Masters Theses
Theses, Essays, and Senior Honors Projects

Spring 2015

THE EFFECTS OF NITROGEN AVAILABILITY AND COMPETITION ON FIRST YEAR PERRENNIAL BUNCHGRASS GROWTH AND ALLOCATION

Jeffrey T. Walker
John Carroll University, jwalker10@jcu.edu

Follow this and additional works at: http://collected.jcu.edu/masterstheses

Part of the Biology Commons

Recommended Citation

Walker, Jeffrey T., "THE EFFECTS OF NITROGEN AVAILABILITY AND COMPETITION ON FIRST YEAR PERRENNIAL BUNCHGRASS GROWTH AND ALLOCATION" (2015). *Masters Theses*. 11.
http://collected.jcu.edu/masterstheses/11

This Thesis is brought to you for free and open access by the Theses, Essays, and Senior Honors Projects at Carroll Collected. It has been accepted for inclusion in Masters Theses by an authorized administrator of Carroll Collected. For more information, please contact connell@jcu.edu.

THE EFFECTS OF NITROGEN AVAILABILITY AND COMPETITION ON FIRST
YEAR PERRENNIAL BUNCHGRASS GROWTH AND ALLOCATION

A Thesis Submitted to the
Office of Graduate Studies
College of Arts & Sciences of
John Carroll University
in Partial Fulfillment of the Requirements
for the Degree of
Master of Science

By
Jeffrey T. Walker
2015

The thesis of Jeffrey T. Walker is hereby accepted:

_____   _____
Reader – Christopher A. Sheil   Date

_____   _____
Reader – Jeffrey R. Johansen   Date

_____   _____
Reader – Jeremy J. James   Date

_____   _____
Advisor – Rebecca E. Drenovsky   Date

I certify that this is the copy of the original document

_____   _____
Author – Jeffrey T. Walker   Date

Acknowledgements

I would like to extend special thanks to Dr. Jeremy J. James and the EOARC for
supporting my Master's research and to Lori Zeigenhagen and EOARC interns for
nurturing my seedlings prior to my arrival in Burns, OR, and for harvesting those that
still remained once I left. I would also like to thank the John Carroll undergraduates in
the Drenovsky lab who sacrificed their time and fingers helping me cut and grind extra-
rigid, dried plant matter. Furthermore, I would like to extend an extra special thank you
to my advisor, Dr. Rebecca Drenovsky, who patiently encouraged me and provided
enlightening advice and feedback throughout the duration of this study. And finally, I
would like to thank the other two members of my Master's committee, Dr. Jeffrey
Johansson and Dr. Christopher Sheil, who served as great teachers and mentors
throughout my time at John Carroll University. Of course, this work would not have
been possible without the support of John Carroll University and the John Carroll
University Biology department, which provided a teaching assistantship and
opportunities to carry out my research.

Table of Contents:

CHAPTER 1

Nitrogen uptake, allocation and recycling during the first year of growth in two perennial bunchgrass species

Abstract

Improving seedling survival of perennial bunchgrasses is a key goal of restoration programs in the Intermountain West. Two perennial bunchgrass species commonly used in restoration programs (*Agropyron desertorum* and *Pseudoroegneria spicata*) were exposed to two levels of N and competition treatments in a randomized complete block study in a pot study in eastern Oregon. I documented uptake, allocation and resorption of N in plants during the first year of growth. *Agropyron desertorum* had significantly higher rates of N uptake than *P. spicata,* but *A. desertorum* maintained lower tissue N concentrations, suggesting that *P. spicata* was more likely to enter into a period of luxury consumption. Results indicated that there may be an inherent trade-off between luxury consumption and resorption, in which high tissue N concentrations due to luxury consumption prevent plants from realizing more complete resorption. Plants of both species experiencing competition realized near or complete resorption, but also had plant-wide tissue concentrations near the minimum values attainable prior to death. These plants also had severely stunted growth. This study demonstrated that early competition results in compounding negative feedbacks for slow growing species, and that the slightly more plastic species (*A. desertorum*) may be better at coping with strong competitive stress. However, if either species is to be successful in a restoration setting, a strong focus should be placed on seeding times and methods, as well as seeding for communities with high functional trait diversity.

Introduction

In the Intermountain West of the United States, areas previously dominated by native perennial bunchgrasses are increasingly threatened by nonnative, invasive, annual grasses (Miller et al. 1986, D'Antonio and Vitousek 1992, James et al. 2013). Prolific biomass production by invasive annual grasses results in high fuel loads for grassland fires (Miller et al. 1986). High thatch load, in turn, creates a positive feedback mechanism promoting non-native annual grass dominance over native, perennial vegetation (D'Antonio and Vitousek 1992). Type conversion from perennial- to annual-dominated grasslands has reduced native plant diversity, decreased rangeland quality for grazing animals, and caused increased fire cycle frequency (Mack 1981). Thus, determining the factors that promote invasion resistance, native plant dominance, and successful ecosystem restoration is critical to breaking the cycle of annual grass invasion and spread.

Many restoration programs in annual grass-infested systems depend on direct seeding efforts, due to the spatial extent of annual grass invasions. However, a recent meta-analysis of seeding projects in the Intermountain West documented that <5% of these efforts were successful (Sheley et al. 2011). Seedling emergence is a key life stage transition for successful seedling establishment in these systems (James et al. 2013). During seedling emergence and early seedling growth, annual and perennial grasses face similar challenges in nutrient poor, aridland soils. Although perennial grasses are predicted to be favored in the long-term in nutrient poor soils (due to efficient nutrient conservation and storage), at the seedling stage they may be at a disadvantage (James et al. 2011). At this stage, perennial seedlings must forage for all nutrients directly from the

soil, as they have not yet developed nutrient reserves. As a result, perennial grass seedlings experience similar reductions in growth rates as annual grasses under nutrient limitations (James 2008b). Additionally, at the end of the growing season, perennial grasses must allocate a portion of their nutrient budget to storage or towards developing long-lived tissues, whereas annual grasses are able to invest all available nutrients into reproduction (James et al. 2011). Thus, successful establishment of perennial grasses will depend on soil nutrient availability, plant nutrient uptake, efficient nutrient use and recycling, and competition for soil resources.

Three key factors influencing nitrogen (N) uptake capacity are N availability, plant N demand, and root allocation (Chapin 1980). N demand represents the amount of N that a plant is using at any given time and is dependent on plant size, allocation, and growth rate (Lambers and Poorter 1992, James and Richards 2005). N demand should be equal to or less than the amount of N that a plant is absorbing from soil at that given time, which can be defined as the critical N concentration ($\%N_{crit}$) (Ulrich 1952, Jeoffroy et al. 2002). If more N is taken up than required to meet N demand ($\%N > \%N_{crit}$), the excess N can be stored (Jeoffroy et al 2002). Luxury consumption of N occurs when uptake exceeds N demand significantly. Stored N can be used to meet N demand at times when current rates of uptake cannot easily match the amount of N required to maintain maximum growth rates, or to recover from catastrophic events and to support reproduction (Bloom et al. 1985, Chapin et al. 1990).

It has been suggested that in low nutrient environments, greater allocation of available resources to roots may increase nutrient uptake by increasing root surface area (Aerts and Chapin 2000). However, uptake is closely tied to growth rate (Rogers and

3

Barneix 1988, James and Richards 2005), and a shift in allocation to roots corresponds to a shift away from new leaf and stem growth (Lambers and Poorter 1992), potentially lowering overall growth rates and nutrient demand. A shift in allocation towards roots may be advantageous in scenarios in which N demand could otherwise exceed N uptake. In cases where demand has exceeded supply, leaves are senesced, growth rates are reduced, and, under extreme conditions, meristematic tissues die (Chapin 1980). Thus, under low nutrient conditions, a shift in allocation of resources to roots might be viewed as an acclimation response to avoid depleting nutrient reserves, rather than a mechanism for increasing uptake.

Root allocation may be either a constitutive or a plastic response (Lambers and Poorter 1992, Aerts and Chapin 2000). Low nutrient adapted species (LNAPs) often have high root allocation, even under high resource conditions. In low nutrient soils, high constitutive root allocation may promote nutrient uptake over the long-term, due to low ion diffusion rates (Chapin 1980, Aerts 1999). However, when nutrient supply in soils is high, low growth rate (due to lower biomass allocation to photosynthetic tissues relative to roots) may lead to strong negative feedbacks (i.e. severe long-term reductions to fecundity), as growth rates may not be sufficient to create the demand that meets the levels of potential nutrient uptake (Rodgers and Barneix 1988, Lambers and Poorter 1992). On the contrary, highly plastic species may alter their biomass allocation to favor root mass under reduced nutrient availability, while maintaining the ability to shift resources back to aboveground biomass in times of soil nutrient abundance (Funk 2008).

Due to the high energy and resource costs of absorbing nutrients, retention is of critical importance to plants in low nutrient habitats (Chapin 1980, Killingbeck 1996).

The ability of LNAPs to reduce nutrient losses through longer-lived tissues, greater nutrient resorption from senescing tissues, and overall greater nutrient use efficiency (NUE) makes them successful in low nutrient habitats (Chapin 1980, Aerts 1996). Nutrient retention is thought to be of critical importance, as nutrients remaining in senesced leaves are lost to soil nutrient pools. Although nutrients in the leaf litter are eventually released via decomposition and mineralization, these processes can be very slow in semi-arid or arid systems (Noy-Meir 1973), and plants may have to compete with others to reabsorb the N from the soil. Additionally, there are high costs to assimilation (Millard 1988). An increased capacity for resorption reduces the plants dependence on soil nutrient uptake and assimilation (Killingbeck 1996). Resorption can be sensitive to changes in environmental conditions, such as drought (Marchin et al. 2010) and nutrient availability (Rejmánková 2005). Thus, resorption measurements may help elucidate the short-term effects of competition and nutrient stress on whole-plant nutrient budgets.

