The Effects of Chronic Nitrogen Application on a Mature Tallgrass Prairie Restoration and Nitrogen Mobilization into Above-ground Biomass of *Andropogon gerardii* [1]

Ann M. O'Neill[2,3], Paul E. Rothrock, and Robert T. Reber

Department of Earth and Environmental Science

Taylor University

Upland, IN 46989, USA

O'NEILL, A.M., P.E. ROTHROCK, AND R.T. REBER (Department of Earth and Environmental Science, Taylor University, Upland, Indiana 46989). The Effects of Chronic Nitrogen Application on a Mature Tallgrass Prairie Restoration and Nitrogen Mobilization into Above-ground Biomass of *Andropogon gerardii*. J. Torrey Bot. Soc. XXX: 000 000. 20XX. The Upland Prairie Restoration was initiated on a 25-acre old field in 1993 in Upland, IN. It has matured into a community dominated by *Andropogon gerardii* and forbs, *Monarda fistulosa* and *Ratibida pinnata*. Prior research on the site found that acute nitrogen enrichment promoted weed cover in early stages of establishment and inhibited the establishment of native prairie species. This current study examined the effects of nitrogen enrichment applied to a mature prairie restoration. In 2002, a complete randomized block design experiment was implemented with annual enrichment using nitrogen-urea fertilizer at 4 levels – 0g/m^2, 5g/m^2, 10g/m^2, and 20g/m^2. Density and percent cover of all plant species was recorded annually over a five year time period. In year five, leaf level nitrogen concentrations in *Andropogon gerardii* were measured. The objectives of this multi-year study were to: 1) quantify the effects of chronically applied nitrogen at various concentrations on the density and percent cover of native prairie species, and 2) quantify the fraction of nitrogen that moves into the above-ground biomass of the dominant grass *Andropogon gerardii* at various levels of applied nitrogen and its potential relationship to changes in community structure. Our results indicate that chronic nitrogen enrichment did significantly decrease prairie grass cover and increase weed cover in the 10g/m^2 and 20g/m^2 transects. However, chronic nitrogen enrichment did not impact prairie forb cover. Correspondingly, we found that there is a significantly higher amount of nitrogen in the above-ground biomass of *Andropogon gerardii* in the 10g/m^2 and 20g/m^2 transects, than in the control and 5g/m^2 transects. Our results suggest that *Andropogon gerardii* buffered the prairie restoration against the negative effects of chronic nitrogen enrichment at lower concentrations, but that community structure degraded when N enrichment was 10g/m^2 or more.

Key Words: nitrogen fertilization, above-ground biomass, chronic nutrient enrichment, *Andropogon gerardii*, prairie restoration.

[1] Funding was provided by Avis Industrial Corporation, Taylor University Undergraduate Research Training Program, Indiana Academy of Sciences, and Creek Run Environmental of Indiana.

[2] Additionally, we would like to thank Janna McIndoe, Michelle Misurac, Mandy Ross, Melissa Werner, and David O'Neill for valuable field and laboratory assistance, Ken Konstantine for his assistance with statistics, and Alice Heikens for helpful comments on the manuscript.

[3] Author for correspondence. E-mail: ann.ebert.oneill@gmail.com

Development and agriculture have led to a near destruction of the North American prairie biome, with losses estimated at 99.9% in Indiana, Illinois, and Wisconsin (Samson & Knopf 1994). Tallgrass prairies are now considered by many ecologists to be North America's most endangered ecosystem (Samson & Knopf 1996). While the wholesale loss of prairie habitat can never be reversed, attempts have been made to restore small examples of the American Midwest biome. The Curtis Prairie, located at the University of Wisconsin Arboretum is the world's oldest restored prairie. Many experiments regarding prairie fire management and planting techniques have been conducted there (www.uwarboretum.org). The 8,600-acre Konza Prairie in Kansas, a joint effort by the Nature Conservancy and the University of Kansas, was established to promote "long-term ecological research, education, and prairie conservation" (www.nature.org). In Indiana, restoration efforts are being pursued by the Division of Nature Preserves, the Nature Conservancy, regional land trusts, parks, and educational institutions. The 25-acre Upland Prairie Restoration, a joint effort by Avis Industrial Corporation and Taylor University, is among the earliest of these restoration efforts. Past research on the Avis prairie has focused on acute nutrient enrichment and its effects on the establishment of a prairie planting (Rothrock and Squiers 2003), and the impact of nitrogen on a young restoration (Ross 2005). These studies and others (Houston and Hyder 1975; Risser and Parton 1982; Foster and Gross 1998) demonstrate the importance of studying the effects of chronic nitrogen enrichment in a prairie restoration.

Several studies, ranging from old fields to mature forests, have examined the effects of nutrient availability in terrestrial ecosystems and their potential for improving the quality of an ecosystem restoration. For example, a study using nitrogen, phosphorous, and potassium on an old field ecosystem concluded that nutrient addition has an impact on ecosystems because they alter competition strategies by expanding the niches of a few opportunistic species (Bakelaar and Odum 1978). When limiting nutrients are added to a system, it can decrease species diversity and increase primary productivity (Bakelaar and Odum 1978, Foster and Gross 1998). It also has been observed that fertilization with various nutrients in herbaceous plant communities can decrease the species richness (Gough et al. 2000; Bakelaar and Odum 1978; Tilman 1993).

Nitrogen enrichment, in particular, can produce both desirable and undesirable responses in grassland ecosystems (Houston and Hyder 1975; Risser and Parton 1982; Turner and Knapp 1996). Nitrogen enrichment has been shown to increase overall biomass (Foster and Gross 1998; Heyel and Day 2006), specifically, allocation to above-ground biomass, due to decreased nutrient resource competition and increased light competition (Power 1972; Tilman 1988). However, nitrogen application also is shown to decrease plant species richness (Tilman 1987; Risser and Parton 1982; Foster and Gross 1998), and increase weed content (Huenneke et al. 1990; Maron and Jeffries 2001). Nitrogen accumulation in the soil is considered to be problematic in prairie restoration efforts because soil nitrogen pools tend to favor fast-growing exotics rather than prairie species adapted to low nitrogen conditions (Blumenthal et al. 2003; Averett et al. 2004).

The effects of nitrogen application on community structure may be influenced by ecological succession. Nitrogen applied at the beginning of a prairie restoration in Upland, Indiana produced a detrimental effect on the successful establishment of both prairie forbs and grasses, a result attributable to increased weed cover (Rothrock and Squiers 2003). A few years later on this prairie it was determined that nitrogen continued to have a detrimental effect on forb cover and density (Ross 2005), but that grasses were more tolerant to the nitrogen application. However, on the mature Konza prairie in Kansas, nitrogen application on a native prairie did not increase weed content (Seastedt et al. 1991). Furthermore, in North Dakota it was concluded that more nitrogen can be taken up in a mature prairie than a young prairie, to a point, although the amount varied greatly with water supply (Power 1972).

