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SOIL PROCESSES AND PLANT SPECIES: DOES THE RE-INTRODUCTION OF NATIVE GRASSES ALTER SOIL CARBON AND NITROGEN CYCLING?

presented by

Wendy Mae Mahaney

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Katherine J. Gross
Major Professor's Signature

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SOIL PROCESSES AND PLANT SPECIES: DOES THE RE-INTRODUCTION OF NATIVE GRASSES ALTER SOIL CARBON AND NITROGEN CYCLING?

By

Wendy Mae Mahaney

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ABSTRACT

SOIL PROCESSES AND PLANT SPECIES: DOES THE RE-INTRODUCTION OF NATIVE GRASSES ALTER SOIL CARBON AND NITROGEN CYCLING?

By

Wendy Mae Mahaney

Human activities have altered biodiversity on a global scale, but the ecological implications of shifts in plant species distributions and abundances are poorly understood. While a number of studies have shown that exotic species can dramatically and rapidly alter ecosystem properties (Vitousek and Walker 1989, Evans et al. 2001, Mack and D'Antonio 2003a), little is known about how reintroductions of extirpated species may impact ecosystem properties in restored systems. My dissertation research focuses on how the reintroduction of native prairie C_4 grasses into abandoned agricultural fields (old-fields) influences soil carbon (C) and nitrogen (N) cycling compared to non-native C_3 grasses typical of successional communities in southwestern Michigan, USA.

In this dissertation, I explore three main aspects of plant species controls on soil processes: 1) What are the decadal scale impacts of a shift from a C_3- to a C_4-dominated system on soil properties and processes (Chapter Two), 2) How quickly do differences in species traits and soil conditions arise (Chapter Three), and 3) Which plant traits are responsible for differences in decomposition rates (Chapter Four)? I addressed these questions in several old-fields at Michigan State University's W. K. Kellogg Biological Station, using previously established experimental plots of C_4 grasses in Chapters Two and Four, and setting up new experimental studies in Chapter Three.

In Chapters Two and Three, I found that C_4 species had significantly greater shoot biomass and more recalcitrant tissue compared to the dominant C_3 species, and

these differences became apparent within two years after the species were established. In contrast, species differences in surface litter and root biomass took longer than two years to develop but were apparent after 11 years. While there was some evidence to suggest that the C_4 species had reduced soil inorganic N levels relative to the C_3 species after just two years, many of the changes in soil properties took longer than two growing seasons to develop. After 11 years, soils under C_4 species had significantly lower inorganic N levels, and slightly lower *in situ* net N mineralization and nitrification rates when compared to soils under C_3 species. I also found limited evidence for increasing soil C pools under C_4 species 11 years after reintroduction. Nevertheless, the $\delta^{13}C$ signal of the C_4 species became measurable in the soil within two years.

I examined how litter quality and microclimate affected litter decomposition rates, and found that while *Andropogon gerardii* (a C_4 prairie grass) differed from C_3 species in its effect on soil moisture and temperature, these differences did not correspond to differences in decomposition rates. Instead, species litter quality was more important than microclimate in determining decomposition rates of both C_3 and C_4 species.

Overall, my results demonstrated that reintroduction of C_4 species into old-fields can alter soil processes related to C and N cycling on relatively short timescales. Process rates changed first, with changes in pool sizes of C and N taking longer to become measurable. Improving our understanding of how plant species impact ecosystem properties and what species traits are driving these changes is imperative if we hope to predict the ecosystem-level consequences of changes in species distribution or composition that could occur, and are occurring, as a consequence of changes in agricultural and land use practices, global change, and species introductions.

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CHAPTER ONE

INTRODUCTION

Human activities, such as land use change and the introduction of exotic species, have altered biodiversity on a global scale (Lawton and May 1995, Pimm et al. 1995, Chapin et al. 2000, Hooper et al. 2005). However, the ecological implications of shifts in plant species distributions and abundances are poorly understood. While a number of studies have shown that exotic species can dramatically and rapidly alter ecosystem properties (Vitousek and Walker 1989, Evans et al. 2001, Mack and D'Antonio 2003a), little is known about how reintroductions of extirpated species may impact ecosystem properties in restored systems. My dissertation research focused on how the reintroduction of C_4 grasses into abandoned agricultural fields (old-fields) influenced soil carbon (C) and nitrogen (N) cycling compared to exotic C_3 grasses typical of successional communities in southwestern Michigan, USA on both short (1-2 years) and decadal timescales.

Total soil C and N loss associated with conversion of prairie soils to agriculture is dramatic (Burke et al. 1995, Camill et al. 2004, DeGryze et al. 2004), and grassland cultivation in the Midwestern United States has resulted in a 30-60% loss of soil organic C and N (Burke et al. 1995). There is some evidence that prairie restorations can begin to restore soil processes (Baer et al. 2002, Camill et al. 2004) and hasten the buildup of soil C and N pools. However, it is unknown how quickly soil conditions will return to pre-agricultural levels, and whether these changes can be accelerated by using particular species.

Plant species influence soil processes in a variety of ways, and changes in species composition can have dramatic impacts on C and N cycling (Figure 1). One of the most commonly examined ways in which plants alter soil processes is through differences in litter quality (i.e., tissue chemistry) and quantity (Hobbie 1992, Wardle et al. 1998, Ehrenfeld 2003, Lovett et al. 2004, Hooper et al. 2005, Dijkstra et al. 2006). While the importance of species litter chemistry in controlling nutrient cycling is well accepted, how plants impact these processes via their effect on soil microclimate has received less attention. Several recent papers (Mack and D'Antonio 2003b, Eviner 2004, Eviner et al. 2006) suggest that such effects can be an important determinant of soil process rates.

To examine how plant traits are linked to soil properties, I chose to examine C_3 and C_4 grass species that differ significantly in tissue production and chemistry. The C_4 grasses that I examined typically produce more biomass (both root and shoot) and have more recalcitrant tissue (i.e., higher C:N, lignin:N) than the C_3 grass species (Wedin and Tilman 1990, Baer et al. 2002, Camill et al. 2004). These differences led me to hypothesize that soil C and N pools and cycling rates would differ under the two groups of species. However, I did not know how quickly these soil differences would arise, or if species within a group would differ in their effects. Because these C_3 and C_4 species differ in litter quality, quantity and their influences on soil microclimate, it difficult to determine if soil process changes are due to one or a combination of these factors.

The next three chapters explore three main aspects of plant species controls on soil processes in old-fields: 1) What are the decadal scale impacts of a shift from a C_3-dominated system to a C_4-dominated system on soil properties and processes (Chapter Two), 2) How quickly do differences in species traits, and the soil conditions observed

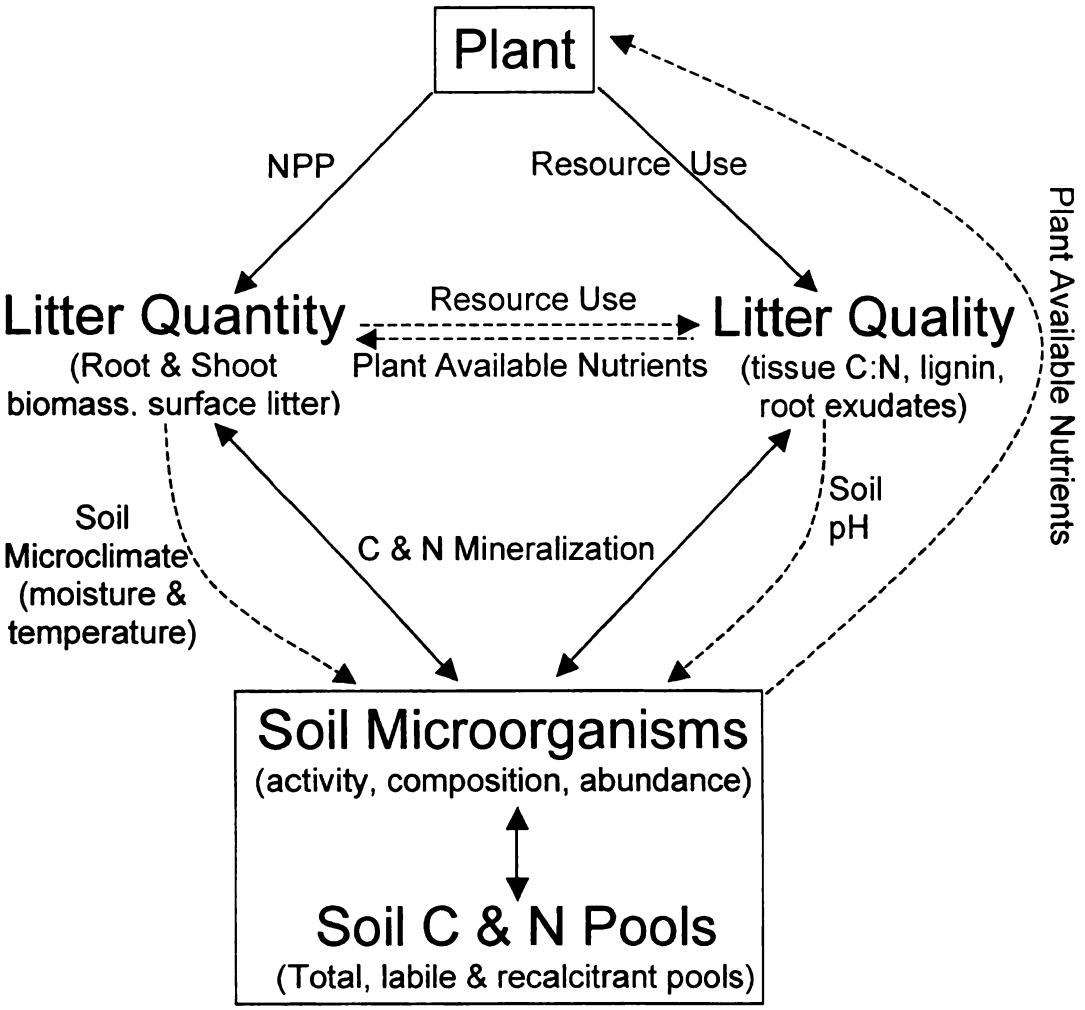


Figure 1. Conceptual diagram of how changes in plant species composition can influence ecosystem processes in terrestrial ecosystems. Plant species may directly (via differences in litter quality and quantity) and indirectly (via effects on soil microenvironment) exert important controls over the functioning of microbial communities that determine nitrogen (N) and carbon (C) cycling.

after 11 years, arise (Chapter Three), and 3) Which plant traits are responsible for differences in decomposition rates (Chapter Four)? This research was performed at several old-fields located at the W. K. Kellogg Biological Station (Appendix 1). All sites were abandoned more than 35 years ago following decades of row crop agriculture (Burbank et al. 1992, Foster and Gross 1997). The old-fields were dominated by non-native C_3 grasses, but had patches of C_4 grass monocultures that were established in 1995 to examine plant competition (Foster 1996, 1999). The C_4 monoculture plots were still intact in 2005 at two sites, allowing me to compare how C_4 versus C_3 grass-dominated communities impact soil properties on a decadal time scale at two old-fields.

In Chapter Two, I examine the decadal-scale effects of the reintroduction of C_4 grasses on soil C and N cycling in the two old-fields with intact patches of C_4 grasses. I utilized the C_4 monocultures established in 1995 to compare plant traits and soil properties in the C_4 monocultures to the surrounding C_3 grass-dominated matrix community. I expected that: 1) the three C_4 species would produce greater biomass and consequently would have greater surface litter and total soil C than the surrounding C_3-dominated community, and 2) slowed microbial process rates associated with the recalcitrant tissue of the C_4 species would result in larger total C and N pools, and inorganic N levels would be lower due to greater microbial N immobilization.

In Chapter Three, I examine whether trait differences between C_4 and C_3 grass species develop within the first two growing seasons, and if soil processes begin to reflect these species differences immediately. I established experimental monocultures in all three old-field sites in 2005, and related tissue production and chemistry to differences in soil properties after two growing seasons to determine the short-term effects of plant

species on soil properties. I predicted that species biomass and tissue chemistry differences would be detected within 1-2 yrs after establishment, and that these differences would translate rapidly into detectable changes in soil inorganic nitrogen pools. In contrast, I expected that changes in soil C and N pools would show a lag between when process rates change and when the total pool would begin to reflect those changes. I hypothesized that the magnitude of species biomass and tissue chemistry differences would determine the rate at which the soil changes are detected.

In Chapter Four, I evaluate the ways in which different traits associated with C_4 and C_3 species can influence litter decomposition to determine the relative importance of litter quality and soil microclimate on plant decomposition rates. I performed two experiments to separate the effects of litter quality and soil microclimate on decomposition. In the first experiment, the decomposition rates of the two litter types (C_3 and C_4) in a common location (either under C_3 species or under C_4 species) were compared to examine both litter quality and soil microclimate effects on decomposition rates. I expected C_4 litter to decompose more slowly than C_3 litter because C_4 litter is more recalcitrant (higher C:N, lignin:N). I also predicted that decomposition rates would be slower under C_3 than C_4 species because the smaller litter layer and lower aboveground biomass of the C_3 species would make these environments less advantageous for microbial activity (i.e., drier and hotter) in the summer than under C_4 species. In the second experiment, I measured the soil microclimate (soil moisture and temperature) under C_3- and C_4-dominated plots, with and without surface litter, to determine if differences in surface litter and aboveground biomass impacted microclimate in a manner that affected decomposition of a standard substrate, cellulose. Summer and

fall soil moisture levels and soil temperature were expected to follow a gradient of relative ground cover, with highest soil moisture and lowest temperatures under the C_4 species with litter intact, followed by C_4 species without litter, then C_3 species with litter and finally C_3 species without litter. I expected cellulose decomposition to be faster under C_4 species if soil moisture limited decomposition and the decomposition rates to be fastest in the C_3 species plots without litter if temperature was the primary factor determining decomposition rates.

The next three chapters in this dissertation describe the results of field experiments that explore how introductions, or re-introductions, of species that differ in functionally important traits (e.g., litter quantity and quality) can alter soil processes, and the timescales over which such changes can be expected to occur. I focused on C_3 versus C_4 grass comparisons as a model system to examine how different plant traits influence soil processes, which has specific application to a variety of areas, including prairie restoration, climate change alterations of C_4-dominated grassland distribution, and the recent focus on using a C_4 prairie grass (switchgrass or *Panicum virgatum* L) as a biofuel source. For example, the current interest in biofuels could result in large expanses of the Midwestern United States being converted from agriculture, old-fields, or conservation properties (i.e., Conservation Reserve Program lands) into C_4 grass monocultures. Given the magnitude of the landscape that may be affected by such changes in species distributions, it is important that we understand how those changes may alter soil properties and processes.

Further, the relationships I show between plant traits and soil processes extend beyond simply native and non-native comparisons or C_3 and C_4 comparisons, and are

generally applicable to other species and systems where a "new" dominant species differs from the previous dominant species by functionally important traits. Improving our understanding of how plant species impact ecosystem properties and what species traits are driving these changes is imperative if we hope to predict the ecosystem-level consequences of changes in species distribution or composition that are predicted to occur, and are occurring, as a consequence of changes in agricultural and land use practices, global change, and species introductions.

CHAPTER TWO

DECADAL SCALE IMPACTS OF C_4 GRASS REINTRODUCTIONS ON SOIL CARBON AND NITROGEN CYCLING IN SUCCESSIONAL ECOSYSTEMS

ABSTRACT. While much recent research has focused on the effects of exotic plant species on ecosystem properties, little is known about how reintroductions of native species may impact these processes in restored systems. I examined how the reintroduction of three native C_4 grasses into old-fields affected soil carbon (C) and nitrogen (N) cycling 11 years after their reintroduction compared to unmanaged successional communities dominated by non-native C_3 grasses in southwestern Michigan, USA. The C_4 species (*Andropogon gerardii* (Vitman), *Sorghastrum nutans* (L), *Schizachyrium scoparium* (Michx)) and C_3 species (*Bromus inermis* (Leyss), *Elymus repens* (L)) examined in this study differ significantly in many traits that are expected to influence soil C and N cycling, and led me to hypothesize that soil C and N pools and cycling rates would differ under the two groups of species. As predicted, the three C_4 species had significantly greater root and shoot biomass, and more recalcitrant tissue compared to the dominant C_3 species. Soils under the three C_4 species had significantly lower inorganic N levels, and *Andropogon* had slightly lower *in situ* net N mineralization rates than soils under C_3 species. I also found little evidence for increasing soil C pools under C_4 species 11 years after their reintroduction. Overall, these results show that reintroduction of C_4 species into grasslands can result in alterations of soil processes related to C and N cycling on relatively short timescales. Improving our understanding of how plant species impact ecosystem properties and what species traits are driving these changes is imperative if we

hope to predict the ecosystem-level consequences of changes in species distribution or composition that could occur, and are occurring, as a consequence of changes in agricultural and land use practices, global change, and species introductions.

INTRODUCTION

Human activities, such as land use change and the introduction of exotic species, are altering biodiversity on a global scale (Lawton and May 1995, Pimm et al. 1995, Chapin et al. 2000, Hooper et al. 2005). However, the ecological implications of such shifts in plant species distributions and abundances are poorly understood. A number of studies have shown that the introduction of an exotic species can dramatically and rapidly alter ecosystem properties (Vitousek and Walker 1989, Evans et al. 2001, Mack and D'Antonio 2003a), but few studies have focused on the reverse: how the reintroduction of native species affects ecosystem properties (sensu Hooper et al. 2005). This study examines how the reintroduction of native prairie grasses into abandoned agricultural fields alters soil carbon (C) and nitrogen (N) cycling after 11 years.

Agriculture has substantially reduced and fragmented prairie systems throughout the United States (Mlot 1990, Samson and Knopf 1994), altering plant communities and ecosystem properties (Camill et al. 2004, DeGryze et al. 2004). In southwestern Michigan, agricultural development has restricted once-common, native C_4 grasses (Gotshall 1972) to prairie remnants, and these species are now rarely found in abandoned agricultural fields (old-fields). Old-fields throughout the Midwest are typically colonized by a successional trajectory of C_3 species, many of which are non-native (Inouye and Tilman 1988, 1995, Foster and Gross 1997, Averett et al. 2004, Gross and Emery 2007).

While C_3 and C_4 functional groups typically differ in many traits that are expected to influence ecosystem properties such as C and N cycling, little is known about the ecosystem-level impacts of the loss of C_4 grasses, in part because their extirpation is confounded with agricultural disturbance. Currently, large tracts of former agricultural land are being reverted to C_4-dominated communities (e.g., prairie restoration and the USDA Conservation Reserve Program) and many climate change models predict shifts in C_4 species distribution (Collatz et al. 1998, Winslow et al. 2003). In addition, the growing interest in biofuels as an alternative energy source is likely to increase the acreage planted to native C_4 grass monocultures such as *Panicum virgatum* (Samson et al. 2005, Sanderson et al. 2006, Tilman et al. 2006). Thus, it is important to understand how the re-establishment, or introduction, of particular C_4 species influences ecosystem properties compared to a common C_3-dominated old-field community.

