Stoichiometric Homeostasis in Two Native and Two Invasive South Dakotan Grasses

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STOICHIOMETRIC HOMEOSTASIS IN TWO NATIVE AND TWO INVASIVE
SOUTH DAKOTAN GRASSES

BY
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STOICHIOMETRIC HOMEOSTASIS IN TWO NATIVE AND TWO INVASIVE SOUTHERN GRASSES

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Biological Sciences degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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</tr>
<tr>
<td>N</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NUE</td>
<td>Nitrogen use efficiency</td>
</tr>
<tr>
<td>P</td>
<td>Phosphorus</td>
</tr>
<tr>
<td>PAR</td>
<td>Photosynthetically active radiation</td>
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ABSTRACT

STOICHIOMETRIC HOMEOSTASIS IN TWO NATIVE AND TWO INVASIVE SOUTH DAKOTAN GRASSES

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Increased nutrient availability has been widely linked to the success of invasive plants, however a general mechanism explaining these observations is lacking. Stoichiometric homeostasis ($H$), which is the regulation of internal nutrient concentrations, has been used to explain changes in plant community diversity under alterations in nutrient availability. One hypothesis holds that plants with high regulation (larger $H$) decrease in abundance in nutrient enriched conditions but are stable in nutrient deficient and drought conditions, likely due to extensive root systems. Additionally, plants with low regulation (lower $H$) increase in abundance under nutrient enriched conditions but are sensitive to drought conditions. I tested the hypotheses that $H$ would be higher in native grasses than in invasive grasses, that $H$ would be modulated by environmental conditions, and that differences in $H$ would be associated with differences in growth and biomass allocation. I calculated $H$ and measured plant growth and growth traits in two native (Pascopyrum smithii and Elymus canadensis) and two invasive (Bromus inermis and Agropyron cristatum) grasses grown in two experiments. Both experiments contained a range of N:P fertilizer supply concentrations and the first experiment contained a two-level drought treatment while the second experiment contained a two-level mycorrhizal inoculation treatment.
In the first experiment, I found support for the hypothesis that $H$ is higher in native than invasive plants, that environmental conditions (i.e. water availability) affect the value of $H$, and that differences in $H$ were associated with differences in growth. In the second experiment, there was no successful mycorrhizal inoculation, resulting in no differences in $H$ between mycorrhizal treatment groups. There were significant differences in total growth between the second experiment native and invasive grasses, despite there being no significant differences in $H$. Differences in $H$ values between control-treated grasses in the two experiments may be due to differences in greenhouse temperature and light conditions. These results show first, significant differences exist in $H$ between invasive and native grasses, with invasive grasses expressing lower values of $H$, second, environmental conditions effect the expression of $H$, and third, that differences in the expression of $H$ are matched by differences in growth.
INTRODUCTION

Identifying the mechanisms behind invasive species success has been both a major objective of and challenge to invasion ecology (van Kleunen et al. 2018). The co-occurrence of global environmental changes, biological invasions, and native biodiversity losses has led researchers to question the ultimate mechanisms underlying invasions (Thompson and Davis, 2011). Do invasive plants have particular traits or trait values which allow them higher competitive or reproductive advantages, leading to the eventual competitive exclusion of native species? Or do changes to the environment, such as alterations to climate or disturbance regimes, make room for alien plants? As the increasing spread of invasive plants threatens the integrity of ecosystem services (Diaz et al. 2006; Pejchar and Mooney, 2009; Pyšek and Richardson, 2010), the need to understand the mechanisms of invasive plant success, in order to develop efficacious risk assessments and management strategies (Rejmánek 2000; Finnoff and Tschirhart 2005; Pyšek and Richardson, 2010), is becoming ever more salient.

Invasion is a complex process that is likely the result of interactions between plant traits and environmental characteristics. Frameworks such as ‘invasion syndromes’ (Kueffer et al. 2013; Perkins and Nowak, 2013) offer conceptualizations of interacting traits or suites of traits within various environmental contexts. This is likely a more fruitful approach to studying plant invasions than either a wholly trait-centric or environment-centric view. Supporting the position of environmental causation, research has shown invasive species often benefit from environmental change drivers like nutrient deposition and global warming (Hellman et al. 2008; Bradley et al. 2010). A history of research has also repeatedly demonstrated ties between invasive plant spread and
disturbance (Hobbs and Huenneke 1992; Lozon and MacIsaac 1997; Mack et al. 2000; Leffler et al. 2016), illustrating the causal role of environment in shaping invasive plant success. The connections between environmental characteristics and invasive plant success has led some to argue that invasive plants have become a global problem simply because of environmental change, with no influence from traits (Thompson and Davis 2011). Alternatively, proponents of a trait-based approaches have shown invasive plants frequently exhibit similar traits that may explain their invasive success such as shorter time to reproductive age, smaller and more numerous seeds, vegetative reproduction, and higher specific leaf area (Hamilton et al. 2005; Pyšek and Richardson 2007; Thompson and Davis 2011).

The use of traits to predict invasive success have been efficacious to a degree (e.g. Reichard and Hamilton 1997; Küster et al. 2008, Hovick et al. 2012) and physiological traits may be especially useful for elucidating the mechanisms behind invasive success. First, continuous physiological traits (e.g. nutrient uptake rate) can be examined in response to continuous environmental variables (e.g. soil pH) in order to explain plant performance, unlike qualitative traits like clonality or geographic origin (Nijs et al. 2004). This capability allows researchers to extract insights into the role of environment on trait expression and the functioning of traits across contexts (Chown and Gaston 2008). Second, physiological traits are often key components in the derivation of global plant strategy schemes, such as the C-S-R theory (Grime 1977) and the leaf economics spectrum (Wright et al. 2004). Physiological traits can be associated with plant strategies from which predictions about plant behavior in different environmental contexts can be formed. Lastly, physiological traits provide mechanistic explanations of ecological
phenomenon by operating at lower scales of integration, focusing on the biochemical, molecular, and physical processes that underlie higher level phenomenon (Lambers et al. 2008).

Experimental evidence has repeatedly shown enhanced invasive plant performance as a result of N enrichment, a critical environmental change (Lowe et al., 2003; Vasquez et al., 2009; Dukes et al. 2011; He et al., 2011; Seastedt and Pyšek, 2011; Vallano et al. 2012; Li et al. 2014). In contrast with invasive plants, native plant species often suffer losses in population abundance and cover due to N eutrophication (Stevens et al. 2004; Bobbink et al. 2010; Clark et al. 2013). In a world of increasing N deposition (Galloway et al. 2008), plant invasions may therefore become more pronounced, to the detriment of native plant communities. Physiological traits may provide for a mechanistic understanding of these performance responses in invasive plants to N enrichment and as a result provide crucial insight for management strategies. In particular, leaf [N] may be a critical physiological trait for understanding these performance responses. First, N is a limiting nutrient for most ecosystems (Lebauer and Treseder, 2008; Bobbink et al. 2010), and an increase in available N results in an increase of leaf [N]. Leaf [N] can be indicative of plant performance since it is linked to relative growth rate (Poorter et al 1990; Yu et al. 2012; Yan et al. 2015) and photosynthetic capacity (Field and Mooney 1983; Evans 1989; Poorter and Garnier 1999). Leaf [N] can describe plant responses to environmental conditions beyond eutrophication, specifically drought. Increasing leaf [N], especially when resulting in increasing RuBisCO abundance, can reduce water loss during photosynthesis through strengthened CO₂ diffusion gradients (Adams et al. 2015). Finally, leaf [N], a continuous trait, could easily be examined under the experimental
framework of Nijs et al. (2004) by examining its response to continuous environmental conditions (e.g. nutrient availability, water availability) and the performance of the plant across those conditions.

While leaf [N] has many attractive features as a trait of potential study, the complexity underlying the expression of leaf [N] warrants the use of a trait that incorporates the cumulative effects of the various physiological mechanisms that control the value of leaf [N]. Leaf [N] is the result of myriad physiological and ecological processes that control the acquisition of N (Miller and Cramer 2005) and its allocation to plant organs (Pillbeam 2011). Therefore, quantifying the mechanisms that determine why leaf [N] takes a particular value in a plant can become an arduous task. A trait that expresses leaf [N] relative to available N in the environment would indicate the impact of assimilatory mechanisms on leaf [N] when examined across a range of N availability. Stoichiometric homeostasis ($H$) is this trait – it denotes the regulatory strength that plant traits exercise on the concentrations or ratios of internal nutrients relative to the concentrations or ratios of available nutrients in the soil. $H$, like relative growth rate (Poorter and Garnier 1999), can be thought of as a higher order parameter that is controlled by multiple underlying traits. $H$ incorporates both environmental condition (soil nutrient concentrations) and physiological trait response (leaf nutrient concentration) and thereby fits the experimental framework of Nijs et al. (2004). All that remains is to link $H$ to plant performance.

An essential characteristic of biological life is homeostasis, the ability of an organism to maintain its internal states (e.g. pH, nutrient ratios, etc.). Stoichiometric homeostasis is the regulation of internal nutrient concentrations or ratios of an organism
relative to the source of its nutrition (Elser et al. 2010; Yu et al. 2015). Organisms with higher degrees of $H$ exhibit more stable ratios (e.g. N:P ratios, denoted $H_{N:P}$) or concentrations of internal elements (e.g. [N], denoted $H_N$) than organisms with lower degrees of $H$ when subjected to variation in element supply. $H$ is defined mathematically as the inverse of the exponent $1/H$ in the equation:

$$y = cx^{1/H} \quad \text{(eq. 1)}$$

where $y$ is either an elemental ratio or concentration within an organism’s tissue, $c$ is an undefined constant, and $x$ is either the elemental ratio or concentration of bioavailable soil elements (Sterner and Elser 2002). When log-transformed the x-y relationship becomes linear and is described by the equation:

$$\log(y) = \log(c) + (1/H)\log(y) \quad \text{(eq. 2)}$$

Log($c$) is assumed to be 0, and $H$ is calculated algebraically. Plants with a high value for $H$ have higher expression of stoichiometric homeostasis than plants with a lower value of $H$ and vice versa. An organism with no expression of homeostasis ($H = 1$, i.e. a straight line with a slope of 1) would have internal elemental ratios or concentrations that would exactly match those of its supply of elements. $H$ only ever restricts internal ratios or concentrations since elements cannot be accumulated beyond their rate of supply. The higher the expression of $H$ in an organism, the less of the available elements it incorporates into its tissues (Sterner and Elser 2002).
Research shows that $H$ can be influenced by environmental factors and predict performance responses in plants to environmental factors. Water availability can influence the expression of $H$ (Sardans et al. 2016). Data presented in Güsewell (2004) showed *Carex curta* plants in low light conditions expressed higher $H$ than did *C. curta* plants grown in high light conditions in both low and high nutrient treatments. Changes in plant tissue stoichiometry in response to drought (e.g. Dijkstra et al. 2012, Urbina et al. 2015) may result in changes to $H$ in plants species. $H$ was predictive of plant population change in response to 25 years of N and P fertilization (Yu et al. 2015). The population size of lower $H$ plants increased in response to eutrophication while the population size of higher $H$ plant species decreased. Additionally, lower $H$ plants were susceptible to drought while higher $H$ plants were more resistant to drought. Yu et al. (2015) hypothesized higher $H$ plants would deploy more extensive root systems to forage for scarce nutrients in order to maintain higher $H$, which would also buffer them against drought. However, Mariotte et al. (2017) showed that the higher foraging capacity of larger root systems in higher $H$ plants becomes inefficient when drought limits N mobility. Meanwhile, lower $H$ plants with smaller root systems can tolerate larger tissue N:P variations and potentially spend more C on mycorrhizal associations to improve N capture. This suggests that both environmental conditions (water availability, nutrient mobility) and morphology (root system size) impinge on the relationship between $H$ and plant performance in response to other environmental conditions, such as eutrophication.

