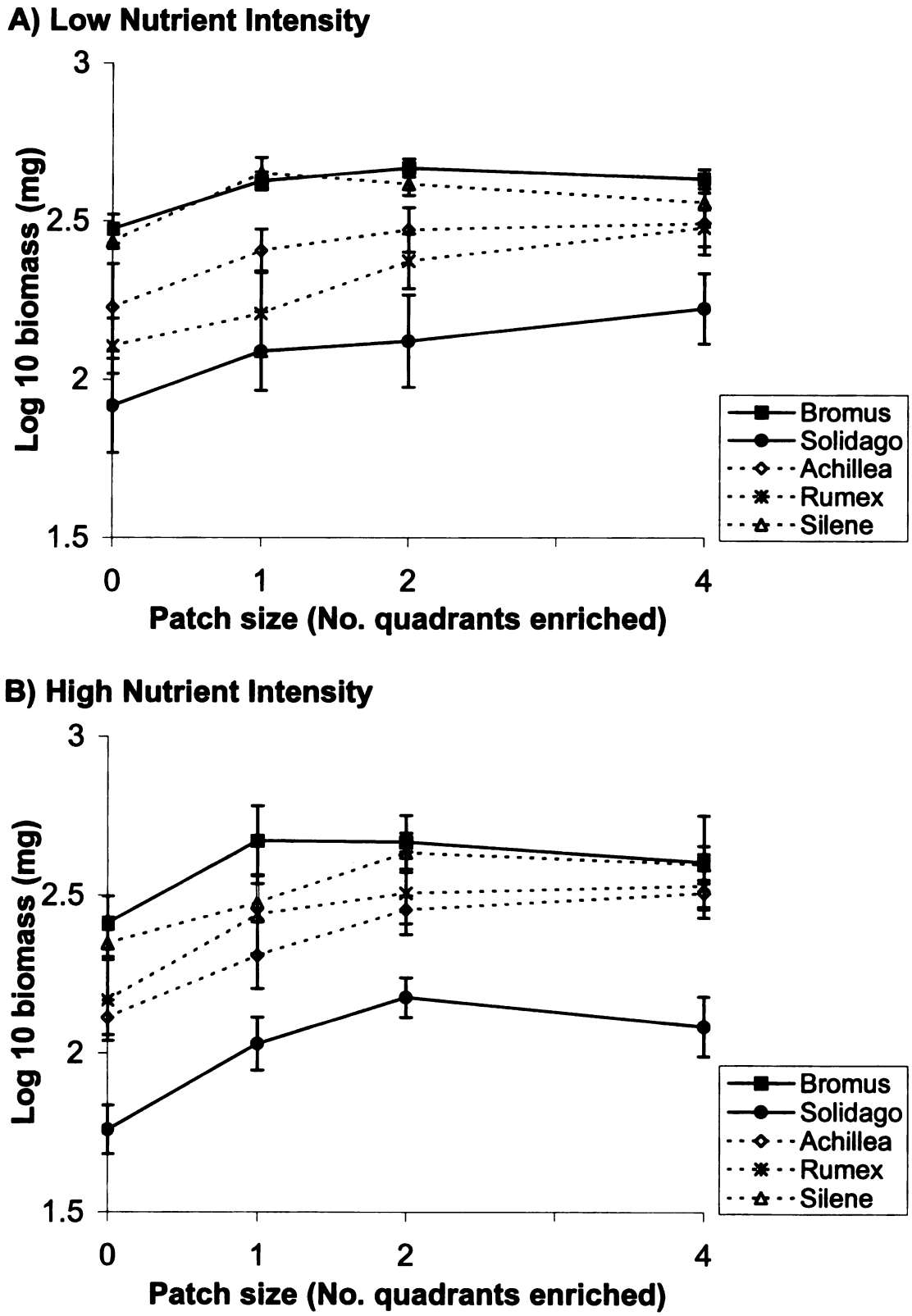

A) Low Nutrient Intensity
Log 10 biomass (mg)
3
2.5
2
1.5
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
Log 10 biomass (mg)
3
2.5
2
1.5
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 2.4

Despite the two-fold difference in fertilizer concentration in the high vs. low nutrient intensity treatments, there was no significant effect of nutrient intensity on biomass (Table 2.1).

Shoot Nitrogen Concentration

The ANOVA for shoot N concentration revealed a significant nutrient intensity x species x patch size interaction (Table 2.1), which indicates that the pattern of response to patch size and nutrient intensity differed among species. Unfertilized control plants (0 quadrants enriched) always had lower N concentrations than plants in the fertilized treatments (1, 2 and 4 quadrants enriched, Figure 2.5). At the high nutrient intensity level, all species, except *Rumex,* had increased shoot N concentrations as patch size increased from small (1 quadrant enriched) to moderate (2 quadrants enriched). In contrast, *Rumex* had a relatively constant N concentration across the three fertilized patch size treatments. In the low nutrient intensity treatments, all five species had similar N concentrations at the larger two patch sizes (2 and 4 quadrants enriched), but *Rumex* and *Bromus* tended to have lower N concentrations at the smallest patch size. There was sufficient variation in shoot N concentration among replicates to produce a significant block effect in the analysis.

Plant Nitrogen Content

Plant nitrogen content is a function of both nitrogen acquistion and growth rate. Differences among species in growth rates, or in the rate of conversion of nitrogen to biomass, can result in biomass or N concentrations alone giving an incomplete assessment of plant response to nutrient treatments. Thus, I also examined the total N incorporated by each species during the course of the experiments. I used total plant N content as an estimate of total N acquired. The ANOVA of plant N content showed significant species x patch size and species x nutrient intensity interactions, indicating that species differed in their pattern of

Figure 2.5 - Nitrogen concentration (%N per g dry weight) in shoot tissue $\pm$ S.E. for two dominant (solid line) and three subordinate (dashed line) species at a) low and b) high nutrient intensity levels.

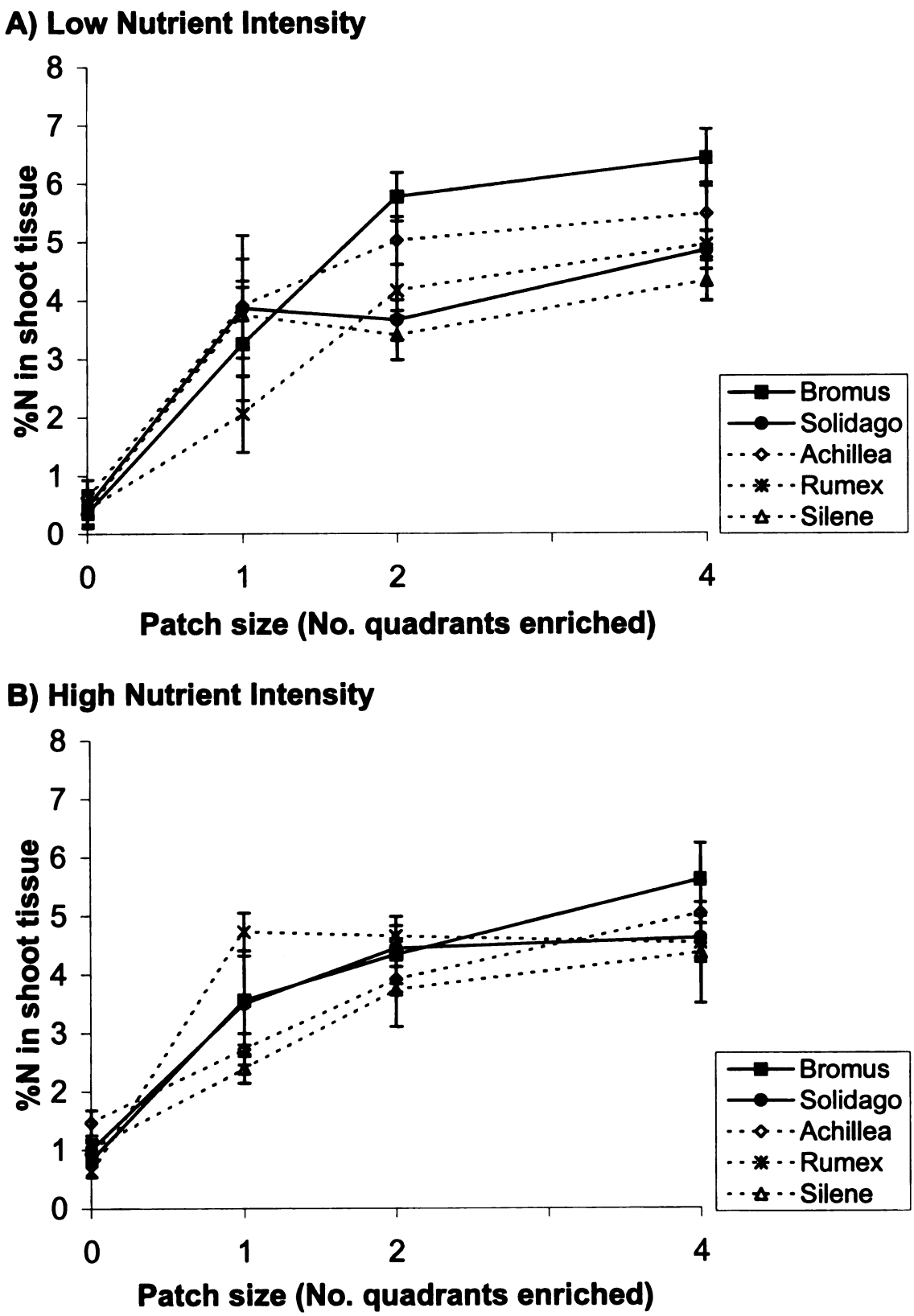

A) Low Nutrient Intensity
%N in shoot tissue
0
1
2
3
4
5
6
7
8
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
%N in shoot tissue
0
1
2
3
4
5
6
7
8
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 2.5

response to patch size and to nutrient intensity. *Bromus* acquired the most nitrogen of any species (Figure 2.6), and it obtained more N at the high nutrient intensity level than the low nutrient intensity level (2.29 vs 1.16 mg, 2.62 vs 2.31 mg and 3.62 vs. 2.50 mg at small, medium and large patch sizes respectively). The other four species acquired about the same amount of total nitrogen at both nutrient intensities (ranging from 0.4-1.8 mg).

The amount of N acquired by *Bromus* also varied with patch size. *Bromus* acquired less N at the small patch size (1 quadrant enriched) in the low nutrient intensity level, and in the small and medium patch sizes (1 and 2 quadrants enriched) at the high nutrient intensity level, than at the largest patch size (4 quadrants enriched; Figure 2.6). *Solidago* acquired the least nitrogen of any species, but the amount of N it obtained was constant regardless of patch size (1, 2 or 4 quadrants enriched) or nutrient intensity (Figure 2.6). *Rumex*, *Achillea*, and *Silene* were intermediate between *Solidago* and *Bromus* in their N acquisition, and in general tended to show a gradual increase in N content as patch size increased.

DISCUSSION

There were differences among these five old field species in the pattern of response to patch size and intensity. However, the species differences were not consistent with the hypothesis that dominant species will exhibit a scale-foraging strategy and subordinate species exhibit a precision-foraging strategy. The magnitude and pattern of response to patch size depended on which parameter was used to assess the response. There were no differences among species in the pattern of biomass response to patch size or nutrient intensity (i.e. no significant species x patch size or species x nutrient intensity interactions), but there were differences among species when shoot nitrogen concentration or plant nitrogen content were used to assess the pattern of response to nutrient heterogeneity. Of the measured parameters, nitrogen content was the most informative measure of plant performance, as it combined

Figure 2.6 - Total plant nitrogen content $\pm$ S.E. for two dominant (solid line) and three subordinate (dashed line) species at a) low and b) high nutrient intensity levels.

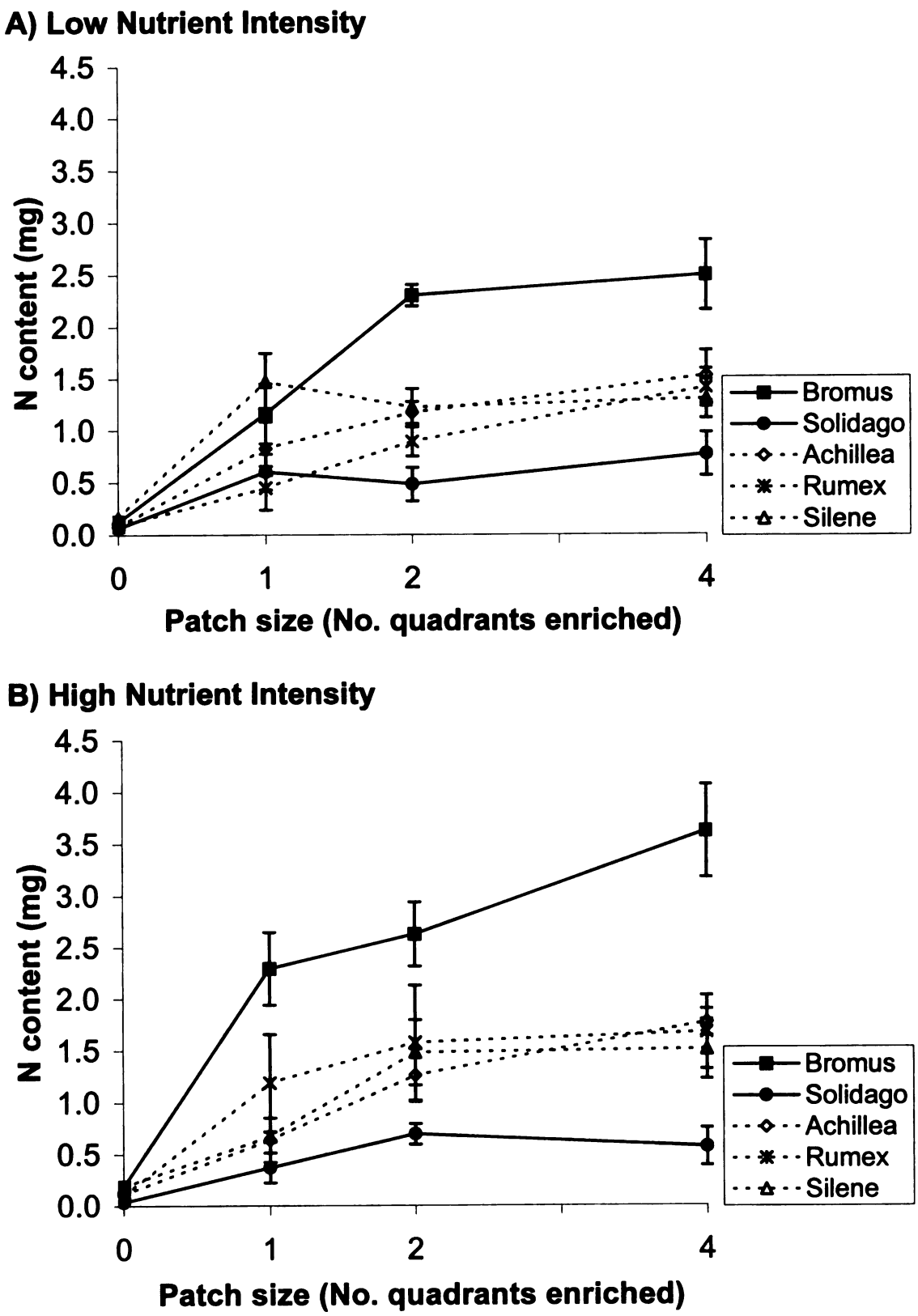

A) Low Nutrient Intensity
N content (mg)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
N content (mg)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 2.6

biomass and nitrogen concentration to give an index of the total nitrogen acquired by the plant.

While I found significant differences among species in biomass and nitrogen acquired, they were not consistently related to the species' biomass abundance in old fields at KBS. My initial expectation was that dominant species would be scale foragers, but only one of the dominant species (*Bromus*) exhibited the pattern of performance across changing patch sizes that was expected of a scale forager. The other dominant species (*Solidago*) and the three subordinate species (*Achillea, Rumex,* and *Silene*) had patterns of response to patch sizes that were consistent with precision foraging. The scale forager (*Bromus*) had a "partially responsive" pattern of performance (see Figure 2.1). *Bromus* acquired the most N of all the species, but the amount of N acquired declined as patch size decreased. In contrast, the four species that were precision foragers had a "responsive" pattern of performance (see Figure 2.1), acquiring the same amount of N across all patch sizes, but not acquiring as much N in total. There were indications that the three subordinate species might not have as responsive a performance pattern as originally expected. *Achillea* had slightly lower performance in the small patch size at both nutrient intensities and *Rumex* had slightly lower performance when patch size and nutrient intensity were both lower. *Silene*'s performance decreased only when nutrient concentrations in soil were very high (as in the 1 quadrant patch of the high intensity level). Improved statistical power and/or an expanded range of patch sizes and intensities would bring these patterns into sharper focus. The trade-off between scale and precision in foraging appears to be a trade-off between ability to acquire a large amount of nutrients vs. ability to maintain constant acquisition levels across a range of patch sizes.

