ABSTRACT

ZHANG, CHENXI. Nitrate Uptake of Kentucky Bluegrass as a Determinant of Nitrogen Use Efficiency. (Under the direction of Dr. Daniel C. Bowman).

Nitrate (NO$_3^-$) leaching from fertilized land can be detrimental to aquatic ecosystems and human health. Although NO$_3^-$ leaching potential is generally found to be low in turfgrass, certain conditions can occur that result in increased leaching loss of nitrogen. Kentucky bluegrass (*Poa pratensis* L.) is the most widely used cool-season turfgrass species in the temperate and subarctic climate zones in the United States. Due to its popularity, many new cultivars are bred and released each year. Despite the ample amount of information characterizing the agronomic features of Kentucky bluegrass genotypes, little is available documenting their physiological characteristics related to nitrogen use efficiency and how these might affect the NO$_3^-$ leaching potential of the genotypes. Such information would be of great value for both scientists and customers.

In this comprehensive study, a screening procedure was developed using nutrient solution culture to evaluate differences in NO$_3^-$ uptake among sixty Kentucky bluegrass genotypes. Two cultivars were selected from the sixty to represent genotypes having high vs. low capacity for NO$_3^-$ uptake. These two cultivars were then used to examine the relationship between NO$_3^-$ uptake efficiency and competitiveness for soil nitrogen. Finally, these two cultivars were compared to determine if NO$_3^-$ uptake efficiency affected NO$_3^-$ leaching potential.

There were significant differences in NO$_3^-$ uptake at both high (1 mM) and low (0.05 mM) N concentrations among sixty Kentucky bluegrass genotypes, with a strong correlation between uptake rates at high and low N concentrations. Julia and Midnight were selected as
representing cultivars with efficient and inefficient nitrate uptake, respectively. In nutrient solution culture, Julia exhibited 56% higher NO$_3^-$ uptake rates than Midnight. In a root competition study, in which the root systems of the two cultivars occupied the same soil volume, Julia was more competitive for nitrogen acquisition (absorbed 20 to 71% more NO$_3^-$) than Midnight. However, the higher NO$_3^-$ uptake capacity and better competitiveness for soil N in Julia did not result in lower NO$_3^-$ leaching potential. These results suggest that differences in root morphology and architecture may play a more important role than uptake capacity in determining nitrate leaching potential among Kentucky bluegrass genotypes.
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Nitrate Uptake of Kentucky Bluegrass as a Determinant of Nitrogen Use Efficiency

by

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DEDICATION

To my parents,

Dun Zhang and Aiping Zhao,

for their dedication to my education.
BIOGRAPHY

Chenxi Zhang was born in Chengdu, China. Under the influence of his parents - both working in a scientific research institution, Chenxi developed a strong interest in science at a young age. After graduating from Chengdu Qi-Zhong High School, Chenxi was admitted by Shanghai Jiao Tong University in 2003 to study plant biotechnology and later concentrated in turfgrass physiology. Under the direction of Dr. Yali He, Chenxi conducted his undergraduate research investigating the shade tolerance of tall fescue as affected by plant growth retardant. Upon college graduation, Chenxi decided to pursue a higher degree across the Pacific Ocean. In 2007, he was admitted as a doctoral student at North Carolina State University, which boasts a nationally-recognized program in turfgrass science.

Chenxi’s Ph.D. work at NC State University was mentored by turfgrass physiologist Dr. Daniel Bowman. Chenxi conducted a series of studies investigating the variations in nitrate uptake capacities of Kentucky bluegrass genotypes and how does the variations affect the nitrogen use efficiency and nitrate leaching potential of genotypes. Meanwhile, Chenxi served as the teaching assistant to Introduction to Turfgrass Management throughout his graduate career.

Chenxi received the Provost’s Fellowship in 2007 as an outstanding doctoral student entering NC State University. He was awarded the Art Bruneau Golf Tournament Scholarship twice in 2010 and 2011 for demonstrating academic excellence in his graduate program. Chenxi also completed additional coursework to earn a Ph.D. minor in Statistics.

In addition to the strong commitment to academics, Chenxi was actively involved in extra-curriculum activities. He served as the vice president of Chinese Students and Scholars
Friendship Association from 2008 to 2010. He has been an active member of American Society of Agronomy. He was also invited to join Delta Sigma Gamma (the honor society of agriculture) and Phi Kappa Phi (the honor society for all academic disciplines).

Upon graduation, Chenxi will apply his knowledge in plant physiology and statistical analysis to physiological research in crop plants. He looks forward to becoming one of the brainpower contributing to solve the giant project of feeding the world.
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TABLE OF CONTENTS

LIST OF TABLES...........................................................................................................viii

LIST OF FIGURES.........................................................................................................x

CHAPTER 1

Literature Review........................................................................................................1
Milestones in the history of studying plant inorganic nutrition.................................1
Nitrogen as a plant essential mineral nutrient..............................................................7
Plant Uptake and Assimilation of Ammonium and Nitrate.........................................10
  Root Absorption of Ammonium (NH4+)..................................................................11
  Ammonium Transporters.......................................................................................14
  Root Absorption of Nitrate (NO3−).......................................................................15
  Nitrate Transporters............................................................................................19
  Assimilation of Ammonium and Nitrate.................................................................20
Nitrogen Loss from Managed Turfgrass....................................................................21
  Pathways of Nitrogen Loss from Turfgrass..........................................................22
  Nitrate Leaching in Managed Turfgrass.................................................................25
Improving Nitrogen Use Efficiency of Kentucky Bluegrass to Limit Nitrate Leaching via Cultivar Screening...............................................................31
References............................................................................................................37

CHAPTER 2

Nitrate Uptake Rates of Kentucky Bluegrass Genotypes and Their Effect on Nitrate Absorption under Competitive Conditions........................................58
Abstract................................................................................................................58
Introduction............................................................................................................59
Materials and Methods........................................................................................62
Results and Discussion.........................................................................................68
References............................................................................................................77

CHAPTER 3

Nitrate Leaching from Two Kentucky Bluegrass Cultivars as Affected by Nitrate Uptake Capacity and Subsurface Soil Compaction...........................................90
Abstract..............................................................................................................90
Introduction..........................................................................................................91
Materials and Methods.......................................................................................93
Results and Discussion.......................................................................................97
References..........................................................................................................106
LIST OF TABLES

CHAPTER 2

Table 1  Biomass and NO₃⁻ uptake rate by 60 Kentucky bluegrass cultivars at high N concentration…………………………………………………………………………………..81

Table 2  Biomass and NO₃⁻ uptake rate by 60 Kentucky bluegrass cultivars at low N concentration…………………………………………………………………………………..83

Table 3  Biomass and NO₃⁻ uptake rate by 10 Kentucky bluegrass cultivars in confirmational screening at high N concentration………………………………85

Table 4  Tissue N content, harvested dry mass (DM) and NO₃⁻ uptake of Julia and Midnight Kentucky bluegrass at high N condition, Trial 1…………………..86

Table 5  Type 3 ANOVA for NO₃⁻ uptake rate of Julia and Midnight Kentucky bluegrass at high N condition, Trial 1……………………………………………….86

Table 6  Tissue N content, harvested dry mass (DM) and NO₃⁻ uptake of Julia and Midnight Kentucky bluegrass at low N condition, Trial 1………………….87

Table 7  Type 3 ANOVA for NO₃⁻ uptake rate of Julia and Midnight Kentucky bluegrass at low N condition, Trial 1……………………………………………….87

Table 8  Recovery of applied N in lysimeters of Trial 1………………………………………………………………………………………………………………………….87

Table 9  Tissue N content, harvested dry mass (DM) and NO₃⁻ uptake of Julia and Midnight Kentucky bluegrass at high N condition, Trial 2…………………..88

Table 10 Type 3 ANOVA for NO₃⁻ uptake rate of Julia and Midnight Kentucky bluegrass at high N condition, Trial 2……………………………………………….88

Table 11 Tissue N content, harvested dry mass (DM) and NO₃⁻ uptake of Julia and Midnight Kentucky bluegrass at low N condition, Trial 2…………………..89

Table 12 Type 3 ANOVA for NO₃⁻ uptake rate of Julia and Midnight Kentucky bluegrass at low N condition, Trial 2……………………………………………….89

Table 13 Recovery of applied N in lysimeters of Trial 2………………………………………………………………………………………………………………………….89
CHAPTER 3

Table 1      Mean comparisons of clipping yields……………………………………………….111
Table 2      Comparisons of biomass harvested at the end of Exp.1……………………………..112
Table 3      Comparisons of biomass harvested at the end of Exp.3……………………………..112
Table 4      ANOVA of cumulative N leaching loss from Julia and Midnight and orthogonal contrast F-test in Exp.1……………………………………………………………………………….113
Table 5      ANOVA of cumulative N leaching loss from Julia and Midnight and orthogonal contrast F-test in Exp.2……………………………………………………………………………….114
Table 6      ANOVA of cumulative N leaching loss from Julia and Midnight and orthogonal contrast F-test in Exp.3……………………………………………………………………………….115
# LIST OF FIGURES

## CHAPTER 3

| Figure 1 | Clipping yields of Julia and Midnight Kentucky bluegrass as affected by subsurface compaction layer (SCL) | 116 |
| Figure 2 | Biomass of Julia and Midnight harvested from lysimeters at the end of Exp.1 | 117 |
| Figure 3 | Biomass of Julia and Midnight harvested from lysimeters at the end of Exp.3 | 118 |
| Figure 4 | NO$_3^-$-N concentration in the leachate from Julia and Midnight Kentucky bluegrass, as affected by subsurface compaction layer in Exp.1 | 119 |
| Figure 5 | NO$_3^-$-N concentration in the leachate from Julia and Midnight Kentucky bluegrass, as affected by subsurface compaction layer in Exp.2 | 120 |
| Figure 6 | NO$_3^-$-N concentration in the leachate from Julia and Midnight Kentucky bluegrass, as affected by subsurface compaction layer in Exp.3 | 121 |
CHAPTER 1

Literature Review

I. MILESTONES IN THE HISTORY OF STUDYING PLANT INORGANIC NUTRITION

“The young of today do not respect their elders. But this lack of respect is not their fault, for in their science courses, they are no longer taught any history of science. ... But in my day, we knew the history of science, and we admired those who enriched it by their work.”

-Jean-Baptiste Boussingault, in Mémoires de J.-B. Boussingault, 1903

It is estimated that plant and animal biomass (defined as organically bound carbon) on earth totals around 560 billion tons. Although oceans make up over 70% of the earth surface, over 98% of the aforementioned biomass is found on land (Groombridge, 2000), and over 99.9% of the terrestrial biomass is retained in plants (Whitman et al., 1998). Being the most important primary producers on the terrestrial ecosystem, photosynthetic plants serve as the foundation of the ecological pyramid and support higher trophic levels in the system by generating about 100 billion tons of organic carbon annually (Field et al., 1998). The green autotrophs do not create biomass out of the void. As a processor, rather, they harness energy from solar radiation, capture carbon dioxide from the atmosphere, and extract mineral nutrients from the soil.
It all started with roots. When Greek philosopher Empedocles proposed the famous cosmogenic theory of the four classical elements over 2400 years ago, he used “roots” to designate the four ultimate elements (fire, air, water, and earth) structuring the world (Bronowski, 1973). Ironically, it was the wide acceptance of these “roots” on the philosophical side (although some modification and advance ensued thereafter) that hindered man’s understanding of plant roots on the physiological side.

More than a century before the first observation of photosynthesis by Jan Ingenhousz in 1778 (Magiels, 2010), a few skeptical pioneers endeavored to put thought into quantitative experimentation to investigate what factors contributed to plant growth. Among the documented works is that famously conducted by van Helmont in the mid-17th century (Gabriel, 1955) and repeated in a more meticulous fashion by Robert Boyle in the 1640’s (Clericuzio, 1993; Hoff, 1964). Although they incorrectly attributed biomass generation to water absorbed by roots, their demonstration offered an invaluable alternative for the long-believed dogma, and opened a gate for later studies in plant nutrition.

With the abandonment of the “phlogiston theory” and the advance of analytical chemistry in the late 18th century thanks to French chemist Antoine Lavoisier (Kerr, 1801), research in plant physiology gained momentum. The first recognition of plant mineral nutrition was made in 1699 by English naturalist, physician, and geologist John Woodward. Aside from demonstrating transpiration by growing spearmint in water culture (a prototype of modern solution culture), he also found that plants exhibited better growth in less pure water compared with distilled water. He thus proposed that it was the dissolved solids that provided plants with nutrients (Eyles, 1965). Unfortunately, his findings drew limited
attention (Stanhill, 1986). Swiss scientist Nicolas-Theodore de Saussure was the first plant physiologist to take full advantage of the innovative concept of chemical elements first proposed by Lavoisier. He determined that accumulation of plant biomass derived not only from chemical elements in water and air, but also in the soil solution (Arnon and Hoagland, 1944). In his book *Recherches chimiques sur la vegetation* published in 1804, de Saussure introduced the concept of “essential nutrients”, specifically that several minerals, found in small quantity from plant ash, were essential for maintaining plant health. Four of those identified (C, H, O, and N) are among the essential elements recognized today (Glass, 1989). In addition, de Saussure indicated that plant demand for the various essential nutrients was not in equal amount (Hart, 1930).

The importance of minerals to plant health was gradually realized towards the mid-19th century. In a time when famine was a world-wide threat and fear of overpopulation generated by the Malthusian theory was widespread (Jungk, 2009), plant scientists started to link crop productivity with mineral nutrition (Epstein and Bloom, 2005). At the same time, publication of *Chemistry in its Application to Agriculture and Physiology* in 1808 by German chemist Justus von Liebig finally shattered the erroneous “humus theory”, which assumed that organic compounds are of utmost importance in the nutrition of plants, and firmly announced the importance of mineral nutrients to plants. The Law of the Minimum independently described by Liebig and German botanist Carl Sprengel became one of the foundations in plant mineral nutrition (Browne et al., 1942; Jungk, 2009). While the “mineral theory” (as opposed to the “humus theory”) was gaining popularity, French scientist Jean-Baptiste Boussingault performed enlightening studies in mid-1800s to prove the importance
of nitrogen. His data from well-designed and conducted field and laboratory experiments were among the first to document the role of animal manure as a fertilizer source of N. Furthermore, using a prototypic sand culture, Boussingault verified that nitrate in soil solution was key to plant growth. Later, Boussingault was the first to demonstrate atmospheric nitrogen fixation by leguminous plants, and his extensive work in the subject of nitrogen forms the basis of our understanding of the nitrogen cycle (Arnon and Hoagland, 1944; Aulie, 1970; Epstein and Bloom, 2005).

As progress was being made in the laboratory, plant mineral nutrition field trials became more sophisticated and scientifically based. In 1843, a 16th-century English manor was converted by inheritor John Lawes into what is now the oldest agriculture research institution – Rothamsted Experiment Station. Improving plant growth with nutrient addition was a major subject of study. One of the first experiments conducted on Rothamsted farms investigated the effect of superphosphate on crop yield. Research projects soon included inorganic nitrogen and potash (Russeli, 1942). Rothamsted Experiment Station’s fame grew, and its annual report appeared regularly in *Nature*, as important notes for both farmers and scientists. In addition, Rothamsted Experiment Station bolstered the association between statistical theory and application in biological research for the first time – marked by Ronald Fisher’s work in design of experiment and analysis of variance between 1919 and 1933 (Box, 1978).

Thanks to the collective efforts of numerous scientists in the 19th century, the importance of inorganic nutrients was gaining public recognition. However, the complexity of the natural soil environment finally became an obstacle to understanding essentiality and
effects of individual nutrients (Arnon and Hoagland, 1944). Various attempts were made to develop alternatives to natural soil for scientific research, including the nutrient solution culture system; this important tool has been pivotal to advancing our knowledge of plant mineral nutrition.

The first “recipes” for nutrient solution culture were developed independently by German scientists Julius von Sachs and J. A. L. W. Knop in the 1860’s. Except for the subsequent addition of micronutrients and aeration, major elements from Sachs and Knop’s formula remain largely unchanged (Arnon and Hoagland, 1944). In the early 20th century, the technique of solution culture quickly migrated from research labs into soilless crop production systems (Gericke, 1929; Gericke and Tavernetti, 1936; Gericke, 1940). Studies by Arnon and Hoagland stimulated the final maturation of modern solution culture. They evaluated the role of soilless culture in crop production and devised guidelines for a dependable and highly functional solution culture system (Arnon, 1937; Arnon, 1938; Arnon and Hoagland, 1939; Arnon and Hoagland, 1940; Arnon and Hoagland, 1944; Hoagland and Arnon, 1950; Hoagland and Broyer, 1936). Our current understanding and practical use of nutrient solution culture is based on several important findings from their research: (1) the four-salt system (KNO₃, KH₂PO₄, CaNO₃, and MgSO₄) which provides six mineral macronutrients; (2) a generous flexibility in nutrient concentration; (3) nutrient proportions that mirror plant removal as opposed to plant mineral composition; (4) nitrate used as the major N source, although small addition of ammonium was beneficial in terms of pH stabilization; and (5) avoiding micronutrient deficiency and providing efficient aeration.
Except for the inclusion of later-recognized micronutrients, their recipe is still widely used today.

The definition for plant essential nutrients was first proposed in 1939 by Arnon and Stout. Essentaility was defined as ensuring complete life cycle, correcting deficiency symptoms, and being directly involved in nutrition (Arnon and Stout, 1939b). After being widely accepted for over 60 years, this definition was revised by Epstein and Bloom. Their definition redefined an essential nutrient as being a structural or metabolically-intrinsic component, and/or causing abnormality during life cycle when severely deprived (Epstein and Bloom, 2005).

A tentative list of mineral nutrients (N, P, K, S, Ca, Mg and Fe) vital for plant growth was first proposed in the 1860s. Iron was the only micronutrient and no additions were made to the list prior to the 20th century (Epstein, 2000; Epstein and Bloom, 2005). A series of discoveries in the 1920s and 1930s led to a quick expansion of the list, with the addition of Mn, B, Zn, and Cu (Lipman and Mackinney, 1931; McHargue, 1923; Sommer and Lipman, 1926; Sommer, 1931; Warington, 1923). The “A to Z” solution formulated by Hoagland was a powerful tool in investigating the essentiality of micronutrients (Stout, 1956), including Mo and Cl (Arnon and Stout, 1939a; Broyer et al., 1954; Stout and Johnson, 1956). With Ni joining the list of micronutrients in 1983 (Eskew et al., 1983), there are currently 17 essential mineral nutrients required by higher plants. Additional studies continue to reveal that certain species of plants require other elements that are generally not considered essential. For example, Na, normally an undesirable mineral, was proven to be essential for a halophytic species as a macronutrient (Williams, 1960).
Nutrient management has gradually been recognized as vital to agricultural production. Nutrients are considered deficient when they are not present in adequate amount in soil or present in an unavailable form. As deficiency often poses a high risk for considerable economic losses, supplemental amounts are provided accordingly via fertilization. Modern fertilization programs have evolved from arbitrary guesswork to ones that are based on science and technology. Various tools are available to make fertilization accurate, economical, and environmentally safe. These include identification of deficiency symptoms, extraction-based soil tests, analytical tissue tests, and even digital image analysis. Of all the nutrients required by plants, nitrogen (N) is often considered as the most important. It will be discussed in detail in the following sections.

II. NITROGEN AS A PLANT ESSENTIAL NUTRIENT

“The world might be better off without Microsoft and CNN, and neither nuclear reactors nor space shuttles are critical to human well-being. But the world’s population could not have grown from 1.6 billion in 1900 to today’s 6 billion without the Haber-Bosch process”

- Vaclav Smil, in Detonator of the Population Explosion, 1999

Nitrogen (N) is the next most abundant element in plant tissue after H, C and O – ranging from about 2% to 6% in dry mass, with 1.5% as a common critical value. The presence of N in plant tissue was detected as early as the mid-17th century (Glauber, 1689) yet the recognition of its physiological importance came gradually. Now we understand that
N is a component of all proteins and nucleic acids, and a large fraction of plant N functions directly to harness energy and produce biomass (Evans and Seemann, 1989).