There is considerable evidence that species' functional characteristics drive important ecosystem properties (Hooper and Vitousek 1998, Reich et al. 2004, Wardle et al. 2004, Hooper et al. 2005). Two common ways in which plant species influence soil processes is through the quality and quantity of their litter (Hobbie 1992, Wardle et al. 1998, Dijkstra et al. 2006). Changes in litter quantity (via net primary productivity) and quality (via allocation and partitioning of C and N into various tissues, determining tissue chemistry) can directly and indirectly influence microbial community activity, abundance, and composition (Zak et al. 2003, Carney and Matson 2005, Hooper et al. 2005, Zavaleta and Hulvey 2007), thereby altering nutrient cycling rates, and potentially feeding back to alter the plant community (Figure 1).

The C_3 and C_4 grasses examined in this study provide an opportunity to examine how plant traits are linked to soil properties because they have similar growth forms yet differ in a number of functionally important traits. C_4 grasses typically produce more biomass (above- and below- ground) and have more recalcitrant tissue (i.e., C:N, lignin:N) than their C_3 grass counterparts (Wedin and Tilman 1990, Baer et al. 2002, Camill et al. 2004). These traits can directly influence soil processes via changes in the amount and form of substrates available for microbial utilization (Zak et al. 2003, Carney and Matson 2005, Hooper et al. 2005, Zavaleta and Hulvey 2007). C_3 and C_4 species also may indirectly impact the soil microbial community via differential effects on the soil microenvironment and timing of resource uptake and release back to the soil. Indeed, several studies have found lower net N mineralization and higher C mineralization rates over relatively short timescales in grasslands as C_4 species become dominant (Baer et al. 2002, Camill et al. 2004).

Here, I examined the decadal-scale effects of the reintroduction of three C_4 grasses on soil C and N cycling in two southwestern Michigan old-fields. Both fields were abandoned from agriculture over 35 yrs ago and were used for an experiment in 1995 to examine colonization and growth of three native C_4 species (Foster 1999). The C_4 monoculture plots were still intact in 2006, allowing me to compare plant traits and soil properties of the C_4 monocultures to the surrounding C_3 grass-dominated matrix community after 11 years. Given the expected differences in functional traits of the C_3 and C_4 grasses, I predicted that: 1) the C_4 species would produce greater biomass (root and shoot), which would cause an increase in surface litter and total soil C compared to the C_3-dominated communities, 2) the C_4 species would have more recalcitrant tissue,

which would slow microbial process rates and result in a buildup of total soil C and N pools, and 3) the more recalcitrant tissue of the C_4 species would cause microbial N immobilization and therefore result in smaller inorganic N pools.

METHODS

Study Sites

I compared experimental monocultures of three C_4 species (*Andropogon gerardii* (Vitman) or Big bluestem, *Sorghastrum nutans* (L) or Little bluestem, and *Schizachyrium scoparium* (Michx) or Indian grass) to the surrounding old-field community dominated by C_3 grasses in two old-fields at Michigan State University's W. K. Kellogg Biological Station (KBS) in southwestern Michigan, USA. Non-native C_3 grasses dominated both fields, though the dominant species identity differed: the Turkey Meadow site is dominated by *Bromus inermis* (Leyss; Smooth brome), while McKay Field is dominated by *Elymus repens* (L; Quackgrass) (Table 1). Nomenclature for all species follows the USDA Plants Database (plants.usda.gov). Both fields were abandoned over 35 years ago following decades of row crop agriculture (Burbank et al. 1992, Foster and Gross 1997). This area of Michigan had extensive prairies and savannas prior to agricultural development, and the C_4 species examined in this experiment were common components of those grasslands (Gotshall 1972, Burbank et al. 1992).

The experimental monocultures were established in 1995 for competition experiments described in Foster (1999). Species were transplanted into clipped plots with minimal soil disturbance, weeded for one year, and then abandoned in 1996. After 11 years, the experimental monoculture plots in both fields were still dominated by the

Table 1. Plant community characteristics of the plots at harvest in August 2006. Shoot biomass (mean ± SE; n=9) and species percentages of the total biomass are based on the average of nine samples for each dominant vegetation plot. Species richness is given as the mean of the plots (n=9; 0.5 m x 0.5 m) and as the total across plots (mean, Σ). Plot names are abbreviated as follows: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum*, Er=*Elymus*, and Bi=*Bromus*. A designation as C_3 or C_4 species and as monocot (**M**) or Forb (**F**) follows the species names.

Site	Plot	Shoot biomass ($g\ m^{-2}$)	Dominant species	% Shoot biomass	Species richness (mean, Σ)
McKay	Ag	1637 ±162	*Andropogon gerardii* (Vitman) **C4 M**	97.5	2, 4
			Elymus repens (L) **C3 M**	1.6	
	Ss	577 ± 68	*Schizachyrium scoparium* (Michx) **C4 M**	85.5	3, 9
			Elymus repens **C3 M**	9.8	
	Sn	442 ± 49	*Sorghastrum nutans* (L) **C4 M**	76.7	4, 10
			Elymus repens **C3 M**	16.3	
	Er	307 ± 18	*Elymus repens* **C3 M**	98.7	2, 5
			Achillea millefolium (L) **C3 F**	1.0	
Turkey	Ag	1570 ±129	*Andropogon gerardii* **C4 M**	95.8	3, 8
			Bromus inermus (Leyss) **C3 M**	2.8	
	Ss	577 ± 56	*Schizachyrium scoparium* **C4 M**	68.9	8, 22
			Bromus inermus **C3 M**	8.7	
			Solidago canadensis (L) **C3 F**	5.0	
	Sn	959 ±108	*Sorghastrum nutans* **C4 M**	84.4	7, 13
			Poa pratensis (L) **C3 M**	5.0	
			Bromus inermus **C3 M**	4.7	
	Bi	282 ± 27	*Bromus inermus* **C3 M**	74.4	4, 13
			Poa pratensis **C3 M**	21.3	

C_4 species, and the surrounding matrix remains dominated by C_3 species (Table 1). Both fields have sandy loam soils (Foster and Gross 1997), although McKay Field has a higher sand fraction and appears more drought-prone than Turkey Meadow. By using two overall similar sites with different dominant C_3 species, I hope to increase my ability to generalize about the results.

Field Sampling

In 2006, I randomly selected nine of the monoculture plots (0.5m x 0.5m) dominated by each of the C_4 species, *Andropogon gerardii* (*Andropogon* plots), *Sorghastrum nutans* (*Sorghastrum* plots) and *Schizachyrium scoparium* (*Schizachyrium* plots) in each in field. Nine additional plots were established in the surrounding C_3 matrix community (C_3 plots), within 10-12m of the C_4 plots. I determined aboveground biomass production in August 2006 by clipping all vegetation at ground level and separating individual species. I then collected surface litter from the plots. Two soil cores (0-20cm deep, 3.8cm diameter) were collected from each plot for soil chemical analyses. Both cores were split into two depths (0-10, 10-20cm), and the soil from each depth interval was combined and refrigerated until processed in the lab. I determined root biomass by taking a single core (6.35cm diameter) from a subset of plots (n=6), split it into two depths (0-10 and 10-20cm), and refrigerated it until roots could be separated from the soil. All soil cores were taken immediately after sampling the litter.

Seasonal patterns of species effects on N-availability were determined using repeated 28-day *in situ* net N mineralization incubations throughout the 2006 growing season. *In situ* incubations were done in the *Andropogon* and C_3 plots (0-10cm cores,

n=5) in both sites. At the onset of each incubation period, two PVC pipes (3.8cm diameter, sharpened on one end) were pounded into the ground to a 10cm depth. The first core (t_0) was removed and taken to the lab for soil inorganic N analyses. The second core (t_{final}) was removed, capped on the top and bottom, and placed back into its original hole (modified from Robertson et al. 1999). After 28 days, the t_{final} core was removed from the ground, sealed in a plastic bag, and refrigerated until processed in the lab. On this same day, a new set of t_0 and t_{final} cores were collected and installed, respectively. This process was repeated every 28 days from June through November 2006.

Laboratory Analyses

Aboveground biomass (separated by species) and surface litter were dried for at least 72h at 65C and weighed (±0.01g). Green tissue samples were taken from a subset of the harvested biomass (n=5 plots) for each dominant species —*Andropogon, Schizachyrium, Sorghastrum, Bromus* (in Turkey Meadow), *Elymus* (in McKay Field). The dried tissue was coarse ground in a Wiley Mill, then ground to <2mm on a Cyclotech Grinder, and re-dried for 48 hours at 65C. Oven-dried tissue (2-3mg) was then packed in tin capsules for C, N and isotope analyses (analyzed at the UC Davis Stable Isotope Facility). Acid Detergent Fiber (ADF; recalcitrant compounds, primarily lignin and hemicellulose) analyses of ground tissue (~0.5g) were performed on an Ankom 2000 Fiber Analyzer (Macedon, NY) at Michigan State University.

Root cores were washed in tap water and roots were floated in a pan, removed with tweezers, and rewashed to remove any remaining soil. Root material was then placed in metal weigh boats, dried for 48h at 65C, and weighed (±0.0001g). Root:shoot

was calculated by dividing root biomass (scaled to g m^{-2} for 0-20cm depth) by shoot biomass (g m^{-2}) in each plot. Total plant N was estimated by multiplying total biomass (root + shoot) by shoot %N value of the dominant species. I used the dominant species shoot %N to approximate total plant N because it comprised the majority (69-99%) of the total shoot biomass and I did not have root %N data. While root and shoot %N data may differ, Wedin and Tilman (1990) show that %N for *Elymus*, *Andropogon* and *Schizachyrium* shoots was generally similar to that for roots.

Soil cores collected for chemical analyses were sieved through a 2mm soil sieve to homogenize and remove large debris. Inorganic N was extracted from a 20g sub-sample of soil using 50ml of 1M KCl, within 24h of sample collection. Extracts were placed in the freezer until analysis on an O.I. Analytical Flow Solution IV analyzer. Gravimetric soil moisture was determined on another sub-sample of soil (~25g fresh weight) by drying soils at 105C for 48h. The remaining soil was air-dried and stored in the laboratory. For C, N and isotope analyses, ~50g of air-dried soil was ground to a flour-like texture using a roller mill and oven-dried at 65C for 48h. 20-50mg sub-samples were then packed into tin capsules and sent to the UC Davis Stable Isotope Facility for determinations of total C, N and $\delta^{13}C$ (relative to PeeDee Belemnite (PDB)). Because C_3 and C_4 species differ markedly in $\delta^{13}C$, the percentage of soil C contributed by C_4 species could be calculated for each plot using a simple end-member mixing model (using the $\delta^{13}C$ of the dominant C_4 and C_3 species in each plot):

$$\%C_4 \text{ signal} = (C_4 \text{ Soil } \delta^{13}C - C_3 \text{ Soil } \delta^{13}C)/(C_4 \text{ plant } \delta^{13}C - C_3 \text{ Soil } \delta^{13}C)*100$$

Soil bulk Density was determined at each site under the *Andropogon* and C_3 plots (n=3) using an Eijkelkamp root corer (8cm diameter *10cm deep). Bulk density was calculated

as oven-dried mass/volume (g cm^{-3}). Bulk density was used to convert surface soil C and N to a mass basis.

For each *in situ* net N mineralization incubation, t_0 and t_{final} samples were taken back to the lab and processed according to the inorganic N extraction procedure described above. To calculate final N pool sizes, the sum of inorganic N in the t_{final} core was divided by the mass of the soil in the core (calculated using bulk density). Net N-mineralization and nitrification rates were calculated as the change in total inorganic N (NH_4^+ and NO_3^-) and NO_3-N (in μgN per gram of dry soil), respectively, over the incubation period.

Statistical Analyses

All data were checked for normality and equal variance, and appropriate transformations were performed prior to analysis. Plant and soil variables were compared between dominant species (C_3, *Andropogon, Schizachyrium*, and *Sorghastrum*) using a Two-Way ANOVA with Site and Species (or Plot) as main effects using SigmaStat 3.5. Pearson correlations were performed to determine whether plant traits were correlated with soil variables. In addition, *in situ* N mineralization and nitrification rates were compared between *Andropogon* and C_3 plots using Repeated Measures ANOVA on Systat 11. For any ANOVA indicating a significant interaction, post-hoc contrasts were made using Tukey comparisons.

RESULTS

Species Traits: Biomass and Tissue Characteristics

There were significant differences among the dominant species in shoot biomass and surface litter with both typically greater in plots dominated by a C_4 species than by C_3 species (Table 2; Figure 2). *Andropogon* plots had significantly greater root biomass (0-20cm depth) than C_3 and *Schizachyrium* plots in both sites (Table 2, Figure 2). Analyses of plant tissue chemistry separated C_3 from C_4 species. Plant tissue C:N was significantly higher for the C_4 species compared to the C_3 species, and %N was significantly lower for the C_4 species (Table 3). Isotope analyses showed that C_4 species had a significantly higher $\delta^{13}C$ (relative to PDB) compared to the C_3 species, indicating a greater discrimination by C_3 species against ^{13}C (Table 3). All C_4 species had higher %Acid Detergent Fiber (ADF) and ADF:N than the C_3 species, except *Sorghastrum* in McKay Field (Table 3). Estimates of total plant N (shoot + root) on an area basis were not significantly different between species in McKay Field ($p>0.05$), but in Turkey Meadow, *Andropogon* had more total N than both *Schizachyrium* and *Sorghastrum* ($p\leq0.009$), and *Schizachyrium* had more than *Bromus* ($p=0.029$). C_3 species had significantly greater root:shoot compared to all C_4 species, with *Andropogon* having the lowest ratio of the C_4 species ($p<0.001$).

There were also site differences in the growth and tissue chemistry of the species (Figure 2, Tables 2 and 3). All species had significantly greater root biomass and root:shoot in McKay Field than Turkey Meadow, and C:N and $\delta^{13}C$ was significantly higher for all species in Turkey Meadow. *Sorghastrum* had higher shoot biomass and ADF:N in Turkey Meadow, and significantly higher %C and N in McKay Field.

Table 2. ANOVA results for various measures of plant production, using Site and dominant species Plot as main effects. Natural log transformations were used to normalize the data for all variables. Significant p-values are indicated in **bold.**

Variable	Factor	F	Degrees of freedom	p-value
Shoot Biomass	Site	6.14	1, 64	**0.016**
	Plot	113.43	3, 64	**<0.001**
	Site*Plot	9.71	3, 64	**<0.001**
Surface Litter	Site	10.05	1, 64	**0.002**
	Plot	39.38	3, 64	**<0.001**
	Site*Plot	3.72	3, 64	**0.016**
Root Biomass (0-20cm)	Site	33.22	1, 40	**<0.001**
	Plot	5.59	3, 40	**0.003**
	Site*Plot	1.64	3, 40	0.195
Root : Shoot	Site	26.20	1, 40	**<0.001**
	Plot	16.63	3, 40	**<0.001**
	Site*Plot	2.61	3, 40	0.064

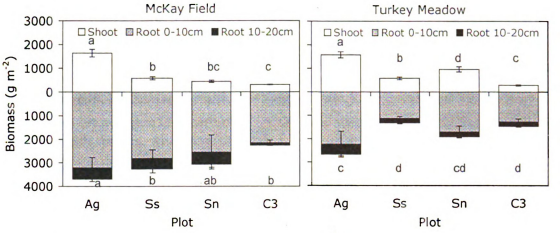


Figure 2. Root and shoot biomass (mean ±SE in g m^{-2}) in August 2006 at both sites. Root biomass is shown separately by depth intervals here for additional detail, but statistical results are reported for total root biomass from 0-20cm. Lowercase letters denote significant differences ($p<0.05$) between plots for shoot (n=9) and root (n=6) biomass. Species names are abbreviated as follows: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum*, and C_3=*Elymus* in McKay Field and *Bromus* in Turkey Meadow.

Table 3. ANOVA model for plant tissue chemistry, using Site and the dominant Species as main effects. Mean (±SE) values are also shown for the dominant species at each site. When Species effects are present, superscript numbers indicate significant differences ($p<0.05$) between species. For significant interactions, superscript letters denote significant differences ($p<0.05$). The Species names are abbreviated as follows: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum*, Er=*Elymus*, and Bi=*Bromus*. Sample sizes for nitrogen (N), carbon (C), C:N, Acid detergent fiber (ADF):N and $\delta^{13}C$ were 4 for each species, while n=6 for ADF.

Site	Species	%N	%C	C:N	%ADF	ADF:N	$\delta^{13}C$
	Site	$F_{1,24}=17.54$	$F_{1,24}=83.58$	$F_{1,24}=28.99$	$F_{1,32}=32.47$	$F_{1,24}=14.79$	$F_{1,24}=4.67$
		p<0.001	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.041**
Model	Species	$F_{3,24}=29.97$	$F_{3,24}=56.21$	$F_{3,24}=14.40$	$F_{3,32}=31.23$	$F_{3,24}=21.62$	$F_{3,24}=1451.36$
		p<0.001	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
	Site* Species	$F_{3,24}=3.42$	$F_{3,24}=35.72$	$F_{3,24}=2.15$	$F_{3,32}=3.10$	$F_{3,24}=5.59$	$F_{3,24}=2.61$
		p=0.033	**p<0.001**	p=0.120	**p=0.040**	**p=0.005**	p=0.075
McKay	Ag	0.80 ± 0.08^{ab}	45.5 ± 0.2^{a}	58.5 ± 6.3^{1}	79.0 ± 1.3^{ab}	100.4 ± 10.9^{a}	-12.7 ± 0.1^{1}
	Ss	0.90 ± 0.08^{a}	45.3 ± 0.3^{a}	51.0 ± 4.4^{1}	81.1 ± 0.4^{b}	91.3 ± 7.7^{a}	-13.5 ± 0.5^{2}
	Sn	1.01 ± 0.09^{a}	44.7 ± 0.5^{a}	45.2 ± 4.7^{1}	77.5 ± 1.1^{ac}	77.3 ± 7.5^{ab}	-14.0 ± 0.5^{2}
	Er	1.26 ± 0.03^{c}	45.7 ± 0.4^{a}	36.5 ± 0.6^{2}	74.6 ± 0.8^{c}	59.7 ± 1.9^{b}	-28.7 ± 0.1^{3}
Turkey	Ag	0.74 ± 0.06^{ab}	49.4 ± 0.5^{b}	67.7 ± 5.1^{3}	74.3 ± 0.5^{d}	101.9 ± 8.2^{ac}	-12.5 ± 0.1^{4}
	Ss	0.67 ± 0.04^{b}	46.2 ± 0.8^{a}	69.5 ± 4.5^{3}	79.1 ± 1.4^{b}	118.8 ± 7.5^{c}	-13.9 ± 0.4^{5}
	Sn	0.64 ± 0.05^{b}	43.6 ± 0.3^{c}	69.3 ± 4.6^{3}	75.9 ± 1.0^{bd}	121.8 ± 8.8^{c}	-12.8 ± 0.4^{5}
	Bi	1.21 ± 0.10^{c}	51.9 ± 0.2^{d}	43.5 ± 3.4^{4}	68.3 ± 1.1^{e}	57.9 ± 4.9^{b}	-28.1 ± 0.2^{6}

Schizachyrium had significantly higher %N and surface litter in McKay Field, and higher ADF:N in Turkey Meadow. Both *Andropogon* and the C_3 species had significantly higher %ADF in McKay Field and significantly higher %C in Turkey Meadow. Plant total N was significantly greater in McKay Field (except *Andropogon*). Plant shoot biomass was highly correlated with surface litter ($n=48$, $p<0.001$, $r=0.70$), and plant $\delta^{13}C$ values were highly correlated with ADF:N ($n=32$, $p<0.001$, $r=0.74$), %N ($n=32$, $p<0.001$, $r=-0.79$), C:N ($n=32$, $p<0.001$, $r=0.65$) and ADF ($n=32$, $p<0.001$, $r=0.62$)

Soil Properties

Surface (0-10cm) and subsurface (10-20cm) soils sampled from under the different species did not significantly differ in total soil C content (Tables 4 and 5). However, both surface and subsurface soil $\delta^{13}C$ values were significantly enriched under the C_4 than C_3 species, and there were also differences between the soil $\delta^{13}C$ values among the C_4 species (Tables 4 and 5). The proportion of both surface and subsurface total C pools contributed by C_4 species was significantly higher in plots that had C_4 species planted to them a decade ago compared to the C_3 plots (Figure 3).