$H$ may be a trait capable of explaining performance differences between native and invasive species in response to environmental conditions. $H$ incorporates leaf [N], which exerts strong influence on plant responses to environmental conditions (e.g. Nijs et
al. 1995; Weih and Karlsson 2002). $H$ is predictive of plant population responses to
eutrophication (Yu et al. 2015), climate warming (Gu et al. 2017) and drought (Yu et al.
2015; Mariotte et al. 2017). $H$’s ability to predict plant responses to environmental
conditions, connection to physiological traits, and its potential to correlate with growth as
a result of leaf nutrient concentrations make it a potentially useful trait for comparing
native and invasive plants. However, a comparison of $H$ between native and invasive
plants has not yet been made. The research presented in this thesis aims to determine if
there are differences in $H$ between two native and two invasive grasses.

While $H$ may be used to describe differential performance responses between
native and invasive plants to environmental factors (e.g. eutrophication), its relationship
to plant performance may be modulated by other environmental conditions (e.g. drought),
their interplay with morphological traits (e.g. root system size), and their potential impact
on the expression of $H$ itself (e.g. as a response to light availability). This warrants the
need for research to describe the relationship between trait values of $H$, plant
performance, and environmental conditions (sensu Nijs et al. 2004). In addition to
comparing $H$ between native and invasive plants, this study examines the relationship
between $H$ and plant growth and morphology as well as the effect of water availability
and mycorrhizae on the value of $H$ itself.

For the first objective, comparing $H$ between native and invasive plants, I
hypothesized that $H$ would be lower in invasive plants compared to native plants. This
hypothesis was derived from research showing invasive plants and lower $H$ plants
exhibiting higher performance advantages in eutrophic environments when compared to
native and higher $H$ plants, respectively (Lowe et al. 2003; Frankow-Lindberg 2012; Yu
et al. 2015; Peltzer et al. 2016). For the second objective, determining what relationships exist between plant growth and morphology and $H$, I hypothesized that larger root mass fractions would be associated with higher values of $H$ and that lower values of $H$ would be associated with overall higher total growth, based on the findings and hypotheses of Yu et al. (2015). I additionally hypothesized that higher $H$ would be associated with lower numbers of leaves and tillers, which are indicative of plant performance (i.e. resource acquisition capability and reproduction, respectively). For the final objective, determining if $H$ is stable with changes in soil abiotic (i.e. water availability) and biotic (i.e. mycorrhizal inoculation) conditions, I hypothesized that the different treatments would have different impacts on $H$. Specifically, in an experiment with a water availability treatment, drought treated plants would exhibit higher values of $H$, and in an experiment with a mycorrhizal inoculation treatment, inoculated plants would exhibit lower values of $H$. This assumes that drought would reduce the assimilation of N, thereby reducing the slope of the regression line relating leaf [N] to soil [N] (thereby increasing $H$) and that mycorrhizal inoculation would increase the assimilation of N and thus raise the regression slope (thereby decreasing $H$).
EXPERIMENT 1

I. INTRODUCTION

Invasive plant species are among the leading causes of global environmental change and a threat to biodiversity (Hejda et al. 2009; Bellard et al. 2016; Mollot et al., 2017), and the prevention and management of plant invasions is necessary to safeguard ecosystem services (Pejchar and Mooney, 2009; Pyšek and Richardson, 2010). Predicting invasive plant response to environmental conditions and changes is a primary goal of invasion ecology (Schmidt and Drake 2011), and functional traits are often used to predict invasive plant responses to environmental conditions (Moles et al. 2008; Van Kleunen et al. 2010). Nutrient enrichment is one environmental change driver that has been repeatedly demonstrated to enhance invasive plant performance (Lowe et al., 2003; Vasquez et al., 2009; He et al., 2011; Seastedt and Pyšek, 2011). Complementing the links between plant invasions and nutrient enrichments are studies showing invasive plants’ limited success in some nutrient poor sites (Kolb et al. 2002; Zefferman et al. 2015). In contrast with invasive species, native plant species often decrease in abundance due to N eutrophication (Stevens et al. 2004; Bobbink et al. 2010; Clark et al. 2013). Identifying traits that are responsible for invasive and native plant responses to nutrient enrichment would therefore aid managers in crafting invasive plant preemption, monitoring, and management strategies.

The prediction of invasive and native plant responses to changing environmental conditions may benefit most from a focus on traits which are simple to quantify (Hamilton et al. 2005), informative in multiple environmental contexts (Moles et al. 2008, Bradley et al. 2010), applicable to plants of any or most taxa, and have either direct
or indirect bearing on the correlates of plant fitness (e.g. Feng and Fu, 2008). One potential trait fitting these criteria is stoichiometric homeostasis. Stoichiometric homeostasis (denoted by the coefficient of homeostasis, $H$) is a measure of an organism’s strength of regulation exercised on its internal concentrations or ratios of nutrients relative to the environment (Sterner and Elser 2002). Plants with a higher value of $H$ exercise more regulation on their internal nutrient concentrations or ratios than plants with lower values of $H$. $H$ is derived by regressing the log-transformed leaf nutrient concentration against the log-transformed soil nutrient concentrations and calculating the inverse of the regression line slope (Sterner and Elser 2002). The same calculations can be applied to nutrient ratios. Yu et al. (2015) showed $H$ to predict long term plant population responses to 25 years of eutrophication, where higher $H$ plants decreased in cover while lower $H$ plants increased in cover.

$H$ is also predictive of plant response to other environmental conditions, notably drought. In Yu et al. (2015), higher $H$ plant species were buffered against the effects of drought and precipitation variability, maintaining higher cover than lower $H$ species. In Mariotte et al. (2017), the relationship between $H$ and drought tolerance was flipped, with lower $H$ species maintaining similar mean biomass in control and drought treatments, while higher $H$ species decreased in biomass in drought plots. In both studies the authors attributed these responses to the role of roots. Yu et al. (2015) hypothesized that larger root systems in the higher $H$ plants would allow them to scavenge scarce nutrients in order to maintain homeostasis, which would also buffer them against the effects of drought. Mariotte et al. (2017) suggested that larger root systems needed for nutrient foraging in higher $H$ plants would become inefficient as drought limited nutrient
mineralization and mobilization. The resulting decreased N uptake would limit plant growth. Flexible stoichiometry and mycorrhizal symbioses allowed lower H species to maintain biomass even in drought like conditions. While both studies did not quantify belowground investment, their results highlight the role root system size may play in both the regulation of H and plant responses to drought conditions.

Research on H has yet to be performed with a plant invasion context. Given the striking results of Yu et al. (2015), this study aims to determine if H is lower in invasive species than in native species. Such a result would suggest that under long term eutrophication, invasive populations would benefit from higher cover compared to native species. Such a pattern would be consistent with research showing positive responses to N enrichment in invasive plants (e.g. Dukes et al. 2011; Li et al. 2014; Vallano et al. 2012). As drought differentially impacts plants of varying H, it may differentially impact the growth of native and invasive plants. Additionally, as drought can influence tissue stoichiometry (e.g. Urbina et al. 2017), drought may impact the values of H themselves in native and invasive plants. This study includes a drought treatment in order to assess if H is stable across different conditions of water availability, as well as if growth reductions caused by the drought treatment diverge between plants of differing H. Given the potential role of roots in buffering plants against drought and maintaining H, this study tracks root investment to determine if differences in H are associated with differences in biomass allocation. Whole plant growth and biomass allocation patterns are assessed to determine if differences in H are associated with overall differences in performance (e.g. growth) across levels of nutrient and water availability. In short, I ask three questions: 1) is H different in native and invasive plants, 2) is H stable across levels of water
availability, and 3) are differences in $H$ associated with differences in growth and morphology?

II. MATERIALS AND METHODS

I conducted a greenhouse sand culture study to determine the values of $H$ in two native and two invasive grasses in well-watered and drought-like conditions.

Model Species

To test for differences in $H$ between native and invasive grasses, I selected two native ($Pascopyrum smithii$ [western wheatgrass], $Elymus canadensis$ [Canada wildrye]) and two invasive ($Bromus inermis$ [smooth bromegrass], $Agropyron cristatum$ [crested wheatgrass]) cool-season perennial species. These species were selected due to the local availability of seed, their widespread use, and the unique characteristics of each species. Seeds were purchased from Millborn Seeds (Brookings, SD). Descriptions of each cultivar are summarized in Table 2.1.

a. $P. smithii$ ‘Rosana’

$P. smithii$ is a common component of the Northern Mixed Prairies and often contributes highly to the total productivity of grassland swards. It is one of the dominant grasses in the Northern Mixed Prairie along with blue grama ($Bouteloua gracilis$) and green needlegrass ($Nassella viridula$) (Singh et al. 1983), and it is often one of the first perennial grasses to dominate abandoned fields due to its ability to spread through rhizomes (Tolstead 1941). In a floristic survey of the tallgrass prairies in eastern South Dakota, $P. smithii$ was found in 50 out of 63 survey sites (Higgins 1999). $P. smithii$ is a drought resistant species (Austin 1968).

b. $E. canadensis$ ‘Mandan’
E. canadensis is a less frequently occurring native grass. It co-occurs with big bluestem (Andropogon gerardii) in tallgrass prairies acting as an important matrix-forming species (Hartnett 1993). E. canadensis occurred in 34 out of 63 sites across eastern South Dakota (Higgins 1999). E. canadensis is facultatively mycorrhizae-dependent (Hartnett 1993) and has shown positive correlations between soil N and root biomass in previous experiments (Anderson 2008). E. canadensis is a drought-resistant species (Walton 1983).

c. B. inermis ‘Rebound’

