Turfgrass and Environmental Research Online

…Using Science to Benefit Golf

Researchers at the University of Rhode Island are studying how efficient different cool-season turfgrasses are at absorbing and utilizing nitrogen.

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PURPOSE

The purpose of *USGA Turfgrass and Environmental Research Online* is to effectively communicate the results of research projects funded under USGA's Turfgrass and Environmental Research Program to all who can benefit from such knowledge. Since 1983, the USGA has funded more than 215 projects at a cost of $21 million. The private, non-profit research program provides funding opportunities to university faculty interested in working on environmental and turf management problems affecting golf courses. The outstanding playing conditions of today's golf courses are a direct result of ***using science to benefit golf***.

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Differences in Nitrate Uptake and Metabolism Among Perennial Ryegrass and Creeping Bentgrass Cultivars

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SUMMARY

- Nitrate uptake and reduction, as well as biomass partitioning, was compared among nine cultivars each of perennial ryegrass and creeping bentgrass as these factors might contribute to preventing summer decline in cool-season turfgrasses.
- Nitrate uptake rates for solution-grown perennial ryegrass cultivars averaged 2.5 times greater than those for creeping bentgrass.
- Turf cultures of creeping bentgrass partitioned more than twice as much of their total biomass to roots as did perennial ryegrass cultures.
- Root specific nitrate reductase activity (NRA) was similar for both turfgrass species, but shoot-specific NRA in perennial ryegrass was more that twice that of creeping bentgrass.
- These findings demonstrated that creeping bentgrass metabolized 13.5% of the nitrate it absorbed within its roots while perennial ryegrass metabolized only 4% of its nitrate within its roots.
- If partitioning more total biomass and nitrate metabolism to its roots makes a turfgrass more tolerant of summer conditions, creeping bentgrass should experience less summer decline than perennial ryegrass.

To maintain high quality turf, the grounds manager must annually apply approximately 3 lbs of nitrogen per thousand square feet. This is equivalent to the nitrogen used for the production of many food crops where a substantial annual harvest is removed. Unless clippings are collected, nothing is harvested from turf and this raises the question of why nitrogen need be applied each year. Nitrogen must be lost in some way or it accumulates within the turf-soil ecosystem. This simple logic has prompted some ecologists to conclude that about 61% of all nitrogen applied to turf migrates as nitrate below the root zone and leaches into ground water (23).

This assumption is not supported by a substantial number of detailed studies that conclude little more than 5% of fertilizer nitrogen leaches as nitrate from turf receiving normal rates of fertilizer (18). The volatilization of nitrogen gas or nitrous oxide when turf becomes anaerobic (denitrification) can be significant, but this has not been demonstrated under field conditions (16). Similarly, loss of ammonia gas following applications of ammonium fertilizers or urea can occur, but this is easily prevented if these materials are not applied to wet turf and irrigation or rain follows application within a few hours (22).

If nitrogen losses from turf are small, where does the nitrogen go? A limited number of research reports indicate that nitrogen can accumulate within the turf-soil ecosystem. In his review of the fate of nitrogen applied to turf, Petrovic (18) cites studies that measured the amount of soil organic nitrogen under turf of different ages. During a 25-year period after turf

Figure 1. With the exception of greens, the golf course turf-soil ecosystem will accumulate substantial amounts of organic nitrogen that should, if properly exploited, meet the fertilizer needs of turf.

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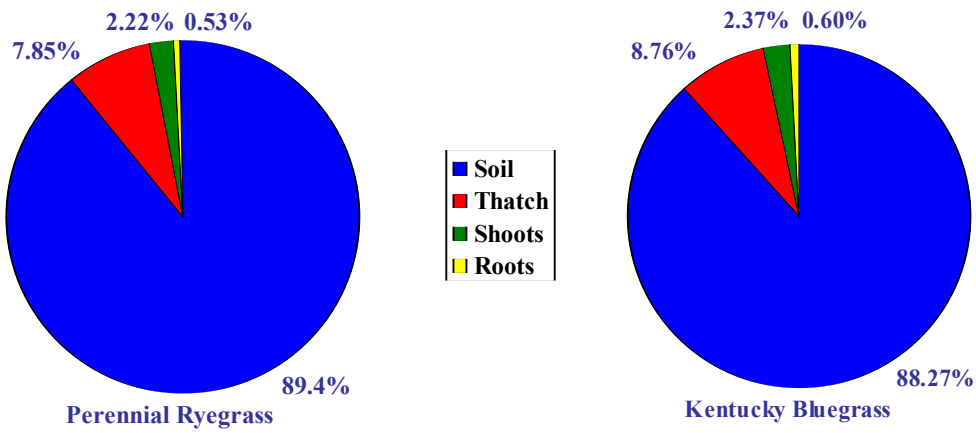

Figure 2. Nitrogen distribution in a long-established turf-soil ecosystem. The soil organic nitrogen amounted to about 2,300 lbs/acre in the upper six inches of soil.

establishment, the total nitrogen within the upper four inches of soil increased from less than 900 lbs/acre to more than 2100 lbs/acre. In some situations, similarly aged turf contained more than 4000 lbs/acre of soil organic nitrogen. Our investigation (11) of a fine silt loam soil planted to turf for more than 30 years also revealed total organic nitrogen levels in the upper six inches of soil at more than 2000 lbs/acre (Fig. 2). In this case, the turf had been fertilized erratically for much of its history and had received 150 lbs N/acre during the previous four years. A recent study by Qian and Koski (19) analyzed soil organic matter increase of golf course soils in Colorado, and they also observed a progressive increase for 25-30 years after establishment.