Soil inorganic N was significantly lower in both surface and subsurface soils under C_4 species than under the C_3 species in both sites (Tables 4 and 5). Both NH_4^+ and NO_3^- typically were higher in soils under C_3 species compared to C_4 species (data not shown). *In situ* net nitrification and N mineralization rates (performed in C_3 and *Andropogon* plots) varied over the growing season ($p \leq 0.002$ using Greenhouse-Geisser adjustment), and typically were higher in soils under C_3 species than under *Andropogon*. However, these differences were only marginally significant ($p=0.09$ and 0.06,

respectively; Figure 4) in part due to high variability among replicates. Total subsurface soil N pools did not significantly differ between species, but surface N pools were significantly greater under *Andropogon* compared to *Sorghastrum* (Tables 4 and 5).

In addition to species effects on soil properties, the sites also differed in soil properties (i.e., significant Site effect; Tables 4 and 5). Across plots, surface soil C and N pools were significantly larger in Turkey Meadow, although subsurface pools did not differ between sites. In Turkey Meadow, surface soils had significantly higher $\delta^{13}C$, and subsurface soils had significantly lower C:N compared to McKay Field. *In situ* net nitrification differed through time but was significantly higher in McKay Field than Turkey Meadow for all incubations ($p=0.04$ using Greenhouse-Geisser adjustment). The calculated proportion of total surface soil C contributed by C_4 species also was significantly higher in Turkey Meadow than McKay Field ($F_{1,40}=4.41$, $p=0.042$).

Several plant traits were highly correlated with particular soil variables. Shoot biomass was correlated with both soil $\delta^{13}C$ (surface soil: $n=23$, $p<0.001$, $r=0.61$; subsurface: $n=21$, $p<0.001$, $r=0.63$) and $\%C_4$-carbon contribution to the soil C pool (surface: $n=23$, $p<0.001$, $r=0.59$; subsurface: $n=21$, $p<0.001$, $r=0.62$), and subsurface soil N ($n=21$, $p=0.01$, $r=0.56$) and C content ($n=21$, $p=0.03$, $r=0.48$). Shallow (0-10cm) root biomass was positively correlated with $\%C_4$-carbon in surface soils ($n=35$, $p=0.04$, $r=0.35$), and root biomass from 10-20cm was positively correlated with $\%C_4$-carbon in subsurface soils ($n=30$, $p=0.03$, $r=0.41$) and subsurface soil $\delta^{13}C$ ($n=30$, $p=0.01$, $r=0.50$). Tissue C:N was negatively correlated with inorganic N (surface soil: $n=21$, $p=0.03$, $r=0.48$; subsurface: $n=21$, $p=0.03$, $r=0.48$), and tissue N content was positively correlated with inorganic N (surface: $n=32$, $p=0.02$, $r=0.41$; subsurface: $n=32$, $p=0.01$, $r=0.46$).

Table 4. ANOVA model results for surface soil (0-10cm) carbon (C) and nitrogen (N) variables, using Site and Plot (dominant vegetation) as main effects. Mean (±SE) values are shown below the ANOVA. When Species effects are present, superscript numbers indicate significant species differences ($p<0.05$). For significant interactions, superscript letters denote significant differences ($p<0.05$). The Plot names are abbreviated as follows: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum*, Er=*Elymus*, and Bi=*Bromus*. The data shown are untransformed values, but inorganic N was ln-transformed prior to analysis.

Site	Plot	Inorganic N (μgN g dry soil^{-1})	C:N	C (kg m^{-2})	N (kg m^{-2})	δ^{13}C
Model	Site	$F_{1,64}=3.21$	$F_{1,40}=1.90$	$F_{1,40}=22.97$	$F_{1,40}=21.07$	$F_{1,40}=4.21$
		$p=0.078$	$p=0.176$	**$p<0.001$**	**$p<0.001$**	**$p=0.047$**
	Plot	$F_{3,64}=9.37$	$F_{3,40}=7.33$	$F_{1,40}=3.08$	$F_{1,40}=3.68$	$F_{1,40}=20.39$
		$p<0.001$	**$p<0.001$**	**$p=0.038$**	**$p=0.020$**	**$p<0.001$**
	Site* Plot	$F_{3,64}=1.81$	$F_{3,40}=3.20$	$F_{1,40}=3.11$	$F_{1,40}=2.71$	$F_{1,40}=0.95$
		$p=0.154$	**$p=0.034$**	**$p=0.037$**	$p=0.058$	$p=0.428$
McKay Field	Ag	3.91 ± 0.43^{1}	11.3 ± 0.2^{ab}	1.94 ± 0.18^{a}	0.17 ± 0.02^{1}	-24.6 ± 0.6^{1}
	Ss	3.55 ± 0.47^{1}	11.2 ± 0.1^{ab}	1.49 ± 0.21^{a}	0.13 ± 0.02^{12}	-25.8 ± 0.5^{2}
	Sn	4.11 ± 0.43^{1}	11.5 ± 0.1^{a}	1.42 ± 0.11^{a}	0.12 ± 0.01^{2}	-25.7 ± 0.3^{2}
	Er	7.08 ± 0.60^{2}	11.2 ± 0.1^{ab}	2.08 ± 0.20^{ab}	0.19 ± 0.02^{12}	-27.1 ± 0.1^{3}
Turkey Meadow	Ag	3.12 ± 0.37^{1}	11.4 ± 0.3^{a}	2.71 ± 0.34^{b}	0.24 ± 0.03^{3}	-23.3 ± 0.8^{4}
	Ss	3.95 ± 0.81^{1}	10.9 ± 0.3^{a}	2.18 ± 0.13^{b}	0.20 ± 0.01^{34}	-25.5 ± 0.3^{5}
	Sn	4.17 ± 0.39^{1}	12.4 ± 0.3^{b}	2.39 ± 0.10^{b}	0.19 ± 0.01^{4}	-24.9 ± 0.4^{5}
	Bi	4.83 ± 0.55^{2}	11.2 ± 0.2^{a}	2.04 ± 0.15^{ab}	0.18 ± 0.01^{34}	-27.1 ± 0.1^{5}

Table 5. ANOVA model for subsurface soil (10-20cm) carbon (C) and nitrogen (N) variables, using Site and Plot (dominant vegetation) as main effects. Mean (±SE) values are shown below the ANOVA. For significant Species effects, superscript numbers indicate significant species differences ($p<0.05$). The Plot names are abbreviated as follows: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum*, Er=*Elymus*, and Bi=*Bromus*. The data shown are untransformed means, but the following variables were transformed prior to analysis: inorganic N (ln), N (square root) and $\delta^{13}C$ (square root).

Site	Plot	Inorganic N (μgN g dry soil^{-1})	C:N	C (g kg^{-1})	N (g kg^{-1})	$\delta^{13}C$
	Site	$F_{1,64}=2.89$	$F_{1,32}=124.66$	$F_{1,32}=0.11$	$F_{1,32}=1.52$	$F_{1,32}=3.44$
		$p=0.094$	**$p<0.001$**	$p=0.745$	$p=0.227$	$p=0.073$
Model	Plot	$F_{3,64}=5.83$	$F_{3,32}=0.76$	$F_{1,32}=0.97$	$F_{1,32}=1.15$	$F_{1,32}=17.31$
		$p=0.001$	$p=0.527$	$p=0.421$	$p=0.344$	**$p<0.001$**
	Site* Plot	$F_{3,64}=0.79$	$F_{3,32}=1.81$	$F_{1,32}=1.20$	$F_{1,32}=1.32$	$F_{1,32}=1.24$
		$p=0.505$	$p=0.165$	$p=0.326$	$p=0.285$	$p=0.312$
	Ag	1.15 ± 0.25^{1}	11.5 ± 0.1	8.83 ± 1.29	0.77 ± 0.12	-24.5 ± 0.3^{1}
McKay	Ss	0.92 ± 0.10^{1}	12.0 ± 0.2	7.70 ± 1.46	0.65 ± 0.13	-25.1 ± 0.2^{2}
Field	Sn	0.86 ± 0.08^{1}	11.8 ± 0.2	6.32 ± 0.95	0.12 ± 0.53	-24.8 ± 0.3^{12}
	Er	1.82 ± 0.22^{2}	11.5 ± 0.2	8.26 ± 0.96	0.72 ± 0.08	-25.7 ± 0.1^{3}
	Ag	0.83 ± 0.19^{1}	10.4 ± 0.1	7.51 ± 0.22	0.72 ± 0.02	-23.7 ± 0.5^{1}
Turkey	Ss	1.07 ± 0.27^{1}	10.3 ± 0.3	6.997 ± 0.57	0.68 ± 0.05	-24.9 ± 0.1^{2}
Meadow	Sn	0.93 ± 0.22^{1}	10.5 ± 0.2	7.74 ± 0.48	0.74 ± 0.05	-24.5 ± 0.2^{12}
	Bi	1.29 ± 0.19^{2}	10.5 ± 0.2	7.58 ± 0.38	0.72 ± 0.04	-25.8 ± 0.2^{3}

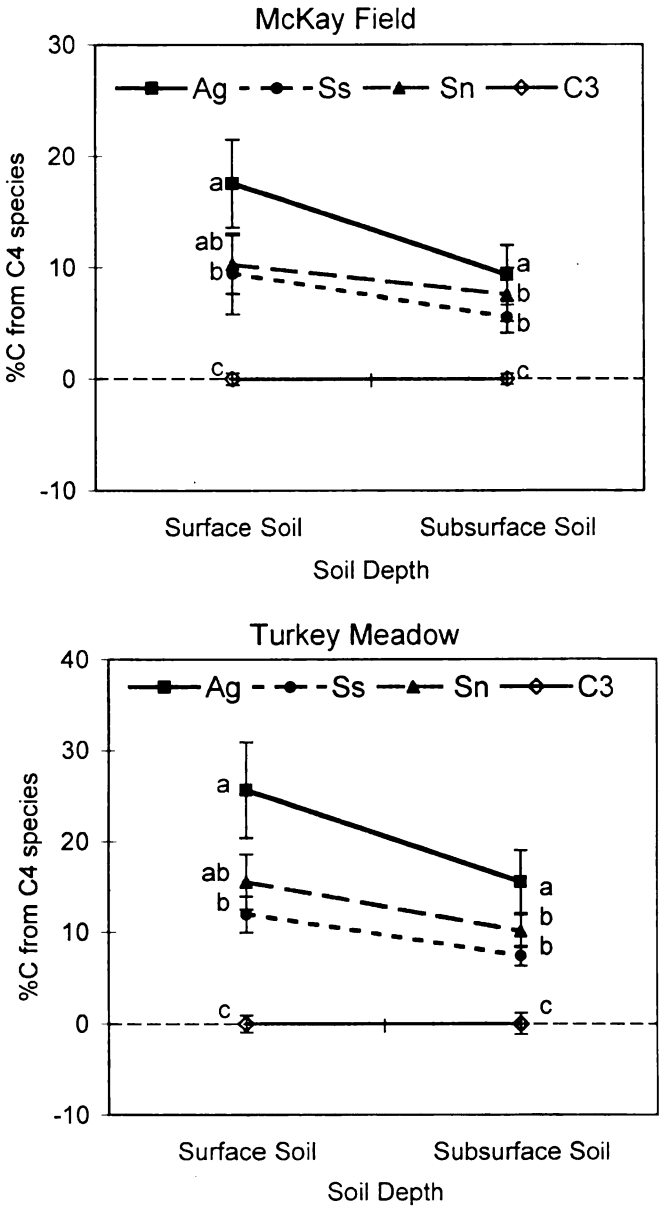


Figure 3. Percentage of total soil carbon (mean ±SE) contributed by C_4 species at both surface (0-10cm; n=6) and subsurface (10-20cm; n=5) soil depths at both sites. Lowercase letters denote significant differences ($p<0.05$) between plots separately for each depth. Plot names are abbreviated as follows: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum* and C_3=*Elymus* in McKay Field and *Bromus* in Turkey Meadow.

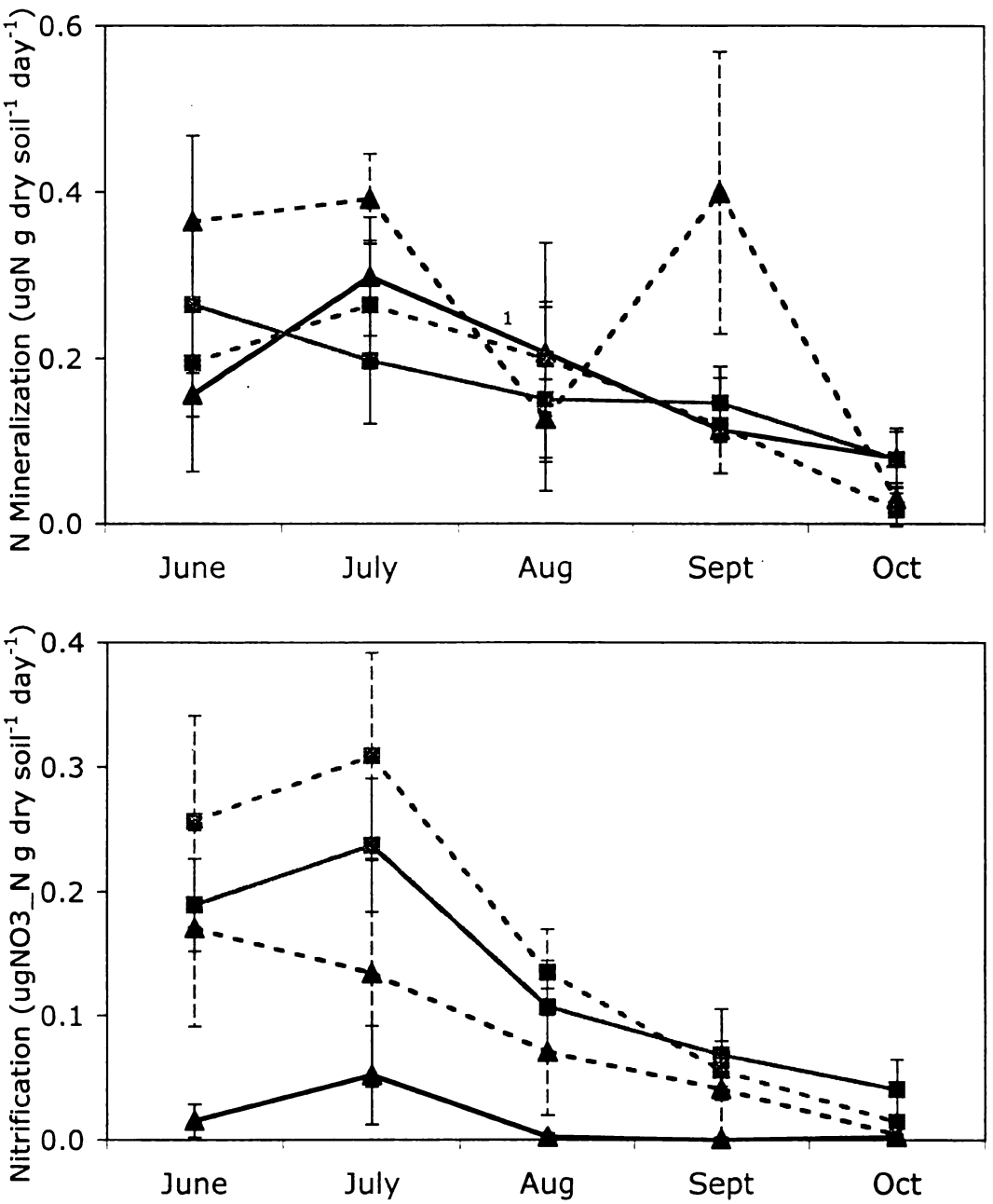


Figure 4. *In situ* net nitrification and N mineralization rates (mean ± SE; n=5) for *Andropogon* plots (solid lines) and C_3 plots (dashed lines) at both Turkey Meadow (black lines) and McKay Field (gray lines). Rates are calculated from 28-day *in situ* incubations performed consecutively from June to November 2006.

DISCUSSION

Eleven years after the establishment of native C_4 species, there were detectable differences in soil processes in areas planted as C_4 monocultures compared to the surrounding C_3-dominated matrix community. These differences correspond to differences in biomass production and tissue chemistry between these two functional groups of grasses. While the magnitude of the differences in soil properties and species characteristics between the dominant species plots sometimes varied between the two sites, the qualitative interpretations (i.e., the relative differences between species) of the results were remarkably similar. This suggests that the differences I observed between species can be generalized across sites. In addition, members of each functional group (C_3 and C_4 species) were typically similar to one another for both plant characteristics and impacts on soil properties, suggesting that the species within each functional group may be functionally equivalent (for the particular species examined in this study).