B. inermis is an invasive grass originally introduced into North America from Eurasia for purposes of soil retention and livestock grazing (Larson et al. 2001). B. inermis has escaped purposefully planted patches and has become established in native prairie remnants (D’Antonio and Vitousek 1992, Dillemuth et al. 2009). In a floristic survey of the tallgrass prairies in eastern South Dakota, B. inermis occurred in all sampled sites (Higgins 1999). In addition to being a widespread invader, B. inermis is a strong competitor (Nernberg and Dale 1997) capable of decreasing plant community diversity and increasing homogeneity (Otfinowski et al. 2007), increasing patch extinction rates of matrix forming native grasses (Dillemuth et al. 2009), and causing local extinctions of endangered flora (Williams and Crone, 2006). B. inermis is resistant to short term drought (Dong et al. 2012, Ott et al. 2017) but is susceptible to longer term droughts which can reduce aboveground dry biomass (Dibbern 1947) and limit population establishment in new ranges (Otfinowski et al. 2007).

d. A. cristatum ‘Hycrest’
*A. cristatum* is a commonly planted Eurasian grass that was initially introduced in 1898 (Rogler and Lorenz, 1983). As part of the Conservation Reserve Program, several million acres of *A. cristatum* have been planted since 1985 (DeLuca and Lesica, 1996). Native prairies often are purposefully replaced with these monoculture plantings (DeLuca and Lesica, 1996), and other native prairie stands have been invaded by *A. cristatum* (Klement et al., 2001). *A. cristatum*’s dispersal ability and dominance over and exclusion of native flora defines it as an invasive species, despite being purposefully planted (Henderson, 2005). *A. cristatum* is a strong competitor that can exclude native plants (McHenry and Newell 1947; Looman and Einrichs 1973; Wilson 1989) and weeds (Knowles and Buglass 1980), take up N (Leffler et al. 2011) and P (Caldwell et al. 1985; Black et al. 1994) faster than competing native plants, and produce greater aboveground biomass than mid-grass prairies (Smoliak et al. 1967; Smoliak and Dormaar 1985; Redente et al. 1989; Dormaar et al. 1995). *A. cristatum* is considered drought tolerant (Dormaar et al. 1995), although drought does reduce its growth (Busso et al. 1989).

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Species Name</th>
<th>Growth Form</th>
<th>Cultivar</th>
<th>Provenance</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Wheatgrass</td>
<td><em>Pascopyrum smithii</em></td>
<td>Rhizomatous</td>
<td>Rosana</td>
<td>Native</td>
<td>Greater seedling vigor and establishment</td>
</tr>
<tr>
<td>Canada Wildrye</td>
<td><em>Elymus canadensis</em></td>
<td>Caespitose</td>
<td>Mandan</td>
<td>Native</td>
<td>Greater seedling vigor and establishment</td>
</tr>
<tr>
<td>Crested Wheatgrass</td>
<td><em>Agropyron cristatum</em></td>
<td>Caespitose</td>
<td>Hycrest</td>
<td>Invasive</td>
<td>Higher establishment and productivity</td>
</tr>
<tr>
<td>Smooth Bromegrass</td>
<td><em>Bromus inermis</em></td>
<td>Rhizomatous</td>
<td>Rebound</td>
<td>Invasive</td>
<td>Fast regrowth after grazing or having</td>
</tr>
</tbody>
</table>

*Table 2.1* Descriptions of species growth form, cultivated variety used, and description of the traits of the cultivated varieties.
**Study Site and Conditions**

All plants were cultivated in the South Dakota State University Horticulture and Forestry Greenhouses (44.32, -96.78) located in Brookings, South Dakota. Greenhouse temperature, humidity, and PAR were measured with a coupled thermometer and hygrometer (Model CS215, Campbell Scientific, Logan, UT) and a PAR sensor (SQ-110, Apogee Instruments, Logan, UT). Greenhouse temperature was set at 18° C. The grasses were supplementally lit for 16 hours daily with two 400 W bulbs in electronic ballasts (WEX120, Growers Supply, Dyersville, IA) suspended above the greenhouse table. The daily average PAR calculated for the 16-hour period of illumination was 92.60 µmol m⁻² s⁻¹, the average daily maximum PAR was 254.62 µmol m⁻² s⁻¹, and the daily average temperature was 22° C.

**Experimental Design and Sampling**

I carried out a fully factorial experiment with a two-level watering treatment and five-level fertilization treatment across four species. Levels of the watering treatment were control (‘well-watered’) and drought-like conditions, and levels of nutrient treatment were fertilizer [N] concentrations of 3, 6, 9, 12, and 15 mM. The multiple levels of the fertilizer treatment are necessary to calculate \( H \). Individual plants were grown in 40 in.³ ‘Deepots’ (Stuewe and Sons, Tangent, OR) filled with silicon quartz sand (Unimin Granusil®). Two paper coffee filters were taped to the bottom of each ‘Deepot’ to prevent loss of sand from the pots while still allowing for drainage. 20 ‘Deepots’ were arranged into trays with 5 x 4 cells. Each tray contained only one species. Plants in the first two columns of each tray were well-watered and plants in the last two columns were exposed to drought-like conditions. Plants in the first row of pots received
fertilizer with the highest N:P ratio, with each succeeding row receiving the fertilizer with
the next lowest N:P ratio. Trays were rotated weekly to prevent microclimatic effects on
growth. Multiple seeds were planted per pot and seedlings were thinned until each pot
contained one seedling. Seeds were planted on May 24, 2017, first fertilized on July 12,
2017 when each pot had one seedling, and harvested on August 23, 2017, for a total
experimental duration of six weeks. During the experiment, fungal infections of the
coffee filters on the bottom of the pots caused tears in the filters, resulting in the sudden
loss of sand from pots in which the sand was sufficiently dry. Coffee filters were replaced
as necessary, however 91 out of 400 plants died before the completion of the experiment
due to either sudden loss of substrate and subsequent drying of the plant or due to the
effects of the drought treatment itself. Data distinguishing death from loss of substrate or
from the effects of the drought treatment were not recorded.

Fertilizer was applied as a modified Hoagland’s solution. Each solution contained
the same concentrations of all reagents except for NH₄NO₃. The highest value of fertilizer
[N], 15 mM, represents the same molarity of N in a standard, full-strength Hoagland’s
solution. The range of fertilizer [N] is necessary to calculate $H$ while the watering
treatments allow us to determine if $H$ is stable under different water regimes. During each
application, plants received approximately 150 mL of fertilizer solution, which was
sufficient to saturate each pot. Two soil moisture probes (EC5, Decagon Devices,
Pullman, WA) per species were placed in 9 mM N treated well-watered and drought-like
condition pots to track substrate volumetric water content (VWC). To determine the
severity of the drought treatment, publicly available data from a Natural Resources
Conservation Service SCAN (Soil Climate Analysis Network) site near Sioux Falls,
South Dakota (site number 2072) was used to estimate the lowest levels of soil water availability that occur in local tallgrass prairies. This value, estimated at 15% soil moisture, was used as the minimum value of soil moisture allowed in the control-treated grasses. Plants in the well-watered treatment received fertilizer solution frequently enough to keep the average value of the pots above 15% VWC. Drought-treated pots were allowed to dry down to an average pot VWC of 7% before being irrigated with fertilizer solution.

At the first application of fertilizer solution, shoot height, number of leaves, and number of tillers were recorded for each plant. On the last day of the sixth week of treatment applications, all plants were harvested. Aboveground biomass was clipped at the crown of the roots and was stored in labelled paper bags, which were then oven dried at 60°C for 72 hours. Belowground biomass samples were separated from sand substrate with a water rinse over a 10 mesh soil sieve, placed into labelled plastic bags, and stored in a freezer until they were processed.

Sample Preparation and Analysis

After drying, aboveground biomass samples were counted for number of leaves, tillers, and fully senesced leaves. Senescent aboveground tissue in the dried samples was manually separated from green tissue, which were both weighed separately. The green portions of the aboveground biomass were shredded in a laboratory mill (Model 4 Wiley® Mill, Thomas Scientific, Swedesboro, NJ) for approximately one minute then were further ground with a ball mill (Mini-Beadbeater 16, BioSpec Products, Bartlesville, OK) for 1.5 min. Approximately 2.5 mg of the resultant samples were analyzed by SDSU Soil and Plant Analysis Laboratory for percent C and N (hereafter [C] and [N],...
respectively) using an elemental analyzer (Vario MAX CNS, elementar, Langenselbold, Germany). The remaining ground leaf tissue was digested with HNO$_3$ in a microwave and analyzed for P concentration through inductively coupled plasma spectrometry in the same laboratory. Belowground tissues were then dried at 60° C for 72 hours and weighed for total belowground mass.

**Data Analysis**

All statistical analyses were performed in the R computing environment (R Core Team, 2015). To determine $H_N$ and $H_{N:P}$ differences between native and invasive grasses and between control-treated and drought-treated grasses, I constructed an initial set of linear mixed models explaining log(leaf [N]) and log(leaf N:P) with the R package nlme (Pinheiro et al. 2017) using the maximum likelihood method for parameter estimation. Each model contained a random species effect, a fixed fertilizer treatment effect, and one of each possible combination of a fixed watering treatment and a fixed provenance (i.e. native or invasive) effect. AIC value weights from these models were used to determine the best fit model. For both log(leaf [N]) and log(leaf N:P) the best fit model included the random species effect and fixed fertilizer, water, and provenance effects and their interactions. Predicted mean log(leaf [N]) and log(leaf N:P) values were derived from the best fit models were calculated at each N:P treatment level for each provenance-watering treatment grouping. These predicted mean values were regressed against the levels of the fertilizer treatment and the $H_N$ and $H_{N:P}$ values were calculated as the inverse of the slope (Sterner and Elser 2002). In order to test for significant differences in $H_N$ and $H_{N:P}$ between provenance-watering treatment groups, I tested for differences in regression line slopes using a pairwise least-squares adjusted mean comparison with the R package
lsmeans (Lenth 2016). For comparisons of \( H \) values between provenance groups to be meaningful, species within each group must have comparable \( H \) values. In order to test the assumption that \( H \) values within provenance groups are comparable, I used the methods described above to calculate and compare \( H_N \) values for each species in separate subsetted data sets of all drought-treated and all control-treated plants. Total aboveground N and P (g) were calculated by multiplying total aboveground biomass by aboveground [N] and [P] respectively. Nitrogen use efficiency (NUE) was calculated by dividing total biomass by total N (Lambers et al. 1998). In order to determine the relationships between \( H \), growth, and biomass allocation, significant differences in mean aboveground [N] and [P], total, aboveground, and belowground biomass were each tested for in a series of linear models containing fixed terms for provenance and watering treatments which where analyzed with ANOVA. (The interactions of the provenance and watering treatment terms defined the provenance-watering treatment groups for which \( H \) values were calculated). To determine how the responses of provenance-watering treatment groups varied with nutrient availability, the same analyses but with the addition of a fixed fertilizer treatment term were performed for all biomass variables, number of leaves and tillers, and aboveground [P]. Mean differences in number of leaves and number of tillers were also tested in models containing fixed provenance and watering treatment effects and in models containing fixed provenance, water, and fertilizer treatments. These models were constructed as generalized linear models with Poisson error structure and were again analyzed with ANOVA. For all ANOVAs, both with and without the fixed fertilizer effect, post-hoc analyses were performed when applicable with a Tukey’s HSD test.
Best fit models for number of leaves and number of tillers were determined by comparing AIC values of multiple general linear models with Poisson error structure. Each model contained a fertilizer effect and either water or provenance effects or both. Best fit models for biomass variables, root mass fraction, aboveground [P], NUE, and phosphorus use efficiency (PUE) were determined in the same manner as described for log(leaf[N]) and log(leaf N:P) above. When assessing the significance of model components within the best fit models, the results from t-tests reported from R’s ‘summary’ function were utilized. Standardized major-axis regressions and a multiple pairwise comparison with Sidak adjustment were used to construct and compare the relationship between the number of tillers and number of leaves of each provenance-watering treatment group using the R package smatr (Warton et al. 2012). Significance for all tests was qualified at alpha = 0.05.

