

## ABSTRACT

BROTHERTON, MARK ADAM. Influence of Cultural Practices on Creeping Bentgrass Putting Green Quality, Disease Incidence, and Organic Matter Dynamics. (Under the direction of Grady L. Miller).

Despite its popularity as a putting surface in North Carolina, creeping bentgrass (*Agrostis stolonifera* L.) is highly susceptible to summer bentgrass decline (SBD) during the hot summer months. Certain cultural practices have been shown to help alleviate the pressure of SBD. The objectives of this study were to detail the impacts of nitrogen (N) fertility, soil moisture content, and hollow- and solid-tine cultivation on 1) summer turfgrass quality and disease and algae incidence and 2) organic matter accumulation, water infiltration rate, soil O<sub>2</sub> and CO<sub>2</sub>, and microbial populations. A two year study was initiated in September 2008 in Raleigh, NC. Cultural treatments included the following: four N rates (97, 195, 293 and 391 kg ha<sup>-1</sup> yr<sup>-1</sup>), four hollow-tine cultivation programs (6.4 mm diameter tines two times yr<sup>-1</sup>, 9.5 mm diameter tines two and three times yr<sup>-1</sup>, and a control that received no core cultivation), two soil moisture levels (low and high), and two summer solid-tine cultivation treatments (spiked and not spiked). Turf quality and normalized difference vegetation index (NDVI) were strongly correlated. A N rate greater than 192 kg ha<sup>-1</sup> was needed to maintain acceptable turfgrass quality. High soil moisture consistently provided better summer turf quality compared to low soil moisture conditions. Nitrogen fertility and soil moisture interacted where higher levels of each resulted in the best quality ratings. Dollar spot incidence was only quantifiable in 2009, where high soil moisture and lower fertility produced the most infection centers. Algae were observed in 2010, reaching unacceptable levels under high soil moisture and 97 kg ha<sup>-1</sup> N rate. Low N fertility and the most intensely core cultivated bentgrass contained the least organic matter at 0 - 2.5 cm. Low soil moisture,

summer solid-tine cultivation and the most intensely core cultivated turf plots had the fastest field infiltration rates. The most intensely core cultivated bentgrass and the high soil moisture treatment also possessed the highest soil CO<sub>2</sub> levels. Microbial biomass was greatest in the surface 2.5 cm of the soil profile. Although weather plays a large role in SBD in North Carolina, results from this study shows that cultural practices can influence its severity, and the secondary stresses that can exacerbate it.

Influence of Cultural Practices on Creeping Bentgrass Putting Green Quality, Disease Incidence, and Organic Matter Dynamics

by  
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## **BIOGRAPHY**

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In 2008, Mark moved to Raleigh, North Carolina. He continued his studies in pursuit of a Masters degree in Crop Science with a focus in turfgrass management at North Carolina State University under Dr. Grady Miller. Mark is looking forward to beginning his career upon completion of his Masters degree.

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## LITERATURE REVIEW

Creeping bentgrass (*Agrostis stolonifera* L.) is a popular putting green surface due to its fine leaf texture, tolerance to low mowing heights and ability to form a very dense stand. Creeping bentgrass has a stoloniferous growth habit promoting vigorous lateral growth (Beard, 1973). It performs well at mowing heights between 3 and 19 mm, with some of the most recently released cultivars able to withstand mowing heights as low as 2.5 mm (Gray and White, 1999).

Since its release in 1955, 'Penncross' has been the industry standard cultivar for creeping bentgrass putting surfaces across the USA (Schlossberg and Karnok, 1999). Its aggressive lateral growth and a wide range of adaptability compared to previously released cultivars were primary reasons for its widespread acceptance (Beard, 1973). In the 1980s and early 1990s, several new cultivars were developed for putting green use. Compared to Penncross these newer cultivars provide higher shoot densities, more upright growth, deeper root system, better climatic tolerance, and an overall higher quality putting surface (Schlossberg and Karnok, 1999; Moeller and Bigelow, 2008; Turgeon 2005). Of those released during that period, 'Penn A'-series, 'Penn G'-series, 'L93', and 'Crenshaw' are among the most popular (Fraser 1998; Landry and Schlossberg, 2001). However, more intense cultural inputs are required to maintain the newer cultivars at a high level, particularly in regards to mowing, N fertility, and cultivation practices (Stier and Hollman, 2003; Totten et al., 2008).

### Management of Creeping Bentgrass Putting Greens

Common turfgrass maintenance practices include mowing, irrigation, fertilization and cultivation. The frequency and intensity each cultural practice is applied is customized to every turf setting. Often, turfgrass quality is directly related to the intensity of the cultural program in place. High quality turf areas such as golf course putting greens require more demanding programs than low quality areas such as grassed roadsides.

#### *Nitrogen Fertility*

Nitrogen (N) is the most important nutrient for turfgrass survival (Beard, 1973). On a molecular level, N is a component of proteins, chlorophyll, hormones and nucleic acids in the plant (Carrow et al., 2001). After carbon, hydrogen, and oxygen, N is the next most abundant macronutrient in the turfgrass plant. Shoots and leaves possess N concentrations on a dry matter basis that range from 20 to 40 g kg<sup>-1</sup> (Carrow et al., 2001). Nitrogen directly influences the plant's color, shoot growth, shoot density, root growth, rhizome and stolon growth, carbohydrate reserves, high temperature stress, cold tolerance, drought resistance, compaction and wear tolerance, thatch accumulation, and recuperative potential. However, excessive N fertilization may negatively affect these responses (Beard, 1973; Christians et al., 1979; Carrow et al., 1987; Carrow et al., 2001).

Nitrogen is taken up by the plant in three forms; nitrate, NO<sub>3</sub><sup>-</sup>, ammonium, NH<sub>4</sub><sup>+</sup>, and urea-N (Carrow et al., 2001). On golf course putting greens, N may be applied in liquid or granular formulations. Although many golf courses are shifting towards exclusive liquid fertilizer programs, granular forms are still used (Totten et al., 2008). Foliar fertilization, also

termed spoon-feeding, is advantageous in that it provides superior control over the actual amount of nutrient taken up by the plant at a given time. Foliar fertilization consists of frequent (every 10-14 days) applications of small quantities of nutrients. Recommended foliar N application rates range from 4.9 to 11.2 kg ha<sup>-1</sup> N (Carrow et al., 2001). Greater rates increase the risk of fertilizer burn. Granular applications are typically made less frequently and at higher N rates, not exceeding 49 kg ha<sup>-1</sup> (Carrow et al., 2001; Turgeon, 2005). Fertilizer programs consisting of both foliar and granular N applications are common (Carrow et al., 2001; Nikolai et al., 2001). Totten et al. (2008) documented that foliar, granular, and combination fertilizer programs can provide equally acceptable turf quality.

Annual N application rates and the response to these rates can be highly variable (Waddington, 1978; Carrow et al., 2001; Turgeon, 2005). Schlossberg and Karnok (1999) found 'Crenshaw' and 'L93' creeping bentgrass exhibited better root and shoot quality than 'Penncross' when fertilized with N at 384 kg ha<sup>-1</sup> yr<sup>-1</sup>, but differences were not as profound when 192 kg ha<sup>-1</sup> yr<sup>-1</sup> was applied. Creeping bentgrass' general N requirement is considered to be 'low-high' which can be explained by current recommendations that may range from 14.6 to 48.8 kg ha<sup>-1</sup> growing month<sup>-1</sup> (Carrow et al., 2001; Dernoeden, 2002; Turgeon, 2005). Totten et al. (2008) found that N rates <195 kg ha<sup>-1</sup> yr<sup>-1</sup> promoted thin turf and algal growth. In a study on a 'Penn A-4' bentgrass putting green, Schlossberg and Schmidt (2007) noted that the turfgrass needed at least 244 kg ha<sup>-1</sup> yr<sup>-1</sup> of N to consistently maintain acceptable turf quality. Moller et al. (2008) reported N rate affected shoot density decline of 'Penn A-4' bentgrass during the summer. Shoot density declined 23% when fertilized with 113 kg ha<sup>-1</sup> yr<sup>-1</sup> N compared to a 17% reduction when 192 kg ha<sup>-1</sup> yr<sup>-1</sup> N was applied.

Reduced N has been shown to increase ball roll (green speed) (Radko, 1985; Hartwiger et al., 2001). As a result, golf course superintendents often reduce N applications, potentially endangering the health of the turfgrass in order to increase green speed (Radko, 1985; Hartwiger et al., 2001).

### *Cultivating to Manage Soil Organic Matter*

Thatch is the accumulation of living and dead grass leaves, stems and organic debris between the soil surface and the green vegetation in a turf, and mat is defined as partially decayed thatch with intermixed sand or soil as a result of regular topdressing. Mat is found directly below the thatch and above the original soil surface (Beard, 1973). Organic matter includes any carbon containing material in the entire soil matrix.

