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# **Invasion of Texas Rangelands by King Ranch Bluestem (*Bothriochloa ischaemum*): The Role of Nutrient Availability, Niche Partitioning, and Mycorrhizal Fungi**

Claire Afflerbach

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**Invasion of Texas Rangelands by King Ranch Bluestem (*Bothriochloa ischaemum*): The Role of Nutrient Availability, Niche Partitioning, and Mycorrhizal Fungi**

**Chapter 1**

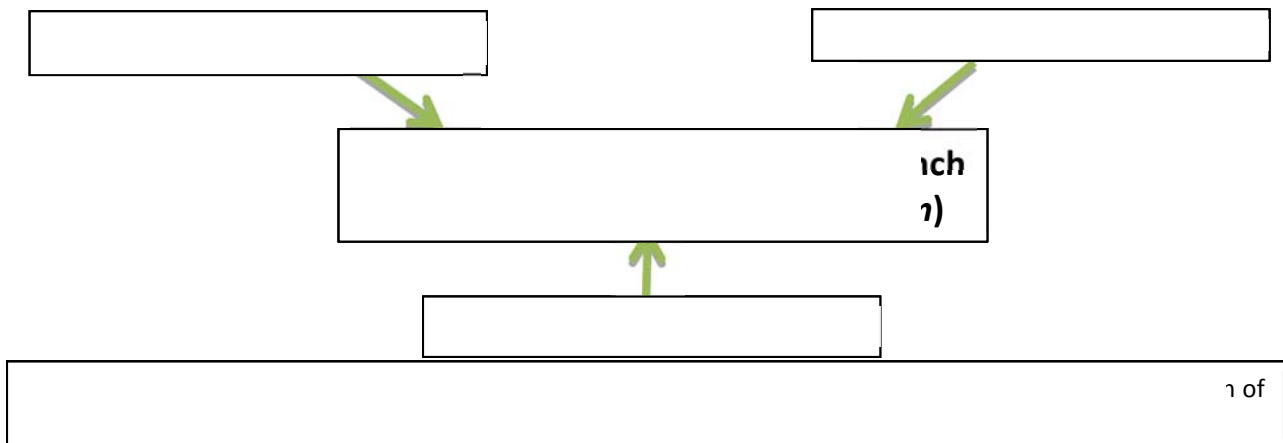
Texas is facing a conservation crisis with invasion of native rangelands by homogenizing non-native grasses. This is especially true for the Old World bluestem species King Ranch Bluestem (*Bothriochloa ischaemum*), which was introduced in the mid-20<sup>th</sup> century for pasture and hay production and for erosion control on roadsides. The monoculture stands produced by King Ranch bluestem (hereafter referred to as KR) have negative effects on the overall health of plant and animal communities by decreasing the biodiversity and destroying habitat occupied by native wildlife (Schreiber 2011). As a result of this widespread trend, there is interest in re-introduction of the native flora and characterization of the mechanisms that allow KR to be a successful invader. The ultimate goal is not only understanding the invasion process but also slowing and reversing it. To this end, this thesis considers three mechanisms that may affect the competitive and invasive abilities of KR: soil nutrient availability, niche partitioning, and the presence of mycorrhizal fungi.

Resource-competition models predict that plant population dynamics and competitive interactions are mediated by a species' ability to compete for resources (Tilman 1986). Therefore, nutrient acquisition forms the basis of the interspecific competition that either allows or resists invasion by non-native species. As a result, nutrient availability may affect the competitive dynamics of a system and therefore change the system's vulnerability (Kolb et al. 2002). Similarly, the degree of niche partitioning affects invasibility of a system, as well as the degree of coexistence between resident species and a focal invader (Dukes 2002). Finally, mycorrhizal fungi have been shown to regulate community dynamics and mediate competition (e.g., Jin et al. 2011). Because of this, fungal inoculants have recently received much interest as a potential tool for restoration (Herrera et al. 1992). Through

the consideration and testing of these factors in a greenhouse experiment, we aim to assess the role mycorrhizal fungi play in the invasion process and identify potential strategies for controlling invasion.

### Nutrient Availability

At the heart of invasion theory lays interspecific competition, the interaction between different species that results in an increase in the abundance of one population at the expense of another (Tilman 1986). In its simplest form, competition either results in competitive exclusion of one species by another or coexistence of both of the species in the same community. In this way, competition affects community structure as a determinant of richness and diversity. In many cases, the negative results of competitive interactions, such as growth inhibition, are likely not a result of direct interspecific



interactions, but rather an indirect result of the fact that both species are competing for the same pool of limiting resources (Tilman 1986). Given the importance of resource acquisition to plant growth and survival, it is theoretically likely that resource availability affects the competitive dynamics between species, ultimately influencing the likelihood of successful invasion of highly competitive and homogenizing species.

The importance of resource availability to population dynamics is clearly realized in Tilman's resource-competition models that predict the likelihood of coexistence (as opposed to dominance) between multiple plant species in long-term equilibrium. At their simplest, these and similar models, such as MacArthur's consumer-resource model (MacArthur 1970; Carroll et al. 2011), consider

interspecific competition between two species competing for a single limiting resource. Competition is modeled as the function of the two or more species' ability to reduce the concentration of the limiting nutrient(s) as they utilize it for growth relative to the influx of the nutrient(s) into the system. The lowest concentration to which a species can reduce an available resource while continuing to exist in a stable monoculture is known as its  $R^*$  value. As a result,  $R^*$  values have been suggested as a measure of a species' competitive ability (Tilman 1999).

Theory predicts that a species with the lowest  $R^*$  will outcompete a species with a higher  $R^*$  by reducing the resource availability below the threshold at which the competitor could survive. Therefore, resource-competition models predict that better competitors are those that use resources more efficiently and are able to reduce the available resource to a level too low to be used by inferior competitors (have a lower  $R^*$ ) and survive (Tilman 1999). This ability allows the species with the lowest  $R^*$  for a given resource to outcompete and exclude other species (Tilman 1986). As related to invasion theory, Tilman's models can theoretically be used to predict the outcome of competition between an invasive species and the natives occupying the physical habitat.

Coexistence in the  $R^*$  model is possible in systems containing multiple limiting resources when each competing species is most limited by a different resource (Tilman 1986). This creates a system of tradeoffs between competitor growth strategies, and therefore overall growth rates under any given set of conditions that effectively prevents one from completely out-competing the other. Tradeoffs in general support stable equilibria (and co-existence) between species within diverse communities. These mathematical models may be overly simplified in that they predict that coexistence is possible only when there are equal or greater possible limiting resources than number of species competing so that each species can be most limited by one of those resources. However, in real systems, there are not an infinite number of limiting resources to create the tradeoffs that these models predict should exist,

suggesting that another factor must be influencing these stable communities with high species richness (Tilman 1986).

In order for these models to be applicable to more complicated natural systems they must take into account the spatial and temporal heterogeneity of resource abundance that occurs in real life ecosystems. Therefore, infinite discrete resources are not necessary for coexistence of many species when fewer resources vary throughout time and space (Tilman 1986). This again creates the potential for tradeoffs that prevents one species from becoming competitively dominant at every spatial and temporal combination of resources. The application of these models to algae populations showed that they were able to explain 75% of the growth and competitive dynamics between the species, as observed by Tilman (Tilman 1997). This supports the central role that resource availability, especially as it varies through time and space, has on the competitive dynamics that structure communities and create stable equilibria.

The application of Tilman's  $R^*$  theory and resource-competition models to the process of invasion may allow us to predict the outcome of competitive interactions between invasive and native species, which forms the heart of the process of invasion. In this way, the invasibility and vulnerability of a system may be predicted based on the resource availability and  $R^*$  values of the native species living there. As a result of  $R^*$  theory, we would predict that an invasive species would be successful in competition with natives when it has the lowest  $R^*$  value over at least some of the spatial and temporal resource combinations, making it the most competitive and efficient in reducing resource concentrations in those conditions. However,  $R^*$  theory alone only considers the effect of limiting resources, and thus may not be complex enough to fully characterize the invasive patterns that we observe in the field.

Invasion is a complex process that involves not only the competitive ability of the invader relative to the natives – which determines its ability to persist in the environment – but also how likely

the invader is to establish in the first place. Ultimately, the ability of the invader to establish in the system may be just as important, if not more so, than its ability to compete. If it cannot establish in the first place, then its competitive abilities are meaningless. The invasibility of a system likely depends on factors overlooked by the resource-competition models, such as the evolutionary history of the system or presence of a disturbance. Nutrient availability may play a role in invasibility, but it may not be predictive in the same way that Tilman found for straight interspecific competition. As a result, the application of  $R^*$  theory to invasion is too simple to allow us to generalize the role of nutrient availability and acquisition in predicting the invasibility of the system.

In many cases, invasive success is correlated with increased soil nutrients, which seems to be the opposite of what Tilman's  $R^*$  theory would predict. Both theoretical and empirical work have demonstrated a positive link between ecosystem invasibility and high nutrient availability, especially in systems where the level of nutrients is historically low (James et al. 2011; Vasquez et al. 2008). For instance, measures of plant cover in coastal grasslands suggest that non-native cover is significantly higher in response to increased nitrogen from N-fixing shrubs, though the effects of confounding factors such as increased light and decreased acidity in these areas can't be ruled out (Kolb et al. 2002). Not only was native cover higher at low nutrient levels, but established native communities were more invasion resistant at low nutrient levels (Kolb et al. 2002). This trend suggests that even though natives may be more competitive than invasives, as defined by Tilman's  $R^*$  theory, they have less ability to respond positively to increased nutrient availability. This likely reflects a tradeoff in growth rate and strategy, and therefore fitness at varying nutrient levels: invasives with higher growth rates can take up more nutrients and thus more quickly increase their biomass in response to increasing nutrients, but are less competitive at lower nutrient levels because their quick growth strategies utilize the available nutrients too quickly. Native species, with slower growth strategies, are thus better able to persist at low nutrient levels (Vasquez et al. 2008). The same can be said of a system where annual and perennial

grasses are in competition – annuals have faster growth rates, and thus are more competitive at higher nutrient levels, while perennials tend to be favored in low nutrient environments (James et al. 2011). Here,  $R^*$  theory is not predictive of invasive success because it fails to take into account systems where the historically limiting resource is no longer limiting, such as is the case after disturbances that create high nutrient environments.

Many successfully invading species have certain characteristics or growth strategies that facilitate their quick positive response to increases in available nutrients, allowing them to out-compete the native species. A field study across multiple sites in northwest Europe observed the impact of seven highly invasive species on nutrient availability in soil and community composition and biomass in an attempt to find consistent traits that may result in invasive success. The invaded plots, regardless of which invader was present, consistently showed increased above ground biomass and nutrient stock in standing biomass when compared with sites consisting only of native species (Dassonville et al. 2008). These findings are suggestive of the possibility that successful invasive species may have an increased ability to respond to changes in nutrient availability by changing their growth strategy and increasing their nutrient acquisition. However, while the invasive species were assimilating more nutrients from the soil than the native overall, the nutrient tissue density for the invader was lower than that found in the resident species (Dassonville et al. 2008). This suggests that the growth strategy of the invader was allowing them to make “cheaper” leaves – that is, to grow in a way that utilizes less energy, allowing for greater biomass expansion. Therefore, invaders are, as a whole, growing faster and out-competing the natives because they assimilate fewer nutrients per leaf and thus have higher growth rates than their competitors. The correlation between competition for nutrients and competitive success makes plasticity in nutrient acquisition likely to have positive influences in growth by allowing the species to respond and adapt more quickly to disturbances and changing environments. This may be a key factor in



allowing them to outcompete and exclude native species, and thus the presence of such traits may be predictive of invasive success in high nutrient environments.

Nonetheless, invasive success does not always show a positive correlation with increased nutrient availability. As a result, the relationship between nutrient availability and invasive success is not so easy to generalize. Invasion success can vary significantly depending on the species and system, evolutionary history, resource examined, response variable measured, and presence or absence of confounding factors (ie. disturbances). For example, European explorers brought a host of invasive species with them when they arrived in Chile, both plants and animals, which have since integrated into the local flora and fauna. An observational study showed that the majority of introduced herbs were most able to successfully colonize nutrient-poor soils. In other words, native species in this system had a larger competitive advantage than introduced species in high nutrient systems (Holmgren et al. 2000).

