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Heat stress and N fertilization affect soil microbial and enzyme activities in the creeping bentgrass (*Agrostis Stolonifera* L.) rhizosphere

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ABSTRACT

High summer temperatures often cause damage to bentgrass on putting greens in transition zone regions. One of the most damaging effects is a depression of rooting. Although heat stress effects on plant functions are considered as a main reason for the damage, heat stress also may be related to organic matter (OM) accumulation and poor gas exchange into the rhizosphere. The OM accumulation and the often-observed root dieback suggest that soil microbial processes play a role in summer bentgrass decline. In this study, the impact of high temperature on soil microbial properties and enzyme activities was examined using creeping bentgrass (*Agrostis stolonifera*) growing in a phytotron controlled environment chamber. The high temperature exposures (34/30 °C versus 22/18 °C for controls) lasted for four weeks and the bentgrass cultures received mineral N at two rates. Our working hypothesis was that not only did high temperatures stimulate overall soil microbial and enzyme activity but also selectively modified microbial catabolic functions. To test this hypothesis, we compared temperature sensitivities and Q_{10} values of microbial substrate utilization patterns using a Biolog plate approach and soil enzyme activities. The results indicated that soil enzyme activities had similar responses to assay temperatures and their Q_{10} values averaged ~2 with changes of laboratory assay temperatures from 12 to 22 °C and from 22 to 34 °C. Such positive responses of microbial activity to high temperatures were supported by parallel increases in rates of microbial substrate utilization. Total substrate availability in Biolog plates also increased with laboratory assay temperatures. This enhancement could not be explained by the overall stimulation of high temperature on microbial activity, but instead by selective modification of microbial community functions. Nitrogen fertilization significantly changed soil biological activities. Phenol oxidase activity was reduced by the high rate of N fertilization, whereas β-glucosidase and β-glucosaminidase activities were increased. Interactions on soil enzyme activities between growth chamber temperatures and N fertilization rates also occurred. Soil peroxidase activity was ~three-fold greater for bentgrass subjected to heat stress and the low rate of N fertilization. Our results indicated that summer heat stress and the associated increases in root and OM degradation in bentgrass systems are related with overall temperature stimulations on soil microbial and enzyme activities as well as with modifications in functional components of the microbial community.

1. Introduction

Turfgrass putting greens represent a unique ecosystem that requires intensive management to maintain a visually acceptable, high-quality playing surface. In transition zones such as North Carolina, USA where bentgrass (*Agrostis stolonifera*) constitutes most putting greens, one of the greatest management challenges is posed by summer bentgrass decline (SBD). With exposure to high temperatures, bentgrass quality decreases, along with photosynthesis, carbohydrate metabolism, and shoot and root growth (Huang et al., 1998; Xu and Huang, 2000, 2001; Pote et al., 2006). Despite efforts to develop bentgrass varieties with more tolerance to heat and altering management strategies, SBD remains a concern.

Maintaining root function is a critical factor in attempts to sustain healthy bentgrass during the high temperatures of summer. Root dieback is commonly observed and considered to be a primary factor causing SBD (Huang and Liu, 2003; Huang et al., 2005). The decreases in root growth can be attributed to several factors. One is a direct heat effect on root growth processes. Another is the decline in photosynthesis, where lower amounts of fixed C are partitioned to the root system (Xu and Huang, 2000). It has also been suggested that a main culprit in SBD is the accumulation of soil

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organic matter (OM) and chemical transformation of OM at the soil surface (Carrow, 2004). The OM effects lead to restrictions of water permeability and gas exchange into the rhizosphere, compounding the physiological effects. Thus, understanding OM degradation is a key part of understanding the underlying mechanisms controlling SBD.

Soil microbes mediate OM decomposition. Similar to many other plant–soil ecosystems, turfgrasses appear to support abundant and diverse soil microbial populations (Mancino et al., 1993; Bigelow et al., 2002; Wang and Skipper, 2004; Yao et al., 2006; Dell et al., 2008, 2010; Elliott et al., 2008). A few studies have specifically described microbial populations in the rhizosphere of bentgrass putting greens (Mancino et al., 1993; Bigelow et al., 2002; Elliott et al., 2003, 2004, 2008). General observations include: (1) Soil microbes populate quickly soon after putting green establishment; (2) Soil microbial populations reach a level similar to that of unmanaged ecosystems; (3) The soil microbial community is dominated by *Bacillus* and *Pseudomonas* spp.; and (4) Soil microbes in the thatch layer are more abundant than those in the lower sand–peat layer, probably because of high root density and the associated production of exudate.

It is unclear how a microbial population might respond during SBD. Root exudate and its provision of C substrate appear to be an important factor controlling microbial activity (Grayston et al., 1998) and, ordinarily, exudation by plant roots is substantial. A meta-analysis that compiled data from 95 ^{14}C-labeled plant studies indicated that 5–10% of photosynthetically fixed C can be recovered in soil (Farrar et al., 2003). Accordingly, with root dieback during SBD, a decrease in root exudation might be expected, causing microbial growth and activity to decline. On the other hand, it is conceivable that dying root material may serve as a significant source of organic C and nutrients, and therefore enhance microbial growth and activity. The few studies that monitored particular characteristics of the microbial population over extended time periods have found that microbial populations appear to remain relatively stable over the summer months (Bigelow et al., 2002; Elliott et al., 2003, 2004). Those studies, however, used cultural techniques to enumerate a selected group of soil microbes, primarily bacteria.