Creeping bentgrass, and in particular the newer cultivars, is an aggressive organic matter producer. Organic matter in the soil provides benefits to a turfgrass stand as it increases soil cation exchange capacity, buffering capacity, and water retention. (McCoy, 1992; Murphy et al., 1993; Carrow et al., 2001; Carrow, 2003). Accumulation of excessive surface organic matter can be detrimental to a putting green, as it may lead to increased disease and insect activity, proneness to mower scalping and footprinting, decreased in ball roll, saturated hydraulic conductivity and gas exchange rates (Turgeon 2005). Research has suggested that maintenance of healthy organic matter concentrations range from 30 – 50 g kg<sup>-1</sup>. Managing soil organic matter requires increased frequency and/or intensity of cultivation and topdressing practices (Carrow, 2003; Stier and Hollman, 2003; Landerth et al., 2008).

Organic matter accumulates when the rate at which the turf stand produces organic matter exceeds the rate at which it is decomposed. The newer creeping bentgrass varieties generate more organic matter as a result of their higher shoot density, denser root systems, and more aggressive stolon growth and production (Fraser, 1998; Stier and Hollman, 2003). Goodman (2009) found that a hyperbolic relationship exists between putting green age and organic matter accumulation in the surface 2.5 cm. In the first 5 years organic matter increased from 5 to 45 g kg<sup>-1</sup>. Over the next 20 years organic matter content increased to 63 g kg<sup>-1</sup>. Carrow (1998) showed similar findings. Organic matter is removed from a turfgrass system primarily through microbial decomposition or physical removal via cultivation.

Among other benefits, the two main objectives in cultivating turfgrass systems are to remove thatch-mat and organic matter, to improve soil physical properties such as soil aeration, air-soil gas exchange, saturated hydraulic conductivity, and to reduce soil compaction (Beard, 1973; Canaway et al., 1986; Carrow et al., 1987; Turgeon, 2005; Baldwin et al., 2006; Sorokovsky et al., 2007). Turf cultivation involves impacting the turf soil without complete destruction of the turf (Beard, 1973). However, it does disrupt the playing surface and cause mechanical injury to the plant. Cultivation of turfgrass systems has always posed a problem to golfers and consequently golf course superintendents. Cultivation creates voids in the turfgrass stand, which inhibits the true roll of the golf ball. As a result, it is seen as a highly unfavorable practice by the golfing community. Nonetheless it is an imperative agronomic practice for sustained turf health and soil physical properties (Beard, 1973; Carrow, 2003; Turgeon, 2005).



If conducted with adequate frequency and aggressiveness, verticutting and hollow-tine core cultivation will reduce and/or prevent net accumulation of thatch/mat and organic matter (Eggens, 1980; Murphy et al., 1993; Callahan et al., 1997; McCarty et al., 2007; Landreth et al., 2008; Fu and Dernoeden, 2009c). Murphy et al. (1993) reported hollow-tine aerification three times yearly reduced organic matter content by  $29 \text{ g kg}^{-1}$ , compared to turfgrass that received no cultivation. Callahan et al. (1997) reported vertical mowing plus core aeration was the most effective program, reducing thatch depth by 2 mm when compared to no cultivation applications. Verticutting 4 – 8 times annually and core cultivation 4 times annually reduced thatch depth by  $\sim 1.5$  and  $0.6$  mm, respectively. Over the course of a two year study, Landreth et al. (2008) measured organic matter in the surface 2.5 cm of the rootzone, and found it was reduced from 48 to  $\sim 40 \text{ g kg}^{-1}$  when hollow-tine aerification was applied twice yearly. The authors also found verticutting to a 2 mm depth reduced organic matter to  $30 \text{ g kg}^{-1}$ . In another two year study, McCarty et al. (2007) reported that organic matter increased from 19 to  $25 \text{ g kg}^{-1}$ , a 32% increase, in the surface 5.1 cm of an uncultivated turfgrass system. Organic matter content was reduced from 20 to  $18 \text{ g kg}^{-1}$ , a 10% reduction, when turfgrass was core cultivated four times annually. Identical results were found in two other verticutting programs that consisted of four annual applications to a 6.4 mm depth and two annual applications to a 19.1 mm depth. Contrary to these studies, Sorokovsky et al. (2006) found uncultivated systems had similar organic matter content to greens core-cultivated twice per year. The author contributes the lack of organic matter reduction in cultivated systems to relatively low ( $< 5\%$ ) surface area impact.

### *Cultivating to Manage Water Infiltration and Soil Atmosphere*

Solid-tine cultivation is another common cultural practice applied to creeping bentgrass putting greens (Beard, 1973; Murphy et al., 1993; Carrow, 2003). Known advantages of this practice include; increased saturated hydraulic conductivity and soil porosity and reduced soil electric conductivity and compaction (Murphy et al., 1993; Carrow, 1998; Green et al., 2001; Carrow, 2003). Unlike hollow-tine cultivation, solid-tine cultivation uses small (less than 10 mm diameter) tines, and does not remove soil and turfgrass. As a result, there is less surface disruption, turf damage, and recovery time. This makes it a common summer cultivation technique when plant stress is high and growth is slowed (Carrow, 2003; Turgeon, 2005).

Research has demonstrated that any cultivation practice that penetrates through the turf canopy and into the soil profile improves water infiltration rates. Green et al. (2001) reported water-injection and solid-tine cultivation increased infiltration rates by an average of 5.2 cm h<sup>-1</sup> compared to turf receiving no treatment. Carrow (2003) observed regular summer water-injection cultivation increased water infiltration rates by an average of 36 cm h<sup>-1</sup> and spring and fall hollow-tine cultivation increased infiltration rates by 10 cm h<sup>-1</sup>, compared to uncultivated turf.

Poor soil aeration and high temperatures have been shown to reduce oxygen diffusion rates to critical levels ( $< 0.20 \mu\text{g O}_2 \text{ cm}^{-2} \text{ min}^{-1}$ ) (Huang et al., 1998a, 1998b; Carrow, 2003). If gas cannot freely diffuse into and out of the soil, O<sub>2</sub> and CO<sub>2</sub> may reach critical levels, and damage or even cause root mortality. Research conducted by Murphy et al. (1993) and Green

et al. (2001) questions whether cultivation of sand-based putting greens is necessary to maintain healthy oxygen diffusion rates,  $> 0.40 \mu\text{g O}_2 \text{ cm}^{-2} \text{ min}^{-1}$ .

### *Irrigation Management*

Unlike many non-golf course turf settings, putting greens are equipped with irrigation systems and water may be applied whenever needed. Turfgrass managers may opt between two distinct irrigation application strategies; deep, infrequent and light, frequent (Fry and Huang, 2004). The light and frequent method maintains soil at field capacity by applying smaller amounts of water daily (or as often as necessary). In this scenario irrigation is applied to prevent any signs of wilt. The deep, infrequent strategy schedules irrigation when signs of wilt are present, and more water is applied in order to replenish the entire rootzone (Fry and Huang, 2004).

Deep, infrequent irrigation programs may inhibit shoot growth, enhance rooting depth, lower leaf water and osmotic potentials, leach salts, and produce better turf quality (Fry and Huang, 2004). Advantages of light, frequent irrigation are less potential for nutrient and pesticide leaching, fewer problems with localized dry spot and maintenance of turf quality when water resources are limited (Fry and Huang, 2004). Specifically on creeping bentgrass putting greens, Jordan et al. (2003) reported higher turf quality, shoot density, and root length density when irrigating every four days versus every one or two days. Each irrigation treatment replaced its respective time frame's ET loss. Fu and Dernoeden (2009a) found deep and infrequent irrigation resulted in less thatch but produced unacceptable turf quality in one of two years of their study, compared to light, frequent irrigation.

### *Pest Pressure*

Compared to 'Penncross', the newer 'Penn A-' and 'Penn G-' series bentgrasses have excellent disease tolerance (Fraser, 1998). However dollar spot (*Sclerotinia homeocarpa* F.T. Bennett) is still a common disease on these newer bentgrasses. Dollar spots are white or light tan in color, and are approximately the size of a US dollar coin. The disease is brought on by drought stress, N deficiencies, excessive thatch, and extended leaf wetness (Beard, 1973; Vargas, 1994). Watkins et al. (2001) saw reduced dollar spot incidence when N fertility was increased from 150 to 300 kg ha<sup>-1</sup> yr<sup>-1</sup> and observed no differences among daily irrigation treatments based on 80 and 100% ET. McDonald et al. (2006) observed dollar spot to be more severe in turfgrass experiencing drought stress as a result of deep, infrequent irrigations. Ellram et al. (2007) found that longer durations of leaf wetness from dew increased dollar spot activity. In another study, Davis and Dernoeden (2002) found that turf plots with increased organic matter content also intensified dollar spot incidence.

Algae are another troublesome invasive organism to putting greens (*Cyanobacteria*), which appear as dark green to black growth in the turf canopy. Algae do not infect the grass plant itself, but it grows as a dense conglomeration of cells, effectively choking out the turf plant. Algae encroach in thinned turf stands, and once established can be difficult to eradicate. The best control approach is to maintain dense healthy turf. Although algae has been shown to be suppressed by frequent applications of chlorothanil, mancozeb and copper-containing products (Beard, 1973; Nus, 1994; Carrow, 1996).

