ABSTRACT

WHERLEY, BENJAMIN GEORGE. Nitrogen Relations in Bermudagrass During Growth and Dormancy Cycles. (Under the direction of Drs. Thomas W. Rufty, Jr. and Daniel C. Bowman).

Use of recycled water for turfgrass irrigation is increasing in the southeastern U.S. because of population growth and interest in protecting water quality. Turfgrass systems are perceived to be well suited for effluent dispersal due to their proximity to waste treatment facilities, in-ground irrigation systems, and ability to efficiently absorb (i.e. filter) nutrient contaminants when actively growing. However, effluent generation is continuous and bermudagrass growth is seasonal in the southeastern U.S. Clearly, there is a need to more thoroughly understand the capacity of bermudagrass, the turfgrass most often involved with effluent dispersal, for receiving effluent irrigation.

This series of experiments was designed with the overall intent of examining the capacity of a bermudagrass turf/soil system for handling effluent applications. Experiments involved 1) characterizing seasonal changes in nitrate assimilation efficiency of the system, 2) determining the effects of prolonged soil saturation on nitrate uptake efficiency, and 3) characterizing internal nitrogen relations during the spring emergence period.

While it is difficult to extend the results of these experiments, quantitatively, to situations where effluent is being applied in the field, the evidence does support a few basic observations. Bermudagrass appears to be capable of assimilating large amounts of nitrogen when growing, an ability that may well extend into transition months when little vertical shoot growth is occurring. Furthermore, although reduction in quality occurred, shoot growth and nitrate uptake efficiency of bermudagrass and centipedgrass was

relatively unaffected by prolonged soil saturation, a condition that may be likely with effluent irrigated sites.

Nitrogen Relations in Bermudagrass During Growth and Dormancy Cycles

by

Benjamin George Wherley

A dissertation submitted to the Graduate Faculty of
North Carolina State University
In partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Crop Science

Raleigh, NC

2007

APPROVED BY:

_____	_____
Dr. Wei Shi	Dr. Fred H. Yelverton
_____	_____
Dr. Daniel C. Bowman Co-chair of Advisory Committee	Dr. Arthur H. Bruneau

_____

Dr. Thomas W. Rufty
Chair of Advisory Committee

BIOGRAPHY

Benjamin George Wherley was born in Dover, OH in 1975, to Benjamin and Suzanne Wherley. The youngest of five children, Benjamin graduated from New Philadelphia High School in 1994. He attended Otterbein College in Westerville, OH, where he majored in Life Sciences. During both his high school and college years, Benjamin worked on the greens staffs at a number of golf courses, developing a strong interest and appreciation for the agronomic challenges involved with turfgrass management. After 2 ½ years at Otterbein, Benjamin transferred to The Ohio State University, where he enrolled in the turfgrass science program. In 1998, Benjamin married his high school sweetheart, Amy (McCluggage) Wherley. A year later, Benjamin graduated from Ohio State with a B.S. in Agronomy, and was employed by Wedgewood Golf and Country Club as an assistant golf course superintendent.

Benjamin began his graduate school career in the Department of Horticulture and Crop Science at The Ohio State University in January 2001 under the direction of Dr. David Gardner, with significant guidance from Dr. Karl Danneberger and Dr. James Metzger. Benjamin's Masters project concentrated on the influence of light intensity and the spectral composition on turfgrass development. After completing his Masters degree in June 2003, he attended North Carolina State University, where he pursued a Ph.D. in Environmental Turfgrass Physiology under the direction of Dr. Thomas Rufty.

In addition to his strong commitment to academics, Benjamin was actively involved in teaching, research, and extension activity during his graduate career. He presented his research at numerous scientific conferences, and was an active member of

American Society of Agronomy, National Association of Colleges and Teachers of Agriculture, Golf Course Superintendents Association of America, Turfgrass Council of North Carolina, and Ohio Turfgrass Foundation. Benjamin is a member of Gamma Sigma Delta (National Agriculture Honor Society), Pi Alpha Xi (National Horticulture Honor Society), and was named both the 2005 GCSAA Watson Fellow and the 2007 TCNC Eagle's Award Scholar.

ACKNOWLEDGEMENTS

I wish to sincerely thank my advisors, Drs. Thomas Rufty Jr., Daniel Bowman, Wei Shi, Fred Yelverton, and Arthur Bruneau for their guidance and support throughout the past four years. Their commitment to both my scholarly activity and professional development is greatly appreciated.

I am also thankful to Mike Jennette, William Blount, Anna Pardo, and Roxanne Fagan, who contributed substantially to the success of my research projects. Furthermore, I am thankful for the relationships I have developed with fellow graduate students during this time, including Adam Hixson, Gerald Henry, James Kerns, and David Goodman.

I am most grateful for my loving and supportive wife, Amy, and son, Grant. Without their sacrifice and support, none of this would have been possible. Most importantly, I am grateful to God for giving me the perseverance, strength, and character needed to fulfill this major accomplishment.

TABLE OF CONTENTS

LIST OF TABLES

CHAPTER I

CHAPTER II

CHAPTER III

LIST OF FIGURES

CHAPTER I

LIST OF FIGURES (continued)

CHAPTER II

CHAPTER III

CHAPTER I

NITROGEN RELATIONS IN BERMUDAGRASS DURING EMERGENCE FROM WITNER DORMANCY

ABSTRACT

In temperate regions, bermudagrass (*Cynodon dactylon* (L.) Pers.) has a distinct winter dormancy period when growth ceases, followed by re-growth in the spring. In this experiment, we investigate N relations during the initial days of re-growth as new shoots are developing from existing nodes. Single-node fragments (phytomers) of dormant bermudagrass were obtained from field plots and placed in nutrient solution culture in a controlled environment chamber. Nitrogen dynamics were determined using $^{15}NO_3^-$ labeling and tissue analysis. Shoots emerged on most plants within 2-3 days, and new adventitious roots emerged alongside existing roots within 5-6 days. Developing shoots were supplied N from internal and external sources, with internal N originating from nodes, internodes, and old roots. Total %N in the tissues remained relatively stable, indicating that mobilized N from internal pools was replaced by N acquired from the external media. Both old and new roots absorbed N from the solution, with uptake by new roots timed with their development. While internal N in isolated nodes and internodes were major contributors of N for shoot growth, alone they could only support shoot growth at a relatively slow rate.

INTRODUCTION

The bermudagrasses (*Cynodon ssp*.) are popular species in the southern U.S. for pastures, lawns, and recreational areas. They typically undergo a cycle of growth and dormancy in temperate regions, growing most rapidly during the heat of summer and transitioning to a state of winter dormancy as shoot growth stops and existing leaves senesce (Lee et al. 2003). The dormancy phase occurs when soil temperatures remain consistently below ~10˚ C and is characterized by near complete inactivity in meristematic regions of roots, rhizomes and stolons (Horowitz 1972; DiPaola 1982; Satorre et al., 1996). Following dormancy, a spring regrowth period signals the start of a new cycle.

DiPaola et al. (1982) characterized the seasonal growth and dormancy of bermudagrass turf. During dormancy, shoot growth stopped but much of the root system appeared to remain alive. Soon after shoot growth resumed in the spring, existing roots turned brown and died, and were replaced by completely new root systems within a period of 2-3 weeks. Shoot re-growth appeared to be driven by internal pools of carbon from old roots. This would imply that nitrogen remobilized from old roots may have contributed to growth of developing shoots.

Within many species (Chapin et al. 1980; Bausenwein et al. 2001b) including the grasses (Gloser 2002; Bausenwein et al. 2001a), remobilization of nitrogen from internal storage structures into new shoots and roots is essential for re-growth in early spring. In the rhizomatous grass *Calamagrostis epigeios*, the content of amino acids (which have a primary role in N storage) increased during the autumn, remained stable through winter, and declined

rapidly in early spring (Gloser 2002), reflecting their remobilization into new tissues. The extent to which remobilized N from existing roots, stems, and nodes is used to support new shoot and root growth in bermudagrass has not been examined in detail.

In this study, we characterize N relations of bermudagrass during emergence from winter dormancy. Of particular interest were internal N pools and external N as each contributed to new shoot development. The experiment involved supplying plants ^{15}N-labelled NO_3^- throughout the green-up process and monitoring changes in N pools in different plant parts as growth occurred. The experiments included an evaluation of the role of new and old roots during the recovery period.

MATERIALS AND METHODS

Stolons (~25 to 50 cm in length) were harvested in late January and early February 2006 from plots of dormant common bermudagrass (*Cynodon dactylon* (L.) Pers.). The bermudagrass had previously been maintained at ~2 cm and received granular fertilizer N (from a complete fertilizer) annually at a rate of 147 kg N ha^{-1} divided into three equal applications at six week intervals from mid-May through early September. Soil was carefully removed from stolons and subtending adventitious roots by gently rinsing. Stolons were then cut with a razor blade halfway between nodal sections to create single-node stolon fragments referred to as phytomers. Phytomers were selected for uniformity based on primary roots between 3 and 8 cm in length and grouped into subsets based on treatments (refer to Fig. 1). Treatments were applied by excising predetermined tissues as follows:

treatment 1) phytomers remained intact with old and new roots; 2) new roots were excised daily during the experiments, so phytomers developed with only old roots; 3) old roots were excised, so phytomers were dependent on new roots only; 4) all old roots and new roots were excised, so shoot growth occurred without contributions from roots; and 5) all tissues were excised from the node, so the new shoot developed without subtending tissues.

Dormant bermudagrass phytomers were placed into plastic culture containers (2.5 cm diameter x 2.5 depth) with plastic mesh bottoms that were held at the surface of culture solutions. A small opening was created in the mesh that allowed careful extension of old roots downward. These cups were randomly assigned to openings in the tops of five 12 L continuous-flow hydroponics units, each having a flow rate of ~ 2.5 L per minute and temperature/ pH control. Throughout the duration of experiments, solution temperature was maintained at 26˚C and pH at 6.5 +/- 0.2. Solution volume was maintained by adding distilled water daily to offset loss by evaporation.

The solution culture units were located in a controlled environment chamber programmed for a 10/14h light/dark period and aerial temperature of 30/22˚C. A photosynthetic photon flux of 1000 μmol m^{-2} sec^{-1} was maintained by a combination of metal halide and incandescent lamps (Phillips Halogena, Maddox Supply). The complete nutrient solution contained (in μmol L^{-1}): 800 $CaSO_4$, 300 $MgSO_4$, 600 KNO_3 (20 atom % ^{15}N), 75 KH_2PO_4, 0.055 $ZnSO_4$, 0.06 $MnCl_2$, 0.3 $B(OH)_3$, 0.065 $CuSO_4$, and 25 $FeSO_4$. The NO_3 concentration in the hydroponics was monitored and never declined more than 15% in any experiment.

Tissues were harvested after 1, 3, 6, 10, or 14 days of growth on the ^{15}N solutions. Phytomers were removed from hydroponics solution and thoroughly rinsed in 1.0 mM $CaSO_4$ to completely remove external nitrate. The tissues were then dissected into shoot, node, internode, old root, and new root fractions. A separate set of phytomers had been harvested prior to the start of the experiment, dissected similarly, and tissues frozen. All harvested tissues were oven dried at 65˚C for 72 hours, weighed, and pulverized to a fine powder with an oscillating ball mill (2000 Geno/Grinder, SPEX Certiprep Inc., Metuchen, NJ.). Dried samples were analyzed for total N and atom % ^{15}N using a Thermo Finningan DeltaPlus continuous-flow isotope ratio mass-spectrometer (CF-IRMSA, Bremen, Germany).

The treatments were arranged in a completely randomized design with four replications per treatment. The experiment was run three consecutive times. Data were subjected to analysis of variance procedures in SAS (ANOVA, SAS Institute, 2007). No experiment, experiment by harvest, or experiment by treatment interactions were detected at the $P= 0.05$ level, with regard to any of the parameters of interest, so a single analysis was carried out using the pooled data from the three experiments (ANOVA, SAS).

RESULTS AND DISCUSSION

This experiment examined how bermudagrass phytomers develop as growth resumes following dormancy, and specifically focuses on the sources of N (internal and extermal) that support new shoot growth. Remobilized N has been shown to provide 70% and 82% of the total N in early-spring shoot growth of *Festuca rubra* and *Agrostis capillaris*, respectively

(Bausenwein et al. 2001a), and 58% to the total above-ground early spring growth of *Rumex acetosa* (Bausenwein et al. 2001b). Little is known, however, about bermudagrass development and its dependence on internal or external pools of N.

The initial signs of shoot development were seen within 2-3 days of transfer to the growth chamber (Fig. 2a). Thereafter, shoot dry matter increased exponentially through the remainder of the study to a maximum of ~17 mg per node on day 14. Overall biomass production occurred primarily in shoots, as ~88% of the whole plant mass accumulated by the end of the study was in the shoot (data not shown).

Total N accumulation in new shoots showed a similar exponential increase, as shoots acquired ~630 μg total N by the end of the study (Fig. 2b). Remobilization of internal N (^{14}N) was fairly constant and supplied the majority of the N driving shoot growth during the initial 10 days. By contrast, ^{15}N absorbed from solution increased exponentially and by day 14 was the dominant source of shoot N.

The results generally agree with previous reports that new leaf tissue of grasses is the primary sink for remobilized N during the re-growth period (Ourry et al. 1988; 1990; Thornton et al. 1993). Shoot tissues also are the primary sink for carbohydrates and mineral reserves (Clement et al. 1978; Satorre et al., 1996). Because bermudagrass depends so heavily on internal N supplies during the initial 10 days of greenup, spring re-growth may be affected by environmental and/or management factors of the previous year (Gloser 2005).

Another method to assess the potential of internal N pools to supply new growth is to calculate the amount of N incorporated into new shoot and root growth over 14 days and compare it with the amount of N present in existing tissues (node, internode, and old roots).

Dormant phytomers harvested at the beginning of the experiment contained ~590 μg N (Table 1). The incorporation of N into new tissues was obtained from periodic harvest data. The hypothetical drop in internal N reserves (Fig. 3) indicates that internal N would be capable of supplying growth for a maximum of ~13 days. This assumes that all pre-existing tissue N is mobile, which is obviously unlikely. Further, although growth would likely be reduced in the absence of external N, the calculations shed light on the relative capacity of internal pools to support growth through the transition period. Clearly, external N must be acquired early in the green up period if a rapid growth rate is to be sustained.

Given the significance of remobilized N for new growth, it is important to consider the extent to which various tissues supply N. Previous research with other grasses indicates that old roots are the primary source of N for new growth, although all tissues provide some N (Gloser 2002; Bausenwein et al. 2001b). To address this question for bermudagrass, we analyzed individually the old roots, nodes, and internodes. Changes in %N, total N, total ^{15}N, and total ^{14}N of each of the tissues were determined from day 0 (dormancy) through the end of the experiment. Due to experimental variability, it was difficult to detect clear changes in %N of the tissues. Nonetheless, several trends were evident. With node tissue, %N and total N increased slightly over time (Fig. 4). Concurrently, the content of internal ^{14}N was decreasing slowly, likely due to translocation to the young shoot.

The N dynamics with internodes and old roots were somewhat different (Figs. 5 and 6). In both cases, %N and total N remained stable or decreased slightly, with internal ^{14}N being mobilized and replaced by ^{15}N from the external media. Thus, the accumulation of N in bermudagrass shoots reflected contributions from all of the tissues in the plant.

These data clearly show that there is substantial mixing of N in bermudagrass tissues during green up. Nitrogen within the dormant tissue is mobilized, even while newly absorbed N is being incorporated. From these analyses, we cannot know the types of N molecules being mobilized internally and those being formed with accumulation of external N, nor can we know if the same rate of mobilization of internal N would occur without an external N source present. There are no obvious precedents for these types of results in the published literature. Mixing of N from internal and external sources evidently occurred with roots during shoot re-growth of *Panicum maximum*, while roots of *Poa trivialis* tended to act as a net sink for mobilized N, irrespective of whether an external N supply was present (Santos et al. 2002).

The relative roles of new vs. old roots in N absorption is crucial to understanding N dynamics, since bermudagrass may lose the old root system during spring transition (Stuckey 1941; DiPaola 1982). To address this question, we evaluated NO_3^- uptake by phytomers possessing only old roots, only new roots, or both old and new roots (intact phytomer). Nitrogen uptake by intact phytomers amounted to ~ 550 μg N by the end of the study (Fig. 8). Surprisingly, phytomers with old roots alone absorbed similar amounts of N. Uptake was lower (~ 40%) by phytomers with only new roots, probably reflecting the time required for new root initiation and growth. However, once they were growing, new roots had higher uptake efficiencies compared to old roots (Fig. 9). When ^{15}N uptake was expressed per root dry wt, values for new roots were ~ 5 to 10 times greater than those for old roots. The most logical explanation is that old roots possessed senescent cell areas or tissues that were inherently inefficient because of aging or suberization. New roots were supporting ~ 60% as

much shoot mass as old roots (data not shown), which might suggest that feedback controls coordinating uptake with shoot sink demand were released.

Interestingly, uptake efficiency of old roots began to increase around day 10 (Fig. 9), coincident with root growth from the previously quiescent apical meristems of old roots. These new roots were white and possessed much finer texture than the old roots from which they developed (refer to Fig. 12d). More importantly, their development indicates that the over-wintering root system of bermudagrass, although initially low in uptake efficiency, is capable of resuming growth and function following dormancy despite its brown, weathered appearance.

One assumption throughout this work is that there are two sources of N, the internal tissue pool and the external soil solution. But it should be considered that the external pool may be extremely limited for some rhizomes perched within the upper thatch layer. What, then, might shoot growth be like if it were dependent only on internal N pools? In comparing the intact phytomers and those with no roots (treatments 4 and 5), it is clear that lack of a root system leads to considerable reductions in shoot growth (Fig. 10). Although little difference could be detected both in the timing of shoot emergence, or in shoot mass through day 6, by day 14, intact phytomers produced ~ 4 times more shoot mass than those lacking roots. Similarities in shoot growth through day 6 between the treatments were likely the result of both treatments relying primarily on internal supplies of N early on, but as roots developed, the importance on external N for sustained shoot growth became clear.

Summary Model

Based on our tissue analyses, it is possible to assemble a general model quantifying the contributions of N to growing shoot tissues, in effect an N budget. When tissues from all treatments are considered, allowing for greater replication, the mass and %N of nodal tissues was found to be stable over time. Therefore, for modeling purposes, masses and %N were normalized across treatments for each date (refer to Fig. 7). The mean node mass was 14.83 mg and %N was 1.37 %; thus, total N was 203 μg (Table 2). With greater replication, internode mass slightly decreased from 14 mg to 12 mg, %N from 0.95 to 0.80, and total N from 130μg to 96μg (Fig. 7). Normalized internode data were calculated from regression equations across harvest dates. When old roots existing at the beginning of the experiments are plotted in a similar manner, a mass of 21 mg, %N of 1.15, and total N 242 μg can be estimated (data not shown; refer to Table 2).

With the inclusion of shoot mass and A% ^{15}N enrichment data, net changes in total N, ^{14}N, and ^{15}N of various plant tissues from day 0 through 14 can be calculated, describing the flow of remobilized N (^{14}N pool) and absorbed N (^{15}N pool) that accumulate in shoot tissues. Whole plant N increased by an estimated 643 μg N from day 0 to day 14. By comparison, the total plant ^{15}N content accounts for an increase of 551 μg N, due to absorption. Thus, the model appears to be relatively accurate in predicting N flow into the tissues (~86%).

This model shows that substantial amounts of N are allocated to growth of new shoots and roots during the initial two weeks of spring emergence (refer to Table 2), as also indicated by the primary data. Over the 14-day period, shoots received ~570 μg N while new roots received ~110 μg N. Nodes, which remained fairly stable at ~200 μg total N

throughout the study, supplied 73 μg internal ^{14}N to other tissues and accumulated the same amount of N from uptake. Internodes lost ~62 μg of internal ^{14}N, while gaining ~29 μg ^{15}N, for a net decrease of ~33 μg of N. Old roots remained stable at 242 μg total N, providing 70 μg internal N to growing tissues and gaining 70 μg of N through uptake. In addition to the internal N coming from these tissues, a substantial amount (320 μg) of ^{15}N from external solution beyond that used to supply other tissues accumulated in the new shoots. The model shows that each tissue lost a similar amount of N with remobilization (~ 70 μg N) to the newly developing roots and shoots over the 14-day study. However, as a percentage of the internal N present, internodes sacrificed relatively more N (48%) than nodes (36%) and old roots (29%).

CONCLUSIONS

Bermudagrass turf grown in temperate climates is subjected to seasonal cycles of growth and dormancy. As rising spring temperatures trigger previously dormant nodes to gradually activate growth processes, the turf canopy slowly re-greens as new shoots begin to develop. Even in the presence of an external N source, bermudagrass plants remobilized considerable amounts of stored N from existing tissues to support growing shoots, and to a lesser extent new roots, during the early transition from dormancy. Thereafter, growth relies primarily on external N. These relationships imply that very early spring re-growth can be strongly influenced by management and environment conditions in the previous season prior to dormancy. Old roots, nodes, and internode tissues all served as sources of N transferred to the developing shoot. And, all tissues accumulated recently-absorbed N, indicating extensive mixing of the various tissue pools. During the initial phase of the green-up period, roots take

up only small amounts of external N. Formation of new roots (both new adventitious and extensions to old roots) is associated with, and presumably responsible for, a noticeable increase in nitrate uptake during the second week of green up.

REFERENCES CITED

Bausenwein, U., Millard, P., and J.A. Raven. 2001a. Remobilized old-leaf nitrogen predominates for spring growth in two temperate grasses. New Phytol. 152: 283-290.

Bausenwein, U., Millard, P., Thornton, B., and J.A. Raven. 2001b. Seasonal nitrogen storage and remobilization in the forb *Rumex acetosa.* Funct. Ecol. 15: 370-377.

