



Microbial and soil properties in bentgrass putting greens: Impacts of nitrogen fertilization rates

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ABSTRACT

Nitrogen fertilization is important for maintaining the quality of golf course putting greens, but causes environmental concerns and affects soil organic matter buildup. Belowground biology and processes are vital to address both environmental and organic buildup issues. We examined microbial and soil properties in sand-based bentgrass putting greens that had been unfertilized or fertilized at the rates of 195, 244, and 305 kg N ha⁻¹ yr⁻¹ for over one year after turf establishment. Nitrogen fertilization increased soil organic C by ~10% and slightly modified microbial community as revealed by denaturing gradient gel electrophoresis, but had no effects on microbial biomass or C and N mineralization. We observed that changes in soil pH and enzyme activities were the functions of fertilization rates. Soil pH was reduced by ~0.3 to 0.8 units as fertilization rates increased. The activities of soil enzymes (β -glucosidase, N-acetyl- β -glucosaminidase, chitinase, and cellulase) were enhanced by fertilization at 195 or 244 kg N ha⁻¹ yr⁻¹, but was equivalent to or even lower than those in the unfertilized control when fertilization rate reached 305 kg N ha⁻¹ yr⁻¹. Results indicated that the activity of soil enzymes could be used as an important metric to diagnose the impacts of fertilization rates on soil. Fertilization rate at approximately 200 kg N ha⁻¹ yr⁻¹ appeared to be appropriate for managing putting greens.

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1. Introduction

Turfgrass systems, especially golf courses, represent intensively managed ecosystems with high levels of fertilization, pesticide application, and irrigation. Although putting greens occupy only a small area of a golf course, they consume a disproportionate amount of resources, especially fertilizers (Schlossberg and Schmidt, 2007). Intensive N fertilization is essential for supporting and maintaining putting green quality including color, vigor, root-to-shoot ratio, and disease resistance (Turgeon, 1999).

Two issues are often associated with N fertilization of putting greens. First, there are concerns over surface and ground-water pollution via N leaching or over the release of global warming gasses, N oxides via nitrification and denitrification. Best management practices, such as controls on the rate and the timing of fertilization and irrigation, may reduce leaching to minimal levels (Braun and Stahnke, 1995; Johnston et al., 2003; Shuman, 2004). Second, N fertilization has been considered to cause the buildup of soil organic matter due to enhanced primary production and thus soil C input through root exudates as well as grass clippings. This buildup may

decrease water permeability, create anaerobic environment in the root zone, and thus damage putting green quality (Carrow, 2003). While soil microbial community is a vital component dictating soil N transformations and organic matter decomposition, its response to N fertilization has been overlooked in putting greens.

Soil and microbial properties have been acknowledged as useful metrics for evaluating management impacts on soil sustainability and health. Often-used microbial attributes include microbial biomass, CO₂ respiration, soil enzyme activity, and microbial community composition (Bandick and Dick, 1999; Biederbeck et al., 1987; Wolters, 1991; Wu and Brookes, 2005). Generally, an increase in microbial biomass and activity is thought to be beneficial to soil C and nutrient cycling and thus ecosystem productivity.

In the case of N fertilization, multifaceted effects may simultaneously act on soil microbial community. Nitrogen fertilization may stimulate microbial growth and activity via its positive controls on primary production and thus soil organic C input (Liang and MacKezie, 1996; Raiesi, 2004). However, if N fertilization considerably increases soil osmotic tension and/or soil acidity, microbial biomass and activity may decline (Treseder, 2008; Yevdokimov et al., 2004). Nitrogen fertilization may also directly influence the physiology of microbial community and therefore its ability of organic matter decomposition. For example, when soil N availability is increased by fertilization, soil microbes may reallocate resource from producing N-

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acquiring enzymes to C- and other nutrient-acquiring enzymes (Sinsabaugh and Moorhead, 1994). It has been reported that in forest systems, N fertilization could stimulate C-acquiring enzymes such as cellulase and glucosidase but suppress oxidative enzymes such as phenol oxidase (Gallo et al., 2004; Saiya-Cork et al., 2002; Sinsabaugh et al., 2005). Such changes in soil enzyme activities have been found to be correlated with soil organic C dynamics in response to soil N availability (Carreiro et al., 2000; Waldrop et al., 2004). Furthermore, N fertilization may cause a shift in soil microbial community composition and subsequently alter microbial production of extracellular soil enzymes. The direction and magnitude of influences depend on microbial properties with some being more sensitive to N fertilization than others. Our previous research in a hay production field found that soil enzyme activity responded to N fertilization rates while CO₂ respiration and N mineralization remained unaffected (Iyyemperumal and Shi, 2008).

The objective of this study was to determine soil and microbial properties most responsive to the different rates of N fertilization in turfgrass putting greens. We hypothesized that, as in the hay production field (Iyyemperumal and Shi, 2008), soil enzymes were more responsive to the rates of N fertilization than other traditional microbial metrics. Because enzyme-catalyzed depolymerization is an important and perhaps rate-limiting step of organic matter decomposition, detection for the change of soil enzyme activity may facilitate an early prediction on potential change in soil organic matter. Thus, a better understanding on the impacts of N fertilization rates on soil microbial properties can help make informed management decisions to minimize organic matter buildup in putting greens. To better evaluate the impacts of fertilization rates on the putting green soil, the compositions of dissolved or extractable soil organic matter and microbial community were also included in the examination.

