

Diversity Relationships in Native, Warm-Season Plant Communities used for Agriculture

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ABSTRACT

Studies suggest that diverse mixtures of plants may improve forage productivity and also be suitable as bioenergy crops. The objectives of this research were: 1) to measure the effects of native, warm-season perennial (NWSP) forage mixtures and management methods on productivity, weed biomass, nutritive value, and community composition, and 2) to identify mechanisms that generate any positive diversity-productivity relationships over a three-year establishment period. In 2008, two experiments were established to evaluate the use of native, warm-season plants in forage-livestock systems using a pool of ten native species. The first, a large-scale, three-year, experiment tested three different NWSP mixtures (switchgrass monoculture, a four-grass mixture, and a ten-species mixture) and two management methods (grazed or biomass crop). Switchgrass monocultures had the lowest forage yield and highest weed biomass in both grazed areas and biomass crop exclosures. Analysis of forage nutritive value did not show many differences among mixtures, although the monoculture tended to have higher crude protein and lower fiber concentrations than the polycultures. Management method affected community composition, with NWSP richness higher and weed species richness lower in biomass crop exclosures than in grazed areas. A second experiment examined if species richness would enhance yields through a positive biodiversity effect. It employed additive partitioning to separate the selection effect (SE) from the complementarity effect (CE) by sowing random assemblages of NWSPs at five levels of richness into small plots. Species richness was associated with increased yields in the first year only, but overyielding and positive diversity effects were present in all three years. On average, over 50% of multi-species plots overyielded and 64% exhibited a positive biodiversity effect. Both SE and CE contributed to the biodiversity effect and the importance of each effect changed over time as communities became better established. All ten species were also individually analyzed for their yield potential, nutritive value, and elemental composition. Warm-season grasses tended to be higher-yielding but of lower nutritive value. All ten NWSPs contained sufficient concentrations of eleven elements to support nonlactating cows. These experiments demonstrated that diverse NWSP mixtures may be a valuable addition to both forage and bioenergy agroecosystems.

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LIST OF ABBREVIATIONS:

ADF	Acid detergent fiber
CE	Complementarity effect
CP	Crude protein
NDF	Neutral detergent fiber
NWSP	Native, warm-season perennial
PLS	Pure live seed
RYT	Relative yield total
SE	Selection effect

Chapter One

General introduction

Overall hypothesis

A diverse assemblage of native, warm-season grasses and associated forbs could provide many advantages to grazing systems due to their high productivity, drought tolerance, summer growth, and nutritive value. The experiments in this dissertation test how increasing NWSP diversity in a forage-livestock grassland system affects certain ecosystem-level properties. The hypothesis to be tested is that diversity (drawing from a pool of ten species and four functional groups) will increase forage productivity and improve nutritive value for cattle while reducing weed biomass. In addition, a second hypothesis is that these relationships (e.g., diversity contribution to aboveground productivity, reduced weed invasibility) should become more biologically important over time as communities become established.

Overall objectives

- I) To evaluate how forage species mixtures and management methods affect herbage mass, nutritive value, weed biomass, and plant species composition within NWSP communities.
- II) To determine if NWSP species richness can affect herbage mass, if so, and elucidate what mechanisms may cause increased yields in diverse mixtures.

Dissertation organization

This research is divided into four chapters written in manuscript style for publication. The first two chapters are based on research performed in a large-plot experiment. The first of the research chapters, Chapter Three, examines the effects of NWSP mixture composition and management type (grazing or biomass crop) on forage plant biomass, forage nutritive value, and weed biomass. Chapter Four studies how management of NWSP mixtures may impact plant species composition over time. The last two chapters describe the results of the small-plot diversity experiment. Chapter Five focuses on testing the biodiversity-productivity relationship and determining the mechanisms causing the observed biodiversity effects. Chapter Six analyzes

the productivity, nutritive value, and elemental composition of each of the ten native plant species used in both the large- and small-plot experiments. A final chapter provides some overall observations and conclusions about the dissertation work.

Chapter Two

Literature review: Evaluating plant diversity effects in native, warm-season forage mixtures

Biodiversity and ecosystem services

Ecosystem services, defined as benefits that people receive from ecosystem functions, include water and climate regulation, soil formation, nutrient cycling and food production. Globally, these services are valued at an estimated \$33 trillion per year (Costanza et al. 1997), but many of these ecosystem services are at risk. Of the 24 ecosystem services evaluated by the Millennium Ecosystem Assessment (2005), 14 were found to be declining. These negative trends have prompted a growing demand to manage areas to enhance a variety of ecosystem services, and one system in which this can be done in is agricultural grasslands. Worldwide, nearly 3.5×10^9 ha are currently in pasture, and that area is expected to increase by almost 16% (5.4×10^8 ha) by 2050 (Tilman et al. 2001a).

Grasslands are multi-functional areas that can be managed to provide a variety of ecosystem services beyond the traditional food, fiber, and fuel. Many important ecosystem-level processes such as productivity (Tilman et al. 1996, Bullock et al. 2007, Kirwan et al. 2007), soil carbon and nitrogen accumulation (Fornara and Tilman 2008), nutrient cycling (Tilman et al. 1996), system stability (Schwartz et al. 2000, Hector et al. 2010), and resistance to invasion (Tracy and Sanderson 2004a) have been linked to plant diversity. The relationship between diversity and ecosystem functioning and services is the subject of frequent study. While many studies have found that diversity may have a positive relationship with ecosystem attributes like productivity, nutrient cycling, and weed invasion (Elton 1958, Tilman et al. 1997a, Hector et al. 1999, Hector et al. 2001), others suggest that plant species composition could explain more variation than diversity when looking at traits like productivity and nitrogen dynamics (Hooper and Vitousek 1997). This suggests that both diversity and the attributes of particular functional groups or species play a role in explaining changes in ecosystem processes. Functional groups can be defined as a collections of species based on shared traits, such as morphology, ecological role, or response to environmental conditions (Lavorel et al. 1997).

The term “diversity” can encompass several attributes, including the number of species, genotypes, and functional traits. In many studies, including this dissertation, diversity is defined

by species richness and numbers, but functional trait diversity is increasingly receiving more attention. Research has demonstrated that functional diversity and/or functional composition may exert a greater influence than species richness on ecosystem services such as productivity, plant nutrient content, and litter decomposition (Hooper and Vitousek 1997, Tilman et al. 1997a, Diaz and Cabido 2001, Scherer-Lorenzen 2008). As a functional group, legumes are often cited as a major explanatory variable in diversity-productivity and diversity-nitrogen relationships (Spehn et al. 2002, Scherer-Lorenzen et al. 2003). However, others have found a long-term positive relationship even when legumes are excluded (van Ruijven and Berendse 2005, 2009). Functional diversity may be crucial for a community to survive through environmental changes (Diaz and Cabido 2001) and resist invasion (Pokorny et al. 2005).

Some of the earliest studies on biodiversity have suggested that more diverse mixtures are more productive. In one of the first ecological experiments, Darwin noted that diverse plots of grasses yielded more biomass than grass monocultures (Darwin 1859). While Darwin used this experiment to explain his theories of evolution and natural selection, modern ecologists use similar experiments when trying to understand the biodiversity-ecosystem functioning debate (Hector and Hooper 2002). When mixtures produce more herbage mass than monocultures, they are said to “overyield.” There are two types of overyielding: nontransgressive, in which mixtures yield more than the average yield of monocultures, and transgressive, in which mixtures yield more biomass than the monoculture of the highest yielding species present in the mix (Hector et al. 2002).

While frequently studied, the relationship between diversity and productivity may be complicated. In a 28-site experiment, Kirwan et al. (2007) found that three of the four species-groups tested exhibited strong evidence of transgressive overyielding. Cardinale et al. (2007) evaluated 44 diversity-productivity experiments and found that while the majority of diverse assemblages produced more aboveground biomass than the average monoculture, only 12% produced more than the most productive monoculture. In addition, they found that long-term experiments were more likely than short-term experiments to exhibit transgressive overyielding. Hector et al. (2002) observed that although mixtures in general did not yield more than the most productive monoculture, they did transgressively overyield when compared to monocultures of the dominant species, and recommended a new framework for evaluating overyielding. Other experiments have found weak or no evidence linking diversity and productivity (Huston et al.

2000, Tracy and Sanderson 2004b). Nevertheless, even in situations where a monoculture is found to be the most productive, species-rich plantings may still be beneficial because diversity may simultaneously enhance more services than just increased aboveground biomass.

The mechanisms behind any positive relationships between diversity and productivity, as well as many other ecosystem processes, are not well understood yet. Loreau and Hector (2001) used additive partitioning as a way to separate out the effects of selection and complementarity. The net biodiversity effect, which is the difference in yields observed in mixtures from that expected based on monocultures, can be separated into the two mechanisms, either selection or complementarity. The selection effect (SE) occurs when a mixture contains a species that is very high-yielding. As species richness increases in artificially assembled communities, the likelihood that such species are present also increases, so mixtureoveryielding is due to an experimental artifact and not necessarily diversity per se. In contrast, the complementarity effect (CE) occurs when there is facilitation and more complete resource use in diverse mixtures. In this instance, diversity increases positive interactions, resulting in increases in productivity.

Although greater aboveground productivity is generally correlated to increased diversity, less is known about belowground productivity. Studies that have examined belowground production and diversity have been equivocal, with some finding a positive relationship (Fornara and Tilman 2008), and others finding no relationship (Gastine et al. 2003). Functional diversity may impact belowground attributes and functions such as root mass loss and nitrogen (N) and carbon (C) sequestration (Fornara et al. 2009). Skinner et al. (2006) found that compared to two- and three-species mixtures, an 11-species mixture had a greater root biomass and exhibited a deeper rooting distribution, which may alleviate water stress. Improving our understanding of belowground dynamics is important in grasslands, where the majority of biomass is located belowground. Jackson et al. (1996) calculated a root:shoot ratio of 3.7 for grasslands, while Dugas et al. (1999) found that root biomass was up to six times greater than shoot biomass in a native prairie. Warm-season grasses in particular have extensive root systems; switchgrass (*Panicum virgatum* L.) contains 27% of its biomass in roots alone, and this number increases to 84% when crown tissue is included (Frank et al. 2004).

Although some research has questioned the true effects of increased diversity, growing evidence suggests that diverse species assemblages provide advantages in managed grasslands.

However, grazing systems in the northeastern United States are typically dominated by just a few cool-season forage species, and the majority of diversity is comprised of weedy species that provide little forage value (Tracy and Sanderson 2000, Sanderson et al. 2007). Manging for increased species diversity in agroecosystems may improve a variety of ecological services and provide agronomic value, such as increased forage yield, added flexibility to the grazing system (Moore et al. 2004, Sanderson et al. 2004b), reduced toxin consumption and the creation of a more balanced diet for livestock (Tracy and Faulkner 2006). Bullock et al. (2007) compared species-rich and species-poor grasslands eight years after establishment and determined that the hay yield of species-rich plots was 43% greater than species-poor plots while still meeting or exceeding nutrient requirements for livestock. The authors conjectured that the increased cost in establishing species-rich pastures would be recovered over time through higher profits from these increased yields.

In grassland systems, where much of the aboveground biomass is removed by grazing, roots are the main source of soil C (Johnson and Matchett 2001). The soil C pool, estimated to be 2550 Gt, is 3.3 and 4.5 times larger than the atmospheric and biotic C pools, respectively (Lal 2004). In temperate grasslands, approximately 105 Pg soil organic carbon (SOC) in total are stored in the top 1 m of soil, with nearly two-thirds in the top 40 cm (Jobbagy and Jackson 2000). The large root systems in grasslands may dramatically increase SOC, resulting in higher rates of C sequestration that could help mitigate greenhouse gas emissions. Along with its C sink capacities, SOC also plays an important role in maintaining soil quality, water quality and retention, and plant productivity (Lal 2004). Adding diversity to grasslands may have a positive effect on the amount of C stored in the soil (Tilman et al. 2006a, Fornara and Tilman 2008) and may further enhance C sequestration.

There is also evidence that more species-rich communities resist invasion (Elton 1958, Tilman 1997, Picasso et al. 2008). Enhanced resistance to invasion is often associated with more complete resource use and competition which reduces availabilities of resources such as light, nutrients, and water (Davis et al. 2000, Hector et al. 2001). Highly diverse communities may be able to employ resource use complementarity to reduce resource levels enough so that invaders have a difficult time establishing. High levels of diversity may also increase the chance that a native species especially suited to suppressing invasion is present in the community (Levine and D'Antonio 1999). Whether diversity itself or a sampling effect is the cause of the observed

increased invasion resistance is still debated (Wardle 2001). Other studies have suggested a positive relationship between diversity and invasion that can be explained by facilitation through “diversity promoters” that create niches in diverse communities (Palmer and Maurer 1997), or factors other than species richness, such as resource availability, may determine the invasibility of a community (Stohlgren et al. 1999a). Functional groups may impact invasion susceptibility, as grasses and legumes have opposite effects on the invasion of unsown species (Roscher et al. 2009). However, most experiments indicate that diversity suppresses invasion; in a review by Hector et al. (2001) of 18 studies studying diversity and invasion, 15 provided evidence of a negative relationship between diversity and invasion. In addition to species diversity, species evenness, defined as the relative abundances of species within a community, may also be important in reducing the invasion of weedy species (Tracy et al. 2004, Tracy and Sanderson 2004a).

Species diversity depends on more than simply the number of species sown or present in the seed bank. Variation in topography may also affect species composition and diversity. Grasslands in southwest Virginia and elsewhere frequently do not have uniform topography, which can create a landscape with a range of soil conditions, including soil moisture, soil organic matter and nutrient levels. Several studies have shown that species diversity was highest on backslopes when compared to toeslopes and summits (Harmony et al. 2001, Guretzky et al. 2007). However, others have found that diversity was highest on summits, where productivity was the lowest, and also noted that species dominance shifted along the slope (Broughton and Gross 2000). Topography may also help increase overall grassland diversity because it can provide more niches for species to occupy. Other forms of environmental heterogeneity may have roles in explaining biodiversity effects. For example, although there were no significant differences in biomass production in heterogeneous soil nutrient plots and uniform plots, when soil nutrient levels were varied, CE was of greater importance, while in uniform soil conditions SE explained more of the observed biodiversity-productivity relationship (Wacker et al. 2008).

Native, warm-season grasslands

Until recently, most research on diversity in grasslands has centered on cool-season species, with much less focus on native, warm-season perennials (NWSPs). Native, warm-

season perennial species, adapted for growth during the hottest part of the year, have a potential for high productivity while being relatively drought tolerant and requiring low nutrient inputs. Species such as switchgrass, big bluestem (*Andropogon gerardii* Vitman) and indiagrass (*Sorghastrum nutans* (L.) Nash) are warm-season grasses native to North American tallgrass prairies that can be used in forage systems and enhance ecosystem services. In most cases, European settlers replaced native grasses and forbs with non-native cool-season forage species that can be continuously grazed (Jackson 1999), but interest in establishing and managing warm-season grasslands is gaining momentum. The addition of highly diverse NWSP pastures to a rotational grazing system could provide an opportunity for the enhancement of ecosystem services and functions in forage-livestock grasslands.

Using NWSPs to complement cool-season grasslands may provide many benefits to foraging systems. Livestock could graze on warm-season forage species during the summer when cool-season species productivity is low, allowing cool-season pastures time to recover from spring grazing. Although warm-season forage species are highly productive, they typically possess lower nutritive value when compared to cool-season species. Moore et al. (2004) found that cattle gained less weight when rotated onto warm-season grasslands than when they remained on cool-season pastures due to the lower nutritive values of the warm-season grasslands. However, with appropriate management, warm-season species can sustain beef cow grazing (Roberts and Gerrish 1999a). Increasing grassland diversity through the addition of N-rich forage legumes and other forbs to pastures may also improve nutritive quality of pastures along with a variety of ecosystem benefits such as nutrient cycling and wildlife habitat.

Forage legumes can fix atmospheric N, lowering N-fertilizer input requirements and providing forage of greater nutritive value. Legumes may also positively interact with warm-season grasses to accumulate more soil C and N and increase warm-season grass biomass (Posler et al. 1993, Fornara and Tilman 2008). Posler et al. (1993) tested grass-legume binary mixtures using three warm-season grasses and six legumes and found that nearly all legume-grass mixtures produced more forage and contained more crude protein than when grass was grown in monoculture. Including native legumes in diverse NWSP assemblages may enhance forage quantity, quality, nutrient cycling and C sequestration.

The presence of N-fixing legumes in diverse assemblages to provide nutrient-enrichment and the resulting increase in productivity may be just one way that diversity affects nutrient cycling. As species richness increases, nutrient capture can increase, reducing nutrient losses from the system (Tilman et al. 1996). Nutrient use efficiency may also be impacted by diversity because as diversity increases the allocation of various nutrients also changes, potentially impacting productivity (Nijs and Impens 2000). Using the ratio of aboveground biomass to aboveground N, Van Ruijven and Berendse (2005) estimated N use efficiency (NUE) and observed a positive relationship between NUE and productivity in mixtures that did not contain legumes. Nutrient cycling may additionally be impacted by changes in microbial community composition caused by increased plant diversity, where more diverse systems have increased rates of N mineralization and thus more available soil N (Zak et al. 2003).

Diverse NWSP grazing lands may also benefit wildlife, in particular grassland birds. Grassland bird populations throughout North America are rapidly declining because of habitat loss and intensification of agricultural practices. Due to their phenology, the bulk of native, warm-season grass growth (70%) occurs after June 1 and thus mowing or grazing does not begin until July or August (Giuliano and Daves 2002). Giuliano and Daves (2002) concluded that the warm-season growth habit allows grasslands birds to nest in undisturbed grasslands for a longer period of time compared to cool-season grasses and increases nest success and the rate of fledging. The vigorous growth and bunchgrass morphology of native, warm-season grasses, in combination with other forbs, may provide suitable habitat for a variety of insects, birds, and mammals.

Biofuel production from native, warm-season grasslands

Recent research on developing methods to use plant biomass for bioenergy has intensified as the global demand for more energy combines with demands for a sustainable and less polluting energy source. Presently ethanol is the most common form of biofuel, typically produced by fermenting corn. However, corn is not an optimal choice for a bioenergy crop as it requires large energy inputs. If all the corn grown in the United States were converted to ethanol, it could still only provide for 12% of the nation's gasoline demand (Hill et al. 2006). Studies examining the reduction of greenhouse gas emissions due to corn-based ethanol

production are also equivocal, and have varied from a 20% increase to a 32% decrease (Farrell et al. 2006). Any advantages in greenhouse gas emission reductions could actually be negated as farmers convert forests and grasslands to croplands for biofuels (Searchinger et al. 2008). Because of these concerns researchers are examining more efficient technology and sources of biofuel. The future of biofuels will most likely be in improving the efficiency of emerging cellulosic technology and using “second generation” bioenergy crops containing high levels of lignocellulose (Sanderson and Adler 2008). In addition to conversion to ethanol, plant biomass can be compressed into pellets, which increases the specific density of the plant material to improve ease of handling, storage and use. Pellets are a compact form of bioenergy that is suitable for cooking and heating (Samson et al. 2005).

Warm-season grasses have been under intense scrutiny as a source of bioenergy because they are able to produce high yields on marginal soils with low inputs. Switchgrass in particular has been the focus of many biofuel studies because of its high yield potential and tolerance of a range of environmental conditions (Lemus et al. 2002, Wullschleger et al. 2010). Switchgrass pellets are estimated to have a net energy production of 168 GJ ha⁻¹, approximately 60% greater than that of corn, and an energy input:output ratio of 14.6 GJ t⁻¹ (Samson et al. 2005). With appropriate management, switchgrass yields of approximately 14 Mg ha⁻¹ can be sustained for years (Fike et al. 2006). Although switchgrass has received most of the attention, the forage and yield potential of other native, warm-season grasses have also been evaluated in monocultures (Cuomo et al. 1996) and in mixtures (Mulkey et al. 2008).

While most biofuel crops are grown as monocultures, Tilman et al. (2006a) observed that a high-diversity assemblage produced higher bioenergy yields than any of the monocultures tested, including switchgrass. They described the mixtures as “low-input high-diversity” and asserted that these mixtures were also C negative. However, Tilman et al.’s experiment was conducted on N-poor, sandy soils, and their plots were burned in the spring to remove biomass, both factors which may have influenced the observed results. Even still, C-negative plantings using NWSPs could provide a sustainable way to produce energy and reduce greenhouse gas emissions while providing other ecosystem services.

Establishment of native warm-season grasses

Native, warm-season grasses have the potential to yield high quantities of biomass for forage and bioenergy applications. These perennial species also can be productive with require low inputs, and with proper management, stands can be maintained for years (Jung et al. 1990). However, establishment is slow, and stands do not typically reach full maturity until several years after establishment. Switchgrass, one of the most studied of the native, warm-season grasses, is slow to reach full productivity due to initial high allocation to roots, taking three years to produce a mature stand (McLaughlin and Kszos 2005). In addition to a slow aboveground growth rate, high levels of seed dormancy and intense competition with weeds can also delay or prevent warm-season grass stand establishment.

Due to their slow aboveground growth, switchgrass and other warm-season grasses are particularly vulnerable to competition from weeds in their first year, especially from crabgrass (*Digitaria* spp.) and foxtail (*Setaria* spp.) and other summer annuals (Wolf and Fiske 1996, McLaughlin and Kszos 2005). Many farmers may be hesitant to plant warm-season grasses for forage and hay because of the slow rate of establishment and uncertainty of success since without some form of weed control, there may be no harvestable yield in the first year (Martin et al. 1982). While many studies cite weed competition as a major impediment to seedling establishment, Wilson et al. (2004) observed that controlling neighboring plants did not impact the establishment of native grasses and instead recommended taking environmental conditions such as soil moisture and soil N into account to improve restoration success.

Seed dormancy can also hinder rapid establishment. Immediately after harvest, seed dormancy can be as high as 90% in some cultivars (Sanderson et al. 1996). There are several methods that may help alleviate this problem. Seed storage, stratification and other treatments have been shown to reduce dormancy (Beckman et al. 1993, Haynes et al. 1997), but these can be expensive or difficult to apply to large batches of seed. Increasing the seeding rate can also improve the rates of stand establishment, but the cost of additional seed may be prohibitive in some cases (Masters 1997). A third option is modifying planting dates. If weed levels can be controlled, planting highly dormant seed early in the season, when conditions are cool and moist, may naturally stratify seeds and increase seedling emergence (Sanderson et al. 1996, Wolf and

Fiske 1996). Breeding programs are also underway to select for plants with improved rates of germination (Sanderson et al. 1996).

Stand density of less than 40% in the first year limits second-year biomass production and may delay grazing or harvesting of switchgrass by several years (Schmer et al. 2006). Launchbaugh and Owensby (1970) observed that a first-year frequency for mixed native species of 11 to 21 plants m⁻² was suitable for second-year grazing. To improve stand establishment and control weeds, various techniques including no-till practices, herbicides, cover or smother crops, sawdust addition, and mowing have been recommended. With appropriate weed control, stands could establish more readily and reach full productivity sooner.

The establishment of warm-season grasses using a variety of herbicides has been studied. However, herbicides that control undesirable cool-season species can often also have an impact on certain warm-season grasses, so both effective weed control and potential grass stand reductions must be taken into account when considering herbicides. One example is atrazine, which controls weeds but also reduces the stand growth of some species, including indiangrass, while switchgrass and big bluestem are more tolerant (Martin et al. 1982). Imazapic is another example of an herbicide to which species have a range of tolerances, as it can be damaging to switchgrass but not big bluestem (Sanderson et al. 2004a). Both forage yield and stand purity can improve with herbicide use; big bluestem treated with pre-emergent herbicides tended to increase stand frequency and yields by up to 2.4 Mg ha⁻¹ (Masters 1997), and herbicide-treated switchgrass had nearly pure stands one year after planting (McKenna et al. 1991). With improved weed control, it may also be possible to reduce seeding rates and still establish vigorous grass stands, decreasing costs of seeds and potentially increasing in the value of the increased forage yield (Masters 1997). Along with improving grass stand establishment, herbicides have also been shown to improve the establishment of native forbs (Masters et al. 1996). However, because relatively few herbicides have been registered for use in perennial grass systems and many herbicides that could be used have the potential to injure perennial grasses, identifying an appropriate herbicide for use in diverse mixtures of grasses and forbs may be a challenge (Buhler et al. 1998).

Many warm-season grass plantings are established on previous cropland in N-rich soils. Quickly-growing weeds are able to take advantage of the high N availability and are extremely

competitive with the slower-growing native grass seedlings. Increased nutrient levels have been shown to make sites more susceptible to invasion by non-native species (Huenneke et al. 1990). If N could be removed from the soil, making soil conditions more similar to undisturbed perennial grass habitat, the competitive ability of these weeds should decrease, favoring slower, less nutrient-demanding species. Carbon addition to the soil has been suggested as a method to reduce N availability by increasing the soil C:N ratio, causing an increase in decomposer microbes that immobilize N (McLendon and Redente 1992). However, not all studies have found that C amendments enhance native plant establishment and growth, and the effectiveness of C additions may depend on the community studied along with the form and amount of C applied (Wilson and Gerry 1995, Corbin and D'Antonio 2004).

Other techniques used to control weeds include cover crops, companion crops and mowing. Cover crops can suppress weed growth through competition for resources such as light and N prior to establishment of the crop (Liebman and Davis 2000). Companion crops work to reduce weed growth without excessive competition against the crop it is planted with. A species that could act as a companion crop for warm-season grasses would be beneficial to growers while the stand is still establishing. While some work has been done using corn as a companion crop for the establishment of switchgrass and big bluestem (Hintz et al. 1998), there are currently no companion crops specifically developed for use with warm-season perennial grasses (Buhler et al. 1998). Weeds can also be controlled through repeated mowing or clipping. The height and timing of mowing is also important, and mowing near flowering when allocations to shoots are greatest has reduced weed cover and vigor (Wilson and Clark 2001). It is critical to mow above the height of the native grasses, and because of this, mowing may be most beneficial early in establishment.

