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Characterization of dissolved organic nitrogen in leachate from a newly established and fertilized turfgrass

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ABSTRACT

Understanding the mechanisms of nitrogen (N) retention and loss from fertilized urban turfgrass is critical to develop practices that mitigate N transport and protect water quality in urban ecosystems. We investigated the fate of N in lysimeters sodded with St. Augustine turfgrass and amended with labeled ^{15}N from either ammonium sulfate or urea. Fourier transform ion cyclotron resonance mass spectroscopy (FTICR-MS) was employed to identify various biomolecular classes in the leached dissolved organic N (DON) from one lysimeter for each treatment and the control. Mean DON concentrations, over 92 days, were 88, 94, and 94% of total N in the leachate from the control, urea, and ammonium sulfate treatments, respectively. Isotopic analysis showed that <3% of N in the leachate originated from newly applied N fertilizer, suggesting that the remainder of the N in the leachate was derived from the lysimeter soil or sod biomass pools. The ^{15}N fertilizer recovery was greatest in soil (44–48%), followed by sod+thatch (18–33%), grass clippings (10–13%), and leachate (<3%). Despite isotopic evidence of little contribution of N from fertilizers in the leachate, a fraction of ammonium sulfate fertilizer was recovered as DON in the leachate, likely after uptake and conversion of inorganic fertilizer to organic plant exudates and/or microbial byproducts. FTICR-MS identified N-bearing organic molecular formulas in the leachate from urea and ammonium sulfate treatments, providing evidence of N leaching from newly established turfgrass of DON compounds in a range of biomolecular compositions such as lipid-, protein-, carbohydrate-, and lignin-like molecules.

1. Introduction

Sources of anthropogenic nitrogen (N) pollution in watersheds are diverse, but an increasing density of human populations due to urbanization is often one cause of elevated N in aquatic ecosystems (Fields, 2004; Lu et al., 2015). Nitrogen fertilizers used to maintain urban turfgrass are a major N input in urban landscapes and may be a source of N loss to water bodies since turfgrass is the dominant urban land cover covering ~16 million ha or 35% of total urban land in the United States (Milesi et al., 2005). Therefore, understanding of the mechanisms of N retention and loss from fertilized urban turfgrass is needed to develop practices that mitigate N transport and protect water quality in urban ecosystems.

A number of field-scale studies, using ^{15}N-labeled fertilizer to determine the fate of N added to turfgrass, have shown that N loss via leaching from fertilized turf is minimal (Carey et al., 2012; Erickson et al., 2001). However, many studies have typically failed to account for and recover all the added ^{15}N even after considering gaseous N loss via volatilization and denitrification (Engelsjord et al., 2004; Frank et al., 2006; Horgan et al., 2002). None of these studies measured dissolved organic nitrogen (DON) in leachate, but instead analyzed only leached inorganic N. Since DON is a major pathway of N loss from agricultural ecosystems (Van Kessel et al., 2009; Willett et al., 2004) and from natural grasslands (Dijkstra et al., 2007; Leimer et al., 2016), we *hypothesize* that DON also likely constitutes a significant N loss pathway from fertilized turfgrass.

There are scarce studies in the literature on DON generation, release, and transport from fertilized urban turfgrass systems, and those that have been done looked mostly at ecotypes of bluegrass, a

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cool season turf (Barton et al., 2009; Lu et al., 2015; Pare et al., 2008). In two of the studies quantifying DON leaching from fertilized turf, Pare et al. (2008) found that DON was a significant portion of the total N leached from golf-green bluegrass, and Barton et al. (2009) observed that DON constituted 43–53% of all N leached from Kikuyu turfgrass (*Pennisetum clandestinum* Hochst. ex Chiov), a warm-season grass, during the first 12 months of growth. Pare et al. (2008) applied ^{15}N fertilizer (as NH_4 and NO_3) and found that the majority (~75%) of leached DON was attributed to soil organic matter and the remainder (~25%) was from mineral fertilizer N that was taken up by plants and/or microbes and released as DON in substances such as plant root exudates or microbial metabolites.

The application of inorganic N fertilizer stimulate plant roots and soil microbes to produce N-enriched organic products over relatively short time-scales (Murphy et al., 2000). At the field scale, a number of researchers (Kalbitz et al., 2000; Magill et al., 2000) have concluded that inorganic N fertilizer applied to landscapes is assimilated into vegetation and soil organic matter and is subsequently released as DON, thus, suggesting that DON is a significant form of N loss even when the N inputs are largely inorganic. For example, Magill et al. (2000) applied inorganic fertilizer N to plots in the Harvard Forest and observed increased DON flux below the soil. Likewise, Kalbitz et al. (2000) provided evidence that plant incorporation of inorganic fertilizer N was followed by increased release of DON to groundwater. Pare et al. (2008) found ^{15}N–DON in turfgrass leachate within 14 days after application of labeled inorganic N fertilizer. They attributed the short time period of transformation from inorganic N to non-humified DON in the form of fresh root exudates and microbial metabolites, though they did not conduct further tests to confirm this suggestion.

From these handful of studies on the leaching losses of DON from turfgrass, it is clear that a failure to account for N export as DON will underestimate the total N loss from turfgrass as well as the full extent of fertilizer N transport in urban watersheds. Since additions of anthropogenic fertilizers to turf systems may produce N-enriched organic products such as microbial exudates (Kalbitz et al., 2000; Magill et al., 2000; Murphy et al., 2000), it is also likely that urbanization and subsequent shifts to high input N fertilizer regimes may alter the biomolecular character of DON leaching from these systems. The use of FTICR-MS is a promising tool for identifying qualitative shifts in the DON molecular character under different fertilizer regimes, but we are not aware of any studies in the literature that have addressed this. The main objective of this work was to quantify the leaching loss of DON from fertilized St. Augustine (*Stenotaphrum secundatum* (Walter) Kuntze) turfgrass, a warm-season grass that is the dominant urban land cover in Florida (Erickson et al., 2001) and for which we have no known reports in the literature on N loss via DON leaching. We asked the following research questions: (1) how does the magnitude of DON loss by leaching from fertilized St. Augustine compare to the leaching losses of inorganic N?, (2) what portion of applied fertilizer N is recovered as leachate DON?, and (3) what biomolecular compound groups are present in the DON fraction of the leachate? The research reported here used soil lysimeters sodded with St. Augustine turfgrass and fertilized with a^{15}N fertilizer label of either ammonium sulfate or urea. Fertilizer was added one month after the sod was planted, therefore, the results are for a young turfgrass system, an important distinction, since turfgrass systems typically lose more N as they age (Lu et al., 2015). A novel component of this study is that we investigated DON as well as inorganic N forms (NO_3 and NH_4) and characterized end-of-study DON in leachate by Fourier transform ion cyclotron resonance mass spectroscopy (FTICR-MS) to provide molecular-level characterization of leached DON.