Understanding how N uptake, use, resorption, and storage impact plant performance may help us better understand the factors influencing success or failure of restoration projects. By comparing species that vary in growth rate and nutrient conservation strategy (*Pseudoroegneria spicata* and *Agropyron desertorum*), one can identify how nutrient availability and competition pressure influence plant nutrient budgets, the trade-offs between growth and nutrient conservation, and overall plant performance. The objectives of this study are three-fold: (1) Document the plant N cycle through the acquisition, growth, and recycling stages in two species of perennial grasses under ideal conditions (sufficient nutrients, monoculture); (2) Determine if and how the nutrient budgets of perennial bunchgrasses change if competitive, non-native, annual

5

neighbors are present, or if N is scarce; (3) Compare the nutrient budgets of *P. spicata* and *A. desertorum*, in order to assess the potential for success of these species in restoration projects. Because *A. desertorum* has a higher RGR than *P. spicata* (James 2008), I hypothesized *A. desertorum* would have a higher N uptake rate than *P. spicata*. Additionally, in treatments with added N, I expected that *A. desertorum* would increase its growth rate to a greater degree than *P. spicata*, as *A. desertorum* directs fewer resources to building stress-tolerant tissues. However, I expected that *P. spicata* would exceed N demand by acquiring more N than the minimum requirement for maximum growth. Both plants were expected to resorb more N from leaves under low N conditions, but the more stress tolerant *P. spicata* was expected to show more complete N resorption under low N than the faster-growing *A. desertorum.* Under competitive stress, it was expected that both species would experience reductions in acquisition and RGR, and that plants experiencing both low N and competition would have a stronger resorption response than those experiencing high N and competition.

MATERIALS AND METHODS

Study location and study species

   The experiment was conducted at the Eastern Oregon Agricultural Research Center (EOARC, Burns, OR, US) using a target-neighbor model to simulate competition. The target plants were two perennial bunchgrass species: *Pseudoroegneria spicata* (Bluebunch Wheatgrass) (Pursh) A. Löve; and *Agropyron desertorum* (Crested Wheatgrass) (Fisch. ex Link) J.A. Schultes. *Bromus tectorum* L. (Cheatgrass) served as the neighbor species in all competition treatments. *Pseudoroegneria spicata* is a native,

late-successional, perennial bunchgrass species found throughout the Intermountain West (Mack 1981, Miller et al. 1986). *Pseudoroegneria spicata* seeds will often germinate in the fall under adequate conditions of soil moisture, and seedlings will spend the winter dormant; in such cases, seedlings will resume active growth in the late spring (Miller et al. 1986). *Agropyron desertorum* is a non-native, non-invasive perennial grass species native to parts of Eurasia that was introduced into the Intermountain West in 20th century as a rangeland forage species. *Agropyron desertorum* is phenologically very similar to *P. spicata* (Caldwell et al. 1981, Nowak and Caldwell 1986). Both of these species have similar germination times, but *A. desertorum* has a greater ability to respond to nutrient pulses (Cui and Caldwell 1997), as *A. desertorum* may allocate more carbon to roots than *P. spicata*. *Agropyron desertorum* has a faster overall growth rate (James 2008a) and a lower N use efficiency (NUE) then *P. spicata* (measured as the biomass production / N concentration) under similar growing conditions (Cui and Caldwell 1997). *Bromus tectorum* is a non-native annual grass known to be a serious invader in the region. Populations of this species were established in the late 19th century and spread rapidly through the early 20th century, most prominently in overgrazed regions that were once dominated by *P. spicata* (Mack 1981). *Bromus tectorum* has long been documented as a serious competitor to *P. spicata*, with roots growing approximately 50% faster than those of *P. spicata* (Harris 1967). *Bromus tectorum* is capable of altering fire regimes and establishing monocultures; additionally, it dies off earlier in the growing season than the native bunchgrasses, severely decreasing the amount of available herbaceous understory later in the season (Rau et al. 2011).

Experimental Design and Measurements

The experiment was carried out in individual pots set within a gravel garden plot using a randomized complete block design. Each block contained 2 target species X 2 levels of nutrients X 2 levels of competition; additionally, three harvests (early, middle, and late) were incorporated into the block design for a total of 24 target plants per block. Each block was replicated eight times.

Seeds of target species were planted on April 20, 2011 in Cone-Tainers (2.56 cm diameter X 18 cm deep; Stuewe and Sons, Inc.) containing a 2:1 mixture of coarse sand and field soil; three seeds were planted in each Cone-Tainer. *Pseudoroegneria spicata* seeds were acquired from the Washington State Department of Agriculture; *A. desertorum* seeds were bought from Bruce Seed Farm, Inc. (Townsend, MT). Seedlings received 1/4 strength Hoagland's on May 5, 11 and 20 to stimulate growth. During this time, seedlings were moved outside in the daytime (excluding windy or rainy days), but kept inside during the nights to cold-harden the seedlings. On May 16, seedlings were thinned to one plant per Cone-Tainer. Germination and early growth occurred in the greenhouse at the EOARC.

Both the initial harvest and transplant events took place on June 8, 2011. Eight seedlings of each species were harvested for initial biomass, root, shoot, and leaf tissue N concentrations (methods later). Harvested plants were rinsed with deionized water, separated into roots, stems and leaves, dried in an oven at 55°C for 48 hours and weighed. Concurrently, 96 seedlings of each species were transplanted into individual experimental pots (25 cm diameter X 19 cm deep) filled with a 2:1 mixture of coarse sand and sandy loam field soil (as per James 2008b). Plants were promptly watered

following transplantation. Plants randomly assigned to the competition treatments were transplanted into pots that had been seeded with *B. tectorum* seeds on May 26 (50 seeds per pot). *Bromus tectorum* seedlings were <3 cm in height when transplanting occurred.

All plants received periodic modified Hoagland's solutions (Epstein 1972); those in the nutrient treatment received modified Hoagland's with reduced N (1% N; only for the first pulse) or 0% N (for later pulses), whereas all others received modified Hoagland's solution throughout the experiment as either 1/10 strength (early harvest period) or ¼ strength (middle and late harvest period) as 1 L pulses twice per harvest period (early and middle) or once per harvest period (late). Nutrient concentrations were increased prior to middle and late harvests in an attempt to strengthen N soil pools for high N treatments.

Transplanted seedlings were randomly assigned to one of three harvests: early (June 7-8), middle (August 8-9), or late (January 7-8). The three resulting inter-harvest periods captured the fast-growth acquisition phase (between the initial and early harvest), a period of steady growth (between the early and middle harvest), and a final period, during which plants were gradually water-stressed to simulate seasonal drought and force the senescence of leaves (between the middle and late harvest). Targeting these specific periods of time allowed us to assess nutrient uptake, use, recycling and storage throughout the first season of growth for both species.

During each harvest, above- and belowground biomass of target plants was collected and separated into leaves, stems, and roots. Additionally, throughout the experiment, senescing leaves were collected and composited for each replicate. Samples were rinsed with deionized water, oven-dried at 55°C for 48 h, and weighed. After

weighing, samples were finely ground and analyzed for total N concentration (all target plants) with a CN analyzer.

Statistical analysis

Rates of N uptake were calculated as:

$$\text{N uptake} = (\Delta \text{ total N pool} / t) * ((\ln(\text{Root wt}_{final}) - \ln(\text{Root wt}_{initial})) / \Delta \text{ root wt}).$$

N uptake was calculated for the periods of initial to early harvest and early to mid-harvest. RGR was calculated for the same time periods:

$$\text{RGR} = (\ln(\text{biomass}_{final}) - \ln(\text{biomass}_{initial}) / t).$$

Univariate ANOVAs were run for N uptake and RGR for both time periods; effects in the model included species, N treatment, competition treatment, and block, as well as the interactions between treatment factors. N uptake data were weighted by the inverse of the variance due to unequal variances. N resorption proficiency (N_{prof}) was determined after the late season harvest and reported as the concentration of nutrients in senesced leaves (Killingbeck 1996). A single, weighted univariate ANOVA was run for analysis of N_{prof}, using the same model design as for N uptake and RGR. Linear contrasts were used to determine relationships between individual treatments. N pools for roots, stems, and leaves were related to total N pools for all plants via linear regression. In conjunction, root, stem, and leaf mass were compared with total plant mass for all plants via linear regression. Similar regressions were used to relate root N concentration with green and senesced leaf N concentrations. ANCOVAs were run to test for differences in slope and intercept between harvests. Assumptions of normality and equal variance were

tested using the Shapiro Wilks test and Levene's test, respectively. All statistical tests were run on SAS Institute software (v9.2).

RESULTS

Allocation of Carbon and N

For both species in the monoculture treatment, the leaf N pool decreased ($P<0.0001$) relative to total N pool, the stem N pool remained constant ($P=0.05$), and the root N pool increased from mid to late harvest ($P<0.0001$) (Fig. 1), as determined by ANCOVA. From mid to late harvest, leaf mass decreased ($P=0.0015$), stem mass remained constant ($P=0.77$), and root mass had a tendency to increase ($P=0.07$) relative to total biomass for both species in monoculture (Fig. 2), as determined by ANCOVA. At each of the three harvests, average total N pools were largest for high-N *A. desertorum* monoculture plants, followed by low-N *A. desertorum* monoculture plants, then by high-N *P. spicata* monoculture plants and low-N *P. spicata* monoculture plants. Differences in total N pools, for monoculture plants, were most extreme at the mid harvest time period (Fig. 3). Among competition plants, high-N *A. desertorum* had the largest total N pool (0.025 g). All other competition plants had similar total N pools averaging only 0.0060 g.

N Uptake

By early harvest (Table 1), monoculture plants (-C) acquired nutrients at significantly higher rates than plants with competitors (+C). The magnitude of this effect differed significantly between species (P<0.0001) and between N treatments (P=0.0027). Early-season uptake rates for *A. desertorum* grown in monoculture were approximately 2-fold greater than *P. spicata* growing in monoculture. For plants experiencing competition, uptake rates for *A. desertorum* were 1.5-fold greater than *P. spicata*. Among monoculture plants receiving high N (-C +N), uptake rates were approximately 1.25-fold greater than those receiving low N (-C -N). For competition plants receiving high N (+C +N), uptake rates were 1.8-fold greater than those receiving low N (+C -N).

Over the time period from early- to mid-harvest, competition continued to have a significant impact on uptake rates (P<0.0001). Of the competition plants, only *A. desertorum* in the high N treatment showed positive rates of uptake. During this time period, the two species differed in their response to N treatments (P=0.02). Low N resulted in a greater reduction to uptake rates in *A. desertorum* than in *P. spicata*. For monoculture plants, uptake rates for *P. spicata* remained relatively consistent over the first two harvest periods, whereas uptake rates for *A. desertorum* decreased between the early and mid-harvest periods.

