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The extent and pathways of nitrogen loss in turfgrass systems: Age impacts

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Abstract

Nitrogen loss from fertilized turf has been a concern for decades, with most research focused on inorganic (NO_3^-) leaching. The present work examined both inorganic and organic N species in leachate and soil N_2O emissions from intact soil cores of a bermudagrass chronosequence (1, 15, 20, and 109 years old) collected in both winter and summer. Measurements of soil N_2O emissions were made daily for 3 weeks, while leachate was sampled once a week. Four treatments were established to examine the impacts of fertilization and temperature: no N, low N at 30kgNha^{-1} , and high N at 60kgNha^{-1} , plus a combination of high N and temperature (13°C in winter or 33°C in summer compared to the standard 23°C). Total reactive N loss generally showed a “cup” pattern of turf age, being lowest for the 20 years old. Averaged across all intact soil cores sampled in winter and summer, organic N leaching accounted for 51% of total reactive N loss, followed by inorganic N leaching at 41% and N_2O -N efflux at 8%. Proportional loss among the fractions varied with grass age, season, and temperature and fertilization treatments. While high temperature enhanced total reactive N loss, it had little influence on the partitioning of loss among dissolved organic N, inorganic N and N_2O -N when C availability was expected to be high in summer due to rhizodeposition and root turnover. This effect of temperature was perhaps due to higher microbial turnover in response to increased C availability in summer. However when C availability was low in winter, warming might mainly affect microbial growth efficiency and therefore partitioning of N. This work provides a new insight into the interactive controls of warming and substrate availability on dissolved organic N loss from turfgrass systems.

Keywords

Reactive N; Microbial activity; N_2O ; Leaching; Turfgrass; Chronosequence

1. Introduction

Nitrogen is the most limiting nutrient for plant growth in terrestrial ecosystems and thus increased N input often leads to increase in plant biomass and yields (Conant et al., 2013). Over the past 50 years, synthetic N application has increased by ~10 fold from ~12 Tgyr⁻¹ in the 1960s to ~100 Tgyr⁻¹ in the 2000s (Fields, 2004; Mulvaney et al., 2009; Tilman et al., 2001), and this trend is unlikely to change. It is undeniable that fertilizer N helps support an increasing world population (Mulvaney et al., 2009; Tilman et al., 2001), but this benefit comes at a cost. On a worldwide scale, fertilizer N recovery efficiency (i.e., N in harvested plant biomass per N input) has remained <50% (Bouwman et al., 2005; Conant et al., 2013), implying that a sizable amount of N is potentially free to move through the environment – soil, water, and air. Indeed, reactive N in the environment has increased steadily since the 1960s and its negative impacts on natural resources, human health, and ecosystem services are broadly recognized (Fields, 2004; Vitousek et al., 1997; WHRC, 2007). Society is aware that controlling N loss from an ecosystem is crucial for sustaining the environment, and yet questions about how to effectively accomplish this control remain.

Turfgrass, planted on athletic fields, parks, golf courses, and home lawns, is popular for both aesthetic and recreational purposes. According to Milesi et al. (2005), turfgrass in the USA is the largest single ‘crop’ and occupies roughly 1.9% of the total land. There are a variety of grass species available, including C₃ (cool-season) and C₄ (warm-season) grasses, but essentially all require relatively high fertility (up to 200–300 kg N ha⁻¹) to maintain health and facilitate recovery from damage (Carey et al., 2012). Concerns have been raised for decades that such high amounts of N must certainly result in considerable loss, especially via leaching of inorganic N. Investigations designed to examine fertilizer N use efficiency in turf systems, typically employing either a mass balance approach or the use of ¹⁵N, have reported that N recovery in grass clippings was moderate (De Nobili et al., 1992; Henning et al., 2013; Miltner et al., 1996; Raciti et al., 2008); and yet most data indicate fairly low amounts of inorganic N leaching from turf (Frank et al., 2005; Miltner et al., 1996; Raciti et al., 2008).

Because turf systems accumulate considerable soil organic N over time (Shi et al., 2006b), it is logical that mineralization would supply more N in older vs. younger turf. As predicted by the N-retention hypothesis – a principle underlying biological controls on inorganic N loss (Vitousek and Reiners, 1975), ecosystem N conservation will surge over time until it hits a steady state, and from this point of succession forward, within-system N will likely provide sufficient available N to support biotic growth. Based on this hypothesis, N applied at a constant rate may lead to high rates of N loss during the early development of a turf when the capacity for biotic N retention via plants and microbes is relatively small, and during the late development of turf when internal N supply is large. Although turfgrass growth will vary depending on management inputs (Milesi et al., 2005), soil organic matter accumulates rapidly during the first 20–30 years after turfgrass establishment and thereafter slows down markedly towards a steady state (Bandaranayake et al., 2003; Carley et al., 2011; Milesi et al., 2005; Qian and Follett, 2002). Thus, “young turf” (i.e., <20–30 years) has been deemed as a sink of external N input, whereas “the old turf” may receive sufficient inorganic N via mineralization and thus any additional fertilizer N would be more subject to significant loss