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Chapter Three

Forage yield, nutritive value, and weed biomass in three native, warm-season perennial plant mixtures managed for grazing and biomass

ABSTRACT: Native, warm-season perennials (NWSP) have been suggested for use in forage-livestock grazing systems and as a source for bioenergy. The goal of this study was to analyze the forage herbage mass, weed biomass, and nutritive value of three different NWSP mixtures (a switchgrass monoculture, a four-grass mixture, and a ten-species mixture of grasses, forbs, and legumes) to determine if increased species diversity will enhance NWSP forage and biomass crop potentials. Forage mixtures were tested under two management methods (grazing or as a biomass crop). Switchgrass monocultures yielded less target forage mass than the more diverse mixtures. In grazed areas, diverse mixture forage yielded four to five times more herbage than monocultures in 2009 and 2010. Grazing appeared to negatively affect NWSP forage mass accumulation, as early-season grazed yields were slightly lower than yields in biomass crop exclosures. As stands established, weed biomass tended to decrease as mixture diversity increased, especially in the biomass crop exclosures, where by October 2010 the weed biomass of the two diverse mixtures was less than one-fifth of that in switchgrass monocultures. Forage nutritive analyses showed few differences among the three mixtures, and in general NWSP forage nutritive value was sufficient for nonlactating cows. Results suggest that planting more diverse NWSP mixtures may help accelerate stand establishment, increase forage yields, and reduce weed biomass. NWSPs show great promise for both grazing and bioenergy purposes, although it might not be possible to manage a stand both purposes simultaneously.

INTRODUCTION

Native, warm-season perennials have the potential to be used both as forage and as a biomass crop for bioenergy or biofuel. Currently, many eastern U.S. grasslands are established with introduced cool-season species like tall fescue (*Festuca arundinacea* Schreb.) which require regular nutrient inputs to remain productive. Native, warm-season perennials (NWSPs) have been suggested as a possible alternative or addition to these traditional cool-season forage systems (Moore et al. 2004). Native grasses and forbs, such as switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* Vitman) and indiagrass (*Sorghastrum nutans* (L.)

Nash), were historically present in American tallgrass prairies and in parts of the eastern U.S. but have largely been replaced with cool-season species (Jackson 1999). NSWPs have a variety of attributes that make them suitable for use in a forage system. They are adapted for growth during the hottest part of the year and are tolerant of drought and low nutrient conditions while being highly productive. Switchgrass yield responses to N inputs has been studied frequently, and while some studies have shown little to no N impact on yield, others suggest that N applications of 40 to 50 kg ha⁻¹ year⁻¹ are sufficient to produce sustainable harvests (Thomason et al. 2004, Parrish and Fike 2005). With these traits, NWSP grasslands have great potential to supply ample and adequate quality herbage during the summer, especially during years of drought.

Switchgrass is a frequently used native species for both forage and biomass crop but is typically grown in monocultures for these purposes. However, recent interest in incorporating additional plant species to increase diversity and enhance ecosystem services has been the basis for several studies testing the effect of diversity in agricultural settings. Diversity has frequently been linked to higher productivity (Hector et al. 1999, Fornara and Tilman 2009). Aboveground productivity is one of the most commonly studied traits that researchers use to understand how biodiversity affects a community. In most cases, more diverse assemblages are higher yielding than areas sown with just one or two species, although it is still unclear how many species may be needed to maximize productivity or whether diverse mixtures can be maintained for long periods of time (Tilman et al. 1996, Tracy and Sanderson 2004b, Roscher et al. 2007, Picasso et al. 2008).

Plant diversity is already often used in grazing systems. Producers recognize that diverse mixtures improve forage nutritive values and increase forage yields. The presence of legumes in a mixture tends to increase forage nutritive values over that of grass alone as legumes have generally higher protein content and are more easily digested by grazing animals (Rochon et al. 2004). In one study, unfertilized species-rich hay meadows produced up to 60% higher yields than species-poor mixtures and over four years produced an average of 2.5 T ha⁻¹ (Bullock et al. 2001b). The productivity increase found in diverse mixtures is often attributed to the fertilizing effect of legumes through N fixation (Sleugh et al. 2000, Spehn et al. 2002). However, increases in yields have also been found in diverse mixtures without legumes, suggesting that this positive diversity effect is due to more than the N-fixing abilities of legumes (van Ruijven and Berendse

2003). It is likely that legumes explain part, but not all of the increases in productivity seen in diverse plantings, with complementarity and positive interactions among other species also playing a role (Tilman et al. 2001b).

Diversity can cause overyielding, where mixtures produce more biomass than expected based on monoculture productivity (Trenbath 1974). While a review of 44 studies showed that a majority of multi-species mixtures produced more biomass than the average monoculture, few produced more than the most productive monoculture (Cardinale et al. 2007). This suggests that care should be taken to design specific mixtures that are highly productive, rather than using random assemblages. As mentioned above, producers are already aware of the benefit of incorporating legumes into their grasslands, as legume-grass binary mixtures are more productive and of better forage quality than grasses alone (Sleugh et al. 2000). However, recent work on native grassland diversity has yet to be fully integrated into studies involving livestock grazing and biomass crop production.

The typical warm-season perennial biomass production system for biofuels typically uses fertilized grass monocultures, and a great deal of research has gone into switchgrass in particular (Parrish and Fike 2005). Diversity in a bioenergy production system is much less common, and there have been conflicting results as to their suitability in such a system. Some research suggests that “low-input high-diversity” grassland species mixtures can produce greater than 200% more bioenergy than monocultures (Tilman et al. 2006a). There are concerns that sowing a more diverse mixture of species may reduce the quality and quantity of the biofuel feedstock. In another experimental grassland, biofuel yields decreased as species richness increased, which the authors attributed to a decrease in warm-season grass cover and effects of different species’ chemical compositions (Adler et al. 2009). In the experiment by Adler et al. (2009), the highest ethanol yields were obtained from grasslands dominated by grasses, with relatively few species but higher total biomass and greater fiber concentrations.

Another purported effect of diversity in plantings is reduced weed invasion (Elton 1958). Many species of weeds are undesirable in pastures because they are less palatable, are of low nutritional value, or may even contain toxins that are detrimental to livestock (Bailey 1978, Uva et al. 1997). Because of this, weed management is a top concern in forage management, and identifying forage mixtures that naturally reduce weed biomass would benefit grazing systems.

One idea is that diverse communities will have more niches filled and resources used by desirable species, making it more difficult for invasive weeds to invade (Levine and D'Antonio 1999, Dukes 2001a, Hector et al. 2001). The related “diversity resistance hypothesis” proposes that increased diversity may be an important ecological barrier to weed invasion by decreasing the number and size of invading plants (Kennedy et al. 2002). Accordingly, diversity may increase interspecific competition, making it more difficult for weeds to obtain needed resources (Naeem et al. 2000).

Others contest the negative diversity-weed invasion relationship, suggesting instead that “diversity begets diversity” and that a species-rich community will result in a more heterogeneous environment for weeds to invade (Palmer and Maurer 1997, Foster et al. 2002). In this scenario, weed invasion is enhanced by facilitation caused by diversity. One explanation for these two conflicting diversity-invasibility viewpoints is the impact of extrinsic factors such as disturbance, fire, nutrient levels, climate, and human populations, all of which may obscure the direct effect of diversity on invasion (Naeem et al. 2000).

One concern about using NWSPs for forage and grazing is their low nutritive value, which can make producers cautious about using them as forage. Compared to cool-season species, native, warm-season grasses are less digestible and typically have lower protein contents (Balasko and Nelson 1995, Tracy et al. 2010). Due to their lower dry matter digestibility, warm-season grasses may not meet the energy requirements of all classes of grazing livestock (Collins and Fritz 1995). However, with appropriate management warm-season species can sustain beef cattle grazing (Roberts and Gerrish 1999a). Incorporating legumes into mixtures has been recommended to improve forage nutritive value, as legumes are nutritious and contain large amounts of protein. Some work has been done comparing warm-season grass-legume mixtures for compatibility, forage yield and nutritional quality and results show that in general the warm-season grasses produced more biomass but have lower protein concentrations than legumes or grass-legume mixtures (Springer et al. 2001).

Producers rely on grasslands to remain productive and to provide sufficient amounts of forage year after year, but when challenged by stresses like drought and heat, many pastures will suffer losses in yield. Increased forage diversity may help mitigate yearly fluctuations by increasing community stability. The “insurance hypothesis” proposes that diversity will buffer a

plant community against environmental changes because each species will respond differently to the change (Yachi and Loreau 1999, Loreau et al. 2001). As a community encounters environmental variation, some populations will decline, but will be compensated for within a community by increases in the abundance of other, functionally equivalent groups. In this way, diversity promotes the stability of ecosystem properties like productivity and nutrient cycling while resulting in the instability of individual populations (Hector et al. 2010). Diverse grassland mixtures may produce more stable yields across years (Tilman et al. 2006b) and to produce better yields during times of stress, when less diverse mixtures suffer larger production losses (Richardson et al. 2010). However, other research suggests that species identity and community composition may be more important than richness in explaining yield stability over time (Sanderson 2010).

One of the biggest challenges that producers face in using NWSPs is their establishment. Warm-season stands often do not typically reach full productivity until several years after establishment. In addition to a slow aboveground growth rate, high levels of seed dormancy and intense competition with weeds also prevent rapid establishment of warm-season grasses. Switchgrass, one of the most studied native, warm-season grass, is slow to reach full productivity due to initial high allocation to roots and takes three years to produce a mature stand (McLaughlin and Kszos 2005). Due to their slow growth in cool weather, switchgrass and other warm-season grasses are particularly vulnerable to competition from weeds, especially summer annuals such as crabgrass (*Digitaria* spp.) and foxtail (*Setaria* spp.), in their first year (Wolf and Fiske 1996, McLaughlin and Kszos 2005). Many producers may be hesitant to plant warm-season grasses for forage and hay because of their reputation for slow establishment and uncertainty of success; even with some form of weed control, there may be no harvestable yield in the first year (Martin et al. 1982). Seed dormancy can also hinder rapid establishment, where seed dormancy can be as high as 90% in some cultivars immediately after harvest (Sanderson et al. 1996). It is possible that using a diverse mix of NWSPs may help alleviate some of the risks of stand establishment. By using a mix of species, a producer may still be able to produce a viable stand even if one or two planted species have poor establishment.

The purpose of this chapter is to describe an experiment that evaluated the forage and weed biomass in fields sown with three NWSP forage mixtures of differing species diversity and under grazing and biomass crop management during the first three years of establishment, a

critical time for NWSP stand development. For this experiment, diversity is defined as species richness. The hypotheses tested were, that in comparison to monocultures, more diverse NWSP mixtures would 1) be higher-yielding, 2) have less weedy biomass, and 3) have greater nutritive value.

MATERIALS AND METHODS

Site

The experiment was located at Kentland farm near Blacksburg in Montgomery County, VA (37°12'0" N latitude and 80°34'40" W longitude). Soils were largely moderately- to well-drained Unison and Braddock cobbly soil, Unison and Braddock soils, and Guernsey silt loam, with a slopes from relatively flat to moderately steep (2 to 25 percent slopes). Climate data were collected each year from the Kentland Farm weather station. Historical climate data (averaging period of 1971 through 2000) were obtained from the National Oceanic and Atmospheric Administration's National Climatic Data Center (NCDC) using the weather station Pulaski 2 E, located at approximately 37°3' N latitude and 80°47' W longitude (NOAA Climatological Data Annual Summary 2009).

Experimental design

The experiment was established 2 to 4 June 2008 using four pastures (each between 1 and 1.1 ha) previously planted to endophyte-free tall fescue. The fields were first prepared by spraying vegetation with 2.3 l ha⁻¹ glyphosate one month before seeding. After vegetation was killed, each pasture was divided into three equal-sized plots. Each plot then was randomly assigned one of three seed mixtures: 1) a switchgrass monoculture, 2) a four-grass species mixture, or 3) a ten-species mixture (Tables 3.1 and 3.2) that resulted in a randomized complete block design with four replications. These three mixes were selected as ones that could reasonably be adopted by producers in forage systems. The ten species chosen for use in this study were taken from a similar mixture that has been used for establishing wildlife habitat, and are also regarded as hardy species that are generally easy to establish.

Seed mixtures were planted using a Haybuster® notill seed drill at a seeding density of 12.8 PLS kg ha⁻¹. Sown seed weights for each species in the multi-species plots were not evenly distributed (Table 3.2). Pelleted lime was incorporated with seed to improve flow through the drill. Weeds were controlled through an herbicide application in early June 2008 using Journey®, a mixture of imazapic (8.13%) and glyphosate (21.94%), applied at 0.88 l ha⁻¹ as a pre-emergent treatment to control weeds. All plots were mowed to a height of ~30 cm in early August to control weeds such as horsenettle (*Solanum carolinense* L.), thistles (*Cirsium* spp.), and crabgrass (*Digitaria* spp.). Due to poor establishment of switchgrass in 2008, two plots were reseeded on 11 March 2009 at 5.6 kg ha⁻¹ using a no-till drill. Thistles were controlled using a spot treatment of Forefront® (mixture of 2,4-D and aminopyralid) on 17 and 20 April 2009.

At the start of the 2009 growing season, two management methods (grazing or biomass production) were imposed on each treatment plot. For the biomass crop production treatment, one 5-m x 10-m enclosure was installed in each treatment plot using a single strand of electrified wire. The enclosures were randomly located within plots and excluded cows from these areas when they grazed. Vegetation within each enclosure was managed as a biomass crop for bioenergy production by allowing biomass to accumulate during the growing season and then harvesting the plots in late fall. Outside of the enclosures, warm-season pastures were grazed twice per year, using a stocking density of three cows ha⁻¹. Cows began grazing the plots in 2009 on 4 June for a length of two weeks and then again 3 August for one month. In 2010, cows grazed the plots starting 28 May for 16 days and 2 August for three weeks. Grazing began when forage height reached a minimum of 60 cm and continued until canopy height was reduced to 16 cm. Average cow weight prior to grazing was 687.8 kg in 2009 and 616.6 kg in 2010. Warm-season pastures were mowed between graze periods to a 30 cm canopy height and biomass crop treatments were mowed to 30 cm in late October each year.

Soil and vegetation sampling

Soil samples were collected in late fall 2008 for nutrient and organic matter content analyses. Plant biomass was initially sampled on 18-19 September 2008 using ten randomly placed 0.5-m² quadrats per plot. In 2009 and 2010, the size and number of quadrat samples were reduced to 0.25-m² and six quadrats per plot for the grazed areas, respectively. Three, 0.25-m²

quadrats were used to sample plant biomass in exclosures. In all three years, vegetation was sampled by cutting to 10 to 12 cm stubble height, sorting to NWSP species or weeds, and then drying at 55°C for 48 to 72 hours and weighed. In this experiment, it is important to note that the term “weed” was used to describe any unsown species. Weeds may therefore include both plant species of low nutritive value and low producer desirability as well as traditionally desirable cool-season forage species. Grazed area biomass sampling occurred 1 to 2 days before cows were allowed to graze plots. Biomass was also sampled once on 23 April 2010, when grazing and biomass crop plots were sampled at the same times.

Nutritive value analysis

Nutritive value analysis was performed on samples collected from the grazed areas prior to each grazing event: three samples per plot ($n = 36$) and two samples per plot ($n = 24$) were analyzed from 28 May and 31 July sampling dates in 2009, respectively. In 2010, samples collected from 25 May and 28 July were tested. Due to low biomass in harvested samples, five to eight samples of forages and weeds were tested for each mixture. In 2009, samples were representative of the entire quadrat used, with both sown and weedy species intermixed, while the 2010 samples were separated into either sown forage biomass or weedy biomass. Samples were ground in a Wiley Mill to pass through a 1-mm screen prior to analysis for ash content, neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP) concentrations (Goering and Van Soest 1970, PerkinElmer 1999, AOAC 2000). Samples were tested in duplicate and results were averaged. Sample dry matter was determined by drying and reweighing 0.5 g of each ground sample. The dried sample, also used in ash determination, was then placed in a cool muffle furnace, heated to 500°C and ashed for two hours at temperature, placed in a desiccator for 45 minutes, and reweighed.

Ankom 200 fiber analyzers were used for ADF and NDF determination. Approximately 0.5 g ground sample was placed into a filter bag and sealed. Samples were placed in the Ankom fiber analyzer along with two L Neutral Detergent Solution, 20 g sodium sulfite, and four ml heat-stable alpha-amylase, heated to 100°C, agitated for 75 minutes, and washed three times with two L hot dH₂O for five minutes each time. Four ml of alpha-amylase were added to each of the first two washes. Samples were then soaked in acetone for five minutes, dried and weighed.

Once processed for NDF, the same samples were used for ADF determination. Samples were placed in the Ankom fiber analyzer with two L Acid Detergent Solution, heated to 100°C agitated for 60 minutes, then rinsed three times with two L hot dH₂O for five minutes each time. Samples were then soaked in acetone for five minutes, dried and weighed.

Crude protein was determined using a combustion nitrogen determination procedure. A 40 to 60 mg ground sample was weighed into an aluminum combustion boat, which was then shaped into a small ball. Samples were loaded into a nitrogen analyzer (Perkin Elmer 2410 Series II) and analyzed along with controls (40 to 60 mg EDTA as an analytical standard) and blanks.

In 2009 bulked samples from each mixture, which included both forage and weed species, were analyzed for CP, ADF, and NDF. In 2010, samples were separated into sown NWSP forage species and unsown weedy species prior to nutritive value analysis to determine forage values along with total sample nutritive values. The total nutritive value, titled “Plot” on Tables 3.8 through 3.10, which represents both sown and weed species within each mixture was also estimated by multiplying the average treatment plot nutritive value by dry biomass proportion for both weeds and NWSP forages and adding these together. Some treatment plots were omitted if there was not sufficient NWSP or weed biomass present for analysis and due to this, plot values may not be directly comparable to NWSP or weed values.

Data analysis

Herbage mass was analyzed using a randomized complete block design, with the treatment factor being sown mixture (n = 3 treatments) and blocked by pasture (b = 4 blocks), with no replications of treatments within a block. Because the data describe NWSP stands as they were establishing, it was assumed that stands were not in equilibrium. Therefore, data from each year were analyzed separately.

Comparisons between grazed and biomass crop treatments were analyzed using a paired t-test. For this comparison, results were first averaged within each pasture or enclosure prior to statistical analysis, with four replications of each grazed area-enclosure pair per mixture. Plant community comparisons between matched grazed areas and biomass crop enclosures were analyzed using paired t-tests. In order to help identify the impact of NWSPs in 2009, nutritive

value was analyzed by regression, and CP, NDF, and ADF were individually regressed against the relative amount of NWSP grass cover within plots.

Data comparing the effect of mixture or management method were analyzed with an analysis of variance using SAS statistical software (SAS SAS Institute, Inc. 2008) to test for significant differences among treatments. Following the analysis of variance, means were separated using the macro pdmix800 (Saxton 1998). Data were checked for normality and equal variance and transformed when necessary. The most common transformation was taking the square root of dry weight biomass (i.e., sown, weedy and total) to address unequal variance. Responses for each treatment were separated using Fisher's test for least significant differences (LSD), with $\alpha = 0.05$.

RESULTS

Soil and weather

Soil nutrient concentrations, pH and organic matter content are listed in Table 3.3. No fertilizers or other soil amendments were added to the plots for the duration of the experiment.

Historic records from a nearby weather station show that the temperature from April through September averages 17.9°C in this area, while total precipitation averages 52.1 cm. In 2008, the average temperature and total precipitation from April through September were 17.7°C and 45.2 cm, respectively (Table 3.4). It was slightly warmer and wetter than average in 2009, with temperatures averaging 18°C and total precipitation accumulation of 58.7 cm from April-September. 2010 was the warmest and driest of the three years, with April through September temperatures averaging 19.6°C and precipitation totaling 36.6 cm. The spring and early summer of 2010 were relatively dry, with nearly half of the rain for the season falling in August and September.

Plant establishment

In 2008, forage grass species were relatively slow to establish, and overall forbs established earlier than grasses. Establishment of Virginia wild rye (*Elymus virginicus* L.) and switchgrass were poor. By the end of June some forbs and a very small number of grasses were

beginning to emerge. In early August the plots had many more forbs growing, and grasses were starting to establish as well. By harvest in September, many of the forbs were flowering, along with several switchgrass, indiangrass, and big bluestem plants. Indiangrass and big bluestem growth were better than the other perennial grasses in the large plots, and some plants were over 1 m tall by harvest. Little bluestem (*Schizachyrium scoparium* (Michx.) Nash) tended to remain small but frequently appeared in plots.

Total biomass

In 2008, total plant biomass, including both forages and weeds, was greatest for the four- and ten-species mixtures, and was two to three times higher than switchgrass monocultures (Table 3.5, $P < 0.0001$). By May 2009, total biomass was equal in all three mixtures ($P = 0.1482$). Biomass crop exclosures were installed and grazing began after this sampling date. With the exceptions of April and May 2010, biomass crop exclosures contained more biomass than grazed areas, but trends were similar within both management systems. Switchgrass monocultures contained significantly less total biomass than the diverse mixtures on nearly all sampling dates. The exceptions included April and May 2010, along with July 2010 biomass crop exclosures, where all three mixtures produced equal amounts of biomass ($P = 0.1557$). Within exclosures, total biomass tended to increase over the growing season for both years, although herbage mass was typically higher in 2009 than it was in 2010.

Sown biomass

Sown NWSP forage mass followed a similar trend to the total biomass, with monocultures containing less sown species biomass than mixtures in most cases (Table 3.6). In September 2008 and May 2009, all ten-species mixtures produced more forage biomass than four-species mixtures ($P < 0.0001$ for both dates). In 2008 the ten-species plots had the greatest yield and contained more than 15 times the amount of forage biomass than monocultures. By July 2009 and continuing for most of the duration of the experiment, the four- and ten-species mixtures produced equivalent amounts of sown biomass. The one exception was that of the grazed areas for April 2010, where sown biomass was once again different for all three mixtures

($P = 0.0003$). In this month, low amounts of switchgrass were present in the monoculture plots in April: 3.65 g/m^2 dry weight, accounting for only 2.6% relative cover.

Biomass crop exclosures usually contained more sown biomass later in the growing season, while spring (April and May) forage biomass was often much more similar between the two management types. In July 2009, exclosures contained on average 100-200% more NWSP biomass than grazed areas did. By July 2010, compared to biomass crop exclosures, the monoculture grazed areas had nearly 260 g m^{-2} less biomass, four-species mixture had 400 g m^{-2} less, and ten-species mixture contained 300 g m^{-2} less NWSP biomass. While sown biomass in grazed areas was typically greater in 2009 than in 2010, 2010 sown herbage mass was equal to or greater than 2009 data collections in biomass crop exclosures.

Weed biomass

Weed biomass did not differ across the three forage mixtures in 2008 and 2009, although in some instances there was a tendency for monocultures to contain more weed biomass, e.g., May 2009 (Table 3.7, $P = 0.0870$). In 2010 weed biomass was generally equal in mixtures subjected to grazing, with differences only seen in July 2010 ($P = 0.0110$). In this case, weed biomass was higher in switchgrass monocultures than in ten-species mixtures. In contrast, weed biomass was frequently significantly lower in monocultures than diverse mixtures in the biomass crop exclosures. Interestingly, only one 2010 biomass crop sampling date, July 2010, had a P -value above 0.10.

Comparisons between the two management systems showed that in many cases weed biomass was equal, although there were exceptions. However, it is important to note that while measured weed biomass may have often been equivalent in grazed areas and biomass crop exclosures, weed biomass in the grazed areas was most likely less than its potential total biomass due to tissue removal from grazing and mowing events. In both management systems, weed biomass was lower in 2010 than in 2009, especially later in the season. Late fall 2009 and 2010 biomass crop exclosure weed mass differences were particularly great. The October 2009 weed mass was over 5 times, 14 times, and 36 times greater than October 2010 monoculture, four- and ten-species weed biomass, respectively.

Forage nutritive value

Crude protein

Crude protein (CP) concentrations were not different for the three forage mixtures samples collected from May and July 2009, as well as from May 2010 (Table 3.8). It was only in July 2010 that differences were first seen. Both sown NWSP and estimated mixture CP were higher in monocultures than in four- and ten-species plots. Weed CP differences, when measured in 2010, were also evident ($P = 0.0511$), with weeds in monocultures also tending to have higher CP.

Crude protein decreased as stands matured in 2009. In 2010, forage CP also decreased from May to July, while weed CP remained the same or slightly increased over the same time period. Changes to the estimated plot CP in 2010 was dependent upon the forage:weed biomass ratio. For example, since switchgrass monocultures contained a higher proportion of weed biomass, estimated monoculture CP was higher in July.

Fiber concentration

In May 2009, switchgrass monocultures contained less neutral detergent fiber (NDF) than diverse mixtures ($P = 0.0005$), a trend that was also weakly significant in July 2009 (Table 3.9, $P = 0.0818$). When plants were separated into forages and weeds in 2010, NWSP NDF tended to also be lower in monocultures ($P = 0.0879$ in May, $P = 0.0319$ in July). In contrast, weed NDF did not differ in among the three mixtures. Estimated total plot NDF values were lower in monocultures in July 2010. Neutral detergent fiber typically increased as the season progressed. The two exceptions were weed NDF from monocultures and four-species mixtures, where NDF was greater in May 2010 compared to July 2010.

For most of the comparisons, acid detergent fiber (ADF) concentration did not vary among the three forage mixtures (Table 3.10). The three times where ADF was different was in May 2009, along with July 2010 forage species and estimated plot mixture values. In all three cases, the monoculture had lower ADF than the four- and ten-species mixtures.

Forage species and weed species nutritive value

Fiber concentrations typically were higher in the diverse mixtures in 2009, but there was no direct way of determining how much of this was due to forage versus weed species. Forage forbs and legumes only made up a small proportion of the forage biomass in the ten-species plots, which would reduce the impacts these species had on nutritive values. In order to understand any possible connection, a regression of fiber content against relative forage grass cover was run (Table 3.11). As the proportion of ground covered by sown grass species increased, NDF and ADF increased in both May and July 2009. The R^2 correlations were relatively small for all variables tested, between 0.24 and 0.37. These results suggest that the proportion of warm-season perennial grasses present in plots was associated with forage nutritive value, regardless of the mixture planted.

In 2010 the contributions of forage species and weed species to nutritive value were tested specifically (Table 3.12). In May, forages and weeds had similar CP and ADF, while NDF was greater in forages ($P = 0.0009$). In July, weed species had higher CP and lower fiber concentrations. While forage CP decreased by 5.4 g kg^{-1} from May to July, weed CP increased by 3.3 g kg^{-1} , to 17.3 g kg^{-1} .