Chapin, F.S., Johnson, D.A., and J.D. McKendrick. 1980. Seasonal movement of nutrients in plants of differing growth form in an Alaskan tundra ecosystem: implications for herbivory. J. Ecol. 68: 189-209.

Clement, C.R., Hopper, M.J., Jones, L.H.P., and E.L. Leafe. 1978. The uptake of nitrate by *Lolium perenne* from flowing nutrient solution. II. Effect of light, defoliation, and relationship to CO_2 flux. J. Exp. Bot. 29: 1173-1183.

DiPaola, J.M., Beard, J.B., and H. Brawand. 1982. Key events in the seasonal root growth of bermudagrass and St. Augustinegrass. Hort. Sci. 17(5): 829-831.

Gloser, V. 2002. Seasonal changes of nitrogen storage compounds in a rhizomatous grass *Calamagrostis epigeios.* Biol. Plant. 45(4): 563-568.

Gloser, V. 2005. The consequences of lower nitrogen availability in autumn for internal nitrogen reserves and spring growth of *Calamagrostis epigejos.* Plant Ecol. 179: 119-126.

Horowitz, M. 1972. Development of *Cynodon dactylon* (L.) Pers. Weed Res. 12: 207-220.

Lee, D.J., Bowman, D.C., Cassell, D.K., Peacock, C.H., and T.W. Rufty. 2003. Soil inorganic nitrogen under fertilized bermudagrass turf. Crop Sci. 43(1): 247-257.

Ourry, A., Boucaud, J., and J. Salette. 1988. Nitrogen mobilization from stubble and roots during re-growth of defoliated perennial ryegrass. J. Exp. Bot. 39(6): 803-809.

Ourry, A., J. Boucoud, and J. Salette. 1990. Partitioning and remobilization of nitrogen during re-growth in nitrogen-deficient ryegrass. Crop Sci. 30: 1251-1254.

Santos, P.M., Thornton, B., and M. Corsi. 2002. Nitrogen dynamics in the intact grasses *Poa trivialis* and *Panicum maximum* receiving contrasting supplies of nitrogen. J. Exp. Bot. 53(378): 2167-2176.

Satorre, E.H., F.A. Rizzo, and S.P. Arias. 1996. The effect of temperature on sprouting and early establishment of *Cynodon dactylon.* Weed Res. 36: 431-440.

Stuckey, I. 1941. Seasonal growth of grass roots. Am. J. Bot. 28: 486-491.

Thornton, B., Millard, P., Duff, E.I., and S.T. Bucklant. 1993. The relative contribution of remobilization and root uptake in supplying nitrogen after defoliation for regrowth of laminae in 4 grass species. New Phytol. 124: 689-694.

Table 1. Dry mass, percent N, and total N of dormant bermudagrass phytomers analyzed prior to the beginning of the experiment. Values are means +/- standard errors.

	Dry Mass (mg)	N (% of dry mass)	Total N (µg)
Node	15.18 +/- 1.80	1.25 +/- 0.08	190 +/- 20
Internodes	15.12 +/- 0.29	0.74 +/- 0.01	110 +/- 3
Old Roots	30.62 +/- 4.41	1.07 +/- 0.07	290+/- 30

Table 2. A summary model of changes in tissue N content in response to externally supplied (^{15}N pool) and internally supplied (^{14}N pool) nitrogen from dormancy (day 0) through day 14 of spring transition. Values were calculated from changes in tissue A%^{15}N enrichment of intact bermudagrass phytomers, and using tissue mass and %N values from all treatments (see text). Model accounts for 86% (551 μg) of the net 643 μg increase in plant ^{15}N.

Tissue	Dormant N Supply, μg	Net Gain/ Loss of Total N, μg	External ^{15}N Gain, μg (% of total uptake)	Internal ^{14}N Gain/ Loss, μg	Internal N Delivered to New Shoots & Roots (% of dormant supply)
Shoot	0	+567	320 (58%)	+247	-----
Node	203	stable	73 (13%)	-73	73 (36%)
Internode	129	-33	29 (5%)	-62	62 (48%)
Old Roots	242	stable	70 (13%)	-70	70 (29%)
New Roots	0	+110	59 (29%)	+51	-----

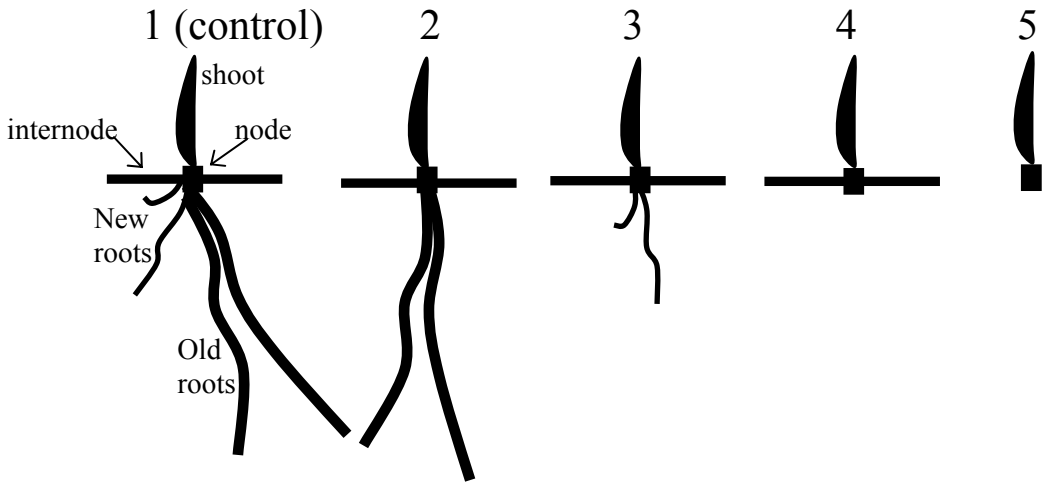

Figure 1. Illustration of bermudagrass phytomer treatments maintained through the experiment.

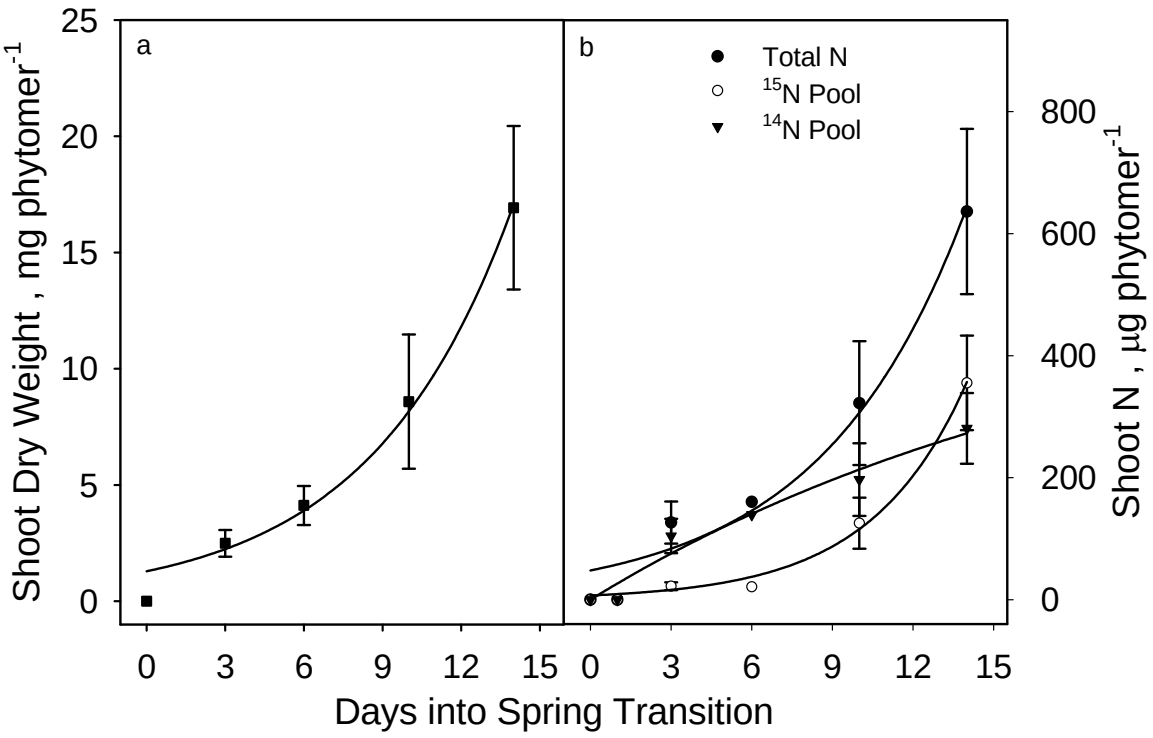

Figure 2. a) Shoot dry weight and b) total N, ^{15}N pool N, and ^{14}N pool N for shoots of intact phytomers. Error bars denote one standard error of the mean.

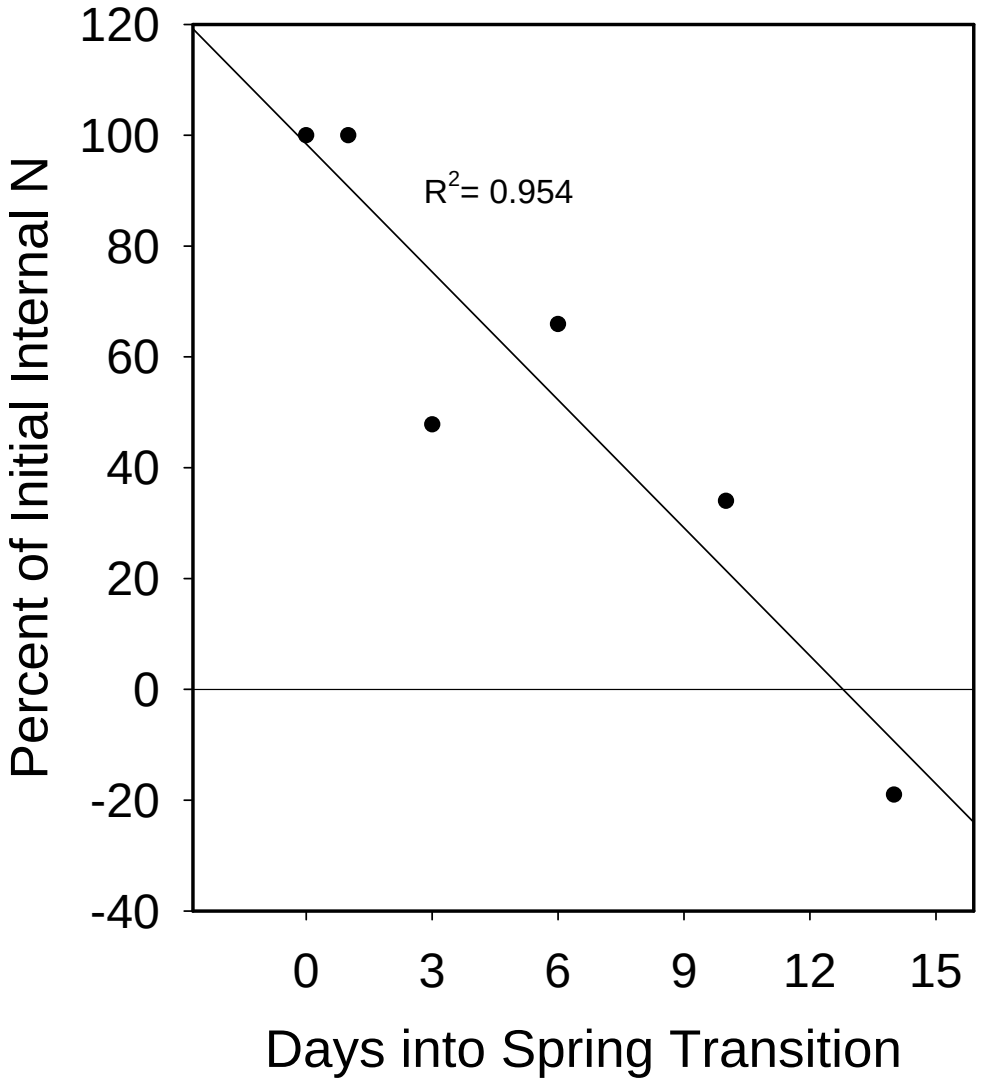

Figure 3. Percent of N supply remaining in existing tissues which could potentially support new shoot and root development. Initial N present in a single dormant phytomer was 590 μg N, determined from the average total N contained in twenty-six dormant phytomers harvested at the beginning of the experiments.

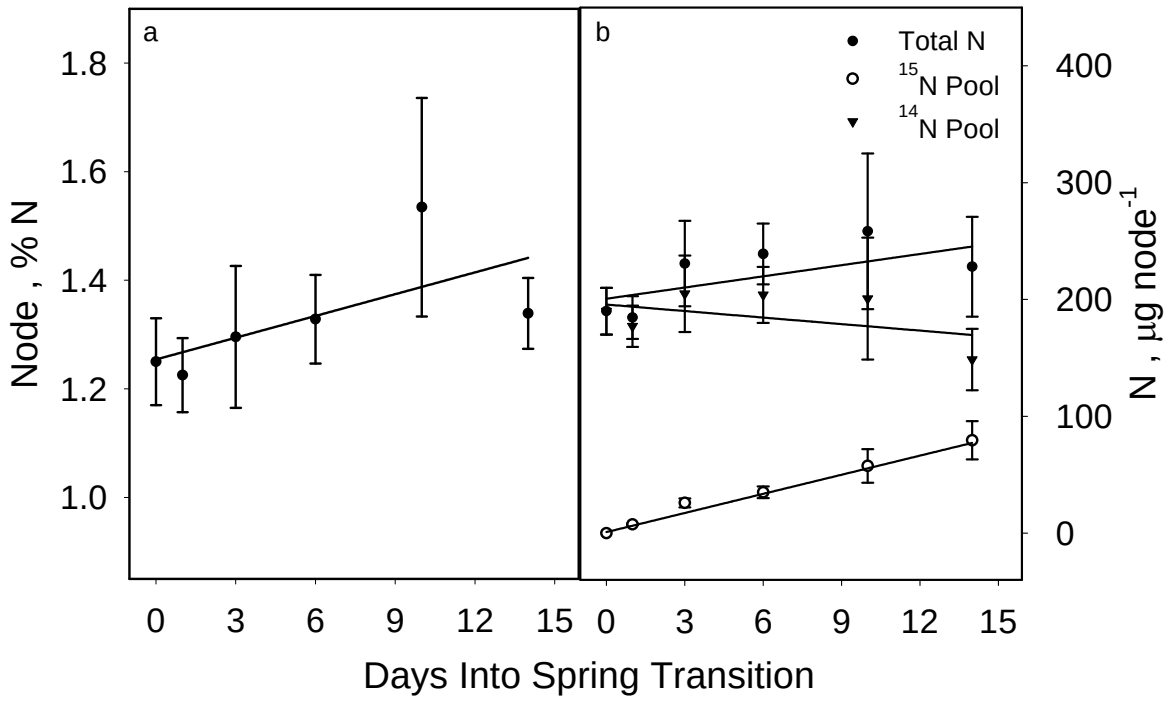

Figure 4. a) Percent N and b) total N, ^{15}N pool N, and ^{14}N pool N in node tissues of control plants. Error bars denote one standard error of the mean.

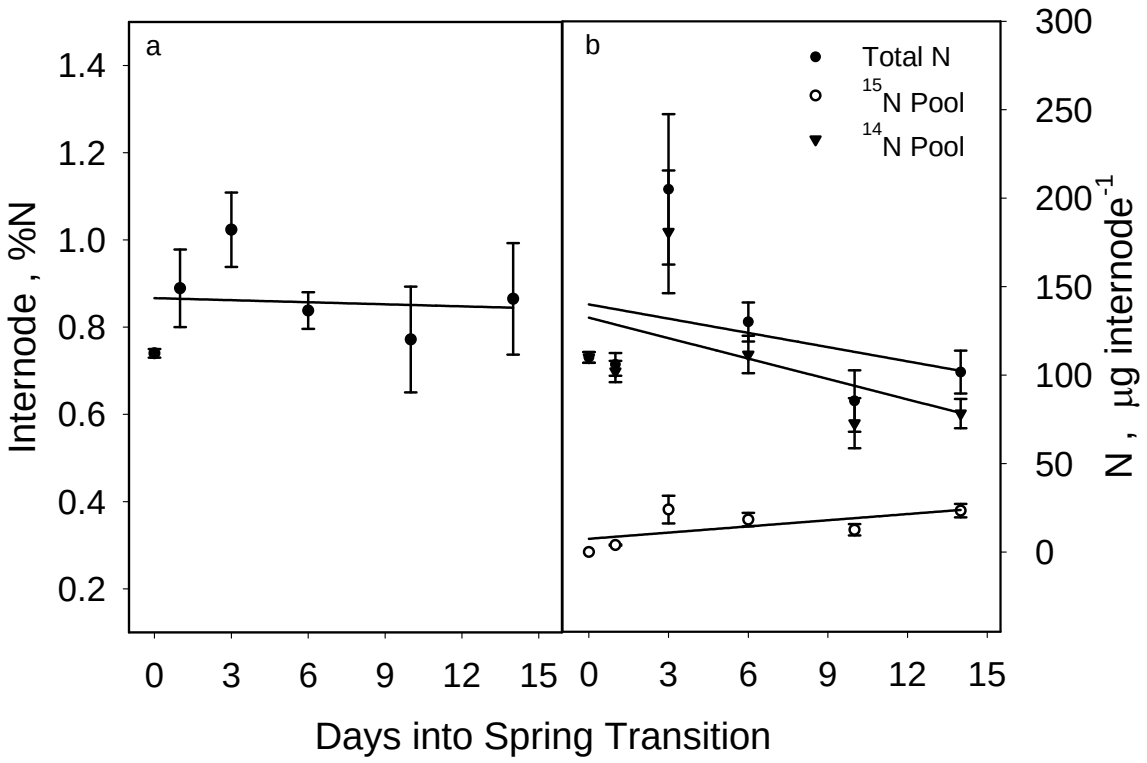

Figure 5. a) Percent N and b) total N, ^{15}N pool N, and ^{14}N pool N in internode tissues of control plants. Error bars denote one standard error of the mean.

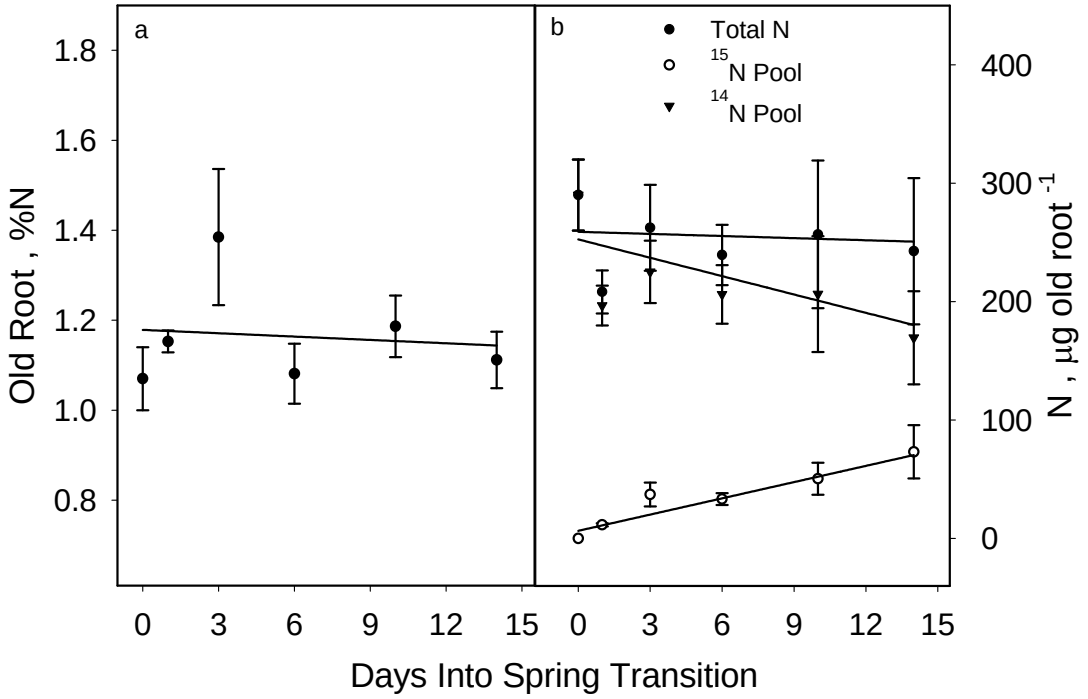

Figure 6. a) Percent N and b) total N, ^{15}N pool N, and ^{14}N pool N in old root tissues of control plants. Error bars denote one standard error of the mean.