If nitrogen does accumulate within the turf-soil system to the levels indicated above, it might be asked why a mature turf requires any fertilizer nitrogen. Most turf managers would agree that high quality mature turf can be maintained using less nitrogen than would be needed for a recent installation. Annual nitrogen rates are often reduced by 1/3 to 1/2 with no loss of turf quality. However, even applying 50 to 75 lbs N/acre/year might seem unnecessary if several thousand pounds of nitrogen are already present within the turf-soil system. The continued need for fertilizer nitrogen probably reflects a failure of soils to deliver nitrogen when cool-season turfgrasses need it most, or turfgrasses are not efficient in recovering and storing nitrogen when it is available.

Nitrogen Availability from Soil

In our study, organic matter in the upper four inches of soil was present as complex organic molecules that contributed about 0.25% nitrogen and 3.5% carbon (~6.6% organic matter) to the soil mass. The resulting C:N ratio of 14 is sufficiently low that this organic matter should be an excellent substrate for soil microbes. However, these molecules are highly resistant to microbial decomposition and are slowly degraded thereby gradually releasing their nitrogen as ammonium ions (mineralization).

This soil organic matter can be viewed as a slow-release nitrogen source. Plant residues including dead roots, rhizomes and degradation products from thatch are more rapidly metabolized by soil microbes, but they represent a small portion of total nitrogen present (Fig. 2). However, these residues do contribute to the mineralized ammonium released into the soil. Ammonium ions are absorbed by plant roots and soil microbes, but most is used as an energy

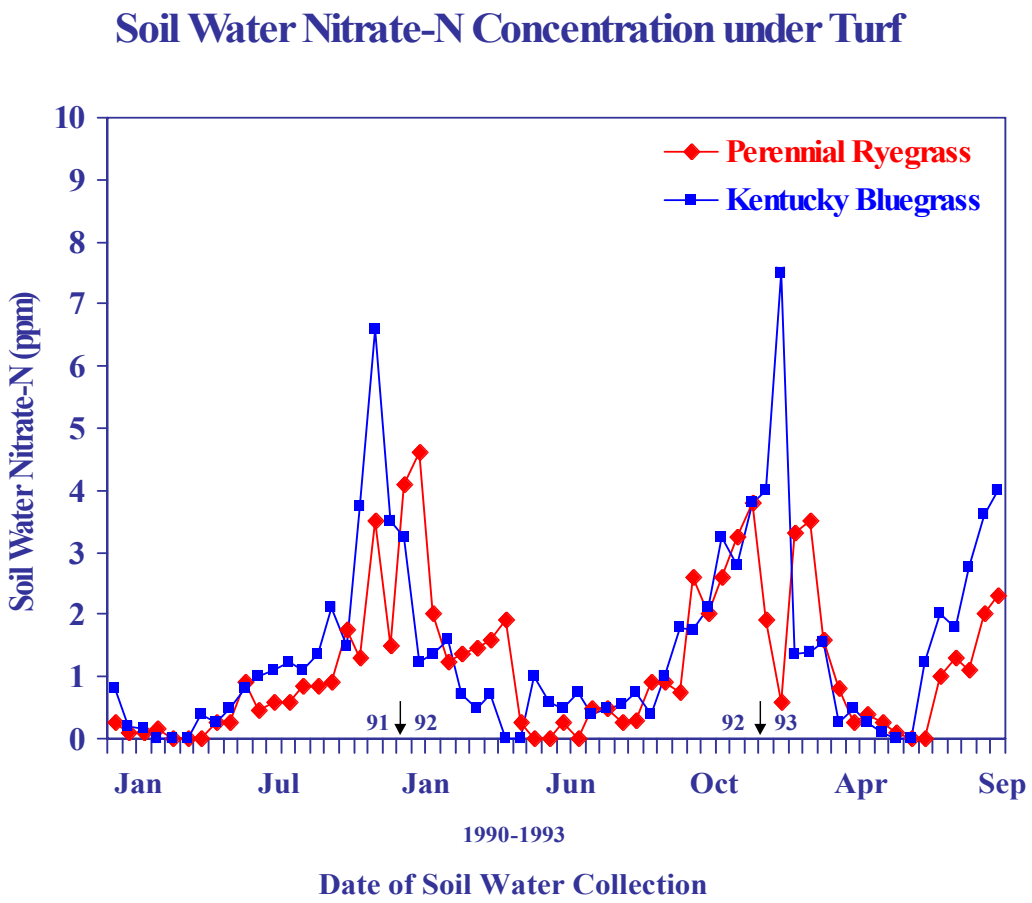

Figure 3. Nitrate-N concentrations in soil water two feet under turf during the period Dec. 1990 through Sept. 1993.

source by chemoautotrophic bacteria. There, ammonium is oxidized to nitrate (nitrification). Because these bacteria are active in most soils, there is little inorganic nitrogen other than nitrate available to turfgrasses.