DISCUSSION

This experiment provides evidence that more diverse forage mixtures may be advantageous over monocultures in systems managed for both grazing and biomass. The hypothesis that diverse plantings will have higher forage yields was confirmed in all three years and for both management systems, with switchgrass monocultures yielding less than the four- and ten-species mixes. The second hypothesis, that of reduced weed biomass in diverse NWSP mixtures, was true for several sampling dates in 2010, but varied by date and management system. The hypothesis that diversity increased forage nutritive value was not supported, however, with monocultures either having comparable nutritive values or even lower fiber concentrations in both 2009 and 2010.

Hypothesis one: increased forage productivity

Diverse mixtures performed better both when grazed and also when managed for just biomass. Switchgrass monocultures produced significantly less forage biomass than the four- and ten-species mixtures in all three years and in both grazing and biomass crop systems. This was especially true in the first year, when the diverse plantings yielded on average 2.5 times more total biomass and almost 12 times more NWSP forage biomass than the switchgrass monocultures. These trends continued in later years, with the four- and ten-species mixtures in grazed areas yielding on average nearly four times more forage biomass in 2009 and five times more in 2010. The hypothesis that there would be greater herbage mass in more diverse mixtures was also supported in biomass crop exclosures.

These results are in agreement with other grassland studies that have also found a positive relationship between diversity and productivity (Tilman et al. 1996, Hector et al. 1999). The diversity-productivity relationship has been attributed to several potential mechanisms, including positive species interactions and the increased odds of a high-yielding species being present in more species-rich plantings.

However, since only three specific mixtures were used, it was not possible to test for the general effects of diversity and determine whether species interactions or the presence of certain species influenced results. Although we cannot say for certain whether diversity per se affected results, it is likely that the performance of individual species may help explain at least some of the differences observed in the productivity of the three mixtures. Switchgrass herbage mass most likely had a large role in causing the differences between the monocultures and the more diverse plantings.

Diverse forage mixes may have additional benefits beyond that of increased productivity. Research suggests that diversity may help improve a plant community's resilience and resistance to environmental fluctuations (Elton 1958, McNaughton 1977, Hector et al. 2010). This can result in more consistent yields over time, as species more tolerant or suited to the particular environmental conditions will compensate for species that will not be as productive under the same conditions. Diversity may thus have a "portfolio effect," akin to the idea that a diverse set of investments can help reduce economic instability and protect an investor from any single major loss (Lehman and Tilman 2000).

The results suggest that diversity may be similarly important during the establishment stage of warm-season stand production, a time when neither community composition nor production has stabilized. Stand establishment is a primary concern for producers interested in growing NWSPs. Fields put into NWSP production have a reputation for taking longer to become fully productive than cool-season pastures, representing a loss in time and productive pasturage that could be grazed or harvested. Producers are also concerned about establishment success of these stands, as due to dormancy, low seedling vigor, slow growth, and weed competition, many NWSPs are difficult to establish (Beckman et al. 1993, Balasko and Nelson 1995). In general, a minimum plant density of 10 plants m⁻² is recommended for grazing (Launchbaugh and Owensby 1970). With seedling emergence estimated at 27 to 34% for untreated seed (Haynes et al. 1997), switchgrass is notorious for poor establishment and producers may need to decide between increasing seeding rates or risking stand failure.

At an early stage of stand development, planting more species may allow for more rapid stand establishment, as species adapted to disturbance and growth during the early stages of warm-season stand development can produce forage until the slower-growing species fully establish. This idea is analogous to using a more diverse mix of plants in prairie restoration to speed up the time to 100% vegetative ground cover (Piper and Pimm 2002). In this experiment, switchgrass establishment was relatively poor compared to the establishment of the other native species: the 2008 monoculture yields were very low, while the switchgrass proportion of forage dry weight was lower than its seeding proportion for both mixtures in that year. Stands that establish poorly may never produce high yields of NWSP forage due to weed competition (Launchbaugh and Owensby 1970). The presence of other species that established more rapidly such as big bluestem and black-eyed susan (*Rudbeckia hirta* L.) may have helped mitigate poor switchgrass establishment in more diverse mixtures, as evident by the higher forage yields in the more diverse plots.

Hypothesis two: reduced weediness

Warm-season stand establishment is frequently hindered by competition with weeds. While there was no difference in weed biomass among the three forage mixtures for the first two years, by 2010 the ten-species mixtures typically had significantly lower weed biomass in both

management systems. Unlike forage biomass, trends in weed biomass were not as consistent across management systems or sampling dates. This suggests that both environment and management, along with species diversity, may all impact weed biomass.

In addition, weed species richness (see Chapter Four) was higher in the switchgrass monocultures compared to the polycultures in all three years. Results support the prediction that over time, the more diverse forage mixes may reduce both weed biomass and richness. As the more diverse stands establish, they appear to become increasingly competitive against weeds, indicating establishing more diverse grasslands may reduce the time to the generation of relatively pure NWSP stands.

The potential negative relationship between diversity and weed invasion that was observed may be explained by two possible mechanisms (Wardle 2001). Firstly, NWSP diversity may help reduce weed invasion and persistence by using resources more completely and thus reducing resources available for weeds. Alternatively, increased diversity could increase the chance of including a competitive species that effectively excludes weeds. This idea is akin to the “selection effect” that is often used in studying the productivity-diversity relationship, as well as other measurable ecosystem properties (Huston 1997, Loreau and Hector 2001, Spehn et al. 2005). If this second mechanism, that of the inclusion of a species that excels at excluding weeds, is true, increased functional diversity may also help increase resistance to weed invasion more than just species richness (Pokorny et al. 2005), although some dispute this hypothesis (Wardle 2001).

Other factors may also help explain grassland invasibility. Some research suggests that species evenness is more important than diversity in explaining weed abundance, as more evenly distributed communities may have more complete resource use (Tracy et al. 2004). In addition to plant community composition, Weed invasion may also be affected by disturbance such as grazing livestock (Renne et al. 2006, Renne and Tracy 2007).

Results from this experiment show that both the four-species grass mixture and the more functionally diverse ten-species mixture had equivalent amounts of weed biomass and weed species richness. The ten-species plots did not have even species richness, as NWSP forbs and legumes comprised only a small part of total biomass. By 2010, four- and ten-species plots resembled each other more closely than they did at the time of establishment and therefore may

behave similarly in terms of total resource use and competitiveness. The amount of bare ground available for weed colonization has also been shown to affect invasion (Burke and Grime 1996). The switchgrass monocultures yielded low herbage mass throughout the experiment, leaving a large amount of ground potentially available for weed colonization. In September 2008, monocultures also had a significantly higher proportion of bare ground (25%, see Chapter Four) than diverse plantings, which may have promoted weed invasion and further reduced switchgrass productivity due to weed competition. Throughout the course of the experiment the more diverse mixtures had higher NWSP herbage mass and plant cover than the monocultures, which may have reduced weed access to resources such as nutrients and light and resulted in lower weed presence in the diverse mixtures.

Hypothesis three: improved nutritive values

Experimental results did not support the third hypothesis, which was that the more diverse mixtures would have higher nutritive values. The three mixtures had equivalent NDF, ADF, and CP levels in July of 2009 and late May 2010. When the nutritive values among the three mixtures varied, the switchgrass monoculture generally had higher CP and lower fiber contents. The lower NDF and ADF concentrations of switchgrass monocultures than other mixtures may be a result of the relatively high amount of weedy biomass present in these plots, which could reduce the contribution of the high fiber switchgrass. However, nutritive values were generally sufficient to maintain nonlactating cows, as well as some other classes of grazing livestock, in all three mixtures and over both growing seasons, suggesting that NWSPs can still be a viable forage choice. Both forage and total plot CP were above the threshold of 60 to 70 g kg⁻¹ CP required by livestock (Dougherty and Collins 1995). While diversity did not improve nutritive values, it is important to note that the more diverse mixtures had higher yields, which may still make them preferred forage choices over monocultures.

Sampling date and the amount of native, warm-season grasses impacted nutritive values more than forage mixture. Forage in early summer tended to have lower fiber concentrations and higher protein levels compared to samples taken in mid-summer. This is not unexpected, as fiber concentrations increase and protein levels decrease with plant maturation (Collins and Fritz 1995). Samples from plots with a high cover of sown grasses also tended to have higher NDF

and ADF levels in 2009, when samples were bulked and included both weeds and sown species. From May to July 2010, NWSP fiber concentrations increased by over 10% in diverse mixtures. The July 2010 forage NDF values were too high to maintain lactating cows, and approached or surpassed maximum NDF concentrations to meet the energy requirements of nonlactating animals (Mertens 1987). Even though lower nutritive values of NWSP pastures are predicted to reduce animal performance, studies have shown that livestock gain more weight than expected (Tracy et al. 2010). In addition, the inclusion of weed species may help reduce NDF concentrations to values where animal intake could potentially provide sufficient energy for nonlactating cows.

Interestingly, forage tests from 2010, when forage species were separated from weed species, show that neither NWSP forage nor weed nutritive values were affected by sown mixture in early summer. By July, forage nutritive values, but not weed values, differed between monocultures and the other two mixtures. In addition, as the season progressed NWSP samples generally were of lower nutritive value than weeds. Despite concerns about toxicity, several weed species contain high CP concentrations and digestible energy levels and only low levels of anti-quality compounds (Marten and Andersen 1975, Carlisle et al. 1980). In this experiment, some unsown “weed” species had high nutritional values, such as clovers (*Trifolium* spp.) and crabgrass. Other weeds also mature later in the season than NWSPs and may therefore have higher protein and lower fiber concentrations in mid-summer. Pasture nutritive value estimates based on both forage and weed species in July showed that there were still significant differences among pastures of different sown mixtures but once again, CP concentrations were adequate to support most classes of grazing livestock in all three mixtures. One limitation of the nutritive value portion of this study is that livestock plant choices were not monitored and therefore only potential nutritive values based on unbiased consumption of herbage can be reported, rather than values based on actual consumption by grazing animals.

Because sampling date impacted nutritive values significantly, careful management of NWSP stands could help maintain higher quality forage for grazing animals (Tracy et al. 2010). Grazing earlier in the summer and mowing to delay the formation of reproductive structures may both slow NWSP maturation and subsequent reduction in forage quality.

Grazing and biomass crop management

This experiment also tested how managing for either grazing or biomass accumulation might impact mixtures of varying diversity differently. The only time that the herbage mass from the two management systems could be directly comparable was in spring of 2010, when the plants began growing for the season and after having been mowed to the same height in fall of 2009. After just one year under different management, there were few differences in total, forage, or weed biomass between grazed areas and biomass crop exclosures. In April 2010, biomass crop exclosures sown with the more diverse mixtures contained more NWSP biomass. In May 2010, biomass crop exclosures still tended to have higher NWSP biomass. Weed biomass tended to be higher in grazed areas in both months but also was not significantly different.

One year of different management is most likely too short of a time to see significant, long-term differences in forage and weed biomass. However, the initial data suggest that grazing could potentially reduce early season forage mass and allow greater growth of invading weeds. This could be due to the weakening of forage plants caused by grazing, which would reduce their ability to compete with weeds. Grazing can impact competition among species and species abundances, and results can vary depending on species, climate, and grazing history (Milchunas et al. 1988, Bullock et al. 2001a). Biomass crops, which were only harvested at the end of the growing season, may therefore be better able to competitively exclude weeds from the plots.

Management choice and environmental conditions can also affect plant biomass. For example, precipitation explained up to 45% of the variation in annual forage production of semi-arid shortgrass regions (Lauenroth and Sala 1992). Furthermore, grazing may interact with precipitation to affect forage production. Shortgrass steppe that was grazed to a 60% consumption level had reductions in forage yields of 3% in wet years but 12% in dry years (Milchunas et al. 1994). In the present study, the July 2009 and 2010 biomass levels in the exclosures much more similar to each other in the grazing areas. In biomass crop exclosures, switchgrass yields in 2010 were 80% of 2009 yields, while the more diverse mixtures 2010 yields were approximately 66% 2009 yields. In contrast, the grazed area 2010 July yields were 25% and 33% of 2009 monoculture and mixture yields, respectively. The timing and amount of seasonal precipitation was most likely a large factor in explaining yield differences between

2009 and 2010. The summer of 2010 was much drier than 2009, especially from April through July, when warm-season species exhibit the bulk of their growth. As a result, plants in the grazed area remained green but did not grow significantly after the first grazing period in June 2010. Plants managed for biomass crops did not undergo this early season defoliation and were thus less affected by the dry summer.

CONCLUSIONS

Questions and challenges still remain in using diverse NWSP plantings as forage for grazing animals. This work suggests that monocultures may not be the optimal choice for livestock forage for a variety of reasons. Compared to the diverse mixtures, switchgrass monocultures performed poorly, with forage mass remaining significantly lower throughout the experiment. Diverse NWSP mixtures may help reduce the risk of poor establishment and low initial productivity, while also reducing weed biomass. The diverse mixtures were able to establish more quickly than the monocultures because they contained species that were able to grow and be productive in conditions that did not favor switchgrass establishment. The three mixtures also varied in their ability to produce vegetative cover, which may have a considerable impact on weed invasion. A high amount of bare ground in the first summer, as seen in the monoculture plots, may have created an environment where weeds could invade and compete for resources with sown forages more effectively. Finally, while NWSPs have been suggested for use as both forage and bioenergy crops, it may not be sustainable to manage a single stand for both objectives. Just a single year of grazing appeared to stress NWSP stands, relative to ungrazed areas managed for biomass.

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TABLES

Table 3.1: Ten-species pool used in experiment.

Functional group	Species name		Abbrev.
C4 grass	Switchgrass	<i>Panicum virgatum</i> L.	SG
C4 grass	Big bluestem	<i>Andropogon gerardii</i> Vitman	BB
C4 grass	Indiangrass	<i>Sorghastrum nutans</i> (L.) Nash	IG
C4 grass	Little bluestem	<i>Schizachyrium scoparium</i> (Michx.) Nash	LB
C3 grass	Virginia wild rye	<i>Elymus virginicus</i> L.	VWR
Legume	Illinois bundleflower	<i>Desmanthus illinoensis</i> (Michx.) MacMill. ex B.L. Rob. & Fernald	ILB
Legume	Showy tick trefoil	<i>Desmodium canadense</i> (L.) DC.	TF
Legume	Partridge pea	<i>Cassia fasciculata</i> Michx.	PP
Forb	Black-eyed susan	<i>Rudbeckia hirta</i> L.	BES
Forb	Oxeye sunflower	<i>Heliopsis helianthoides</i> (L.) Sweet	SUNF

Table 3.2: Grazing experiment seeding mixture composition by percent pure live seed (PLS) sown by weight. Sowing rate was 12.4 PLS kg ha⁻¹ for each treatment.

Species	One species	Four species	Ten species
	-----%-----		
SG†	100	20	10
BB		40	20
IG		30	25
VWR		10	10
LB			10
ILB			5
TF			5
PP			5
BES			5
SUNF			5

†See Table 3.1 for species abbreviations.

Table 3.3: Soil nutrient levels, soil pH, and organic matter content (OM) in 2008.

Mixture	P	K	Ca	Soil pH	OM (%)
	----- kg ha ⁻¹ -----				
One	26.8	186.1	1417.8	5.6	3.8
Four	32.9	191.6	1433.6	5.8	3.7
Ten	32.9	165.3	1586.5	5.8	4.1
Average	30.9	181.0	1479.3	5.7	3.9

Table 3.4: Historic and monthly average temperatures and total precipitation for April through September 2008, 2009, and 2010 in the Kentland Farm region.

Month	Historic Averages		2008		2009		2010	
	Temp (°C)	Precip (cm)	Temp (°C)	Precip (cm)	Temp (°C)	Precip (cm)	Temp (°C)	Precip (cm)
April	15.6	10.2	11.6	9.7	11.5	6.8	12.8	3.4
May	19.7	10.0	15.0	5.3	16.4	18.9	17.6	6.3
June	21.9	9.4	20.9	5.6	20.9	9.2	22.6	3.7
July	21.2	7.2	21.2	14.1	20.2	9.4	23.6	6.1
August	17.7	7.9	19.7	7.1	21.4	7.4	22.9	8.8
September	11.5	7.4	18.0	3.4	17.4	6.9	18.5	8.3
Avg Temp	17.9	--	17.7	--	18.0	--	19.6	--
Total Precip	--	52.1	--	45.2	--	58.5	--	36.6

Table 3.5: Mean total dry herbage mass, including sown species and weeds, from each of the three forage mixtures and two management systems. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
		----- g m ⁻² -----			
Sept. 2008†	---	41.4 \pm 8.5 ^a	89.8 \pm 9.4 ^b	123.5 \pm 16.4 ^b	< 0.0001
May 2009	---	67.4 \pm 8.7	87.8 \pm 10.5	85.7 \pm 6.3	0.1482
July 2009	Grazing	216.5 \pm 29.6 ^a	331.1 \pm 28.0 ^b	284.6 \pm 22.9 ^a	0.0021
	Biomass	365.0 \pm 42.3 ^a	671.9 \pm 92.2 ^b	742.1 \pm 93.6 ^b	0.0034
	P-value	0.0128	0.0148	0.0528	
Oct 2009§	---	635.3 \pm 72.1	828.2 \pm 83.3	793.7 \pm 119.1	0.2956
April 2010	Grazing	38.1 \pm 5.8	34.5 \pm 6.5	47.2 \pm 8.9	0.3639
	Biomass	38.8 \pm 7.4	50.8 \pm 7.8	57.5 \pm 8.9	0.1986
	P-value	0.9583	0.0346	0.3691	
May 2010	Grazing	104.7 \pm 18.2	130.5 \pm 18.0	135.2 \pm 18.2	0.2163
	Biomass	87.3 \pm 19.1	106.2 \pm 16.4	117.0 \pm 26.9	0.5097
	P-value	0.5829	0.5717	0.5166	
July 2010	Grazing	57.9 \pm 8.0 ^a	112.7 \pm 14.2 ^b	100.4 \pm 48.8 ^b	0.0032
	Biomass	294.5 \pm 58.0	500.3 \pm 66.0	405.6 \pm 62.1	0.0863
	P-value	0.0439	0.0024	0.0054	
Oct. 2010	Grazing	88.3 \pm 22.4	133.8 \pm 30.4	128.0 \pm 21.4	0.2741
	Biomass	333.4 \pm 65.4 ^a	507.5 \pm 49.0 ^b	550.5 \pm 62.4 ^b	0.0230
	P-value	0.0152	0.0276	0.0165	

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

§ October 2009 data is from the biomass crop enclosure.

Table 3.6: Sown native, warm-season perennial dry herbage mass from each of the three forage mixtures and two management systems. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
----- g m ⁻² -----					
Sept. 2008†	---	5.2 \pm 1.1 ^a	39.1 \pm 5.6 ^b	81.4 \pm 12.7 ^c	< 0.0001
May 2009	---	3.6 \pm 1.0 ^a	20.8 \pm 2.4 ^b	28.9 \pm 1.4 ^c	< 0.0001
July 2009	Grazing	48.6 \pm 15.0 ^a	192.2 \pm 35.0 ^b	172.8 \pm 28.9 ^b	0.0004
	Biomass	125.2 \pm 37.8 ^a	365.6 \pm 59.9 ^b	538.6 \pm 93.8 ^b	0.0005
	P-value	0.1574	0.0922	0.0610	
Oct 2009§	---	144.3 \pm 36.9 ^a	458.7 \pm 100.8 ^b	508.6 \pm 83.6 ^b	0.0014
April 2010	Grazing	3.6 \pm 1.4 ^a	10.3 \pm 3.1 ^b	17.1 \pm 3.1 ^c	0.0003
	Biomass	10.1 \pm 2.9 ^a	34.1 \pm 7.3 ^b	48.0 \pm 8.0 ^b	0.0010
	P-value	0.1143	0.0123	0.0200	
May 2010	Grazing	13.02 \pm 5.0 ^a	53.2 \pm 8.8 ^b	68.3 \pm 15.0 ^b	< 0.0001
	Biomass	32.5 \pm 13.5 ^a	84.1 \pm 16.2 ^b	96.5 \pm 25.6 ^b	0.0064
	P-value	0.1295	0.1771	0.4165	
July 2010	Grazing	16.0 \pm 5.7 ^a	83.8 \pm 12.3 ^b	84.2 \pm 11.0 ^b	< 0.0001
	Biomass	274.6 \pm 60.2	487.0 \pm 68.7	381.3 \pm 65.2	0.1557
	P-value	0.0344	0.0033	0.0133	
Oct. 2010	Grazing	28.1 \pm 13.5 ^a	74.8 \pm 17.0 ^b	95.6 \pm 20.1 ^b	0.0053
	Biomass	240.7 \pm 74.4 ^a	481.5 \pm 58.9 ^b	542.7 \pm 67.0 ^b	0.0032
	P-value	0.0009	0.0213	0.0115	

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

§ October 2009 data is from the biomass crop enclosure.

Table 3.7: Weedy (unsown) dry herbage weights from each of the three forage mixtures and two management systems. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
----- g m ⁻² -----					
Sept. 2008†	---	36.2 \pm 8.0	50.7 \pm 10.8	42.0 \pm 8.6	0.7743
May 2009	---	53.1 \pm 5.6	43.1 \pm 5.8	40.6 \pm 1.6	0.0870
July 2009	Grazing	167.9 \pm 23.5	138.9 \pm 18.7	111.8 \pm 22.2	0.1511
	Biomass	239.8 \pm 55.5	306.3 \pm 89.3	203.5 \pm 63.9	0.5781
	P-value	0.0485	0.2623	0.2998	
Oct 2009§	---	491.0 \pm 84.0	369.5 \pm 109.7	285.1 \pm 151.4	0.1207
April 2010	Grazing	34.5 \pm 5.7	24.2 \pm 5.7	30.1 \pm 9.2	0.2913
	Biomass	28.6 \pm 7.9 ^a	16.6 \pm 4.6 ^{ab}	9.5 \pm 2.0 ^b	0.0314
	P-value	0.6948	0.3827	0.1718	
May 2010	Grazing	91.7 \pm 18.2	77.3 \pm 18.3	67.0 \pm 17.7	0.4337
	Biomass	54.8 \pm 18.4	22.1 \pm 5.7	20.5 \pm 7.1	0.0652
	P-value	0.1531	0.1398	0.1781	
July 2010	Grazing	41.9 \pm 7.2 ^a	28.9 \pm 9.6 ^{ab}	16.2 \pm 4.5 ^b	0.0110
	Biomass	20.0 \pm 7.3	13.3 \pm 6.1	24.2 \pm 12.0	0.8136
	P-value	0.1700	0.2111	0.7537	
Oct. 2010	Grazing	60.2 \pm 18.6	59.0 \pm 22.7	32.4 \pm 13.1	0.2590
	Biomass	92.7 \pm 25.9 ^a	26.0 \pm 17.6 ^b	7.8 \pm 6.8 ^b	0.0003
	P-value	0.4189	0.0237	0.1094	

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

§ October 2009 data is from the biomass crop enclosure.

Table 3.8: Crude protein (CP) values for each forage mixture. For 2010 data, native, warm-season perennial (NWSP) values are for sown forages, weed values are for unsown species, and plot represents the values of all plants within the mixture, taking NWSP forage and weed biomass proportions into account. Values are mean \pm one standard error.

Date	Sample Type	One species	Four species	Ten species	P-value
		----- g kg ⁻¹ -----			
May 2009†	--	174 \pm 13	151 \pm 12	158 \pm 11	0.2780
July 2009	--	117 \pm 6	98 \pm 7	119 \pm 12	0.1321
May 2010	NWSP	160 \pm 12	165 \pm 9	138 \pm 9	0.1201
	Weed	140 \pm 5	133 \pm 15	146 \pm 14	0.7781
	Plot	138 \pm 4	147 \pm 9	146 \pm 9	0.7414
July 2010	NWSP	113 \pm 2 ^b	98 \pm 5 ^a	94 \pm 4 ^a	0.0300
	Weed	198 \pm 19	154 \pm 17	151 \pm 11	0.0511
	Plot	173 \pm 14 ^b	117 \pm 9 ^a	106 \pm 6 ^a	0.0029

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD; corresponding P-values are listed for each row.

Table 3.9: Neutral detergent fiber (NDF) concentration for each forage mixture. For 2010 data, native, warm-season perennial (NWSP) values are for sown forages, weed values are for unsown species, and plot represents the values of all plants within the mixture, taking NWSP forage and weed biomass proportions into account. Values are mean \pm one standard error.

Date	Sample Type	One species	Four species	Ten species	P-value
		----- g kg ⁻¹ -----			
May 2009†	--	411 \pm 46 ^a	585 \pm 36 ^b	559 \pm 33 ^b	0.0005
July 2009	--	577 \pm 20	668 \pm 28	614 \pm 38	0.0818
May 2010	NWSP	611 \pm 7	597 \pm 11	586 \pm 27	0.0879
	Weed	497 \pm 30	541 \pm 46	462 \pm 46	0.9543
	Plot	520 \pm 20	566 \pm 16	508 \pm 54	0.5257
July 2010	NWSP	645 \pm 10 ^a	675 \pm 7 ^b	680 \pm 9 ^b	0.0319
	Weed	390 \pm 27	480 \pm 63	506 \pm 63	0.1068
	Plot	420 \pm 48 ^a	608 \pm 36 ^b	645 \pm 17 ^b	0.0043

Note: Plot values are estimates based on average values from each treatment plot. Some plots were omitted if there was not sufficient NWSP or weed biomass present for analysis and due to this, plot values may not be directly comparable to NWSP or weed values.

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD; corresponding P-values are listed for each row.

Table 3.10: Acid detergent fiber (ADF) concentration for each forage mixture. For 2010 data, native, warm-season perennial (NWSP) values are for sown forages, weed values are for unsown species, and plot represents the values of all plants within the mixture, taking NWSP forage and weed biomass proportions into account. Values are mean \pm one standard error.

Date	Sample Type	One species	Four species	Ten species	P-value
		----- g kg ⁻¹ -----			
May 2009†	--	259 \pm 20 ^a	318 \pm 15 ^b	306 \pm 14 ^b	0.0034
July 2009	--	340 \pm 7	379 \pm 13	356 \pm 18	0.1554
May 2010	NWSP	278 \pm 6	285 \pm 4	298 \pm 8	0.8304
	Weed	286 \pm 13	285 \pm 16	280 \pm 14	0.4112
	Plot	281 \pm 7	286 \pm 6	283 \pm 14	0.9563
July 2010	NWSP	319 \pm 9 ^a	341 \pm 5 ^b	350 \pm 6 ^b	0.0243
	Weed	272 \pm 20	321 \pm 42	298 \pm 21	0.2893
	Plot	278 \pm 19 ^a	331 \pm 16 ^b	340 \pm 9 ^b	0.0402

Note: Plot values are estimates based on average values from each treatment plot. Some plots were omitted if there was not sufficient NWSP or weed biomass present for analysis and due to this, plot values may not be directly comparable to NWSP or weed values.