2. Materials and methods

2.1. Soil lysimeter packing

Nine soil lysimeters were built by cutting 30 cm internal diameter polyvinyl chloride (PVC) pipe into 55 cm sections. Each PVC lysimeter (total surface area: 730 cm^2) was packed with approximately 34 kg of soil taken from the A horizon (23–55 cm; bulk density = 1.52 Mg m^{-3}) of a Seffner fine sand (Sandy, siliceous, hyperthermic Aquic Humic Dystrudept). This soil was collected in summer 2014 from the University of Florida Gulf Coast Research and Education Center in Wimauma, Florida from a field that was taken out of citrus production in 2000, and had been since maintained as a mowed field. Soil was air dried and sieved through a standard No. 10 (2 mm) sieve and analyzed for basic physical and chemical properties (Table 1). After packing the lysimeters with Seffner fine sand soil from 23 to 55 cm depth, approximately 17 kg of commercially fine sand from a lawn and garden store was packed from 6 to 23 cm depth (bulk density = 1.40 Mg m^{-3}). Turfgrass, St. Augustine, was placed over the fine sand in each lysimeter (0–6 cm). The bottom end cap of each lysimeter was filled with a mixture of 5 kg pea gravel and 3 kg coarse sand to promote free drainage. A piece of cheesecloth was placed below the gravel–sand layer to prevent any material (sand) loss from the lysimeters. A hole was drilled at the bottom of each end cap, and plastic tubing was attached to allow leachate collection. Leachate was directed via the tubing into dark glass bottles. Lysimeters were placed outside and subjected to natural rainfall and were manually irrigated with deionized water as necessary to maintain at least 2.54 cm (0.11 pore volume, PV) of water input per week, per University of Florida/Institute of Food and Agricultural Sciences Extension recommendations for irrigating St. Augustine turf in the region.

2.2. Turf establishment and treatments

Prior to the experiments, lysimeters were equilibrated by applying approximately 1 PV (16.15 L) of deionized water to remove entrapped air. The PV was determined by calculating the product of soil porosity and volume of each layer (sand and subsoil) and then summing the two. The lysimeters were then allowed to freely drain for 14 days before sodding.

After 28 days of sod establishment (on 9 August 2014), three treatments, with three replicates for each treatment, were established: ammonium sulfate [AS, $(NH_4)_2SO_4$], urea [$CO(NH_4)_2$], and control (no fertilizer). The day of fertilization was designated as experiment day 1. Fertilizer solutions were prepared in 2 L (equivalent to 2.54 cm or 0.11 PV) deionized water and applied over the top of each lysimeter.

Treatments were made with 10 atom% ^{15}N–AS (Sigma Aldrich Product #348473) or 10 atom% ^{15}N–urea (Sigma Aldrich Product

Table 1
Basic physical and chemical properties (±S. D.) of soils used to construct experimental lysimeters.

	Sand	Subsoil
Depth in lysimeters, cm	6 to 23	23 to 55
Bulk density, Mg m^{-3}	1.40 ± 0.04	1.52 ± 0.07
Particle density, Mg m^{-3}	2.70 ± 0.06	2.69 ± 0.08
Porosity, %	48.1 ± 1.2	43.5 ± 1.3
pH_{water}	7.32 ± 0.04	5.85 ± 0.6
pH_{KCl}	6.96 ± 0.08	4.89 ± 0.1
% organic matter	0.80 ± 0.01	4.3 ± 0.1
NH_4–N, mg Kg^{-1}	0.91 ± 0.11	2.62 ± 0.27
NO_3–N, mg Kg^{-1}	0.66 ± 0.18	12.08 ± 1.5
Total N, mg Kg^{-1}	79.97 ± 2.3	210.18 ± 3.7

#490970). Treatments were applied to achieve 1 pound N per 1000 square feet of turfgrass (equivalent to 49 kg/ha), which is the current recommended fertilizer application rate for lawns in central Florida (Sartain, 2000). This amount was equivalent to 355 mg of applied N per lysimeter, based on the molar weights of 10 atom% ^{15}N–AS (MW = 132.34 g mol^{-1}) or 10 atom% ^{15}N–urea (MW = 60.25 g mol^{-1}). After fertilizer application, the experiment was conducted for 92 days from 9 August to 7 November 2014.

2.3. Leachate collection and analysis

Leachate was passed through anti-microbial plastic tubing that drained from the bottom of each lysimeters into 4-L amber glass bottles that were housed in a cooler modified to allow insertion of the tubing. Leachate volume was measured after each rain or irrigation event and a 250-mL subsample was collected and stored at 4 °C for analysis. At the time of analysis, all the samples from a given time interval were used to create flow-weighted composites for that interval i.e. days 0–3; 4–7; 8–14; 15–28; 29–49, and 50–92.

Samples were analyzed for NO_x–N, NH_3–N, total N (TN), total dissolved N (TDN), and for ^{15}N recovery in the dissolved inorganic N (DIN: NO_x–N + NH_3–N) and DON pools. For NH_3–N and NO_x–N analysis, 0.45 μm-filtered aliquots were analyzed using a discrete analyzer (AQ2+, Seal Analytical Inc., Mequon, WI). For TDN analysis, a subsample of the filtered leachate was oxidized with the alkaline persulfate oxidation method described by Hosomi and Sudo (1986) and then analyzed for NO_x–N with a discrete analyzer as above. For TN analysis, an aliquot of unfiltered sample was likewise oxidized and analyzed; particulate organic N (PON) was calculated as the difference between TN and TDN, and DON was calculated as the difference between TDN and NO_x–N + NH_3–N. The persulfate oxidation method used for TDN and TN analysis involved preparation of a digestion reagent made by dissolving 20 g recrystallized K_2SO_4 and 3 g NaOH in 1-L of deionized water. The samples were oxidized by adding digestion reagent to a sample (1:2) in a loosely capped screw-cap glass culture tube and autoclaving at 110 °C for 30 min. Determination of ^{15}N recovery in the DON pool in leachate required quantifying recovery in TDN pool and then subtracting the recovery in the DIN pool.