III. RESULTS

Stoichiometric Homeostasis

$H$ values calculated on the basis of species were all similar in drought-treated grasses. In the control-treated grasses, $E. canadensis$ and $P. smithii$ had comparable values of $H$, while $P. smithii$, $B. inermis$, and $A. cristatum$ were all comparable in their values of $H$. The best fit model for log(leaf[N]) and log(leaf N:P) contained the fixed log(fertilizer [N]), watering treatment, provenance effects, their interactions, and the random species effect. Values for the stoichiometric homeostasis coefficient calculated from these best-fit models ranged from 2.69 to 5.35 for $H_N$ and ranged from 1.78 to 3.47 for $H_{N:P}$ (Figs. 2.1 and 2.2). Comparisons of $H_N$ and $H_{N:P}$ values resulted in the same pattern of significance groupings (Figs. 2.1 and 2.2). Native-control, native-drought, and
invasive-drought plants had similar regression slopes for calculations of aboveground [N] and N:P (and therefore similar values of $H_N$ and $H_{N:P}$) and invasive-control had the highest regression slopes (and therefore the lowest values of $H_N$ and $H_{N:P}$). In both the best fit models for $H_N$ and $H_{N:P}$, fertilizer and watering treatment effects were significant ($p < 0.01$). Provenance effects were only significant in the $H_N$ model ($p < 0.01$), but fertilizer:provenance interactions were significant in both the $H_N$ and $H_{N:P}$ models ($p < 0.01$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model</th>
<th>df</th>
<th>AIC</th>
<th>Δ(AIC)</th>
<th>w(AIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_N$</td>
<td>Fertilizer<em>Water</em>Provenance</td>
<td>10</td>
<td>640.37</td>
<td>0</td>
<td>0.99924</td>
</tr>
<tr>
<td></td>
<td>Fertilizer*Water</td>
<td>6</td>
<td>625.97</td>
<td>14.39</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Fertilizer*Provenance</td>
<td>6</td>
<td>616.52</td>
<td>23.85</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Fertilizer</td>
<td>4</td>
<td>600.71</td>
<td>39.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$H_{N:P}$</td>
<td>Fertilizer<em>Water</em>Provenance</td>
<td>10</td>
<td>438.88</td>
<td>0</td>
<td>0.99806</td>
</tr>
<tr>
<td></td>
<td>Fertilizer*Water</td>
<td>6</td>
<td>426.39</td>
<td>12.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Fertilizer*Provenance</td>
<td>6</td>
<td>408.92</td>
<td>29.96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Fertilizer</td>
<td>4</td>
<td>398.88</td>
<td>39.99</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 2.2 AIC values and weights for the set of mixed models explaining leaf [N] and leaf N:P and used to calculate $H_N$ and $H_{N:P}$, respectively.

C:N:P stoichiometry and nutrient use efficiency

There were no significant relationships between aboveground [N] and [P] across the whole data set, nor across any level of $H_N$ (Fig. 2.3). Mean [P] was similar across all provenance-water groups except for the native-drought group which had a slightly lower mean. Mean N:P however was significantly higher in drought-treated groups than in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean [P]</th>
<th>Grouping</th>
<th>Mean N:P</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive Drought</td>
<td>0.355</td>
<td>a</td>
<td>15.517</td>
<td>a</td>
</tr>
<tr>
<td>Native Drought</td>
<td>0.318</td>
<td>b</td>
<td>14.128</td>
<td>a</td>
</tr>
<tr>
<td>Invasive Control</td>
<td>0.368</td>
<td>a</td>
<td>11.894</td>
<td>b</td>
</tr>
<tr>
<td>Native Control</td>
<td>0.354</td>
<td>a</td>
<td>11.058</td>
<td>b</td>
</tr>
</tbody>
</table>

Table 2.3 Mean aboveground [P] and N:P values and their associated significance groupings. Different letters in the grouping columns indicate a significant difference, while similar letters indicating a lack of difference.
control groups (Table 2.3, Fig. 2.5). When analyzed across fertilizer treatment levels, only control-treated native grasses had consistent mean [P] values, while all other groups’ mean [P] values varied idiosyncratically across the range of the fertilizer treatment (Fig. 2.6). The lack of significant relationships between aboveground [N] and [P] (Fig. 2.3), supplied N and aboveground [P] (Fig. 2.6), and the similarities between $H_N$ and $H_{N:P}$ significance groupings suggests group $H_{N:P}$ values may merely reflect changing values of [N] between groups, rather than differences in P homeostasis. Aboveground [P] had no significant relationship with aboveground [C] ($p = 0.346$) or total biomass ($p = 0.414$). However, the significant effects of aboveground [N] on total biomass ($p < 0.001$) were reflected in the significant effect of plant N:P on total biomass ($p = 0.003$). There were no significant differences in aboveground [C] between treatment groups (Fig. 2.7) nor was there a significant relationship between aboveground [C] and [N] ($p = 0.169$).

Aboveground [N] significantly differed between watering treatment-provenance groups (Fig. 2.8), and the same patterns of treatment group differences appear in aboveground C:N ratios (Fig. 2.9, Table 2.4). Aboveground [N] had a significant relationship with total plant biomass across the whole data set ($p < 0.001$). Water-limited growth in the drought groups resulted in overall less total aboveground N than the control-treated groups (Fig. 2.10), despite having higher aboveground [N] (Fig. 2.8).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean [N]</th>
<th>Mean C:N</th>
<th>Grouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive Drought</td>
<td>4.87</td>
<td>9.46</td>
<td>a</td>
</tr>
<tr>
<td>Native Drought</td>
<td>4.54</td>
<td>10.13</td>
<td>ab</td>
</tr>
<tr>
<td>Invasive Control</td>
<td>4.37</td>
<td>10.35</td>
<td>bc</td>
</tr>
<tr>
<td>Native Control</td>
<td>3.91</td>
<td>11.74</td>
<td>c</td>
</tr>
</tbody>
</table>

*Table 2.4* Mean aboveground [N] and C:N values and their associated significance groupings.
NUE was best fit by a mixed model containing a fixed fertilizer treatment effect, a fixed provenance effect, and a random species effect. An ANOVA of this model showed the provenance and fertilizer:provenance interaction were not significant ($p=0.572$ and $0.087$, respectively). A Tukey’s HSD test showed that grasses grown at the lowest level of the fertilizer treatments had the highest mean NUE, followed by plants grown in the second lowest level of fertilizer treatment. Grasses grown in the highest three levels of fertilizer treatments shared the lowest mean values. PUE was best fit with a model containing fixed fertilizer and water effects with a random species effect. However, an ANOVA showed that only the watering treatment effect was significant. A Tukey’s HSD test showed that drought-treated grasses had a significantly higher mean PUE than control-treated grasses.

*Biomass*

The best fit models for total, aboveground, and belowground biomass included the same parameters for the best fit model for $H_N$ (Table 2.5). Only the water treatment effect was significant within the best fit models of each biomass variable. The fertilizer effect had a significant effect on aboveground biomass, and the interactions of fertilizer with the effects of water and provenance both had marginally significant impacts on aboveground biomass (Table 2.6). The best fit models for total, aboveground, and belowground biomass lacked significant watering treatment:provenance:fertilizer treatment interactions. This was reflected by similar significance group assignments derived from a Tukey’s HSD between control-treated and drought-treated grasses within the native and invasive provenance groups (Figs. 2.12a – 2.12c), with the only exceptions being control-treated native and invasive plants at the fertilizer level of N:P = 5.43 for
total and belowground biomass (Figs. 2.12a and 2.12c). The fertilizer effect was itself only significant in the aboveground biomass model. The aboveground biomass model also contained significant watering treatment:fertilizer treatment and provenance:fertilizer treatment interactions.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Best fit model parameters</th>
<th>df</th>
<th>AIC</th>
<th>w(AIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Biomass</td>
<td>Fertilizer<em>Water</em>Provenance</td>
<td>10</td>
<td>1586.59</td>
<td>0.994</td>
</tr>
<tr>
<td>Aboveground Biomass</td>
<td>Fertilizer<em>Water</em>Provenance</td>
<td>10</td>
<td>16.52</td>
<td>0.892</td>
</tr>
<tr>
<td>Belowground Biomass</td>
<td>Fertilizer<em>Water</em>Provenance</td>
<td>10</td>
<td>1631.71</td>
<td>0.979</td>
</tr>
<tr>
<td>Root Mass Fraction</td>
<td>Fertilizer</td>
<td>10</td>
<td>-828.91</td>
<td>0.864</td>
</tr>
</tbody>
</table>

*Table 2.5* Best fit model parameters, AIC values, and AIC weights for the set of mixed models explaining biomass variables and root mass fraction.

Drought-treated groups had the lowest mean total, aboveground, and belowground biomass (Fig. 2.11a – 2.11c). Native, control-treated grasses had lower mean total, aboveground, and belowground biomass than invasive, control-treated grasses, which had the highest mean total, aboveground, and belowground biomass (Figs. 2.11a – 2.11c). When analyzed across the range of supplied fertilizer levels, consistent

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Total</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Aboveground</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>•</td>
<td>•</td>
<td>NS</td>
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<tr>
<td>Belowground</td>
<td>NS</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Table 2.6* Significance levels for the model component factors of each best fit biomass model; • indicates significance at $\alpha = 0.1$, *** indicates significance at $\alpha = 0.01$, and NS indicates the factor was not significant. The three-way interaction between the water, fertilizer, and provenance effects was not significant in each biomass variable model.
mean differences in biomass were not found between drought-treated native and invasive plants (Fig. 2.12a – 2.12c).