Because mineralization and nitrification occur through the action of soil microbes, these processes depend upon soil conditions that favor microbial activity: abundant moisture, available oxygen and mild temperatures. During cold winters and dry summers, soils do not support the microbial release of nitrogen from organic matter and the supply of available nitrogen to turf can become limiting. For cool-season turfgrasses, this is most likely to be a problem during the spring when demand for nitrogen by turf is high, but soils are still cold and mineralization is suppressed. This is evident from the low amounts of nitrate detected in soil water (Fig. 3) during the spring months (6).

During hot summer conditions, most cool-season turfgrasses experience a marked reduction in root growth and the death of many roots (12). This summer decline in turfgrass root mass is the result of many factors including drought and reduced root-zone oxygen levels (5), but much is related directly or indirectly to the sensitivity of roots to high temperatures. Thus, when soil temperatures increase during late spring and early summer, they quickly pass through the optimum range for root growth and into the inhibiting range. This will affect shallow roots more than deep roots because soil temperatures remain cooler with increasing depth.

As the root system becomes less effective in absorbing water and nutrients, the leaves become subjected to greater heat and drought stress that eventually causes a cessation of growth and even death. With fewer functioning turfgrass roots available to absorb nitrate, nitrogen can accumulate within the soil to levels that may permit substantial nitrate leaching during periods of

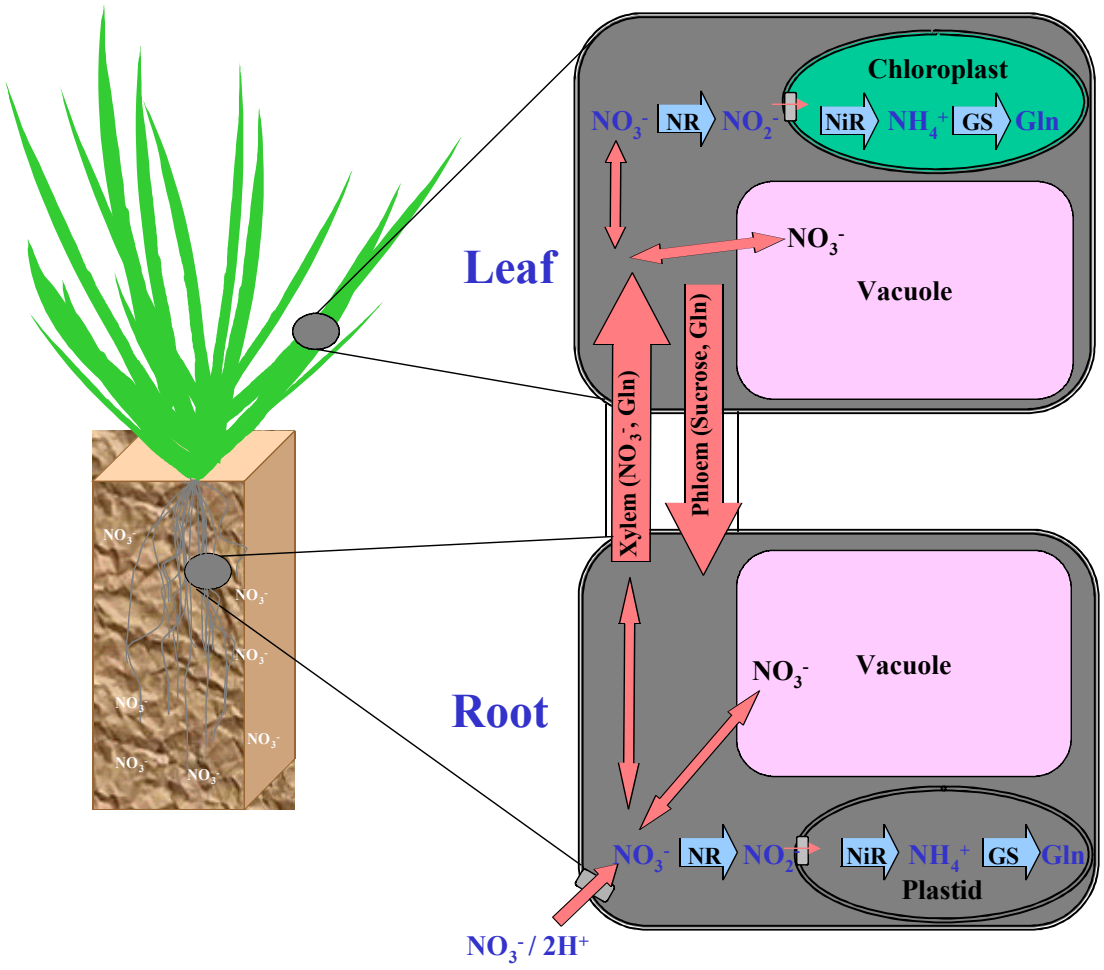

Figure 4. Outline of nitrate metabolism and transport between roots and shoots of cool-season turfgrasses.

heavy rain or irrigation.