Although being the major component of our atmosphere (78%), gaseous N was discovered after O and CO₂. In 1772, Englishman Daniel Rutherford first isolated this unbreathable and non-combustible portion in air after removing O₂ and CO₂; it was named “noxious air” or “phlogisticated air” (Weeks, 1968). The elemental nature of N₂ was later clarified by Antoine Lavoisier in 1789 (Kerr, 1801). In addition to the huge atmospheric pool of N, ~ 100 billion kilograms of N is contained, mostly as organic forms, in terrestrial organisms and the soil (Schlesinger, 1997). The dynamic interaction between organic and inorganic pools of N forms a complex network in the biosphere that supports all fauna and flora populations. The discussion here will be limited to the relationship between N, plant, and soil.

The essentiality of N for plant growth was discovered concurrently with C, O and H by Nicolas-Theodore de Saussure in 1804. After examining humus for its mineral composition, de Saussure suggested that the majority of the nutritional effects of humus were due to the mineral nutrients it released, with NO₃⁻ being primary (Pennazio, 2005). Combined with his previous finding that accumulation of plant biomass was not matched to carbon fixation in photosynthesis, he concluded that N was another essential nutrient that plants used to generate biomass. De Saussure characterized N as one of the most important nutrients controlling plant growth and proposed that fertilizers be judged based on their N content (Aulie, 1970).
In the early 19th century, most people believed that atmospheric N was the only N source for plants and farmers need not worry about supplying N. De Saussure was skeptical; he thought atmospheric N was inert and plant N was absorbed in mineral form by roots (Hart, 1930). De Saussure’s speculation was backed by Jean-Baptiste Boussingault’s experiments in 1837, which showed that the soil solution was an important N source. Working at the Rothamsted Experiment Station, John Lawes and Henry Gilbert concluded that continuous crop production required additions of N, as well as some other nutrients (Lawes, 1847; Lawes and Gilbert, 1855). Nitrogen is now recognized as the most limiting nutrient for plant growth in most terrestrial ecosystem (Lee et al., 1983; Miller and Cramer, 2004; Tilman, 1988; Vitousek and Howarth, 1991).

Various and diverse materials have been used to supply plants with N even before the science of plant nutrition was established. Bird guano, for example, has been used as an N fertilizer for centuries. And saltpeter (containing KNO₃) is one of the oldest forms of inorganic N fertilizer. Ammonium salts became the most popular form of inorganic N fertilizer used in the mid 1800’s. It was later demonstrated by Jean-Baptiste Boussingault that the nitrate form of N could carry out the same function (Aulie, 1970). Although the importance of N fertilizers in agriculture was accepted, their availability was limited. That changed with the invention of the Haber-Bosch process in the early 20th century. This industrial process reduces N₂ gas to NH₃, thus converting N from an inert to a chemically reactive and biologically available form. Haber-Bosch ushered in the era of synthetic N fertilizer. Since that time, the use of N fertilizers has increased over 20 fold (Glass, 2003). As much as half of the crop yield increase in the 20th century is estimated to derive from
increased fertilizer input, especially the N-containing fertilizers (Baligar et al., 2001). According to the International Fertilizer Industry Association, global annual fertilizer N consumption was over 80 Tg (1 Tg = 1×10^{12} g) in 2000, and about half of the total N accumulated in plant biomass is due to fertilizer application (Smil, 1999). It is thus not surprising that the Haber-Bosch process is considered by many to be the single most important invention of the twentieth century.

III. PLANT UPTAKE AND ASSIMILATION OF AMMONIUM AND NITRATE

“Plant performance – be it fitness, yield, nutrient efficiency, or susceptibility to biological or environmental stress – generally hinges on the ability of the plant to obtain ammonium and nitrate”

- Emanuel Epstein and Arnold Bloom, in Mineral Nutrition of Plants, 2005

Nutrient acquisition is one of the most fundamental biological activities. Even the simplest prokaryote organisms devote a great amount of their limited genome to code for nutrient absorption and utilization-related processes (Epstein and Bloom, 2005).

Plant physiologists have been studying the mechanisms of nutrient uptake for nearly a century. In the late 1800’s, the diffusion-osmosis theory (passive transport) was thought to be the dominant mechanism of mineral acquisition by plant roots (Epstein and Bloom, 2005). It became obvious that uptake was more complicated when Hoagland found that giant kelp accumulated K^+, I^- and Br^- in its cells against the concentration gradient (Hoagland and Davis, 1929). Further, anti-gradient transport of cations and anions in algal cells were all related to cell metabolic activities (Brooks, 1940; Hoagland and Broyer, 1942). Similar
findings were soon reported in higher plants, although not in root tissue at first (Steward, 1933). In his lecture on plant mineral nutrition, Dennis Hoagland first termed this metabolic-dependent anti-gradient solute accumulation as “active transport” (Hoagland, 1944). Ensuing evidence from ion uptake kinetics, biochemistry, and molecular biology demonstrated that active transport was common in higher plants (Assmann, 2001; Assmann, 2001; Doyle et al., 1998; Epstein, 1973a; Hirsch et al., 1998; Mitchell, 1961). Currently the so-called passive movement of nutrients is primarily of importance in the apoplast, while most membrane transport of nutrients in the plant, including root uptake, is considered to be mediated by carriers (Epstein and Bloom, 2005).

Most terrestrial plants rely on their root system to acquire N from the soil solution. And ammonium and nitrate are the two primary forms of N absorbed by roots (Haynes and Goh, 1978; Pardo, 1935). Opinions vary as to which one is superior. Arguments in favor of ammonium focus on reduced environmental impact, more rapid absorption by plants, more efficient assimilation, and higher nitrogen use efficiency at elevated CO₂ concentration. On the other hand, possible soil fixation, greater microbial competition, higher risk of Al toxicity and unavoidable nitrification are negative factors of feeding with ammonium (Bloom, 1997; Haynes and Goh, 1978; Miller and Cramer, 2004). It was also suggested that supplying plants with both ammonium and nitrate helped to stabilize rhizospheric pH and ion balance inside the plant body (Bloom, 1997).

**Root Absorption of Ammonium (NH₄⁺)**

The cross-membrane absorption of NH₄⁺ is considered to be an active and carrier-mediated process. This is supported by several lines of evidence. First, NH₄⁺ entry into root
cells is against a concentration gradient. Root cell NH$_4^+$ concentrations were found in the millimolar range (Lee and Ratcliffe, 1991; Wang et al., 1993b), while surveys indicated that most agricultural soil has NH$_4^+$ concentrations between 10 and 50 μM (Novoa and Loomis, 1981). Second, although NH$_3$ (interconvertible with NH$_4^+$) has high membrane permeability, the cross-membrane chemical potential gradient was not in favor of NH$_3$ passive transport into root cells (Kronzucker et al., 1995a). Third, NH$_4^+$ absorption is inhibited by anaerobic conditions, low temperature, and metabolic inhibitors (Bloom, 1997). And finally, root uptake of NH$_4^+$ follows enzyme kinetics.

The enzyme kinetics, or Michealis-Menten kinetics, was first presented by Epstein and Hagen (1952) to describe root uptake of K$^+$ and Rb$^+$ by barley plants. They suggested that there are important similarities between enzymatic catalysis and root ion transport, namely an ion must undergo a transient phase in combination with an energy dependent carrier before being transported into root cells. Therefore, at a given number of available carriers, absorption rate of K$^+$ was initially linear at low rhizospheric concentrations, but was gradually limited by carrier saturation with increasing concentrations of K$^+$. Subsequently, Epstein et al. (1963) reported biphasic uptake kinetics for potassium, with the first phase saturating at about 0.2 mM and the second at about 1mM. This served to confirm their early speculation that several transport systems might be operational for a single ion. These two distinct systems are now referred to as High Affinity Transport System (HATS) in the low external ion concentration range and Low Affinity Transport System (LATS) in the high external ion concentration range (Elzam et al., 1964; Epstein, 1973b).
Ammonium uptake kinetics was originally thought to resemble that of K⁺ (Hassan and VanHai, 1976; Lycklama, 1963; Smith and Epstein, 1964), but the similarity was later confined to the HATS (Bloom and Chapin, 1981; Kronzucker et al., 1996; Wang et al., 1993a). HATS of NH₄⁺ uptake follows a Michaelis-Menten pattern, with Kₘ values reported from about 10 to 170 μM (Bowman and Paul, 1988; Glass and Siddiqi, 1995; Kronzucker et al., 1996). However, at high NH₄ concentrations, root absorption appears to be non-saturable. Wang et al. reported in 1993 that NH₄⁺ uptake by rice exhibited a linear pattern at concentrations above 1 mM. The linear pattern of NH₄⁺ uptake (LATS, at [NH₄⁺] = 1 mM to 40 mM) had little dependency on metabolic energy and was thus more likely to be passive.

Tissue NH₄⁺ status has an effect on NH₄⁺ uptake kinetics. HATS of N-deficient rice roots exhibited low Kₘ and higher Vₘₐₓ than roots pretreated with NH₄⁺ (Bowman and Paul, 1988; Kronzucker et al., 1996; Wang et al., 1993a). On the other hand, LATS of NH₄⁺-pretreated rice roots exhibited greater linear slope than N-deficient roots at [NH₄⁺] > 1 mM (Wang et al., 1993a; Wang et al., 1993b). Similar results were reported by others (Cerezo et al., 2000; Min et al., 1999).

Upon resupply of NH₄⁺ after a period of deprivation, NH₄⁺ uptake initially appeared to increase for a very brief period before declining (Goyal and Huffaker, 1986; Kronzucker et al., 1995a; Mack and Tischner, 1988; Morgan and Jackson, 1988; Nicoulaud and Bloom, 1996; Nicoulaud and Bloom, 1998). This increase was originally thought to be the induction for NH₄⁺ uptake (Goyal and Huffaker, 1986; Mack and Tischner, 1988), although Kronzucker et al. (1998) argued that his was rather a strategy to relieve N deficiency before root N influx became synchronized with up-regulated root to shoot translocation of N.
After the initial stage, \( \text{NH}_4^+ \) uptake is subject to feedback regulation. As cytoplasmic and vacuolar \( \text{NH}_4^+ \) plus assimilative products build up, \( \text{NH}_4^+ \) influx is suppressed and efflux increases (Kronzucker et al., 1995a; Lee et al., 1992; Ullrich et al., 1984; Wang et al., 1993b). The regulating agents include accumulated \( \text{NH}_4^+ \) and amino acids, such as glutamine (Feng et al., 1994; Glass et al., 1997; Glass et al., 2002; Rawat et al., 1999; Ryan and Walker, 1994).

Efflux of \( \text{NH}_4^+ \) could offset an appreciable portion of influx at high external \( \text{NH}_4^+ \) concentrations (Glass, 2003). Wang et al. (1993b) determined that \( \text{NH}_4^+ \) efflux in rice roots at 1mM of external \( \text{NH}_4^+ \) concentration offset about 30% of influx. This magnitude dropped to about 10% of influx if roots were N-deficient (Wang et al., 1993b). The mechanism of \( \text{NH}_4^+ \) efflux is unclear. Britto and Kronzucker (2001) suggested that \( \text{NH}_4^+ \) efflux at high external concentration could be achieved via active extrusion. If so, influx results in a futile cycling of ammonium (Britto and Kronzucker, 2001; Feng et al., 1994). Nevertheless, appreciable efflux was only evident at high external \( \text{NH}_4^+ \) concentration. In agricultural soil, \( \text{NH}_4^+ \) efflux is probably minimal; net uptake is thus mainly determined by influx rate and feedback regulation (Kosola and Bloom, 1994).

**Ammonium Transporters**

Membrane transport of \( \text{NH}_4^+ \) is considered to be via an \( \text{NH}_4^+ \) uniport, based on strong depolarization of the plasmalemma after exposure to \( \text{NH}_4^+ \) (Ullrich, 1987). However, an \( \text{NH}_4^+/\text{H}^+ \) cotransport scheme was also proposed by others (Ayling, 1993; Wang et al., 1994). Cells involved in transport are challenged to maintain charge balance and in response employ
several strategies: rapid assimilation of cytoplasmic ammonium, vacuolar loading, proton export via ATPases, and stimulated anion influx (Bloom, 1997).

The \( \text{NH}_4^+ \) transporter is highly specific. Although \( \text{NH}_4^+ \) can enter cells via \( K^+ \) channels and thus interfere with \( K \) uptake (Hassan and VanHai, 1976; Rufty et al., 1982; Smith and Epstein, 1964; White, 1996), \( K^+ \) apparently has little effect on \( \text{NH}_4^+ \) absorption (Scherer et al., 1984; Smart and Bloom, 1988). Two distinct families of \( \text{NH}_4^+ \) transporters, AMT1 and AMT2, have been identified in higher plants. AMT1 transporters function as high affinity transporters at lower concentrations, while AMT2 transporters probably function at high \( \text{NH}_4^+ \) concentrations (~1 mM) (Epstein and Bloom, 2005). There are at least five AMT1 transporters identified so far in higher plants (Epstein and Bloom, 2005; Glass et al., 2002; von Wiren et al., 2000). Low affinity transporters for \( \text{NH}_4^+ \) are generally recognized as ion channels (Williams and Miller, 2001). The electrically neutral ammonia molecule has good membrane permeability and can enter cells passively at high external concentrations (Howitt and Udvardi, 2000; Niemietz and Tyerman, 2000). Few studies have investigated the \( \text{NH}_4^+ \) efflux transporter, which has been characterized as either an ATPase linked transporter or an \( \text{NH}_4^+\text{-H}^+ \) antiport (Britto and Kronzucker, 2001; Miller and Cramer, 2004).

**Root Absorption of Nitrate (NO\(_3^\text{-}\))**

Root uptake of \( \text{NO}_3^- \) follows biphasic kinetics similar to that of \( \text{NH}_4^+ \). Siddiqi et al. (1990) demonstrated that two distinct types of \( \text{NO}_3^- \) uptake systems are present in roots: a saturable HATS is mainly functional at concentrations of less than 0.5 mM, and the uptake curve at these low concentrations can be characterized by Michaelis-Menten kinetics. A non-
saturable LATS is mainly functional at concentrations higher than 1 mM, with uptake increasing linearly with concentration.

The HATS for NO$_3^-$ uptake was thought to have a constitutive component and an inducible component (Siddiqi et al., 1990). The kinetic parameters $K_m$ and $V_{max}$ of induced HATS range from 20 to 100 μM and 3 to 8 μmol·g$^{-1}$·h$^{-1}$, respectively (Crawford and Glass, 1998). Uninduced, nitrogen deprived roots exhibited very slow initial uptake upon N supply, with the duration of this lag phase lasting from several hours to days depending on species (Bowman and Paul, 1988; Bowman et al., 1989a; Kronzucker et al., 1995b; Siddiqi et al., 1989; Siddiqi et al., 1990). Concentrations as low as 10 μM NO$_3^-$ will induce NO$_3^-$ uptake in N-deprived roots of corn and barley when given enough exposure time. Rapid induction can be achieved using higher concentrations, such as 250 μM for one hour (Siddiqi et al., 1989). Nitrate itself appeared to be the inducing agent (Ingemarsson et al., 1987; King et al., 1993), although down-stream products (amino acids) were also considered as possible regulating agents (Glass et al., 2002; Lee and Clarkson, 1986; Lee et al., 1992; Muller and Touraine, 1992; Vidmar et al., 2000; Zhuo et al., 1999). Induction is generally considered to be the initiation of protein synthesis upon the transition from N-scarce to N-abundant environment (Hole et al., 1990; Jackson et al., 1973). Proteins produced would include membrane transporters on the root epidermis and stele loading transporters on the endodermis (Siddiqi et al., 1991). Given this complexity, some investigators suggest that HATS should be separated into cHATS (actively constitutive) and iHATS (substrate induced) (Aslam et al., 1992). By contrast, LATS for NO$_3^-$ uptake is not thought to be inducible (Glass and Siddiqi, 1995; Glass, 2003; Siddiqi et al., 1990).
Nitrate uptake is negatively correlated with tissue N status. After induction, $V_{\text{max}}$ of nitrogen deficient barley roots increased about 5 times compared to N-sufficient roots (Siddiqi et al., 1990). Similar enhancement of NO$_3^-$ influx was also reported in N-deficient perennial ryegrass and Kentucky bluegrass (Bowman et al., 1989a). High root NO$_3^-$ concentration was shown to down-regulate NO$_3^-$ influx and up-regulate its efflux (Deane-Drummond and Glass, 1983; Siddiqi et al., 1989). NO$_3^-$ uptake was negatively regulated by several other internal nitrogen pools, including NO$_2^-$, NH$_4^+$, and other assimilation products (King et al., 1993); this regulation was thought to be imposed on both NO$_3^-$ uptake and reduction (Lee et al., 1992; Shiraishi et al., 1992). LATS of NO$_3^-$ uptake is also regulated by tissue N status, but the difference between N-deficient and N-sufficient roots is not as dramatic as that in HATS (Siddiqi et al., 1990).

Nitrate uptake via HATS is carrier-mediated active transport, while LATS appears to be passive (Glass et al., 1990). Based on compartmental analysis, cytosolic NO$_3^-$ concentrations were found in the millimolar range for various species (Lee and Clarkson, 1986; Macklon et al., 1990; Presland and McNaughton, 1984; Siddiqi et al., 1991). These data indicate that absorption of NO$_3^-$ is energetically uphill at concentrations less than 0.5 mM (Glass et al., 1990; Siddiqi et al., 1991).

While NO$_3^-$ concentrations in the soil solution can be quite variable, they generally range from ~ 0 to 1 mM (Crawford and Glass, 1998; Crowley, 1975; Haynes, 1986; Miller and Cramer, 2004; Nye and Tinker, 1977; Reisenaur, 1966). This is especially true for areas with ample rainfall or irrigation. This indicates that active transport via HATS is the major
pathway for NO$_3^-$ acquisition in most agricultural soils (Glass et al., 1992; Glass et al., 1992; Glass, 2003; Zhen et al., 1991).

Nitrate uptake by roots is rapidly suppressed by the presence of ammonium (Frith, 1972; Lee and Drew, 1989; Lycklama, 1963; Minotti et al., 1969; Schrader et al., 1972; Shen, 1969). For example, in a nutrient solution where NH$_4^+$ accounted for 10% of total N, 50% of root absorbed N came from NH$_4^+$ (Siddiqi et al., 2002). Various hypotheses have been proposed to explain the mechanism. Lycklama (1963) attributed the inhibitory effect of NH$_4^+$ to be suppression of NO$_3^-$ reduction, which was later confirmed by Shen (1969) and Frith (1972). Frith (1972) also added that NH$_4^+$ could have a direct effect on NO$_3^-$ uptake. Rufty et al. (1982a) found the inhibitory effects of NH$_4^+$ were on both uptake and reduction of NO$_3^-$ in detopped corn roots, with inhibition of uptake being more limiting. In addition, assimilation of absorbed NH$_4^+$ may have imposed some inhibitory effects. Others have suggested the inhibitory effect of NH$_4^+$ is on the plasmalemma, as NH$_4^+$ dissipates the membrane potential which reduces the proton motive force driving the NO$_3^-$ transporter (Ayling, 1993; Lee and Drew, 1989). This mechanism sounds straightforward, but direct evidence is lacking. As an example, temporary depolarization of membrane electrical potential can also be triggered by K$^+$ uptake, but K$^+$ had no effect on NO$_3^-$ uptake (Wang et al., 1996). Bloom and Finazzo (1986) summarized possible causes of NO$_3^-$ uptake inhibition by NH$_4^+$, which include reduced synthesis of the NO$_3^-$ uptake system, inhibition of NO$_3^-$ reduction, and stimulation of NO$_3^-$ efflux.
Nitrate Transporters

Electrophysiology studies suggest that NO$_3^-$ ions are actively cotransported into the cytosol with two H$^+$ (Crawford and Glass, 1998; Glass et al., 1992; Ullrich and Novacky, 1981). This is consistent with data showing the optimum pH for NO$_3^-$ uptake is < 6 (Haynes and Goh, 1978; Munn and Jackson, 1978; Vessey et al., 1990). The energy requirement for NO$_3^-$ influx mainly comes from the need to pump out H$^+$ in order to maintain a trans-plasmalemma H$^+$ gradient (McClure et al., 1990a; McClure et al., 1990b).