Consistent with my expectations, the C_3 and C_4 species differed in both the quantity and quality (C:N, ADF:N) of biomass and litter produced. Total shoot biomass was 0.5 to 8-fold higher in plots with C_4 compared to C_3 species, and C_4-dominated plots tended to have much larger total root biomass and more investment in deeper root systems than C_3-dominated plots (Figure 2). Many studies (e.g., Baer et al. 2002, Camill et al. 2004) have found higher biomass (root and shoot) and surface litter accumulation in plots as C_4 species abundance increases. Overall, the predicted increase in tissue quantity with C_4 species reintroduction was supported by my results, as was the prediction that C_4 species have more recalcitrant tissue (higher C:N and ADF:N) than the C_3 species.

I found a strong positive correlation between biomass (both root and shoot) and the percentage of soil C contributed by C_4 species, suggesting that perhaps C_4 species are affecting soil C. The increase in tissue quantity associated with the C_4 species, and therefore potential contributions to the soil C pool, might be expected to stimulate microbial activity; however, the greater recalcitrance of this tissue may suppress C mineralization rates. The interaction of litter quality and quantity differences on soil processes in this study confounds my ability to independently examine how litter quality and quantity impact C cycling, and thus how C pools may change. Several studies have shown that decomposition is highly correlated with tissue C:N and lignin:N (Wardle et al. 1997, Vinton and Goergen 2006), so C_4 tissue should decompose slower than C_3 tissue and result in increased soil C. However, recent work in restored prairies has found higher C mineralization rates in sites that have high levels of C_4 species dominance (Baer et al. 2002, Camill et al. 2004). One explanation for this apparent contradiction is that the larger rooting system of C_4 species, compared to C_3 species, results in larger quantities of labile material being released to the microbial community (e.g., fine root turnover and exudation), subsequently stimulating C mineralization in the rooting zone (Baer et al. 2002). At the same time, the greater total production and recalcitrance of C_4 species shoot and coarse root tissue acts to slow its decomposition relative to C_3 species. Thus, while C mineralization rates may be higher in the rooting zone of C_4 species, overall decomposition is slower, and soil C subsequently accumulates faster in C_4-dominated plots compared to the C_3-dominated plots.

Despite these differences in tissue quality and quantity, I did not find significant increases in total soil C in the C_4 plots compared to the C_3 plots in this study. It may

take longer than 11 years for soil pools to respond to plant-driven changes in microbial process rates. Kindscher and Tieszen (1998) found no evidence of C accumulation after 5 and 25 years following the re-establishment of C_4 species dominance in prairie restorations in Kansas. Camill et al. (2004) saw a similar lack of soil C accumulation after 6-8 years of C_4 species dominance in restored prairies in Minnesota. In contrast, McLauchlan et al. (2006) found increased soil C in grasslands on decadal timescales after agricultural abandonment, regardless of whether they were planted as C_3- or C_4-dominated communities. Conventionally tilled agricultural fields in close proximity to my sites have much lower soil C (~690 g m^{-2} in 0-5cm depth, Grandy et al. 2006) than in the old-fields in my study (~2000 g m^{-2} in 0-10cm depth), suggesting that soil C pools likely are increasing under both C_3 and C_4 species in my sites, potentially making it difficult to detect species differences after just 11 years. I did detect slightly higher surface soil C in C_4 plots compared to C_3 plots in Turkey Meadow, suggesting that soil C is increasing under C_4 species, but that more time is needed for the higher production and slower decomposition of C_4 species tissue to result in measurably larger soil C pools.

While I did not find evidence of increasing total soil C pools, species may still be affecting the stability of the soil C present in those soils. Two factors that can have a large influence on soil aggregate structure and stability are the amount of roots and the presence of mycorrhizae (Jastrow et al. 1998, Rillig et al. 2002, Rillig and Mummey 2006, Jastrow et al. 2007). The C_4 species examined in this study are known to be mycorrhizal and tended to have more root biomass (0-20cm), both of which may contribute to greater soil C physiochemical protection and thereby longer-term sequestration of C relative to the C_3 species. Jastrow (1987) found that prairie graminoid

aboveground biomass was the most significant predictor of percent aggregates >0.2mm diameter and >2mm, and that a 14 year old pasture had significantly fewer small aggregates (>0.2mm) than an 11 year old restored prairie. In addition, McLauchlan et al. (2006) found that soils under C_4 species tended to have larger aggregates than soils under C_3 species. Direct investigation of how individual species may differ in their ability for direct and indirect physiochemical protection of soil organic matter is needed.

At both sites, C_4 species are contributing 9-26% of the total surface soil C pool and 6-16% of the total subsurface soil C pool after just 11 years. This suggests a relatively rapid turnover of the soil C pools, and could indicate that greater quantities of C are entering the soil C pool in C_4 plots. This fast turnover of C suggests that any increases in total soil C by the C_4 species are likely to become apparent relatively quickly. Based on the C_4 species contributions, complete turnover of surface soil C could happen in 50 to 100 years, and any increase in total soil C under C_4 species should become measurable prior to complete turnover.

As predicted, the re-introduction of C_4 grass species into these old-fields significantly altered N cycling. My measurements of significantly lower total inorganic N availability in plots dominated by C_4 species compared to C_3 plots are consistent with patterns found in several studies from a broad range of temperate grasslands. Wedin and Tilman (1990) found that after only three years, inorganic N levels sampled under monocultures of C_4 species were significantly lower than monocultures of C_3 species. Evans et al. (2001) found that establishment of an invasive C_3 grass (*Bromus tectorum*) with greater biomass and more recalcitrant tissue compared to the native C_3 species (*Bromus tectorum* is the functional analog to the C_4 species in my study) significantly

altered N cycling within two years in an arid grassland in Utah. Decreased potential net N mineralization rates in that study were linked to changes in litter quality and quantity, which resulted in increased microbial N immobilization (Evans et al. 2001). The reduced levels of inorganic N in my C_4 plots compared to the C_3 plots could be a result of slower N mineralization rates causing more of the N in the system to be immobilized by microbes, greater N uptake and storage by C_4 species, or a combination of both factors.

Differences in N uptake among species inherently confound the effects on microbial communities, so it is difficult to determine which mechanism might exert a stronger influence on N cycling. In my study, the total plant N stocks of C_4 and C_3 species did not differ on an area basis. This suggests that C_4 species are not taking up more N than C_3 species, and thus uptake differences may not be a determining factor for N availability at these sites. Despite the high variability, *in situ* net N mineralization and nitrification rates tended to be higher in C_3 plots compared to *Andropogon* plots (C_4), particularly from June-September. I also found significant positive correlations between tissue %N and C:N and inorganic N levels, suggesting that the species with the highest %N (the C_3 species) may have increased N cycling rates to increase inorganic N availability. Vinton et al. (2006) suggested that the lower C:N of *Bromus inermus* may encourage rapid and efficient N cycling, which could increase inorganic N availability in the soil. Even though *Andropogon* was the only C_4 species examined for N cycling rates, one might expect similar results for the other C_4 species because all three C_4 species had similar tissue chemistry and productivity. Other studies have found similar results when comparing C_3 and C_4 dominated plots (e.g., Wedin and Tilman 1990, Baer et al. 2002, Dijkstra et al. 2006), supporting the hypothesis that C_4 species alter inorganic N

availability by slowing mineralization rates and/or increasing microbial immobilization. Total soil N was not significantly different in soils under C_4 and C_3 species, and a lag in pool responses to rate changes would be expected given the large size of the soil N pool. Total soil N was slightly higher under C_4 species than in C_3 plots in Turkey Meadow, which provides some evidence to support the prediction that total N pools are increasing in soil under C_4 species relative to C_3 species.

Net N mineralization and nitrification rates varied seasonally for both *Andropogon* and C_3 plots and were higher in the early summer. Mineralization rates are often high in spring and early summer as a consequence of litter inputs from the previous fall that are available for microbial utilization, combined with warmer soil temperatures and adequate moisture to stimulate microbial activity (Eviner et al. 2006). Net nitrification and mineralization rates were low by October for all plots, however, the *Andropogon* plots continued to process N later into the fall than the C_3 plots. The higher surface litter in the *Andropogon* plots may insulate the soil in these plots and thereby sustain microbial activity later into the fall. My evidence suggests that *Andropogon* is altering the timing and rate of N transformations, but more detailed studies are needed to examine the community- and ecosystem-level importance of such changes, as well as the generality of these results to other C_4 species.

Overall, this study demonstrates that the introduction of a species with different functional traits than the surrounding community can alter soil properties after ten years. However, because the C_3 and C_4 grasses examined in this study differ in three main ways—litter quality, litter quantity, and timing of resource use/release—it is difficult to determine which traits, or combination of traits, influence particular processes in the

field. In this study, I provide evidence that differences between C_3 and C_4 species in tissue quantity, tissue quality, and plant phenology are likely to influence soil processes. Further study is needed in more a controlled setting to tease apart the direct and indirect impacts of these trait differences on soil properties and processes.

My results also have important implications for understanding the effects of restoring native species on ecosystem processes, and provide insights into the challenges of re-establishing ecosystem structure and functions in prairie restorations. Years of agricultural activity create a legacy of low soil C and N pools, small inorganic N pools, and poor soil aggregate structure (Camill et al. 2004, DeGryze et al. 2004). Most efforts to restore old-fields to prairie and savanna have focused primarily on re-establishing native plant assemblages using seeds or propagules, however, the success of these restoration efforts may be limited due to the failure to consider how changes associated with past land use have altered both soil properties and species interactions (Suding et al. 2004). Reintroduction of specific species or functional groups that can facilitate restoration of soil processes may be integral to the success of restoration projects (Suding et al. 2004). The extent of human impacts on plant communities are ever increasing, and a mechanistic understanding of how plant species introductions or losses are likely to impact ecosystem properties is needed to evaluate which species changes are likely to have the greatest effect on ecosystem function and properties.

CHAPTER THREE

SHORT-TERM IMPACTS OF C_4 PRAIRIE GRASS RE-ESTABLISHMENT ON SOIL CARBON AND NITROGEN IN OLD-FIELDS

ABSTRACT. Prairie restorations often focus on the re-establishment of diverse native plant communities rather than on the restoration of soil properties and processes that have been altered by decades of agricultural activities. C_3 species typically dominate abandoned agricultural fields, likely because they are superior competitors in the disturbed conditions created by agricultural activities compared to the native C_4 prairie grasses that were common prior to agriculture. Differences in aboveground production and tissue chemistry between the C_3 and C_4 grasses examined in this study support the expectation that C_4 grasses will accelerate soil carbon (C) and nitrogen (N) buildup compared to C_3 grasses, although the immediacy of this effect is unknown. I compared newly established monocultures of three native prairie C_4 grasses to similar stands of C_3 grasses that typically dominate successional old-fields in the Midwestern United States to determine whether, and how quickly, C_4 grasses affect soil properties. I related species differences in productivity and tissue chemistry to changes in soil properties at three sites in southwestern Michigan, USA after two growing seasons. I examined root production of these species in a separate study to determine how the development and distribution of roots throughout a soil profile might influence C, N cycling and pools. I found that the C_4 species consistently produced more shoot biomass, which was more recalcitrant than C_3 tissue (i.e., higher C:N, lignin:N). Although there were few species' differences in root biomass, C_4 species typically produced more biomass deeper in the soil profile

(below 20cm) compared to C_3 species. Although inorganic N was significantly lower in soils under the C_4 functional group, there was little evidence to suggest that soil total C or N pools differed after two years. However, based on observed species differences in productivity and tissue chemistry, I expect that inorganic N levels from soils beneath C_3 and C_4 species will continue to diverge over the next several years as the plants mature, but decades may be needed before soil pools reflect the plant species effects on these soil processes.

INTRODUCTION

While agricultural activities have dramatically changed both plant community composition and soil properties, most restoration efforts focus primarily on restoring plant communities and species composition (Howe 1994, Sluis 2002, Averett et al. 2004, Blumenthal et al. 2005, Martin et al. 2005, Williams et al. 2007) and have not considered restoring pre-agricultural soil conditions (but see Brye et al. 2002, McLauchlan et al. 2006). Total soil carbon (C) and nitrogen (N) loss associated with agriculture is dramatic (Burke et al. 1995, Camill et al. 2004, DeGryze et al. 2004), and grassland cultivation in the Midwestern United States has resulted in a 30-60% loss of soil organic C and N (Burke et al. 1995). There is evidence that prairie restorations begin to restore these pools and processes (Baer et al. 2002, Camill et al. 2004), however, it is unknown how quickly soils will return to pre-agricultural levels, and whether changes in these processes can be accelerated by using particular plant species.

After abandonment, old-fields are often dominated by a successional sequence of non-native C_3 species; native C_4 prairie grasses that once were dominant are slow, or fail,

to establish in these sites (Foster and Gross 1997, Averett et al. 2004, Emery and Gross 2006, Gross and Emery 2007). In this study, I examined how individual species common in native prairie or old-field communities influence soil properties and the rate at which change might occur. C_3 and C_4 grass species differ in several traits expected to affect C and N cycling, and there is evidence that re-establishment of particular 'keystone' prairie species (C_4 grasses) may help to restore soil processes to pre-agricultural levels (Baer et al. 2002, Camill et al. 2004, Chapter Two). Native prairie C_4 grasses typically have higher biomass and more recalcitrant tissue (i.e., high C:N and lignin:N) compared to the non-native C_3 grasses that characterize successional old-fields in the Midwestern USA (Chapter Two). These differences in species traits have been shown to affect soil C and N cycling (Wedin and Tilman 1990, Evans et al. 2001, Baer et al. 2002, Chapter Two). However, it is unknown how quickly these species' differences will affect soil process rates, or how long it will take for those process rates to restore total soil C and N pools.

While productivity and tissue chemistry differences between C_3 and C_4 grasses support the expectation of long-term effects on soil ecosystem processes, the short-term temporal dynamics of this effect is unknown. In Chapter Two, I found that there were detectable differences in soil properties and processes in soils collected from stands of C_4 species that were established in 1995 (decadal timescale) compared to soils from the surrounding community dominated by non-native C_3 species. I established monocultures of several C_3 grass species that typically dominate old-fields in southwestern Michigan and three C_4 grass species that were common in the region prior to agricultural development (Gotshall 1972) to determine whether species can affect soil properties and processes soon after establishment (i.e., after two growing seasons). To test the

generality of these results, I examined these differences in three old-field sites, with similar agricultural histories, at Michigan State University's W. K. Kellogg Biological Station (KBS) in southwestern Michigan. I measured differences in productivity and tissue chemistry and related these differences to changes in soil properties at three sites after two growing seasons. Based on known differences in C_3 and C_4 species productivity and tissue chemistry, I expected that there would be detectable changes in inorganic N availability within 1-2 years. However, I expected that changes in soil C and N pools may take longer than a few years to become apparent because there is a lag between when rates change (i.e., input and output rates) and the pool sizes (i.e., total stocks) begin to reflect those changes. I also hypothesized that the magnitude of species differences in biomass and tissue chemistry within and among functional groups would determine the rate at which the soil changes occur and can be detected. Given the relative similarity of the sites, I did not expect many differences in the qualitative results between sites.

METHODS

Monoculture Experiment

Species and Study Sites

I established experimental monocultures of six species, three C_4 (*Andropogon gerardii* (Vitman) or Big bluestem, *Sorghastrum nutans* (L) or Little bluestem, *Schizachyrium scoparium* (Michx) or Indian grass) and three C_3 grasses (*Bromus inermis* (Leyss) or Smooth brome, *Poa pratensis* (L) or Kentucky bluegrass, *Elymus repens* (L) or Quackgrass planted only in one site)) in spring 2005 at three old-fields at KBS in

southwestern Michigan. The three C_4 species were all common in the area prior to agricultural development (Gotshall 1972), and the three C_3 grasses are all commonly found in old-fields in the area (Burbank et al. 1992, Foster 1999). All three sites were abandoned more than 35 years ago following decades of row crop agriculture (Burbank et al. 1992, Foster and Gross 1997). Site characteristics are summarized in Table 6.

I established experimental monocultures of the six species in late spring 2005 in three randomized blocks (11 m by 14 m) at each site. I treated each block with a glyphosate herbicide (Roundup®) in early spring 2005, and then removed surface litter and standing dead tissue by hand clipping. To minimize soil disturbance, I left highly fragmented surface litter in place. I planted five species in all sites: *Andropogon, Sorghastrum*, *Schizachyrium*, *Bromus* and *Poa*. I wanted to include monocultures of *Elymus* because it was the dominant species at McKay Field, but its status as a noxious weed prevented me from purchasing seed and I had only enough seed to plant it at one site (Turkey Meadow). In each block, I randomly selected six (1 m^2) plots for each species, with plots separated by 0.5 m. In May 2005, I covered the plots with landscape fabric to minimize weed growth, and planted 7-week old transplants (16 plants m^{-2}), started from seed in the greenhouse, into holes cut in the fabric. The landscape fabric was kept in place for several months following transplanting and the transplants were watered as needed for several weeks to aid establishment. In total, each site had 18 replicate monocultures of each species, six in each of three blocks.

Table 6. Site characteristics of old-fields used for the Monoculture Experiment. All data are means (±SE) from data collected in September 2005; biomass data (g m^{-2}) was from harvested plots (n=9) and soil carbon (g m^{-2}) and nitrogen were from soils cores (0-10cm deep; n=4). Species percent of the total shoot biomass is given in parentheses after the species name. Nomenclature for all species follows the USDA Plants Database (plants.usda.gov).

Turkey Meadow	**McKay Field**	**Louden Field**
Dominant Vegetation		
Bromus inermis (72%)	*Elymus repens* (94%)	*Bromus inermis* (50%)
Poa pratensis (23%)	*Achillea millefolium* L (5%)	*Solidago canadensis* L (20%)
		Dactylis glomerata L (14%)
Shoot Biomass (g m^{-2})		
362±30	241±27	386±26
Soil type		
Sandy loam	Sandy loam	Loam
Soil Carbon (g m^{-2})		
2815±150	1638±225	--
Soil Nitrogen (g m^{-2})		
288±12	169±18	--

Field Sampling

Throughout 2005 and 2006, I maintained the monocultures by removing (clipping at ground level) species that were not planted in that particular plot (hereafter referred to as weeds). In 2005, weeding was minimal as the landscape fabric effectively prevented weed growth. In 2006, I clipped and removed weeds every 6-7 weeks; weed biomass from each plot was bagged, dried, and weighed (±0.01 g). In August 2006, three plots of each species were randomly selected in each block to sample plant and soil properties (n=9 per species, 3 per block). I established subplots (0.25 m x 0.25 m) within each plot for vegetation and soil sampling. All vegetation was clipped at ground level and separated into the monoculture species and weed biomass. I also collected the surface litter from these subplots. I then estimated root biomass in a subset of plots at McKay Field and Turkey Meadow (n=6 per species, 2 per block), by collecting one soil core (0-20 cm deep, 6.35 cm diameter) from the subplot immediately after vegetation sampling. The core was split into two depths (0-10 and 10-20 cm) and refrigerated until processed in the laboratory. After sampling for root biomass, I collected two additional cores (0-10 cm deep, 3.8 cm diameter) from all subplots in each site for soil chemical analyses; the two cores were combined and refrigerated until processed in the lab.