Disturbances that cause changes to the 'normal' levels of nutrients in a system are likely the key to successful invasion and the largest predictive factor. These disturbances can come in many forms – natural and human-made (or anthropogenic) – and their relative impact on the invasion of ecosystems by non-native species often depends on the type, intensity, and timing of the disturbance (Hobbs and Huenneke 1992). In some systems, disturbance is a result of a change to the historical regime or environmental pattern. For instance, many systems experience a certain level of grazing or frequency of wildfires naturally. It is thus the change (either an increase or decrease) that causes a disturbance in the system (Hobbs and Huenneke 1992). Much like Goldilocks and the three bears, there is often a level of disturbance that is “just right” in an ecosystem to support the greatest species diversity. This “intermediate disturbance hypothesis” suggests that species diversity is highest at moderate frequency and intensity of disturbance (Hobbs and Huenneke 1992). Common disturbances that affect community structure include changes in fire, herbivory, and grazing, which often directly lead to soil disturbances like increased nutrients or soil biota change, and/or change in input or cycling of nutrients, among

others (Vasquez et al. 2008). Therefore, successful invasion is a result of changing soil conditions, which disrupts the stability of established communities to allow invasion.

Therefore, successful invasion seems most strongly predicted by a change to historic levels of soil resources. The species native to that environment would have adapted through evolution to the historical conditions at that site, and thus theoretically be a strong competitor under those conditions. Invasive success, then, is most likely when these conditions change, disrupting the established community structure and function. These changes to the historical 'norm' can be the result of human activities and capitalized on by the invasive species, or a direct impact of the invasive species itself. Considering the pattern of invasion across Europe, it is clear that the process of invasion is either being facilitated by or causing changes in soil nutrients. However, the impact of the invader on the nutrient concentration of the topsoil was site-specific and therefore not consistent, but seems to follow a general pattern (Dassonville et al. 2008). Essentially, the result of invasion is a change in soil nutrient availability depending on the initial condition of the site. Sites where nutrient availability increased in response to invasion were those that had low initial starting concentrations. Conversely, sites that showed a negative soil nutrient concentration response to invasion were those that started at high nutrient availability (Dassonville et al. 2008). The invaders considered in this study occupied different niches and likely exhibited different invasion characteristics, but no correlation was found between the specific invader and the impact that its invasion had (whether it tended to have a positive or negative effect) on soil nutrients. In other words, the success of each invasion event is more a result of the change itself than the direction (positive or negative) of the change. It is also not clear whether the successful invader is merely being opportunistic in this system, utilizing windows left open by an unrelated disturbance that destabilized the system, or if the process of invasion itself is causing the disturbance. Through the process of invasion, many species can create positive feedback loops that further facilitate their invasion

– altering soil microbes (Stinson et al. 2006), for instance, or changing the rates of other disturbances to create synergistic effects on the community (Vasquez et al. 2008).

Nutrient availability alone may not explain invasive success. A test of native and invasive response to increased nutrients found that all responded similarly with biomass, survival, and root:shoot ratio increases as a result of increased nutrients (Hastwell and Panetta 2005). This suggests that most plant species may have the potential for positive biomass response to nutrient increase, but the presence of confounding factors in the field affect how likely any given species is to actually show its potential greatest growth rate increase. In these cases, invasive success may be the result of specific interactions between a variety of factors, of which nutrient availability is only one, that vary by site and species.

Ultimately, it seems to be that a change in environmental conditions, of which nutrient availability may be one of the key elements, is a greater predictor of invasive success (whether disturbance per se is a result of the invasion or invasion involves some third-party process). This change seems to supercede any specific nutrient change or invader trait. However, traits that make a species more resistant to disturbance, such as high resistant seed output and ability to survive harsh conditions, may increase an invaders' success at colonizing these disturbed sites (Duda et al. 2003).

The invasion of native Texas grasslands by King Ranch Bluestem may be largely facilitated by disturbance, likely as a result of agricultural processes such as grazing, burning, and fertilization that create environmental conditions to which native grasses are not adapted (Baer et al. 2009). KR may also respond more positively to nutrient addition than its native grass competitors, but there are no clear trends to suggest that this is always the case for successful invaders. In conclusion, changing soil resource availability is likely a key factor in the invasion of KR, especially due to the species' ability to colonize and establish in disturbed systems. Once established through the invasion windows left open by these changes, we hypothesize that KR is likely a better competitor in high nitrogen environments

than the native Texas grasses. This may be due to the fact that KR has traits that make it more responsive to increased nitrogen, and thus better competitors at high nutrient levels.

### **Niche Partitioning**

A species' niche is formally defined as an n-dimensional space composed of multiple axes of all levels of resource use and/or environmental conditions under which a species is able to maintain a net growth rate  $\geq 1$  (Silvertown 2004). This can be thought of less formally as a combination of the physical space that a species can occupy, the resources that it can access and utilize, and temporal changes involved with its life cycle, such as when reproductive growth occurs. Often the niche of one species will overlap wholly or partially with that of a similar species. When this occurs, interspecific competition with the species will force the first to either occupy a smaller subset of their possible niche, called the realized niche, or be subjected to high levels of competition that often result in competitive exclusion of one species from the environment entirely. In this way, differences in how species utilize their available niche – whether or not they partition the niche so as to reduce competition between themselves – can predict whether two species are likely to coexist.

There are multiple theories of coexistence related to the use of the niche that have been largely unexplored (Silvertown 2004). A big component of a species' niche involves its resource acquisition and composition for the limiting resource, making resource-based models like MacArthur's consumer-resource model a potential tool for examining the role of niche partitioning in reducing direct competition for the limiting resource, leading to either coexistence or competitive exclusion (Carroll et al. 2011). MacArthur's model defines the dynamics of multiple species in terms of their reduction on the available resources in the system – this assumes that species in competition negatively affect the growth of one another by utilizing resources that the other species needs. In this way, one species indirectly negatively affects its competitor. Since the degree of competition can be mitigated by the degree of

niche partitioning between the two species – a high degree of niche partitioning resulting in reduced competition for the same subset of the limiting resource – modifications on the MacArthur model can allow us to examine and quantify the niche difference between two species as a function of one species' reduction of the growth of the other (Carroll et al. 2011). Theoretically, a high degree of niche difference between two competing species should lead to coexistence of the two due to reduced competition between them. Coexistence is often a function of tradeoffs, especially in systems with multiple resources to compete over. This leads to tradeoffs in niche partitioning which gives one species better access to one group of resources while the other species has better access to the other group of resources, preventing one species from being competitively dominant (Tilman 1994).

Almost all theories of how niche partitioning leads to coexistence involve some form of tradeoff that prevents one species from competitively excluding the other (Silvertown, 2004). For instance, three annual species inhabiting California grasslands are able to coexist due to niche differences in their spatial and temporal water access and use (Gulmon et al. 1983). The lifespan of *Plantago erecta* coincides with the rainy months when water is not scarce in this system, so *P. erecta* experiences reduced competition for water due to its temporal niche. However, the other two species *Clarkia rubicunda* and *Hemizonia luzulifolia* both reproduce during the summer when they are dependent on stored water resources. The niche partitioning that allows both these species to survive on this limited water resource involves physiological differences that allow each species to access the water in different ways. *H. luzulifolia* likely accesses deeper water resources stored in decomposed rock below the soil layer, especially later in the summer, and thus has a higher root:shoot ratio (that increases during the summer months) than the other two species. However, as a result of placing more energy into root growth to reach deeper water stores, *H. luzulifolia* reproduces later in the season and tends to suffer pre-productive mortality as a result of significant water stress. *C. rubicunda* also reproduces during the water-limited summer months, but tends to inhabit regions on north-facing slopes with less evaporative

water loss, resulting in soil moisture that stays closer to the surface longer into the summer. As a result, *H. luzulifolia* and *C. rubicunda* are able to coexist even though both are reproductively active around the same time period because they are not directly competing for the same water source (Gulmon et al. 1983). Ultimately, these three species are able to coexist in a water-limiting environment because their realized niches differ in time and physical space, taking them out of direct competition with each other. Spatial and temporal niche partitioning often allows for coexistence between multiple species with different rooting depths, such as between trees and grasses, or between individuals of the same species at different stages in their developmental maturity (seedlings vs. saplings). Indeed, due to their greater rooting depth, trees have access to deeper supplies of water than grasses do, resulting in little competition between the two and facilitating coexistence (Weltzin and McPherson 1997).

A similar method of coexistence based on niche partitioning and competitive tradeoffs exists between two winter annual *Erodium* species which exhibit varying amounts of resource partitioning during the water-stress caused by potential spring and fall droughts. The timing of the drought, whether spring or fall, affects which species is more likely to increase in abundance the following season – autumn droughts occur directly after germination, and spring droughts reduce production of seed (Rice and Menke 1985). A competitive reversal occurs due to slight timing differences in seed formation and germination between the two species, resulting in a change in dominance as a result of the fact that each species is more negatively affected by drought at a different time of year. This competitive reversal creates a nonequilibrium coexistence that complements the equilibrium coexistence predicted by the reported niche partitioning. The overall result is stable coexistence (Rice and Menke 1985). In allowing coexistence between multiple competing species, niche partitioning creates systems that can support multiple species and multiple functional groups in the same physical environment as long as each utilizes a distinct niche. This creates systems with high biodiversity, in which the available resources in the system are being optimally used by multiple interacting species and types of species. High

biodiversity often results in increased ecosystem stability, due to a greater ability of the environment to compensate for loss of one or more species without resulting in gaps or openings that would leave a system vulnerable to invasion by non-native species (Spehn et al. 2005). In this way, biodiversity can be predictive of ecosystem stability, function, and resistance to invasion as a result of multiple species with overlapping niches of resource acquisition, which leaves no openings for an invader to establish.

Decreases in biodiversity, perhaps following a disturbance such as changes to the fire or grazing regime that result in multiple species being excluded from the system likely results in open “windows” or spaces where an invader could establish a foothold in the community. Sometimes this effect is clear, such as when disturbances that directly reduce species diversity, like flooding, allow for invasion as a result of the increased space and light availability left by the species that no longer occupy those areas (Kercher and Zedler, 2004). Other disturbances, such as grazing, can have either positive or negative effects on species diversity, depending on the spatial and temporal scale, size, or type of herbivore. Herbivores may increase biodiversity through local colonization events or by consumption of dominant species to increase relative abundances of less dominant species. Extinctions that decrease biodiversity may also result from herbivory (Olf and Ritchie, 1998). Ultimately, changes to species diversity are one mechanism by which disturbances may change the invasibility of a system.

There are two main ways in which biodiversity may make a community resistant to invasion. The first is complementarity, that a community with greater diversity of species and functional groups more completely utilizes the available resources and occupies the available spaces, leaving no openings for invasion. The second is sampling effect, that more diverse systems are more likely to contain highly competitive species capable of outcompeting most invasive species, thus resisting invasion (Spehn et al. 2005). Thus biodiversity theoretically reduces both the invasibility and vulnerability of a system. Even though theory predicts a clear link between increased biodiversity and decreased invasibility of a system, manipulative studies have reached conflicting conclusions as to the direction or presence of this

trend, and observational studies tend to report positive correlations between diversity and invisibility (Dukes 2002). This unexpected result could be due to confounding factors that are masking the true causes of invasion in these systems, as it is rarely possible in observational studies to separate and control for the effect of each possible confounding factor. For example, invasion may be higher in systems with high biodiversity if these systems are those that receive a larger influx of species, making them more likely to come in contact with a highly competitive invader capable of establishing in the system. An experimental setup involving the invasion of *Centurea solstitialis*, a common noxious weed, utilized controlled plots to test how species composition, species richness, and community age affects invasibility of grassland communities. The growth of *Centurea* was most reduced in the presence of a monoculture of a functionally similar summer time herb that likely exhibited a large amount of niche overlap with *Centurea*, increasing competition between the two and allowing a larger decrease in overall growth due to interspecific competition. While the reduction in the growth of *Centurea* was directly related to increasing community complexity or functional diversity, the invasibility of the system was unaffected by community complexity. When growing with competitors such as annual grasses, evidence suggests that *Centurea* partitions resources, which may help explain its successful coexistence in North American grasslands. As a result of this partitioning, annual grasses experienced little negative feedback by growing in the presence of *Centurea*, even though many other types of competing species were suppressed. This suppression decreased with the declining success of *Centurea* in more species and functionally rich communities (Dukes 2002). Overall, *Centurea* responds to differences in both species composition and functional diversity, which influence the vulnerability of the system, or what affect the invasive species has on the native plant growth. In this system, increased species richness decreased the vulnerability of the system, reducing *Centurea's* ability to negatively affect this system (Dukes 2002).