Relative Growth Rate

From the initial harvest to the early harvest, RGRs were significantly different between N treatments for all plants (P=0.02); (Table 1). High N plants grew at an average rate of 0.052 g g^{-1} d^{-1} compared with low N plants at 0.046 g g^{-1} d^{-1}. The effect

of competition on growth rate was significantly different between species (P=0.02). The difference in RGRs between monoculture and competition plants was much larger for *A. desertorum* than *P. spicata;* however, on average, *A. desertorum* competition plants still grew at a 1.25-fold faster rate than *P. spicata* plants. *A. desertorum* monoculture plants grew at a 1.42-fold faster rate than *P. spicata* in monoculture.

From the early to mid-harvest, RGRs were significantly different between species (P=0.008) and competition treatments (P<0.0001). In monoculture, *A. desertorum* plants grew at a similar rate to *P. spicata* (0.082 and 0.076 g g^{-1} d^{-1}, respectively). In competition, *Agropyron desertorum* in the high N treatment grew at an average rate of 0.024 g g^{-1} d^{-1}, a rate over 3-fold greater than the average of any other competition plants.

Tissue N Concentrations and Resorption

Root and leaf N concentrations were positively correlated across monoculture treatments and over time (Fig. 4A, B), and no significant differences in slope or intercept were detected between harvest periods. This positive linear correlation between root and leaf N concentrations remained consistent regardless of species. Over the course of the experiment, for monoculture plants, total plant N concentrations in all tissues decreased (Table 2). Whole plant N tissue concentrations for all competition plants were relatively low by the early harvest and remained low throughout the experiment. N concentrations in roots and leaves across all competition plants and time were also positively correlated with no significant differences in slope or intercept detected (Fig. 4C).

Through the end of the experiment, for both species in monoculture, green leaf N concentration ranged from 1.93 – 4.40% and root concentration from 1.08 – 1.96%. For

both species in competition, green leaf concentrations ranged from 0.92 – 2.29%, and root concentration from 0.78 – 0.91%. Mid-season green leaf N concentrations differed significantly between N treatments (P<0.0001), as well as competition treatments (P<0.0001) (Fig. 6A). High N treatments had 1.3-fold higher concentrations of leaf N than low N treatments. Monoculture plants had 2.6-fold greater N concentrations than those experiencing competition. Green leaf N did not differ significantly between species (P=0.672), nor were interactions between treatments significant. For N_{prof}, there was a significant three-way interaction between species*soil N availability*competition (P=0.0285) (Fig. 6B). Using linear contrasts it was determined that though level of N had a significant impact on N_{prof} in monoculture plants (P<0.0001), no significant differences were detected in competition plants. The impact of N on monoculture plants was significant within both *P. spicata* (P=0.0002) and *A. desertorum* (P=0.0174). For both species, leaves of low N plants had more complete N_{prof} than leaves of high N plants.

DISCUSSION

Effects of N Treatments on Monoculture Plants

Consistent with our hypotheses, in monoculture treatments, *A. desertorum* had greater soil N uptake rates and a greater average RGR than *P. spicata,* and for both species, higher soil N was correlated with increased N uptake rates per unit root mass during early and mid-growing season time periods. However, patterns of uptake differed between the two species. For *P. spicata,* uptake rates remained constant relative to root mass from early- to mid-growing season, whereas *A. desertorum* uptake rates decreased

significantly, and high soil N levels had less of an impact on uptake rates for *P. spicata* in comparison to *A. desertorum.* Additionally, whereas growth rates of both species increased significantly in high N treatments during the early-growing season, growth rates were similar among both species and soil N treatments during the mid-growing season.

Comparing the phenology and growth strategies of the two species provides context for these differences in seasonal N uptake and growth rate. N uptake per unit root mass was significantly higher for *A. desertorum* than *P. spicata,* even though *A. desertorum* RMR was lower*,* indicating that *A. desertorum* has stronger uptake kinetics and/or that aboveground traits, such as SLA or LMR, also impacted uptake rates (Poorter et al. 1990). These data are consistent with literature linking uptake closely with growth rate (Rogers and Barneix 1988, Garnier et al. 1989, James and Richards 2005), as rapid shoot growth increases N demand. From an economics perspective, greater investment in shoot tissue provides a greater return on investment with respect to growth rates (Bloom et al. 1985, James and Drenovsky 2007) and also increases N demand. Conversely, greater investment in root tissue lowers growth rate and thus lowers N demand. Species with lower RMRs may be better suited to taking advantage of prolonged increases in soil N. The relatively muted response to increased soil N levels by *P. spicata,* as compared to *A. desertorum,* supports this concept, and suggests that *P. spicata* seedlings are not able to take full advantage of large N pulses, even early in the growth season.

Despite early season differences in growth rates, both species, regardless of N treatment, had similar RGRs later in the growing season, which were significantly higher than the early season RGRs for both species; however, as *A. desertorum* individuals were

already much larger at this point in time, a similar RGR (relative to the much

significantly smaller *P. spicata* plants) corresponds to an exponential increase in absolute

biomass. Between the early and mid-season harvests, average biomass for *A. desertorum*

increased from 0.83 – 9.95 g, whereas *P. spicata* increased from 0.50 – 5.19 g. Because

overall plant mass is often correlated closely with uptake rates (Rogers and Barneix

1988), exponential increases in biomass can result in exponential increases in N uptake.

Thus, growth rate early in the season may have compounding effects on uptake and

growth rates later in the season. If some plants are unable to take advantage of excess

amounts of soil N (e.g., *P. spicata*), whereas others are able to take advantage of these

excesses (e.g., *A. desertorum*), the result may represent a compounding negative feedback

loop (Lambers and Poorter 1992). These feedbacks can have a strong negative impact on

survival, growth, and seed production in years to come (Humphrey and Schupp 2004).

For both species and N treatments, N tissue concentrations declined over time.

While it is common for leaves to dilute N concentrations during senescence (Killingbeck

1996), dilution of N concentrations during the growing season is not often discussed in

regards to the whole plant. Furthermore, as overall tissue concentrations decreased, leaf

N concentrations remained significantly proportional to root N concentrations, suggesting

a close relationship between the two traits. If this relationship holds true across a larger

suite of species, we may gain a greater understanding of belowground plant nutrient

status, simply by observing leaf nutrient concentrations. Plants with large root systems

proportional to their respective root N pools ought to have lower N concentrations in

leaves. Whereas N concentrations in leaves do not directly indicate the size of the root

system, or the total N pool, leaf N concentrations may indicate whether or not root

systems are nutrient stressed. Low-N *P. spicata* and *A. desertorum* both had lower N

concentrations in green leaf tissue at the middle harvest. High-N *P. spicata* plants had

significantly higher N concentrations in roots by the late-harvest, but root systems were

not significantly larger than low-N plants. These data suggest that for high-N *P. spicata*

plants, plant N uptake had exceeded N demand, and plants were undergoing luxury

consumption, whereas low tissue concentrations in low-N *P. spicata* plants may have

triggered the plants to increase RMR and continue to more actively forage for N. In this

scenario, increased allocation to roots increases surface area for potential nutrient uptake

and reduces nutrient demand that would result from new leaf formation, ensuring that

demand does not exceed supply. *Agropyron desertorum* did not appear to have a strong

luxury consumption response; high-N *A. desertorum* were predictably larger with slightly

higher tissue N concentrations than low-N plants.

　　　　Regardless of species or N treatment, RMR increased by the end of the

experiment. Whereas overall root N pools were larger, root N concentrations were lower,

as were N concentrations in all other tissues. This trend may indicate that plants prepare

for the next season of growth by biasing allocation of carbon towards roots to either: (1)

maximize root surface area for N uptake in the following year; or (2) to increase the roots

as an N sink (due to lower root N concentrations), thereby enabling a greater percentage

of N to be extracted from the leaves and decreasing N losses for the following season.

These leaf resorption data supports this second hypothesis. Both species had similar N

resorption responses to decreased N. When soil N was lower, plants realized more

complete resorption. As with green leaf N concentrations, senesced leaf tissue N

concentrations (resorption values) were strongly correlated with root N concentrations.

In contrast to my initial hypotheses regarding nutrient concentration patterns between the species, *P. spicata* had higher concentrations of N in both roots and senesced leaves than *A. desertorum,* irrespective of N treatment. It was expected that *P. spicata,* the LNAP, would be more likely to maximize resorption, both under low N and high N treatments. Whereas LNAPs may be adapted to maximizing N returns from leaves when soil N is limiting, they are also known to be luxury consumers. If N supply is in abundance, N uptake may outpace N demand, thus resulting in higher N concentrations in roots. This may inhibit the ability of plants to realize more complete resorption from leaves at the end of the season. This is consistent with past studies, which found that plants that have accumulated nutrients to luxury levels resorbed less nutrients (Shaver and Melillo 1984). It is possible that in our study, the low N treatments were not far below the optimum levels of N for *P. spicata.* Had we been able to lower N to an even greater degree, we may have seen more complete resorption in these plants. Our study suggests that controls on resorption proficiency may be sink-source related, as suggested by Chapin and Moilanen (1991), with the sink strength of the roots as a controlling mechanism in our study species. It would also explain why a significant number of past studies have found decreased resorption as a response to increased nutrient availability (Aerts 1996, Killingbeck 2004). Sink-source relationships would also explain why plants with high N concentrations in green leaves tend to realize less complete resorption values for senesced leaves. Plants have high concentrations leaf N concentrations because root N concentrations are high, and high root N concentrations decrease sink strength and N resorption proficiency. This process may occur instead of, or in addition to, the

hypothetical scenario in which high green leaf N concentrations may have incurred higher amounts of recalcitrant nitrogen-containing compounds (Killingbeck 2004).

Impact of Competition

Competition had a strong negative impact on all aspects of first year growth for both species and regardless of N treatment. Uptake rates for plants experiencing competition were nearly an order of magnitude lower than monoculture plants between the initial and early harvest. Across both species, plants experiencing competition at the early harvest of both species maintained similar (though notably smaller) root masses as the monoculture plants, yet recorded disproportionately low rates of uptake per unit of root mass. By mid harvest, root mass was significantly lower for all competition plants and uptake rates for both species were even lower. Three of the four treatments had negative average uptake rates from early to mid-harvest indicating a net loss of N. High-N, *A. desertorum* competition plants were the exception to this trend. This does not, however, suggest that *A. desertorum* would benefit competitively from increases in soil nitrogen. Increases in soil N have been shown to increase annual grass growth rates to an even greater degree than for perennial species (James 2008b).