### *Soil Microbial Community*

Soil microorganisms are very important to overall soil health, but are often overlooked by golf course superintendents. Soil microbes are especially important in organic matter stabilization and decomposition, nutrient transformations, and soil structure. Root exudate and organic matter are microbes primary food source (Mueller and Kussow, 2005; Yao et al., 2006). Consequently, soil carbon (C) content and putting green age have been found to have a direct, positive correlation with microbial population and diversity (Mancino et al., 1993; Kerek et al., 2002; Yao et al., 2006). Similarly, Mancino et al. (1993) and Raturi et al. (2004) observed that the thatch layer of creeping bentgrass putting greens housed >100 times greater microbial populations than the underlying sand-based rootzone. Groffman et al. (1996) demonstrated microbial population differences with varying soil types. Of the soils tested, silt loam soils contained greater microbe numbers than a coarser loamy sand soil. Despite high sand content, putting green rootzones may have similar microbial populations to finer texture native soils (Mancino et al., 1993).

Microbial metabolism increases with temperature; therefore microbial activity should be high during the summer months. However, Mueller and Kussow (2005) and Bigelow et al. (2002) found a decline in microbe populations in creeping bentgrass putting greens as summer progressed. Likely, summer bentgrass decline (SBD) resulted in decreased root activity and organic matter production, and food sources for soil microorganisms were limited (Mueller and Kussow, 2005). Research has indicated that the addition of biostimulants, organic amendments, and fertilizers have small or no influence on long term

microbial communities and populations (Mancino et al., 1993; Groffman et al., 1996; Bigelow et al., 2002; Kaminski et al., 2004; Mueller and Kussow, 2005).

#### Summer Bentgrass Decline on Golf Course Putting Greens

Summer bentgrass decline (SBD) has been described as a reduction in turf color, density, vigor, and overall quality observed during hot summer months in geographic locations not suited for optimal cool-season turfgrass growth (Carrow, 1996; Huang, 2001; Dernoeden, 2002; Lucas, 1996). Summer bentgrass decline is often used as a vague term referring to a complex of biotic and abiotic stresses such as supra-optimal temperature and humidity, excessive shade, soil compaction, drought, and pest pressures (Lucas, 1996; Carrow, 1996; Beard, 1997; Huang, 2001). Prolonged supra-optimal temperature is the primary contributing factor to SBD and the start of a chain of reactions leading to secondary stresses (Carrow, 1996). Increased heat leads to physiological stress, first compromising root function and growth, and eventually resulting in root death. Rapid root death alters both physical and chemical soil properties. When death occurs in this manner cell membranes rupture causing the root to become succulent and expand in diameter. Because the majority of roots are located in the surface 5 - 7.5 cm of the soil profile, a dense layer of fresh organic matter may be formed (Beard, 1973). The new layer holds more water, reduces water infiltration rates, reduces gas exchange between the soil and atmosphere and is a food source for pathogens, all of which add secondary stresses to the plant, contributing to SBD (Carrow, 1996). As SBD persists, a thinned turf stand can succumb to algae invasion and may further inhibit gas exchange and water infiltration.

### *Climatic Influence*

Optimal temperatures for creeping bentgrass shoot growth ranges from 15 - 24°C and 10 - 18°C for root growth (Beard, 1973;). Creeping bentgrass thrives in the cool, moist northern regions of the US where it experiences a climate nearer to that of its native Eurasia. In the northern US, it is commonly planted on golf course fairways, greens and tees. Creeping bentgrass has excellent cold tolerance, giving it the ability to survive winters with little detrimental effects (Fry and Huang, 2004). Summertime temperatures in the northern regions may exceed creeping bentgrass' optimal growing temperature, but for limited periods. Temperatures in the southeast consistently exceed 30°C in the summer months, putting extensive physiological stress on the plant (Huang and Gao, 2000; Xu and Huang, 2000a and 2000b). Grass species such as bermudagrass are better suited for warmer climates but are not as popular for putting greens because they are thought to have a lower quality putting surface than bentgrass (Casler and Duncan, 2003). Another advantage of bentgrass putting greens is year round green color. Playing on a green putting surface is preferred by the average golfer over the straw color of dormant bermudagrass (Long, 2006). Despite unfavorable warm temperatures, creeping bentgrass putting greens are common throughout the southeastern region of the USA (Toubakaris and McCarty, 2000).

In the southeast US, SBD is typically brought on by hot, humid conditions (Carrow, 1996; Huang, 2001; Fry and Huang, 2004). Excessive, prolonged periods of hot weather (>30°C) increase the turfgrass' need for transpirational cooling. High humidity inhibits evapotranspiration (ET), reducing the plant's ability to cool itself, leading to physiological stress. Photosynthetic efficiency is reduced, and carbohydrate synthesis is restricted. In an

attempt to restore maximum photosynthetic rates, photoassimilates are predominantly retained in the shoots to maintain leaf production. Carbohydrate partitioning to the roots is limited, slowing and eventually ceasing root growth. Prolonged exposure to supra-optimal temperatures causes roots to metabolize stored carbohydrate reserves and eventually leads to root death (Carrow, 1996; Beard, 1997; Huang and Gao, 2000; Huang et al., 2005).

Recent research concluded high soil temperatures are far more detrimental to the health of a turfgrass system than high air temperatures (Lucas, 1996; Beard, 1997; Xu and Huang, 2000a and 200b; Huang, 2001). Xu and Huang (2000a) demonstrated that turf quality, canopy photosynthetic rate, and fresh root weight decreased only slightly when exposed to optimal (20°C) soil temperature and high (35°C) air temperature compared to optimal air and soil temperatures. However, significantly larger decreases occurred at soil temperatures above 20°C, regardless of air temperature.

### *Secondary Stresses*

A compromised root system may prevent the plant from acquiring adequate amounts of water and nutrients from the soil (Carrow, 1996; Huang, 2001). During periods of high evapotranspiration, the plant experiences water stress when the water loss from the leaves exceeds water uptake by roots. Under such conditions, leaf stomata close, ET declines, and canopy temperature increases. Cell death may occur if temperatures exceed 40°C are reached (Beard, 1997). Finally, a decline in turf quality is observed in the form of decreased shoot growth, density, and yellowing of the leaf tissue (Xu and Huang, 2000a and 200b).



Roots require O<sub>2</sub> for respiration, and root tips have an especially high O<sub>2</sub> demand. Temperature and root respiration rates are directly related, and root O<sub>2</sub> demand peaks in the summer (Huang et al., 2005). If macropores near the soil surface, previously filled with air, are plugged with water and fresh organic matter, O<sub>2</sub> is unable to diffuse into the soil. Depleted soil O<sub>2</sub> levels can lead to root death (Carrow, 1996).

Carbon dioxide is a product of cellular respiration. As respiration rates increase so does CO<sub>2</sub> production. A dense turf canopy and surface organic layer may trap CO<sub>2</sub> in the soil. Rodriguez et al. (2005), Bunnell et al. (2002), and Chong et al. (2004) have shown that elevated soil CO<sub>2</sub> levels occur concurrent with reductions in creeping bentgrass quality. Bunnell et al. (2002) observed a decrease in root mass and depth at soil CO<sub>2</sub> concentrations  $\geq 2.5\%$ , and turf quality decreased at 10% soil CO<sub>2</sub>. In a field experiment, Chong et al. (2004) reported unacceptable bentgrass quality when  $\geq 5\%$  soil CO<sub>2</sub> was detected. Rodriguez et al. (2005) observed a decrease in photosynthesis and increase in respiration at higher soil CO<sub>2</sub> concentrations. Also, soil CO<sub>2</sub> levels tend to be highest in the midst of summer resulting from increased root and microbial respiration (Wolfenden and Diggle, 1995; Bunnell et al., 2002; Chong et al., 2004). Rodriguez et al. (2005) reported increasing day/night temperatures from 26.5/21 to 32/26.5C further reduced photosynthetic rates and root dry weights. Contrary to these studies, Ervin and Corwin (1999) did not observe a decline turfgrass quality when soil CO<sub>2</sub> concentrations consistently exceeded 10%. Additional research on cultural practices' impacts on soil CO<sub>2</sub> levels is limited.

There has been some debate whether certain fungal diseases (*Sclerotinia homeocarpa*, *Pythium* spp. and *Rhizoctonia solani*) species contribute to SBD. Lucas (1996)

found active isolates of *Pythium* and *Rhizoctonia solani* on roots and stolons from bentgrass greens that experienced SBD, and concluded that their presence were major contributors to SBD. Carrow (1996) and Dernoeden (1998) disagree, proclaiming neither *Pythium*, dollar spot (*Sclerotinia homeocarpa*), nor brown patch are contributing factors to SBD.

#### *Cultural Practices to Alleviate SBD*

Unfortunately, golf course superintendents are unable to control air and soil temperatures, the primary factors contributing to SBD. Installation of low velocity fans and syringing may temporarily reduce canopy temperatures (Bennett and Peacock, 1995; Beard, 1997; Fu and Dernoeden, 2009a and 2009b). Irrigation can also temporarily lower soil temperatures, but repeated, frequent applications are not recommended and may lead to a host of other turf and environmental problems (Linde, 1997; DaCosta and Huang, 2006).