However, species diversity doesn't necessarily mean functional diversity. In another study of plant community invasibility, California poppy (*Eschscholzia californica*) seeds were released into



experimental plots of varying species richness. Contrary to the predicted inverse relationship between richness and invasibility, the more species-rich plots showed higher levels of invasive establishment and reproduction (Robinson et al. 1995). However, the results also showed small mammal disturbance had a positive correlation with invasive success – as is often the case of disturbances. Some plots also contained a single highly competitive native species had a strong negative effect on invasion – as predicted from the sampling effect – but which may also have excluded native species, resulting in plots that have low invasibility and low species richness. Other factors that may affect this result include available soil moisture, grassland composition (perennial vs. annual), or complexity of community structure. A highly complex community is one with great functional diversity, which results from different types of species utilizing overlapping niches. Therefore, it may be functional diversity more than straight species diversity that results in reduced invasibility of native systems (Robinson et al. 1995; Dukes 2002), or this study may be completely confounded by site factors that determine both invasion and diversity.

It has been somewhat widely accepted that increased biodiversity is positively correlated with ecosystem function, health, and stability. As a result, many conservation efforts are directed towards maintaining ecosystem biodiversity. However, the conflicting conclusions of various observation and manipulative studies suggest that the link between biodiversity and stability – in the sense of resistance towards invasion – may not be as clear cut as some restoration ecologists would like. It now seems more likely that functional group diversity, rather than straight species diversity, is more accurately predictive of the invasibility of a system, though increased species diversity may regulate the overall impact that the invader has on the native species already present.

The application of the theoretical link between niche partitioning and species coexistence may be enlightening to the predicted outcome of the competition, and thus invasion, between King Ranch Bluestem and native Texas grasses. In this way, a lack of functional diversity may play an important role

in the invasion of Texas ecosystems by KR. A study involving the invasion of KR specifically suggests that on a large scale, invasion success may increase with increasing species richness (Alofs and Fowler 2013). These rangelands, which are relatively rich in number of species present, may lack the functional diversity in order to truly occupy all available niches and thus prevent KR from having a window through which to invade. It is also possible that KR niche partitions with the native grasses present, decreasing its competition with native species which may under other circumstances be strong enough competitors to resist its invasion. A lack of direct competition with native grasses would therefore facilitate coexistence. However, since we often see KR competitively exclude all native species in the area during a process of homogenization, a strong effect of niche partitioning is unlikely. There must be other factors that are influencing its competition with the native grasses as well. While we are not studying biodiversity in particular, we do predict that KR will be at least somewhat negatively affected by competition from the native species, and thus that there will be a difference between with and without.

### **Mycorrhizal Fungi and Soil Biota**

Competition, resource partitioning, and varying tolerance of predation, herbivory and other disturbances, do not adequately explain the observed community dynamics and structure of most ecosystems, invaded or natural (Klironomos 2002). To truly understand competitive dynamics between species, you must understand plant-soil relationships, which are largely determined by interactions between functional groups, such as mycorrhizal fungi, in soil biota (Andrade et al. 1997). Arbuscular mycorrhizal fungi are endomycorrhizal fungi that exhibit branching structures within the cortical cells of roots called arbuscles, named due to their 'tree-like' appearance. These structures increase the contact between fungi and plant root in order to further facilitate the bidirectional transfer of nutrients – carbon from plant to fungi, and nitrogen, phosphorous, or other essential minerals from fungi to plant (Bever et al. 2001). The presence of these arbuscles inside the roots themselves is the primary difference

between the two main classes of mycorrhizal fungi: endo- and ecto-mycorrhizal fungi. Endomycorrhizal fungi, like AMF, have hyphae capable of penetrating root cells and are generally associated with a great variety of plant taxa, including C4 grasses. Ectomycorrhizal fungi, in contrast, have hyphae that remain extracellular, and are primarily associated with trees and shrubs (Bonfante and Anca 2009). These interactions between soil biota and plants in the community form a gradient from extremely mutualistic to extremely parasitic, including all interactions in between, though the focus in both literature and restoration efforts is often on those that have symbiotic relationships (Hoeksema et al. 2010). These symbiotic relationships, where both partners benefit from the interaction, such as those between arbuscular mycorrhizal fungi (AMF) and the roots of C4 grasses, are often a key to determining the structure of the plant community, as well as impacting the abundance and diversity of other biota in the soil, such as bacteria. This sets up complex systems of highly specific three-way interactions between plant roots, mycorrhizal fungi, and other soil biota, generally bacteria (Bonfante and Anca 2009). While mycorrhizal fungi were originally thought to be functionally similar and thus to exhibit little ecological diversity, recent interest in their role in ecosystem function has led to the discovery that they are actually an extremely diverse group, both morphologically and physiologically, which many different species that exhibit great genetic, and thus functional, diversity as well (Bever et al. 2001; Oehl et al. 2003). As a result of this diversity, plant roots and AMF have formed highly species-specific interactions that often benefit both partners. In fact, a large majority of plants use some kind of mycorrhizal fungi to increase their access to scarce or immobile resources in order to increase their growth rate (Bever et al. 2001). Plants benefit by gaining access to inorganic minerals present in small amounts in the environment and increasing uptake of nitrogen and/or phosphorus. In return, the fungi gains access to a reliable source of organic carbon synthesized through plants' photosynthetic mechanisms (Bonfante and Anca 2009). Mycorrhizal fungi also form strong associations with soil bacteria in natural conditions. The spatial structure of bacterial communities depends largely on the fungal community present (along with

soil characteristics such as pH or moisture), while the fungal community is most influenced by the plant community present. In this way, the fungal community forms the link that connects the visible ecosystem function above ground with that below ground (Bonfante and Anca 2009; Andrade et al. 1997). Due to the fact that fungi play a key role in this overall relationship, AMF are often targets for regenerative and restorative efforts of the community as a whole.

Multiple studies suggest that mycorrhizal fungi can contribute to both nitrogen and phosphorous uptake through interactions between the arbuscules of the fungi and cortical cells of the plant, which may be facilitated by highly specific transporters (Harrison et al. 2002). These interactions between plant and fungi may be essential to plant growth. In some systems, the mycorrhizal fungi are the dominant route of phosphorous supply for plant (Smith et al. 2003). Fungi also play important roles in ecosystem function and nitrogen cycling (Hodge et al. 2001), and as a result there are similar trends between ecosystem function and biodiversity in soil communities as there are for above ground communities – that is, that greater biodiversity increases ecosystem function and stability (Spehn et al. 2005; Van der Heijden et al. 1998). Similarly, decreased biodiversity, such as is found as a result of agricultural disturbances in European sites, results in decreased community stability and increased risk of invasion (Oehl et al. 2003). Sampling of fungal species present in communities suggests that fungal species diversity is often comparable to that found in the plant ecosystems. For instance, 37 different species of mycorrhizal fungi were recovered from 1 hectare of grassland, and this may not even be representative of the true functional diversity, given that even within a species there was an extreme amount of genetic diversity found (Bever et al. 2001). Due to the strong links between above and below ground communities, the structure, stability, and function of each community is regulated by the other (Van der Heijden et al. 1998) through a process of multiple feedback loops. The complexity of these interactions is further enhanced by the fact that multiple species of fungi can colonize multiple species of plants in different ratios in order to form optimal colonization for both plant and fungi in terms of

their resulting growth rates (Bever et al. 2001). Differences in the ratios of fungal species colonization on different host species roots results in positive or negative feedback loops that either promote or inhibit, respectively, the increase in abundance of certain species in the system. Positive regulation occurs when the fungi that most promotes the growth of a host species is also the major colonist on that species. Therefore, growth of both species increases, leading to local loss of plant and fungal community diversity, but overall contributing to wide spatial structuring. Negative regulation occurs when the fungus that promotes growth in one plant species is not the major symbiont associated with that species. Thus, the increase of the host species changes the relative abundances of the fungal species in the community by increasing the abundance of its fungal symbionts, which indirectly decreases the abundance of the fungus that has the greatest positive affect on it. This in turn leads to a decrease in the host plant's growth rate, which results in a return to the established ecosystem equilibria (Bever et al. 2001). In this way, complex and diverse fungal communities are major contributors to overall ecosystem function and stability (Van der Heijden et al. 1998).

The species-specific nature of plant-fungi interactions is a result of co-evolution between plants and fungi with a long sympatric history. It is widely expected that these symbioses are susceptible to the introduction of novel colonizers species that may influence or disrupt these interactions. The range of plant responses to colonization by mycorrhizal fungi tends to be largest when both the fungi and the plant species are local, which is reflective of their shared evolutionary history. Combinations of either exotic plant or fungal species with native partners decrease the magnitude of the host species' response to the symbiont, and make that response more likely to be negative (Klironomos 2003). This is especially important considering the use of commercially available mycorrhizal fungal inoculants, which often contain mixes of species that are not native to the systems in which they are marketed. A test of these products in New Zealand showed that the presence of the exotic species of fungus either had no effect or a negative effect on the growth of the native plants, compared to the positive effect shown in

interactions between native fungi and native plants (Williams et al. 2012). However, these positive interactions between native fungi and plants may not assist them in competition (Williams et al. 2012) with other natives or invasive species due to other factors that negatively regulate growth. Most plants typically respond positively to mycorrhizal fungi in the absence of other factors, but in natural systems native species are generally more strongly negatively affected by pathogens and other enemies than the invasive species. Therefore the overall positive response of native species to fungal colonization tends to be lower than the response of invasive species, who, as predicted by the 'enemy escape' hypothesis, are more free from pathogens and other enemies in the invaded environment (Klironomos 2002).

Many of the same factors that lead to invasion in above ground systems, such as decreased biodiversity and disturbance, also affect the stability and invasibility of below ground systems. The invasion of above ground systems often mirrors changing in the soil biota, but whether one of these changes causes the other, or both are simultaneously the result of a third factor, is harder to determine. However, multiple systems report correlations between changes in soil biota in response to invasion or between successfully invaded and native systems. As predicted by the relationship between biodiversity and ecosystem invasibility, newly invaded sites in southeastern Utah show lower overall fungal species richness and increased numbers of bacteria. Some of the bacterial species in these newly invaded plots are also found associated with invaded soils 50 years prior, suggesting that certain soil microbes may facilitate invasion or strongly associate with this particular species. The fungal community (species composition) also changed with invasion. The number of "generalist" fungi increased relative to the fungi that have specialized interactions with native species, which may constitute a long-term environmental change that is an impediment to reintroduction of native plant species (Belnap and Phillips 2001). In some systems, experimental data from a study of relative growth rates between native and invasive wetland species on sterilized soil suggests that a soil biota change precedes successful invasion, ultimately facilitating it by differentially benefiting the invasive species over native. Successful

invasion may not just be a result of changes in soil biota; it may directly or indirectly cause these changes in order to create a positive feedback loop for itself that it can then utilize to facilitate its own invasion (Scharfy et al. 2010). For instance, disturbing evidence considering *Alliaria petiolata*, garlic mustard, suggests that it directly disrupts mutualistic interactions between mycorrhizal fungi and native trees with the release of an unknown toxic anti-fungal agent into the soil. In this way, the invader is creating the environmental disturbance that it can then utilize to invade the system by directly disrupting necessary mutualisms that maintain the stability of the ecosystem (Stinson et al. 2006).

Due to the important role that mycorrhizal fungi play in maintaining system stability and function, restoration efforts of various disturbed ecosystems could be positively influenced by the appropriate regulation of soil biota communities. One disruptive process that mycorrhizal fungi may play a role in alleviating is desertification, which is characterized by damage to physical, chemical, and biological soil properties that limit reestablishment (Requena et al. 2001). Mycorrhizal fungi may be particularly effective in restoring desertified systems as a result of the ability of fungi to increase access to resources, including water, and aid the plant in resisting stressors. Often time species establish better when introduced in combination with an appropriate mutualistic fungal species (screening must first occur due to high host/fungi specificity) (Herrera et al. 1992). Over time, inoculation of these systems with fungi, combined with the successful reestablishment of the native species, may increase soil fertility and productivity in the long-term (Requena et al. 2001).

