TURFGRASS TRENDS

NITROGEN ASSIMILATION

Leaf Analysis Offers Window Into Proper Fertilization Schedule

By Zhongchun Jiang

Proper nitrogen (N) management is critical for maintaining high-quality turf while conserving resources and protecting the environment. This is in part because N applications affect turfgrass growth directly and disease susceptibility indirectly. N application can be costly, not only because of the expenditures on N fertilizers. More importantly, costs rise because of the maintenance costs associated with the excessive shoot growth, poor root growth, and high disease susceptibility that often result. Such ill-timed and excessive N applications can potentially cause runoff, ammonia volatilization, nitrate leaching and groundwater contamination.

Turfgrass maintenance professionals have often relied on empirical knowledge-based fertilization programs to decide when and how often to apply N fertilizers. Chlorophyll meters and near infrared reflectance spectroscopy have been used in research to schedule N applications (Rodriquez and Miller, 2000a, 2000b).

Here is a surprising discovery: Turfgrass assimilates nitrate at high rates during the night.

These technologies provide the means to estimate the content of total N in turfgrass leaves. However, readings of a chlorophyll meter or a near infrared reflectance spectrometer do not indicate how much N is stored in the vacuoles of leaf cells and how fast the stored, unassimilated N will be assimilated into chlorophylls and other N compounds. The amount of the leaf-stored unassimilated N and the rate of N assimilation must be known before one can attempt to estimate when and how much a turfgrass needs supplemental N. Research on nitrogen assimilation will help turfgrass managers in this regard.

Nitrate assimilation, not ammonium

There are numerous brands of N fertilizers with different chemical analyses, but the inorganic N forms that turfgrasses take up from the soil water are only two: ammonium (NH_4^+) and nitrate (NO_3^-) (Figure 1).

In addition to fertilizers such as ammonium sulfate that provide ammonium directly, ammonium is produced through hydrolysis from urea and through mineralization from organic fertilizers and organic matter in the soil. Ammonium is an ion that carries one positive charge, which is attracted to negatively charged soil colloids. When the soil is anaerobic, ammonium stays in the soil and turfgrass roots can absorb it when oxygen becomes available. When the soil is acidic (pH above 8), some of the ammoni-

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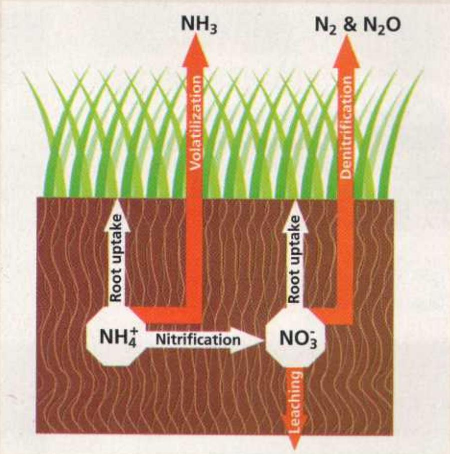
FIGURE 1

Fate of nitrogen fertilizers in turf systems



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um ions become ammonia gas (NH_3) and can be lost as ammonia escapes into the atmosphere (Figure 1).

Turf soils are often aerobic and mildly acidic. Under such conditions, most of the ammonium is converted to nitrate through nitrification (Figure 1). Some quick-release fertilizers provide nitrate directly. In general, turfgrass roots see more nitrate ions than ammonium ions, and nitrate is the predominant form of inorganic N available to turfgrass roots. In addition, nitrate is safer to the turfgrass than ammonium because high concentrations of ammonium are toxic to plant cells (Jiang et al., 2001b).

Nitrate can be accumulated at high concentrations in turfgrass leaves without apparent toxicity (Jiang and Hull, 2000).

Nitrate assimilation pathway

Once the roots have absorbed nitrate from the soil water, the turfgrass can store and assimilate nitrate in the roots (Figure 2). Nitrate is stored in the vacuole of the cell. If roots cannot assimilate nitrate at a rate that meets the needs of the turfgrass for N compounds, such as amino acids and proteins, the turfgrass transports nitrate to the leaves and assimilates it there to meet its needs.