*Root Mass Fraction*

Regardless of $H$ values, plants of different provenance-watering treatment groups had similar mean root mass fraction. Log(total biomass) had a significant relationship to RMF ($p < 0.01$), in which the number of observations of RMF and their variation taper off at higher levels of total biomass (Fig. 2.14). The lowest level of the fertilizer treatment had the highest mean value of RMF while all other groups had comparable means (Fig. 2.16). When compared across provenance-watering treatment groups, mean RMF values were comparable. However, when also examined across fertilizer levels there was some variation in mean RMF between provenance-watering treatment groups (Fig. 2.17). Control-treated native grasses were the only group that had consistent mean RMF values across fertilizer treatment levels (Fig. 2.17).

While there was some variation in the provenance-watering treatment groups’ mean RMF values across fertilizer treatments, the best fit model for RMF contained only a fixed fertilizer effect (Table 2.5). The higher mean in the lowest fertilizer level however may be caused by lower aboveground biomass without changes in belowground biomass, potentially as a result of nutrient limitation (see Figs. 2.12b and 2.12c).

*Plants Leaves and Tillers*

The best fit models for both number of leaves and number of tillers ($w(AIC) > 0.9999$ each) both contained the terms for watering treatment, nutrient treatment, and provenance effects. In both best fit models, the residual deviance was much greater than the degrees of freedom, indicating overdispersion in the models. In the best fit model for
number of leaves, only the fertilizer treatment (p < 0.01), watering treatment (p < 0.01), and their interaction (p < 0.01) showed significant effects. In the best fit model for number of tillers, only the watering treatment (p = 0.016) and fertilizer treatment (p = 0.009) showed significant effects.

When analyzed across provenance-watering treatment groups only, control-treated invasive grasses had the highest mean number of leaves followed by the control-treated native grasses; native drought-treated grasses had the lowest mean number of leaves (Fig. 2.20). A similar pattern emerged for tillers (Fig. 2.23) in which control-treated invasive grasses had the highest mean number of tillers, followed by control-treated native grasses with drought-treated natives again exhibiting the lowest mean. When analyzed across fertilizer levels, drought-treated grasses shared similar significance group pairings for mean number of leaves at fertilizer treatment levels N:P = 2.71 and 8.14, but at all other levels, drought-treated invasive grasses had higher mean number of leaves. Drought-treated native plants had overlapping significance groupings with control-treated native plants for mean number of tillers at fertilizer treatment levels N:P = 2.71 and 8.14 and had overlapping groupings with drought-treated invasive plants at all fertilizer treatment levels (Fig. 2.22). In control-treated native and invasive grasses and drought-treated invasive grasses, the mean number of leaves were higher at the highest level of fertilizer treatment than at the lowest level. Only control-treated grasses had higher mean numbers of tillers at the highest fertilizer treatment than at the lowest (Fig. 2.22).

Morphometric models were constructed relating number of tillers to number of leaves. When morphometric models were constructed to compare control and drought-
treated groups across nutrient treatment levels the morphometric relationship between control and drought groups only differed in native species at [N] = 12 mM and in invasive species at [N] = 15 mM (Fig. not included). When analyzed across the levels of fertilizer treatment, there were significant differences in the morphometric relationship between tillers and leaves in all watering treatment provenance groups except for the drought-treated native plants (Fig. 2.24). Number of leaves was a significant predictor of total (p = 0.012) and aboveground (p < 0.001) biomass across groups. Tillers alone were not a significant predictor of total or aboveground biomass, but a leaf:tiller interaction was significant in predicting aboveground biomass across groups (p = 0.021).

IV. DISCUSSION

This study presents novel evidence of lower values of \( H_N \) and \( H_{N:P} \) in invasive grasses when compared to native grasses grown in comparable conditions. This shows invasive grasses are less regulatory in tissue N stoichiometry than native plants, resulting in tissue [N] that more closely matches soil [N] than native plants. This study also shows that the expression \( H_N \) and \( H_{N:P} \) is resistant to changes in water availability. While the drought-treated native and invasive grasses had higher values of aboveground [N] than their control-treated counterparts, these differences were not large enough create significant differences in \( H_N \) and \( H_{N:P} \) between drought and control-treated provenance groups. Lastly, this study demonstrates several connections between \( H \) and the allocation of growth in both native and invasive plants. Lower values of \( H \) were associated with higher biomass, number of leaves, and number of tillers. Research linking plant-nutrient relationships to invasion (Daehler, 2003; Van Kleunen et al. 2010; Sardans et al. 2017) suggests lower \( H \) in invasive plants compared to competing native plants may be
Invasive grasses had significantly lower $H$ values than native grasses when grown in well-watered conditions. Yu et al. (2015) showed that increased nutrient availability would benefit lower $H$ grasses. Results from this experiment in conjunction with Yu et al.'s findings suggest that invasive grasses would have higher performance responses to nutrient enrichment than native grasses, absent environmental stressors such as drought. This interpretation of our results fits with past research demonstrating positive responses to nutrient enrichment in invasive plants (e.g. Lowe et al. 2003; Dukes et al. 2011; Vallano et al. 2012; Li et al. 2014; Sardans et al. 2017). As $H$ is the result of morphological (e.g. specific root length) and physiological (e.g. instantaneous root uptake rate) traits that control nutrient acquisition and status, differences in $H$ between native and invasive plants are the result of differing morphology and physiology. While $H$ is promising as a predictor of plant responses to environmental changes, it is not a static parameter itself, and we should not fully suspect that differences in $H$ between native and invasive species are consistent across environments.

This study showed that $H$ is resistant to changes in environmental conditions, namely water availability. The drought treatment did not significantly alter the expression of $H$ in either native or invasive grasses, in spite of higher aboveground [N] in drought-treated grasses. This [N] enrichment was not the result of decreased [C], as can be discerned from the similar mean aboveground [C] between groups (Fig. 2.7), and lower C:N ratios in drought-treated groups (Fig. 2.9). While drought is generally seen to reduce plant [N] as a result of reduced N mass flow due to lower transpiration (He and Dijkstra,
increasing plant [N] in response to drought has been reported (Wright et al. 2001; Huang et al. 2009; Drenovsky et al. 2012; Khasanova et al. 2013). With higher [N] in drought-treated groups not resulting from any reduction in [C], it may be that drought-treated grasses upregulated their rate of N capture or concentrated N in less tissue. Increasing leaf [N] can be beneficial to drought stressed plants by increasing water use efficiency through an increase in carboxylation capacity (Wright et al. 2001; Adams et al. 2016). As I measured neither nutrient uptake, water use, or photosynthesis, I cannot support these conjectures. However, it has been shown that drought can both positively and negatively impact aboveground [N] and PNUE in dryland grasses (Khasanova et al. 2013). It is therefore not outside the realm of possibility that the increase in aboveground [N] in this study’s drought-treated grasses was an adaptive response to water limitation. Altogether, these findings show stable expressions of H when exposed to environmental stress.

Differences in H often reflected differences in growth. When biomass values for each provenance-watering treatment group were viewed across the range of fertilizer treatment, there were no differences in mean biomass at each fertilizer level in the drought-treated grasses (Figs. 2.12a – 2.12c). Furthermore, within each provenance-watering treatment group there was no clear trend of increasing total or belowground biomass with increasing fertilizer [N] (Figs. 2.12a and 2.12c, Table 2.6). Altogether, differences in biomass accumulation between significantly different provenance-watering treatment groups cannot be explained by N availability, and some other mechanism explaining these differences is needed. NUE and nitrogen productivity have been used in other research to distinguish growth differences between native and invasive plants (Funk
and Vitousek 2007; James 2008). Yet in this study there were no significant differences in NUE between provenance-watering treatment groups despite differing $H$ values between the groups (Fig. 2.25), nor was there any clear pattern relating aboveground [N] to total biomass (Fig. 2.13). Ultimately, $H$ is a factor relevant to nutrient assimilation, while metrics like nitrogen productivity are concerned with nutrient utilization. With no differences in N utilization (i.e. NUE) and no differences in the effect of available N on growth, there are likely other physiological factors underlying growth differences between control-treated native and invasive grasses.

This study failed to find any significant differences in RMF between provenance-watering treatment groups. This was an unexpected result since differences in root:shoot partitioning between plants with differing $H$ was previously hypothesized (Yu et al. 2015). Differences in RMF were seen however between fertilizer treatment levels, with all grasses receiving the lowest fertilizer treatment level exhibiting higher mean RMF values (Fig. 16). Root:shoot partitioning is a plastic trait that is well known to respond to nutrient availability, especially when limited (Gedroc et al. 1996; Marschner et al. 1996; Mokany et al. 2006). These results simply reaffirm the view that plants should invest more into belowground structures when nutrients are limited (Bloom et al. 1985). Yu et al. (2015) suggested that plants with higher $H$ values would exhibit larger root systems, since larger root systems would allow them to scavenge scarce nutrients in order to maintain proper levels of nutrient homeostasis. There are serious limitations in applying the results of this study to the hypothesis of Yu et al. Grasses in this study grew in a constrained rooting environment, in a pure sand media, and with all nutrients being supplied through irrigation. Naturally occurring grasses are likely to have different
rooting architectures than grasses grown in this study. Limited transpiration in drought like conditions can limit mass flow of N through the soil in natural environments (Mariotte et al. 2017). In this study, this effect may not have been as impactful as in natural systems as all N was supplied to plants through irrigation. Despite these limitations to this study, the results still show significant differences in $H$ with no concurrent differences in RMF, which in this case suggests that some root morphological traits (e.g. RMF, total root system length) do not directly impinge on the expression of $H$. Still, demonstrated links between root:shoot allocation, plant nutrient status, and acquisition (Gedroc et al. 1996; Marschner et al. 1996) hints at potential connections between root:shoot allocation and the expression of $H$ in plants. The relationship between $H$ and root:shoot allocation deserves more attention, especially with experimentation that utilizes plants grown in their natural environments.