As previously noted, root mass of competition plants was comparable in size to monoculture plants at the early harvest, but competition plants of both species had significantly decreased green leaf tissue mass, and thus, a higher RMR. This allocation pattern is a clear example in which high RMR does not indicate an increased ability to capture soil nutrients. In this experiment, RMR increased under intense competitive stress. In other studies, RMR has increased allometrically as a function of plant size in

high nutrient scenarios (Muller et al. 2000). This contradiction highlights the fact that similar changes to RMR can indicate responses to different environmental cues and further emphasizes the necessity of obtaining a whole plant perspective on traits over time and in various scenarios. Herein, the increase of RMR in response to competitive stress appears more so as a survival strategy for plants that are accustomed to enduring periods of low nutrient availability, and less like a competitive strategy (sensu Goldberg 1990) to deplete common resources. It is possible that *A. desertorum* and *P. spicata* lack the ability to distinguish between competitive stress and periods of abiotic soil N deficiency.

As with monoculture plants, decreases in green leaf tissue were consistent with decreases in whole-plant tissue N concentrations in plants experiencing competition. Regardless of species, average root N concentrations for plants experiencing competition never exceeded 10 g kg^{-1}, and did not drop below 7.5 g kg^{-1}. This may indicate a minimum root N concentration (i.e., the N concentration below which roots would die). For these plants, leaf N concentrations fluctuated across a much larger range (8.5 g kg^{-1} and 23 g kg^{-1}), but remained significantly correlated with root N concentrations. The lowest of these green leaf values were similar to values found in senesced leaves, again indicating that plant N concentrations were, on occasion, near the minimum N concentrations for leaves. These values may be indicative of maximum potential resorption concentrations (Killingbeck 2004). Low-N, *P. spicata* plants realized less complete resorption than high-N, *P. spicata* plants. The opposite was true for *A. desertorum.* All competition plants realized more complete resorption than monoculture plants, and both species realized similar minimum N concentration values in senesced

leaves. As with monoculture plants, N resorption proficiency was closely linked with N concentrations in roots, and may, therefore, be an indicator of plant root N status and sink strength.

Addressing Restoration

Our experiment indicates that *A. desertorum* seedlings possess more competitive traits than *P. spicata*; however, neither of these species demonstrated a strong ability to compete with *B. tectorum.* It is unlikely that either of these species would, alone, contribute strongly to restoration success at the seedling stage. This is evidenced by low success rates of restoration projects in these regions (James et al. 2013) and field experiments that have shown poor success rates of promising restoration species, such as *Elymus elymoides* (Humphrey and Shupp 2004). Whereas *P. spicata* may have once been the dominant bunchgrass species in the Intermountain West, its dominance was supported by long-term conservation of nutrients (an N storage strategy that appears to be present even at the seedling stage), not through the possession of traits that would otherwise make it a strong first-year competitor. Recent advances in the literature suggest that restoration success may be improved by focusing on restoration species with similar functional traits to those of the invading species (Drenovsky et al. 2012), and/or by varying seeding times and methods (Boyd and James 2013). If restoration success is to be improved in the Intermountain West, I suggest focusing efforts initially on seeding a mixture of species, the majority of which share traits with *B. tectorum,* and only shifting seeding strategies towards an increase in LNAPs, such as *P. spicata,* once *B. tectorum* populations appear to be in decline.

21

CONCLUSION

The major physiological implication of this study is that regardless of whole plant N concentrations throughout the season (which had a tendency to decrease as plants aged), N concentrations in leaf tissues remained proportional to N concentrations in root tissues. Furthermore, when leaves senesced at the end of the season, N concentrations in senesced leaf tissues were also proportional to N concentrations in roots. These patterns suggest that luxury consumption and more complete resorption, two traits hypothesized to be indicative of LNAPs, may be mutually exclusive. When nutrients were abundant, luxury consumption was high and resorption proficiency was low. In conditions where nutrients were sufficiently scarce, luxury consumption was low and resorption proficiency was high; however, even in conditions of extremely low N, we did not observe more complete resorption in *P. spicata,* an LNAP, than *A. desertorum,* an HNAP.

In terms of ecological and restoration significance, our study supports the hypothesis that slow growing, non-plastic, perennial seedlings experience severe negative feedbacks to growth, seed production and survivorship due to low RGR early in the season. Whereas a high RMR may be beneficial for mature plants, the inability of seedlings to adjust biomass allocation to more closely resemble a fast growing species appears to be a major barrier to competitive ability and overall success in the first year of growth.

REFERENCES

Aerts, R. 1996. Nutrient resorption from senescing leaves of perennials: Are there general
patterns? *Journal of Ecology* 84:597-608.

Aerts, R. 1999. Interspecific competition in natural plant communities: mechanisms,
trade-offs and plant-soil feedbacks. *Journal of Experimental Botany* 330:29-37.

Aerts, R. and F.S. Chapin. 2000. The mineral nutrition of wild plants revisited: A re-
evaluation of processes and patterns. *Advances in Ecological Research* 30:1-67.

Bloom, A.J., S. Chapin III, and H.A. Mooney. 1985. Resource limitation in plants – An
economic analogy. *Annual Review of Ecology and Systematics* 16:363-392.

Boyd, C.S. and J.J. James. 2013. Variation in timing of planting influences bluebunch
wheatgrass demography in an arid system. *Rangeland Ecology & Management*
66:117-126.

Caldwell, M.M., J.H. Richards, D.A. Johnson, R.S. Nowak and D.S. Dzurec. 1981.
Coping with herbivory: Photosynthetic capacity and resource allocation in two
semiarid *Agropyron* bunchgrasses. *Oecologia* 50:14-24.

Chapin, F.S. III. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology
and Systematics* 11:233-260.

Chapin, F.S. III, E.D. Schulze and H.A. Mooney. 1990. The ecology and economics of
storage in plants. *Annual Review of Ecology and Systematics* 21:423-447.

Chapin, F.S. III and L. Moilanen. 1991. Nutritional controls over nitrogen and
phosphorus resorption from Alaskan birch leaves. *Ecology* 72:709-715.

Cui, M. and M.M. Caldwell. 1997. Growth and nitrogen uptake by *Agropyron desertorum* and *Pseudoroegneria spicata* when exposed to nitrate pulses of different duration. *Australian Journal of Plant Physiology* 24:637-642.

D'Antonio, C.M. and P.M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual Review of Ecology and Systematics* 23:63-87.

Drenovsky, R.E., B.J. Grewell, C.M. D'Antonio, J.L. Funk, J.J. James, N. Molinari, I.M. Parker and C.L. Richards. 2012. A functional perspective on plant invasion. *Annals of Botany* 110:141-153.

Epstein, E. 1972. *Mineral nutrition of plants: principles and perspectives*. John Wiley & Sons, New York.

Funk, J.L. 2008. Differences in plasticity between invasive and native plants from a low resource environment. *Journal of Ecology* 96:1162-1173.

Garnier, E., G.W. Koch, J. Roy, and H.A. Mooney. 1989. Responses of wild plants to nitrate availability – Relationships between growth rate and nitrate uptake parameters, a case study with two *Bromus* species. *Oecologia* 79:542-550.

Goldberg, D.E. 1990. Components of resource competition in plant communities. *Perspectives in Plant Competition* (Ed. by J. Grace and D. Tilman) 27-49. Academic Press, New York.

Harris, G.A. 1967. Some competitive relationships between *Agropyron spicatum* and *Bromus tectorum. Ecological Mongraphs* 37:89-111.

Humphrey, L.D. and E.W. Schupp. 2004. Competition as a barrier to establishment of a native perennial grass (*Elymus elymoides*) in alien annual grass (*Bromus tectorum*) communities. *Journal of Arid Environments* 58:405-422.

James, J.J. and J.H. Richards. 2005. Plant N capture from pulses: effects of pulse size, growth rate, and other soil resources. *Oecologia* 145:113-122.

James, J.J. and R.E. Drenovsky. 2007. A basis for relative growth rate differences between native and invasive forb seedlings. *Rangeland Ecology and Management* 60:395-400.

James, J.J. 2008a. Leaf nitrogen productivity as a mechanism driving the success of invasive annual grasses under low and high nitrogen supply. *Journal of Arid Environments.* 72:1775-1784.

James, J.J. 2008b. Effect of soil nitrogen stress on the relative growth rate of annual and perennial grasses in the Intermountain West. *Plant and Soil* 310:201-210.

James, J.J., R.E. Drenovsky, T.A. Monaco and M.J. Rinella. 2011. Managing soil nitrogen to restore annual grass-infested plant communities: effective strategy or incomplete framework? *Ecological Applications* 21:490-502.

James, J.J., C.S. Boyd and T. Svejcar. 2013. Seed and seedling ecology research to enhance restoration outcomes. *Rangeland Ecology & Management* 66:115-116.

Jeoffroy, M.H., B. Ney and A. Ourry. 2002. Integrated physiological and agronomic modeling of N capture and use within the plant. *Journal of Experimental Botany* 53:809-823.

Killingbeck, K.T. 1996. Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. *Ecological Society of America* 77:1716-1727.

Killingbeck, K.T. 2004. Nutrient resorption. *Plant Cell Death Processes* (ed. by L.D. Noodén) 215-226. Elsevier, Amsterdam.

Lambers, H. and H. Poorter. 1992. Inherent variation in growth rate between higher plants: a search for physiological causes and ecological consequences. *Advances in Ecological Research* 34:187-261.

Mack, R.N. 1981. Invasion of *Bromus tectorum* L. into western North America: An ecological chronicle. *Agro-Ecosystems* 7:145-165.

Marchin, R., H. Zeng and W. Hoffmann. 2010. Drought-deciduous behavior reduces nutrient losses from temperate deciduous trees under severe drought. *Oecologia* 163:845-854.

Millard, P. 1988. The accumulation and storage of nitrogen by herbaceous plants. *Plant, Cell, and Environment* 11:1-8.