Sample Processing and Laboratory Analyses

Aboveground biomass and surface litter was dried for at least 72h at 65C and species biomass, weed biomass, and surface litter mass was recorded (±0.01 g). For each species in each site, shoot tissue was coarse ground in a Wiley Mill, then ground to <2mm in a Cyclotech Grinder, and re-dried for 48h at 65C. Tissue (2-3 mg) was then

packed in tin capsules for C, N and $\delta^{13}C$ analyses (relative to Peedee Belemnite; run by UC Davis Stable Isotope Facility). Roots >0.6 mm were removed from soil cores by floatation in water and collected using tweezers, and then rewashed to remove remaining soil. Roots from each soil depth were dried for 48h at 65C and weighed (±0.0001 g). I estimated plant total N for each plot by multiplying total biomass (root+shoot) by shoot tissue %N. Using the tissue %N of the monoculture species was appropriate, as weed biomass was a minor component of the total shoot biomass (typically less than 15%). While root and shoot %N data may differ, Wedin and Tilman (1990) showed that %N for *Elymus*, *Andropogon* and *Schizachyrium* shoots was generally similar to that for roots.

Soils collected for soil chemistry were passed through a 2 mm sieve prior to analysis to homogenize the sample and remove large roots and rocks. I extracted inorganic N from 20 g subsamples processed within 24h of sample collection using 50ml of 1M KCl. The extractions were stored at 3C until analysis on an O.I. Analytical Flow Solution IV analyzer. I determined gravimetric soil moisture on a subsample of soil (~25g fresh weight) by drying soils at 105C for 48h. A subsample of air-dried soil (~50 mg) from six replicates was ground to a flour-like texture on a roller mill, oven-dried, and analyzed for C, N and $\delta^{13}C$ at UC Davis. C_3 and C_4 species $\delta^{13}C$ differs, so I calculated the percent soil C contributed by C_4 species with the model:

$$\%C_4 \text{ signal} = (C_4 \text{ Soil } \delta^{13}C - C_3 \text{ soil } \delta^{13}C)/(C_4 \text{ plant } \delta^{13}C - C_3 \text{ soil } \delta^{13}C)*100,$$

using the plot mean $\delta^{13}C$ from the *Bromus* plots as the C_3 soil $\delta^{13}C$.

Plant Trait Experiment

To obtain more detailed information about the traits of the species used in the Monoculture Experiment, I grew individuals of all six species in open-bottom "pots" constructed from PVC pipe (15.24 cm diameter, 100 cm deep) filled with a sand:soil mixture. I buried the pots to a depth of ~97 cm in a fenced area at KBS, on a grid (10.5 m x 9 m) with a 1.5 m buffer between pots. The pots were filled with a 3:1 mixture of pure sand and sandy loam topsoil collected from an old-field at KBS. Prior to filling the pots, the soil was sieved (6.35 mm) to remove large rocks and roots and then combined with the sand in a cement mixer. After filling, the soil was supersaturated with water to settle the soil and achieve similar bulk densities among pots (Craine et al. 2003), and refilled with the sand:soil to within 3cm of the top of the pot. I randomly assigned five replicate pots per species and transplanted one 7-week old individual to the center of each pot in June 2006. Pots were watered as necessary throughout the summer to prevent desiccation, with all pots receiving equal amounts at each application.

In late-September 2006, I clipped plants at ground level, dried the material for 72h at 65C, and weighed it (±0.01 g). Using the methods described in the Monoculture Experiment, I analyzed plant tissue for total C and N using an Elemental Analyzer (Costech Analytical, Ventura, CA). In addition, I performed Acid Detergent Fiber analyses (recalcitrant compounds composed primarily of lignin and hemicellulose) on the ground plant tissue (~0.5 g) using an Ankom 2000 Fiber Analyzer (Macedon, NY). To determine root biomass, I removed the pots from the ground, cut them in half lengthwise without disturbing the soil core, and separated the core into four depth intervals (0-10, 10-20, 20-40, and 40-80 cm). I placed the soils from each depth interval in plastic bags

and kept them at 3C until processed using the methods described in the Monoculture Experiment. For each species, I determined the absolute biomass and proportion of total root biomass in each depth interval to examine whether species exhibited differential patterns of root biomass production and depth distribution.

Data Analysis

I checked all data for normality and homoscedasity of variance, and made appropriate transformations prior to analysis. I used ANOVA to examine species differences and for any ANOVA indicating a significant interaction, I performed post-hoc contrasts using Tukey comparisons. For the Monoculture Experiment, I compared plant biomass and soil inorganic N using a nested ANOVA with Site and Species as main effects, with Block nested in Site. I was primarily interested in between (and not within) site variation, so I ran analyses excluding Block from the model to test for Site and Species effects on tissue and soil C, N and $\delta^{13}C$. Weed biomass was a minor component of the total shoot biomass collected in August 2006, so I used the combined monoculture species shoot biomass and weed biomass (total shoot biomass) for statistical analysis. I did not include *Elymus* in the analyses because it was present at only Turkey Meadow, but I show the data for comparison purposes. To test for species differences in the Plant Trait Experiment, I used a One-Way ANOVA with post-hoc Tukey comparisons. Pearson's Product Moment correlations were performed to determine if the plant traits were highly correlated with soil variables. I used SigmaStat 3.5 for all analyses except the nested ANOVA, where I used Systat 11.

RESULTS

Monoculture Experiment

Plant Productivity and Chemistry

The differences among the three sites in soil fertility (Table 6) were reflected in plant production and tissue chemistry differences of species across sites. However, I still found consistent differences in plant and soil variables between C_3 and C_4 species. $p<0.001$). C_4 species had significantly higher aboveground biomass than C_3 species, and there were also significant differences among species for surface litter production and subsurface root biomass (Table 7, Figure 5). Species differed in surface litter production across sites, but *Poa* generally had significantly lower surface litter than all other species (Tables 7 and 8). I did not detect differences among species for shallow root biomass within a site, but *Poa* had significantly less subsurface (10-20cm) root biomass than all three C_4 species and *Brome* had significantly less subsurface root biomass than *Sorghastrum* (Figure 5, Table 7). McKay Field had significantly higher surface litter and root biomass (both surface and subsurface) than Turkey Meadow (Table 7). Louden Field had significantly more shoot biomass than McKay Field, which had significantly more than Turkey Meadow (Table 7). C_4 species typically had significantly higher tissue C:N, and lower %N content than C_3 species (Table 9), and tissue %N and C:N differed across sites ($F_{2,45}$=12.72, 14.49, respectively; $p<0.001$ for both). Species had significantly higher tissue %N and C:N at McKay Field (%N=1.15±0.11, C:N=44.25±3.27) than at Turkey Meadow (%N=0.86±0.07, C:N=57.86±4.16) or Louden Field (%N=0.94±0.11, C:N=55.65±4.22). Individual species comparisons showed no

Table 7. ANOVA results for aboveground variables for all five species in Louden Field McKay Field, and Turkey Meadow, using Site and Species as main effects, with Block (Blk) nested within Site. Significant differences are indicated in **bold**.

	Model	Site	Species	Site*Species	Blk(Site)
Total Shoot	F	8.91	162.15	1.66	4.03
Biomass ($g\ m^{-2}$)	d.f.	2,111	4,111	8,111	6,111
	P value	**<0.001**	**<0.001**	0.177	**0.001**
Surface Litter	F	4.71	23.06	0.99	3.80
($g\ m^{-2}$)	d.f.	2,111	4,111	8,111	6,111
	P value	**0.011**	**<0.001**	0.450	**0.002**
Surface Root	F	19.94	1.22	2.73	1.14
Biomass 0-10cm	d.f.	1,45	4,45	4,45	4,45
($g\ m^{-2}$)	P value	**<0.001**	0.314	**0.041**	0.352
Subsurface Root	F	9.47	9.18	0.46	2.36
Biomass 10-20cm	d.f.	1,45	4,45	4,45	4,45
($g\ m^{-2}$)	P value	**0.004**	**<0.001**	0.762	0.068
Soil Inorganic	F	37.05	3.10	1.27	6.25
Nitrogen	d.f.	2,111	4,111	8,111	6,111
($\mu gN\ gdry\ soil^{-1}$)	P value	**<0.001**	**0.018**	0.267	**<0.001**

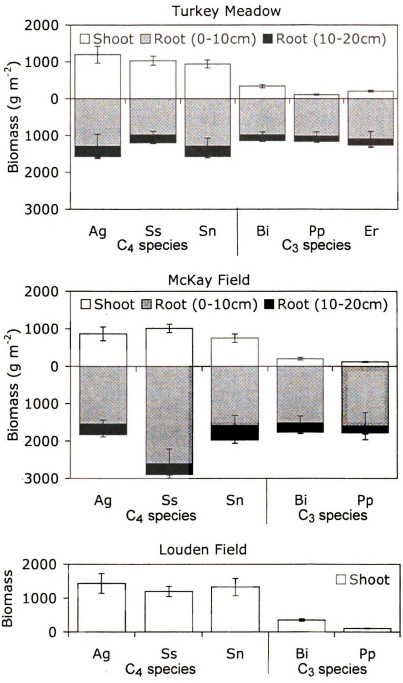


Figure 5. Shoot and root production estimates of each species in each site in the Monoculture Experiment, averaged across the three blocks in each site. Mean (±SE) root biomass for each depth interval (n=6) and shoot biomass (n=9) in August 2006 is shown separately for each site. Root biomass was not collected at Louden Field. Species names are abbreviated: Ag=*Andropogon*, Ss=*Schizachyrium*, Sn=*Sorghastrum*, Bi=*Bromus*, Pp=*Poa*, and Er=*Elymus*.

significant differences in total plant (root+shoot) N stocks at either Turkey Meadow or McKay Field ($p>0.14$).

Soil Properties

Soil properties varied among dominant vegetation plots and sites, with no Site x Plot interactions (Tables 7 and 10). Soil inorganic N was significantly higher in soil under *Bromus* than under *Sorghastrum* (Figure 6, Table 7). There were a few significant differences between species in total soil C or N, and soil under *Andropogon* and *Sorghastrum* tended to have significantly higher C:N and $\delta^{13}C$ than C_3 species (Table 9). Soil $\delta^{13}C$, N content, and C content were strongly correlated with surface root biomass (Pearson $r=0.49, -0.44, -0.43$, respectively; $p<0.02$ for all). C_4 species contributed an average of 2.3±0.52% (Range: 0 to 9.6%) of the total soil C in C_4 monoculture soils after just two years.

The three sites differed significantly for many soil properties. Soil N and C content (g kg^{-1}) was higher at Louden Field (means: N=0.19±0.01; C=2.02±0.07) than at the McKay Field (means: N=0.12±0.01; C=1.34±0.08) or Turkey Meadow (means: N=0.13±0.01; C=1.47±0.06) sites ($F_{2,60}=38.77, 32.34$, respectively; $p<0.001$ for both). Soil C:N was significantly higher in McKay Field (mean of 11.5±0.1) than in Turkey Meadow (mean of 11.1±0.1) and Louden Field (mean of 10.8±0.1; $F_{2,60}=22.82$, $p<0.001$). Soil $\delta^{13}C$ also differed between sites ($F_{2,60}=3.90$, $p=0.026$) and was significantly more depleted in Louden Field (mean of -26.9±0.1) than in Turkey Meadow (mean of -26.7±0.1). Soil inorganic N was also significantly higher in Louden Field than at McKay Field or Turkey Meadow (Table 7).

Table 8. Mean (±SE) surface litter mass (g m^{-2}) for each species at each site. Superscript numbers denote significant differences ($p<0.05$) between species within each Site. In addition, McKay Field had significantly more surface litter than Turkey Meadow ($p=0.011$).

Plot	Turkey Meadow	McKay Field	Louden Field
Andropogon	76 ± 18^{a}	78 ± 10^{a}	91 ± 15^{a}
Schizachyrium	43 ± 9^{ab}	70 ± 10^{ab}	61 ± 9^{ab}
Sorghastrum	53 ± 13^{ab}	59 ± 7^{ab}	52 ± 8^{ab}
Bromus	37 ± 10^{b}	63 ± 15^{b}	35 ± 6^{b}
Poa	21 ± 9^{c}	27 ± 6^{c}	19 ± 6^{c}
Elymus	35±6	--	--

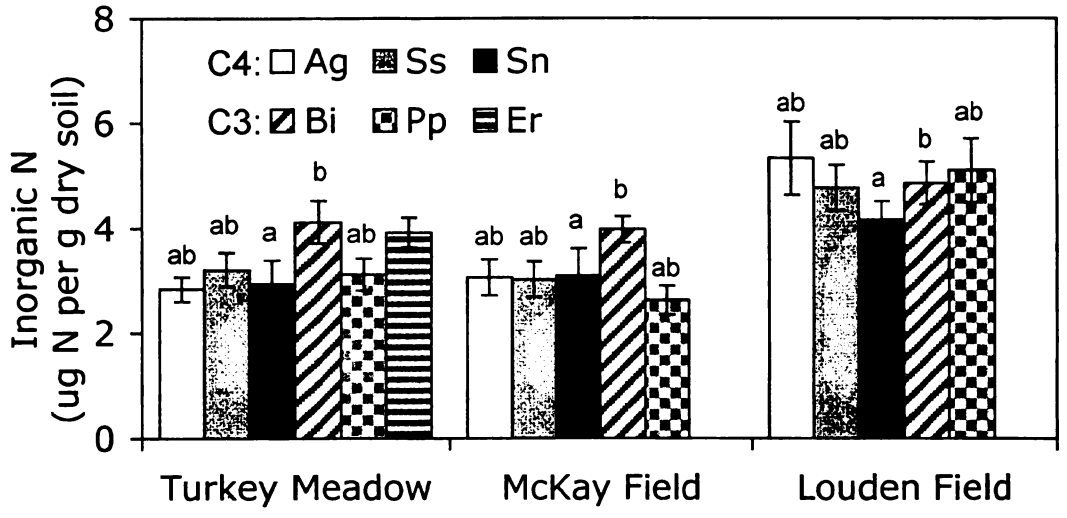


Figure 6. Species effects on inorganic N (mean ± SE) concentrations in the Monoculture Experiment, averaged across blocks, for the three sites. Species names are abbreviated: Ag=*Andropogon*, Ss= *Schizachyrium*, Sn=*Sorghastrum*, Bi=*Bromus*, Pp=*Poa*, and Er=*Elymus*. Solid bars represent soils under C_4 species and patterned bars represent soils under C_3 species. Lowercase letters denote significant differences ($p<0.05$) between plots (*Elymus* plot data is shown for comparison purposes only).

Table 9. Plant tissue chemistry (mean±SE) for each species (n=4), averaged across sites. *Elymus* data is shown for comparison only. For variables with significant Species main effects, lowercase letters denote significant differences ($p<0.05$) determined from post-hoc Tukey comparisons.

Species	Group	%N	%C	C:N	$\delta^{13}C$
Andropogon	C4	0.72 ± 0.05^{a}	45.1 ± 0.1^{a}	65.7 ± 4.3^{a}	-12.4 ± 0.2^{a}
Schizachyrium	C4	0.71 ± 0.04^{a}	45.0 ± 0.2^{a}	65.1 ± 3.1^{a}	-12.6 ± 0.1^{a}
Sorghastrum	C4	0.80 ± 0.04^{ab}	44.3 ± 0.2^{ab}	57.2 ± 3.0^{ab}	-12.7 ± 0.1^{a}
Bromus	C3	0.98 ± 0.09^{b}	44.6 ± 0.2^{a}	48.4 ± 3.3^{b}	-28.0 ± 0.2^{b}
Poa	C3	1.71 ± 0.11^{c}	43.8 ± 0.3^{b}	26.6 ± 1.7^{c}	-27.8 ± 0.2^{b}
Elymus	C3	0.93±0.08	45.3 ± 0.9	49.4 ± 4.3	-28.3 ± 0.2

Table 10. Soil chemistry data (mean±SE) for soils under each species, averaged across sites. For variables with significant Species effects, superscript letters denote significant differences ($p<0.05$) determined from post-hoc Tukey comparisons. *Elymus* is shown for comparison only.

Species	Group	N (g kg^{-1})	C (g kg^{-1})	C:N	$\delta^{13}C$
Andropogon	C4	1.3±0.1 ^{a}	14.9±1.3 ^{ab}	11.6±0.2 ^{a}	-26.5±0.1 ^{a}
Schizachyrium	C4	1.5±0.1 ^{ab}	16.2±0.9 ^{ab}	11.1±0.1 ^{bc}	-26.8±0.1 ^{abc}
Sorghastrum	C4	1.5±0.1 ^{ab}	16.7±1.2 ^{ab}	11.4±0.1 ^{ab}	-26.7±0.1 ^{ab}
Bromus	C3	1.6±0.1 ^{b}	18.1±1.4 ^{b}	11.1±0.1 ^{bc}	-27.1±0.1 ^{c}
Poa	C3	1.3±0.1 ^{ab}	14.7±0.9 ^{a}	11.0±0.1 ^{c}	-26.9±0.2 ^{bc}
Elymus	C3	1.5±0.4	16.3±2.6	10.9±0.2	-27.0±0.2

There were also several significant correlations between plant and soil variables. Plant tissue C:N was significantly correlated with soil C ($n=39$, $p=0.02$, $r=0.36$) and N content ($n=39$, $p=0.03$, $r=0.35$), and total root biomass was positively correlated with soil $\delta^{13}C$ ($n=29$, $p<0.001$, $r=0.55$), and negatively correlated with both soil C ($n=29$, $p=0.01$, $r=-0.45$) and N content ($n=29$, $p=0.01$, $r=-0.47$). Surface litter was positively correlated with soil $\delta^{13}C$ ($n=75$, $p<0.001$, $r=0.41$).

Plant Trait Experiment

Growing these species as individuals in pots allowed me to more accurately assess plant traits, particularly root characteristics, than I could in the field monocultures. I again found that the three C_4 species tended to have greater shoot biomass than the C_3 species (Figure 7), and tissue chemistry also separated the C_3 and C_4 species (Figure 8). There were significant differences between species in root biomass at each depth interval, but few clear trends emerged (Table 11). While all species had over 50% of their root biomass in the top 20cm of soil, the three C_3 species tended to have a larger proportion of their total root biomass (62-68%) in the surface soils (0-20cm) than the C_4 species (51-60%). The C_4 species produced a larger percentage of root biomass (18-21%) deeper in the soil (20-40cm) than did the C_3 species (10-13%).