Maintaining root function

The above analysis suggests that the prevention of summer turf decline and the protection of ground water quality depend on extending root vitality and growth throughout the summer period. This is obviously a complex problem involving both turfgrass management and physiological factors. Carrow (5) has provided a good discussion of turf management practices that will minimize summer decline of bentgrass turf, but the physiological remedies are less well understood. A simplistic approach would suggest that maintaining the transport of adequate photosynthetic product from leaves to roots throughout the summer should do much to prevent root decline. During the winter and early spring, more than 20% of current photosynthetic products are translocated to turfgrass roots, but this declines to <1% during mid-late summer (10).

There are many causes for this seasonal shift in energy partitioning within cool-season turfgrasses, but one obvious contributing factor is the dramatic stimulation of shoot growth when temperatures become favorable and nitrogen is readily available. This marked increase in shoot growth normally occurs at the expense of root growth, i.e. less photosynthetic product is transported to the roots. The problem is aggravated by the wasteful stimulation of photorespiration in leaves by high temperatures (9), but it would be less of a problem if the growth of roots rather than shoots was promoted during summer conditions.

The stimulation of shoot growth during summer conditions is caused to a considerable extent by elevated soil nitrate concentrations. Nitrate availability in summer is inevitable in a turf environment due to the stimulated mineralization of soil organic nitrogen by elevated temperatures. It doesn't matter if fertilizer nitrogen is applied during the summer or not, soil microbial activity will degrade organic matter more rapidly and increase the supply of available nitrogen mostly as nitrate.

Healthy turfgrass roots are efficient in absorbing soil nitrate both from thatch and soil. Bowman et al. (3) observed that one pound of nitrate-nitrogen irrigated into 1000 sq-ft of a Kentucky bluegrass turf was 70% absorbed within 24 hours and within 48 hours, it was all absorbed. This efficient uptake of nitrate has been demonstrated in several cool-season turfgrasses (15). In the spring when soils are cool and roots are healthy, nitrate uptake is rapid and soil nitrate levels are kept low thereby reducing the chance for nitrate leaching (Fig. 3). As root systems increase in size, nitrate uptake will be more complete.

However, much of the nitrate absorbed by roots is rapidly transported via the xylem to the leaves where is acts as a signal diverting photosynthate from sucrose or starch to organic acids (21). These organic acids are required to assimilate nitrogen into amino acids where they promote vegetative growth. Sucrose is the sugar most often transported to roots where it provides energy for root function and growth. When nitrate in leaves promotes amino acid synthesis instead of sucrose, leaf growth is stimulated while roots are starved. As the season progresses and soils warm further, mineralization increases, nitrate becomes increasingly available, uptake by roots in excess of need triggers the nitrate signaling scenario outlined above, and the decline of roots occurs rapidly. The secret for maintaining healthy turf during hot weather is preventing this nitrate stimulated decline of the grass root system.

Nitrate use by turf

Nitrate absorbed by roots can follow one of three paths. It can be stored in the vacuoles of root cells, it can be transported to the leaves or it can be reduced to ammonium and assimilated into amino acids (17 & Fig. 4). Nitrate cannot be used directly by plants, but must first be reduced to ammonium that is then assimilated into amino acids used for the synthesis of proteins and all nitrogenous compounds required by plants. This process requires much energy in the form of reducing power and a carbon compound that binds with ammonium to form amino acids. If produced in the roots, these amino acids can stimulate root growth providing sufficient resources from leaves are available, or they can be transported to leaves.

If sufficient energy for nitrate metabolism is not available in root cells, the nitrate can be loaded into vacuoles and stored or transported into xylem vessels and carried to the leaves via the transpiration stream. Once in leaf cells, nitrate either can be reduced to ammonium and assimilated into amino acids, or stored in vacuoles. In leaves, photosynthetic energy would be available to reduce and assimilate nitrate during daylight hours or respiratory energy during darkness. In any event, nitrate is likely to be metabolized in leaves with storage occurring only when delivery from roots exceeds the rate of leaf nitrate reduction.

In both roots and leaves, nitrate reduction is initiated by the enzyme nitrate reductase (NR) that catalyzes formation of nitrite. Within the cell, nitrite is transported into plastids where it becomes ammonium. Without exiting the plastid, ammonium is bound to glutamic acid forming glutamine that is the first organic form of nitrogen produced in a plant from inorganic nitrogen. Through a series of transfer reactions, this glutamine-nitrogen can be used as substrate for the synthesis of most nitrogen compounds present in plants.

