JASON BROSSARD WEST

The effects of dominant bunchgrass species on sandhill longleaf pine savanna ecosystem function: a comparison of wiregrass to the bluestems.

(Under the direction of Dr. Lisa Alayne Donovan)

A key question in understanding plant-soil relationships is to what degree are these relationships species-specific? The research presented in this dissertation is a comparison of several bunchgrass species native to, and dominant in, the fall-line sandhill *Pinus palustris* savannas. I compared relevant plant traits, as well as components of C and N cycling to determine whether these species differ in their effects on those processes and what traits may account for those differences. In a greenhouse experiment I demonstrated performance differences between *Aristida stricta* (wiregrass) and *Schizachyrium scopariu*m (little bluestem), as well as differences in their responses to added N. *Aristida stricta* grew more slowly and responded less to N addition than did *S. scoparium,* suggesting differences in nutrient use strategies. In a field study differences in plant tissue quality as well as species-specific influences on C and N cycling were found at the scale of an individual plant, especially between *A. stricta* and the “bluestems” (*S. scoparium* and *Andropogon* spp.). In a study of planted monocultures of these species, I showed species-specific root proliferation responses, as well as species-specific depletion of inorganic N pools following N addition. *Aristida stricta* surprisingly showed the greatest proliferation response to N addition. The ability of the bluestems to deplete the available N pool depended on its size and to a certain extent its form (ammonium versus nitrate). In an observational study of fine root dynamics and soil resources I showed a clear gradient in resource availability that corresponded with a gradient in soil type, as well as vegetation dominance. Finally, a comparison of patch types within the middle of the gradient dominated by either wiregrass or a mix of bluestem species showed little difference in fine root dynamics or soil resource availability between these patches. An interesting pattern in root production did emerge, however, that is consistent with the root production phenology of *S. scoparium* versus *A. stricta*. Although these relationships can be complex, I conclude generally that species-specific effects on belowground ecosystem function are common and may be related to differences among species in adaptation to soil conditions.

Index words: *Aristida stricta*, savanna, roots, nitrogen, carbon, ecosystem function, bluestems, *Pinus palustris*, functional group

THE EFFECTS OF DOMINANT SANDHILL BUNCHGRASS SPECIES ON SANDHILL LONGLEAF PINE SAVANNA ECOSYSTEM FUNCTION: A COMPARISON OF WIREGRASS TO THE BLUESTEMS

by

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DEDICATION

I humbly dedicate this dissertation to my family. In particular, I want to thank my mother Rosemary West for her very early and continued support of my academic achievement, my father Thomas West for showing me the unending beauty that is found in nature, my grandfather Stratford Brossard for a handful of morning walks through dew-covered grass and for his long letters - too intellectual for me at the time, and all of the rest of my family who, whether they knew it or not, were fostering a keen fascination and a desire to understand.

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INTRODUCTION AND LITERATURE REVIEW

Plants are intimately linked to belowground ecosystem functions (e.g., C fixation, respiration, productivity, nutrient cycling, water cycling). The relationship is reciprocal since plants not only respond to soil resource availability but also cause changes in it (Wedin and Tilman 1990, Hobbie 1992, Chapin et al. 1998, Chapin et al. 2000). Plant effects on belowground ecosystem function may occur directly through carbon allocation, transpiration, nutrient uptake, and tissue senescence or indirectly through effects on soil fauna and aboveground herbivores (Johnson et al. 1992, Steltzer and Bowman 1998). Although plants can affect ecosystem function, there is little empirical understanding of how plant adaptive strategies interact with the environment to affect ecosystem level processes (Solbrig 1994).

Ecosystem functions are large-scale processes that integrate all of the biotic and abiotic components of, for example, the movement of nitrogen through a savanna. An important question, then, is do ecologists need to understand how every individual species in the savanna affect a given process in order to understand that process? Or, can we understand it by knowing what kinds of species (=functional groups: trees, shrubs, grasses, N-fixers, etc.) occupy that savanna? An important, associated question is does the loss of species from a forest result in changes in ecosystem function? These questions, and those related to them, have become increasingly important to both ecologists and conservationists because of the human-caused reductions in earth’s biological diversity and attempts to predict the effects of those losses on ecosystem function (Chapin et al. 1998). The focus of my research was generally on the interactions between fine roots and soil nitrogen cycling. Although the many linkages between roots and soil nitrogen are obvious, due primarily to the technical difficulties associated with studying roots, these have not generally been well studied in natural systems (Nadelhoffer 2000). Root distributions, growth, death, turnover and nutrient uptake rates are all important to both plant success and plant effects on ecosystem function (Nadelhoffer et al. 1985, Vogt et al. 1996, Pregitzer et al. 1995, Hendrick and Pregitzer 1997, Bassirirad 2000, Gill and Jackson 2000). Although there are obvious differences between roots and leaves, because of the large dataset available for leaves and the much smaller one for roots, it has been suggested that fine root dynamics may be predicted from analogies to leaves (Eissenstat et al. 2000). However, a recent review showed that root decomposition may be most strongly controlled by root chemistry rather than, as is found in leaves, chemistry having a secondary role to climatic and other abiotic factors (Silver and Miya 2001). Other characteristics may reveal fundamental differences between leaves and roots.

It is clear that individual species can differentially affect ecosystem function (Wedin and Tilman 1990, Hobbie 1992, Hooper and Vitousek 1997, Van der Krift and Berendse 2001). However, in some cases functional groups appear to be adequate descriptors of ecosystem function (Chapin et al. 1996) and in others, the effects of plant species appear to be considerably less important than an abiotic controller (Craine et al. 2001). A clarification of these issues may come from comparisons of species *within* apparent functional groups. If naturally occurring, morphologically and physiologically similar species differ substantially from each other then those differences are likely to be larger for more dissimilar species. On the other hand, if these species are largely similar it suggests that placing species into functional groups is an appropriate way of reducing ecosystem complexity. As a step in this direction, the broad objective of my dissertation was to quantify the effect of co-occurring, C4 bunchgrass species on belowground ecosystem functions in *Pinus palustris* (longleaf pine) savannas. The species we studied are dominant members of what may be considered the "C4 perennial bunchgrass" functional group.

Pinus palustris savanna ecosystems often exhibit strong soil resource limitations and are dependent on frequent (<10 year return interval) fires to maintain a savanna-like vegetative structure (Christensen 1988). These pine savannas once dominated the southeastern coastal plain of the U.S., but due to land use changes and fire suppression currently cover less than two percent of their former range (Noss 1989). *Aristida stricta* (wiregrass) is a C4 bunchgrass that dominates the understory of fire-maintained savannas. Because of its historical dominance and ability to carry the requisite ground fires, a significant effort is currently underway to restore *A. stricta* (Walters et al. 1994, Glitzenstein et al. 1995, Gordon and Rice 1998). There are indications that *A. stricta* may play an important role in the water and nutrient dynamics across edaphic gradients. Similar to grasses in other savannas, *A. stricta* constitutes a significant portion of the net annual primary productivity (Mitchell et al. 1999). *Aristida stricta* roots are the primary source of the shallow humus layer in xeric sandhills (Wells and Shunk 1931), which increases the nutrient and water retention of these sandy soils (Woods 1957, Outcalt and Lewis 1988). Alternatively, *A. stricta* may prevent other species from taking up that moisture through its uptake (Woods 1958). The root biomass of these savannas is comparable to that of grasslands, but the annual fine root turnover (as estimated from sequential coring) is low compared to other ecosystems (Saterson and Vitousek 1984). Saterson and Vitousek (1984) also noted a high capacity of *A. stricta* to retranslocate nitrogen and phosphorous out of senescing leaves. This capacity to retranslocate would lead to very low tissue quality (high carbon:nitrogen ratio). The low fine root turnover combined with low tissue quality suggests that *A. stricta* has a conservative nutrient use strategy, which would drive a slow nutrient cycle with low nutrient availability.

Several co-occurring C4 bunchgrass species have been discussed as potential functional analogs (i.e., have the same effects on ecosystem function) to *A. stricta* (Duever 1989). They include *Schizachyrium scoparium* and *Andropogon* spp. (collectively the bluestems). It is thought that the bluestems have not historically dominated pine savannas on the eastern coastal plain. However there are pine savannas dominated by *S. scoparium* on the western gulf coastal plain (Bridges and Orzell 1989). Following a soil disturbance that removes the herbaceous layer (e.g., farming or logging practices) the bluestems will often be found colonizing, but *A. stricta* will not (Grelen 1962). The reason for this difference in colonizing ability is not known. The potential for these species to differ from *A. stricta* in terms of ecosystem function has not been explored. Since they are native species with similar morphologies, photosynthetic pathways, and phenologies they provide an ideal comparison for tests of functional redundancy in ecosystems.

My approach to the study of species differences within the C4 bunchgrass group was to quantify trait differences among them that are relevant to nitrogen cycling and to determine whether these species have differential effects on ecosystem function. Both manipulative and observational studies were employed at various scales.

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CHAPTER 1

SEEDLING GROWTH DIFFERS WITHIN AND AMONG C4 GRASS SPECIES NATIVE TO *PINUS PALUSTRIS* SAVANNAS. [1]

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[1] West, J. B. and L. A. To be submitted to the *Journal of the Torrey Botanical Society*

ABSTRACT

Longleaf pine (*Pinus palustris*) savannas are endangered ecosystems of the eastern U.S. coastal plain that are characterized by low soil resource availability and frequent ground fires. The native herbaceous layer of these savannas is dominated by C4 bunchgrasses, most commonly wiregrass (*Aristida stricta* and *A. beyrichiana*). We compared variation in growth among populations of both species of wiregrass, and compared the responses to variation in soil nutrient availability of the northern species (*A. stricta*) to two other native C4 grasses (*Schizachyrium scoparium*, and *A. tuberculosa*). When grown in a common environment, *A. stricta* seedlings grew more slowly than those of *A. beyrichiana*, suggesting divergence of the two species probably in response to latitudinal gradients. In addition, wiregrass individuals from xeric habitats grew more slowly than did individuals from mesic habitats, suggesting ecotypic adaptation in both species of wiregrass. *Aristida stricta* also differed dramatically from two early successional species, *S. scoparium* and *A. tuberculosa,* in growth response to high and low nutrient availability. *Schizachyrium scoparium,* and *A. tuberculosa* responded to increased nutrient availability by increasing total biomass and allocating less to roots, whereas *A. stricta* was not affected by changing nutrient availability. This difference may explain the inability of wiregrass to reinvade disturbed sites that are likely to have relatively high nutrient availabilities. Growth differences among wiregrass populations, and between wiregrass and earlier successional grasses, have important consequences for conservation and restoration of longleaf pine savannas.

INTRODUCTION

Longleaf pine (*Pinus palustris* Miller) savanna ecosystems exhibit strong soil resource limitations and are dependent on frequent (<10 year return interval) fires to maintain their savanna-like vegetative structure (Christensen 1988). They are characterized by an open overstory of longleaf pine, a sparse midstory of oaks (*Quercus* spp.), and a dense herbaceous layer dominated by C4 bunchgrasses (Glitzenstein et al. 1995). These endangered ecosystems once dominated the southeastern coastal plain of the U.S., but due to land use changes and fire suppression currently cover less than two percent of their former range (Noss 1989). Conservation of what remains of this endangered ecosystem, as well as its restoration, are important components of the agenda of many southeastern land managers.

Two grasses in the genus *Aristida*, both commonly referred to as wiregrass, dominate the herbaceous layer of fire-maintained longleaf pine savannas. *Aristida stricta* Michaux is restricted to the northern coastal plain and *A. beyrichiana* Trinius & Ruprecht is found in the south (Figure 1.1). The description of *A. beyrichiana* as a distinct species from *A. stricta* follows Peet (1993). Both *Aristida* species provide fuel for the frequent ground fires common to this system (Christensen 1977, Clewell 1989) and have important influences on soil resource availability (Wells and Shunk 1931, Woods 1957, Outcalt and Lewis 1988). Restoration of longleaf pine savannas often focuses on the reintroduction of wiregrass (Noss 1989, Walters et al. 1994, Glitzenstein 1995, Gordon and Rice 1998). Understanding variation within and among *Aristida* species may be critical to restoration efforts. If species and populations are locally adapted, then reintroduction of the appropriate plant material is likely to yield the highest degree of success (Fahselt 1988). Large scale latitudinal gradients may drive differences between the two *Aristida* species and smaller scale variation in edaphic factors are likely to cause more local adaptation of populations. Although evidence for genetically-based differentiation among populations of *A. beyrichiana* exists (Kindell et al. 1996, Gordon and Rice 1998), support for ecotypic differentiation has been inconsistent (Gordon and Rice 1998), and to our knowledge no study has compared populations from both *A. stricta* and *A. beyrichiana*.

The objectives of the wiregrass population experiment were to determine whether there are genetically based differences in seedling growth 1) between *A. stricta* and *A. beyrichiana*, and 2) between populations occupying mesic and xeric habitats. We expected differences in growth to be consistent with adaptation to broad geographic range and local soil resource environments. This experiment compared seedlings from mesic and xeric habitats within each of two *A. stricta* and two *A. beyrichiana* collection sites (i.e. eight total populations).

In addition to wiregrass, the understory of these savannas can be dominated by other C4 grasses such as *Schizachyrium scoparium* (Michaux) Nash (perennial bunchgrass) and *Aristida tuberculosa* Nuttall (annual). These early successional grasses often colonize following a soil disturbance that removes a large portion of the herbaceous layer, such as with site preparation before pine planting (Hebb 1971, West personal observation). In contrast, wiregrass does not readily colonize these disturbed sites (Noss

1989, Grelen 1962), which is surprising given its large range and common association with *P. palustris* (Myers 1990). Although some research suggests that the low colonization ability of wiregrass may be due to consistently low seed output (Myers 1990), this seems unlikely given that wiregrass produces large quantities of viable seed following growing season fires (van Eerden 1997, Outcalt 1994). Alternatively, increases in soil nutrient availability following a disturbance may favor colonization of the early successional species that can exploit the high resources for greater biomass accumulation and competitive ability (Gleeson and Tilman 1994, Chapin et al. 1993).

The objective of the nutrient response experiment was to determine if *A. stricta, S. scoparium* and *A. tuberculosa* seedlings differ in their response to nutrient availability. We expected the early successional species, *S. scoparium* and *A. tuberculosa*, to have a greater capacity to exploit increased soil nutrient availabilities. This experiment compared the species for seedling allocational shifts and biomass accumulation in response to two levels of soil resources.

METHODS

Wiregrass population experiment

Seeds of *A. stricta* and *A. beyrichiana* were collected in December 1997 from two sites each (Figure 1.1). Seeds of *A. stricta* were collected from two northern sites: Ft. Bragg Army base (Ft. Bragg, NC) and Carolina Sandhills National Wildlife Refuge (McBee, SC). Seeds of *A. beyrichiana* were collected from two southern sites: Jones Ecological Research Center (Newton, GA) and Apalachicola National Forest (Liberty, FL). All of the collection sites are were sandhills with very open canopies dominated by *P. palustris* and *Quercus* spp., with the exception of the Apalachicola National Forest site which was a flatwood with open *P. palustris* canopy. Within each site, seeds were collected from a mesic (relatively wet) and xeric (relatively dry) habitat giving a total of eight sampled populations. The mesic and xeric habitats were selected on the basis of associated vegetation and soil information. The proportion of more mesic oak species (e.g., *Q. marilandica*) relative to more xeric species (e.g., *Q. laevis*) was generally used to indicate soil moisture availability (Christensen 1988). Seeds were collected from greater than 50 individuals per population. All populations were burned in the spring or early summer prior to seed collection and have a history of regular prescribed burns. After collection, 20 lots of 20 seeds each were weighed for each population to obtain mean seed biomass.

The glasshouse experiment was conducted at the University of Georgia, Athens, GA. The seeds were thoroughly mixed within each population and then placed in separate trays of wet, sterilized sand in January 1998. Two weeks after germination, seedlings were transplanted into 625 cm^3 D40 Deepots (Steuwe and Sons, Inc., Corvallis, OR, USA) filled with sand. Approximately 30 seedlings died within 5-10 days of transplant and were replaced. All seedlings were watered and received one-quarter strength modified Hoagland’s solution (Epstein 1972) as needed until treatment initiation. The D-pots were assigned to positions in a factorial, randomized block design with two blocks, two watering treatments, two species, and two habitat types.

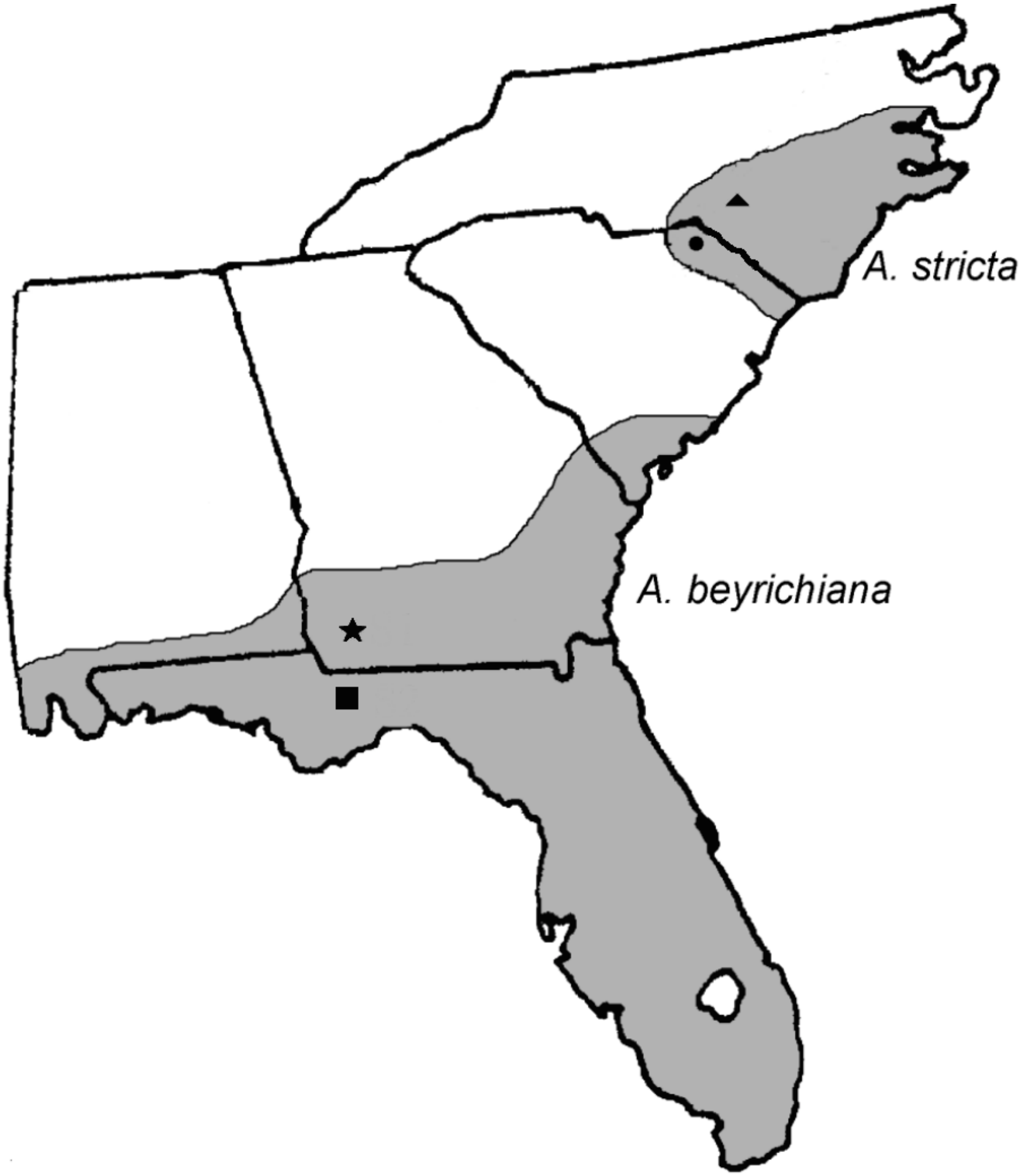

Figure 1.1 Seed collection sites. *A. stricta*: ▲ - Ft Bragg, ● - Carolina Sandhills National Wildlife Refuge; *A. beyrichiana*: ★ - Jones Ecological Research Center, and ■ - Apalachicola National Forest. Shading shows the range of *A. stricta* and *A. beyrichiana*. Within each site, seeds were collected from populations growing in mesic and xeric habitats for a total of eight populations.

The blocks were centered under two 400 watt sodium vapor lamps that were on 13 hours per day to supplement sunlight. On March 1, 1998, approximately five weeks after germination, treatments were initiated and four seedlings from each population were harvested for determination of initial biomass. Seedlings in the high water treatment received one-quarter strength Hoagland's every other day, whereas those in the low water treatment received the same solution every fourth day.

Over the course of the 8-week experiment, the glasshouse was monitored for air temperature (copper-constantan thermocouples), photosynthetically active radiation (LI-190SZ quantum sensor, LiCor Inc., Lincoln, NB, USA), and relative humidity (HMP45C temperature and relative humidity probe, Campbell Scientific Inc., Logan, UT USA). The sensor data were logged every half hour to a CR-10 datalogger (Campbell Scientific). The mean daytime minimum/maximum (and overall mean) temperature was 25/34 ^{o}C (30 ^{o}C), relative humidity was 29/59% (40%), and PAR was 681/1250 μmol m^{-2} s^{-1} (441μmol m^{-2} s^{-1}).