Transporters of HATS have high specificity for NO$_3^-$ at low external concentrations. Common soil solution anions like Cl$^-$ and SO$_4^{2-}$ have little effects on HATS. Molecular studies report that two families of membrane proteins, nitrate-nitrite porters (NNP or NRT1, coded by NRT1 genes) and peptide transporters (PTR or NRT2, coded by NRT2 genes), function as NO$_3^-$ transporters (Forde, 2000). Genes encoding for 11 NO$_3^-$ transporters have been identified in higher plants (Glass et al., 2002). Most transporters in the NRT1 family appear to be LATS transporters, while a few NRT1 transporters, such as AtNRT1.1 coded by CHL1 gene, exhibited dual affinity, switched by a phosphorylation mechanism (Liu et al., 1999; Liu and Tsay, 2003). Most NRT2 transporters are HATS transporters (Epstein and Bloom, 2005). Based on spatial expression of NO$_3^-$ transporters, LATS could be of vital importance in NO$_3^-$ acquisition for young roots as they intercept available NO$_3^-$ during elongation, and HATS would be responsible for scavenging low levels of NO$_3^-$ left in the region of mature roots (Glass, 2003).

Nitrate efflux transporters function separately from influx. It was proposed as a nitrate-inducible anion channel, transporting down the concentration gradient (Aslam et al.,...
The efflux system is considered to be under negative feedback regulation by amino acids, especially glutamine (Pal'ove-Balang and Mistrik, 2002). An additional NO$_3^-$ transporter is responsible for vacuole loading. Miller and Smith (1992) investigated cross-tonoplast transport of NO$_3^-$ in barley and suggested the mechanism was a NO$_3^-$/$H^+$ antiport.

**Assimilation of Ammonium and Nitrate**

Absorption of NO$_3^-$ and NH$_4^+$ is typically followed by assimilation. Nitrate is converted to NH$_4^+$ in a series of reduction steps before being assimilated into amino acids; this can occur in either or both roots and shoots. The site of nitrate reduction differs greatly among species. As reviewed by Pate (1973), some plants directly reduce acquired NO$_3^-$ in the root, or translocate NO$_3^-$ to the shoot for reduction. Others, such as barley, reduce about half the absorbed NO$_3^-$ in roots and translocate the rest to shoots. The site of reduction also depends on tissue N status, as plants tend to retain more N in the root system when N source is scarce (Marschner, 1995; Tolley-Henry and Raper, 1986; Vessey and Layzell, 1987).

Before assimilation, NO$_3^-$ is first reduced to NO$_2^-$, catalyzed by nitrate reductase (NR). This reaction uses 3 ATPs and 2 electrons, donated mainly by NADH or occasionally NADPH (Oaks, 1994; Warner and Kleinhofs, 1992). Following NO$_3^-$ reduction, the chemically active and potentially toxic NO$_2^-$ is co-transported with a proton across the chloroplast (or plastid) membrane (Oaks and Long, 1992). Inside the organelle, NO$_2^-$ is reduced to NH$_4^+$ by NO$_2^-$ reductase (NiR), at an expense of 7 ATPs and 6 electrons, donated by reduced ferridoxin. In roots, reduced ferridoxin is generated via NADPH produced from oxidative pentose phosphate pathway; whereas in shoots, reduced ferridoxin is generated via
photosynthetic electron transfer. The NH$_4^+$ produced in chloroplasts then enters assimilation reactions (Epstein and Bloom, 2005).

Most plants assimilate NH$_4^+$ in roots soon after absorption to avoid high concentrations of free NH$_4^+$. During assimilation, NH$_4^+$ combines with glutamate to form glutamine, catalyzed by glutamine synthetase (GS) at the expense of 1 ATP. Glutamine then combines with oxoglutarate to produce two glutamates, catalyzed by glutamate synthase (GOGAT), with NADH or reduced ferridoxin providing electrons, depending on site of assimilation (Lea et al., 1992). Glutamates in NH$_4^+$ assimilation can be used to generate other amino acids via aminotransferases (Epstein and Bloom, 2005).

Nitrogen assimilation is clearly an energy-intensive process. Aside from energy consumed in cross-membrane transport, roughly 10 ATPs and 2 ATPs are consumed by nitrate reduction and ammonium assimilation, respectively. This corresponds to a combined 40% of the energy and reducing power generated from respiration or photosynthesis (Bloom, 1997). Therefore, nitrogen assimilation is a major consumer of available carbon pool, especially in roots. In fact, when roots are supplied with high levels of N, carbohydrate supply often becomes limiting (Marschner, 1995).

IV. NITROGEN LOSS FROM MANAGED TURFGRASS

“... human activity alone now generates more than 160 million tons (reactive nitrogen) per year ... and more than 100 million tons from the industrial production of fertilizer. The trouble is that a lot of this nitrogen doesn’t end up where it is meant to be.”

- Nicola Nosengo, in Fertilized to Death, 2003
Agricultural input of N has greatly increased since the start of the 20th century, and significant loss of the N has been documented, as reviewed by Scharf and Alley (1988). A general estimate for N recovery from applied fertilizers in the cropping system is around 50% (Baligar et al., 2001; Hardy and Havelka, 1975), although other research indicated lower numbers (Raun and Johnson, 1999). Because N loss in agricultural lands leads to both economic loss and environmental concerns (Tilman et al., 2002), various efforts have focused on developing systematic strategies to curtail N loss as well as incorporate environmental stewardship (Fageria and Baligar, 2005; Garnett et al., 2009; Miller and Cramer, 2004).

Pathways of Nitrogen Loss from Turfgrass

The urban landscape, especially turfgrass, is an important N fertilizer sink in many developed countries. It was estimated in 2005 that there were roughly 20 million hectares (50 million acres) of managed turfgrass in the United States, including ~ 80 million lawns and 17 thousand golf courses (Emmons, 2008). Assuming an overall annual fertilization rate of 50 kg N ha⁻¹, 2 Tg (1 Tg = 10¹² g) of N is applied to US turfgrasses per year. This accounts for about 10% of the total annual N input across the US (Galloway et al., 2003).

One might assume that N loss from turf systems would account for 10% of the total fertilizer loss in the US. However, differences between turf and cropping systems must be considered. First, most crop plants are annuals whereas turfgrasses are often perennials. Annual root systems are only functional during the growing season, whereas turfgrass roots could function throughout the year. In a study with hybrid bermudagrass, Wherley et al. (2009) showed that even roots under dormant shoots maintain some nitrogen uptake function.
Second, many crop plants are grown in rows while turfgrass is a solid canopy. Although turfgrass roots may be shallow, they often occupy the topsoil at a much higher density than that of crop plants, giving the turf great capacity to scavenge nutrients. Third, crops are often fertilized only once or twice early in the season, and at fairly high rates. In contrast, turfgrass fertilization programs are more flexible, and annual N demand can be split into multiple applications at lower rates. Fourth, studies show that crop plants can lose appreciable amount of N following flowering and seed set (Francis et al., 1993; Harper et al., 1987; Raun and Johnson, 1999). For aesthetic reasons, turfgrasses are managed to prevent flowering and seed production.

Applied N can be lost from the plant-soil system through four main pathways – volatilization, denitrification, surface runoff and leaching. Volatilization refers to the loss of applied N in the form of gaseous NH₃ and can be significant when NH₄⁺-N is applied to calcareous soil or urea is surface applied to relatively dry soil (Fageria and Baligar, 2005; Scharf and Alley, 1988). Significant volatilization can occur in turfgrass. Maximum volatilization was found to occur soon after application when no follow-up irrigation was administered (Bowman et al., 1987; Knight et al., 2007; Wesely et al., 1987). In an extreme case, Titko et al. (1987) reported that of 60% of the urea applied to Kentucky bluegrass was lost via volatilization. However, when irrigation was applied after fertilization, volatilization loss of N was greatly reduced to less than 2% (Bowman et al., 1987; Titko et al., 1987). Other strategies, including using liquid urea (Bowman and Paul, 1990; Stiegler et al., 2011), slow-release N fertilizer (Knight et al., 2007; Nelson et al., 1980; Torello et al., 1983), urease
inhibitor (Joo et al., 1991), and controlling thatch layer (Bowman et al., 1987; Nelson et al., 1980), can also reduce volatilization.

Denitrification also involves gaseous loss of N. It occurs when soil microorganisms utilize NO$_3^-$ as an electron acceptor and thereby reduce it to NO, N$_2$O, or N$_2$ (Broadbent and Clark, 1965). Denitrification is an important ecological process in the global nitrogen cycle, transforming reactive N mostly to non-reactive N$_2$ gas (Galloway et al., 2003). However, the smaller amount of N$_2$O produced has a damaging effect on the stratospheric ozone and is viewed as an environmental concern (Vitousek, 1994; Vitousek et al., 1997). Research shows that denitrification is relatively high on irrigated and heavily fertilized agricultural lands (Ryden and Lund, 1980), averaging ~ 12% of applied N in cropping system (Scharf and Alley, 1988), although losses up to 40% of applied N have been reported (Rolston et al., 1978; Ryden and Lund, 1980). Limited information is available documenting denitrification losses in turfgrass, but available data indicate denitrification to be insignificant. Mancino et al. (1988) examined denitrification in Kentucky bluegrass as a function of soil moisture. When soil was 75% saturated, less than 0.4% of applied NO$_3^-$ (52 kg N ha$^{-1}$) was lost via denitrification. When fully saturated and at 22°C, the soil lost only slightly more N (< 5.4%). However, at 30°C, the saturated turf soil lost a large amount of applied N (46%) via denitrification. Horgan et al. (2002a, b) used $^{15}$N to discern losses from fertilizer vs. soil N. They reported that 4.3% and 6% of applied KNO$_3$ (49 kg N ha$^{-1}$) was lost as N$_2$ and N$_2$O, respectively in Kentucky bluegrass during spring. Loss of N$_2$ increased to 15.0% in summer due to higher soil temperature and heavy rainfall, whereas N$_2$O loss was unchanged.
Surface runoff can occur when water is loaded onto a soil surface at a rate exceeding infiltration. The moving water is capable of carrying soil particles as well as nutrients. Runoff loss of N in cropping system was reported to range from 1 to 13% of applied N (Raun and Johnson, 1999). Conservation tillage (Blevins et al., 1996; Chichester and Richardson, 1992) and vegetative filter strips (Mayer et al., 2007) are effective strategies to restrict surface runoff. Runoff is generally not a major concern on turfgrass. For example, Moss et al. (2006) reported N runoff losses of 0.5 to 1.5% from a bermudagrass fairway prone to runoff.

Nitrate Leaching in Managed Turfgrass

Leaching losses of N occur when NO$_3^-$ moves below the root zone and further into groundwater. It is considered to be the primary avenue by which N is lost from fertilized land (Keeney, 1986; Nolan and Stoner, 2000; Nolan, 2001; Puckett et al., 1999; Spalding and Exner, 1993; Turner and Rabalais, 1991), and NO$_3^-$ is now identified as the most common nutrient in groundwater (Burkart and Stoner, 2001; Nolan and Stoner, 2000). Nitrate in groundwater can be further carried to surface freshwater and coastal ecosystems, causing secondary impacts such as stream acidification, eutrophication, and hypoxia of water bodies (Galloway et al., 2003; Howarth et al., 2002; Nosengo, 2003; Rabalais, 2002; Smith et al., 1987).

Aside from impacts on the environment (Vitousek, 1994; Vitousek et al., 1997; Walker and Branham, 1992), NO$_3^-$ discharged from fertilized land can have serious consequences on human health (Townsend et al., 2003). Ingesting water with a high nitrate concentration can cause methemoglobinemia in humans (Follett and Follet, 2001).
concentration of 10 mg L\(^{-1}\) is set by World Health Organization as the maximum allowed level of NO\(_3\)\(^{-}\)-N in drinking water.

Because turfgrasses are regularly fertilized and irrigated, they have long been suspected to have a high potential for NO\(_3\) leaching (Flipse et al., 1984). However, most investigations report that very little NO\(_3\)\(^{-}\) actually leaches from turfgrass managed as home lawns, landscapes, or golf courses. Gold et al. (1990) examined NO\(_3\)\(^{-}\) leaching below eight New England home lawns receiving relatively heavy annual N fertilization of 224 kg ha\(^{-1}\) and found the leachate contained less than 1.7 mg L\(^{-1}\) of NO\(_3\)\(^{-}\)-N at all locations. In addition, except for early spring, leachate NO\(_3\)\(^{-}\) concentrations showed no difference between fertilized and unfertilized lawns. Duff et al. (1977) monitored NO\(_3\)\(^{-}\) leaching from a 25-year-old Kentucky bluegrass turf over 19 months. With various annual N fertilization rates up to 257 kg ha\(^{-1}\), NO\(_3\)\(^{-}\)-N concentrations in leachate were all below 10 mg L\(^{-1}\), except for two samples. Erickson et al. (2001) compared NO\(_3\)\(^{-}\) leaching from sand rootzones of newly established St. Augustinegrass turf and mixed-species landscape in south Florida. While receiving comparable rates of N fertilizer (150 or 200 kg ha\(^{-1}\) yr\(^{-1}\)), annual leaching loss was only 4.1 kg NO\(_3\)\(^{-}\)-N ha\(^{-1}\) from turfgrass, much less than 48.3 kg NO\(_3\)\(^{-}\)-N ha\(^{-1}\) in the mixed-species landscape. Pannkuk et al. (2011) compared nutrient leaching from six different irrigated landscapes receiving annual N applications of 147 kg ha\(^{-1}\); both dissolved organic N and NO\(_3\)\(^{-}\) concentration were lowest below turf covers. Lee et al. (2003) reported an in situ study monitoring soil NO\(_3\)\(^{-}\) concentrations under hybrid bermudagrass fairways on two golf courses in eastern North Carolina. Over a period of two years, soil NO\(_3\)\(^{-}\) concentrations in the upper 120 cm were mostly below 4 mg kg\(^{-1}\), almost identical to those obtained from adjacent
native, and unfertilized, vegetation. Similar results have been reported from other studies conducted on golf courses (Cohen et al., 1999; Kunimatsu et al., 1999).

Various factors affect NO$_3^-$ leaching from turfgrass systems. For example, Mosdell and Schmidt (1985) fertilized Kentucky bluegrass with N at 74 kg ha$^{-1}$ and found leaching loss dropped from 2.6 to 1.2% of applied N when daily irrigation rate was reduced from 7.2 to 3.6 mm. Morton et al. (1988) reported a similar drop in leaching when irrigation was cut from 3.75 to 1.2 cm water per week. Brown et al. (1977) monitored leachate NO$_3^-$ concentration below a bermudagrass putting green receiving N at 163 kg ha$^{-1}$. Nitrate-N concentration was < 1 mg L$^{-1}$ throughout the experiment with irrigation of 6 to 8 mm every other day. However, a 2 mm increase resulted in leachate NO$_3^-$-N concentration above 10 mg L$^{-1}$ for 20 days after fertilization. Snyder et al. (1984) investigated the effects of two irrigation regimes on NO$_3^-$ leaching potential. When a hybrid bermudagrass putting green was watered to compensate for daily evapotranspiration, 24.0% of N was leached out. However, when irrigation was given based on soil moisture measurement, leaching loss declined to 7.9%. Leachate NO$_3^-$-N concentrations averaged 5.4 and 3.7 mg L$^{-1}$ for two irrigation regimes, respectively. Barton et al. (2009) irrigated sandy soil-grown Kikuyugrass to compensate for 60% of daily evapotranspiration and found NO$_3^-$ leaching from the established turf accounted for less than 1% of applied N (N was given from 50 to 150 kg ha$^{-1}$ yr$^{-1}$). In another study where precipitation was the only irrigation source, Starr and DeRoo (1981) detected only trace amount of NO$_3^-$ leaching from a mixed turfgrass stand.

Fertilization rate also affects leaching potential from turf. Mangiafico and Guillard (2007) examined the quantitative relationship between fertilization rate and leaching
potential in irrigated Kentucky bluegrass, and found leachate NO₃⁻ concentration exhibited an exponential relationship with fertilization rate. Leachate NO₃⁻-N concentration increased slowly from a trace to 10 mg L⁻¹ when NH₄NO₃-N fertilization rate was raised from 4.9 to 49 kg ha⁻¹ month⁻¹. Higher rates resulted in a sharp rise in leachate NO₃⁻-N concentration to nearly 50 mg L⁻¹.

Nitrate leaching potential may be affected by soil texture. Leaching is typically low when turfgrass is grown on relatively fine soils, such as sandy loam or silt loam (Modsdell and Schmidt, 1985; Petrovic et al., 1986; Starr and DeRoo, 1981), while coarser-textured soils generally have higher leaching potentials (Brown et al., 1977; Brown et al., 1982; Rieke and Ellis, 1974; Sheard et al., 1985). However, in order to improve drainage and resist compaction, many intensively managed turf sites are constructed with sand rootzones. Leaching in sand rootzones can be excessive when soluble N fertilizers are applied at high rates, especially when followed by heavy irrigation. As reviewed by Petrovic (1990), when quickly-available N sources (e.g., NH₄NO₃, (NH₄)₂SO₄, and urea) were applied to turfgrass with sand rootzones or grown in sandy soils, leaching loss of N ranged from 16 to 56%, with most over 20%. Such leaching often resulted in leachate NO₃⁻-N concentrations above 10 mg L⁻¹. Nevertheless, severe leaching can be effectively controlled in turf with constructed rootzones. Research indicates that splitting N fertilizations into more frequent and lighter applications with carefully managed irrigation reduces NO₃⁻ leaching to very low levels. For example, Snyder et al. (1981) applied urea and Ca(NO₃)₂ at a low N rate of 39 kg ha⁻¹ to a sand-based hybrid bermudagrass putting green. When irrigation was given as needed to avoid drought, leaching loss was less than 5% and NO₃⁻-N concentrations in leachate were less than
1 mg L\(^{-1}\). Mancino and Troll (1990) reported less than 0.5% of leaching loss when monthly N fertilization of 39 kg ha\(^{-1}\) on a sand-based creeping bentgrass putting green was split into 2 or 4 applications.

Using slow-release N fertilizers (IBDU, ureaformaldehyde, sulfur-coated urea, polymer-coated urea and Milorganite) can greatly reduce leaching potential, even at high rates and with heavy irrigation. Brown et al. (1977) compared leachate NO\(_3^-\) concentration under a sand-based bermudagrass putting green receiving various N sources. Fertilized at N rate of 146 to 224 kg ha\(^{-1}\) yr\(^{-1}\) and put under heavy irrigation (6 to 12 mm daily), NO\(_3^-\)-N concentration remained below 1 mg L\(^{-1}\) with ureaformaldehyde, ranged about 3 to 5 mg L\(^{-1}\) with Milorganite, but exceeded 10 mg L\(^{-1}\) over a prolonged period with NH\(_4\)NO\(_3\). In a subsequent study, quantitative leaching loss from a sand-based bermudagrass putting green totaled 0.9, 5.3, 0.5, and 17.5% of N applied as IBDU, Milorganite, ureaformaldehyde, and NH\(_4\)NO\(_3\), respectively (Brown et al., 1982). Snyder et al. (1984) made two applications of N at 49 kg ha\(^{-1}\) to a sand-based hybrid bermudagrass putting green using either NH\(_4\)NO\(_3\) or sulfur-coated urea (SCU). Total N loss and leachate NO\(_3^-\) concentration from SCU were \(~\)1/3 of that from NH\(_4\)NO\(_3\). Petrovic (1986) evaluated N leaching loss from Kentucky bluegrass receiving N at 98 kg ha\(^{-1}\) from various N sources. While up to 47% was lost from urea, less than 4% was lost from ureaformaldehyde or Milorganite. Similar effects of slow-release N fertilizers have been documented in other studies (De Nobili et al., 1992; Guillard and Kopp, 2004; Mancino and Troll, 1990; Quiroga-Garza et al., 2001; Snyder et al., 1981). However, it was noted that slow-release N fertilizers, if used alone, may not release sufficient N to support acceptable turf quality (Arrobas et al., 2011).
Matching N additions to turf growth and demand should increase N uptake and reduce leaching (Barton and Colmer, 2006; Beard, 1982; Emmons, 2008; Madison, 1971; Petrovic, 1990; Turgeon, 2012). For example, Snyder et al. (1984) applied N at 49 kg ha\(^{-1}\) to hybrid bermudagrass in February, April, and June and found 25.5, 12.8, and 9.7% of the applied N in the leachate, respectively. In a long-term monitoring program, Devitt et al. (2008) measured soil solution NO\(_3^-\)-N concentrations over 100 mg L\(^{-1}\) through the winter months, while values during summer were always much lower. Mosdell and Schmidt (1985) investigated effects of temperature regimes on NO\(_3^-\) leaching from Kentucky bluegrass. When receiving N at 74 kg ha\(^{-1}\), leaching was only observed from grasses grown under 30/23°C day/night, but not 16/4°C day/night. Therefore, in turfgrass management, higher N rates are often practiced in spring and summer for cool and warm-season species, respectively. Meanwhile, late fall application of N to cool-season grass is also popular among turf managers, as it has been considered to enhance winter color, promote root but not shoot growth, improve winter hardiness, and shorten winter dormancy. However, recent reviews found most of the supposed benefits were poorly supported by research (Bauer et al., 2012). Moreover, many studies indicated that late fall N fertilization resulted in considerable NO\(_3^-\) leaching, due to slow root uptake and low evapotranspiration (Frank et al., 2006; Geron et al., 1993; Mangiafico and Guillard, 2006; Mangiafico and Guillard, 2007; Miltner et al., 1996).