At the onset of SBD, changes to irrigation scheduling may be required. As root length decreases, the depth at which water can be accessed decreases. Deep and infrequent irrigations may not maintain enough water near the surface where the majority of roots have exist (Fry and Huang, 2004; Huang et al., 2005). Instead, a light, infrequent irrigation strategy may be needed to maintain water near the surface of the profile (Fry and Huang, 2004; Fu and Deroeden, 2009a and 2009b).

Photosynthetic rate slows during the hot summer months, and nutrient demand is less. As a result, fertilizer programs should be modified for the summer months. Spoon feeding with 4.9 to 6.1 kg ha<sup>-1</sup> N every 10 to 14 days provides adequate N to the plant during a period of slow growth (Beard, 1973; Radko, 1985; Carrow et al., 2001; Nikolai et al., 2001; Moeller

et al., 2008). Excess N can promote algae invasion and disease. In a study where annual N rates were divided equally in applications made every 14 days, Totten et al. (2008) found annual N rate of 392 kg ha<sup>-1</sup> produced unacceptable turf quality during the summer months; whereas annual rates of 196 and 293 kg N ha<sup>-1</sup> maintained acceptable turf quality.

Greater ball roll distances have become an obsession of golfers. One of the easiest, most effective ways for a superintendent to accomplish this is by lowering mowing heights (Beard and Daniel, 1965; Beard, 1973). Research has found that mowing creeping bentgrass below 4 mm reduces turf quality and growth, particularly in the summer months (Salaiz, 1995; Beard, 1997; Lui and Huang, 2003). Lui and Huang (2001) found that raising daily mowing height from 3 mm to 4 mm in the summer increased turf quality, total root length, and rooting depth.

In hot humid weather massive root death occurs may occur at which point there is a sharp increase in fresh organic matter. This may reduce macroporosity near the soil surface (Carrow, 1996; Carrow, 2003). Macropores are important in air-soil gas exchange and water infiltration. Cultivation is a popular practice to create new macropores and/or remove organic matter (Beard, 1973; Carrow, 1998; Carrow, 2003). Carrow (1996) found hollow-tine cultivation beneficial if performed in early summer, but does not recommend it in the midst of summer when the plant is in a weakened state and growing slowly. Fu et al. (2009) found core-cultivation in the summer reduces turf quality and color initially, though it is an effective practice to remove organic matter. In contrast, Murphy et al. (1993) found summer core cultivation provided equal or better turf quality compared to solid-tine cultivation and no cultivation. Solid-tine, air-injection or water-injection can be used to break through the

canopy and into the soil. Carrow (1996) reported an increase in shoot density following cultivation with solid-tines or water injection. These practices tend to be less disruptive and create new macropores to increase water infiltration rates and air-soil gas exchange (Murphy et al., 1993; Lucas, 1996; Carrow, 1996).

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## CHAPTER 1

### **Influence of Cultural Practices on Creeping Bentgrass Putting Greens: Summer Bentgrass Decline and Disease Susceptibility**

Despite its popularity as a putting surface in North Carolina, creeping bentgrass (*Agrostis stolonifera* L.) is highly susceptible to summer bentgrass decline (SBD) during hot summer months. Cultural practices have been shown to help alleviate the pressure of SBD. The objectives of this study were to detail the impacts of nitrogen (N) fertility, soil moisture content and hollow- and solid-tine cultivation on creeping bentgrass quality and disease incidence. A two year study was initiated in September 2008 in Raleigh, NC. Cultural treatments included four N rates (97, 195, 293 and 391 kg ha<sup>-1</sup> yr<sup>-1</sup>), four hollow-tine cultivation programs (6.4 mm diameter tines two times yr<sup>-1</sup>, 9.5 mm diameter tines two and three times yr<sup>-1</sup> and a control that received no core cultivation), two soil moisture levels (low and high), and two summer solid-tine cultivation treatments (spiked and not spiked). Visual turf quality, normalized difference vegetation index (NDVI), leaf tissue chlorophyll content and disease and algae incidence were measured. Turf quality and NDVI were strongly correlated in 2009 and 2010,  $r = 0.628$  and  $0.783$ , respectively. Overall turfgrass quality was higher in 2009. A N rate greater than 192 kg ha<sup>-1</sup> was needed to maintain acceptable turfgrass quality. High soil moisture consistently provided better summer turf quality compared to low soil moisture conditions. Nitrogen fertility and soil moisture interacted where higher levels of both resulted in the best quality ratings. Neither hollow- nor solid-tine cultivation practices

influenced turfgrass quality or NDVI. No differences were observed in leaf tissue chlorophyll content. Dollar spot incidence was only quantifiable in 2009, where high soil moisture and lower fertility produced the most infection centers. Algae were observed in 2010, reaching unacceptable levels under high soil moisture and  $97 \text{ kg ha}^{-1} \text{ N}$ . Although weather plays a large role in SBD in North Carolina, results from this study show that cultural practices can influence its severity.

## Introduction

The game of golf is a \$5.3 billion industry in North Carolina (SRI, 2007). Represented in every county, most of the 560+ golf courses in North Carolina manage creeping bentgrass (*Agrostis stolonifera* L.) putting greens. Creeping bentgrass is a cool-season turfgrass characterized by its fine leaf texture, vigorous lateral growth, and ability to form a very dense stand when closely mowed. The aforementioned traits are ideal for creating a high quality putting surface.

Optimal air and soil temperatures for bentgrass growth range from 18 to 24°C and 10 to 18°C, respectively (Beard, 1973). In North Carolina and most of the southeastern US, summer temperatures generally exceed that range (Figures 1 and 2). As a result of heat-induced plant responses, a reduction in turf quality is often observed, often referred to as summer bentgrass decline (SBD) (Carrow, 1996; Beard, 1997).

Soil temperature is more critical than air temperature in the turfgrass plant's response to heat stress (Beard and Daniel, 1966; Huang, 2001). SBD is more prevalent later in the summer because an extended period of warm air temperatures is required to raise soil temperature. Once soil temperature exceeds 18°C, normal physiological processes in the plant become compromised, preventing adequate transpirational cooling (Carrow, 1996; Beard, 1997). When soil temperatures rise above 25°C root growth ceases and eventually root death occurs (Huang et al., 1998; Huang, 2001; Pote et al., 2006). A damaged root system prevents acquisition of adequate amounts of water and nutrients from the soil (Carrow, 1996; Xu and Huang, 2000a and 2000b; Huang, 2001). Finally, a decline in turf quality is observed in the form of decreased shoot density and yellowing of the leaf tissue.



Temperature, the primary contributing factor to SBD, cannot be readily controlled in a golf course setting. However, sound cultural management programs may alleviate secondary stresses resulting in SBD including water retention by the soil, reduced air-soil gas exchange, excessive organic matter, and pathogenic infections. Proper fertility, irrigation, and cultivation may help bentgrass better survive hot summer temperatures.

Annual recommended N rates vary significantly, but it is generally accepted that all cool-season grasses should be minimally fertilized during the summer (Radko, 1985; Carrow et al., 2001; Nikolai et al., 2001; Dernoeden, 2002; Moeller et al., 2008). Totten et al. (2008) reported turfgrass receiving N at  $391 \text{ kg ha}^{-1} \text{ yr}^{-1}$  annually produced unacceptable turf quality during the summer months, while lesser rates of 195 and  $293 \text{ kg ha}^{-1} \text{ yr}^{-1}$  maintained acceptable levels.

Conflicting views exist regarding irrigation and soil moisture strategies and their affects on turfgrass quality. Jordan et al. (2003) observed that turf performed better when irrigated every 4 days compared to every 1 and 2 days. In one of two years of their study, Fu and Dernoeden (2009) found deeply irrigating at the first signs of wilt, produced unacceptable turf quality.

Research has demonstrated cultivating turfgrass systems improves soil properties but its direct affect on turf quality is unclear. Murphy et al. (1993) observed better or equal turf quality from core cultivation compared to no cultivation. McCarty et al. (2007) found core cultivation generally reduced turf quality compared to verticutting and topdressing only. However, bentgrass quality was above acceptable levels for all treatments. Landreth et al. (2008) did not find that different core cultivation intensities had any effect on turfgrass

quality. Fu et al. (2009) reported higher turf quality in late summer after applying hollow-tine cultivation; however initially turf quality was reduced.

Solid-tine cultivation, or spiking, may serve as an alternative to core cultivation during the hot summer months. Spiking provides some of the benefits of core cultivation with minimal surface disruption, and results in faster recovery (Murphy et al., 1993; Green et al., 2001). Murphy et al. (1993) found that on average turf quality was higher when spiking was applied compared to no spiking. Carrow (1996) found cultivating with solid-tines in early-summer, improved turf quality, but became detrimental when applied in mid- or late-summer.