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD; corresponding P-values are listed for each row.

Table 3.11: Relationship between sown grass and forage nutritive values for May and July 2009. Regression is based on forage grass cover, relative to total vegetated cover.

Date	Variable	Equation	P-value	R ²
May 2009	NDF†	393 + 4.1x	0.0002	0.3433
	ADF	252 + 1.4x	0.0026	0.2374
July 2009	NDF	540 + 2.1x	0.0015	0.3749
	ADF	329 + 0.8x	0.0107	0.2612

† NDF: neutral detergent fiber; ADF: acid detergent fiber

Table 3.12: Comparison between NWSP forage and weed samples for May and July 2010. Values are mean \pm one standard error.

Date		Forage	Weed	P-value
		----- g kg ⁻¹ -----		
May 2010 ^{†‡}	CP	154 \pm 6	140 \pm 7	0.1502
	NDF	596 \pm 11	500 \pm 24	0.0009
	ADF	288 \pm 4	284 \pm 8	0.6552
July 2010	CP	100 \pm 3	173 \pm 11	<0.0001
	NDF	670 \pm 6	447 \pm 29	<0.0001
	ADF	339 \pm 4	293 \pm 16	0.0009

[†]Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD.

[‡] CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber

Chapter Four

Changes in plant species community composition of three native, warm-season perennial mixtures managed for grazing or biomass

ABSTRACT: In typically managed forage-livestock grasslands, plant diversity tends to be low, and any diversity present is often a result of weedy, transient species. Diverse forage mixtures can potentially provide several advantages, such as increased productivity and reduced weed biomass. The objective of this experiment was to study how management (grazing or biomass crops) and forage mixture diversity affect native, warm-season perennial (NWSP) community composition during the first three years of establishment. Increasing NWSP diversity increased forage species cover and reduced weed species cover. With grazing, forage cover of diverse mixtures was four to five times higher than cover in switchgrass monocultures. Grazing tended to increase forage cover and reduce weed cover and bare ground. Forage species richness was greater in biomass crop exclosures, while weed species richness was highest in switchgrass monocultures and in grazed areas. Forage species composition shifted over the three years of study, as quickly-establishing forbs which contributed over 50% of forage biomass in the first year were replaced by perennial grasses. Unexpectedly, grazing did not increase forb or legume abundance. Although community composition changes occurred, forage mixture had little effect on measurements of community dissimilarity, the Shannon index of diversity (H'), or evenness. Grazed areas tended to have higher H' values than biomass crop areas, largely due to increased weed richness. More diverse NWSP mixtures may reduce weed species richness and cover. These findings also suggest that grazing, even just for two years, can have impacts on NWSP forage communities.

INTRODUCTION

Ecological studies have indicated that plant species diversity may have a relationship with many ecosystem properties and functions. Research suggests that incorporating diversity into forage systems may improve agronomic value along with a variety of other services, such as increased forage yield, providing flexibility to the grazing system (Moore et al. 2004, Sanderson et al. 2004b), reduced toxin consumption, and creating a more balanced diet for livestock (Tracy and Faulkner 2006). Other positive effects of diversity may include reduced weed invasibility

(Tracy and Sanderson 2004a), increased system stability (Schwartz et al. 2000), increased yield reliability (Milcu et al. 2010), and enhanced nutrient cycling (Tilman et al. 1996). However, many northeastern U.S. pastures are dominated by just a few cool-season forage species and any diversity present is typically from transient weedy species that provide little forage value (Tracy and Sanderson 2000, Sanderson et al. 2007). In addition, while most studies have used cool-season species, ones that have included native prairie plants have produced similar results (Tilman et al. 2001b).

While research suggests there are benefits to managing grasslands for greater levels of diversity, diverse plant mixtures provide management challenges when compared to monocultures. Planting may be more complicated, and weed control can be difficult if not all sown plants are tolerant to the same herbicides. Diversity, defined in this case as species richness, may be difficult to maintain over long periods of time due to poor establishment or local extinctions, although other experiments have shown only small reductions in species richness several years after establishment (Tilman et al. 2001b, Tracy and Sanderson 2004b, Spehn et al. 2005). Plant community richness and species abundances depend on several factors, including plant interactions like competition and facilitation, along with external variables such as weather and disturbance (Hobbs and Huenneke 1992, Hacker and Bertness 1999, Renne and Tracy 2007, Kreyling et al. 2008). Plant diversity is typically expected to be low when community perturbation levels are either low or high, and to peak at intermediate levels of stress, disturbance or herbivory (Hobbs and Huenneke 1992).

In addition to affecting species richness, disturbance may impact community aspects such as species composition, productivity and invasibility (Gibson 1989, Hobbs and Huenneke 1992, Bartolome et al. 2004, Renne and Tracy 2007). The impacts of disturbance may shift over time, as one study found that high disturbance initially promoted species invasion, while over time invaders persisted best at low disturbance levels (Clark and Johnston 2011). In forage or biomass crop systems, disturbance events include haying, mowing and livestock grazing. The actions of grazing livestock are a major source of disturbance in grasslands and have been implicated in impacting several aspects of community composition (Hobbs and Huenneke 1992).

Grazing plays an important part in maintaining grassland diversity. In grasslands, grasses are the dominant species and in the absence of grazing would normally competitively exclude

many other species (Olf and Ritchie 1998, Hickman et al. 2004). Grazing has been shown to increase species diversity, richness, and evenness and cause changes in species dominance (Collins et al. 1998, Altesor et al. 2005), although other studies have found few or no plant community differences caused by grazing (Stohlgren et al. 1999b, Scimone et al. 2007, Lewis et al. 2008). Ungulates on the Serengeti have been credited with increasing plant diversity by increasing spatial pattern and reducing interspecific competition among plants (McNaughton 1983). In addition to reducing dominance by grasses, grazing animals can alter community composition through selection preferences, lowering the competition for less-preferred forage species (Howe 1994). Community composition is also affected by the intensity of grazing, and species abundances may shift when grasslands are subjected to light, moderate, or heavy levels of grazing (Howe 1994).

Both plant species traits and the timing of grazing are important in understanding changes in community composition over time (Bullock et al. 2001a). While grazing is often considered an action that reduces species abundance, many grassland species are adapted to frequent defoliation events and have coevolved with grazing animals. “Grazophils” require grazing animals to persist and can include normally dominant species that will disappear in the absence of grazing (McNaughton 1979). Grazing intensity may result in the selection of species with specific growth habits that increase tolerance of frequent defoliation, including prostrate growth habits, annual life cycles, stoloniferous or rosette growth forms, increased tillering, and meristems found close to the ground (Belsky 1986, Altesor et al. 2005, Diaz et al. 2007). However, such traits may make grazing-tolerant species poorer competitors in less disturbed environments, where competition for space and light are limiting factors (Belsky 1986).

Diversity has also been proposed as a mechanism of community resistance to weed invasion (Elton 1958). The precise relationship between community diversity and weed invasion remains unclear and is further complicated by comparing natural versus constructed communities as well as including other factors that covary with diversity such as stress, disturbance, and competition, along with weather events (Levine and D'Antonio 1999). In one study, when these external factors were removed, plant diversity suppressed weed invasion (Naeem et al. 2000). Other research suggests that while both weather events and diversity can affect invasibility, weather events such as a severe drought or rainfall event can have similar effects on communities regardless of species richness level (Kreyling et al. 2008). Similar to the potential

positive grazing-diversity effects, grazing can also promote weed colonization by generating a heterogeneous habitat through the creation of openings in the canopy and bare patches of soil (Bergelson et al. 1993). Research from Argentina provides evidence that non-native grazers (cattle and horses) increased weed invasion in natural grasslands (Alejandro et al. 2010). In another study, a single intense cattle trampling event following rain altered pasture composition, which temporarily reduced forage cover and increased weedy cover but also had a longer-term impact of increased weed species richness in the seed bank (Renne and Tracy 2007).

We were interested in comparing the effects that management had on species composition for three different forage mixtures. Because managing native, warm-season perennial species (NWSPs) for either grazing or biomass may impact the types and intensities of disturbances, there is ample reason to believe that community composition will not remain the same under these two management methods.

The primary objective of this study was to evaluate how plant species composition of three NWSP mixtures would change over the first three years of stand establishment. A second objective was to determine how management for either grazing or biomass might affect species composition. Any changes to both forage and weed species community components were investigated. Two hypotheses were tested. The first was that more diverse mixtures would have higher forage cover and lower weed cover and richness. The second hypothesis was that communities sown with identical NWSP mixtures but placed under different management methods would diverge, so that grazed areas would have greater total species richness (both forage and weed species) than biomass crop exclosures

MATERIALS AND METHODS

See Chapter Three for information on site location and experimental design. Tables 3.1 and 3.2 describe NWSP species used and mixture seed compositions.

Data Collection

Data on species biomass and ground cover were collected on 18-19 September 2008 using ten randomly placed 0.5-m² quadrats per plot. In 2009 and 2010, the number of quadrat

samples was reduced to six, 0.5-m² quadrats per plot for the grazed areas and three, 0.5-m² quadrats were used to collect data in biomass exclosures. Grazed area biomass was sampled prior to grazing in late May and again in late July for both 2009 and 2010, with an additional sampling period in April 2010. Biomass crop exclosures were sampled in July and October 2009 and then again in April, May, July and October 2010. At each sampling date, a visual botanical assessment was performed before animal entry using randomly placed 0.5-m² quadrats. Each species within the quadrat was identified and its percent of ground cover recorded. After visual assessment, one-half of each 0.5-m² quadrat was randomly harvested to 10 to 12 cm and sorted to NWSP species or weeds, dried at 55°C for 48 to 72 hours, and then weighed.

Measures of Community Similarity and Evenness

Plant community compositional similarity and evenness (based on relative abundances of species in a community) were estimated between associated grazed areas and biomass crop exclosures using data from July 2009, May 2010, and July 2010. The sampling dates provide the closest representation of how the areas differ in terms of sown species, along with any weeds present. Grazed areas and exclosures within each treatment plot were used to calculate the Bray-Curtis dissimilarity index (BC_{ij}), based on vegetated relative cover (McCune et al. 2002):

$$BC_{ij} = 100 * \sum |x_{ik} - x_{jk}| / \sum (x_{ik} + x_{jk})$$

where BC_{ij} is the difference between exclosure i and grazed area j, and x_{ik} and x_{jk} represent the relative vegetated cover for species k in exclosure i or grazed area j, respectively. Because vegetated relative cover is used, $\sum (x_{ik} + x_{jk})$ always equals 200. As the value for BC_{ij} approaches 100, communities become increasingly different from each other.

Plant community richness was calculated for each forage mixture in the grazed areas and biomass crop exclosures based on vegetated relative cover using the Shannon index of diversity, H' and evenness, E_H (Magurran 1988):

$$H' = -\sum p_i \ln(p_i)$$

$$E_H = H' / \ln(S)$$

where p_i is the proportion of vegetated cover contributed by species i and where S is the number of species present. As H' increases, communities are generally more species rich. When E_H equals 1, the abundance of each species in the community becomes equal.

Data analysis

Species composition, cover, and percent herbage mass were analyzed using a two-way factorial randomized complete block design, with the treatment factors being sown mixture (the three forage mixtures) and management (grazing or biomass crop), and blocked by pasture. Comparisons between management methods were analyzed using a paired t-test, pairing each grazed area with the enclosure contained within it. For this comparison, results were first averaged within each pasture or enclosure prior to statistical analysis, with four replications of each grazed area-enclosure pair. Plant community comparisons between matched grazed areas and biomass crop enclosures were analyzed using paired t-tests.

All data were analyzed using SAS statistical software (SAS SAS Institute, Inc. 2008) to test for significant differences. The macro pdmix800 was used to investigate results from the two-way factorial portion of the experiment (Saxton 1998). Data were checked for normality and equal variance and transformed if necessary. Responses for each treatment were separated using Fisher's test for least significant differences (LSD), with $\alpha = 0.05$.

RESULTS

Soil and weather data have been presented in Chapter Three, in Tables 3.3 and 3.4.

Forage cover

Forage cover (relative to the cover of other species) was differed across the three mixtures on every sampling date and in both management methods, with the switchgrass (*Panicum virgatum* L.) monoculture having the lowest forage ground cover (Table 4.1). Compared to more diverse mixtures, initial NWSP cover was very poor in monocultures, with only 3% to 4% of ground covered by switchgrass in September 2008 and May 2009, compared to 20% to 38% NWSP cover in diverse mixtures for the same period. Additionally, the ten-

species plots had greater initial NWSP ground cover compared to four-species plots. After May 2009, four- and ten-species plots usually had equivalent proportions of forage cover.

In the grazed areas, switchgrass monoculture NWSP cover was always lower than the diverse mixtures. Forage ground cover never exceeded 10.3% in grazed monocultures, while the ten-species mixtures nearly reached 50% cover in July 2009. Biomass crop enclosure relative cover was frequently greater than that in the grazed areas. Monoculture NWSP cover (30.6%) was greater than for grazed plots in July 2010 and not significantly different from that of the four-species mixtures (59.7%). Ten-species plots also produced the highest amount of forage cover in July 2010, at 67.6%.

Weed cover

Trends in weed cover were opposite those of forage cover (Table 4.2). With the exception of September 2008, switchgrass monoculture plots had the greatest weed cover of the three mixtures under both management methods ($P = 0.52$ for September 2008, and $P < 0.10$ for all other sampling dates). In September 2008, weed cover was 30% to 35% for all three mixtures. Weed relative cover tended to increase during the growing season, reaching maximum values in July 2009 and May 2010.

Management effects on weed relative cover were variable. Throughout 2009 and 2010, biomass crop enclosures tended to have lower amounts of weed cover in five cases ($P < 0.10$). However, of these five instances, three occurred in the switchgrass monoculture. In the grazed areas, weed cover was greater than 80% in monocultures in both July 2009 and May 2010. Weeds covered nearly 50% or more of the ground in grazed monocultures throughout 2009 and 2010. Weed cover in grazed diverse mixtures was approximately 50% to 60% through July 2010. In July 2010, weed cover was nearly half that of May 2010, with weed cover in switchgrass monocultures also showing marked decreases.

While biomass crop enclosure weed cover was greater than 50% for most of 2009 and 2010 in monoculture plots, cover in either of the diverse mixtures was only greater than 50% once during the same time period. As in the grazed areas, weed cover dropped sharply by July 2010.

Bare ground

Bare ground was greatest at the start of the experiment (Table 4.3). Switchgrass monocultures had 25% bare ground in September 2008, more than either of the diverse mixtures ($P = 0.0282$). After this, bare ground decreased as NWSPs and weed species continued to establish. Bare ground was greatest in spring, prior to the main part of the NWSP summer growth period. After 2008, the only time that mixture affected bare ground was in the grazed areas in July 2010, when once again, monocultures had the most bare ground, with nearly three times more bare ground than diverse mixtures ($P = 0.0115$).

While sown forage mixture did not usually affect bare ground, management did. In 2010, biomass crop exclosures contained more bare ground than grazed areas, with one exception of July 2010 monocultures ($P = 0.5571$ for July monocultures, $P < 0.10$ for all others). Whereas bare ground in grazed areas was nearly always less than 10% in 2009 and 2010, bare ground in biomass crop exclosures ranged from 10% to 25%.

Species richness

Total species richness was low in September 2008 (Table 4.4). Monocultures had only 3.2 species per 0.5 m² quadrat, while ten-species plots were the most species-rich at 6.8 species per quadrat ($P = 0.0007$). By May 2009, total richness had increased for all mixtures, although ten-species plots still were more diverse ($P = 0.0068$). In most cases, total species richness increased as forage mixture diversity increased. The two exceptions were for grazed areas in April 2010 and July 2010, where all three mixtures were equally species-rich. While forage mixture usually affected richness, management type did not seem to have major impacts. In July 2010, there was a tendency for biomass crop exclosures to contain approximately 0.5 to 1.5 fewer species per 0.5 m² quadrat, P -values were near 0.10, making the trend weak.

Sown forage NWSP richness always differed by forage mixture and also frequently with management (Table 4.5). Monocultures typically contained 0.8 to 1 species per quadrat, with four-species plots and ten-species plots containing 1.8 to 2.8 species and 2.8 to 5.0 species, respectively. In 2010, NWSP species richness in the two diverse mixtures was typically greater in biomass crop exclosures than in grazed areas.

The effects of forage mixture and management on weed species richness were more variable (Table 4.6). Weed species richness was not affected by forage mixture in 2008 and spring 2009 ($P = 0.3063$ in September 2008 and $P = 0.4079$ in May 2009). As stands established and matured, weed species richness was often lower in more diverse mixtures. Differences were especially noticeable in July 2009 and 2010, where monocultures in both grazed areas and biomass crop exclosures contained 1.4 to 3 weed species per quadrat more than diverse mixtures. By July 2010, management affected weed species richness in all three mixtures, although only weakly for monocultures ($P = 0.0639$). Biomass crop exclosures sown with diverse forage mixtures had approximately two fewer weed species per quadrat compared to grazed areas.

Species composition

Dry weight proportions

Biomass proportions were used as a general method to compare mixtures and management methods. In 2008, the proportion of dry herbage mass due to weed species in switchgrass monocultures was nearly double that of more diverse mixtures (Table 4.7a). Four-species plots contained the most sown grasses ($P < 0.0001$), although ten-species plots had a similar proportion of sown biomass if forage forbs were included. Weed and forage species proportions in grazed plots in 2009 differed for all three mixtures, with monocultures having the lowest sown proportion and the greatest weed proportion. Forage forb proportions were much lower in grazed areas in 2009, at only 3.4%, compared to 30% in 2008 (Table 4.7b). In biomass crop exclosures, monocultures tended to have a lower forage grass proportion ($P = 0.1294$) and a higher proportion of weed biomass ($P = 0.0477$). There were no differences when comparing grazed areas to biomass crop exclosures in 2009, although in biomass exclosures more biomass tended to be due to forage species than in grazed areas.

Just as in 2009, in 2010 grazed area biomass contributions from forage species were greater in more diverse mixtures, while weed proportions were lower (Table 4.7c, $P < 0.0001$ for both forages and weeds). Forage species contributions were nearly 3.5 times higher in grazed diverse mixtures than in monocultures, and in monocultures the bulk of plant biomass, nearly 80%, was from weed species. Dry weight proportions of weeds in biomass crop exclosures were not different among the three mixtures, with sown forage species contributing 80% to 90% of

biomass. Management method only affected biomass proportions of switchgrass monocultures, with biomass exclosures having a greater amount of biomass contributed by switchgrass than grazed areas, and less by weed species ($P = 0.0262$). While not significant, forage forb biomass proportions also tended to be greater in biomass crop exclosures.

Four-species mixtures

Switchgrass establishment in the four-species mixture was poor in 2008, providing only 5% of forage species biomass in 2008 (Table 4.8a). Big bluestem (*Andropogon gerardii* Vitman) established better than the other three grasses and produced the most biomass in 2008, producing 62% of forage biomass, while indiagrass contributed 33% of forage biomass. Virginia wild rye (*Elymus virginicus* L.) was not common in 2008.

In 2009, switchgrass proportions increased, to 9% in grazed areas and nearly 19% in biomass exclosures. Big bluestem proportions dropped by nearly half in both management methods, while indiagrass (*Sorghastrum nutans* (L.) Nash) biomass proportions increased. Virginia wild rye remained relatively rare in grazed areas and was nearly nonexistent in biomass crop exclosures. Switchgrass biomass proportions continued to increase in 2010 under both management methods, to over 20% in biomass crop exclosures. In grazed areas, big bluestem proportions were reduced to one third that of 2010, while indiagrass increased to 75% of forage biomass. In biomass exclosures big bluestem proportions increased and indiagrass proportions decreased from 2009 to 2010.

Ten-species mixtures

In 2008, black-eyed susan (*Rudbeckia hirta* L.) contributed the bulk of the forage biomass, at nearly 50% (Table 4.8b). Indiagrass comprised 22% of forage biomass, while the rest of the species provided less than 10%. Biomass from black-eyed susan dropped sharply in 2009, to less than 1% under both management methods. In 2009 the biomass proportions of all five grass species increased in both management systems, in many cases more than doubling 2008 proportions. Forb and legume contributions were variable and generally low, at under 4% for any species.

Grazed areas and biomass crop enclosure biomass proportions began to differ more in 2010. The two dominant species were big bluestem and indiangrass. As in the four-species plots, indiangrass was the most common species in grazed areas and big bluestem in biomass crop enclosures. Both switchgrass and Virginia wild rye biomass proportions increased from 2009 to 2010 in biomass enclosures but decreased in grazed areas. In both areas, oxeye sunflower (*Heliopsis helianthoides* (L.) Sweet) contributed the bulk of forb biomass, but in biomass crop enclosures it provided over 12% of total forage biomass.

Weed species

Weed species comprised a significant proportion of vegetative cover and total biomass in both grazed areas and enclosures for much of the experiment. Weed species richness was also frequently greater than NWSP species richness, especially in spring. Most weed species were found in many blocks, forage mixtures, and in both grazed areas and enclosures. This included white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), nimblewill (*Muhlenbergia shreberi* J.F. Gmel.), horseweed (*Conyza Canadensis* (L.) Cronquist), and crabgrass (*Digitaria* spp.). A few species were particularly common within certain blocks of forage treatments. For example, while common ragweed (*Ambrosia artemisiifolia* L.) was occasionally found in other blocks, it was most frequently found in the second block and was present within most grazed areas and enclosures for the duration of the experiment. Horseweed, while present in all three years, was particularly abundant in 2009, but then declined in 2010. In one enclosure, horseweed made up 63% of vegetative cover in July 2009, but dropped to less than 3% in July 2010.

Measurements of diversity

Community composition similarity was assessed for each pair of enclosures and grazed areas using the Bray-Curtis dissimilarity index (Table 4.9). Neither forage mixture nor management method affected the Bray-Curtis values in either 2009 or 2010. In 2009, grazed areas and enclosure plant communities tended to differ from each other with an average index value of 42.6. However, the Bray-Curtis index values between grazed areas and enclosures were

not significantly different among the three forage mixtures ($P > 0.5$). In May 2010, the Bray-Curtis dissimilarity values between grazed areas and their corresponding biomass crop enclosure had an average of 43.6, with no significant differences across forage mixtures, while in July, values averaged 49.1 for monocultures, 42.7 for four-species plots, and 50.4 for ten-species plots, with an average difference of 47.4 across mixtures. Although the overall index value increased slightly from May to July 2010, this change was not significant ($P = 0.3677$).

July was the only sampling date in 2009 in which there were any differences in diversity, based on the Shannon index of diversity (H') and evenness (E_H), among the three forage mixtures for either management method (Table 4.10). In July, the ten-species grazed plots had a larger Shannon index (H') than the monoculture or four-species plots, although species evenness was not different. When comparing H' and E_H between grazed areas and enclosures, in most cases grazed areas and biomass crop enclosures tended to be similar. The only differences in 2009 were that four-species mixtures in grazed areas tended to have a larger Shannon index (H') ($P = 0.0771$), and biomass crop monocultures varied more in term of evenness (Table 4.10a and b). In general, grazed areas tended to have higher cover in clovers and crabgrass, while biomass crop enclosures had higher tended to have greater cover in warm-season grasses.

As with 2009, in May 2010 the only significant difference among Shannon index values was for four-species plots, where once again H' was larger in grazed areas ($P = 0.0137$). Ten-species mixtures that were grazed also tended to have higher H' than those managed for biomass crops. Both Shannon diversity index values and evenness also tended to be highest for all three forage mixtures in both grazed areas and enclosures in May 2010. July 2010 Shannon index (H') values tended to be greater in grazed areas than biomass crop enclosures for all three mixtures, although this was not significant for four-species mixtures. Evenness also varied amongst grazed and biomass crop monocultures, with grazed monocultures having greater evenness in July 2010.

DISCUSSION

The results show that there are differences among plant communities due both to forage mixture and management type even with just two years under different management methods. As predicted, forage species cover and species richness was lowest in the switchgrass

monoculture and highest in the ten-species plots. Results also support the hypothesis that diverse mixtures would have lower weed abundance. As stands matured, weed cover and species richness were highest in switchgrass monocultures in both the grazed areas and biomass crop exclosures. In contrast, the prediction that grazed areas would have higher NWSP and weed species richness was only partially true. When there were differences among the two management types, biomass crop exclosures tended to contain higher NWSP forage species cover and richness, while grazed areas had higher weed species cover and richness.

Hypothesis one: increased forage richness and cover

Forage mixture affected forage relative cover throughout the experiment, with cover increasing as sown diversity increased. The positive relationship between cover and diversity has been observed by others and is likely correlated with increases in biomass (Hector et al. 1999). In contrast to work by Tracy and Sanderson (2004b) which found that forage species richness decreased over time, forage richness was relatively stable from 2008 through 2010, although numbers decreased slightly for ten-species grazed mixtures. Diverse mixtures retained higher numbers of sown species, although July 2010 forage species present per 0.5 m² quadrat in the most diverse mixture were less than half of the original ten species.

Community composition did not remain the same throughout the experiment, in agreement with ecological theory, which states that composition will shift until communities reach equilibrium (Loucks 1970). As these grasslands have only been established for three years, it is extremely unlikely that they have reached equilibrium, and therefore compositional shifts are expected. Warm-season grasses, which can take up to three years to produce a mature stand (McLaughlin and Kszos 2005), became more abundant in 2009 and 2010, while forb abundance and biomass declined. Most of the species richness present in the ten-species plots was due to warm-season grass species, with forbs and legumes relatively rare by 2010.

In the first growing season, quickly establishing forbs such as black-eyed susan were especially important in providing cover in ten-species plots, compared to the more slowly-establishing warm-season grasses. In 2008 the forbs and legumes accounted for over 60% of forage biomass in the diverse plots, and also produced more biomass than weed species did in these plots. However, the presence of these additional species over that of the four-species grass

mixture did not affect weed biomass or cover in 2008. Throughout the experiment, the four- and ten-species mixtures almost always had equivalent weed cover, richness, and biomass proportions. Forage grass biomass proportions, along with absolute forage biomass (see Chapter Three, Table 3.6) were also generally equal in four- and ten-species plots. From an agronomic standpoint, sowing forbs did not improve forage yields or reduce weed biomass, even in the initial year when forbs were dominant, making an argument for not including them in future forage mixtures. However, it is possible that sowing warm-season polycultures could potentially benefit producers by reducing the time needed for stand establishment. It may be advantageous to include high-yielding, disturbance-tolerant species in a mixture in order to provide cover and forage early in establishment.