Unlike RMF, number of leaves and number of tillers were correlated with $H$. Provenance-watering treatment groups with higher values of $H$ had lower mean number of leaves and tillers compared to treatment groups with lower $H$ values (Figs. 2.20 and 2.23). Across nutrient treatments there was little variation in mean number of leaves (Fig. 2.19) and some variation but no clear patterns in number of tillers (Fig. 2.22). When the morphometric relationship between the number of tillers and the number of leaves were examined for each provenance-watering treatment group across fertilizer treatment levels, only drought-treated native grasses had a consistent relationship between the number of leaves and tillers (Fig. 2.24). Furthermore, within these groups the fertilizer levels responsible for both the highest and lowest number of leaves per tiller were different in each group (Fig. 2.24). This indicates morphological variability occurs regardless of the
value of $H$. While the lack of relationship between RMF and $H$ suggests that morphological traits do not directly impinge on the expression of $H$, the variability in leaf-tiller relationships across provenance-watering treatment groups suggests that group level values of $H$ do not directly influence morphological traits. There are a few reasons for considering the relationship between $H$ and morphology. First, $H$ may set limits to the proportions of tissue types that a plant can deploy. Different plant organs (e.g. stems, leaves) are associated with different stoichiometric signatures and construction costs (Sterner and Elser 2002), and a plant’s homeostatic capacity may limit it to certain ranges in morphological ratios (e.g. RMF, leaf mass fraction). Second, differential nutrient capture by plants of varying $H$ could translate into different absolute amounts of certain organs. Agricultural and ecological research have demonstrated the role nutrient availability plays in some plants in determining investment in leaves, quantified as both number of leaves and leaf weight (e.g. Walker and Aplet, 1994; Santamaria et al. 2002; Huang et al. 2009; Zhang et al. 2015). These morphological traits can be informative about plant performance, as with number of leaves and its impacts on photosynthetic capacity (Valladares and Pearcy, 2000), and in some cases as fitness correlates, as with number of tillers. Elucidating the relationships between $H$ and morphology may help researchers better unravel the ties between $H$ and plant performance in changing environments.

While assessing how $H$ may have changed with either an extended or more limited growth period is beyond the capabilities of this study, it is worthwhile considering the potential impact of plant development on $H$. Studies that examine changes in $H$ with plant development are few and provide mixed results. Peng et al. (2016) saw no
significant changes in $H_N$ across seedling, flowering, and seed-filling stages in *A. mangostanus* but did find significantly higher $H_{N:P}$ at the flowering stage compared to the seedling stage. Yan et al. (2016) found that in *A. thaliana*, $H$ for N, P, and N:P significantly decreased for leaves, stems, and siliques across three stages of development. $H$ has also been shown to increase in plant age over the course of several weeks in Yu et al. (2011). $H$ appears to be stable across growing seasons as Yu et al. (2010) reported no significant differences in $H$ values of 12 species grown across two growing seasons. These first three studies (Yu et al. 2011; Peng et al. 2016; Yan et al. 2016) suggest that the expression of $H$ can be linked to plant development, while Yu et al. (2010) shows $H$ to be stable across growing seasons. The contradictory evidence of the effect of age on $H_N$ from Peng et al. (2016) and Yan et al. (2016) makes speculation regarding the impact of plant age and stage of development on $H_N$ in the plants of this study difficult. As all grasses in this study were in a vegetative growth stage and very close to the same age, I do not suspect there were any significant impacts of plant age or developmental stage on the analysis and comparison of $H$. Comparing $H$ values of plants in this study to those in others (e.g. Dijkstra et al. 2012) is likely complicated by differences in plant age and developmental stage.

Of final note, I found differences in one other paper which reports a value of $H_{N:P}$ for a species used in this study: Dijkstra et al. (2012) reported a value of 9.6 for $H_{N:P}$ in *P. smithii*. This value was derived from wild plants which were part of the Prairie Heating and CO$_2$ Enrichment (PHACE) study near Cheyenne, WY (Dijkstra et al. 2012). A calculation following the methods used above provided $H_{N:P}$ values of 2.81 and 3.34 for the control and drought-treated *P. smithii* in this study, respectively. Several explanations
may account for these differences in $H_{N:P}$ between the $P.\ smithii$ grasses in this study and in those in Dijkstra et al. (2012). First, there is likely unaccounted for genetic differences between the grasses in this study and those in the PHACE experiment. $P.\ smithii$ grasses grown for this study were a cultivated variety while those in the PHACE experiment were a wild type population. Second, differences in climatic conditions experienced by the grasses at PHACE and the grasses in this study may account for the differences in $H_{N:P}$. This study shows that drought like conditions can alter the expression of $H_{N:P}$. Differing magnitudes of pressure exerted by drought on the plants in this study and those at PHACE may have led to the observed differences in $H_{N:P}$. Yu et al. (2011) showed that as some plants age their measured value of $H_{N:P}$ increases. Grasses in this study were only grown for six weeks while the age of the grasses at PHACE, while not reported, are likely much older. If the effect on aging on $H_{N:P}$ also exists for $P.\ smithii$, then this may have a role as well in the observed difference. While leaves turn over each year in these grasses, older plants that have developed larger root systems may show higher $H$ values as proposed by Yu et al. (2015). Finally, other environmental factors may have played a role in ways that have not yet been assessed e.g. soil texture (quartz sand vs. fine loamy soil) or soil biotic conditions (pasteurized, sterile sand vs. natural soil with microbiota). Testing the role of these and other environmental variables in the expression of $H_N$ may provide novel insight into the role of abiotic and biotic conditions in the expression of $H_N$ and local adaptation in plant-nutrient relationships. While there were large differences in $P.\ smithii$ $H_{N:P}$ values between this study and Dijkstra et al. (2012), differences between other reported species’ $H_{N:P}$ were less pronounced ($H_{N:P}$ Bouteloua gracilis = 4.28 and $H_{N:P}$ Heterstipa comata = 4.81). Regardless, all $H_{N:P}$ values reported in Dijkstra et al.
(2012) were larger than those reported in this study, raising the question as to the sources of these differences.

V. CONCLUSION

This study demonstrates lower stoichiometric homeostasis in invasive grasses than in native Northern Great Plains grasses in well-watered conditions. Stoichiometric homeostasis showed inverse relationships with biomass, number of leaves, and number of tillers but did not relate to root:shoot biomass allocation. With prior research demonstrating connections between long term population responses to eutrophication and stoichiometric homeostasis, this study suggests that invasive grasses should have greater performance than native plants in eutrophic environments when not facing environmental stressors like water limitation. I suggest new avenues of research for stoichiometric homeostasis, including elucidating the connections between stoichiometric homeostasis, biomass allocation, morphometry, and nutrient use variables.
VI. FIGURES

**Figure 2.1** Linear relationships between log-transformed fertilizer [N] and aboveground [N] values in native and invasive grasses. Letters by the regression lines indicate significant differences in the slopes of the regression lines, with similar letters indicating a lack of significance. The inverse of the regression slopes produce the $H_N$ values, which are indicated near their respective regression lines.
Figure 2.2 Linear relationships between log-transformed fertilizer N:P and aboveground N:P values in native and invasive grasses. Letters by the regression lines indicate significant differences in the slopes of the regression lines, with similar letters indicating a lack of significance. The inverse of the regression slopes produce the $H_{N:P}$ values, which are indicated near their respective regression lines.
**Figure 2.3** The relationship between aboveground \([N]\) and aboveground \([P]\). Colors of the data points represent treatment groups as indicated in the legend. There were no statistically significant relationships between \([N]\) and \([P]\) across either the whole data set or within the different provenance-watering treatment groups.
Figure 2.4 Boxplot of aboveground [P] values by provenance-watering treatment groups. Letters denote significant differences based on Tukey’s HSD test, with similar letters indicating a lack of significant difference. Notches in the boxplot show the 95% confidence interval around the mean.

Figure 2.5 Boxplot of N:P values by provenance-watering treatment groups. Letters denote significant differences based on Tukey’s HSD test, with similar letters indicating a lack of significant difference. Notches in the boxplot show the 95% confidence interval around the mean.
Figure 2.6 Aboveground [P] by provenance-watering treatment across levels of the fertilizer treatment. Points along the lines represent mean values and the bars across the means represent a 95% confidence interval of the mean.
Figures 2.7 to 2.10. Group differences in C and N stoichiometry. For each plot significant differences are denoted with letters above each boxplot, with similar letters indicating a lack of significant difference between means. Notches in each boxplot show the 95% confidence interval around the mean. Group $H_N$ values are indicated below each boxplot. Clockwise from top left: Fig 2.7 [C] across provenance-watering treatment groups. Fig 2.8 Aboveground [N] across provenance-watering treatment groups. Fig. 2.9 Aboveground C:N ratios across provenance-watering treatment groups. C:N is calculated by dividing [C] by [N]. Fig. 2.10 Total aboveground N across provenance-watering treatment groups. Total aboveground N is calculated with the product of total aboveground biomass and aboveground [N] values.
Sub-plots are distinguished by letters in the bottom left corner of each sub-plot. From top: Fig. 2.11a Total aboveground biomass across provenance-watering treatment groups, Fig. 2.11b Total belowground biomass across provenance-watering treatment groups, and Fig. 2.11c Total biomass across provenance-watering treatment groups. Significant differences are denoted with letters above each boxplot, with similar letters indicating a lack of significant difference. Notches in each boxplot show the 95% confidence interval around the mean (darkened horizontal line). Treatment group $H_N$ values are indicated at the bottom of the graph.
Figure 2.12a – 2.12c Sub-plots are distinguished by letters in the top left corner of each sub-plot. Clockwise from top left: Fig. 2.12a Total biomass between provenance-watering treatment groups across fertilizer levels. Fig. 2.12b Total aboveground biomass between provenance-watering treatment groups across fertilizer levels. Fig. 2.12c Total belowground biomass between provenance-watering treatment groups across fertilizer levels. In each graph, points along the lines represent mean values and the bars across the means represent a 95% confidence interval of the mean. Letters next to each mean point represent significance groups based on a Tukey’s HSD test, with points sharing similar letters indicating a lack of significant difference.
Figure 2.13 Total biomass in relation to aboveground [N], fertilizer treatment level, and provenance-watering treatment groups. Symbol shapes represent the different levels of the fertilizer treatment, while the symbol colors represent the provenance-watering treatment group (shown as their respective $H_N$ to which the respective observation belongs).
Figures 2.14 – 2.16. Clockwise from left: Fig. 2.14 Root mass fraction as described by total biomass and fitted with a log-curve. Symbol shapes represent fertilizer treatment levels and colors represent the $H_N$ values of their respective provenance-watering treatment groups. Fig. 2.15 Distribution and mean values of root mass fraction across fertilizer treatment levels. Fig. 2.16 Distribution and mean values of root mass fraction across fertilizer treatment levels. The dark lines in the boxplots of Figures 15 and 16 represent the mean values and the notches in the boxplots show the 95% confidence interval of the mean.
Figure 2.17 Root mass fraction by provenance-watering treatment groups. Points along the lines represent mean values and the bars across the means represent 95% confidence intervals of the mean. Letters next to each mean point represent significance groups based on a Tukey’s HSD test, with points sharing similar letters indicating a lack of significant difference.
Figures 2.18 to 2.20. Clockwise from left: Fig 2.18 Number of leaves in relation to aboveground [N], provenance-watering treatment group H_N values, and fertilizer treatment. Symbol shapes represent levels of the fertilizer treatment while symbol colors represent the H_N values of the provenance-watering treatment groups. Fig. 2.19 Mean values for the number of leaves for each provenance-watering treatment group across fertilizer treatment levels. Points represent mean values while bars represent 95% confidence intervals of their respective means. Letters next to each mean point represent significance groups based on a Tukey's HSD test, with points sharing similar letters indicating a lack of significant difference. Fig. 2.20 Boxplots for the number of leaves in each provenance-watering treatment group. Bars represent mean values while notches represent 95% confidence intervals around the mean.
Figures 2.21 to 2.23. Clockwise from left: Fig. 2.21 Number of leaves in relation to aboveground [N], provenance-watering treatment group $H_N$ values, and fertilizer treatment. Symbol shapes represent levels of the fertilizer treatment while symbol colors represent the $H_N$ values of the provenance-watering treatment groups. Fig. 2.22 Mean values for the number of leaves for each provenance-watering treatment group across fertilizer treatment levels. Points represent mean values while bars represent 95% confidence intervals of their respective means. Letters next to each mean point represent significance groups based on a Tukey's HSD test, with points sharing similar letters indicating a lack of significant difference. Fig. 2.23 Boxplots for the number of leaves in each provenance-watering treatment group. Bars represent mean values while notches represent 95% confidence intervals around the mean.
Figure 2.24 Standardized major axis regressions for the relationship between the number of tillers and the number of leaves across fertilizer and watering treatments for invasive and native provenance groups. Colors of each line and point signify their respective fertilizer treatment. Significant differences between the regression slopes for control and inoculated group lines are indicated by an asterisk (\(*\)) in the top left of a sub-plot.
Figure 2.25 Nitrogen use efficiency in relation to aboveground [N], fertilizer treatment, and provenance-watering treatment groups. Symbol shapes represent the different levels of fertilizer treatment, while the symbol colors represent the provenance-watering treatment group.
CONCLUSION