(Petrovic, 1990). Zhang et al. (2013) reported that Kentucky bluegrass turf in Colorado changed from a sink to a weak source of greenhouse gas emission 20–30 years after establishment; by gradually reducing the rate of N fertilization as turf aged, N₂O emissions could be reduced ~40% compared to N fertilization at a constant rate of 150 kg N ha⁻¹ yr⁻¹. Bremer (2006) also reported that low N application (50 kg N ha⁻¹ yr⁻¹) reduced N₂O loss 63% compared to high N (250 kg N ha⁻¹ yr⁻¹). It should be noted that some consider that turfgrasses, regardless of age, will always require some fertilizer N, because constant N leaking through harvest and cycling processes restricts ecosystem N conservation, causing N limitation even after the steady state (Neff et al., 2003; Vitousek et al., 1998). Therefore, a better understanding of N leaking pathways and their regulation modes, specifically in pertaining to turf of different ages, may help prevent excessive N loss.

Nitrate leaching has long been emphasized as the primary route of N loss from turf, leading to eutrophication and acidification of water body (Flipse et al., 1984; Geron et al., 1993; Keeney and Olson, 1986; Petrovic, 1990). However, a growing body of evidence from natural and agricultural ecosystems suggests that organic N runoff and leaching must be considered when assessing the total N loss from an ecosystem (Christou et al., 2005; Murphy et al., 2000; Perakis and Hedin, 2002; Siemens and Kaupenjohann, 2002; Van Kessel et al., 2009). A 22-month lysimeter study showed that 25 to 78% of N leached from a turfgrass system was in the organic form (Barton et al., 2009). By examining 17 bermudagrass systems, Lu et al. (2015) also showed that water- and salt solution-extractable soil organic N was comparable to soil inorganic N, implying that N leaching or runoff via organic form could be as high as inorganic species.

Soil N₂O efflux has received considerable attention due to N₂O potent effects on global warming and ozone depletion (IPCC Climate Change, 2007; Ravishankara et al., 2009). The U.S. EPA reported that roughly 79% of total anthropogenic N₂O emissions in the USA are from the soil and systems under management (U.S.EPA, 2016). Fertilized turf may be an important source of atmospheric N₂O (Bremer, 2006; Groffman et al., 2009; Li et al., 2013; Maggiotto et al., 2000). As shown by Kaye et al. (2004), turf contributed 30% of N₂O emissions in a studied region where it occupied only 6.4% of the total land area. So far, most investigations on N loss from lawn have centered on individual processes and control factors. While simultaneous measurements of N loss via leaching and gas emissions can help better identify the major pathway of N loss and associated biotic and abiotic controls, systematic data collection is lacking.

Given rapid global urbanization and the concurrent rise in turfgrass acreage, mitigating N loss from turf remains a challenge and a goal. Although there is no easy solution for N efflux, the knowledge gained from natural ecosystems – forests and grasslands, suggests that the degree of biotic N retention as well as ecosystem N leak must be taken into account when designing an environmentally sound N fertilization program. We used a turfgrass chronosequence, and specifically three events or conditions (grass dormancy versus active growth; seasonal change in temperature; fertilization regime with zero fertilization versus fertilization at low and high rates) to address the fundamental question, how does turf age affect the extent and pathways of N loss?

2. Materials and methods

2.1. Site description and soil sampling

Four turf sites in or near the renowned Pinehurst Resort and Country Club, Sandhills region, North Carolina, USA were selected for sampling. Turfgrass was established on these sites in 1907, 1996, 2001, and 2015 and were 109, 20, 15, and 1 years old, respectively, when intact soil cores were taken. We did not choose additional turf sites between 20 and 109 year old turfs, as we assumed that additional turf sites of >20–30 years old might not affect the age pattern of N loss. This is because soil organic matter is the major factor influencing soil N processes, and often soil C and N contents stabilize 20–30 years after turfgrass establishment (Qian and Follett, 2002; Bandaranayake et al., 2003; Shi et al., 2006b). Furthermore, turf sites having similar grass species, professional management, and environmental conditions, such as climate and soil texture were limited in the region. These sites were in close proximity and had similar or identical surface soils, with ~ 90% sand, 5% silt, and 4% clay (Shi et al., 2006b). All sites were planted to hybrid bermudagrass (*Cynodon dactylon* × *transvaalensis*), a warm-season perennial, although the oldest site had been replanted with different grasses or varieties over years. All sites also received N fertilization at approximately 150 kg N ha⁻¹ yr⁻¹ in split applications (i.e., 2–4 times per year). Turfgrasses were also fertilized with phosphorus (P) and potassium (K), and limed to maintain a close-to-neutral soil pH. Additional standard turfgrass management included mowing, irrigation, and chemical pest control.