During the course of the experiment, seedling growth rates were estimated from non-destructive measurements. Total leaf length was measured on March 11, 13, 25 and April 1. Total plant height, number of tillers and number of leaves were measured on March 13, 25, April 1, 9, and 16. On seedlings not included in the growth rate measurements, predawn water potential (ψ_{pd}) was measured using a pressure chamber (model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) in March and April, a morning before the low water plants were to be watered (n=8 per treatment). On

May 2, seedlings measured for growth rate were harvested, separated into aboveground (leaves and tillers) and belowground (roots and rhizomes) biomass, dried at 65^{o}C and weighed.

Initial data analyses indicated that watering treatment had no effect on seedling ψ_{pd}, relative growth rates, and biomass (data not shown). It is likely that the watering regime produced no significant difference in soil moisture availability. Accordingly, the watering treatment effect was dropped from all analyses. Three-way mixed-model ANOVAs with species (*A. stricta* and *A. beyrichiana*) and habitat (mesic and xeric) as fixed effects, and block as a random effect (SAS 1989) were performed on seed biomass (20 lots of 20 seeds each) and seedling biomass at treatment initiation (n=4 replicate individuals per population). For this and all subsequent SAS analyses, data were transformed as necessary to satisfy model assumptions.

Non-destructive growth rates were calculated on a per-pot basis using linear regressions of plant height, tiller number, leaf number, and total leaf length against time (n=12 replicate individuals per population). A linear approximation of growth rate was considered adequate for the short (8 wk) duration of the experiment. The slopes from these regressions (i.e. the growth rates) were then analyzed using three-way mixed-model ANOVAs to test for species and habitat effects. Final aboveground, belowground, total biomass and belowground:total ratios at final harvest were also analyzed using mixed-model ANOVAs.

Nutrient response experiment

Seeds of *A. stricta*, *S. scoparium* and *A. tuberculosa* were collected from a xeric sandhill habitat at the Carolina Sandhills National Wildlife Refuge near McBee, South Carolina in December 1997. This site was burned in the spring of 1997 and had been regularly prescribed burned prior to that. Seed sowing and seedling transplanting occurred at the same time and manner as in the wiregrass population experiment. At treatment initiation on March 1, 1998, four seedlings of each species were harvested for determination of initial biomass. The nutrient treatments were one-quarter and one-sixteenth strength modified Hoagland's solution. On May 2, the plants were harvested, dried, and weighed as above (n=12 per species/treatment combination). Three-way mixed-model ANOVAs were performed to test for block, species and nutrient treatment effects.

RESULTS

Wiregrass population experiment

There was significant variation for seed biomass among populations, attributable to both species and habitat (Table 1.1, Figure 1.2). Seeds of *A. stricta* were heavier than those of *A. beyrichiana*. In addition, seeds from xeric populations were heavier than those from mesic populations for both species. At initial harvest, there was no significant effect of species or habitat (mesic versus xeric) on aboveground biomass, belowground biomass, total biomass or belowground:total biomass ratio (Table 1.1).

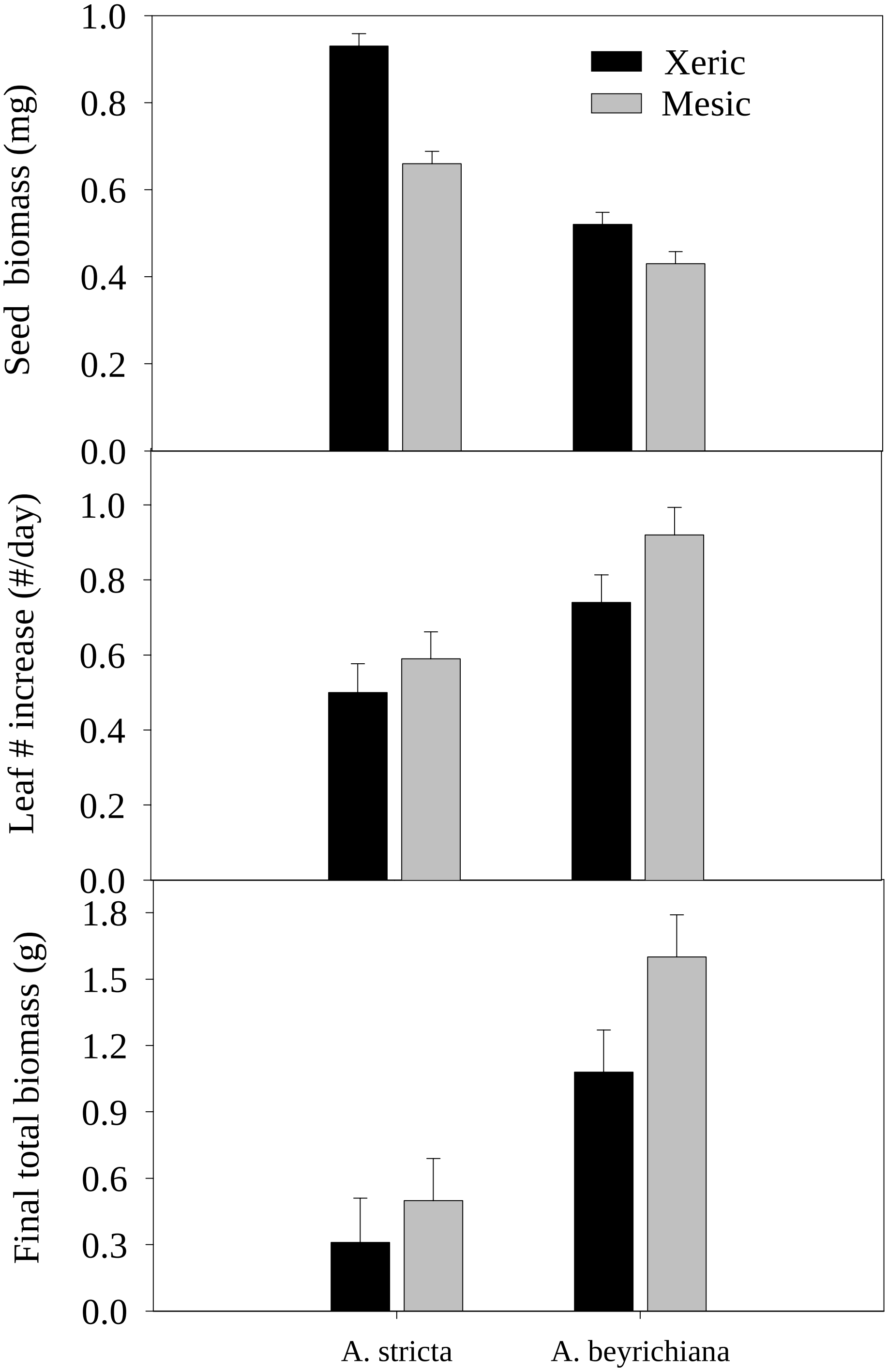

Figure 1.2. Measures of performance comparing populations from xeric to those from mesic habitats for *A. stricta* and *A. beyrichiana* (least squares means +1 S.E.). See Table 1.1 for statistical analysis.

During the experiment, seedlings of *A. stricta* grew more slowly than those of *A. beyrichiana*, based on increases in plant height, tiller number, leaf number, and total leaf length (Table 1.1, Figure 1.2). In addition, seedlings from xeric populations generally grew slower than those from mesic populations. At final harvest *A. stricta* seedlings had significantly lower aboveground, belowground, and total biomass than did those of *A. beyrichiana*. In addition, seedlings grown from xeric habitat seed acquired less biomass than those from mesic habitats for both species (Table 1.1, Figure 1.2). There was no significant interaction between species and habitat for any of the variables measured.

Nutrient response experiment

Comparing seedlings of *A. stricta*, *S. scoparium*, *A. tuberculosa* at the initial harvest, there were significant species differences (P<0.001) for all biomass measures except belowground:total ratio (P=0.065). *Aristida stricta* had less total biomass (0.01g than *S. scoparium* (0.14g and *A. tuberculosa* (0.10g). Given these pre-treatment differences, it is not surprising that at final harvest, *A. stricta* seedlings had much less biomass than did *A. tuberculosa* and *S. scoparium* (Table 1.2, Figure 1.3).

TABLE 1.1 Means and results of ANOVAs of mesic and xeric populations of *A. stricta* and *A. beyrichiana*. Seeds were collected from a total of eight populations and plants were grown in individual pots in a randomized block design. Least squares means are organized by species (*A. stricta* and *A. beyrichiana*) and habitat (X and M). *F*-statistics are from three-way (species, habitat, block) mixed model ANOVAs (*P*<0.05*, *P*<0.01**, *P*<0.001***).

	Species*habitat means				*F* (ndf ddf)		
Source of variation	*A. stricta* (X)	*A. stricta* (M)	*A. beyr*. (X)	*A. beyr*. (M)	Species	Habitat	Spp*Habitat
Seed biomass (mg)	0.926	0.658	0.522	0.433	210.33 (1,156)***	57.98 (1,156)***	0.01 (1,156)
Initial aboveground biomass (g)	0.0062	0.0078	0.0074	0.0088	1.04 (1,2)	2.85 (1,25)	0.01 (1,25)
Initial belowground biomass (g)	0.0043	0.0071	0.0049	0.0057	0.14 (1,2)	3.98 (1,25)	0.69 (1,25)
Initial total biomass (g)	0.011	0.015	0.012	0.015	0.14 (1,2)	4.01(1,25)	0.41 (1,25)
Initial belowground:total biomass ratio	0.39	0.47	0.37	0.39	1.47 (1,2)	1.65 (1,25)	0.70 (1,25)
Plant height increase (mm/day)	1.78	2.49	4.60	5.38	107.18 (1,83)***	6.88 (1,83)**	0.23 (1,83)
Tiller number increase (#/day)	0.14	0.17	0.23	0.31	26.93 (1,85)***	4.68 (1,85)*	0.13 (1,85)
Total leaf number increase (#/day)	0.50	0.59	0.74	0.92	22.29 (1,85) ***	4.81 (1,85)*	0.67 (1,85)
Total leaf length increase (mm/day)	19.76	29.87	48.10	65.40	45.53 (1,85)***	9.46 (1,85)**	0.62 (1,85)
Aboveground biomass at final harvest (g)	0.25	0.39	0.84	1.23	16.18 (1,83) ***	6.94 (1,83) **	0.22 (1,83)
Belowground biomass at final harvest (g)	0.06	0.10	0.24	0.37	58.88 (1,83)***	7.72 (1,83)**	0.59 (1,83)
Total biomass at final harvest (g)	0.31	0.50	1.08	1.60	30.16 (1,83)***	8.35 (1,83)**	0.53 (1,83)
Root:total biomass ratio at final harvest	0.18	0.19	0.22	0.22	9.25 (1,83)**	0.31 (1,83)	0.13 (1,83)

TABLE 1.2 Analysis of variance of final harvest biomass of *A. stricta*, *S. scoparium*, and *A. tuberculosa* in response to high ($^1/_4$) and low ($^1/_{16}$ Hoagland's) nutrient availabilities ($P<0.05$*, $P<0.01$**, $P<0.001$***).

	F (ndf, ddf)		
Source of Variation	Species	Nutrient level	Species*Nutrient
Aboveground biomass	178.99 (2,31)***	79.67 (1,31)***	23.87 (2,31)***
Belowground biomass	20.78 (2,30)***	2.99 (1,30)	1.14 (2,30)
Total biomass	26.14 (2,31)***	14.56 (1,31)***	7.70 (2,31)***
Belowground:total ratio	0.17 (2,30)	4.88 (1.30)*	21.24 (2,30)***

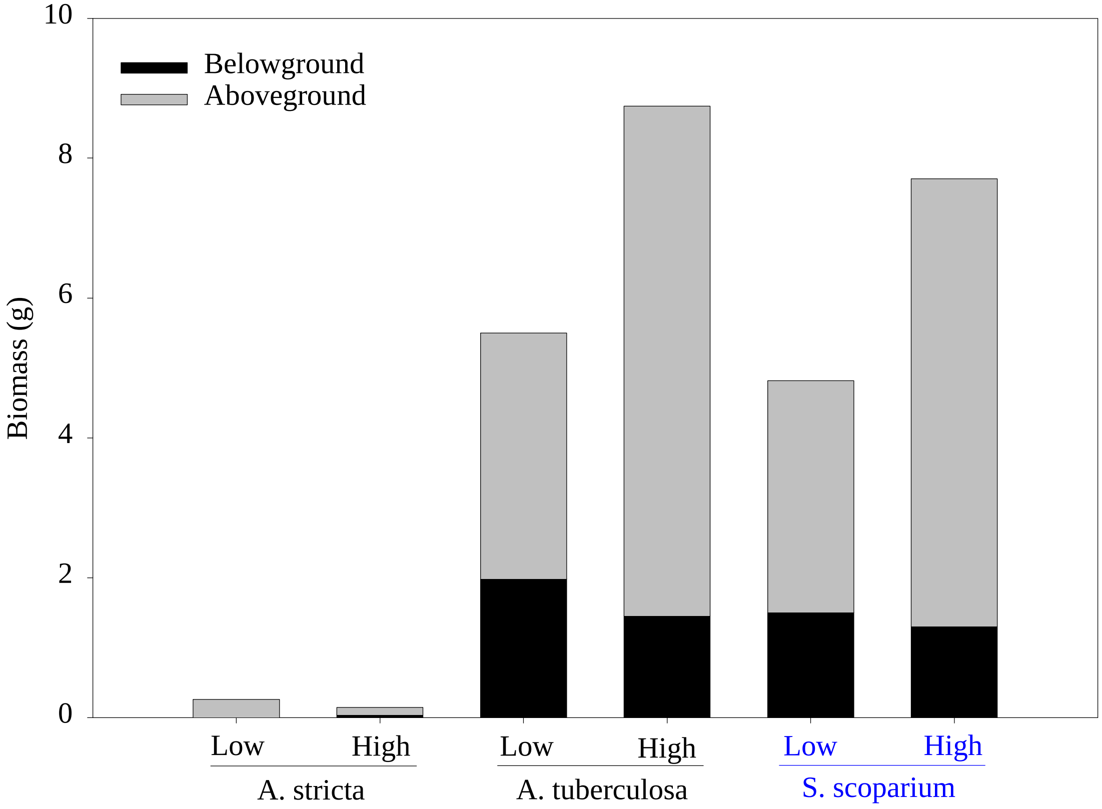

Figure 3. Biomass accumulation and allocation of *A. stricta*, *A. tuberculosa*, and *S. Scoparium* in response to high and low nutrient availability (least squares means). See Table 2 for statistical analysis.

However, the species by treatment interaction is the important effect for testing our hypothesis that the species responded differently to the nutrient treatment. There was a significant interaction between species and nutrient treatment for aboveground and total biomass and belowground:total biomass ratio (Table 1.2). When provided with increased nutrients, *A. tuberculosa* and *S. scoparium* responded by allocating a lower proportion to belowground biomass and producing higher total biomass. In contrast, *A. stricta* did not appear to change biomass accumulation or allocation patterns in response to different levels of available nutrients (Figure 1.3).

DISCUSSION

When grown in a common environment, *A. stricta* and *A. beyrichiana* consistently differed in growth*. Aristida stricta* had heavier seeds, but grew more slowly and produced smaller seedlings by the end of the experiment. Although we cannot rule out parental environment effects (Wulff 1986; Mazer and Gorchov 1996), our results strongly suggest that there is a genetic component to the growth differences expressed by these two species in the relatively uniform greenhouse environment. The expressed differences between *A. stricta* and *A. beyrichiana* are consistent with adaptation to environmental variation driven largely by latitude (Li et al. 1998).

In addition to the large differences found between *A. stricta* and *A. beyrichiana*, habitat (xeric vs. mesic) also affected seed and seedling performance. Seeds from xeric habitats had greater biomass than those from mesic habitats, again perhaps reflecting differences in maternal environment. Reproductive output can be lower for *A. stricta* on mesic as compared to xeric habitats (van Eerden 1997), although the opposite pattern has also been reported (Platt, Evans, and Davis 1988). Seed biomass did not, however, predict seedling growth, since the xeric habitats produced the heaviest seeds but seedlings with a slower growth rate that had acquired less biomass by the end of the experiment. This suggests that seedlings from hydrologically divergent habitats differ in early life-cycle physiology, although other potentially important seed quality factors such as nutrient content may also play a role (Parrish and Bazzaz 1985). In addition to the consistent differences in growth between habitats, some individuals collected from the Apalachicola site produced tillers with nodes along their length by the end of the growing period (data not presented). Since these tillers are typically reproductive (Parrott 1967), this suggests that population-specific differences in phenology may occur as well. These results suggest that seedlings of *A. stricta* and *A. beyrichiana* are locally adapted to low soil moisture availability in the xeric habitats since lower potential growth rates are often a consequence of adaptation to low resource environments (Chapin 1980).

Local adaptation of *A. beyrichiana* populations to sandhill (dry) and flatwood (wet) sites has been demonstrated with reciprocal transplant experiments, based on “home site” advantage of an older age class (Kindell et al. 1996). These authors suggested that soil moisture was the likely selective agent. Our results comparing populations from xeric and mesic habitats provide additional evidence for the existence of ecotypic adaptation to soil moisture in these two species. In another experiment (Gordon and Rice, 1998), populations of *A. beyrichiana* collected from both mesic and xeric habitats were shown to be genetically differentiated, although reciprocal transplants among xeric habitats revealed no local adaptation, as would be evidenced by “home site” advantage in the different xeric habitats (Gordon and Rice, 1998).

Taken together, these patterns suggest that wiregrass populations can be locally adapted to soil moisture. Other factors such as genetic drift (Wright 1931) and responses to other selective factors such as fire, competition, or pathogens (e.g., the smut *Sporisorium consanguineum*, Vanky 1988) may be important as well and deserve further investigation.

Aristida stricta's lack of response to increased nutrient availability differentiates it from two other C4 grasses from the same ecosystem. *Aristida stricta* did not increase in biomass or shift allocation patterns, whereas *A. tuberculosa* and *S. scoparium* responded to the increased nutrient availability in a manner similar to plants from higher resource habitats, by allocating less to roots and increasing growth (Levin et al. 1989). The very different nutrient relations of *A. stricta* demonstrated here have important ecological consequences. Although other characteristics such as seed dispersal may also be important (Tilman and Wedin 1991) and have not been compared for these species, these results may partially explain why both *A. tuberculosa* and *S. scoparium* are found on sites recovering from recent soil disturbances, whereas *A. stricta* is not. Since soil disturbances likely increase nutrient availability (Vitousek and Matson 1985), disturbances would favor species like *S. scoparium* and *A. tuberculosa* that can exploit increased nutrient availability. *Aristida stricta*, on the other hand, would not be competitive until soil fertility decreased to levels which inhibited the earlier successional species (Rice and Penfound 1954).

Growth differences expressed by both species of wiregrass and their ecotypes in this, as well other studies, demonstrates the importance of seed source in restoration projects. Wiregrass reintroduction efforts will likely benefit from the recognition of the existence of ecotypic and species-level variation. Success of wiregrass reintroduction may also be related to competition from plants adapted to higher resource conditions. If soil fertility is relatively high, competition from grasses such as *S. scoparium* or *A. tuberculosa* may limit wiregrass establishment. Conservation of the remaining native longleaf pine savannas may also depend on maintaining the low resource conditions to which wiregrass has adapted. Human caused increases in nitrogen availability are significant and continue to have dramatic impacts on many ecosystems (Vitousek et al. 1997). In longleaf pine savannas, increased nitrogen availability may decrease the abundance of low resource adapted species such as wiregrass, relative to others. A decrease in the abundance of these species may negatively effect other species such as the endangered red cockaded woodpecker since its success has been shown to be directly related to ground cover composition (James et al. 1994). The adaptations of longleaf pine savanna grasses and their ecological consequences deserve further investigation.

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CHAPTER 2

C4 PERENNIAL BUNCHGRASSES AFFECT C AND N CYCLING OF SANDHILL SAVANNA ECOSYSTEM SOILS.[1]

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Summary

- We investigated the influence of co-dominant, C4 perennial bunchgrasses (*Aristida stricta*, *Schizachyrium scoparium*, and *Andropogon ternarius*) on the spatial heterogeneity of C and N cycling in a fire-maintained *Pinus palustris* savanna.
- In a cross-site comparison, leaf tissue and soils were sampled from a xeric and a subxeric site. At a third site, paired, spatially mapped samples were taken under plants and adjacent (15 cm) locations. Net C and N mineralization was estimated from laboratory incubations.
- Principal components analysis revealed differences in plant characteristics among species, but no species-specific influence on N mineralization. The spatial analysis revealed spatial autocorrelation in biomass, but not in nutrients. A decoupling of C from N mineralization and higher inorganic N in *A. stricta* soils suggested important species-specific effects, although C and N mineralization showed no direct effect of species identity.
- Co-dominant species of the same physiomorphotype differentially affect C and N mineralization likely through changes in microbial communities.

Key words: *Aristida stricta*, sandhills, savanna, C mineralization, N mineralization, spatial, C:N ratio, plant-soil feedbacks

INTRODUCTION

Nutrient pools and cycling rates are known to exhibit substantial spatial variability in soils (Gross et al., 1995). Plants are important causes of some of this heterogeneity, and it is clear from observational and experimental work that individual species can have dramatically divergent effects (Wedin and Tilman, 1990; Vinton and Burke, 1995; Hooper and Vitousek, 1997; Vinton and Burke, 1997; Steltzer and Bowman, 1998; Chen and Stark, 2000; Van der Krift and Berendse, 2001).