Stressed turfgrass plants can be prone to pathogen infections and pest infestations such as dollar spot (*Sclerotinia homeocarpa* F.T. Bennett), brown patch (*Rhizoctonia solani* Kühn), *Pythium* spp., and algae invasion. There is still some debate whether pathogens cause SBD or are another contributing factor (Lucas, 1996; Carrow, 1996; Dernoeden, 1998). Prolonged disease infection reduces turfgrass quality and may result in a thinned stand. Voids in the turf canopy can provide an opportunity for algae to establish.

Many researchers have evaluated common cultural practices individually for their effects on SBD. Few have combined multiple practices in attempt to develop an ideal program. The objectives of this study were to detail the impacts of N fertility, core cultivation, soil moisture and summer spiking on turfgrass quality and disease and algae incidence on creeping bentgrass putting greens.

### Materials and Methods

The study was conducted on two, 2-year old creeping bentgrass putting greens at the Lake Wheeler Turf Field Laboratory at North Carolina State University in Raleigh, NC. The greens were built to United States Golf Association (USGA) specifications (USGA Greens Section Staff, 2004). The soil was a 90:10 (sand:peat) mixture by volume with a pH of 6.1. The greens were seeded in November 2006 with ‘Penn A-1’ creeping bentgrass and were fully grown-in before treatments were initiated in fall 2008. Putting greens were mowed five times per week, June through September at 4 mm. The remainder of the year, the greens were mowed at 3.5 mm three to five times per week.

The experiment was arranged in a complex strip-strip block design. Each green measured 25.9 x 25.9 m, and were separated into four equal quadrants. Quadrants were separated by a plastic barrier installed through the depth of the rootzone, which allowed for isolated irrigation and drainage. Individual plots within each quadrant measured 2.4 by 2.7 m.

Zoned irrigation and drainage by quadrant enabled the creation of two moisture levels. Soil moisture treatments were maintained mid-May through October via scheduled irrigation applications. An irrigation audit was performed to ensure the irrigation system had acceptable distribution uniformity. Distribution Uniformity (lower quarter) test showed 0.60 uniformity, and Christiansen’s Uniformity test resulted in a uniformity distribution of 0.76. Soil moisture treatments will be referred to as *high soil moisture* and *low soil moisture* from this point forward. High soil moisture was maintained via daily irrigation equivalent to 80% of daily evapotranspiration (ET) based on a thirty year historical average. The low soil

moisture treatment received irrigation every four to five days equal to 40% historic ET. Actual irrigation amounts ranged from 3.2 - 4.6 mm. In the event of rainfall, scheduled irrigation was withheld. 'Hotspots' and localized dry spots were lightly hand watered to prevent turf mortality. Otherwise, both greens were lightly syringed as needed to alleviate noticeable signs of wilt ('foot-printing' and/or purplish tint).

Each quadrant was equipped with a Toro Turf Guard Wireless Sensor (The Toro Company Riverside, CA) providing real-time monitoring of soil moisture, temperature and salinity. Soil sensors were installed parallel to the surface, 5 cm deep. Due to equipment failure, soil temperature and moisture data were unavailable late-July to late-August 2009. Soil moisture data were pooled by week. There were 17 weeks of available sensor data in 2009 and 21 weeks in 2010. Air temperature and precipitation data were obtained from a nearby (<1200 m) weather station maintained by the State Climate Office of North Carolina.

Four nitrogen-based fertilizer treatments were applied in strips within each quadrant, running west-east. The fertilizer programs were based of annual N rates of 97, 195, 293, or 391 kg ha<sup>-1</sup>. Both foliar and granular fertilizers were used. Contec DG 18N-9P-18K (Andersons Golf Products, Maumee, OH) greens-grade granular fertilizer was applied immediately following hollow-tine cultivation. Fertilizer treatments were pre-weighed for individual plots and applied using shaker bottles. A 0-52-34 and 0-0-30 fertilizer was applied to turfgrass plots receiving a lesser rate of the 18-9-18 fertilizer so all plots received equal amounts of the P and K. Foliar fertilizer applications were made using a CO<sub>2</sub> powered boom sprayer calibrated to apply 374 L ha<sup>-1</sup>. Green Flo 30-0-0 (John Deere Landscapes, Troy, MI) was used for foliar applications that were applied on the first or fifteenth day of each month,

+/- 4 days due to weather adjustments. In both the foliar and granular fertilizers, half of the total N was quick-release urea-N and the other half was slow-release urea-Triazone™-N.

Table 1 outlines the schedule and rate of fertilizer applications.

Four hollow-tine cultivation programs were also stripped within each quadrant, running north-south. Two of the cultivation treatments used 9.5 mm diameter tines two and three times yearly (9.5 mm x 2 and 9.5 mm x 3). A second cultivation treatment used 6.4 mm diameter tines twice per year (6.4 mm x 2). The last treatment was a control that received no coring. The control treatment was initiated 18 September 2009. Prior to this date, bentgrass was cultivated following the 9.5 mm x 2 schedule. The control was only applied in four of the eight quadrants. A Toro ProCore 648 (The Toro Company, Bloomington, MN) was set to remove cores 8.9 cm deep on a 5.1 cm by 5.1 cm spacing. Cores were harvested and removed from the site. Greens were immediately topdressed with straight quartz sand and sand was brushed in until all holes were completely filled. Greens were aerified on 8 October in 2008, 19 March and 18 September in 2009, and 26 March and 15 September in 2010. The lone treatment receiving three aerification applications per year was cored on 15 May in 2009 and 14 May in 2010.

The final cultural program involved solid-tine cultivation, or spiking. From June through mid-September, the two southern quadrants of each green were spiked twice per month and the northern half was not spiked. A Toro ProCore 648 aerator was used and equipped with bayonet tines. Tines were spaced 5.1 cm by 7.6 cm and penetrated the soil to an 8.9 cm depth.

In 2009, curative fungicide applications of chlorothalonil were tank-mixed with either iprodione or boscalid were made for dollar spot control on 9 April, 5 May, 29 June, 17 and 31 July, 4 and 14 September, and 20 October. On 12 June only boscalid was applied and 18 August iprodione only was applied. Fungicide treatments were effective at reducing dollar spot infections to minimal levels. One curative application of propiconazole was made for brown patch on 17 July.

In 2010, algae were the most prominent pest problem and chlorothalonil was curatively applied on 30 April, 14 and 27 May, 7 and 28 June and 6 July. Dollar spot was curatively sprayed with chlorothalonil and iprodione on 14 May, 28 June and 12 August. Brown patch was curatively sprayed on 7 June, 2 July and 18 August.

#### *Data Collected*

Visual assessment of turfgrass quality was based on a scale of 1 - 9 where 1 = brown/dead turf, 7 = minimally acceptable turf color and quality and 9 = dark green/excellent quality turf (Morris and Sherman, 2008). Ratings were taken weekly from March through November and twice monthly December - February.

Immediately following visual ratings, turfgrass canopy spectral reflectance was measured using a Crop Circle ACS-210 Plant Canopy Reflectance Sensor (Holland Scientific, Inc., Lincoln, NE). The device emits visible and near infrared light (NIR) simultaneously from a single light emitting diode, closely mimicking natural light. Canopy reflectance is then measured over those wavelengths. Normalized difference vegetation index (NDVI) values were determined by the device as  $(R_{\text{NIR}} - R_{\text{red}})/(R_{\text{NIR}} + R_{\text{red}})$  where  $R_{\text{NIR}}$  is the

reflectance in NIR light at 860 nm and  $R_{\text{red}}$  is the reflectance in the red light at 650 nm. The Crop Circle sensor was held about 1 m above the turf surface and between 50 and 70 individual readings were averaged per plot area. In 2009, equipment malfunctions prevented data acquisition from late-September to late-October.

Turfgrass leaf tissue was harvested and analyzed for chlorophyll *a* and *b*. In the field, representative samples of freshly mowed turf leaves (clippings) were collected, individually bagged, and transported to the laboratory. From each sample, 0.0325 g of fresh green tissue was digested in a test tube containing 3 mL of N,N Dimethylformamide (DMF). Digestion occurred within 5 hours of clipping collection. After digestion for 20 h minimum, a UV spectrophotometer was used to measure the absorbance of the extract at 647 nm and 664 nm. Chlorophyll *a* and *b* were calculated using the following formulas as described by Inskeep and Bloom (1985);

$$\text{Chlorophyll } a = 12.70*(A_{664\text{nm}}) - 2.798*(A_{647\text{nm}}) \quad [1]$$

$$\text{Chlorophyll } b = 20.70*(A_{647\text{nm}}) - 4.62*(A_{664\text{nm}}) \quad [2]$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b \quad [3]$$

Chlorophyll content was analyzed on 14 May, 22 July and 15 September in 2009, and 12 May, 14 July, and 6 September in 2010.

Disease ratings were taken weekly coinciding with visual ratings. Dollar spot incidence was measured by a count of individual infection centers per plot area. Brown patch and algae was rated on a scale of 1 to 6 where >3 indicated unacceptable levels of each. Disease and algae ratings were taken weekly from March through November and twice monthly from December - February.

Treatment and interaction effects were determined using PROC MIXED of the Statistical Analysis System (SAS Institute, Cary, NC). Means were separated using the LSMEANS statement and were subject to Fisher's protected least significance test ( $p < 0.05$  or  $0.01$ ).