Andropogon gerardii Vitman is the dominant grass in the eastern tallgrass prairie ecosystem (Turner et al. 1995; Knapp and Gilliam 1985). Because of this, its responses to drought, grazing, nutrient enrichment, and fire have been studied from the leaf level up through the population level (Knapp 1984,1985; Knapp and Gilliam 1985; Svejcar and Browning 1988; Wallace 1990). A study comparing early to late successional grasses found that, when competing in soils with high nitrogen, early successional grasses such as *Agrostis scabra* Willd. and *Schizachyrium scoparium* (Michx.) Nash have inferior competitive abilities to absorb nitrogen compared to their later successional counterparts, such as *Andropogon gerardii* (Tilman and Wedin 1991). Chronic nitrogen application at a rate of $10g/m^2$ in the form of ammonium nitrate (NH_4NO_3) has been found to increase the soil pools of nitrogen on the Konza prairie up to ten fold (Silletti and Knapp 2001). This chronic nitrogen enrichment led to an increase in *Sorghastrum nutans* (L.) Nash leaf-level photosynthesis, but did not significantly increase the leaf-level photosynthesis in *Andropogon gerardii* (Silletti and Knapp 2001). A long-term nitrogen fertilization experiment in Minnesota concluded that specific leaf area (ratio of leaf area to leaf mass) was increased with higher levels of nitrogen enrichment (Knops and Reinhart 2000).

Although literature on nitrogen metabolism by native prairies is available, little addresses the effects of chronic nitrogen enrichment on mature prairie restorations and their management implications. Thus, this study sought to determine the impact of chronic nitrogen enrichment on prairie community structure and weed content. One question that arose from preliminary findings is why, compared to earlier studies on the site, was chronic nitrogen addition having minimal effects on the prairie forbs and grasses? Was something retaining nitrogen so that its application had a minimal effect? Because of these preliminary questions, a second experiment was initiated to analyze nitrogen allocation within a tallgrass prairie ecosystem at different levels of chronic nitrogen enrichment. Because the dominant biomass was *Andropogon gerardii,* shoot nitrogen levels in this species were measured. The two objectives of this multi-year study were to: 1) determine and quantify the effects of chronically-applied nitrogen at various concentrations on the density and percent cover of native prairie species and weed species and 2) quantify the fraction of nitrogen that moves into the above-ground biomass of *Andropogon gerardii* at various levels of applied nitrogen, and determine how the *Andropogon gerardii* nitrogen budget is related to changes in community structure within the prairie restoration.

Materials and Methods. THE STUDY SITE. In 1993, Taylor University, working with Avis Industrial Corporation, began the 25-acre Upland Prairie Restoration in Upland, Indiana. Previously, the land had been used for pasture and contained weedy and old field forbs and grasses including *Festuca spp*., *Poa pratensis* L., *Taraxacum officinale* Weber, and *Daucus carota* L. Approximately 30 years ago, prior to its use as a pasture, it was an agricultural field that produced row crops (corn and soybeans). The prairie was seeded with a mix of approximately 50 grass and forb species (Appendix A, O'Neill et al. 2007). Dominant forbs included wild bergamot (*Monarda fistulosa* L.), and prairie coneflower (*Ratibida pinnata* (Vent.) Barnh.), as well as compass plant (*Silphium laciniatum* L.), false sunflower (*Heliopsis helianthoides* (L.) Sweet), and showy tick trefoil (*Desmodium canadense*). Big blue stem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans* (L.) Nash) were the dominant grasses in the mix. The prairie has reached a mature stage of development (Rothrock and Squiers 2003) and is maintained by an annual spring burn.

EXPERIMENTAL DESIGN. In 2002, a study was initiated to determine the effects that chronic N enrichment has on a mature prairie planting. Three 36 x 17 m blocks were established for this study, forming a randomized complete block design. Blocks 2 and 3 were placed in the forb rich zone on the western side of the prairie, while block 1 was placed in an area of low forb content in the central part of the prairie. Each of the blocks contained four randomly assigned 15 m treatment strips. Three strips received an annual application of 5 g/m^2, 10 g/m^2, or 20 g/m^2 using nitrogen-urea fertilizer (46% nitrogen by weight); the fourth transect in each block served as a control. The nitrogen-urea fertilizer was applied in pellet form each spring, after the annual burn. The annual burn before the year 4 (2005) sampling season occurred in the fall, while the annual burn was in the spring the other four years. In each treatment strip, 18 random 0.25m^2 plots were sampled each summer from 2002-2006. The plots were sampled for density (number of shoots) and percent aerial cover (the percentage of the plot that each species covers) for each species present. Plant species were grouped into the following guilds – prairie forbs, prairie grasses, and non-prairie weeds (Averett et al. 2004). Weeds were defined as any species not present in the original prairie planting (Appendix B, O'Neill et al. 2007). Only cover results are presented since they produced similar results to the density analysis, and cover results had fewer problems meeting statistical assumptions. Appendices C-F (O'Neill et al. 2007) present block by block cover and density results and can be accessed online at http://www.taylor.edu/academics/graduate/mes/students.shtml.

PERCENT NITROGEN UPTAKE. Plant shoots were sampled at intervals along the length of the transects in May 2006 when the plants were approximately 40 cm tall and the above-ground N concentrations were at or near the seasonal peak (Hayes 1985; Li and Redmann 1992). All plant samples were gathered on the same day to reduce potential temporal variation in leaf N content. Fifteen samples (~10g) of above-ground biomass of *Andropogon gerardii* were collected at random in each of the four transects across the three blocks. After drying at 70ºC (Houston 1975), the samples were ground with a Wiley mill (40-mesh) and stored in a desiccator until being analyzed for total N content by the Waters Agricultural Laboratories, Inc. (Camilla, GA), using a LECO FP 528 Total N analyzer (Etheridge et al. 1998; Brye and Slaton 2003). In order to estimate the N uptake per m^2, the average density of *Andropogon gerardii* shoots were measured in each transect. An average shoot weight for each transect was determined by measuring the dry weight of shoots and dividing by the number of shoots collected. Appendix G (O'Neill et al. 2007) presents block by block data of the percent N uptake and can be accessed online.

STATISTICAL ANALYSIS. MINITAB® Release 14.13. software was used for all statistical analyses. Year 5 data alone, and the change from year 1 to year 5 data were analyzed to test for both impacts of the long-term N enrichment on prairie species, as well as the change over time. Because the experimental design was a randomized, complete block, the effects of varying levels of N enrichment on density and cover of prairie species were determined using a two-way analysis of variance ($P < 0.05$). The main effects of treatment and block were analyzed, as well as block × treatment interaction. N uptake was also analyzed by a two-way ANOVA. When an interaction was significant, main effect F-statistics and p-values are presented for descriptive purposes, not statistical inference. Significant main effects were separated using Tukey simultaneous confidence intervals. When block effects or interaction were present, a one-way

ANOVA was used. When data did not meet ANOVA assumptions of normality and homogeneity, they were transformed (square root, natural log, or arcsine square root). Since forbs were not found in approximately 66% of quadrats measured in block 1, the normality of forb density and cover were skewed, reflecting the high number of zeros in the data. Because of this, all forb data reported includes only blocks 2 and 3. All other analyses included all 3 blocks. All F-statistics and p-values reported are of the data that met ANOVA assumptions. All graphs presented depict original, untransformed data.