Twenty intact soil cores (5 cm diameter × 10 cm length) were collected from each turfgrass system in January and August 2016, respectively, using an AMS soil core sampler kit with removable butyrate plastic retaining cylinders of 15 cm length. Grass leaves were kept during soil sampling and also during the following incubation experiment. Most roots were within top 7.5 cm of soil where microbial activity and processes were significant (Shi et al., 2006b), and thus grass root might be little damaged in terms of nutrient uptake. Further, coarse-textured soil, i.e., sandy soil in this study, was prone to drainage. As such, soil core of 10 cm could be reliable to illustrate fundamental principles of microbial controls on pool size of soluble N and then N loss via leaching. The intact soil cores were capped, transported in an ice-packed cooler to the laboratory, and stored at 4 °C for ~ two days before initiating leaching and gas sampling experiments.

2.2. Intact soil core setup for collecting leachate and gas sample

A glass fiber filter paper was fitted to the bottom of each intact soil core and then the core was tightly placed onto the base of EMD Millipore Sterifil™ vacuum filter system (Fig. 1). The top of intact soil core was sealed with a cap having one-way luer stopcock for periodic gas sampling. This setup allowed for simultaneous measurements of reactive N effluxes via leaching and gas emission.

For each turfgrass system, a total of 12 intact soil cores (four treatments × 3 replicates) were used to measure N effluxes during a 23-d incubation. The treatments were (1) no N input and 23 °C, (2) low N input (i.e., addition of 5.9 mg N as NH₄NO₃, equivalent to 30 kg N ha⁻¹) and 23 °C, (3) high N input (i.e., addition of 11.8 mg N as NH₄NO₃, equivalent to 60 kg N ha⁻¹)

and 23°C, and (4) high N input and 13°C for winter samples or 33°C for summer samples. On day 0, water and NH_4NO_3 solution were added to the respective soil cores to bring soil moisture to 60% water filled pore space (WFPS). Here, we estimated soil bulk density and moisture content from the other eight intact soil cores and then calculated soil porosity assuming that soil particle density was 2.65gcm^{-3} . These soil cores were also used for soil pH measurement.

Soil N_2O and CO_2 effluxes were measured ~ daily during the 23-d incubation. The top of each soil core was capped for 2h to withdrawing 30-ml gas sample from the headspace using a gas-tight push button syringe. Leachates were collected only on d 4, 11, and 18 following N_2O efflux measurement. Roughly 80ml leachate was collected under 2h free drainage and then vacuum from each soil core after addition of 80ml distilled water to soil surface.

2.3. Measurements of N efflux rates

Nitrous oxide was measured using a dual quantum cascade laser (QCL) N_2O measurement system (Model CWQCL-200-D, Aerodyne, Billerica, MA, USA) (Chen et al., 2016). The efflux rate of N_2O ($\mu\text{gm}^{-2}\text{h}^{-1}$) was calculated as: $((C_{\text{sample}} - C_{\text{air}}) \times M \times V) / (r \times m \times t)$, where C_{sample} and C_{air} are the N_2O concentrations in the headspace of intact soil core and ambient air (ppbv), respectively; V is the volume of intact soil core headspace (l); M is the molar mass of N_2O (gmol^{-1}); r is the molar volume at 1atm (23.47lmol^{-1} at 13°C, 24.29lmol^{-1} at 23°C, and 25.11lmol^{-1} at 33°C); m is soil core surface area (m^2); and t is the incubation time (h).

The QCL system also provided the mixing ratio of natural abundance of ^{15}N in the center and edge position of N_2O , i.e., $^{15}\text{N}^{\alpha}$ and $^{15}\text{N}^{\beta}$, respectively. After normalized to total N_2O -N concentration, these ratios were used to calculate the natural abundance of $^{15}\text{N}^{\alpha}$ and $^{15}\text{N}^{\beta}$ of N_2O . Then, ^{15}N site preference was estimated by $\delta^{15}\text{N}^{\alpha} - \delta^{15}\text{N}^{\beta}$ and used to help probe the biological source of N_2O efflux (Chen et al., 2016).

Inorganic N (NH_4^+ -N and NO_3^- -N) of leachate was measured using a FIA QuikChem 8000 auto-analyzer (Lachat Instruments, Loveland, CO, USA). Total N of leachate was determined using a TOC- V_{CPN} analyzer combined with TNM-1 (Shimadzu Scientific Instruments, Japan). Organic N of leachate was then calculated as the difference between total N and inorganic N.