### Results and Discussion

Both temperature and precipitation patterns differed between 2009 and 2010 (Figure 1). April – November in 2009 average daily minimum and maximum air temperature was  $14.7$  and  $25.4^{\circ}\text{C}$ , respectively. During the same period in 2010, average daily minimum and maximum air temperature was  $15.6$  and  $27.5^{\circ}\text{C}$ , respectively. June through October, when SBD typically occurs in the southeast, daily minimum and maximum air temperature was  $27.6$  and  $17.2^{\circ}\text{C}$  in 2009, and  $30.5$  and  $19.0^{\circ}\text{C}$  in 2010, respectively.

Like air temperatures, soil temperatures in 2010 were greater than 2009 (Figure 2). June – October of 2009, mean daily maximum and minimum soil temperature was  $27.7$  and  $20.8^{\circ}\text{C}$ , respectively. In 2010 those data were  $29.8$  and  $21.8^{\circ}\text{C}$ , respectively. These data indicate roots up to 5 cm deep in the soil profile experience daily maximum temperature similar to the maximum air temperature. However, the rootzone did not cool as much as air temperature during the night. Average minimum soil temperature was on average  $3.2^{\circ}\text{C}$  greater than minimum air temperature. Based on results from Huang and Gao (2000) and Pote et al. (2006) SBD should have been present as turfgrass experienced supra-optimal air ( $>30^{\circ}\text{C}$ ) and soil ( $>25^{\circ}\text{C}$ ) temperatures.

In a twenty-one week span, June through October, 44 cm of precipitation fell in 2009 and 53 cm in 2010 (Figures 1 and 3). Despite receiving more total precipitation in 2010, there



were only seven weeks in which greater than 1.3 cm of rain fell. The majority of 2010's total precipitation occurred during three, nonconsecutive weeks that totaled 38.3 cm. Though there were dry weeks, 2009 had a more uniform weekly precipitation pattern. In 2009, ten of twenty-one weeks between June and October had greater than 1.3 cm of rain, and only one week received rainfall greater than 4.2 cm. To put it in perspective, the median weekly rainfall in 2009 was 1.4 cm and 0.4 cm in 2010.

In 2009, no differences in soil moisture existed between the two soil moisture treatments in four of sixteen weeks (Figure 3). From June through October of 2009, mean soil moisture was 16.9 and 13.5% for the high and low soil moisture treatments, respectively. During the same period in 2010, there were no differences in soil moisture content in six of twenty-one weeks. Mean soil moisture in 2010 was 17.5 and 14.6% for the high and low soil moisture treatments, respectively. Changes in soil moisture in the low soil moisture treatment were obviously related to precipitation more in 2010 than 2009.

Mean weekly visual quality ratings across all treatments ranged from 3.2 to 8.4 from March through November of 2009 (Figure 4). During the same period in 2010, quality ratings ranged from 4.0 to 8.1. Beginning in March, turf quality increased until it peaked in June. Spring weather in Raleigh is ideal for bentgrass growth. Daytime temperatures are mildly warm with cool night temperatures. In 2009, average turf quality decreased from 8.3 in early June to a low of 6.8 by the end of August. In 2010, SBD was more pronounced as average turfgrass quality decreased from 8.1 in early June to a low of 5.0 in late September. In June daily high air and soil temperatures began to consistently exceed 30 and 25°C, respectively. These temperatures are too high for optimal bentgrass growth and usually result in SBD.

During the fall in each year turf quality increased ~2.5 rating units from the summertime low. As summer heat subsides bentgrass growth increases, and results in a sharp increase in turfgrass quality.

Exposure to supra-optimal air and soil temperatures is the primary contributing factor to SBD (Carrow, 1996; Huang et al., 1998; Huang, 2001). Differences in weather patterns between 2009 and 2010 may have contributed to differences observed in turfgrass quality. Compared to 2009, prolonged higher temperatures from June through October in 2010 exposed the turf to greater heat stress (Figure 1 and 2). Initially, differences in turf quality were minimal. Prolonged supra-optimal temperature in September and October of 2010 caused large continual decreases in turf quality during that time. In September and October of 2009, temperatures were ~3.4°C cooler than 2010. Cooler temperatures likely promoted turf recovery beginning in September in 2009. However in 2010, turf quality continued to decline until late-September while supra-optimal temperatures persisted. Under supra-optimal air temperatures Huang (2001) also found differences in turfgrass quality across a small temperature gradient. The author observed reduced bentgrass quality when soil temperature increased from 29 to 32°C and from 32 to 35°C. These differences were observed after 21 days. Prolonged exposure to those conditions over the course of an entire summer may result in continued decline of turf quality

In both years, SBD (visual rating < 7) was not observed until August. Raleigh-area golf courses typically experience SBD earlier in the season. In our setting, we did not have the added wear stress imposed by golfing activities on the putting surface. Mowing five times weekly was the only considerable wear stress applied to the turfgrass. Also, mowing height

was raised to 4 mm in the summer months because maintenance of high green speeds was unimportant. This mowing height is higher than those maintained on many golf courses. Lui and Huang (2003) observed higher turf quality when bentgrass was mowed at 4 mm compared to 3 mm, and differences were more pronounced in the summer months. We suspect the plant was able to better combat heat stress due to reduced physiological plant stress from raised mowing height and limited wear stress. As a result, higher turf quality persisted well into the summer before severe SBD was observed. In 2010, this logic was especially evident, but in 2009 high temperatures did not persist long enough into September and October to see a severe decline in turf quality compared to 2010.

Visual turf quality ratings separated by N rate showed differences at each rating date in both 2009 and 2010 ( $p < 0.0001$ ) (Table 2) and (Figure 5). Turf that received  $391 \text{ kg ha}^{-1}$  N consistently possessed the highest turf quality. Acceptable turf quality ( $>7$ ) was never achieved with the  $97 \text{ kg ha}^{-1}$  N program. Totten et al. (2008) had similar findings with a  $97 \text{ kg ha}^{-1}$  N program, but found N rates of  $195 - 293 \text{ kg ha}^{-1}$  N provided higher turf quality in the summer than a  $391 \text{ kg ha}^{-1}$  N program. In 2009 turfgrass that received  $195 \text{ kg ha}^{-1}$  N maintained acceptable turf quality until early-August, when it fell slightly below the acceptable quality threshold until mid-September. Turf that received 293 and  $391 \text{ kg ha}^{-1}$  N demonstrated acceptable turf quality throughout 2009. However, turf under the  $293 \text{ kg ha}^{-1}$  N program bordered of unacceptable quality from mid-August to mid-September, when it received an average rating of 7.1. In 2010, turfgrass under the  $195 \text{ kg ha}^{-1}$  N program maintained acceptable quality until mid-July before receiving ratings  $<7$ . Turfgrass that received 293 and  $391 \text{ kg ha}^{-1} \text{ yr}^{-1}$  N followed a similar pattern except those programs

provided acceptable turf quality until mid-August. Acceptable turf quality lasted one week longer in the 391 kg N ha<sup>-1</sup> program compared to 293 kg ha<sup>-1</sup> N. All treatments reached a low in turf quality in late-September. Each program except 97 kg ha<sup>-1</sup> N quickly recovered back to acceptable turf quality levels just two weeks later. The sharp increase in overall turf quality was likely due to a combination of significantly cooler temperatures (~ 10°C), a rain event, and response to a granular fertilizer application made on 27 September.

June – October 2009 had four weeks, and in 2010 there were three weeks, when soil moisture did not affect turfgrass quality ( $p < 0.0001$ ) (Figure 5). During that period in 2009, visual turf quality ratings averaged 7.3 and 7.7 for the low and high soil moisture treatments, respectively. Turfgrass maintained under high soil moisture status never fell below acceptable levels in 2009, while the low soil moisture treatment had unacceptable turf quality from August through September. In 2010, differences were more pronounced. June – October average ratings for the low and high soil treatments were 6.4 and 7.2, respectively. High soil moisture maintained acceptable turfgrass quality until mid-August, a full month longer than turfgrass maintained under drier soil conditions. Similarly, Fu and Dernoeden (2009) found frequent irrigation promoted higher turf quality throughout the summer compared to deep, infrequent irrigation. Contrary to these results, Jordan et al. (2003) observed higher turfgrass quality when irrigation was applied every four days compared to bentgrass irrigated every one and two days.

Interactions between N fertility and soil moisture resulted in differences in turfgrass quality from June – October in both 2009 and 2010 ( $p = 0.005$  and  $0.0002$ , respectively) (Figure 6). In 2009, turfgrass that received 97 and 293 kg ha<sup>-1</sup> yr<sup>-1</sup> N demonstrated higher turf

quality under the high soil moisture treatment than low soil moisture status. In 2010, differences in turf quality existed between each soil moisture treatment under N fertility programs except 391 kg ha<sup>-1</sup> N. Turfgrass quality was not different in plots receiving 391 kg ha<sup>-1</sup> N under low soil moisture conditions and 293 kg ha<sup>-1</sup> N with high soil moisture. The same trend was found between the 195 and 293 kg N ha<sup>-1</sup> programs. Results suggest higher N rates combined with low soil moisture can attain similar turfgrass quality as lower N rates and increased soil moisture.