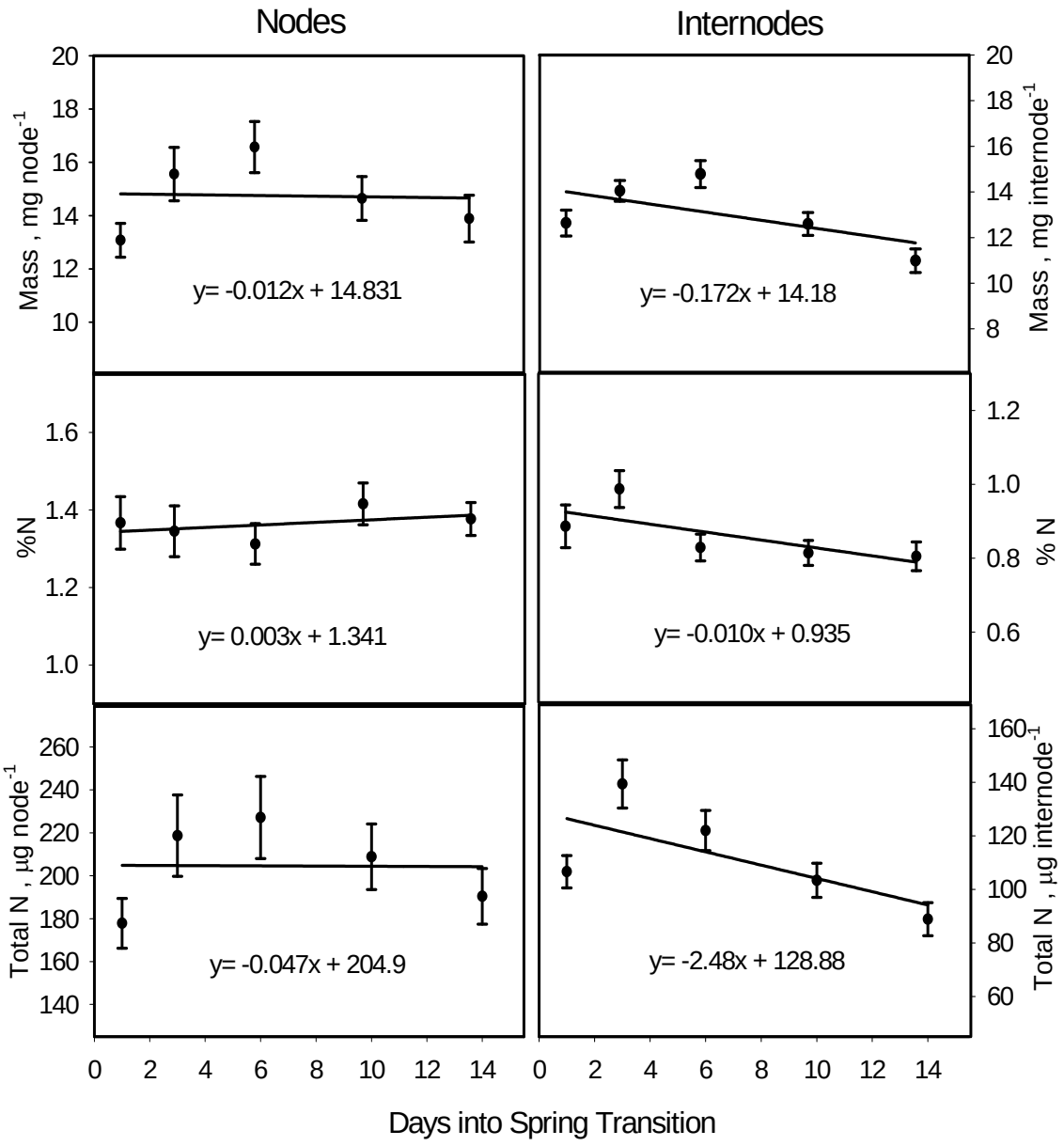

Figure 7. Mass, %N, and total N of nodes and internodes for the combined treatments over the 14-day experiment. Pure A% ^{15}N data and values derived from regression analysis at each harvest date were used to develop N flow model for bermudagrass emerging from dormancy.

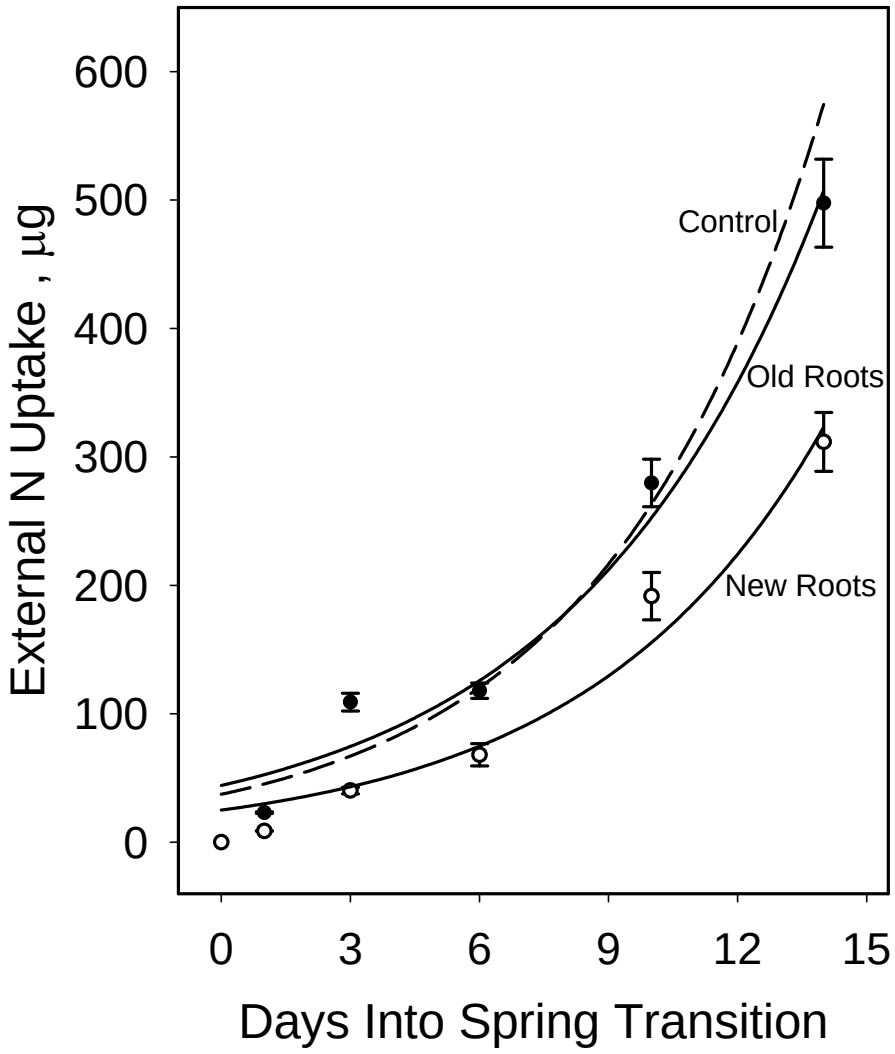

Figure 8. Total uptake from ^{15}N pool by phytomers having complete root system (dotted line), new root system only (hollow circles), or old root system only (solid circles). Error bars denote one standard error of the mean.

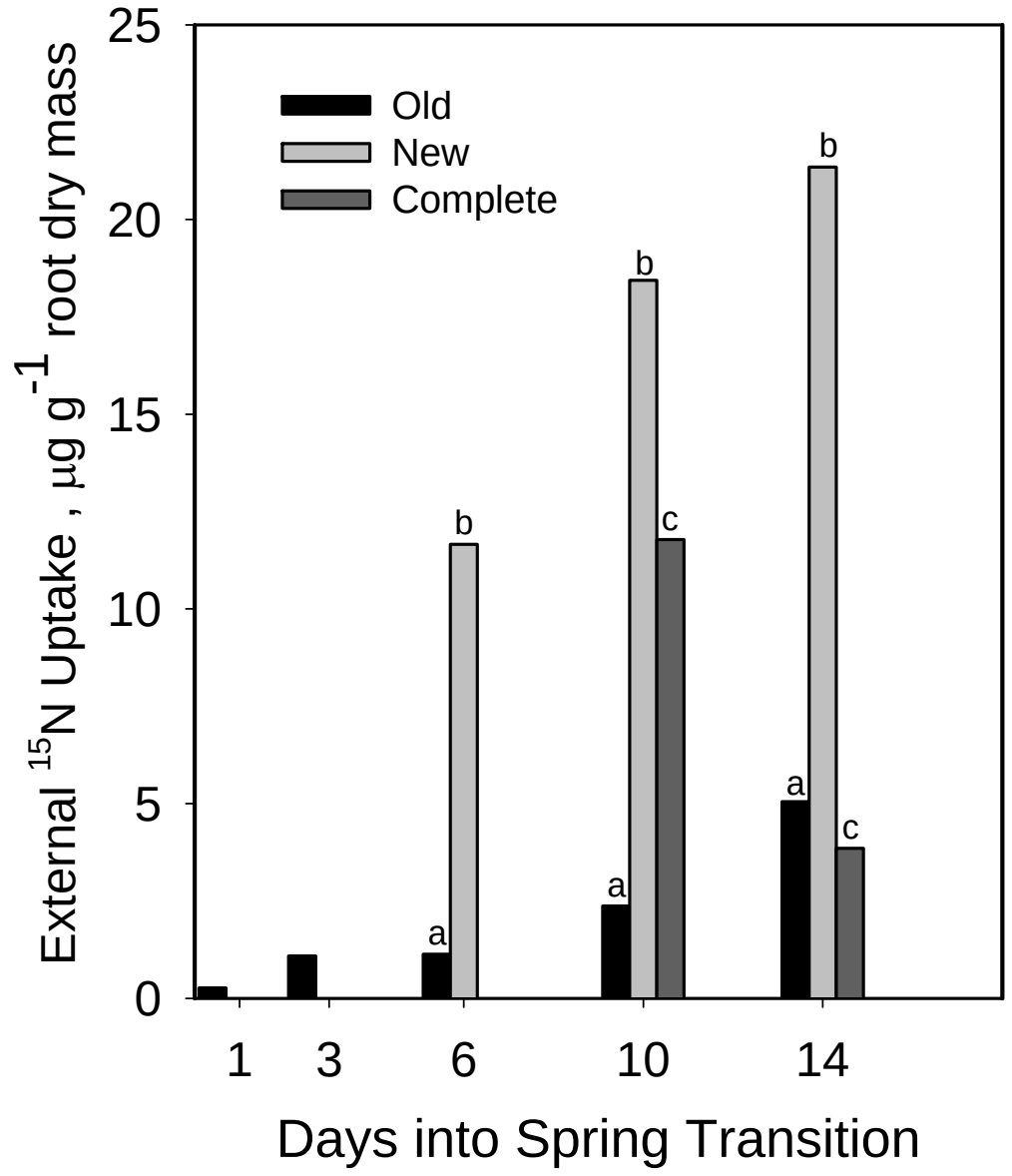

Figure 9. ^{15}N-nitrate uptake efficiency of bermudagrass phytomer tissues maintained through the experiment with old, mixed (old and new), or new root systems. Differences are significant at the $P < 0.05$ level based on analysis of log-transformed data (ANOVA, SAS).

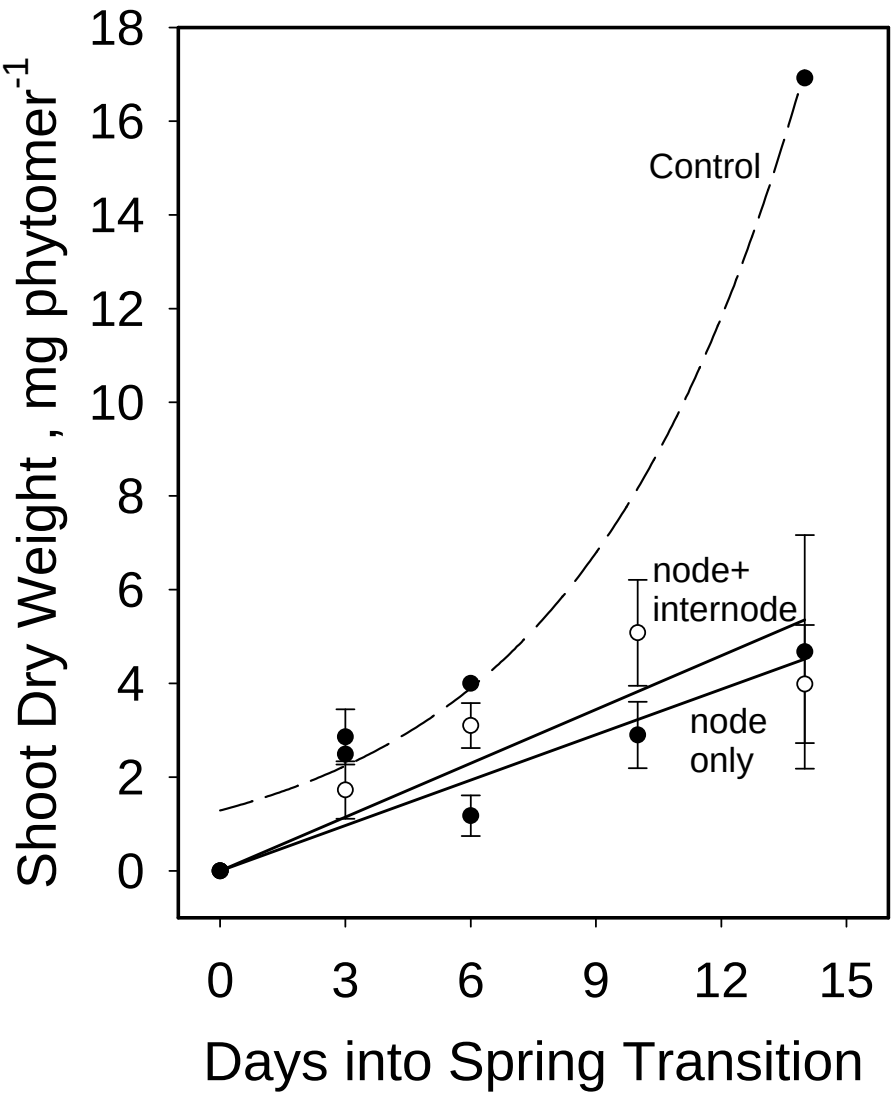

Figure 10. a.) Shoot dry matter production in treatments lacking roots. Treatment 5 (node only) represented by solid circles, and treatment 4 (node and internode) represented by hollow circles. Error bars represent one standard error of the mean.

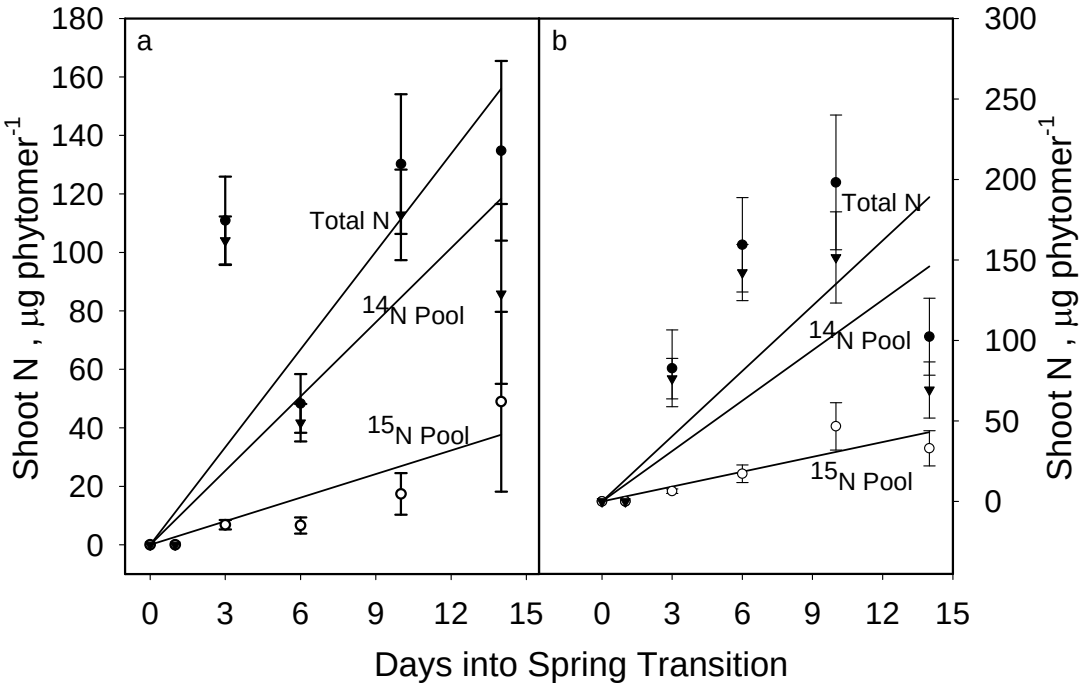

Figure 11. Shoot N accumulation in treatments lacking root systems. a) treatment 1 (node only) and b) treatment 2 (node and internode). Error bars denote standard error.

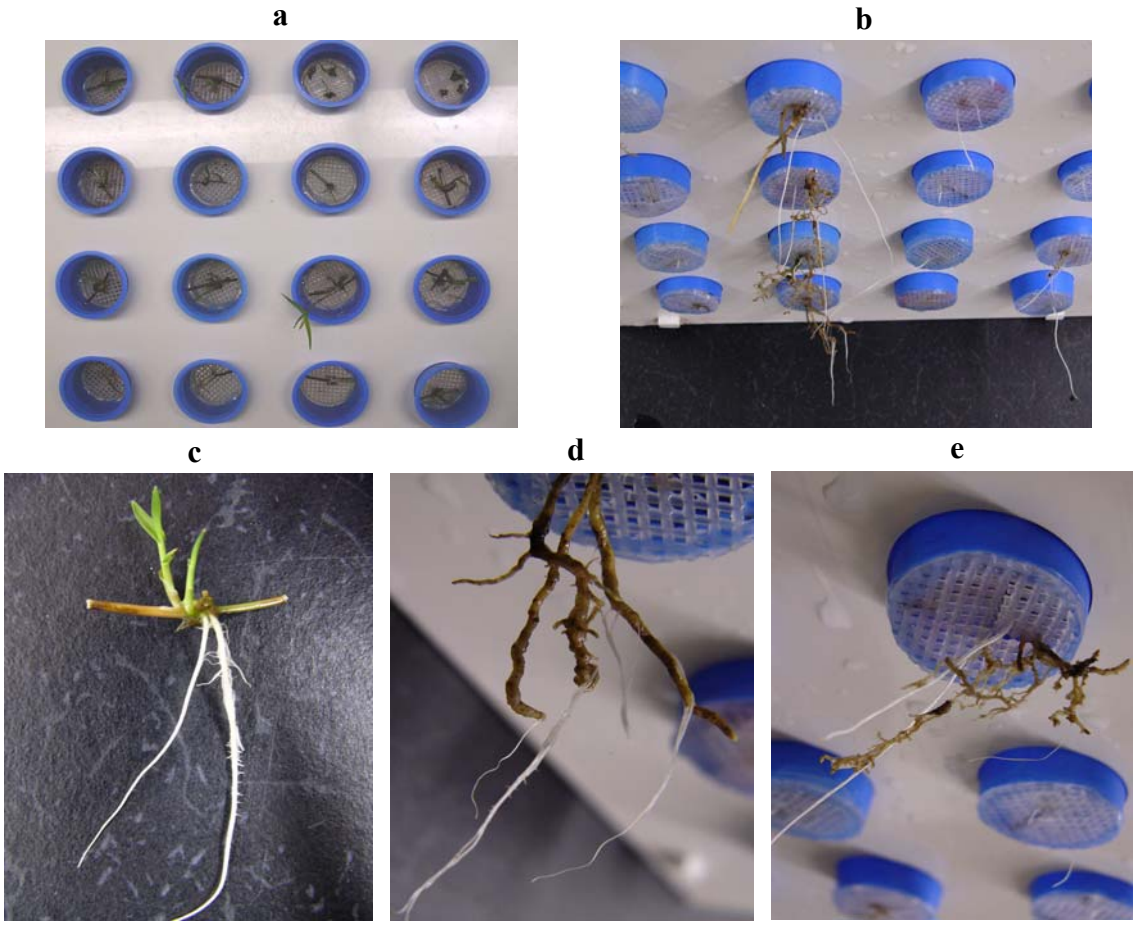

Figure 12a-e. a.) Surface view of experimental hydroponics unit containing bermudagrass tissue treatments. b.) View from underneath experimental hydroponic unit with old and new roots extending through mesh bottoms of containers. c,d,e.) Treatment 3, treatment 2, and treatment 1, respectively.

CHAPTER II

SEASONAL CHANGES IN NITRATE ASSIMILATION EFFICIENCY OF A BERMUDAGRASS SYSTEM

ABSTRACT

Pressures to protect water quality and water shortages are leading to increased applications of effluent water on turfgrass systems. In the southeastern U.S., there is a need to more thoroughly understand nitrogen use efficiency by bermudagrass, the turfgrass most often involved with effluent dispersal. The purpose of this series of experiments was to investigate changes in NO_3^- assimilation efficiency in 'Tifway' bermudagrass during different seasons of the year as it moves through growth and dormancy cycles. A model system used bermudagrass turf/soil cores obtained from field plots that were placed in controlled environment chambers and fed solutions containing 40 A% ^{15}N- NO_3^-. Periodic leaching and sampling of plant tissues and soil over 16 days revealed that NO_3^- uptake was, as expected, highest in summer months when plants were growing rapidly, as ~ 67% was taken into the plant within 3 days and >80% within 10 days. Even though the microbial population was elevated, little ^{15}N could be found in the soil microbial or organic fractions. The system was very inefficient in winter months when bermudagrass was dormant, as ~80% to 90% of the NO_3^- remained in soil for the duration of the 16-day experiment. The assimilation patterns were very different during the transition months of May and October. The $^{15}NO_3^-$ assimilation efficiency was higher than expected, with ~80 to 90% assimilated within 1 week, even though little bermudagrass shoot growth was occurring. A greater proportion of the ^{15}N was

sequestered belowground in rhizomes and roots and also in soil microbial and organic fractions. The apparent competitiveness of the microbial population was noticeably higher than that during rapid bermudagrass growth. Although differences in competitive interactions could be distinguished during various seasons, bermudagrass roots were consistently more competitive than the microbial population for applied $^{15}NO_3^-$, an observation contrary to that observed in other grass systems.

INTRODUCTION

Grass systems have long been utilized for disposal of animal waste in the southeastern U.S. Traditionally, this has involved applications of effluent generated from the anaerobic lagoons of farming operations onto nearby pastures (Burns et al., 1990). In recent years, landscape dispersal of effluent generated from municipal waste treatment facilities also has become common. The main drivers for this are legislation to protect water quality and water shortages usually resulting from population growth (refer to Harivandi, 2004). Applying effluent to the landscape allows filtering of potential contaminants by the vegetation, and it provides an alternative irrigation source. Because of the extensive acreages available and a need for irrigation, managed turfgrass systems are often being used as effluent dispersal sites.