Concentrations of NH_3–N in the leachate were negligible and typically below the detection limit of 0.02 mg/L. Therefore, we only determined ^{15}N recovery in NO_3^- for the DIN pool. In this way, ^{15}N–DON was calculated as: ^{15}N–TDN − ^{15}N–NO_3–N. To quantify ^{15}N–TDN, bulk filtered samples were first lyophilized to produce a powder that could produce the isotopic signatures of TDN. The powder was transferred to a tin capsule for analysis of isotopic composition with a Costech model 4010 elemental analyzer (Costech Analytical Industries, Valencia, CA) coupled to a Finnigan MAT DeltaplusXL mass spectrometer (continuous flow isotope ratio mass spectrometry; Thermo Finnigan, San Jose, CA). The next step was to determine the isotopic composition of the leachate NO_3^-, using the microbial denitrifier method (Sigman et al., 2001). The recovery % of labeled fertilizer was calculated for leachate N pools as follows:

$$\%\,15N\ recovered = 100\,x\,\frac{p(c-b)}{f(a-b)} \tag{1}$$

where.

p = moles of N in the given leachate pool (e.g., NO_3, DON)
f = moles of N in the fertilizer input
c = atom % ^{15}N in the given leachate pool
a = atom % ^{15}N in the fertilizer = 10
b = atom % ^{15}N in the control

2.4. Molecular characterization of leachate

At the conclusion of the experiment (day 92), a flow-weighted composite sample of leachate from each treatment and control was used to provide insights on the molecular character of DON in leachate. We accomplished this through FTICR-MS at the National High Magnetic Field Laboratory in Tallahassee, Florida. Methods used for this analysis were from Lusk and Toor (2016b) and, briefly, involved concentrating 75 mL of 0.45 μm-filtered leachate that had been acidified to approximately pH 2.0 with hydrochloric acid (HCl) through solid phase extraction (SPE) with 1 g (6 ml) Varian Bond Elut PPL SPE cartridges. Samples obtained through SPE were analyzed by FTICR-MS via positive mode atmospheric pressure photoionization (+APPI) with toluene as the dopant on a modified Thermo Fisher APPI source (Thermo Fisher Corp., San Jose, CA). We used +APPI because it has been shown to generate more N-bearing molecules than electrospray ionization and can ionize both polar and nonpolar molecules (Osborne et al., 2013; Podgorski et al., 2012).

2.5. Soil and plant analysis

The sand and subsoil used to pack the lysimeters were characterized for basic properties, including bulk density, particle density, porosity, pH, organic matter, and N content. Bulk density was measured from undisturbed soil cores. Particle density was measured through the pycnometer method (Blake and Hartge, 1986). We used bulk density and particle density to calculate soil porosity. Organic matter content was determined by loss on ignition (Ball, 1964). The TN content was determined with an elemental analyzer (Thermo Flash EA 1112) coupled to a mass spectrometer.

During the experiment, grass in the lysimeters was mowed (clipped) regularly to maintain ~6 cm above-ground biomass. Clippings were dried (60 °C), weighed, and pooled over the course of the experiment and analyzed at the end of the study period for TN and ^{15}N abundance with an elemental analyzer (Thermo Flash EA 1112) coupled to a mass spectrometer. At the end of the experiment, the sod and thatch layers were removed from each lysimeter and also dried, weighed, and analyzed for TN and ^{15}N abundance in the same manner as the grass clippings. We considered the sod+thatch layer to be the actively growing turfgrass, surface layer roots, and the dead stems and rhizomes that were not yet decayed. Further, at the end of the experiment, each lysimeter was emptied and a composite soil sample from the sand and subsoil layers of each lysimeter was collected, field sieved (2-mm mesh) to remove coarse roots, and analyzed for TN and ^{15}N abundance as above for grass, sod, and thatch. The % recovery of added fertilizer N in soil and plant tissue pools (clippings, sod/thatch) was calculated with Equation (1) above.

2.6. Statistical analyses

We conducted a one-way ANOVA using the statistical software JMP Pro 11 to compare the means of cumulative leached TN, DIN, and DON among treatments (control, AS, and urea), using a significance level of 0.05. As reported in the Results section, the ANOVA showed no statistically significant differences among treatment means; thus, no post-hoc tests were conducted to confirm where significant group differences occurred.

3. Results

3.1. Nitrogen leaching from the turfgrass

Cumulative TN and DIN were highest in leachate from the control, followed by the urea and AS treatments (Table 2). However, these differences were not shown by the ANOVA to be statistically significant, nor were there any significant differences in DON concentrations among groups. Consistent with findings of other researchers (Erickson et al., 2001; Trenholm et al., 2012), leaching losses of inorganic N were low, and DON was the dominant form leached from the newly established turfgrass system (Table 2). For example, over the 92-day experiment, the cumulative mean % DON in leachate was 88, 94, and 94 of TN for the control, urea, and AS treatments, respectively.

Flow-weighted DIN concentrations in leachate did not exceed 0.14 mg/L and were often below 0.05 mg/L (Fig. 1a). Concentrations of DIN in the leachate peaked at 7 days after fertilizer application for the AS treatment and at 14 days for the control and urea treatment. By the end of the experiment (92 days), DIN concentrations were ≤0.04 mg/L in all treatments, and differences in DIN concentrations between treatments were not significant. In contrast, the DON concentrations were much higher and ranged from 1.0 to 2.2 mg/L (Fig. 1b). Flow-weighed DON concentrations were variable but in general peaked between 7 and 14 days after fertilizer application and then decreased with time for the fertilized lysimeters.