Miller, R.F., J.M. Seufert and M.R. Haferkamp. 1986. The ecology and management of bluebunch wheatgrass (*Agropyron spicatum*): A review. Agricultural Experiment Station, Oregon State University.

Muller, I., B. Schmid and J. Weiner. 2000. The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3/2:115-127.

Nowack, R.S. and M.M. Caldwell. 1986. Photosynthetic characteristics of crested wheatgrass and bluebunch wheatgrass. *Journal of Range Management* 39:443-450.

Noy-Meir, I. 1973. Desert ecosystems: Environment and producers. *Annual Review of Ecology and Systematics* 4:25-51.

Poorter, H., C. Remkes and H. Lambers. 1990. Carbon and nitrogen economy of 24 wild species differing in relative growth rate. *Plant Physiology* 94:621-627.

Rau, B.M., D.W. Johnson, R.R. Blank, A. Lucchesi, T.G. Caldwell and E.W. Schupp. 2011. Transition from sagebrush stepe to annual grass (*Bromus tectorum*): Influence on belowground carbon and nitrogen. *Rangeland Ecology and Management* 64:139-147.

Rejmánková, E. 2005. Nutrient resorption in wetland macrophytes: comparison across several regions of different nutrient status. *New Phytologist* 167:471-482.

Rogers, C.O. and A.J. Barneix. 1988. Cultivar differences in the rate of nitrate uptake by intact wheat plants as related to growth rate. *Physiologia Plantarum* 72:121-126.

Shaver, G.R. and J.M. Melillo. 1984. Nutrient budgets of marsh plants: efficiency concepts and relation to availability. *Ecology* 65:1491-1510.

Sheley, R. L., J. J. James, M. J. Rinella, D. M. Blumenthal, and J. M. DiTomasso. 2011. A scientific assessment of invasive plant management on anticipated conservation benefits. In: D. D. Briske [ed.]. Conservation benefits of rangeland practices: assessment, recommendations, and knowledge gaps. Lawrence, KS, USA: Allen Press. p. 291–335.

Ulrich, A. 1952. Physiological bases for assessing the nutritional requirements of plants. *Annual Review of Plant Physiology* 3:207-228.

Table 1: Rates of N Uptake (mg g^{-1} d^{-1}), RGR (g g^{-1} d^{-1}), and RMR for both species. Data are averages ± SD ($N = 8$, or $N = 7$ for early harvest *P. spicata* in the low-N monoculture treatment where the only case of mortality in this experiment occured). Plants were grown in either monoculture (-C) or with competitors (+C) under high N (+N) or low N (-N) conditions. Negative values for uptake indicate a net loss of nitrogen.

Trait	Harvest period	P. spicata				A. desertorum			
		-C +N	-C -N	+C +N	+C -N	-C +N	-C -N	+C +N	+C -N
Uptake	early	4.80 ± 0.84	3.92 ± 1.72	0.79 ± 0.29	0.55 ± 0.32	8.83 ± 1.03	7.06 ± 1.44	1.04 ± 0.40	0.94 ± 0.30
	mid	4.69 ± 1.50	4.14 ± 1.53	-0.08 ± 0.50	-0.09 ± 0.12	6.94 ± 1.84	4.28 ± 2.10	0.39 ± 0.59	-0.20 ± 0.23
RGR	early	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
	mid	0.08 ± 0.02	0.08 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
RMR	early	0.33 ± 0.05	0.35 ± 0.05	0.54 ± 0.07	0.53 ± 0.06	0.24 ± 0.03	0.27 ± 0.05	0.39 ± 0.04	0.42 ± 0.03
	mid	0.30 ± 0.10	0.32 ± 0.07	0.33 ± 0.14	0.48 ± 0.09	0.22 ± 0.06	0.24 ± 0.03	0.31 ± 0.06	0.34 ± 0.07
	late	0.39 ± 0.07	0.46 ± 0.13	0.90 ± 0.04	0.90 ± 0.04	0.39 ± 0.05	0.39 ± 0.08	0.70 ± 0.14	0.70 ± 0.17

Table 2: Total plant nitrogen concentrations expressed in % N. Data are averages ± SD ($N = 8$, or $N = 7$ for early harvest *P. spicata* in the low-N monoculture treatment). Plants were grown in either monoculture (-C) or with competitors (+C) under high N (+N) or low N (-N) conditions.

Trait	Harvest period	*P. spicata*				*A. desertorum*			
		-C +N	-C -N	+C +N	+C -N	-C +N	-C -N	+C +N	+C -N
Total plant	early	3.18 ± 0.39	3.02 ± 0.75	1.28 ± 0.22	1.16 ± 0.18	3.51 ± 0.52	3.27 ± 0.54	1.10 ± 0.20	1.09 ± 0.10
nitrogen	mid	2.03 ± 0.29	1.87 ± 0.42	1.03 ± 0.27	0.85 ± 0.15	2.01 ± 0.40	1.46 ± 0.40	0.82 ± 0.19	0.68 ± 0.11
concentrations	late	1.31 ± 0.27	0.99 ± 0.23	0.87 ± 0.13	0.94 ± 0.19	0.97 ± 0.14	0.91 ± 0.15	0.99 ± 0.18	0.83 ± 0.16

Figure 1: Scatter plots of leaf, stem, and root N pools (expressed in g) of all monoculture plants to total N pools. Slope and intercepts of leaf and root N pools both differed significantly from mid to late harvest periods.

Figure 2: Scatter plot of leaf, stem, and root biomass (expressed in g) of all monoculture plants to total biomass. Slope and intercepts of leaf biomass differed significantly from mid to late harvest periods.

Figure 3: Detailed N pools (expressed in mg) for *P. spicata* (PSSP) and *A. desertorum* (AGDE) for all treatments over the three major harvest periods. *Agropyron desertorum* monoculture plants were the only target plants to produce seed heads.

Figure 4: (**A**) Scatter plot showing the positive linear relationship between green leaf N concentrations and root N concentrations (expressed as %N) for *P. spicata* and *A. desertorum* for monoculture (0), competition (x), high-N (+), and low-N (-) treatments. Data points are individual plants from the three different harvest periods (harvest period not distinguished in this figure). (**B**) Scatter plot for the relationship between root and leaf N concentrations (expressed as %N) for monoculture plants of both species across nutrient treatments. No significant differences in slope or intercept were found among harvests. Data points represent values for individual plants. (**C**) Scatter plot for the relationship between root and leaf N concentrations (expressed as %N) for competition plants of both species across all treatments. No significant differences in slope or intercept were found amongst harvests. Data points represent values for individual plants.

Figure 5: Regression analysis for the relationship between leaf (green or senesced) and root N concentrations. No significant difference was found in slope, but significant

differences in intercept were detected. Data points are individual plants of both species across all treatments.