Bermudagrass (*Cynodon ssp.)* is widely utilized in turfgrass systems that receive effluent throughout the southern U.S. From observations with other grass and pasture systems (Dillaha et al., 1989; Magette et al., 1989; Burns et. al, 1990; Lowrance and Sheridan, 2005), it is known that bermudagrass can efficiently take up (i.e. filter) large amounts of NO_3^- and P in applied effluent, which evidently are the primary causes of water quality degradation (Carpenter et al., 1998). Bermudagrass is a warm season grass, however, that is adapted to tropical and subtropical climates (Taliafarro, 1995). Throughout much of its growth range, it enters dormancy as winter approaches and may remain dormant for up to half of the year. Because bermudagrass cycles between growth and dormancy, it seems likely that seasonal NO_3^- uptake efficiency might also vary

considerably. Indeed, variations in leachate NO_3^- concentrations have been observed when bermudagrass received fertilizer or effluent during different times of the year (Snyder et al., 1984; Thomas et al., 2006). Large seasonal variations in NO_3^- uptake have frequently been observed with cool season grasses (Brown et al., 1982; Miltner et al., 1996; Liu et al., 1997; Frank et al., 2006).

The emergence of the effluent application issue highlights the need for understanding seasonal changes in NO_3^- uptake by bermudagrass managed as a turfgrass. The present work was designed for that purpose. A model system was developed that involved turf/soil cores removed from bermudagrass plots in the field and feeding of a relatively high level of ^{15}N-NO_3^-. The approach allowed evaluation of changes in NO_3^- uptake efficiency and fate of ^{15}N within different plant and soil fractions during different growth and dormancy cycles.

Of particular interest were interactions of bermudagrass with the soil microbial fraction. Past studies with annual grasslands and pasture grasses have indicated that soil microbes are capable of rapidly acquiring (immobilizing) a large portion of applied fertilizer nitrogen (Jackson et al., 1989; Kaye and Hart, 1997; Davidson et al., 1990). Often times, soil microbes release N after several days and weeks, serving as a temporary reservoir or 'storage pool' for N. It is unclear whether this storage pattern exists when bermudagrass is managed as a turfgrass, but it could have important implications for N retention efficiency.

MATERIALS AND METHODS

This study was carried out over two years (August 2004- May 2006). Turf/soil cores were periodically harvested from a six-year old stand of 'Tifway' bermudagrass (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burtt Davy) at the Sandhills Research Station, Jackson Springs, NC. The bermudagrass was growing on a native sand-textured soil (91% sand, 6% silt, and 3% clay; sandy thermic Arenic Kanhapludult). The plot was maintained at fairway height (~1.9 cm) and received a granular, complete fertilizer. Yearly N additions of 147 kg N ha^{-1} were split into three equal applications at six-week intervals from mid-May through early September. Organic matter content for the 0-15 cm depth of soil was ~1.5%, soil CEC was 3.9 meq 100 cm^{-3}, and pH was 5.9.

The turf/soil cores were removed from the field plot using a soil-sampling tool and cylinders (AMS Inc., American Falls, ID), which allowed a 5 cm diameter x 15 cm deep core to be removed from the field plots with minimal disturbance. Cores were obtained from the plot periodically during the growth and dormancy cycle: early May, early August, mid-October, and mid-January (refer to fig. 2). Prior to each core removal event, irrigation was applied to the turf to prevent wilting during transfer.

Twenty-four turf/soil cores were moved into growth chambers in the Southeast Plant and Environment Laboratory on the N.C. State University campus at each specified time during the year. Transfer occurred within 24 hours of removal from the field plots. Chamber conditions were programmed to simulate existing field conditions at that time of year, based on 40-year historical climate data obtained from North Carolina Climate

Records Database (refer to Table 1).

Experimental Approach

A thin fiberglass filter disk was placed at the bottom end of each core cylinder to prevent soil loss during leaching. A tight-fitting 5 cm female PVC bushing (Lasco Fittings, Inc., Brownsville, TN) was fitted onto the cylinder bottom. A ¼" I.D. threaded coupler was fitted into the bushing opening, providing an air-tight connection between the core and polypropylene drain tubing. The cores were then set into 5 cm diameter openings in the top of a 52cm x 30cm x 30cm PVC box, with only the top 1 cm of cores exposed to light. Thus, bermudagrass shoots and the soil surface were open to the chamber environment, but light exposure to the below ground portions was blocked. The drain tubing was connected to a manifold on the box exterior, which in turn was connected to a vacuum pump. With an inline vacuum gauge, the vacuum pump provided a tension of 0.15 bars evenly distributed over all cores, which approximates field capacity for sands. Each core was connected to 125 ml Erlenmeyer flasks by the drain tubing, allowing collection of leachate from individual cores.

On the first day of a 7 day pretreatment period, the turf/soil cores were watered heavily, followed by an immediate application of vacuum to bring the soil to field capacity. Twenty-four hours later, 33 ml of a complete nutrient solution containing 2.5 mg NO_3^--N was applied to the grass at the top of each core; equivalent to 1.2 g N m^{-2} applied in 1.5 cm of water. The cores were maintained at or slightly below field capacity by replacing water lost through ET on a daily basis (based on average water mass loss from several reference cores) for the next 5 days. On day 6, each core was thoroughly

flushed with 500 ml (~3 pore volumes) of re-distilled water and drained under vacuum. This removed residual soil NO_3^- prior to the start of the experiment. Preliminary experiments indicated that this volume removed > 95% of the residual soil NO_3^- from the soil (data not shown). Water was withheld on day 7 to allow soil moisture to decline below field capacity in preparation for application of ^{15}N solutions.

^{15}N Pulse-Chase

At the onset of the experimental period, the bermudagrass was clipped and $^{15}NO_3$ was applied at 9.9 mg N per core (4.9 g N m^{-2}) in 33 ml of solution. Sets of turf/soil cores were harvested periodically over the next 16 days to determine the fate of the applied ^{15}N.

Sample processing involved first flushing each core with 500 mls of re-distilled water, aided by the 0.15 bar tension. The tension was applied until leachate ceased to drip from the turf/soil cores (~10 minutes). The leachate was frozen until further analysis. Next, bermudagrass shoots were cut at 1.9 cm, with the clippings dried and weighed to determine growth. The remaining plant and soil material was removed from the core sleeve. Verdure (remaining above ground biomass) was excised cut at the soil surface and rinsed with 1.0 mM $CaSO_4$ to remove residual NO_3^-. Rhizomes and roots were separated from soil by hand, with root capture enhanced by use of a 2 mm sieve. The rhizomes and roots were then rinsed in 1.0 mM $CaSO_4$ as before. All plant tissues were oven dried at 65˚C for 72 hours and weighed. Dried plant samples were then milled to a fine powder, encapsulated in tin capsules, and analyzed for total N and A% ^{15}N using a Thermo Finningan DeltaPlus ratio mass spectrometer (CF-IRMSA, Bremen, Germany).

Following removal of plant tissues, soil was thoroughly homogenized and subsampled for analysis of soil inorganic ^{15}N, microbial biomass ^{15}N, dissolved organic ^{15}N, and ^{15}N in the solid fraction of soil organic matter (Fig. 1). Soil NO_3- was extracted with 0.5 M K_2SO_4 and determined colorimetrically using a Lachat flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). A diffusion method was used to prepare samples for ^{15}N analysis (Stark and Hart, 1996).

Microbial biomass ^{15}N was determined by the $CHCl_3$ fumigation-extraction procedure (Brookes el al., 1985). Briefly, 15 g moist soil samples were fumigated with $CHCl_3$ for 24 hours and extracted with 0.5 M K_2SO_4. Soluble N was oxidized to NO_3 with alkaline persulfate (Cabrera and Beare, 1993). Total NO_3-N of the extracts was determined colorimetrically. All extracts and samples were then analyzed for total N and % ^{15}N using the diffusion procedure described above. Microbial biomass N was calculated by dividing the N flush, i.e., the difference between fumigated and unfumigated samples, by an extraction coefficient of 0.54 (Brookes et al., 1985).

After fumigation-extraction, two additional 0.5 M K_2SO_4 extractions were performed on these soil subsamples to thoroughly remove any remaining soil NO_3^-. These soil samples were then analyzed for total N and ^{15}N as described previously. The N in these samples represented the solid fraction of soil organic nitrogen.

RESULTS

The seasonal growth pattern of bermudagrass was established based on clippings harvested from the field plots (Fig. 2). Growth was most rapid at the time the August

cores were removed. Acceleration to the most rapid growth coincided with mean soil temperatures reaching ~ 28 °C (refer also to Lee et al. 2003). On the other extreme, bermudagrass was completely dormant at the January sampling and no clipping mass could be collected. In May, a solid green cover was present even though little vertical growth was detectible with the clipping harvest. The bermudagrass was still green in October, but vertical shoot growth was even lower than in May. Overall, the sampling 'windows' provided cores with bermudagrass in very different growth states.

Seasonal leaching of NO_3^-

From the amount of applied NO_3^- recovered in leachate with the August cores, it was evident that NO_3^- was efficiently assimilated (Fig. 3). Of the 10.0 mg of NO_3^- applied added to the columns, only ~3.0 mg could be recovered with the washout after one day, < l.0 mg on day 3, and < 0.2 mg after the first week. Assimilation of NO_3^- was markedly lower with cores taken in January, with 7.4 to 8.2 mg of NO_3^- recovered in leachate throughout the 16 day experiment. The efficiency of NO_3^- assimilation in cores from the transition periods in May and October was noticeably lower than that in August during the first week after NO_3^- addition. Nonetheless, NO_3^- was steadily assimilated in cores taken in May; only very low amounts were detected after the first week. With cores sampled in October, NO_3^- in leachate followed a similar pattern. Some nitrate could be detected in leachate after the first week, but low amounts (~ 1 to 1.5 mg) were washed out later on.

Rapid Growth - August

At four of the sampling dates, the applied NO_3^- was labeled with 40 A% ^{15}N. Thus, columns being supplied with 10 mg of N were receiving 4 mg of ^{15}N. As is most often the case with ^{15}N experiments of this type, the total amount of ^{15}N recovered in plant, soil, and leachate fractions was variable. Thus, the fate of applied ^{15}N is most easily seen when data in individual fractions are expressed as a % of the ^{15}N recovered in the column (cf. Bristow et al. 1987; Jackson et al. 1989; Horgan et al. 2002; Dell and Rice 2005).

On day 1, about 3023 μg of ^{15}N were recovered in the grass and soil fractions, and 48% of the recovered ^{15}N was in plant and soil fractions other than the inorganic (Table 3, bold). About 42% of the recovered label was in plant tissues, and that percentage increased steadily to 67% on day 3, 83% on day 10, and 90% on day 16. The proportion of the recovered ^{15}N in shoots of plants increased as the ^{15}N chase period progressed, from 15% on day 1 to 49% on day 16. About 20-25% of the recovered ^{15}N was consistently present in rhizomes. Root ^{15}N tended to increase with time from 6% to 18-20%.

The microbial soil fraction contained about 7 to 8% of the recovered ^{15}N through day 10, after which the percentage dropped by half. The solid and dissolved soil organic matter fractions in the soil contained little of the ^{15}N present in the cores, always < 4% of the label in the system.

Dormancy - January

As indicated above, the cores in January were fully dormant, with no measurable

bermudagrass shoot growth occurring. The ^{15}N distribution patterns were very different than that in August, as a much smaller amount of ^{15}N was found in plant tissues (Table 4). Most of the plant ^{15}N accumulated in rhizomes, and none was found in shoots over the course of the experiment. The % of the recovered ^{15}N present in the soil microbial fraction was always low (< 3%), until the last sample date when the microbial fraction contained 7% of the ^{15}N label.

Transitions – May and October

The May samples were representative of Spring transition from dormancy to rapid growth. A substantial amount of recovered ^{15}N (~ 70 to 75%) was present in the bermudagrass plants after week 1 (Table 5). In contrast to the ^{15}N accumulation patterns in August, however, a smaller portion of the ^{15}N was found in shoot tissues (<30%), coinciding with the low clipping yields (cf. Fig. 2). Rhizomes and roots accumulated about the same portions of recovered ^{15}N as in August, with rhizomes being the main tissues accumulating ^{15}N, particularly early in the experiment.

The soil microbial fraction contained somewhat greater proportions of recovered ^{15}N than in August and January, with values ranging from 8 to 11% after day 3. ^{15}N in the solid and dissolved organic matter fractions also were somewhat elevated compared to the other months.

The October samples represent the seasonal transition from active growth to dormancy. With the exception of the dormant turf in January, ^{15}N accumulation by bermudagrass was lowest in October (Table 6). Although 21% of the recovered label was in the bermudagrass on day 1, the percentage increased to only 52% after 16 days.

This reflected lower ^{15}N accumulations in all the plant parts, compared to the proportions of ^{15}N recovered in the plant parts in August and May.

The decline in ^{15}N accumulation in bermudagrass in October coincided with higher accumulations in the soil organic fraction. The microbial fraction ^{15}N was similar to that in May, containing 8 to 12% of the recovered ^{15}N from days 7 to 16. The ^{15}N in the solid organic soil fraction was elevated, accounting for 7 to 11 % of recovered ^{15}N. The result was that the total ^{15}N accumulation in the organic fraction approached 25%, which was noticeably higher than that at other times of year.

Whole Plant Distributions of ^{15}N

From data presented in Tables 3 – 6, it is apparent that absorbed ^{15}N was distributed differently within the plants in the different seasons. The main seasonal effect was a change in the proportion of whole plant ^{15}N that was accumulated in shoot tissues. Re-calculation of the ^{15}N data from the bermudagrass plant, separate from the soil and leachate fractions, reveals that the proportion of the whole plant ^{15}N accumulated in the shoot was greatest during August, when plants were growing rapidly (Fig. 4), and lowest in January when plants were dormant and almost all of the ^{15}N taken up was retained below ground in rhizomes and roots. The individual data points for the months of May and October show, however, that shoot accumulation of ^{15}N was noticeably lower than in August.

Microbial Activity

As part of the ^{15}N analyses, measurements were made of total microbial biomass N ($^{14}N + {}^{15}N$; Table 3). It can be assumed that total microbial biomass N is correlated with microbial population size. The total N measurements indicate that microbial N was much larger in August than in other months (Fig. 4A). The mean values for all sample dates for each season were 48 in August, 22 in January, 14 in May, and 13 in October.

Using microbial biomass N as an estimate of the microbial population size, one can calculate a microbial activity index that reflects how effectively the microbial population competed for applied ^{15}N with the bermudagrass plants. Microbial assimilation of ^{15}N first involves accumulation by the microbes themselves and then release into the soil organic N pools. Thus, the best estimate of ^{15}N assimilation by soil microbes is the entire organic fraction. Expressing organic ^{15}N per mg of total microbial N (i.e. microbial population) indicates that the most effective microbial activity was in the transition months of May and October (Fig. 4). Microbial assimilation of ^{15}N was low in August, when the population was high, and very little occurred in January when temperatures were very low.

DISCUSSION

The main purpose of these experiments was to determine seasonal changes in NO_3^- uptake efficiency in a bermudagrass system. The leachate analyses and the ^{15}N analyses from plant and soil fractions indicated that large differences occurred in the fate of applied NO_3^- during different seasons. The most obvious contrast was between August

and January, one period when bermudagrass was growing rapidly and NO_3^- assimilated efficiently and the other when bermudagrass was dormant and NO_3^- capture was low. One of the most interesting observations was that NO_3^- uptake and ^{15}N retention by the system was relatively high in the transition periods of May and October, when vertical shoot growth of bermudagrass was relatively low. As discussed below, the N fate profiles during transitions were noticeably different than in other times of year, and also from each other.

Various studies have indicated that bermudagrass is highly efficient in taking up NO_3^- when growing rapidly. That was the case in constructed field plots (Snyder 1984) and lysimeters in a greenhouse (Bowman et al. 2002), and is implied by the very low levels of NO_3^- found beneath bermudagrass *in situ* on golf course fairways (Lee et al. 2003). The general caveat has been that fertilizer additions must not coincide with excess rainfall or irrigation, which would increase the likelihood of leaching (Snyder et al., 1984). As shown in the ^{15}N recoveries in our experiments, the high capture efficiency of the system reflects, primarily, uptake by the bermudagrass roots and incorporation of N into shoot tissues. Leachate analyses indicated that little NO_3^- could be washed out of the columns after 3 days, and almost 70% of the recovered ^{15}N was in the plant after 3 days and greater than 90% after 2 weeks. The majority of the remaining ^{15}N was present in organic fractions. Leaching studies with ^{15}N and cool season turfgrass species have found similar results. When perennial ryegrass, tall fescue, and creeping bentgrass were growing under ideal conditions, almost all of the soluble fertilizer N was assimilated by the turf/soil system within 2 days of application (Bowman et al. 1989). Also, application

of ^{15}N-labelled ammonium sulfate to Kentucky bluegrass and perennial ryegrass turf resulted in minimal nitrogen movement below the root zone (Engelsjord et al., 2004).

Our results indicate that bermudagrass takes up NO_3^- at a somewhat slower rate during the transition months, but > 90% of added NO_3^-N was assimilated after one week in cores from May, and 80% was assimilated by the end of two weeks with cores from October. In both cases, there was greater retention of absorbed NO_3^-in below ground plant tissues and soil organic fractions compared to that in August.

It is likely that two physiological factors are responsible for the high uptake efficiency of bermudgrass in August. First is the very high root length density, which allows full access to water and mobile nutrients within the rhizosphere volume (Bowman et. al 2002). It has been estimated that a mature turf may possess more than 100,000 roots and 1 million root hairs per liter of topsoil (Dittmer 1938). The second factor is the high activity of the NO_3^- uptake system. Even with fertilizer N additions like that supplied in our experiments, bermudagrass and other turfgrasses are generally growing far below their maximal capacities and thus are N deficient (Bowman 1989). Uptake of NO_3^- per unit of root is strongly regulated by an internal feedback control system that coordinates plant growth potential and acquisition (Clarkson 1986; Imsande and Touraine 1994; Glass 2003). With a sub-optimal N supply, NO_3^- transporters at root cell membranes would be expected to be de-repressed and able to rapidly take up NO_3^- as molecules approach the root surface. The ^{15}N accumulation pattern during transition months indicates that bermudagrass had the ability to efficiently take up NO_3^-, but the accumulation of ^{15}N in rhizomes and roots suggests that translocation out of the root into

vascular tissues was restricted. Cause and effect are always difficult to sort out in this type of response, but it is known that xylem transport is a sensitive control point in feedback responses (Pitman 1977; Rufty 1997). It is conceivable that xylem transporters could be responding to signals originating in the shoot, where vertical shoot growth may be limited by the number of active nodes in the less than ideal conditions, or they could be responding to cool temperatures in the root zone, which are known to inhibit movement of substances upward (Rufty et al. 1981; Engels et al. 1992).

Microbial N Assimilation

Most available evidence indicates that soils beneath turfgrasses contain relatively high levels of organic matter and high microbial activity (for a recent review, see Shi et al. 2007). This results from rapid plant growth rates (i.e. high primary productivity), cycling of clippings (Shi et al. 2006a), and substantial allocation of plant mass to below ground structures (Kaye et al. 2005). With turfgrasses in general, organic matter (Qian and Follet 2002; Qian et al. 2003; Bandaranayake et al. 2003) and microbial biomass (Kaye et al. 2005) increase with age of the turfgrass system; a relationship that clearly holds with bermudagrass (Shi et al. 2006b).

The changes in microbial biomass N in our experiments indicate that the microbial population beneath bermudagrass turf varied during the year. Much larger microbial biomass N levels were present in August cores, presumably reflecting the high rate of carbon generation in the system. Yet, ^{15}N capture by microbes, estimated by the microbial activity index, was much lower in August than in the transition months of May

and October. The implication is that the microbial population was less competitive for available $^{15}NO_3^-$ than roots and rhizomes during bermudagrass' rapid growth phase. It logically follows that higher indices in May and October reflected decreased bermudagrass competitiveness.

Regardless of the time of year, the proportion of the ^{15}N associated with microbial activity was noticeably lower than that observed in experiments with other grass systems. Studies with ^{15}N have not always measured the same soil components, so direct comparisons are not as precise as one might prefer. Estimates from ^{15}N in the microbial biomass alone have indicated an initial assimilation of 37% in an established ryegrass sward (Bristow et al. 1987), 37 to 61% in a native grassland savanna (Jackson et al. 1989), and 35 to 80% in a natural tall grass prairie (Dell and Rice 2005). All were much higher than the 13% found in our experiments when the microbial fraction is considered alone, and the ^{15}N proportion was still less than < 20% if the entire organic fraction is taken into account (with one exception in October). This observation again suggests that the below ground structures in bermudagrass turf out-compete microbes for available NO_3^-. In addition, there was no evidence of a decline in the ^{15}N microbial pool with time, as has been observed in some other studies. Once assimilated by the microbial system, ^{15}N remained relatively stable in the microbial fraction itself or the downstream soil organic fraction at least over the time frame of our experiments. Some caution is required when considering these observations. The bermudagrass stand was relatively young, having been established for about 6 years at the time the experiment began. It is conceivable that capture of ^{15}N by the microbes would be considerably higher at a more

advance age when soil organic matter and the microbial population would have been larger (Shi et al. 2007).

Implications for Effluent Dispersal

It is difficult to extent the results of these experiments, quantitatively, to situations where effluent is being applied in the field. Nonetheless, the evidence does support a couple of basic observations. The nitrogen load on bermudagrass will reflect N in effluent and N applied in fertilization (Thomas et al. 2006). The amount added to cores was ~ equivalent to that added during individual fertilizations. Because of the low amount of assimilation of NO_3^-, it is clearly inappropriate to apply effluent during dormancy because of environmental concerns. Although this may seem obvious, few studies of this type have been conducted previously, and with the year-round generation of effluent, it can be tempting to disperse effluent year-round as well. From our results, it is also is evident that substantial amounts of effluent can be applied during transition periods when bermudagrass growth is slow. Other than during dormancy, the results of our experiments clearly indicate that bermudagrass has the capability of assimilating large amounts of N and the ability may well extend into transition months when little vertical shoot growth is occurring. We cannot know what repeated applications over weeks or months might do to the bermudagrass uptake efficiency, a question that will require additional experimentation.