Results. RESPONSE OF FORBS. An analysis of forb cover data from year 5 indicated that the chronic N enrichment did not have an impact on native prairie forbs over the 5-year study ($P = 0.686$, Table 1). Transect means for percent cover (± S.E.) for the control transects (17.42 ± 4.41) were nearly identical to the 5g/m^2 transects (17.58 ± 4.81), and not significantly different from the 10g/m^2 (12.03 ± 3.47) or 20g/m^2 (16.25 ± 4.49) transects (Fig. 1A). The range of total forb cover data was lowest in the 10g/m^2 transects (75%) and highest in the 20g/m^2 transects (120%). Forb relative cover in year five showed a similar pattern (Table 2), with no significant differences between the control, 5g/m^2,10g/m^2, and 20g/m^2 treatments ($P = 0.753$, Table 1). Block effect and interaction also were insignificant in forb cover and relative cover results. Treatment effects for changes in forb cover and relative forb cover between years 1 and 5 showed similar results to the data collected in year 5. Changes in forb cover over the five year period (Table 2) resulted in no significant differences between treatments ($P = 0.237$, Table 3). Likewise, changes in forb relative cover (Table 2) were not significant over the five years of chronic nitrogen enrichment ($P = 0.485$, Table 3). The changes in relative forb cover due to treatment ranged from a 1% decrease to a 7% increase (Table 2). Negative means indicated an average loss in cover over the five year study.

There were 8 prairie forb species present in both blocks: *Ratibida pinnata, Desmodium canadense* (L.) DC., *Tradescantia ohienses* Raf., *Silphium integrifolium* Michx., *Heliopsis helianthoides, Solidago rigida* L., *Silphium perfoliatum* L., and *Monarda fistulosa*. *Solidago rigida* appeared in only 2 of the 8 total transects, and was only present in low N treatment transects (control and 5g/m^2). *Monarda fistulosa* appeared in 7 transects, the highest frequency. Another forb, *Silphium perfoliatum*, was only present in high N treatment transects (10g/m^2 and 20g/m^2), perhaps indicating its preference for high levels of nitrogen. Blocks 2 and 3 contained a total of 9 and 12 different forb species across the four treatments. The number of species in each treatment group across blocks 2 and 3 did not show a trend related to treatment. The 5g/m^2 transects had the highest number of average native forb species per transect, with 7. The 10g/m^2 transects had the lowest average of just 4.5 species per transect, while the control and 20g/m^2 treatments had an average of 6.5 and 6 species per transect, respectively.

RESPONSE OF GRASSES AND NATIVE PRAIRIE SPECIES. Grass cover in year five showed significant treatment effects (P = 0.022, Table 1). The mean percent grass cover (± S.E.), largely *Andropogon gerardii*, was highest in the control (73.07 ± 2.93), and decreased in the 5g/m^2 (70.93 ± 3.54), 10g/m^2 (61.96 ± 3.95), and 20g/m^2 (58.96 ± 4.67) treatments (Fig. 1B). The Tukey analysis of grass cover indicated that only the control and 20g/m^2 treatments were significantly different (P = 0.0437). Percent native prairie cover also decreased with increasing nitrogen application. The control (85.11 ± 2.57) and 5g/m^2 (85.39 ± 2.74) treatments were both higher in native species cover than the 10g/m^2 (70.11 ± 3.61) and 20g/m^2 (70.28 ± 4.03) treatments. Although prairie cover did show a significant treatment effect ($P = 0.000$), only limited inferences could be drawn from the results due to site × treatment interaction ($P = 0.026$) and block effect ($P = 0.011$). In the Tukey analysis (Table 4), the control and 5g/m^2 (low treatment) transects showed essentially identical prairie cover ($P = 0.999$), and the 10g/m^2 and 20g/m^2 (high treatment) transects behaved identically ($P = 1.00$), with a very significant difference between the low treatment and high treatment groups ($P < 0.0062$ in all pairwise comparisons between the two groups).

When year five and year one differences were analyzed, significant treatment effects were determined for grass and prairie cover ($P = 0.002$ for both, Table 3). There was a decrease in grass cover in all treatment groups over the 5-year study (Fig. 1C). However, the decrease in grass cover was the least in the control transects (-8.00 ± 4.29), compared to -19.35 or more in the N enriched transects. The Tukey analysis of grass cover indicated a significant difference between the control transects and both the 10g/m^2 and 20g/m^2 transects ($P = 0.0436, 0.0009$, respectively). The change in prairie cover showed similar statistical trends (Table 3). Means (± S.E.) decreased slightly in the control (-12.87 ± 4.22) and 5g/m^2 (-18.93 ± 3.87) transects, while means decreased more dramatically in the 10g/m^2 (-31.50 ± 4.68), and 20g/m^2 (-31.13 ± 3.63) treatments. The Tukey analysis indicated that the 10g/m^2 and 20g/m^2 treatments responded significantly different from the control transects ($P = 0.0077, 0.0095$, respectively). Chronic N application on this mature tallgrass prairie restoration resulted in a decrease in grass and overall native prairie cover with increasing rates of N application.

RESPONSE OF WEEDS. Percent weed cover (±S.E.) in year 5 generally increased with greater nitrogen concentration. The control (3.981 ± 0.991) transects were lower than the 5g/m^2

(8.81 ± 2.05), 10g/m^2 (24.11 ± 3.62), and 20g/m^2 (24.50 ± 3.90) transects. The ANOVA indicated that the response of weed cover depended upon block location (i.e. there was block × treatment interaction) in year 5 ($P = 0.000$, Table 1). According to the interaction plot (Fig. 2A), weed cover in the 20g/m^2 transects were unexpectedly lower in blocks 1 and 3 compared to block 2. Because of interactions, limited inferences could be drawn from the treatment effects. The Tukey analysis on weed cover yielded no difference between the control and 5g/m^2 (low treatment) transects ($P = 0.3672$), and no difference between the 10g/m^2 and 20g/m^2 (high treatment) transects ($P = 0.7425$). When comparing individual treatments between the low treatment groups (control and 5g/m^2) and high treatment groups (10g/m^2 and 20g/m^2), the largest p-value in any Tukey pairwise comparison between the two groups (i.e., control vs. 10, control vs. 20, 5 vs. 10, 5 vs. 20) was $P < 0.0014$ (Table 4). Examination of the raw data indicated probable microsite variation between the blocks. For example, when comparing identical treatments, block 2 had greater increases in weed cover over the 5 year study than block 3, and block 3 had greater increases in weed cover than block 1. In light of the significant interaction (P < 0.000) and microsite variation, the blocks were pooled and a one-way ANOVA was performed to test for treatment significance ($P = 0.000$). Tukey analyses comparing means of treatments indicated significant differences between low treatment (control and 5g/m^2) and high treatment (10g/m^2 and 20g/m^2) transects (Table 4, Fig. 2B), with the greatest weed cover found in the most N-enriched transects.

The change in weed cover between years one and five did not meet the assumption of normality, even after attempting many standard transformations. Because of this an ANOVA was not performed. A graph of the data shows some interesting trends over the five-year study (Fig. 3). In year one, the control, 5g/m^2 and 20g/m^2 treatment transects all had statistically similar weed cover. One year of nitrogen application did not have a notable effect. By year 5, however, the control and 5g/m^2 weed cover had decreased, while the 20g/m^2 weed cover increased markedly. It appears that high levels of chronic nitrogen enrichment have increased weed cover, while the control and 5g/m^2 treatments led to a decrease in weed cover. The three weed species that contributed the majority of weed cover in year 5 were *Asclepias syriaca* L. (milkweed), *Solidago altissima* L. (tall goldenrod), and *Ambrosia trifida* L. (giant ragweed). Based upon occurrences where at least one of these species had a cover of 10% or greater, these 3 species occurred in 3 quadrats in the control transects, 13 times in the 5g/m^2 transects, 27 times in the 10g/m^2 transects, and 37 times in the 20g/m^2 transects. The control transects had a slightly more diverse array of weeds relative to the other treatments, with an average of 7.7 species per transect. The 5g/m^2 and 10g/m^2 had an average of 7 weed species per transect, and the 20g/m^2 transects had an average of 6.33 weed species. Other frequent weeds included *Taraxacum officinale* (common dandelion), *Erigeron annuus* (L.) Pers. (daisy fleabane), and *Verbascum thapsus* L. (great mullein).