Figure 6: Average green leaf N (expressed in g kg^{-1}) $\pm$ SD ($N = 8$) for mid-harvest period (**A**) *P. spicata* and (**B**) *A. desertorum* plants; And average N$_{prof}$ (expressed in g kg^{-1}) $\pm$ SD ($N = 8$) for final-harvest period *P. spicata* and *A. desertorum* plants.
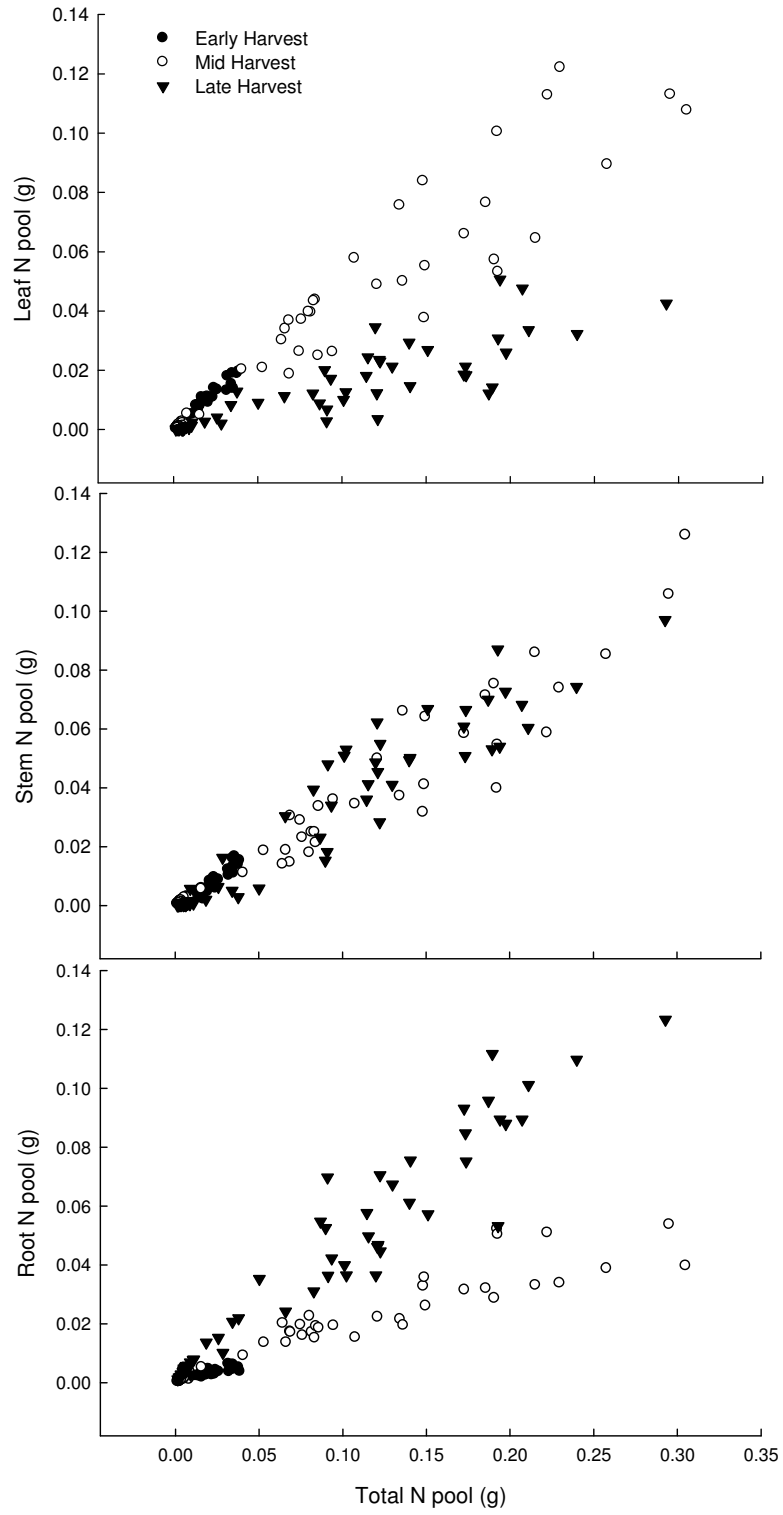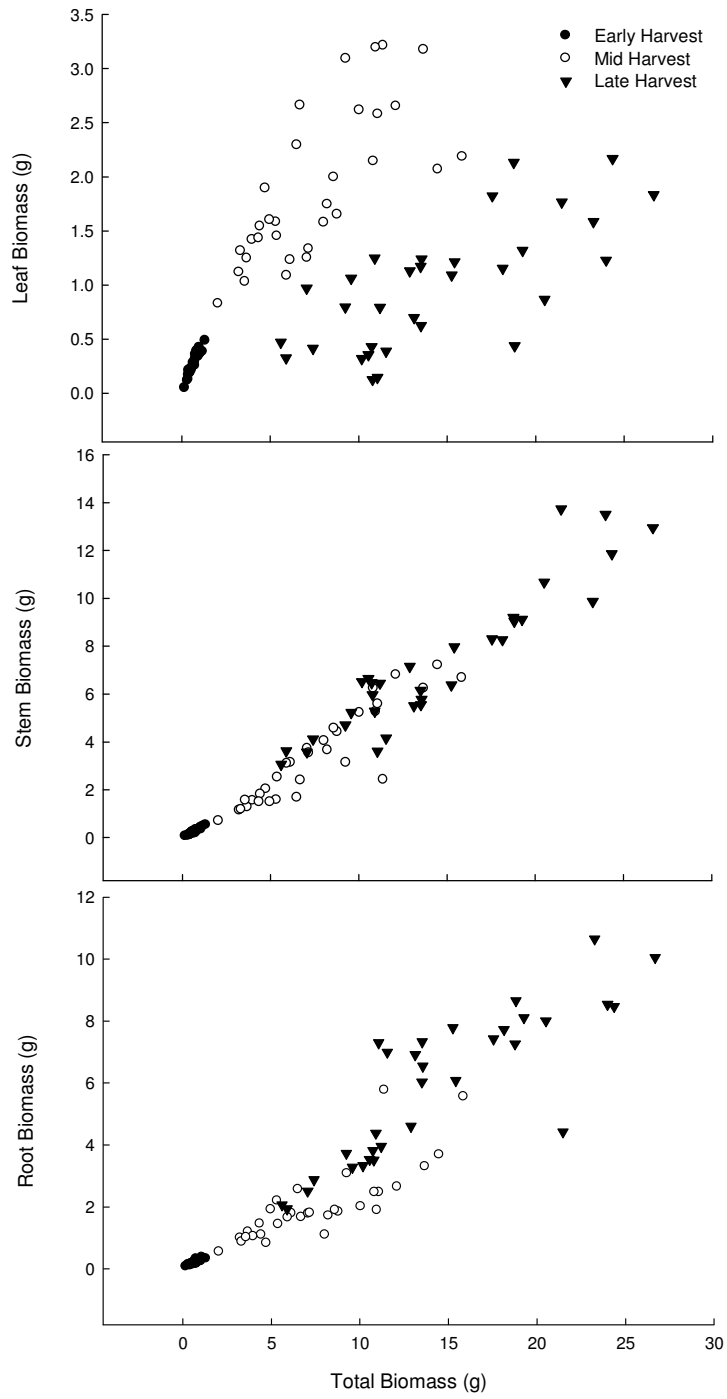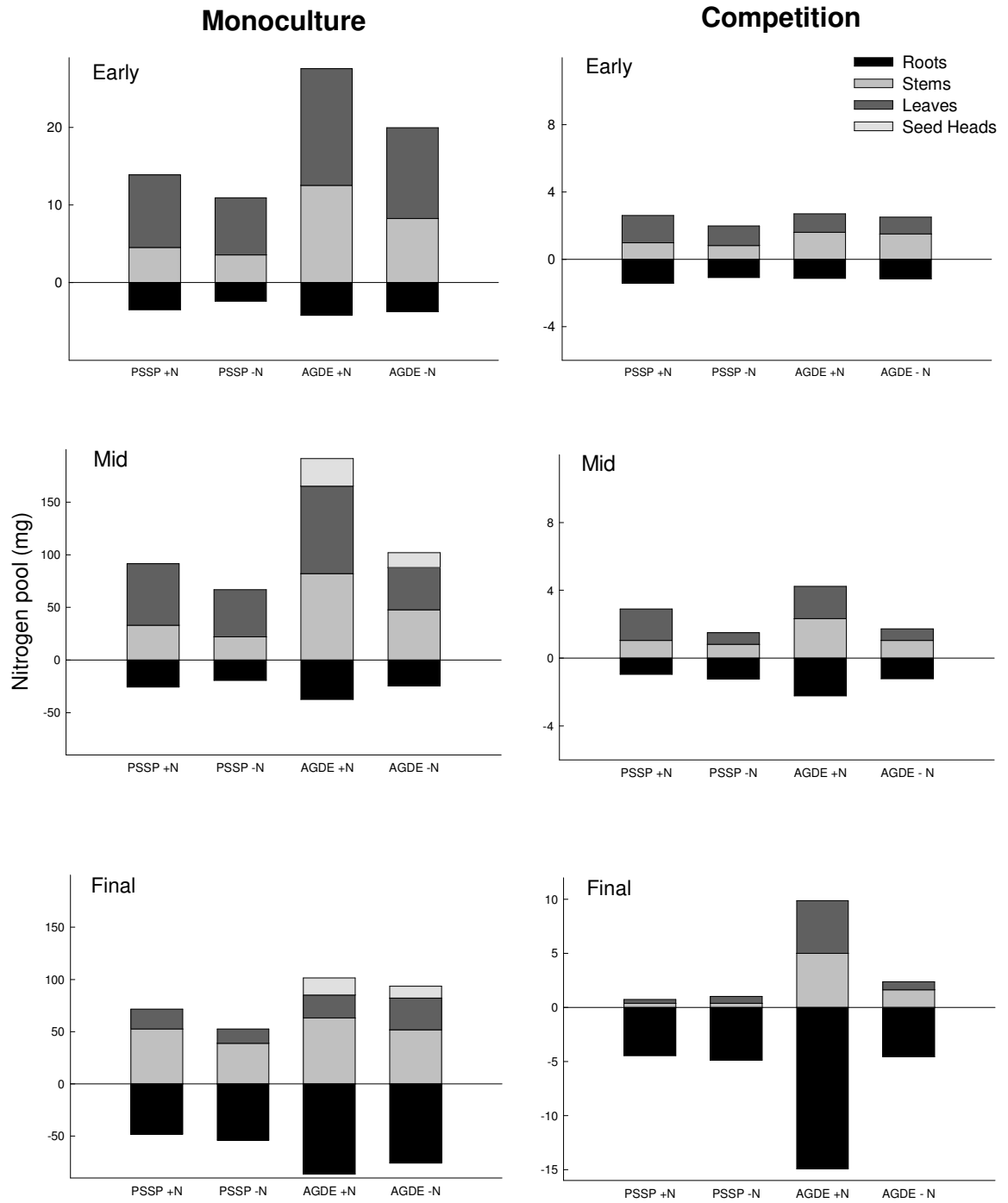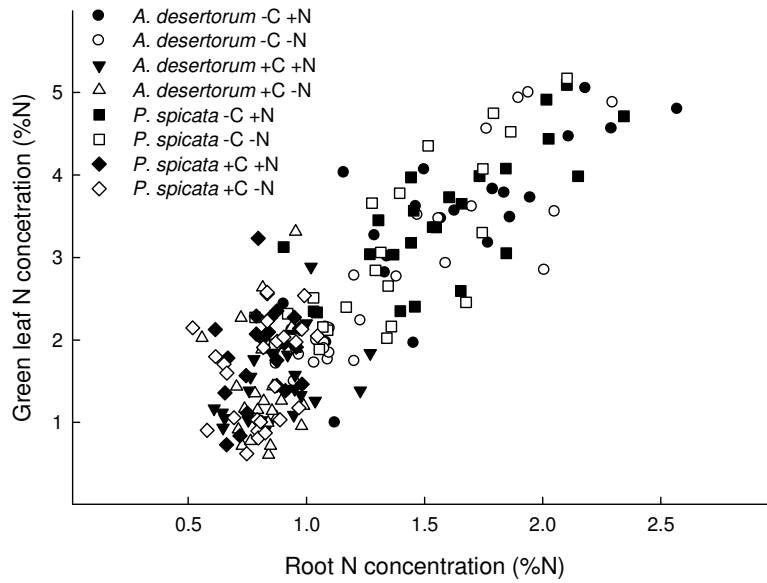
Figure 1.

32

Figure 2.

Figure 3.

Figure 4A.
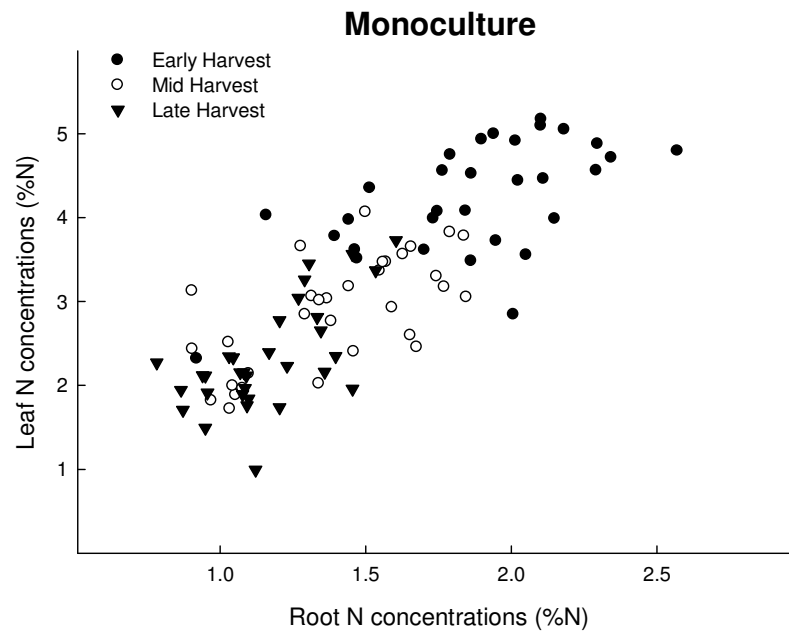
Figure 4B.