In fact, research shows that cool-season turfgrasses cannot assimilate nitrate in roots at a sufficient rate (Jiang and Hull, 1998, 1999, 2000; Jiang et al., 2001a; Bushoven et al., 2002). Leaves are the primary sites of nitrate assimilation in these turfgrass species. For example, in Kentucky bluegrass, leaf blades contribute as much as 96 percent to 98 percent of the plant total nitrate reduction at nitrate concentrations above .15 micromoles (*mM*) or 2.1 parts per million. Efforts can be made to develop new varieties that assimilate nitrate in roots at rates high enough to meet the plant needs. In the meantime, we can practically focus on leaf nitrogen assimilation potential (NAP) alone to ascertain the NAP of the whole turfgrass plant.

In leaf cells, nitrate is first converted to nitrite in a chemical process called oxidation-reduction (Figure 3). Oxidation is the chemical process in which a chemical compound loses one or more electrons, often to the oxygen atom. On the other hand, reduction is the process in which a chemical compound accepts one or more electrons. When nitrate is reduced to nitrite, the N atom in the nitrate ion gains two electrons and the N in the nitrite ion is reduced by the same number. These two electrons come from the reduced form of nicotinamide adenine dinucleotide or NADH. When NADH gives its electrons to nitrate, NADH is oxidized and becomes NAD^+. This oxidized NAD^+ goes into the mitochondrion, during which NAD^+ gains two electrons from carbohydrates and is reduced to NADH. The NADH produced in mitochondria can be exported to the cytosol and be used again to reduce another nitrate ion.

The product of nitrate reduction is nitrite, which is immediately transported into the chloroplast where nitrite is reduced to ammonium (Figure 3). The enzyme nitrite reductase (NiR) catalyze the reduction of nitrite to ammonium. Here, the N atom in nitrite gains six electrons from six molecules of reduced ferredoxin (Fd red). Fd red is produced in the chloroplast only under light.

During the day, if leaves are placed in total darkness, no new Fd red can be produced and the reduction of nitrite to ammonium cannot be sustained. Therefore, on cloudy days, nitrate reduction is slowed and eventually stopped due to nitrite accumulation and feedback inhibition.

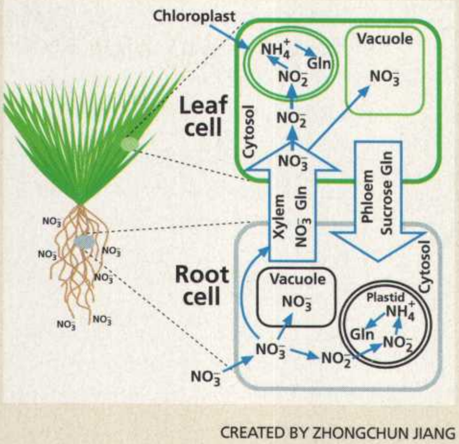
When there is sufficient light, ammonium produced in nitrite reduction and in photorespiration is immediately assimilated into glutamine, one of the 20 amino acids in living organisms. In leaves, ammonium assimilation occurs in chloroplasts. The enzyme glutamine synthetase (GS) catalyzes ammonium assimilation into glutamine. This step is not an oxidation-reduction reaction and no electron donors are needed, but it requires adenosine triphosphate (ATP), the energy currency in living organisms. Glutamine carries the N throughout the plant and donates it to the production of other molecules such as chlorophylls and proteins. Until a new molecule of glutamine is synthesized from ammonium and organic acids, nitrate-N is not assimilated.

Nitrite is a smaller ion than nitrate ion and is toxic to plant cells at high concentrations. Nitrite is also toxic to humans because it binds with hemoglobin to form methemoglobin. Hemoglobin carries oxygen throughout our bodies but methemoglobin does not. When young babies are fed with food or water that contains high concentrations of nitrate, it is converted to nitrite in the gastrointestinal tract. Nitrite is then absorbed into blood, reacting with hemoglobin and interfering with oxygen supply. The baby turns blue, a symptom referred to as blue baby syndrome.

Ammonium is also toxic at high concentrations. In fact, L-phosphinothricin-based herbicides kill plants by inhibiting GS, blocking ammonium assimilation and causing rapid accumulation of ammonium to lethal concentrations in growing leaves. As a result, the plant dies. This effect has been observed in creeping bentgrass (Jiang et al., 2001b).

FIGURE 2

Fate of nitrate in turfgrass plants



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Due to the toxicities of nitrite and ammonium ions, the plant cell must control the reduction of nitrate to nitrite precisely so that it meets its needs for assimilated N forms such as amino acids and proteins while causing no harm to itself.