A conceptual model of plant-soil interactions predicts the importance of species identity to increase with increasing precipitation (Burke et al., 1998). Low precipitation supports discontinuous plant cover where plant presence ("islands of fertility") is more important that species identity. High precipitation supports more continuous cover and species identity becomes important. The predictions for habitats receiving intermediate precipitation amounts (e.g., subhumid grasslands and savannas) are less clear. These systems support species with a wide range of adaptation to soil resource availability and often-discontinuous cover. This results in a case where both plant presence and species identity may be important. An important indication of differential adaptation to low nitrogen availability is high tissue C:N ratio (Chapin, 1980). This estimate of tissue quality has also been shown to be an important predictor of plant feedbacks on element cycling in soils (Paul and Clark, 1989; Bloemhof and Berendse, 1995; Vinton and Burke, 1997; Steltzer and Bowman, 1998; Nicolardot et al., 2001), although its predictive power may vary across habitats (Vinton and Burke, 1997).

In this study we compared three co-dominant species with the same photosynthetic pathway and life form (C4 perennial bunchgrass) that are native to longleaf pine (*Pinus palustris*) savannas of the southeastern US. These savannas are fire-maintained and are the subject of significant restoration efforts (Duever and Noss, 1988). One aspect of this effort is the reintroduction of an herb layer dominated by *Aristida stricta*. However, other bunchgrasses such as *Schizachyrium scoparium* have been discussed as possible functional analogs to *A. stricta* (Duever, 1989). Understanding the degree of variation within the native bunchgrasses, especially with respect to their effects on soil processes, must be understood prior to accurately treating these species as functionally redundant. This system, therefore, provides an opportunity to address species-specific effects on element cycling in the context of human-induced change in vegetation structure.

Our broad objectives were to establish the degree of variation between these species in tissue C:N ratios and to determine whether this variation has consequences for inorganic N pool sizes, and net C and N mineralization (Cmin and Nmin). *Aristida stricta* retains green leaves year-round, whereas the bluestems (*S. scoparium* and *A. ternarius*) senesce aboveground at the onset of winter. This evergreen character (Aerts, 1995), as well as successional and performance differences observed between these species (Lemon, 1949; Grelen, 1962; Walker, 1985; West and Donovan unpublished), led us to predict that *A. stricta* would have higher tissue C:N ratios that would therefore reduce net Nmin while having little effect on Cmin.

MATERIALS AND METHODS

Site description

The fieldwork for this study was conducted at the Carolina Sandhills National Wildlife Refuge (McBee, South Carolina). The refuge is composed of resource-limited fall line sandhills that are dominated by *P. palustris* and receive frequent prescribed burns. Average annual temperature is 15°C and the average annual rainfall is 1200 mm with little seasonality in precipitation. Although the edaphic gradients are complex in these systems, there is a topographic gradient that generally corresponds to soil moisture availability (Jacqmain et al., 1999). The ridge tops are relatively dry (xeric), the side slopes are intermediate (subxeric) and the foot slopes are relatively wet (mesic). The herbaceous layer of these savannas is dominated by C4 perennial bunchgrasses. The most common of these on our sites is wiregrass (*A. stricta*), with bluestems such as *S. scoparium* and *A. ternarius* co-dominating.

Cross-site sampling of tissue quality and Nmin

This sampling was conducted in June 2000 on one subxeric and one xeric site. Both sites were burned in spring 1998. The soils of the subxeric site are sandy, siliceous, thermic Arenic Paleudults and those of the xeric site are thermic, coated Typic Quartzipsamments. Ten individuals each of *A. stricta*, *S. scoparium*, and *A. ternarius* were randomly selected at each site. The aboveground biomass of each was clipped and placed in a paper bag. After clearing the soil surface of litter, two 3.5 cm diameter soil cores were taken directly adjacent to the outermost tiller to a depth of 6.5 cm. One core was quickly placed in a plastic bag and then stored in an ice-filled cooler until it was transported to a 3° C cold room (<10 hours later). The second core was sealed in a soil tin for gravimetric soil moisture content determination.

The clipped aboveground biomass was dried to constant weight at 65°C and weighed. A subsample of this biomass was then ground with a ball-mill grinder and analyzed for C and N content on a Finnigan MAT isotope ratio mass spectrometer (Thermo Finnigan MAT, Bremen, Germany).

Laboratory incubations were conducted to determine potential net N mineralization (Nmin) rates (Hart et al., 1994b). The bagged, moist soil samples were sieved (2mm diameter mesh) to remove large roots and litter prior to extraction. Four-gram subsamples were placed in flasks with 20 ml of 2 M KCl. These were shaken for 1 hour and then filtered through Whatman filters into scintillation vials. Flasks without soil were also shaken and filtered to determine the degree of N contamination in solutions or apparatus. The vials were stored at 3°C until analysis by continuous flow colorimetry (Alpkem RFA300, OI Corp., College Station, TX). A second subsample (~25 g) was placed in a gas-permeable bag with enough distilled water to bring the soil to approximately field capacity. These bags were then incubated in the dark at 23°C for 30 days. Following the incubation, a 4 g subsample was extracted and analyzed as before and a second subsample was used to determine gravimetric moisture content. Potential Nmin was calculated as the inorganic N accumulated during the incubation on a dry soil weight basis:

$$N_{\min}(\mu g \cdot g^{-1} \cdot d^{-1}) = \frac{(NH_4{}^{+} + NO_3{}^{-})_{post-incubation} - (NH_4{}^{+} + NO_3{}^{-})_{pre-incubation}}{Time(days)}$$

Spatially explicit sampling of Nmin and Cmin

In order to test hypotheses generated by our cross-site results, we selected an additional subxeric site to sample more intensively. This site was burned in spring 2000. The soils of this site are loamy, siliceous, thermic Grossarenic Kandiudults. In April 2001, 20 blocks were located along an approximately 100 m transect that ran roughly east to west. Each block contained one individual of each species (*A. stricta, S. scoparium, and A. ternarius*) located <1.5 m from each other. Each sampling location was mapped using a TOPCON CTS-2 Total Station (Topcon America Co., Paramus, NJ). As before, the aboveground biomass was clipped and placed in paper bags. Soil was sampled from surface to a depth of 10 cm (5.5 cm dia.) in a paired design. A core was taken from the center of each plant and another from an unvegetated location (15 cm away from the edge of the target plant and >10 cm from any other stem). Contrary to what is seen in arid and semi-arid grasslands (Burke et al., 1999), no raised hummocks are evident in our system, so the surface of the soil "under" plants is at the same elevation as those in the "open". Cores were stored as for the cross-site sampling.

Pre- and post-incubation extractions were done as before, with one exception. The solutions were centrifuged and the supernatant pipetted, rather than filtered, to improve accuracy. For the incubations, approximately 20 g of soil were placed in a 25 ml flask, brought to field capacity with distilled water, and then placed in an incubation chamber. To allow determination of potential Cmin, incubations were conducted in glass incubation chambers. The chambers consisted of 400 ml Mason jars that included a vial with 4 ml of 1.5 M NaOH to serve as a CO_2 trap (Zibilske, 1994). To ensure the atmosphere in the chamber remained water saturated, 10 ml of distilled water was added to the bottom of the jars. “Blank” chambers were also set up and incubated without soil for baseline CO_2 determination. All chambers were closed tightly and incubated in the dark at 23°C for 31 days.

Following incubation, the soils were extracted as before, and potential Nmin was calculated in the same manner. The NaOH traps were titrated with standardized 1% HCl and the quantity of CO_2 evolved was calculated as the difference between the sample vial and the mean of the “blanks.” Potential Cmin is calculated as:

$$C_{\min}(\mu g \cdot mg^{-1} \cdot day^{-1}) = \frac{(B-V)(N \cdot E)}{Time(days)}$$

where B is the volume of the standardized HCl required to titrate the trap solution from the “blank” chambers, V is the volume of HCl required to titrate the solution from sample vials, N is the normality of the acid and E is the equivalent weight of C in CO_2 (E=22), expressed on a dry soil weight basis.

Statistical analyses

In order to generate hypotheses about the relationship between plant species and soil processes, principal components analysis was conducted on the cross-site data set (MVSP; Kovach Computing Services, Anglesey, Wales). Because soil and plant variables were included in this analysis, all variables were standardized and centered prior to analysis (Pielou, 1984; Dunteman, 1989). Unless otherwise noted, all other statistical analyses were done using SAS v 8.2 (SAS Institute, Cary, NC).

To determine the extent of spatial autocorrelation between sampling points we used semivariance analysis on the spatially explicit data set. This technique analyses the variance between measurements at increasing distances (Robertson and Gross, 1994). The semivariance (γ) at a given distance interval (h) is calculated as:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [y(x_i) - y(x_i + h)]^2$$

where $N(h)$ is the number of intervals at distance h, and $y(x_i + h)$ is the value of a variable at a distance h from x_i. When appropriate, large-scale patterns were removed prior to semivariance analysis by inputting residuals from ordinary least-squares multiple regression of x,y against the variable rather than the variable itself. A weighted, non-linear exponential model was fitted to the semivariograms:

$$f = C_0 + C_e(1 - e^{-ah})$$

where C_0 is the "nugget" effect, C_e the "sill," and $1/a$ the "range" (SigmaPlot, SPSS Science, Chicago, IL). The nugget is the variance present at zero distance. This represents essentially residual variance not explained by spatial correlations. The sill is maximum between point variance, and the range is the distance over which sampling points are spatially auto-correlated. If the model adequately describes the spatial dependence of the variable, the asymptote of the variogram (the sill) of a given variable should be roughly equivalent to the total variance of that variable (Robertson and Gross, 1994).

A mixed-model ANOVA (block as random and location as fixed effects) was carried out to determine whether there was an effect of plant presence on Cmin or Nmin. For all ANOVA-based analyses data were transformed as necessary to satisfy model assumptions.

Following the determination of a "plant presence" effect, a more detailed analysis of species-specific effects was carried out. We first determined whether there was a relationship between biomass and the soil variable of interest (initial ammonium concentration, Nmin or Cmin). When plant biomass was correlated with the soil pool or process, ANCOVA was performed to determine the direct effect of individual species on the process after correcting for differences due to biomass. If biomass was not correlated, ANOVA was performed to test for species effects. Finally, species-specific differences in the relationship between Cmin and Nmin were determined with the use of ANCOVA: after correcting for differences in biomass, we tested for an interaction between species identity and the relationship between Nmin and Cmin.

RESULTS

Cross-site sampling of tissue quality and Nmin

The principal components analysis showed strong separation of *A. stricta* from the other species in multivariate space (Figure 2.1). The three PCA axes explained a total of 94% of the variation. The separation along axis I was due primarily to plant characteristics and clearly separated the species (Table 2.1), whereas axis II appeared to be driven primarily by soil moisture, with subxeric sites showing a trend of higher soil moisture.

Table 2.1. Principal Components Analysis of the cross-site sampling of *A. stricta*, *S. scoparium*, and *A. ternarius*.

	Axis 1	Axis 2	Axis 3
Eigenvalues	2.417	1.454	0.839
Cumulative Percentage	48.341	77.425	94.213
Variable			
Biomass	0.486	-0.353	0.390
Tissue C:N ratio	0.554	0.399	0.061
Tissue percent N	-0.560	-0.372	0.032
Soil moisture	-0.250	0.702	-0.194
N mineralization	-0.283	0.291	0.898

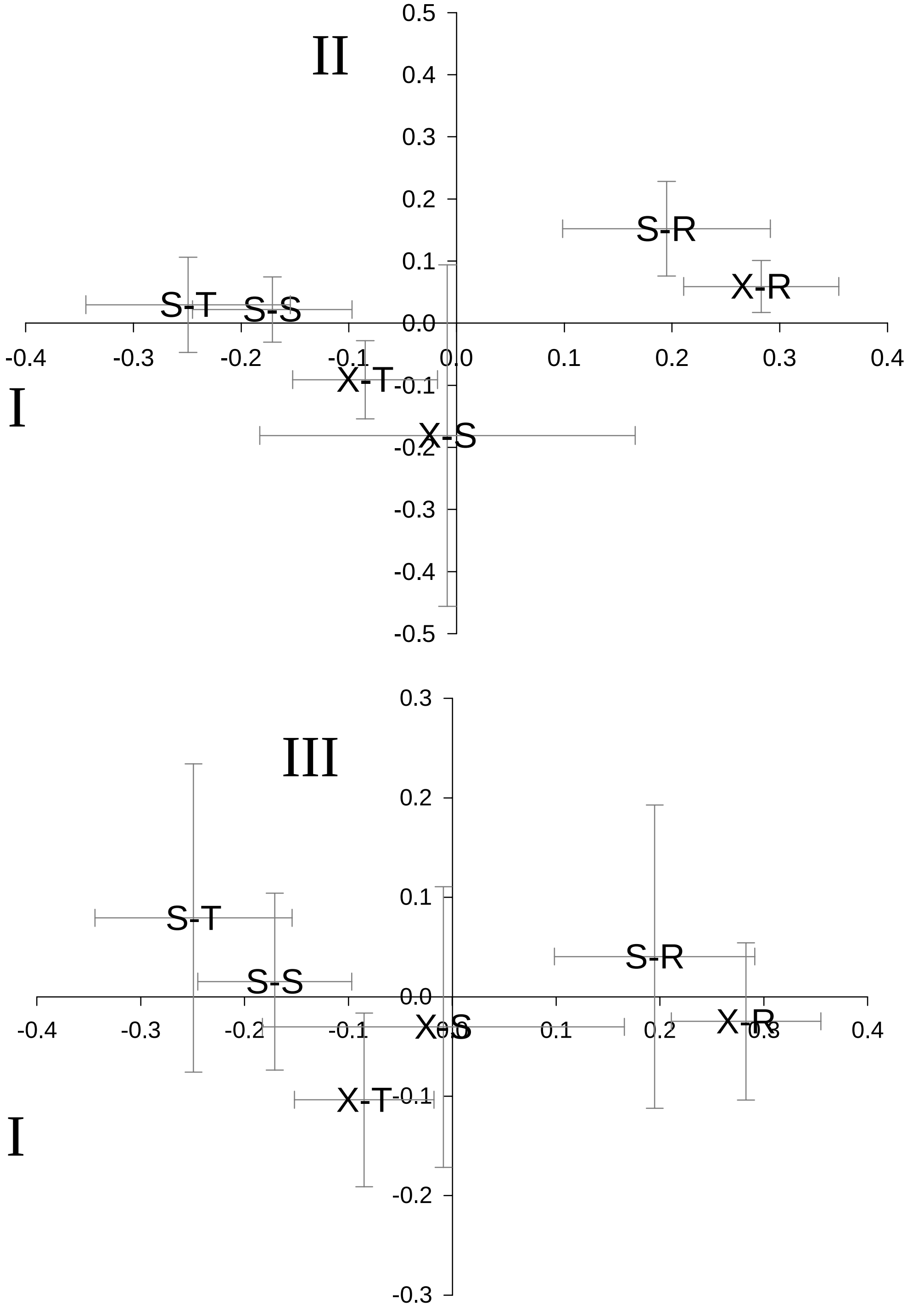

Figure 2.1. Principal Components Analysis of the cross-site sampling. Shown are mean axis scores +/- 1 S.D. for axes I vs. II and I vs. III (S-subxeric, X-xeric; R-*A. stricta*, S-*S. scoparium*, T-*A. ternarius*).

Nmin primarily drove axis III, again showing clear differences in sites, but only weak support for differences among species. Plant tissue characteristics were clearly different between these species and invariant between sites, with *A. stricta* exhibiting higher biomass and C:N ratios over twice those of the bluestems (Figure 2.2). This difference in tissue quality, however, did not result in differential effects on Nmin.

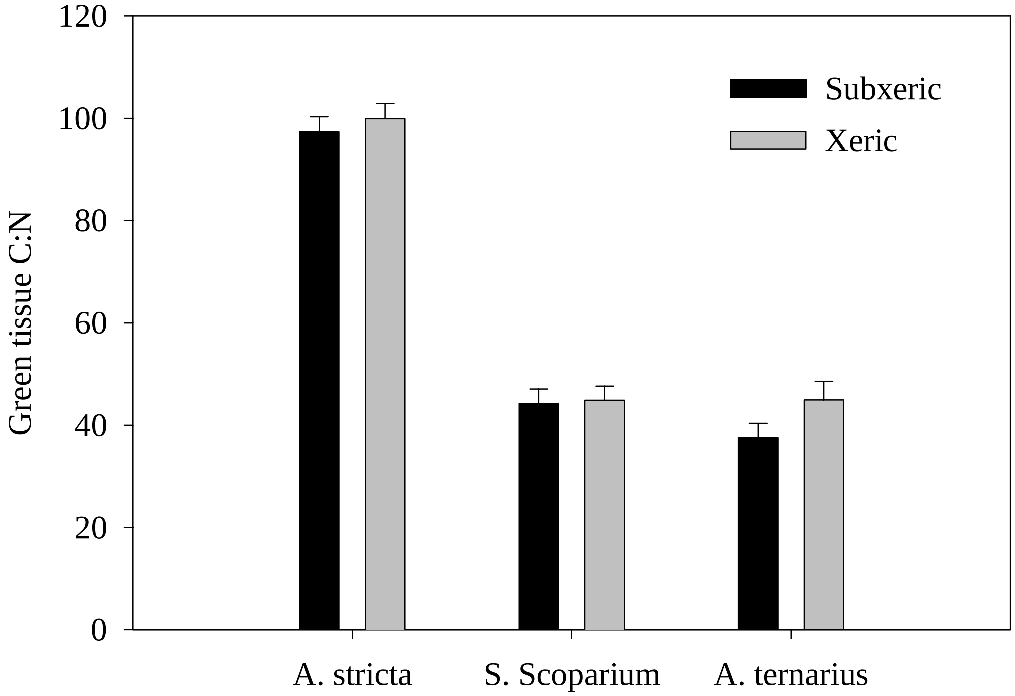

Figure 2.2. Mean C:N ratio (± 1 SE) of aboveground green tissue; two-way ANOVA showed a significant effect of species ($F_{2,48}$=96.58, p<0.0001), no significant effect of site or species*site interaction.

Spatially explicit sampling of Nmin and Cmin

At our sampling scale there was no spatial dependence for Cmin or Nmin (Figure 2.3a,b). There was also no spatial dependence for initial extractable ammonium

($NH_4^+{}_{initial}$, data not presented). Aboveground biomass, on the other hand, did show spatial dependence. The non-linear weighted least-squares fit of the exponential model indicated that the range of spatial dependence was 200-476 cm, the nugget effect was 16.6 and the sill was 248.5 (Figure 2.3c). The variance for biomass was 256.3, close to the asymptote of 248.5, suggesting the model adequately describes the spatial dependence in this variable (Robertson and Gross, 1994).

Both Cmin and Nmin were greater for soils directly under individual plants, although the effect on Nmin was not as strong (Figure 2.4). Aboveground plant biomass was positively related to Cmin (R^2=0.22, p<0.001), but no relationship was found between biomass and either $NH_4^+{}_{initial}$ or Nmin (Figure 2.5). Initial ammonium availability was higher under *A. stricta* individuals than under *S. scoparium* or *A. ternarius* (Figure 2.6). Nitrate levels were typically below the limits of detection (data not presented). After correcting for biomass differences among species, there was no significant effect of species identity on Cmin. There was also no significant effect of species on Nmin (Table 2.2).

The relationship between Cmin and Nmin differed between species (Figure 2.7). There was a positive relationship between biomass-corrected Cmin and Nmin for *S. scoparium* (R^2=0.32, p<0.01) and *A. ternarius* (R^2=0.23, p=0.03), but no significant relationship for *A. stricta* (p=0.41).

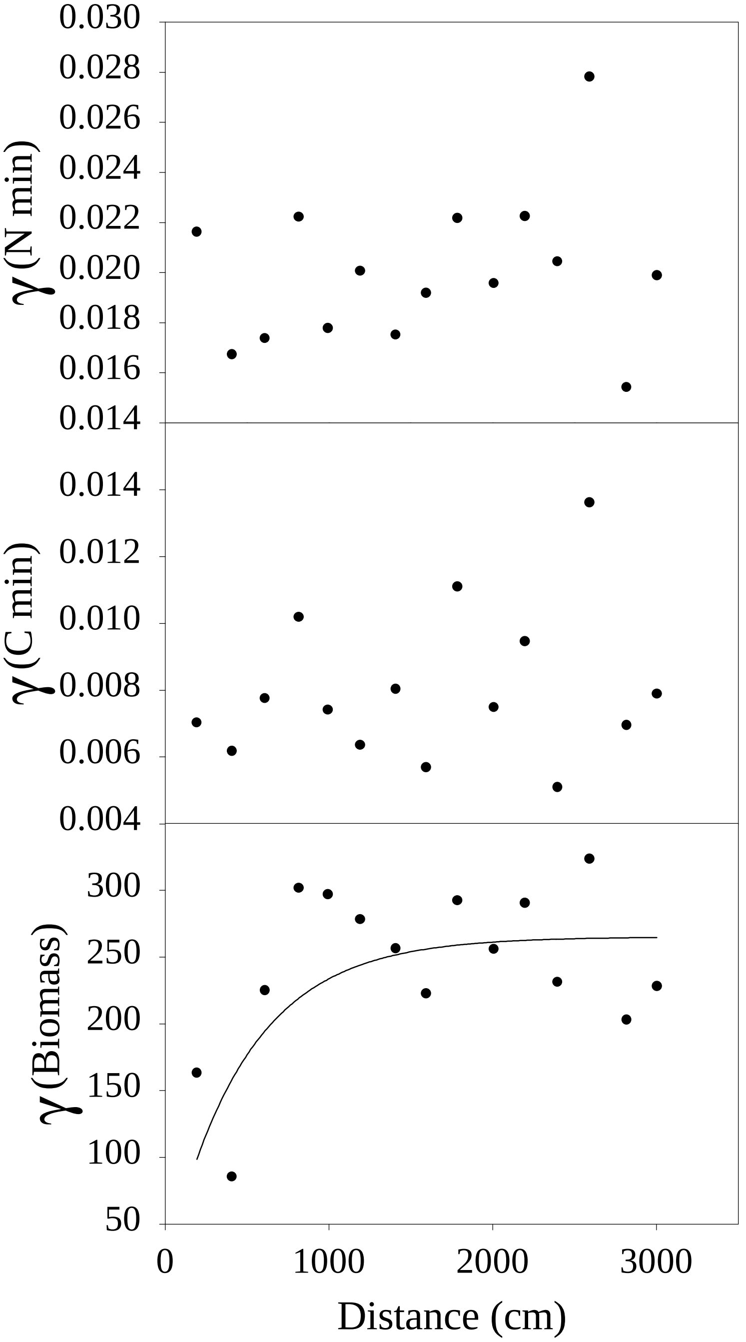

Figure 2.3. Semivariograms of "plant" locations for Nmin, Cmin, and aboveground biomass. Only aboveground biomass showed spatial autocorrelation (R^2=0.51: nugget=16.6, sill=248.5, and range=476.2). No spatial autocorrelation for any variable was detected for "open" locations (data not presented).