Figure 4C.

Figure 5.

Mid

Figure 6A.

Senesced
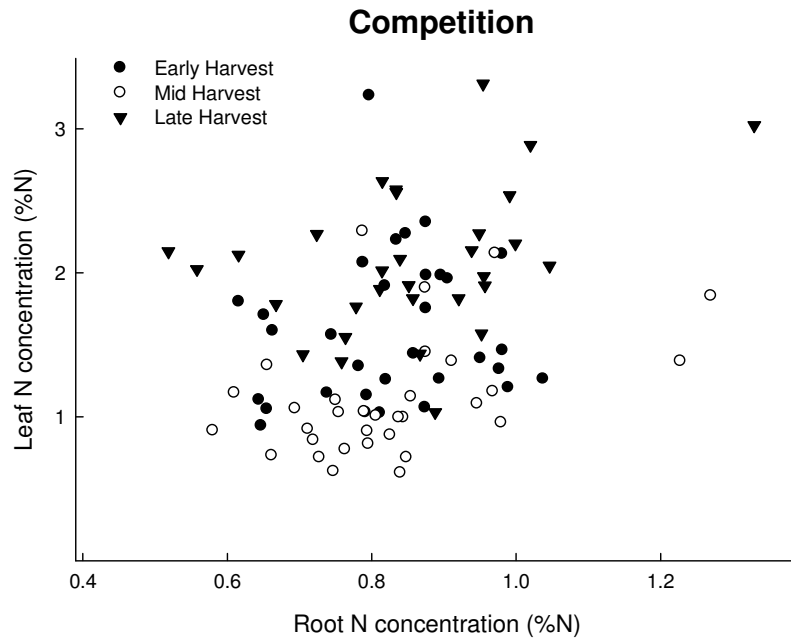
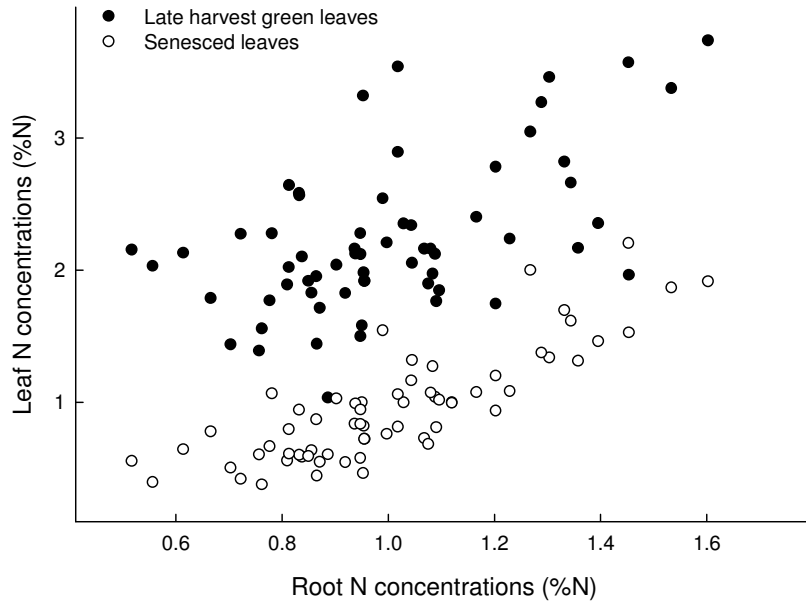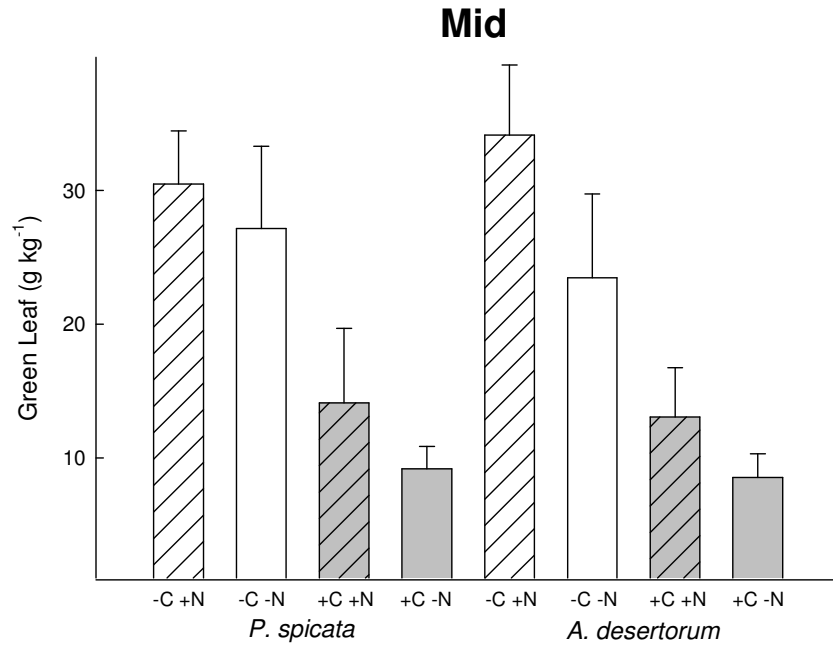**Figure 6B.**
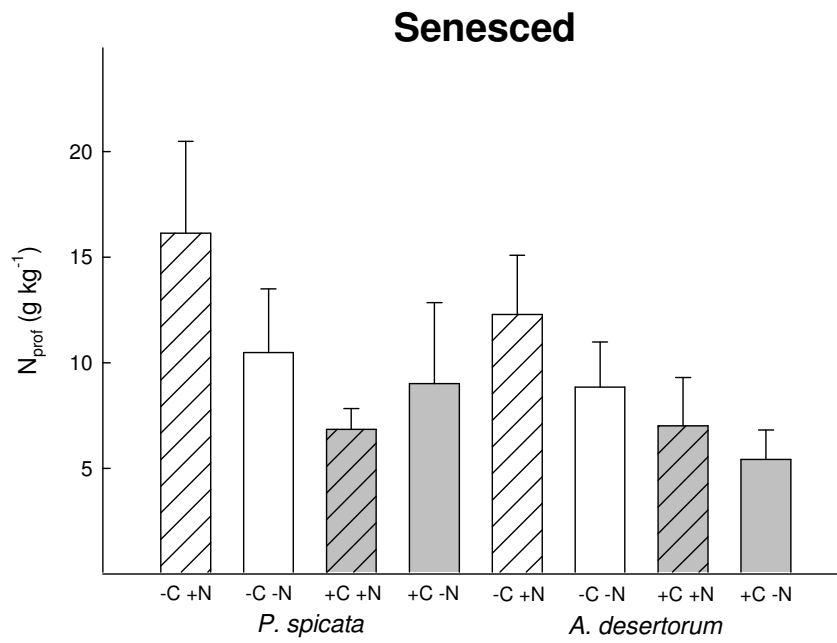**CHAPTER 2 (A supplemental N use model)**

The complicated matter of defining nitrogen use efficiency (NUE) for juvenile

perennials and non-perennial species

A Brief History of Mean Retention Time (MRT)

Nitrogen use efficiency (NUE) was originally proposed by Chapin (1980) as the

inverse of plant nitrogen (N) concentration in living tissues. Based on this ratio, lower N

concentrations would indicate more efficient N use because more dry mass had been

produced per unit nitrogen in the plant. Vitousek (1982) later proposed that NUE should

be calculated as the inverse of the nitrogen concentration in senesced tissues and wood.

With this ratio, a more efficient plant was one that lost less nitrogen, per unit biomass, to

its surroundings. However, later authors indicated that these measurements may not have

a strong ecological significance and sought a more satisfying metric for NUE (Berendse

and Aerts 1987).

Berendse and Aerts (1987) introduced the concept that NUE could be broken into

component parts to illustrate the apparent trade-off between nitrogen productivity (NP)

and the mean retention time of nitrogen (MRT). NP is the amount of biomass produced

per unit nitrogen per unit time (Ingestad 1979), whereas MRT is the average amount of

time that any given unit of N spends in the plant. They hypothesized that low nutrient

adapted species (LNAPs) would be more likely to minimize losses (i.e., maximize MRT)

and that this would lead to slower growth. In contrast, high nutrient adapted species

(HNAPs) would be more likely to maximize growth rate (i.e., maximize NP), which

would result in greater losses of nitrogen and a lower MRT. Whereas data from

proceeding studies tend to support this hypothesis (Aerts 1990, Vázquez de Aldana and

Berendse 1997, Silla and Escudero 2004), the accuracy of the MRT measurement has

come under question (Hirose 2011). In many experiments, MRT has only been

calculated for the aboveground portion of plants, resulting in two major drawbacks: (1) N

losses due to root turnover are not considered; and (2) root N pools can constitute a very

high proportion of the total N pool with these proportions differing significantly between

species. Though the previous points do merit consideration, perhaps the greatest

drawback of the MRT measurement is the assumption of a **steady state**. The steady state

assumption presupposes that over a given period of time, the amount of N absorbed is

equal to the amount of N lost. The steady state assumption may be true for some plants

(e.g., those that are not in a rapid growth phase) or, more likely, whole populations;

however, this assumption is problematic when it comes to measuring MRT over shorter

periods of time or for plants that are actively growing. For example, plants experiencing

rapid growth (e.g., seedlings or juvenile plants) are building biomass faster than they are

senescing biomass; as a result, they grow larger but their nutrient gains and losses are not

in equilibrium.