Other than previously mentioned volatilization, denitrification, surface runoff, and leaching, N is also lost from the turf system via clipping removal. Bowman et al. (2002) indicated that from 5.5 to 38.9% of fertilizer N was lost from warm-season turfgrass through clipping removal. By returning clippings, labile organic N can be recycled and made
available for turfgrass use. Starr and DeRoo (1981) estimated that returned clippings supplied at least 20 kg ha\(^{-1}\) of N per growing season to a Kentucky bluegrass and red fescue mixed stand. Kopp and Guillard (2002) suggested that N fertilization rate could be cut in half if clippings were returned to a cool season lawn, without sacrificing quality. Lee et al. (2003) estimated that mineralization of returned clippings and dead roots could supply N up to 154 kg ha\(^{-1}\) yr\(^{-1}\) on a hybrid bermudagrass fairway in eastern North Carolina.

V. IMPROVING NITROGEN USE EFFICIENCY OF KENTUCKY BLUEGRASS TO LIMIT NITRATE LEACHING VIA CULTIVAR SCREENING

With a combination of the appropriate management practices discussed above, leaching loss of NO\(_3^-\)-N from turf should be less than 10% of applied N, usually below 15 kg ha\(^{-1}\) a year (Hull and Liu, 2005). On the other hand, managing NO\(_3^-\) leaching in turfgrass might also be achieved via improving turfgrass N use efficiency. Nitrogen use efficiency in the turf system is defined and studied differently from that in a cropping system. Crop N use efficiency is often related to yield, which can be separated into two components - the ability to absorb applied N, and the ability to translocate acquired N to grains. This definition usually makes little sense in turfgrass since a crop is not harvested (sod being the exception). Therefore, the ability to absorb applied N is often used to characterize turfgrass N use efficiency (Liu et al., 2008).

Studies indicate that turfgrasses are generally very efficient in scavenging applied N (Gold et al., 1990; Groffman et al., 2009; Raciti et al., 2008; Trenholm and Sartain, 2010). While NO\(_3^-\) and NH\(_4^+\) concentrations in agricultural soils average 4.5 and 0.75 mM,
respectively (Glass, 2003), those in turf soils are much lower, averaging 0.15 and 0.05 mM, respectively (Hull and Liu, 2005). In a field study, Bowman et al. (1989) applied Ca(NO$_3$)$_2$ and (NH$_4$)$_2$SO$_4$ at an N rate of 49 kg ha$^{-1}$ to a mature Kentucky bluegrass turf in a well-drained loam soil. Twenty-four hours after fertilization, 78 to 80% of the applied N had been depleted from the inorganic N pool, with most absorbed by roots. After another 24 hours, soil inorganic N had dropped to the unfertilized level. Similar rates of nitrogen depletion were also observed by Bowman et al. (1989) in perennial ryegrass, tall fescue and creeping bentgrass. Due to this feature of turfgrass, the US Environment Protection Agency has recommended remediating reuse water by applying it to golf courses (EPA, 1992). This recommendation is supported by studies (Devitt et al., 2008; Tesfamariam et al., 2009) documenting the ability of turfgrass to reduce NO$_3^-$ loads in reuse water.

Although turf is generally efficient at absorbing applied N, there is substantial variation among turfgrass species, as well as among cultivars within a species (Hull and Liu, 2005). Morphological differences in root architecture and physiological differences in root NO$_3^-$ uptake kinetics are the two major determinants causing the interspecific and intraspecific variations in turfgrass N use efficiency.

Managed turfgrasses often have shallow root systems due to regular mowing, frequent irrigation, and soil compaction (Madison, 1971). Investigations have indicated that root characteristics differ among species and cultivars (Boeker, 1974; Ensign and Weiser, 1975). Lehman and Engelke (1991) reported high heritability in several root architecture characteristics of creeping bentgrass. The authors concluded that these traits could be included in breeding programs to select for drought tolerant creeping bentgrass cultivars.
Similar traits might be used to breed for turfgrass N use efficiency. Bowman et al. (1998) compared leaching potential between a shallow-rooted (SR) genotype and a deep-rooted (DR) genotype of creeping bentgrass. When grown in sand columns under worst-case conditions, 38% of applied N leached out from the SR genotype, while only 18% of applied N leached out from the DR genotype. The authors also mentioned that both rooting depth and root density contributed to leaching potential. Sullivan et al. (2000) examined the relationship between root morphology and NO$_3^-$ uptake rate of six Kentucky bluegrass cultivars in solution culture. Nitrate uptake rate of the cultivars was positively correlated with its root biomass, root length, root surface area and rhizome biomass. In a lysimeter study, Bowman et al. (2002) found significant differences in NO$_3^-$ leaching among species, with cumulative N loss being negatively correlated with a species’ average root length density.

Turfgrass germplasm with higher N uptake efficiency have the potential to reduce NO$_3^-$ leaching (Morton et al., 1988; Petrovic, 1990). Root NO$_3^-$ uptake kinetic parameters ($V_{\text{max}}$ and $K_m$) can be used to characterize NO$_3^-$ uptake efficiency. Germplasm with ideal NO$_3^-$ uptake would possess high $V_{\text{max}}$ and low $K_m$ values. Nitrate uptake kinetic parameters have been determined for turfgrass species and cultivars in previous studies. While interspecific variations were often found, intraspecific variations were also evident in certain species. Cisar et al. (1989) measured $V_{\text{max}}$ and $K_m$ of root NO$_3^-$ uptake for Kentucky bluegrass (2 cultivars), perennial ryegrass (1 cultivar) and chewing fescue (1 cultivar). With interspecific differences evident for both parameters, Kentucky bluegrass exhibited the highest $V_{\text{max}}$ (5.5 to 6.0 μmol NO$_3^-$ g$^{-1}$ fresh root h$^{-1}$), and lowest $K_m$ (18.1 to 19.7 μM) among the three species, leading the authors to conclude that Kentucky bluegrass had the
highest N utilization capacity. No intraspecific differences were found between the 2 Kentucky bluegrass cultivars. Interspecific differences in NO$_3^-$ uptake kinetic parameters were also identified among four warm-season turfgrasses (bermudagrass, centipedegrass, St. Augustinegrass and zoysiagrass) (Bowman et al., unpublished data). In that study, St. Augustinegrass was found to have the highest values in both $V_{\text{max}}$ and $K_m$, while centipedegrass was found to have the lowest values for both. These data are consistent with the common understanding that St. Augustinegrass is better adapted to high N fertility while centipedegrass is more competitive in low N fertility. Liu et al. (1993) determined NO$_3^-$ uptake kinetic parameters in solution culture for Kentucky bluegrass, perennial ryegrass and tall fescue. While $K_m$ was not different among three species (range: 33.4 $\mu$M to 42.2 $\mu$M), interspecific differences were identified in $V_{\text{max}}$. Perennial ryegrass $V_{\text{max}}$ (7.22 $\mu$mol g$^{-1}$ fresh root h$^{-1}$) was higher than both tall fescue and Kentucky bluegrass (5.43 $\mu$mol g$^{-1}$ fresh root h$^{-1}$ and 5.15 $\mu$mol g$^{-1}$ fresh root h$^{-1}$, respectively). On the other hand, variations of $V_{\text{max}}$ and $K_m$ among cultivars were found to be even greater than those among species. In a follow-up field study, Liu et al. (1997) examined soil water NO$_3^-$ concentrations and percolation N loss from field grown Kentucky bluegrass, tall fescue and perennial ryegrass. Again, both interspecific and intraspecific variations among investigated turfgrasses were evident for soil water NO$_3^-$ concentration as well as percolation N loss. Certain cultivars that exhibited favorable kinetic parameters in solution culture showed low NO$_3^-$ leaching in field condition as well. Based on these data, the authors suggested that a screening program to select cultivars with favorable $V_{\text{max}}$ and $K_m$ values would increase turfgrass N use efficiency and reduce NO$_3^-$ leaching.
Kentucky bluegrass is the most widely used cool-season turfgrass species in the temperate and subarctic climate zones in the US. Due to its popularity, many new Kentucky bluegrass cultivars are bred and released each year. These cultivars are grouped into several types according to various characteristics related to visual quality (Bonos et al., 2000; Shortell et al., 2009). However, little information is available regarding the N use efficiency of the cultivars. Such information could be valuable when cultivars are chosen for high fertility situations.

Kentucky bluegrass has the interesting feature of being a highly apomictic species, resulting in individuals within a cultivar having a high degree of genetic uniformity. Other open-pollenated species have a relatively high variability between individuals within a given cultivar. This is an important consideration because it means that Kentucky bluegrass germplasm can be studied by sampling a small number of tillers compared to other turfgrass species. Therefore a rapid cultivar screening procedure might be developed using less plant materials, less time, and less money. Such a procedure, if successfully established, could rapidly quantify N use efficiency of Kentucky bluegrass cultivars, and possibly have an application in reducing NO$_3^-$ leaching. Solution culture is a convenient technique for such a screening program. A large number of plants can be processed and, with the use of $^{15}$N labeled fertilizer, very precise measurements of N absorption can be obtained.

The goal of this investigation is to develop a screening procedure in solution culture to evaluate differences in N uptake by Kentucky bluegrass cultivars. One germplasm exhibiting high nitrate uptake efficiency and one exhibiting low nitrate uptake efficiency were identified after screening 60 cultivars. The two selected cultivars were further examined
to determine their competition for soil N and the environmental significance of N absorption efficiency.
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CHAPTER 2

Nitrate Uptake Rates of Kentucky Bluegrass Genotypes and Their Effect on Nitrate Absorption under Competitive Conditions

ABSTRACT

Nitrate leaching from turfgrasses continues to be a concern. It is proposed that selecting turfgrass genotypes with higher NO$_3^-$ absorption abilities could reduce NO$_3^-$ leaching. This study examined the intraspecific difference in NO$_3^-$ absorption among Kentucky bluegrass (Poa pratensis L.) genotypes, and how such a difference affects N absorption when roots are in competition for soil N. A nutrient solution screening procedure was used to identify Kentucky bluegrass genotypes having high vs. low NO$_3^-$ uptake capacity. Tillers of 60 Kentucky bluegrass cultivars were rooted and transferred to a continuous flow-through solution culture system. After establishment, plants were treated to develop moderate N deficiency. $^{15}$N-labeled KNO$_3$ was introduced at high (1 mM) and low (0.05 mM) concentrations to screen for differences in NO$_3^-$ uptake. After a brief uptake period, plants were harvested, dried, and analyzed for $^{15}$N content to determine N uptake rate. There were significant and substantial differences among the cultivars for uptake rate at both high and low N concentrations. The 60 genotypes exhibited a wide range of uptake rates, with strong correlation between rates at high and low N. The cultivars Julia and Midnight were selected as representing cultivars with efficient and inefficient NO$_3^-$ uptake, respectively. Julia had NO$_3^-$ uptake rates averaging 56% higher than Midnight. A subsequent
lysimeter study examined whether higher N uptake capacity would translate into increased N absorption under competitive conditions. Tillers of the two cultivars were planted as a mixed stand in soil/sand column lysimeters. After establishment, $^{15}$N-labeled KNO$_3$ solution at high (2 or 1mM) and low (0.05mM) concentrations was applied to each column. After an uptake period, individual plants of each cultivar were harvested for $^{15}$N analysis. Results indicated that Julia absorbed 20 to 50% more NO$_3^-$ than Midnight at the high N concentration, and 25 to 71% more NO$_3^-$ more than Midnight at the low N concentration. Most differences were statistically significant. This indicates that differences in NO$_3^-$ absorption by Kentucky bluegrass identified in solution culture translate into differences in absorption of soil N.

**INTRODUCTION**

Plant-soil ecosystems are described as either open or closed with regards to nutrient recycling and retention. Most are open with regards to N fertilizer inputs, with losses occurring via water movement (leaching and runoff) and as a gas (volatilization and denitrification). Due to the vast acreage planted to turfgrasses (Emmons, 2008) and their high profile in urban environments, considerable public concern and scrutiny has focused on NO$_3^-$ leaching and water pollution from turfgrass fertilizers (Baier and Rykbost, 1976; DeRoo, 1979; Flipse et al., 1984; Geron et al., 1993; Petrovic, 1990; Trenholm et al., 2012). While most studies have found relatively low potential for NO$_3^-$ leaching (Cohen et al., 1999; Duff et al., 1997; Erickson et al., 2001; Gold et al., 1990; Kunimatsu et al., 1999; Lee et al., 2003; Pannuk et al., 2011), some report higher concentrations and amounts, resulting from heavy irrigation (Brown et al., 1977; Madsdell and Schmidt, 1985; Morton et al., 1988; Roy
et al., 2000; Snyder et al., 1984; Starr and DeRoo, 1981), heavy fertilization (Brown et al., 1982; Mangiafico and Guillard, 2007; Petrovic et al., 1986; Rieke and Ellis, 1974; Sheard et al., 1985; Snyder et al., 1981), sandy soil (Brown et al., 1982; Petrovic, 1990; Rieke and Ellis, 1974), and age of the turf (Frank et al., 2006; Petrovic, 1990; Porter et al., 1980).

Unlike many traditional row crops, established turfgrass systems, particularly when young, are remarkably efficient at immobilizing applied N and behave as an N sink (Raciti et al., 2008). This has been attributed to a very dense root system (Bowman et al., 1989b; Murphy et al., 1994), a high uptake capacity for both NO$_3^-$-N and NH$_4^+$-N (Bowman et al., 1989a; Liu et al., 1993; Morton et al., 1988; Petrovic, 1990), and high microbial activity (Bristow et al., 1987). There is an obvious advantage to the turf in retaining N in the system, thus minimizing losses, and young turf may represent as “closed” a plant ecosystem for N as exists.

Various management practices can contribute to N retention within and prevention of N loss from the turf. For example, scheduling irrigation based on soil moisture status minimizes leachate and prevents leaching (Blonquist et al., 2006; Pathan et al., 2007; Snyder et al., 1984). Use of slow release fertilizers and proper timing of their application also control losses (Guillard and Kopp, 2004; Mancino and Troll, 1990; Petrovic et al., 1986; Quiroga-Garza et al., 2001). Perhaps the most important factor, however, is the turfgrass root system which is central to the efficient capture and immobilization of applied N. Turfgrass root systems are extremely dense (Beard, 1999), and inter-root distances are quite small (< 1 mm on average) which minimizes diffusion as a limitation to uptake. Additionally, roots develop
an enhanced capacity for N uptake when subject to N deficiency (Bowman and Paul, 1988; Bowman et al., 1989a). Turfgrasses are routinely and continuously managed with suboptimal levels of N, and their root systems are in the deficiency-enhanced uptake state most or all of the time. As a result, typical applications of soluble N are very quickly immobilized and the potential for significant leaching events eliminated (Bowman et al., 1989b).

Numerous studies have examined N absorption by turfgrass root systems. Uptake at the root level has been characterized by the kinetic parameters $V_{\text{max}}$ (capacity factor) and $K_m$ (affinity factor) and differences between turfgrasses have been reported both at the species (Bowman et al., 1989a; Cisar et al., 1989) and cultivar levels (Bertauski et al., 1997; Liu et al., 1993). For example, Liu et al. (1993) measured both $V_{\text{max}}$ and $K_m$ for six cultivars each of Kentucky bluegrass, perennial ryegrass ($Lolium perenne$ L.) and tall fescue ($Festuca arundinacea$ Schreb.) and found differences among cultivars exceeded differences among species. In a subsequent study of the same three species, Liu et al. (1997) found significant differences in NO$_3^-$ leaching both between cultivars and species, leading them to suggest that screening and selecting genotypes for better N uptake efficiency could reduce NO$_3^-$ leaching from turf. It is certainly logical that improving N absorption by roots would benefit both the plant and environment, but there are scant data to document the relationship. This study was conducted to determine if variation in N uptake by Kentucky bluegrass cultivars translates into discernible differences in N acquisition and retention.
MATERIALS AND METHODS

Screening for N uptake Efficiency of 60 Kentucky Bluegrass Genotypes

Plant Culture: This study was conducted during 2007-2009 in a controlled environment using field-grown plant material. It was designed to evaluate a wide selection of Kentucky bluegrass genotypes for NO$_3^-$ uptake efficiency at high (1mM) and low (0.05 mM) NO$_3^-$-N. These concentrations are associated, at least loosely, with $V_{\text{max}}$ and $K_m$ in turfgrasses, respectively. We were not attempting to quantify either kinetic parameter; 1 mM was chosen since $V_{\text{max}}$ for NO$_3^-$ uptake typically approaches maximum close to this value, and 0.05 mM was chosen as being relatively close to published values of $K_m$ for turfgrass species (Bowman et al., 1989a; Jiang and Hull, 1998; Liu et al., 1993).

Tillers of selected Kentucky bluegrass genotypes were harvested from a cultivar evaluation trial at the NCSU Turfgrass Research Lab (Raleigh, NC). Roots and older leaves were excised to provide uniform tillers with 2-3 young, expanded leaves. Tillers were planted in greenhouse flats filled with a porous ceramic medium (Profile Greens Grade, Profile Products LLC, Buffalo Grove, IL) and placed under artificial lighting (PPFD of 450 $\mu$mol m$^{-2}$ s$^{-1}$ provided by a combination of high pressure sodium and mercury vapor lamps). Flats were watered 2-3 times per day. After 3 weeks in the flats, rooted tillers were transplanted to a custom-built continuous flow-through nutrient solution culture system. This system consisted of 8 separate chambers constructed of polyvinyl chloride (PVC) sheet, each with an upper and lower reservoir. A pump circulated solution from the lower to the upper reservoir, and solution flowed back via a spillway. Each chamber held a total
of 13 L. The upper reservoir was covered with a PVC lid (30 cm × 30 cm) in which ten 1.5-cm diameter holes were cut to hold tillers.

A total of 60 genotypes (both commercial and experimental) were initially selected for this study. Due to space limitations in the chambers, only ten could be screened at a time. Thus a series of 6 runs, each screening 10 genotypes, was undertaken. For each run, rooted tillers were washed free of the rooting medium; roots were trimmed to a uniform 2 cm and developing rhizomes, if present, were excised. The base of each tiller was wrapped in a foam plug and inserted into one of the chamber lid holes. Each of the 8 chambers was planted with one each of 10 different genotypes, per run.