NITROGEN UPTAKE. N uptake was evaluated in three ways: total % N, g of N per shoot, and g of N per m^2. On a percent N (by weight) basis, N was lower in the control (1.8084 ± 0.0295) and 5g/m^2 (1.8396 ± 0.0231) transects, compared to the 10g/m^2 (2.2617 ± 0.0437) and 20g/m^2 (2.2656 ± 0.0348) transects (Fig. 4). Using the percent N, the g of N per shoot was calculated from the average *Andropogon gerardii* shoot weight in each transect. Mean g of N per shoot were again lower in the control (0.0103 ± 0.0005) and 5g/m^2 (0.0111 ± 0.0007), and higher in the 10g/m^2 (0.0147 ± 0.0009) and 20g/m^2 (0.0163 ± 0.0010) transects. Finally, the average g of N per shoot was multiplied by the density of *Andropogon gerardii* across the transect to obtain an estimate of g of N per 1 m^2. The amount of N per 1 m^2 did not show the same pattern as the first two analyses. This was due to lower initial densities in the 10g/m^2 transects, providing less above-ground biomass into which the N could be potentially taken up. Not surprisingly, the control had the lowest mean N per m^2 (2.0128 ± 0.0615), followed by the10g/m^2 (2.1986 ± 0.0524), 5g/m^2 (2.411 ± 0.1040), and 20g/m^2 (2.915 ± 0.1120) transects. The ANOVA (Table 5) showed significant block × treatment interaction in all 3 analyses. A closer examination of the percent nitrogen interaction (Fig. 4A) indicated that the interaction is on 2 levels: an interaction is present between the control and the 5g/m^2, and between the 10g/m^2 and 20g/m^2. The 10g/m^2 and 20g/m^2 both had higher amounts of N, both on a percent N and g of N per shoot basis, compared to the control and 5g/m^2 treatments ($P < 0.05$ in all pairwise comparisons between the 2 groups). An inspection of the raw data in all three categories indicated that there is microsite variation between the blocks. For example, the average tissue percent N, when comparing identical treatment transects between the blocks, was lowest in block 2 and highest in block 1. An observation of the g of N per shoot indicated that block 3 had the lowest results, and again block 1 was highest. In the g of N per 1m^2 analysis, transects in block 3 were lower than the same treatment transects in blocks 2 and 1.

In light of the probable microsite variation between the blocks, which is demonstrated here by the significant block effect ($P < 0.000$), a one-way ANOVA was performed to test for treatment significance. All three one-way ANOVAs were significant based upon treatment. The distinction between the low treatments (control and 5g/m^2) and high treatments (10g/m^2 and 20g/m^2) was again evident (Fig. 4B). Tukey analyses for both percent N and g of N per shoot confirm the differences between high and low treatment transects (Table 6). Analysis of the g of N per 1m^2 used the density and percent N of *Andropogon gerardii* shoots to determine the amount of total N. Although the 10g/m^2 transects showed lower total amounts of N than the 5g/m^2 transects, the Tukey analyses indicated that the difference is not significant (P = 0.308). The difference between the control and the 10g/m^2 transects were also insignificant (P = 0.427). All other Tukey pairwise comparisons indicated significant differences (Table 6).

Discussion. EFFECTS OF NITROGEN ENRICHMENT ON COVER. The data analysis indicated that five years of N application at rates as high as 20g/m^2 did not have a significant impact on forb cover in this study. This is dramatically different from previous studies conducted earlier on the Avis prairie where acute N application in the first year, at rates up to 40g N/m^2, resulted in few prairie forbs and grasses (Rothrock and Squiers 2003). Furthermore, a study initiated in year 3 on the Avis prairie demonstrated that acute nitrogen application caused a significant decrease in forb growth, but did not cause a significant decrease in native prairie grasses (Ross 2005). Foster and Gross (1998) also determined that N enrichment inhibits forb seedling growth in young prairie restorations. In contrast to earlier studies on the Avis prairie, our results recorded less impact to the prairie community and structure with N applied chronically for five years on a mature prairie. Native prairie forbs were present in all treatment levels, and significant differences due to N enrichment were not observed. Species common to the low N transects were also present in the high N transects. This study indicates that the forbs in the Avis prairie restoration have become resilient to chronic N enrichment, which is consistent with the results obtained by Turner and Knapp (1996). They observed that increased N availability did not limit forb physiological processes in spite of higher tissue N concentrations with increased N availability. Their study added to the consensus that C_3 forbs have higher N requirements than dominant C_4 grasses (Risser and Parton 1982; Turner et al. 1995). Because of this, N application may have a greater positive effect on forbs than on grasses (Turner and Knapp 1996). They also postulated that high grass production can limit the light available to forbs. Thus, a reduction in grasses may help to promote forb growth. Our results support the idea that N application on a mature prairie restoration does not inhibit forb growth.

The negative effects of N enrichment on native grass species that we obtained from the Avis prairie is consistent with results from others who determined that native prairie species richness is generally highest in low-N settings, where there is competition for N (Risser and Parton 1982; Tilman and Wedin 1990; Knapp and Seastedt 1986). An increase in N can shift the competitive advantage to fast-growing exotics, which depend on high levels of N for rapid growth (Buckland and Grime 2000), and allow weedy species to displace low-N tolerant prairie species relatively quickly (Maron and Jeffries 2001). Previous research has determined that N enrichment has a negative impact on the survival of *Andropogon gerardii* (Foster and Gross 1998; Karel et al. 2003). N applications of 10g N/m^2 (applied as ammonium nitrate) did increase leaf-level photosynthesis in some prairie species, but not in *Andropogon gerardii* (Silletti and Knapp 2001). This shift in competitive advantage from stress-tolerant, native prairie species (largely *Andropogon gerardii*) to fast-growing ruderals (such as *Taraxacum officinale* and *Asclepias syriaca*) with high levels of N application also was demonstrated in this study. However, it is possible that the reduced effects of N in this study are a result of the mature stage of development of the native prairie grasses and the established prairie ecosystem helped to buffer the impact of the chronic N application.

NITROGEN UPTAKE. The significance of both main effects (treatment and block) as well as the interaction in all three analyses of N uptake (percent N, g of N/shoot, and g of N/m^2) must be carefully considered in any discussion of the results. The significant block and interaction effects indicate microsite variation between the blocks. One clear trend based on non-statistical observation of the data was that block 1 had the highest amounts of N in all three N uptake analyses. We attribute this to the fact that block 1 is located in a lower topographic position than the other two blocks, and it contains more N-containing organic matter in the soil. Soil samples from the prairie confirm these observations. Bulked soil samples from each block indicate that block 1 had the highest soil % N (0.213 % N), followed by block 2 (0.209 % N) and then block 3 (0.179 % N). If block 1 is removed from the analysis, the interaction would be reduced in the percent N analyses (Fig. 4A), perhaps making the significant treatment differences more conclusive.