The effect of use of mycorrhizal fungi to combat invasion and resulting homogenization of systems are less clearly positive. Multiple studies have suggested that mycorrhizal fungi do not equally impact the growth of all species in an environment, and therefore may be able to mediate the effects of competition between natives and invasives. This regulatory function, however, may be limited to interspecific competition between multiple native competitors (Jin et al. 2011). Through evolutionary history, the fungi may have specifically evolved to regulate this interaction – some evidence suggests

that when taken out of context and applied to competition between a native and invasive species, the fungi may not have the same mediating effect on competition (Williams et al. 2012). In fact, the fungi may even provide more benefits to the invader, and thus further increase its growth (Emery and Rudgers 2012). This is especially likely when attempting to utilize commercial fungal inoculants in the restoration process – these often contain generalist species that are more likely to react positively with the invasive species or not at all (Williams et al. 2012), and thus will often hurt restoration, not help it. The affect that mycorrhizal fungi will have in mediating the competition between natives and invasives in the process of invasion is often unclear, and depends strongly on the system. However, soil microbial communities are strongly involved in the invasion process, and often must experience some kind of change in order to allow or facilitate invasion.

The wide scale application of commercially available generalist mycorrhizal fungi to Texas rangelands in an attempt to increase crop yield may be actively facilitating the invasion of these areas by King Ranch Bluestem. These fungal inoculants have become something of a “snake-oil” miracle cure to increase yield of native species, when in reality their use may be altering soil communities, creating a positive feedback loop for further invasion. The effect of agricultural processes is often a reduction in the soil biota biodiversity (Oehl et al. 2003). Thus, to promote the reestablishment of native in these areas it may very well be necessary to artificially inoculate to restore previous species. However, it is unclear if the use of cosmopolitan microbial generalists that are likely non-indigenous to a region will increase or decrease establishment of species targeted for restoration in the context of management for a non-indigenous, invasive species with similar life-history strategies. These reintroduced fungal species need to be those that are already native to the area, or the effect they are likely to have is an unwanted negative one.

In considering multiple possible mechanisms that may be increasing ecosystem invasibility and vulnerability, the most consistent predictive factor for invasion seems to be changes in established



nutrient availability and soil communities that upsets the stability of the system and thus opens invasion windows that facilitate invader establishment. The effects of nutrients, soil biota, or degree of niche partitioning alone may only cause ungeneralizable trends, but when these factors occur synergistically, they are much more predictive of the likely community dynamics, including invasion. The experiment described in this thesis is designed to test the effects of these three factors on the invasion and competitive ability of King Ranch Bluestem in Texas rangelands.

## Chapter 2

The invasion of Texas rangelands by King Ranch Bluestem (*Bothriochloa ischaemum*) has become the focus of restoration and conservation efforts due to the species' highly homogenizing nature. Though KR was purposely introduced for rangeland management and erosion control, it has since spread uncontrolled, leading to the creation of monospecific stands that threaten former native grassland communities (Schreiber 2011). Attempts to remove KR and restore these rangelands have been, thus far, largely unsuccessful. As a result, we consider the synergistic effects of three factors that commonly affect competition and invasion – nutrient availability, niche partitioning, and mycorrhizal fungi – in order to identify the key factors facilitating the invasion by KR.

Competition for nutrients forms the basis of inter-specific competition (Tilman 1986; Wilson and Tilman 1993). Nutrient availability within a system, combined with resident species' abilities to acquire nutrients when scarce, acts as a determinant of species' competitive dynamics and, thus, the vulnerability of a system to invasion (Tilman 1977). Resource-competition theory predicts that the most competitive species are those that can persist at the lowest resource levels (Tilman 1986). Field studies support this theory by demonstrating correlations between high levels of resource availability and invasive success (Maron and Connors 1996). This is potentially a result of a greater ability of invasive species to respond positively to increased nutrients by altering their growth strategies (Maurer and

Zedler 2002). Nitrogen is often the limiting resource in Texas systems and is also the nutrient most affected by agricultural processes such as burning or pollution.

Since interspecific competition is the indirect effect of competition for limiting resources, the ability of two species to niche partition can both decrease the competition between them and therefore predict that they will be more likely to coexist (Tilman 1999). Since we observe in the field that KR outcompetes the native grasses and homogenizes the system, it seems unlikely that any large degree of niche partitioning occurs between KR and the natives.

Mycorrhizal fungi play an essential role in a plant's uptake of nutrients by directly associating with the roots of a host species to increase nutrient uptake (Bonfante and Anca 2009). Fungi form very host-specific interactions, and play a key role in maintaining ecosystem function and stability (Bever et al. 2001). As a result, there is the possibility that the presence of a fungus will mediate competition between the native and invasive species, thus assisting the native species in competition and decreasing the invasibility of the system (Jin et al. 2011). As a result, the addition of mycorrhizal fungi may be a key tool in the restoration process of degraded communities (Herrera et al.1992).

In this experiment we address the role that nutrient availability and the presence of mycorrhizal fungi have on the competitive dynamics between KR and the Texas native sideoats grama (*Bouteloua curtipendula*, hereafter referred to as SOG), and whether either factor singly or in combination can explain the invasive success of KR. We also consider the degree of niche partitioning between KR and SOG to assess the level of competition between the two. Because the native species are typically displaced by KR and are also C4, perennial grasses, we expect that little niche partitioning is occurring and that KR will be more responsive to increased nutrients. We do expect, however, that the addition of mycorrhizal fungi may mediate the effect of the presence of KR on SOG providing some benefit to SOG.

## **Materials and Methods**

We employed a three-way factorial design to manipulate species compositions, soil available nitrogen, and the presence or absence of mycorrhizal fungi. Our two focal species were King Ranch bluestem (*Bothriochloa ischaemum*), an invasive, C4 grass, and sideoats grama (*Bouteloua curtipendula*), a C4 grass native to Texas often found growing sympatrically with KR. Three combinations of our two focal species were tested – 100% KR, 100% SOG, and 50:50 KR:SOG. Pots were initially seeded for a target density of 20 individuals per pot; however, low germination rates required reseeding after approximately two weeks. Pot densities were held at 16 individuals per pot – in the competition pots, this involved eight individuals of each species in the 50:50 KR:SOG treatments. SOG seeds were acquired from Native American Seed (Junction, Tx) and KR seeds were acquired from Douglas King.

Nutrients were added to the pots at three weeks after initial seeding using a modified Hoagland's solution (Table 1) containing all essential nutrients that plants need for growth with incrementally higher levels of nitrogen added as ammonium nitrate (Hoagland and Arnon 1950). These ranged in even increments: 0 g/pot, 0.0192 g/pot, 0.0385 g/pot, 0.0577 g/pot, and 0.0769 g/pot. Our aim was to use concentrations of nitrogen representing a range from severe nitrogen limitation, to agricultural levels of nitrogen, to pollution. A total of 24 mL of Hoagland's solution was added in two 12 mL waterings over the course of a day. Following each addition of nutrient solution, the pots were watered with 50 mL of distilled water to decrease the likelihood of burning the leavings of the seedlings. In order to test the persistence of the nutrients in the sand, eight control pots were set up without grasses (four with fungal inoculant and four without) and one of each treatment was harvested at each of the harvest dates.

In order to create treatments with the presence and absence of mycorrhizal fungi, half the pots were inoculated with commercially available fungal inoculant per the manufacturer's instructions. Fifteen grams of inoculant was mixed with the seedling mix before it was added to the pots. The fungal inoculant was a commercially available granular mix of five species of endomycorrhizal fungi (*Glomus*

*mosseae*, *G. aggregatum*, *G. intraradices*, *Pisolithus spp.*, and *Rhizopogon spp.*) purchased from MycorrhizalProducts.com (Ft. Myers, Fl). These species are commonly found in agricultural systems in the Americas, as well as Europe and Asia.

We planted three replicates of each of the 30 treatment combinations for four harvest dates, for a total of 360 pots. Grasses were grown in black plastic pots (7 cm in diameter x 25.4 cm tall) manufactured by Deepots (Hummert International, catalog #14-2673-1), and placed randomly within trays ensuring adequate space, approximately 10 cm, between pots. The pots were filled with Pavestone play sand (Home Depot, SKU #715646) to 2 cm from the top in order to provide a nutrient-free growing environment and further control nutrient addition. The last 2 cm was filled with Jiffy Organic Seedling Mix (Home Depot, SKU # 427249) to aid the seeds in germinating. Polyester mesh fabric (10.2 by 15.2 cm) was placed at the bottom of the pots to prevent the loss of sand. The experiment was watered several times before initial seeding to leach any remaining nutrients from the soil. With the exception of nitrogen, we aimed to insure that all pots received the same amount of nutrients. The experiment was carried out in a greenhouse with a controlled environment at approximately 21°C, and ran for approximately eight weeks over the months of June and July in San Antonio, Tx.

Four total harvests were conducted at one week intervals. The first harvest occurred four weeks after initial seeding and one week after nutrient addition. For each pot, data was collected on plant height (of the tallest plant), root length after washing (of two randomly chosen individuals), total dry weight of above ground and below ground biomass, separated by species where appropriate, and specific leaf area for two randomly chosen leaves. Roots were separated from the sand with gentle running water and teased apart to separate the roots of each species while minimizing loss of belowground biomass.

Data from the various response variables was standardized to control for relative number of individuals between the monoculture and competition pots by multiplying the data from the

competition pots by two for the 50:50 KR:SOG treatment. A multi-variate MANOVA was run with R to analyze the relative effects of the treatments on the response variables over time. The effects of the treatments were considered alone, and in 2-way and 3-way crosses with the other treatments.

Regression analyses were also performed where appropriate to characterize trends through time or across nitrogen levels. The soil was also analyzed for nutrient level using KCl extractions. Following protocols outlined by Robertson et al. (1999), ten grams of soil was combined with 100 mL of 1 M KCl for 24 hours. The filtrate was sent off to the A&L Analytical Laboratories, Inc. in Memphis, TN and analyzed for total nitrate and ammonia, as well as phosphorous.

## **Results**

### *Biomass*

As expected, overall biomass showed a general trend of growth through time at all nutrient and fungal levels, though this effect became less clear at later time points (Fig. 2; Table 2). Nitrogen level had only a statistically significant impact on KR's total pot biomass at the first time point (Fig. 3; Table 2).

Nonetheless, when split into above ground and below ground biomass, the results suggested that only the roots of KR were significantly responsive to the nitrogen level. As with total biomass there was a strong effect at the first time point ( $p=0.019$ ) but below ground biomass also showed a weak effect at the fourth time point ( $p=0.044$ ). Conversely, SOG showed a weaker, later response to the manipulated nitrogen. The only significant effect was seen in the total pot biomass at the 3<sup>rd</sup> time point ( $p=0.022$ ). This effect was lost when the biomass was split into above and below ground (Table 2).

Both SOG and KR showed significant responses to being grown in competition versus in monoculture, though SOG responded much more negatively in both above ground and below ground biomass than KR (Fig. 4;  $p<0.005$  for all harvests). In total pot biomass, KR responded significantly to competition at all four time points ( $p<0.05$ ). When biomass was split into above and below grown biomass, KR demonstrated differences in response to competition only at the first two time points

(Table 2;  $p < 0.01$ ). SOG showed a strong negative response to competition at all four time points for the above ground biomass (Fig. 5; Table 2;  $p < 0.005$  at all harvests) and the last three time points for below ground biomass ( $p < 0.001$ ). For belowground biomass of SOG in particular, competition was the only factor to have a clear significant influence (Table 2).

The presence of fungi positively affected biomass in both species (Fig. 6), but influenced KR more strongly than SOG. KR's below ground biomass was affected across all time points (Fig. 7;  $p < 0.05$ ), while SOG's below ground biomass was only affected at the 2<sup>nd</sup> time point (Table 2;  $p = 0.004$ ). The fungi had a similarly strong effect on the above ground biomass of both KR and SOG (Table 2; KR:  $p < 0.05$  for H1 and H4; SOG:  $p < 0.05$  for H2, H3, and H4). The main difference occurred in the roots, where KR responded much more positively than SOG. In KR, there also appeared to be an interaction between fungi and nitrogen level and fungi and species composition (Table 2). This appeared in the split measures of biomass but not the total biomass. This is the opposite of SOG, where no cross effects were present except in the total biomass, where the effect of fungi appeared mediated by the level of nitrogen and the presence of competition (Table 2).