I also predicted that precision foragers would be responsive at both high and low nutrient intensity, while the performance of scale foragers in patches would drop relative to the performance in the equivalent homogeneous environment at lower nutrient intensities. I

did not observe this pattern. Although the pattern of N acquisition across patch sizes did not vary between nutrient intensity levels (no significant intensity x species x patch size interaction), *Bromus*, the scale forager, was the only species to acquire less N in total at the lower nutrient intensity than the higher nutrient intensity.

Species with similar growth forms (e.g. functional groups), particularly those that are closely related species, could be expected to be more similar in the traits they possess, and thus have more similarity in ability to respond to heterogeneous nutrient supply. However, in this study the two Asteraceae species (*Achillea* and *Solidago*) were not more similar in their pattern of performance than any of the other species. *Bromus*, which performed very differently from the other species, is a grass, and grasses can have different patterns of response than dicotyledons (Grime 1994, Taub and Goldberg 1996). However, preliminary data from *Poa compressa*, a grass species found as a subordinate in the same communities, suggests that its pattern of response to patch size and nutrient intensity is more similar to that for the other subordinate species in this study than it is to *Bromus* (see Appendix).

Another factor that may influence a species' ability to respond to nutrient heterogeneity is whether or not a plant forms mycorrhizal associations. Species that form mycorrhizal associations are often less able to adjust their root systems to patches in heterogeneous environments (Hetrick *et al.* 1991, Hetrick 1991). My results do not support the expectation of amyccorhizal species being more precise in their foraging. Both of the amycorrhizal species I used (*Rumex* and *Silene*) were not more responsive than the other species. Furthermore, *Rumex* and *Silene* differed from one another in their responsiveness to nutrient intensity.

Conclusions

This study provides some support for the hypothesis of Campbell *et al.* (1991) that there is a trade-off between scale and precision in resource foraging by plants in heterogeneous environments. In these experiments, I found that species patterns of nitrogen acquistion fell into only two categories: those species that acquired more nitrogen overall, but whose nitrogen acquisition dropped as patchiness increased (scale foragers) and those species that acquired less total nitrogen, but whose nitrogen acquisition stayed constant as patchiness increased (precision foragers). There were no species that were able to both acquire high amounts of nitrogen and keep their acquisition constant across the range of patch sizes. This basic trade-off could lead to differences in competitive ability or performance in the field. If the precision forager is maintaining a constant or improved performance under heterogeneous conditions while a scale forager's performance declines, then the precision forager may experience less interspecific competitive effects from the scale forager under heterogeneous conditions. So a trade-off in foraging strategy may provide a means by which less competitive subordinate species can persist: increased plasticity and precision versus the faster growing but less precise dominant species.

However, for the species I studied, the differences in response to nutrient heterogeneity are not related to biomass abundance in the field. I classified species as dominant or subordinate by using field biomass abundance, whereas Campbell *et al.* (1991) used a lab competition experiment to determine a competitive hierarchy. It could be that some of the species I used have higher or lower abundance in the field for reasons other than their ability to compete for nutrients, or that some other factor besides abundance influences whether or not a given species exhibits a scale or precision foraging strategy. The trade-off may not be exhibited by differences between dominant and subordinate species, *per se*, but in large versus small species. In plant communities, dominant species are also usually the larger

species, so a strategy that is correlated with large plant size will also appear to be correlated with dominance. In this experiment, the seedlings of one of the dominant species (*Solidago*) were also the smallest, and it was the species with the most precise foraging. It may be that the precision with which a species can forage will decrease as the species grows larger and forages over a wider scale. In field conditions, plants of a given species may be of different sizes (and ages), so the ability to forage with precision while small will help the plant survive until it grows large enough that precision no longer is necessary. A patch of a small absolute size will be relatively larger to a small plant than to a large one, so the relative benefit of exploiting the patch will be larger for a small plant than a large one.

This study also demonstrated the importance of the scale and magnitude of heterogeneity in determining the response of a species. When patches are larger in size and higher in concentration, these five species will perform as well as if the nutrients were homogeneously distributed around the roots. In environments with a coarse scale of heterogeneity plants could compete with each other to exploit patches in a similar fashion as they would compete in a homogeneous environment. However, as the size and intensity of patches decrease, differences among species in ability to detect and respond will become more important. The trade-off between scale and precision in resource foraging could be key in reducing competition between species. Large dominant scale foragers would have relatively poorer performance under highly heterogeneous conditions, while the precision foragers would be able to maintain their performance and thus be relatively more competitive in heterogeneous environments.

Chapter 3

MECHANISMS OF RESPONSE TO NUTRIENT HETEROGENEITY

INTRODUCTION

The ability of plants to obtain nutrients in patchy environments is well documented (Robinson 1994 and references therein). However, species differ in their ability to respond to nutrient heterogeneity at scales smaller than individual plant root systems (Campbell *et al.* 1991, also see Chapter 2). Differences in the ability of species to adjust to heterogeneously distributed resources may influence interspecific competitive interactions and community structure in spatially heterogeneous environments. A vital component of linking the responses of individual plants to resource heterogeneity to community structure is understanding the mechanisms of plasticity used by plants and relating those mechanisms to plant performance (Casper and Jackson 1997).

Plants can use a variety of mechanisms to increase nutrient acquisition in response to nutrient heterogeneity. It is well documented that plant species can proliferate roots in nutrient patches (see review by Robinson 1994 and references therein) and some species adjust the nutrient uptake rate of roots in enriched patches (Drew and Saker 1975, Robinson and Rorison 1983, Jackson *et al.* 1990, Caldwell *et al.* 1992, Caldwell 1994, Robinson 1994, van Vuuren *et al.* 1996). Plants also may change the rate of root turnover (Gross *et al.* 1993) or the branching architecture of the root system (Fitter 1994) in response to nutrient heterogeneity.

It will not be advantageous for a plant to expend energy to exploit a nutrient patch

unless there is sufficient benefit (on average) in the amount of nutrients acquired to compensate for the cost of obtaining those nutrients (Robinson 1996, Gleeson and Fry 1997). The construction of new roots, or new cells within a root, likely involves a greater expenditure of carbon and energy than to adjust the function of cells or roots that already exist. Physiological responses would be expected to occur before responses involving new root growth (Robinson 1996). Thus, the type of response mechanism a plant utilizes will influence the cost of acquiring nutrients. The concentration, size and duration of an enriched nutrient patch will influence the potential nutrients available to the plant and thus the benefit to the plant. The net benefit will be determined by how well a plant can maximize nutrient uptake while minimizing costs (Eissenstat 1992, Robinson 1996). For example, if a plant allocates energy to increase root biomass, it faces a trade-off between producing short, highly branched roots that cover a smaller soil volume, and thoroughly spread throughout that volume, versus producing longer, less branched roots that exploit a larger soil volume, but with less intense packing of roots within that volume. Campbell *et al.* (1991) suggested that this is a trade-off that characterizes whether a plant will be a precision or scale forager. An increased scale of foraging allows a plant to encounter more patches in a heterogeneous environment, while increased precision of foraging is a means of more thoroughly exploiting those patches that are encountered.

If the trade-off between precision and scale of foraging is important for plants, then one could expect there to be differences between precision- and scale-foraging species in the mechanisms they use to adjust to small scale nutrient heterogeneity (Fitter 1994, Grime 1994). A plant using a highly precise foraging strategy would be expected to have greater plasticity of response. Thus, it should be more plastic in both the mechanisms it can use to respond to patches and the range of conditions under which it utilizes those mechanisms. Plants using a scale foraging strategy would be less likely to detect patches they encounter and instead

expand the root system into the largest possible area. Scale foragers would be expected to have less plasticity in foraging, and so would use fewer types of mechanisms to respond to nutrient patches and would not respond to patches that are small, relative to the size of the root system, or of lower nutrient intensity, relative to the nutrient levels in the rest of the soil.

In this study, I compared five perennial species (*Achillea millefolium, Bromus inermis, Rumex acetosella, Silene latifolia* and *Solidago canadensis*) that commonly occur in Southwest Michigan old-fields in a series of greenhouse experiments to determine their responses to varying nutrient patch sizes and intensities. *Bromus* and *Solidago* are dominant species in these communities and *Achillea, Rumex*, and *Silene* are subordinate species. Based on the pattern of nitrogen acquisition across a range of patch sizes (see chapter 2), I classified *Bromus* as a scale forager and the other four species (*Solidago*, *Achillea*, *Rumex* and *Silene*) as precision foragers. Of these four species, *Achillea*, *Rumex* and *Silene* have a tendency to be less precise than *Solidago*.

In this chapter, I investigated three possible mechanisms of response to nutrient heterogeneity: root branching architecture, biomass allocation and nutrient uptake rates. Based on the hypothesis that there is a fundamental trade-off between the scale and precision of root foraging that leads to differences in the plasticity of root systems, I expected that:

1) Scale and precision foragers would differ in the mechanisms used to respond to heterogeneous nutrients. Precision-foraging species were also expected to use more mechanisms that scale-foraging species.

2) Precision-type species would respond across all patch sizes and intensities, scale-type species would only respond at larger patch sizes (or homogeneous enrichment).

3) For a mechanism used by both precision- and scale-foraging species, precision species were expected to have a finer degree of control within the root system. That is, the degree to which roots in enriched patches differ from roots in unenriched areas would be

greater in precision foragers than scale foragers.

METHODS

Each of the five species was exposed to four patch size treatments: control, small, medium and large (0, 1, 2 and 4 quadrants enriched, respectively; see Chapter 2, Figure 2.3) at two nutrient intensity levels. The experiment was repeated six times at each nutrient intensity level. Chapter 2 contains a detailed description of the growth conditions and experimental apparatus.

Root Architecture

To assess if root architecture patterns change in response to patch size and nutrient intensity, I sampled the largest unbroken piece of root harvested from two randomly selected quadrants in the control and large patch treatments (0 and 4 quadrants enriched, respectively) and from one patch and one background quadrant for small and medium patch treatments (1 and 2 quadrants enriched, respectively). Roots were dyed using Safranin O and then laid out on clear acetate sheets. I used forceps to tease apart roots so that their complete branching structure was visible. A second acetate sheet was placed on top of each sample for protection and the samples were allowed to air dry. I photographed each sample using black and white print film (Kodak TMAX 400) and developed the images onto CD-ROM. These digitized images were processed using Adobe Photoshop version 3.0 (Adobe Systems Inc. 1994). Architectural indices were calculated using the BranChing program (Bernston 1992, Online).

I used both topological (arrangement of links or branches within the root system, following Fitter 1987) and geometrical (link size or density of branching) indices of root branching to quantify architecture. Root system topology can vary between two extremes: perfectly herringbone and completely dichotomous (see Figure 3.1) with a random pattern of

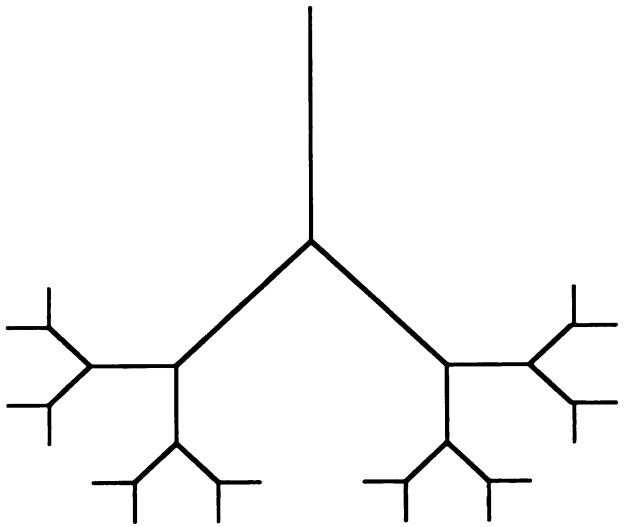
branching as an intermediate. Herringbone root systems are predicted to minimize the overlap of root depletion zones in the soil, while dichotomous systems have more efficient nutrient transport within the plant (Fitter 1985, 1987). As nutrient levels increase, depletion zones are less critical (especially for mobile nutrients) and producing a root system with better transport efficiency will benefit the plant (Fitter 1987). This would lead to the expectation that plants using this response mechanism move from more herringbone to more dichotomous branching. Fitter (1994) found evidence that faster-growing species are more likely to use changes in root topology as a response to nutrient patches, so I expected that scale-foraging species would use this mechanism and precision-foraging species would not.

I used a topological index of a/E(a) where a= altitude (# links in longest path from exterior link to top of root system) and E(a) is the expected altitude of a root system of that size (total # links) given random growth (Fitter 1994). A herringbone root branching pattern gives the highest a/E(a) index and a dichotomous branching pattern gives the lowest (Fitter 1987, Fitter *et al.* 1991). After measuring the altitude of a sample, the expected altitude was calculated by the software program BranChing (Bernston 1995, Online) using formulae based on Werner and Smart (1973). If a plant responds to nutrient patches by changing its branching pattern to be more dichotomous, a/E(a) will decrease. Although topological indices are usually calculated for whole root systems, sub-samples can be assessed if they are cut randomly from the system and one assumes that topology is consistent across the whole system (Van Pelt and Verwer 1984, Fitter and Stickland 1992).

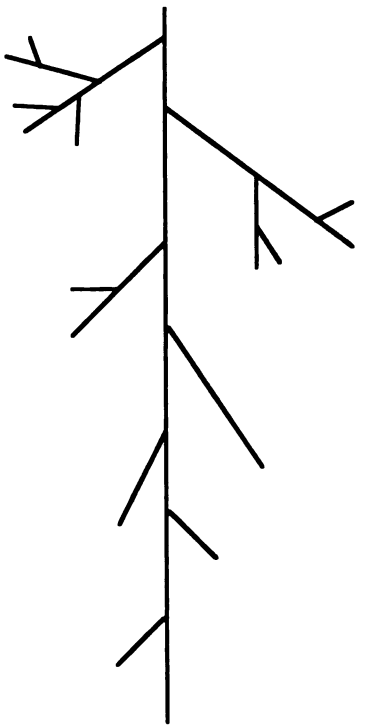
I calculated mean segment length (MSL) on the same root samples used for the topological index, by dividing total root length by the number of segments (or links) as measured by BranChing. MSL is an index of root system geometry that assesses the density of branching in the roots; as MSL decreases, the root system becomes more densely

Figure 3.1 - Types of branching possible in root systems (adapted from Taub and Goldberg 1996). Magnitude = total number of external links. Altitude = maximum pathlength possible from an external link to the top of the root system.

Dichotomous Branching
magnitude = 16
altitude = 5



Random Branching
magnitude = 16
altitude = 8



Herringbone Branching
magnitude = 16
altitude = 16

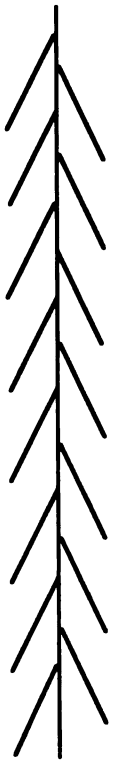


Figure 3.1

branched. I expected that precision foraging species would be more likely to use this mechanism of response to nutrient patches.

Biomass Allocation

The root biomass from each quadrant of the pot was kept separate as it was harvested, and the total root dry weight plus that of any sub-samples used in other analyses were added to give total root biomass per pot quadrant. I summed the root biomass from each quadrant to get the total root biomass for each plant. I calculated root:shoot ratio from dried shoot and root biomass to test whether allocation between roots and shoots changed across treatments. To assess whether plants were selectively allocating more root biomass to enriched patches, I compared the biomass of roots from two different quadrants for each plant. I randomly selected two quadrants from each pot in the control and large patch treatments (0 and 4 quadrants enriched, respectively). I compared the enriched patch to a randomly selected background quadrant in the small patch treatment (1 quadrant enriched). In the medium patch treatment (2 quadrants enriched), I compared a randomly selected patch quadrant to a randomly selected background quadrant.

Nutrient Uptake Rates

To assess if resource heterogeneity affected nutrient uptake rates, I took a sub-sample of root from two quadrants when the plants were harvested. For the control and large patch treatments (0 and 4 quadrants enriched, respectively), I sampled a randomly chosen quadrant and its counter-clockwise neighbor. For the small and medium patch treatments, I sampled the enriched patch quadrant (or randomly chose one of the two patch quadrants), and its neighboring background quadrant in a counter-clockwise direction. These excised roots were then used immediately to conduct an ^{15}N enrichment assay (adapted from Kosola and Bloom

1994, 1996 and Jackson and Reynolds 1996). I placed the excised roots in an aerated equilibrating solution (2 mM $CaSO_4$) for 10 minutes, then added labelled $K^{15}NO_3$ (0.5 ml of 0.1 M $K^{15}NO_3$ in 500 mL of 2 mM $CaSO_4$) and let the roots soak for 30 min. The samples were aerated and kept in a 25°C water bath to maintain constant temperatures and keep solutions well mixed. I then rinsed the root samples in water to remove excess solution, and soaked them for 2 min in an unlabelled nitrogen solution (0.5 mL 0.1M KNO_3 in 500 mL of 2mM $CaSO_4$) to rinse labelled N off the exterior surfaces of the roots. I allowed samples to air dry before placing them in a drying oven for several days at 45°C. I ground the samples in microfuge tubes with two ball bearings inside using a dental amalgamator and then weighed out sub-samples of 1-5 mg into tin cups for analysis on an ANCA-MS mass spectrometer (Harris and Paul 1989). I was only able to analyze two of the six replicates for each nutrient intensity level, due to budget and time constraints.