2.4. Measurements of auxiliary soil properties

Carbon dioxide was measured using an infrared gas analyzer LI-840A (LI-COR, Lincoln, NE, USA), and soil CO_2 efflux rate was calculated using the same equation as for N_2O efflux rate. To better understand controls on N loss and its pathways, soil chemical and biological properties were determined. After three-week incubation, the intact soil cores were manually crushed and sieved ($<4\text{mm}$), and analyzed for soil total C and N by dry combustion method using a Perkin-Elmer 2400 CHN analyzer (Perkin-Elmer Corporation, Norwalk, CT, USA). Soil extractable inorganic and organic N were measured on 0.5M K_2SO_4 extract (1:5 soil (g)/solution (ml) ratio). Soil microbial biomass C and N were determined by the chloroform fumigation extraction method with extraction coefficient 0.38 for biomass C and 0.54 for biomass N (Brookes et al., 1985; Vance et al., 1987). The

activities of soil exoglucanase (EC 3.2.1.91), β -glucosidase (EC 3.2.1.21), and β -glucosaminidase (EC 3.2.1.30) were determined using 2mM *p*-nitrophenyl- β -D cellobioside, 10mM *p*-nitrophenyl- β -D-glucopyranoside, and 2mM *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide as the substrates, respectively (Tian and Shi, 2014), and the activity of peroxidase was measured following the method of Johnsen and Jacobsen (2008). The assay of each soil enzyme activity included two types of controls (i.e., substrate alone and soil alone). After subtracting the controls, hydrolase activity was calculated against the standard curve of *p*-nitrophenol; and oxidase activity was calculated using the extinction coefficient $59,000\text{M}^{-1}\text{cm}^{-1}$.

2.5. Data analysis

Significant differences of all the data among treatments were analyzed using ANOVA of a completely randomized block design (SAS 9.3, SAS Institute Inc. Cary, NC, USA), since treatments were randomized within three bench/incubator places (blocks). Linear correlation analyses were also used to evaluate the relationships of N loss and its pathways with soil chemical and biological properties.

3. Results

3.1. N losses from turfgrass systems of different ages

Regardless of turfgrass systems, inorganic N and organic N in leachates as well as N_2O effluxes were relatively stable for no N treatment, but were greatest in the first week of the incubation for low N and high N treatments (Fig. 2), suggesting that mineral N input and microbial processes interactively controlled pool sizes of different N species and thus N loss via leaching and gas emission. However, total reactive N loss, i.e., sum of inorganic N and organic N in leachate plus N_2O -N efflux over 23-d incubation, differed significantly among the four turfgrass systems (Fig. 3). Irrespective of grass growing stages (i.e., winter vs. summer samples), and temperature and fertilization treatments, total reactive N loss generally showed a “cup” pattern of turfgrass age, being lowest for the 20-year old turf site. Compared with no N treatment, low N and high N enhanced total reactive N loss by ~2 and 3.5 folds, respectively, on average across the four turfgrass systems. The increase in loss was equivalent to ~70% of respective mineral N inputs, suggesting that ~30% of added N remained in the grass-soil system, perhaps due to grass N uptake, microbial N assimilation, and N adsorbed onto soil particles and organic matter. A reduction in temperature from 23°C to 13°C for winter samples did not affect total reactive N loss, but an increase in temperature from 23°C to 33°C for summer samples increased average total reactive N loss ~2 folds across the four turfgrass systems.

Despite the same surface area and volume, soil mass varied among intact soil cores due to variations in soil bulk density (Table 1). Soil dry weight across all intact soil cores used for leaching and gas emission measurements averaged 212g with 20% coefficient of variation. Expressed on a soil dry weight basis, total reactive N losses were similar to those in Fig. 3 (data not shown), indicating that our results were consistent between area- and mass-based measurements.

3.2. N loss pathways as affected by turf age, season, and temperature/fertilization treatments

On average across all intact soil cores sampled in winter and summer, organic N leaching accounted for ~51% of total reactive N loss, followed by 41% from inorganic N leaching and 8% as N₂O-N efflux. However, their relative contributions varied with turf age, season, and temperature and fertilization treatments (Fig. 4). Twenty year old turf had the lowest fraction of inorganic N leaching, but the largest fraction of organic N leaching in winter and also the largest fraction of N₂O-N efflux in summer ($P<0.05$, Fig. 4A). Independent of turf age, the fraction of organic N leaching was significantly larger ($P<0.05$) in summer than in winter, i.e., 63% and 42%, respectively, whereas the fraction of inorganic N leaching was greater in winter (50% compared to 28%).

Decreasing the temperature from 23°C to 13°C for winter samples reduced the fractions of organic N leaching and N₂O-N efflux, but enhanced the fraction of inorganic N leaching ($P<0.05$) (Fig. 4B). By contrast, increasing the temperature from 23°C to 33°C for summer samples had no effect on the relative contributions of organic N leaching, inorganic N leaching and N₂O-N efflux to the total reactive N loss.

Compared to no N addition, low N and high N increased the fraction of inorganic N leaching or N₂O-N efflux, but reduced the fraction of organic N leaching ($P<0.05$) (Fig. 4C). However, these fractional changes, especially for inorganic N leaching or organic N leaching were relatively small, i.e., ~10% on average across the four turfgrass systems.