Table 2.2. Results of ANCOVA or ANOVA for the test of species-specific effects on Cmin and Nmin.

Source	DF_n	DF_d	F	P
Cmin				
Biomass	1	36	9.93	0.0033
Species	2	36	0.20	0.8157
Nmin				
Species	2	35	0.29	0.7493

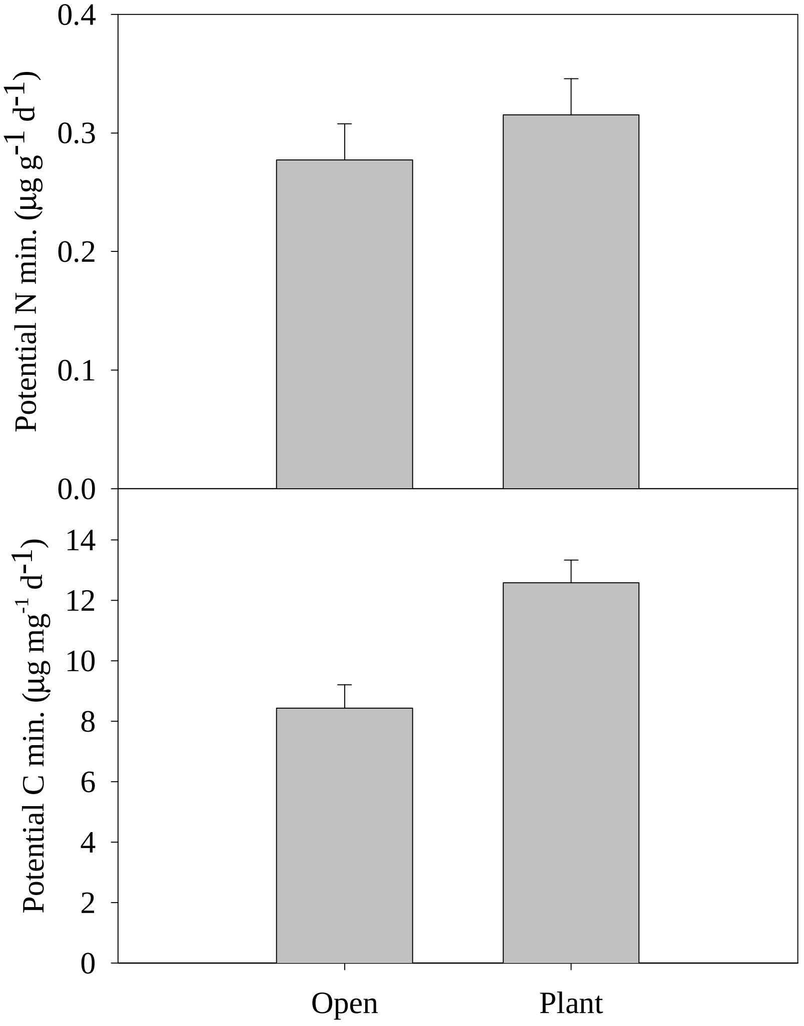

Figure 2.4. Potential N mineralization (± 1 SE; $F_{1,95}$=3.71, $p<0.06$) and C mineralization ($F_{1,94}$=27.47, $p<0.0001$) for locations under individual plants (plant) and paired unvegetated locations (open).

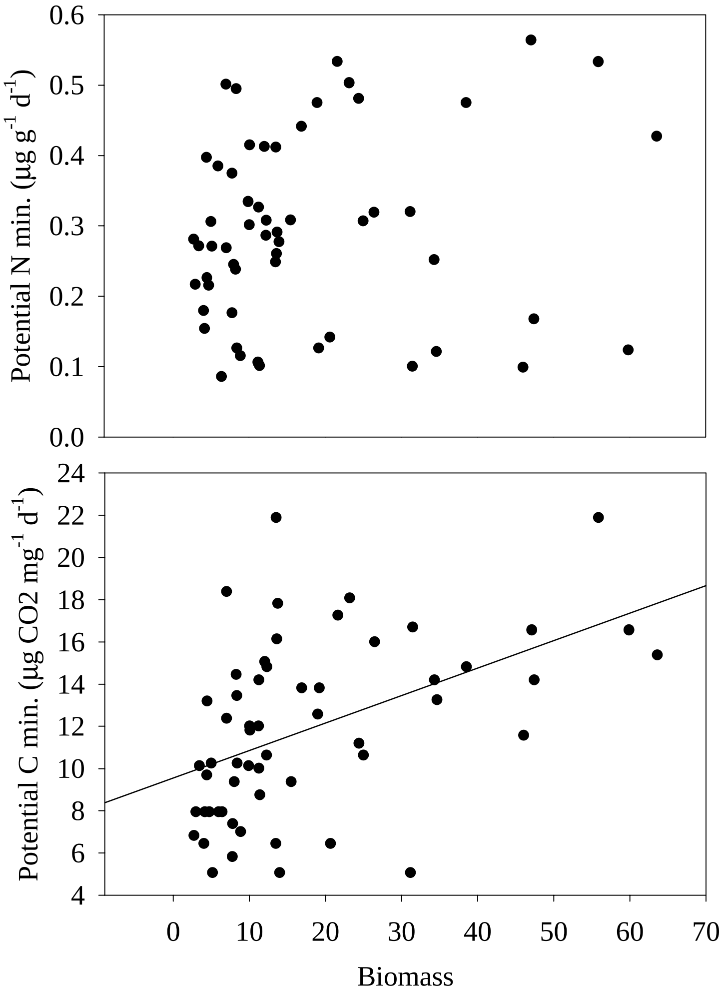

Figure 2.5. Effect of biomass on Cmin (R^2=0.22) and Nmin (not significant).

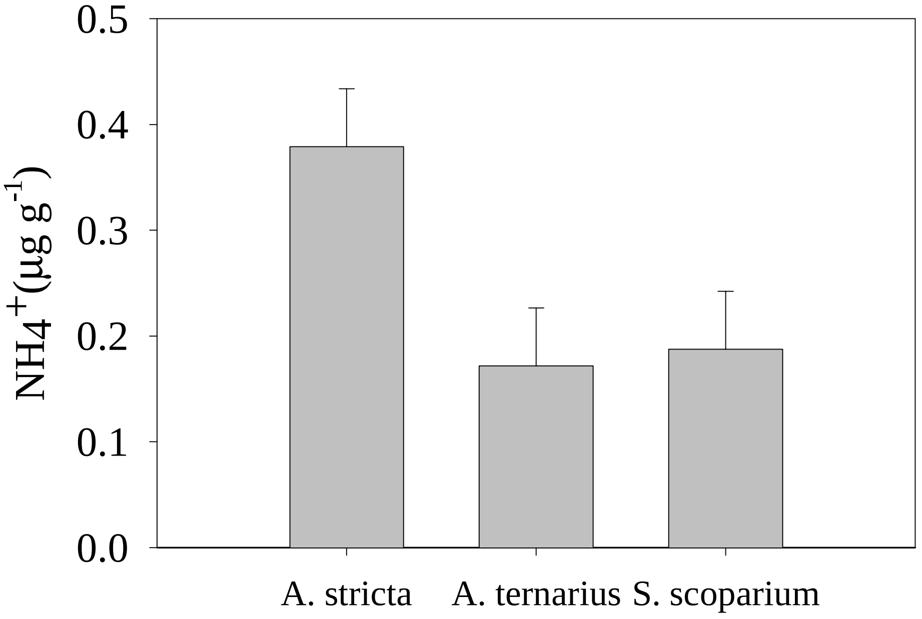

Figure 2.6. Mean ammonium availability (± 1 SE); one-way ANOVA showed a significant effect of species ($F_{2,35}=3.88$, $p<0.05$).

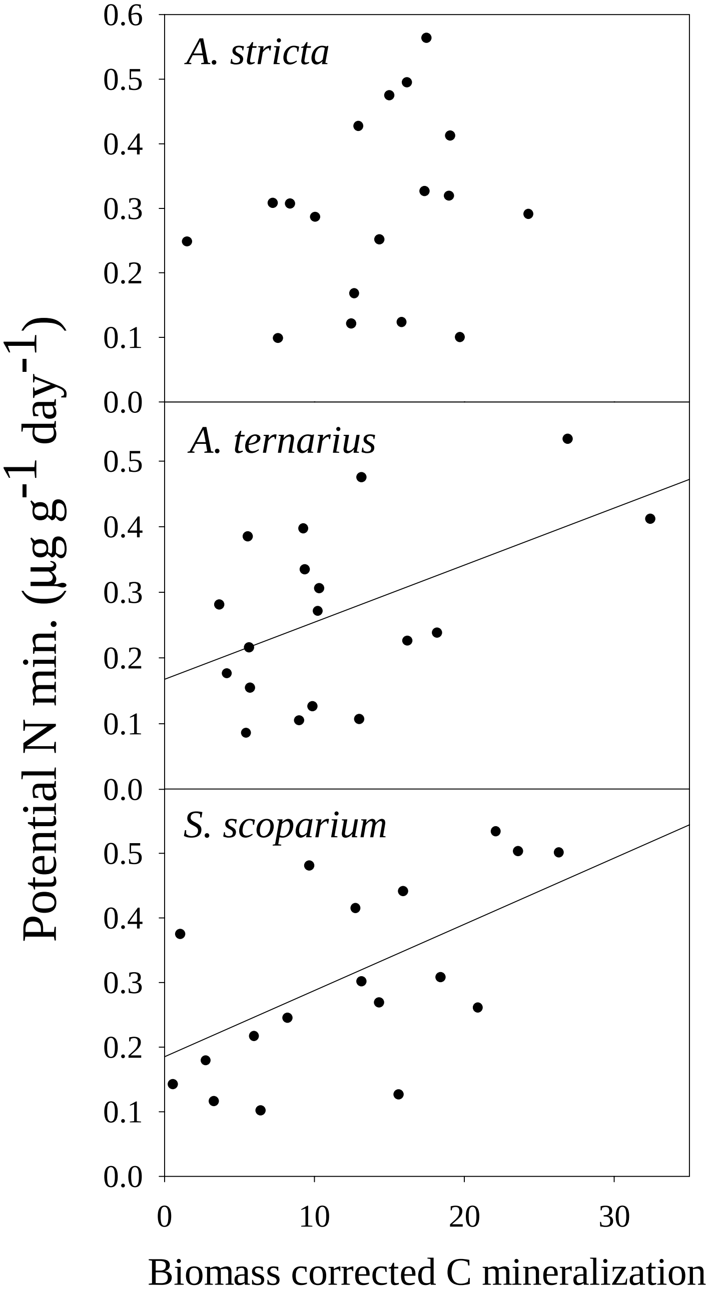

Figure 2.7. Relationships between Cmin and Nmin for *A. stricta* (not significant), *S. scoparium* (R^2=0.32), and *A. ternarius* (R^2=0.23). Cmin was corrected for biomass by adding the residuals of the regression of Cmin against biomass (see Fig. 6) to individual Cmin values.

DISCUSSION

Our cross-site analysis revealed strong tissue quality differences between *Aristida stricta* and those of both *S. scoparium* and *A. ternarius* that were invariant with respect to site (Figure 2.2). The much higher C:N ratio of *A. stricta* leaf tissue is consistent with higher nitrogen use efficiency relative to the other species (Aerts and Chapin, 2000). If, as expected, this is representative of the quality of organic matter being decomposed in these soils, we might expect net immobilization under *A. stricta* and net mineralization under the bluestems (Stevenson and Cole, 1999). Surprisingly, however, these differences in tissue quality did not appear to result in species-specific differences in net production of inorganic N (Figure 2.1). Indeed the most important Nmin differences were between sites, with the subxeric site exhibiting higher rates. The apparent lack of species-specific effects in the cross-site analysis was surprising given the very large difference between these species in tissue quality and the negative relationship often seen between tissue quality and Nmin (Nicolardot et al., 2001).

Our more detailed study of a single, subxeric site revealed interactions between individual species and C and N mineralization that suggest more complex species-specific effects. The geostatistical analysis showed a clear pattern of spatial dependence for biomass up to 4.8 m but no such pattern in either C or N mineralization (Figure 2.3). Although some authors have found spatial dependence of N mineralization at fairly large scales (e.g., 1-20 m; Robertson et al., 1988), our results are similar to Jackson and Caldwell (1993a) who found no spatial dependence for net N mineralization or microbial respiration even at a scale of 12.5 cm. The lack of spatial dependence at our sampling scale suggests that factors at smaller scales, such as within the rhizosphere (Robertson et al., 1988; Jackson and Caldwell, 1993a,b), have important effects on C and N mineralization.

Predictably, Cmin was positively correlated with the size of the plant under which the soil core was taken (Figure 2.4). Larger plants would be expected to return larger amounts of carbon to the soil, causing increased microbial activity as was seen in our incubations. In our analysis we removed the effect of plant size in order to determine the effect of other plant traits. However, the largest plants at all three sites were consistently *A. stricta*. Although the ages of these plants are unknown, considerably higher leaf C:N ratios, its lack of winter senescence and low root mortality (West, Espeleta, and Donovan *unpublished*) likely provide a competitive advantage to *A. stricta* in this very nutrient-limited system. These traits, then, would be expected to feed back on nitrogen cycling.

After correcting for plant size, however, we did not detect any effect of species identity on Cmin or Nmin (Table 2.2). The soils collected under each species exhibited nearly identical mean rates of inorganic N production in our 30-day laboratory incubations. A potential explanation for this may be that the primary organic matter decomposing under individual plants is not leaf tissue, but roots (Burke et al., 1998) and the root tissue C:N may not vary in similar ways. However, previous studies of *A. stricta* (Woods et al., 1959) and our preliminary results (data not presented) reveal similar differences in root tissue C:N ratios among these species. Our data suggest, on the other hand, that this convergence in net inorganic nitrogen production of soils with very different organic matter sources happened as a result of differences in the microbial communities. The positive relationship between Cmin and Nmin seen for the bluestem species demonstrates that as microbial activity increases in these soils, net production of inorganic N increases. In *A. stricta* soils, however, there is no such relationship. We suggest that a logical explanation for this pattern is divergence in the microbial C:N ratios in the different soils. Gross rates of N mineralization typically increase linearly with increasing microbial respiration (Hart et al., 1994a). Given the much higher C:N ratios of *A. stricta* tissues, it is likely that the C:N ratio of the microbial biomass in those soils is also significantly higher compared to bluestem soils (Hassink et al., 1994). Since Cmin is not likely to be affected by microbial C:N ratios (Hunt et al., 1987), and higher microbial C:N ratios will produce more inorganic N per unit biomass (Hassink, 1994), changes in microbial C:N ratios may explain the different relationships between Cmin and Nmin for these species (Hart et al., 1994a).

Surprisingly, the ammonium concentrations in soils under *A. stricta* were much greater than those under the bluestems. Clearly, either net production of inorganic N is greater in the field under *A. stricta* compared to the bluestems, or plant uptake is lower, or some combination of both. Given the clear differences in tissue quality between these species, we suggest that the microbial communities associated with this very nutrient use efficient species are also themselves very nutrient use efficient, resulting in relatively high rates of inorganic N production. Regardless of the cause, however, these results suggest clear divergence between the species in their influence on inorganic N availability in the field.

Plants undoubtedly influence their belowground environment, and they do so in ways that may not be easily predicted from simple traits such as tissue C:N ratios. As has been demonstrated here and in other studies (Jackson and Caldwell, 1993b; Gross et al., 1995), soil resources exhibit significant heterogeneity at very small spatial scales (on the order of centimeters). Clearly individual plants interact with many other mechanisms that generate this heterogeneity. Plant presence has important influences on the spatial heterogeneity of soil element cycling, and, at least for C cycling, plant size is clearly important in this system. The C4 bunchgrass species of this system differ in C:N ratios and biomass and differ in their effects on C and N cycling even though no differences were apparent in Cmin or Nmin alone. In terms of these ecosystem functions then, these species are not functionally redundant. Caution, therefore, should be exercised in making conclusions about the presence or absence of species-specific effects on C or N cycling based solely on net production of inorganic N (Hart et al., 1994a). Detailed studies of the relationships between ecophysiological adaptation to soil resource limitation and its effects on element cycles and pool sizes, especially gross rates of inorganic N production (Hart et al., 1994a) are necessary to improve our understanding of these processes and we are currently pursuing these questions.

ACKNOWLEDGEMENTS

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CHAPTER 3

DIFFERENTIAL NITROGEN DEPLETION AND ROOT PROLIFERATION OF THREE FALL-LINE SANDHILL BUNCHGRASSES IN RESPONSE TO AN INORGANIC N PULSE.[1]

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[1] West, J. B. and L. A. Donovan. To be submitted to *New Phytologist*

ABSTRACT

Soil nutrients are often made available to plants in short-duration pulses. The differential ability of plants to respond to these pulses may affect competitive balances among species and their effects on soil N. We studied the responses of three co-occurring fall-line sandhill C4 bunchgrasses to a single pulse of inorganic NH_4NO_3. Because of apparent differences in nutrient use strategies, we predicted that *Schizachyrium scoparium* and *Andropogon gyrans* would respond to the nutrient pulse with significant root proliferation, whereas less of a response would be seen in *Aristida stricta*. We also predicted that *A. stricta* would be better able to reduce the nutrient pulse through significant nutrient uptake. Neither prediction was supported by the results. *Aristida stricta* exhibited the most pronounced difference between treatments in root production, showing a large proliferation response to the nutrient pulse. *Schizachyrium scoparium* exhibited an increased proliferation, but not to the same degree and *A. gyrans* exhibited no proliferation response. The ability to deplete the supplied pulse of N was dependent on species and differed for NH_4^+ and NO_3^-. *Schizachyrium scoparium* depleted the $NH4^+$ pulse to the lowest levels and exhibited the greatest difference in ability to deplete the $NH4^+$ pool between the two treatments. *Aristida stricta* reduced the $NH4^+$ pool by the same proportion in both treatments. Both *A. stricta* and *S. scoparium* depleted most of the NO3- pool in both treatments, whereas *A. gyrans* depleted a greater proportion in the fertilized treatment as compared to the unfertilized treatment. These results do not support a hypothesized tradeoff between adaptation to low nutrient conditions and morphological plasticity. However, they do suggest a tradeoff between the ability to

deplete nutrient pools of different sizes and a dependence on the form of available N. *Aristida stricta* more successfully depletes $NH4^+$ at small pool sizes, whereas *S. scoparium* most effectively depletes large $NH4^+$ pools. These conclusions have implications for both competitive interactions between these species, as well their effects on such ecosystem functions as nitrogen retention.

INTRODUCTION

Pulses of mineralization, such as may occur after fire or soil wetting-drying cycles, are characteristic of low nutrient systems (Lewis 1974, Chapin et al. 1990, Gebauer and Ehleringer 2000). Although comparisons among co-existing species are rare, the one study that has made this comparison demonstrated important species-specific differences in the ability to exploit nutrient pulses (Bilbrough and Caldwell 1997). Predicting differential abilities of species to deplete nutrient pulses may come from predicted strategies of plants exhibiting differential adaptation to nutrient availability (Aerts and Chapin 2000). Species adapted to relatively high nutrient availability are likely to be more plastic in their response to variation in nutrient availability (Lambers and Poorter 1992). However, species can exhibit plasticity in both morphology and physiology and it is not yet clear how species may differ in such morphological responses as root proliferations as compared to such physiological responses as increased uptake rates (Bassirirad 2000).

Pinus palustris ecosystems are fire-maintained savannas that exhibit strong soil resource limitations (Wilson et al. 1999). A significant proportion of the biomass of these systems is belowground and is dominated by grasses (Mitchell et al. 1999). Post-fire nutrient pulses are frequently documented (Christensen and Muller 1975, Hurlbert 1988) and are likely to be important factors in controlling the vegetative structure of these savannas since return intervals of 3 years are not uncommon. However, it has been argued that these particular savannas may not experience post-fire nutrient pulses, although the vegetation does respond generally by increased post-fire biomass production (Abrahamson 1984, Anderson and Menges 1997). Unfortunately, the existing literature reports soil nutrient conditions no less than three months following fire. My results have shown significant post-fire increases (< 1 week) in inorganic ammonium (West and Donovan, unpublished) that may be rapidly assimilated into microbial or plant biomass.

Comparisons between *Aristida stricta* Michx. (wiregrass) and several co-occurring bunchgrasses (collectively the bluestems, e.g., *Schizachyrium scoparium* (Michx.) Nash and *Andropogon ternarius* Michx.) have suggested differences among these species in nutrient use strategies. *Aristida stricta* has a lower potential relative growth rate, higher tissue C:N ratios, has longer root lifespans and tends to increase in relative abundance on drier, more nutrient-poor sites relative to the bluestems (West and Donovan, unpublished). These results all strongly suggest that *A. stricta* is better adapted to low nutrient availability than are the bluestems.