Diversity through the inclusion of forbs may have additional ecological benefits. In agricultural settings, biodiversity is typically managed for its utilitarian, or commercial, benefits, and often at levels below those required for some ecosystem functions (Swift et al. 2004). The forbs and legumes helped increase forage relative cover in 2008, which also helped to reduce bare ground in ten-species mixtures. Increased vegetative cover is important for many processes, including soil quality and protection, water quality, and wildlife habitat. Native plants with deep root systems and the ability to produce large amounts of ground cover could help protect soils and water. In one study, areas dominated by the invasive weed spotted knapweed (*Centaurea stoebe* L.) had greater amounts of water and sediment runoff, compared to areas dominated by bunch grasses, largely due to the better infiltration capabilities of the bunchgrasses (Lacey et al. 1989). Forb cover has also been shown to affect bird abundances in Conservation Reserve Program lands, as forbs can enhance grasslands for many breeding and wintering bird species (Delisle and Savidge 1997).

Hypothesis two: reduced weed species richness and cover

Weed richness and cover were affected by both forage mixture and management method, as predicted. Weed species richness and abundance were greater in grazed areas and in monoculture plots in particular. Community invasibility is strongly tied to traits such as biomass, available light and available space, all of which will affect resource availability and the competitiveness of the native community (Naeem et al. 2000). Higher forage biomass and cover

in the more diverse mixtures and ungrazed areas could have made it more difficult for weeds to invade and persist in these areas.

Many of the cool-season weed species found in NWSP plots may have been able to grow because of the summer growth habits of warm-season species. A variety of factors may have impacted these results, including sown species richness, soil and seedbank differences, management, time, and weather. The more diverse plots had greater sown NWSP cover, which would have reduced resource availability for colonizing weed species. As stands established, NWSP cover continued to increase, possibly increasing competition and reducing resources further. Grazing, on the other hand, tended to create open space for weed invasions and may have also reduced grass competitiveness. Finally, weather has been proposed to affect species richness by altering species interactions; in particular, variations in moisture may impact the abilities of species to competitively exclude other plants (Lewis et al. 2008). The weather was very different in 2009 and 2010, as the summer of 2009 was cooler and much wetter than 2010 (see Chapter Three). Hotter and drier conditions in 2010 also may have contributed to early senescence of many cool season weed species.

The results agree with those of other studies examining the effects of diversity on weed communities (Elton 1958, Picasso et al. 2008, Frankow-Lindberg et al. 2009). Noxious weed cover was positively correlated with plant species diversity in Conservation Reserve Program pastures sown with switchgrass, although pastures also had desirable volunteer cool-season species present (Jewett et al. 1996). Some research suggests that species evenness is more important than diversity in explaining weed abundance, as more evenly distributed communities may have more complete resource use (Tracy et al. 2004). Findings from this study did not support this idea; although both weed richness and cover varied among the three forage mixtures and both management methods, evenness was similar. The results suggest that productivity, not species evenness, may play a large role in explaining weed suppression.

Weed colonization and suppression have been correlated with above-ground biomass and while diversity tends to increase yields, community composition is also important (Van der Putten et al. 2000). Pastures yielding less than 150 g m⁻² may not be productive enough to reduce resources available for weed invasion (Tracy and Sanderson 2004a). Based on this number, no grazed monocultures ever produced forage yields this high, while diverse grazed

mixtures only surpassed this number in July 2009. In contrast, diverse mixtures in biomass crop areas exceeded this minimum in both July and October of 2009 and 2010, with monocultures exceeding it by 2010 (see Chapter Three).

Forage and weed community composition

Both forage and weed communities were affected by sown forage mixture and by management method. Forage relative cover and richness tended to be greater in diverse mixtures and in biomass crop areas. Weed relative cover and richness were greatest in monocultures and frequently in grazed areas. Nevertheless, in most situations neither mixture nor management made any difference when analyzing the communities using three different measurements: the Bray-Curtis dissimilarity index and the Shannon indices of diversity (H') and evenness (E_H). It was surprising that, with the exception of the July 2009 grazed areas, sown mixture had little to no effect on any of these three community measurements.

While grazed areas and biomass crop enclosures did exhibit some differences in composition, as evident by the Bray-Curtis dissimilarity index values ranging from 40 to 50, forage mixture had no significant effect. The inclusion of up to nine more species in sown mixtures only increased the Shannon index (H') in the first year, and did not affect evenness at all in this experiment. Grazing tended to increase H' , but even this effect was only significant ($P < 0.05$) in three of nine comparisons over the course of the experiment. Grazing reduced evenness (E_H) only twice in the experiment, for monocultures in July 2009 and 2010. Part of the increases seen in grazed plots or monocultures might be explained by weed species invasion, as higher weed species richness in monoculture plots may have partially compensated for low sown species richness.

The findings are in partial agreement with those of other researchers such as McIntyre et al. (1995) and Hart (2001), as results supported the idea that grazing increases species richness; however there were no indications that Shannon index measures or evenness also increased due to grazing. It is important to note that these results were based on only two seasons of grazing of recently established NSW grasslands, in contrast to many studies that use results comparing grazed sites to long-term grazing enclosures. It is possible that with more time, grazed areas and biomass crop areas would become more different in terms of species richness and evenness.

After the two management methods were initiated, comparisons of plant community composition suggested that NWSP forbs were more negatively affected by grazing and mowing in summer than NWSP grasses were. This was also surprising, as grazing generally reduces grass dominance and increases forb and legume abundance. Oxeye sunflower biomass contributions were much higher in biomass crop exclosures than in grazed areas, nearly four times higher in 2010. Even though cows typically did not graze this species, preferring grasses and legumes instead (personal observation), the summer mowing most likely stunted the growth and production of this tall forb. In contrast, NWSP grasses, with growing points found much closer to the ground early in the summer, were probably less affected by top removal of vegetation.

Research suggests that without grazing, warm-season grasses can competitively exclude other species, including potentially both weeds and desirable forbs and legumes (Collins 1987). The ten-species pastures typically contained approximately four to five NWSP forage species per 0.5 m² quadrats, a decrease from the original ten species sown, due largely to the reduction in forb and legume abundance. By 2010, most of the forage present in ten-species plots was from NWSP grasses, so that sown species composition of the four- species and ten-species plots did not differ as much as in the first year. This may help explain why these two more diverse forage mixtures often performed similarly. Oxeye sunflower was the primary forb remaining in the ten-species plots in 2010, while the other four species each contributed less than 2% of sown biomass. The loss in NWSP forb abundance in grazed ten-species plots was somewhat surprising, as moderate levels of grazing is hypothesized to prevent competitive exclusion by grasses and promote diversity (Hobbs and Huenneke 1992). Grazing has been shown to increase total species richness, along with increasing richness in different functional groups (Collins et al. 1998, Pykala 2004). Based on published literature, we had predicted that disturbance by grazing animals would create an environment where some forbs would be better competitors with the NWSP grasses, but this did not appear to be true. This suggests that grazed NWSPs must be managed carefully in order to retain sown forage species.

CONCLUSIONS

Community composition of the three NWSP mixtures tested differed from each other and varied over time. Overall, switchgrass monocultures had lower forage cover, higher weed cover, and higher weed species richness when compared to the two diverse mixtures. Initial bare ground measurements were also greatest in monoculture plots. Even though trends for relative cover and species richness were similar across mixtures under either management method, grazing reduced forage abundance and richness while increasing weed species cover and richness. Contrary to what was expected, grazing did not increase the abundances of forage forbs and legumes. There were surprisingly few differences found among treatments using three measurements of diversity (Bray-Curtis index, Shannon index of diversity and evenness), even though the compositions of monoculture plots were different from the compositions of more diverse plots. These findings suggest that monocultures, initially less species-rich than more diverse plots, can become more diverse through the invasion of unsown weed species. In contrast, the more diverse mixtures, while not retaining their original sown species levels may have reduced weed invasions through increased forage productivity caused by higher species richness.

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TABLES

Table 4.1: Native, warm-season perennial species cover in each of the three forage mixtures and two management methods. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
		----- % -----			
Sept. 2008†	---	4.1 \pm 9.1 ^a	22.6 \pm 4.2 ^b	38.4 \pm 4.1 ^c	0.0013
May 2009	---	3.6 \pm 1.0 ^a	20.8 \pm 2.4 ^b	28.9 \pm 1.4 ^c	< 0.0001
July 2009	Grazing	10.3 \pm 1.6 ^a	39.8 \pm 5.7 ^b	49.5 \pm 6.3 ^b	0.0021
	Biomass	11.9 \pm 3.5 ^a	52.1 \pm 16.6 ^b	66.7 \pm 11.6 ^b	0.0243
	P-value	0.7283	0.4118	0.0890	
April 2010	Grazing	2.6 \pm 0.8 ^a	10.9 \pm 1.3 ^b	18.1 \pm 3.3 ^c	0.0052
	Biomass	9.1 \pm 3.2 ^a	24.6 \pm 1.2 ^{ab}	33.3 \pm 7.7 ^b	0.0256
	P-value	0.0980	0.0035	0.0838	
May 2010	Grazing	6.5 \pm 2.1 ^a	28.7 \pm 2.6 ^b	36.5 \pm 5.1 ^b	0.0014
	Biomass	10.8 \pm 2.1 ^a	35.5 \pm 4.7 ^b	49.8 \pm 6.4 ^c	0.0008
	P-value	0.0746	0.1025	0.2009	
July 2010	Grazing	7.3 \pm 2.6 ^a	38.1 \pm 6.1 ^b	38.1 \pm 2.2 ^b	0.0016
	Biomass	30.6 \pm 3.9 ^a	59.7 \pm 11.9 ^{ab}	67.6 \pm 8.5 ^b	0.0481
	P-value	0.0293	0.1230	0.0398	

†Means within rows having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

Table 4.2: Weed species cover in each of the three forage mixtures and two management methods. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
		----- % -----			
Sept. 2008†	---	29.9 \pm 8.3	35.0 \pm 11.3	31.2 \pm 7.4	0.5200
May 2009	---	53.1 \pm 5.6	43.1 \pm 5.8	40.6 \pm 1.6	0.0870
July 2009	Grazing	85.8 \pm 1.2 ^a	54.7 \pm 5.7 ^b	47.8 \pm 6.8 ^b	0.0014
	Biomass	73.3 \pm 4.9 ^a	35.2 \pm 9.3 ^b	27.1 \pm 7.0 ^b	0.0077
	P-value	0.0600	0.1424	0.0053	
April 2010	Grazing	72.3 \pm 4.0 ^a	52.1 \pm 5.5 ^b	50.1 \pm 2.4 ^b	0.0170
	Biomass	59.4 \pm 4.1 ^a	37.2 \pm 4.8 ^b	33.1 \pm 8.2 ^b	0.0430
	P-value	0.0364	0.0844	0.1134	
May 2010	Grazing	83.2 \pm 3.7 ^a	61.7 \pm 2.6 ^b	57.3 \pm 6.2 ^b	0.0111
	Biomass	76.4 \pm 4.1 ^a	52.2 \pm 6.1 ^b	40.6 \pm 9.8 ^c	0.0005
	P-value	0.2553	0.1146	0.2226	
July 2010	Grazing	49.0 \pm 3.9 ^a	25.2 \pm 4.5 ^b	31.1 \pm 3.1 ^b	0.0006
	Biomass	32.7 \pm 1.0	10.3 \pm 5.1	14.3 \pm 8.8	0.0752
	P-value	0.0241	0.1083	0.1879	

†Means within rows having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

Table 4.3: Bare ground in each of the three forage mixtures and two management methods. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
		----- % -----			
Sept. 2008†	---	25.2 \pm 4.6 ^b	17.5 \pm 1.3 ^{ab}	12.3 \pm 0.7 ^a	0.0282
May 2009	---	43.3 \pm 5.8	36.1 \pm 4.2	30.5 \pm 1.7	0.1556
July 2009	Grazing	3.9 \pm 0.7	5.6 \pm 4.0	2.7 \pm 1.1	0.7186
	Biomass	14.9 \pm 5.0	12.8 \pm 8.1	6.1 \pm 4.6	0.5907
	P-value	0.1322	0.4570	0.5652	
April 2010	Grazing	8.1 \pm 2.1	6.9 \pm 0.4	5.5 \pm 1.5	0.4976
	Biomass	21.6 \pm 3.3	25.5 \pm 4.0	24.4 \pm 1.6	0.6791
	P-value	0.0697	0.0173	0.0004	
May 2010	Grazing	3.6 \pm 1.7	1.9 \pm 1.0	0.2 \pm 0.2	0.1607
	Biomass	10.8 \pm 4.2	10.7 \pm 2.9	9.6 \pm 3.7	0.9644
	P-value	0.0767	0.0836	0.0860	
July 2010	Grazing	15.4 \pm 2.6 ^b	5.2 \pm 1.9 ^a	4.7 \pm 2.1 ^a	0.0115
	Biomass	18.0 \pm 2.9	23.8 \pm 6.8	17.3 \pm 1.9	0.5433
	P-value	0.5571	0.0341	0.0035	

Sown NWSP cover, weed cover, and bare ground may not always add up to 100% due to rounding error and the presence of standing dead plant material (data not shown).

†Means within rows having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

Table 4.4: Total species richness from each of the three forage mixtures and two management methods. Values are mean \pm one standard error.

Date	Management	One species \ddagger	Four species	Ten species	P-value
		----- species 0.5m ⁻² -----			
Sept. 2008 \dagger	---	3.2 \pm 0.6 ^a	4.2 \pm 0.2 ^a	6.8 \pm 0.3 ^b	0.0007
May 2009	---	7.0 \pm 0.6 ^a	7.7 \pm 0.4 ^a	10.0 \pm 0.2 ^b	0.0068
July 2009	Grazing	7.0 \pm 0.3	7.0 \pm 0.3	8.3 \pm 0.5	0.0788
	Biomass	6.3 \pm 0.7 ^a	5.6 \pm 0.8 ^a	8.3 \pm 0.7 ^b	0.0355
	P-value	0.3312	0.1269	0.9374	
April 2010	Grazing	8.5 \pm 0.3	8.3 \pm 0.5	9.1 \pm 0.4	0.3128
	Biomass	6.8 \pm 0.60 ^a	9.1 \pm 0.63 ^b	10.3 \pm 0.3 ^b	< 0.0001
	P-value	0.1498	0.4684	0.2190	
May 2010	Grazing	8.5 \pm 0.34 ^a	9.6 \pm 0.5 ^{ab}	10.7 \pm 0.5 ^b	0.0022
	Biomass	8.8 \pm 0.6 ^a	9.7 \pm 0.5 ^{ab}	10.8 \pm 0.6 ^b	0.0371
	P-value	0.6641	0.9310	0.8823	
July 2010	Grazing	6.4 \pm 0.5	6.0 \pm 0.3	7.0 \pm 0.4	0.2603
	Biomass	5.8 \pm 0.2 ^a	4.6 \pm 0.4 ^b	6.4 \pm 0.5 ^a	0.0095
	P-value	0.1086	0.1063	0.0907	

\dagger Means within rows having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD; corresponding P-values are listed for each row.

\ddagger P-values within columns represent paired t-test results comparing grazing and biomass areas.

Table 4.5: Sown native, warm-season perennial species richness from each of the three forage mixtures and two management methods. Values are mean \pm one standard error.

Date	Management	One species‡	Four species	Ten species	P-value
		----- species 0.5m ⁻² -----			
Sept. 2008†	---	0.8 \pm 0.1 ^a	2.2 \pm 0.3 ^b	4.5 \pm 0.3 ^c	0.0002
May 2009	---	0.9 \pm 0.1 ^a	2.2 \pm 0.1 ^b	4.6 \pm 0.1 ^c	< 0.0001
July 2009	Grazing	0.9 \pm 0.1 ^a	2.4 \pm 0.2 ^b	4.3 \pm 0.2 ^c	< 0.0001
	Biomass	0.8 \pm 0.1 ^a	2.7 \pm 0.4 ^b	5.0 \pm 0.7 ^c	0.0024
	P-value	0.1817	0.5002	0.4033	
April 2010	Grazing	0.6 \pm 0.1 ^a	1.8 \pm 0.2 ^b	2.8 \pm 0.2 ^c	< 0.0001
	Biomass	0.7 \pm 0.1 ^a	2.8 \pm 0.3 ^b	4.3 \pm 0.4 ^c	< 0.0001
	P-value	0.8832	0.0251	0.0089	
May 2010	Grazing	0.8 \pm 0.1 ^a	2.5 \pm 0.2 ^b	4.1 \pm 0.3 ^c	< 0.0001
	Biomass	0.8 \pm 0.1 ^a	2.3 \pm 0.3 ^b	5.0 \pm 0.5 ^c	< 0.0001
	P-value	0.9727	0.4472	0.0106	
July 2010	Grazing	0.8 \pm 0.1 ^a	2.1 \pm 0.2 ^b	3.2 \pm 0.2 ^c	<.0001
	Biomass	1.0 \pm 0.0 ^a	2.8 \pm 0.3 ^b	4.7 \pm 0.6 ^c	0.0010
	P-value	0.2504	0.0351	0.0438	

†Means within rows having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas.

Table 4.6: Weedy (unsown) species richness from each of the three forage mixtures and two management methods. Values are mean \pm one standard error.

Date	Management	One species \ddagger	Four species	Ten species	P-value
----- species per 0.5m ⁻² -----					
Sept. 2008 \dagger	---	2.5 \pm 0.5	2.0 \pm 0.3	2.4 \pm 0.5	0.3063
May 2009	---	6.1 \pm 0.6	5.5 \pm 0.3	5.3 \pm 0.2	0.4079
July 2009	Grazing	6.1 \pm 0.3 ^a	4.7 \pm 0.5 ^b	3.9 \pm 0.4 ^b	0.0184
	Biomass	5.7 \pm 0.7 ^a	2.9 \pm 0.7 ^b	3.3 \pm 0.4 ^b	0.0197
	P-value	0.5008	0.0178	0.1919	
April 2010	Grazing	7.8 \pm 0.1 ^a	6.5 \pm 0.5 ^b	6.3 \pm 0.4 ^b	0.0112
	Biomass	6.2 \pm 0.6	6.3 \pm 0.5	6.1 \pm 0.3	0.9759
	P-value	0.2042	0.7766	0.8237	
May 2010	Grazing	7.6 \pm 0.3	7.1 \pm 0.3	6.6 \pm 0.5	0.1421
	Biomass	7.9 \pm 0.6 ^a	7.3 \pm 0.5 ^a	5.8 \pm 0.5 ^b	0.0124
	P-value	0.6859	0.7518	0.5499	
July 2010	Grazing	5.5 \pm 0.5 ^a	3.9 \pm 0.5 ^b	3.8 \pm 0.4 ^b	0.0380
	Biomass	4.8 \pm 0.2 ^a	1.8 \pm 0.5 ^b	1.8 \pm 0.5 ^b	0.0045
	P-value	0.0639	0.0566	0.0041	

\dagger Means within rows having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD; corresponding P-values are listed for each row.

\ddagger P-values within columns represent paired t-test results comparing grazing and biomass areas.

Table 4.7: Dry weight proportions of sown species separated into forbs and grasses and weed species for the three forage mixtures in a) September 2008, b) July 2009, and c) July 2010.

a) September 2008

Mixture	Grass	Forb	Weed
	----- % -----		
One†	20.3 ^a	-	79.7 ^a
Four	57.3 ^b	-	42.7 ^b
Ten	34.6 ^a	30.0	35.4 ^b
P-value	<0.0001	--	< 0.0001

b) July 2009

Mixture	Grasses			Forbs			Weeds		
	Grazing	Biomass	P-value	Grazing	Biomass	P-value	Grazing	Biomass	P-value
	----- % -----			----- % -----			----- % -----		
One	23.6 ^a	37.2	0.2280	-	-		76.4 ^b	62.8 ^b	0.2270
Four	52.3 ^b	58.2	0.6897	-	-		47.8 ^a	41.8 ^{ab}	0.6867
Ten	53.5 ^b	61.7	0.4937	3.9	8.6	0.4850	42.6 ^a	29.8 ^a	0.2803
P-value	0.0053	0.1294					0.0024	0.0477	

c) July 2010

Mixture	Grasses			Forbs			Weeds		
	Grazing	Biomass	P-value	Grazing	Biomass	P-value	Grazing	Biomass	P-value
	----- % -----			----- % -----			----- % -----		
One	21.0 ^a	80.8	0.0262	-	-		79.0 ^b	19.2	0.0262
Four	71.9 ^b	89.5	0.2450	-	-		28.1 ^a	10.5	0.2433
Ten	75.8 ^b	73.6	0.8875	4.6	14.3	0.4873	19.6 ^a	12.1	0.5707
P-value	<0.0001	0.4709					< 0.0001	0.7247	

† P-values within row represent paired t-test results comparing grazing and biomass areas. Means within columns having unlike letters differ statistically ($P < 0.05$), using Fisher's LSD.

Table 4.8: Forage biomass proportions for each species (i.e., not including weed biomass) for both grazing and biomass management methods in a) the four-species mixture and b) the ten-species mixture. Note: Monoculture data is not presented as switchgrass comprises 100% of sown forage biomass.

a) Four-species mixture

Year	Management	SG	BB	IG	VWR
		----- % -----			
2008	--	4.9	62.0	33.0	0.1
2009	Grazing	9.0	33.9	54.7	2.5
2010		18.6	35.0	46.4	0.0
2009	Biomass	12.2	12.6	75.2	0
2010		22.6	44.5	30.2	2.6

b) Ten-species mixture

Year	Management	SG	BB	IG	VWR	LB	ILB	TF	PP	BES	SUNF
		----- % -----									
2008	--	4.4	8.3	22.1	0.1	2.5	3.7	1.0	4.5	49.8	3.5
2009	Grazing	13.6	15.7	52	4.6	8.4	2.5	2.3	0.1	0.8	0.0
2010		10.2	32.3	46.3	1.5	4.4	0.8	1.3	0.0	0.0	3.2
2009	Biomass	7.5	29.6	45.3	5.8	5.7	0.5	0.8	1.1	0.0	3.8
2010		17.9	32.2	23.5	10.9	2.2	0.2	0.7	0.1	0.3	12.1

Table 4.9: Bray-Curtis dissimilarity index values for each forage mixture in July 2009, May and July 2010.

Date	One species‡	Four species	Ten species	P-value
July 2009†	41.3 ± 4.4	45.6 ± 5.5	40.8 ± 4.1	0.7410
May 2010	45.4 ± 4.8	41.4 ± 4.4	43.9 ± 4.0	0.8182
July 2010	49.1 ± 5.4	42.7 ± 5.5	50.4 ± 2.5	0.4939
P-value	0.5505	0.8467	0.2158	

† Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD; corresponding P-values are listed for each row.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas across all three years.

Table 4.10: a) Shannon index of diversity (H') and b) evenness index for each forage mixture and both management methods in July 2009, May and July 2010.

a) Shannon index of diversity (H')

Date	Management	One species‡	Four species	Ten species	P-value
July 2009†	Grazing	2.0 ± 0.1 ^a	2.0 ± 0.1 ^a	2.4 ± 0.0 ^b	0.0129
	Biomass	2.0 ± 0.1	1.6 ± 0.2	2.1 ± 0.3	0.2874
	P-value	0.6803	0.0771	0.2995	
May 2010	Grazing	2.4 ± 0.1	2.5 ± 0.0	2.7 ± 0.1	0.1174
	Biomass	2.3 ± 0.1	2.2 ± 0.1	2.4 ± 0.0	0.1401
	P-value	0.1624	0.0137	0.0930	
July 2010	Grazing	2.2 ± 0.1	2.0 ± 0.2	2.3 ± 0.1	0.3297
	Biomass	1.6 ± 0.1	1.5 ± 0.3	1.8 ± 0.1	0.5206
	P-value	0.0224	0.1352	0.0137	

†P-values within rows represent results comparing mixtures using Fisher's LSD.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas across all three years.

b) Evenness index

Date†	Management	One species‡	Four species	Ten species	P-value
July 2009	Grazing	0.75 ± 0.03	0.77 ± 0.03	0.83 ± 0.02	0.1433
	Biomass	0.83 ± 0.01	0.73 ± 0.05	0.76 ± 0.08	0.5000
	P-value	0.0445	0.4908	0.4934	
May 2010	Grazing	0.82 ± 0.03	0.84 ± 0.01	0.82 ± 0.02	0.8665
	Biomass	0.82 ± 0.03	0.81 ± 0.02	0.84 ± 0.01	0.5276
	P-value	0.9354	0.2053	0.3081	
July 2010	Grazing	0.79 ± 0.05	0.74 ± 0.05	0.80 ± 0.02	0.6452
	Biomass	0.66 ± 0.02	0.79 ± 0.05	0.72 ± 0.04	0.1134
	P-value	0.0340	0.3858	0.2997	

†P-values within rows represent results comparing mixtures using Fisher's LSD.

‡ P-values within columns represent paired t-test results comparing grazing and biomass areas across all three years.

Chapter Five

Productivity,overyielding, and additive partitioning in diverse native, warm-season perennial mixtures

ABSTRACT: The relationships between biodiversity and ecosystem properties have been of increasing interest due to global declines in species richness. The objective of this study was to examine the impact of native, warm-season perennial (NWSP) forage species richness on productivity and identify mechanisms behind the diversity-productivity relationship. Ten NWSP species were grown in random assemblages of one, two, four, six, and all ten species for three years. Species richness was positively associated with forage biomass in the first year, while in the following two summers, yields were equivalent across all five richness levels. Although species-poor NWSP plantings can be as productive as species-rich ones, increased richness may improve yield stability. Even though diversity only affected yields in one year, a net biodiversity effect, where mixtures yielded more than expected based on monoculture yields, was seen in all three years with an average of 64% of multi-species plots exhibiting a positive biodiversity effect. Biodiversity effects were separated into selection effects (SE) and complementarity effects (CE) through additive partitioning to measure the underlying mechanisms of the effect. In the first year, SE played a more prominent role as the high-yielding black-eyed susan (*Rudbeckia hirta*) dominated multi-species plots. In the second year, positive CE became more prevalent as perennial grasses established. In the third year SE once again became more important, averaging 22 in multi-species plots, compared to an average CE of 2. These findings suggest that several factors, including climate and species succession, may affect the magnitude of CE and SE.