Chambers were initially filled with 13 L of 0.1-strength Hoagland solution (Hoagland and Arnon, 1950) amended with full strength micronutrients and 1 mg Fe L⁻¹ as Fe-EDDHA. Supplemental Fe as FeSO₄·7H₂O was periodically added at a rate of 0.2 mg Fe L⁻¹ to prevent chlorosis, and the pH was automatically maintained at 6.0 ± 0.3. All solutions were changed weekly. Air temperature was controlled at approximately 29/18°C day/night, with solution temperatures fluctuating between 20°C and 25°C. Light was supplied at a PPFD of 500 μmol m⁻² s⁻¹ with a 14-h photoperiod (0600 to 2000 h).

Shoots were trimmed at 5 cm 1-2 times per week; tillering was permitted but rhizomes were excised when observed. After 3 weeks growth the plus-N solutions were changed to 0.1-strength minus-N formulation to induce moderate N deficiency. Plants were grown in the new solution for 3 and 4 weeks before screening for high and low N uptake, respectively.
Screening Procedure: The 8 chambers were randomly divided into two groups of 4; one group was used to screen at a high (1 mM) and the other group at a low (0.05 mM) N concentration. Shoots were trimmed one day prior to screening. To initiate screening, at 0600 h, roots were exposed for a period of 6 h to either 0.1 mM or 0.05 mM KNO₃-N for the high and low N concentration screening, respectively. This was designed to induce the NO₃⁻ uptake system (Bowman et al., 1989a). After induction, the solutions were replaced with minus-N solution amended with 1.0 mM (high N) or 0.05 mM (low N) ¹⁵N-labeled KNO₃ (enriched to 10 atom %). Uptake of the labeled N proceeded for a period of 8 and 4 h for the high N and low N screening, respectively. At the end of the uptake period, plants were rinsed several times and placed in minus-N nutrient solution for 1 h. They were then harvested, subdivided into root and shoot tissue, dried at 60° C and weighed. Shoot and root tissue were then combined, milled to a fine powder and prepared for commercial ¹⁵N analysis (UC Davis Stable Isotope Facility). Natural abundance of tissue ¹⁵N atom % was assumed at 0.3663 (Vose, 1980). Nitrate uptake rate was calculated as:

\[
\frac{(\text{Shoot dry mass} + \text{Root dry mass}) \times \text{Sample N\%} \times (\text{Sample ¹⁵N atom \% - 0.3663\%}) \times 10}{\text{Root dry mass} \times \text{Uptake duration}}
\]

Confirmational Screening of 10 Kentucky Bluegrass Genotypes

Based on the initial uptake data from the 60 genotypes examined, 5 were selected as being the most efficient and 5 as the least efficient in terms of N uptake rate. These 10 were then rescreened. The procedure was as above with the exception that the plants were grown in the culture chambers with plus-N nutrient solution for 6 weeks rather than 3 prior to
switching to the minus-N solution. Additionally, an uptake period of 8 h was used for both the low and high $^{15}\text{NO}_3^-$ concentrations. Results were used to select 2 final genotypes - one being efficient and the other inefficient for $\text{NO}_3^-$ uptake.

Statistical analysis was performed on data obtained in each run. The four high N concentration and four low N concentration chambers in each run were treated as independent experiments. The experimental design was a Randomized Complete Block Design with 10 treatment levels (genotypes) and four blocks (culture chambers). Analysis was performed using Statistical Analysis Software 9.2 (SAS Institute, Inc., 2008). Data were analyzed by ANOVA with significant means separated via Tukey’s Honestly Significant Difference test.

**Nitrate Absorption under Competitive Conditions**

This study was conducted in 2009 - 2010 to evaluate the effects of $\text{NO}_3^-$ uptake efficiency on absorption of soil N. Based on the screening results, tillers of Julia and Midnight Kentucky bluegrass were harvested from the field and rooted as described above. After 3 weeks, rooted tillers were transplanted to 10 polypropylene column lysimeters (58.4 cm tall and 15.2 cm in diameter). One porous ceramic suction cup was placed at the bottom of each lysimeter and covered with a 4 cm layer of diatomaceous earth. Suction cups were connected to 5-L glass bottles, which were in turn connected via manifold to a vacuum pump. Lysimeters were then filled with growing media (detailed below), irrigated and drained via the ceramic cups at a tension of 0.03 MPa. Afterwards, a $6 \times 6$ plastic grid of thirty-six $1.5 \times 1.5$ cm cells was inserted into the soil surface of each lysimeter, and 18 tillers
each of Julia and Midnight were transplanted to each column. The two genotypes were planted in alternating cells in the grid to create a mixed canopy and an intermingling of the developing root systems.

Two trials were conducted. In the first trial, lysimeters were filled with Wagram loamy sand (an Arenic Hapludult; pH = 6.5; 4.2% clay, 10.8% silt, 85% sand) packed to a bulk density of 1.60 g cm⁻³. Tillers were transplanted and columns were irrigated with 2 cm tap water daily for two weeks. Subsequently, 0.1-strength Hoagland solution amended with 6 mM KNO₃ was supplied at 2 cm, three days per week, with tap water used the remaining four days. This provided the equivalent of 24.5 kg N ha⁻¹ per week, and a predicted leaching fraction of approximately 0.5. A suction of 0.03 MPa was applied to each lysimeter to drain the columns. Shoots were trimmed at 5 cm 1-2 times per week. Air temperature was controlled at approximately 29/18 °C day/night. Light was supplied at a PPFD of 500 μmol m⁻² s⁻¹ with a 14-h photoperiod (0600 to 2000 h). After 4 weeks of N supply, solutions were changed to tap water for 4 weeks to induce moderate N deficiency. The ten lysimeters were then randomly split into two groups of 5 to evaluate absorption of soil N at both high and low NO₃⁻ concentrations.

Nitrate uptake was induced in the high NO₃ group with four sequential 500 mL aliquots of 0.1 mM KNO₃ one day before treatment. The following day, four sequential 500 mL aliquots of 2 mM K¹⁵NO₃ (enriched to 10 atm %) were applied to each lysimeter. After a 24 h uptake period, 2 L of unlabeled 2 mM KNO₃ was added in four equal portions. New growth was clipped and collected on a per plant basis 2 days after ¹⁵N introduction. Biomass
from the center 16 cells (4 × 4 grid) was harvested as 16 samples four days after $^{15}$N introduction. Each sample included tillers and rhizomes, and shallow roots which could be identified as clearly being part of the plant. Harvested tissues were combined with the previously collected clippings and dried at 60 °C.

The low N group was induced as above with 0.05 mM KNO$_3$ and then treated with a 10-day schedule of K$^{15}$NO$_3$ addition. On day 1, four 500 mL aliquots of 0.05 mM K$^{15}$NO$_3$ were applied. On day 2, two 500 mL aliquots of 0.05 mM K$^{15}$NO$_3$ were applied, and on days 3 through 10, a single 500 mL addition of labeled solution was applied. This schedule was designed to represent the low concentrations and longer time periods of N made available through mineralization. Finally, the columns were flushed with unlabeled 0.05 mM KNO$_3$. After an additional four days of growth, the plants were harvested as described above. The total amount of N applied to the high and low N columns was equivalent to 30.7 and 2.7 kg ha$^{-1}$.

Several changes were made in the second trial based on the results from the first. Instead of the field soil, which drained slowly, a double-washed topdressing sand (2% very fine, 17% fine, 59% medium, 22% coarse sand) packed to a bulk density of 1.50 g cm$^{-3}$ was used. Additionally, the periods during which the columns were irrigated with plus-N and minus-N Hoagland solution were reduced from 4 to 3 weeks each.

Nitrate uptake was induced in the high N group by providing each column with a total of 7 L of 0.1 mM KNO$_3$ over a 6-h period. Immediately following induction, 3 L of 1 mM K$^{15}$NO$_3$ was applied to each lysimeter. This was followed 1 h later by 2 L of 1 mM K$^{15}$NO$_3$,
with an additional six aliquots of 1.5 L 1 mM K\textsuperscript{15}NO\textsubscript{3} applied hourly. As a result, 14 L of 1 mM K\textsuperscript{15}NO\textsubscript{3} was “flushed” through each lysimeter over an 8-h period. Finally, 3 L of 0.1-strength minus-N Hoagland solution was applied to each lysimeter to flush out the remaining K\textsuperscript{15}NO\textsubscript{3}. Plants were allowed to grow for four days prior to being harvested as described above.

For the low N group, uptake was induced as above with 0.05 mM KNO\textsubscript{3}. This was followed by addition of 0.05 mM K\textsuperscript{15}NO\textsubscript{3} at the same schedule as described above. Flushing the columns with 0.1-strength minus-N Hoagland solution and subsequent harvesting of plant materials were as above. The total amount of N applied to the high and low N columns was equivalent to 107.5 and 5.4 kg ha\textsuperscript{-1}.

Tissues were analyzed for $^{15}$N enrichment as described above. The experimental was conducted as a Random Complete Block Design (1 factor (cultivar), 5 blocks (lysimeters), and 8 subsamples of each cultivar in a block). Statistical analysis was performed with Statistical Analysis Software 9.2 (SAS Institute Inc., 2008) using a mixed model with cultivar treated as fixed, and others as random. Mean comparison was based on single degree of freedom orthogonal contrasts.

RESULTS AND DISCUSSION

Screening of 60 Kentucky Bluegrass Genotypes

The methodology, in particular concentration and time of uptake, was designed to avoid severe depletion of $^{15}$N from the nutrient solution, especially at the lower
concentration. Depletion ranged from 3 to 9% for the high N group and from 29 to 51% for the low N group (data not shown). Nitrate concentration remained above 0.9 mM throughout the uptake period in the high N groups, while NO$_3^-$ in the low N groups declined to as low as 0.025 mM. It should be noted that the minimum NO$_3^-$ concentration at which net uptake by Kentucky bluegrass falls to zero ($C_m$) is roughly 0.005 mM (Liu et al., 1993), indicating that NO$_3^-$ was being absorbed even at the lowest concentration of 0.025 mM. Nitrate depletion varied among batches (data not shown), possibly due to differences in group NO$_3^-$ uptake capacities or other factors such as plant vigor for the various tiller harvests.

Significant differences in NO$_3^-$ uptake were found in all six batches of 10 cultivars at both the high and low N concentration (Table 1 and 2). Nitrate uptake rate at 1mM ranged from 0.59 to 1.61 mg N g$^{-1}$ root dry wt h$^{-1}$, and from 0.30 to 0.96 mg N g$^{-1}$ root dry wt h$^{-1}$ at 0.05 mM NO$_3^-$. Liu et al. (1993) reported the $V_{max}$ for NO$_3^-$ uptake by Kentucky bluegrass to be 5.15 μmol N g$^{-1}$ root fresh wt h$^{-1}$. Assuming 85% water content in fresh root tissue, this is equivalent to approximately 0.5 mg N g$^{-1}$ root dry wt h$^{-1}$. The higher values in our study may be due to the higher uptake concentration and the longer N deprivation period. Previously measured $K_m$ values for Kentucky bluegrass ranged from 8.0 μM to 76.2 μM, averaging 42.2 μM (Liu et al., 1993). Thus the NO$_3^-$ concentration range (from initial to depleted) in our low N treatment was within the reported range of $K_m$, and the values for uptake rate were in fact about one half of the corresponding uptake rates ($V_{max}$) for the high N treatment.

Nitrate uptake rate data for the 60 genotypes were pooled and ranked to select candidates for a second conformational screening. A significant positive rank correlation
(Spearman rank-order correlation) of the genotypes was found between the high and low N conditions ($\rho = 0.68$, $p < 0.0001$). We thus proceeded to select genotypes that ranked highest and lowest in terms of N absorption at both high and low N concentrations. Selection was also based on similarity of root mass and shoot:root ratio, and we attempted to include genotypes from the various batches. As a result, Julia, ThermalBlue, Dynamo, CPP822, and Bandera were selected as genotypes that were efficient in NO$_3^-$ uptake, and Kenblue, Everest, Juliet, Midnight, and AKB449 were selected as cultivars that were inefficient.

**Confirmational Screening of 10 Kentucky Bluegrass Genotypes**

Due to the longer uptake period used for the low N group in the confirmational screening, N depletion (66 ± 2%) was higher than in the previous screenings. Nitrate concentration in the low N group declined to a low of 0.017 mM, still higher than the reported $C_m$ of 0.005 mM. Significant differences between genotypes for uptake rate were found at both high and low N concentrations (Table 3). Spearman rank-order correlation analysis reaffirmed that NO$_3^-$ uptake rates of the 10 genotypes were positively correlated between high and low N conditions ($\rho = 0.75$, $p = 0.0133$). Furthermore, NO$_3^-$ uptake rank sequence of the 10 genotypes in the confirmational screening correlated well with their relative rank sequence in the 1st round screening (high N condition: Spearman rank-order correlation $\rho = 0.79$, $p = 0.007$; low N condition: Spearman rank-order correlation $\rho = 0.76$, $p = 0.011$).

Although variations in NO$_3^-$ uptake rates among the 10 genotypes were confirmed by statistical analysis, the values had a narrower range than that in the 1st screening, and
differences between the efficient and inefficient genotypes were less dramatic. This is not surprising, as grouping and screening the 10 genotypes in one batch would eliminate some of the inherent variation (temperature during establishment, vigor of plants at field harvest, etc.) between the six batches in the first screening. As the data range narrowed in conformational screening, several cultivars that previously were identified as distinct in \( \text{NO}_3^- \) uptake were found not to differ (Table 3).

Nitrate uptake rates at high N concentration ranged from 0.69 to 1.20 mg N g\(^{-1}\) root dry wt h\(^{-1}\) in the confirmational screening, compared to a range of 0.61 to 1.67 mg N g\(^{-1}\) root dry wt h\(^{-1}\) in the 1st round. Similarly, \( \text{NO}_3^- \) uptake rates at low N concentration ranged from 0.34 to 0.52 mg N g\(^{-1}\) root dry wt h\(^{-1}\) in the confirmational screening, whereas they ranged from 0.42 to 0.96 mg N g\(^{-1}\) root dry wt h\(^{-1}\) in the first round screening. Uptake rates from the low N confirmational screening were generally lower than measured in the 1st round screening. This may be due to the extended uptake period in the confirmational screening. Bowman et al. (1989) reported that N-deficient Kentucky bluegrass exhibited the highest \( \text{NO}_3^- \) uptake rate immediately after induction, after which it decreased.

Based on data from the confirmational screening, cultivars Julia and ThermalBlue were considered as being more efficient in \( \text{NO}_3^- \) absorption than cultivars Midnight and Everest. Based on availability of healthy plant material, Julia and Midnight were selected as representative of genotypes having high and low \( \text{NO}_3^- \) uptake efficiency, respectively. For comparison, \( \text{NO}_3^- \) uptake rates for Julia were 68 and 44% higher than those for Midnight at high and low N concentrations, respectively.
Nitrate Absorption under Competitive Conditions

Rooted tillers of Julia and Midnight were planted in alternating cells of a 6 × 6 grid to promote intermingling of roots and establish competitive conditions for nutrients. Because the soil rootzone had relatively low hydraulic conductivity, irrigation was with a higher (2 mM) NO₃⁻ concentration than used in the screening study. It was impossible to separate the roots of the two cultivars, and the majority of the root system was not included in the harvest and tissue analysis. Thus, uptake is presented on a per plant instead of a per root basis. It was inevitable that some ¹⁵N remained in the root system; the effect of this on ¹⁵N recovery was minimized by allowing a 4-day chase period with non-labeled N after administering the ¹⁵N to allow recently absorbed labeled N to be transported to the shoot.

Over a period of 24 h, N uptake per plant was 20% higher in Julia (0.59 mg N plant⁻¹ day⁻¹) than Midnight (0.49 mg N plant⁻¹ day⁻¹) (Table 4). However, this difference was not statistically significant (Table 5). For perspective, these N uptake rates are equivalent to approximately 2.6 and 2.2 g m⁻² day⁻¹ for Julia and Midnight, respectively. They are very similar to that (3.5 g m⁻² day⁻¹) determined by Bowman et al. (1989), after field grown Kentucky bluegrass was fertilized at a rate of 50 kg N ha⁻¹.

A total N of 30.7 kg ha⁻¹ was applied to the columns as 2 mM K¹⁵NO₃, with 66.5% of the applied N recovered in shoot tissue four days after application (Table 8). A wide range in N recovery rate has been reported by previous studies employing ¹⁵N. Bowman et al. (1989) applied ¹⁵N-labeled ammonium sulfate to Kentucky bluegrass at an N rate of 50 kg ha⁻¹, and determined that up to 75% of applied N was rapidly absorbed by roots in the first 5 days after
application. Miltner et al. (1996) fertilized a 1-year-old Kentucky bluegrass turf with $^{15}$N-labeled urea at 39.2 kg N ha$^{-1}$ and found 36 to 39% of applied N recovered in clippings and verdure 18 days after application. In a subsequent study on the same turf, Frank et al. (2006) reported up to 16% recovery of applied N in clippings plus verdure 15 days after $^{15}$N-labeled urea was applied at 24.5 kg N ha$^{-1}$.

With the low N concentration, NO$_3^-$ uptake rate over the 10-day uptake period was 71% higher in Julia ($0.036$ mg N plant$^{-1}$ 10 day$^{-1}$) than Midnight ($0.021$ mg N plant$^{-1}$ 10 day$^{-1}$) (Table 6). The difference was significant at $\alpha = 0.05$ (Table 7). However, these values were much lower than expected. First, 41% of applied N was recovered in shoot when 4.9 mg $^{15}$NO$_3$-N was applied at 0.05 mM (equivalent to 2.7 kg N ha$^{-1}$) (Table 8). This recovery rate was lower than that determined in high N condition, which was somewhat unexpected. Second, NO$_3^-$ uptake rates determined for Julia and Midnight in low N condition were considerably lower than that of high N condition. Similarly, when converted to area based values ($0.016$ and $0.009$ g N m$^{-2}$ day$^{-1}$ for Julia and Midnight, respectively), NO$_3^-$ uptake rates were an order of magnitude lower than the average daily N uptake rate found in field-grown Kentucky bluegrass ($0.084 - 0.14$ g N m$^{-2}$ day$^{-1}$) reported by Liu et al. (1993). These data suggest that our Kentucky bluegrass may have been under stress, most likely caused by poor soil aeration. Despite the suction cups positioned at the bottom of each lysimeter, the loamy sand soil drained slowly and remained near saturation for long periods after irrigation. This was particularly true after applying the large volumes of $^{15}$N solution. An inspection of the root systems at the end of the experiment confirmed that they were relatively shallow.
(<10 cm). This prompted us to change the rootzone material from field soil to a topdressing sand in the second trial.

The sand rootzone used in the second trial improved drainage and promoted better root growth (all columns had roots deeper than 30 cm at the end of experiment). It also facilitated a faster introduction of the $^{15}$N treatment solution as well as faster “flushing” of the columns after the uptake period.

Measured at 1 mM K$^{15}$NO$_3$, NO$_3^-$ uptake rate by Julia (0.37 mg N plant$^{-1}$ 8h$^{-1}$) was 50% higher than Midnight (0.25 mg N plant$^{-1}$ 8h$^{-1}$) (Table 9). This difference was significant at $\alpha = 0.05$ (Table 10). These rates are approximately half those obtained using nutrient solution culture (calculated from Table 3 and Table 9). Higher uptake rates in solution culture are probably due to the flowing solution minimizing depletion zones around the roots. Using the 8 h uptake data and extrapolating to a daily value, N uptake by Julia and Midnight determined at 1 mM K$^{15}$NO$_3$ was equivalent to 4.9 and 3.3 g N m$^{-2}$ day$^{-1}$, respectively. These values compare well with reported N uptake rate (3.5 g N m$^{-2}$ day$^{-1}$) by field grown Kentucky bluegrass after fertilized at 50 kg N ha$^{-1}$ (Bowman et al., 1989).

When measured at 0.05 mM K$^{15}$NO$_3$, NO$_3^-$ uptake by Julia (0.060 mg N plant$^{-1}$ 8h$^{-1}$) was 25% higher than Midnight (0.048 mg N plant$^{-1}$ 8h$^{-1}$) (Table 11). The difference was again significant at $\alpha = 0.05$ (Table 12). Compared with Trial 1 (Table 6), uptake rates at 0.05 mM K$^{15}$NO$_3$ were much higher in this trial, supporting speculation that NO$_3^-$ uptake in Trial 1 was probably inhibited by stress. However, the rates were much lower than those
determined in nutrient solution culture, probably due to depletion of the 0.05 mM NO$_3^-$ solution in the rootzone between additions.