3.3. N losses in relation to soil chemical and biological properties

The pattern of total reactive N loss as a function of age (Fig. 3) was not associated with soil organic matter content, soil pH or bulk density. Despite significant differences in total reactive N loss between 20 and 109year old turfgrass, soil C and N contents and soil bulk density were similar between the two systems (Table 1). Further, this cup-shaped pattern of loss was not correlated with soil microbial biomass C or CO₂ respiration. Similar to total soil C and N content, microbial biomass C and CO₂ respiration were also greater in 20 and 109year old turfs than the younger ones.

The promotive effect of mineral N input on total reactive N loss (Fig. 3) was positively and significantly correlated with its effect on soil exoglucanase activity (Table 2, Pearson's correlation coefficient $r=0.73$, $P<0.001$). However, N input had no consistent positive effect on microbial biomass C or CO₂ respiration (Fig. 5). The positive effect of summer/high temperature on total reactive N loss (Fig. 3) was highly correlated with its effect on CO₂respiration (Fig. 5, Pearson's correlation coefficient $r=0.52$, $P<0.01$). By contrast, winter/low temperature treatment had little effect on total reactive N loss despite associated reductions in CO₂ respiration and soil hydrolytic enzyme activities (Fig. 5, Table 2).

The effect of turf age on N loss (Fig. 4A) was not correlated with soil C and N content, microbial biomass C, soil respiration or soil enzyme activities. However, winter/low temperature treatment significantly reduced organic N leaching and N₂O-N efflux fractions (Fig. 4B); this was significantly correlated with reduced CO₂ respiration (Pearson's correlation coefficient $r=0.85$, $P<0.001$), soil glucosidase activity (Pearson's correlation

coefficient $r=0.51$, $P<0.05$), and soil glucosaminidase activity (Pearson's correlation coefficient $r=0.54$, $P<0.05$). Although mineral N inputs enhanced the fraction of N_2O -N efflux (Fig. 4C), the ^{15}N site-preferences of N_2O emitted from differently treated turfgrass systems were similar, $\sim 4.15\text{‰}$ on average over 288 data points where soil N_2O flux was $>280\mu\text{gN-N}_2\text{O m}^{-2}\text{h}^{-1}$. This suggests that N input and temperature treatments did not alter biological source of N_2O emissions.

Differences in soil bulk density among the four turfgrass systems (Table 1) might affect leaching and N_2O diffusion via difference in tortuosity, thereby the partitioning of soil N between loss and retention. We measured salt solution-extractable inorganic and organic N after leaching and gas emission experiments and found that the ratio of salt-extractable N to total reactive N loss varied significantly among the four turfgrass systems (Fig. 6A). Therefore, we summed total reactive N loss and extractable soil N as another metric to further evaluate N loss potential. Similar to total reactive N loss, sum of total reactive N loss and soil extractable N also showed a “cup” pattern of turfgrass age (Fig. 6B), and such a “cup” pattern was strengthened to be more clear after normalized to soil microbial biomass N (Fig. 6C).

4. Discussion

As opposed to unmanaged and natural ecosystems, the ecological succession of turfgrass systems manifests mainly belowground. The community compositions of soil heterotrophs, nitrifiers, and denitrifiers changed as a function of turf age, although these changes were moderate (Dell et al., 2008; Dell et al., 2010; Yao et al., 2006). Microbial community functional traits, e.g., apparent C and N use efficiency, also respond to turf age (Shi et al., 2006b). In agreement with other reports (Bandaranayake et al., 2003; Qian and Follett, 2002; Shi et al., 2006a), our data showed that soil C and N as well as microbial community biomass and activity stabilized approximately 20–30 years after turfgrass establishment. However, despite these successional changes and ultimate stabilization, the bermudagrass turf continued to discharge N to the environment.

4.1. Regulation modes of N loss in turfgrass ecosystems

Understanding the controls on pool size of soil soluble N is central to predicting N loss from soil to water and air. It's well known that microbial processes, and specifically soil microbial community activity, turnover and resource use efficiency, mediate soil dissolved N (Fig. 7) (Schmidt et al., 2007; Mooshammer et al., 2014). However, these microbial functional traits may respond divergently to various edaphic factors, hence creating diverse extents and regulatory modes of soil N loss. Our chronosequence data showed that total reactive N loss from soil to water and air was not associated with soil C or N, microbial community biomass C or N, or CO_2 respiration. This suggests that during turfgrass ecosystem succession there is a complex response pattern of microbial functional traits.

During the early successional stage in turf, ecosystem productivity was increasing and resource would be ample to support rapid growth of the soil microbial community. The so-called “r-strategy” microbes, such as bacteria and some fungi could quickly respond to available resources and occupy the territory by rapid proliferation (Blagodatskaya et al.,

2011; Howell et al., 2014; Rousk and Bååth, 2011). Their fast growth rate would like be tied to rapid turnover, thereby increasing the flux of N from biomass to the soil. Although a significant portion of released N could be recycled back into the microbial biomass, the remainder would accumulate over time, eventually resulting in a sizable pool of soluble N. Another consequence of rapid microbial growth is low resource use efficiency (Lipson, 2015); more C would be partitioned into CO₂ respiration than to increased biomass and thus less N would be assimilated by the microbial community. This low C use efficiency, together with rapid microbial turnover might explain why there was a large amount of soluble N in leachate during the time when the turf was still below its capacity as N sink.