As an indirect assessment of uptake rate, I measured root N concentrations in four of the six replicates at the high nutrient intensity level and in five of the six replicates at the lower nutrient intensity level. I used the CN protocol described in Chapter 2 to measure root nitrogen concentration (%N per g dry weight). Again I chose samples randomly where possible, from two quadrants in the control and large patch treatments, or a patch and a background quadrant in the small and medium patch treatments. As this was the last set of sub-samples taken from the root systems, in many cases there were only two quadrants that had sufficient tissue left to provide a sample for CN analysis. These measures of root N concentrations are the result of both uptake into the roots and transport from the roots, and so N concentrations will not provide as robust a measure of uptake. Nevertheless, the data will allow estimation of general differences between species and treatments.

Statistical Analyses

Statistical analyses were performed using Systat 5.0 for Windows (Systat Inc. 1992). I performed a 4-factor repeated measures mixed model ANOVA. Nutrient intensity, patch size and species were fixed effects, while block was random. Block effects were nested within nutrient intensity level, because blocks were replicated through time and the blocks at high nutrient intensity were run at different times than the blocks at low nutrient intensity. The model was left unbalanced where data was missing. Root biomass and MSL were log transformed to improve normality and reduce heteroscedasticity.

I used a repeated measures analysis because every experimental unit (= 1 plant) was measured twice for each root system variable. I sampled either one patch and one background for small and medium patch treatments (1 and 2 quadrants enriched) or two quadrants for control and large patch treatments (0 and 4 quadrants enriched). For each variable I measured, the between-subjects component of the analysis is the average of the two quadrants and estimates of differences between species, nutrient intensity and patch size treatments are based on this average. The between-subjects component thus provides information as to how the whole root system of these species responded to resource heterogeneity. The within-subjects components of the analysis measures the variation between the two samples from different quadrants of a pot. This provides information on how individual root systems varied across species, patch size and nutrient intensity levels.

RESULTS

Whole Root System Architectural Indices

For both of the architectural indices (altitude/expected altitude (a/E(a)) and mean segment length (MSL)), the values calculated for the two sampled quadrants in a pot were averaged for the between-subjects component of the ANOVA, providing an estimate of the

mean architectural index for the whole root system. This analysis thus provides information on any differences in the architectural indices among species, patch size or nutrient intensity. There was a significant species effect on root systems topology (a/E(a); Table 3.1), but the effect was driven by *Bromus*, the scale forager, having a higher index (or more herringbone-like root systems) than the other species (Figure 3.2). There was also a significant patch size effect on root topology. The a/E(a) index was slightly lower (indicating a more random branching topology) in the three fertilized treatments (small, medium and large patch size) than in the unfertilized control treatment (Figure 3.2). The decrease in a/E(a) index in response to enriched patches appears to be driven by *Silene* at the low nutrient intensity (Figure 3.2a) and *Bromus* at the high nutrient intensity (Figure 3.2b). However, there were no significant interactions, indicating that species did not differ significantly in the pattern of a/E(a) values across patch sizes.

MSL was more variable among species and there were significant species x patch size interactions, indicating that some species responded differently to the patch size treatments (Table 3.1). Both *Achillea* and *Solidago* had lower MSL, and thus increased branching density in the fertilized treatments (small, medium and large patch size), while the other three species did not change in MSL relative to the unfertilized control (Figure 3.3). *Achillea* and *Solidago* also had shorter MSL in the control treatment at the lower nutrient intensity, giving a significant species x nutrient intensity interaction. *Bromus*, *Rumex* and *Silene* all had shorter MSL in the unfertilized control treatment than *Achillea* and *Solidago*. This suggests that *Bromus*, *Rumex*, and *Silene* have high root densities that did not allow them to adjust their roots to decrease MSL further in the fertilized treatments.

Table 3.1 - F-values from repeated measures analyses of variance presented with degree of significance (*** $P<0.001$; ** $P<0.01$; * $P<0.05$). Between-subjects values summarize differences between root systems of individual plants. Within-subjects values estimate differences between quadrants within plants. † df=8 for Rep (Nutrient intensity) and Quadrant x Rep (NI) because only 9 of the 12 experimental replications were analyzed for nitrogen concentrations.

		Variable			
Between-subjects	df	Log_{10} Root Biomass	a/E(a)	Log_{10} MSL	Root N Concentration†
Nutrient intensity	1	0.816	2.404	8.423 **	2.217
Rep (Nutrient intensity)	11	4.952 ***	0.899	0.642	3.309 **
Species	4	47.914 ***	34.640 ***	152.196 ***	22.853 ***
Patch Size	3	1.130	3.714 *	7.502 ***	175.818 ***
NI x S	4	3.473 **	1.011	5.182 **	1.115
NI x PS	3	0.357	2.058	1.083	6.960 ***
S x PS	12	0.934	0.853	2.080 *	6.633 ***
NI x S x PS	12	0.597	0.872	1.108	1.260
Within-subjects					
Quadrant	1	9.744 **	1.853	2.041	15.574 ***
Quadrant x NI	1	3.061	0.307	0.939	0.010
Quadrant x Rep (NI)	11	0.933	0.891	1.111	0.486
Quadrant x S	4	0.227	3.216 *	2.758 *	3.284 *
Quadrant x PS	3	4.560 **	0.932	2.659 *	16.875 ***
Quadrant x NI x S	4	0.218	0.878	3.752 **	0.095
Quadrant x NI x PS	3	2.145	1.753	1.351	4.455 **
Quadrant x S x PS	12	1.417	0.916	0.806	1.730
Quadrant x NI x S x PS	12	1.348	0.889	0.724	1.025

Figure 3.2 - Root architecture index a/E(a) $\pm$ S.E. in relation to patch size for high and low fertilizer levels for each species. The expected altitude is the altitude that would occur if branching was random at a given magnitude (number of root links). a/E(a) $= 1$ means random branching did occur. a/E(a) > 1 indicates more herringbone branching. a/E(a) < 1 indicates more dichotomous branching. Filled symbols with solid lines are dominant species, hollow symbols with dashed lines are subordinate species.

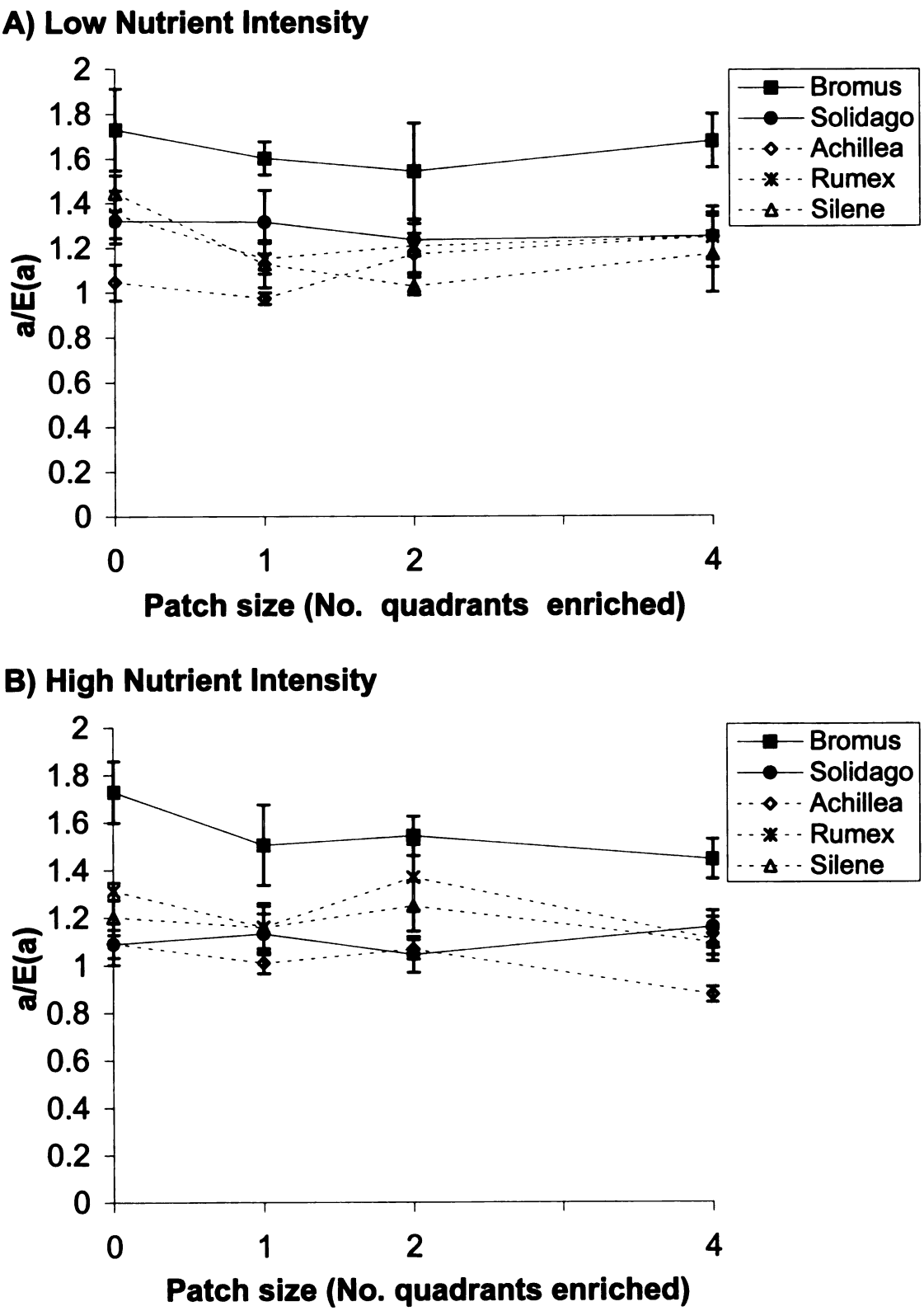

A) Low Nutrient Intensity
a/E(a)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
a/E(a)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 3.2

Figure 3.3 - Mean root segment length $\pm$ S.E. in root samples across patch size treatments at high and low patch intensities for each species. Filled symbols with solid lines are dominant species, hollow symbols with dashed lines are subordinate species.

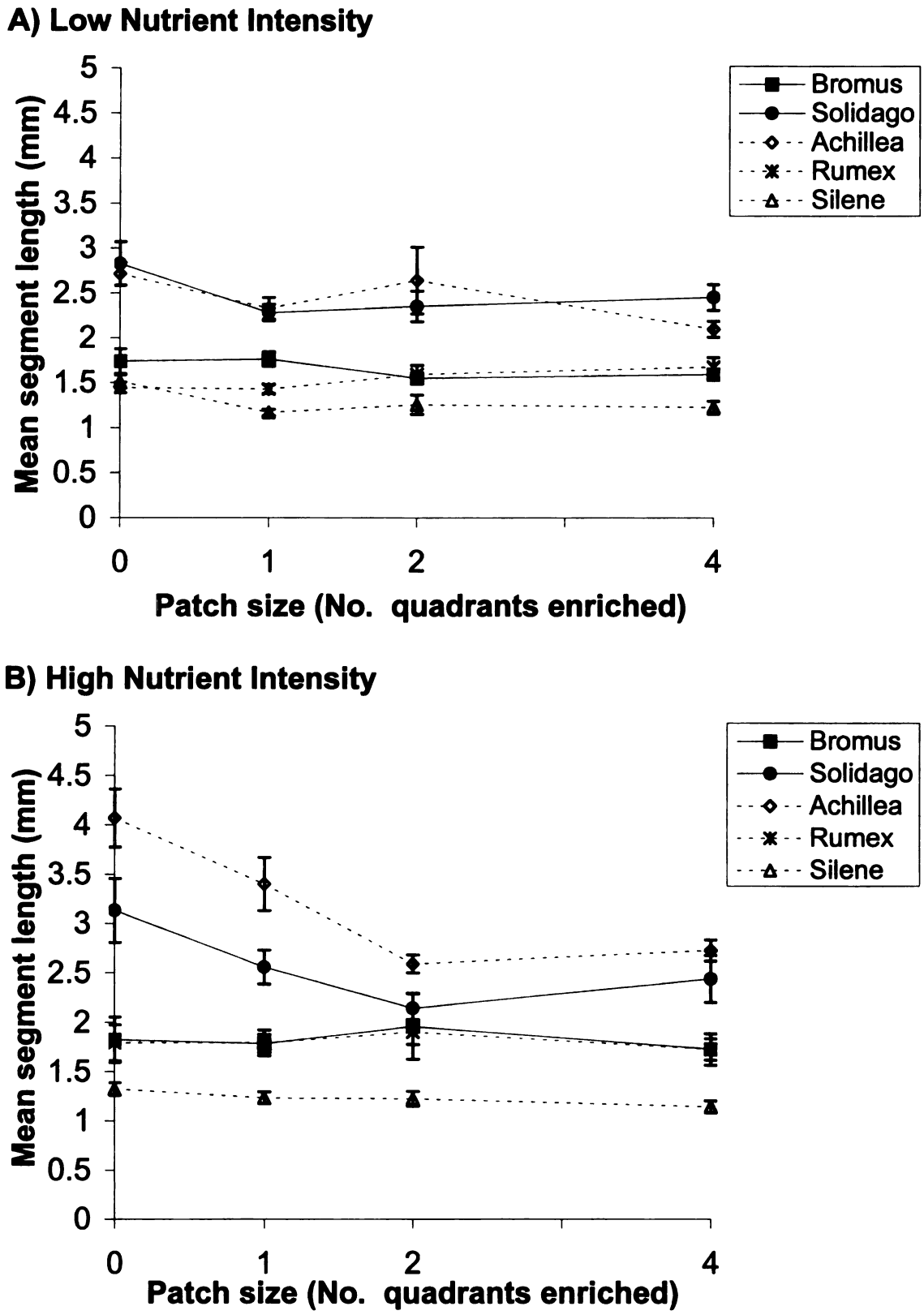

A) Low Nutrient Intensity
Mean segment length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
Mean segment length (mm)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 3.3

Resource heterogeneity effects on root architecture

The within-subjects component of the ANOVA for the topological index is an estimate of the amount of variation between the two quadrants sampled in each pot and indicates whether sub-sections of a root system have different topologies. Within subjects, there was a significant quadrant x species interaction for a/E(a) (Table 3.1), indicating differences among species in variability of a/E(a) between quadrants. In addition to having higher a/E(a) index values, *Bromus* had more variability in a/E(a) within its root systems than the other species (Figure 3.4a, b). There were no significant interactions between species and patch size or nutrient intensity, indicating there was no significant pattern in the a/E(a) index related to either patch size or nutrient intensity. Although there was not a significant pattern, both *Bromus* and *Achillea* had lower a/E(a) values in enriched vs. unenriched sectors at the high nutrient level. This raises the possibility that they might be able to selectively adjust their root architecture in only part of the root system as a response to nutrient heterogeneity.

The within-subjects component of the ANOVA for MSL indicated significant quadrant x species and quadrant x nutrient intensity x species interactions. These interactions were driven by *Achillea* and *Solidago*, both of which had more variation in MSL within their root systems than *Bromus*, *Rumex*, or *Silene* (Figure 3.5). *Achillea* had lower MSL values in the enriched patches than in background quadrants of heterogeneous patch size treatments (1 and 2 quadrants enriched) at both nutrient intensity levels (Figure 3.5e, f). *Solidago* also selectively decreased MSL in enriched patches at the higher nutrient intensity (Figure 3.5d), however, the reverse was found for the 1-quadrant-enriched treatment at low nutrient intensity, where MSL was greater in enriched patches than in the background quadrants (Figure 3.5c).

Figure 3.4 - Topological index [a/E(a)] values $\pm$ S.E. for patch and background quadrants within root systems at high and low nutrient intensity levels for each species. Expected altitude is that generated by random branching at a given magnitude (# of root links). a/E(a) $= 1$ means random branching occurred (dashed line). a/E(a) > 1 means more herringbone branching. a/E(a) < 1 means more dichotomous branching. Open diamonds are the means of root samples from unenriched quadrants. Solid squares are means of root samples from enriched quadrants. All quadrants in the patch size 0 treatment were unenriched. All quadrants in the patch size 4 treatment were enriched.