3.2. *Fate of nitrogen derived from fertilizer*

Of the total DIN in the leachate, DIN-DFF was 0.6 and 0.7% of TN for the urea and AS treatments, respectively (Table 2). Of the total DON in leachate, the DON-DFF was 1.6 and 1.8% of TN for the urea and AS treatments, respectively (Table 2). Thus, a small amount of TN leached from each treatment during the 92-day experiment (≤2.5%) was derived from fertilizer, and of this, most of it was in the DON form. As such a small amount of the TN leached was derived from fertilizer, we suggest that most of N that leached during the 92 days was derived from soil and/or the initial sod biomass.

We determined ^{15}N recovery in other pools to quantify the fate of fertilizer N (Table 3). We could account for 75.7 and 90.6% of the 355 mg of fertilizer N added with the urea and AS treatments, respectively. The soil was the dominant ^{15}N recovery pool, followed by sod + thatch, and clippings. The remainder (~9–24%) of the ^{15}N was estimated to be lost via ammonia volatilization and/or denitrification.

In both urea and the AS treatments, DIN-DFF and DON-DFF were detected as early as after 0–3 day in the leachate (Fig. 2). The mass of N derived from fertilizer (NDFF) in both DIN and DON leachate pools increased with time and was still increasing when the experiment ended at 92 days.

3.3. *Molecular characterization of leached DON*

Our FTICR-MS data characterized only end-of-experiment DON at one point in time, thus, we do not attempt to make broad conclusions about statistically significant differences in DON composition between the treatments. We instead describe the similarities and differences observed in the FTICR-MS data for one temporal point. The FTICR-MS spectra contained 8,740, 8,190, and 16,249 peaks for which molecular formulas were assigned in end-of-experiment leachates from the control, AS, and urea treatments, respectively. In all samples, the relative percent spectral abundance was highest for the heteroatom group CHO (36.1–49.1), followed in order by the CHON (24.5–33.2), CHOS (4.3–7.6), and CHONS (1.4–4.7) (Table 4). Isolating only N-bearing formulas (those in the CHN, CHON, CHONS groups) allows characterization of the DON molecular groups found in each sample. There were 3,960, 4,304, and 8124 N-bearing organic compounds in leachate from the control, AS, and urea treatments, respectively. The majority of N-bearing formulas were in the CHON group (Table 4). Fig. 3 compares the treatments by relative spectral abundances of CHN and CHON formulas. Among the three treatments, the urea amended lysimeters had a higher relative abundance of high O compounds (O6 to O13), whereas the AS amended lysimeters had a higher relative abundance of low O compounds (O1 to O5).

We used van Krevelen diagrams to provide visual representations of the molecular character of compounds present in the bulk leached DON (Fig. 4). A van Krevelen diagram plots the identified compounds according to their O/C and H/C atomic ratios (Kim et al., 2003) and can be used to classify compounds into biomolecular regions, such as a lipid-like region, a protein-like region, a lignin-like region, or a carbohydrate-like region (Ohno et al., 2010). In all three cases, these compounds fall within a range of biomolecular compounds (Fig. 4). Lipid-, protein-, and lignin-like compounds were the most common in all samples, whereas carbohydrates and unsaturated hydrocarbons (UHC) accounted for less than 0.1% of assigned formulas. The urea treatment contained a region of UHC not present in the other two samples (Fig. 4).

The control and both treatments contained several thousands of leached DON compounds not found in either of the other samples. There were 2518 (64%), 6866 (85%), and 3035 (71%) unique N-bearing formulas in the control, urea, and AS treatments, respectively (Fig. 5a–c).

4. Discussion

4.1. *Importance of leaching of organic nitrogen from turfgrass*

Understanding and properly managing N fertilizers in urban landscapes is an important part of urban nutrient management. In Florida, a rapidly urbanizing state with numerous freshwater, marine, and estuarine water resources, the fate of excess N in the environment is especially critical and is often a focus of regulatory activity aimed at preventing cultural eutrophication and water quality degradation. For example, more than 50 Florida counties and municipalities currently have summer wet season (June to September) “blackout periods,” or bans on the application of N-bearing lawn fertilizers, under the premise that summer rains drive fertilizer N into runoff and groundwater (Hartman, 2008).

Our wet season application of either AS or urea fertilizers to newly established St. Augustine turfgrass did not result in any significant differences, when compared to unfertilized controls, in

Table 2
Initial fertilizer N input, mean cumulative leached DIN and DON (±S.D.), and mean cumulative N derived from fertilizer (DFF) in DIN and DON pools in leachate collected from three experimental treatments.

Treatment	Fertilizer N input, mg N/lysimeter	Cumulative leached DIN, mg N/lysimeter	Cumulative leached DON, mg N/lysimeter	DIN-DFF, μg N/lysimeter	DON-DFF μg N/lysimeter
Control	0	4.63 ± 3.02	33.70 ± 1.67	–	–
Urea	355	2.12 ± 0.71	32.69 ± 4.18	13.2 (±4.8)	520 (±103)
Ammonium Sulfate	355	1.86 ± 0.58	28.71 ± 5.71	12.7 (±3.4)	495 (±87)