Tissue % N results ranged from 1.81% N in the control to 2.27% N in the 20g/m^2 transects. These results were similar to a study that measured the shoot % N of *Andropogon gerardii* (45 days after planting) at 2.3% (Miller et al. 2002). However, Miller's experiments were conducted in a controlled greenhouse. Our results also were similar to a study on a tallgrass prairie in Oklahoma, dominated by warm-season grasses including *Andropogon gerardii*, which measured a live-shoot N at 2.0% across all prairie species (Risser and Parton 1982). A study on the Konza Prairie Research Natural Area (KPRNA) in the Flint Hills of northeastern Kansas estimated May N concentrations in *Andropogon gerardii* at about 1.9%, nearly identical to our control results (Turner et al. 1995).

The % N uptake and g of N/shoot results indicated that the high treatment transects (10g/m^2 and 20g/m^2) contained significantly higher amounts of N in the *Andropogon gerardii* shoots than the low treatment transects (control and 5g/m^2). It appears that a threshold is reached with a N application of 10g/m^2 or more. *Andropogon gerardii* is able to take up more N as it is applied, to a point, which we estimate at 10g/m^2. Beyond that level of application, this dominant prairie grass is unable to take up more N into its above-ground biomass. Possibly, as the capacity of *Andropogon gerardii* to buffer this prairie system against the effects of chronic nitrogen enrichment is reached at about 10g/m^2, the prairie system experiences degradation of native grasses and total native prairie species. We postulate that until the 10g/m^2 threshold is reached, *Andropogon gerardii* is able to absorb the added N. This was demonstrated in the fact that 17% of the total applied N was accounted for in the above-ground biomass of the 5g/m^2 transects (Table 7). Only 1.9 g of N were unaccounted for in the 5g/m^2 transects, while this increased to 4.4 g of N in the 10g/m^2 and 8.3 g of N in the 20g/m^2 transects. If *Andropogon gerardii* is absorbing significant portions of the applied N in the 5g/m^2 transects, there is a relatively low amount available for other plants, including weeds, to take up and alter the prairie system environment. The considerable N uptake by *Andropogon gerardii* in the 5g/m^2 transects may be the reason that these transects behaved quite similarly to the control in most analyses of grass, prairie, and weed cover, while the 10g/m^2 (4.6g N/m^2) and 20g/m^2 (9.2g N/m^2) treatments were negatively affected by the N application. A study on the native tallgrass Konza prairie is similar to this threshold level (Rains et al. 1975). That study found that 4.48g N/m^2 (applied as urea in year 1 and ammonium nitrate in year 2) increased the production of *Andropogon gerardii*. However, an addition of 8.96 g N/m^2 led to an accumulation of N, as it exceeded the needs of the plants.

The N, once applied, has many potential fates. The N may be absorbed into the above-ground biomass to promote above-ground growth and competition for light (Morgan and Smith 1979; Smith 1982; Schmid and Bazzaz 1994). Other potential fates include absorption by the roots, leeching, runoff, or gaseous losses (Brye et al. 2003; Risser and Parton 1982). The N cycle could be affected by the annual burn, as volatilization of the N with combustion is a major pathway of N loss in a prairie ecosystem (Seastedt and Ramundo 1990). If significant amounts of N are lost, chronic application of N may have less of an impact. Additionally, because *Andropogon gerardii* has relatively low N requirements compared to other species, we would expect relatively low concentrations in above-ground biomass (Escudero et al. 1992). In light of this, we were not surprised that less than 17% of the N found in the above-ground tissue of *Andropogon gerardii* can reasonably be attributed to the treatments themselves (Table 7). And yet, it appears that a change in N concentration of this magnitude can produce significant effects in the physiology, function, and competitive abilities of *Andropogon gerardii* in a mature prairie restoration. The results demonstrate that N should be monitored on a prairie restoration, and influxes over approximately 10g/m^2 of urea will exceed the buffering capacities of *Andropogon gerardii*. Further research is needed to determine the true threshold level and corresponding effects, as well as account for the local fate of the applied N in both low and high treatment transects in this mature prairie restoration.

Literature Cited

AVERETT, J.M., R.A. KLIPS, L.E. NAVE, S.D. FREY AND P.S. CURTIS. 2004. Effects of soil carbon amendment on nitrogen availability and plant growth in an experimental tallgrass prairie restoration. Restor. Ecol. 12:568-574.

BAKELAAR, R.G. AND E.P. ODUM. 1978. Community and population level responses to fertilization in an old-field ecosystem. Ecology 59:660-665.

BLUMENTHAL, D.M., N.R. JORDAN AND M.P. RUSSELLE. 2003. Soil carbon addition controls weeds and facilitates prairie restoration. Ecol. Appl. 13:605-615.

BRYE, K.R., J.M. NORMAN, S.T. GOWER AND L.G. BUNDY. 2003. Methodological limitations and N-budget differences among a restored tallgrass prairie and maize agroecosystems. Agriculture, Ecosystems and Environment 97:181-198.

BRYE, K.R. AND N. SLATON. 2003. Carbon and nitrogen storage in a typic albaqualf as affected by assessment method. Communications in Soil Science & Plant Analysis 34:1637-1657.

BUCKLAND, S.M. AND J.P. GRIME. 2000. The effects of trophic structure and soil fertility on the assembly of plant communities: a microcosm experiment. Oikos 91:336-352.

ESCUDERO, A., J.M. DEL ARCO, I.C. SANZ AND J. AYALA. 1992. Effects of leaf longevity and retranslocation efficiency on the retention time of nutrients in the leaf biomass of different woody species. Oecologia 90:80-87.

ETHERIDGE, R.D., G.M. PESTI AND E.H. FOSTER. 1998. A comparison of nitrogen values obtained utilizing the Kjeldahl nitrogen and Dumas combustion methodologies (Leco CNS 2000) on samples typical of an animal nutrition analytical laboratory. Animal Feed Sci. and Tech. 73:21-28.

FOSTER, B.L. AND K.L. GROSS. 1998. Species richness in a successional grassland: Effects of nitrogen enrichment and plant litter. Ecology 79:2593-2602.

GOUGH, L., C.W. OSENBERG, K.L. GROSS AND S. L. COLLINS. 2000. Fertilization effects on species density and primary productivity in herbaceous plant communities. Oikos 89:428-439.

HAYES, D.C. 1985. Seasonal nitrogen translocation in big bluestem during drought conditions. J. of Range Manage. 38:406-410.

HEYEL, S.M. AND F.P. DAY. 2006. Long-term residual effects of nitrogen addition on a barrier island dune ecosystem. J. Torrey Bot. Soc. 133(2):297-303.

HOUSTON, W.R. AND D.N. HYDER. 1975. Ecological Effects and Fate of N Following Massive N Fertilization of Mixed-Grass Plains. J. of Range Manage. 28:56-60.

HUENNEKE, L., S. HAMBURG, R. KOIDE, H. MOONEY AND P. VITOUSEK. 1990. Effects of soil resources on plant invasion and community structure in California (USA) serpentine grassland. Ecology 71:478–491.

KAREL, I., V. SMITH, B. FOSTER, A. BROTHERS, M. RAMSPOTT AND K. PRICE. 2003. Poster, Ecological Society of America Annual Meeting; August 3-8, 2003, Savannah, GA. Effects of experimental nutrient manipulations on a restored grassland community. Poster #124.