A More Logical Assumption

I propose a model built on a more logical assumption: **it is most *probable* that**

nitrogen absorbed earlier will be lost from the plant earlier. From this assumption, it

can be deduced that the most probable retention time (time between acquisition and loss

of a given unit of nitrogen) is equal to amount of time (Δt) it takes the plant to lose an amount of nitrogen equal to the total amount of nitrogen in the plant (total plant N pool) at the given time of absorption (Fig. 1). To clarify; if we define T_0 as the time at which a molecule of nitrogen is acquired and N_0 as the nitrogen pool at the time of acquisition, and if we define T* as the time that must pass for an amount of nitrogen equal to N_0 to be *lost* through senescing tissue, then the most probable retention time (RT) of a molecule of nitrogen absorbed at T_0 is the amount of time elapsed between T_0 and T*; thus, a molecule of nitrogen that enters the plant at T_0 has the highest probability of exiting the plant when Δt = RT. It must be stressed, that *all* molecules of nitrogen absorbed by the plant at T_0 are not lost from the plant when Δt = RT. There may be a large window of time in which nitrogen absorbed at T_0 may leave the plant (a range of possible retention times); however, the greater the difference between a given time and RT, the less probable it is that a molecule of nitrogen that had been absorbed at T_0 will leave the plant at that time. It then follows that the highest frequency of loss of N absorbed at T_0 will occur nearest to Δt = RT.

Thus, **if** the function g(t) represents the rate of nitrogen loss:

$$\textbf{then } RT = T^* - T_0 \textbf{ when } \int g(t)dt \text{ from } T_0 \text{ to } T^* = N_0$$

If the rate of N loss is constant, and equal to the rate of uptake (i.e. a steady state) then the integral of g(t) is simply the rate of loss X RT and therefore N_0 = rate of N loss X RT (Fig. 1). Rearranging this equation gives us RT = N_0 / (rate of N loss), which

mirrors the equation ($1/L_n$ where $L_n = g\ N_{lost} / (g\ N_{in\ plant} \times time)$) proposed by Berendse and Aerts (1987); but, while the latter *only* holds true when losses equal gains, the newly proposed model works just as well for a non-steady state (Fig. 2). There are, however, two caveats: (1) we must be able to accurately depict or estimate the function g(t); and (2) we must take into consideration the fact that in a non-steady state, RT will vary depending on the time chosen to represent T_0.

Addressing Caveat (1): There may be several methods of determining g(t), or rate of N loss. One option is to collect senescing tissues throughout the growing season and, if applicable, seasonal periods of defoliation. The more often material is collected, the more accurate g(t) will become. Unfortunately, as with many other approaches, this approach ignores root turnover (which may or may not be substantial). A second approach would be to use ^{15}N labeling, but would likely be limited to juvenile plants or hydroponic systems. In this approach, we would label a plant with ^{15}N and determine the ^{15}N pool at this time with an initial harvest of some of the labeled plants. With the remaining plants, senescing tissues would be collected and analyzed for ^{15}N; the value of Δt when concentrations of ^{15}N relative to ^{14}N in litterfall are highest would estimate RT. Additionally, if living biomass is harvested at any point and analyzed for ^{15}N, then initial $^{15}N -$ (senesced ^{15}N + living ^{15}N) would allow us to estimate the amount of nitrogen lost to root turnover.

Addressing Caveat (2): Under non-steady state, RT depends on T_0; that is, if the instantaneous rate of acquisition is either greater, or less than, the rate of loss, then the nitrogen pool is either shrinking, or growing, and N_0 will depend upon the time we have

chosen to represent T_0. If RT depends on an ever-changing N_0, then RT measurements will be most accurate to a true MRT when changes in N_0 are relatively small. This is not the case for seedlings or juvenile perennials.

Implications of This Model

Recently, it was proposed that MRT should be calculated with a strong emphasis on uptake, and a minimal consideration of losses (Hirose 2011); this is in stark contrast to my model, which suggests that rate of loss is a highly significant aspect of MRT. In determining MRT, both uptake and losses merit equal consideration. Minimizing losses often results in the trade-off of decreased growth rates (Berendse and Aerts 1987); whereas environmental conditions often limit potential rates of acquisition, plants often have a greater element of biological control over rates of loss (i.e. increased leaf longevity, resorption, or production of defensive compounds)**.**

My model contains an additional implication: the larger the N pool prior to acquisition, the longer the RT. Thus, if net uptake remains greater then net loss, RT will increase with the lifespan of the plant. Additionally, the longer a plant lives, the longer the possible MRT of the plant. For example, annual plants that reproduce and die within the span of a year cannot have a MRT of greater than a year. Biennials cannot achieve a MRT of greater than two years. Theoretically, a molecule containing N could remain with a plant for its entire lifetime, thus RT of individual atoms could exceed hundreds of years for some perennial species; however, these atoms may be the outliers and not have a significant influence on the MRT of such plants. By estimating the proportion of N that

43

ends up in long-lasting tissues on a yearly basis, one could gage the impact N trapped in long-lasting tissues on MRT.

I believe that it is possible to gain an accurate value of MRT through this model for long-lived plants; however, seedlings and juveniles still pose a problem. For these plants, it is possible to find a value for RT, but this value will likely be much lower than MRT for the life of the plant, and timing of the measurement could have a large impact on accuracy. Because of the large N pool variability early in the plants life, these values should be kept distinct from values found later in the plants life. Through further thought exercises on how RT may relate to and vary among different seedling strategies (and with the addition of experimental data), it may be possible to gain insight into this critical period of plant establishment.

[**side note** *The fundamental theorem of calculus tells us that the area under the curve of a function (the integral) of the rate of change of something is equal to the total change in the amount of that something over the specified period of time. Thus, if we graph two functions over the same period of time: (1) $f(t)$ = rate of uptake, and (2) $g(t)$ = rate of loss, then subtract the integral of $g(t)$ from the integral of $f(t)$, the remainder will be the net increase or decrease to the total N pool over the given period of time.]

REFERENCES

Aerts, R. 1990. Nutrient use efficiency in evergreen and deciduous species from heathlands. *Oecologia* 84:391-397.

Berendse, F. and R. Aerts. 1987. Nitrogen-use-efficiency: A biologically meaningful definition? *Funtional Ecology* 1:293-296.

Chapin, F.S. III. 1980. The mineral nutrition of wild plants. *Annual Review of Ecology and Systematics* 11:233-260.

Hirose, T. 2011. Nitrogen use efficiency revisited. *Oecologia* 166:863-867.

Ingestad, T. 1979. Nitrogen stress in birch seedlings. II. N, P, Ca and Mg nutrition. *Physiologia Plantarum* 45:149-157.

Silla, F. and A. Escudero 2004. Nitrogen-use efficiency: Trade-offs between N productivity and mean residence time at organ, plant and population levels. *Functional Ecology* 18:511-521.

Vázquez de Aldana, B.R. and F. Berendse. 1997. Nitrogen-use efficiency in six perennial grasses from contrasting habitats. *Functional Ecology* 11:619-626.

Vitousek, P. 1982. Nutrient cycling and nutrient use efficiency. *The American Naturalist* 119:553-572.

Figure 1: Figure represents a steady state of uptake and loss. $g(t)$ represents N loss and is equal to $f(t)$. Because the two are equal, the integral of $g(t)$ minus the integral of $f(t)$ equals zero, and N pool is neither increasing or decreasing (N_0 is equal for all values of t). $RT = N_o / $ (Rate of N loss). $h(t)$ represents rate of loss for N absorbed at time T_0, and the integral of $h(t)$ is equal to the total amount of N absorbed at time T_0

Figure 2: More realistic cycle of uptake and loss. $f(t)$ is the function representing rates of uptake, whereas $g(t)$ is the function representing rates of loss. Uptake rates and rates of N loss are increasing and decreasing seasonally, thus N_0 is in a constant state of flux.
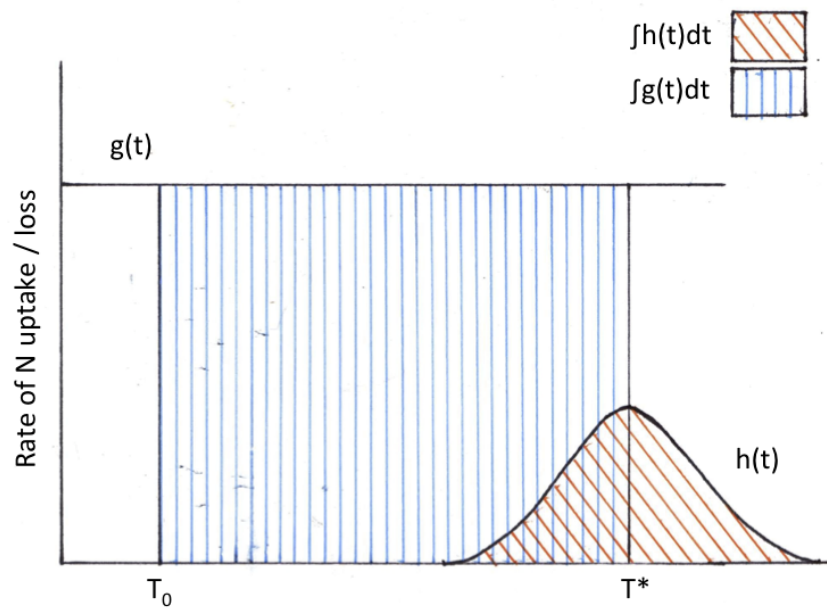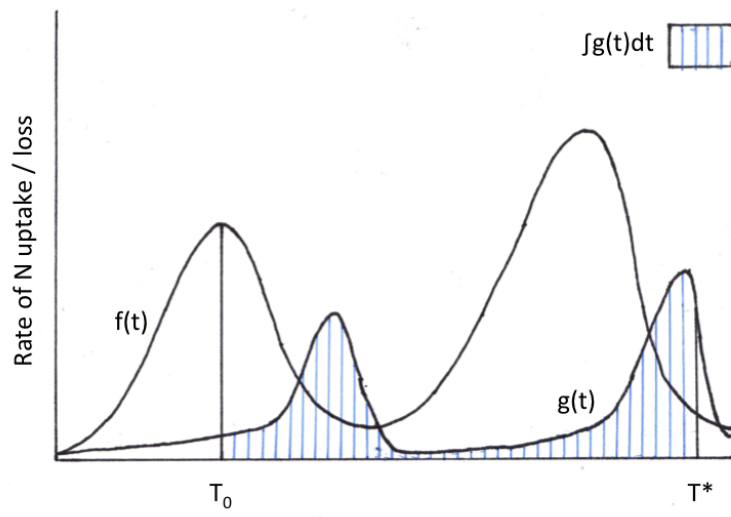
Figure 1.

Figure 2.