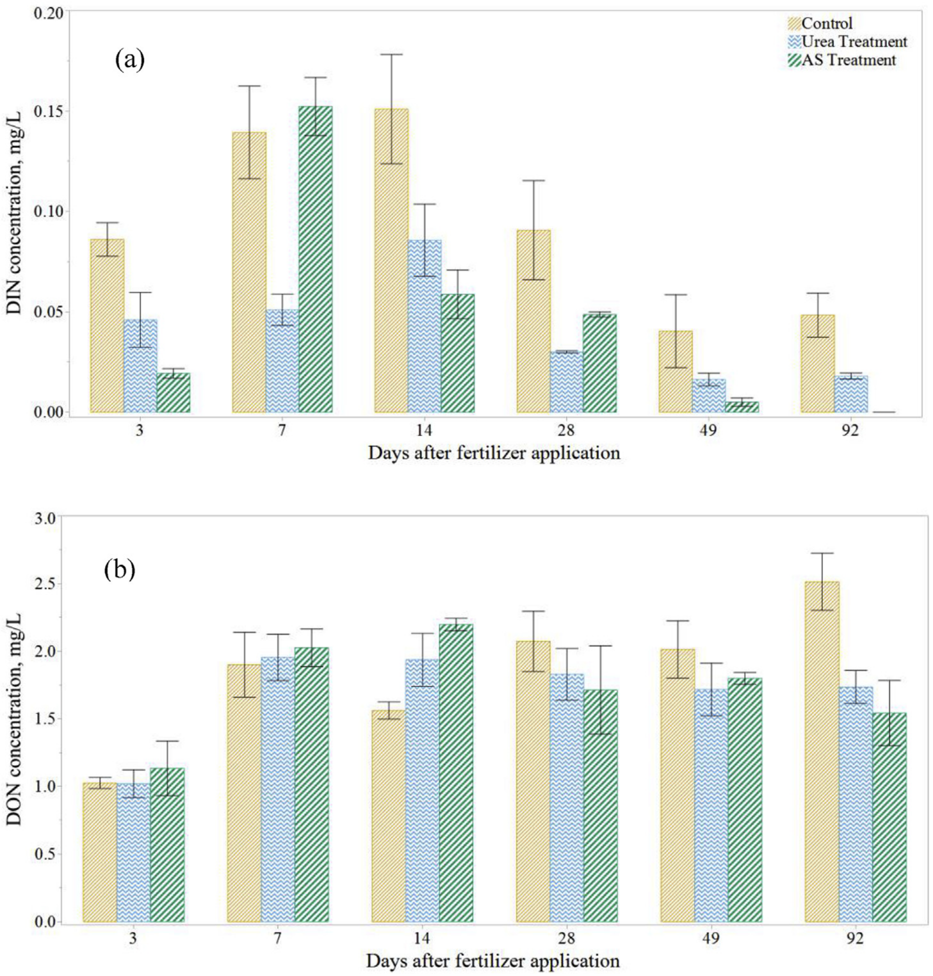

Fig. 1. Flow-weighted DIN (a) and DON (b) concentrations in leachate for six sampling intervals (days 3, 7, 14, 28, 49, and 92). The concentrations of NH_3–N were below the detection limit and hence DIN only includes NO_3–N. Values represent means with standard errors of replicates. AS = Ammonium Sulfate.

Table 3
Mean % ^{15}N recovery in various pools after 92 days study period (±S.D. of three replicates).

	Urea Treatment	Ammonium Sulfate Treatment
Leachate DIN	<0.01	<0.01
Leachate DON	0.15 (±0.9)	0.14 (±0.9)
Clippings	9.7 (±2.1)	13.4 (±1.1)
Sod + Thatch	17.7 (±3.9)	33.2 (±2.8)
Soil	48.2 (±3.1)	43.9 (±6.0)
Total	75.7 (±3.8)	90.6 (±4.4)

leachate NO_3 or DON concentrations during the first 92 days after fertilizer application. Organic N represented the dominant portion (88–94%) of TN leached from both fertilized treatments and the control (Table 2). Our results concerning inorganic N loss being much less important than organic N loss are consistent with those cited in reviews of turfgrass leaching by Qian and Follett (2012), who found that turf plots less than 10 years old presented a little risk for inorganic N leaching. Similarly, Petrovic (1990) concluded that leaching loss of inorganic fertilizer in turfgrass is almost always less than 10% of TN.

Our study is also consistent with the sparse other studies that have looked at DON from turfgrass systems. For example, Barton et al. (2009) observed that 43% of all N lost via leaching from 20-week old turfgrass was DON; that number increased to 53% for 20-year old turfgrass. Pare et al. (2008) found that 10–70% of the N that leached from newly established bluegrass was DON. They also used a ^{15}N fertilizer label to conclude that most leached DON was derived from residual soil pools, and ^{15}N-DON was present in leachates as early as 14 days after application. Our study highlights the leaching loss of DON from newly established St. Augustine turf, a warm-season turfgrass for which we are aware of no other studies that have investigated DON leaching during establishment.

In the scientific literature, most studies of N leaching from turfgrass have ignored leaching loss via DON (Petrovic, 1990). Our study shows that failure to account for DON in leachate greatly underestimates N loss from urban residential landscapes. We know from studies in natural ecosystems that DON is an important pathway of N loss for forested watersheds regardless of soil N stock (Neff et al., 2003; Perakis and Hedin, 2002; Scott and Rothstein, 2017). This observation leads to the characterization by some authors of DON as an ecosystem "N leak," because, unlike DIN which is tightly cycled when biotic demand is high, DON is observed to leach across a wide range of N availabilities (Neff et al., 2003). The observation that DON leaches from soils even during times of high biotic demand is likely due to the compositional variability of DON, it being comprised of both labile and refractory compounds that function ecologically in different ways (Perakis and Hedin, 2002). Aside from this, one physico-chemical process that can limit DON flux from soils is the formation of strong mineral-organic matter