### *Niche Partitioning*

Nitrogen, species composition, and the presence of mycorrhizal fungi also affected traits such as root length (Fig. 8), height, root mass ratio (Fig. 9; Fig.10), and specific leaf area that may be associated with niche partitioning between KR and SOG (Table 2). While there appeared to be no effect of nitrogen on root length or plant height, the nitrogen level did significantly impact the root mass ratio of both species (Fig. 9; Table 2;  $p < 0.05$ ). Specific leaf area showed no response to nitrogen level for either species.

Species composition significantly affected the height and root length of both KR and SOG, as well as significantly decreased their root mass ratio (Fig. 8; Fig. 9; Fig. 10;  $p < 0.001$ ). At the first time

point the specific leaf area of KR was significantly impacted by a competitor, but this was lost in later time points.

Both the root length and height of KR showed the strongest positive response to the addition of fungi ( $p < 0.001$  for both). The height of SOG also responded to the addition of the fungi, but not as strongly as KR (Table 2). KR's root mass ratio also responded to the fungi over all four time points, unlike that of SOG which only responded to the fungi at the first time point (Table 2).

#### *Nutrient Use*

Analysis of the changes in soil nitrogen over time (as shown by the KCl extractions performed on the sand the grasses were grown in) showed, as expected, a significant difference in level of nitrogen depending on how much nitrogen was originally added (Table 1; Table 2;  $p < 0.05$ ). However, these differences between the nitrogen treatments groups were lost after the first week (for nitrate) or after the second week (for ammonium). There were no other significant differences associated with either species composition or fungi, suggesting that neither of these factors affect the rate of nitrogen absorption. However, absorption of nitrogen is affected by the form in which it is found (Table 2).

#### **Discussion**

Our results suggest that KR is ultimately more positively responsive than SOG to the presence of fungi and better able to utilize increased nutrients for growth. Conversely, the presence of a competitor has very little effect on the growth of KR, while SOG is strongly negatively affected by being grown in competition with KR.

Nitrogen level did not have a strong consistent effect on the biomass of either KR or SOG. As a result, there was no clear effect of nitrogen addition that would confirm or deny the previous observation that invasion is correlated with high nutrient systems (Vasquez et al. 2008). However, these results should not alleviate concerns over whether agricultural practices like over fertilization or

burning, which create high nitrogen environments, may be contributing to the homogenization of Texas grasslands by KR (Baer et al. 2009). Per our experimental design, nutrients were only added once prior to the first harvest, and thus likely were depleted or leached out of the sand quickly. The weak response of KR to increased nitrogen could therefore be explained by the fact that the high nitrogen levels were depleted quickly, as well as the fact that a seedling study such as ours may not be sufficient to study overall response to nutrients levels over longer periods of time.

There were no significance differences between the root lengths or root mass ratio of KR and SOG when grown in competition, which suggests that there is no significant niche partitioning occurring among the roots of KR and SOG. This may be a result of the relatively recent invasion of KR that has not allowed sufficient time or opportunities for evolution toward niche partitioning between SOG and KR to allow for species coexistence. Therefore, competition for resources between the two species is likely high and the competitive dominance of KR is likely due to its overall higher competitive fitness.

Nonetheless, relative to their growth in monoculture, both KR and SOG appear to be responding to competition by altering their growth strategies. Both species allocated fewer resources to the growth of roots when grown in competition. This suggests that an important aspect of their competition occurs above ground, such as competition for light, which is supported by changes in plant height for both SOG and KR in response to competition. These results suggest that this lack of niche partitioning between SOG and KR, and overall higher fitness of KR, will make management and restoration of homogenized grasslands difficult as SOG, a relatively good competitor under most restoration conditions, will be out-competed through time. Observations of KR across the Edwards Plateau of central Texas support this, suggesting that KR is competitive in almost every habitat considered (except those that are extremely light-limited), and that KR appeared to be excluding the otherwise dominant native species (Garrard and Fowler 2006).



The biomass of SOG was strongly negatively influenced by the presence of KR as a competitor. This is consistent with the results of similar greenhouse competition studies involving KR and native grasses – KR and other old world bluestems significantly inhibit the growth of the native species, one of which was SOG, while the native species had no inhibitory effect on KR growth. In fact, growth of KR was actually enhanced when grown in competition with one of the natives, little bluestem (*Schizachyrium scoparium*), suggesting the possibility of KR being facilitated by competition with less competitive species (Schmidt et al. 2008).

The strong evidence that KR is more positively affected by the presence of mycorrhizal fungi than SOG is particularly troubling in terms of rangeland management. The fungal inoculant that was tested is widely used commercially. Our results suggest that addition of these fungal inoculants will facilitate the spread of KR at the expense of SOG. This may be due to the fact that the fungi tested are non-indigenous to Texas ecosystems. Therefore, these fungi have not co-evolved a mutualistic relationship with native Texas grasses like SOG and thus it is unlikely to preferentially associate with native species to resist invasion. Recent evidence also suggests that the invasion of KR is directly altering the native mycorrhizal community in a way that suppresses the growth of native species that depend on these interactions (Wilson, Hickman, and Williamson 2012). This consequence of KR's invasion is likely combining synergistically with the widespread abuse of these non-native fungal inoculants to further disrupt the mycorrhizal community and increase invasibility of the system. As a result, we recommend research designed to identify and isolate native Texas fungi that might be a better partner to native Texas grasses.

## **Conclusion**

Our results suggest that nitrogen addition alone does not have a large impact on the ability of KR to invade Texas systems. We suggest that nitrogen increases may act synergistically with other

factors, such as disturbance and soil microbial effects, to further facilitate invasion. KR is, however, very positively responsive to the generalist mycorrhizal fungal inoculant, at least at the seedling stage, to the disadvantage of SOG. Application of mycorrhizal inoculants may disrupt established below ground microbial communities and facilitate KR success. This may make later restoration efforts more difficult if the disruption to the below ground diversity prevents the reestablishment of the native species. Our work with SOG also suggests that the native species could be more sensitive to competition than KR. SOG responded negatively in overall biomass to the presence of a competitor, though KR did show a much weaker negative response to competition, primarily at early time points. Based on our results, the strongest influence on the biomass of SOG was the presence of KR as a competitor (negative), while the strongest influence on KR's biomass, especially the belowground biomass, was the fungi (positive). This suggests an interaction between KR, SOG, and mycorrhizal fungi that may explain widespread homogenization observed under field conditions where the indigenous microbial community may be disturbed or novel generalist microbes have been introduced.

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## Appendix

Solute	Amount
0.5 M K <sub>2</sub> SO <sub>4</sub>	5 mL/L
1 M MgSO <sub>4</sub>	2 mL/L
0.05 M Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	10 mL/L
0.01 M CaSO <sub>4</sub>	100 mL/L
H <sub>3</sub> BO <sub>3</sub>	2.86 mL/L
MnCl <sub>2</sub> 4H <sub>2</sub> O	1.81 mL/L
ZnSO <sub>4</sub> 7H <sub>2</sub> O	0.22 mL/L
CuSO <sub>4</sub> 5H <sub>2</sub> O	0.08 mL/L
H <sub>2</sub> MoO <sub>4</sub> H <sub>2</sub> O	0.02 mL/L
0.5% solution FeCl <sub>3</sub>	1 mL/L
NH <sub>4</sub> NO <sub>3</sub>	0 g/pot to 0.0769 g/pot

Table 1. Hoagland's Solution (Hoagland and Arnon 1950). The ammonium nitrate was used to create a nitrogen gradient. 5 levels of ammonium nitrate were added in increments of 5. Nitrogen level 0 had 0 grams; level 1 had 5 grams; level 2 had 10 grams; level 3 had 15 grams; level 4 had 20 grams.

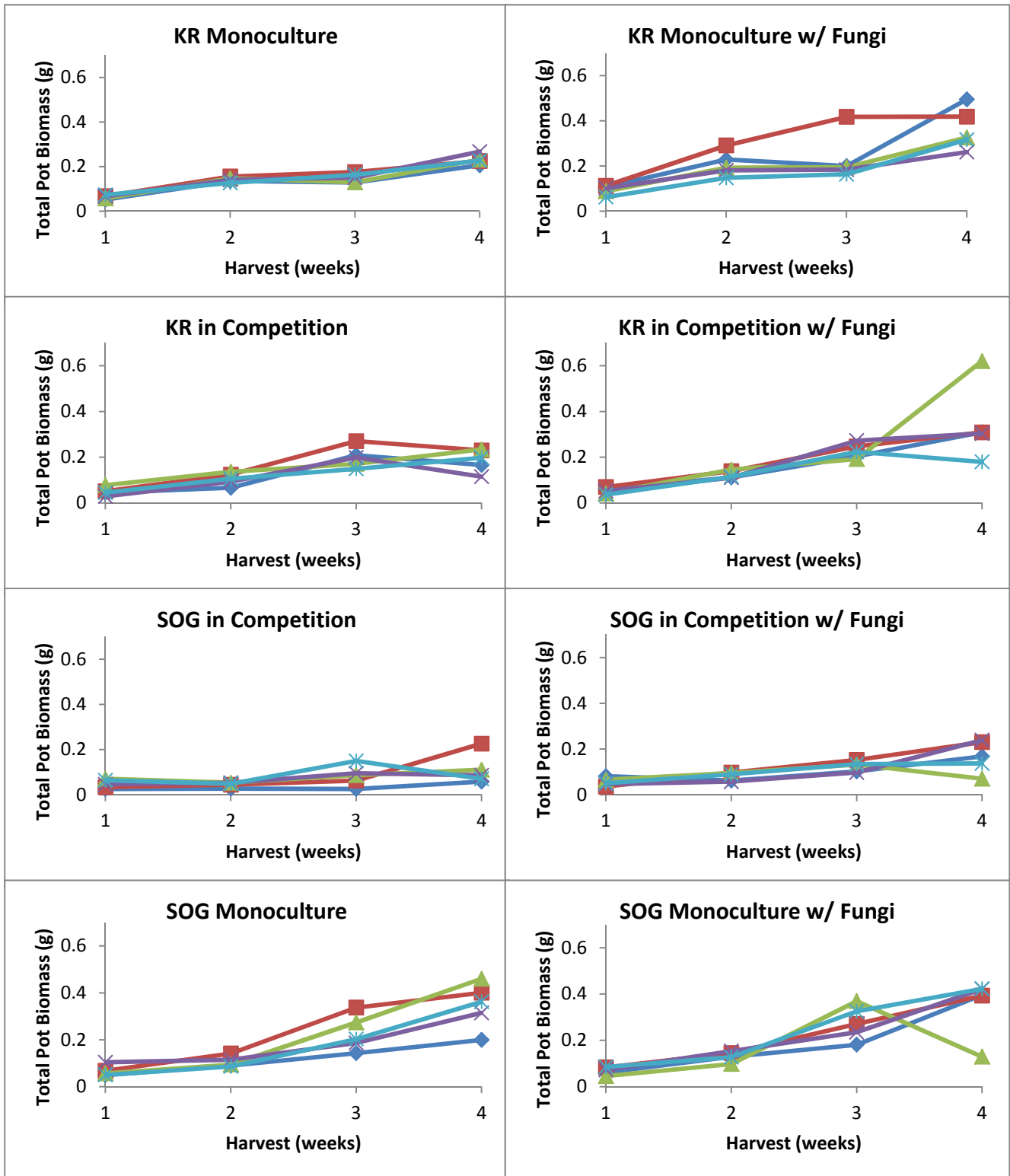


Figure 2. Total biomass (g) for all treatment combination through time (4 weekly harvests). Different nitrogen levels are shown as different colors: N0 – blue; N1 – red; N2 – green; N3 – purple; and N4 – light blue.

