



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 ¹⁵N 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 ¹⁵N 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.

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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 ¹⁵N-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 ¹⁵N 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, $(\text{NH}_4)_2\text{SO}_4$], urea [$\text{CO}(\text{NH}_4)_2$], 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 (\pm 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
$\text{NH}_4\text{-N}$, mg Kg^{-1}	0.91 ± 0.11	2.62 ± 0.27
$\text{NO}_3\text{-N}$, mg Kg^{-1}	0.66 ± 0.18	12.08 ± 1.5
Total N, mg Kg^{-1}	79.97 ± 2.3	210.18 ± 3.7

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