2. Materials and methods

2.1. Field plots and soil sampling

Bentgrass putting greens were established in 2004 on sand-based topsoil made by mixing 10% peat and 90% sand, at Lake Wheeler Turfgrass Research Station, North Carolina State University, NC, USA. The fertilization experiment was initiated in spring 2006 for studying the impacts of fertilization rates on bentgrass growth and organic matter buildup in soil. Sixteen-field plots, each occupying ~5.6 m² and representing one of four N treatments, were arranged into four blocks based on a randomized block design. The N treatments were an unfertilized control and three rates of N application: 195, 244, and 305 kg N ha⁻¹ yr⁻¹. Fertilizers were applied as a 20–20–20 liquid fertilizer on a bi-weekly basis from June through September using a backpack sprayer (Pro-spray, Cleveland, OH) and as a granular 15–15–15 IBDU in October, November, February, March, and April using a hand-held shaker. Herbicides, fungicides and insecticides were also applied for preventative controls of weeds, fungal pathogens and insects. Irrigation was provided every week but varied in volume from 0.9 cm to 9 cm per plot dependent on weather and turfgrass growth phase. Bentgrass was mown to 0.30 cm–0.46 cm, with a greater height during environmentally stressful periods in summer and grass clippings were always removed. Topdressing sand was applied with a drip spreader (Gandy, Owatonna, MN) every 2–3 weeks from March through November at a rate of 5.38 m³ ha⁻¹ based on USGA recommendations. A push broom was used to incorporate the topdressing sand into the turf canopy. During the period from spring 2006 to August 2007, grass clipping weights and N in grass tissue were measured in six sampling dates and were found to increase significantly with fertilization rates. Soil organic matter contents in 0–2.54 cm depth and 2.54–7.62 cm depth were also measured in seven sampling dates. Generally, soil organic matter content was greater in 0–2.54 cm depth than 2.54–7.62 cm depth and the

differences in soil organic matter content among fertilization treatments were more pronounced in 0–2.54 cm depth. Effects of fertilization rates on clipping production, N in grass tissue and soil organic matter content were independent of our sampling times.

Soils were sampled by coring technique in September 2007, approximately two weeks after a scheduled fertilization. This sampling time allowed us to examine the consequence of one-year-long fertilization treatments. In addition, this sampling time minimized the impacts of newly-produced plant biomass, such as root exudates on soil properties because bentgrass grew slowly in September, as shown by the low weight of grass clippings. A deep soil sampling was made to further minimize the impacts of belowground plant biomass on soil properties. Six cores (2 cm in diameter × 12 cm in length) were randomly taken from each plot and then mixed to represent a composite soil sample. After sieving (<2 mm) and removal of visible roots and plant residues, soil samples were stored at 4 °C until analyses of soil and microbial properties.

2.2. Soil properties

Total soil C and N were determined by dry combustion using Perkin-Elmer Series II CHNS/O-2400 analyzer (Perkin Elmer Corp., Norwalk, CT, USA). Soil inorganic N (NO₃⁻ and NH₄⁺) was extracted with 1 M KCl and then analyzed colorimetrically using a Lachat flow-injection auto-analyzer (Lachat Instruments, Mequon, WI, USA). Soil pH was measured in soil slurry with a soil (g) to water (ml) ratio of 1:2.5.

Chemical compositions (i.e., functional groups) of extractable and soil organic matter were assessed with Fourier transform infrared (FTIR) spectroscopy. Fifteen grams of moist soil were extracted with 75 ml of 0.5 M K₂SO₄. After shaking at 250 rpm for 30 min, the soil slurry was centrifuged at ~2000 × g for 10 min. The liquid and solid fractions were separated, freeze-dried, and an aliquot (10 mg) was mixed with 0.5 g KBr, ground to fine powder, and pressed into a translucent pellet. The pellets were scanned from wavenumber 4000 to 400 cm⁻¹ with a Nexus 470 FTIR spectrophotometer (Thermo Nicolet Corporation, Madison, WI, USA). Chemical functional groups, as defined by Johnston and Aochi (1996), were identified and semi-quantified using the ratio of peak height at a certain wavenumber to the peak height at the polysaccharide peak near 1060 cm⁻¹ (Gressel et al., 1995b).

2.3. Soil microbial biomass, activity, and composition

Microbial biomass C and N were determined by the chloroform fumigation method (Brookes et al., 1985; Vance et al., 1987) and extraction coefficients 0.38 and 0.54 were used for biomass C and N calculation, respectively. Carbon and nitrogen mineralization potentials were measured as a cumulative production of CO₂ and inorganic N over a two-month incubation as described previously (Shi et al., 2006b).

Activities of soil enzymes including β-glucosidase, N-acetyl-β-glucosaminidase, cellulase, chitinase, and peroxidase were determined using colorimetric assays. Glucosidase and N-acetyl-β-glucosaminidase activities were determined using p-nitrophenol-glucopyranoside and p-nitrophenol-glucosiminide as substrates, respectively (Parham and Deng, 2000; Turner et al., 2002). Cellulase activity was determined using carboxymethyl cellulose (i.e., CM-cellulose) as the substrate according to the method of von Mersi and Schinner (1996). Chitinase activity was measured via the appearance of N-acetyl-glucosamine after soil incubation with chitin (Rössner, 1996). Peroxidase activity was measured using L-dihydroxy-phenylalanine (L-DOPA) as the substrate (Saiya-Cork et al., 2002). Enzyme activities were expressed as pkat g⁻¹ soil with one kat defined as 1 mol of product appearance or substrate disappearance per second.

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