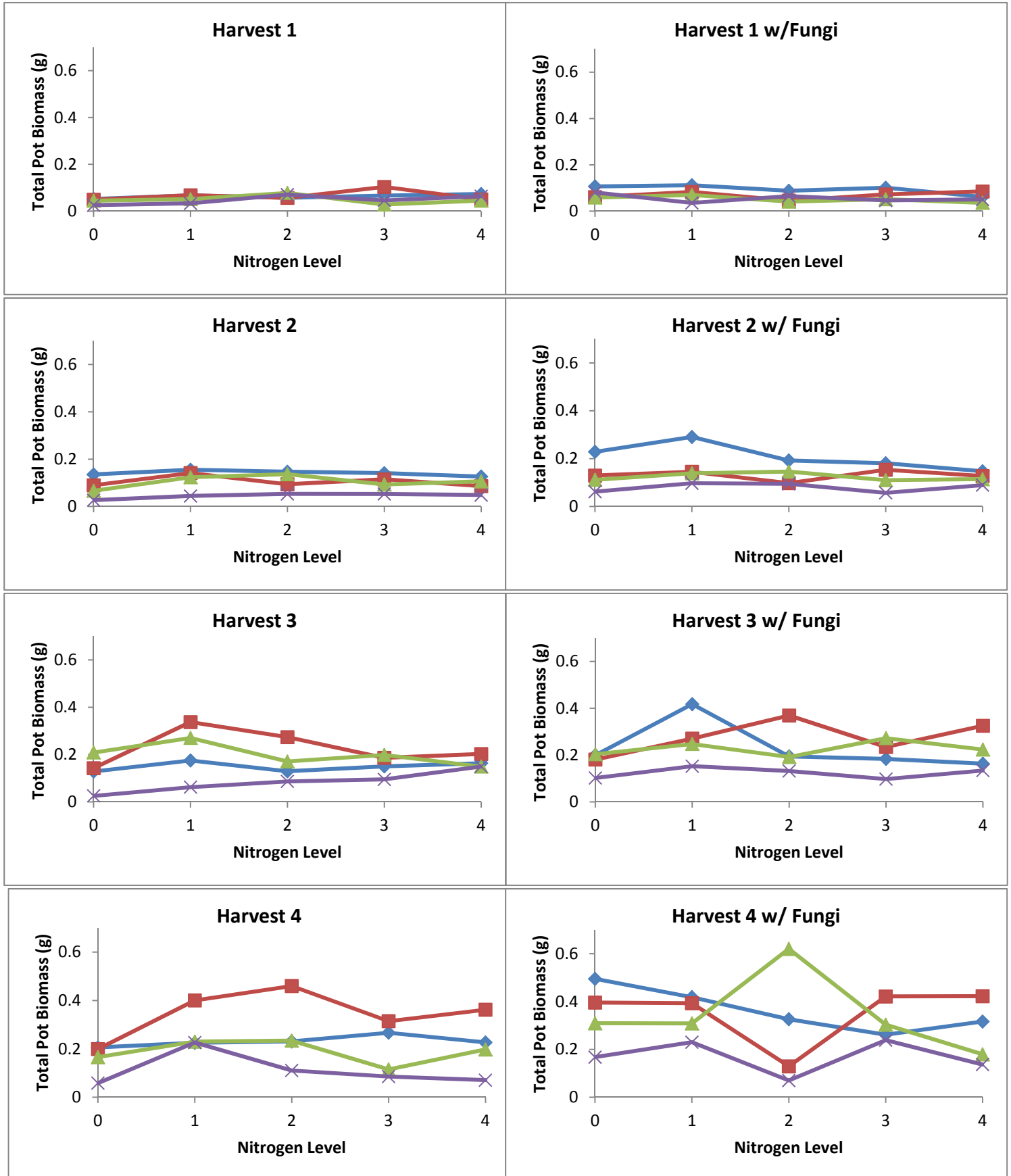


Figure 3. Total biomass (g) at each nitrogen level at each harvest. KR and SOG in monoculture are blue and red respectively, and KR and SOG in competition are green and purple, respectively.



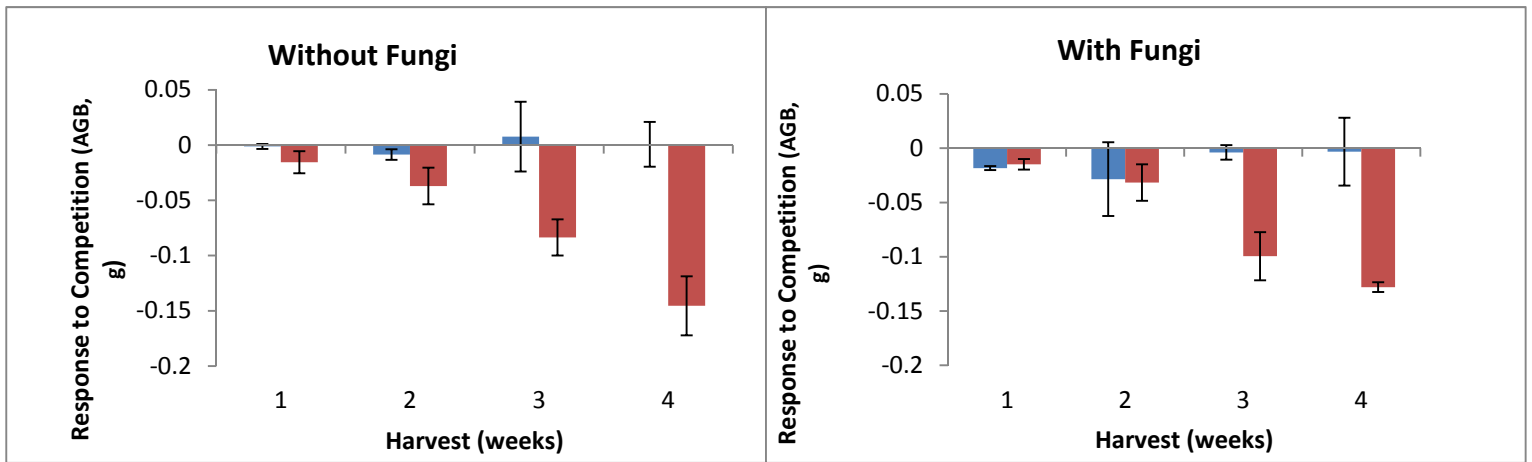


Figure 4. Above ground biomass response (g) to competition for KR (blue) and SOG (red). Values reported are above ground biomass in competition pots minus above ground biomass in monoculture pots; error bars are standard deviation.

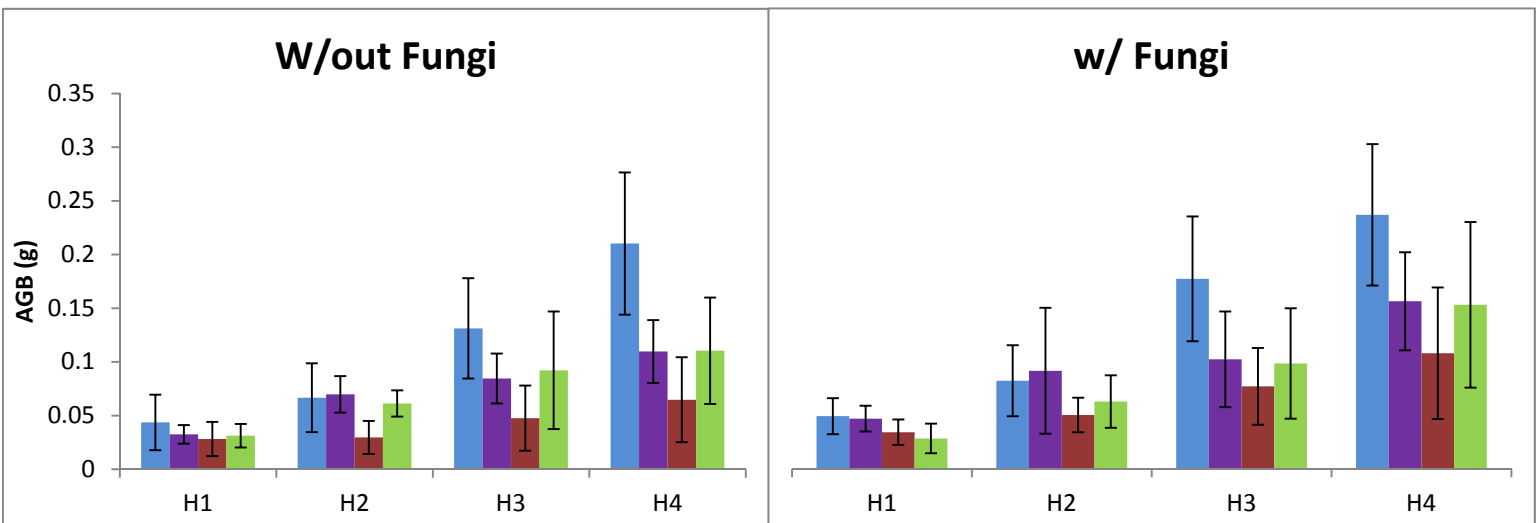


Figure 5. Above ground biomass (g) at each harvest, averaged across the different nitrogen levels; error bars are standard deviation. Blue and purple represent SOG and KR in monoculture, respectively. Red and green represent SOG and KR in competition, respectively.

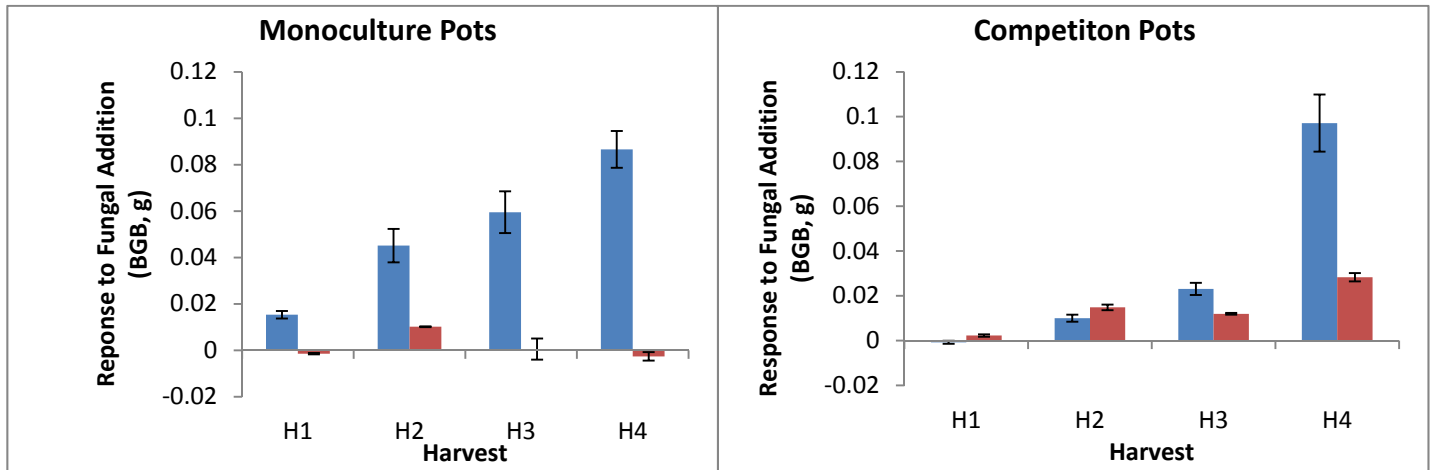


Figure 6. Below ground biomass (g) response to fungi for KR (blue) and SOG (red). Values reported are the average below ground biomass with fungi minus the average below ground biomass without fungi; error bars are standard deviation.