The first experiment demonstrated three significant findings: $H$ is lower in invasive plants than native plants in well-watered conditions, the expression of $H$ is resilient to environmental conditions (i.e. water availability), and differences in $H$ are associated with differences in growth. This study is the first to specifically test for differences in $H$ between native and invasive plants. While significant differences in $H$ between the native and invasive plants specifically apply to the model species of this study, this result may be indicative of a larger trend of less homeostatic nutrient relationships in invasive plants compared to native plants. The first experiment demonstrated that water availability does not impact the expression of $H$. As there was no mycorrhizal inoculation in the second experiment, any potential impact of mycorrhizal symbiosis on $H$ could not be addressed. Regardless, I suspect that mycorrhizal symbioses would impact the expression of $H$ in plants. Lastly, I show that differences in $H$ are associated with differences in growth. Few studies examined $H$ as well as growth traits (root biomass in Peng et al. 2016; relative growth rate in Yan et al. 2014), and comparisons between growth traits in these papers were across nutrient treatment levels rather than between groups of plants based on $H$ values. This paper specifically compared biomass, biomass allocation, and other morphological traits between provenance-watering treatment groups and found differences in $H$ were associated with differences in biomass and aboveground morphological traits.

The lack of successful mycorrhizal infection in the second experiment severely limited my ability to explore the relationship between symbiosis, plant provenance, and $H$. However, some insights were still gleaned from the results. First, there was an
unexpected lack of significant differences in $H$ between the invasive and native grasses. With no detected mycorrhizal infection, all grasses in this study were essentially in the ‘control’ group. With the same nutrient treatment levels and target minimum sand moisture levels, the second study only differed from the first noticeably in their greenhouse conditions. Daily average PAR, average daily maximum PAR, and average daily temperature were higher in the first experiment than the second. Güsewell (2004) presented unpublished data that showed light availability altering the expression of $H$ in $C.\ curta$, with lower light availability increasing the expression of $H$. $H$ values in the second experiment were lower than those in the first experiment. While light availability may have impacted the expression of $H$ in the second experiment, such a change would have the opposite impact as shown in Güsewell (2004). To my knowledge, no other study has examined the impact of light availability on $H$ in plants, and any potential impact of light availability on $H$ in these two studies can only remain speculative. It may be possible that the cooler greenhouse temperatures in the second experiment impacted the expression of $H$ in the study grasses.

Identifying traits that can explain and predict performance challenges between native and invasive plants remains an active challenge to researchers. The first chapter shows a promising characteristic for the utility of $H$ as an explanatory and predictive trait; that is, differences in $H$ were associated with differences in growth. If this pattern is consistent across other taxa, then researchers would be able to quantify $H$ for plant species in a community and derive scenarios for growth differences between species with changing nutrient conditions. While this would be a highly desirable outcome, researchers should account for the potential influence that environmental conditions (e.g.
light availability, mycorrhizal symbioses) could have on the expression of \( H \), as well as the amount of growth achieved by plants of different \( H \) values. Predicting plant growth responses to changing nutrient conditions would therefore require an understanding of what other environmental conditions impact the community of interest and how those conditions influence the expression of \( H \) and growth. Lastly, differences in growth between invasive and native grasses may persist despite similarities in \( H \), as was seen in the second experiment, indicating the role of other unidentified traits in biomass accumulation. No single trait will ever explain or predict performance differences between native and invasive plants, and it would be unreasonable for \( H \) to do just that. However, my research shows that \( H \) is associated with species provenance and biomass accumulation, making \( H \) a trait worthy of consideration when studying and modelling alien plants and their invasions.
APPENDIX: EXPERIMENT 2

I. METHODS

Study Site and Conditions

All plants were cultivated in the South Dakota State University Horticulture and Forestry Greenhouses (44.32°, -96.78°) located in Brookings, South Dakota. Greenhouse temperature, humidity, and PAR were measured with a coupled thermometer and hygrometer (Model CS215, Campbell Scientific, Logan, UT) and a PAR sensor (SQ-110, Apogee Instruments, Logan, UT). Greenhouse temperature was set at 18°C and the grasses were supplementally lit for 16 hours daily with two 400 W bulbs in electronic ballasts (WEX120, Growers Supply, Dyersville, IA) suspended above the greenhouse table. The daily average PAR calculated for the 16-hour period of illumination was 19.39 µmol m⁻² s⁻¹, average daily maximum PAR was 45.87 µmol m⁻² s⁻¹, and the daily average temperature was 14.32°C.

Experimental Design and Sampling

The experimental design and sampling methods were similar to those in Chapter 2. The model species, fertilizer treatment and treatment levels, and sand culture method in this experiment are the same in both implementation and materials used as in Chapter 2. In place of a drought treatment, a mycorrhizal inoculation treatment was applied. With two levels of the mycorrhizal (control and inoculated), five levels of fertilizer treatment, and four species, there were 40 treatment combinations with 10 replicates for each treatment combination.

Newly purchased quartz sand (Unimin Granusil®) was steam pasteurized for 45 minutes with a soil aeration wagon (Lindig Soil Cart, Cornelius Equipment Company,
Oshkosh, WI). The pasteurized sand was placed into ‘Deepots’ (Stuwe and Sons, Tangent, OR) with synthetic landscaping fabric taped over the pots’ bottoms to prevent loss of sand while still allowing for drainage. Seeds for plants in the inoculated group were wetted then dusted with an endomycorrhizal powder (Micronized Endomycorrhizal Inoculant, BioOrganics, New Hope, PA) prior to planting. Seeds in both mycorrhizal treatment groups were planted on October 31, 2017. Pots were irrigated to maintain moist conditions until all pots contained at least one seedling, at which point all pots were thinned so that there was one seedling per pot. After thinning, all inoculated pots received 100 mL of a mycorrhizal drench made up of approximately 121 g of inoculant in one gallon of distilled water. Control-treated pots received 100 mL of distilled water. Plants were first fertilized on December 2, 2017, and fertilization treatments continued until February 1, 2018 when harvesting began, for a total experimental duration of 9 weeks.

The fertilizer used in this experiment followed the same levels of concentrations and was applied in the same manner as described in Chapter 2. Two soil moisture probes (EC5, Decagon Devices, Pullman, WA) per species were placed in 9 mM N treated well-watered and drought-like condition pots to track substrate volumetric water content (VWC). Plants received fertilizer solution frequently enough to keep the average value of the pots above 15% VWC. Fertilizer application ceased at the beginning of harvesting, which lasted from February 1 to February 16, and plants received irregular and minimal water in order to maintain their vigor during this period until they were harvested. At harvest, total number of leaves, tillers, and shoot height were quantified. Aboveground biomass was clipped at the crown of the roots and was stored in labelled paper bags, which were then oven dried at 60° C for 72 hours. Belowground biomass was separated
from the sand substrate using a 10 mesh soil sieve, and placed into labelled plastic bags. After harvesting, approximately 1 g of fresh weight roots were subsampled from all inoculated root systems and several control root systems, rinsed of sand, and individually stored in labelled centrifuge tubes with 50% ethanol sufficient to cover the subsamples. The remainder of each sample was rinsed with water over a 10 mesh soil sieve to remove the remaining sand, placed in labelled plastic bags, and stored in a freezer until they were processed.

An initial set of 20 randomly selected inoculated root subsamples and 5 control root subsamples were stained for determination of mycorrhizal colonization. The selected samples had the ethanol drained off and were cleared in 10% KOH for three days, acidified in 5% HCl for 30 min., then stained with a 0.05% solution of Trypan blue in lactoglycerol (Weremijewicz and Seto, 2016). These samples were examined for fungal structures under a dissecting microscope. No fungal structures were detected, and as a result, there was no follow-up with any quantitative method for determining mycorrhizal infection.

**Sample Preparation and Analysis**

After drying, senescent aboveground tissue was manually separated from green tissue, which were weighed separately. The green portions of the aboveground biomass were shredded in a coffee bean grinder until reaching a fine consistency. Samples were then pulverized with a ball mill (Mini-Beadbeater 16, BioSpec Products, Bartlesville, OK) for 1.5 min. Approximately 2.5 mg of the resultant samples were analyzed by SDSU Soil and Plant Analysis Laboratory for [C] and [N] using an elemental analyzer (Vario MAX CNS, elementar, Langenselbold, Germany). The remaining ground leaf tissue was
digested with HNO₃ in a microwave and analyzed for P concentration through inductively coupled plasma spectrometry in the same laboratory. Belowground tissues were then dried at 60° C for 72 hours and weighed for total belowground biomass.