Following the initial phase of turf ecosystem succession, microbial growth rate declines (Shi et al., 2006a) and the increase in soil microbial biomass and activity begin to level off. As a result, microbial turnover rate would likely decline whereas microbial C use efficiency would increase. These changes would favor microbial N retention and therefore reduce soil soluble N. This framework provides a mechanistic explanation of the decline in reactive N loss during successional states prior to steady state, but doesn't explain why the oldest turf deviated from projected patterns of reactive N loss.

One possible explanation is that the quantity and quality of soil organic matter did not develop synchronously as turfgrass ecosystems aged. The chemistry of soil organic matter might continue to change after total soil C and N content peaked. We did observe that soil exoglucanase activity differed between the two older turfgrass systems, although their organic C contents were similar. Soil peroxidase activity also tended to be greater in the oldest turfgrass system. Therefore, enzyme-catalyzed degradation of soil organic matter might continue to increase even after steady state, generating more soluble organic matter. This enzyme-catalyzed contribution was greatly enhanced by mineral N input, and specifically for the oldest turfgrass system (data not shown), leading to significant correlation between exoglucanase activity and total reactive N loss. Our data suggest that enzyme- and microbial activity-mediated processes increasingly contributed to soluble N, and impeded microbial N retention during turf succession. It's possible that these antagonistic processes regulating soluble N peaked at different time points, thereby resulting in the cup-shaped pattern of total reactive N loss.

We used intact soil cores in this study to investigate reactive N loss from turfgrass ecosystems and found a cup-shaped pattern of reactive N loss vs. time. This differs from data obtained using sieved and thoroughly mixed soils (Lu et al., 2015) where reactive N (i.e., soil dissolved organic N, inorganic N and N₂O-N) increased with soil organic C and N, suggesting a positive relationship between reactive N loss and turfgrass age. Such discrepancy may be due to their experiment conditions favoring enzyme and microbial activity and therefore intensifying enzyme and microbial activity-mediated production of soluble N. When vegetation was included, as in the present study, microbial turnover and growth efficiency might play more important roles in regulating soluble N (Blagodatskaya et al., 2014; Cheng, 2009; Schmidt et al., 2007).

4.2. Pathways of N loss in turfgrass ecosystems

Ammonia volatilization represents one of the pathways of N loss, specifically in calcareous and basic soils with urea as N fertilizer (Avnimelech and Laher, 1977; Craig and Wollum, 1982). However, this pathway was perhaps less significant in our study, given that soil pH was close to the neutrality and that NH_4NO_3 rather than urea was used as the input of mineral N. Our data document that dissolved organic N was an important component of N cycling and dominated N loss from turfgrass ecosystems, specifically during periods of active growth (summer). This sizable pool of dissolved organic N could be caused by fast microbial turnover, uncoupling between organic matter decomposition and microbial N demand, or root efflux.

It's well known that microbial turnover rate is fast and life cycles as short as a couple of days (Rousk and Bååth, 2011). As such, turnover rates of microbial biomass C could be hundreds times greater than those of soil organic C (Blagodatskaya et al., 2011). Besides this broad sense of microbial turnover, associated with death and proliferation, compounds within living cells are also subjected to replacement and renaissance (Gunina et al., 2017; Malik et al., 2015). Nitrogen-containing cellular compounds, such as RNA and DNA could be renewed in about one day (Malik et al., 2015). This, together with turfgrass root uptake and also immediate N loss right after mineral N input through leaching and gas emissions, perhaps explained why added inorganic N did not remain in soil, but rather was rapidly transformed into dissolved soil organic N. Interestingly, we found that the C-to-N ratio of soluble organic matter differed between winter and summer samples, being 6.6 and 2.4, respectively, on average across all the treatments. These relatively "low" C-to-N ratios were closer to the averaged C-to-N ratios of twenty amino acids (8.2) and four nucleobases (2.8), than to other cellular compounds. This seems to suggest that protein turnover might play a more important role in winter, whereas nucleotide turnover dominated in summer in regulating soil N cycling and the environmental fate of N in turfgrass ecosystems.

Decoupling between organic matter decomposition and microbial C and N utilization could also enhance the pool size of soil dissolved organic N. Enzyme-catalyzed depolymerization of soil organic matter leads to the production of soluble organic compounds. However, further mineralization of these soluble organic compounds depends not only on their degradability but also on soil microbial activity. Lower degradability and/or less microbial demands for C and nutrients could cause the accumulation of soluble organic compounds in soil. Given the increased pool size and recalcitrance of soil organic matter as well as increased enzyme activities along the turf chronosequence (Shi et al., 2006a), the enzyme-catalyzed production of soluble organic compounds would likely be greater, but less degradable in older turfgrass systems than the younger ones and therefore the decoupling mechanism might be more important in older turfgrass systems. We did find that mineral N addition stimulated microbial biomass C of older turfgrass systems but not younger ones for summer samples, suggesting some divergence in microbial activity and nutrient demands between old and younger turfs.