KNAPP, A.K. 1984. Water relations and growth of three grasses during wet and drought years in a tallgrass prairie. Oecologia 65:35-43.

KNAPP, A.K. 1985. Effect of fire and drought on the ecophysiology of *Andropogon gerardii* and *Panicum vergatum* in a tallgrass prairie. Ecology 66:1309-1320.

KNAPP, A.K. AND F.S. GILLIAM. 1985. Response of *Andropogon gerardii* (Poaceae) to fire-induced high vs. low irradiance environments in a tallgrass prairie: leaf structure and photosynthetic pigments. Am. J. of Bot. 72:1668-1671.

KNAPP, A.K. AND T.R. SEASTEDT. 1986. Detritus accumulation limits productivity of a tallgrass prairie. Oecologia 65:35-43.

KNOPS, J.M.H. AND K. REINHART. 2000. Specific leaf area along a nitrogen fertilization gradient. Am. Mid. Nat. 144:265-272.

LI, Y.S. AND R.E. REDMANN. 1992. Nitrogen budget of *Agropyron dasystachyum* in Canadian mixed prairie. Am. Mid. Nat. 128:61-71.

MARON, J.L. AND R.L. JEFFRIES. 2001. Restoring enriched grasslands: effects of mowing on species richness, productivity, and nitrogen retention. Ecol. Appl. 11:1088-1100.

MILLER, R.M., S.P. MILLER, J.D. JASTROW AND C.B. RIVETTA. 2002. Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. New Phyt. 155:149-162.

MORGAN, D.C. AND H. SMITH. 1979. A systematic relationship between phytochrome-controlled development and species habitat, for plants grown in simulated natural radiation. Planta 145:253-258.

O'NEILL, A.M., P.E. ROTHROCK AND R.T. REBER. 2007. The effects of chronic nitrogen application on a mature tallgrass prairie restoration and nitrogen mobilization into above-ground biomass of *Andropogon gerardii*. MS Thesis. Taylor University, Upland, IN. < http://www.taylor.edu/academics/graduate/mes/students.shtml>

POWER, J.F. 1972. Presentation, American Society of Range Management annual meeting; February 18, 1971, Reno, NV. Fate of fertilizer nitrogen applied to a Northern Great Plains rangeland ecosystem. USDA, Mandan, ND, USA.

RAINS, J.R., C.E. OWENSBY AND K.E. KEMP. 1975. Effects of nitrogen fertilization, burning, and grazing on reserve constituents of big bluestem. J. of Range Manage. 28: 358-362.

RISSER, P.G. AND W.J. PARTON. 1982. Ecosystem analysis of the tallgrass prairie: nitrogen cycle. Ecology 63:1342-1351.

ROSS, A.J. 2005. Degradation and recovery of a prairie restoration following acute nitrogen fertilization. MS Thesis. Taylor University, Upland, IN.

ROTHROCK, P.E. AND E.R. SQUIERS. 2003. Early succession in a tallgrass prairie restoration and the effects of nitrogen, phosphorous, and micronutrient enrichments. Proc. of the Indiana Acad. of Sci. 112:160-168.

SAMSON, F.B. AND F.L. KNOPF. 1994. Prairie conservation in North America. BioScience 44:418-421.

SAMSON, F.B. AND F.L. KNOPF. (EDS.). 1996. *Prairie Conservation: Preserving North America's Most Endangered Ecosystem.* Island Press, Washington, D.C.

SCHMID, B. AND F.A. BAZZAZ. 1994. Crown construction, leaf dynamics, and carbon gain in two perennials with contrasting architecture. Ecol. Monographs 64:177-203.

SEASTEDT, T.R., J.M. BRIGGS, AND D.J. GIBSON. 1991. Controls of nitrogen limitation in tallgrass prairie. Oecologia 87:72-79.

SEASTEDT, T.R. AND R.A. RAMUNDO. 1990. The influence of fire on below-ground processes of a tallgrass prairie, pp. 99-177 *In* S.L. Collins and L.L. Wallace [eds.], Fire in North American grasslands. University of Oklahoma Press, Norman, OK, USA.

SILLETTI, A.M. AND A.K. KNAPP. 2001. Responses of the codominant grassland species *Andropogon gerardii* and *Sorghastrum nutans* to long-term manipulations of nitrogen and water. Am. Mid. Nat. 145:159-167.

SMITH, H. 1982. Light quality, photoperception, and plant strategy. Ann. Rev. of Plant Phys. 33:481-518.

SVEJCAR, T.J. AND J.A. BROWNING. 1988. Growth and gas exchange of *Andropogon gerardii* as influenced by burning. J. of Range Manage. 41:239-244.

TILMAN, D. 1987. Secondary succession and the pattern of plant dominance along experimental nitrogen gradients. Ecol. Monographs 57:189-214.

TILMAN, D. 1988. Plant strategies and the dynamics and structure of plant communities. Princeton University Press, Princeton, NJ, USA. 360 p.

TILMAN, D. 1993. Species richness of experimental productivity gradients: how important is colonization limitation? Ecology 74:2179-2191.

TILMAN, D. AND D. WEDIN. 1990. Proceedings, Twelfth North American Prairie Conference; University of Northern Iowa, Cedar Falls, IA. Nitrogen cycling, plant competition, and the stability of tallgrass prairie.

TILMAN, D. AND D. WEDIN. 1991. Dynamics of nitrogen competition between successional grasses. Ecology 72:1038-1049.

TURNER, C.L. AND A.K. KNAPP. 1996. Responses of a C_4 grass and three C_3 forbs to variation in nitrogen and light in a tallgrass prairie. Ecology 77:1738-1749.

TURNER, C.L., J.R. KNEISLER AND A.K. KNAPP. 1995. Comparative gas exchange and nitrogen responses of the dominant C_4 grass *Andropogon gerardii* and five C_3 forbs to fire and topographic position in a tallgrass prairie during a wet year. Int. J. of Plant Sci. 156:216-226.

WALLACE, L.L. 1990. Comparative photosynthetic responses of big bluestem to clipping versus grazing. J. of Range Manage. 43:58-61.

Table 1. ANOVA results: The effects of chronic N enrichment on cover (forbs, grasses, prairie species, weeds, and forb relative cover) in year 5.

Main Effects	Forb Cover[a]			Grass Cover			Prairie Cover			Weed Cover			F.R.C.[b]		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Block(B)	1	1.97	0.163	2	1.70	0.185	2	4.58	0.011	2	2.81	0.063	1	2.33	0.129
Treatment(T)	3	0.50	0.686	3	3.27	0.022	3	7.49	0.000	3	18.29	0.000	3	0.40	0.753
B × T	3	0.55	0.651	6	1.71	0.121	6	2.44	0.026	6	7.34	0.000	3	0.99	0.402
Error	136			204			204			204			136		
Total	143			215			215			215			143		

[a] Because block 1 was dominated by grasses, it was excluded from all forb analyses.

[b] Forb Relative Cover is forb cover/total cover, expressed as a percent.

Table 2. Forb cover and relative cover results (± standard error). Both main effects (treatment and block) and interaction were insignificant for all analyses.