INTRODUCTION

Biodiversity has been credited with enhancing a plethora of ecosystem services and functions. In the wake of these findings, some research has questioned whether traditional monoculture agricultural systems may need to be redesigned to incorporate more multi-species production areas as a method of maintaining productivity and ecosystem health (Kirschenmann 2007). In a review of 20 experimental studies by Schwartz et al. (2000), 19 demonstrated a positive relationship between diversity and ecosystem functions such as productivity, nutrient

use or retention, and percent cover. Aboveground productivity is a frequently measured variable when testing the effects of diversity and is a major concern in grazing lands, where the amount of available forage may impact livestock weight gains. Some research suggests that high diversity mixtures may have higher productivity and provide more biomass bioenergy than monocultures, while others suggest that careful selection of a few perennial species can provide yields similar to more diverse mixtures (Tilman et al. 2006a, DeHaan et al. 2010). However, in both instances these experiments propose that polycultures, from simple bicultures to more diverse mixes, may be higher-yielding than monocultures.

Recent research tends to show a positive relationship between productivity and diversity. Diverse assemblages may overyield, or perform better than monocultures (Trenbath 1974, Vandermeer 1989). There are two kinds of overyielding: non-transgressive, where a mixture performs better than average monoculture of the species in the mixture, and transgressive, where a mixture surpasses that of the best-performing member species' monoculture.

When diverse mixtures produce more biomass than expected based on monoculture yields, there is a positive biodiversity effect. The mechanisms behind this positive biodiversity effect may be separated into two effects that are not mutually exclusive, the complementarity effect (CE) and the selection or sampling effect (SE), which can be separated by the additive partitioning method developed by Loreau and Hector (2001). A positive complementarity effect occurs when diverse assemblages have increased productivity due to species interactions, such as facilitation and niche differentiation, resulting in more a complete use of resources (Loreau and Hector 2001, Tilman et al. 2001b). In the case of a selection effect, if a highly productive species planted in diverse assemblage dominates the plot, the positive relationship between diversity and productivity is due to the increased likelihood of including that species in a diverse mixture and is not attributed as a diversity effect. Another suggested variation of the selection effect, the "context-specific sampling effect," occurs when a species performs unusually well in mixtures is more likely to be present at higher levels of diversity (Dukes 2001b).

Some researchers suggest that only CE is a true biodiversity effect, while SE is an experimental artifact or a "hidden treatment" resulting from the artificial design of experimental communities (Huston 1997, Wardle 1999), although others contest that it is a true diversity effect (Tilman et al. 1997b). The debate over the identity of SE lies in the nature community assembly,

where experiments are often populated with randomly generated assemblages, while in real ecosystems, communities are unlikely to be random (Wardle 1999). If a mixture does transgressively overyield, it provides evidence that more than just SE is at work and that species may be using facilitation and niche differentiation to increase productivity. In addition, understanding what causes a net biodiversity effect may be complicated, as it can be caused by one or both mechanisms and can change over time (Cardinale et al. 2007, Fargione et al. 2007).

Because CE and SE can occur simultaneously, Loreau and Hector (2001) developed additive partitioning as a way to differentiate between the two mechanisms. When they tested this model in the BIODEPTH experiment, they found that CE had positive effects on productivity, while SE was more variable and had no significant impact across sites. This finding is corroborated by other studies and provides support for the role of facilitation and niche differentiation in enhancing the productivity of diverse assemblages (van Ruijven and Berendse 2003). The impact that a particular effect has on productivity and other ecosystem functions depends on species composition as well as diversity. When Jiang et al. (2007) used additive partitioning to study productivity-diversity and productivity-invasion relationships, they discovered that SE played a significant role in an annual community, while in a perennial community CE was the main driver behind the observed relationships, and suggested that differences in plant traits and life history may explain this difference.

While both species and functional group diversity have been shown to impact ecosystem processes, it is also true that individual species can also play a role. High productivity is often linked to the presence of legumes, and in some cases, even just one especially well-adapted legume species (Spehn et al. 2002, DeHaan et al. 2010). Other species besides legumes can also act as “driver species” and impact productivity more than species richness alone (Picasso et al. 2008). Along with diversity, species composition has been shown to explain changes in ecosystem properties like productivity and N cycling (Hooper and Vitousek 1997, Tilman et al. 1997a). Species interactions, in particular that between legumes and warm-season grasses, have been associated with rates of high productivity (Tilman et al. 2001b, DeHaan et al. 2010). Positive diversity effects have also been observed even in the absence of legumes or when the presence of legumes has been taken into account (van Ruijven and Berendse 2003, Spehn et al. 2005).

Because it has been shown that certain species or species combinations can have significant effects on yield, if non-random assemblages are used, differences among mixtures may due to particular species instead of diversity (Huston 1997). Another way to test the effects of diversity is by comparing random combinations of species at different richness levels. Random assemblages, while commonly used in experiments, have also been accused of containing a “hidden treatment:” the selection effect, or a sampling bias, and it is debated whether this is an experimental artifact or a diversity effect (Aarssen 1997, Tilman et al. 1997b, Loreau et al. 2001).

Using native, warm-season perennials (NWSPs) may provide several benefits when established as part of a diverse agroecosystem. Due to their physiological adaptations, warm-season grasses have higher water use and N use efficiencies that allow them to be highly productive during the hottest part of the year which allow them to produce large quantities of biomass while requiring less water and nutrients than cool-season species (Wedin and Tilman 1990, MacAdam and Nelson 1995). Native, warm-season perennial grasslands may also provide additional benefits such as enhanced soil C storage (Knops and Tilman 2000). Fields dominated by native, warm-season grasses stored significantly more C than non-native cool-season fields in a low N environment, and soil C increases in the warm-season fields averaged 10-21% compared to 1% in cool-season fields (Wedin and Tilman 1996). However, most diversity-productivity experiments use cool-season species or a mix of cool- and warm-season species.

Native, warm-season perennial species have additional roles and advantages in both agriculture and environmental conservation. Compared to cool-season fields, Conservation Reserve Program (CRP) fields planted with warm-season species have a different vegetative community structure and are suitable wildlife habitat for some bird species, including ring-necked pheasants, common yellowthroats, and sedge wrens (Delisle and Savidge 1997). Warm-season pastures and hayfields may also support more species of birds, as well as have higher nesting rates and fledging successes, results of the later grazing and haying management practices that increase vegetated cover (Giuliano and Daves 2002). If managed correctly, a grazing system could create heterogeneous vegetation patches and community structure that could support more species of grassland birds and may help reduce the decline in many grassland bird populations (Walk and Warner 2000). CRP lands established with warm-season species could also be used for biofuel production and have the potential to supply nearly 5800 L ha⁻¹

ethanol (Adler et al. 2009). Soil erosion rates could also be greatly reduced under perennial crops, compared to annual row crops (Hohenstein and Wright 1994).

There were two objectives to this study. The first objective was to test the relationship between NWSP species richness and herbage mass over a three-year establishment period. The second objective analyzed any net biodiversity effects and separate it into its two components, CE and SE, over this same time. Because replication of random assemblages is important to ascertain whether particular species or diversity itself is the cause of any changes in yields from multi-species plots, the biodiversity effects CE and SE can only be summarized as means, with trends suggested. The two hypotheses tested in this study were: 1) that more diverse species mixtures would yield higher amounts of biomass, and 2) net biodiversity effects of diverse mixtures would initially be due primarily to SE but shift to CE over time.

MATERIALS AND METHODS

Site description

The experiment was located at Kentland farm near Blacksburg in Montgomery County, VA (37°12'0" N latitude and 80°34'40" W longitude). Soil in the small plots was primarily well-drained Unison and Braddock cobbly soil (NRCS data) on a relatively even surface.

Experimental design

The experiment was planted on 15 May 2008 and consisted of five levels of species richness and used a pool of ten native species. The five levels of species richness were one, two, four, six, or all ten species, and the ten-species pool contained four warm-season grasses, one cool-season grass, three legumes, and two non-legume forbs (See Chapter 3, Table 3.1). All ten species were also planted in monoculture and replicated four times. Species composition for each of the two-, four- and six-species plots were randomly chosen from the pool of ten species, so that each plot contained a different assemblage of species. There was no attempt to regulate functional groups in multi-species assemblages. Six assemblages of each of the multi-species plots were sown, with no repetition of any unique assemblage, i.e. the composition of each of the

randomly-drawn multi-species plots was different from the others, with the exception of the ten-species plots, which contained all possible species.

Seeds were broadcast at a seeding density of ~8.4 g PLS per plot (1.4 g m⁻²) into 2x3m plots and rolled to increase seed contact with soil. Plots were separated from each other by 1-m alleys, and no fertilizers were applied to the plots. The plots were hand-weeded three times over the summer of 2008 at two- to three-week intervals to remove unsown species and allow sown species to establish. After the first summer, weeds were allowed to grow in the small plots and were not removed.

Data collection

Soil samples were collected on 20 November 2008 for nutrient and organic matter analyses to determine existing soil conditions. Each plot was divided into three sections and five randomly placed cores were taken to a depth of 15 to 18 cm in each section. The five cores from each subsection were mixed and the composited sample was analyzed.

Species compositional data were collected by harvesting 0.25-m² quadrats from each plot on 21 August 2008, 19 August 2009 and 20 August 2010. After clipping vegetation to 10 to 15 cm, plants were hand-sorted to species, dried at 55° for 48 to 72 hours, then weighed. Unsown species were also harvested and collected without sorting, as weeds. In fall of each year, all plots were mowed to remove any standing vegetation and reduce grassy weed seed production.

Diversity calculations

Berger-Parker dominance index

In each plot, the dominant species was identified as the sown species that produced the highest yield. The Berger-Parker dominance index (d), calculated as the proportion of the entire plot yield due to the dominant species was also determined annually in August for each plot (Berger and Parker 1970, Magurran 1988):

$$d = N_{\max}/N$$

where N_{\max} is the dry herbage mass of the most abundant NWSP and N is the total plot herbage mass. As d approaches 1, dominance by a species increases and diversity decreases.

Overyielding and net biodiversity effects

Overyielding and the net biodiversity effect were calculated, and the net effect was separated into complementarity and selection effects as described by Loreau and Hector (2001). In the following calculations, weed biomass was not included, as plots were weeded in the establishment year and weed biomass was low in general in the second year. Since multi-species plot assemblages were not replicated, statistical analyses on overyielding and the partitioning into SE and CE could not be performed. For each multi-species plot, an analysis of overyielding and additive partitioning was performed. Table 5.1 lists the variables used for the following equations.

Relative yield total (RYT) is the sum of the relative yields for all species in a mixture and is a measure of overyielding:

$$RYT = \sum RY_{oi},$$

D_{\max} determines transgressive overyielding, where positive species interactions allow a mixture to be more productive than the best performing species in the mixture:

$$D_{\max} = [Y_o - \max(M_i)] / \max(M_i)$$

Individual species performance within mixtures was measured using D_i :

$$D_i = (O_i - E_i) / E_i, \text{ where } E_i = M_i / S$$

RYT cannot be used to identify mechanisms causing overyielding. Instead, the net biodiversity effect (ΔY) can be used to determine mechanisms that cause deviations from expected yields:

$$\Delta Y = Y_o - Y_e = CE + SE$$

When ΔY is zero, the observed yield equals the expected yield and there is no effect of diversity.

Net biodiversity effects can be positive, where mixtures yield more than expected, or negative,

where mixtures yield less than expected. The net biodiversity effect is partitioned into two other effects, complementarity and selection.

The complementarity effect (CE) quantifies the change in yield due to effects such as complementation, facilitation, inhibition, and suppression. Complementation occurs when niche partitioning results in reduced interspecific competition, while facilitation is when one species improves the environmental conditions or provides a needed resource that promotes the survival of another species; both of these may cause overyielding (Hooper et al. 2005). In contrast, inhibition occurs when one species competes for and reduces resources to a level that reduces the growth of another species, while inhibition can include the production of allelochemicals (Turner and Rabinowitz 1983, Wardle et al. 1998). The complementarity effect is calculated as:

$$CE = S \times \text{mean}(\Delta RY_i) \times \text{mean}(M_i)$$

where ΔRY_i is the deviation in relative yield from expected:

$$\Delta RY_i = RY_{oi} - RY_{ei}$$

A positive CE occurs when a mixture produces more biomass than expected, and is due to positive interactions such as facilitation and niche partitioning. A negative CE occurs when a mixture yields less than expected due to negative species interactions.

The selection effect (SE) measures whether overyielding is due to the presence of particular species:

$$SE = S \times \text{covariance}(\Delta RY_i, M_i)$$

The SE will be positive when a species that is most productive in monocultures also overyields the most when in a mixture. There will be a negative SE when a species that produces low biomass in monoculture overyields the most in mixture.

Data analysis

Biomass was analyzed using a randomized complete design with species richness as the treatment factor (n = five treatment levels), and replications at each richness level (four replications of each species in monoculture and six plots each of two-, four-, six- and ten-species richness levels), but no replications of specific multi-species mixes. All data were analyzed by

an analysis of variance using SAS statistical software (SAS SAS Institute, Inc. 2008) to test for significant differences among treatments. Means from each richness level were separated using the macro pdmix800 (Saxton 1998). Data were checked for normality and equal variance and transformed when necessary. The most common transformation was taking the square root of dry weight biomass to address unequal variance. Responses for each treatment were separated using the test for least significant differences (LSD), with $\alpha = 0.05$.

RESULTS

Initial (November 2008) soil nutrients measurements showed that average soil P was 16.8 kg ha⁻¹, soil K was 123.7 kg ha⁻¹, and soil Ca was 1023.0 kg ha⁻¹. Soil pH averaged 6.3 and organic matter was at 2.0%.

In 2008, sown forbs in general established earlier and produced higher yields than the more slowly-establishing native grasses. Many of the forbs were emerging by mid-June and rapidly increased in size, while the grasses began to emerge in early July but were much slower to grow. By the August harvest several switchgrass (*Panicum virgatum* L.), indiangrass (*Sorghastrum nutans* (L.) Nash), and big bluestem (*Andropogon gerardii* Vitman) plots had inflorescences, compared to Virginia wild rye (*Elymus virginicus* L.) and little bluestem (*Schizachyrium scoparium* (Michx.) Nash), which remained vegetative and smaller. The forbs generally were still more productive than the perennial grasses. Because of the differences in growth rate, forbs, especially the vigorously growing black-eyed susan (*Rudbeckia hirta* L.) and partridge pea (*Cassia fasciculata* Michx.), produced denser plots and tended to have fewer co-occurring weedy species.

Total herbage mass

In 2008, total yields generally increased with increasing species richness, with monocultures producing less biomass than six- or ten-species mixtures ($P = 0.0005$, Table 5.2). The six-species plots were the most productive richness level, producing slightly more herbage mass than the ten-species plots. Significant differences in total yields disappeared in 2009 and 2010, with monocultures being as productive as the multi-species plots.

Forage biomass

Just as with total herbage mass, in 2008 sown forage yields were lowest in the one-species and two-species plots and greatest in the six- and ten-species mixtures (Table 5.3). No differences in yield were observed in 2009 or 2010. When comparing each richness level across the three years, compared to 2008, 2009 and 2010 monoculture yields were over 2.75 times larger, two-species yields were 3.7 times larger, and four-species plots tended to be twice as large. Only the six-species richness level showed a decrease in forage biomass in 2010, with yields approximately two-thirds of those of previous years. The ten-species plots maintained a consistent yield for all three yields of the experiment.

Weed biomass

Plots were weeded in 2008, so weed biomass was equal across all five richness levels (Table 5.4). However, in 2009, weed biomass showed a tendency to be higher in the monoculture plots than in the more diverse plots ($p = 0.0606$). By 2010, weed biomass did not differ by richness level. When comparing individual richness levels by year, weed biomass decreased within two richness levels, two-species and ten-species, while remaining the same for the other three levels.

Species grown in monoculture differed greatly in the amount of weeds present. In 2008 weed biomass was highest in grass plots, but declined to only about 5% of plot biomass by 2010. Weed biomass in forb and legume monocultures was variable. Within partridge pea monoculture plots, weeds contributed 4% of total biomass in 2008, but nearly 100% in 2010. In comparison, weed contributions in Illinois bundleflower monoculture plots were similar in 2008 and 2010, while weed contributions in oxeye sunflower plots decreased from 31% in 2008 to nearly 0% in 2010. This variation in monoculture weed biomass prevented trends in weediness from being significant.

Forage species richness

Species richness, measured by the number of forage species per 0.25-m² quadrat, increased as sown species numbers increased in all three years (Table 5.5). Species richness

changed in the more diverse plots over time. From 2008 to 2009, species richness nearly doubled to five species per quadrat in six-species plots, and remained higher in 2010. In contrast, species richness for ten-species plots was greatest in 2009, when an average of 7.5 species were identified, although richness in 2010 dropped back to near 2008 levels ($P = 0.0039$). This increase in the number of sown species present in 2009 was largely due to increased abundance of sown grasses within polycultures. Even though forage species richness increased temporarily in 2009 in the ten-species mixtures, by 2010 only approximately half of the species originally sown were identified in the harvested quadrats.

Berger-Parker dominance indices

In 2008, the observed Berger-Parker dominance index tended to increase as sown species richness increased (Table 5.6). Dominance analysis showed some shifts from August 2008 to August 2009. As the warm-season grasses continued to establish, monoculture and biculture dominance values also tended to increase, while values from more diverse mixtures remained the same or decreased. As in 2009, 2010 average Berger-Parker dominance values tended to be higher in the lower-diversity mixtures. Changes over time for Berger-Parker dominance values varied depending on sown richness. Less diverse plots showed increases in dominance over time, with monoculture and two-species plots increasing to 0.75 and 0.80, respectively. Four-species mixtures remained relatively constant, while six- and ten-species plots tended to decrease in dominance over time.

In 2008, black-eyed susan was the dominant species in all the multi-species plots it was sown in and comprised a large proportion of each plot based on Berger-Parker index values (average BP = 0.82, Table 5.7a). With one exception, a grass species was only dominant in multi-species plots if only grass species were sown; in the one exception oxeye sunflower (*Heliopsis helianthoides* (L.) Sweet) was sown but was not present in the quadrat sampled. Dominance index values were generally lower for grasses and higher for forbs. The increased probability of black-eyed susan occurring in more species-rich plots most likely accounts for the corresponding increase in the observed dominance index.

While Berger-Parker values tended to increase for grasses and decrease for forbs from 2008 to 2009, black-eyed susan still remained the dominant species in most of the multi-species

plots it was sown in and was the only dominant forb in 2009 (Table 5.7b). By 2010, dominance shifted from forbs to grasses, as grasses were the dominant species in 21 of 24 plots. Both indianguass and big bluestem were dominant in at least half of the plots they were sown in (Table 5.7c). Unlike in past years, black-eyed susan was never a dominant species in any multi-species plots it was sown in, although two other forbs, showy tickfoil (*Desmodium canadense* (L.) DC.) and oxeye sunflower, were dominant in a total of three of the 24 multi-species plots.

Shifts in species dominance over time were evident. Black-eyed susan was the dominant species in both 2008 and 2009, while grasses became the dominant functional group in 2010. Even though black-eyed susan was the dominant species in most or all the plots it was sown in for both 2009 and 2010, its Berger-Parker value decreased in 2009 by nearly 25%. In contrast, Berger-Parker values for the grass species more than doubled from 2008 to 2010.

Overyielding and additive partitioning

2008

In 2008 ten of the 24 multi-species plots overyielded ($R_{YT} > 1$), and of those ten, six displayed evidence of transgressive overyielding ($D_{max} > 0$) (Tables 5.8a and b). Most plots had at least one species performing better than expected ($D_i > 0$); in particular, black-eyed susan always yielded more than expected when sown in mixtures, as did partridge pea in eight of the 13 plots it was sown in. The average R_{YT} was close to 1 for all four richness levels.

When looking at all multi-species plots, CE values were positive in ten plots, while SE was positive in 21 plots. All four richness levels had the same patterns when analyzing diversity mechanisms in 2008; multi-species plots tended to have a negative CE and positive SE, and the net deviation from expected yield (ΔY) was positive. The magnitude of CE was small, compared to SE. Average CE for each richness level ranged from -5.6 to 2.7, with individual plot values ranging from -24.1 to 19.7. In contrast, SE was much larger, with averages ranging from 6.7 to 95.0 and individual plot values ranging from -34.8 to 154.9. Both SE and ΔY were greater in the six- and ten-species plots when compared to the less diverse mixtures.

2009

By August 2009, 16 out of 24 plots exhibited overyielding, and five of those displayed transgressive overyielding (Tables 5.9a and b). All species except oxeye sunflower overyielded in mixture at least once in August. Once again, black-eyed susan and Virginia wild rye overyielded the most number of times, in 13 plots and ten plots, respectively.

There was a positive SE in nine plots, while CE was positive in 16 plots in 2009. All richness levels generally exhibited similar trends when partitioning the diversity effect, with multi-species plots tending to have a positive CE and negative SE, and a positive net deviation from expected yield. In general the magnitude of SE was smaller than that of CE. When averaged within species richness levels, CE ranged from 31.2 to 117.0, with individual plot values from -51.6 to 231.0. Values for SE averaged within treatments ranged from -81.9 to -7.9, with individual plot values from -222.0 to 62.3. More diverse plots also appeared to have stronger positive CE values and negative SE values in August 2009 than in 2008.

2010

In August 2010, 50% of the multi-species plots overyielded, but only four exhibited transgressive overyielding (Table 5.10a and b). Only two species, Illinois bundleflower and black-eyed susan, did not individually overyield. Indiangrass was the species that overyielded the most, overyielding in over 50% of the multi-species plots it was sown in.

Out of the 24 plots, SE was positive in 17 and CE was positive in 12 plots. Multi-species plots tended to have positive SE, while CE values were more variable, although in general, SE values tended to have a greater magnitude than CE values. The net deviation from expected yield (ΔY) tended to be greater than zero. When averaged within species richness levels, CE was weaker in 2010, and averages for each richness level ranged from -8.4 to 11.7, with individual plot values from -73.9 to 121.1. SE values were more positive in 2010, compared to 2009. Averaged values within richness levels ranged from -19.0 to 42.9, with individual plot values from -150.6 to 114.1. Interestingly, the two-, four- and ten-species plots all followed the same tendencies for CE, SE and ΔY , although the six-species richness level departed from these

trends. While the average magnitude of CE was relatively small, both SE and ΔY tended to be negative at the six-species richness level.

The year 2009 had both the most number of plots with a positive CE and of the greatest magnitude, so the species composition of mixtures with a large positive CE were identified in an attempt to ascertain if any the presence of any particular species in mixture could be associated high complementarity. Nine plots containing between two and six species and which had a CE of greater than 50 were isolated. Nine of the ten species were represented between three and seven times. Only one species, big bluestem, appeared a single time. Based on species representation within these plots, there did not appear to be any specific combination of species that caused such a strong positive CE. However, Virginia wild rye was present in seven of the nine plots and switchgrass in six of the nine plots that met criteria for analysis, suggesting that these two species may have some effect in fostering positive CE.

DISCUSSION

While previous diversity-productivity studies have included NWSPs in their pool of species, such as Tilman et al.'s diversity experiments (Tilman et al. 2006a, Fornara and Tilman 2009, DeHaan et al. 2010), this experiment is one of the first to focus primarily on the ability of diverse NWSP assemblages to affect herbage mass. Adler et al. (2009) noted a negative relationship between diversity and productivity in their assessment of 34 CRP warm-season grasslands but did not control species richness or seeding rates in an experimental setting. While this experiment lacked replication of each random assemblage, preventing a full analysis of the effects of species composition, some trends related to diversity were still observed.

Although by 2009 all five richness levels produced equivalent amounts of forage herbage mass, only ten-species mixtures yields were stable throughout all three years. This suggests that diversity may help improve community stability over time, even if species composition changes. Diversity may help improve community temporal stability through several mechanisms includingoveryielding, the “portfolio effect,” where a total community productivity will fluctuate much less than individual species' yields, and by fluctuations in the productivity of individual species (Lehman and Tilman 2000, Isbell et al. 2009). Changes in individual species populations are expected over time, as each population responds differently to annual environmental variations.

In diverse communities, this “population asynchrony” has been shown to help stabilize yields (Hector et al. 2010). The ten-species mixtures may have had the most stable forage yields over time due to all three mechanisms, as there was evidence of overyielding and of asynchronous species productivities.

Stability is a valuable trait for a community. Diversity-related stability has been shown to help maintain productivity during both major events, such as droughts, along with minor annual environmental fluctuations (Tilman 1996). While drought is one commonly studied disturbance, diversity also stabilizes productivity when a system is challenged with nutrient pulses and grazing (McNaughton 1977). Species-rich grasslands may also be more resilient and recover more quickly from perturbations to the system (Tilman and Downing 1994). These findings suggest that in agricultural settings, more diverse forage plant communities could be desirable to help ensure that sufficient forage is produced each year to meet grazing livestock requirements. However, other work proposes that diversity may not increase stability and that careful selection of a few well-suited species may maximize productivity and stability (Tracy and Sanderson 2004b).

Weed biomass tended to be highest in monocultures in 2009 and 2010, when the plots were not weeded, and the average monoculture weed biomass was nearly 4.5 times greater than in more diverse mixtures. The weed-suppression ability of a mixture is most likely closely tied to its forage productivity. In 2009 and 2010, monocultures tended to contain greater amounts of weedy biomass due to low forage yields from species such as partridge pea, Illinois bundleflower, and to a lesser extent, showy tick trefoil. Mixtures including these species did not typically contain as much weed biomass due to the presence of other higher-yielding forage species.

While species-rich plots had higher NWSP richness throughout the duration of the experiment, dominance, as represented by the Berger-Parker index, changed. In 2008, Berger-Parker values were lower in low diversity plots and higher in species-rich plots. Low dominance values in monocultures and binary mixtures may be explained by slow native grass establishment, while high values in the six- and ten-species plots could be attributed to the productive forbs and legumes, especially black-eyed susan. Berger-Parker dominance values changed in 2009 and 2010, with higher dominance values in the low diversity plots and lower

values in species-rich plots. While less diverse mixtures ended up becoming more dominated by a single species, more diverse mixtures actually increased in diversity over time. Shifts in species dominance also occurred, with grasses becoming the dominant functional group in 2010, and increases in grass abundances helped lower the Berger-Parker dominance values in diverse plots. About 40% of the biomass of six- and ten-species plots was due to non-dominant species in 2010, compared to only 15 to 25% in 2008.