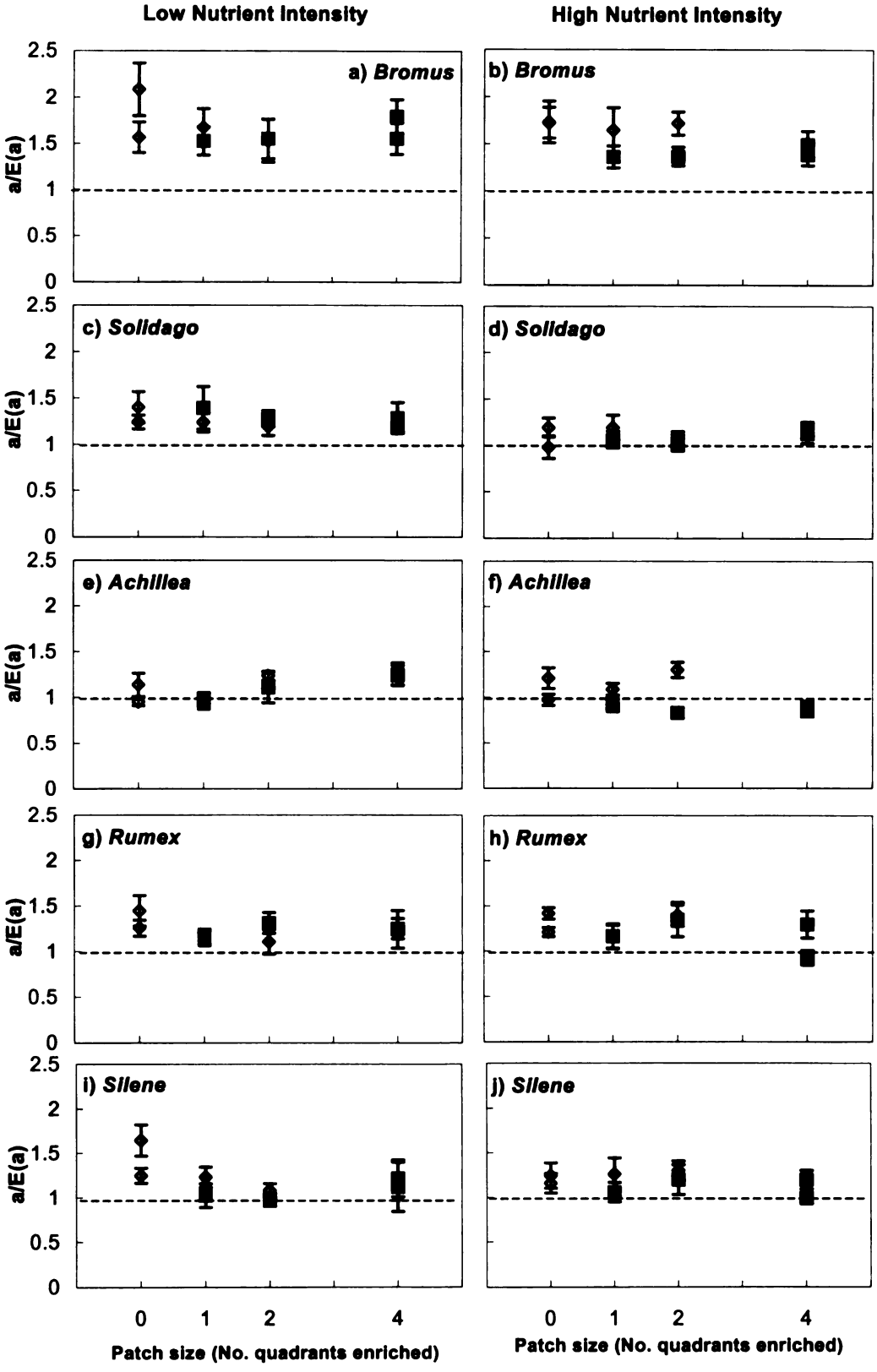

Low Nutrient Intensity
High Nutrient Intensity
a) Bromus
b) Bromus
c) Solidago
d) Solidago
e) Achillea
f) Achillea
g) Rumex
h) Rumex
i) Silene
j) Silene
a/E(a)
2.5
2
1.5
1
0.5
0
0
1
2
4
Patch size (No. quadrants enriched)

Figure 3.4

Figure 3.5 - Patch and background quadrant values of mean root segment length $\pm$ S.E. within root systems at high and low nutrient intensity levels for each species. Open diamonds are the means of root samples from unenriched quadrants. Solid squares are means of root samples from enriched quadrants. All quadrants in the patch size 0 treatment were unenriched. All quadrants in the patch size 4 treatment were enriched.

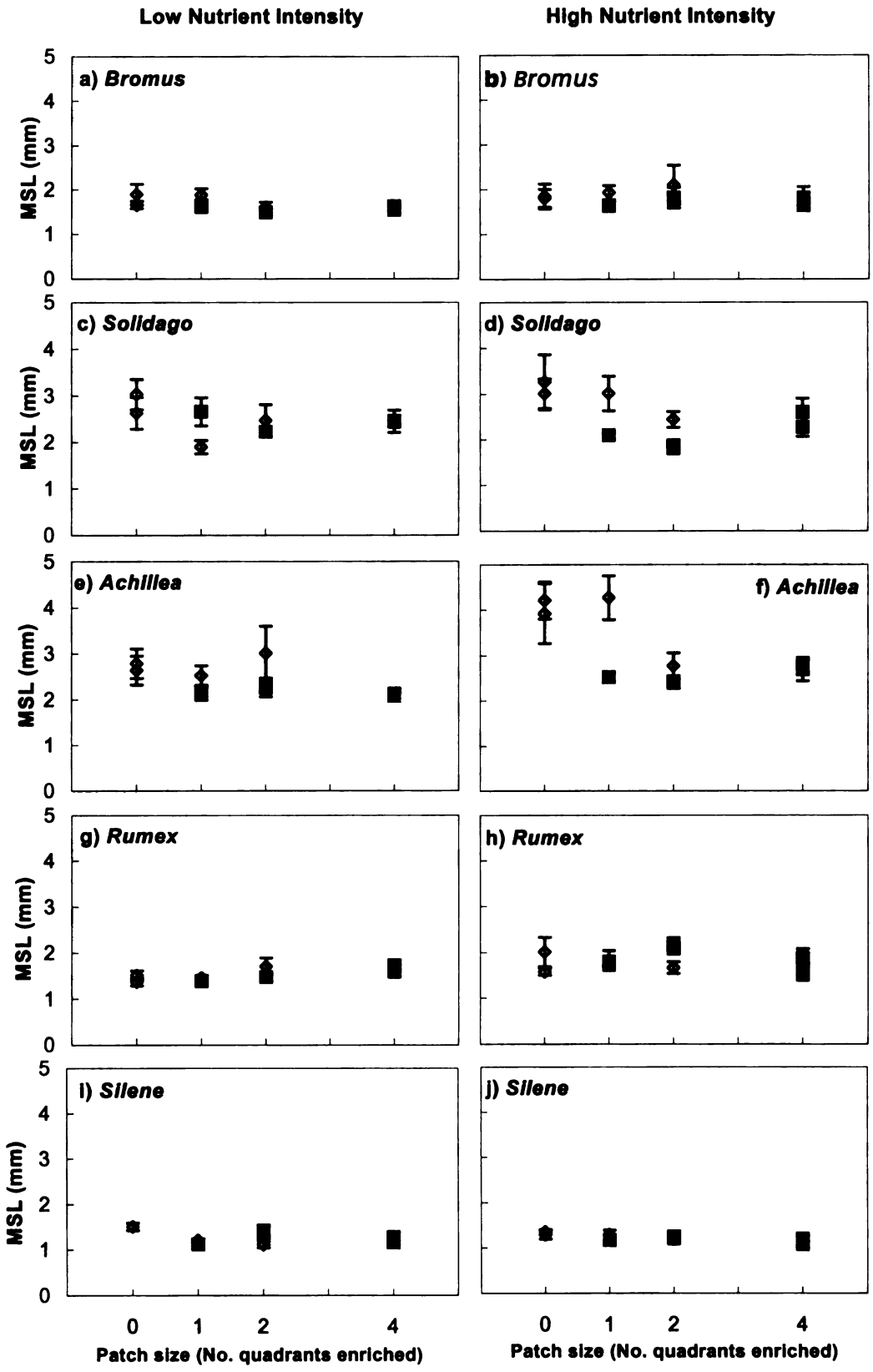

Low Nutrient Intensity
High Nutrient Intensity
a) Bromus
b) Bromus
c) Solidago
d) Solidago
e) Achillea
f) Achillea
g) Rumex
h) Rumex
i) Silene
j) Silene
MSL (mm)
0
1
2
3
4
5
0
1
2
4
Patch size (No. quadrants enriched)

Figure 3.5

Root Biomass

The between-subjects component of the repeated measures ANOVA on $\log_{10}$ root biomass provides information on whether root system biomass differed among species, or across patch size or nutrient intensity. There were significant differences in root biomass between species (Table 3.1); *Bromus* and *Silene* had larger root biomass than *Rumex* and *Silene*, and *Solidago* had the smallest root biomass (Figure 3.6). There was a significant species x nutrient intensity interaction (see Table 3.1), driven by one species (*Achillea*) having less root biomass at high nutrient intensity, and one species (*Rumex*) having more root biomass at high nutrient intensity. The other three species did not differ in root biomass at the two resource intensity levels. There were no differences in total root biomass between patch size treatments for any of the species (no patch size effect and no species x patch size interaction). Although the total root biomass of these species did not change between fertilized and unfertilized treatments, all of the species had larger total plant biomass in the fertilized treatments (see Chapter 2). All species increased shoot and not root production in response to nutrient enrichment. Consequently, the R:S ratio decreased in all species with fertilization (Table 3.1, Figure 3.7).

Biomass allocation within the root system

Although the total root biomass of these species did not vary across the patch size treatments, there were differences among patch sizes in how root biomass was allocated among quadrants in a pot. The within-subjects component of the root biomass ANOVA provides an estimate of the variation between the two quadrants sampled in each pot. There was a significant quadrant x patch size treatment interaction (see Table 3.1, within-subjects effects), suggesting that the variation in root biomass between quadrants varied across patch size treatments. All the species had greater root allocation in the enriched patches of the

Figure 3.6 - Average root biomass per plant $\pm$ S.E. at high and low patch intensities for each species. Filled symbols with solid lines are dominant species, hollow symbols with dashed lines are subordinate species.

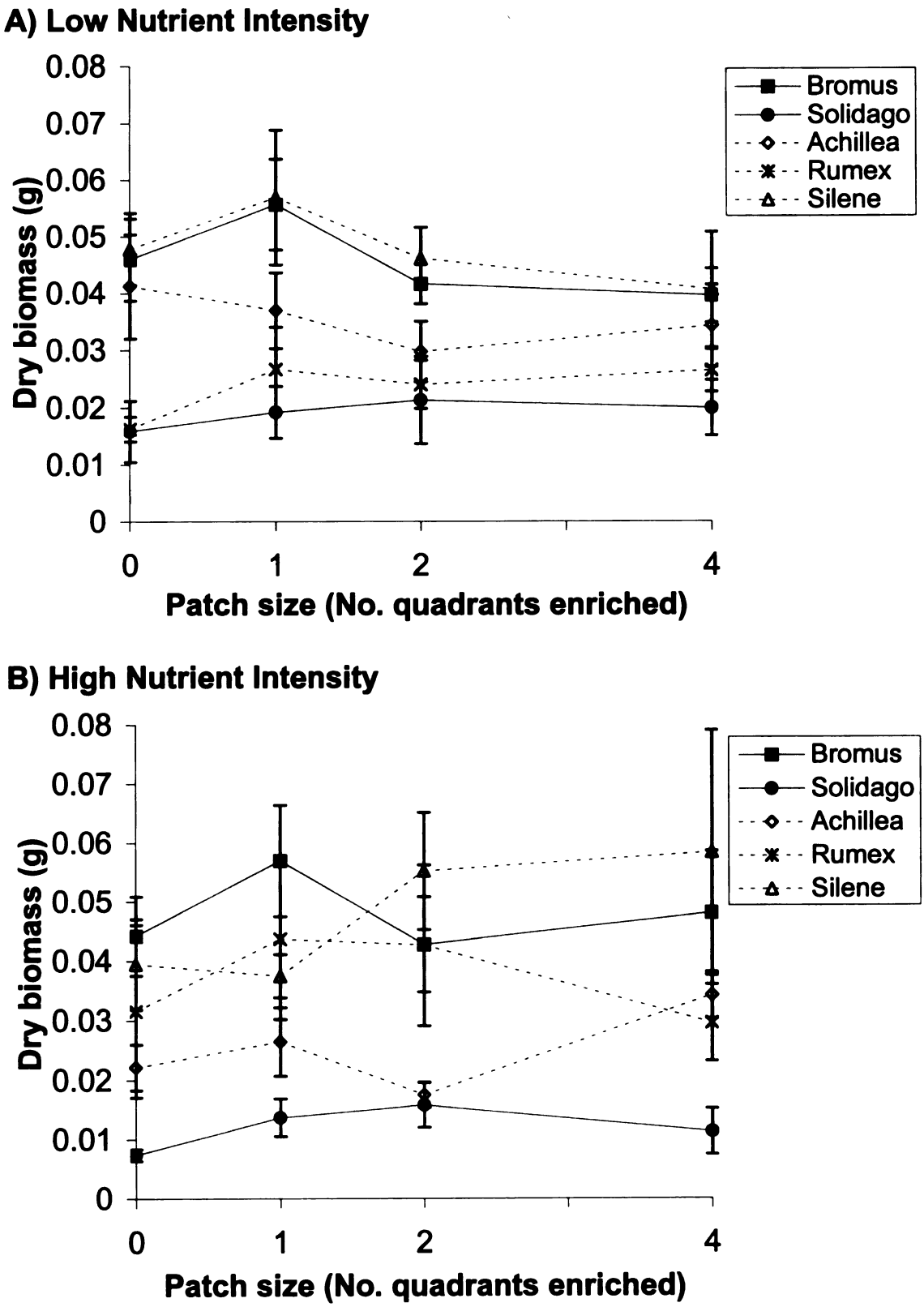

A) Low Nutrient Intensity
Dry biomass (g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
Dry biomass (g)
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 3.6

Figure 3.7 - Root:shoot ratios $\pm$ S.E. at high and low fertilizer levels for each species. Filled symbols with solid lines are dominant species, hollow symbols with dashed lines are subordinate species.

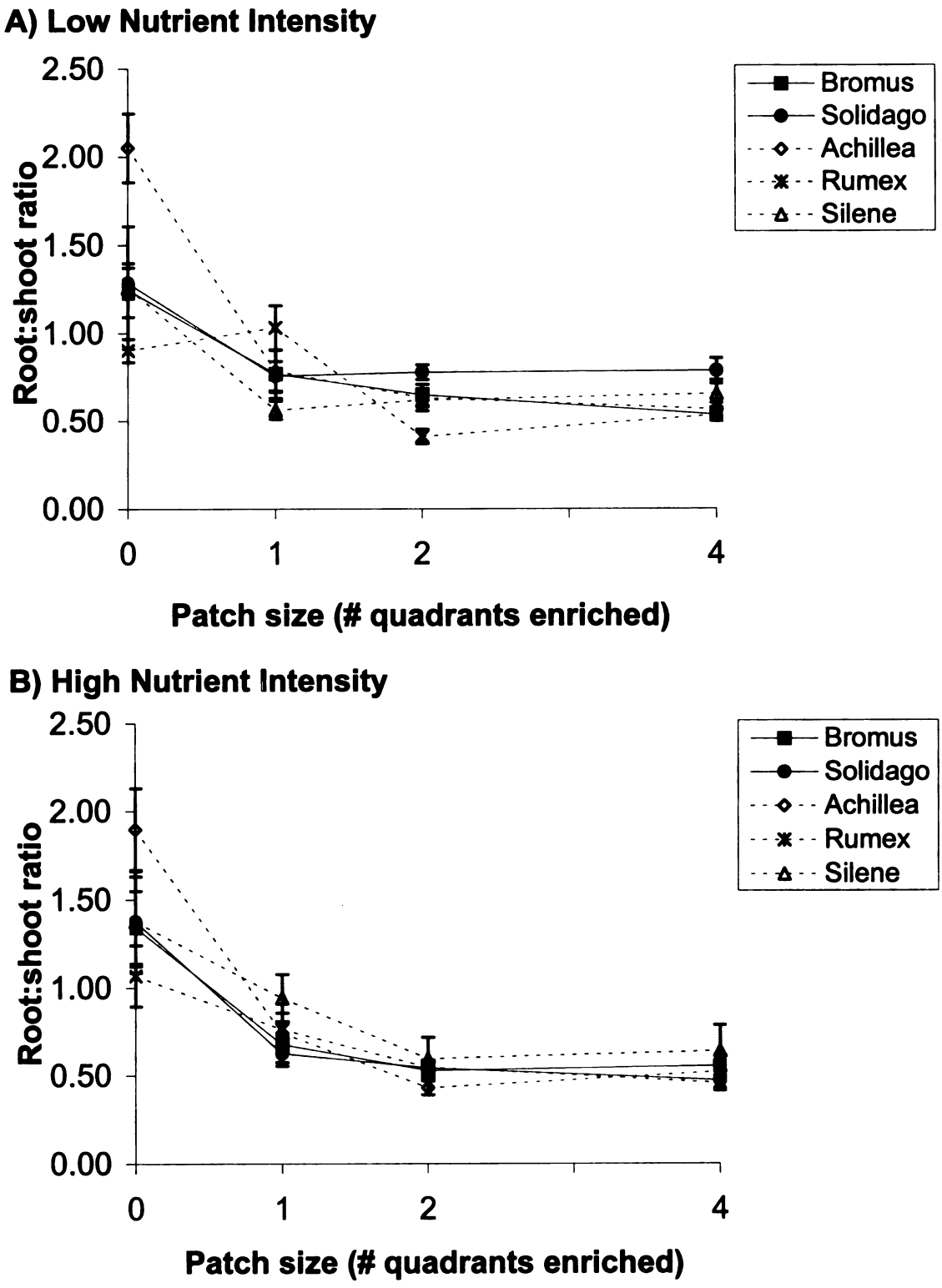

A) Low Nutrient Intensity
Root:shoot ratio
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
4
Patch size (# quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
Root:shoot ratio
2.50
2.00
1.50
1.00
0.50
0.00
0
1
2
4
Patch size (# quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 3.7

small and medium patch size treatments (1 and 2 quadrants enriched) and so greater within-subjects variation (Figure 3.8). There was generally less variation between quadrants in the control and large patch size treatments than in the small and medium patch treatments for all five species. The lack of significant quadrant x species x patch size effect (Table 3.1), indicates that the five species responded the same way to resource heterogeneity by shifting allocation to the enriched quadrants in heterogeneous treatments (ie. root biomass in background < patch), but having approximately even allocation among quadrants in homogeneous treatments (Figure 3.8).