Microbial population density or biomass measures often do not provide sufficient detail on the microbial responses to soil C and related nutrient dynamics. Microbial properties such as community composition and functionality provide more relevant information. This is because different microbial genera and species may have dissimilar preferences and competitive abilities in using organic compounds. And, the preferences and competitive abilities are strongly influenced by the rhizosphere environment. Microbial production of extracellular soil enzymes, for example, is highly dependent on soil C substrate and nutrient dynamics (Tabatabai, 1994; Sinsabaugh et al., 2008), and soil pH and temperature (Burns, 1982; Wallenstein and Weintraub, 2008; Shi, 2011).

In this study, we examined the impacts of summer heat stress on microbial communities and on soil enzyme activities in a bentgrass rhizosphere. Because N fertility has been linked with severity of SBD (Brotherton, 2011), microbial responses to summer heat stress were examined with bentgrass supplied with different N fertilization rates. It is common for bentgrass greens to receive lowered rates of N fertilization during hot summer months.

2. Materials and methods

2.1. Experimental design and soil sampling

Sod pieces of creeping bentgrass were collected from a 3-year-old A-series USGA-spec putting green by using soil core samplers (AMS, Inc., American Falls) to extract intact sod plugs of 5 cm wide × 15 cm long. Roots were then cut at 5 cm and sod pieces were transplanted into a volume-based mixture of 90% sand and 10% peat in Deepots (Stuewe and Sons, Inc., Tangent, OR) that were 6.4 cm diameter and 25.4 cm in length. These bentgrass pots were cultured in phytotron growth chambers at 22/18 °C aerial day/night temperatures. Day light was provided by a combination of incandescent and fluorescent lamps with a photosynthetic photon flux density of 500–600 μmol m^{-2} s^{-1} for a photoperiod of 9 h/day, and night light was given by incandescent lamp for only 3 h/day. Pots were irrigated daily with the amount of deionized water that led to free draining from bottoms of the pots. The turfgrass was clipped at 3 mm height weekly and clippings were removed.

After an eight-week stabilization period, heat stress treatments were imposed. Bentgrass pots were separated into two phytotron growth chambers where aerial day/night temperatures were 22/18 °C and 34/30 °C, representing non-heat-stress or heat-stress environments, respectively. Within each growth chamber, bentgrass pots were fertilized twice a week with 40 ml of Hoagland's solution (Hoagland and Arnon, 1950) or with a mixture of 7 ml of Hoagland's solution and 33 ml of deionized water (equivalent to ~340 and 60 kg N ha^{-1}) for high or low N fertilization rates, respectively.

This experiment was carried out as a 2 × 2 factorial design (i.e., two temperature regimes and two rates of fertilization) with three replications and runs twice. Sod pieces were collected at different times within 1 year for the two runs, but phytotron conditions remained the same. The first run was used as a preliminary experiment to examine if temperature and fertilization treatments could generate subtle differences in bentgrass quality as well as soil properties. Therefore, we only measured a few soil parameters in the first run.

After four weeks, bentgrass pots were removed from growth chambers. Turf quality was visually rated on a scale of 1–9 with 1 being a completely dead or brown canopy and a rating of 9 representing dark green plants with a dense uniform canopy (Turgeon, 1999). Visual qualities of bentgrass were similar between the two runs, but subtle differences appeared among treatments. Bentgrasses subjected to the non-heat stress treatment were rated as 4.9 and 6.4 for low and high rates of N fertilization, respectively. However, visual qualities of bentgrass subjected to the heat stress treatment decreased to 3.0 and 3.5 for low and high rates of N fertilization, respectively. After the leaf tissue was removed, the top 4 cm of soil was collected and homogenized. Then following the removal of most roots and plant debris, soil samples were immediately used for the analyses of microbial substrate utilization patterns and soil enzyme activities. Microbial biomass and other chemical analysis were conducted within 2 weeks by using soil samples stored at 4 °C.

2.2. Soil chemical and microbial properties

Microbial biomass C (MBC) and N (MBN) were determined using the chloroform-fumigation extraction method (Brookes et al., 1985; Vance et al., 1987). Soil inorganic N was measured colorimetrically using a Lachat flow-injection autoanalyzer (Lachat Instruments, Mequon, WI) after samples were extracted with 0.5 M K_2SO_4 solution and filtered. Soluble organic C was also determined from 0.5 M K_2SO_4 extracts using a total C analyzer (TOC-5000, Shimadzu).

2.3. Microbial substrate utilization patterns

Microbial community metabolic diversity and composition were determined using substrate-utilization patterns of Biolog® microplates. Ecoplates (ECO) containing 31 C substrates and a non-substrate control per replicate were used to analyze