Using total N absorbed over 8 h from the low N solution and extrapolating to daily uptake, Julia and Midnight absorbed 0.27 and 0.21 g N m$^{-2}$ day$^{-1}$, respectively. These values are slightly higher than the average daily N recovery by field-grown Kentucky bluegrass (0.084 to 0.14 g N m$^{-2}$ day$^{-1}$) estimated by Liu et al. (1993). For the purposes of this study, the sand rootzone in combination with the flushing technique appears to be a reasonable approximation of a native soil rootzone.

The amount of K$_{15}$NO$_3$ applied in Trial 2 was equivalent to N rates of 108 and 5.4 kg ha$^{-1}$ for high and low N conditions, respectively. Four days after application, N recoveries were 11 and 38% for high and low N, respectively. The recovery rate (Table 13) at high N condition was in the lower range of previous reported 16 to 75% N recovery values (Bowman et al., 1989b; Frank et al., 2006; Miltner et al., 1996; Starr and DeRoo, 1981). This was likely due to the high N rate used, as well as the rapid movement of the solution through the columns in Experiment 2.

**Conclusion**

The procedure developed to screen Kentucky bluegrass genotypes for NO$_3^-$ uptake efficiency seems robust but is fairly time consuming and relatively expensive if the $^{15}$N analyses are outsourced. Out of 60 genotypes screened, Julia and Midnight were identified as having high and low NO$_3^-$ uptake efficiency, respectively. The significance of higher vs.
lower uptake efficiency was evaluated in terms of soil N absorption. The results document that higher uptake capacity in Julia compared to Midnight translated into greater acquisition of a nutrient resource. Until now, there was little to document the relationship between uptake rate and resource acquisition under highly competitive rooting conditions. Our results also suggest that a cultivar screening program can identify Kentucky bluegrass genotypes distinct in N acquisition, and possibly other nutrients where a labeled form is available.
REFERENCES


Table 1. Biomass and NO$_3^-$ uptake rate by 60 Kentucky bluegrass cultivars at high N concentration. Nitrogen was supplied as $^{15}$N-labeled KNO$_3$ at 1 mM for a period of 8 h. Values shown are means of 4 replications ± SE.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shoot dry mass mg</th>
<th>Root dry mass mg</th>
<th>Uptake rate mg N g$^{-1}$ root dry wt h$^{-1}$</th>
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<tbody>
<tr>
<td>Baron</td>
<td>114 ± 8</td>
<td>138 ± 19</td>
<td>0.85 ± 0.06 a†</td>
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<td>103 ± 10</td>
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81
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† Values followed by the same letter are not different according to Tukey’s HSD (0.05).
Table 2. Biomass and NO$_3$- uptake rate by 60 Kentucky bluegrass genotypes at low N concentration. Nitrogen was supplied as $^{15}$N-labeled KNO$_3$ at 0.05 mM for a period of 4 h. Values shown are means of 4 replications ± SE.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shoot dry mass mg</th>
<th>Root dry mass mg</th>
<th>Uptake rate mg N g$^{-1}$ root dry wt h$^{-1}$</th>
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<td>Excursion</td>
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<td>Kenblue</td>
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<td>66 ± 17</td>
<td>119 ± 36</td>
<td>0.67 ± 0.05 b</td>
</tr>
<tr>
<td>A98-689</td>
<td>80 ± 6</td>
<td>144 ± 16</td>
<td>0.65 ± 0.05 b</td>
</tr>
<tr>
<td>Rugby II</td>
<td>58 ± 10</td>
<td>136 ± 23</td>
<td>0.61 ± 0.09 b</td>
</tr>
<tr>
<td>MTV TX F1</td>
<td>62 ± 4</td>
<td>115 ± 17</td>
<td>0.61 ± 0.05 b</td>
</tr>
<tr>
<td>Madison</td>
<td>36 ± 4</td>
<td>78 ± 14</td>
<td>0.59 ± 0.07 b</td>
</tr>
<tr>
<td>Barvette HGT</td>
<td>51 ± 12</td>
<td>105 ± 17</td>
<td>0.59 ± 0.09 b</td>
</tr>
<tr>
<td>Alexa II</td>
<td>51 ± 11</td>
<td>100 ± 11</td>
<td>0.57 ± 0.08 b</td>
</tr>
<tr>
<td>- batch 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Julia</td>
<td>108 ± 4</td>
<td>156 ± 14</td>
<td>0.96 ± 0.09 a</td>
</tr>
<tr>
<td>ThermalBlue</td>
<td>104 ± 11</td>
<td>155 ± 20</td>
<td>0.87 ± 0.11 ab</td>
</tr>
<tr>
<td>Diva</td>
<td>101 ± 5</td>
<td>158 ± 17</td>
<td>0.79 ± 0.05 abc</td>
</tr>
<tr>
<td>America</td>
<td>108 ± 7</td>
<td>167 ± 3</td>
<td>0.73 ± 0.04 abc</td>
</tr>
<tr>
<td>Prosperity</td>
<td>104 ± 9</td>
<td>134 ± 20</td>
<td>0.73 ± 0.07 abc</td>
</tr>
<tr>
<td>Glenwood</td>
<td>114 ± 12</td>
<td>224 ± 32</td>
<td>0.68 ± 0.06 bcd</td>
</tr>
<tr>
<td>Award</td>
<td>106 ± 2</td>
<td>204 ± 15</td>
<td>0.66 ± 0.06 bcd</td>
</tr>
<tr>
<td>Bluestone</td>
<td>88 ± 3</td>
<td>200 ± 17</td>
<td>0.63 ± 0.06 bcd</td>
</tr>
<tr>
<td>Skye</td>
<td>80 ± 5</td>
<td>129 ± 15</td>
<td>0.58 ± 0.06 cd</td>
</tr>
<tr>
<td>Washington</td>
<td>53 ± 6</td>
<td>82 ± 15</td>
<td>0.45 ± 0.09 d</td>
</tr>
</tbody>
</table>
(Table 2. continued)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shoot dry mass mg</th>
<th>Root dry mass mg</th>
<th>Uptake rate mg N g(^{-1}) root dry wt h(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>- batch 4 -</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bariris</td>
<td>120 ± 11</td>
<td>185 ± 21</td>
<td>0.70 ± 0.06 a</td>
</tr>
<tr>
<td>Starburst</td>
<td>115 ± 13</td>
<td>182 ± 19</td>
<td>0.60 ± 0.09 ab</td>
</tr>
<tr>
<td>Mystere</td>
<td>108 ± 9</td>
<td>216 ± 32</td>
<td>0.60 ± 0.02 ab</td>
</tr>
<tr>
<td>A00-247</td>
<td>113 ± 10</td>
<td>181 ± 14</td>
<td>0.57 ± 0.05 abc</td>
</tr>
<tr>
<td>PST-1A1-899</td>
<td>94 ± 12</td>
<td>130 ± 20</td>
<td>0.57 ± 0.06 abc</td>
</tr>
<tr>
<td>Shiraz</td>
<td>107 ± 34</td>
<td>160 ± 29</td>
<td>0.54 ± 0.03 bc</td>
</tr>
<tr>
<td>Beyond</td>
<td>140 ± 9</td>
<td>268 ± 21</td>
<td>0.46 ± 0.03 bcd</td>
</tr>
<tr>
<td>Rhythm</td>
<td>150 ± 17</td>
<td>283 ± 42</td>
<td>0.46 ± 0.05 bcd</td>
</tr>
<tr>
<td>Reveille</td>
<td>127 ± 18</td>
<td>234 ± 40</td>
<td>0.42 ± 0.04 cd</td>
</tr>
<tr>
<td>Everglade</td>
<td>120 ± 20</td>
<td>102 ± 26</td>
<td>0.36 ± 0.06 cd</td>
</tr>
<tr>
<td><strong>- batch 5 -</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Horse</td>
<td>99 ± 9</td>
<td>187 ± 16</td>
<td>0.57 ± 0.08 a</td>
</tr>
<tr>
<td>H94-305</td>
<td>84 ± 5</td>
<td>219 ± 11</td>
<td>0.53 ± 0.10 a</td>
</tr>
<tr>
<td>Bewitched</td>
<td>104 ± 22</td>
<td>240 ± 30</td>
<td>0.51 ± 0.04 a</td>
</tr>
<tr>
<td>Belissimo</td>
<td>90 ± 6</td>
<td>174 ± 18</td>
<td>0.50 ± 0.06 a</td>
</tr>
<tr>
<td>Arrowhead</td>
<td>79 ± 4</td>
<td>168 ± 15</td>
<td>0.48 ± 0.06 ab</td>
</tr>
<tr>
<td>H98-701</td>
<td>71 ± 12</td>
<td>162 ± 19</td>
<td>0.48 ± 0.06 ab</td>
</tr>
<tr>
<td>Blue Note</td>
<td>100 ± 14</td>
<td>225 ± 28</td>
<td>0.46 ± 0.08 ab</td>
</tr>
<tr>
<td>Impact</td>
<td>87 ± 8</td>
<td>167 ± 29</td>
<td>0.44 ± 0.08 ab</td>
</tr>
<tr>
<td>Nu Destiny</td>
<td>81 ± 9</td>
<td>177 ± 15</td>
<td>0.40 ± 0.06 ab</td>
</tr>
<tr>
<td>POPR 04594</td>
<td>76 ± 12</td>
<td>123 ± 17</td>
<td>0.30 ± 0.04 b</td>
</tr>
<tr>
<td><strong>- batch 6 -</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>J-3429</td>
<td>62 ± 6</td>
<td>162 ± 13</td>
<td>0.58 ± 0.02 a</td>
</tr>
<tr>
<td>SW AG 514</td>
<td>67 ± 1</td>
<td>217 ± 11</td>
<td>0.56 ± 0.01 ab</td>
</tr>
<tr>
<td>Harmonie</td>
<td>55 ± 2</td>
<td>139 ± 11</td>
<td>0.56 ± 0.01 ab</td>
</tr>
<tr>
<td>Avid</td>
<td>56 ± 4</td>
<td>181 ± 31</td>
<td>0.50 ± 0.04 abc</td>
</tr>
<tr>
<td>Zinfandel</td>
<td>63 ± 5</td>
<td>117 ± 6</td>
<td>0.49 ± 0.01 abc</td>
</tr>
<tr>
<td>Everest</td>
<td>65 ± 11</td>
<td>159 ± 11</td>
<td>0.46 ± 0.03 bc</td>
</tr>
<tr>
<td>NuGlade</td>
<td>62 ± 5</td>
<td>192 ± 18</td>
<td>0.46 ± 0.02 bc</td>
</tr>
<tr>
<td>Barrister</td>
<td>65 ± 5</td>
<td>185 ± 15</td>
<td>0.46 ± 0.02 bc</td>
</tr>
<tr>
<td>DLF76-9075</td>
<td>45 ± 6</td>
<td>143 ± 18</td>
<td>0.45 ± 0.03 bc</td>
</tr>
<tr>
<td>AKB449</td>
<td>51 ± 4</td>
<td>114 ± 18</td>
<td>0.42 ± 0.02 c</td>
</tr>
</tbody>
</table>

† Values followed by the same letter are not different according to Tukey’s HSD (0.05).
**Table 3.** Biomass and NO₃⁻ uptake rate by 10 Kentucky bluegrass cultivars in confirmational screening. Nitrogen was supplied as ¹⁵N-labeled KNO₃ at initial concentration of 1 or 0.05 mM. Values shown are means of 4 replications ± SE.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Shoot dry mass (mg)</th>
<th>Root dry mass (mg)</th>
<th>Uptake rate (mg N g⁻¹ root dry wt h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>- high concentration screening (1 mM) -</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Julia†</strong></td>
<td>123 ± 11</td>
<td>185 ± 19</td>
<td>1.20 ± 0.04 a‡</td>
</tr>
<tr>
<td><strong>ThermalBlue</strong></td>
<td>145 ± 3</td>
<td>210 ± 8</td>
<td>1.09 ± 0.05 ab</td>
</tr>
<tr>
<td>Juliet</td>
<td>101 ± 4</td>
<td>137 ± 5</td>
<td>0.90 ± 0.04 bc</td>
</tr>
<tr>
<td>Bandera</td>
<td>148 ± 9</td>
<td>257 ± 24</td>
<td>0.90 ± 0.06 bc</td>
</tr>
<tr>
<td>CPP822</td>
<td>110 ± 7</td>
<td>206 ± 37</td>
<td>0.85 ± 0.07 bc</td>
</tr>
<tr>
<td>Dynamo</td>
<td>142 ± 23</td>
<td>230 ± 43</td>
<td>0.84 ± 0.09 bc</td>
</tr>
<tr>
<td>Kenblue</td>
<td>93 ± 4</td>
<td>110 ± 2</td>
<td>0.84 ± 0.11 bc</td>
</tr>
<tr>
<td>AKB449</td>
<td>108 ± 14</td>
<td>160 ± 21</td>
<td>0.83 ± 0.04 bc</td>
</tr>
<tr>
<td>Midnight</td>
<td>115 ± 5</td>
<td>240 ± 20</td>
<td>0.72 ± 0.07 c</td>
</tr>
<tr>
<td>Everest</td>
<td>122 ± 6</td>
<td>236 ± 9</td>
<td>0.69 ± 0.03 c</td>
</tr>
<tr>
<td><strong>- low concentration screening (0.05 mM) -</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ThermalBlue</strong></td>
<td>130 ± 6</td>
<td>192 ± 11</td>
<td>0.52 ± 0.01 a</td>
</tr>
<tr>
<td><strong>Julia</strong></td>
<td>118 ± 8</td>
<td>159 ± 29</td>
<td>0.48 ± 0.02 ab</td>
</tr>
<tr>
<td>Bandera</td>
<td>143 ± 18</td>
<td>194 ± 9</td>
<td>0.45 ± 0.01 abc</td>
</tr>
<tr>
<td>Kenblue</td>
<td>89 ± 3</td>
<td>116 ± 9</td>
<td>0.43 ± 0.01 abc</td>
</tr>
<tr>
<td>Dynamo</td>
<td>140 ± 14</td>
<td>216 ± 9</td>
<td>0.43 ± 0.04 abc</td>
</tr>
<tr>
<td>CPP822</td>
<td>126 ± 26</td>
<td>190 ± 23</td>
<td>0.40 ± 0.01 abc</td>
</tr>
<tr>
<td>AKB449</td>
<td>103 ± 11</td>
<td>152 ± 20</td>
<td>0.40 ± 0.05 bc</td>
</tr>
<tr>
<td>Juliet</td>
<td>84 ± 3</td>
<td>134 ± 7</td>
<td>0.39 ± 0.01 bc</td>
</tr>
<tr>
<td>Everest</td>
<td>136 ± 18</td>
<td>230 ± 22</td>
<td>0.36 ± 0.02 c</td>
</tr>
<tr>
<td>Midnight</td>
<td>125 ± 9</td>
<td>227 ± 22</td>
<td>0.34 ± 0.03 c</td>
</tr>
</tbody>
</table>

† Cultivars in bold denote genotypes identified as efficient in NO₃⁻ uptake in the 1st round screening.

‡ Values followed by the same letter are not different according to Tukey’s HSD (0.05).
Table 4. Tissue N content, harvested dry mass (DM) and NO$_3^-$ uptake of Julia and Midnight Kentucky bluegrass at low N condition, Trial 1. Nitrogen was applied as KNO$_3$ solution at 1 mM. Uptake rate was calculated per plant (harvested shoots, rhizomes, and shallow roots attached to the tillers in each grid). Values are means of 40 samples, followed by SEs.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Tissue N %</th>
<th>Harvested DM mg</th>
<th>NO$_3^-$ Uptake Rate mg N plant$^{-1}$ d$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julia</td>
<td>1.43 ± 0.04</td>
<td>218 ± 9</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Midnight</td>
<td>1.52 ± 0.03</td>
<td>196 ± 9</td>
<td>0.49 ± 0.04</td>
</tr>
</tbody>
</table>

$p = 0.2380^\dagger$

† Mean comparison was based on single degree of freedom orthogonal contrasts.

Table 5. Type 3 ANOVA for NO$_3^-$ Uptake Rate of Julia and Midnight Kentucky bluegrass at high N condition, Trial 1.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cultivar</td>
<td>1</td>
<td>0.1825</td>
<td>0.1825</td>
<td>1.92</td>
<td>0.2380</td>
</tr>
<tr>
<td>block</td>
<td>4</td>
<td>4.5956</td>
<td>1.1489</td>
<td>12.09</td>
<td>0.0166</td>
</tr>
<tr>
<td>block×cultivar</td>
<td>4</td>
<td>0.3800</td>
<td>0.0950</td>
<td>3.36</td>
<td>0.0143</td>
</tr>
<tr>
<td>residual</td>
<td>70</td>
<td>1.9819</td>
<td>0.0283</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6. Tissue N content, harvested dry mass (DM) and NO$_3^-$ uptake of Julia and Midnight Kentucky bluegrass at low N condition, Trial 1. Nitrogen was applied as KNO$_3$ solution at 0.05 mM. Uptake rate was calculated per plant (harvested shoots, rhizomes, and shallow roots attached to the tillers in each grid). Values are means of 40 samples, followed by SEs.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Tissue N</th>
<th>Harvested DM</th>
<th>NO$_3^-$ Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg</td>
<td>mg N plant$^{-1}$ 10 d$^{-1}$</td>
</tr>
<tr>
<td>Julia</td>
<td>0.92 ± 0.01</td>
<td>229 ± 11</td>
<td>0.036 ± 0.002</td>
</tr>
<tr>
<td>Midnight</td>
<td>0.97 ± 0.01</td>
<td>200 ± 13</td>
<td>0.021 ± 0.002</td>
</tr>
</tbody>
</table>

$^\dagger$ Mean comparison was based on single degree of freedom orthogonal contrasts.

Table 7. Type 3 ANOVA for NO$_3^-$ Uptake Rate of Julia and Midnight Kentucky bluegrass at low N condition, Trial 1.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cultivar</td>
<td>1</td>
<td>0.0049</td>
<td>0.0049</td>
<td>29.1</td>
<td>0.0057</td>
</tr>
<tr>
<td>block</td>
<td>4</td>
<td>0.0048</td>
<td>0.0012</td>
<td>7.11</td>
<td>0.0419</td>
</tr>
<tr>
<td>block×cultivar</td>
<td>4</td>
<td>0.0007</td>
<td>0.0002</td>
<td>1.7</td>
<td>0.1593</td>
</tr>
<tr>
<td>residual</td>
<td>70</td>
<td>0.0069</td>
<td>0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Recovery of applied N in lysimeters of Trial 1. Calculation was based on N applied to and tissues harvested from the center 4 × 4 grid of each lysimeter. Values are means of 5 replications ± SEs.

<table>
<thead>
<tr>
<th>N applied per column</th>
<th>Estimated N applied to center 4×4 grids</th>
<th>N Recovery in harvested tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>mg</td>
<td>%</td>
</tr>
<tr>
<td>High N (2 mM)</td>
<td>56.0</td>
<td>13.0</td>
</tr>
<tr>
<td>Low N (0.05 mM)</td>
<td>4.9</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Table 9. Tissue N content, harvested dry mass (DM) and NO$_3^-$ uptake of Julia and Midnight Kentucky bluegrass at high N condition, Trial 2. Nitrogen was applied as KNO$_3$ solution at 1 mM. Uptake rate was calculated per plant (harvested shoots, rhizomes, and shallow roots attached to the tillers in each grid). Values are means of 40 samples, followed by SEs.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Tissue N</th>
<th>Harvested DM</th>
<th>N Uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg</td>
<td>mg N plant$^{-1}$ 8h$^{-1}$</td>
</tr>
<tr>
<td>Julia</td>
<td>1.65 ± 0.04</td>
<td>127 ± 6</td>
<td>0.37 ± 0.02</td>
</tr>
<tr>
<td>Midnight</td>
<td>1.50 ± 0.03</td>
<td>121 ± 6</td>
<td>0.25 ± 0.01</td>
</tr>
</tbody>
</table>

$p = 0.0198^{†}$

† Mean comparison was based on single degree of freedom orthogonal contrasts.