	Year Five		Change between Years 1 and 5	
Treatment	Forb Cover	Forb Relative Cover [a]	Forb Cover	Forb Relative Cover [a]
Control	17.42 ± 4.41	0.1772 ± 0.0398	-7.83 ± 5.89	-0.0106 ± 0.0439
5g/m^2	17.58 ± 4.81	0.1689 ± 0.0434	2.86 ± 3.84	0.0442 ± 0.0318
10g/m^2	12.03 ± 3.47	0.1361 ± 0.0394	-8.00 ± 4.33	-0.0036 ± 0.0353
20g/m^2	16.25 ± 4.49	0.1794 ± 0.0477	2.00 ± 4.96	0.0664 ± 0.0500

[a] Forb Relative Cover is forb cover/total cover, expressed as a percent.

Table 3. ANOVA results: The change in cover between years one and five (forbs, grasses, prairie species, and relative forb cover). Data for weed cover that did not meet assumptions of homogeneity and normality are not presented.

Main Effects	Forb Cover[a]			Grass Cover			Prairie Cover			F.R.C.[b]		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Block(B)	1	0.00	0.998	2	1.07	0.344	2	1.60	0.205	1	0.72	0.398
Treatment(T)	3	1.43	0.237	3	5.23	0.002	3	5.14	0.002	3	0.82	0.485
B × T	3	0.20	0.900	6	0.78	0.585	6	1.61	0.147	3	0.55	0.650
Error	136			204			204			136		
Total	143			215			215			143		

[a] Because block 1 was dominated by grasses, it was excluded from all forb analyses.

[b] Forb Relative Cover is forb cover/total cover, expressed as a percent.

Table 4. A Tukey analyses of prairie cover in year 5 based on a two-way ANOVA, and a Tukey analysis of weed cover in year 5 based on a one-way ANOVA (treatment was significant at p=0.000). Numbers reported are p-values. The prairie cover and weed cover analyses indicated significant differences between the low (control and 5g/m^2) and high (10g/m^2 and 20g/m^2) treatment transects.

	Prairie Cover				Weed Cover			
Treatment	Control	5g/m^2	10g/m^2	20g/m^2	Control	5g/m^2	10g/m^2	20g/m^2
Control	---	0.9999	0.0055	0.0062	---	0.640	0.000	0.000
5g/m^2	---	---	0.0045	0.0051	---	---	0.001	0.001
10g/m^2	---	---	---	1.000	---	---	---	0.999
20g/m^2	---	---	---	---	---	---	---	---

Table 5. ANOVA results: The effects of chronic N enrichment on the average percent N in the above-ground biomass of *Andropogon gerardii*, g of N per shoot, and g of N per m^2 in year 5.

Main Effects	% Nitrogen			g of nitrogen/shoot			g of nitrogen/m^2		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Block(B)	2	24.88	0.000	2	16.10	0.000	2	292.81	0.000
Treatment(T)	3	80.87	0.000	3	19.31	0.000	3	127.74	0.000
B × T	6	4.12	0.001	6	5.73	0.000	6	74.59	0.000
Error	168			168			168		
Total	179			179			179		

Table 6. Tukey analyses based on one-way ANOVAs of % N (by weight), g of N/shoot, and g of N/m^2. All one-way ANOVA results for treatment were significant at p=0.000. Numbers reported are p-values. Significant differences between the high and low treatment transects are evident in the % N and g of N/shoot analyses. Only the 5g/m^2 and 10g/m^2 are not significantly different from each other in the g of N/m^2 analysis.

	%N				gN/shoot				gN/m^2			
Treatment	C	5g/m^2	10g/m^2	20g/m^2	C	5g/m^2	10g/m^2	20g/m^2	C	5g/m^2	10g/m^2	20g/m^2
Control	---	0.914	0.000	0.000	---	0.903	0.001	0.000	---	0.007	0.4271	0.000
5g/m^2	---	---	0.000	0.000	---	---	0.006	0.000	---	---	0.308	0.000
10g/m^2	---	---	---	0.999	---	---	---	0.510	---	---	---	0.000
20g/m^2	---	---	---	---	---	---	---	---	---	---	---	---

Table 7. Grams of nitrogen added per m^2 and percent of application taken up into the above-ground biomass of *Andropogon gerardii*.

Measurement	Control	$5 g/m^2$	$10 g/m^2$	$20 g/m^2$
Applied g of N/m^2 [a]	0.0	2.3	4.6	9.2
g of N/m^2 in above-ground biomass [b]	2.0	2.4	2.2	2.9
g of N adjusted for control [c]	---	0.4	0.2	0.9
% of application uptake [d]	---	17%	4%	10%
g of unaccounted N [e]	---	1.9	4.4	8.3

[a] Percent of N-containing urea application that is N (46% of application)

[b] Calculated from: % N × average shoot weight (g) × density/m^2

[c] Calculated from: (average g of N/m^2 in each of the non-control treatments) - (control g of N/m^2)

[d] % of N that is accounted for in the above-ground biomass.

Calculated from: c/a

[e] g of N that is unaccounted for in the above-ground biomass.

Calculated from: a-c

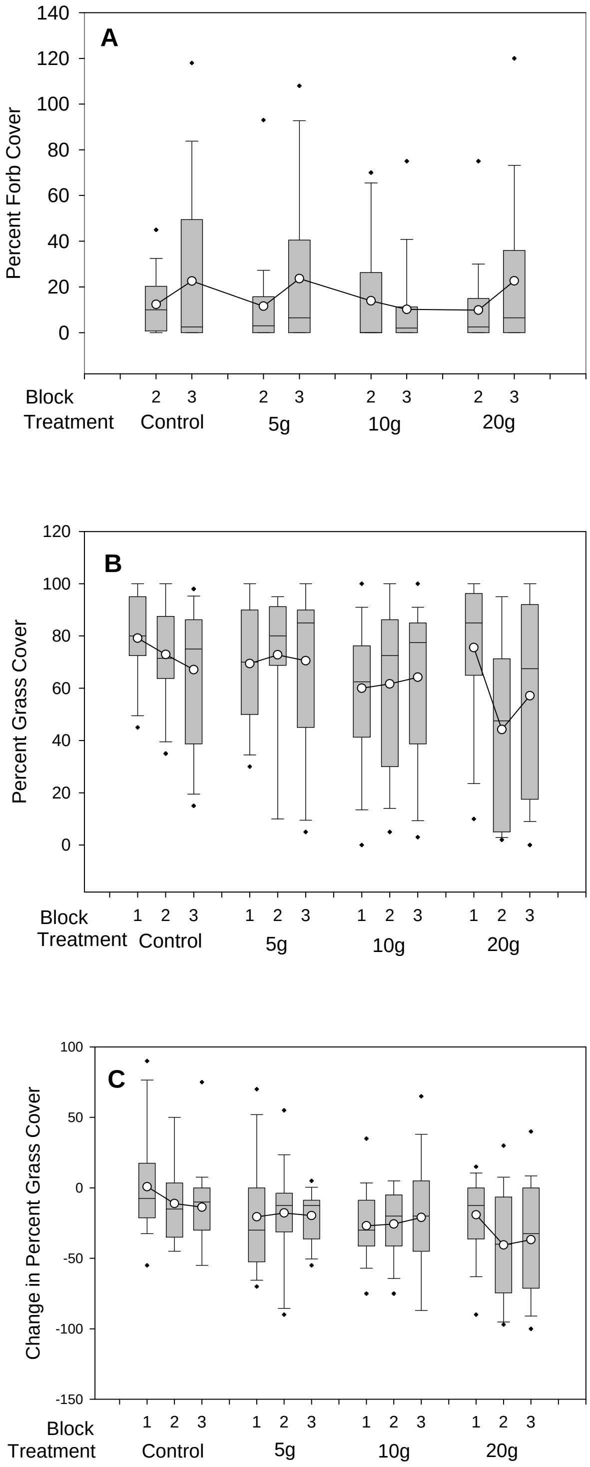

FIG. 1. Boxplots displaying the effects of 5 years of chronic N enrichment on native species cover on the AVIS prairie restoration in Upland, Indiana. The gray boxes display the 25th-75th

percentiles. The solid horizontal line in the middle represents the 50th percentile (median), and the white circle represents the mean. Lines above and below the boxes extend to the 90th and 10th percentile data. A: The ANOVA for forb cover indicated no significant differences ($p>0.05$) across all treatments and blocks in year five. B: The ANOVA for year five grass cover indicated a significant treatment effect ($p=0.022$). C: The ANOVA for the change in grass cover over the five years was also significant ($p=0.002$). Negative numbers indicate a decrease in cover.