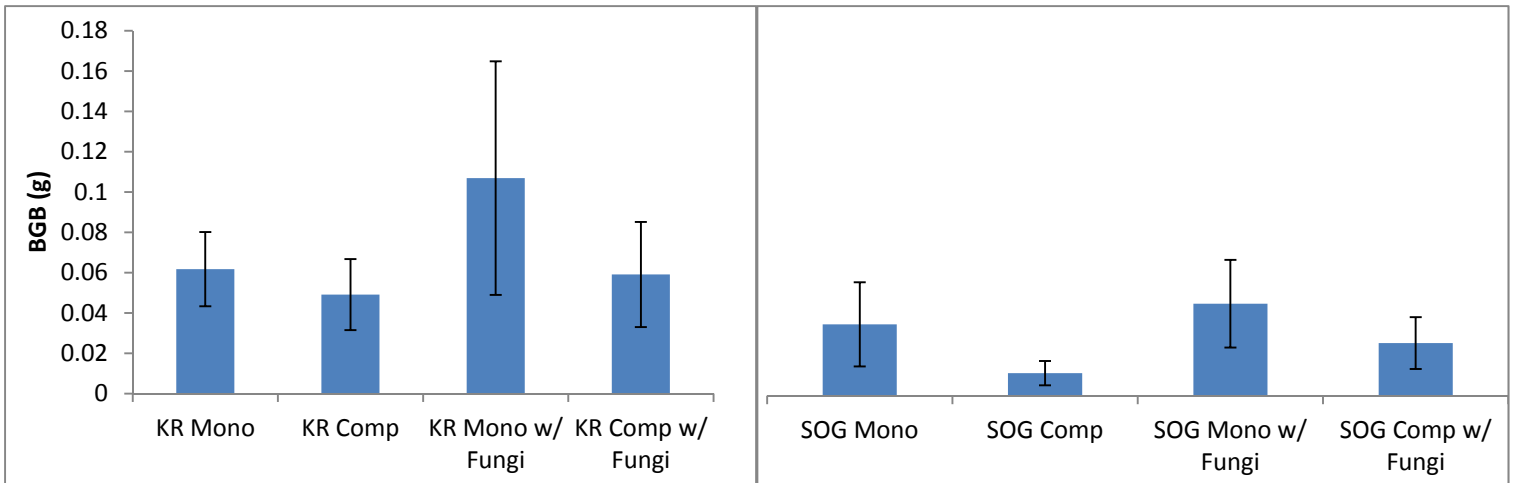


Figure 7. Below ground biomass (g), averaged across all nitrogen levels, for each species composition with and without fungi. Error bars are standard deviation.

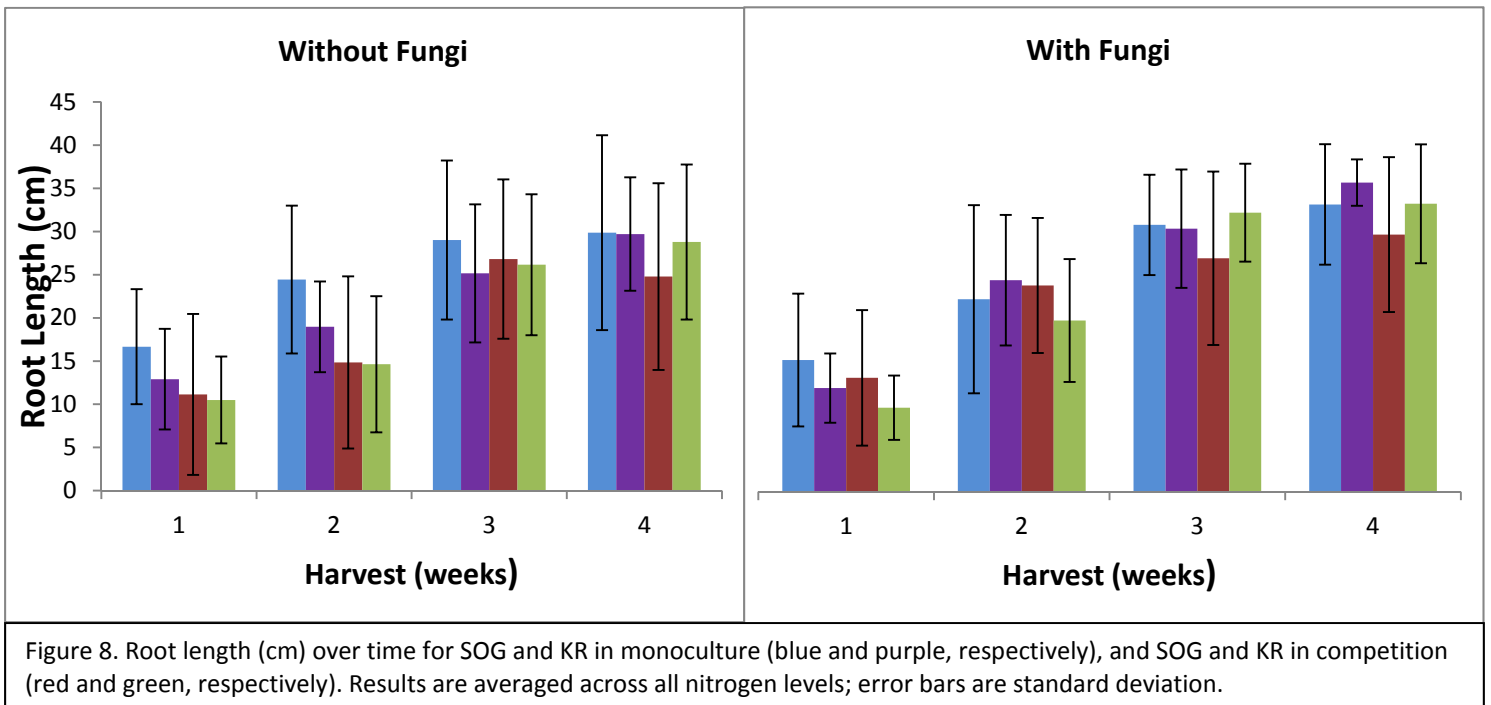


Figure 8. Root length (cm) over time for SOG and KR in monoculture (blue and purple, respectively), and SOG and KR in competition (red and green, respectively). Results are averaged across all nitrogen levels; error bars are standard deviation.

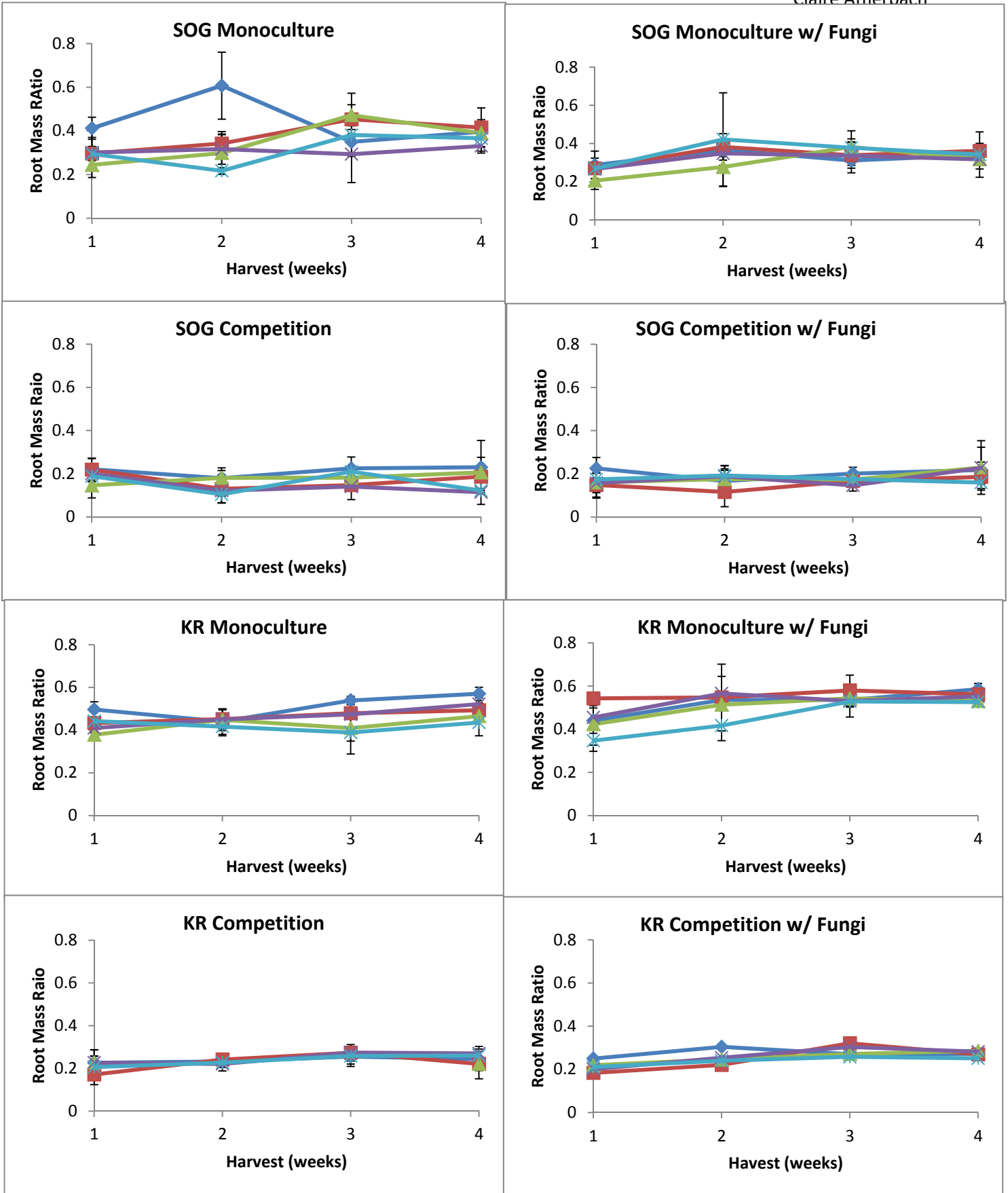


Figure 9. Change in root mass ratio (g/g) through time for each treatment combination. Different nitrogen levels are shown as different colors: N0 – blue; N1 – red; N2 – green; N3 – purple; and N4 – light blue. Error bars are standard deviation.

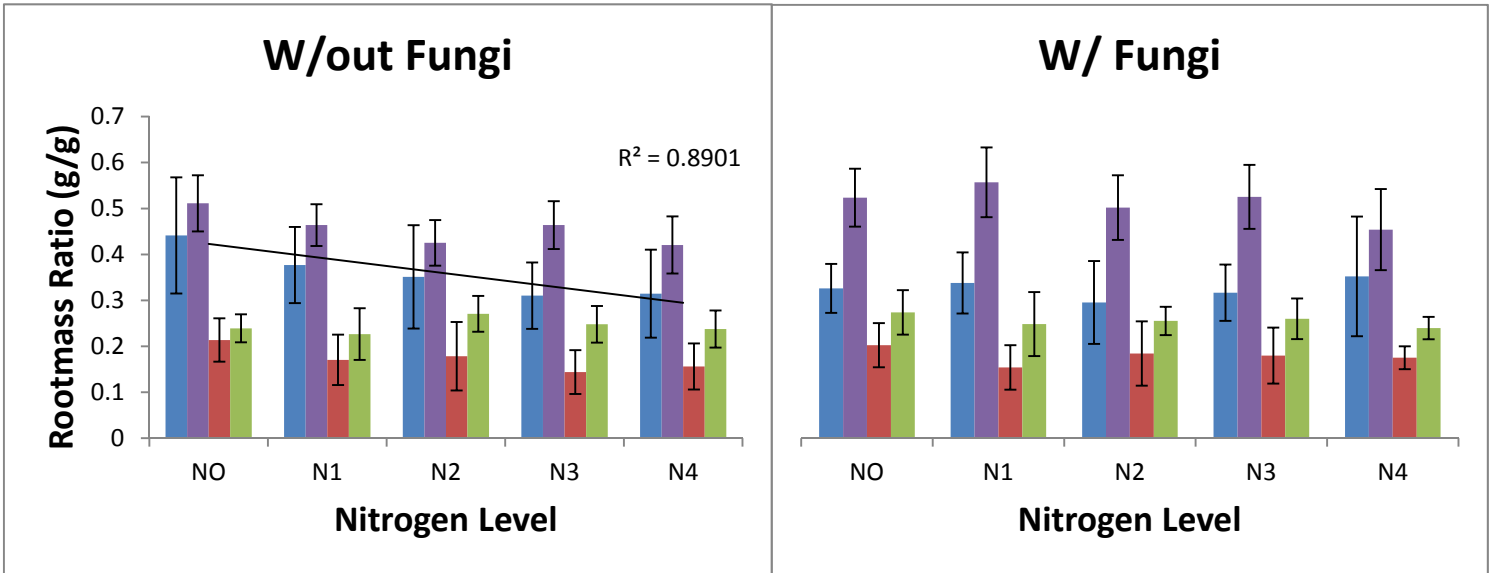


Figure 10. Root mass ratio (g/g) across the different nitrogen levels, values were averaged across all time points; error bars are standard deviation. Blue and purple represent SOG and KR in monoculture, respectively, and red and green represent SOG and KR in competition, respectively. Trendline shows a decrease in root mass ratio as nitrogen increases.