4.3. Temperature impacts on reactive N loss

Temperature affects the soil microbial community across the entire spectrum from structure to function. It regulates organic matter decomposition with a Q_{10} temperature coefficient (the measure of decomposition rate change as a consequence of increasing the temperature by 10°C), averaging ~2 across the temperature range of 5–35°C (Kätterer et al., 1998). However, Q_{10} temperature coefficients varied substantially with not only organic matter quantity and quality, but also biochemical processes (Bracho et al., 2016; Gershenson et al., 2009; Koch et al., 2007). Koch et al. (2007) reported an inverse relationship between substrate quality and Q_{10} coefficient and also higher Q_{10} values for microbial respiration than for N mineralization. In this study, we also found that Q_{10} temperature coefficients of microbial respiration for winter samples increased significantly from ~1.5 in young turfs to ~2.7 in older turfs, as soil organic matter quality declined with the succession of turfgrass ecosystems (Shi et al., 2006a). This suggests that organic matter decomposition was more sensitive to an increasing temperature for older than young turfgrass ecosystems.

Interestingly, we found that the two temperature treatments had opposite effects on the extent and pathways of reactive N loss. Increasing the temperature from 13 to 23°C in winter samples had little effect on the total amount of reactive N loss, but altered the partitioning of N loss via soluble organic N, inorganic N and N_2O -N. By contrast, increasing the temperature from 23 to 33°C in summer samples increased reactive N loss, but had no effect on the partitioning of N loss species. Such divergent temperature responses were perhaps due to interactive controls of temperature and substrate availability on microbial biomass turnover and growth efficiency.

A number of studies have focused on microbial C use efficiency in response to rising temperatures to explore underlying mechanisms of soil CO_2 efflux feedback to global warming. While an inverse relationship between temperature and microbial C use efficiency has been accepted as the norm (Manzoni et al., 2012), a few studies have argued that rising temperature only accelerates microbial biomass turnover, but has no effect on microbial C use efficiency (Dijkstra et al., 2011; Hagerty et al., 2014). Recent work has led to some reconciliation over this debate by emphasizing the regulatory roles of substrate availability and degradability on relationships between microbial C use efficiency and temperature (Frey et al., 2013). It seems that the more labile and available substrates are, the less responsive microbial C use efficiency is to temperature. For example, Frey et al. (2013) found no temperature response of microbial C use efficiency when glucose and oxalic acid are the main C and energy sources for microbial growth.

For winter samples with dormant turfgrass, C flow from plant to soil was probably low, and stabilized soil organic C was expected to be the major substrate to sustain the microbial community. With rising temperature, soil enzyme activities would increase and stimulate soil organic C decomposition. However, oligomers that were generated from enzyme-catalyzed depolymerization or dissociation of complex soil organic matter are biochemically diverse and the average degradability was most likely low compared to monomers, such as glucose (Blagodatskaya et al., 2009). In this situation, microbial C use efficiency was likely negatively associated with rising temperature. Therefore, for winter samples, rising temperature not only might accelerate microbial turnover, but also likely reduced microbial

C use efficiency. While fast turnover led to a shift of N loss from inorganic N to organic N, reduced microbial C use efficiency might explain why this shift was moderate compared to that for summer samples.

For summer samples with actively growing turfgrass, root efflux of labile compounds would contribute a significant portion of available substrates to the soil microbial community (Hütsch et al., 2002; Kuzyakov and Schneckenberger, 2004). Compared with stabilized soil organic matter, C compounds released by plants are often more labile and degradable. This might lead to little temperature response of microbial C use efficiency. As a consequence, rising temperature for summer samples did not affect the partitioning of N loss via dissolved organic N, inorganic N, and N₂O-N.

4.4. Conclusions

Our results demonstrate that dissolved organic N leaching was a dominant pathway of reactive N loss from turf, specifically during the summer period of active growth. Several hypothetical modules were proposed to explain reactive N loss along with ecosystem succession and as a function of fertilization and rising temperature. While microbial growth efficiency, turnover and activity-mediated organic matter degradation could all regulate the pool size of soil soluble N, their trends over time differed. While microbial turnover was expected to decline with the succession of turfgrass ecosystems, growth efficiency and biochemical process rates might increase over time. The combined effects perhaps led to a time delineated cup-shaped pattern of reactive N loss from turf. Fast microbial biomass and biomolecule turnover was likely the cause for the large contribution of dissolved organic N to reactive N loss. Our data further suggest that substrate availability (e.g., rhizodeposition and root biomass turnover between winter and summer samples) reacts to warming to affect extent and pathways of reactive N loss. When substrate availability was high, as in summer, rising temperature was expected to mainly affect microbial turnover and therefore enhanced total reactive N loss. In contrast, when substrate availability was low, warming might mainly affect microbial growth efficiency and therefore the partitioning of N loss among dissolved organic N, inorganic N, and N₂O-N. Our work also highlights the importance of including vegetation to reliably predict reactive N loss from turfgrass ecosystems.