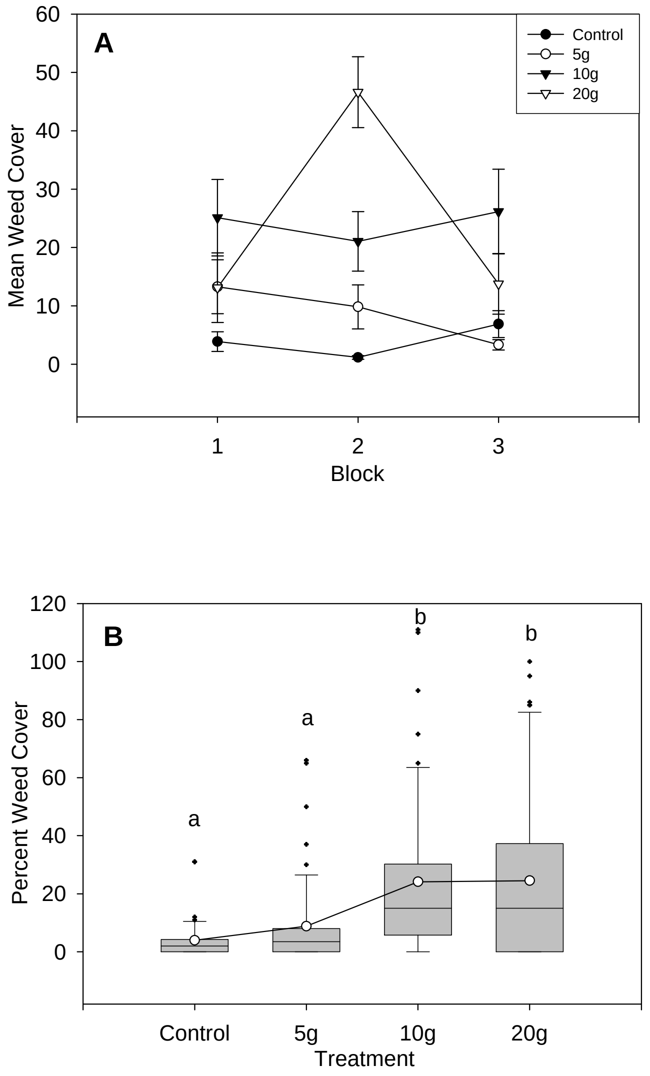

FIG. 2. Responses of weed cover to various levels of chronic N enrichment in year 5. A: The two-way ANOVA indicated significant interaction (p=0.000). Standard error bars are displayed. B: When blocks were combined, the one-way ANOVA indicated a significant treatment effect (p=0.000). Lower case letters indicate significant differences.

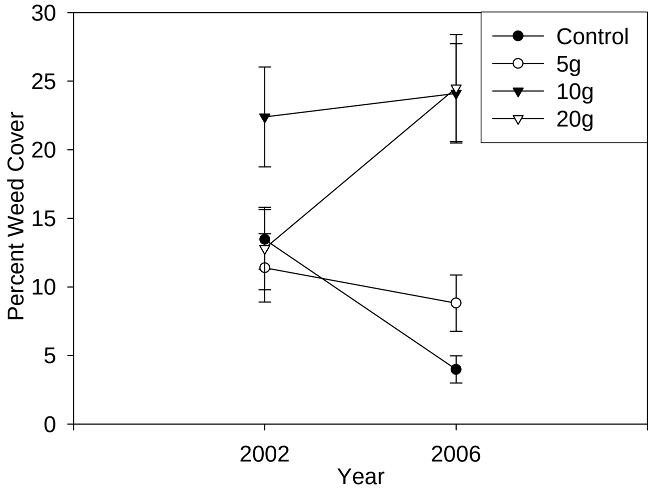

FIG. 3. Changes in percent weed cover due to chronic N enrichment over five years on the AVIS prairie restoration. The low treatment transects (control and 5g/m^2) decreased in weed cover, while the high treatment transects (10g/m^2 and 20g/m^2) increased in weed cover.

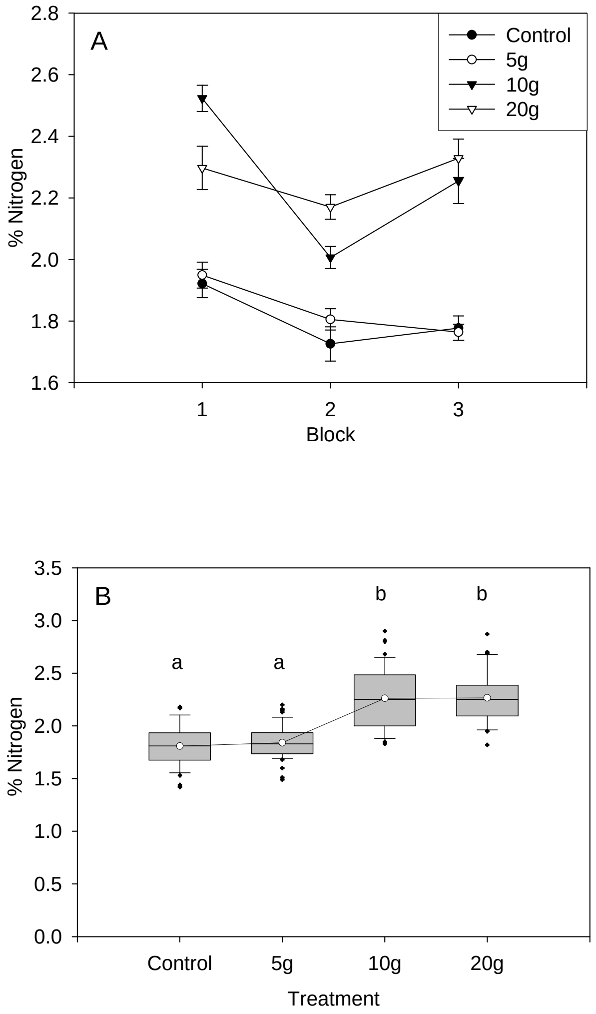

FIG. 4. The effects of nitrogen enrichment on percent N concentrations in the above-ground biomass of *Andropogon gerardii*. A: The two-way ANOVA indicated significant interaction (p=0.001) in the total percent N data. The interaction was between the control and 5g/m^2 transects, and between the 10g/m^2 and 20g/m^2 transects. B: Significantly higher amounts of N were taken up with the two higher applications of N than the control and 5g/m^2 treatments. Lower case letters indicate significant differences.