Nitrogen uptake assessments

There are indications that uptake rates changed in response to nutrient heterogeneity. All species had higher and more variable uptake in the small or medium patch treatments in at least one level of nutrient intensity (Figure 3.9). A lack of replication in the ^{15}N uptake analyses, however, limited the power to detect differences across patch sizes or among species.

Root N concentration increased significantly across patch size treatments (Figure 3.10, Table 3.1) and was always higher in the fertilized treatments (small, medium and large patch) than in the unfertilized control, for all species at both levels of nutrient intensity. There was a significant species x patch size interaction, indicating differences between species in the pattern of response to the different treatments (Table 3.1). At both levels of nutrient intensity, *Achillea* and *Bromus* had higher root N as the patch size increased (Figure 3.10). This is an indication that these species are less able to adjust N uptake in response to heterogeneity, because the plants have less root nitrogen at smaller patch sizes (even though the total amount of fertilizer in the pot was constant). Both *Silene* and *Rumex* were able to better adjust their uptake to nutrient heterogeneity, and had just as much root N regardless of

Figure 3.8 - Root biomass values $\pm$ S.E. for patch and background quadrants within root systems at high and low nutrient intensity levels for each species. Open diamonds are the means of root samples from unenriched quadrants. Solid squares are means of root samples from enriched quadrants. All quadrants in the patch size 0 treatment were unenriched. All quadrants in the patch size 4 treatment were enriched.

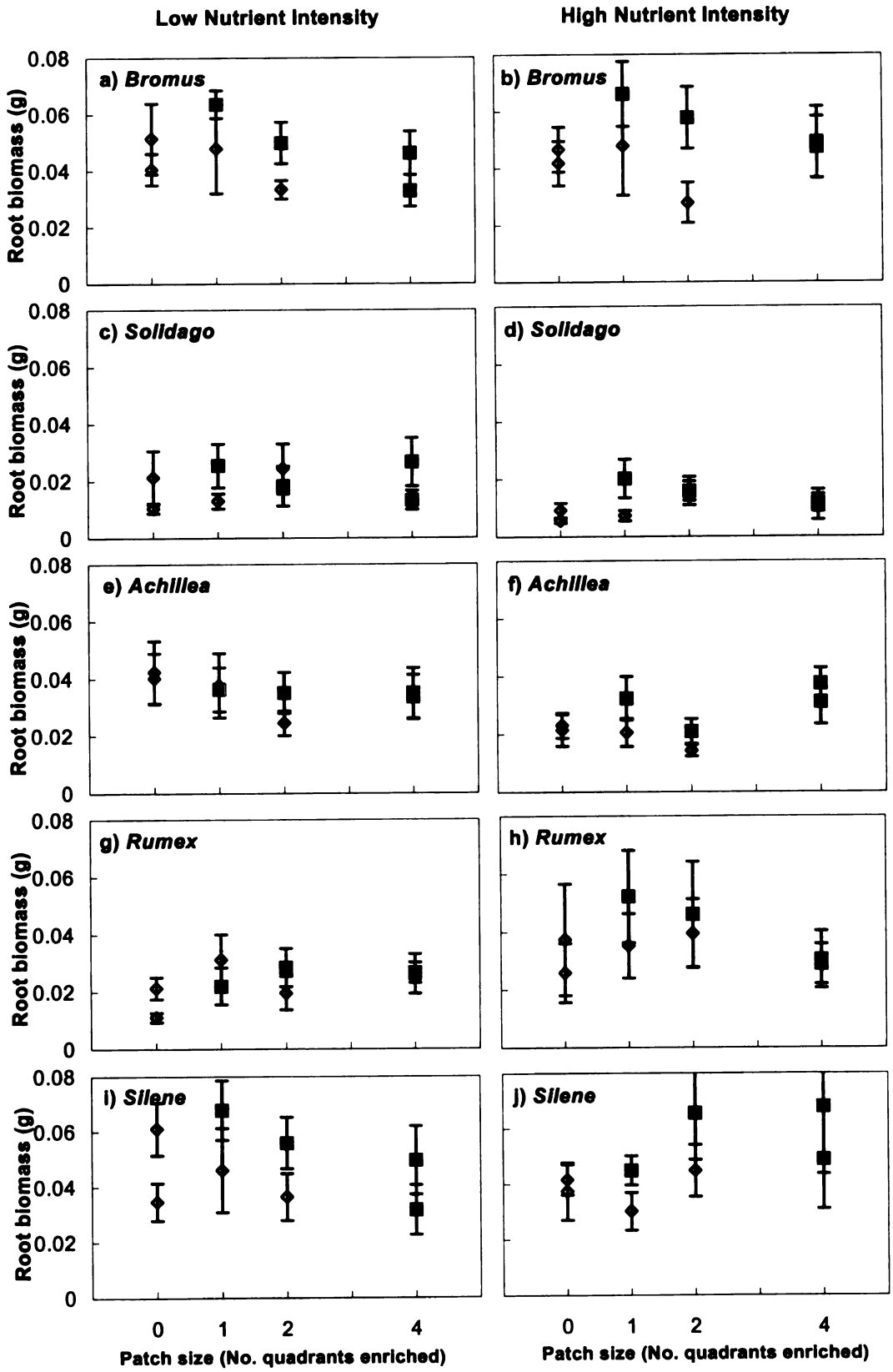

Low Nutrient Intensity
High Nutrient Intensity
a) Bromus
b) Bromus
c) Solidago
d) Solidago
e) Achillea
f) Achillea
g) Rumex
h) Rumex
i) Silene
j) Silene
Root biomass (g)
0.08
0.06
0.04
0.02
0
0 1 2 4
Patch size (No. quadrants enriched)

Figure 3.8

Figure 3.9 - Nitrogen uptake rate $\pm$ S.E. for the five species as determined by ^{15}N assay with excised roots. Filled symbols with solid lines are dominant species, hollow symbols with dashed lines are subordinate species.

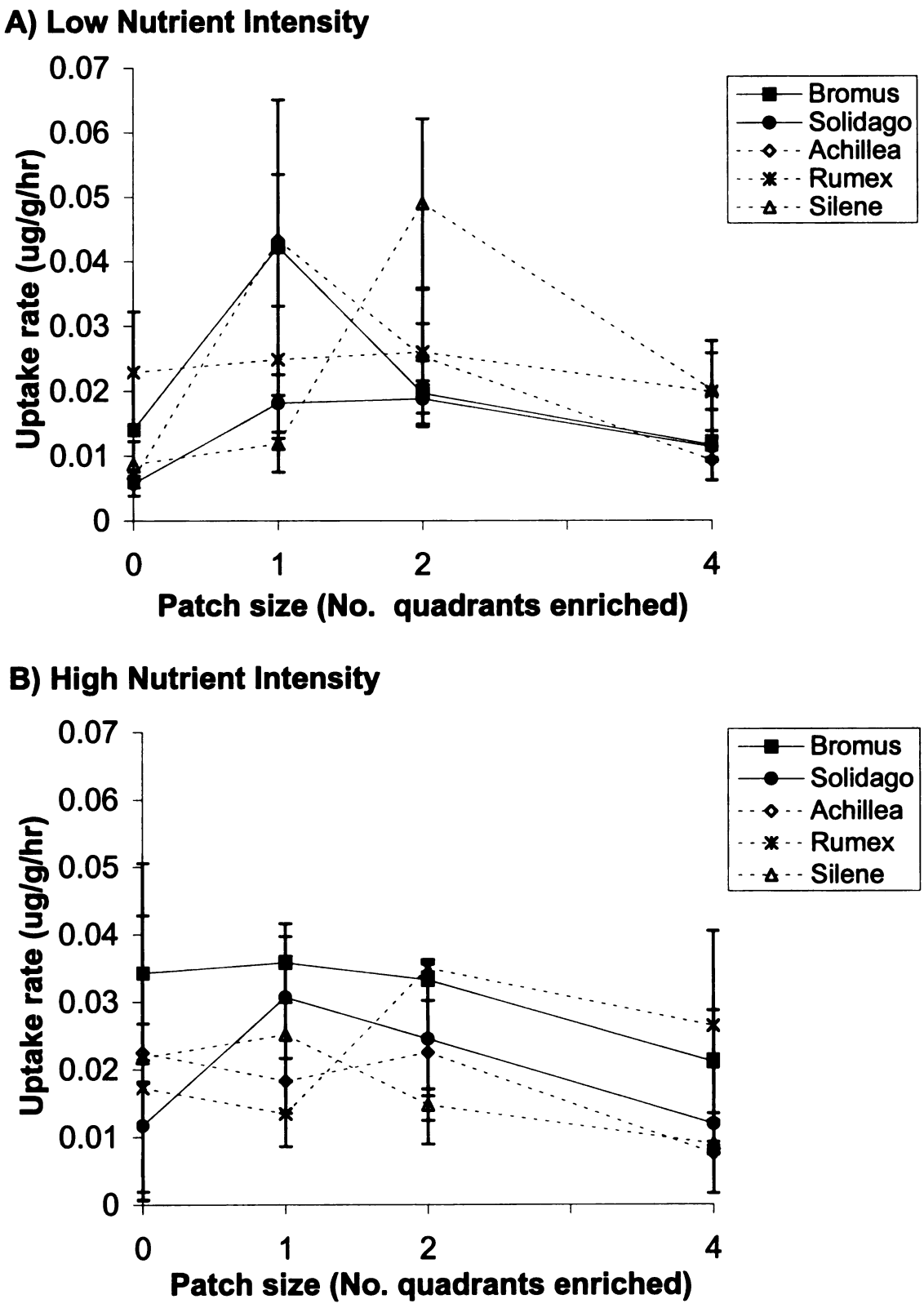

A) Low Nutrient Intensity
Uptake rate (ug/g/hr)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
Uptake rate (ug/g/hr)
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 3.9

Figure 3.10 - Average %nitrogen concentration of whole roots systems $\pm$ S.E. at high and low patch intensities levels for each species. Filled symbols with solid lines are dominant species, hollow symbols with dashed lines are subordinate species.

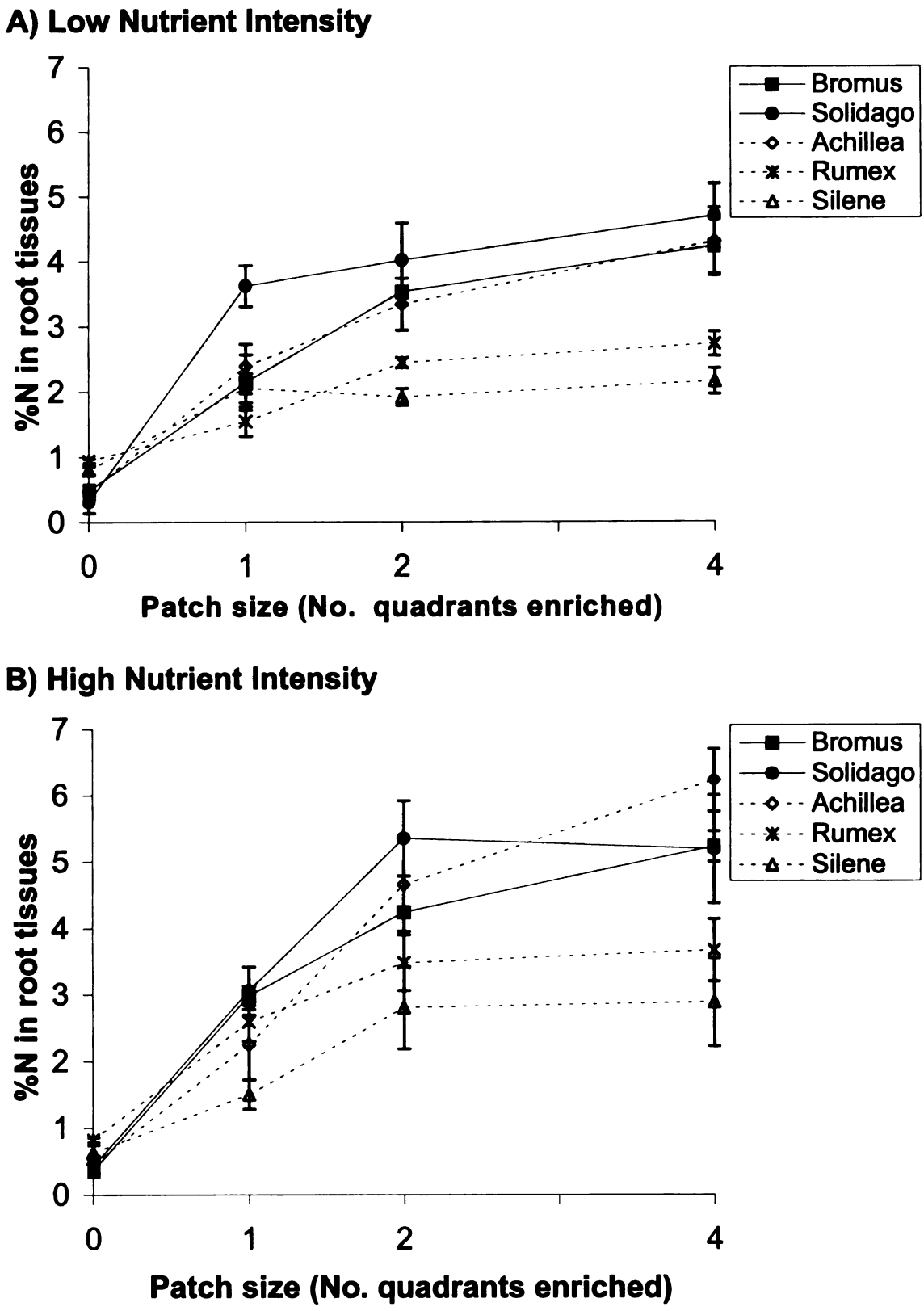

A) Low Nutrient Intensity
%N in root tissues
0
1
2
3
4
5
6
7
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene
B) High Nutrient Intensity
%N in root tissues
0
1
2
3
4
5
6
7
0
1
2
4
Patch size (No. quadrants enriched)
Bromus
Solidago
Achillea
Rumex
Silene

Figure 3.10

the spatial arrangement of enrichment at both nutrient intensity levels. The highest root N concentration of *Rumex* and *Silene* was lower than those obtained by the other three species (Figure 3.10). *Solidago* also adjusted uptake to nutrient heterogeneity, although its ability to adjust to the smallest patch size was diminished at the higher nutrient intensity. Nitrogen concentrations for all species were higher in the largest two patch sizes (2 and 4 quadrants enriched) at the higher nutrient intensity (Figure 3.10), as suggested by a significant patch size x nutrient intensity interaction.

Patch vs. background differences in N uptake

There were indications that uptake rates between quadrants for some of the patch size treatments differed. There was generally less variability in uptake in the control and large patch size treatments (0 and 4 quadrants enriched, respectively) than in the small and medium patch size treatments (1 and 2 quadrants enriched; see Figure 3.11).