In order to test predictions about how these species would respond to a nutrient pulse, we subjected field-grown monocultures of *A. stricta*, *S. scoparium*, and *Andropogon gyrans* Ashe to a pulse of inorganic nitrogen and monitored fine root proliferation and nitrogen depletion ability of each species. Given the nutrient use characteristics of *A. stricta*, we predicted that *A. stricta* would exhibit less root proliferation in response to the pulse relative to the other two species. Predictions about the differential abilities of these species to deplete the inorganic N pulse are less simple to make given the limited dataset currently available. However, given the dominance of A. stricta over much of the savannas that receive very frequent burning, we predicted that the active root system of *A. stricta* would be better able to deplete the N pulse than would the other species.

METHODS

Seeds of *A. stricta*, *A. ternarius*, *A. gyrans*, and *S. scoparium* were collected from the Carolina Sandhills National Wildlife Refuge (CSNWR, McBee, SC) in 1998 and stored at room temperature in paper bags until germination. Seeds were germinated on wet filter paper in December 1999 and plants were grown in a glasshouse in Athens, GA in conetainers filled with a mix of 60% steam-sterilized sand, 20% fritted clay (Turface, Buffalo Grove, IL), and 20% sieved (1.7mm) field soil. In May 2000 each plant was divided in half to provide clones and replanted in the same soil mix. While growing in the glasshouse, plants were kept well-watered and fertilized with 1/10 modified Hoagland's solution (Epstein 1972) when necessary.

The experimental plots are located on a cleared field on nutrient poor Candor soils (Sandy, siliceous, thermic Arenic Paleudults) at the CSNWR. A randomized block design was employed, in which each plot was a monoculture of one of the four species or was left unvegetated. Half of the plots (n=5) were allocated at random to either soil sampling (no minirhizotron tube) or root monitoring plots (installed minirhizotron tube). Prior to planting, minirhizotron tubes (100 cm long x 5.1 cm diameter) were installed in half of the field plots, selected at random. Each tube was scribed with rectangular frames (0.9 x 1.3 cm) along its length and every fifth frame was numbered consecutively. The minirhizotron tubes were installed at a 30° angle from horizontal and reached a depth of approximately 35cm. Care was taken to minimize gaps between the minirhizotron surface and the soil surface (Johnson et al. 2001), and the portion of the tube that extended above ground was wrapped in reflective tape and covered with a white cap.

After the plants had established in the glasshouse, they were transplanted into the field plots (May 17, 2000). Each monoculture plot consisted of nine individual plants spaced 20cm apart in a regular, square pattern and plots were separated by 40cm. The plants were watered when transplanted, but did not receive any additional irrigation after that. In early June, approximately 14% of the plants had died presumably from transplant shock and were replaced. The plants were allowed to establish for 7 months and weeds were removed by hand from all plots when necessary. Several *A. ternarius* plants died during that period, perhaps because of the drought, and as a result had to be removed from the analysis.

The minirhizotron tubes were imaged on December 5, 2000, March 13, May 23, August 17, and October 1, 2001 using a Hi-8 camcorder attached to a Bartz Minirhizotron Research camera (Bartz Technology Co., Santa Barbara, CA). Because of logistical problems, a June or July imaging date is not available. For the first date, the videotaped images were analyzed using the PC-based software program ROOTS (Hendrick and Pregitzer 1992). Using this software, root lengths and diameters of every individual root were calculated. Unvegetated plots had minirhizotrons installed and were imaged to ensure root absence, but were not digitized. Following Crocker and Hendrick (unpublished), total root length (per minirhizotron) was regressed against the total number of roots per minirhizotron. Given the very tight relationship between these two variables, and to allow simple and rapid root counting of the other dates, after the first date only root number was recorded. In order to improve the speed of this process, digital “snapshots” of each frame were taken using a PC-based video capture card. The roots were then counted while simultaneously viewing digital images of two dates (previous date and date currently being counted) in order to determine the appearance of new roots, as well as the disappearance of pre-existing roots between imaging dates. For all dates, roots were classified as new, brown, white, or missing.

On May 23, 2001 nitrogen fertilizer was applied at random to half of the plots at a rate of 30 g m^2. It was applied as dry NH_4NO_3, directly to the soil. Available $NH4^+$ and $NO3^-$ was estimated using cation and anion exchange membranes placed at a vertical depth of 3 cm in the center of the soil-sampling plots (AR204-SZRA and CR67-HMR, Ionics, Inc., Watertown, MA). These membranes are functionally identical to ion exchange resins placed in bags (e.g., Giblin et al. 1991), but are flat and therefore cause significantly less soil disturbance (Cooperband and Logan 1994). The membranes were cut into 5cm x 5cm squares, attached to fishing line and were individually inserted into the ground using a putty knife. The membranes were left in the ground for a period of approximately three weeks and were allowed to accumulate N. After this, they were removed, rinsed with deionized water, and extracted in a 2 M KCl solution for 2 hours. The extractant was then analyzed for NH_4^+ or NO_3^- by continuous flow colorimetry (Alpkem RFA300, OI Corp., College Station, TX). A pre-treatment estimation (March

12-April4, 2001) and two post-treatment estimations (June 10-July 2 and August 17-September 18, 2001) were conducted.

The June data were normalized to the maximum concentrations of NH_4^+ and NO_3^- in each of the fertilized and unfertilized, unvegetated plots and are expressed as relative N depletion: ([Max unvegetated N]-[Vegetated N])/[Max unvegetated N]. Mixed-model ANOVAs (block as random effect; Littell et al. 1996) were then used to examine the effects of species and treatment on relative N depletion. Mixed-model ANOVAs were also conducted on the March and August sampling to examine the effects of species and treatment (August only) on nitrogen availability. Because of high mortality (>4 dead plants), two *A. stricta* plots and one *S. scoparium* minirhizotron plot, as well as one *A. gyrans* soil-sampling plot were removed.

Mean root diameter was compared among species by averaging the root diameters recorded for a given minirhizotron and then comparing species with the plot (=minirhizotron) as the experimental unit in a mixed-model ANOVA. A mixed-model repeated measures analysis was used to examine the effects of species and treatment on cumulative root production and mortality, as well as standing live roots after treatment, with the plot again as the experimental unit. Separate analyses were conducted for the dates prior to fertilization and for those after. Data were transformed as necessary to meet ANOVA assumptions. Means are adjusted for other terms in the model, as well as unbalances in the design (e.g., incomplete blocks) are presented (LS Means).

RESULTS

Air temperature was average and rainfall was approximately 70% of normal, with significant early spring and late summer droughts during the study period (Figure 3.1). There were no significant differences among plots in available NH_4^+ or NO_3^- prior to fertilization ($F_{4,40}=1.04^{ns}$). The fertilizer produced a significant short-term increase in NH_4^+ and NO_3^-availability (Figure 3.2). The fertilizer effect was not present by August (NH_4^+: $F_{1,25}=0.16^{ns}$, NO_3^-: $F_{1,25}=2.44^{ns}$). There was a significant species*treatment interaction for both relative NH_4^+ and NO_3^- depletion after fertilizer application (Table 1). *Aristida stricta* depleted approximately 60% of the maximum NH_4^+ availability under both unfertilized and fertilized conditions (Figure 3.3). *Schizachyrium scoparium*, however, exhibited almost undetectable depletion in unfertilized conditions and nearly 100% depletion of available NH_4^+ when fertilized. *Andropogon gyrans* showed trends intermediate to both in both conditions. Both *A. stricta* and *S. scoparium* depleted most of the available NO_3^-; however *A. gyrans* showed significantly decreased NO_3^- depletion under unfertilized conditions.

A significant relationship was found between total root number and length per minirhizotron ($R^2=0.96$, $P<0.0001$, Figure 3.4). The relationship between root number and length were similar among species (data not presented). All species showed significant root production and death prior to treatment and did not differ from each other for either variable (Table 2a). There was a significant species*treatment interaction for both cumulative root production and death following treatment application (Table 2b). *Aristida stricta* exhibited a significant stimulation of root production with the application of fertilizer, as did *S. scoparium* but to a lower degree (Figure 3.5a). *Andropogon gyrans*, however, exhibited no root production response. There was greater root death under fertilization for both *A. stricta* and *S. scoparium*, however *A. gyrans* exhibited greater death in unfertilized conditions relative to fertilized (Figure 3.5b). At the end of the study, *A. stricta* had significantly higher standing live roots in the fertilized plots relative to unfertilized, whereas there was no significant difference between treatments for either *A. gyrans* or *S. scoparium*. No significant difference in root diameter was found between species (overall mean = 130 μm; $F_{2,23}=1.93^{ns}$).

Fertilizer was applied on May 23. Dark symbols indicate fertilized and open symbols indicate unfertilized. Data were analyzed using a repeated measures, mixed-model ANOVA in separate analyses before and after fertilizer application. There were no differences in root production or death among species prior to treatment ($p>0.5$). There were significant species*treatment interactions for both production and death ($p<0.05$, see Table 3.2 for statistical comparisons).

DISCUSSION

The surprising results documented here highlight the potentially complex nature of belowground responses even when comparing morphologically similar, and co-occurring plant species. Contrary to expectations, *A. stricta* was not more capable of depleting the supplied resource pulse. In addition, it was apparently more morphologically plastic than the other two species.

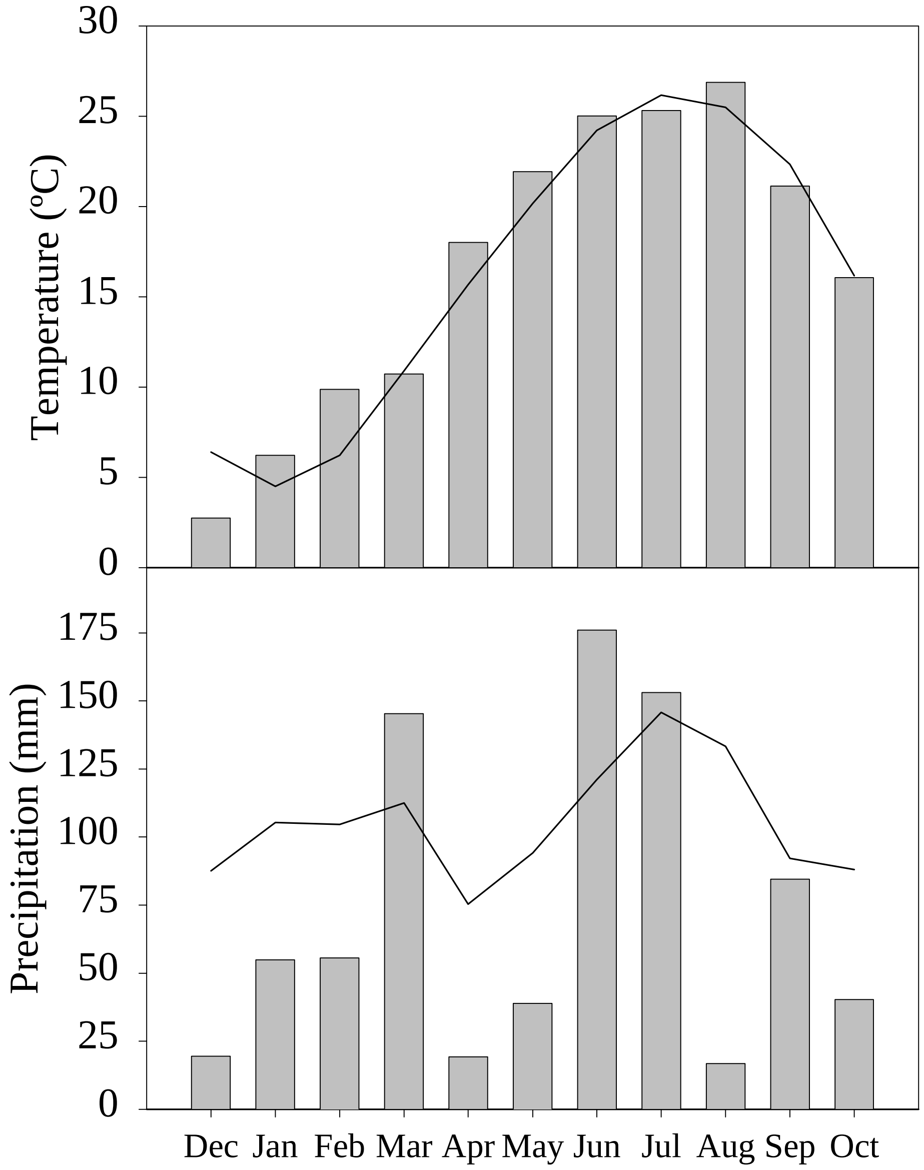

Figure 3.1. Mean monthly temperature and total monthly rainfall for the study period (vertical bars) plotted onto 30-year averages. The monthly data was collected at the CSNWR headquarters, immediately adjacent to the field site (<0.5 km). The average temperature and precipitation curves are from 30 years of data (1961-1990) collected in Cheraw, SC (approx. 20 km from the field site).

Table 3.1. Mixed-model analysis of variance for relative N depletion.

Source	$F_{nDF,dDF}$
Ammonium	
Species	$F_{2,14} = 0.39$
Treatment	$F_{1,14} = 10.36$**
Species*Treatment	$F_{2,14} = 6.85$**
Nitrate	
Species	$F_{2,17} = 1.16$
Treatment	$F_{1,17} = 0.50$
Species*Treatment	$F_{2,17} = 4.25$*

** Significant at $P<0.01$; * significant at $P<0.05$

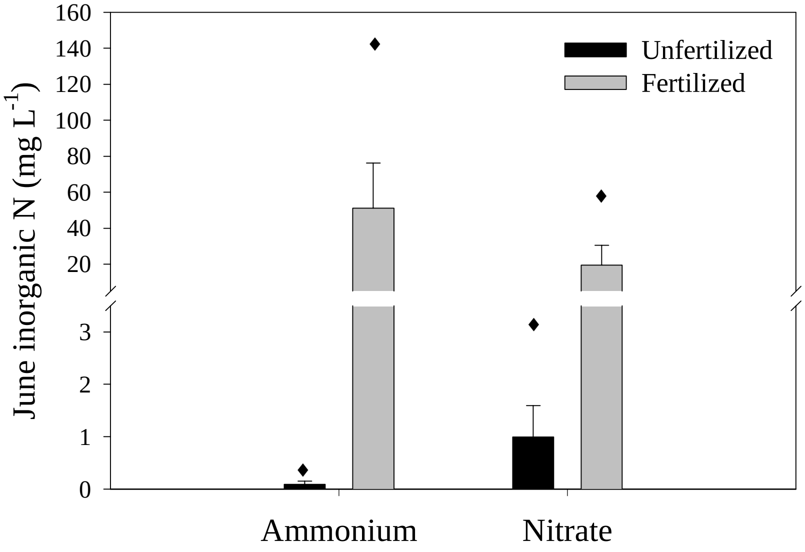

Figure 3.2. Inorganic N concentrations from extractions of ion exchange membranes (IEMs) placed in unvegetated plots (LS mean +1 SE). The membranes were placed approximately two weeks after the application of the fertilizer treatment (June 10) and remained in the soil for three weeks. The symbols above the bars (♦) indicate the maximum value used in the calculation of relative nitrogen depletion.

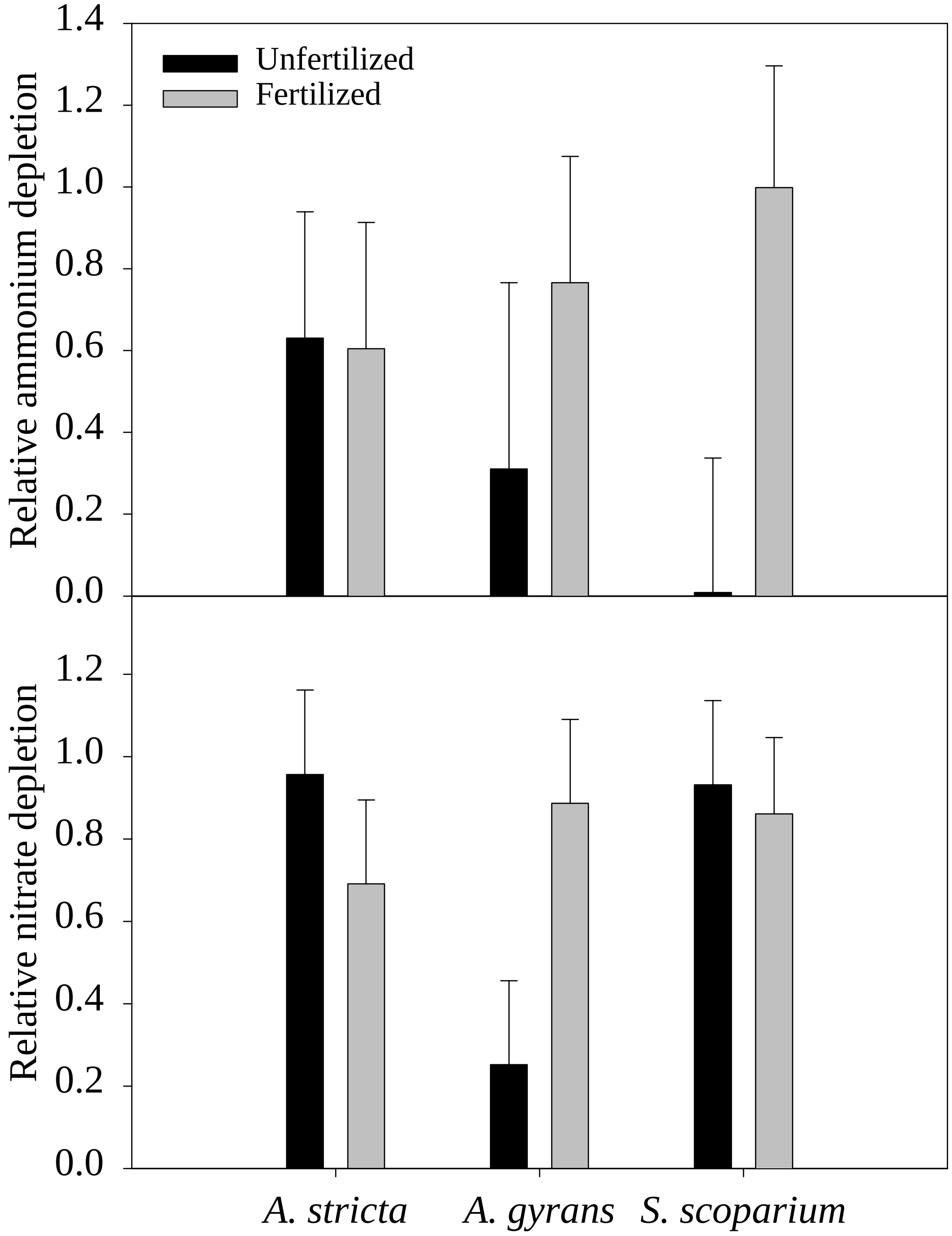

Figure 3.3. Relative depletion of inorganic N (LS mean +1 SE) based on IEM extractions. The membranes were placed approximately two weeks after the application of the fertilizer treatment. Relative depletion was calculated as: ([Max unvegetated N] – [Vegetated N])/[Max unvegetated N]. There was a significant species*treatment interaction for both NH4+ and NO3- ($p<0.05$, see Table 3.1 for statistical comparisons).

Table 3.2a. Repeated-measures analysis of pre-treatment root production and death. The analysis includes December 5, 2000, March 13, and May 23 2001.

Source	$F_{nDF,dDF}$
Cumulative production	
Species	$F_{2,68} = 0.4$
Time	$F_{2,68} = 25.96$***
Species * Time	$F_{4,68} = 0.88$
Cumulative death	
Species	$F_{2,68} = 0.17$
Time	$F_{2,68} = 40.2$***
Species * Time	$F_{4,68} = 0.08$

Table 3.2b. Repeated measures analysis of post-treatment root production and death. The analysis includes August 17 and October 1. Fertilizer was applied on May 23.

Source	$F_{nDF,dDF}$
Cumulative production	
Species	$F_{2,39}$ = 1.95
Treatment	$F_{1,39}$ =18.10***
Species * Treatment	$F_{2,39}$ = 5.15**
Time	$F_{1, 39}$ = 1.39
Species * Time	$F_{2, 39}$ = 0.17
Time * Treatment	$F_{1, 39}$ = 0.06
Species * Time * Treatment	$F_{2, 39}$ = 0.16
Cumulative death	
Species	$F_{2, 39}$ = 4.82**
Treatment	$F_{1, 39}$ = 0.91
Species * Treatment	$F_{2, 39}$ = 4.11*
Time	$F_{1, 39}$ = 3.49
Species * Time	$F_{2, 39}$ = 0.07
Time * Treatment	$F_{1, 39}$ = 0.29
Species * Time * Treatment	$F_{2, 39}$ = 0.09

***Significant at P<0.0001; **significant at P<0.01; *significant at P<0.05

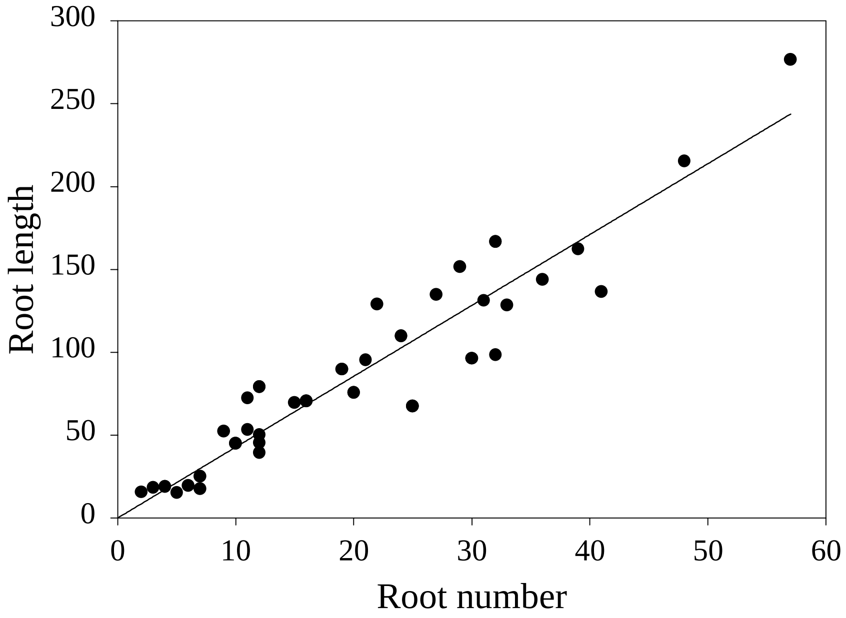

Figure 3.4. Root length versus root number (y = 4.28x, $R^2 = 0.96$, $p<0.0001$). Each point represents the total for an individual minirhizotron tube.