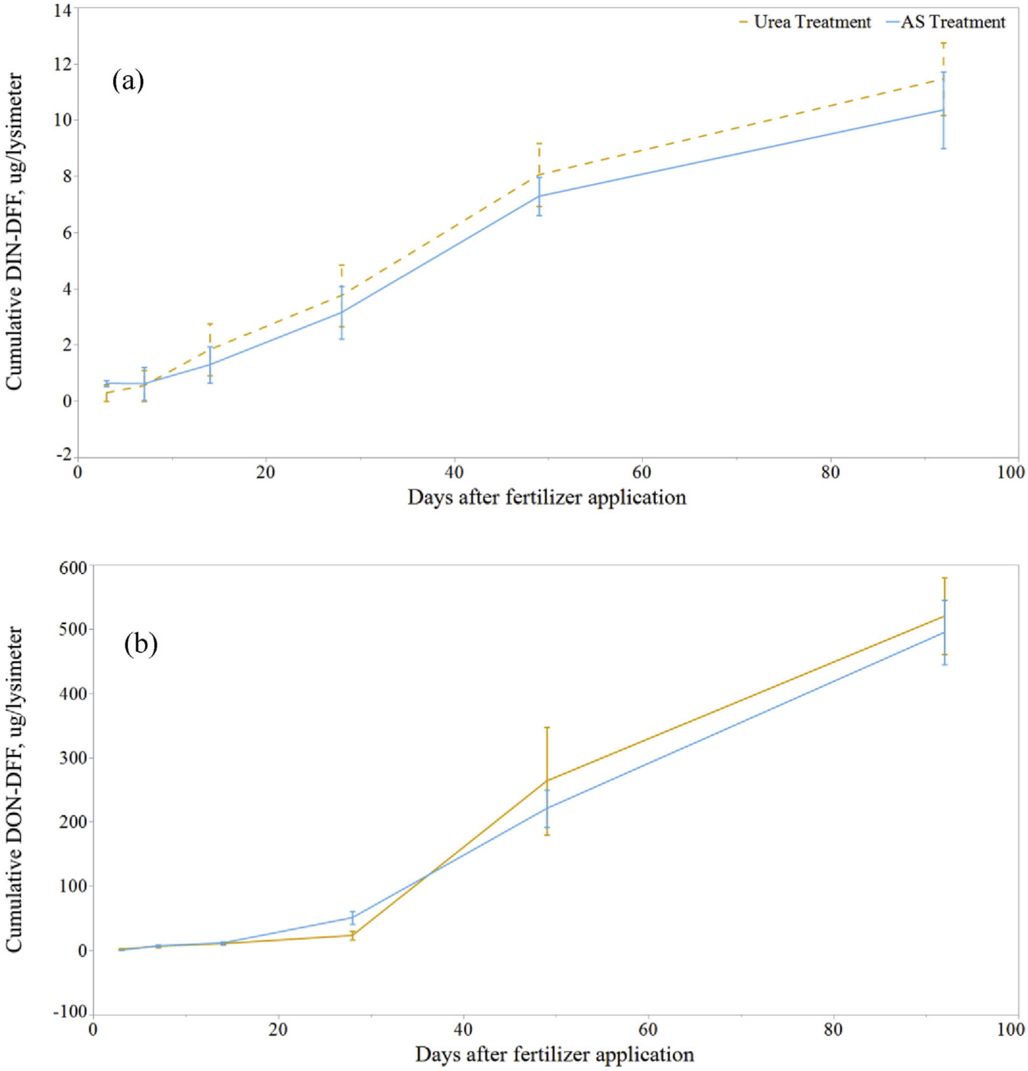

Fig. 2. Cumulative leachate losses of N derived from fertilizer (DFF) of DIN (a) and DON (b) for urea and ammonium sulfate (AS) treatments. Values represent means with standard deviations of replicates. Note differences in y-axis scales.

Table 4
Relative spectral abundance of heteroatom classes in day 92 leachate from each treatment.

Heteroatom Class	Control	Ammonium Sulfate Treatment	Urea Treatment
CHO	49.1	43.7	36.1
CHON	24.5	33.2	29.5
CHOS	7.6	4.3	5.6
CHONS	1.4	1.7	4.7
CHN	0.00	0.04	0.17

associations, with DON fluxes higher in sandy textured soils than in fine textured soils (Campbell et al., 2000; Qualls et al., 2002). The lysimeter soils used in this study were sandy textured, with likely little capacity to retain DON because of reduced soil solution contact time and few sorption opportunities. Our previous study of a highly urbanized Florida watershed (56–83% urbanized) showed that N in local streams is predominantly DON (64–72% of TN) (Lusk and Toor, 2016b), and we believe the high DON leaching observed in this study is representative of N fate in the turf-dominated landscape.

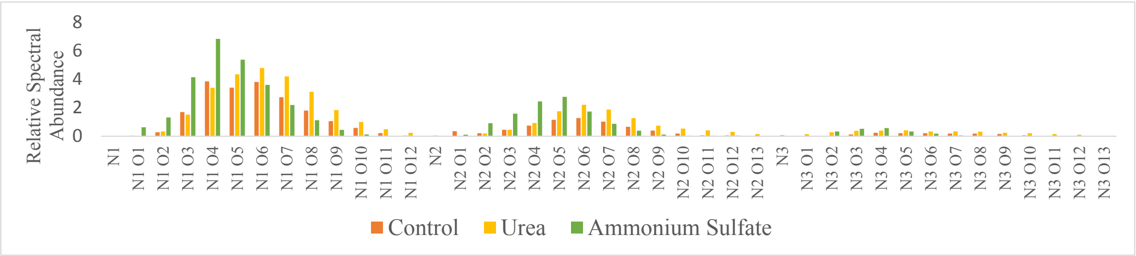

Fig. 3. Relative spectral abundances of CHN and CHON classes in control and fertilizer treatments (urea, ammonium sulfate) determined with FTICR-MS.

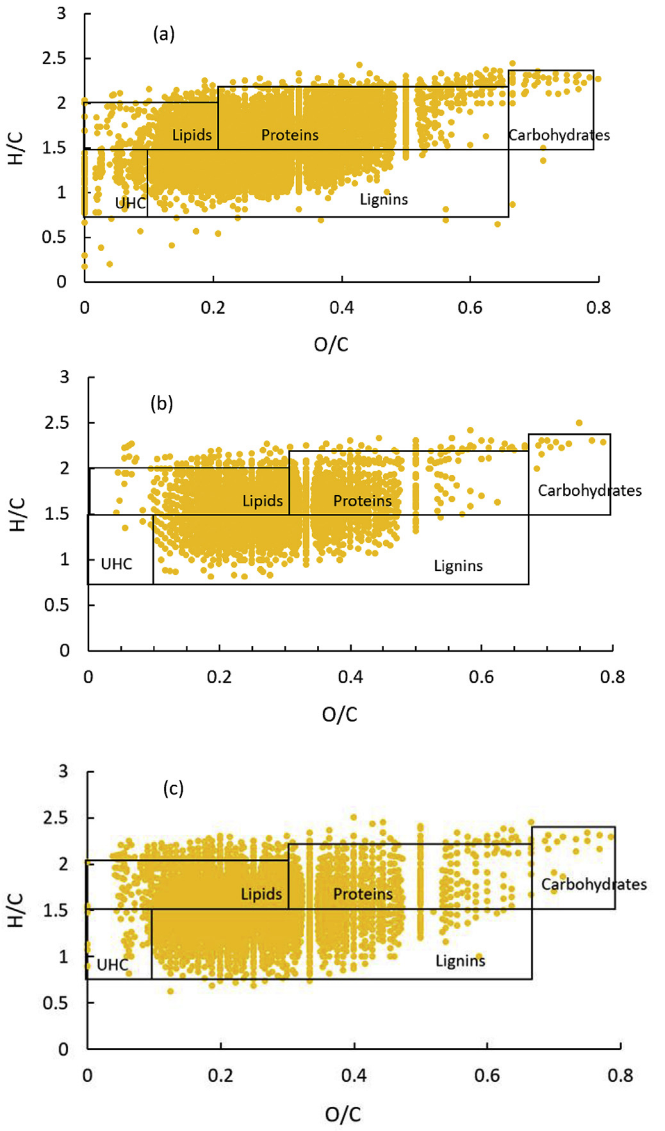

Fig. 4. van Krevelen diagrams of all N-bearing organic compounds in day-92 leachates for (a) control, (b) urea, and (c) ammonium sulfate treatments. UHC = unsaturated hydrocarbons.