Differences in turfgrass quality existed among hollow-tine cultivation regimes on some of the rating dates (data not presented). The majority of those dates were within 3 - 4 weeks following core aeration. The largest reduction in turf quality was seen in plots cultivated with the largest diameter tines. Bentgrass that received three annual hollow-tine cultivations had reduced turfgrass quality in May when it received an additional application. Besides those few dates, core cultivation had no effect on turfgrass quality. There were no differences related to turfgrass quality and summer spiking.

NDVI can be a useful measurement to objectively assess turfgrass quality, and is often used to complement visual ratings (Fitz-Rodríguez and Choi, 2002). Weekly mean NDVI values ranged from 0.6246 to 0.8209 (Figure 7). Despite small differences in values, results showed strong correlations with visual quality ratings in both years. Turf quality and NDVI had a Pearson Product Moment Correlation Coefficient of 0.628 in 2009 and 0.783 in 2010 across all treatments.

Differences in NDVI existed among N fertility programs at each rating date in both years ( $p < 0.0001$ ). Turfgrass that received 97 and 391 kg ha<sup>-1</sup> N consistently held the lowest

and highest NDVI values, respectively. Correlation coefficients for NDVI and visual turfgrass quality under the 97, 195, 293 and 391 kg ha<sup>-1</sup> N programs were 0.406, 0.568, 0.703 and 0.451 in 2009 ( $p < 0.0001$ ), and 0.753, 0.875, 0.738 and 0.779 in 2010 ( $p < 0.0001$ ), respectively.

Contrary to reports by Fitz-Rodriguez and Choi (2002) and Fenstermaker-Shaulis et al. (1997), our NDVI values were not consistently different between soil moisture regimes (data not presented). NDVI values were only different among soil moisture treatments in 2010 ( $p = 0.0172$ ). When separated by date, four of fifteen dates, from June – October were different ( $p < 0.05$ ). Mean NDVI values during that time period were 0.7333 and 0.7320 for the high and low soil moisture treatments, respectively. Similar to visual turf quality ratings, NDVI values were only different between core cultivation treatments on rating dates immediately following applications (data not presented). No differences were observed as a result of summer spiking.

After July 2009, turf plots fertilized with 97 kg ha<sup>-1</sup> N, had areas of semi-dormant grass that never fully recovered to uniform density and color for the remainder of the study. These areas remained yellow/brown in color and had slow growth, but remained a mostly uniform stand of turf. However, other areas remained lush green and actively grew throughout the year. In the other bentgrass plots, turf quality and color remained fairly uniform across the stand as visual ratings fluctuated. Under these circumstances leaf tissue chlorophyll data obtained were inconsistent and inconclusive.

Dollar spot incidence was consistent throughout 2009, but was not present after mid-June 2010, likely due to consistent hot weather. Dollar spot incidence was influenced by N

fertility ( $p < 0.0001$ ) and soil moisture ( $p = 0.0025$ ) (Table 2). An interaction between N fertility and soil moisture also impacted the number of dollar spot infection centers ( $p = 0.0011$ ) (Figure 8). At every collection date, from June 2009 forward, the most dollar spots were observed in plots receiving  $97 \text{ kg ha}^{-1}$  N. Fewer infection centers were counted in turf plots with each increment in N fertility rate. Dollar spot incidence was affected by soil moisture level beginning in mid-June when moisture levels were established. Turf plots under the high soil moisture regime exhibited more dollar spots than under low soil moisture. Dollar spot infection centers for the low soil moisture treatment ranged from 8 to 21, while incidence under the high soil moisture treatment ranged from 14 to 65. Prolonged periods of leaf wetness from daily irrigation likely resulted in higher dollar spot incidence in bentgrass maintained under high soil moisture compared to low soil moisture.

Interactions among N fertility and soil moisture showed high soil moisture combined with  $97$  and  $195 \text{ kg ha}^{-1}$  N encouraged an average of  $\sim 60$  infection centers per plot ( $6.7 \text{ m}^2$ ) (Figure 8). Similar dollar spot incidence was observed in turf plots fertilized with  $97$ ,  $195$  and  $293 \text{ kg ha}^{-1}$  N that were also subject to low soil moisture and bentgrass under high soil moisture that received  $391 \text{ kg ha}^{-1}$  N. Bentgrass plots that received  $391 \text{ kg ha}^{-1}$  N and low soil moisture averaged the fewest infection centers per plot. Results suggest soil moisture played a larger role in dollar spot incidence than annual N rate.

Algae were not present in 2009, and in 2010 were only found in plots that received  $97$  and  $195 \text{ kg ha}^{-1}$  N (Table 2 and 3). Totten et al. (2008) also did not observe algae in bentgrass fertilized with  $>195 \text{ kg ha}^{-1}$  N. Differences were observed as a result of N fertility, soil moisture and hollow- and solid-tine cultivation ( $p < 0.0001$ ). Though differences occurred,

no one factor alone promoted unacceptable algae ratings ( $>3$ ). Algae favor moist conditions and are more likely to establish on thinned putting greens, or in voids in the turf canopy (Nus, 1994). Findings were consistent with these principles. High soil moisture, low N fertility, and increased cultivation all promoted the greatest algae incidence.

Brown patch (*Rhizoctonia solani*) was observed in July, August and September in 2010, but no treatment effects were observed. However, brown patch was only observed in turf plots receiving 293 and 391 kg ha<sup>-1</sup> N and high soil moisture.

### Conclusions

Though newer creeping bentgrass cultivars have been shown to be better adapted to the climate of the southeast, bentgrass putting greens still struggle to maintain acceptable turf quality throughout the summer. A golf course superintendent's job often depends on how well the putting greens perform throughout the summer and peak golfing season. Results from this research suggest N fertility and irrigation management can aid in maintaining acceptable quality putting greens over the length of a summer in the southeast USA.

Higher annual N rates produced higher turfgrass quality. Greater than 195 kg ha<sup>-1</sup> yr<sup>-1</sup> N was needed to maintain acceptable turf quality. Adequate water in the soil profile, particularly the surface 5 cm, played a large role in providing better turf quality. Interactions between soil moisture and N fertility affected turf quality. Results from this study may allow golf course superintendents some flexibility in their management strategy regarding annual N rates and irrigation programs. Under water restrictions, turf managers may increase N input to promote greater turf quality. NDVI, correlated well with visual quality ratings, especially regarding N fertility. NDVI may be a legitimate method to assess turfgrass quality.



Dollar spot incidence was magnified under low N fertility levels and moist soils. Prolonged leaf wetness played the larger role in increased dollar spot presence. Similar to dollar spot, algae presence was dominated by moist soil conditions and when  $\leq 195 \text{ kg ha}^{-1}$  N was applied. Algae severity progressively increased with increased hollow- and solid-tine cultivation intensity.

This research simulated golf course conditions in most aspects except one, traffic. Daily traffic from golfers has shown to stress the turfgrass plant. The addition of this factor could have affected the results from this study. Additional research exploring traffic intensities should be conducted to best simulate golf course conditions and help further understand SBD.

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Table 1. Yearly schedule of granular and foliar N fertilizer applications. Monthly foliar rates are split between two separate application made on the 1<sup>st</sup> and 15<sup>th</sup> of each month (+/- 4 days).

Month	Total N applied (kg ha <sup>-1</sup> yr <sup>-1</sup> )							
	97		195		293		391	
	Foliar	Granular	Foliar	Granular	Foliar	Granular	Foliar	Granular
Monthly N Rate (kg ha <sup>-1</sup> )								
Feb			6.1†		9.8†		24.4	
Mar	4.9†	12.2	12.2	24.4	19.5	36.6	24.4	36.6
Apr	9.8		12.2		19.5		24.4	
May	9.8		12.2	24.4	12.2	36.6	24.4	36.6
June	9.8		12.2		12.2		24.4	
July	9.8		12.2		12.2		24.4	
Aug	9.8		12.2		12.2		24.4	
Sept	9.8	12.2	12.2	36.6	19.5	36.6	24.4	36.6
Oct	9.8		12.2		19.5		24.4	
Nov			6.1†		9.8†	36.6	24.4	36.6

† Indicates only one scheduled application in that month

Table 2. Mean squares from combined analyses of variance for turf quality, NDVI, Dollar Spot incidence and algae incidence.