REFERENCES CITED

Bandaranayake, W., Y.L. Qian, W.J. Parton, D.S. Ojima, and R.F. Follett. 2003. Estimation of soil organic carbon changes in turfgrass systems using the CENTURY model. Agron. J. 95: 558-563.

Bowman, D.C., C.T. Cherney, and T.W. Rufty, Jr. 2002. Fate and transport of nitrogen applied to six warm-season turfgrasses. Crop Sci. 42:833-841.

Bowman, D.C., J.L. Paul, W.B. Davis, and S.H. Nelson. 1989. Rapid depletion of nitrogen applied to kentucky bluegrass turf. J. Amer. Soc. Hort. Sci. 114(2):229-233.

Bristow, A.W., J.C. Ryden, and D.C. Whitehead. 1987. The fate at several time intervals of ^{15}N-labelled ammonium NO3- applied to an established grass sward. J. Soil. Sci. 38:245-254.

Brookes. P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17(6): 837-842.

Brown, K.W., J.C. Thomas, and R.L. Duble. 1982. Nitrogen source effect on NO3- and ammonium leaching and runoff losses from greens. Agron. J. 74:947-950.

Burns, J.C., L.D. King, and P.W. Westerman. 1990. Long-term swine lagoon effluent applications on 'coastal' bermudagrass: I. yield, quality, and element removal. J. Environ. Qual. 19:749-756.

Cabrera, M.L., and M.H. Beare. 1993. Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. Soil Sci. Soc. Am. J. 57(4):1007-1012.

Carpenter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Ecol. App. 8(3): 559-568.

Clarkson, D.T. 1986. Regulation of the absorption and release of nitrate by plant cells: a review of current ideas and methodology. In: Lambers H, Neeteson JJ, Steulen I, eds. *Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants.* Martinus Nijhoff Publishers, 3-27.

Davidson, E.A., J.M. Stark, and M.K. Firestone. 1990. Microbial production and consumption of NO3- in an annual grassland. Ecology 71(5):1968-1975.

Dell, C.J. and C.W. Rice. 2005. Short-term competition for ammonium and nitrate in tallgrass prairie. J. Soil. Sci. 69: 371-377.

Dillaha, T.A., R.B. Reneau, S. Mostaghimi, and D. Lee. 1989. Vegetative filter strips for agricultural nonpoint source pollution control. T ASAE 32:513–519.

Dittmer, H.J. 1938. A quantitative study of the subterranean members of three field grasses. Am. J. Bot. 25: 654-657.

Engels, C., L. Munkle, and H. Marschner. 1992. Effect of root zone temperature and shoot demand on uptake and xylem transport of macronutrients in maize (*Zea mays* L.). J. Exp. Bot. 43(4): 537-547.

Engelsjord, M.E., Branham, B.E., and B.P. Horgan. 2004. The fate of nitrogen-15 ammonium sulfate applied to kentucky bluegrass and perennial ryegrass turfs. Crop Sci. 44: 1341-1347.

Frank, K.W., K.M. O'Reilly, J.R. Crum, and R.N. Calhoun. 2006. The fate of nitrogen applied to a mature kentucky bluegrass turf. Crop Sci. 46:209-215.

Glass, A.D.M. 2003. Nitrogen use efficiency of crop plants: Physiological constraints upon nitrogen absorption. Critical Rev. in Plant Sci. 22: 453-470.

Harivandi, M.A. 2004. Considerations in retrofitting a golf course for recycled water irrigation. USGA Green Section Record. Nov/Dec 2004.

Horgan, B.P., B.E. Branham, and R.L. Mulvaney. 2002. Mass balance of ^{15}N applied to kentucky bluegrass including direct measurement of denitrification. Crop Sci. 42:1595-1601.

Imsande, J., and B. Touraine. 1994. N demand and the regulation of nitrate uptake. Plant Physiol. 105: 3-7.

Jackson, L.E., J.P. Schimel, and M.K. Firestone. 1989. Short-term partitioning of ammonium and NO3- between plants and microbes in an annual grassland. Soil Biol. Biochem. 21(3):409-415.

Kaye, J.P. and S.C. Hart. 1997. Competition for nitrogen between plants and soil microorganisms. Trends Ecol. Evol. 12(4):139-143.

Kaye, J.P., R.L. McCulley, and I.C. Burke. 2005. Carbon fluxes, nitrogen cycling, and soil microbial communities in adjacent urban, native and agricultural ecosystems. Global Change Biol. 11: 575-587.

Lee, D.J., D.C. Bowman, D.K. Cassel, C.H. Peacock, and T.W. Rufty, Jr. 2003. Soil inorganic nitrogen under fertilized bermudagrass turf. Crop Sci. 43:247-257.

Liu, H., R.J. Hull, and D.T. Duff. 1997. Comparing cultivars of three cool-season turfgrasses for soil water NO_3^- concentration and leaching potential. Crop Sci. 37:526-534.

Lowrance R. and J.M. Sheridan. 2005. Surface runoff water quality in a managed three zone riparian buffer. J. Environ. Qual. 34:1851-1859.

Magette, W.L., R.B. Brinsfield, R.E. Palmer, and J.D. Wood. 1989. Nutrient and sediment removal by vegetated filter strips. T ASAE 32(2):663-667.

Miltner, E.D., B.E. Branham, E.A. Paul, and P.E. Rieke. 1996. Symposium on pesticide and nutrient fate under turfgrass golf course conditions. Crop Sci. 36:1427-1433.

Pitman, M.G. 1977. Ion transport into the xylem. Annu. Rev. Plant Physiol. 28: 71-88.

Qian Y.L., and R.F. Follett. 2002. Assessing soil carbon sequestration in turfgrass systems using long-term soil testing data. Agron. J. 94: 930-935.

Qian, Y.L., W. Bandaranayake, W.J. Parton, B. Mecham, M.A. Harivandi, and A.R. Mosier. 2003. Long-term effects of clipping and nitrogen management in turfgrass on soil organic carbon and nitrogen dynamics: the CENTURY model simulation. J. Env. Qual. 32: 1694-1700.

Rufty, T.W. 1997. Probing carbon and nitrogen interactions: A whole plant perspective. In CH Foyer, P Quick, eds, *A Molecular Approach to Primary Metabolism in Higher Plants*. Taylor and Francis, London, UK, pp 255-273.

Rufty, T.W., C.D. Raper, W.A. Jackson. 1981. Nitrogen assimilation, root growth and whole plant responses of soybean to root temperature and to $C0_2$ and light in the aerial environment. New Phytol. 88:607-619.

Shi, W., S. Muruganandam, D. Bowman. 2006a. Soil microbial biomass and nitrogen dynamics in a turfgrass chronosequence: a shot-term response to turfgrass clipping addition. Soil Biol and Biochem 38: 2032-2042.

Shi, W., H. Yao, D.C. Bowman. 2006b. Soil microbial biomass, activity, and nitrogen transformations in a turfgrass chronosequence. Soil Biol and Biochem 38: 318-319.

Shi, W., Bowman, D.C., and T.W. Rufty. 2007. Soil microbial community composition and function in turfgrass ecosystems. Biodiversity and Bioavailability 1: 72-77.

Sidle, R.C. and G.V. Johnson. 1972. Evaluation of a turfgrass-soil system to utilize and purify municipal waste water. In: Hydrology and Water Resources in Arizona and the Southwest, vol. 2. Proceedings of Meetings of the Arizona Section, American Water Resources Assn., and the Hydrology Section, Arizona Academy of Science, May 5-6, Prescott, AZ. 277-289.

Snyder, G.H., B.J. Augustin, and J.M. Davidson. 1984. Moisture sensor-controlled irrigation for reducing N leaching in Bermudagrass turf. Agron. J. 76: 964-969.

Stark, J.M., and S.C. Hart. 1996. Diffusion technique for preparing salt solutions, kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Sci. Am. J. 60:1846-1855.

Taliaferro, C.M. 1995. Diversity and vulnerability of bermuda turfgrass species. Crop Sci. 35:327–332.

Thomas, J.C., R.H. White, J.T. Vorheis, H.G. Harris, and K. Diehl. 2006. Environmental impact of irrigating turf with type I recycled water. Agron. J. 98:951-961.

Table 1. Growth chamber conditions within each growth and dormancy cycle phase. Chamber conditions were based on 40-year average climatic conditions.

Growth Phase	Temperature (°C)		PPF (μmol m^{-2} s^{-1})	Daily PAR (mols m^{-2} day^{-1})	Photoperiod (light/dark)
	Light	Dark			
August	32.5	19.5	1000	38	14/ 10
January	5	5	450	15	10/ 14
May	26	10.5	750	37	13.5/ 10.5
October	24	8.5	550	22	11.25/ 12.75

Table 2. Dry mass of plant tissues and N content of the organic fraction averaged across harvests for each ^{15}N experiment. Standard errors are denoted in parentheses.

	August		**January**		**May**		**October**	
	mg	%N	mg	%N	mg	%N	mg	%N
Shoots	642 (47)	2.1 (0.1)	243 (32)	1.4 (0.1)	457 (28)	2.5 (0.1)	517 (43)	1.9 (0.1)
Rhizomes	1952 (81)	1.6 (0.1)	1100 (60)	1.5 (0)	1564 (68)	1.7 (0)	970 (57)	1.6 (0)
Roots	1061 (84)	1.4 (0.1)	561 (30)	1.5 (0)	796 (31)	1.7 (0)	922 (51)	1.4 (0)
Organic N $\mu g\ g^{-1}$ soil	545 (31)		327 (30)		745 (28)		576 (38)	

Table 3. August recovery and mass balance of ^{15}N. Values are absolute μg ^{15}N recovered of the 3940 μg applied to core +/- standard error. Values in parentheses represent % of the total ^{15}N recovered.

August	Day1	Day 3	Day 10	Day 16
Plant	1255.0 +/- 277.5 **(42)**	2332.0 +/- 202.6 **(67)**	2637.8 +/- 240.1 **(83)**	4115.2 +/- 188.6 **(90)**
Shoots	436.5 +/- 85.5 **(15)**	1202.0 +/- 210.4 **(34)**	1109.0 +/- 59.8 **(35)**	2216.0 +/- 168.8 **(49)**
Rhizomes	638.3 +/- 142.4 **(21)**	765.9 +/- 86.4 **(22)**	860.0 +/- 208.4 **(27)**	1092.5 +/- 68.5 **(24)**
Roots	180.2 +/- 61.0 **(6)**	364.1 +/- 27.7 **(11)**	668.8 +/- 65.5 **(21)**	806.7 +/- 88.0 **(17)**
Organic	195.3 +/- 19.1 **(6)**	289.2 +/- 65.1 **(8)**	328.8 +/- 24.7 **(10)**	343.2 +/- 51.1 **(8)**
Microbial	192.9 +/- 20.6 **(6)**	226.0 +/- 17.7 **(6)**	246.1 +/- 20.9 **(8)**	170.2 +/- 27.5 **(4)**
Solid	2.0 +/- 2.0 **(0)**	62.5 +/- 52.2 **(2)**	82.7 +/- 36.7 **(2)**	166.1 +/- 35.0 **(4)**
Dissolved	0.4 +/- 0.4 **(0)**	0.7 +/- 0.7 **(0)**	0 **(0)**	6.9 +/- 2.1 **(0)**
Inorganic	1573.0 +/- 119.0 **(52)**	884.7 +/- 64.1 **(25)**	232.1+/- 30.4 **(7)**	87.4 +/- 8.8 **(2)**
Leachate	1007.6 +/- 97.7 **(33)**	638.0 +/- 55.4 **(18)**	61.3 +/- 15.9 **(2)**	36.3 +/- 4.8 **(1)**
Soil extr.	565.4 +/- 72.3 **(19)**	246.7 +/- 12.3 **(7)**	170.7 +/- 16.2 **(5)**	51.1 +/- 6.2 **(1)**
^{15}N recovered ***% of applied***	3023.3 +/- 228.6 ***77***	3505.9 +/- 196.5 ***89***	3198.7 +/- 250.0 ***81***	4545.7 +/- 191.2 ***115***

Table 4. January recovery and mass balance of ^{15}N. Values are absolute µg ^{15}N recovered of the 3940 µg applied to core +/- standard error. Values in parentheses represent % of the total ^{15}N recovered.

January	Day 1	Day 3	Day 7	Day 10	Day 13	Day 16
Plant	250.9 +/- 27.6 **(8)**	216.6 +/- 62.0 **(7)**	274.8 +/- 49.0 **(9)**	440.5 +/- 75.2 **(15)**	417.2 +/- 23.4 **(13)**	639.6 +/- 153.7 **(21)**
Shoots	0 **(0)**	0 **(0)**	0 **(0)**	0 **(0)**	0 **(0)**	0 **(0)**
Rhizomes	225.1 +/- 25.1 **(7)**	154.8 +/- 77.2 **(5)**	248.3 +/- 42.7 **(8)**	409.0 +/- 73.8 **(14)**	249.0 +/- 98.0 **(8)**	588.9 +/- 145.2 **(20)**
Roots	25.8 +/- 2.8 **(1)**	61.8 +/- 15.6 **(2)**	26.5 +/- 7.6 **(1)**	30.5 +/- 3.5 **(1)**	168.2 +/- 75.1 **(5)**	50.7 +/- 8.8 **(1)**
Organic	183.8 +/- 20.0 **(6)**	178.0 +/- 3.5 **(6)**	203.6 +/- 79.8 **(6)**	171.7 +/- 8.4 **(6)**	172.1 +/- 38.6 **(5)**	311.2 +/- 27.7 **(10)**
Microbial	76.2 +/- 26.1 **(2)**	59.5 +/- 25.2 **(2)**	80.9 +/- 22.6 **(3)**	91.3 +/- 6.0 **(3)**	107.4 +/- 40.0 **(3)**	201.8 +/- 15.0 **(7)**
Solid	47.8 +/- 7.3 **(2)**	55.3 +/- 17.2 **(2)**	42.4 +/- 13.7 **(1)**	37.5 +/- 9.1 **(1)**	30.9 +/-10.9 **(1)**	57.4 +/- 4.2 **(2)**
Dissolved	59.8 +/- 23.2 **(2)**	63.2 +/- 17.4 **(2)**	80.3 +/- 44.7 **(2)**	42.9 +/- 8.8 **(2)**	33.8 +/- 7.9 **(1)**	51.90 +/- 26.8 **(1)**
Inorganic	2620.7 +/- 96.0 **(86)**	2686.5 +/- 55.4 **(87)**	2661.7 +/- 42.6 **(85)**	2408.4 +/- 233.4 **(79)**	2639.0 +/- 85.4 **(82)**	2145.5 +/- 108.5 **(69)**
Leachate	2613.1 +/- 95.7 **(86)**	2676.0 +/- 55.2 **(87)**	2652.6 +/- 41.6 **(85)**	2397.2 +/- 234.0 **(79)**	2619.3 +/- 88.5 **(81)**	2128.8 +/- 110.6 **(69)**
Soil extr.	7.6 +/- 1.0 **(0)**	10.5 +/- 0.5 **(0)**	9.1 +/- 1.1 **(0)**	11.2 +/- 1.1 **(0)**	19.7 +/- 4.4 **(1)**	16.7 +/- 4.0 **(0)**
^{15}N recovered ***% of applied***	3055 +/- 79 ***78***	3081 +/- 49 ***78***	3140 +/- 73 ***80***	3033 +/- 163 ***77***	3228 +/- 72 ***82***	3096 +/- 64 ***79***

Table 5. May recovery and mass balance of ^{15}N. Values are absolute µg ^{15}N recovered of the 3940 µg applied to core +/- standard error. Values in parentheses represent % of the total ^{15}N recovered.

May	Day1	Day 3	Day 7	Day 10	Day 13	Day 16
Plant	646.9 +/- 70.0 **(27)**	2336.1 +/- 251.9 **(59)**	2273.6 +/- 235.7 **(63)**	2768.7 +/- 71.4 **(74)**	2813.5 +/- 90.8 **(77)**	2601.7 +/- 175.0 **(72)**
Shoots	247.0 +/- 28.7 **(10)**	886.4 +/- 95.0 **(23)**	856.9 +/- 110.9 **(24)**	1065.5 +/- 145.6 **(28)**	790.7 +/- 84.5 **(22)**	837.5 +/- 115.1 **(23)**
Rhizomes	321.5 +/- 25.0 **(14)**	946.4 +/- 103.0 **(24)**	933.3 +/- 122.2 **(26)**	1044.6 +/- 102.4 **(28)**	1266.6 +/- 70.9 **(35)**	980.0 +/- 89.6 **(27)**
Roots	78.4 +/- 24.6 **(3)**	503.3 +/- 155.0 **(12)**	483.4 +/- 73.9 **(13)**	658.6 +/- 49.4 **(18)**	756.2 +/- 83.5 **(20)**	784.3 +/- 60.9 **(22)**
Organic	120.3 +/- 14.5 **(5)**	327.1 +/- 129.7 **(8)**	608.1 +/- 123.4 **(17)**	603.3 +/- 50.6 **(16)**	506.5 +/- 101.3 **(14)**	629.7 +/- 58.1 **(17)**
Microbial	40.5 +/- 5.3 **(2)**	148.9 +/- 28.2 **(4)**	368.4 +/- 79.4 **(10)**	472.3 +/- 95.4 **(13)**	389.7 +/- 137.0 **(11)**	252.9 +/- 47.2 **(7)**
Solid	50.8 +/- 8.8 **(2)**	171.9 +/- 122.5 **(4)**	141.8 +/- 47.3 **(4)**	131.0 +/- 51.6 **(4)**	116.8 +/- 48.0 **(3)**	307.7 +/- 27.7 **(8)**
Dissolved	29.0 +/- 7.0 **(1)**	6.3 +/- 6.3 **(0)**	97.9 +/- 40.7 **(3)**	0 **(0)**	0 **(0)**	69.1 +/- 26.1 **(2)**
Inorganic	1623.2 +/- 137.6 **(68)**	1271.3 +/- 127.5 **(33)**	704.2 +/- 76.3 **(20)**	386.0 +/- 23.9 **(10)**	347.0 +/- 34.7 **(9)**	378.2 +/- 48.9 **(11)**
Leachate	1400.5 +/- 113.5 **(59)**	1000.5 +/- 122.9 **(26)**	217.8 +/- 64.0 **(6)**	19.5 +/- 6.9 **(1)**	4.9 +/- 1.4 **(0)**	4.2 +/- 1.2 **(0)**
Soil extr.	222.7 +/- 33.8 **(9)**	270.8 +/- 37.7 **(7)**	486.4 +/- 23.8 **(14)**	366.5 +/- 26.5 **(10)**	342.1 +/- 34.8 **(9)**	374.0 +/- 49.7 **(11)**
^{15}N recovered ***% of applied***	2390.4 +/- 187.7 ***61***	3934.5 +/- 252.0 ***100***	3585.9 +/- 314.4 ***91***	3758.0 +/- 76.0 ***95***	3667.0 +/- 127.5 ***93***	3609.6 +/- 144.1 ***92***

Table 6. October recovery and mass balance of ^{15}N. Values are absolute μg ^{15}N recovered of the 3940 μg applied to core +/- standard error. Values in parentheses represent % of the total ^{15}N recovered.