DISCUSSION

As expected, I was able to detect significant differences in plant production among species within two years of establishment in all three sites. The greater shoot biomass for the C_4 species seemed to be a consistent pattern for all C_4 species, and *Poa* had the

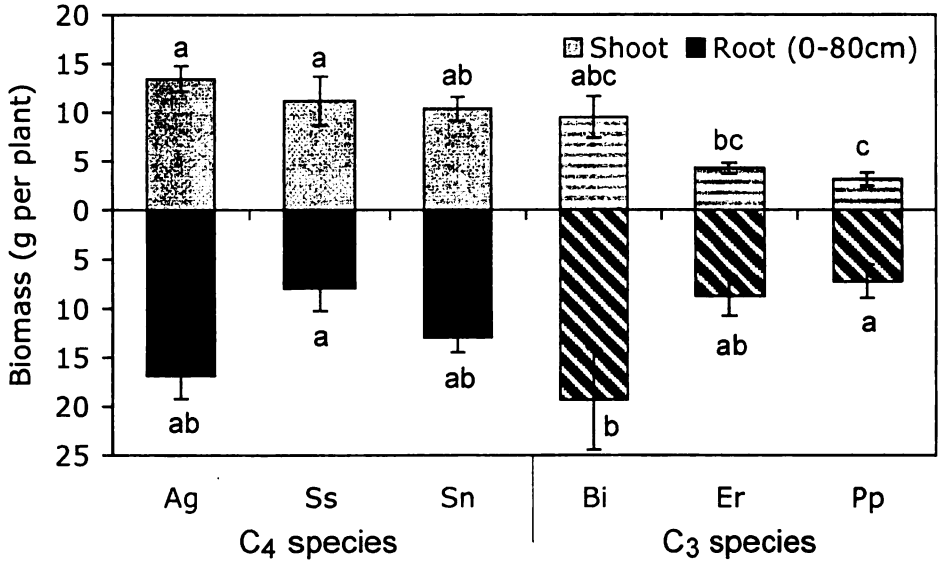


Figure 7. Above- (n=5) and below- (0-80 cm; n=4) ground biomass per plant for each species (mean ±SE) in the Plant Trait Experiment. Species names are abbreviated: Ag=*Andropogon*, Ss= *Schizachyrium*, Sn=*Sorghastrum*, Bi=*Bromus*, Pp=*Poa*, and Er=*Elymus*. Solid bars represent C_4 species and patterned bars represent C_3 species. Lowercase letters denote significant ($p<0.05$) differences between species for shoot biomass and total root biomass.

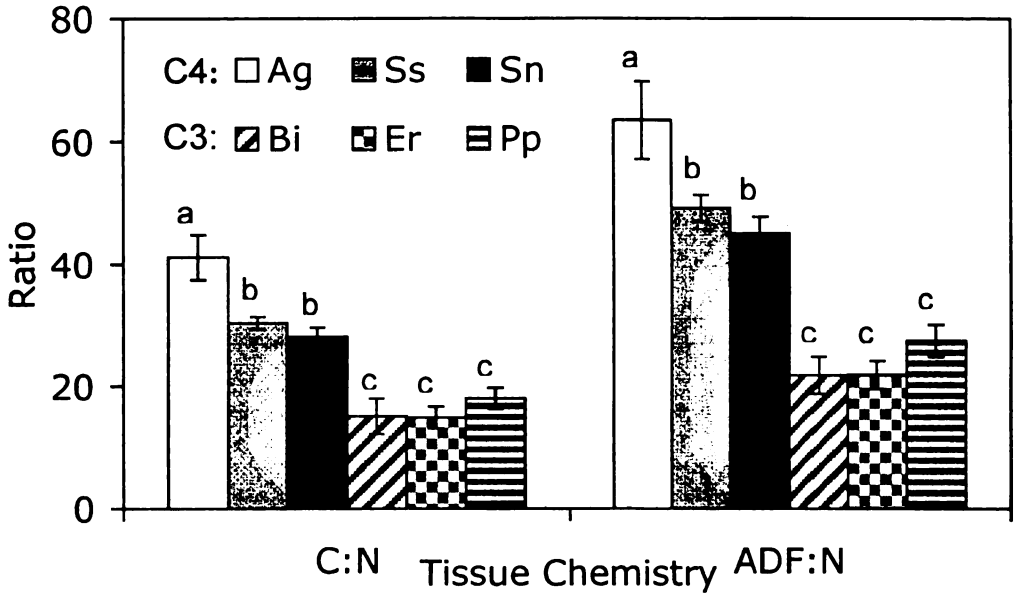


Figure 8. Tissue chemistry data for species grown in the Plant Trait Experiment; values are mean (±SE; n=4). Species names are abbreviated: Ag=*Andropogon*, Ss= *Schizachyrium*, Sn=*Sorghastrum*, Bi=*Bromus*, Pp=*Poa*, and Er=*Elymus*. Acid detergent fiber (ADF) is a measure of recalcitrant compounds, primarily lignin and hemicellulose. Solid bars represent C_4 species and patterned bars represent C_3 species. Lowercase letters denote significant differences ($p<0.05$) between species.

Table 11. Plant Trait Experiment root biomass at each depth interval (mean±SE; n=4). For each depth interval, significant differences ($p<0.05$) between species are indicated by superscript lowercase letters, as determined from post-hoc Tukey comparisons. Biomass values without letters indicate no significant difference from any of the other species.

	Root Biomass (g per plant)			
	0-10 cm	10-20 cm	20-40 cm	40-80 cm
Andropogon	6.21±0.98	3.47±0.33^{a}	3.58±0.63^{a}	3.60±0.71^{a}
Schizachyrium	2.79±0.93^{a}	1.33±0.35	1.68±0.54	2.16±0.63
Sorghastrum	6.47±1.11	1.38±0.09	2.34±0.41^{ab}	2.78±0.40
Bromus	10.08±2.90^{b}	2.32±0.71^{ab}	2.64±0.81^{ab}	4.26±0.83^{a}
Poa	4.29±1.33	0.80±0.18^{c}	0.71±0.10^{c}	1.44±0.23^{b}
Elymus	4.64±1.18	0.81±0.15^{bc}	1.12±0.27^{bc}	2.15±0.50

lowest shoot biomass and surface litter layers. The surface litter in this experiment reflects a single growing season and so should strongly reflect difference in shoot production. *Andropogon* had 8 to 27% higher aboveground biomass than the other C_4 species and this difference was clearly reflected in litter levels, where *Andropogon* had 10 to 80% more litter mass than the other C_4 species. All else being equal (e.g. similar phenology and litter quality), I would expect that the significantly higher shoot biomass levels for C_4 species would result in faster litter accumulation in C_4 plots after a relatively short period of time. I found in Chapter Two that surface litter mass in plots of these C_4 species was significantly greater than in the surrounding C_3-dominated old-fields of Turkey Meadow and McKay Field after 11 years.

There were few differences in root biomass among species after two growing seasons in the Monoculture Experiment. The Plant Trait Experiment, which allowed me to examine species rooting patterns in greater detail, also did not reveal clear differences in root biomass among species after just one year. Craine et al. (2003), in a three-year study of 11 grassland species in Minnesota, also found it difficult to generalize about functional group differences in rooting dynamics. While I did not see clear trends in root production, I did see differences in allocation patterns in the Plant Trait Experiment. While the majority of root biomass for all species was in the top 20cm of soil, C_4 species allocated on average 40-49% of root biomass to deeper soils (20-80cm depth), compared to 32-38% for C_3 species. Craine et al. (2003) also found comparable results for *Bromus*, *Poa*, *Andropogon*, and *Schizachyrium* in a similar study. Sampling for roots in the top 20cm may have disproportionately underestimated root biomass for C_4 species because of their higher allocation (almost half) in deeper soil. This greater investment in deeper

roots suggests that C_4 species may be accessing soil nutrients and water that are unavailable to the shallower rooted C_3 species and potentially gaining a competitive advantage during periods of drought. This deeper root investment also suggests that C_4 species may affect soil processes at greater depth than C_3 species, with implications for C sequestration in deeper soil.

The lack of clear differences between species root biomass may reflect the fact that these were relatively short-term experiments and the rooting systems of the species may not have fully developed after 1-2 growing seasons. In a related study of 11-year-old monocultures of the same C_4 species in McKay Field and Turkey Meadow, I found higher overall root biomass values, and significantly greater root biomass (0-20cm) for the C_4 species than for *Elymus* and *Bromus* (Chapter Two). I expect that the differences between C_3 and C_4 species will become more pronounced as the plants mature and belowground biomass accumulates.

The C_4 species produced more aboveground biomass (potential litter) and this tissue was higher in C:N, ADF:N and had lower %N than C_3 species. As a result, litter produced by C_4 species would be expected to decompose slowly and thus increase soil organic matter buildup. Indeed, I found strong, positive correlations between soil $\delta^{13}C$ and both surface litter and root biomass, suggesting that the soil is beginning to reflect the C inputs of the C_4 species after just two growing seasons. In contrast, while C_4 species generally had lower soil inorganic N compared to C_3 species, these differences were not statistically significant except for between *Sorghastrum* and *Bromus*. Many studies have shown lower levels of inorganic N and/or reduced N mineralization rates in communities dominated by C_4 species (Wedin and Tilman 1990, Wedin and Pastor 1993, Baer et al.

2002, Camill et al. 2004, Chapter Two). Based on the results in Chapter Two I expect that inorganic N levels in soils associated with C_3 and C_4 species will continue to diverge and produce detectable difference in the next 10 years.

The mechanism behind these slight reductions in inorganic N is unclear. C_4 species could be reducing inorganic N levels by taking up more N than C_3 and therefore have larger tissue N stocks, or through reduced N cycling rates of its recalcitrant litter. Evidence from the 11-year-old C_4 monoculture stands of these species in McKay Field and Turkey Meadow supports slowed N cycling as the more likely explanation (Chapter Two), and data from that study and the current study suggest that C_4 species do not contain more total N in their combined root and shoot tissue than C_3 species. Craine et al. (2003) found similar results; there were no significant differences in aboveground biomass N between *Andropogon, Schizachyrium, Poa* and *Elymus*, although *Poa* had significantly lower belowground biomass N content than the other three species.

While there was little indication that species had impacted soil total C and N pools after two years, there was a significant, positive correlation between plant tissue C:N and both soil C and soil N pools. This suggests that the higher C:N species (i.e., the C_4 species) are increasing total soil C and N pools. In addition, the isotopic signature of C_4 species was detectable in the soils after just two growing seasons, indicating that C_4 species had contributed a significant amount of C to the soil C pool in a short time period. C_4 species contributed an average 2.3% of the total soil C (0-10cm) in C_4 monoculture soils after just two years. Most of this contribution is likely from roots, as little surface litter was likely incorporated into the soil after such a short time. I showed in Chapter Two that C_4 species contributed 9-26% of the total soil C pool after 11 years, which

suggests that C replacement in my study will steadily increase in the next decade. However, I do not have clear evidence as to whether this replacement will result in faster rates of soil C accumulation for C_4 species. Baer (2002) showed some evidence of total soil C levels increasing in C_4 dominated restored prairies after 12 years, but McLauchlan et al. (2006) found that total soil C increased with time since abandonment regardless of whether the community was dominated by C_4 or C_3 species. Soil C:N was higher under several C_4 species than C_3 species, suggesting that C and N pools may be changing slowly to reflect the higher C:N ratios of the C_4 species.

The results of this study demonstrate that species differences in biomass and tissue chemistry can be detected rapidly in the field, but the effects of these species differences on soil processes are slower to emerge and will depend on the magnitude of the differences between species. Many of the plant and soil variables were similar for species within a functional group, including *Elymus*, which typically fell between *Poa* and *Bromus* in terms of plant traits and soil properties at Turkey Meadow. Although inorganic N pools were just beginning to show species effects, I did not see significant changes in soil total C or N pools after two years. I expect that inorganic N differences between species will continue to diverge over the next several years as the plants mature and differences in surface litter and root production become more pronounced. However, a decade or more may be needed before total soil pools reflect species effects on soil processes.

CHAPTER FOUR

EFFECTS OF LITTER QUALITY AND SOIL MICROCLIMATE ON DECOMPOSITION

ABSTRACT. Plant species can affect soil processes in a variety of ways, including through the quantity of biomass produced and the chemistry, or quality, of biomass. In this study I examined whether species differing in litter characteristics may alter decomposition directly via tissue chemistry differences and indirectly via effects on microclimate. I set up a reciprocal transplant experiment to compare the decomposition rates of two C_3 grasses (*Bromus inermis* and *Elymus repens*) and *Andropogon gerardii* (a C_4 grass) in two old-field communities in southwest Michigan. My findings suggest that seasonal controls (i.e., temperature and moisture) on decomposition are stronger than the effects of litter quality, and litter quality differences become important when examining decomposition within a common environment (i.e., within a site). Examination of the effects of these species on microclimate (soil moisture and temperature) indicated that while soils were warmer under C_3 than C_4 species, there was no evidence to suggest that this affected decomposition rates. This suggests that litter chemistry was the controlling factor determining the observed differences in decomposition rates between C_3 and C_4 species. These results indicate that restoring native C_4 prairie grasses into C_3-dominated old-fields will slow decomposition rates of aboveground plant tissue and may ultimately lead to changes in soil C and N cycling and storage.

INTRODUCTION

Plant species that differ in above- and below-ground biomass and tissue chemistry can influence soil processes in various ways, and changes in species composition can have dramatic impacts on carbon and nutrient cycling. One of the most frequently examined ways by which plants alter soil processes is through differences in litter quality (i.e., tissue chemistry) and quantity (Hobbie 1992, Wardle et al. 1998, Ehrenfeld 2003, Lovett et al. 2004, Hooper et al. 2005, Dijkstra et al. 2006). Vitousek and Walker (1989) demonstrated that the invasion of a novel nitrogen fixer (*Myrica faya*) dramatically alters N cycling in young Hawaiian forests. Other studies (Evans et al. 2001, Mack et al. 2001, Drenovsky and Batten 2007) have shown that plant invasions alter N cycling via production of more recalcitrant tissue, and that species with higher C:N or lignin:N typically have slower decomposition rates (Ehrenfeld et al. 2001, Xu and Hirata 2005, Hobbie et al. 2006, Drenovsky and Batten 2007).

While the importance of species litter chemistry in controlling decomposition rates is well accepted, whether plants can impact soil processes via their effect on soil microclimate has received less attention. However, several recent papers (Mack and D'Antonio 2003b, Eviner 2004, Eviner et al. 2006) suggest that such effects can be as or more important than litter chemistry as a determinant of soil process rates. Soil temperature and moisture are two factors that exert strong controls on soil microbial function (Aerts 2006). Plants can influence these factors in a variety of ways (reviewed in Eviner and Chapin 2003), including the rate and timing of water uptake, surface litter effects on evaporation from the soil, and aboveground production, all of which can buffer soil temperature fluctuations. Bengston et al. (2005) found that soil water is positively

related to soil respiration and N mineralization rates, which suggests that a shift to a species with higher water use efficiency or greater litter (i.e., reducing evaporation) could alleviate microbial water limitation and result in higher process rates. Thus, a shift in species composition to a new dominant species that alters microclimate could also influence soil processes such as decomposition.

Even with dramatic differences in litter characteristics among species, it is often difficult to isolate plant traits responsible for observed influences on soil processes. In Chapter Two, where I compared the relative effects of C_3 and C_4 grasses on soil C and N after 11 years, I found that soils under native prairie C_4 grasses, with both greater biomass and more recalcitrant tissue than C_3 grasses, tended to have lower rates of *in situ* net nitrification and N mineralization and significantly higher surface litter accumulation than soils under non-native C_3 grasses typical of old-field communities in the Midwest. Results from that study led me to hypothesize that decomposition rates would be slower in sites dominated by C_4 species. However, because these two functional groups differ in biomass production and litter quality, I expected that they would also affect soil microclimate. Thus it is difficult to determine whether effects on soil processes are due to direct effects of plant traits, microclimate or a combination of these factors.

This study evaluates the ways in which traits of C_4 and C_3 species can influence litter decomposition and turnover. I performed two experiments to separate the effects of litter quality and soil microclimate. In the first experiment, the decomposition rates of the two litter types (C_3 and C_4) were compared in two common locations (both litter types were placed under C_3 species and under C_4 species) in two fields. I expected C_4 litter to decompose slower than C_3 litter because C_4 litter is more recalcitrant (higher

C:N, lignin:N). Comparing decomposition of a particular litter placed in two locations allowed me to examine how differences in soil microclimate influences decomposition rates. I also expected decomposition to be slower under C_3 species than C_4 species because the smaller litter layer and lower aboveground biomass would make these environments less advantageous for microbial activity (i.e., drier and hotter) in the summer.

In a second experiment, I used cellulose filters as a standard substrate to determine decomposition rates under C_3 and C_4 species, with and without litter. Soil moisture and midday temperatures were measured in these plots to determine the effect of aboveground biomass and surface litter on these factors that are important determinants of soil processes such as decomposition. Summer and fall soil moisture levels and soil temperature were expected to vary with ground cover (litter and above-ground biomass) and so I predicted highest soil moisture and lowest temperatures under the C_4 species with litter intact, followed by C_4 species without litter, then C_3 species with litter and finally C_3 species without litter. I expected cellulose decomposition to be faster under C_4 species if soil moisture limited decomposition and to be fastest in the C_3 species plots without litter if temperature was the primary factor determining decomposition rates.

METHODS

Study Sites

To determine the relative importance of litter quality and microclimate associated with different dominant species on decomposition rates, I performed a reciprocal transplant

litterbag decomposition experiment using litter from two C_3 species and *Andropogon gerardii* (Vitman; a C_4 species), in two old-fields located at the W.K. Kellogg Biological Station (KBS) in southwestern Michigan, USA. Both sites were abandoned more than 35 years ago following decades of row crop agriculture (Burbank et al. 1992, Foster and Gross 1997). Plant communities in the two sites were dominated by C_3 non-native species, but the composition of the communities differed: Turkey Meadow is dominated by *Bromus inermis* (Leyss) and McKay Field is dominated by *Elymus repens* (L). Patches of *Andropogon* were established in 1995 for competition experiments described in Foster (1999), and subsequently abandoned in 1996. Nomenclature for all species follows the USDA Plants Database (available online: plants.usda.gov). All soils are sandy loams (Foster and Gross 1997), but McKay Field has a higher sand fraction and seems to be more drought-prone than Turkey Meadow.