Table 3.3. Repeated measures analysis of post-treatment live root number. The analysis includes August 17 and October 1. Fertilizer was applied on May 23.

Source	$F_{nDF,dDF}$
Species	$F_{2,38} = 0.79$
Treatment	$F_{1,38} = 12.47$**
Species * Treatment	$F_{2,38} = 5.30$**
Time	$F_{1, 38} = 0.02$
Species * Time	$F_{2, 38} = 0.23$
Time * Treatment	$F_{1, 38} = 0.06$
Species * Time * Treatment	$F_{2, 38} = 0.01$

***Significant at P<0.0001; **significant at P<0.01; *significant at P<0.05

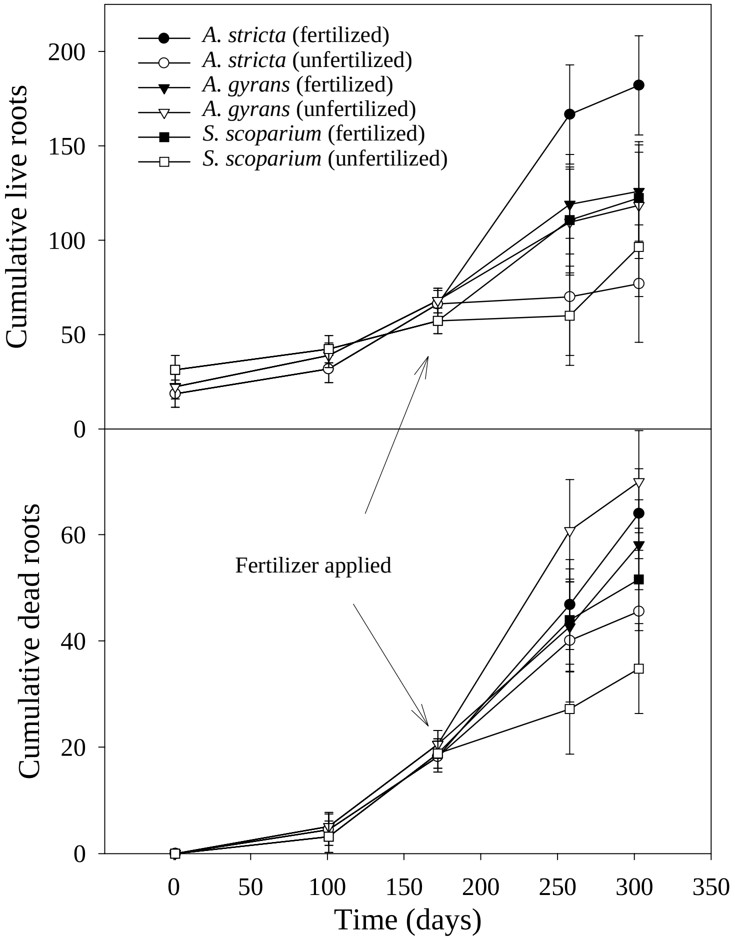

Figure 3.5. Cumulative root production and death (LS mean ±1 SE). Minirhizotron tubes were imaged on December 5, 2000, March 13, May 23, August 17, and October 1, 2001.

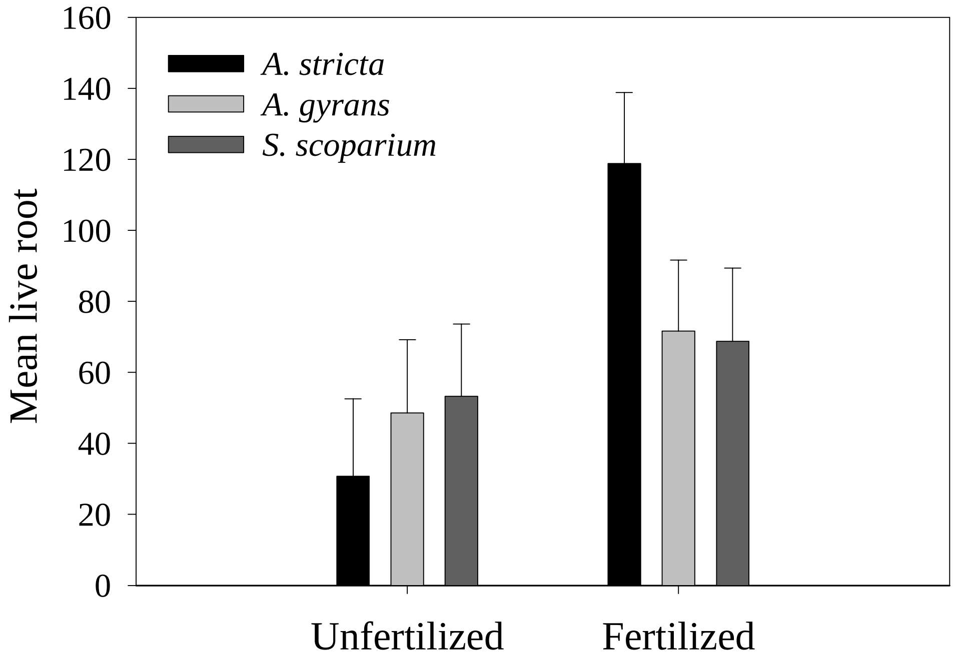

Figure 3.6. Mean standing live root number pooled across the final two observation dates There was no significant time effect and a significant species * treatment interaction (P<0.01, see Table 3.3).

This is inconsistent with the hypothesized tradeoff between adaptation to low resource habitats and morphological plasticity (Grime et al. 1991, Eissenstat and Yanai 1997). Instead these results suggest an alternative tradeoff between the ability to deplete soil resources at high versus low levels of availability.

The relative abilities of these species to deplete the available inorganic N pool depend on the size of that pool and the form of available N. Although nutrient uptake kinetics were not measured here, these results lend support to the argument that high-resource adapted plants have a greater capacity for increased uptake kinetics than do low-resource adapted plants (Bassirirad 2000) since *S. scoparium* depleted nearly 100% of the maximum available NH_4^+ in the fertilized plots. This capacity, however, appeared to result in an inability to deplete available pools when those pools are small. This trend, however, also depended on the form of available nitrogen. Both *A. stricta* and *S. scoparium* showed similar abilities to deplete nitrate when nitrate pools are large and small. *Andropogon gyrans*, however, exhibited an increased capacity for both NH_4^+ and NO_3^- uptake when fertilized.

We have made the assumption that plant uptake was responsible for the difference between vegetated plots and unvegetated plots. It has often been assumed that microbes are better competitors for N than are plants (Hodge et al. 2000), suggesting that this assumption may not be accurate. However new evidence suggests that plants may compete quite successfully with microbes for available N (Hodge et al. 2000). In addition, the variable we measured was a passive sampling of the available inorganic N pool in all plots and the depletion of that pool relative to a maximum value obtained in unvegetated plots. Microbial immobilization, if important to the depletion of the available pool, would have occurred in all plots and no plant or species effect would have been evident. On the other hand, it is likely that the vegetated plots supported higher microbial biomass since root exudation and turnover likely stimulate microbial growth (Hamilton and Frank 2001). In order for microbial uptake to account for the observed pattern, however, the individual plant species would need to have supported different microbial communities since the pool depletion differed substantially between species. We argue that a more parsimonious explanation for the differences among species is plant uptake, although future work is planned to explore this question further.

The differential response among species in root proliferation following the nutrient pulse was surprising since *A. gyrans* did not respond to the treatment at all, *S. schizachyrium* responded with significant root proliferation, but *A. stricta*, the species with the most conservative nutrient use strategy, produced the highest number of roots in the fertilized treatment. The high degree of morphological plasticity in *A. stricta* demonstrated here indicates that low-nutrient adapted plants with long root lifespans may also be quite morphologically plastic in response to pulses of nutrient availability. For these species, the observed differences in plasticity are not linked to differences in root diameter. Although thinner roots are always more efficient than thick roots, no relationship between root diameter and such things as root lifespan or morphological plasticity has yet been established (Eissenstat and Yanai 1997). They may, however, be related to other morphological or physiological characteristics such as specific root length or uptake capacity. The roots of these species are very fine (130 μm diameter), and are likely all very efficient at belowground resource capture. Although we did not quantify mycorrhizal infection in this study, it is interesting to note that *A. stricta* is non-mycorrhizal in Florida sandhills (Anderson and Menges 1997). If this is the case for the plants in this study site, then root characteristics such as the ability to capitalize on nutrient pulses or extract nutrients from very infertile soils will be important determinants of survival and relative competitive dominance.

It is possible that we underestimated the root proliferation response to the applied nutrient pulse. If there was a short-term proliferation of roots, followed by high mortality of that cohort, the long time interval between my pre-treatment sampling and post-treatment sampling would not have recorded that event. This is certainly not an unrealistic expectation since a previous study has showed relatively high mortality of *S. scoparium* roots in intervals of 8 weeks or less (West, Espeleta, and Donovan, unpublished). Although a shorter interval was planned and could not be completed because of logistical difficulties, the data to answer this question strictly are not available. It is interesting to note, however, that although this was a very short-term pulse, there are clearly long-term effects that are species-specific. *Aristida stricta* had significantly more live roots than did the other two species four months after the nutrient application. The long-lived nature of its roots suggests that this higher root number will be retained, giving it a likely future advantage in resource capture. This possibility suggests an additional hypothesis. Although Anderson and Menges (1997) concluded that there was no pulse of inorganic nutrient availability following a prescribed fire, they did show a significant, short-term increase in tissue N concentration in *A. stricta* in response to the fire. This pulse may result in increased proliferation of long-lived roots. The ecosystem in which this species is dominant is dependent on these frequent, low-intensity fires to maintain its savanna-like structure (Glitzenstein et al. 1995). If those fires are associated with pulses in resource availability, *A. stricta* may continue to capitalize on them by producing increased root biomass with each pulse. Experiments are currently planned to test this hypothesis.

The results did not support either of my hypotheses. First, although *A. stricta* is adapted to low-nutrient habitats, it responded to the nutrient pulse with the greatest increase in root number. Second, *A. stricta* was less capable of exploiting the nutrient pulse than were the other species. *Aristida stricta* demonstrated a greater capacity to deplete small inorganic nitrogen pools than either of the other two species studied. These results suggest that a tradeoff instead exists between the ability to deplete small N pools and the ability to deplete large pools. *Schizachyrium scoparium* did not significantly reduce pool sizes under unfertilized conditions, but was capable of depleting nearly all of the maximum potentially available N in the fertilized plots. In addition, the lower amount of root proliferation in fertilized plots relative to *A. stricta* suggests that increased uptake capacity was an important component of the response of *S. scoparium* to the nutrient pulse. These differences may partially explain the relative dominance of the most infertile sites by *A. stricta* and relative increases in abundance of *S. scoparium* on more fertile sites. Given the very sandy nature of the soils of the southeastern Coastal Plain and the frequent occurrence of fire in these ecosystems, pulses of soil resources are likely to be common. In addition to their potential importance to competitive interactions between these species, the species-specific differences demonstrated here are likely to have important consequences for nutrient cycling on sites where they co-occur, potentially increasing the spatial heterogeneity of nutrient availability.

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CHAPTER 4

FINE ROOT DYNAMICS WITHIN AND ACROSS THE COMPLEX EDAPHIC GRADIENT OF THE FALL-LINE SANDHILLS.[1]

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[1] West, J. B., J. F. Espeleta and L. A. Donovan. To be submitted to *Ecology*

ABSTRACT

Across an edaphic gradient in a fall-line sandhill longleaf pine savanna ecosystem, fine root production and death increased with increased soil resource limitation, as described by vegetation structure, soil type, and total soil N. This pattern is consistent with the hypothesis that plants should allocate greater C to roots under conditions of limiting soil resource availability. Fine root production and death occurred year-round with significant growing season peaks in production. No seasonal patterns were evident for root death. Root turnover, however, was highest at the intermediate point of the gradient. This peak in fine root turnover coincided with peaks in potential net N mineralization and microbial biomass N. This pattern suggests that the controls on root turnover differ from those on total root production and death and may be more closely related to sizes of labile N pools or competition with microbes than overall soil resource availability. The ranges of turnover rates reported here cover the spectrum of rates reported for global trends, suggesting much greater within-ecosystem variability in turnover than across-biomes. Within the middle of the gradient, the two grass patch types were largely similar in soil characteristics and fine root dynamics. Within the top 30 cm of soil, the wiregrass patches, however, exhibited significant winter root growth, whereas little was recorded in the bluestem patches. Consistent with aboveground phenology, this pattern suggests important differences between C4 grasses in their effects on belowground ecosystem function.

INTRODUCTION

Ecosystem functions such as carbon and nutrient cycling are tightly linked to fine root production and turnover (Aber et al. 1985, Nadelhoffer et al. 1985). An improved understanding of soil resource effects on fine root dynamics, and the importance of community composition in modulating this response, would significantly improve our ability to predict fine root response to change (Jackson et al. 2000). Root growth is expected to decrease with increasing resource availability (Grime et al. 1991), but responses of ecosystem-scale patterns in fine root dynamics are not consistent (Keyes and Grier 1981, Pregitzer et al. 1995, Burton et al. 2000). Although clear patterns in root turnover are evident at a global scale (Gill and Jackson 2000), the relationships between resource availability and fine root dynamics for any particular ecosystem cannot currently be predicted from these trends (Burton et al. 2000, Gill and Jackson 2000).

The *P. palustris* (longleaf pine) savanna ecosystems of the fall-line sandhills exhibit strong soil resource limitations and are dependent on frequent (<10 year return interval) fires to maintain a savanna-like vegetative structure (Christensen 1988). These savannas once dominated the southeastern coastal plain of the U.S., but due to land-use changes and fire suppression currently cover less than two percent of their former range (Noss 1989).

The objectives of this study were: 1. To determine whether patterns in fine root dynamics (<2cm) *across* edaphic gradients of the sandhills could be explained by nutrient availability at three points along that gradient, and 2. To determine, *within* a portion of the gradient, whether distinct grass patch types exhibited differential nutrient and fine root dynamics.

METHODS

I conducted this study at the Carolina Sandhills National Wildlife Refuge (Lat. 34°33’48” N, Long. 80°13’29” W). The refuge is composed of resource-limited fall line sandhills that are dominated by *P. palustris*. These savannas are maintained by frequent prescribed burning and are characterized by having an open canopy, a sparse midstory of *Quercus* spp., and a relatively dense herbaceous layer dominated by C4 bunchgrasses. The dominant grass species is *Aristida stricta* (wiregrass), but other C4 grasses are often found co-dominating. The bluestems (*Andropogon* spp. and *Schizachyrium scoparium*) often form distinct patches of dominance in the understory. Mean annual temperature is 15°C and the mean annual rainfall is 1200 mm, with little seasonality in precipitation. Although the edaphic gradients are complex in these systems, there is a topographic gradient that generally corresponds to soil moisture availability (Jacqmain et al., 1999). The ridge tops are relatively dry, deep sands (xeric), the side slopes are intermediate (subxeric) and the foot slopes are relatively shallow, moist soils (mesic). The herbaceous layer of these savannas is dominated by C4 perennial bunchgrasses.

100 m^2 plots were established in three replicate stands in February 1999 (April 1999 for mesic plots), at three points along the sandhill gradient (xeric, subxeric, and mesic), for a total of nine sites. All sites have been regularly burned and were burned in the spring of 1998. The gradient is described both by the presence of changes in oak species co-dominance, as well as accompanying changes in soil type. *Quercus laevis*

dominates the xeric portion (typically thermic, coated Typic Quartzipsamments), there is a mix of *Quercus* spp. (*Q. laevis*, *Q. incana, and Q. margaretta*) at the intermediate portion (typically sandy, siliceous, thermic Arenic Paleudults), and species such as *Q. marilandica* are found dominating at the mesic end of the gradient (typically Fine-loamy, siliceous, thermic Aquic Hapludults). Within all sites, single plots were established within wiregrass-dominated patches to allow gradient comparisons. In order to compare herb-layer patches dominated by different grass species, additional plots were established in bluestem-dominated patches within the subxeric sites. The bluestem patches were primarily dominated by *Andropogon ternarius*, *A. gyrans*, and *S. scoparium*.

Within each plot, three clear minirhizotron tubes (2 m long x 5.08 cm inside diameter) were installed at a 30° angle to the soil surface to a vertical depth of approximately 65 cm (see Hendrick and Pregitzer 1992). The tubes are scribed down the length of the tube with regular 0.9 x 1.3 cm rectangles to allow repeated imaging of the same locations. The tubes were installed with the scribed surface pointing upward. The portions of the tubes extending above the soil surface were wrapped in reflective tape and capped to prevent light and water from entering. The tubes were imaged using a Hi-8 camcorder attached to a Bartz Minirhizotron Research camera (Bartz Technology Co., Santa Barbara, CA) on the following dates: June 22, July 15, August 19, September 24, December 18, 1999; February 4, March 18, June 9, July 15, August 28, October 9, December 6, 2000.

The initial imaging date was analyzed using a PC-based image analysis system (ROOTS, Hendrick and Pregitzer 1992). Using a TARGA videographics card (Truevision Inc., Indianapolis, IN) root numbers, widths and lengths were traced to the nearest

0.1mm. This analysis allowed the generation of a regression between root number and root length. Because of the time intensive nature of data collection, following the first date all data is thereafter based on root numbers. Roots were counted by first digitally capturing individual images of each frame from each tube. Roots were then counted as “new,” “brown,” or “unsuberized” (white, but not new). We quantified new root production and disappearance (=death) by simultaneously viewing images from consecutive dates and recording the number of roots that disappeared between dates and those that were new. We were unable to use root color or other characteristics to determine root death, so root death is quantified solely based on root disappearance. Although this does not discriminate between herbivory, pathogens and root senescence, it represents root biomass that is lost and transferred to soil pools.

Two depth classes were established (0-30cm and 30-65cm) and the gradient and patch analyses were conducted separately. New and cumulative root production and cumulative death were analyzed using a mixed-model repeated measures analyses. Fine root turnover was calculated in three ways: 1. Total number of roots produced/maximum number of live roots (Gill and Jackson 2000), 2. Total number of roots produced/mean number of live roots, and 3. Total number of dead roots/mean number of live roots Burton et al. 2000). Separate analyses of the effect of gradient and patch type were conducted using a mixed-model ANOVA.

For all soil sampling, cores were augured at least 1 meter directly behind each minirhizotron tube (three cores per plot). Soil samples collected in February 1999 (April for mesic plots) from two depths (5.5 cm dia. at two depths: 20-30cm and 60-70cm) were analyzed for total carbon and nitrogen (Micro-Dumas combustion analysis). Carbon and nitrogen were undetectable at 70 cm and are therefore not reported. These soils were also sieved (2mm dia. mesh) and analyzed for extractable phosphorous using a double acid (Mehlich #1) extraction followed by continuous flow (con-flow) colorimetric analysis of orthophosphate (Jones 1990). Sieved (2 mm dia. mesh) soil cores taken in September 1999 (3.5 cm dia. at 5-10cm depth) were analyzed for microbial carbon and nitrogen content using a fumigation/direct extraction technique (Vance et al. 1987). Potential net nitrogen mineralization rate was estimated using laboratory incubations of soil collected in August 2000 (3.5 cm dia. at 2-10 cm depth). Incubations were 30 days long and at 23°C in the dark. Pre- and post-incubation extractions were done with a 2 M KCl extract solution. The extractant was filtered and analyzed for either NH_4^+ or NO_3^- by con-flow colorimetric analysis. For both microbial biomass and potential N mineralization, soils were collected, placed in sealable plastic bags in an ice-filled cooler, and then transported to the laboratory where they were stored at 3°C until the analyses could be performed. Loss of nitrogen to 100 cm was estimated by using cation and anion exchange membranes (AR204-SZRA and CR67-HMR, Ionics, Inc., Watertown, MA). These membranes are functionally identical to ion exchange resin beads placed in bags (Cooperband and Logan 1994). At three locations in each plot, a hole was augured to 100 cm. A 5 cm x 5 cm cation and anion membrane was each attached to fishing line and placed at the bottom of the hole that was then re-filled. After one month, the membranes were retrieved, rinsed with distilled water and extracted with 2 M KCl. The extractant was filtered and analyzed for either NH_4^+ or NO_3^- by con-flow colorimetric analysis.

RESULTS

Gradient analyses

Total C and N varied predictably with the gradient (Figure 4.1). Both C and N exhibited the lowest concentrations at the xeric end, although gradient differences in C were only marginally significant (Table 4.1). Phosphorous was more uniformly distributed with depth at the mesic end, but overall had higher availability in subxeric and xeric sites (Figure 4.1). Potential N mineralization and microbial biomass N, however, exhibited distinct peaks at the middle of the gradient (Table 4.2). Leaching of N to 100cm showed no significant change across the gradient.