October	Day1	Day 3	Day 7	Day 10	Day 13	Day 16
Plant	427.5 +/- 58.0 **(21)**	840.9 +/- 152.3 **(35)**	1013.7 +/- 70.4 **(40)**	1244.0 +/-308.3 **(41)**	1201.6 +/-274.5 **(46)**	1634.4 +/-226.5 **(52)**
Shoots	99.9 +/- 24.1 **(5)**	274.0 +/- 110.6 **(11)**	407.0 +/- 89.6 **(16)**	625.3 +/- 308.4 **(19)**	560.6 +/- 190.9 **(22)**	777.1 +/- 203.7 **(25)**
Rhizomes	204.9 +/- 27.4 **(10)**	371.4 +/- 83.8 **(15)**	389.1 +/- 82.8 **(16)**	368.5 +/- 102.9 **(13)**	399.9 +/- 83.6 **(15)**	534.1 +/- 100.8 **(17)**
Roots	122.6 +/- 17.5 **(6)**	195.5 +/- 17.5 **(9)**	217.6 +/- 45.1 **(8)**	250.2 +/- 69.0 **(9)**	241.0 +/- 10.4 **(9)**	323.2 +/- 76.1 **(10)**
Organic	219.8 +/- 41.5 **(10)**	305.7 +/- 32.7 **(14)**	494.7 +/- 108.6 **(19)**	513.6 +/- 119.9 **(17)**	489.6 +/- 88.9 **(19)**	781.2 +/- 55.0 **(25)**
Microbial	116.6 +/- 18.0 **(6)**	124.7 +/- 17.8 **(5)**	283.2 +/- 83.4 **(11)**	260.2 +/- 85.7 **(8)**	226.4 +/- 52.7 **(9)**	339.7 +/- 84.5 **(11)**
Solid	94.70+/- 26.4 **(4)**	147.2 +/-13.52 **(7)**	210.1 +/- 26.0 **(8)**	230.6 +/-40.5 **(8)**	168.7 +/- 9.1 **(7)**	337.2 +/-86.3 **(11)**
Dissolved	8.5 +/- 8.7 **(0)**	33.8 +/- 25.07 **(2)**	1.4 +/- 1.47 **(0)**	22.8 +/- 14.9 **(1)**	94.5 +/- 55.1 **(3)**	104.3 +/- 32.2 **(3)**
Inorganic	1387.6 +/- 60.7 **(69)**	1177.2 +/- 92.2 **(51)**	1039.8 +/- 136.8 **(41)**	1227.1 +/- 107.1 **(42)**	893.9 +/- 91.6 **(35)**	709.8 +/- 183.2 **(23)**
Leachate	1168.0 +/- 65.8 **(58)**	836.6 +/- 81.7 **(36)**	485.2 +/- 171.0 **(18)**	584.3 +/- 52.6 **(20)**	387.8 +/- 31.8 **(15)**	156.2 +/- 68.7 **(5)**
Soil extr.	219.6 +/-13.8 **(11)**	348.6 +/-66.08 **(15)**	442.6 +/- 96.7 **(23)**	642.8 +/-117.3 **(22)**	517.1 +/- 92.9 **(20)**	553.6 +/- 118.4 **(18)**
^{15}N recovered ***% of applied***	2034.9 +/-123.7 ***52***	2323.8 +/-208.3 ***59***	2548.2 +/-221.0 ***65***	2921.6 +/-420.0 ***74***	2585.0 +/-191.9 ***66***	3124.7 +/-117.4 ***79***

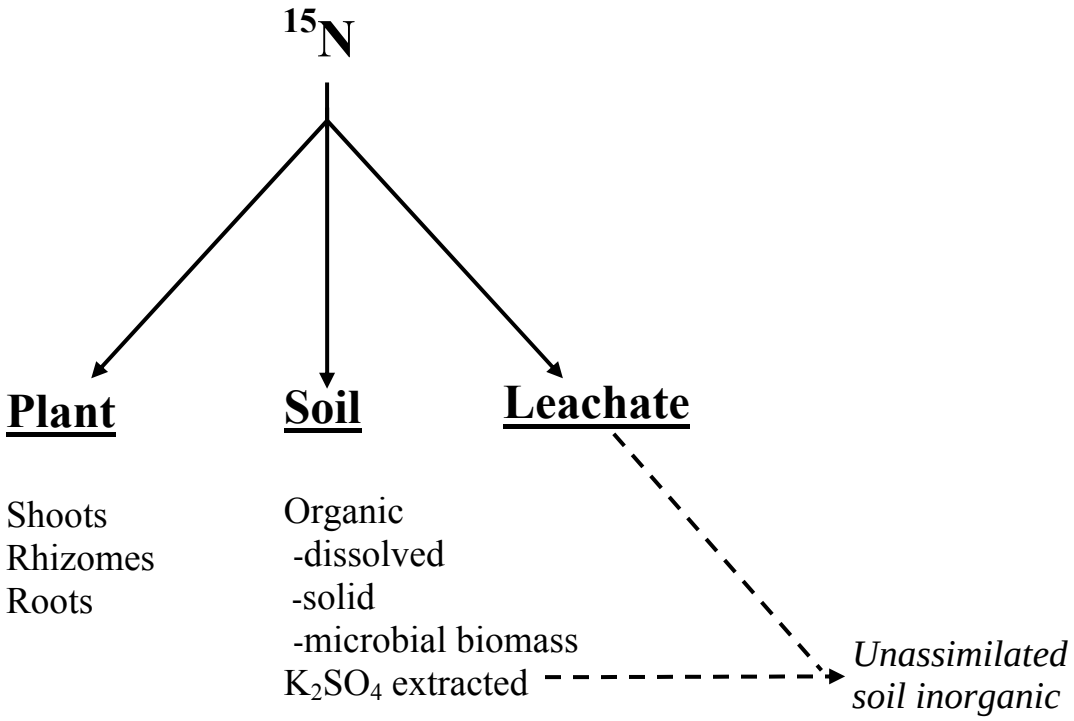

Figure 1. Fractions of the bermudagrass turf/soil system analyzed for ^{15}N fate and mass balance.

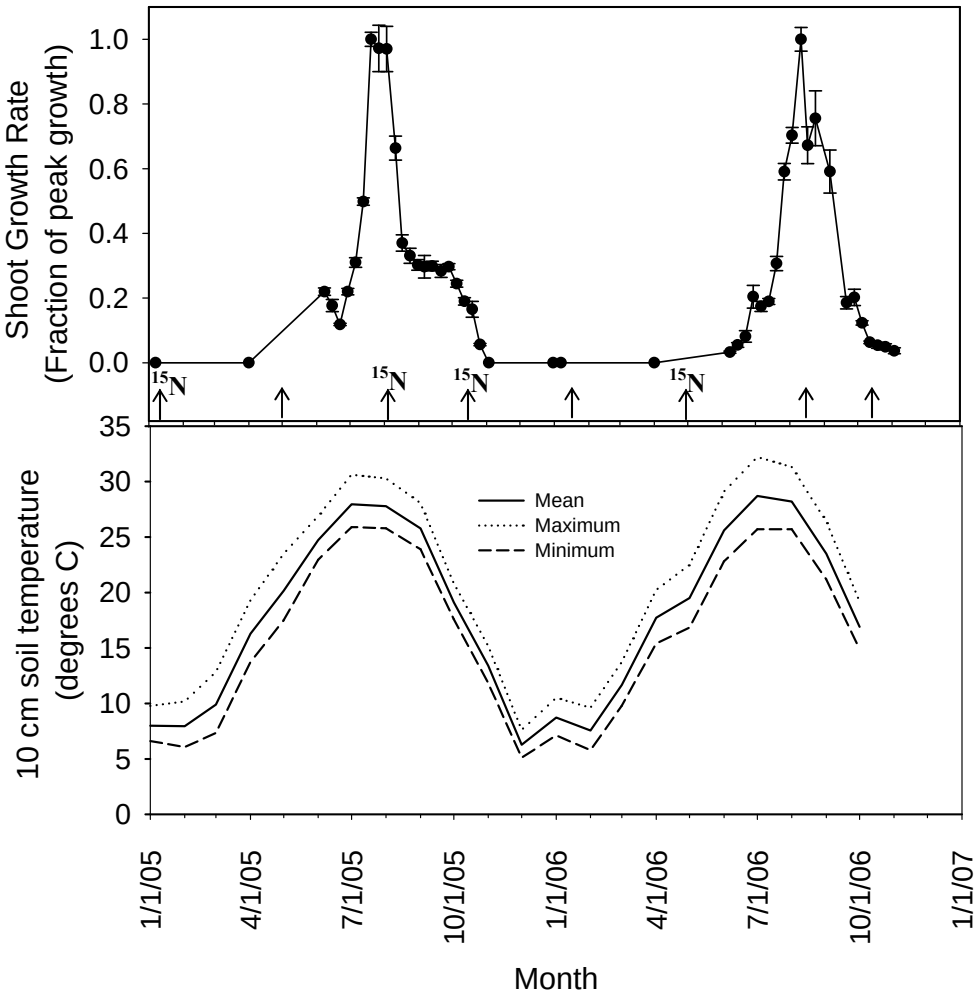

Figure 2. 10 cm soil temperature and shoot growth pattern of Tifway 419 bermudagrass field plot over the course of the experiment (2004-2006) at Sandhills Research Station, Jackson Springs, NC. Arrows indicate sampling periods. Experiments where ^{15}N was used are indicated. Shoot growth rate values are represented as a fraction of the August maximum growth rate of 1.27 mg cm^{-2} d^{-1}. Error bars represent one standard error of the mean.

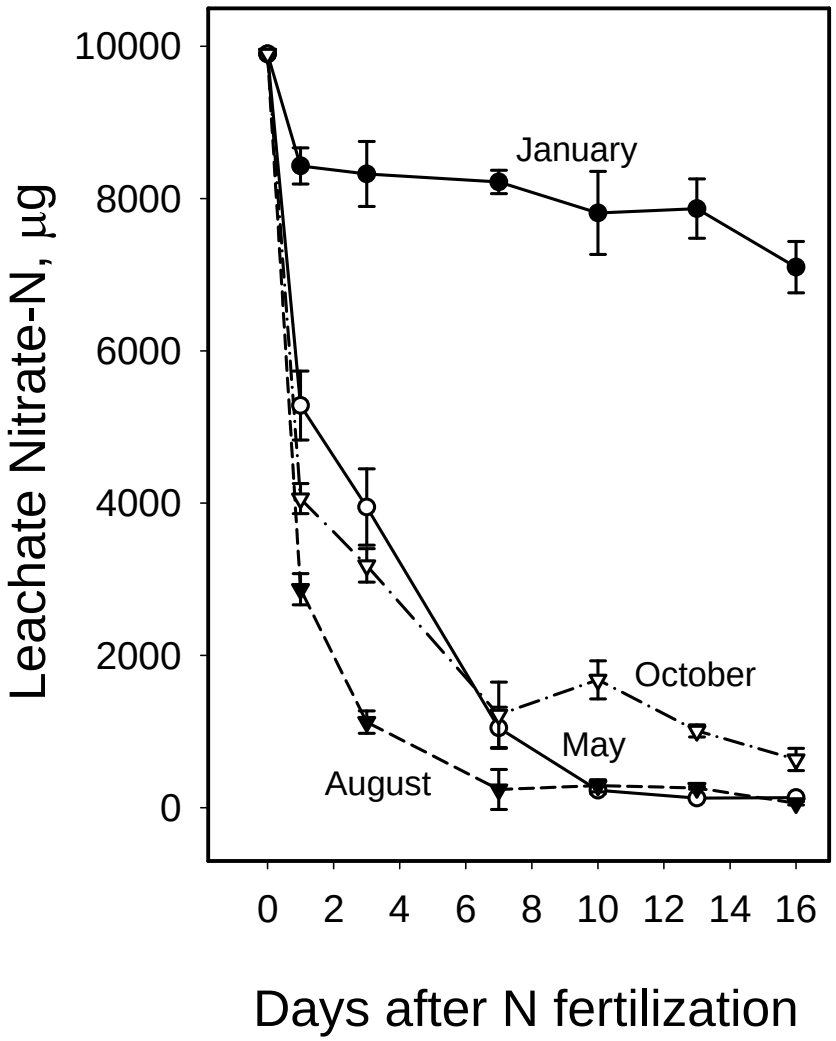

Figure 3. Leachate NO_3^- collected from cores following day 0 application of 9900 µg nitrate-N. Values are pooled averages of two seasons (^{14}N and ^{15}N experiments). Error bars represent standard error of the mean.

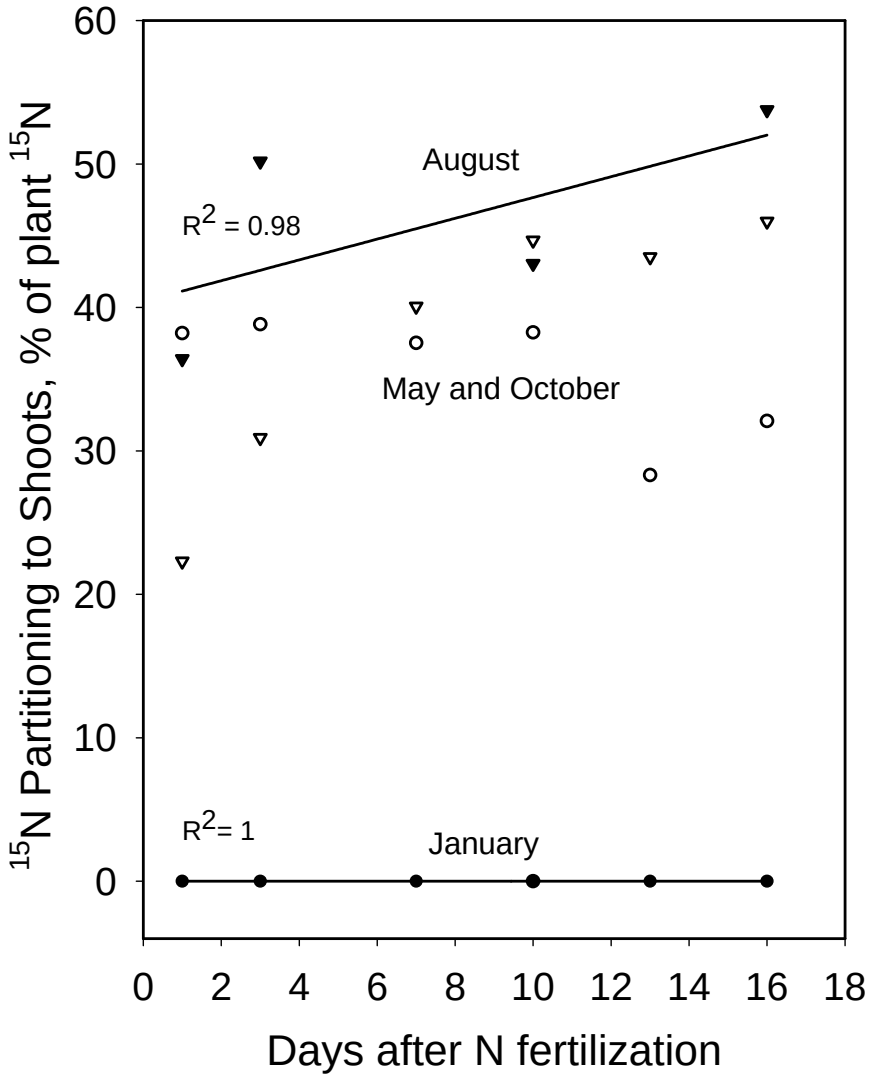

Figure 4. Partitioning of whole plant ^{15}N in the shoot. Plotted lines depict data for August and January. Scatter plots are values from the transition months of May and October.

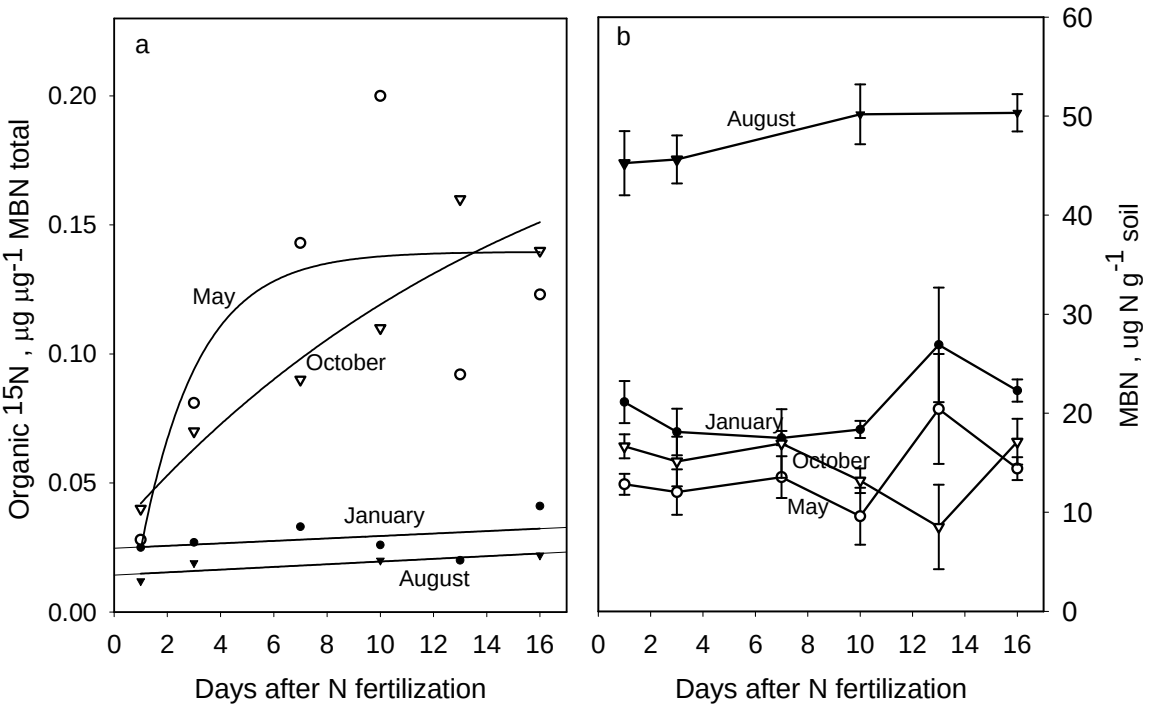

Figure 5. a) Total microbial biomass nitrogen and b) apparent microbial activity (^{15}N assimilation) in cores at each of the four seasonal experiments. Error bars denote standard error of the mean.

EFFECT OF SOIL SATURATION ON DEVELOPMENT AND ^{15}N-NITRATE UPTAKE EFFICIENCY OF TWO WARM-SEASON GRASSES EMERGING FROM DORMANCY

ABSTRACT

Use of effluent irrigation on turfgrass is increasing in the southeastern U.S. due to population growth, environmental regulations, and concerns about limited potable water supplies. Because effluent is generated continuously, turf managers may be forced to over-irrigate during rainy periods or when the turf is dormant, leading to extended periods of soil saturation. Although the nutrients in effluent (nitrate, ammonium, and phosphate) are readily absorbed by healthy turf systems, the effects of prolonged soil saturation on uptake are unknown. This research examined the impact of soil saturation on plant development and nitrate uptake efficiency of two warm-season turfgrasses emerging from dormancy. Dormant grass/soil cores of hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burtt Davy) and common centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) were treated to simulate the spring transition from dormancy, with soil moisture controlled at saturation (~36% v:v) or field capacity (~13% v:v). Cores were fertilized with $K^{15}NO_3$ (40A%) to determine the fate of applied N. Soil saturation reduced canopy development in both species, but shoot growth was affected only in bermudagrass. New roots of centipedegrass were concentrated near the surface of saturated soils. Nitrate uptake by both species was generally unaffected by soil saturation. Our results suggest that while extended periods of soil saturation may alter plant development, they do not appear to adversely affect the ability of these turfgrasses to absorb fertilizer or effluent N.

INTRODUCTION

Use of recycled water for irrigation is increasing in the southeastern U.S. because of population growth and environmental concerns. Until recently, effluent was commonly discharged directly into surface waters, but recent regulations often require it be applied to the landscape to remove contaminants. Turfgrass systems are well suited for effluent dispersal because of their proximity to waste treatment facilities, in-ground irrigation systems, and ability to efficiently absorb (i.e. filter) nutrient contaminants when actively growing (Sidle and Johnson, 1972; Anderson et al., 1981; Thomas et al., 2006).

Effluent has been used for decades to irrigate turfgrasses in the arid southwestern U.S. where it may cause increased soil salinity due to high rates of evapotranspiration (ET) and inadequate leaching (Hayes et al., 1990; Mancino and Pepper, 1992). While salt accumulation is not as serious a concern in the Southeastern U.S., the low permeability of native clay soils increases the potential for saturated root-zone conditions, especially when effluent irrigation plus rainfall exceeds ET. Because effluent is generated continuously, turfgrass managers are often required to take the contractual amount of water each day, regardless of season or soil moisture status (Harivandi, 2007).

Excess water in the root zone can be injurious or even lethal to plants because it blocks the transfer of oxygen and other gases between the atmosphere and the soil, creating an anaerobic environment (Drew, 1997). Grasses grown in soils containing excess water exhibit shallow root development and reduced vigor and quality (Bennett et al., 1960; Doss et al., 1960). Anaerobic soil conditions also impair water and nutrient

uptake in many plant species (Drew and Lynch, 1980; Trought and Drew, 1980). Therefore, a primary concern associated with turfgrasses growing in saturated soils and supplied with effluent is the potential reduction in uptake capacity for nutrient contaminants.

Bermudagrass (*Cynodon* (L.) Pers. X *C. transvaalensis* Burtt Davy) and centipedegrass (*Eremochloa ophiuroides* Munro Hack.) are turfgrasses widely planted in the southeastern U.S. While there are few published data, bermudagrass is considered to possess tolerance to a wide range of conditions including submergence (Rhoades, 1964; Blanch et al., 1999), while centipedegrass may lack tolerance to saturated soils (Horn, 1966; Beard, 1973; Turgeon, 2002).

The specific objective of this research was to determine the impact of soil saturation on plant development and nitrate uptake efficiency of bermudagrass and centipedegrass during emergence from dormancy. In a broader sense, this work addresses the potential environmental impacts of continuous wastewater disposal on turfgrass systems.

MATERIALS AND METHODS

Experiments were conducted over a 72-day period at the Southeastern Plant and Environmental Laboratory (SEPAL), Raleigh, North Carolina. Dormant grass/soil cores of 'Tifway 419' hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. X *C. transvaalensis* Burtt Davy) and common centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) were

harvested from mature field plots (~10 yrs old) at the Sandhills Research Station, Jackson Springs, NC. The soil at the site was characterized as a Candor sand (sandy, siliceous, thermic grossarenic kandiudults) with 91% sand, 6% silt, 3% clay, and a pH of 6.1. Each species was mowed at ~2 cm and received granular fertilizer N (from a complete fertilizer) at a rate of 147 kg N ha^{-1} yr^{-1}, split into three equal applications at six-week intervals from mid-May through early September.

The plant/soil cores (5 cm diameter X 15 cm depth) were harvested from the field plots in late January 2005 using a soil sampler (AMS Inc., American Falls, ID) that collected the cores in individual plastic sleeves. The cores were adjusted within the sleeves such that the soil surface was 1 cm below the top edge of the sleeve, creating a headspace. A thin fiberglass filter disk was then placed at the bottom of each grass/soil core to prevent soil loss and a tight-fitting 5 cm female PVC bushing (Lasco Fittings, Inc., Brownsville, TN) was sealed onto the sleeve bottom. A drain line of polypropylene tubing was attached to the bushing with a threaded coupler. The grass/soil cores were randomly placed into 5 cm diameter openings in the top of four 52 cm x 30 cm x 30 cm PVC growth containers, with the upper 1 cm of the sleeves (the soil surface and shoots) exposed to the chamber lights and the below ground section in darkness. A vacuum pump was connected to each core via a manifold to provide a drainage tension of 15 kPa to the soil (slightly below field capacity for this soil). Each grass/soil core was connected to a 125 ml Erlenmeyer flask by the drain tubing, allowing leachate to be collected from individual cores.