Table 10. Type 3 ANOVA for NO$_3^-$ Uptake Rate of Julia and Midnight Kentucky bluegrass at high N condition, Trial 2.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cultivar</td>
<td>1</td>
<td>0.3102</td>
<td>0.3102</td>
<td>14.14</td>
<td>0.0198</td>
</tr>
<tr>
<td>block</td>
<td>4</td>
<td>0.1191</td>
<td>0.0298</td>
<td>1.36</td>
<td>0.3873</td>
</tr>
<tr>
<td>block×cultivar</td>
<td>4</td>
<td>0.0877</td>
<td>0.0219</td>
<td>2.18</td>
<td>0.0795</td>
</tr>
<tr>
<td>residual</td>
<td>70</td>
<td>0.7028</td>
<td>0.0100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11. Tissue N content, harvested dry mass (DM) and NO₃⁻ uptake of Julia and Midnight Kentucky bluegrass at high N condition, Trial 2. Nitrogen was applied as KNO₃ solution at 0.05 mM. Uptake rate was calculated per plant (harvested shoots, rhizomes, and shallow roots attached to the tillers in each grid). Values are means of 40 samples, followed by SEs.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Tissue N %</th>
<th>Harvested DM mg</th>
<th>N Uptake mg N plant⁻¹ 8h⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julia</td>
<td>0.93 ± 0.01</td>
<td>155 ± 6</td>
<td>0.060 ± 0.003</td>
</tr>
<tr>
<td>Midnight</td>
<td>1.08 ± 0.02</td>
<td>143 ± 6</td>
<td>0.048 ± 0.003</td>
</tr>
</tbody>
</table>

*p = 0.0131†

† Mean comparison was based on single degree of freedom orthogonal contrasts.

Table 12. Type 3 ANOVA for NO₃⁻ Uptake Rate of Julia and Midnight Kentucky bluegrass at low N condition, Trial 2.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>cultivar</td>
<td>1</td>
<td>0.0027</td>
<td>0.0027</td>
<td>18.14</td>
<td>0.0131</td>
</tr>
<tr>
<td>block</td>
<td>4</td>
<td>0.0033</td>
<td>0.0008</td>
<td>5.57</td>
<td>0.0625</td>
</tr>
<tr>
<td>block×cultivar</td>
<td>4</td>
<td>0.0006</td>
<td>0.0002</td>
<td>0.47</td>
<td>0.7594</td>
</tr>
<tr>
<td>residual</td>
<td>70</td>
<td>0.0225</td>
<td>0.0003</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 13. Recovery of applied N in lysimeters of Trial 2. Calculation was based on N applied to and tissues harvested from the center 4 × 4 grid of each lysimeter. Values are means of 5 replications ± SE.

<table>
<thead>
<tr>
<th>N applied per column mg</th>
<th>Estimated N applied to center 4×4 grids mg</th>
<th>N Recovery in harvested tissue %</th>
<th>mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>High N (1 mM)</td>
<td>196</td>
<td>45.4</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Low N (0.05 mM)</td>
<td>9.8</td>
<td>2.3</td>
<td>38 ± 2</td>
</tr>
</tbody>
</table>

89
CHAPTER 3

Nitrate Leaching from Two Kentucky Bluegrass Cultivars as Affected by
Nitrate Uptake Capacity and Subsurface Soil Compaction

ABSTRACT

There are a number of strategies to minimize NO$_3^-$ leaching from turfgrass, including planting turfgrass cultivars with higher NO$_3^-$ absorption abilities. This column lysimeter study was conducted to examine NO$_3^-$ leaching from two Kentucky bluegrass (*Poa pratensis* L.) cultivars differing in NO$_3^-$ uptake capacity. Subsurface soil compaction was included as a second factor. Tillers of Julia and Midnight Kentucky bluegrass, previously identified as having high and low NO$_3^-$-N uptake capacity, respectively, were grown in column lysimeters. Each column was filled with a loamy sand soil with or without a subsurface compaction layer 8.5 cm below the surface. The two cultivars were established and then treated to impose moderate N deficiency. Potassium nitrate was applied in solution at 50 kg N ha$^{-1}$, followed by daily heavy irrigation. Leachate was collected and analyzed for NO$_3^-$-N. In two out of three trials, both cultivars absorbed NO$_3^-$ very efficiently in non-compacted soil, with only trace amounts of NO$_3^-$ leaching. By contrast, approximately 20% of applied N leached from both cultivars in one trial, with peak NO$_3^-$-N concentrations of $\sim$ 30 mg L$^{-1}$. This was probably due to the turf being younger and having a less established root system. Soil compaction increased NO$_3^-$ leaching. Cumulative N loss ranged from 2.6 to 19% and 2.8 to 21% for Julia and Midnight, respectively. Peak NO$_3^-$-N concentrations were $\geq$ 10 mg L$^{-1}$ in
two of the three trials. Results indicated that despite being identified as more efficient for 
NO$_3^-$ uptake, NO$_3^-$ leaching from Julia was similar to or even greater than that from 
Midnight. Because root biomass distribution was similar between the two genotypes, 
differences in root morphology or architecture may have played a more important role than 
uptake capacity in determining NO$_3^-$ leaching from these Kentucky bluegrass genotypes.

**INTRODUCTION**

Due to its mobility in soil, NO$_3^-$ can leach below rootzones and enter surface and 
ground water. High rates of fertilizer N on agricultural lands have been found to increase 
NO$_3^-$ levels in aquatic ecosystems (Howarth et al., 2002; Keeney, 1986; Nolan and Stoner, 
2000; Nolan, 2001; Puckett et al., 1999; Spalding and Exner, 1993; Turner and Rabalais, 
1991). Nitrate is a threat not only to aquatic ecosystems (Galloway et al., 2003; Howarth et 
el., 2002; Nosengo, 2003; Rabalais, 2002; Smith et al., 1987; Vitousek et al., 1997) but also 
to human health (Follett and Follet, 2001; Townsend et al., 2003). There are roughly 20 
million ha of managed turfgrass in the US (Emmons, 2008), which, on average, receive 
approximately 50 kg N ha$^{-1}$ yr$^{-1}$ (Bruce Augustin, personal communication). In fact, 
turfgrass fertilization accounts for 10% or more of total N fertilizer use in the US (calculated 
from Galloway et al., 2003). It is thus not surprising that the public perceives turf as being 
excessively fertilized and a significant source of NO$_3^-$ leaching.

A large body of work indicates that very little NO$_3^-$ leaches from healthy and well-
managed turf (Cohen et al., 1999; Duff et al., 1997; Erickson et al., 2001; Gold et al., 1990; 
Kunimatsu et al., 1999; Lee et al., 2003; Pannkuk et al., 2011; Rufty et al., 2008; Trenholm
and Sartain, 2010). However, significant NO$_3^-$ leaching can occur under certain conditions, such as heavy irrigation (Brown et al., 1977; Modsdell and Schmidt, 1985; Morton et al., 1988; Roy et al., 2000; Snyder et al., 1984; Starr and DeRoo, 1981), heavy or mis-timed fertilization (Brown et al., 1977; Brown et al., 1982; Guillard and Kopp, 2004; Mangiafico and Guillard, 2007; Petrovic et al., 1986; Rieke and Ellis, 1974; Roy et al., 2000; Sheard et al., 1985; Snyder et al., 1981), sandy soils (Brown et al., 1982; Petrovic, 1990; Rieke and Ellis, 1974), and mature turf (Frank et al., 2006; Petrovic, 1990; Porter et al., 1980).

The low potential for NO$_3^-$ leaching in turfgrass is often attributed in large part to efficient N uptake by roots (Bowman et al., 1989b; Morton et al., 1988; Petrovic, 1990). At least two factors are responsible for this efficiency: enhanced uptake capacity in response to N deficiency (Bowman and Paul, 1988; Bowman et al., 1989a) and a very dense root system (Bowman et al., 1989b; Murphy et al., 1994). While most if not all turfgrass species exhibit these two factors, there are differences in N uptake capacity both between species and cultivars (Bertauski et al., 1997; Jiang and Hull, 1998; Liu et al., 1993). We recently reported intraspecific differences among 60 Kentucky bluegrass cultivars in terms of NO$_3^-$ uptake rate, and further identified cultivars Julia and Midnight as being high and low in NO$_3^-$ uptake efficiency, respectively. In addition, the higher uptake capacity of Julia translated into a competitive advantage when the two cultivars were grown in a mixed stand. However, it is unknown if such a difference would lead to different leaching potentials between cultivars. Liu et al. (1997) reported differences in leaching potential among 10 Kentucky bluegrass genotypes in terms of leachate NO$_3^-$ concentration and cumulative N loss. However, no relationship was drawn between leaching potential and NO$_3^-$ uptake efficiency.
Soil compaction is a very common and often severe problem in most turfgrass systems (Madison, 1971). Rooting depth is restricted and turf can suffer from both nutrient and water deficiencies when growing on compacted soils. While surface compaction can be moderated via cultivation, subsurface compaction that results from site construction or subsequent management practices (Murphy et al., 1993; Petrovic, 1979; Raney et al., 1955) is less easily corrected. Deep rooting is an important plant characteristic that helps to reduce NO$_3^-$ leaching (Bowman et al., 1998; Dunbabin et al., 2003). Thus NO$_3^-$ leaching might increase when rooting is restricted by a subsurface compaction layer. However, little information is available documenting this effect. With a root system confined to a relatively shallow layer of soil, it is possible that increased NO$_3^-$ uptake capacity might translate into more efficient and complete removal of N from the soil, and as a result, less NO$_3^-$ leaching. The objective of this study was to examine NO$_3^-$ leaching from two cultivars of Kentucky bluegrass differing in N uptake capacity, as affected by subsurface soil compaction.

**MATERIALS AND METHODS**

**Plant Culture**

Tillers of Julia and Midnight Kentucky bluegrass were harvested in October, 2010 (Experiment 1) and April, 2011 (Experiment 2 and 3) from a cultivar evaluation trial at the NCSU Turfgrass Research Lab (Raleigh, NC). Roots and older leaves were excised to provide uniform tillers with 2-3 young, expanded leaves. Tillers were planted to greenhouse flats filled with a porous ceramic medium (Profile Greens Grade, Profile Products LLC, Buffalo Grove, IL) and placed under artificial lighting (PPFD of 450 μmol m$^{-2}$ s$^{-1}$ provided
by a combination of high pressure sodium and mercury vapor lamps). Flats were watered 2-3 times per day to prevent wilting and promote rooting. After 4 weeks in the flat, tillers were transplanted to 20 custom-built polyvinyl chloride (PVC) column lysimeters (Matthieu et al. 2011). Each lysimeter was constructed by stacking three 12.7 cm diameter PVC pipe sections together and filling the resulting column with Wagram loamy sand soil (an Arenic Hapludult; pH = 6.5; 4.2% clay, 10.8% silt, 85.0% sand). Soil was packed to a bulk density ($\rho_b$) of 1.60 g cm$^{-3}$ in the top (0-8.5 cm) and bottom (14.4-33.4) rootzone. The middle (8.5-14.4 cm) rootzone in half of the columns was compacted at 1.9 g cm$^{-3}$ to simulate subsurface compaction. The other half remained non-compacted at 1.60 g cm$^{-3}$ serving as controls. A barrier flange was glued to the inside wall of the middle PVC segment, and the inside wall of the middle segment was roughened with soil glued to the wall with epoxy. These strategies were undertaken to prevent roots from growing down the smooth PVC sides and bypassing the compaction layer. One porous ceramic suction cup was placed at the bottom of each lysimeter, connected to a 5-L amber glass bottle to collect leachate. The glass bottles were then connected via manifold to a vacuum pump. Lysimeters were flushed several times with tap water, and drained to ~ 0.03 MPa before planting. For both cultivars, twenty-eight tillers were planted to each of five columns with subsurface compaction and five without compaction.

**Experiment 1**

Planted columns were irrigated three times per week with 1 cm of 0.1-strength minus-N Hoagland solution (Hoagland and Arnon, 1950) amended with full strength micronutrients and 1 mg Fe L$^{-1}$ Fe-EDDHA. Tap water was used on the other four days. A tension of 0.03
MPa was applied to each column at night to drain and collect leachate. Air temperature was controlled at approximately 29/18°C day/night. Light was supplied at a PPFD of 500 μmol m⁻² s⁻¹ with a 14-h photoperiod (0600 to 2000 h). Shoots were clipped at 5 cm 1-2 times per week.

Beginning two weeks after planting and continuing for eight weeks, KNO₃ was added to the minus-N nutrient solution to supply the equivalent of 98 kg N ha⁻¹ per month. At ten weeks, irrigation with the minus-N solution was resumed for a period of five weeks to induce moderate N deficiency in the turf. Irrigation depth was increased to 1.6 cm (three times per week) to remove as much soil NO₃⁻ as possible.

Nitrate leaching was initiated with the application of a solution of KNO₃ (0.8 cm of 45.2 mM N, equivalent to 49 kg N ha⁻¹) on day 0. Daily irrigation (tap water only) was subsequently applied at 1.2 cm. Columns were drained under vacuum each day and the leachate volume determined. A subsample was stored under refrigeration before analysis for NO₃⁻-N using a Lachat QC-3000 flow injection auto analyzer (Lachat Instruments, Milwaukee, WI). Samples were collected over a period of one month.

Clipping yields representing 4-days growth were determined three times during Experiment 1: at the end of initial plus-N irrigation, 10 days after N treatment, and 20 days after N treatment. Total biomass was harvested at the end of this experiment. Each lysimeter was separated into the individual segments and roots were washed free of the soil. Verdure plus rhizomes were harvested as shoots, and roots were harvested by segment. Harvested tissues were oven dried at 60 °C before weighing.
Experiment 2

Despite our precautions in Experiment 1, some roots were able to grow along the sides of the column and bypass the compaction layer, growing into the bottom section of the column. To prevent this in Experiment 2, we painted the inside walls of top and middle sections with SpinOut Root Growth Regulator (SePro Corporation, Carmel, IN). Additionally, we shortened the pretreatment period to four weeks of irrigation with plus-N solution followed by three weeks of minus-N irrigation. Finally, the columns were not harvested after thirty days of leachate collection. They were instead used for a third round of leachate collection (Experiment 3), as follows:

Experiment 3

At the conclusion of sampling the leachate in Experiment 2, the columns were irrigated daily for two weeks with 1.6 cm of minus-N nutrient solution (3 times per week) and tap water (4 times per week). Thereafter, irrigation was changed to tap water only and gradually reduced over 7 days to 0.6 cm at the time of N treatment. This was designed to reduce the leaching fraction.

Nitrogen was applied as 80 mL of 56.5 mM KNO₃ solution (equivalent to 49 kg N ha⁻¹) and daily leachate NO₃⁻-N determined for a month. Irrigation was adjusted daily to maintain a leaching fraction of 0.4; the average daily irrigation depth was 0.76 ± 0.11 cm. Clipping yield (4-day growth) was collected 3 days before N treatment, 10 days after N treatment, and 20 days after treatment. Biomass was harvested and processed as described in Experiment 1.
Experimental Design and Statistical Analysis

A Complete Random Design with 2 factors (cultivar, compaction) and 5 replicates was used for each experiment. ANOVA was performed with Statistical Analysis Software 9.2 (SAS Institute Inc., 2008) with both factors treated as fixed. Mean comparison was based on single degree of freedom orthogonal contrasts.

RESULTS AND DISCUSSION

Clipping Yield

Turfgrass growth was closely associated with N status. Not surprisingly, growth was higher in turf supplied with N compared to turf deprived of N (Fig. 1).

Differences in growth between Julia and Midnight were consistently observed, with Julia generally producing more clippings (Table 1). In five out of ten harvests, Julia had greater clipping yield than Midnight, regardless of subsurface compaction. In four other harvests, Julia produced more clippings than Midnight in lysimeters with subsurface compaction, but similar amounts were determined for both cultivars in lysimeters without subsurface compaction. And in one harvest clipping yields did not differ between cultivars regardless of subsurface compaction. The effect of compaction on growth was less consistent. In 4 out of 10 harvests, subsurface compaction reduced growth of both cultivars. In 3 other harvests, subsurface compaction reduced clipping yield of Midnight, but not Julia. In 1 harvest, subsurface compaction reduced clipping yield of Julia, but not Midnight. There were 2 harvests in which clipping yields were not affected by subsurface compaction. In Experiment 1, the effect of compaction tended to disappear towards the end of experiment.
This was probably due to the fact that roots in compacted lysimeters were bypassing the subsurface compaction layer, but it took time for the roots to get into the bottom of the column and access that segment’s water and nutrients.

**Shoot and Root Biomass**

Experiment 1: Shoot biomass at the end of the experiment was significantly greater in Julia than in Midnight, but was unaffected by soil compaction (Fig. 2). Root biomass declined with soil depth, with roughly two thirds located in the top 10 cm of soil. Neither cultivar nor compaction affected root weight in the top section (0-8.5 cm) of the column. In non-compacted soils, Midnight produced more roots in both the middle (8.5-14.4 cm) \( (p = 0.0092) \) and bottom (14.4-33.4 cm) \( (p = 0.0008) \) rootzone compared to Julia. Compaction reduced but did not eliminate root growth into the bottom section of the column. A close examination at the final harvest revealed that most roots had bypassed the compacted layer and entered the bottom section by growing along the lysimeter wall. This occurred in 9 out of the 10 compacted lysimeters. Although the lysimeters were modified specifically to prevent this, it was apparent that the modifications were only partially effective. As will be discussed below, these relatively few roots growing in the bottom section had a considerable impact on \( \text{NO}_3^- \) leaching.

Experiment 3: Tissue data collected at the end of Experiment 3 show that Julia produced more shoot mass than Midnight in compacted soil \( (p = 0.0113) \), but not in non-compacted soil \( (p = 0.4013) \). Almost all roots in lysimeters with subsurface compaction were restricted to the top section (Fig. 3), indicating that the application of SpinOut to the inside walls of the column kept roots from bypassing the subsurface compaction layer. Since there
were few roots in the bottom sections of compacted column lysimeters following Experiment 3, it is inferred that a similar paucity of roots was also present in Experiment 2. Subsurface compaction increased root mass in the top section, compared to non-compacted soils \((p = 0.0002)\) whereas no difference was evident between cultivars. Without compaction, Midnight generated slightly more deep roots than Julia \((p = 0.0044\) in the middle rootzone, \(p = 0.0546\) in the bottom rootzone).

**Nitrate Leaching**

Leaching fraction during Experiment 1 averaged 0.46 ± 0.08 which represents a worst case situation for leaching of a soluble ion like NO\(_3\)\(^-\). Despite this, extremely low amounts of NO\(_3\)\(^-\) leached from lysimeters with non-compacted soil (Figs. 4c and 4d). By contrast, subsurface compaction increased leaching from both cultivars, with peak NO\(_3\)\(^-\)-N concentrations of ~17 and 14 mg L\(^-1\) on Day 8 for Julia and Midnight, respectively (Figs. 4a and 4b). Individual plots rather than means are presented in Fig. 4 due to the high variability observed in lysimeters with subsurface compaction. For instance, little NO\(_3\)\(^-\) leaching was detected in 3 out of the 5 lysimeters planted with Julia, while significant leaching was evident in the other 2 (Figs. 4a). As noted above, roots were found bypassing the compacted middle segments and penetrating into the bottom section of lysimeters with subsurface compaction. Although present in very small amount (Fig. 3), these deeper roots in some lysimeters almost eliminated NO\(_3\)\(^-\) leaching. The random escape of roots into the bottom section confounded the data, but it also illustrates the importance of deeper roots in preventing NO\(_3\)\(^-\) leaching.