There was a significant quadrant x species interaction for N concentrations. Although all five species generally had higher root N concentrations in enriched patches compared with background quadrants, *Bromus*, *Achillea* and *Solidago* had greater magnitudes of difference between patch and background quadrants than *Rumex* and *Silene* (Figure 3.12). There were also significant quadrant x patch size x nutrient intensity and quadrant x patch size interactions, driven by greater variation between quadrants in the heterogeneous than in homogeneous treatments, especially at the high nutrient intensity level. The higher nutrient concentrations in the roots from unenriched quadrants of the heterogeneous treatments (1- and 2-quadrants enriched) than in roots from the treatment with no quadrants enriched are indicative of some transport of nitrogen taking place within the root system.

Figure 3.11 - ^{15}N uptake rate $\pm$ S.E. in patch and background quadrants within root systems at high and low nutrient intensity levels for each species. Open diamonds are the means of root samples from unenriched quadrants. Solid squares are means of root samples from enriched quadrants. All quadrants in the patch size 0 treatment were unenriched. All quadrants in the patch size 4 treatment were enriched.

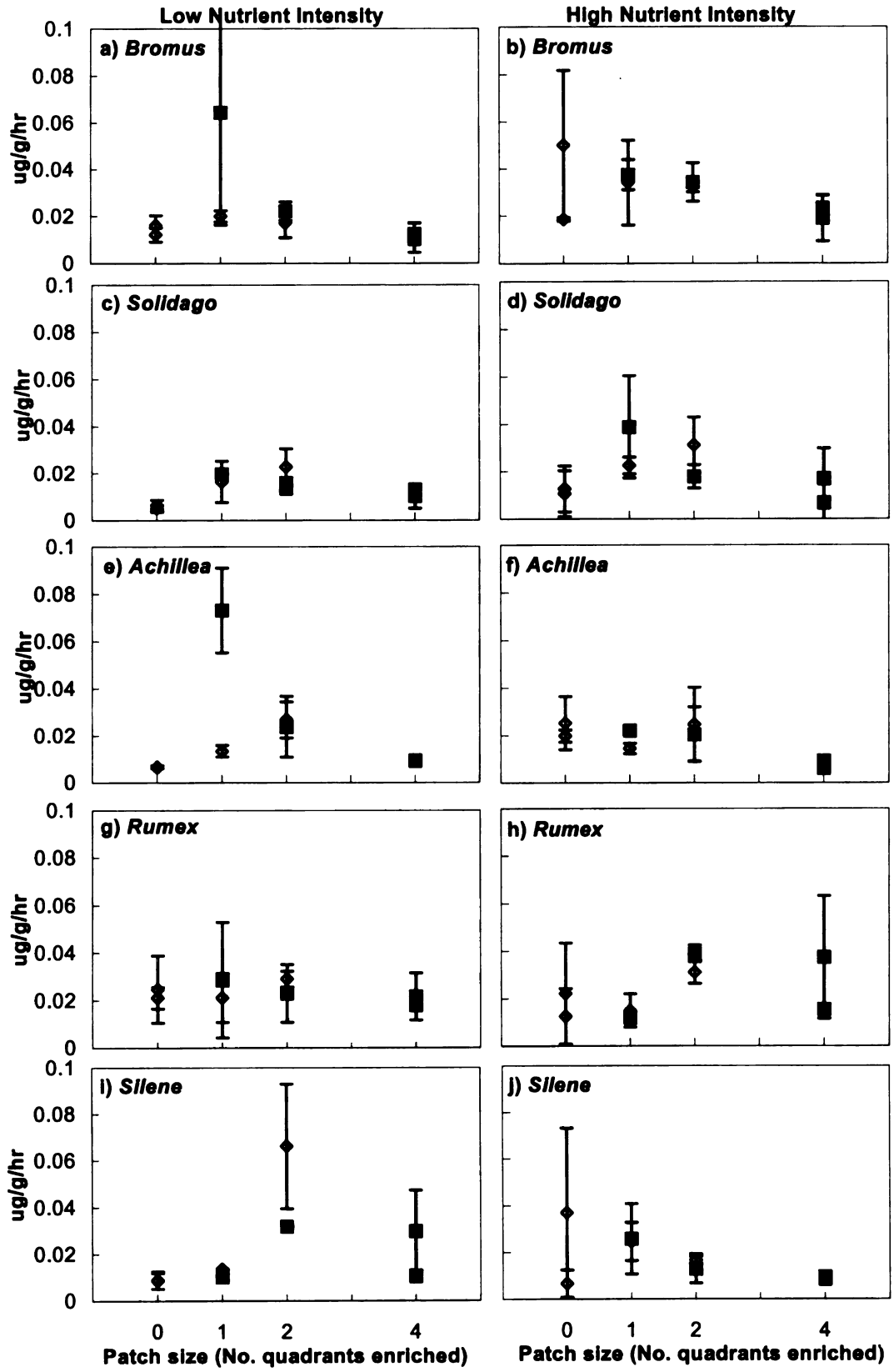

Low Nutrient Intensity
High Nutrient Intensity
a) Bromus
b) Bromus
c) Solidago
d) Solidago
e) Achillea
f) Achillea
g) Rumex
h) Rumex
i) Silene
j) Silene
ug/g/hr
0.1
0.08
0.06
0.04
0.02
0
0 1 2 4
Patch size (No. quadrants enriched)

Figure 3.11

Figure 3.12 - Root nitrogen concentration $\pm$ S.E. in patch and background quadrants within root systems at high and low nutrient intensity levels for each species. Open diamonds are the means of root samples from unenriched quadrants. Solid squares are means of root samples from enriched quadrants. All quadrants in the patch size 0 treatment were unenriched. All quadrants in the patch size 4 treatment were enriched.

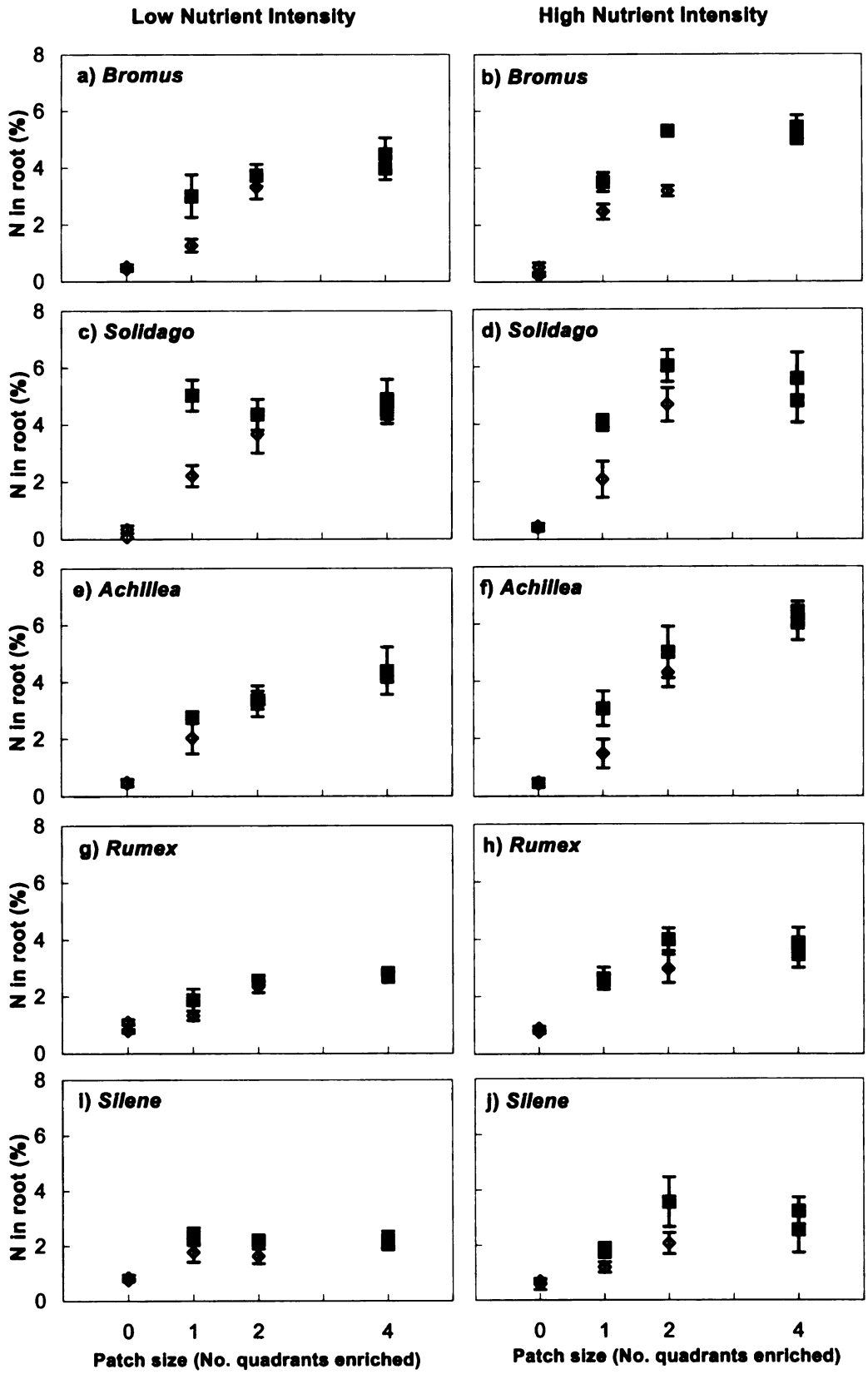

Low Nutrient Intensity
High Nutrient Intensity
a) Bromus
b) Bromus
c) Solidago
d) Solidago
e) Achillea
f) Achillea
g) Rumex
h) Rumex
i) Silene
j) Silene
N in root (%)
0
2
4
6
8
0
1
2
4
Patch size (No. quadrants enriched)

Figure 3.12

DISCUSSION

This study shows that species differ in which mechanisms they used to respond to nutrient heterogeneity, although there were no patterns linking response mechanisms to either foraging strategy or dominance in the field. Species also differed in how many mechanisms they used and in how well they could use a mechanism across the range of patch sizes or nutrient intensity in these experiments (Table 3.2).

Architecture indices

All of the species had topological indices indicative of random branching architecture except for *Bromus*, which had more herringbone root architecture. Although there was no significant species difference in the pattern of how branching architecture changed across patch size or nutrient intensity, there was some indication that *Bromus* might be able to adjust its root architecture to less herringbone branching in enriched patches. Fast growing species, like *Bromus*, are expected to be more likely to adjust their root architecture in response to a patch (Fitter 1994). The fact that *Bromus* was more herringbone than the other species is probably a general trait of grass species. Preliminary data from another grass species, *Poa compressa,* also had higher a/E(a) values than dicot species (Corbett, unpubl.). Taub and Goldberg (1996) also found grasses to have a more herringbone root architecture than dicots.

There were significant differences among species in the pattern of root branching density in response to nutrient heterogeneity, as measured by mean segment length (MSL). Three of the species had no change in MSL across patch treatments: *Bromus, Silene,* and *Rumex* had short MSL in both the unfertilized controls and fertilized treatments. Only *Solidago* and *Achillea* exhibited plasticity in MSL and produced roots with larger MSL in unfertilized soil and short MSL in enriched soil.

Table 3.2 - Summary of species responses by different mechanisms across patch sizes and concentrations. Dom=dominant, Sub=subordinate, Mon=monocot, Di=dicot, Myc=mycorrhizal, Amyc=amycorrhizal *= variables for which there were significant species by patch size or species by nutrient intensity interactions.

	Biomass		a/E(a)		Mean Segment Length *		Root N Content *	
Species	Change in whole root system	Patch vs. background differences	Change in whole root system	Patch vs. background differences	Change in whole root system	Patch vs. background differences	Change in whole root system	Patch vs. background differences
Bromus inermis (Dom, Mon, Myc) Scale forager	none	yes	moderate	some, but not significant	none	none	< as patch size <	yes at higher intensities
Solidago canadensis (Dom, Di, Myc) Precision forager	none	yes	weak	none	< when fertilized	yes	generally = in patch treatments	yes
Achillea millefolium (Sub, Di, Myc) Precision forager	none	yes	weak	some, but not significant	< when fertilized	yes	< as patch size <	moderate
Rumex acetosella (Sub, Di, Amyc) Precision forager	none	yes	weak	none	none	none	= at larger patch size	none
Silene latifolia (Sub, Di, Amyc) Precision forager	none	yes	moderate	none	none	none	= in all patch treatments	weak

Biomass responses

None of the species increased total root biomass across patch sizes, although this could be a function of adjusted root birth and death rates, which were not measured in this study. While root systems had larger biomass at the high nutrient intensity and some species had larger root biomass than others, there was no difference in the pattern of response via root biomass that species exhibited across patch sizes. Instead, all five species responded to nutrient heterogeneity by shifting root biomass allocation to patches. All species had higher root biomass in patch quadrants than in background quadrants of the heterogeneous treatments (small and medium patch size). This result is consistent with the pattern observed by Robinson (1994), where an increase in root biomass in enriched patches resulted in a decrease elsewhere in the root system. There were no differences among species in their ability to selectively allocated biomass to patch quadrants, i.e. species did not differ in how much variation in biomass there was between patch and background quadrants (Figure 3.8).

Uptake rate responses

In spite of the low power of the analysis of the ^{15}N uptake assay, there was some indication that these species had higher uptake rates in heterogeneous treatments vs. homogeneous treatments, and that there was more variability in uptake in heterogeneous treatments. It was not clear if there were different uptake rates between patch and background quadrants, and no indication of whether there might be differences among species.

When root N concentration was used as an estimate of N uptake, there were differences in how plant species responded to nutrient heterogeneity and in the abilities of plant species to selectively use this mechanism within portions of the root system. *Solidago*, *Bromus*, and *Achillea* had lower uptake per root in some of the heterogeneous treatments, as

indicated by lower root N concentrations in those treatments, even though total N in the pot was the same across the enriched patch size treatments. These species, however, were able to compensate for nutrient heterogeneity by having higher N concentrations in roots from enriched patch quadrants than roots from unenriched quadrants. *Silene* and *Rumex* kept the N concentrations in their roots relatively constant as patch size changed, but they had less difference between patch and background quadrants within the root systems of heterogeneous treatments. These differences in N concentration among species and patch size treatments could also be due to differing rates of transport from the root. The fact that all species had higher N concentrations in roots from unenriched quadrants in the heterogeneous treatments (1 and 2 quadrants enriched) than in roots from the unenriched control treatment indicates that some transport of N is occurring. However, since nitrogen was supplied to enriched quadrants right up until the moment of harvest, there will be N acquired through uptake that could not be transported out of the roots before harvest. So the differences between enriched and unenriched sectors are driven by uptake differences.

Summary of species responses

These five species exhibited differences in the mechanisms they used to respond to nutrient heterogeneity (Table 3.2). However, these differences were not associated with whether the species was a scale or precision forager, or a dominant or subordinate species in the field. Instead, mechanisms appear to be more constrained by root system type. The amycorrhizal species (*Rumex* and *Silene*) had lower %N concentrations than the other species, although they were able to keep concentrations constant across treatments. These two species and the grass species (*Bromus* and preliminary data from *Poa*; see Appendix) were densely branched under all nutrient conditions. This may have limited them from exhibiting plasticity in branching density in response to nutrient patches. There was some indication that *Bromus*

might be able to adjust root branching topology to be less herringbone in response to nutrient patches, but it was not significant in this study.

Fitter (1994) found evidence of a negative correlation between biomass allocation adjusters and species that changed root architecture and suggested that these might represent alternative means for species to respond to nutrient heterogeneity. However, I did not find any evidence for trade-offs between the mechanisms I investigated in this study. I found no evidence that species used a response mechanism at only certain patch sizes or nutrient intensities. I also found no evidence of differences in the magnitude of plasticity between precision and scale foragers as patch size changed. If a species used a given response mechanism, it was used with equal effectiveness across the range of patch sizes in the experiments.

There was limited evidence that species differ in how precisely they can allocate a response mechanism within the root system. There were no species differences in how much they shifted biomass allocation within the roots, but there were differences in how species adjusted nitrogen uptake in response to nutrient patches. Contrary to my hypothesis, the scale forager (as well as two precision foragers) had greater difference in uptake between patch and background quadrants than two of the precision foragers.

This study shows that linking the mechanisms of response by root systems to overall plant performance in nutrient patches is a complex process. In my experiments, there was no consistent pattern of individual response mechanisms being linked to foraging strategy, pattern of dominance in the field, or overall performance of the species in patchy conditions. Response mechanisms that were used by a species were determined more by root system morphology and plant growth form. In addition, all species used more than one mechanism when responding to nutrient heterogeneity. Further research would be valuable in determining whether these mechanisms differ in the importance of the contribution they make

to the plant adjusting to nutrient heterogeneity or whether the temporal scale of heterogeneity influences which mechanisms are used to respond to nutrient patches.