As mentioned above, the presence of excess nitrate in leaves promotes a diversion of photosynthate from sucrose that is transported to and supports root growth to amino acids that are the product of nitrate metabolism and stimulate

		Mean quality ratings	
		RI	National
	Cultivar	Mean	Mean
Perennial ryegrass	Palmer III	6.5*	6.3*
	Secretariat	6.3	6.2
	Calypso II	6.3	6.2
	Saturn II 5.9	5.8	
	Manhattan III	5.5	6.0
	Morning Star	5.6	5.8
	Nighthawk	5.4	5.7
	Figaro	4.5	4.9
	Linn	2.4	3.5
	LSD (0.05)	0.6	0.1
Creeping bentgrass	L-93	6.6	6.6
	Penn G-2	6.3	6.3
	Providence	5.7	6.3
	Southshore	5.6	6.1
	Pennlinks	5.2	5.9
	SR 1020	4.9	5.9
	Penncross	4.0	5.5
	18th Green	3.9	5.5
	Seaside	3.4	4.4
	LSD (0.05)	0.7	0.1

* Scores 1-9 (9=Ideal turf)
† USDA, ARS, NTEP Final Report 1995-98 No. 99-11
‡ USDA, ARS, NTEP Final Report 1994-97 No. 98-12

Table 1. Mean quality ratings of nine perennial ryegrass† and nine creeping bentgrass‡ cultivars from the National Turfgrass Evaluation Program

shoot growth (21). This signaling function of nitrate strongly regulates the mass ratio between roots and shoots and in cool-season turfgrasses, is likely a major reason for the dramatic decline in root growth and function during the heat of summer.

Root uptake of soil nitrate must be efficient in order to utilize this valuable resource and minimize leaching of nitrate into ground water. However, if nitrate is translocated to the shoots, it will reduce resources available to the roots causing their decline. It becomes important, therefore, that nitrate be retained in roots and metabolized there so root growth will be stimulated and a large root:shoot mass ratio can be maintained. The research in our laboratory is currently studying the possibility of achieving this favorable pattern of nitrate utilization in turf with a view toward reducing summer turfgrass decline.

Before attempting to alter the distribution of nitrate metabolism and photosynthate partitioning in turfgrasses during summer conditions, it is important to know the magnitude of genetic variation in relevant characteristics of turfgrasses currently in use. We need to know how cultivars of turfgrass species vary in their ability to absorb soil nitrate, to metabolize nitrate in their roots and leaves, and to partition their biomass between roots and shoots. With such information, we will know if the genetic potential currently exists within turfgrass species to breed more nitrogen-efficient cultivars, or if appropriate genes must be introduced from other species. This report attempts to assess the variation in these plant properties among nine cultivars each of creeping bentgrass and perennial ryegrass.

Nitrate uptake capacity

Cultivars of creeping bentgrass and perennial ryegrass were selected based on their performance in the National Turfgrass Evaluation Program (NTEP). Selections were made to include grasses of diverse genetic background that performed well and not so well in the NTEP trials (Table 1). Seeds of each cultivar were germinated under intermittent mist on washed silica sand. After 20 days, seedlings were washed free of sand and groups of thirty were transplanted into 250-ml Erlenmeyer flasks containing quarter-strength, N-free, aerated, modified Hoagland's solution (8). The flasks were first painted black followed by silver to reduce heating of the solution and exclude light to prevent the growth of algae. While all macronutrients were set at quarter-strength, micronutrients were maintained at the full-strength Hoagland's solution. Nitrate concentration was set at 1.0 mM (14 ppm NO_3-N) using $NaNO_3$. Solutions were replaced weekly and tap water was added daily to replace evapotranspiration loss.

These turfgrass cultures were maintained for 30-50 days in a controlled environment room having a photoperiod of 16-hours at a photosynthetic photon flux density (light) of 800 µmole/m2/s at plant canopy level provided by sodium vapor lamps and fluorescent tubes. This was a little more than one-third of full sunlight.

The day/night temperatures were maintained at 77/68°F. Turf cultures were mowed weekly at a two inch height using sharp scissors. The growth room was disinfected thoroughly after each experiment to prevent insect or disease problems.

The rate of nitrate uptake by turfgrass cultures was determined by measuring the depletion of nitrate from their nutrient solution (15). At the beginning of an uptake experiment, turf cultures were transferred to clean flasks containing fresh 1/4-strength Hoagland's solution with the initial nitrate concentration set at 1 mM using $NaNO_3$. Twenty four hours later, the final solution volume and its nitrate concentration was determined as was the mass of roots present. Flasks of nutrient solution lacking turfgrass plants were included in each experiment to determine if nitrate loss would occur in the absence of plants. It did not. Nitrate concentrations were measured using the copperized cadmium reduction method described by Keeney and Nelson (14). Nitrate uptake rates (NUR) were calculated using the equation:

$$\mathbf{NUR = \{[Vi(Ci) - Vf(Cf)]/R\}/t}$$

Where Vi and Vf = initial and final solution volumes, respectively and Ci and Cf = the initial and final nitrate concentrations, respectively. R = root fresh weight and t = duration of uptake = 24 hr. Nitrate uptake was expressed as µmole NO3- absorbed from culture solution per gram of root mass per hour (µmol/g/hr).