There are three ways turfgrass leaves can control the nitrate reduction process: do not supply nitrate, do not supply NADH, and/or deactivate the enzyme nitrate reductase (NR) (Figure 3). NR is an enzyme that catalyzes the reaction between nitrate ion and NADH. If leaf samples do not contain active NR, the reduction of nitrate to nitrite cannot occur. Activation and deactivation are the quickest among the three ways. This is why studies on turfgrass NAP have been focused on the enzyme NR.

NAP research findings

To estimate leaf NAP in turfgrasses, the author of this article modified a method used for wheat, barley and corn leaves (Jiang, 1998). This method is *in vivo*, which in Latin means "within a living organism." First, we weighed a small amount of leaf tissue containing the enzyme NR within the live leaves and incubate the leaf sample in an incubation solution that contains nitrate at a saturating concentration. After proper preparation, the leaf tissue will absorb the nitrate rapidly, and the supply of nitrate will not be limiting. As long as there is enough NADH available inside the leaves, the enzyme will reduce nitrate to nitrite. To determine how fast nitrite is produced, the reduction of nitrite to ammonium is prevented in the dark. By determining how much nitrite is produced during a known period of time, NAP is calculated.

NAP studies have revealed the following, among other findings:

- Turfgrass roots have an extremely low NAP (Figure 1) and the reason is because turfgrass roots often contain limited carbohydrates (Jiang et al., 1998).
- Turfgrass leaves are the primary sites for nitrate assimilation (Figure 1, Jiang and Hull, 1999).
- Large amounts of nitrate — unassimilated N — are found in leaves under certain conditions (Jiang and Hull, 2000). When the grass is mowed, the unassimilated N will be lost from the plant. Nitrate content is more important

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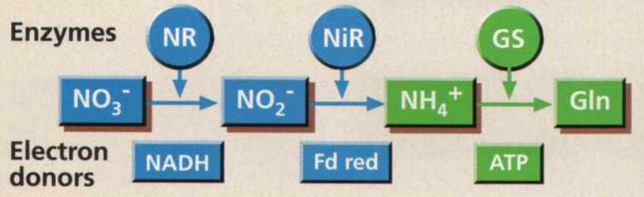
FIGURE 3

Nitrate assimilation pathway



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than total N content of the leaves when predicting fertilization needs.

- Turfgrass assimilates nitrate at high rates during the night (Jiang and Hull, 2000). This discovery is surprising, but it makes sense as turfgrass grows at higher rates during the night and thus needs more assimilated N for growth.
- Turfgrass species differ in NAP. Kentucky bluegrass tends to have greater leaf NAP than perennial ryegrass, tall fescue and bermudagrass (Jiang et al., 2002). Perennial ryegrass tends to have greater leaf NAP than creeping bentgrass (Bushoven et al., 2002).
- Cultivars within the same species differ in NAP. For example, in Kentucky bluegrass, Baron had greater NAP than Princeton 104 when grown at 1 *mM* (Jiang and Hull, 1998). At .1 *mM*, Glade Kentucky bluegrass had a higher NAP than Suffolk (Jiang et al., 2001). In perennial ryegrass, Secretariat had greater NAP than Figaro, Nighthawk, Morning Star, Palmer III, and Linn. In creeping bentgrass, SR 1020 was higher than PennLinks (Bushoven et al., 2002). This new knowledge will help turfgrass breeding programs, enhance N-use efficiency and reduce N fertilizer use.

Future NAP research

Another possible use of NAP knowledge is in the development of models for forecasting turfgrass fertilization needs. Estimating NAP using the *in vivo* method described above is impractical for turfgrass managers. However, we know that leaf NAP is correlated with nitrate concentration and turfgrass variety (Jiang and Hull, 1998). Leaf NAP should also be correlated with environmental variables, such as light intensity and temperature.

When sufficient data is available, it will be possible to construct computer models for predicting NAP based on soil nitrate concentration, turfgrass variety, light, temperature and other factors. By then, turfgrass managers will be able to forecast when and how much to apply N simply by providing the computer program with turfgrass variety and soil data, along with a few readily available weather data.

Jiang is an associate professor of plant science at the State University of New York College of Agriculture and Technology at Cobleskill, N.Y.

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