The growth containers plus cores were placed in the growth chamber within 24

hours of harvest. The chamber was illuminated with a 1:1 ratio of metal halide and high-pressure sodium lamps plus incandescent bulbs (~1000 μmol m^{-2} s^{-1} PPFD) and programmed to a 13-hour photoperiod with light/dark temperatures of 26˚C/ 19˚C to simulate spring conditions.

Grass/soil cores were arranged in a three-factor completely randomized design with three replications per treatment. The factors included species (2), soil moisture (2), and duration of growth prior to ^{15}N application (4). Two soil moisture treatments were imposed throughout the experiment. Half of the cores were maintained at ~70-100% field capacity by watering every other day followed by application of 15 kPa tension to drain soil water content to field capacity. Actual volumetric water content following drainage was ~13%. The other set of cores was irrigated 3x daily (8 am, 12 pm, and 5 pm) to maintain a constant head of water (ranging from 1 to 10 mm) on the soil surface; the top of the canopy remained above the water throughout the experiment. Drainage was prevented from these cores by capping the outlets at the bottom of the cores, resulting in a volumetric soil water content of ~36%. Grasses were clipped to 1.9 cm above the soil surface and fertilized weekly with a complete nutrient solution at a rate of 0.5 g N m^{-2}. Clippings were dried and weighed as a measure of shoot growth. Visual assessment of green canopy cover was made following clipping removal. Ratings were based on a 0-100% scale (0 = no green canopy cover, 100% = complete green canopy cover). Because grasses were dormant at the initiation of the experiment, all grass/soil cores began with a density of 0, increasing as new growth occurred.

On four dates over the course of the experiment (d14, d28, d56, d72), three

grass/soil cores from each treatment were randomly selected and thoroughly leached with 500 ml (~3 pore volumes) of distilled, deionized water to remove residual soil nitrate. Preliminary work determined that 3 pore volumes removed >90% of soil solution nitrate. Leaching was facilitated by applying a tension of 15 kPa until drainage stopped, leaving the soil at field capacity.

Nitrate-uptake was then evaluated by applying 1.8 g N m^{-2} of $K^{15}NO_3$ (40 A %) in solution (15 ml $core^{-1}$, equivalent to a depth of ~1 cm). It was estimated that soil moisture content immediately following this application was 18% (v:v). Drainage outlets were sealed during the uptake period to prevent loss. Following a 24-hour uptake period, cores were again leached with ~3 pore volumes distilled deionized water. Leachate was collected and frozen for analysis, and grass/soil cores were removed for fractionation and ^{15}N analysis.

Grass/soil cores were removed from the plastic sleeves and soil was carefully separated from plant material. Shoots (leaves and verdure) were clipped to the subtending stolon or rhizome. Roots were then removed from rhizomes and stolons. A 2 mm sieve was used to separate fine roots from soil. New roots that had developed in the growth chamber were separated from the previous season's roots, based on differences in color and degree of suberization. All plant tissues were then rinsed in 1 mM $CaSO_4$ followed by distilled-deionized water.

Plant tissues were oven dried at 65˚C for 72 hours, weighed, and milled to a fine powder. Tissue samples were then analyzed for total N and ^{15}N using a Thermo Finningan DeltaPlus continuous flow isotope ratio mass spectrometer (CF-IRMSA,

Bremen, Germany). Following removal of plant tissues, soil was thoroughly homogenized and subsampled for analysis of soil inorganic ^{15}N, microbial biomass ^{15}N, dissolved organic ^{15}N, and ^{15}N in the solid fraction of soil organic matter.

Soil nitrate was extracted with 0.5 M K_2SO_4 and determined colorimetrically using a Lachat flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). A diffusion method was used to prepare samples for ^{15}N analysis (Stark and Hart, 1996). Briefly, Devarda's alloy and MgO were added to extract subsamples (containing ~50 μg total N) and placed in 120 ml plastic specimen containers to catalyze reduction of NO_3 to NH_3. NH_3 was captured on two acidified Whatman #1 filter paper disks (7 μl of 2.5 M $KHSO_4$ per disk) sealed in Teflon tape. After 6-days, disks were dried in a desiccator over concentrated H_2SO_4 for 24 hours. Disks were encapsulated in tin capsules and analyzed for total N content and % ^{15}N as described above.

Microbial biomass ^{15}N was determined by the $CHCl_3$ fumigation-extraction procedure (Brookes el al., 1985). Briefly, 15 g moist soil samples were fumigated with $CHCl_3$ for 24 hours and extracted with 0.5 M K_2SO_4. Soluble N was oxidized to NO_3 with alkaline persulfate (Cabrera and Beare, 1993). Total NO_3-N of the extracts was determined colorimetrically. All extracts and samples were then analyzed for total N and ^{15}N using the diffusion procedure described above. Microbial biomass N was calculated by dividing the N flush, i.e., the difference between fumigated and unfumigated samples, by an extraction coefficient of 0.54 (Brookes et al., 1985).

After fumigation-extraction, two additional 0.5 M K_2SO_4 extractions were performed on these soil subsamples to thoroughly remove any remaining soil nitrate.

These soil samples were then analyzed for total N and ^{15}N as described previously. The N in these samples represented the solid fraction of soil organic nitrogen.

RESULTS

The objective of this study was to determine the impact of soil saturation on growth and development and NO_3 uptake efficiency as bermudagrass and centipedegrass emerge from dormancy. Both grasses responded rapidly to the warmer conditions in the growth chamber, with new shoots appearing by day 3. Over the course of the initial 4-6 weeks, shoot cover developed linearly for centipedegrass but logarithmically for bermudagrass (Fig. 1). The bermudagrass canopy established more rapidly than that of centipedegrass in both moisture treatments (Fig. 1a,b; slopes of plotted lines). Soil saturation reduced canopy development in both species by week 4; at the end of the experiment, canopy cover was reduced ~30% for both species.

Although soil saturation affected canopy development in both species, its effects on shoot growth (measured as clipping dry mass) were mixed (Fig. 2). Centipedegrass shoot growth rate remained relatively constant through the study, unaffected by soil moisture content (Fig. 2a). In contrast, bermudagrass shoot growth rate decreased after several weeks of growth in saturated soil and was significantly reduced in later stages of the experiment (Fig. 2b).

Soil saturation did not significantly affect new root growth in centipedegrass (Fig. 3a), although proportionately more root mass developed near the soil surface (top 5 cm)

compared to the control (Fig. 4a). In contrast, bermudagrass root growth was reduced ~40% by soil saturation (Fig. 3b) while root distribution was unaffected on 3 of the 4 harvest dates (Fig. 4b).

A primary objective of this study was to determine whether soil saturation affected the ability of the turfgrasses to absorb nitrate. Nitrate uptake efficiency was determined on days 14, 28, 56 and 72, using ^{15}N-labelled KNO_3 as a tracer. Total ^{15}N recoveries from plant/soil cores (total ^{15}N recovered/ total ^{15}N applied), did not differ (P= 0.05) between the soil moisture treatments within either species on any date (see % ^{15}N recovery, Tables 1, 2). Therefore, ^{15}N within each fraction (whole plant, unassimilated inorganic, organic, microbial biomass) was calculated as a percentage of the total ^{15}N recovered from each core, rather than as a percentage of the amount applied.

Centipedegrass

Total ^{15}N recoveries averaged ~51% and ~64% of applied ^{15}N for turf grown at field capacity and soil saturation, respectively (Table 1). Soil moisture treatment did not affect N uptake (whole plant ^{15}N) through day 56. However, on day 72, significantly more of the recovered ^{15}N was detected in waterlogged plants (~75%) than those grown at field capacity (~56%). ^{15}N uptake efficiency (mg ^{15}N g^{-1} total root dry mass) was significantly higher in waterlogged plants on the final two harvest dates (Fig. 5a). These data demonstrate that while soil saturation reduced canopy cover and altered plant development, it did not affect the efficiency at which centipedegrass absorbed nitrate.

^{15}N-Nitrate uptake by centipedegrass grown at field capacity was fairly constant over the course of the experiment, with only the second harvest showing slightly lower absorption (Table 1). By contrast, N uptake from saturated soils increased over the 72 day experiment, doubling from ~35% (d14) to ~75% (d72) of the recovered N (Table 1).

Stems (rhizomes and stolons) were the primary sink for absorbed ^{15}N, followed by roots and shoots on all but the final harvest date (Table 1). Soil saturation affected the distribution of ^{15}N on days 56 and 72, as a significantly greater percentage of recovered ^{15}N was present in roots from saturated soil compared to those from field capacity soil (Table 1).

Nitrogen immobilization by soil microbes was unaffected by soil moisture treatment. Nitrogen recoveries in the microbial biomass ranged from 8% to 16% of total recovered ^{15}N in drained soils and 10 to 22% in saturated soils (Table 1). Nitrogen immobilization by the microbial biomass was substantially less than plant uptake on all sampling dates. Microbial ^{15}N uptake efficiency ($\mu g\ ^{15}N\ \mu g^{-1}$ microbial biomass N) was also greater in saturated soils on day 56, although differences were not significant on other harvest dates (Fig. 6a).

Bermudagrass

Total ^{15}N recoveries in bermudagrass averaged ~58% and ~68% of applied N in saturated and drained cores, respectively (Table 2). With the exception of the day 56 harvest, soil moisture status did not affect N acquisition by bermudagrass plants. In

contrast to centipedegrass, saturation reduced plant nitrate uptake by ~21% on day 56 (Table 2). This reduction may be due to decreased root development in saturated soils (refer to Fig. 3b).

Nitrogen uptake increased from the first through fourth harvests, from ~49% to ~69% in drained soils, and from ~44% to ~70% in saturated soils (Table 2). Similar to centipedegrass, the majority of absorbed ^{15}N was in rhizomes and stolons, although a gradual shift in allocation to shoots (above ground biomass) and roots occurred midway through the experiment (Table 2). In turf grown in drained soils, significantly more of the recovered ^{15}N was found in shoots (d14, d28, and d72) and stems (d72) compared to those grown in saturated soils (Table 2). With the exception of day 56, when roots of field capacity plants possessed twice the recovered ^{15}N of saturated plants, ^{15}N content of roots did not differ between soil moisture treatments (Table 2).

^{15}N immobilization by microbes again accounted for only a small fraction (~5% to ~15%) of the total recovered ^{15}N from the system; much less than in plant tissues. As in the centipedegrass system, soil moisture had little affect on immobilization until d72, when it was significantly higher in saturated (~15%) than drained (~5%) soils (Table 2).

DISCUSSION

Turfgrasses are periodically subjected to soil saturation resulting from excessive irrigation, rainfall, and/or soils of low permeability. However, the degree to which this represents a physiological challenge to warm season species is unknown. This study is

one of the first to examine nutrient absorption and partitioning in response to soil saturation, and the data should contribute to ecologically sound management of turfgrasses and preservation of environmental quality.

Both species survived 72 days of soil saturation, although they responded somewhat differently. Canopy development following dormancy was noticeably reduced by soil saturation. Shoot dry matter production was unaffected in centipedegrass, but was reduced later in the experiment in bermudagrass. Root dry matter production was significantly reduced in bermudagrass, but remained unchanged in centipedegrass, where new roots developed primarily near the soil surface. Despite these developmental changes, nitrate uptake by these turfgrasses was relatively unaffected by prolonged growth in saturated soil.

Variable and/or incomplete recovery of labeled fertilizer N is commonly reported in ^{15}N mass balance experiments involving turfgrasses. Recoveries ranging from ~60 to ~80% have been reported in a number of studies (Starr and DeRoo, 1981; Whitehead and Dawson, 1984; Bristow et al., 1987; Miltner et al., 1996; and Horgan et al., 2002a; Engelsjord et al., 2004), although these are generally associated with longer experimental timeframes than were evaluated in the present study. Incomplete recovery is traditionally attributed to gaseous loss, especially where leachate is collected. However, in measuring denitrification from Kentucky bluegrass and creeping bentgrass turf, Horgan et al. (2002a, 2002b) found that ^{15}N deficits were not explained by gaseous losses alone.

Total ^{15}N recoveries in the current study (ranging from ~50 to 70%) were similar to those of Horgan et al. (57 to 73%, 2002a), but slightly lower than the ~60-80%

reported by others (Starr and DeRoo, 1981; Miltner et al., 1996; Horgan et al., 2002a; and Engelsjord et al., 2004). Fewer data are available for warm season turfgrasses, and complete ^{15}N mass balances are lacking. However, plant absorption is reportedly quite efficient, ranging from 63 to 84% of applied N, depending on species (Bowman et al., 2002). This is consistent with our finding that bermudagrass is the primary sink for applied N, independent of soil moisture.

Growth and Development

Soil saturation often reduces plant growth, as has been reported for wheat (*Triticum aestivum* L.) (Huang et al., 1994), winter oats (*Avena sativa* L.) (Cannell et al., 1985) and barley (Drew and Sisworo, 1979). This is not universal, however, since growth of some plants is unaffected or even stimulated by soil saturation (Rubio et al., 1995). Even though turfgrass canopy development was reduced by soil saturation in the present study, shoot dry matter production in both species was relatively unaffected by saturated conditions. Root growth was similarly unaffected in centipedegrass, but significantly inhibited in bermudagrass during the latter half of the study (Fig. 3). Although centipedegrass root mass was unaffected by soil saturation, new adventitious roots proliferated close to the soil surface. This was particularly evident when compared to turf grown at field capacity. It is well documented that flood-tolerant species often respond to saturated conditions by producing adventitious roots near the soil surface (Drew et al., 1979; Hook, 1984; Naidoo and Naidoo, 1992).

Although it might be expected that nitrate uptake would decline following extended growth in saturated soil, our data indicate otherwise. In fact, nitrate uptake by centipedegrass increased in response to soil saturation, likely due to the dense layer of roots that formed near the soil surface, coincident with the applied ^{15}N. Numerous experiments demonstrate that absorption of water and nutrients is reduced by anoxia in plants that are not adapted to low soil oxygen (Drew and Sisworo, 1979). By contrast, flood-tolerant species maintain high rates of N uptake in anoxic soils (Rubio and Lavado, 1999). Furthermore, Webster et al. (1986) reported that winter wheat absorbed the greatest amounts of N from spring fertilizations following very wet winters. The authors attributed enhanced uptake to denitrification during the wet periods, which depleted available soil N and caused a subsequent deficiency-enhanced uptake. It is possible that in the present study, denitrification may have reduced the amount of N available to saturated plants, causing them to compensate once labeled N was applied. It is important to consider that in this experiment; nitrate uptake was measured over a 24 hr period under aerobic soil conditions and does not represent uptake directly under saturated conditions. Even so, the continued shoot growth of plants while growing in saturated soil suggests a functional nitrate uptake system that continued to satisfy shoot demand for N.

Bermudagrass is reportedly capable of tolerating prolonged periods of complete submersion (Blanch et al., 1999). Therefore, it is not surprising that bermudagrass performed well under saturated conditions for the 72-day period of this study. The fact that centipedegrass was largely unaffected by soil saturation suggests that it also tolerates anaerobic soil conditions as long as the shoots are exposed to the atmosphere.

CONCLUSIONS

Turfgrasses commonly experience periods of soil saturation resulting from slowly permeable soils and/or irrigation surplus. The survival and sustained growth of two turfgrasses through a 72-day study demonstrate that each is capable of adapting to excessive soil moisture, despite perceived differences in submergence tolerance. Adaptations to soil saturation common to both species involved proliferation of adventitious root development near the soil surface and formation of aerenchyma, which may have facilitated gas exchange between the atmosphere and roots. More importantly, although turfgrass canopy cover declined in saturated soil, both species retained their capacity for nitrate uptake throughout the study. On the basis of these results, actively growing turfgrasses represent excellent systems for filtering fertilizer or effluent nitrogen, even when growing in saturated soils for extended periods.

REFERENCES CITED

Anderson, E.L., Pepper, I.L. & Kneebone, W.R. (1981). Reclamation of wastewater with a soil-turf filter: I- Removal of nitrogen. *Journal Water Pollution Control Federation, 53(9),* 1402-1407

Beard, J.B. (1973). *Turfgrass Science and Culture*. (Englewood Cliffs, NJ: Prentice Hall)

Bennett, O.L., Orus, L., & Doss, B.D. (1960). Effect of soil moisture level on root distribution of cool-season forage species. *Agronomy Journal, 52,* 204-207

Blanch, S.T., Ganf, G.G., & Walker, K.F. (1999). Tolerance of riverine plants to flooding and exposure indicated by water treatment. *Regulated Rivers: Research and Management, 15,* 43-62

Bowman, D.C., Cherney, C.T., & T.W. Rufty, Jr. (2002). Fate and Transport of Nitrogen Applied to Six Warm-Season Turfgrasses. *Crop Science, 42,* 833-841

Bristow, A.W., Ryden, J.C., & Whitehead, D.C. (1987). The fate at several time intervals of ^{15}N-labelled ammonium nitrate applied to an established grass sward. *Soil Science Society of America Journal, 38,* 245-254

Brookes, P.C., Landman, A., Pruden, G., & Jenkinson, D.S. (1985). Chloroform fumigation and the release of soil nitrogen; a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry, 17(6),* 837-842

Cabrera, M.L. & Beare, M.H. (1993). Alkaline persulfate oxidation for determining total nitrogen in microbial biomass extracts. *Soil Science Society of America Journal, 57(4),* 1007-1012

Cannell, R.Q., Belford, R.K., Blackwell, P.S., Govi, G., & Thomson, R.J. (1985). Effects of waterlogging on soil aeration and on root and shoot growth and yield of winter oats *(Avena sativa* L.). *Plant and Soil, 85,* 361-373

Doss, B.D., Ashley, D.A., & Bennett, O.L. (1960). Effect of soil moisture regime on root distribution of warm season forage species. *Agronomy Journal, 52,* 569-572

Drew, M.C., Jackson, M.B., & Giffard, S. (1979). Ethylene-promoted adventitious rooting and development of cortical air spaces (Aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. *Planta, 147(1),* 83-88

Drew, M.C. & Sisworo, E.J.. (1979). The development of waterlogging damage in young barley plants in relation to plant nutrient status and changes in soil properties. *New Phytologist, 82(2),* 301-314

Drew, M.C. & Lynch, J.M. (1980). Soil Anaerobiosis, Microorganisms, and Root Function. *Annual Review of Phytopathology, 18,* 37-66

Drew, M.C. (1997). Oxygen Deficiency and Root Metabolism: Injury and Acclimation Under Hypoxia and Anoxia. *Annual Review of Plant Physiology and Plant Molecular Biology, 48,* 223-250

Engelsjord, M.E., Branham, B.E., & Horgan, B.P. (2004). The fate of nitrogen-15 ammonium sulfate applied to kentucky bluegrass and perennial ryegrass turfs. *Crop Science, 44,* 1341-1347

Harivandi, A. (2007). Using recycled water on golf courses. *Golf Course Management 75(6),* 98-105

Hayes, A.R., Mancino, C.F., & Pepper, I.L. (1990). Irrigation of turfgrass with secondary sewage effluent: I. Soil and leachate water quality. *Agronomy Journal, 82,* 939-943

Hook, D.D. (1984). Adaptations to flooding with fresh water. (In: T. Kozlowski (Ed)), *Flooding and plant growth* (pp 265-294). New York: Academic Press)

Horgan, B.P., Branham, B.E., & Mulvaney, R.L. (2002a). Mass balance of ^{15}N applied to kentucky bluegrass including direct measurement of denitrification. *Crop Science, 42,* 1595-1601

Horgan, B.P., Branham, B.E., & Mulvaney, R.L. (2002b). Direct measurement of denitrification using ^{15}N-labeled fertilizer applied to turfgrass. *Crop Science, 42,* 1602-1610

Horn, G.C. (1966). Excess Water and turfgrass management. II. *Florida Turf-grass Association Bulletin, 13,* 3-8.

Huang, B., Johnson, J.W., Nesmith, S., & Bridges, D.C. (1994). Growth, physiological and anatomical responses of two wheat genotypes to waterlogging and nutrient supply. *Journal of Experimental Botany, 45,* 193-202

Mancino, C.F., & Pepper, I.L. (1992). Irrigation of turfgrass with secondary sewage effluent: Soil quality. *Agronomy Journal, 84,* 650-654

Miltner, E.D., Branham, B.E., Paul, E.A., & Rieke, P.E. (1996). Symposium on pesticide

and nutrient fate under turfgrass golf course conditions. *Crop Science, 36,* 1427-1433

Naidoo, G. & Naidoo, S. (1992). Waterlogging responses of *Sporobolus virginicus* (L.) Kunth. *Oecologia, 90,* 445-450

Rhoades, E.D. (1964). Inundation tolerance of grasses in flooded areas. *Transactions of the ASAE 7,* 164-169.