Mass distribution between roots and shoots

Each gram of fresh perennial ryegrass roots absorbed more than twice as much nitrate as did a gram of creeping bentgrass roots (Table 2). Relatively little difference in nitrate uptake was noted among cultivars of each grass species. Among perennial ryegrass cultivars, only Secretariat absorbed nitrate more rapidly than Linn and among creeping bentgrass cultivars, only Penncross exhibited an uptake rate greater that that of 18th Green.

Following the completion of each nitrate uptake experiment, turfgrass cultures of each cultivar were separated into roots and shoots (leaves plus stems) and each fraction weighed. The root mass of each culture was divided by its shoot mass providing a root:shoot mass ratio. These values are summarized in Table 3.

The total biomass of perennial ryegrass and creeping bentgrass cultures was similar averaging 6.95 and 7.46 grams fresh weight, respectively. However, the perennial ryegrass cultures partitioned only about half as much of their biomass to roots as did creeping bentgrass cultures, 12.7% and 26.8%, respectively. The greater root production of creeping bentgrass may explain, in part, why this grass can tolerate much closer mowing than can perennial ryegrass even under stressful conditions.

While significant differences in shoot and root growth were observed among cultivars of both turfgrass species, there never was much more than a two-fold difference in shoot or root mass among cultivars of either species. The relationship between biomass partitioning to roots

		NO_3^- uptake rate
	Cultivar	µmole/g FW/hr†
Perennial ryegrass	Secretariat	10.8a*
	Nighthawk	9.7ab
	Figaro	9.3ab
	Calypso II	8.6ab
	Saturn II	8.0ab
	Morning Star	8.0ab
	Manhattan III	7.2ab
	Palmer III	7.0ab
	Linn	5.8b
	Mean	8.29
Creeping bentgrass	Penncross	4.0a
	Penn G-2	3.8ab
	SR 1020	3.7ab
	Seaside	3.2ab
	Pennlinks	3.2ab
	Providence	3.1ab
	Southshore	2.7ab
	L-93	2.7ab
	18th Green	2,6b
	Mean	3.24

† FW = fresh root weight

* Means within a column for each species followed by the same letter are not significantly different at the 5% confi dence level.

Table 2. Nitrate uptake rates for nine cultivars each of perennial ryegrass and creeping bentgrass measured for 24 hours.

(root:shoot ratio) and turf quality was inconsistent. Palmer III perennial ryegrass and L-93 creeping bentgrass exhibited the highest quality scores for their species and there root:shoot ratios were also among the highest observed. This is what we would predict.

However, Calypso II perennial ryegrass and Penn G-2 creeping bentgrass also produced high quality turf but had among the lowest root:shoot ratios for their respective species. This is also not surprising since our plants were grown with adequate nitrogen (14 ppm NO_3-N) and were not subjected to environmental stress. Thus, any limitation in nitrate or water uptake efficiency due to inadequate root mass might not be exhibited under the conditions our turf cultures were grown. While the difference in biomass partitioning between the two grass species was significant, differences among cultivars of either species were only marginally so.

	Root	Shoot	Root:Shoot Ratio
	(grams fresh weight)		
Perennial ryegrass			
Palmer III	1.32a*	6.60ab*	0.20a*
Morning Star	0.78bcd	4.33bc	0.18a
Figaro	0.79bcd	4.67bc	0.17ab
Linn	1.17ab	7.61a	0.15ab
Manhattan III	1.10abc	7.78a	0.14ab
Saturn II	0.92bc	6.37ab	0.14ab
Secretariat	0.50d	3.74c	0.13ab
Calypso II	0.65cd	6.68ab	0.10b
Nighthawk	0.72cd	6.88ab	0.10b
Mean	0.88	6.07	0.15
Creeping bentgrass			
18th Green	1.62ab*	3.33b*	0.49a*
L-93	2.53a	6.23a	0.41ab
Southshore	2.39a	6.40a	0.38ab
Seaside	2.20ab	5.98a	0.38ab
SR 1020	2.28ab	6.12a	0.37ab
PennLinks	1.31b	3.59b	0.36ab
Providence	2.17ab	6.27a	0.35ab
Penncross	1.62ab	4.64ab	0.35ab
Penn G-2	1.84ab	6.62a	0.28b
Mean	2.00	5.46	0.37

* Means within a column for each species followed by the same letter are not significantly different at the 5% confidence level.

Table 3. Root and shoot fresh weights of nine solution cultured cultivars each of perennial ryegrass and creeping bentgrass.

Nitrate reductase activity (NRA)

Since the first step in nitrate metabolism requires the enzyme nitrate reductase to reduce nitrate to nitrite, turfgrass cultivars were evaluated for their nitrate reductase activity (NRA) in leaves and roots. The NRA of a plant tissue should indicate the capacity of that tissue to metabolize nitrate. There are two NRA determinations commonly in use; *in vivo* and *in vitro* assays. The i*n vivo* assay for NRA was used in this investigation because it should best reflect the activity of the NR enzyme as it exists within the cells of plant tissue. We adapted the method of Hageman and Reed (7) to leaf and root tissues of perennial turfgrasses.