Chapter 4

USING SEMIVARIOGRAMS FOR COMPARISON OF SPATIAL PATTERNS IN MULTIPLE ENVIRONMENTS: CHOOSING A SAMPLING SCHEME

(Manuscript co-authored with K.L. Gross and G.P. Robertson)

INTRODUCTION

Determining spatial patterns in environments is important to many ecological questions. Many techniques have been developed for assessing spatial patterns (for example, see Cressie 1991). Of these techniques, geostatistical analyses provide excellent means for both quantifying the scale and magnitude of spatial structure in a variate (from the semivariance analysis; Rossi *et al.* 1992, Robertson and Gross 1994) and for interpolating or mapping the spatial distribution of a variate in an environment (using kriging algorithms; see Robertson 1987).

Geostatistical analyses were originally developed to aid geologists in estimating the locations of ore deposits. Ecologists have adopted geostatistics for many uses, such as assessing the distributions of insects (e.g. Schotzko and O'Keeffe 1990; Liebhold *et al.* 1993); mapping the abundances of migratory song birds (e.g. Villard and Maurer 1996); and evaluating variability of soils (e.g. Robertson *et al.* 1988, Schlesinger *et al.* 1996)

A critical issue in any application of geostatistics is the choice of an appropriate sampling design (Olea 1984, Yfantis *et al.* 1987), especially when there is insufficient background information about the variate or relationship being studied. Many people in both geology and ecology have sought ways to improve sampling designs. In fact, there is a wide body of applied mathematical literature dedicated to the improvement of geostatistical

measures and interpretation. Some have assessed the best geostatistical sampling methods to use for kriging and mapping purposes (Burgess *et al.* 1981, McBratney and Webster 1983, Olea 1984, Trangmar *et al.* 1985, Oliver and Webster 1986, Robertson 1987, Yfantis *et al.* 1987). These assessments require the researcher to already have a semivariogram on which to base his or her calculations of optimal sampling. Others have taken a more theoretical approach, with the aim of minimizing sampling variance (e.g. Cressie 1991; Zimmerman and Homer 1991; Brus and de Gruiter 1994) A few have assessed different sub-sampling strategies within one sampled population (e.g. Fortin *et al.* 1989, Oliver and Webster 1986). All these studies have focussed on customizing sampling strategies to the particular environment being sampled such that a maximum amount of information about spatial patterns in the environment is gained for a minimum of sampling effort.

Even though ecologists have used geostatistical techniques to aid in describing spatial patterns in the environment, a more recent application of geostatistical methods to ecology is to compare patterns of spatial structure in multiple environments, or the same environment over time. This extends the use of geostatistics from the merely descriptive to the testing of hypotheses. For example, Gross *et al.* (1995), studied whether the scale of heterogeneity in soil changed between communities of different successional age. Schlesinger *et al.* (1996) compared spatial distributions of soil nutrients between grassland and shrubland desert ecosystems. Ryel *et al.* (1996) tracked the changes in soil spatial heterogeneity through a growing season.

In the situation where we wish to make comparisons, spatial structure is being determined concurrently in several locations or at the same location through time. The central question we ask when selecting sampling locations then, is not "how do we customize our spatial sampling to this one particular site?", but "what spatial sampling scheme can I use in all of my sites and still obtain a good estimate of spatial structure at each site?" Will the

same set of sampling points need to be used in each environment in order to have methodological consistency? Given that the location of sampling points may affect our estimate of spatial structure, we would like to be confident that the sampling set is maximizing the information that we obtain from the data in all environments.

Our goal was to address this question by creating a simple model that would mimic potential sampling schemes that an ecologist might use and investigating how those sampling schemes fared in assessing the spatial pattern in a variety of environments.

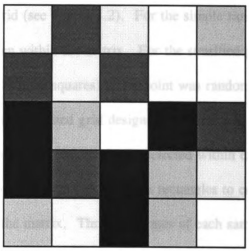
METHODS

We chose three types of sampling regimes that we felt were commonly used by ecologists: simple random (all sampling points randomly chosen); stratified grid (environment divided into a grid and one sampling point randomly chosen within each grid cell); and stratified-nested grid (same as stratified grid, except that, in addition, a sub-set of randomly chosen grid cells will contain multiple sampling points).

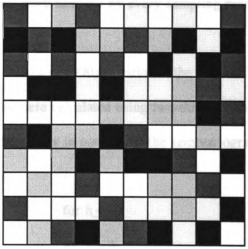
We then generated very simple simulated environments that were squares of 50x50 cells. There were environments at three different scales of heterogeneity: 25 patches of 10x10 cells each, 100 patches (5x5), or 625 patches (2x2). Each patch was randomly assigned a numerical value (0 to 4), with equal numbers of patches of each value in each environment (see Figure 4.1). Five different replicates of each environment were generated by changing the random assignment of the patches. and compared the variogram parameters estimated by three different sampling regimes. We generated replicates of these environments so that we could statistically compare the results of the semivariance analyses and determine which sampling regime gave the most consistent results across a range of environments with different scales of spatial structure.

Figure 4.1 - Examples of the three different environments with different patch sizes and number of patches. Numerical values have been converted to shading for visual effect. Increasing darkness equals increasing numerical value (i.e. white=0, black=4).

A) 10x10 patch size



B) 5x5 patch size



C) 2x2 patch size

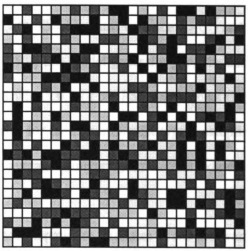


Figure 4.1

We generated 100 sampling points using three different sampling regimes: simple random, stratified grid and stratified-nested grid (see Figure 4.2). For the simple random pattern, 100 points were randomly chosen from within the matrix. For the stratified grid, the matrix was first divided into a grid of 5x5 cells (100 squares) and a point was randomly selected from within each square. In the stratified-nested grid design, the matrix was divided into a grid of 50 rectangular 5x10 cells and one point was randomly selected within each rectangle. An additional 5 points were randomly selected in 10 of the rectangles to create the nested structure of smaller scale sampling of the matrix. Three replicates of each sampling regime were generated and all the environments were sampled by every sampling regime replicate, for a total of 135 data sets.

We used GS+ v2.1 (Gamma Design Software, 1990) to estimate the semivariance parameters for every data set. Semivariograms were calculated using two-thirds of the maximum lag and a step size of two. For consistency in comparing the semivariograms, we fit a spherical model to all the semivariograms:

$$t(h)=C_0+C[1.5(h/A_0)-(h/A_0)^3] \qquad \text{for } h \leq A_0$$

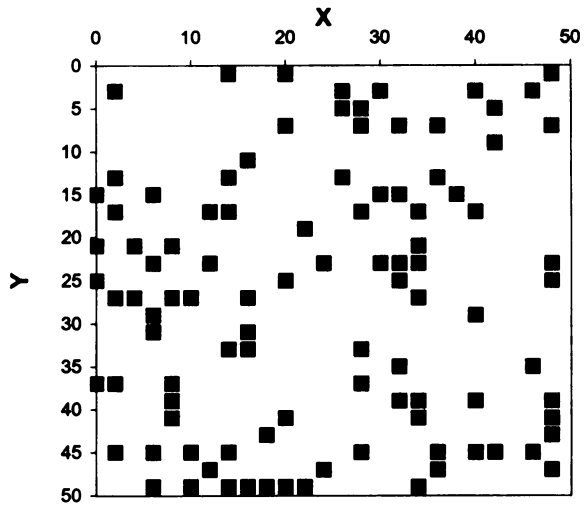
and $$t(h)=C_0+C \qquad \text{for } h > A_0$$

where h=lag interval, C_0=nugget variance (≥ 0), C=structural variance ($\geq C_0$) and A_0=range (see Figure 4.3). In most cases (86.5%) this model generally gave the best fit (by lowest residual sum of squares) or had an RSS within 0.1 of the RSS for the best fit model. The semivariance parameters of the 15 replicates for each environment type x sampling regime were averaged and used to produce an average semivariogram with standard error bars.

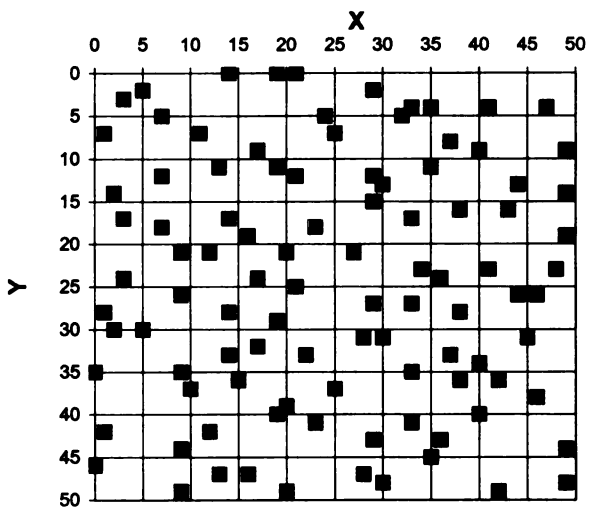
We used repeated measures ANOVA to compare the estimates of the semivariogram parameters (sill, range and nugget) with different patch sizes and sampling regimes. The nugget variance (C_0) is expected to be zero if all the variance is spatially structured, otherwise

Figure 4.2 - Examples of the three different sampling regimes, including the grid divisions that were used (if any).

A) Simple Random



B) Stratified Grid



C) Stratified-nested Grid

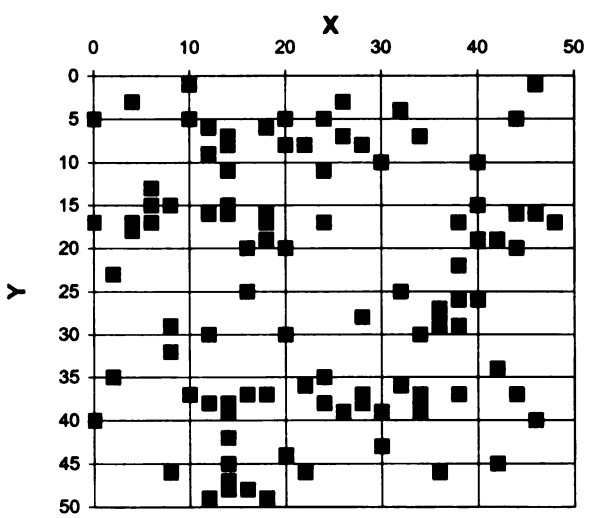


Figure 4.2

Figure 4.3 - Idealized semivariograms showing the parameters of nugget (C_0), range (A_0) and sill ($C+C_0$).

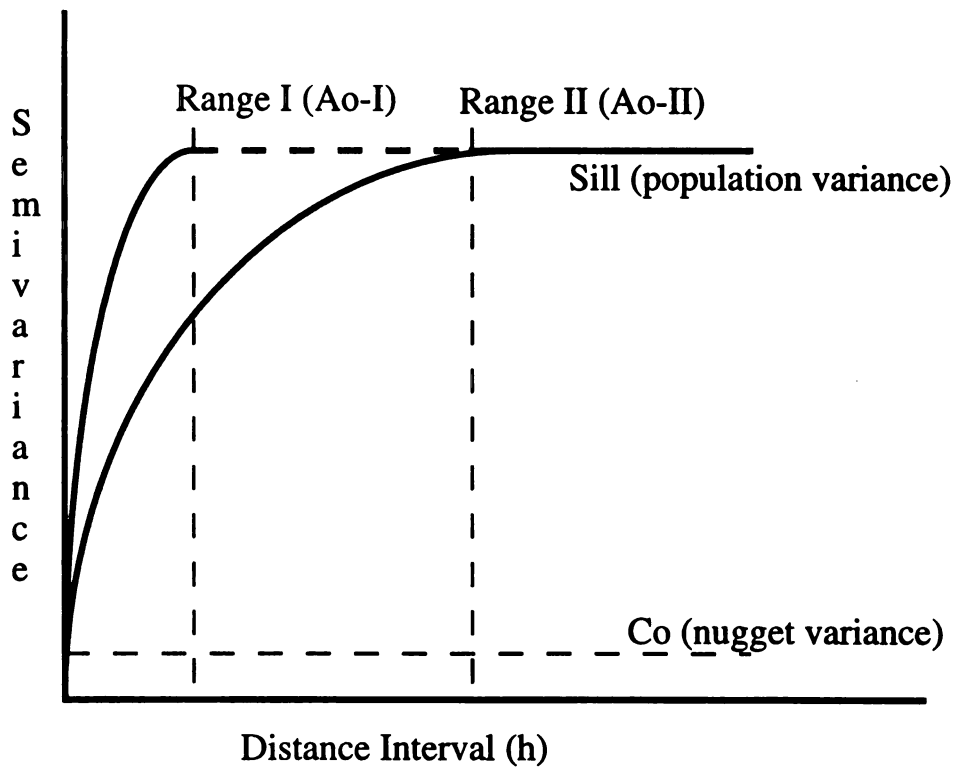

Range I (Ao-I)
Range II (Ao-II)
Sill (population variance)
Co (nugget variance)
Semivariance
Distance Interval (h)

Figure 4.3

it represents the variance that is not spatially structured or is structured at a scale smaller than that sampled. The sill (C_0+C) is expected to be equal to the population variance. The range (A_0) is the distance at which spatial dependence no longer occurs. A_0 can be used to approximate the scale of heterogeneity (or the size of patches) in the environment (see Figure 4.3). All analyses were performed using SYSTAT (Systat Inc. 1992).

To assess whether a sampling regime gave "better" estimates of spatial structure, we used four criteria: a "good" sampling regime will produce consistent estimates of range (A_0); a "good" sampling regime will consistently produce nugget estimates close to zero; a "good" sampling regime will have little variation in the semivariance values at lag distances smaller than the A_0 distance; as a corollary of this, a "good" sampling regime will have more data pairs used in the semivariance calculation at small lag distances.

RESULTS

All three sampling regimes gave similar, and accurate, estimates of the true population mean and variance for patch value (2.00 ± 2.00).

Range estimates

The sampling regimes did not differ significantly in their estimates of the range (A_0), but estimates from the random sampling regime were more variable, especially at the 2x2patch size (see Table 4.1). All three sampling regimes showed a decrease in range as patch size decreased.

Nugget estimates

If all the variance in an environment is spatially structured and the sampling regime is at the appropriate scale, the nugget (C_0) of the semivariogram will be zero. The estimated

Table 4.1 - Average estimates of semivariogram parameters (n=15) for the three sampling regimes. For each parameter, comparisons were made between sampling regimes within each environmental patch size. Significant differences were determined from repeated measures ANOVA, adjusted by the Greenhouse-Geisser value (*=P<0.05, **=P<0.01, ***=P<0.001).

Patch size	Sampling regime	Nugget (C_0)	Range (A_0)	Sill ($C+C_0$)
2x2	Grid	0.645±0.238	4.347±0.434	2.028±0.056
	Nested	0.544±0.164	5.919±1.601	2.031±0.035
	Random	1.731±0.073	19.401±7.676	2.025±0.025
		**	ns	ns
5x5	Grid	1.409±0.246	5.281±1.177	2.177±0.071
	Nested	0.067±0.037	7.837±0.620	1.889±0.071
	Random	0.165±0.093	8.166±1.366	2.060±0.044
		***	ns	**
10x10	Grid	0.585±0.131	15.959±1.391	2.095±0.025
	Nested	0.032±0.022	14.771±0.783	2.069±0.057
	Random	0.172±0.061	15.190±0.667	2.057±0.056
		**	ns	ns

nugget differed among sampling regimes (Table 4.1) and the stratified-nested sampling regime consistently had the lowest, and least variable, estimate of nugget.

Semivariance estimates

The stratified-nested design has more pairs of points at lower lag classes (see Table 4.2). The random sampling regimes has no sampling points in the first lag class, and the grid sampling regime has an average of 3 pairs at the smallest lag distance.

The nested sampling regime provided a less variable estimate of the semivariance at the lowest lag classes (see Figure 4.4). Particularly, at the smallest lag distance (where the random regime has no sampling points), the magnitude of the standard error from the nested sampling regime is lower than that of the grid sampling regime at all three patch sizes. In the second lag class, the standard errors for all three sampling regimes are similar, but the nested sampling regime is slightly less variable than the grid at 5x5 and 10x10 patch sizes, and slightly less than the random regime at 2x2 and 10x10 patch sizes.

DISCUSSION

Range estimates

The range of the semivariogram is defined as the lag distance at which the semivariogram reaches the sill; at distances larger than the range, points are spatially independent of each other (Yfantis *et al.* 1987, Robertson and Gross 1994). Thus the range is equal to the radius of the area over which spatial dependence occurs, and can be considered an estimate of the mean patch size in the environment. While there were not differences between sampling regimes in the estimates they produced, the random regime was less consistent. All three sampling regimes were able to detect the mean patch size in the environments, as shown by the decrease in range as patch size decreased.

Table 4.2 - Average number of pairs used to estimate the semivariance at the first eight lag classes, using a step size of two.