Diversity studies often only report findings from one or two growing seasons (Hector et al. 1999, Dukes 2001b). It may take more time for trends to develop or potentially change as communities establish. The relationship between diversity and biomass yields has been shown to change over time in some experiments (Tilman et al. 2001b, Hooper and Dukes 2004, van Ruijven and Berendse 2005). In general, positive net biodiversity effects tend to increase with the length of the experiment, according to meta-analysis (Cardinale et al. 2007).

In contrast to what many studies have reported (Tilman et al. 2001b, Hooper and Dukes 2004, van Ruijven and Berendse 2005), results from this experiment did not show that the initial diversity-productivity relationship became increasingly positive several years after establishment. The prediction that increased species richness would increase productivity only held true for the first of three growing seasons. More diverse plots had higher yields in the establishment year, but in following years yields in all five levels of species richness were equivalent. Weis et al. (2007) observed that as algal communities proceed through successional stages, diverse assemblages had a temporary positive relationship to productivity followed by a negative relationship, attributing this trend to both intraspecific and interspecific competition. Early in establishment, monoculture yields may be limited by intraspecific competition, while later, the yields from diverse assemblages may be reduced due to interspecific competition among species. While negative intraspecific interactions were most likely small in the first season, as NWSPs were establishing and many species produced relatively small yields, it is possible that interspecific interactions may have affect yields in subsequent seasons.

Results were similar to those of Cardinale et al. (2007), who found that diverse mixtures still had positive diversity effects even though their yields tended to be less than the most productive monocultures. Even though multi-species plots produced as much forage biomass as monocultures, there still was evidence of overyielding and a biodiversity effect in many of the

multi-species assemblages. Approximately 64% of plots exhibited a positive biodiversity effect for each year. Annually, 53% of multi-species plots had a relative yield total greater than 1, signaling overyielding, while fewer plots, only 4-6 per year, exhibited transgressive overyielding. The incongruity between the relatively high frequencies of positive CE with low levels of transgressive overyielding may be a result of a level of complementarity that generates a positive diversity effect but is not strong enough to result in transgressive overyielding (Cardinale et al. 2007).

Multi-species plots showed both selection effects and complementarity effects, and plot SE and CE values shifted over time. While the combination of complementarity and sampling effects help explain the higher yields obtained from diverse mixtures, CE and SE tend to shift based on spatial, climatic, and temporal changes. Location and geographic variation also can impact the relationship between diversity and ecosystem properties, as different sites can have various diversity responses (Spehn et al. 2005). Environmental heterogeneity, such as soil disturbance, can impact partitioning of the net biodiversity effect, with increased CE in more heterogeneous environments and a greater SE in more homogeneous environments (Wacker et al. 2008).

Climate, in particular water availability, also may affect SE and CE partitioning. Increased soil moisture may increase CE and overyielding in diverse communities by increasing complementarity among species (Hooper and Dukes 2004). Increases in overyielding due to complementary resource use and resource partitioning have been observed when other resources such as nutrients and light are not limiting (Fridley 2003, Vojtech et al. 2008). In contrast, other studies have found that higher yields in species-rich communities have been associated with facilitation and more complete use of water under water-limiting conditions (Caldeira et al. 2001). In times of drought stress, diverse mixtures overyield because they may either partition water more efficiently or contain drought-tolerant species (Bulteel et al. 2008).

Shifts in biodiversity mechanisms have been observed in longer-term experiments, with CE becoming increasingly positive and SE remaining constant (Cardinale et al. 2007) or SE becoming more negative (Fargione et al. 2007). When comparing just the 2008 and 2009 diversity effects in this experiment, CE increased and SE became more negative, a change predicted by long-term studies. The considerable selection effect found in 2008 was due to

black-eyed susan, which always yielded more than expected based on monoculture yields. The wettest growing season, defined as April through September, was in 2009, which had 6.4 cm more precipitation than historical averages but 13.3 cm more than 2008 and 21.9 cm more than 2010 (see Chapter Three, Table 3.4). The year 2009 also had the most the number of plots with a positive CE and the fewest number with a positive SE. It is possible that increased water availability in this year reduced interspecific competition, as the greater number of plots exhibiting a positive CE suggests that positive species interactions such as resource partitioning may be occurring. These trends did not continue in 2010, though, which was the driest year. This decrease in precipitation may also help explain the reduction in plots with a positive CE. Based on three years of data there did not appear to be any trends in CE or SE, but this is not unexpected due to the relatively short duration of the experiment.

Another possible explanation for the shifts in additive partitioning components is that successional shifts may also explain changes in CE and SE over time. Diverse plots originally started out being dominated by early successional forbs, black-eyed susan in particular. Over time, the later successional grasses increased in biomass and became dominant species in 2010. This change in species composition may have caused 2009 to be a transitional year, with forbs decreasing in dominance and grasses increasing. This may have allowed CE to be higher, while lowering SE by reducing the dominance of any particular species. Both positive CE and SE were important in 2010, which could be explained by increased dominance of grasses, but not any single species in particular. Finally, based on 2009 additive partitioning, no one species or species mix seemed to be more compatible than others, as no trend stood out among mixtures with a large positive CE.

CONCLUSIONS

This experiment produced several conclusions. It is possible that over time, species-rich NWSP mixtures are as productive as NWSP monocultures. Additionally, more species-rich plots may be more stable over time and tend to have more even communities over time. Although the most productive monoculture outperformed species-rich plots in all three years, there was still evidence of a positive diversity effect. In all three years, both SE and CE were important factors in explaining the diversity effect, although weather and species succession may both play

important roles in causing shifts in the additive partitioning of the net biodiversity effect. This suggests that early positive diversity effects may be primarily due to the increased chance of species-rich plots containing a high-yielding species, in this case, black-eyed susan. Positive effects in later years may be a result of increasing complementarity among establishing NWSPs.

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TABLES

Table 5.1: Variables foroveryielding and additive partitioning

S = number of NWSP species in mixture
 M_i = average yield of species i in monoculture
 $\max(M_i)$ = average monoculture yield of the best performing species found in mixture
 O_i = observed yield of species i in mixture
 E_i = expected yield of a species i in mixture
 Y_o = observed total yield of a mixture
 Y_e = expected total yield of a mixture
 $RY_{oi} = O_i / M_i$ = observed relative yield of species i in mixture
 $RY_{ei} = 1/S$ = expected relative yield of species i in mixture

Table 5.2: Total biomass (dry weight) for each of the five richness levels. Values are mean \pm one standard error.

Year	One species	Two species	Four species	Six species	Ten species	P-value
	----- g m ⁻² -----					
2008†	219.0 \pm 33.6 ^a	269.9 \pm 66.8 ^{ab}	377.6 \pm 120.2 ^{ab}	624.1 \pm 53.6 ^b	575.1 \pm 79.6 ^b	0.0005
2009	595.9 \pm 64.7	604.0 \pm 159.6	642.2 \pm 91.7	579.9 \pm 35.6	637.8 \pm 119.8	0.9653
2010	528.0 \pm 58.7	632.6 \pm 112.7	729.4 \pm 98.3	364.8 \pm 65.8	618.7 \pm 146.6	0.3048

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD.

Table 5.3: Sown native, warm-season perennial biomass (dry weight) for each of the five richness levels by richness for each year. Values are mean \pm one standard error.

Year	One species	Two species	Four species	Six species	Ten species	P-value
----- g m ⁻² -----						
2008†	167.3 \pm 34.9 ^a	160.7 \pm 73.9 ^a	336.0 \pm 127.3 ^{ab}	588.4 \pm 52.3 ^b	517.6 \pm 79.7 ^b	< 0.0001
2009	475.7 \pm 68.8	569.8 \pm 161.7	618.0 \pm 93.3	553.0 \pm 31.5	615.9 \pm 119.2	0.4521
2010	455.2 \pm 65.4	614.3 \pm 113.5	713.6 \pm 101.1	343.8 \pm 65.8	611.2 \pm 147.9	0.1982

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD.

Table 5.4: Mean weed biomass (dry weight) for each of the five richness levels by richness for each year. Values are mean \pm one standard error.

Year	One species	Two species	Four species	Six species	Ten species	P-value
----- g m ⁻² -----						
2008†	51.7 \pm 6.2	109.3 \pm 37.3	41.6 \pm 12.2	35.7 \pm 7.5	57.5 \pm 6.1	0.2688
2009	120.3 \pm 34.1	34.2 \pm 16.0	24.2 \pm 11.2	26.8 \pm 11.2	21.9 \pm 11.4	0.0606
2010	72.7 \pm 21.4	18.3 \pm 5.4	15.9 \pm 10.8	21.0 \pm 7.1	7.4 \pm 1.9	0.4985

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD

Table 5.5: Native, warm-season perennial species richness for each of the five richness levels by richness for each year. Values are mean \pm one standard error.

Year	One species	Two species	Four species	Six species	Ten species	P-value
	----- species 0.25 m ⁻² -----					
2008†	1.0 \pm 0.0 ^a	1.8 \pm 0.2 ^b	2.7 \pm 0.4 ^{bc}	2.5 \pm 0.3 ^{cd}	4.5 \pm 0.6 ^d	< 0.0001
2009	1.0 \pm 0.0 ^a	1.8 \pm 0.2 ^b	3.7 \pm 0.3 ^c	5.0 \pm 0.5 ^d	7.5 \pm 0.3 ^e	< 0.0001
2010	1.0 \pm 0.0 ^a	1.8 \pm 0.2 ^b	2.7 \pm 0.3 ^c	4.7 \pm 0.2 ^d	5.3 \pm 0.7 ^d	< 0.0001

†Means within rows having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD.

Table 5.6: Average Berger-Parker dominance index values for across species richness levels for each year. Values are mean \pm one standard error.

Species richness	2008	2009	2010
One†	0.59 \pm 0.15	0.74 \pm 0.06	0.75 \pm 0.08
Two	0.47 \pm 0.13	0.72 \pm 0.07	0.80 \pm 0.06
Four	0.63 \pm 0.15	0.63 \pm 0.07	0.77 \pm 0.07
Six	0.85 \pm 0.04	0.66 \pm 0.08	0.61 \pm 0.06
Ten	0.74 \pm 0.02	0.52 \pm 0.06	0.60 \pm 0.06
P-value	0.1413	0.3891	0.6452

†Means within columns having unlike letters differ statistically ($P < 0.05$) using Fisher's LSD.

Table 5.7: Berger-Parker dominance index value percentages of multi-species plots dominated by each species for each plot where the species was planted for a) 2008, b) 2009, and c) 2010. See Table 3.1 for explanation of species abbreviations. Values are mean \pm one standard error.

a) 2008

Dominant species	Average dominance	# Plots dominant in	% of sown plots
SG	0.26 ± 0.07	3	20
BB	0.22	1	8
IG	--	--	--
LB	0.09	1	10
VWR	--	--	--
ILB	--	--	--
TF	--	--	--
PP	0.55 ± 0.15	3	23
BES	0.82 ± 0.03	15	100
SUNF	0.8	1	7

b) 2009

Dominant species	Average dominance	# Plots dominant in	% of sown plots
SG	0.71 ± 0.21	2	13
BB	0.44	1	8
IG	0.80 ± 0.03	2	15
LB	0.46 ± 0.02	2	18
VWR	0.76 ± 0.06	4	25
ILB	--	--	--
TF	--	--	--
PP	--	--	--
BES	0.60 ± 0.05	13	87
SUNF	--	--	--

c) 2010

Dominant species	Average dominance	# Plots dominant in	% of sown plots
SG	0.86 ± 0.06	3	20
BB	0.53 ± 0.03	6	50
IG	0.74 ± 0.06	8	62
LB	0.95	1	9
VWR	0.70 ± 0.08	3	19
ILB	--	--	--
TF	0.74	1	8
PP	--	--	--
BES	--	--	--
SUNF	0.58 ± 0.2	2	14

Table 5.8: Overyielding results for August 2008 diversity experiment mixtures.

a) Values for individual plots

Species richness	RYT	D_{\max}	ΔY	CE	SE
Two	0.80	-0.42	-0.05	-1.39	1.33
Two	1.004	-0.02	14.52	0.06	14.46
Two	0.42	-0.57	-11.59	-11.68	0.08
Two	0.81	-0.22	32.54	-17.91	50.46
Two	1.17	-0.63	-23.08	11.72	-34.80
Two	0.95	-0.05	8.21	-0.59	8.80
Four	1.35	0.24	146.88	19.65	127.24
Four	1.20	0.15	103.68	8.56	95.12
Four	1.55	-0.77	-6.76	7.71	-14.47
Four	0.51	-0.49	36.59	-22.85	59.44
Four	1.10	-0.58	5.15	4.76	0.39
Four	0.86	-0.55	-2.30	-1.61	-0.70
Six	1.17	0.04	109.72	10.32	99.40
Six	1.18	0.13	123.60	10.91	112.69
Six	0.73	-0.32	51.04	-15.83	66.88
Six	1.04	0.01	121.38	1.64	119.74
Six	0.70	-0.35	65.15	-12.34	77.49
Six	0.92	-0.08	89.51	-4.51	94.03
Ten	0.80	-0.28	75.79	-8.21	84.00
Ten	0.97	-0.12	101.77	-1.38	103.15
Ten	1.42	0.32	172.66	17.76	154.90
Ten	0.87	-0.24	82.31	-5.41	87.72
Ten	0.71	-0.33	67.78	-12.07	79.85
Ten	0.42	-0.59	25.12	-24.06	49.19

Cells highlighted in gray depict positive values.

b) Overyielding summary tallies based on species richness

Species richness	RYT > 1	$D_{\max} > 0$	+ ΔY	+ CE	+ SE
Two	2	0	3	2	5
Four	4	2	4	4	4
Six	3	3	6	3	6
Ten	1	1	6	1	6
Total	10	6	19	10	21

Relative yield total (RYT) RYT > 1 : overyielding (sum of relative yields for species in mixture) where CE is the complementarity effect and SE is the selection effect

$D_{\max} > 0$: transgressive overyielding

$\Delta Y = CE + SE = Y_{\text{obs}} - Y_{\text{exp}} = \text{net biodiversity effect}$

Table 5.9: Overyielding results for August 2009 diversity experiment mixtures.

a) Values for individual plots

Species richness	RYT	D_{\max}	ΔY	CE	SE
Two	0.82	-0.22	-23.03	-23.03	0.01
Two	1.77	-0.12	57.34	188.31	-130.97
Two	0.52	-0.62	-18.46	-26.49	8.02
Two	0.74	-0.31	-5.71	-17.57	11.87
Two	0.71	-0.29	34.39	-27.87	62.26
Two	1.78	0.51	95.42	94.07	1.34
Four	1.63	0.65	45.38	66.27	-20.89
Four	0.86	-0.62	-24.45	-20.94	-3.51
Four	1.48	-0.53	6.14	75	-68.86
Four	0.63	-0.24	-29.69	-51.64	21.95
Four	3.87	0.17	71.68	120.44	-48.76
Four	1.83	0.84	122.96	116.29	6.67
Six	2.27	0.13	94.41	85.26	9.15
Six	1	-0.59	5.43	0.13	5.3
Six	2.07	-0.66	-8.6	135	-143.6
Six	1.23	-0.57	-4.16	34.69	-38.85
Six	0.85	-0.67	-41.92	-22.57	-19.35
Six	2.52	-0.56	4.66	220.63	-215.98
Ten	2.01	-0.47	62.84	119.98	-57.14
Ten	1.35	-0.66	-2.06	41.95	-44.02
Ten	2.86	-0.16	170.87	221.36	-50.49
Ten	2.94	-0.63	9.05	231.04	-221.99
Ten	0.96	-0.73	-27.73	-4.54	-23.18
Ten	1.77	-0.66	-2.59	92.11	-94.71

Cells highlighted in gray depict positive values.

b) Over-yielding summary tallies based on species richness

Species richness	RYT > 1	$D_{\max} > 0$	+ ΔY	+ CE	+ SE
Two	2	1	3	2	5
Four	4	3	4	4	2
Six	5	1	3	5	2
Ten	5	0	3	5	0
Total	16	5	13	16	9

Relative yield total (RYT) RYT > 1 : overyielding (sum of relative yields for species in mixture) where CE is the complementarity effect and SE is the selection effect

$D_{\max} > 0$: transgressive overyielding

$\Delta Y = CE + SE = Y_{\text{obs}} - Y_{\text{exp}} = \text{net biodiversity effect}$

Table 5.10: Overyielding results for August 2010 diversity experiment mixtures.

a) Values for individual plots

Species richness	RYT	D_{\max}	ΔY	CE	SE
Two	1.05	-0.20	1.15	7.29	-6.14
Two	0.72	-0.48	-37.96	-49.18	11.22
Two	1.18	0.15	135.74	21.64	114.10
Two	1.15	-0.03	10.78	4.28	6.49
Two	0.83	-0.17	63.52	-16.38	79.90
Two	1.05	-0.19	24.38	7.77	16.61
Four	0.87	-0.33	30.79	-15.07	45.86
Four	1.31	0.03	124.90	43.26	81.63
Four	0.77	-0.25	-32.91	-27.56	-5.34
Four	0.92	-0.08	54.34	-10.04	64.38
Four	1.21	-0.23	101.00	14.21	86.79
Four	1.38	0.01	49.69	65.38	-15.69
Six	1.58	-0.41	65.86	37.08	28.78
Six	0.66	-0.64	-36.50	-44.16	7.65
Six	0.96	-0.67	-1.52	-3.80	2.27
Six	0.46	-0.81	-88.41	-73.93	-14.48
Six	0.86	-0.50	-6.81	-19.06	12.25
Six	1.41	-0.88	-97.23	53.37	-150.60
Ten	1.02	-0.59	-7.14	1.98	-9.11
Ten	2.06	0.28	216.26	121.14	95.12
Ten	1.59	-0.39	43.96	67.35	-23.38
Ten	0.79	-0.48	21.20	-24.22	45.43
Ten	0.57	-0.61	-12.73	-49.35	36.62
Ten	0.53	-0.67	-27.55	-53.90	26.35

Cells highlighted in gray depict positive values.

b) Over-yielding summary based on species richness

Species richness	RYT > 1	$D_{\max} > 0$	+ ΔY	+ CE	+ SE
Two	4	1	5	4	5
Four	3	2	5	3	4
Six	2	0	1	2	4
Ten	3	1	3	3	4
Total	12	4	14	12	17

Relative yield total (RYT) RYT > 1 : over-yielding (sum of relative yields for species in mixture) where CE is the complementarity effect, SE is the selection effect

$D_{\max} > 0$: transgressive over-yielding

$\Delta Y = CE + SE = Y_{\text{obs}} - Y_{\text{exp}} = \text{net biodiversity effect}$

Chapter Six

Forage yield, nutritive value, and elemental composition of ten native, warm-season perennial species

ABSTRACT: Native, warm-season perennials (NWSPs) have the potential to provide nutritious summer forage in grazing systems. Although some research has examined NWSP grass yields and nutritive value, there is little documentation on the productivity and nutritive quality of many NWSP forbs and legumes. The objective of this study was to measure the herbage mass, nutritive value, and elemental composition of ten NWSPs. Five perennial grasses, three legumes, and two forbs were grown for three years and harvested annually in August for analysis. Black-eyed susan (*Rudbeckia hirta*) yielded the most biomass in 2008 (6.5 Mg ha⁻¹), but by 2010 the native, warm-season grasses and oxeye sunflower (*Heliopsis helianthoides*) were the highest yielding (8.6 Mg ha⁻¹). Perennial grasses generally had higher fiber concentrations and lower crude protein (CP) concentrations than legumes and forbs. While neutral detergent fiber concentrations of some perennial grasses exceeded 650 g kg⁻¹, potentially limiting available energy, CP in all ten species was high enough to maintain nonlactating cows. Results of elemental analysis showed that concentrations of eleven elements were adequate for nonlactating cows, with only sodium levels below minimum livestock requirements. With appropriate management such as fertilization and the timing of mowing or grazing, NWSPs could be a valuable forage option for grazing cattle.

INTRODUCTION

Native, warm-season perennial (NWSP) species have been recommended as a way to diversify and enhance traditional cool-season grazing systems (Tracy et al. 2010). Warm-season species, adapted for peak productivity during the summer months, produce the bulk of their total yields in mid-summer (Griffin and Jung 1983). Midsummer is also a period when cool-season grasses undergo a “summer slump,” which is a drop in cool-season productivity or even temporary dormancy. This slump can pose a challenge in a solely cool-season grazing system where producers may need to rely on stored hay or else purchase additional feed until grasses resume higher productivity rates (Riesterer et al. 2000). One solution is to use NWSPs as a way

to even out forage availability during the grazing season by providing forage when cool-season species have low productivity.

There are several advantages to using NWSPs in a forage-grazing system. Warm-season species are adapted to a range of soil conditions, are winter hardy and are drought tolerant; some species, such as switchgrass, even have cultivars that are adapted for growth in wet soils (Balasko and Nelson 1995). Due to their physiology, warm-season species have higher levels of water use and N use efficiencies, while their light-use efficiency and overall productivity do not decline as temperatures increase (Monson 1993). They can be highly productive, with sustainable switchgrass yields approaching $15 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ when fertilized with $50 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ (Parrish and Fike 2005). While NWSPs have the potential to be highly productive, their timing of growth and maturation, forage quality, and grazing tolerance varies among species, and management of NWSPs is important for optimizing their forage potential (Balasko and Nelson 1995). Compared to cool-season species at similar maturity stages, NSWPs are lower in protein content, higher in fiber, and are less digestible (Collins and Fritz 1995). Maturity is another concern, since as plants mature they decrease in nutritive quality. As the native, warm-season species eastern gamagrass mature from the boot to anthesis growth stage, neutral detergent fiber (NDF) increased by 20% and crude protein (CP) decreased by 50% (Roberts and Gerrish 1999b).

While data can be found on the nutritional aspects of cool-season forage species, less is known about the mineral concentrations of native, warm-season perennial species (NWSPs). It is generally accepted that NWSPs are lower in nutritional quality than cool-season grasses and typically contain lower protein concentrations and higher fiber concentrations (Balasko and Nelson 1995). As a result, NWSPs are recommended as forage for animals that have lower nutrient requirements, such as beef cattle (Griffin et al. 1980). However, to our knowledge there have been few nutrient and elemental composition analyses performed on potential NWSP forage species. Some work has been done on a few NWSPs, mainly switchgrass (*Panicum virgatum* L.) and Illinois bundleflower (*Desmanthus illinoensis* (Michx.) MacMill. ex B.L. Rob. & Fernald) (Shockley et al. 2004, Fischbach et al. 2005a, Adler et al. 2006, Monti et al. 2008). Illinois bundleflower has even been suggested as a native species that may be suitable for domestication and incorporation into the human diet (Kulakow et al. 1990). In general, the composition and nutritive values of many native plant species have not been widely documented.

The objectives of this study were to analyze the yield, nutritive value, and elemental composition of ten native prairie species grown in monoculture over a period of three years and to compare them to livestock nutritional requirements. This information on NWSPs could be useful for forage producers and may also aid in determining if any NWSPs are suitable for further domestication and breeding.

MATERIALS AND METHODS

Table 3.1 in Chapter Three describes NWSP species tested. See Chapter Five for information on site location and experimental design. For individual species analyses, all ten species were planted in monoculture and replicated four times.

Soil sampling

Soil samples were collected by first dividing the entire area into thirds, then collecting five 15-18 cm deep subsamples. Within each third, subsamples were combined and tested for various soil properties by the Virginia Tech Soil Testing Laboratory (Table 6.1).

Nutritive value analysis

Three samples of each species were collected for nutritive analysis in 2008, 2009, and 2010. When the collected biomass sample from a single plot was too low, samples were pooled to have enough biomass for sampling. Occasionally there was not enough biomass present in the collected samples for some species to allow for three nutritive samples. In 2010 partridge pea (*Cassia fasciculata* Michx.) could not be sampled due to low partridge pea presence. Samples were ground in a Wiley mill to pass through a 1-mm screen and analyzed for crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), and ash content (% ash). Plant samples were processed in the same manner as described for the grazing experiment forage samples in Chapter Three.

The chemical composition of plant samples was assessed by A & L Eastern Laboratories (Richmond, VA). Plant samples were dried, then ground to a fine powder and tested. Nutrients

analyzed included sulfur, phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc, copper, boron, and aluminum. Nitrogen was analyzed with a LECO FP-528, using a combustion furnace at 850°C. Nitrogen content was then evaluated by a thermal conductivity cell. Elemental composition was determined through microwave digestion of samples followed by measurement using an ICP spectrometer.

Data Analysis

Plant data were analyzed using a randomized complete design with species as the treatment factor (n = ten species, with four replications). All data were analyzed using SAS statistical software (SAS SAS Institute, Inc. 2008) to test for significant differences among species using the macro pdmix800 (Saxton 1998). Data were checked for normality and equal variance and transformed when necessary. The most common transformation was taking the square root of dry weight biomass (i.e., sown, weedy and total) to address unequal variance.

RESULTS

Yield results

Herbage mass differed among the ten species in 2008 ($p < 0.0001$, Figure 6.1). The forbs and legumes generally yielded more biomass than the grass species. In this establishment year, black-eyed susan (*Rudbeckia hirta* L.) was the most productive species, yielding over 75 times more biomass than the least productive species, little bluestem (*Schizachyrium scoparium* (Michx.) Nash) and Virginia wild rye (*Elymus virginicus* L.).

Most species were much more productive in the second year. In August 2009, average forage biomass yields were 180% greater than in August 2008. When comparing yields from August 2008 and August 2009, seven of the ten species averages increased; the exceptions were partridge pea, an annual legume that did not re-establish well in 2009 and exhibited a significant decrease, black-eyed susan, which produced slightly less (approximately 4% less) in the second year, and Illinois bundleflower, which yielded equivalent amounts both years. As in 2008, yields varied across species, but biomass rankings were different between years. The perennial grasses and oxeye sunflower (*Heliopsis helianthoides* (L.) Sweet) in particular grew much more

vigorously in 2009. Oxeye sunflower was most productive species, followed by the perennial grasses, while the partridge pea was least productive ($p < 0.0001$).

Trends in species yields that were seen in 2009 continued in 2010. Oxeye sunflower yielded the most biomass, followed by the native, warm-season grasses, some of which were as high-yielding as oxeye sunflower. Switchgrass was a particularly notable species, as its herbage mass more than doubled from 2009 to 2010. Very little partridge pea was present in the plots in 2010. Black-eyed susan biomass continued to decrease from 2009 to 2010, and was much less abundant than in past years. In contrast, warm-season grass biomass tended to increase or remain constant.