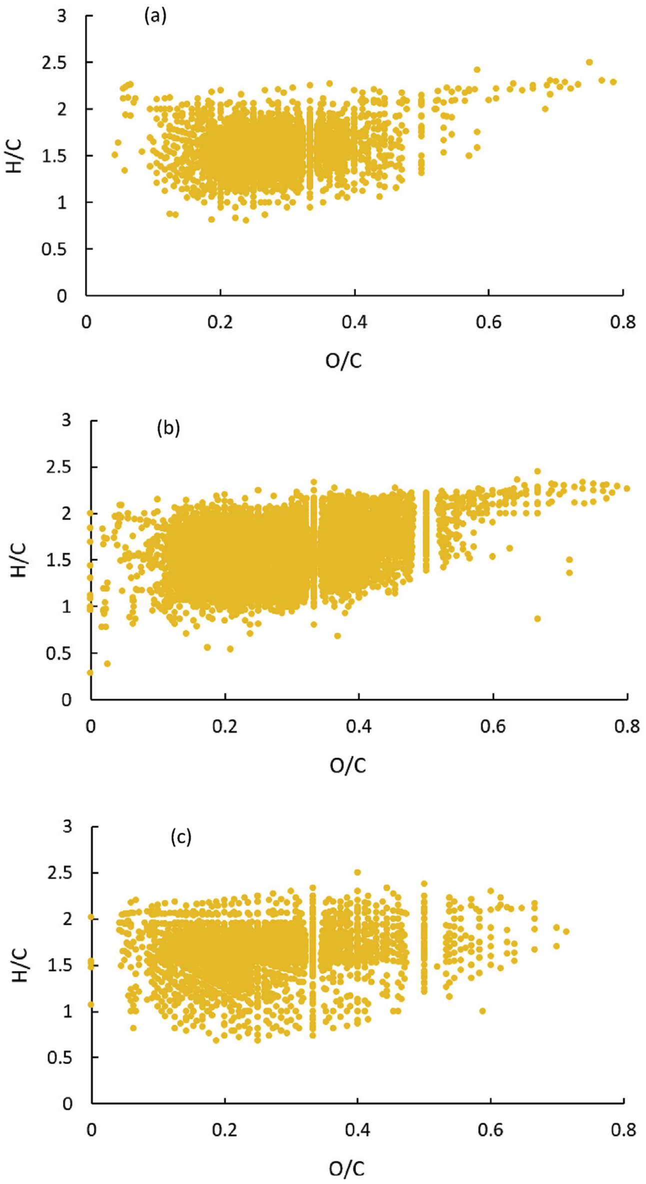

Fig. 5. van Krevelen diagrams of unique N-bearing organic compounds in day-92 leachate for (a) control, (b) urea, and (c) ammonium sulfate treatments.

4.2. Fate of fertilizer nitrogen

Often when ^{15}N tracer studies have been applied to the fate of fertilizer N in turfgrass systems, any ^{15}N unaccounted for at the end of an experiment is attributed to gaseous loss via volatilization or denitrification (Engelsjord et al., 2004; Frank et al., 2006; Miltner et al., 1996), without that assumption having actually been checked by measuring gaseous N loss. Horgan et al. (2002) in a^{15}N tracing study measured N loss via denitrification and found that ^{15}N recovery in the turfgrass system was far from complete. We suggest that a part of the fertilizer N can be converted in turfgrass systems to new organic N and leached quickly (in days to weeks) as DON—making DON-DFF one vector of N loss in urban residential landscapes. This also means that previous assumptions about all unrecovered ^{15}N being attributable to gaseous loss may not hold true.

If we want to accurately determine the fate of added N to urban systems, we need to recognize that a portion of that N is likely being lost as leached DON. It may be in the DON form even if inputs are inorganic, as we saw here for our AS treatment (Table 2) and as corroborated by Pare et al. (2008). Because ^{15}N recovery as DON-DFF occurred rapidly just days after fertilizer was added, we contend that the leached DON-DFF was not humified but instead was from fresh organic matter derived from root and microbial exudates. In this case, mineral N taken up by plant roots or soil microbes would have been rapidly turned over, giving the system a form of fresh, leachable DON.

Although portions of both DIN and DON in leachate were attributable to fertilizer, and that finding is important, when we traced the applied ^{15}N, we observed that less than 1% of the inorganic N and less than 2% of the organic N in leachate was derived from fertilizer (Table 2). This means that the initial soil and/or the sod that was used to plant the experimental lysimeters were the leading source of leachate N—at least during the early ~3 month period of sod establishment looked at in this study.

The sod + thatch was not only a major source of leached N but also a sink for fertilizer N, capturing 18 and 33% of applied fertilizer in the urea and AS treatments, respectively (Table 3). This is consistent with findings by Raciti et al. (2008), who found that 70 days after addition of a^{15}N fertilizer label to turfgrass, 28 and 36% was recovered in thatch and soil pools, respectively. By 365 days after the fertilizer addition in that study, the soil alone dominated as the ^{15}N sink, holding 70% of the added N. We found 44–48% of applied N in the lysimeter subsoils. Though we did not distinguish how much fertilizer N was recovered in organic versus inorganic N pools in the subsoils, we *hypothesize* that much of the ^{15}N recovered was in organic matter pool. In young turfgrass systems like ours, the soil organic matter is an important pool for N retention (Qian and Follett, 2012). When new urban land is developed and converted to turfgrass coverage, soil organic matter pools are quickly built due to the fertilization and irrigation, creating urban turfgrass systems with high root and shoot biomass productivity, and with little tillage, they begin to sequester soil organic carbon (Qian and Follett, 2012). During the first ~20–30 years after turf establishment, this build-up of soil organic carbon favors N immobilization in soil organic matter (Qian and Follett, 2012). During this time, inorganic N loss via leaching is minimal because N input from fertilization is retained by the system, and it is not until generally after 25–30 years that turfgrass become carbon and N saturated, causing N immobilization processes to give way to N mineralization processes (Lu et al., 2015). In this way, older turfgrass has frequently been shown to have lower N fertilizer needs than younger turf because of the soil's release of mineral N via mineralization (Barton et al., 2009; Lu et al., 2015; Qian and Follett, 2012). Given this, it could be argued that N fertilizer recommendation should be lowered for older turfgrass relative to the current recommendation.