Greater fine root production occurred at the xeric end of the gradient, where soil type and total N suggest greatest soil resource limitation (Figure 4.3). Root production occurred year-round with clear peaks in root production during the summer months (Figure 4.4). The seasonal trends in cumulative root production differed, however, along the gradient (Table 4.3). Mesic root production appeared less strongly seasonal than xeric or mesic (Figure 4.2). Root death also exhibited trends consistent with gradient differences, with no seasonal differences (Figure 4.5).

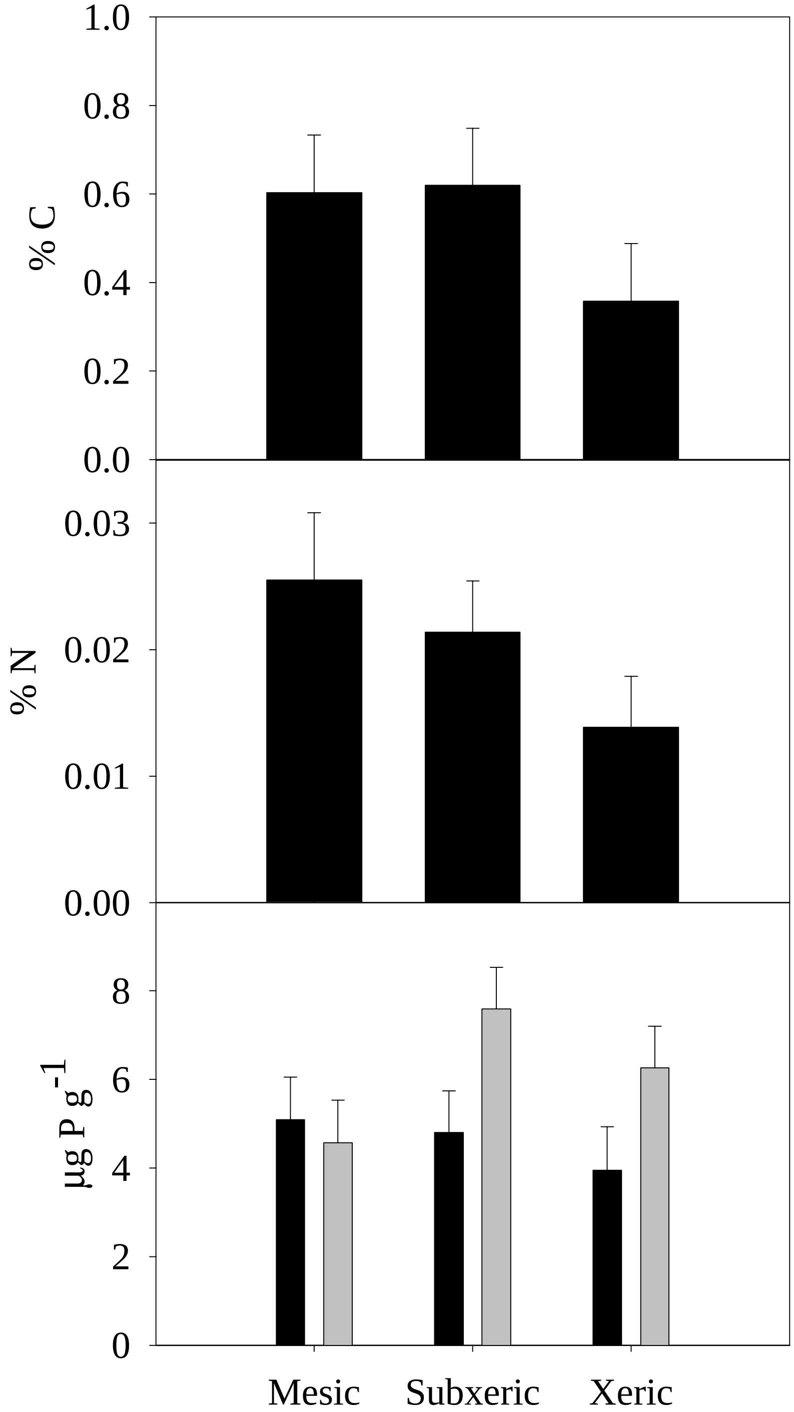

Figure 4.1. Total soil C, N and extractable P. Black bars are 20-30cm soil depth and gray bars are 60-70 cm. C and N were undetectable at 60-70 cm.

Table 4.1. Analysis of soil carbon and nutrient characteristics. Gradient and patch analyses were conducted separately.

Soil nutrient characteristics	nDF	dDF	F
Gradient analysis			
Total soil carbon	2	22	2.64^
Total soil nitrogen	2	16	4.88*
Extractable soil phosphorous			
Gradient	2	23	1.93
Depth	1	22	10.13**
Gradient * Depth	2	22	4.61*
Microbial carbon	2	24	0.34
Microbial nitrogen	2	24	4.95*
Potential nitrogen mineralization	2	16	12.05***
Leaching	2	17	0.32
Patch analysis			
Total soil carbon	1	7	0.17
Total soil nitrogen	1	6	0.42
Extractable soil phosphorous			
Patch	1	24	1.93
Depth	1	24	12.95***
Patch * Depth	1	24	2.24
Microbial carbon	1	16	0.05
Microbial nitrogen	1	14	0.33
Potential nitrogen mineralization	1	13	0.48
Leaching	1	15	0.49

^ $P<0.10$, *$P<0.05$, **$P<0.01$, ***$P<0.001$

Table 4.2. Soil nutrient characteristics across gradient and between patch types.

Soil nutrient characteristics - gradient	Mesic	Subxeric	Xeric
Microbial carbon (µg C g^{-1})	123.40 (32.63)	92.03 (26.64)	93.52 (23.07)
Microbial nitrogen (µg N g^{-1})	20.08 (2.35)	24.89 (1.93)	16.89 (1.67)
Potential N min. (µg NH_4NO_3 g^{-1})	2.43 (1.43)	4.53 (1.43)	0.20 (1.43)
Leaching (mg NH_4NO_3 L^{-1})	9.65 (1.51)	10.11 (1.31)	11.20 (1.51)

Soil nutrient characteristics - patch	Wiregrass	Bluestem
Microbial carbon (µg C g^{-1})	92.03 (34.84)	103.57 (34.84)
Microbial nitrogen (µg N g^{-1})	24.89 (2.04)	26.66 (2.32)
Potential N min. (µg NH_4NO_3 g^{-1})	2.63 (1.63)	3.12 (1.63)
Leaching (mg NH_4NO_3 L^{-1})	8.94 (1.51)	10.11 (1.22)

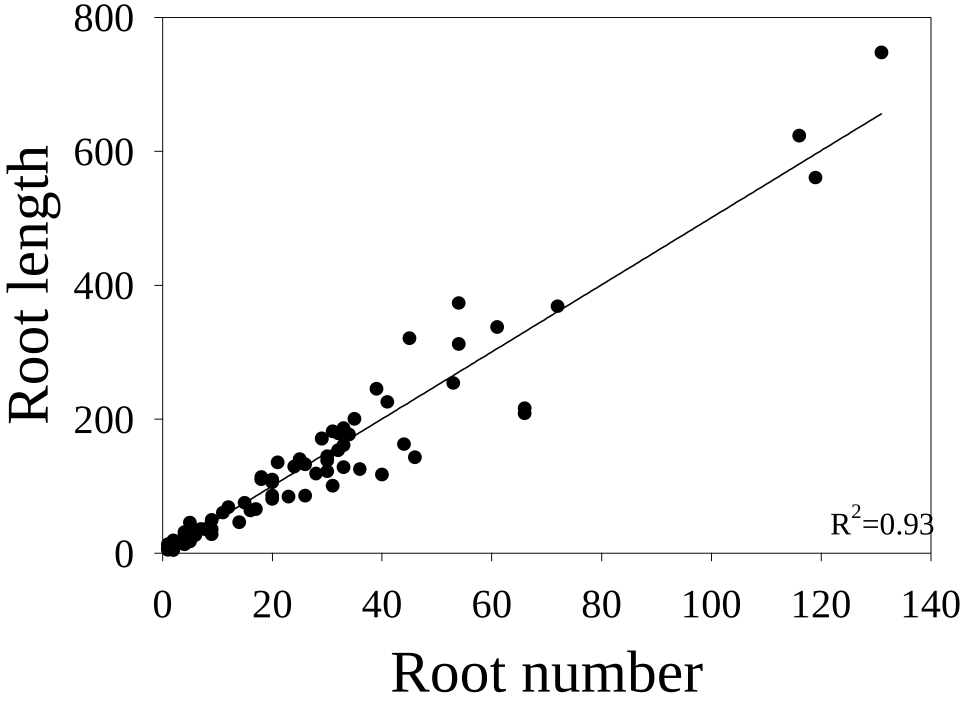

Figure 4.2. Regression of root length against root number. Data are pooled across plots and are from June 1999.

Table 4.3. Repeated-measures analysis of cumulative root production and death. Gradient and patch analyses done separately.

Cumulative Production	dDF	nDF	F
Gradient analysis			
Time	11	138	24.51***
Gradient	2	138	7.08***
Time * Gradient	22	138	3.41***
Depth	1	138	5.10*
Time * Depth	11	138	0.78
Time * Gradient * Depth	22	138	0.87
Patch analysis			
Time	11	94	10.08***
Patch	1	94	0.06
Time * Patch	11	94	1.18
Depth	1	94	0.52
Time * Depth	11	94	0.32
Patch * Depth	1	94	1.56
Time * Species * Depth	11	94	1.00

Cumulative Death	dDF	nDF	F
Gradient analysis			
Time	11	138	14.89***
Gradient	2	138	4.99**
Time * Gradient	22	138	3.10***
Depth	1	138	4.67*
Time * Depth	11	138	2.35**
Gradient * Depth	2	138	0.21
Time * Gradient * Depth	22	138	0.52
Patch analysis			
Time	11	94	10.23***
Patch	1	94	0.63
Time * Patch	11	94	1.69^
Depth	1	94	2.58
Time * Depth	11	94	2.61**
Patch * Depth	1	94	0.59
Time * Patch * Depth	11	94	0.83

^ P<0.10, *P<0.05, **P<0.01, ***P<0.001

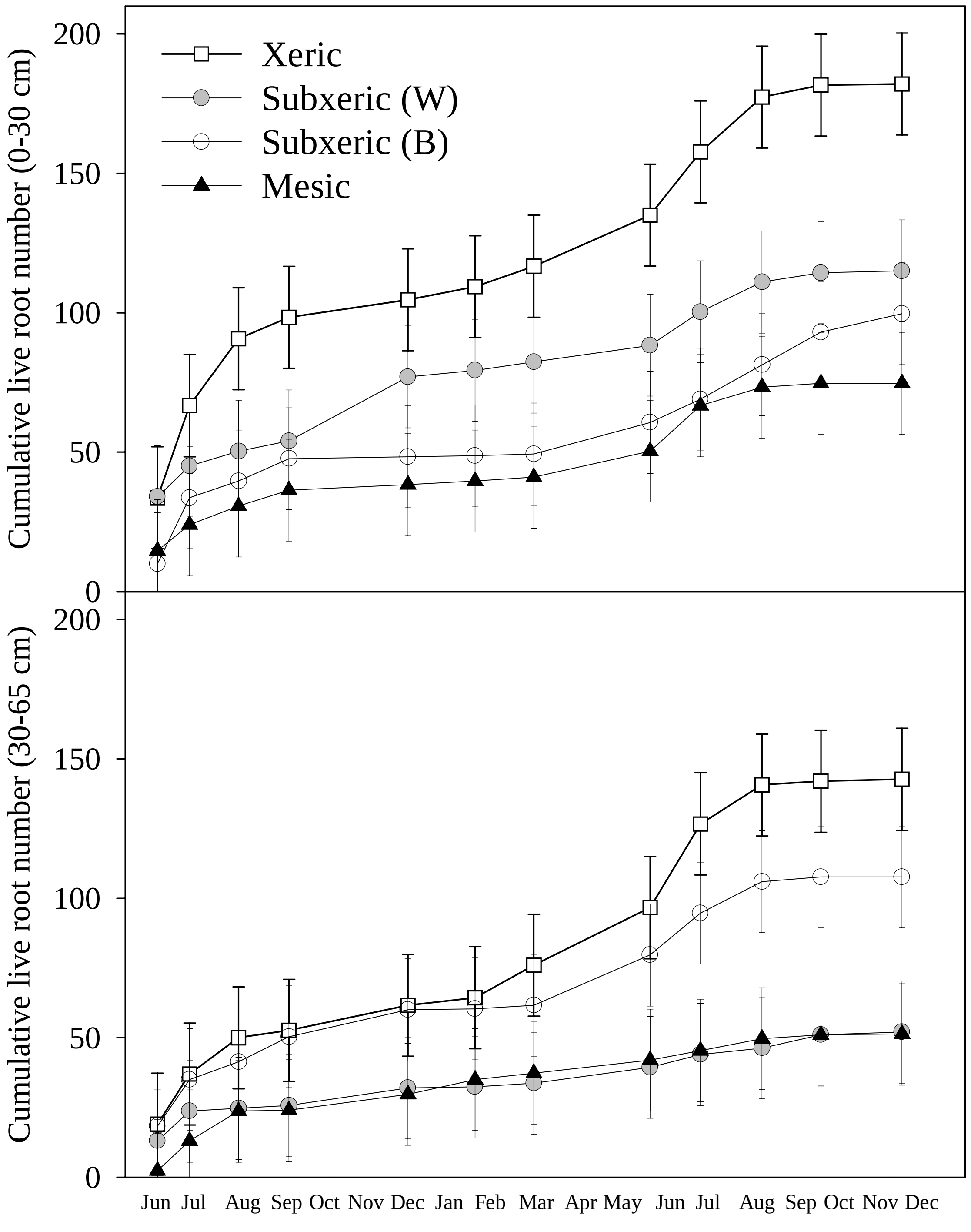

Figure 4.3. Mean cumulative live root number ±1 SE. Separate analyses were conducted for the gradient and patch comparisons (W = wiregrass patch, B = Bluestem patch). See Table 4.3 for statistical comparisons.

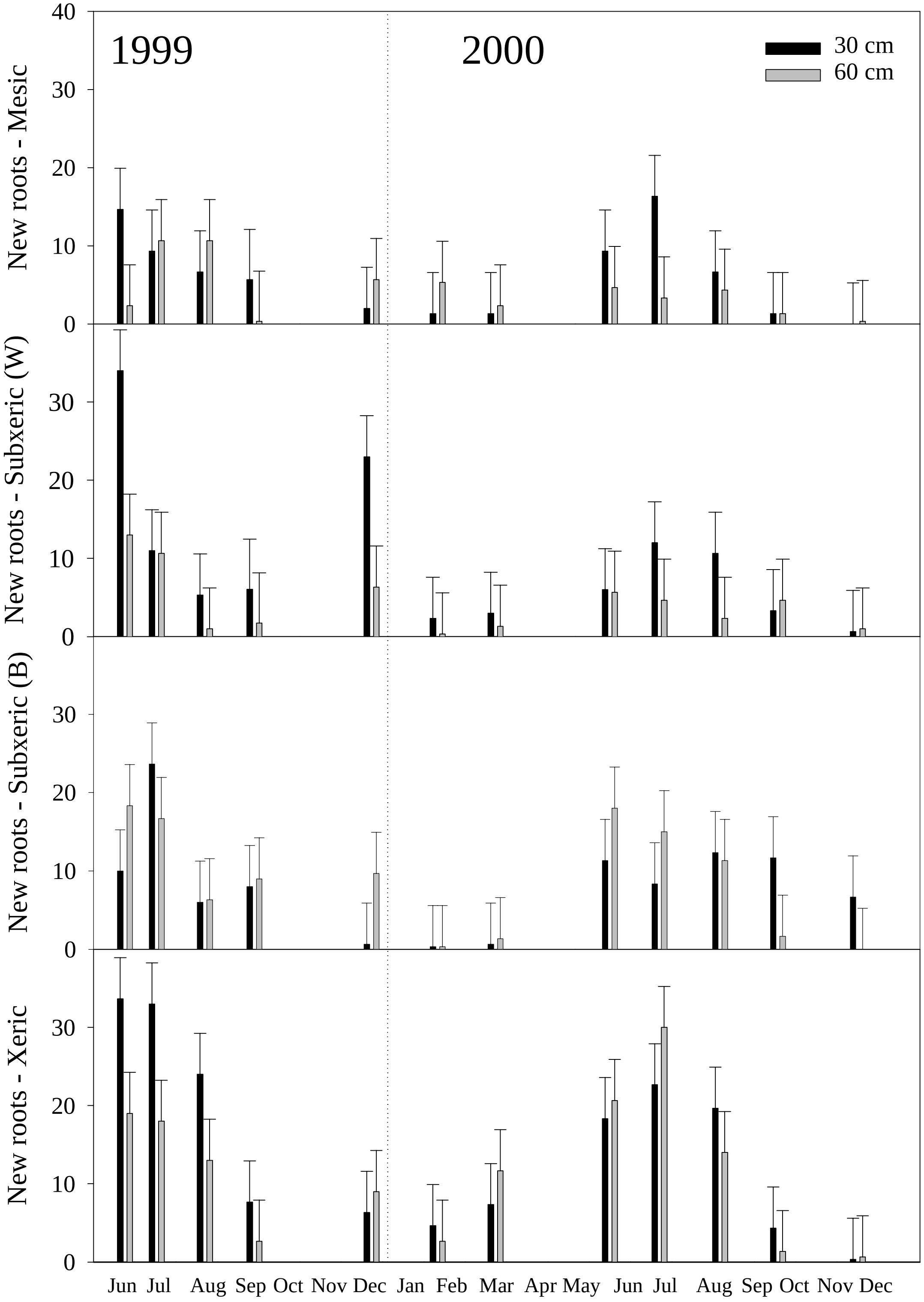

Figure 4.4. Mean monthly root production ±1 SE. Separate analyses were conducted for the gradient and patch comparisons. See Table 4.4 for statistical comparisons.

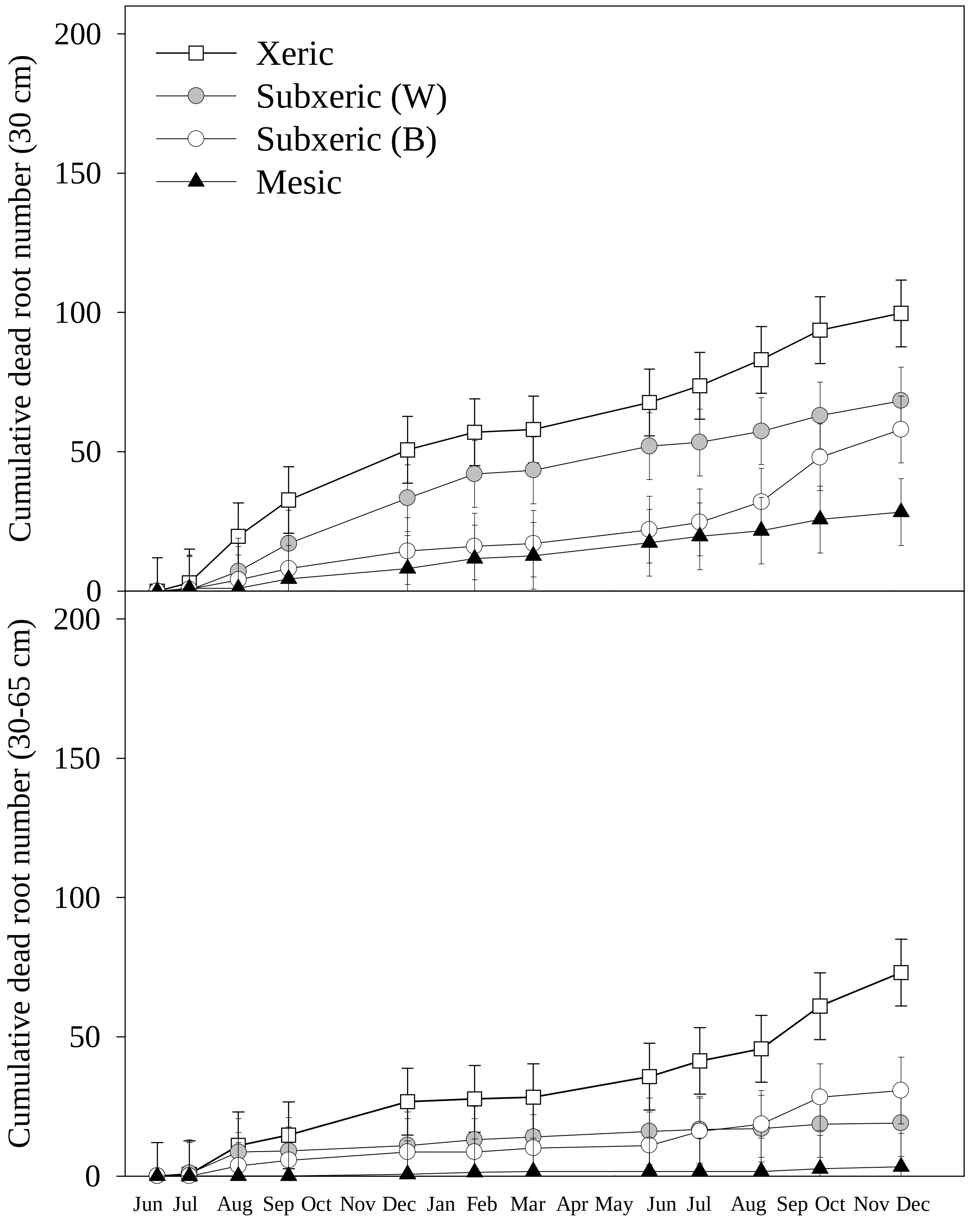

Figure 4.5. Mean cumulative dead root number ±1 SE. Separate analyses were conducted for the gradient and patch comparisons (W = wiregrass patch, B = Bluestem patch). See Table 4.3 for statistical comparisons.