Rubio, G., Casasola, G., & Lavado, R.S. (1995). Adaptations and biomass production of two grasses in response to waterlogging and soil nutrient enrichment. *Oecologia, 102,* 102-105

Rubio, G. & Lavado, R.S. (1999). Acquisition and allocation of resources in two waterlogging-tolerant grasses. *New Phytologist, 143,* 539-546

Sidle, R.C., & Johnson, G.V. (1972). Evaluation of a turfgrass-soil system to utilize and purify municipal wastewater. *Hydrology and Water Resources in Arizona and the Southwest, 2,* 227-289

Stark, J.M., & Hart, S.C. (1996). Diffusion technique for preparing salt solutions, kjeldahl digests, and persulfate digests for nitrogen-15 analysis. *Soil Science Society of America Journal,* 60, 1846-1855

Starr, J.L., & DeRoo, H.C. (1981). The fate of nitrogen fertilizer applied to turfgrass. *Crop Science, 21,* 531-536

Thomas, J.C., White, R.H., Vorheis, J.T., Harris, H.G., & Diehl, K. (2006). Environmental impact of irrigating with type I recycled water. *Agronomy Journal, 98,* 951-961

Trought, M.C.T. & Drew, M.C. (1980). The development of waterlogging damage in wheat seedlings (*Triticum aestivum* L.). I. Shoot and root growth in relation to changes in the concentrations of dissolved gases and solutes in the soil solution. *Plant and Soil, 54,* 77-94

Turgeon, A.J. (2002). *Turfgrass management*. (Upper Saddle River, NJ: Prentice Hall)

Webster, C.P., Belford, R.K., & Cannell, R.Q. (1986). Crop uptake and leaching losses of ^{15}N labeled fertilizer nitrogen in relation to waterlogging of clay and sandy loam soils. *Plant and Soil, 92,* 89-101

Whitehead, D.C. & Dawson, K.P. (1984). Nitrogen, including ^{15}N-labelled fertilizer nitrogen, in components of a grass sward. *Applied Ecology, 21,* 983-989

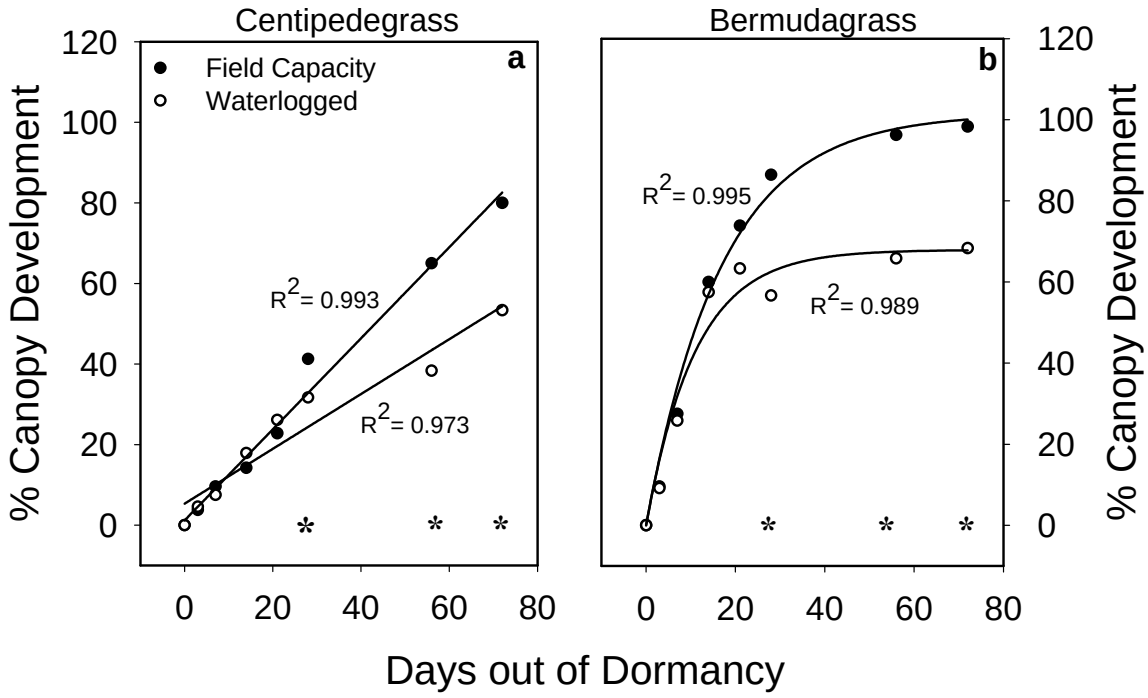

Figure 1. Percent green canopy development on grass/soil cores of a- centipedegrass and b- bermudagrass. Measurements were taken on verdure remaining after clipping. Asterisks denote significance at the P= 0.05 level based on analysis of variance (PROC GLM), SAS.

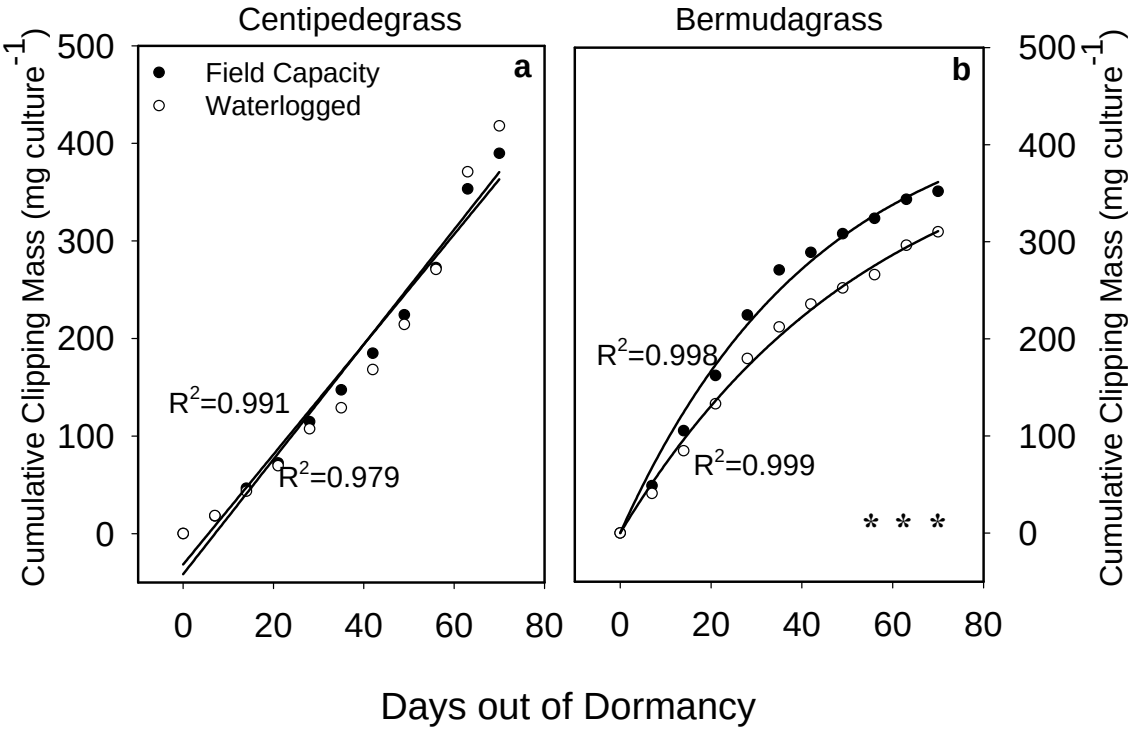

Figure 2. Cumulative clipping dry mass for a- centipedegrass and b- bermudagrass through the experiment. Clippings were collected weekly. Asterisks denote significance at the P= 0.05 level based on analysis of variance (PROC GLM, SAS).

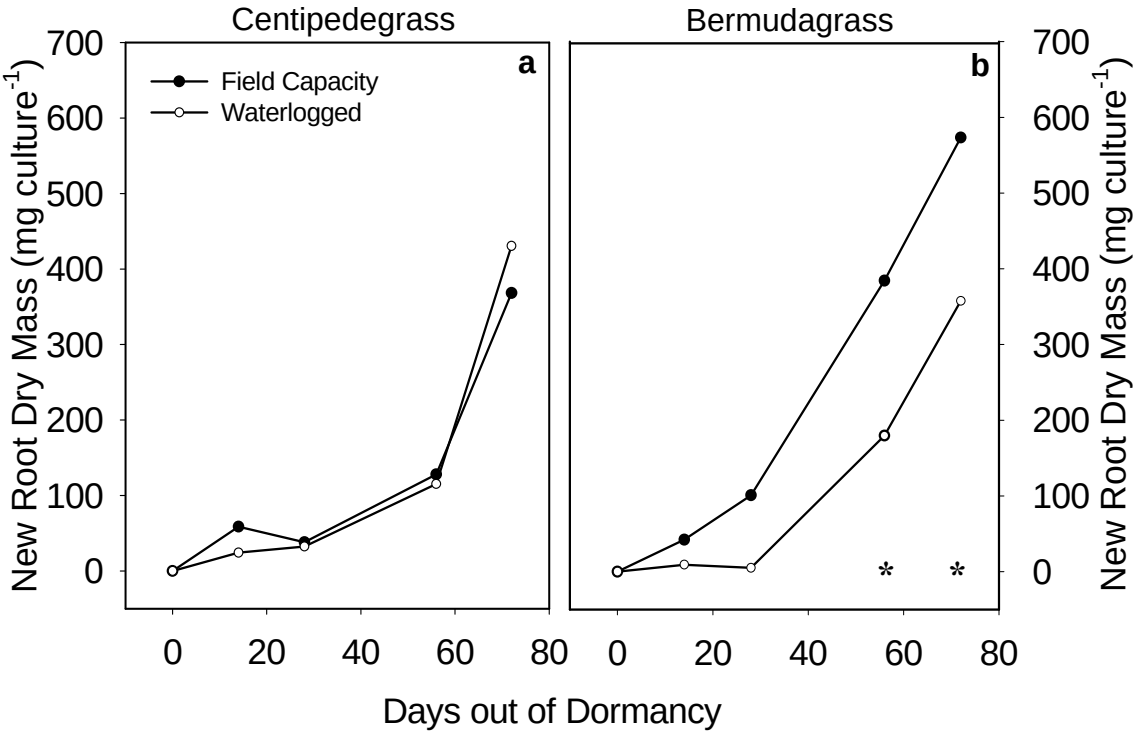

Figure 3. New root production in a- centipedegrass and b- bermudagrass through the experiment. Asterisks denote significance at the P= 0.05 level based on analysis of variance (PROC GLM, SAS).

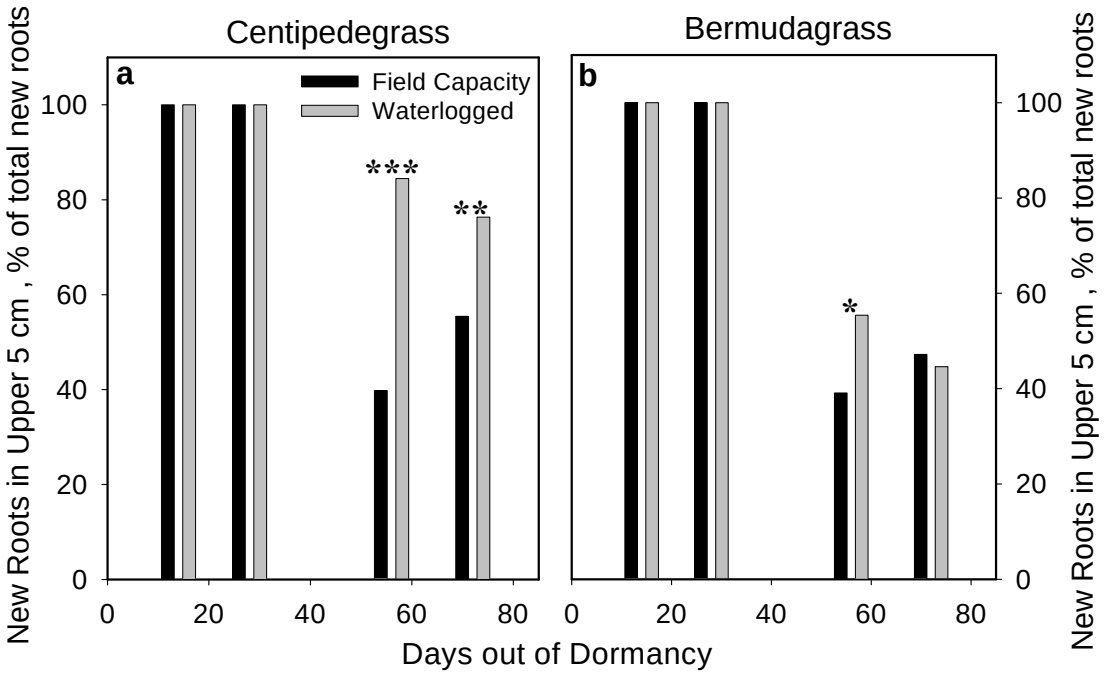

Figure 4. Percentage of new roots at the surface (upper 5 cm) relative to total new root mass (15 cm) in a- centipedegrass and b- bermudagrass. *, **, and *** denote significance at the P= 0.05, P=0.01, and P=0.001 level, respectively, based on analysis of variance (PROC GLM, SAS).

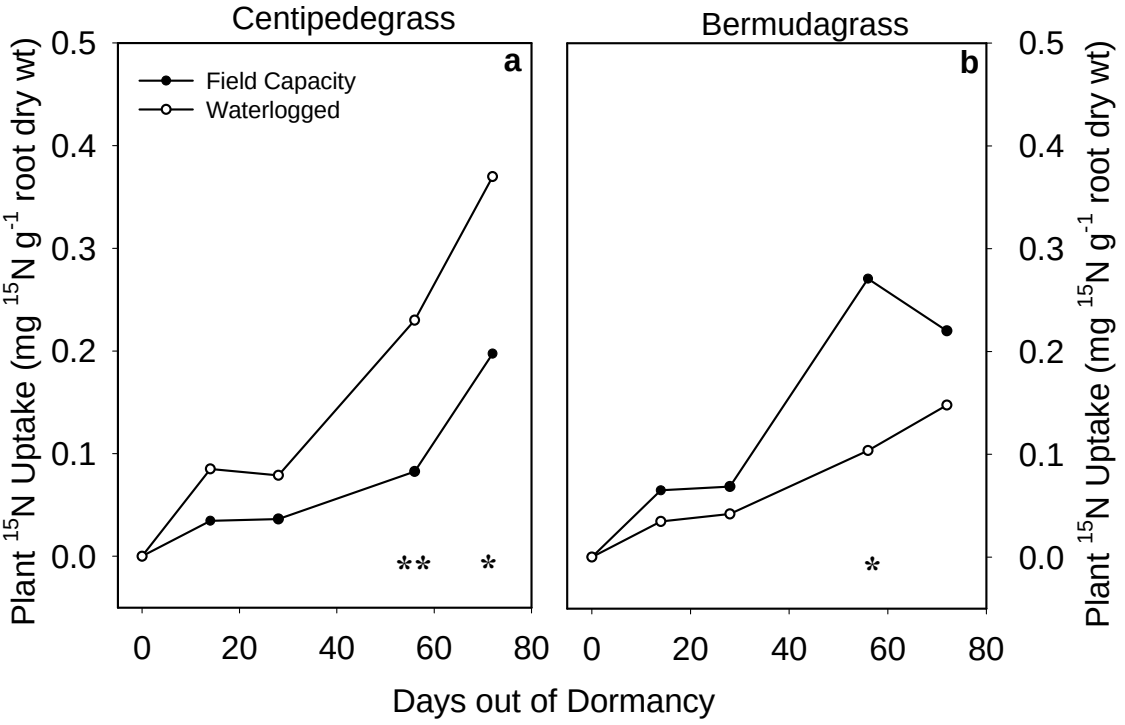

Figure 5. 24-hr ^{15}N uptake efficiency of a- centipedegrass and b- bermudagrass. Uptake efficiency was calculated as a function of total dry mass of all roots in each grass/soil. * and ** denotes significance at the P= 0.05 and 0.001 levels, respectively, based on analysis of variance (PROC GLM, SAS).

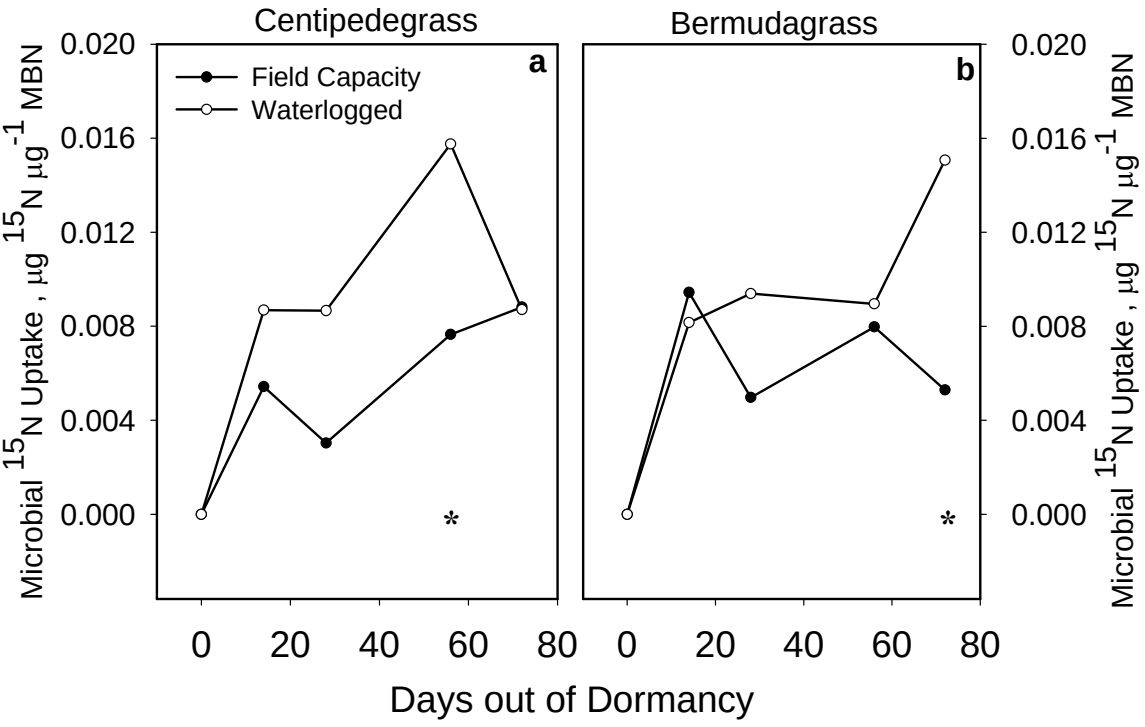

Figure 6. Microbial biomass ^{15}N uptake efficiency following 24-hr uptake period in a- centipedegrass and b- bermudagrass over the course of the experiment. Asterisks denote significance at the P=0.05 level based on analysis of variance (PROC GLM, SAS).

Table 1. Distribution and mass balance of ^{15}N recovered from centipedegrass cores supplied with $^{15}NO_3$. Values represent the percentage of total recovered ^{15}N that was obtained from each fraction. Values within the same row on each date divided by an asterisk are significantly different at the P= 0.05 level based on analysis of variance (PROC GLM, SAS). LSDs are provided for comparisons between all data within a row across harvest dates and are calculated at the P= 0.05 level.

Centipedegrass	*LSD P=0.05*	14		28		56		72	
		FC	WL	FC	WL	FC	WL	FC	WL
% ^{15}N Recovery	*21.4*	47.3	57.9	43.3	59.4	51.5	66.3	61.8	71.1
Whole Plant	*17.2*	44.1	35.3	25.9	40.1	44.0	53.0	56.2 *	74.9
Shoots	*6.0*	3.6	4.5	6.1	5.9	12.0	8.3	16.5	15.2
Stems	*13.0*	35.6	21.2	14.4	25.4	21.6	21.8	18.0	26.0
Roots	*7.9*	4.9	9.5	5.3	8.8	10.4 *	22.9	21.7 *	33.7
Unassimilated	*18.5*	43.4	46.5	64.8 *	43.2	38.2 *	20.6	26.5	12.8
Leachate	*17.3*	36.1	41.0	60.6 *	36.9	35.5 *	10.3	23.8 *	7.2
K_2SO_4 extracted	*4.8*	7.3	5.4	4.3	6.3	2.7 *	10.3	2.8	5.6
Organic	*4.8*	0.6	0	1.2	2.6	2.3	4.7	2.7	1.9
Microbial Biomass	*11.2*	11.9	18.3	8.1	14.1	15.5	21.7	14.6	10.4

[a] Values reported are means of three replications. Grass/soil cores were fertilized with KNO_3 (40 atom % ^{15}N, 1.8 g N m^{-2})

[b] 'K_2SO_4 extracted' represents ^{15}N extracted from soil following leachate flush and collection

[c] 'Organic' represents ^{15}N from soluble and solid fraction of soil organic fractions

Table 2. Distribution and mass balance of ^{15}N recovered from bermudagrass cores supplied with $^{15}NO_3$. Values represent the percentage of total recovered ^{15}N that was obtained from each fraction. Values within the same row on each date divided by an asterisk are significantly different at the P= 0.05 level based on analysis of variance (PROC GLM, SAS). LSDs are provided for comparisons between all data within a row across harvest dates and are calculated at the P= 0.05 level.

Bermudagrass	*LSD P=0.05*	14		28		56		72	
		FC	WL	FC	WL	FC	WL	FC	WL
% ^{15}N Recovery	*18.9*	63.6	58.7	68.3	53.2	63.9	60.0	74.3	60.1
Whole Plant	*17.7*	49.0	43.8	63.9	54.9	84.8 *	67.2	68.7	70.0
Shoots	*13.0*	15.1 *	5.3	16.9 *	6.7	27.7	19.7	25.8 *	13.7
Stems	*19.9*	27.1	34.4	39.8	42.9	28.1	35.2	23.1 *	39.8
Roots	*8.3*	6.8	4.1	7.2	5.3	29.0 *	12.3	19.8	16.4
Unassimilated	*15.0*	39.2	43.1	30.1	31.3	5.7	15.6	22.8	12.3
Leachate	*14.0*	32.0	35.4	26.2	23.5	4.6	9.9	20.6	7.0
K_2SO_4 extracted	*4.2*	7.2	7.6	3.9	7.8	1.1 *	5.7	2.3	5.3
Organic	*6.1*	0.1	0.9	1.0	0	4.8	6.3	3.6	3.4
Microbial Biomass	*8.2*	11.7	13.0	5.9	13.8	4.6	10.9	4.9 *	14.5

[a] Values reported are means of three replications. Grass/soil cores were fertilized with KNO_3 (40 atom % ^{15}N, 1.8 g N m^{-2})

[b] 'K_2SO_4 extracted' represents ^{15}N extracted from soil following leachate flush and collection

[c] 'Organic' represents ^{15}N from soluble and solid fraction of soil organic fractions