Lag Class	Grid	Nested	Random
1	3	14	0
2	36	92	54
3	106	111	108
4	142	154	142
5	171	191	148
6	195	234	226
7	250	231	197
8	227	246	245

Figure 4.4 - Average semivariograms estimated for the three environmental spatial structures using the different sampling regimes. Values were averaged from the semivariance analyses for each lag distance category across environmental and sampling replicates (n=15). Standard errors at each distance interval are shown. The fitted curve is the spherical model with the parameters (C_0, $C+C_0$ and A_0) obtained from the averages in Table 4.1.

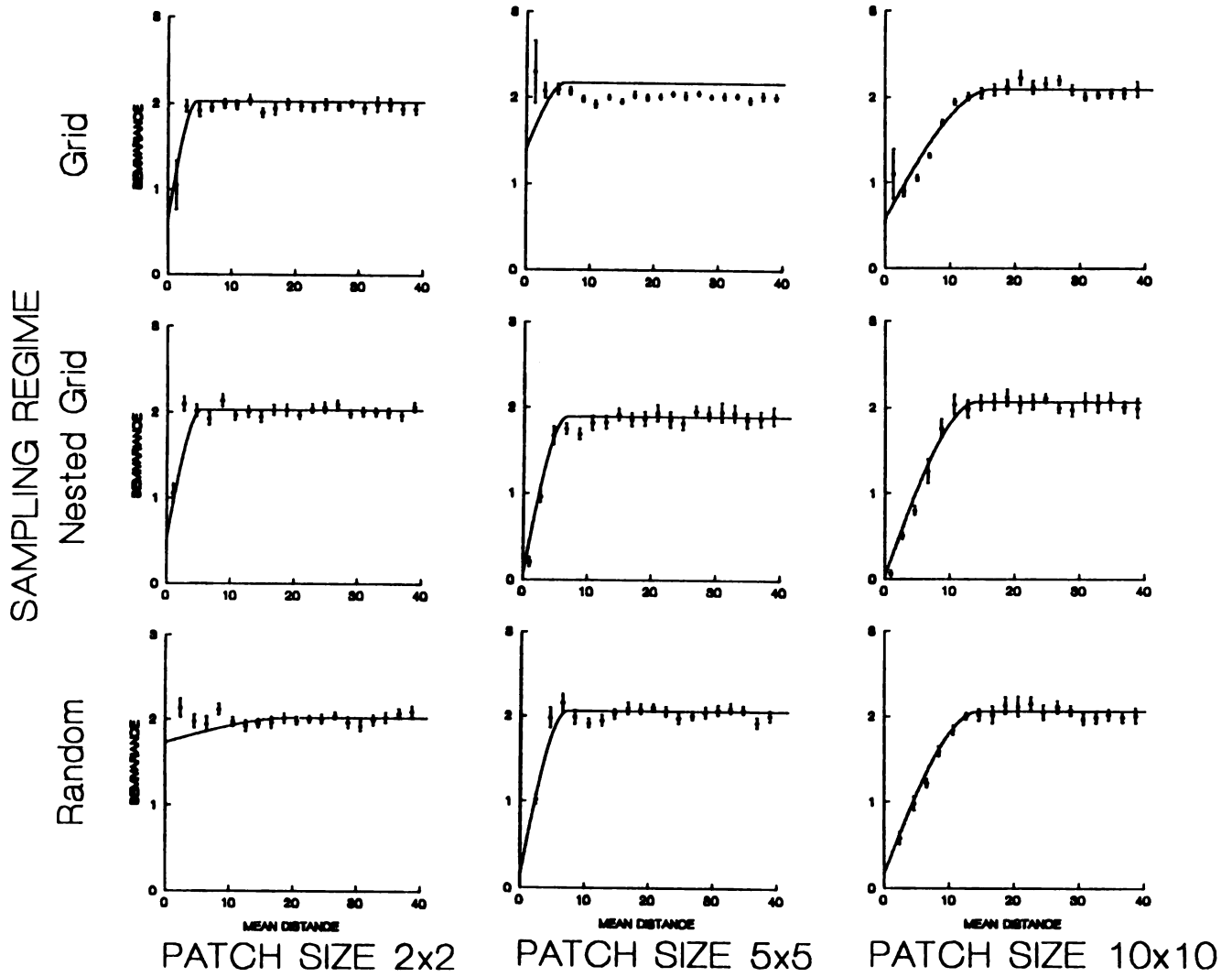

SAMPLING REGIME
Grid
Nested Grid
Random
SEMIVARIANCE
MEAN DISTANCE
PATCH SIZE 2x2
PATCH SIZE 5x5
PATCH SIZE 10x10

Figure 4.4

Note that the range provides an estimate of mean patch size only. In the simulated environments we sampled for these analyses, all patches are the same size in a given environment and there are sharp boundaries between patches. So although the range provides a good estimate of patch size in our examples, they are very simplistic compared to natural environments. In natural environments, patch sizes can vary considerably, and boundaries will be much more gradual, such that the range does not wholly represent patch size. For example, an environment could consist of a mixture of small and large patches which would result in an estimated "intermediate range" over which there is spatial dependence. Alternatively, if the sampling is sufficiently fine-scaled, it may be possible to detect this as a "nested structure" with the semivariogram (c.f. Robertson and Gross 1994; Gross *et al.* 1995).

Nugget estimates

If all the variance in an environment is spatially structured and the sampling regime is at the appropriate scale, the nugget (C_0) of the semivariogram will be zero. Low and consistent estimates of the nugget are important in semivariance analysis, because it is used to estimate the magnitude of spatial structure in a variate ($C_0/C+C_0$; e.g. Gross *et al.* 1995). The stratified nested sampling regime was able to consistently provide low estimates of the nugget over a range of patch sizes, while the other two sampling regimes produced higher nugget values. This indicates that the nested sampling regime is more likely to capture the spatially-structured variance in environments when the same set of sampling points is used across several locations.

Semivariance estimates

The level of confidence in a semivariogram depends on the number of sample pairs used in the calculation of each point, particularly at the smaller lag distances, as these determine the nugget (C_0) and the range (A_0) over which there is spatial dependence. Based on this criteria, we are most confident in the estimates produced by the stratified-nested sampling design. The larger number of pairs at shorter lags generated by the stratified-nested design (Table 4.2) probably accounts for this design giving a lower, and more consistent, estimate of the nugget (C_0).

The random sampling regime cannot be used to assess semivariance at scales as small as the other two regimes, because of the lack of sampling points in the first lag class used. We expected more variation in the semivariance estimate when the number of data pairs used in the calculation is small. This was observed with the grid sampling regime, where more variation was observed at the lag class that had only three data pairs.

Conclusions and recommendations

The results of our analyses suggest that a stratified-nested grid sampling regime is better than a random or grid system of sampling when one wishes to use geostatistics to generate a set of semivariograms from several environments sampled concurrently, especially when the scales of heterogeneity are not well known before the sampling takes place . The greater consistency of the stratified-nested grid sampling pattern in detecting spatial structure over a broad range of patch sizes is the result of better estimating the nugget term of the semivariogram. The lower and less variable nugget is due to the increased number of pairs at short lag distances from the smaller scale sampling and thus less variation in the semivariance estimates at those lag distances. The lower end of the semivariogram is the critical area of

interest for semivariance analysis, because it is where the range, nugget and sill are determined.

In addition to providing lower and more consistent estimates of the nugget, the stratified-nested regime could be used to examine spatial structure over a greater distance than the other two sampling regimes. When the spatial scale of greatest interest may not be known it allows the assessment of heterogeneity at more than one level (e.g. are belowground resource levels patterned at the scale of individual roots, whole plants, entire fields, or some combination? Robertson and Gross 1994), using a sampling pattern that incorporates a broad range of spatial scales (such as the stratified-nested grid sampling pattern).

Other authors have also recommended using a stratified-nested sampling scheme for sampling ecological variables in a single environment (Oliver and Webster 1986, Fortin *et al.* 1989). Our investigation shows that this type of sampling regime will also provide more consistent estimates than a simple random or stratified grid design of the scale and magnitude of spatial structure when used in a study comparing several different environments. This should be encouragement for ecologists to adapt geostatistical techniques to ecological questions, and to expand the uses of these techniques further, from a range of descriptive studies into the realm of hypothesis testing.

Chapter 5

DISCUSSION

The studies reported in this dissertation demonstrate that there are species differences in ability to respond to nutrient heterogeneity and in which mechanisms they use when responding. These experiments also provides some support for the hypothesis that plants experience a trade-off between scale and precision in foraging strategy. Consistent differences among species in the ability to exploit nutrients in a heterogeneous environment could potentially be important in influencing community structure. The next step is to determine how foraging strategy translates into competitive ability under different conditions. Campbell *et al.*(1991) found evidence that competitive dominants were scale foragers, but in my study only one of the two dominant species exhibited a scale-type foraging strategy. Instead, I found that foraging strategy was not related to dominance in the field *per se*, but to the size of the plant. Large, fast-growing plants were the dominants in Campbell *et al.*'s (1991) study and they were scale foragers, but in my experiments one dominant species was a large scale forager and the other was a small precision forager. In both studies, subordinate species were smaller and scale foragers.

My initial hypothesis was that scale foragers are competitively more dominant in homogeneous conditions, pre-empting resources in the soil. Precision foragers can exploit any small pockets of nutrients left in between the depletion zones created by scale foragers' roots, but I would also hypothesize that precision foragers competitive ability (relative to scale foragers) would improve as the environment becomes more heterogeneous. I found that the

scale forager always obtained more nitrogen than the precision-foraging species. As patch size decreased, however, the amount of nitrogen obtained by the scale forager decreased, while, for the precision foragers, the amount of nitrogen acquired was independent of patch size. These results suggest that when patches become sufficiently small or of low intensity, then precision forager would acquire more N than the scale forager, resulting in a competitive advantage.

The next step would be to conduct a series of experiments comparing the competitive abilities of various scale- and precision-foraging species in environments where soil conditions have different nutrient patch sizes and intensities. Under heterogeneous conditions with many smaller patches, I hypothesize a better performance by precision foragers, while in homogeneous conditions I would expect scale foragers to dominate.

If it is determined that the relative competitive abilities of plant species are influenced by the scale of nutrient heterogeneity in the environment, then determining the scale of heterogeneity in an environment will be an important component of understanding community structure. One way to assess the scale of nutrient heterogeneity is to use geostatistical tools. The modelling used in chapter 4 indicated that a nested-grid sampling design would provide the most consistent assessment of spatial scales of heterogeneity across a range of locations. Consistency in the determination of scale of heterogeneity will become even more important when the modelling of plant responses to heterogeneity is expanded to include a temporal component (Ehrenfeld *et al.* 1997). The same environment would be sampled multiple times through the growing season and the scale at which heterogeneity is important to the plants could change drastically through the season.

APPENDIX

APPENDIX

Table A.1 - Preliminary data for *Daucus carota* and *Poa compressa*. Data values for two additional species used in these experiments, listed as mean ± standard error. Data that are **quadrant means** list first the mean of the background quadrant (patch size of 1 or 2 quadrants) or the first quadrant sampled (patch size of 0 or 4 quadrants) and then the mean of the enriched quadrant (patch size of 1 or 2 quadrants) or the second quadrant sampled (patch size of 0 or 4 quadrants). † For *Daucus* at low nutrient intensity, n=1 where no standard error is given. ‡ For *Daucus* at high nutrient intensity, n=4 for shoot N concentration, total plant N content, and root N concentration. * N/A = no data available.

Variable	No. of quadrants enriched	*Daucus carota* (n=2)† Low nutrient intensity	*Daucus carota* (n=6)‡ High nutrient intensity	*Poa compressa* (n=3) Low nutrient intensity
Total Plant Biomass (g)	0	0.17±0.12	0.12±0.02	0.14±0.04
	1	0.15±0.07	0.21±0.03	0.36±0.07
	2	0.12±0.01	0.27±0.06	0.32±0.03
	4	0.17±0.01	0.22±0.22	0.36±0.04
Shoot N Concentration (%)	0	1.16±0.11	1.66±0.14	0.14±0.10
	1	5.51±0.27	3.89±0.31	4.00±0.61
	2	6.44±0.20	5.25±0.52	4.88±0.25
	4	7.13±0.30	4.57±0.45	4.70±0.46

Table A.1 (cont'd)

Variable	No. of quadrants enriched	*Daucus carota* (n=2) Low nutrient intensity	*Daucus carota* (n=6) High nutrient intensity	*Poa compressa* (n=3) Low nutrient intensity
Total Plant N Content (mg)	0	0.13±0.08	0.14±0.04	0.06±0.02
	1	0.82	0.79±0.07	1.28±0.46
	2	0.81±0.08	1.53±0.29	1.64±0.15
	4	1.12±0.02	1.20±0.13	1.45±0.12
a/E(a) - plant means	0	1.30±0.14	1.46±0.11	2.14±0.19
	1	1.23±0.08	1.42±0.14	1.64±0.19
	2	1.24±0.08	1.32±0.14	1.99±0.12
	4	1.23±0.15	1.07±0.11	1.53±0.21
- quadrant means	0	1.47±0.15 1.14±0.20	1.38±0.12 1.53±0.10	2.01±0.18 2.27±0.35
	1	1.30±0.02 1.15±0.17	1.40±0.13 1.45±0.15	1.60±0.33 1.68±0.28
	2	1.22±0.04 1.25±0.18	1.26±0.13 1.37±0.17	1.95±0.20 2.03±0.19
	4	1.09±0.25 1.37±0.20	1.02±0.09 1.12±0.14	1.36±0.27 1.70±0.35
Mean segment length (mm) - plant means	0	3.36±0.70	3.42±0.50	1.35±0.07
	1	2.58±0.40	2.49±0.30	1.55±0.11
	2	2.47±0.18	2.19±0.22	1.49±0.05
	4	2.73±0.34	2.17±0.20	1.43±0.03
- quadrant means	0	2.70±0.66 4.02±1.27	3.35±0.63 3.49±0.41	1.44±0.12 1.26±0.08
	1	3.00±0.78 2.16±0.00	2.88±0.31 2.10±0.21	1.45±0.18 1.67±0.17
	2	2.75±0.12 2.20±0.19	2.43±0.26 1.94±0.12	1.39±0.05 1.59±0.09

Table A.1 (cont'd)

Variable	No. of quadrants enriched	*Daucus carota* (n=2) Low nutrient intensity	*Daucus carota* (n=6) High nutrient intensity	*Poa compressa* (n=3) Low nutrient intensity
MSL (mm) - quadrant means	4	2.66±0.73 2.80±0.37	2.08±0.05 2.26±0.28	1.44±0.07 1.44±0.05
Root biomass (g) - plant means	0	0.10±0.08	0.07±0.01	0.08±0.02
	1	0.07±0.05	0.10±0.02	0.10±0.01
	2	0.05±0.01	0.10±0.02	0.12±0.08
	4	0.06±0.00	0.08±0.01	0.07±0.01
- quadrant means	0	0.01±0.01 0.05±0.05	0.02±0.01 0.02±0.00	0.03±0.00 0.02±0.01
	1	0.01±0.01 0.01±0.01	0.01±0.00 0.04±0.01	0.03±0.01 0.04±0.01
	2	0.00±0.00 0.03±0.00	0.04±0.01 0.02±0.01	0.02±0.00 0.04±0.01
	4	0.01±0.00 0.01±0.01	0.02±0.01 0.02±0.01	0.04±0.00 0.03±0.00
^{15}N uptake rate (μg/hr)	0	N/A*	0.02±0.01	0.03±0.01
	1	N/A	0.06±0.02	0.03±0.01
	2	N/A	0.04±0.01	0.02±0.00
	4	N/A	0.03±0.01	0.02±0.00
- quadrant means	0	N/A	0.02±0.01 0.01±0.01	0.02±0.01 0.03±0.01
	1	N/A	0.05±0.02 0.07±0.05	0.02±0.00 0.03±0.01
	2	N/A	0.03 0.04±0.02	0.02±0.00 0.02±0.00
	4	N/A	0.05±0.03 0.02±0.01	0.03±0.01 0.02±0.00

Table A.1 (cont'd)

Variable	No. of quadrants enriched	*Daucus carota* (n=2) Low nutrient intensity	*Daucus carota* (n=6) High nutrient intensity	*Poa compressa* (n=3) Low nutrient intensity
Root N concentration (%) - plant means	0	0.49±0.06	0.50±0.06	0.77±0.00
	1	2.66	4.09±0.20	1.92±0.39
	2	5.66±1.26	5.69±0.55	2.60±0.50
	4	6.19±1.07	6.98±0.58	2.71±0.44
- quadrant means	0	0.50±0.07 0.51	0.47±0.14 0.52±0.16	0.76±0.06 0.78±0.06
	1	3.59±1.99 3.73	2.81±0.31 5.37±0.15	1.67±0.45 2.17±0.33
	2	4.31±2.37 7.01±0.15	5.14±0.64 6.23±0.81	2.14±0.63 3.06±0.40
	4	6.34±0.73 6.05±1.40	6.95±0.74 7.00±0.91	2.66±0.40 3.20±0.29

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