Reciprocal Transplant Experiment

I compared the decomposition dynamics of *Andropogon gerardii* and *Elymus repens* at McKay Field, and *Andropogon gerardii* and *Bromus inermis* at Turkey Meadow by collecting recently senesced, standing aboveground tissue from many plants of each species in late October 2005. Litter was air-dried in the laboratory for two weeks, cut into 6-8cm long pieces, and gently mixed to homogenize the litter for each species. Approximately 4g of air-dried litter (3.8g oven-dried equivalent) was placed into 10cmx10cm polyester mesh litterbags (0.17cmx0.17cm mesh), which were sealed with an impulse heat sealer. For both species at each site, I placed six replicate sets of six litterbags in each of two environments (litter under C_4 species and litter under C_3

species) in November 2005. In total, I had four treatments in each site—C_3 litter under C_4 species, C_3 litter under C_3 species, C_4 litter under C_4 species and C_4 litter under C_3 species—with six replicates of each treatment for collection at each of six time intervals. In late November 2005, I gently slid the litterbags under the barely decomposed surface litter layer (to avoid contamination, highly fragmented litter was not placed on the litterbags). Some litterbag replicates were lost to mammal activity, so I collected at only four dates in 2006: April 10, June 19, August 30, and November 28, after 142, 213, 284 and 374 days.

The litterbags were sealed in plastic bags and transported to the laboratory, where soil, root, and green tissue contamination was removed, and then weighed to determine a field-moist weight of the litter (field moisture was used as an indicator of potential microclimate differences between species and sites). The litterbags were then dried for 48h at 65C, and then reweighed. For each bag, I calculated percent moisture using the field-moist weight measurement and the oven-dried measurement for the litter. I calculated the percent mass remaining relative to $time_0$. This data was then used to calculate decay constants (k) for each replicate in each treatment using both a linear and single exponential model (Trofymow et al. 2002). The two models had a similar fit to the data (average R^2 of 0.92 for the linear model and 0.90 for the exponential model), so I used the more biologically realistic exponential model for analysis.

I compared mass remaining between treatments over the one-year period using Repeated Measures ANOVA with Site, Litter type (C_4 or C_3), and Placement (placed under C_4 or under C_3 species) as factors. Decay constants for the exponential model were compared using a Three-way ANOVA with Site, Litter type (C_4 or C_3), and

Placement (placed under C_4 or under C_3 species) as factors. For any ANOVA indicating a significant interaction, I performed post-hoc contrasts using Tukey comparisons. I used Systat 11 for Repeated Measures and SigmaStat 3.5 for all other analyses.

To examine seasonal impacts on decomposition rates, I compared the decomposition of the first 142 days of the experiment above (November 2005-April 2006; winter decomposition) to a second set of litterbags installed in April 2006 (April 2006-September 2006; summer decomposition). Mass remaining and the exponential decay rates (k) for winter and summer periods were compared separately for each site using a Three-way ANOVA with Litter type (C_4 or C_3), Placement (under C_4 or under C_3 species), and Season (Winter or Summer) as factors. For significant interactions, I performed post-hoc contrasts using Tukey comparisons.

To determine initial litter chemistry for each species, I analyzed a finely ground (<2mm on a Cyclotech grinder) litter sub-sample for C and N concentrations using an Elemental Analyzer (Costech Analytical, Ventura, CA), and for Acid Detergent Fiber (recalcitrant compounds composed primarily of lignin and hemicellulose) on an Ankom 2000 Fiber Analyzer (Macedon, NY). Data for Species and Site were compared using Two-way ANOVA.

Decomposition and Soil Microclimate Experiment

The species examined above differ in both their surface litter and aboveground biomass, and these differences may affect soil microclimate, particularly soil moisture and temperature. This experiment determines whether microclimate differences exist between the species, and how these differences affect the decomposition of a standard

cellulose substrate. To examine how surface litter contributes to microclimate differences under C_3 and C_4 grasses, I removed the surface litter by hand from six plots (0.5mx0.5m) for both C_3 and C_4 grasses (-litter treatment) and left the surface litter intact in six plots for both C_3 and C_4 grasses (+litter treatment). I placed one 10cmx10cm polyester mesh litterbag (0.17cmx0.17cm mesh) filled with ~4g of cellulose filter paper (Whatman No.1) into each plot in late May 2006. Cellulose was used as a decomposition standard material to eliminate the confounding factor of litter quality and focus on the microclimate impacts on decomposition. Bags were placed on the soil surface in the -litter treatment and under the surface litter layer in the +litter treatment.

I measured soil moisture and temperature in each plot in mid-June, mid-July, mid-October and mid-November 2006. All measurements were made close to midday on mostly sunny days. To determine gravimetric soil moisture, I combined 3 soil cores (2cm diameter, 0-10cm depth) from each plot and dried them at 105C for 48h. I measured soil temperature (Taylor model 9841) at two depths: surface soil temperature and soil temperature at 10cm below the soil surface. Three temperature readings were averaged for each plot at each depth.

I collected the cellulose bags in mid-May 2007 (after 354 days), placed them in plastic bags for transport to the laboratory, removed soil and plant contamination, and weighed the filters immediately and after oven-drying for 24h at 65C. I calculated the percent mass remaining from $time_0$ and moisture content of the cellulose. Both variables were compared using Three-way ANOVA comparing Site, Placement, and +/- litter as main effects. Repeated Measures ANOVA was used to determine changes in soil moisture and temperature over the one-year period, using Site, Placement (under C_4 or

under C_3), and +/- Litter (+litter and -litter) as factors. For any ANOVA indicating a significant interaction, I performed post-hoc contrasts using Tukey comparisons. I used Systat 11 for Repeated Measures ANOVA and SigmaStat 3.5 for Three-way ANOVA.

RESULTS

Reciprocal Transplant Experiment

Andropogon (C_4) had significantly higher C:N ($F_{1,9}$=61.72, p<0.001) and ADF:N (ADF is primarily lignin and hemicellulose; $F_{1,7}$=49.22, p<0.001) than the C_3 species, and there were no significant differences in tissue quality between the two sites (Table 12).
In both sites, mass loss of *Andropogon* (the C_4 species) was significantly less than for C_3 tissue ($F_{1,39}$=90.49, p<0.001), and mass loss for *Andropogon* was lower in Turkey Meadow compared to McKay Field ($F_{1,39}$=11.76, p=0.002; Figure 9). Using an exponential model, k values were significantly higher for C_3 litter compared to *Andropogon* in Turkey Meadow but not McKay Field (p<0.001 and p=0.054, respectively). Moisture contents of litter at field collection were significantly higher in Turkey Meadow than in McKay Field ($F_{1,39}$=9.03, p=0.005) and significantly higher for litter located under C_4 species than for litter located under C_3 species ($F_{1,39}$=28.51, p<0.001). However, there was no impact of litter type on moisture content ($F_{1,39}$=2.50, p=0.122).

Comparisons of winter and summer decomposition after 142 days showed that mass loss and decay rates were significantly influenced by season, but C_3 litter still decomposed faster than *Andropogon* litter (p<0.001 for all; Figure 10). In McKay Field, litter placement was also important; litter placed under C_3 species decomposed more

Table 12. Chemistry of the senesced shoot tissue for the species used in the Reciprocal Transplant Experiment. Data shown are means±SE, based on fraction of oven-dried weight. Acid detergent fiber (ADF) is primarily lignin and hemicellulose). Superscript lowercase letters denote significant differences ($p<0.001$) between species.

Site	Species	C:N	ADF:N
Turkey Meadow	*Andropogon*	83.17± 6.77[a]	151.57±13.56[a]
	Bromus	47.02± 4.40[b]	80.37±13.67[b]
McKay Field	*Andropogon*	101.14±11.64[a]	178.50±19.74[a]
	Elymus	52.65± 2.86[b]	84.89± 4.87[b]

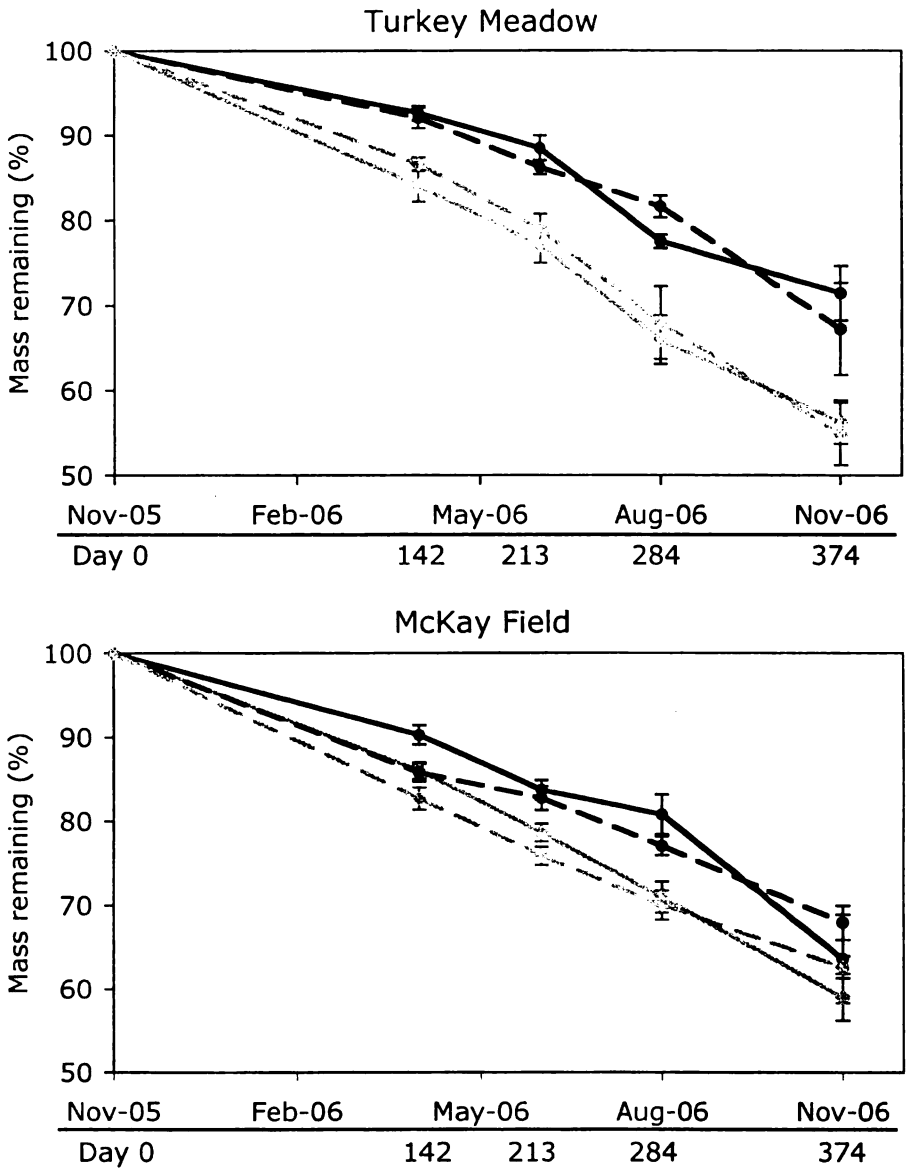


Figure 9. Litter mass remaining (% of initial) across time for the Reciprocal Transplant Experiment. Black lines (both solid and dotted) represent *Andropogon* litter, while Gray lines represent the C_3 species litter (*Bromus* in Turkey Meadow, *Elymus* in McKay Field). Solid lines show decomposition when the litter was placed under *Andropogon* and dotted lines show decomposition when the litter was placed under C_3 species.

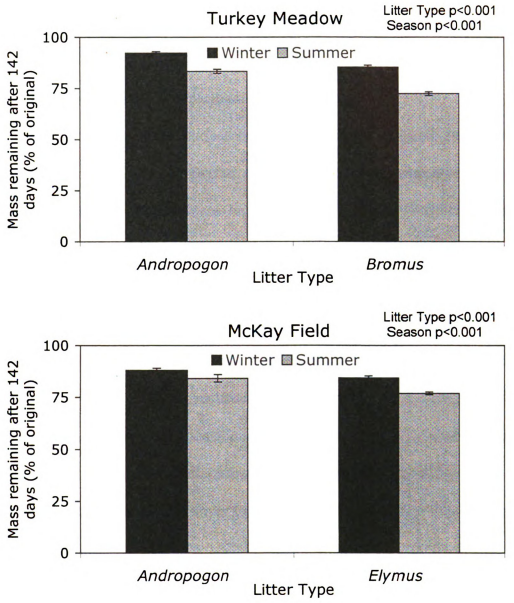


Figure 10. Comparison of the mass remaining (% of initial) after the first 142-days for litter placed in the field from November 2005-April 2006 (black bars) compared to litter placed in the field from April-September 2006 (gray bars). Data are the means (±SE) for each litter type, averaged across placement location (i.e., under C_3 and under *Andropogon*).

quickly than litter placed under C_4 species, regardless of litter type or measure of decomposition ($p=0.004$ for mass loss and $p=0.002$ for k).

Decomposition and Soil Microclimate Experiment

Soil temperatures at both the surface and 10cm depth differed significantly between both sites and species (Figure 11). Surface and 10cm temperatures were typically higher under C_3 species than C_4, and were higher in Turkey Meadow compared to McKay Field (Figure 11). However, there was no significant effect of whether litter was intact or removed on either temperature measure. Soil moisture was generally higher in Turkey Meadow than McKay Field (Figure 12). In contrast, there was no evidence of Placement effects on soil moisture except for one sampling point; after day 213 (June 19, 2006), soil moisture was significantly higher under C_3 species than C_4 species ($F_{1,40}=15.60$, $p<0.001$). In contrast to the microclimate variables, the presence of surface litter (+/-litter treatment) had a significant influence on mass loss of the cellulose ($F_{1,39}=4.74$, $p=0.036$), while mass loss did not differ under C_3 and C_4 species (Placement effect) or between sites (Figure 13).

Discussion

As expected, the C_3 species had significantly lower C:N and ADF:N than *Andropogon*, and these two ratios are often correlated with slower decomposition rates. In a study of 125 fresh leaf litter types, including woody species, forbs and grasses, Cornelissen (1996) found that decomposition was strongly negatively correlated with lignin:N. Indeed, I found that decomposition rates were faster for the C_3 litter than for the *Andropogon* litter.

a) Surface Soil Temperatures

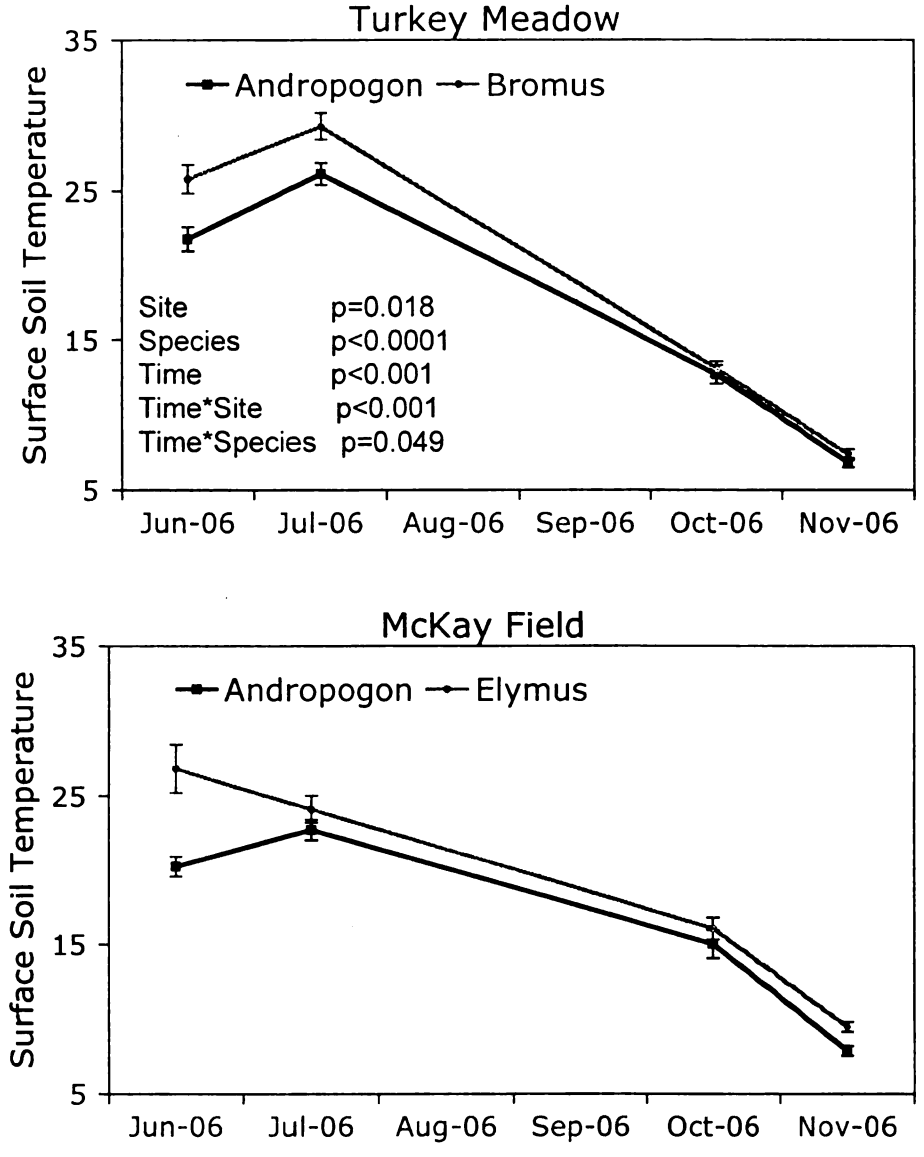
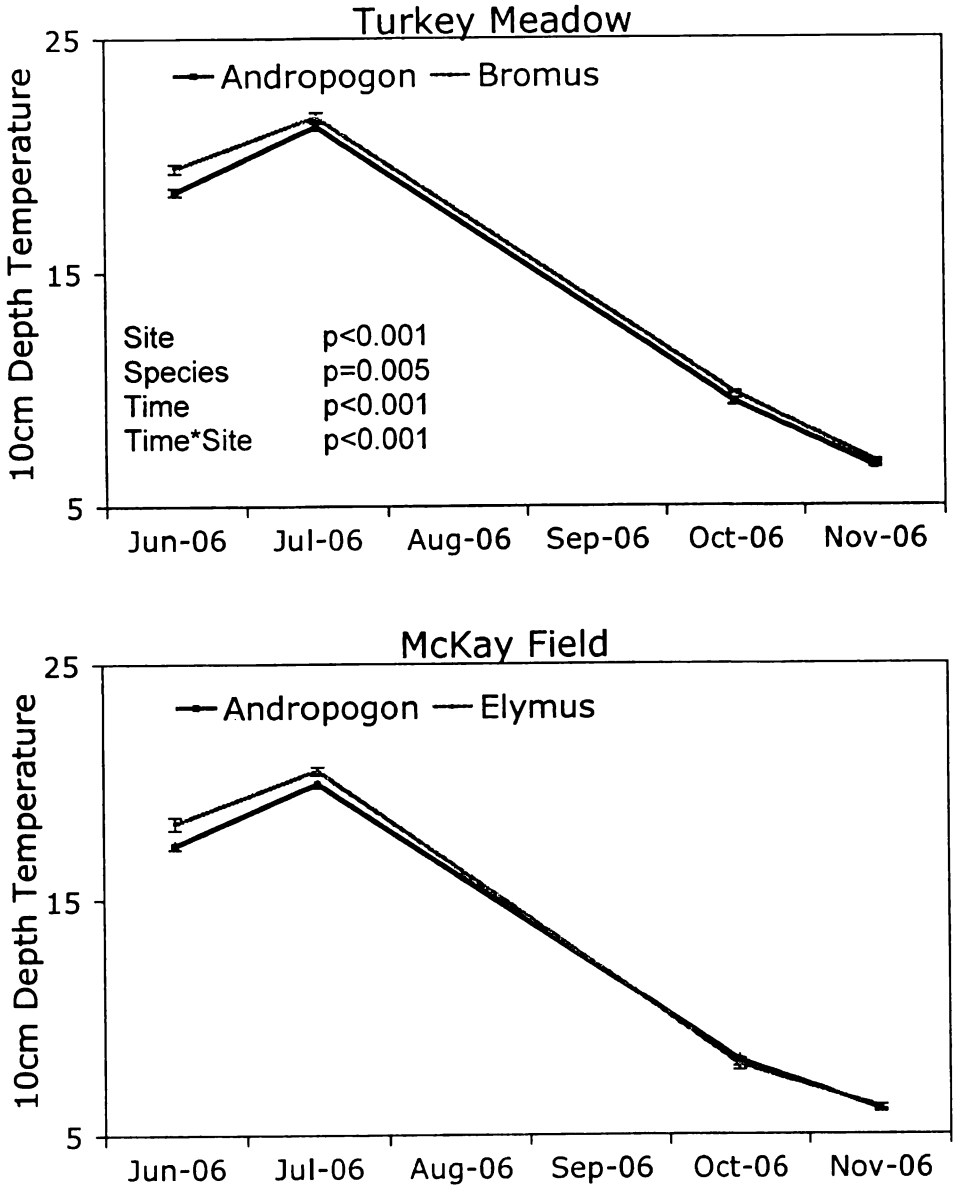


Figure 11. Soil temperature (degrees C) on the soil surface (a) and at 10cm depth (b) for Sites in 2006, averaged across the +/-Litter treatment. Black lines represent *Andropogon* plots, while Gray lines represent C_3 plots. Greenhouse-Geisser adjusted p-values for log-transformed data are reported for time variables, and untransformed data are shown.