Total N leaching loss was very low in Experiment 1. Even with subsurface compaction, average loss from Julia and Midnight amounted to only 2.6 and 3.0% of applied
N, respectively. The difference was not significant (Table 4). The data were highly variable (CV of total N leaching loss = 159%), probably due to roots in some columns bypassing the subsurface compaction layer. In fact, analysis confirmed that the amount of root mass present in the bottom section of compacted lysimeters was negatively correlated with the amount of total N leaching loss (Pearson product-moment \( \rho = -0.81, p = 0.0044 \)). Both cultivars were very efficient at capturing applied N in lysimeters without compaction, with only a trace of applied N being lost.

Daily leaching fractions averaged 0.49 ± 0.04 in Experiment 2. Compared to Experiment 1, there was much less variation in leaching from lysimeters with subsurface compaction (Figs. 5a and 5b), likely due to the successful prevention of roots bypassing the compacted layer. In lysimeters with subsurface compaction, daily leachate NO\(_3^–\)-N concentration of Julia peaked on Day 11, averaging 13 mg L\(^{-1}\) across 5 reps. Peak leachate NO\(_3^–\)-N concentration of Midnight was detected on Day 9, averaging 24 mg L\(^{-1}\). For both cultivars, there were 6 days during which NO\(_3^–\)-N concentration stayed above 10 mg L\(^{-1}\).

Also unlike the results from Experiment 1, considerable NO\(_3^–\) leaching was evident from lysimeters without subsurface compaction (Figs. 5c and 5d). Peak NO\(_3^–\)-N concentration from both Julia and Midnight was detected on Day 7, averaging 32 and 29 mg L\(^{-1}\), respectively. Nitrate-N concentration exceeded 10 mg L\(^{-1}\) for 4-5 days. While unexpected, leaching from non-compacted soil was probably due to the shorter establishment period compared to Experiment 1, which would prevent roots from proliferating as deeply in the columns.
Indicated by higher peak values and rapid rise/decline of concentration, the pattern of NO\textsubscript{3}\textsuperscript{−} breakthrough was more defined and less attenuated from non-compactened compared to compacted soil (Fig. 5). There also seemed to be background levels of NO\textsubscript{3}\textsuperscript{−} in the compacted soil as evidenced by the low concentration of N collected both before and after the peak had passed (Figs. 5a and 5b). This may be from mineralization of soil organic N, especially in the bottom section where the absence of roots would allow leaching to occur.

With 19 and 21% of the applied N being lost from Julia and Midnight, respectively, total N leaching loss from compacted soils was much higher in Experiment 2 than Experiment 1. No difference was evident between the two cultivars in compacted soils ($p = 0.3926$, Table 5). Total N leaching loss was also high from non-compactened soils, averaging 17 and 11% of applied N for Julia and Midnight, respectively ($p = 0.0210$, Table 5). Since the columns were not destructively harvested until after Experiment 3, we can only speculate that differences in root distribution caused the difference in total N lost. The effect of subsurface compaction differed between cultivars (Table 5), promoting NO\textsubscript{3}\textsuperscript{−} leaching in Midnight ($p = 0.0004$) but not in Julia ($p = 0.3217$).

Experiment 3 repeated the procedures used in Experiment 2 with the exception that irrigation was reduced. This resulted in an average leaching fraction of 0.41 ± 0.11. Lysimeters were flushed with 1.6 cm of water daily for two weeks following the conclusion of Experiment 2. This was gradually lowered to 0.6 cm per day prior to applying N. However, this amount resulted in leaching fractions that gradually declining below our target, and irrigation was adjusted higher to correct this. The average daily irrigation applied in Experiment 3 was 0.76 ± 0.11 cm.
One replication of Midnight growing with subsurface compaction was contaminated with high levels of N prior to the scheduled N application and was thus excluded from analysis. Nitrate concentrations in the leachate (Fig. 6) were generally lower than in Experiment 2. Peak concentrations of ~10 mg N L\(^{-1}\) were measured from both cultivars growing in compacted soils. These peaks occurred on Day 9 and were relatively broad, likely due to the lower leaching fraction adopted in this experiment. It is unclear why NO\(_3^-\) concentration increased towards the end of the sampling period. It is highly unlikely that a single application of KNO\(_3\) would separate into two pulses, so the most likely source is mineralization/nitrification in the bottom section. The fact that this late flush of NO\(_3^-\) leaching was not detected in the non-compact ed columns, with roots present in the bottom section, supports the argument.

Nitrate leaching was barely detectable in lysimeters with non-compact ed soils (Figure 6c and 6d) with one exception. A single rep of Midnight produced a spike of NO\(_3^-\)-N (~15 mg L\(^{-1}\)) on Day 10 and 11. We have no good explanation for this other than the possibility of soil or root heterogeneity.

Cumulative N lost from the compacted soil treatments amounted to 4.8 and 2.8% of applied N from Julia and Midnight, respectively. The difference was nearly significant at \( p = 0.055 \) (Table 6). Leaching loss from non-compact ed soil treatments was essentially zero, again with the exception of one replication.

Nitrate leaching in this study varied from a trace amount to 17% and from 2.6 to 21% in non-compact ed and compacted soils, respectively. Peak leachate NO\(_3^-\)-N concentration
ranged from zero to > 30 mg N L\(^{-1}\). These peak concentrations are near the higher end of previous reported NO\(_3^-\) leaching in turf (Hull and Liu, 2005; Petrovic, 1990).

Limited information is available comparing leaching potentials between turfgrass genotypes. Liu et al. (1997) compared 10 genotypes each of three cool-season turfgrasses for leachate NO\(_3^-\)-N concentration and cumulative NO\(_3^-\)-N loss under field conditions. The authors detected intraspecific differences in all three species. Specifically for 10 Kentucky bluegrass cultivars, mean leachate NO\(_3^-\)-N concentration ranged from 2.4 to 10.1 mg N L\(^{-1}\) over 2 years, and cumulative NO\(_3^-\)-N loss ranged from 2 to 19% and 6.7 to 30% of applied N. It was pointed out that Liberty Kentucky bluegrass, which was previously determined as less efficient in N acquisition (Liu et al., 1993), had the greatest leaching loss. However, there was no clear relationship between N uptake efficiency and NO\(_3^-\) leaching potential at the cultivar level. In fact, some discrepancy was observed between N uptake efficiency and NO\(_3^-\) leaching potential. For example, ‘P-160’ tall fescue was identified as having high N uptake efficiency in a previous investigation (Liu et al., 1993), but exhibited high NO\(_3^-\) leaching potential. The authors suggested that experimental conditions favoring higher leaching might help clarify the relationship between uptake and leaching loss. By contrast, our results generally refute a direct linkage between NO\(_3^-\) uptake efficiency and NO\(_3^-\) leaching potential.

Information documenting the effect of subsurface compaction on NO\(_3^-\) leaching from turf is scarce. In a long term field study, Kussow (2000) reported that subsurface compaction had no significant effect on NO\(_3^-\) leaching from a Kentucky bluegrass turf receiving N application at 198 kg ha\(^{-1}\) yearly. However, their turf was in a heavy soil (silt loam), and
watered only upon visible drought stress. Therefore, leaching potential was generally low regardless of soil compaction.

**Conclusion**

In this study, we examined NO$_3^-$ leaching from Kentucky bluegrass cultivars previously characterized as having high and low capacity of NO$_3^-$ absorption. Subsurface compaction was used to isolate the root systems to a fairly confined zone of ~ 10 cm. Under conditions which would promote leaching, both cultivars were very efficient at preventing NO$_3^-$ leaching. In Experiments 1 and 3, leaching from non-compacted soils amounted to only a trace (< 1%) independent of cultivar. When roots were restricted by subsurface compaction, NO$_3^-$ leaching was still less than 5% of applied N. In Experiment 2, however, considerable NO$_3^-$ leaching (10 - 20% of applied N) occurred regardless of cultivar or compaction. The results from these three experiments suggest little difference between Julia and Midnight with regards to NO$_3^-$ leaching. If anything, slightly greater amounts of N were lost from Julia. We previously identified Julia as having more efficient NO$_3^-$ uptake compared to Midnight. In solution culture, Julia had 68 and 44% higher NO$_3^-$ uptake rate than Midnight at high (1 mM) and low N (0.05 mM) concentrations, respectively. In sand lysimeters, Julia again had 43 and 14 % higher NO$_3^-$ uptake rate than Midnight at high (1 mM) and low N (0.05 mM) conditions, respectively. However, this difference did not translate to reduced NO$_3^-$ leaching potential. Both cultivars had similar a similar distribution of root biomass with depth, although Midnight did generate slightly more deep roots in non-compacted soils. Although root biomass was similar, root morphology and architecture could differ between the two cultivars. Characteristics such as fibrous root length/surface/volume and root length density
can directly affect NO$_3^-$ leaching potential (Bowman et al., 2002; Sullivan et al., 2000). Collectively, these results indicate that selecting and planting turfgrass cultivars with high NO$_3^-$ uptake capacity is not a sound strategy to control NO$_3^-$ leaching.
REFERENCES


Table 1. Mean comparisons of clipping yields. Comparisons were based on one degree of freedom orthogonal contrasts.

<table>
<thead>
<tr>
<th>Orthogonal Contrasts</th>
<th>At the End of plus-N Irrigation</th>
<th>At the End of minus-N Irrigation</th>
<th>10d after N Treatment</th>
<th>20d after N Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1 (p-values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Julia vs Midnight w/ SCL†</td>
<td>0.0003</td>
<td>-‡</td>
<td>0.0009</td>
<td>0.0007</td>
</tr>
<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>0.2620</td>
<td>-</td>
<td>&lt;.0001</td>
<td>0.0003</td>
</tr>
<tr>
<td>SCL vs no SCL in Julia</td>
<td>0.5036</td>
<td>-</td>
<td>0.0223</td>
<td>0.1337</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>0.0150</td>
<td>-</td>
<td>0.1789</td>
<td>0.2809</td>
</tr>
<tr>
<td>Exp. 2 (p-values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Julia vs Midnight w/ SCL</td>
<td>0.0289</td>
<td>0.1653</td>
<td>&lt; 0.0001</td>
<td>0.0016</td>
</tr>
<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>0.9332</td>
<td>0.1458</td>
<td>0.1396</td>
<td>0.2374</td>
</tr>
<tr>
<td>SCL vs no SCL in Julia</td>
<td>0.4399</td>
<td>0.0110</td>
<td>0.5172</td>
<td>0.0079</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>0.1473</td>
<td>&lt; 0.0001</td>
<td>0.0042</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Exp. 3 (p-values)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Julia vs Midnight w/ SCL</td>
<td>-</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.0004</td>
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<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>-</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
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<tr>
<td>SCL vs no SCL in Julia</td>
<td>-</td>
<td>&lt; 0.0001</td>
<td>0.1667</td>
<td>&lt; 0.0001</td>
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<tr>
<td>SCL vs no SCL in Midnight</td>
<td>-</td>
<td>&lt; 0.0001</td>
<td>0.0060</td>
<td>0.0030</td>
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</table>

† SCL, subsurface compaction layer.
‡ Data not collected.
**Table 2.** Comparisons of biomass harvested at the end of Exp.1. Comparisons are based on one degree of freedom orthogonal contrasts. (Top root, 0 - 8.5 cm below surface. Middle root, 8.5 - 14.4 cm below surface. Bottom root, 14.4 - 33.4 cm below surface.)

<table>
<thead>
<tr>
<th>Orthogonal Contrasts</th>
<th>Shoot</th>
<th>Top Root</th>
<th>Middle Root</th>
<th>Bottom Root</th>
<th>Total Root</th>
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</thead>
<tbody>
<tr>
<td>Julia vs Midnight w/ SCL†</td>
<td>0.0389</td>
<td>0.1369</td>
<td>0.1522</td>
<td>0.7384</td>
<td>0.1533</td>
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<td>Julia vs Midnight w/o SCL</td>
<td>0.0080</td>
<td>0.3160</td>
<td>0.0092</td>
<td>0.0008</td>
<td>0.0384</td>
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<tr>
<td>SCL vs no SCL in Julia</td>
<td>0.3836</td>
<td>0.3270</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.1305</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>0.9080</td>
<td>0.1426</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.0319</td>
</tr>
</tbody>
</table>

† SCL, subsurface compaction layer.

**Table 3.** Comparisons of biomass harvested at the end of Exp.3. Comparisons are based on one degree of freedom orthogonal contrasts. (Top root, 0 - 8.5 cm below surface. Middle root, 8.5 - 14.4 cm below surface. Bottom root, 14.4 - 33.4 cm below surface.)

<table>
<thead>
<tr>
<th>Contrast</th>
<th>Shoot</th>
<th>Top Root</th>
<th>Middle Root</th>
<th>Bottom Root</th>
<th>Total root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julia vs Midnight w/ SCL†</td>
<td>0.0113</td>
<td>0.7929</td>
<td>0.7213</td>
<td>0.8564</td>
<td>0.7942</td>
</tr>
<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>0.4013</td>
<td>0.2944</td>
<td>0.0044</td>
<td>0.0546</td>
<td>0.0936</td>
</tr>
<tr>
<td>SCL vs no SCL in Julia</td>
<td>0.6144</td>
<td>0.0009</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.1721</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>0.0055</td>
<td>0.0146</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>0.5448</td>
</tr>
</tbody>
</table>

† SCL, subsurface compaction layer.
**Table 4.** ANOVA of total N leaching loss from Julia and Midnight and orthogonal contrast F-test in Exp.1.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>15.28535</td>
<td>5.095117</td>
<td>2.28</td>
<td>0.1185</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>35.75338</td>
<td>2.234586</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compaction</td>
<td>1</td>
<td>14.6877</td>
<td>14.6877</td>
<td>6.63</td>
<td>0.0204</td>
</tr>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>0.0893</td>
<td>0.0893</td>
<td>0.04</td>
<td>0.8434</td>
</tr>
<tr>
<td>Compaction × Cultivar</td>
<td>1</td>
<td>0.0843</td>
<td>0.0843</td>
<td>0.04</td>
<td>0.8478</td>
</tr>
<tr>
<td>Contrasts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Julia vs Midnight w/ SCL†</td>
<td>1</td>
<td>0.1190</td>
<td>0.1190</td>
<td>0.05</td>
<td>0.8205</td>
</tr>
<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>1</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0</td>
<td>0.9967</td>
</tr>
<tr>
<td>SCL vs no SCL in Julia</td>
<td>1</td>
<td>6.6793</td>
<td>6.6793</td>
<td>2.99</td>
<td>0.1031</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>1</td>
<td>8.5444</td>
<td>8.5444</td>
<td>3.82</td>
<td>0.0682</td>
</tr>
</tbody>
</table>

† SCL, subsurface compaction layer.
Table 5. ANOVA of total N leaching loss from Julia and Midnight and orthogonal contrast F-test in Exp.2.

<table>
<thead>
<tr>
<th>Source</th>
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<th>Mean Square</th>
<th>F</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>3</td>
<td>126.8132</td>
<td>42.2711</td>
<td>7.45</td>
<td>0.0024</td>
</tr>
<tr>
<td>Error</td>
<td>16</td>
<td>90.78138</td>
<td>5.6738</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Compaction</td>
<td>1</td>
<td>85.2790</td>
<td>85.2790</td>
<td>15.03</td>
<td>0.0013</td>
</tr>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>8.0093</td>
<td>8.0093</td>
<td>1.41</td>
<td>0.2521</td>
</tr>
<tr>
<td>Compaction × Cultivar</td>
<td>1</td>
<td>33.5249</td>
<td>33.5249</td>
<td>5.91</td>
<td>0.0272</td>
</tr>
</tbody>
</table>

Contrasts

<table>
<thead>
<tr>
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<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julia vs Midnight w/ SCL†</td>
<td>1</td>
<td>4.3808</td>
<td>4.3808</td>
<td>0.77</td>
<td>0.3926</td>
</tr>
<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>1</td>
<td>37.1534</td>
<td>37.1534</td>
<td>6.55</td>
<td>0.0210</td>
</tr>
<tr>
<td>SCL vs no SCL in Julia</td>
<td>1</td>
<td>5.9326</td>
<td>5.9326</td>
<td>1.05</td>
<td>0.3217</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>1</td>
<td>112.8713</td>
<td>112.8713</td>
<td>19.89</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

† SCL, subsurface compaction layer.
Table 6. ANOVA of total N leaching loss from Julia and Midnight and orthogonal contrast F-test in Exp.3.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
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<td>27.1489</td>
<td>9.0496</td>
<td>11.01</td>
<td>0.0004</td>
</tr>
<tr>
<td>Error</td>
<td>15</td>
<td>12.3338</td>
<td>0.8223</td>
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<tr>
<td>Compaction</td>
<td>1</td>
<td>21.5787</td>
<td>21.5787</td>
<td>26.24</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cultivar</td>
<td>1</td>
<td>0.8464</td>
<td>0.8464</td>
<td>1.03</td>
<td>0.3264</td>
</tr>
<tr>
<td>Compaction × Cultivar</td>
<td>1</td>
<td>3.3175</td>
<td>3.3175</td>
<td>4.03</td>
<td>0.0629</td>
</tr>
</tbody>
</table>

Contrasts

<table>
<thead>
<tr>
<th>Contrasts</th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julia vs Midnight w/ SCL†</td>
<td>1</td>
<td>3.5489</td>
<td>3.5489</td>
<td>4.32</td>
<td>0.0553</td>
</tr>
<tr>
<td>Julia vs Midnight w/o SCL</td>
<td>1</td>
<td>0.4317</td>
<td>0.4317</td>
<td>0.52</td>
<td>0.4799</td>
</tr>
<tr>
<td>SCL vs no SCL in Julia</td>
<td>1</td>
<td>22.2159</td>
<td>22.2159</td>
<td>27.02</td>
<td>0.0001</td>
</tr>
<tr>
<td>SCL vs no SCL in Midnight</td>
<td>1</td>
<td>3.7656</td>
<td>3.7656</td>
<td>5</td>
<td>0.0492</td>
</tr>
</tbody>
</table>

† SCL, subsurface compaction layer.
Figure 1. Clipping yields of Julia and Midnight Kentucky bluegrass as affected by subsurface compaction layer (SCL). Bars shown are means of 5 replications, with SEs marked as error bars. a – Exp.1 (clippings harvested 3 times). b – Exp.2 (clippings harvested 4 times). c – Exp.3 (clippings harvested 3 times).
Figure 2. Biomass of Julia and Midnight harvested from lysimeters at the end of Exp.1. Bars are means of 5 replications. Error bars denote SEs. SCL, subsurface compaction layer. Top root, 0 - 8.5 cm below surface. Middle root, 8.5 - 14.4 cm below surface (location of SCL). Bottom root, 14.4 - 33.4 cm below surface.
**Figure 3.** Biomass of Julia and Midnight harvested from lysimeters at the end of Exp.3. Bars are means of 5 replications. Error bars denote SEs. SCL, subsurface compaction layer. Top root, 0 - 8.5 cm below surface. Middle root, 8.5 - 14.4 cm below surface (location of SCL). Bottom root, 14.4 - 33.4 cm below surface.
Figure 4. NO$_3$-N concentration in the leachate from Julia and Midnight Kentucky bluegrass, as affected by subsurface compaction layer in Exp.1. a - Julia with subsurface compaction layer. b - Midnight with subsurface compaction layer. c - Julia without subsurface compaction layer. d - Midnight without subsurface compaction layer. Five curves in each panel represent the 5 reps. Day 0 denotes the day when N treatment was given.
Figure 5. NO$_3$-N concentration in the leachate from Julia and Midnight Kentucky bluegrass, as affected by subsurface compaction layer in Exp.2. a - Julia with subsurface compaction layer. b - Midnight with subsurface compaction layer. c - Julia without subsurface compaction layer. d - Midnight without subsurface compaction layer. Five curves in each panel represent the 5 reps. Day 0 denotes the day when N treatment was given.
Figure 6. NO$_3^-$-N concentration in the leachate from Julia and Midnight Kentucky bluegrass, as affected by subsurface compaction layer in Exp.3. a - Julia with subsurface compaction layer. b - Midnight with subsurface compaction layer. c - Julia without subsurface compaction layer. d - Midnight without subsurface compaction layer. Panel a, c, and d each has 5 curves representing the 5 reps. One rep was dropped in b, thus 4 curves are plotted. Day 0 denotes the day when N treatment was given.