Table 2: results from MANOVA with R in the form of p-values for each response variable x treatment combination. Results for all 4 time points are shown. Significant p-values <0.5 are shown in green.

KR – Total pot biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.043	0.5174	0.359	0.16224
<b>Sp Comp</b>	1.65E-02	0.02704	6.14E-07	4.67E-05
<b>Fungi</b>	0.04668	0.02321	0.0946	8.58E-05
<b>N:Sp Comp</b>	0.27765	0.18516	0.9881	0.81467
<b>N:Fungi</b>	0.05649	0.26345	0.7518	0.16817
<b>Sp Comp:Fungi</b>	0.07866	0.46669	0.7522	0.08302
<b>N:Sp Comp: Fungi</b>	0.60338	0.65645	0.1299	0.8167

SOG – Total pot  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.21892	0.532082	0.02273	0.846233
<b>Sp Comp</b>	5.60E-04	0.863019	0.0503	0.002449
<b>Fungi</b>	0.28788	0.000249	0.01954	0.026269
<b>N:Sp Comp</b>	0.93817	0.326091	0.29339	0.104436
<b>N:Fungi</b>	0.03465	0.797652	0.44248	0.651874
<b>Sp Comp:Fungi</b>	0.50358	0.068021	0.57772	0.188275
<b>N:Sp Comp: Fungi</b>	0.03842	0.46385	0.01956	0.840353

Combined total  
 pot biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.82647	0.401816	0.689896	0.30619
<b>Sp Comp</b>	1.43E-01	0.000642	0.025547	0.06055
<b>Fungi</b>	0.02364	0.000749	0.005828	4.36E-06
<b>N:Sp Comp</b>	0.23383	0.238112	0.203361	0.10229
<b>N:Fungi</b>	0.01778	0.261261	0.756993	0.11175
<b>Sp Comp:Fungi</b>	0.07961	0.209321	0.488456	0.12119
<b>N:Sp Comp: Fungi</b>	0.20717	0.223482	0.097019	0.31604

KR – Above ground  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.204381	0.456359	0.5529	0.181016
<b>Sp Comp</b>	1.74E-06	0.001078	0.793	0.281661
<b>Fungi</b>	0.003293	0.08526	0.233	0.000344
<b>N:Sp Comp</b>	0.31106	0.29942	0.9933	0.653611
<b>N:Fungi</b>	0.044835	0.271099	0.8072	0.098809
<b>Sp Comp:Fungi</b>	0.024269	0.523035	0.4498	0.538999
<b>N:Sp Comp: Fungi</b>	0.277604	0.716471	0.0338	0.231796

SOG – Above ground  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.094586	0.3245	0.070422	0.18555
<b>Sp Comp</b>	1.99E-03	4.26E-06	6.76E-11	3.6E-12
<b>Fungi</b>	0.257163	0.0116	0.001938	0.03264
<b>N:Sp Comp</b>	0.759188	0.9748	0.89849	0.19039
<b>N:Fungi</b>	0.250411	0.2702	0.967081	0.79516
<b>Sp Comp:Fungi</b>	0.949461	0.6913	0.480882	0.5679
<b>N:Sp Comp: Fungi</b>	0.284972	0.8153	0.175229	0.47045

Combined  
 above ground  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.46513	0.98946	0.408836	0.915732
<b>Sp Comp</b>	4.25E-01	0.15625	1.91E-05	2.98E-06
<b>Fungi</b>	0.017	0.00968	0.003252	0.000124
<b>N:Sp Comp</b>	0.28892	0.23974	0.33743	0.128654
<b>N:Fungi</b>	0.05522	0.13725	0.887686	0.21121
<b>Sp Comp:Fungi</b>	0.27601	0.75019	0.423088	0.592757
<b>N:Sp Comp: Fungi</b>	0.36537	0.71091	0.11116	0.491688

KR – Below ground  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.019853	0.136664	0.108438	0.04409
<b>Sp Comp</b>	9.58E-06	0.00083	0.161463	0.32477
<b>Fungi</b>	0.007999	0.002121	0.002483	9.34E-07
<b>N:Sp Comp</b>	0.774143	0.098871	0.498163	0.35854
<b>N:Fungi</b>	0.008541	0.143835	0.810455	0.03871
<b>Sp Comp:Fungi</b>	0.003507	0.044262	0.166123	0.75162
<b>N:Sp Comp: Fungi</b>	0.172468	0.328904	0.157137	0.50657

SOG – Below ground  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.9058	0.34423	0.21535	0.6292
<b>Sp Comp</b>	7.80E-01	2.74E-06	2.92E-07	5.83E-09
<b>Fungi</b>	0.87527	0.00388	0.53926	0.2628
<b>N:Sp Comp</b>	0.74341	0.10293	0.95498	0.2061
<b>N:Fungi</b>	0.08689	0.08154	0.75585	0.9822
<b>Sp Comp:Fungi</b>	0.47578	0.57563	0.57526	0.1792
<b>N:Sp Comp: Fungi</b>	0.08852	0.29949	0.04408	0.9829

Combined  
 below ground  
 biomass

	H1	H2	H3	H4
<b>Nitrogen</b>	0.15957	0.08198	0.70521	0.05675
<b>Sp Comp</b>	3.85E-05	2.3E-07	0.94399	0.10108
<b>Fungi</b>	0.074589	9.78E-05	0.01147	3.32E-06
<b>N:Sp Comp</b>	0.238481	0.29683	0.15305	0.09497
<b>N:Fungi</b>	0.007441	0.5808	0.92355	0.11792
<b>Sp Comp:Fungi</b>	0.017901	0.02782	0.04689	0.01163
<b>N:Sp Comp: Fungi</b>	0.12246	0.03909	0.12133	0.18623

KR Root Length

	H1	H2	H3	H4
<b>Nitrogen</b>	0.554696	0.225373	0.93287	0.9387
<b>Sp Comp</b>	8.29E-03	0.00066	0.28374	0.1748
<b>Fungi</b>	0.363432	4.92E-05	2.48E-05	2.55E-05
<b>N:Sp Comp</b>	0.240646	0.317979	0.09986	0.5455
<b>N:Fungi</b>	0.814339	0.454535	0.50927	0.8412
<b>Sp Comp:Fungi</b>	0.940656	0.892141	0.74729	0.5366
<b>N:Sp Comp: Fungi</b>	0.884104	0.53978	0.56047	0.663



SOG Root Length

	H1	H2	H3	H4
<b>Nitrogen</b>	0.805659	0.689541	0.02432	0.55781
<b>Sp Comp</b>	9.06E-03	0.021076	0.053	0.01824
<b>Fungi</b>	0.800643	0.041782	0.46067	0.01785
<b>N:Sp Comp</b>	0.055414	0.292149	0.17321	0.70673
<b>N:Fungi</b>	0.096047	0.082023	0.96147	0.86286
<b>Sp Comp:Fungi</b>	0.229876	0.001429	0.59231	0.65952
<b>N:Sp Comp: Fungi</b>	0.313709	0.687521	0.1202	0.56399

KR Specific leaf area

	H1	H2	H3	H4
<b>Nitrogen</b>	0.68565	0.964	0.05002	0.78553
<b>Sp Comp</b>	2.20E-02	0.9113	0.09967	0.31683
<b>Fungi</b>	0.32208	0.6045	0.98232	0.07716
<b>N:Sp Comp</b>	0.52681	0.3492	0.05891	0.98342
<b>N:Fungi</b>	0.76352	0.4077	0.78058	0.77228
<b>Sp Comp:Fungi</b>	0.75438	0.3332	0.4759	0.88081
<b>N:Sp Comp: Fungi</b>	0.57639	0.2124	0.95853	0.33804

SOG Specific leaf area

	H1	H2	H3	H4
<b>Nitrogen</b>	0.1536	0.45713	0.074932	0.64293
<b>Sp Comp</b>	8.00E-01	0.88632	0.126169	0.75878
<b>Fungi</b>	0.405	0.60477	0.000145	0.86784
<b>N:Sp Comp</b>	0.4697	0.72753	0.217138	0.08857
<b>N:Fungi</b>	0.9698	0.06743	0.039107	0.18435
<b>Sp Comp:Fungi</b>	0.188	0.10823	0.097996	0.04343
<b>N:Sp Comp: Fungi</b>	0.8185	0.88219	0.172855	0.49064

KR Height

	H1	H2	H3	H4
<b>Nitrogen</b>	0.9615	0.3756	0.304174	0.502084
<b>Sp Comp</b>	8.82E-01	0.1742	0.001497	0.000498
<b>Fungi</b>	3.88E-05	5.65E-08	1.38E-05	1.27E-08
<b>N:Sp Comp</b>	0.7424	0.5841	0.856936	0.411207
<b>N:Fungi</b>	0.6837	0.3364	0.635947	0.093437
<b>Sp Comp:Fungi</b>	0.6467	0.1848	0.443725	0.098809
<b>N:Sp Comp: Fungi</b>	0.3615	0.7706	0.839587	0.907549

SOG Height

	H1	H2	H3	H4
<b>Nitrogen</b>	0.075228	0.141322	0.413748	0.082649
<b>Sp Comp</b>	8.36E-02	0.047971	0.623812	0.008612
<b>Fungi</b>	0.001229	0.002219	0.000184	0.030602
<b>N:Sp Comp</b>	0.771044	0.739263	0.27961	0.172975
<b>N:Fungi</b>	0.850606	0.387551	0.507473	0.334906
<b>Sp Comp:Fungi</b>	0.411789	0.028216	0.590236	0.663745
<b>N:Sp Comp: Fungi</b>	0.61386	0.701548	0.497172	0.081508

KR Root Mass Ratio

	H1	H2	H3	H4
<b>Nitrogen</b>	0.03127	0.063435	0.021244	0.023502
<b>Sp Comp</b>	< 2e-16	< 2.2e-16	< 2.2e-16	< 2.2e-16
<b>Fungi</b>	0.80505	0.003348	0.000114	0.000556
<b>N:Sp Comp</b>	0.08467	0.502352	0.053892	0.002931
<b>N:Fungi</b>	0.29047	0.265553	0.253681	0.991259
<b>Sp Comp:Fungi</b>	0.69196	0.05167	0.004201	0.059426
<b>N:Sp Comp: Fungi</b>	0.75376	0.609232	0.088847	0.105321

SOG Root Mass Ratio

	H1	H2	H3	H4
<b>Nitrogen</b>	0.03701	0.020622	0.7644	0.02219
<b>Sp Comp</b>	1.06E-08	4.78E-12	3.86E-15	2.86E-14
<b>Fungi</b>	0.01898	0.551887	0.1686	0.72048
<b>N:Sp Comp</b>	0.64972	0.034656	0.5696	0.44822
<b>N:Fungi</b>	0.39574	0.000671	0.4405	0.17345
<b>Sp Comp:Fungi</b>	0.34362	0.640023	0.3521	0.02355
<b>N:Sp Comp: Fungi</b>	0.35068	0.067659	0.2941	0.64442

Nitrate

	H1	H2	H3	H4
<b>Nitrogen</b>	0.01213	0.05154	0.3773	0.3379
<b>Sp Comp</b>	4.34E-01	0.07346	0.9324	0.2248
<b>Fungi</b>	0.79236	0.56864	0.2835	0.3124
<b>N:Sp Comp</b>	0.3563	0.15389	0.7073	0.4122
<b>N:Fungi</b>	0.40286	0.50303	0.9084	0.2626
<b>Sp Comp:Fungi</b>	0.33299	0.98605	0.6986	0.1624
<b>N:Sp Comp: Fungi</b>	0.11553	0.51721	0.6842	0.9237

Ammonium

	H1	H2	H3	H4
<b>Nitrogen</b>	0.008312	0.02657	0.286	0.7429
<b>Sp Comp</b>	4.12E-01	0.28091	0.596	0.4929
<b>Fungi</b>	0.64188	0.46435	0.5436	0.0841
<b>N:Sp Comp</b>	0.146401	0.54034	0.9367	0.7873
<b>N:Fungi</b>	0.307628	0.32746	0.2024	0.5346
<b>Sp Comp:Fungi</b>	0.588246	0.25465	0.8864	0.3839
<b>N:Sp Comp: Fungi</b>	0.926276	0.22322	0.9367	0.5939