Figure 11 (cont'd)

b) Soil Temperatures at 10cm Depth



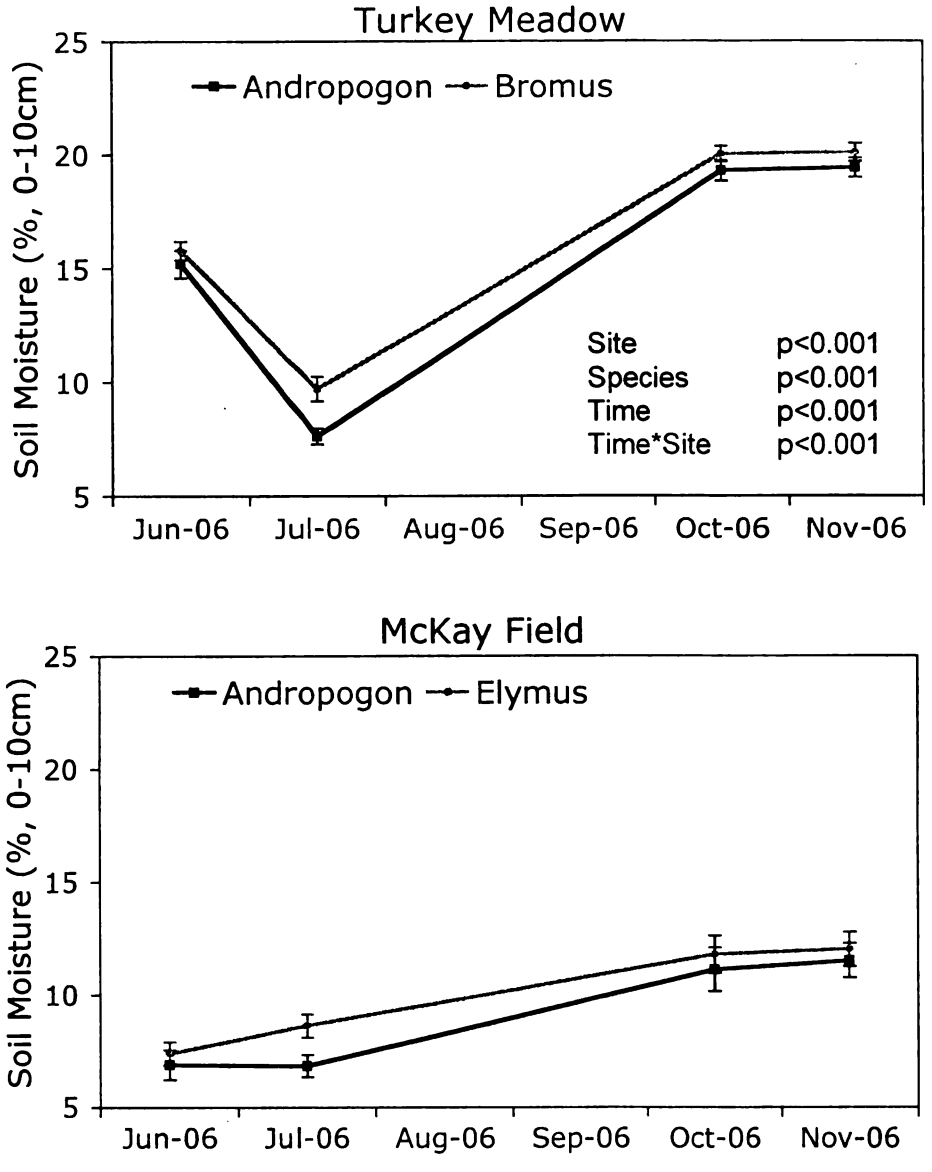


Figure 12. Soil moisture (0-10cm depth) for the two sites in 2006, averaged across the +/-Litter treatment. Black lines represent Andropogon plots, while Gray lines represent the C_3 plots. Greenhouse-Geisser adjusted p-values for ln-transformed data are reported below, and untransformed data are shown.

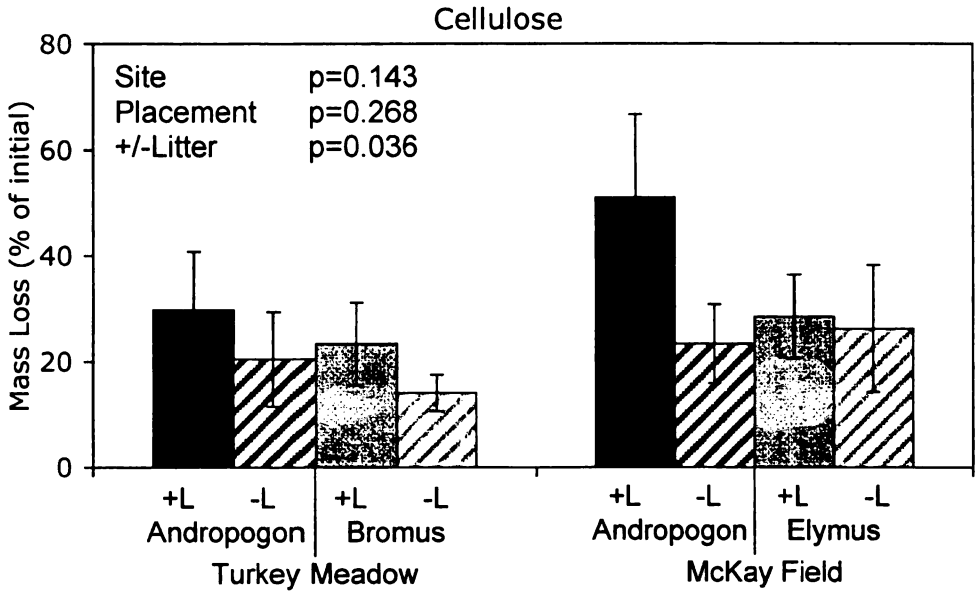


Figure 13. Mass loss (% of initial) for cellulose in both sites (McKay Field and Turkey Meadow), placed under either *Andropogon* or the C_3 species (Placement). Black solid bars represent *Andropogon* plots with litter left intact (+litter), black striped bars represent *Andropogon* plots where the litter was removed (-litter), gray solid bars represent C_3 plots with litter left intact (+litter), and gray striped bars represent C_3 plots where the litter was removed (-litter).

In a study comparing decomposition of *Bromus inermis* and *Panicum virgatum* (a C_4 species similar to *Andropogon*), Vinton and Goergen (2006) related its faster decomposition rates for *Bromus* to its lower tissue C:N. The invasion of *Aegilops triuncialis* into serpentene grasslands slowed C and N cycling rates via slower decomposition rates of its recalcitrant shoot tissue (Drenovsky and Batten 2007). My results follow the patterns seen in these studies; faster decomposition occurs for the species with the lower tissue C:N and ADF:N.

The significantly faster summer decomposition rates (April-Sept 2006) compared to the winter rates (Nov 2005-April 2006) provided evidence of strong seasonal effects on decomposition, regardless of tissue quality. In fact, initial decomposition rates were often twice as fast in summer compared to winter (Figure 10). Decomposition proceeded slowly through the winter months, resulting in low initial mass loss and a more linear trend for mass remaining after one year. This slow initial decomposition in the winter months was likely a result of cold temperatures limiting microbial activity. My findings suggest that seasonal controls (i.e., temperature and moisture) on decomposition are stronger than the effects of litter quality, and litter quality differences become important when examining decomposition within a common environment (i.e., within a site).

The moisture levels of the litter at the time of collection were significantly higher under *Andropogon* plants (i.e., a Placement effect) compared to the C_3 species, suggesting that the microclimate under the species may have differed. However, there were no differences in decomposition rates when the litter was placed in locations with different microenvironments (i.e., Placement under C_3 and under C_4 species), suggesting that litter quality, and not microclimate, was the primary factor controlling the differences

in decomposition between species in this study. However, based on that experiment alone, I could not determine whether there were no microclimate differences between C_3 and C_4 species or whether microclimate differences between C_3 and C_4 species had no impact on decomposition.

The second experiment allowed me to determine whether microclimate differences existed and if so, whether those differences affected decomposition rates. I focused on soil moisture and temperature, as they are two important variables controlling microbial activity. While there were few differences in soil moisture between C_3 and C_4 species, temperatures at the soil surface and at 10cm were significantly higher under C_3 species. These temperature results were expected based on the small amount of surface litter and aboveground biomass associated with the C_3 species (Chapter Two), which would provide less shade to moderate midday temperature increases.

The soil moisture results were surprising; based on the finding of greater moisture content of litter placed under *Andropogon*, I expected to see soil moisture differences between soils under *Andropogon* and soils under the C_3 species. Another unexpected result was that the presence or absence of litter (+/-Litter treatment) had no impact on either soil moisture or temperature. Eviner (2004) found that species had a large influence on soil temperature fluctuations, with lower daily fluctuations in plots with higher graminoid shoot biomass and litter. Summer afternoon temperatures were negatively correlated with litter, and the relationship was reversed in winter (Eviner 2004). It is possible that the high *Andropogon* biomass (Chapter Two) provided a maximum "shading effect" on soil temperatures, and thus surface litter did not provide any additional shading benefit. In addition, the relatively sparse surface litter in the C_3

plots (Chapter Two) may not have shaded the plots enough to alter temperature or moisture. Eviner (2004) found differences in species effects on soil moisture only during the driest months and related those differences to aboveground biomass. Soil moisture was only weakly related to surface litter (Eviner 2004). Any decrease in evaporation due to shading by *Andropogon* litter and biomass could have been offset by higher transpiration losses compared to the C_3 species, and therefore no differences in soil moisture were found between treatments.

Although I found differences in soil temperatures between species and treatments, I found no evidence to suggest that microclimate influenced decomposition rates. There were no significant differences in the decomposition rate of either C_3 or C_4 species between the two microenvironments (placement under their own species versus placed under the opposite species), nor was there a difference in cellulose decomposition between the two environments (under C_3 and C_4). This evidence suggests that while the microbial communities may differ in many aspects, the microbial decomposers were functionally similar under *Andropogon* and the C_3 species. The only factor that affected cellulose decomposition was the presence or absence of surface litter (+/- Litter treatment), and soil moisture and temperature did not differ significantly between these two treatments. Thus, it is unclear whether some aspect of microclimate other than soil moisture and temperature influenced cellulose decomposition.

Results presented here demonstrate that both litter quality and environmental conditions affect decomposition rates. Litter quality controls relative decomposition rates of species in a site, but seasonal differences in environmental conditions also impact decomposition rates. While this study focused on only a few species, these results should

hold for a variety of C_3 species common to old-fields and C_4 species common in prairies. The two C_3 species examined here did not differ in their tissue quality, nor did they differ in their decomposition rate. In Chapter Two, I found no significant differences between *Bromus* and *Elymus* in surface litter, shoot biomass, and tissue C:N or ADF:N between the two sites, suggesting that these two species are similar in their effects on decomposition. In addition, I also found no significant differences in tissue C:N and ADF:N between *Andropogon* and two other C_4 grasses (*Schizachyrium scoparium* and *Sorghastrum nutans*) in Chapter Two, suggesting that those two C_4 grasses would have similar decomposition rates. This research suggests that communities dominated by C_4 grasses will impact C and N cycling via these slower decomposition rates compared to C_3 grass dominated communities. This has particular implications for predicting how prairie restorations will impacts soil C and N recovery processes over time. These results have an application for the prediction of how invasive species may alter ecosystem processes, and therefore which species we should be most concerned about from a belowground ecosystem process perspective.

CHAPTER FIVE

CONCLUSIONS

While much recent research has focused on the effects of exotic plant species on ecosystem properties, little is known about how reintroductions of extirpated species may impact these processes in restored systems. I examined how the reintroduction of C_4 grasses into old-fields influenced soil C and N cycling, and the timeframe over which these changes become apparent. The previous three chapters demonstrated that C_3 and C_4 species differ in litter quality, quantity and their influences on soil microclimate. Biomass production and tissue chemistry traits were related to differences in soil properties and processes, and the magnitude of species differences, as well as the time since the species was established, were important factors influencing the relative changes in soil properties. However, because species differed in several traits, it was difficult to isolate which plant traits are responsible for observed influences on soil processes. In Chapter Two, I examined the decadal scale impacts of a shift from a C_3-dominated system to a C_4-dominated system. In Chapter Three, I explored how quickly differences in species traits, and the soil process changes observed after 11 years, arise. Finally, in Chapter Four, I evaluated which plant traits were responsible for differences in decomposition rates.

In Chapters Two and Three, I showed that C_4 species had significantly greater shoot biomass and more recalcitrant tissue compared to the dominant C_3 species, and these differences became apparent within two years of their establishment. However, differences between the two groups of species in surface litter and root biomass tended to

take longer to develop, but were apparent after 11 years. While there was some evidence to suggest that the C_4 species had reduced soil inorganic N levels relative to the C_3 species after just two years, many of the changes in soil properties took longer than two growing seasons to develop. After 11 years, soils under C_4 species had significantly lower inorganic N levels, and slightly lower *in situ* net N mineralization and nitrification rates when compared to soils under C_3 species. I also found limited evidence for increasing soil C pools under C_4 species 11 years after reintroduction. Nevertheless, the $\delta^{13}C$ signal of the C_4 species became measurable in the soil very quickly. Overall, my results demonstrated that reintroduction of C_4 species into grasslands can result in alterations of soil processes related to C and N cycling on relatively short timescales. These results also indicated that process rates tended to change first, with changes in pool sizes of C and N taking longer to become measurable.

However, because the C_3 and C_4 grasses examined in this study differ in three main ways—litter quality, litter quantity, and timing of resource use/release—it is difficult to determine which traits, or combination of traits, influence particular processes in the field. The decomposition experiments (Chapter Four) took place in a more controlled setting to separate the direct and indirect effects of these trait differences on decomposition rates. I found that while climate controls decomposition on a regional scale, litter quality was the most important factor determining decomposition rates within a site. I also found evidence for differences in soil microclimate under *Andropogon* compared to C_3 species, but these differences did not appear to have a strong influence on decomposition rates of either tissue type or of cellulose. Although the greater shoot biomass of C_4 species is contributing to the larger surface litter layers compared to C_3

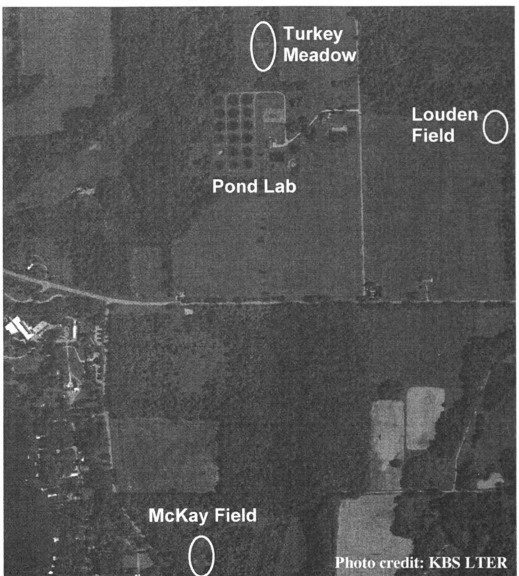
species, slowed decomposition rates of this more recalcitrant C_4-derived litter is also increasing surface litter mass. It is likely that both litter quality and quantity are important factors contributing to increased soil C and reduced inorganic N levels under C_4 species. Soil microclimate differences may have some effect on soil processes, such as extending microbial N mineralization longer into the autumn, but not on surface litter decomposition rates.

In the future, I plan to follow up on these experiments. I hope to sample the 11- and 2-year-old plots in the coming years to follow the changes in soil properties and processes as the plots age. This fall, I will begin a laboratory experiment to examine the soil microbial communities under *Andropogon* and the dominant C_3 species at Turkey Meadow and McKay Field, to see whether the microbial communities under these species have diverged over the last decade. I also will investigate whether the microbial community under *Andropogon* is better adapted to decomposing recalcitrant litter compared to the C_3 species. This will provide an additional component to my research; I did not examine whether there were changes in the microbial community composition, abundance and activity under the different species. I will begin exploring this question as part of my research at the Holden Arboretum in Ohio.

My dissertation research, as well as the research that I plan to continue in these old-field sites, is centered on how plant species can influence soil processes. Improving our understanding of how plant species impact ecosystem properties and what species traits are driving these changes is imperative if we hope to predict the ecosystem-level consequences of changes in species distribution or composition that could occur, and are

occurring, as a consequence of changes in agricultural and land use practices, global change, and species introductions.

APPENDIX



Appendix 1. Location of the three study sites at the W. K. Kellogg Biological Station.

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