Data Analysis

All statistical analyses were performed in the R computing environment (R Core Team, 2015). To determine $H_N$ differences between native and invasive grasses and mycorrhizal treatments, I constructed an initial set of linear mixed models explaining log(leaf [N]) and log(leaf N:P) with the R package nlme (Pinheiro et al. 2017) using the maximum likelihood method for parameter estimation. Each model contained a random species effect, a fixed fertilizer treatment effect, and one of each possible combination of a fixed mycorrhizal treatment and a fixed provenance (i.e. native or invasive) effect. AIC value weights were used to determine the best fit model. For log(leaf [N]), the best fit model included only the random species effect and the fixed fertilizer effect. This best fit model was used to calculate an $H_N$ value across the whole dataset, by calculating the inverse of the slope of the regression line best fitting log fertilizer [N] and log aboveground [N] (Sterner and Elser 2002). In the same manner described in Ch. 2, $H_N$ was also calculated for each provenance-mycorrhizal treatment grouping and tested for significant differences using a pairwise least-squares adjusted mean comparison.

Total aboveground N was calculated by multiplying total aboveground biomass by aboveground [N]. Nitrogen use efficiency (NUE) was calculated by dividing total aboveground biomass by total N (Lambers et al. 1998). While there were no significances in $H_N$ between provenance-mycorrhizal treatment groups, these groups were still compared for differences in biomass, height, number of leaves, and number of tillers
using ANOVA. To determine how provenance-mycorrhizal treatment groups varied with nutrient availability, the same analyses but with the addition of the fertilizer treatment effect were performed for all biomass variables, number of leaves, and number of tillers. In both sets of analyses, ANOVAs for number of leaves and number of tillers were performed on generalized linear models with Poisson error structure. For both ANOVAs with and without the fertilizer effect, post-hoc analyses were performed when applicable with a Tukey’s HSD test.

Best fit models for number of leaves and number of tillers were determined by comparing AIC values of multiple general linear models with Poisson error structure. Best fit models for biomass variables, root mass fraction, and NUE were determined in the same manner as described for log(leaf[N]) and log(leaf N:P) above. Standardized major-axis regressions and a multiple pairwise comparison with Sidak adjustment were used to construct and compare the relationship between the number of tillers and number of leaves of each provenance-watering treatment group using the R package smatr (Warton et al. 2012). Significance for all tests was qualified at alpha = 0.05.
II. TABLES

<table>
<thead>
<tr>
<th>Model</th>
<th>df</th>
<th>AIC</th>
<th>Δ(AIC)</th>
<th>w(AIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer</td>
<td>5</td>
<td>-629.61</td>
<td>0</td>
<td>0.56231745</td>
</tr>
<tr>
<td>Fertilizer*Provenance</td>
<td>7</td>
<td>-628.07</td>
<td>1.543</td>
<td>0.26005789</td>
</tr>
<tr>
<td>Fertilizer*Mycorrhizae</td>
<td>7</td>
<td>-627.18</td>
<td>2.4261</td>
<td>0.16716969</td>
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<tr>
<td>Fertilizer<em>Mycorrhizae</em>Provenance</td>
<td>1</td>
<td>-621.64</td>
<td>7.9699</td>
<td>0.01045497</td>
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</tbody>
</table>

*Table 3.1* AIC values and weights for the set of mixed models explaining aboveground [N] used to calculate $H_N$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$H_N$</th>
<th>Significance groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive Inoculated</td>
<td>2.32</td>
<td>a</td>
</tr>
<tr>
<td>Invasive Control</td>
<td>2.38</td>
<td>a</td>
</tr>
<tr>
<td>Native Inoculated</td>
<td>2.62</td>
<td>a</td>
</tr>
<tr>
<td>Native Control</td>
<td>2.87</td>
<td>a</td>
</tr>
<tr>
<td><strong>Whole Dataset</strong></td>
<td>2.45</td>
<td></td>
</tr>
</tbody>
</table>

*Table 3.2* $H_N$ values for each provenance-mycorrhizal treatment group, their significance groupings, and the grouped $H_N$ value for all grasses. Similar letters in the significance groups column indicates a lack of significant difference.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Best fit model parameters</th>
<th>df</th>
<th>AIC</th>
<th>w(AIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total biomass</td>
<td>Fertilizer</td>
<td>7</td>
<td>781.948</td>
<td>0.764</td>
</tr>
<tr>
<td>Aboveground biomass</td>
<td>Fertilizer</td>
<td>7</td>
<td>410.9</td>
<td>0.691</td>
</tr>
<tr>
<td>Belowground biomass</td>
<td>Fertilizer*Provenance</td>
<td>7</td>
<td>454.87</td>
<td>0.924</td>
</tr>
<tr>
<td>Root mass fraction</td>
<td>Fertilizer<em>Mycorrhizae</em>Provenance</td>
<td>22</td>
<td>-553.083</td>
<td>0.342</td>
</tr>
<tr>
<td>Number of leaves</td>
<td>Fertilizer<em>Mycorrhizae</em>Provenance</td>
<td>370</td>
<td>2640.549</td>
<td>0.999</td>
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<tr>
<td>Number of tillers</td>
<td>Fertilizer<em>Mycorrhizae</em>Provenance</td>
<td>370</td>
<td>1461.975</td>
<td>0.999</td>
</tr>
<tr>
<td>NUE</td>
<td>Fertilizer*Provenance</td>
<td>6</td>
<td>469.905</td>
<td>0.507</td>
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</tbody>
</table>

*Table 3.3* Best fit model parameters, AIC values, and AIC weights for the set of mixed models explaining biomass variables, root mass fraction, and morphological variables. ANOVAs ran on the best fit models for root mass fraction, number of leaves, and number of tillers showed that the mycorrhizal treatment terms were only significant at $\alpha=0.1$. The provenance effect was not significant in the NUE model or belowground biomass model. There was a significant fertilizer:provenance interaction in the belowground biomass model.
III. FIGURES

Figure 3.1 Linear relationships between log-transformed fertilizer [N] and aboveground [N] values in native and invasive grasses. Letters by the regression lines indicate significant differences in the slopes of the regression lines, with similar letters indicating a lack of significance. The inverse of the regression slopes produce the $H_N$ values, which are indicated near their respective regression lines.
Figure 3.2 to Figure 3.5: Group differences in C and N stoichiometry. For each plot significant differences are denoted with letters above each boxplot, with similar letters indicating a lack of significant difference between means. Notches in each boxplot show the 95% confidence interval around the mean. Group $H_N$ values are indicated below each boxplot. Clockwise from top left: Fig 3.2 [C] across provenance-mycorrhizal treatment groups. Fig 3.3 Aboveground [N] across provenance-mycorrhizal treatment groups. Fig. 3.4 Aboveground C:N ratios across provenance-mycorrhizal treatment groups. C:N is calculated by dividing [C] by [N]. Fig. 3.5 Total aboveground N across provenance-mycorrhizal treatment groups. Total aboveground N is calculated with the product of total aboveground biomass and aboveground [N] values.
Figure 3.6a – 3.6c Sub-plots are distinguished by letters in the bottom left corner of each sub-plot. From top: Fig. 3.6a Total aboveground biomass across provenance-mycorrhizal treatment groups, Fig. 3.6b Total belowground biomass across provenance-mycorrhizal treatment groups, and Fig. 3.6c Total biomass across provenance-mycorrhizal treatment groups. Significant differences are denoted with letters above each boxplot, with similar letters indicating a lack of significant difference. Notches in each boxplot show the 95% confidence interval around the mean (darkened horizontal line). Treatment group $H_N$ values are indicated at the bottom of the graph.
Figure 3.7a – 3.7c Sub-plots are distinguished by letters in the top left corner of each sub-plot. Clockwise from top left: Fig. 3.7a Total biomass between provenance-mycorrhizal treatment groups across fertilizer levels. Fig. 3.7b Total aboveground biomass between provenance-mycorrhizal treatment groups across fertilizer levels. Fig. 3.7c Total belowground biomass between provenance-mycorrhizal treatment groups across fertilizer levels. In each graph, points along the lines represent mean values and the bars across the means represent a 95% confidence interval of the mean. Letters next to each mean point represent significance groups based on a Tukey’s HSD test, with points sharing similar letters indicating a lack of significant difference.
Figure 3.8 Total biomass in relation to aboveground [N], fertilizer treatment level, and mycorrhizal treatment. Symbol shapes represent the different levels of the fertilizer treatment, while the symbol colors represent the mycorrhizal treatment group to which the respective observation belongs.
Figure 3.9 Root mass fraction as described by total biomass and fitted with a log-curve. Symbol shapes represent the levels of fertilizer treatment and symbol colors represent provenance-mycorrhizal treatment groups.
Figures 3.10 to 3.12. Clockwise, from left. Fig. 3.10 Number of leaves in relation to aboveground $[N]$, $H_N$, and fertilizer treatment level Symbols’ shapes represent the levels of fertilizer treatment and symbol colors represent provenance-mycorrhizal treatment groups. Fig. 3.11 Mean values for number of leaves for each provenance-mycorrhizal treatment group across fertilizer treatment levels. Bars around each point represent a 95% confidence interval of the mean. Letters next to each mean point represent significance groups based on a Tukey’s HSD test, with points sharing similar letters indicating a lack of significant difference. Colors indicate the provenance-mycorrhizal treatment group to which the observations belong. Fig. 3.12 Boxplots for the number of leaves in each provenance-mycorrhizal treatment group. Dark horizontal bars show the location of the mean, and notches in the boxplot represent a 95% confidence interval around the mean.
Figures 3.13 to 3.15. Clockwise, from left. Fig. 3.13 Number of tillers in relation to aboveground [N], $H_N$, and fertilizer treatment level. Symbols’ shapes represent the levels of fertilizer treatment and symbol colors represent provenance-mycorrhizal treatment groups. Fig. 3.14 Mean values for number of tillers for each provenance-mycorrhizal treatment group across fertilizer treatment levels. Bars around each point represent a 95% confidence interval of the mean. Letters next to each mean point represent significance groups based on a Tukey’s HSD test, with points sharing similar letters indicating a lack of significant difference. Colors indicate the provenance-mycorrhizal treatment group to which the observations belong. Fig. 3.15 Boxplots for the number of tillers in each provenance-mycorrhizal treatment group. Dark horizontal bars show the location of the mean, and notches in the boxplot represent a 95% confidence interval around the mean.
Figure 3.16 Standardized major axis regressions for the relationship between the number of tillers and the number of leaves across fertilizer and mycorrhizal inoculation treatments for invasive and native provenance groups. Colors of each line signify the respective fertilizer treatment. Significant differences between the regression slopes for control and inoculated group lines are indicated by an asterisk (*) in the top left of a sub-plot.
Figure 3.17 Nitrogen use efficiency in relation to aboveground [N], fertilizer treatment, and provenance-mycorrhizal treatment groups. Symbol shapes represent the different levels of fertilizer treatment, while the symbols’ colors represent the provenance-mycorrhizal treatment group.
REFERENCES