Yields of individual species changed over the three years. Yields from seven of the ten species increased, with grass species yields increasing the most. Switchgrass was the grass species that increased the least, at a nearly 10-fold increase, while little bluestem yields increased by over 50 times from 2008 to 2010. Two species, partridge pea and black-eyed susan, decreased in herbage mass by 90% to 100%. Illinois bundleflower and showy tick trefoil (*Desmodium canadense* (L.) DC.) yields remained relatively consistent throughout the three years.

Nutritive values and elemental composition of species

Overall, legumes tended to have a higher crude protein (CP) concentration, lower neutral detergent fiber (NDF) and lower acid detergent fiber (ADF) than the warm-season grasses (Figure 6.2). Forb nutritive values tended to be intermediate between legumes and grasses. ADF concentrations were generally more similar across NSWPs than NDF concentrations were, except for Illinois bundleflower, which tended to be lower than other species. In 2008, warm-season grasses had CP concentrations similar to the forbs and legumes, although showy tick trefoil CP was higher than any other species. In 2009, showy tick trefoil and partridge pea had higher CP than the rest of the species, while the third legume, Illinois bundleflower, had levels similar to that of the other non-leguminous forbs and grasses. In 2010, big bluestem (*Andropogon gerardii* Vitman) CP concentrations dropped below the rest of the warm-season grasses, to 58 g kg^{-1} .

Neutral detergent fiber was higher for grasses than forbs or legumes throughout the experiment. One exception was in 2010 for black-eyed susan, which had a higher NDF than the other forbs and was equal to that of the grasses. Native species had more similar ADF concentrations, and rankings varied each year, making generalizations difficult. Illinois bundleflower typically had lower ADF than other species, while black-eyed susan often had higher ADF.

For most species, CP concentration was highest and fiber concentrations were lowest in 2008, the establishment year. Forage nutritive values for each species typically remained similar in the 2009 and 2010. Between 2008 and 2010, perennial grass CP concentrations declined by approximately 44%. In contrast, black-eyed susan, partridge pea, and Illinois bundleflower showed some increases in CP from 2008 to 2010. Most grass and forb species tended to increase in fiber concentrations from 2008 to 2010. Grass species NDF increased by 10% and ADF increased by 15%, while forb NDF and ADF increased by 29% and 43%, respectively. Legume NDF concentrations did not change from 2008 to 2010, although ADF increased by 30%. Changes were species specific, as fiber concentration for little bluestem, Illinois bundleflower, and showy tick trefoil remained constant throughout the experiment.

Chemical composition of the ten species could be roughly separated using species functional groups, with perennial grasses, legumes, and forbs forming groups (Tables 6.2a and b). In general, the warm-season grasses contained lower levels of many macronutrients such as nitrogen, calcium, and sulfur when compared to legumes and forbs. Forbs and legumes also often had different elemental compositions. Nitrogen concentration was highest in legumes and was nearly 60% higher than in non-leguminous forbs and grasses. The average grass calcium concentration of 0.34 g kg^{-1} was well below the minimum requirement for livestock and were more than four times lower than concentrations in legumes or forbs. In comparison, black-eyed susan had the highest calcium concentration at 2.23 g kg^{-1} . Legumes also tended to contain higher levels of micronutrients with nearly 50% more iron and 42% more manganese than other NWSP functional groups. Manganese concentrations in showy tick trefoil were particularly high, at 225.8 ppm. Forbs contained higher concentrations of boron, nearly 50% more than legumes and 90% more than grass species. Forbs also had the greatest sodium concentrations and contained almost 65% more than the perennial grasses. Grasses, forbs, and legumes contained similar amounts of copper, zinc, and aluminum.

DISCUSSION

Results from analysis of the ten NWSP species show that when grown in monoculture, NWSPs vary in their herbage mass, nutritive value, and elemental composition. The forbs and legumes were higher-yielding than grasses in the first year, but by the end of the third year, the oxeye sunflower was the only species that was as productive as the grasses. While warm-season grasses produced more biomass, their nutritive value was lower than the other species analyzed, and nutritive value typically declined over the three-year period. Analysis of elemental composition showed that once again, the native, warm-season grasses as a group were similar, while individual forbs and legumes had more variation in elemental composition. However, most of the NWSPs tested contained sufficient CP levels and most nutrient concentrations were above minimum livestock requirements for mature, nonlactating cows.

While initial yields were low for the grasses, yields increased in the second and third years. The observed warm-season grass yields of 355 to 883 g m⁻² may be very good for stands that were not fertilized. Other studies have reported unfertilized grass yields ranging from 200 to 400 g m⁻² (Brejda et al. 1995), to approximately 400 g m⁻² (Hall et al. 1982), and up to 600 to 800 g m⁻² (Vogel et al. 2002). In comparison to these reported yields, yields from this current study are at the upper range of yields. Higher yields could occur if NWSPs were fertilized, with one study reporting a yield increase of nearly 1.5 to 2 times when 150 kg N ha⁻¹ was applied (Hall et al. 1982).

Herbage mass from native forbs and legumes were lower than perennial grass yields, with the exception of oxeye sunflower. There are few studies that have reported yields of these species in monoculture for comparison. Additionally, while the initial soil nutrient composition was generally sufficient to maintain switchgrass stands (Table 6.1), little is known about the nutrient requirements of many other NWSPs. Illinois bundleflower herbage mass in this experiment may be somewhat low, as another experiment reported unfertilized yields of 50 to 390 g m⁻² (Fischbach et al. 2005b). Only two of the ten species showed yield decreases from 2008 to 2010: black-eyed susan and partridge pea. Partridge pea is an annual species and black-eyed susan a short-lived perennial, compared to the long-lived perennial nature of the other species. It is possible that due to the combination of life history and a late fall mowing these two species were not able to produce vigorous populations for the duration of the experiment.

Crude protein concentration is often positively correlated with feed energy and negatively associated with fiber concentration (Weiss 1993). All ten species tested were near or above the minimum CP of 60 to 70 g kg⁻¹ CP (60-70 g kg⁻¹) required by livestock (Dougherty and Collins 1995). However, lactating beef cattle will require forage with a higher CP concentration of approximately 90 g kg⁻¹ (National Research Council 2001, Lalman 2004). In comparison, midbloom alfalfa hay CP concentration averages 170 to 220 g kg⁻¹ and midbloom grass hay averages 91 g kg⁻¹ (Sniffen et al. 1992). The CP of the warm-season legumes and forbs tested ranged from 89 to 190 g kg⁻¹, approaching or surpassing requirements for lactating cows. Warm-season grass CP ranged from 108 to 202 g kg⁻¹ in 2008, levels acceptable for cattle, but dropped to 58 to 85 g kg⁻¹ in 2009 and 2010 and would be too low to sustain lactating cattle without supplementation.

Fiber concentration, in particular NDF, is negatively correlated with voluntary dry matter intake and available energy (Mertens 1987, Weiss 1993). As NDF levels increase, dry matter digestibility drops and causing a “fill effect” that reduces daily intake (Collins and Fritz 1995). If NDF concentrations are high enough, maximum daily animal intake will not be able to provide enough energy for animal maintenance. Lactating animals, which require more energy for milk production, will require diets with lower NDF concentrations than nonlactating animals; a cow producing 20 kg of 4% fat content milk per day can consume a diet up to approximately NDF 400 g kg⁻¹ NDF, while a similar nonlactating animal could have a diet over 600 g kg⁻¹ NDF (Mertens 1987). In comparison, midbloom alfalfa hay NDF concentration averages 440 to 460 g kg⁻¹ and midbloom grass hay averages 670 g kg⁻¹ (Sniffen et al. 1992). The higher NDF concentration present in warm-season grasses, which ranges from 624 to 738 g kg⁻¹, may potentially impede the ability for livestock to obtain adequate energy and nutrients if livestock are solely grazing on grasses.

Predictions of forage quality are often based on NDF and ADF, such as the relative feed value (RFV), a prediction of intake rate and energy value of a forage (Ball et al. 2007). However, RFV may be inaccurate as it assumes that fiber digestibility is constant, even though research has shown that degradability within the rumen can vary (Nocek and Russell 1988). Neutral detergent fiber digestibility can affect dry matter intake and milk yield in dairy cows (Oba and Allen 1999). Relative forage quality (RFQ) has been suggested as an alternate forage quality index that addresses the issue of digestibility. In comparison to RFV, RFQ uses total

digestible nutrients (TDN) instead of digestible dry matter (DDM) and should be more accurate in predicting animal performance (Moore and Undersander 2002).

Variation in NWSP fiber digestibility by species can cause actual energy availability to differ and may have caused the discrepancy in predicted versus actual animal performance noted in studies measuring animal weight gains on NWSP grasslands (Reid et al, 1988). Studies have demonstrated that warm-season digestibility varies by species, with switchgrass digestibility lower than that of big bluestem and also declining more rapidly over time (Griffin and Jung 1983). In addition, laboratory tests of warm-season digestibility have underestimated actual digestibility by as much as 17%, compared to animal feeding trials (Griffin et al. 1980). Animal performance on warm-season grass pastures often exceeds predicted performance, and may be due to higher than expected rates of dry matter intake (Reid et al. 1988). Beef steer average daily gains of 0.93 to 0.96 kg day⁻¹ have been recorded on switchgrass, and ADG on indiagrass was 1.08 kg day⁻¹, while big bluestem gains were lower at 0.70 kg day⁻¹ (Krueger and Curtis 1979, Burns et al. 1984).

It is important to note that the NWSP plots were not fertilized, mowed, or grazed during the summer. Plant samples were also taken in August, after many of the species had begun heading. At this late stage of maturity, forage value typically declines, compared to more immature vegetative growth stages (Balasko and Nelson 1995). Management practices such as proper grazing and mowing may help keep plants vegetative for longer periods of time, reduce plant fiber concentration, and maximize animal production on warm-season grasslands (Tracy et al. 2010).

Adequate animal nutrition is vital in maintaining healthy livestock. Although animals can tolerate a range of concentrations for many minerals, in some cases concentrations that are too low or too high can be toxic or deleterious to animal health and growth. At least 17 mineral nutrients are required by beef cattle and are used by animals in a variety of manners, including protein synthesis, maintenance of skeletal, blood and immune system components, and enzyme reactions (Barker and Collins 1995, National Research Council 2000, 2001). Grazing livestock obtain many needed minerals from forage, but any nutrient deficits may need to be supplemented through mineral feed. Elemental composition analysis showed that the ten native species tested provided sufficient amounts of eleven nutrients analyzed to maintain nonlactating cows (Table

6.2a). Only sodium concentrations were too low in all species to maintain cows without mineral supplementation, even though plant sodium concentrations were generally within range of normal plant levels. However, it is also important to note that in this study, species were not fertilized for the duration of the experiment. In addition, entire aboveground portions of plants were sampled for analysis as compared to just leaf tissue.

Mineral elemental composition of plants is dependent on a variety of factors including soil properties, plant species, plant maturity, yield, and climate, as well as grassland management (McDowell 1992). Compared to “normal” forage foliage, elemental composition for these native species was generally low (Barker and Collins 1995). While Table 6.2 lists minimal concentrations at which nutrients cause deficiency, there are also upper limits that may be toxic to livestock (National Research Council 2000). Based on element composition, most nutrients of the NWSPs studied are at sufficient levels to support dry cattle, and many may also support lactating animals. Some exceptions are nitrogen and calcium concentrations of warm-season grasses, which are lower than minimum levels needed for livestock, as well as sodium concentrations for all ten species. Cool-season grasses and legumes and NWSPs appear to differ in nutrient concentrations as well. Compared to typical cool-season species, the NWSPs (in particular the grasses) have lower nitrogen, potassium, and calcium concentrations, but higher manganese and zinc concentrations (Blevins and Barker 2007).

CONCLUSIONS

Yield, nutritive value, and nutrient analyses suggest that NWSPs can provide forage of adequate quantity and quality for grazing nonlactating livestock in summer. As with most forages, NWSP nutritive value and elemental composition provide some limitations to animal nutrition, although NWSPs may provide better forage than expected based on laboratory analyses alone. Forage nutritive values declined over time, so it may be necessary to manage soil fertility to maintain higher quality forages. It is possible that mixing grasses and legumes together, along with mineral supplementation, may provide sufficient forage amounts, as well as alleviate many nutritional concerns. Other management practices, such as the timing of grazing and mowing, that balance forage yields with nutritional aspects may also help improve forage quality.

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TABLES

Table 6.1: Average soil properties for pH, percent organic matter (OM), and nutrient concentration from soil samples collected in November 2008.

Soil pH	6.3	
OM (%)	2.0	
Nutrient	kg ha ⁻¹	Rating†
Phosphorus	16.8	Medium -
Potassium	123.7	Medium
Calcium	1023.0	Medium -
Magnesium	285.4	Very High
Nutrient	ppm	
Iron	5.2	Sufficient
Manganese	9.6	Sufficient
Boron	0.3	Sufficient
Copper	0.3	Sufficient
Zinc	1.1	Sufficient

†Nutrient ratings based on requirements for switchgrass. Soil nutrients testing Medium may respond to fertilization, while nutrients rated Very High generally do not respond to additional fertilization.

Table 6.2: Mineral requirements and elemental composition of a) native, warm-season perennial species, averaged across all three years, and b) grouped by functional group (perennial grasses, forbs, legumes). See Table 3.1 for explanation of species abbreviations.

a) By species

Nutrient	Livestock Deficiency‡			Species									
	Normal†	dry	lactating	SG	BB	IG	VWR	LB	ILB	TF	PP	BES	SUNF
Macro-	----- % -----												
Nitrogen	1 - 4	< 1	< 2.5	1.42	1.34	1.34	1.63	1.60	2.12	2.42	2.52	1.47	1.49
Phosphorus	0.25 - 0.5	< 0.1	< 0.1	0.16	0.17	0.19	0.11	0.19	0.17	0.12	0.15	0.22	0.21
Potassium	2 - 4	< 0.9	< 0.9	1.24	1.22	1.30	1.02	1.10	0.91	1.14	1.08	1.52	1.18
Calcium	0.5 - 2.0	< 0.4	< 0.6	0.34	0.30	0.39	0.28	0.41	1.75	1.32	0.80	2.23	1.15
Magnesium	0.2 - 0.8	< 0.1	0.3	0.24	0.22	0.21	0.11	0.21	0.61	0.35	0.26	0.60	0.95
Sulfur	0.2 - 0.3	< 0.2	< 0.2	0.12	0.10	0.09	0.16	0.11	0.29	0.18	0.16	0.25	0.09
Micro-	----- ppm -----												
Iron	50 - 1000	< 0.1	< 1	66.6	65.3	70.0	63.2	80.1	86.0	84.7	129.9	67.2	66.1
Manganese	30 - 300	< 15	< 15	88.4	103.5	135.8	48.8	129.0	177.9	225.8	90.1	83.4	96.4
Boron	10 - 50	0	0	3.5	5.3	3.8	3.6	4.3	38.5	18.1	18.7	47.7	51.1
Copper	5 - 15	< 0.6	< 0.6	9.1	5.9	6.5	5.0	7.2	9.5	7.3	9.9	8.3	8.0
Zinc	10 - 100	< 4	< 6	20.7	25.0	31.0	20.2	25.3	34.8	23.6	26.9	33.8	18.5
Sodium	100 - 200	< 1000	< 2000	99	82	126	81	101	283	222	154	353	201
Aluminum				7.2	2.4	6.5	11.1	6.4	10.6	5.7	3.6	8.4	10.7

† “Normal” plant element composition of forage foliage and livestock deficiency levels taken from Barker and Collins (1995).

‡ Nutrient concentration minimums that may result in deficiencies for livestock are separated into two general livestock classes: dry (nonlactating) and lactating cows.

b) By functional groups

	Grass	Legume	Forb
Macronutrients	----- % -----		
Nitrogen	1.47	2.35	1.48
Phosphorus	0.16	0.15	0.22
Potassium	1.18	1.04	1.35
Calcium	0.34	1.29	1.69
Magnesium	0.20	0.41	0.78
Sulfur	0.12	0.21	0.17
Micronutrients	----- ppm -----		
Iron	69.0	100.2	66.7
Manganese	101.1	164.6	89.9
Boron	4.1	25.1	49.4
Copper	6.7	8.9	8.2
Zinc	24.4	28.4	26.2
Sodium	97.8	219.7	277.0
Aluminum	6.7	6.6	9.6

FIGURES:

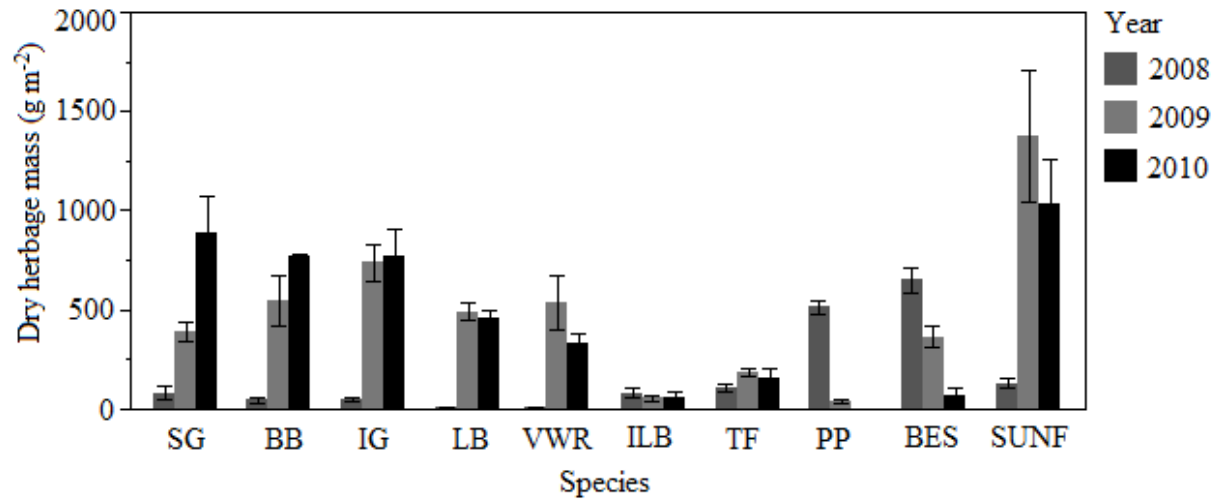


Figure 6.1: Sown species biomass in 2008, 2009 and 2010. Error bars represent one standard error above and below the mean. See Table 3.1 for explanation of species abbreviations.

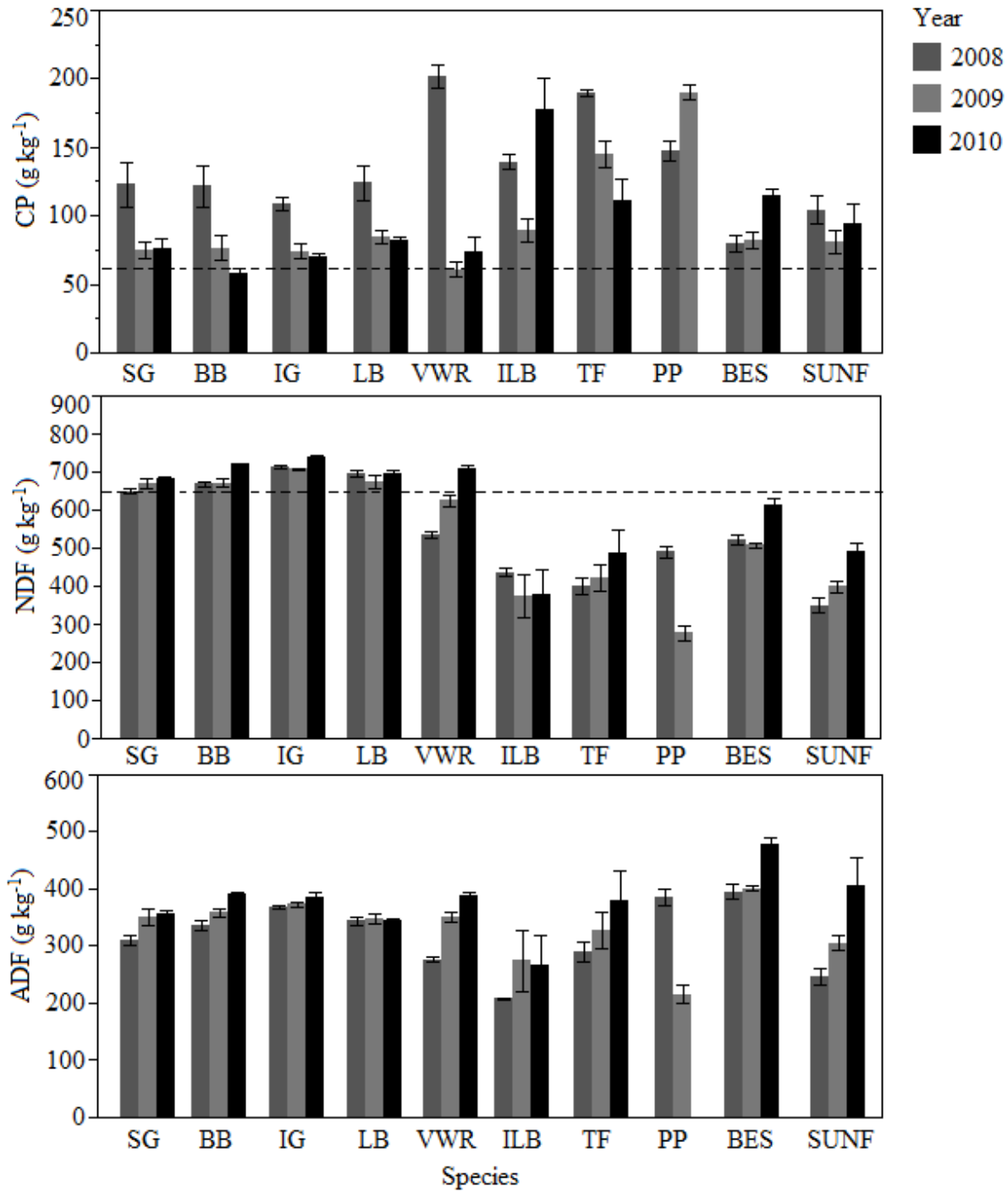


Figure 6.2: Yearly forage nutritive values for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Error bars represent one standard error above and below the mean. The dashed line in the CP bar chart represents minimum CP requirements for livestock. In the NDF chart the dashed line represents the balance point between NDF and animal fill for a nonlactating cow; Note: Partridge pea was not present in plots in 2010 and thus has no values for this year. See Table 3.1 for explanation of species abbreviations.

Chapter Seven

Conclusion: Native, warm-season perennial diversity: implications and applications

Native-warm season perennials (NWSPs) are being suggested for use in a variety of purposes, from agricultural uses such as forage and bioenergy crops, to ecological uses such as wildlife habitat and soil protection. Diverse NWSP mixtures are hypothesized to be advantageous over monocultures through higher yields, reduced weediness, more stable yields, and higher nutritive value. However, NWSP communities behave differently when grazed or left ungrazed. The first part of this research examined how three NWSP mixtures of different levels of species richness would perform under management for grazing or biomass biocrops.

Many hypotheses were supported, including that grasslands sown with diverse mixtures would: 1) produce more herbage mass and contain less weed biomass and 2) support more sown forage species while reducing weed species richness. Compared to biomass crop exclosures, grazing increased weed cover and weed species richness, also supporting hypotheses put forth in this experiment. Several hypotheses were not supported, as 1) forage mixture did not improve nutritive value but tended to decrease it, 2) grazing did not help diverse mixtures retain forbs and legumes, and 3) grazing and forage mixture did not tend to cause differences in measurements of community diversity. Grazing, even just for two seasons, seemed to negatively affect NWSP communities and may have reduced NWSP vigor and competitiveness. In contrast, NWSPs managed for biomass produced much higher forage yields and lower weed yields after two years of management. The work suggests that managing NWSPs for grazing must be done carefully in order to prevent stand damage and reduce weed invasions. However, diverse mixtures may help generate productive stands that can better withstand grazing and weed invasions.

The large-plot grazing experiment provided information on forage yields, nutritive values, and community composition of three different forage mixtures, but did not directly test the effects of NWSP diversity per se. To test the effects of diversity, a second experiment consisting of random assemblages of NWSPs at five different levels of species richness tested the hypothesis that increased diversity would increase yields. Results show that the hypothesis was only supported in the first year, as all richness levels produced equivalent amounts of herbage mass in the following two years. Even though yields were similar in two of three years, many multi-species plots still exhibited a positive biodiversity effect and yielded more than

expected based on monoculture yields. Additive partitioning showed that in 2008, a positive selection effect (SE) was more common than a positive complementarity effect (CE). In 2009, more plots were observed to have a positive CE, which may be due in part to increased precipitation that allowed positive species interactions. However, by 2010 plots with a positive SE once again increased, contrary to the hypothesis that CE would increase over time and SE would decrease. Results suggest that both SE and CE are important in explaining overyielding in diverse plots, and that both species and the environment may affect diversity effects.

Each of the ten species used in both experiments were also analyzed in monoculture to assess their individual potential as forage. Forbs and legumes were higher-yielding initially, but by the second year grass yields exceeded yields of all the forbs and legumes except for oxeye sunflower. Forage nutritive value varied by species and year, but warm-season grasses were typically of lower value, having lower crude protein concentrations and higher fiber concentrations. Warm-season grass protein levels were usually high enough to maintain livestock, although fiber contents were higher than recommended for lactating cows and at the upper range of that for nonlactating animals. Elemental analysis of each species did not show any unexpected deficits in plant nutrient concentrations, although warm-season grasses often had lower concentrations of many minerals, in particular nitrogen and calcium. Most mineral concentrations were above the minimum acceptable levels for nonlactating cows. Nutritive value and elemental composition both suggested that NWSPs could potentially meet nutrient needs of livestock, with some minor mineral supplementation, such as sodium. Yields and nutritive aspects could further be enhanced by improved management such as fertilization and timing of harvesting or grazing to utilize NWSPs at their peak nutritive point.

This work suggests that NWSPs are a viable option for both forage and as a biomass crop. Diversity may be an additional benefit to agroecosystems by reducing the risk of poor stand establishment, increasing productivity early in establishment, and reducing weed biomass. Warm-season grasses were particularly high yielding and tended to outcompete forbs and legumes in mixtures. Maintaining some legume and forb species may be advantageous in grasslands, both to improve forage nutritive quality and to enhance ecosystem services that are not directly tied to productivity such as soil protection and wildlife habitat.