Fine root turnover, however, followed the trends in potential N mineralization and microbial N exhibiting a peak in the middle of the gradient (Table 4.5). All three measures of turnover agree qualitatively, although there were important quantitative differences between them (Table 4.5).

Patch analyses

No significant differences were found between patch types in any of the soil characteristics measured (Figure 4.6, Table 4.1).

Although no significant differences in cumulative root production were found (Table 4.3), new root production showed a significant difference between patches with respect to overall seasonal production (Table 4.4). This is likely due to the greater winter root production in wiregrass patches relative to bluestem patches (Figure 4.4). Cumulative death showed no significant differences between patch types. There were no significant differences between patch types in any of the estimates of turnover.

DISCUSSION

Consistent with previous reports for this system (Saterson and Vitousek 1984), root production and death occurred all year. Year-round root production has been documented in several northern, deciduous forests (e.g., Hendrick and Pregitzer 1992), resulting presumably from stored C reserves (Chapin et al. 1990).

Table 4.5. Estimates of fine root turnover [mean (1 SE)]. Prod/max represents total root productivity divided by the maximum live root number (see Gill and Jackson 2000). Prod/mean is total productivity divided by the mean live root number, and death/mean is the total root death divided by the mean live root number (see Burton et al. 2000).

Fine root turnover	Prod/max		Prod/mean		Death/mean	
Gradient						
Mesic	1.22 (0.19)	$F_{2,9}$=6.48*	1.59 (0.24)	$F_{2,9}$=7.53**	0.36 (0.26)	$F_{2,9}$=5.15*
Subxeric	2.18 (0.19)		2.91 (0.24)		1.47 (0.26)	
Xeric	1.66 (0.19)		2.35 (0.24)		1.23 (0.26)	
Patch						
Bluestem	1.74 (0.25)	$F_{1,8}$=1.48	2.36 (0.35)	$F_{1,8}$=1.58	1.29 (0.36)	$F_{1,8}$=0.12
Wiregrass	2.17 (0.25)		2.91 (0.35)		1.47 (0.36)	

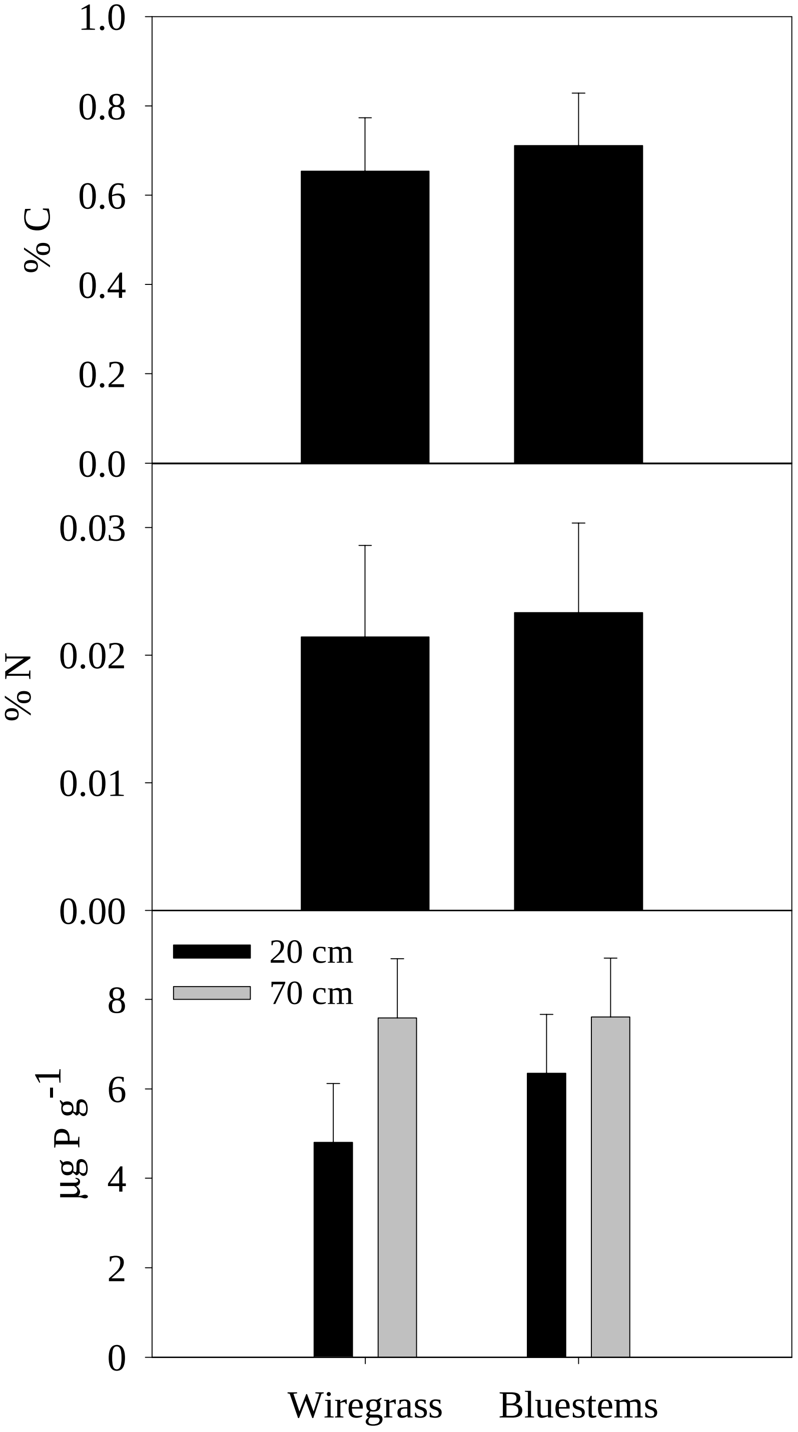

Figure 4.6. Total soil C, N and extractable P. Black bars are 20-30cm soil depth and gray bars are 60-70 cm. C and N were undetectable at 60-70 cm.

Table 4.4. Repeated-measures analysis of new root production. Gradient and patch analyses done separately.

New root production	nDF	dDF	F
Gradient analysis			
Time	11	92	6.66***
Gradient	2	92	6.08**
Time * Gradient	22	92	1.21
Depth	1	92	4.34*
Time * Depth	11	92	1.00
Gradient * Depth	2	92	1.23
Time * Gradient * Depth	21	92	1.33
Patch analysis			
Time	11	57	3.74***
Patch	1	57	0.00
Time * Patch	11	57	2.16*
Depth	1	57	0.18
Time * Depth	11	57	0.72
Patch * Depth	1	57	2.34
Time * Patch * Depth	10	57	1.04

*P<0.05, **P<0.01, ***P<0.001

This is not surprising given the ability of the trees to produce roots from stored reserves, in addition to the retention of green leaves (and therefore continued photosynthesis) by the dominant tree (*P. palustris*) as well as the dominant grass (*A. stricta*) year-round. Root production showed significant peaks during the summer months, consistent with the most active period of photosynthesis. Root death, however, did not exhibit any seasonal trends and showed relatively consistent rates over time. The lack of a seasonal pattern of root death suggests that controls on root death differ substantially from those on leaf senescence, for example (Eissenstat and Yanai 1997). One controlling factor that typically receives little attention in the literature is losses through herbivory or parasitism, although these losses are likely to be significant for most ecosystems (Eissenstat and Yanai 1997). Since we rarely observed gradual root senescence followed by death, we suggest that root death in this system may primarily occur as a result of herbivory (also Mitchell, RJ pers. comm.). Although plants can clearly control losses to herbivores to some extent, the patterns seen here suggest that these losses are significant in this system.

Overall, approximately 52% of the total root number was in the top 30 cm (data not presented). This is consistent with distributional patterns found for tropical savannas or temperate coniferous forests (Jackson et al. 1996). However, greater root production and death was evident in the top 30 cm, reflecting the more dynamic water and nutrient availability likely to occur within that profile.

Although production and death varied predictably across the gradient, root turnover did not. Root turnover exhibited patterns consistent with those found for potential N mineralization and microbial biomass N. Surprisingly, both potential N min and microbial biomass N were highest at the middle of the gradient. For all measures of root turnover, this is also where the highest rates occurred. Because of this disconnect between root production and root turnover, we suggest that the controls on production and total root death are different from those on turnover. It may be that the ratio of annual production to standing crop is more tightly linked to the availability of labile N since high N mineralization and microbial biomass N may indicate relatively large, active N pools (especially in the shallow soil horizons). Overall rates of root production, however, may be more closely linked to total soil resource availability. The pattern of increased rates of fine root production and death with decreased total soil N and more xeric (greater sand content – more highly drained soils) indicates increased root allocation to compensate for lower soil resource availability. These patterns also emphasize that, although turnover is an important component of belowground dynamics, higher turnover does not necessarily mean greater belowground C allocation.

The turnover rates reported here are relatively high and, for the xeric and subxeric sites, are much higher than previous reports for this system (0.39 yr^{-1}; Saterson and Vitousek 1984). Saterson and Vitousek (1984) point out, however, that their estimates are minimum estimates because they are based on "max-min" estimates from sequential soil coring. We suggest that the direct-observation technique allowed by the use of minirhizotrons provides a more accurate estimate of fine root turnover in these systems. Since our estimates of turnover based on production were greater than those based on mortality, it may be that the soil and roots observed by the minirhizotron tubes had not yet fully equilibrated after the disturbance necessary for their installation (Joslin and Wolfe 1999). We expect that over time the two estimates would come to equal each other (Burton et al. 2000). As a consequence, however, we believe the most accurate estimate is that based on root death. This being the case, the estimate from Saterson and Vitousek (1984) may be accurate since the site they sampled is comparatively mesic. In comparison to the global data summarized by Gill and Jackson (2000), the range of longevities shown here within one ecosystem, but across an edaphic gradient, showed the full range of variability seen from high latitude to tropical ecosystems. Although there may be global trends in fine root turnover, the data presented here suggest that those patterns are likely to break down for a given ecosystem.

Within the gradient, the patch types were largely similar with respect to both nutrient characteristics and fine root dynamics. Since the patches are largely similar, except for the species composition of the grasses, this result was not surprising and suggests that individual species differences observed at the scale of individual plants or small monocultures may not be evident at larger scales (West and Donovan, unpublished).

One rather striking pattern that did differ between patches, however, was winter root production. Bluestem patches did not exhibit substantial root growth during the winter months, whereas wiregrass patches showed substantial root growth during winter, especially in the 0-30 cm soil horizon. Since grass roots proliferate primarily in the top 30 cm (personal observation), these differences may be attributable to differences in the dominant grass species. Espeleta and West (unpublished) have shown that *A. stricta* produces roots year-round, whereas *S. scoparium* (a dominant in the bluestem patches) ceases root production over the winter. Since the bluestems are all winter deciduous, the lack of winter photosynthesis apparently prevents winter root growth for these species. Although we were unable to ascribe individual roots to particular species, the differential patterns of root growth by the bluestems versus wiregrass, and the tendency for grasses to dominate shallow soil horizons, suggest that this difference is due primarily to differences between the grasses. The lack of root growth during the winter in the bluestem patch is likely to shift patterns of nutrient availability, away from the herbaceous layer pool and either into trees or microbes or lost as a consequence of leaching. Our data do not support hypothesized leaching losses, although we did not collect soil nutrient data during the winter months. As a consequence, although nearly all of the variables measured showed similar belowground properties, even these very morphologically and physiologically similar species may have important, differential effects on belowground dynamics.

In summary, both root production and death followed a predictable trend across the gradient. With increasing overall soil resource limitation, greater root production and death was observed. Root turnover, however, was highest at the intermediate portion of the gradient, coincident with a peak in potential net N mineralization and microbial biomass N. These patters suggest that the controls on root production and death may differ from those on turnover rates, although they are both linked to soil resource availability. Comparisons between wiregrass and bluestem patch types showed largely similar patterns of root dynamics and soil conditions. However, the wiregrass patches showed significant winter root production, especially in the shallow horizon, whereas the bluestem patches exhibited much less winter root production. These patterns suggest that, although the dominant grasses of this system are C4 bunchgrasses, significant differences likely exist in their effects on belowground ecosystem function.

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CONCLUSION

The dominant members of the “C4 perennial bunchgrass” functional group in the sandhill longleaf pine savannas that I studied exhibited several differences in traits relevant to ecosystem function, as well as differential effects on soil processes. My overall results suggest that these species are adapted to different nutrient availabilities (Aerts and Chapin 2000) and that, with some important exceptions, these adaptations largely explain their effects on ecosystem function.

In Chapter 1, I showed that *A. stricta* did not exhibit any changes in growth or allocation in response to a fertilizer addition; whereas, *S. scoparium* responded in a predictable manner by allocating less biomass to roots and increasing total biomass overall. *Aristida stricta* also exhibited a lower overall relative growth rate than *S. scoparium*. These two characteristics are consistent with the division often cited between inherently fast-growing and inherently slow-growing plants (Lambers and Poorter 1992). Inherently fast-growing species are thought to be adapted to high soil resource conditions, whereas slow-growing species dominate habitats with low resource availability. Growing slowly does not appear to have inherent benefits (Lambers and Poorter 1992), so the dominance of these species in low-resource habitats has presented a paradox. Aerts and Chapin (2000) offered an attractive hypothesis for this dominance, at least with respect to adaptation to low soil nutrient availability. That is, slow growth is simply an unavoidable consequence of increased nutrient retention. Plants adapt to low nutrient availability by increasing nutrient retention, and the mechanisms of this retention result in the inability of the plant to exhibit high relative growth rates.

This framework is consistent with much of the data I have collected. As was seen in Chapter 2, the higher leaf C:N ratios of *A. stricta* are consistent with higher nutrient use efficiency relative to the other two bluestems. Other results (data not presented) have shown that this pattern is consistent for senesced leaves as well as root tissue. In addition, West, et al. (unpublished) have shown that the root lifespans of *A. stricta* are longer than those of *S. scoparium*. The longer lifespans of these root tissues are consistent with the retention of leaves year-round by *A. stricta* and the winter deciduousness of the bluestems and indicate a more pronounced strategy of nutrient retention. Although to my knowledge the term has referred exclusively to woody species, being “evergreen” has been identified as a characteristic of species adapted to low nutrient availability (Aerts 1995). All of these data support the contention that *A. stricta* exhibits a nutrient-retention strategy, whereas the bluestems (especially *S. scoparium*) exhibit a rapid-growth rate strategy that has, as a consequence, increased nutrient losses.

How do these characteristics affect such ecosystem processes as nitrogen cycling? The theory predicting species-specific effects on ecosystem function generally predicts that species should reinforce the nutrient conditions of the habitats to which they are adapted (a positive feedback; Hobbie 1992). So, the effects of a species such as *A. stricta* should result in lower nutrient availability or slower nutrient cycling rates. High-nutrient adapted species such as the bluestems, on the other hand, should foster relatively fertile conditions.

My results suggest that the actual species-specific feedbacks are likely to be more complex than this simplistic view suggests. In Chapter 2, I showed that there were significant, species-specific effects at the scale of individual plants on C mineralization, but no apparent species, or even plant presence, effects on N mineralization. Taken alone, this suggests that the bunchgrasses were having a minimal impact on N mineralization in these soils. There were two lines of evidence, however, that suggested that there are real, species-specific effects obscured by this analysis. First, the initial ammonium pools under *A. stricta* plants were double the size of those under the bluestems. Second, there was a positive relationship between C mineralization and N mineralization for the bluestems, but there was no such relationship for *A. stricta*. Higher ammonium pools under *A. stricta* suggests that wiregrass may not be depleting the available nitrogen as efficiently as the bluestems. This may be consistent with its nutrient-retention strategy since, as is seen in chapter 3, there may be a tradeoff between the ability to deplete N at low levels versus at relatively high levels. The lack of a relationship between C mineralization and N mineralization in *A. stricta* suggests that different microbial communities are associated with this species when compared to the bluestems (Hassink et al. 1994). Although the evidence is primarily indirect, I concluded from these results that there are in fact species-specific differences in their effects on C and N cycling at the scale of the individual plant in this system, but that the microbial communities actually responsible for that mineralization may modulate effects on mineralization rates.

Interpretation of the results from Chapter 2 has the caveat that this study was done using extant plants where cause and effect relationships between plant presence and soil characteristics cannot be explicitly disentangled. It is for this reason that planted monocultures were used to assess the direct effects of these species on soil processes. These monocultures also allowed the experimental addition of nitrogen so that I could determine how species identity and nitrogen availability interacted. The results of root dynamics in this experiment were surprising. *Aristida stricta* exhibited the greatest apparent plasticity in response to the addition of ammonium nitrate by significantly increasing production under enrichment. *Schizachyrium scoparium* exhibited the same trend, but less dramatically. The third species *A. ternarius* showed no root response to the treatment. Species adapted to low soil resource availability should exhibit lower plasticity than those adapted to high resource availability (Lambers and Poorter 1992), although there is very little data with which to judge this hypothesis with respect to fine root dynamics (Eissenstat et al. 2000). My results suggest that low-nutrient adapted species may in fact be capable of a high degree of morphological plasticity in response to changes in nutrient availability.

This experiment also revealed some surprises with respect to plant uptake of inorganic nitrogen. Regardless of the amount of ammonium available in the soil, *A. stricta* depleted approximately 60% of that available. *Schizachyrium scoparium* was apparently incapable of that level of depletion under unfertilized conditions, but depleted nearly 100% of the available ammonium when ammonium availability was elevated. It may be that the ability to efficiently deplete large pools of nutrients comes at the cost of being able to deplete small pools. These apparent tradeoffs have consequences not only for the competitive interactions between these species, but also for the effects individual species have on ecosystem function. Most importantly it suggests that species-specific effects on nitrogen availability may change quite dramatically depending on the absolute amount of nitrogen availability. Clearly, more detailed and species-specific analyses are needed to understand the complex interactions between plants and soils.

A final question in my dissertation was how do these smaller scale processes (individual plant, small monoculture plots) scale to larger scales such as that of distinct vegetation patches in the herbaceous layer of these savannas? In order to address this question I established 100 m^2 plots within herbaceous layer patches of three replicate stands that were dominated either by *A. stricta* or a mix of bluestems (primarily *S. scoparium, A. ternarius,* and *A. gyrans*). Within these plots I monitored fine root dynamics with minirhizotron tubes and several measures of soil nutrient dynamics. The most distinct patches exist within the intermediate portion of the edaphic gradient in the sandhills and so this comparison was made there. In order to place the patch differences within the appropriate context of edaphic variation, however, I also established plots in wiregrass patches in three xeric stands and in three mesic stands. This design allowed me to determine the magnitude of importance of within-stand differences potentially caused by differences among the two patch types versus across-gradient differences caused by a complex of edaphic factors.

The results showed a clear gradient in nutrient availability and fine root dynamics, as well as some interesting complexities with respect to the appropriate descriptor of the edaphic gradient. There were also differences in the responses of root production and death versus the response of turnover to the edaphic gradient. For nearly all root and nutrient characteristics, the different grass patch types did not reveal any significant differences. At this scale, and at the resolution of my sampling design, the overall pattern suggested that the smaller-scale species differences were not important to belowground ecosystem function. This contradicts much of my previous data and suggests that scaling from individual plant to ecosystem may indeed be a challenging endeavor. However, one pattern was consistent with measurements made at the individual plant scale: the wiregrass patches exhibited significant winter root production in the top 30 cm, however the bluestem patches did not show significant production in the same profile during the winter months. Since most of the grass roots occur in the top 30 cm, it is likely that the grasses are significant contributors to this pattern. West et al. (unpublished) observed continuous winter root growth in *A. stricta* in field rhizotrons, whereas (coincident with leaf senescence) root growth essentially stopped for *S. scoparium* during the winter months. It appears that these species-specific seasonal trends in root production result in patch-scale differences in root activity. Since winter nutrient uptake may be an important component of the nutrient cycle in this system (Saterson and Vitousek 1984), these differences reveal important seasonal differences between these patch types in their potential effects on N cycling in this system.

In conclusion, the functional group "C4 perennial bunchgrass" is an inadequate descriptor of the species-specific variation among the dominant bunchgrasses of this sandhill savanna ecosystem. Based on my reading of recent experiments where variation within functional groups was explicitly studied (Hooper and Vitousek 1998, Reich et al. 2001), I believe that this trend will tend to dominate. What, then, would a productive next step be? I suggest reframing the question of the role of plants in ecosystem function, perhaps within the context of Brown's "Complex Adaptive Systems" (Brown 1995). A tremendous amount of work has been done to identify general patterns of plant adaptation to natural environments (Grime et al. 1997, Reich et al. 1997). I believe that linking what is known about *plant traits* to the effects those traits have on ecosystem function would be a productive avenue to pursue. In this way, the focus is on understanding the influences of plant traits on ecosystem function. Species identities are relevant if their traits differ, and not relevant (i.e., they are part of the same functional group) if their traits do not differ. In terms of plant species effects on nutrient cycling this may be done using the framework of nutrient mean residence time (MRT). There is a body of theory that addresses the relevance of different nutrient MRTs in plant tissues to plant adaptation (Berendse and Aerts 1987), and there is a similar body that recognizes the importance of nutrient MRTs in soils (Hart et al. 1994). Understanding the plant-centered forces that structure plant nutrient MRTs and how those relate to soil nutrient MRT will I believe significantly improve our understanding of entire-ecosystem nutrient cycling as well as when and if species-specific differences are relevant.

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