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Fig. 1.

A system setup for simultaneously collecting reactive N via leaching and gas emission. An intact soil core taken from a turfgrass system was installed onto a filter support screen and 250ml receiver flask (EMD Millipore Filtration System). The top of intact soil core was capped only when gas sampling was needed.

Fig. 2.

Inorganic N and organic N losses through leaching that were collected on d 4, 11, and 18 from intact soil cores, and N₂O efflux rates that were measured almost daily during the 23-d incubation. Data are means of four turfgrass systems and bars for N in leachates represent standard errors. For better visualization, standard errors for N₂O effluxes are not given.

Fig. 3.

Total reactive N losses, i.e., sum of inorganic and organic N in leachate and N₂O-N efflux, from intact soil cores sampled in winter and summer from four turfgrass systems of different ages (i.e., 1, 15, 20, and 109 years old). Errors bars represent standard errors for $n=3$.

Fig. 4. Effects of turfgrass age (A), temperature (B), and mineral N input (C) on inorganic N leaching, organic N leaching, and N₂O-N efflux as a fraction of total reactive N loss in intact soil cores sampled in winter and summer. Errors bars represent standard errors for n=3.

Fig. 5. Soil microbial biomass C and CO₂ respiration in intact soil cores sampled in winter and summer from four turfgrass systems of different ages (i.e., 1, 15, 20, and 109 years old). Errors bars represent standard errors for n=3.

Fig. 6. Ratio of soil extractable N to total reactive N (A), sum of soil extractable N and total reactive N (B), and (C) ratio of sum of soil extractable N and total reactive N to soil microbial biomass N in intact soil cores collected in winter from four turfgrass systems of different ages (i.e., 1, 15, 20, and 109 years old). Errors bars represent standard errors for n=3.

Fig. 7.

Microbial functional traits: activity, turnover, and growth efficiency in regulating soil soluble N pool size and thus the loss of reactive N via leaching and gas emissions. Solid lines (a) represent activity-regulated processes, including soil enzyme-catalyzed degradation from organic N to dissolved organic N (DON), biochemical and biological mineralization from DON to inorganic N, and transformations between inorganic N species through nitrification and denitrification. Dash lines (t) represent N flows between microbial biomass N and soil N (organic N, DON, and inorganic N) due to microbial biomolecule and cell turnover. A double solid line (g) represents microbial N uptake that are controlled by organic C available and microbial C use efficiency.

Table 1.

Selected soil properties from four turfgrass systems of different ages (i.e., 1, 15, 20, and 109years old). Bulk density and soil pH were determined from non-leaching intact soil cores, and soil C and N were determined from leaching intact soil cores. Different letters within each row indicate significant differences at $\alpha = 0.05$.

	1-yr	15-yr	20-yr	109-yr
Bulk density (gcm^{-3})	1.33 ^a	1.08 ^b	1.13 ^b	1.07 ^b
Soil pH	7.10 ^a	6.94 ^{ab}	6.99 ^{ab}	6.81 ^b
Soil total C (mgg^{-1})	8.46 ^c	14.20 ^b	29.66 ^a	27.46 ^a
Soil total N (mgg^{-1})	0.63 ^c	1.28 ^b	2.23 ^a	2.45 ^a

*

Because soil properties in winter and summer samples were not different statistically, the means were reported.

Table 2.

Effects of synthetic N input and temperature on the activities of soil exoglucanase, β -glucosidase, β -glucosaminidase, and peroxidase determined in samples collected in winter from four turfgrass systems of different ages (i.e., 1, 15, 20, and 109 years old). Different letters within each column of a factor group indicate significant differences at $\alpha = 0.05$.

	Exoglucanase	β -glucosidase	β -glucosaminidase	Peroxidase
	(μmol g ⁻¹ soil h ⁻¹)			
1-yr	0.182 ^b	0.301 ^a	0.246 ^a	0.450 ^{ab}
15-yr	0.187 ^b	0.324 ^a	0.228 ^a	0.430 ^b
20-yr	0.152 ^b	0.331 ^a	0.216 ^a	0.447 ^{ab}
109-yr	0.272 ^a	0.334 ^a	0.216 ^a	0.474 ^a
No N	0.117 ^b	0.257 ^b	0.217 ^a	0.450 ^{ab}
Low N	0.185 ^b	0.304 ^a	0.234 ^a	0.443 ^b
High N	0.292 ^a	0.339 ^a	0.236 ^a	0.447 ^b
High N-temp	0.199 ^b	0.230 ^b	0.163 ^b	0.478 ^a