On average, the shoot specific NRA of perennial ryegrass was 132% greater than that of creeping bentgrass (Table 4), while the root NRA was similar for both species. Greater variation in NRA was observed in shoots of perennial ryegrass than in creeping bentgrass. Among perennial ryegrass cultivars, shoot NRA of Secretariat was 173% greater than that of Linn, while shoot NRA of SR-1020 creeping bentgrass was only 96% greater than that of Pennlinks. Here highest and lowest values are compared in both species. Root specific NRA varied little among the nine cultivars of both species. No significant differences were observed in root NRA of perennial ryegrass and only two creeping bentgrass cultivars differed in root NRA (SR 1020 > Penncross).

Figure 5 compares NRA in leaves and roots of perennial ryegrass as assayed using *in vivo* and *in vitro* methods. The *in vitro* assay used was very similar to that described by Kaiser and Huber (13). It is evident that the NRA in leaves is substantially greater that that in roots when measured by either method. The *in vitro* NRA in leaves was about three times greater than the *in vivo* activity except in plants cultured at the lowest nitrate concentration where they were about the same. The much reduced NRA in the leaves of plants cultured at 0.14 ppm nitrate-N, especially when using the *in vitro* assay, may indicate incomplete induction of the enzyme by the low nitrate level. The lower leaf NRA measured using the *in vivo* assay may indicate that within the leaf tis-

	Nitrate reductase activity (µmol NO_2^-/g FW/hr)	
	Root	Shoot
Perennial ryegrass		
Secretariat	0.50a*	4.01a*
Saturn II	0.46a	3.49ab
Manhattan III	0.28a	3.29abc
Calypso II	0.39a	3.28abc
Figaro	0.39a	2.79bcd
Nighthawk	0.53a	2.70bcd
Morning Star	0.38a	2.48cd
Palmer III	0.56a	1.95de
Linn	0.38a	1.47e
Mean	0.43	2.83
Creeping bentgrass		
SR 1020	0.69a	1.61a
Southshore	0.59ab	1.49ab
L-93	0.48ab	1.45ab
Penncross	0.27b	1.32ab
Penn G-2	0.48ab	1.27ab
Providence	0.49ab	1.17ab
Seaside	0.38ab	0.95ab
18th Green	0.35ab	0.89ab
PennLinks	0.34ab	0.82b
Mean	0.45	1.22

* Means within a column for each species followed by the same letter are not significantly different at the 5% confi dence level.

Table 4. Nitrate reductase activity in roots and shoots of nine cultivars each of perennial ryegrass and creeping bentgrass based on an in vivo assay method.

sues, reducing power (NADH) was less available under the dark conditions of that assay or more likely the enzyme was partly inhibited (phosphorylated). Whatever the reason for its lower activity, the *in vivo* assay is much less disruptive of the leaf tissue and may more closely approximate the enzyme activity occurring in leaves under field conditions.

Root NRA was very low as measured by the *in vivo* method, but virtually nonexistent when measured by the *in vitro* assay (Fig. 5). Since the *in vitro* assay should normally provide larger NRA values, their absence in perennial ryegrass roots grown in four nitrate concentrations suggests that the *in vivo* values may be erroneous. The *in vivo* assay for root NRA involved culturing roots in 50 mM nitrate for two hours. Over that duration at such a high nitrate level, NR induction may have occurred sufficiently to yield measurable NRA when none actually was present in the roots under the growing conditions of this experiment (24). This would explain the very low root NRAs reported in Table 4 for perennial ryegrass and the lack of any significant variability among the nine cultivars.

We are not ready to dismiss the root NRA we detected using the *in vivo* method as artifact. Bowman and Paul (2) reported that Manhattan II perennial ryegrass, deprived of nitrogen for seven days, reduced most of the nitrate within its roots and little in shoots during the first eight hours after nitrate was again supplied. Between 8 to 16 hours after nitrate addition, the site of reduction shifted to the shoots. Apparently, perennial ryegrass roots have the potential for substantial

	Potential total NRA	
	root:shoot ratio	Percent root contribution to total potential NRA
Perennial ryegrass		
Linn	0.04ab*	5.92a*
Palmer III	0.06a	5.27ab
Figaro	0.02bc	3.96ab
Morning Star	0.03bc	3.32ab
Saturn II	0.02bc	2.36ab
Secretariat	0.02bc	2.05ab
Nighthawk	0.02bc	1.96ab
Manhattan III	0.01c	1.52b
Calypso II	0.01c	1.34b
Mean	0.03	3.87
Creeping bentgrass		
18th Green	0.19a	17.4a
Providence	0.14a	15.8a
SR 1020	0.16a	15.2a
Southshore	0.15a	14.6a
Pennlinks	0.15a	13.5a
Seaside	0.14a	13.0a
L-93	0.13a	12.1a
Penn G-2	0.11a	10.3a
Penncross	0.07a	9.8a
Mean	0.17	13.5

* Means within a column for each species followed by the same letter are not significantly different at the 5% confi dence level.