4.3. *Molecular composition of leachate DON*

A novel component of our study was to investigate the end-of-season molecular character of leachate DON. The single samples analyzed for each treatment and the control showed that leachate DON was present in a wide range of biomolecular compounds, but that there were variations in DON composition among treatments. It is beyond the scope of this study to investigate why those differences are present and how much those differences are due to natural soil heterogeneity versus a response to experimental treatments, as it was our intent to use the FTICR-MS analysis as a preliminary investigation into the general molecular character of leachate DON. Future research should be conducted through replicated experimentation to parse out potential treatment effects and to trace how leachate DON changes with time.

Adding N fertilizer to the system can be expected to change the composition and biomass of the soil microbial community (Hartman and Richardson, 2013; Sarathchandra et al., 2001). This in turn may lead to accumulations of different organic compounds in the soil, as the variable microbial communities exert differing influences on soil organic matter alteration and decomposition (Janssens et al., 2010; Peltre et al., 2017). We cannot state with certainty that this is the case for our study, but future research should be conducted to more closely investigate the effects of fertilizer inputs to turfgrass and microbial communities on leached DON composition. It is also known that root exudates can “prime” soil microbial communities and lead to increased alteration of low quality soil organic matter (Kuzyakov, 2010). We did not quantify root biomass in our fertilized treatments, but doing so would be an important next step in future studies, as differences in root biomass may cause changes in microbial functioning that in turn lead to accumulation of a new suite of organic compounds in the system.

4.4. *Practical implications of managing nitrogen fertilizers in urban landscapes*

Nitrogen added to urban landscapes via fertilizers to managed turfgrass is a subject of intense scrutiny and concern for water quality issues, and regulatory agencies are tasked with addressing those concerns. This research offers insights on the fate of N in newly established St. Augustine turfgrass during the Florida summer rainy season. Our study is representative of what might be expected to happen to fertilizer N soon after previously undeveloped pasture is converted to urban residential land and the ground is newly sodded with turfgrass. Such systems are shown here to be large exporters of N, but the great majority of that N is present as DON derived from the residual soil. Studies that seek to complete mass balances for inputs versus outputs of N in urban landscapes must account for leaching via DON. We have known for some time that DON is a significant N loss vector in agroecosystems (Van Kessel et al., 2009), and we need to begin a new round of studies to quantify such loss in urban landscapes with high percentage of turfgrass coverage. In particular, the first important practical application of this work is that we likely make assumptions that overestimate N losses to the atmosphere (via volatilization and/or denitrification), if we fail to account for leaching losses of DON.

A second important implication of this work is that a portion of the inorganic N added to urban landscapes as fertilizer will be recovered as DON. We observed portions of the fertilizer N in leached DON pools just days after adding fertilizer. Our study was temporally short (92 days), but at the conclusion of the experiment, the mass loadings of DON-DFF were continuing an upward trend, indicating that with time even greater loads of DON-DFF would likely be recovered. This finding is important because the DON derived from fertilizer in the AS treatment could only be originating from mineral N that was taken up by plants and/or microbes and released back to the soil as root or microbial exudates. These N-bearing organic materials, being comprised mostly of non-humic proteins and amino acids, have been shown to be highly bioreactive, and hence have ecological significance in that they may be sources of reactive N to groundwater (Petrone et al., 2009). Important study for future work will be to look at DON-DFF over longer periods of time, coupled with more in-depth investigations of the molecular structure and bioavailability of the DON leached from urban turfgrass, as done in urban waters in our previous work (Lusk and Toor, 2016a, b).

A third important implication from this work is that the newly established turfgrass system leached very little inorganic N, even during the summer rainy season. Although there were small increases in inorganic N leaching after fertilization, there were no significant differences in N loss between the control and treated lysimeters, indicating that adding a wet season application of fertilizer caused no significant increase in N leaching in our young turfgrass system. Understandably, there are concerns about water quality whenever there are inputs of fertilizer N to urban watersheds. This study, however, confirms the findings of other researchers (Baker et al., 2001; Groffman et al., 2004; Raciti et al., 2008; Wollheim et al., 2005) that urban watersheds have a high capacity for inorganic N retention, especially during the season of active turf growth. However, increased N mineralization with time has been reported for turfgrass soils, such that N retention in turfgrass systems may decrease as the turf ages (Qian and Follett, 2012). Other related research in the region has indicated that a part of urban N fertilizers can be subject to losses via stormwater runoff (Yang and Toor, 2016, 2017), thus, whole system studies that investigate N pools in surface runoff and leaching pathways should be considered. We suggest that while a regulatory summer ban on fertilizer application may have little impact on the health of newly established turf, we should recognize that older turf might be more prone to N leaching losses. Hence, N fertilizer recommendations should be developed and fine-tuned for new vs. older turf systems to optimize nutrient management in urban watersheds that keeps N in the plant root zone and reduce losses to receiving waters.

5. Conclusions

This study investigated the fate of fertilizer N to young newly established turfgrass. Here, we determined the role of DON leaching from St. Augustine turfgrass, a warm-season grass, using a^{15}N fertilizer label to quantify the extent of applied fertilizer N loss via leaching. A key finding of the study was that DON is the dominant form in leachate from both fertilized and unfertilized treatments. While most of this DON is derived from the residual soil, it is important to note that both urea and ammonium sulfate fertilized turf leached DON derived from fertilizer, and that the mass loading of this DON was increasing with time and that this DON- likely originated from root and microbial exudates. For urban areas in which we are concerned about the fate of anthropogenic N, we cannot ignore the large N losses that occur via DON leaching from turfgrass, and we need to acknowledge that a portion of fertilizer N may be recovered as potentially bioreactive DON.

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