Table 5. Potential root:shoot ratio of total nitrate reductase activity and percent root contribution to potential total nitrate reductase activity of nine perennial ryegrass and nine creeping bentgrass cultivars.

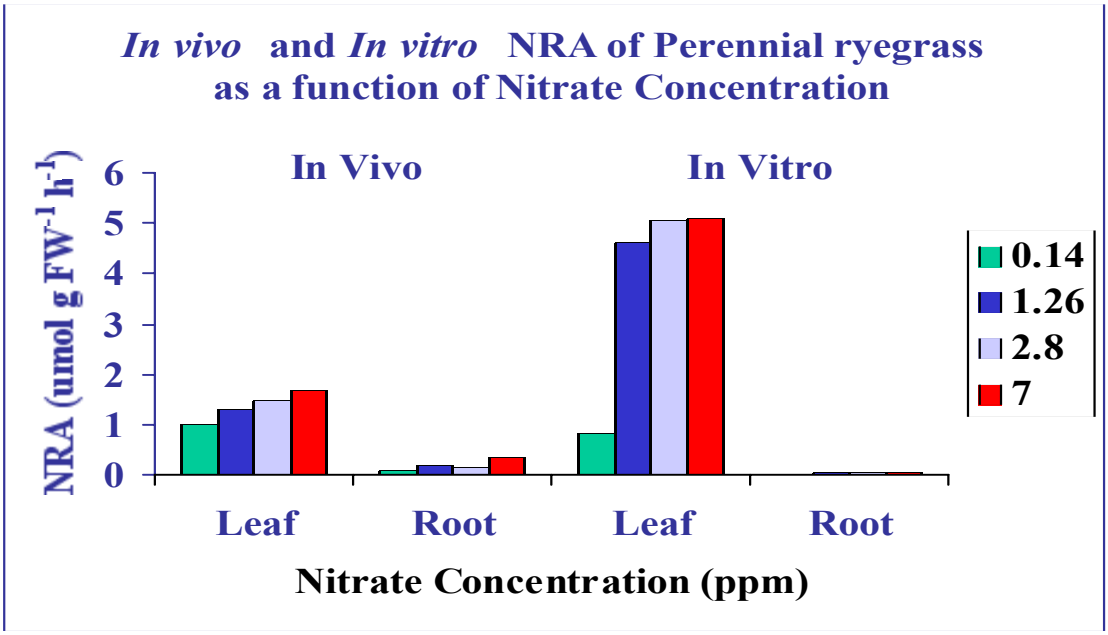

Figure 5. Comparison of *in vivo* and *in vitro* nitrate reductase assays in leaves and roots of perennial ryegrass maintained at four nitrate levels.

nitrate reduction, and their failure to do so is the result of enzyme inhibition under conditions of abundant nitrate.

Partitioning of NRA within turfgrass plants

To determine how NRA is distributed within turfgrass plants, the specific NRA of roots or shoots was multiplied by the total mass of roots or shoots for each turf culture. The total NRA for roots was then divided by the total NRA for shoots to give a total plant NRA root:shoot ratio (Table 5). The total NRA for roots and shoots of a single turf culture were added together and the percent of that total contributed by roots and shoots was calculated (Table 5).

The distribution of NRA between roots and shoots differed between the two species with perennial ryegrass averaging 3.9% of total NRA in its roots, while in creeping bentgrass, roots accounted for 13.5% of the total NRA. The values obtained should be viewed as estimates of potential NRA that may not be realized in the field where nitrate is more likely to be limiting. Since the specific root NRA of both grass species was similar (Table 4), the greater contribution by roots to whole-plant NRA in creeping bentgrass was largely due to its greater root mass.

The nitrate uptake rate per gram of perennial ryegrass roots was more than double that of creeping bentgrass (Table 2). Since the root-specific NRA was similar for both species, perennial ryegrass roots should transport twice as much nitrate to their leaves as do roots of creeping bentgrass. If leaf nitrate levels regulate the partitioning of photosynthate between shoots and roots, the greater root mass of creeping bentgrass might result from less photosynthate diversion to shoot growth because less nitrogen is delivered to shoots as nitrate (4).

Among the nine cultivars of perennial ryegrass, only Linn exhibited more NRA in its roots than did Calypso II and Manhattan III. No significant differences in the partitioning of NRA between roots and shoots was observed among the nine creeping bentgrass cultivars. No positive correlation was observed between the amount of nitrate metabolized in roots and turfgrass performance in the field (quality scores) for either grass species.

However, it should be noted that none of

the grasses evaluated in this study metabolized much nitrate in their roots, and only slight variations in root NRA among cultivars of either species was observed. Therefore, it is unlikely that the contribution of nitrate metabolism in turfgrass roots toward increasing nitrogen use efficiency or stress tolerance of turf was effectively evaluated by this study. It is evident that among cultivars of the two species, little variation in rates or location of nitrate metabolism was observed. Even mass partitioning to roots did not exhibit much variation among cultivars of the two species studied. Therefore, any major alterations in these characteristics will require the importation of genetic traits from external sources.

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