Source of Variation†	df	Mean Square			
		Quality	NDVI	Dollar Spot	Algae
Spiking, S	1	20.7	0.0003	4864.3	48.2*
Moisture, M	1	306.4**	0.007	466917.2**	357.4**
S x M	1	0.01	0.028*	8622.2	12.8
Quad(S x M)	4	7.9**	0.008	10353.9	2.5
Fertility, F	3	2037.0**	2.141**	94160.1**	220.5**
F x S	3	1.7	0.002	1715.3	4.1
F x M	3	20.0**	0.0007	36309.9**	60.6**
F x S x M	3	0.9	0.002	4060.4	0.0
F x Quad(S x M)	12	1.0	0.001	3136.4**	0.5
Cultivation, C	3	38.9**	0.04**	8291.1	18.1**
C x S	3	0.8	0.001	705.4	1.6
C x M	3	0.2	0.002	4475.9	13.8**
C x S x M	3	0.7	0.00003	1004.7	0.6
C x Quad(S x M)	8	0.3	0.0007	2212.9*	0.5
C x F	9	4.5**	0.004	780.0	2.3
C x F x S	9	0.2	0.0002	446.2	0.5
C x F x M	9	0.3	0.0003	363.1	0.6
C x F x S x M	9	0.3	0.0007	400.6	0.7
C x F x Quad(S x M)	24	0.3	0.0004	586.8	1.0
CV %		14.0	12.1	60.3	78.8
Mean‡		128.5	0.118	34179.3	39.3

\*, \*\* Indicates significance at  $p < 0.05$  and  $0.01$ , respectively.

† S = summer solid-tine cultivation, M = soil moisture, F = N fertility program and C = hollow-tine cultivation, Quad = quadrant

‡ Units = Quality, scale 1 – 9; NDVI, index 0 – 1; Dollar Spot, no. of infection centers plot<sup>-1</sup>;

Algae, scale 1 - 6

Table 3. Algae incidence in response to hollow-tine aerification programs, summer spiking, two N rates, and two soil moisture conditions averaged June through October in 2010.

Algae Incidence (1 = no algae to 6 = >50% algae, with 3 = maximum acceptable algae rating)								
Cultivation Method	Not Spiked				Spiked			
	97 kg ha <sup>-1</sup> yr <sup>-1</sup> N		195 kg ha <sup>-1</sup> yr <sup>-1</sup> N		97 kg ha <sup>-1</sup> yr <sup>-1</sup> N		195 kg ha <sup>-1</sup> yr <sup>-1</sup> N	
	High SM	Low SM	High SM	Low SM	High SM	Low SM	High SM	Low SM
6.5 mm x 2	2.8 a†	1.4 bcd	1.3 bcd	1.1 cd	3.2 a	1.8 b	1.6 bc	1.1 d
9.5 mm x 2	2.7 b	1.6 cde	1.5 def	1.0 f	4.0 a	2.0 cd	2.1 c	2.2 ef
9.5 mm x 3	4.1 b	1.5 de	2.0 def	1.1 f	4.6 a	1.9 d	2.7 c	1.1 ef
control	2.4 b	1.2 d	1.6 cd	1.0 d	3.6 a	1.5 cd	2.1 bc	1.2 d

† Differences in letters within row indicate statistical differences within N rate according to Fisher's LSD<sub>0.05</sub>.



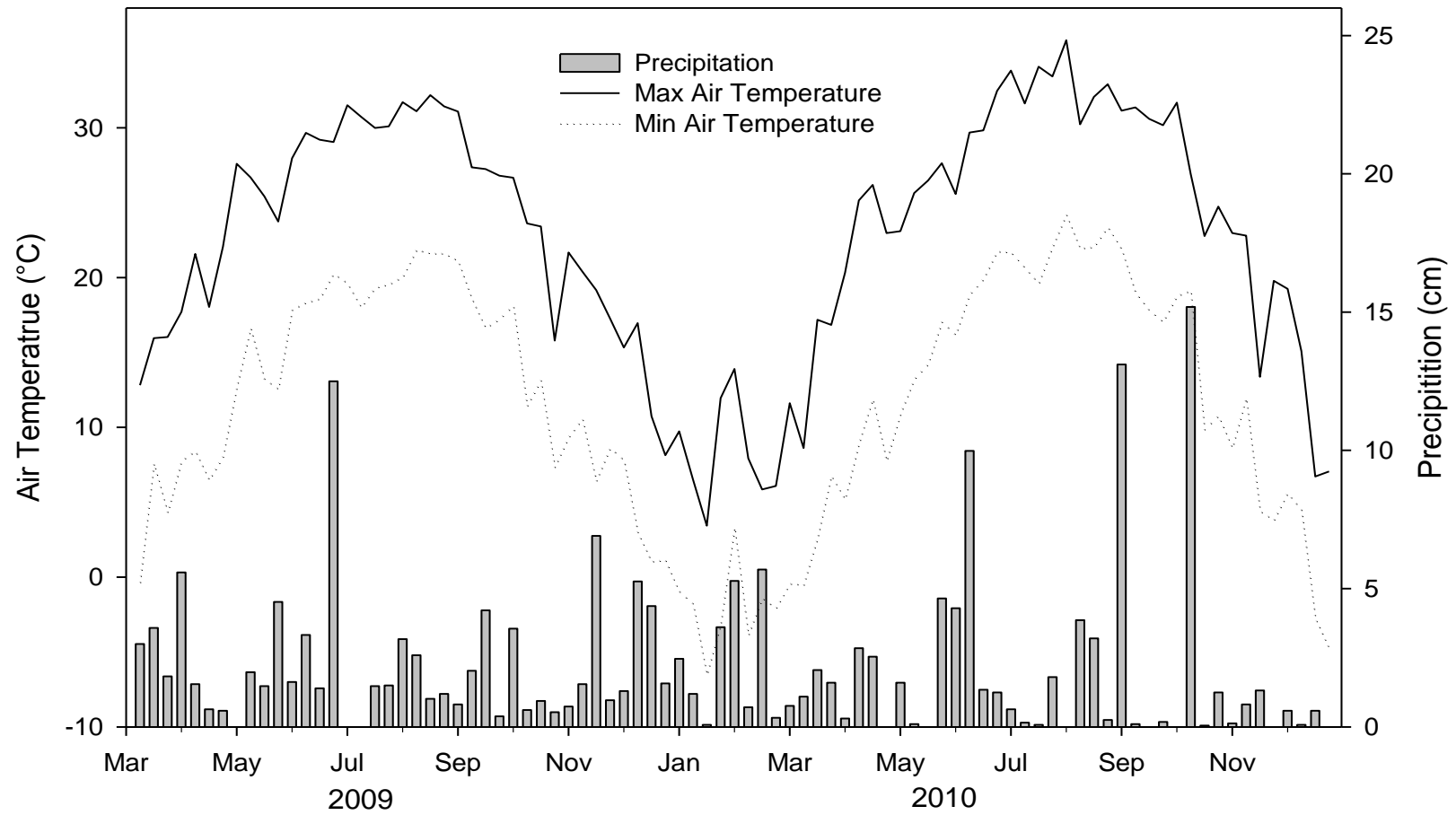


Figure 1. Weekly average precipitation (cm), minimum and maximum air temperature (°C) recorded 1 March 2009 to 31 December 2010 at Lake Wheeler Field Laboratory in Raleigh, NC.

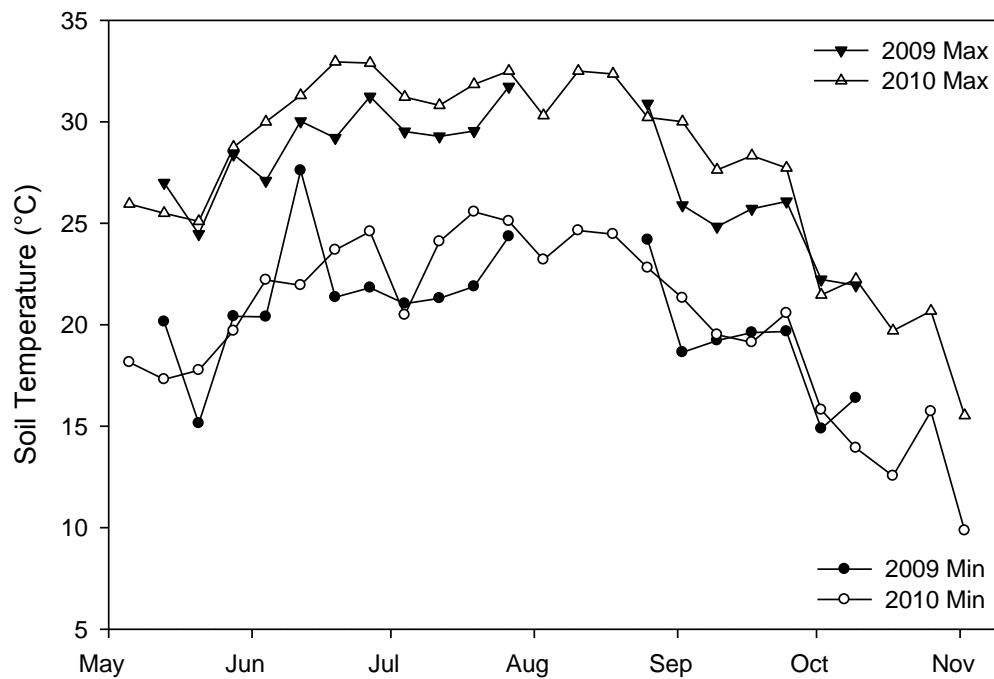


Figure 2. Weekly average minimum and maximum soil temperature (°C) recorded May to November 2009 and 2010. Temperatures were measured every 5 minutes at a 5 cm depth by Toro Turf Guard® Wireless Soil Sensors.

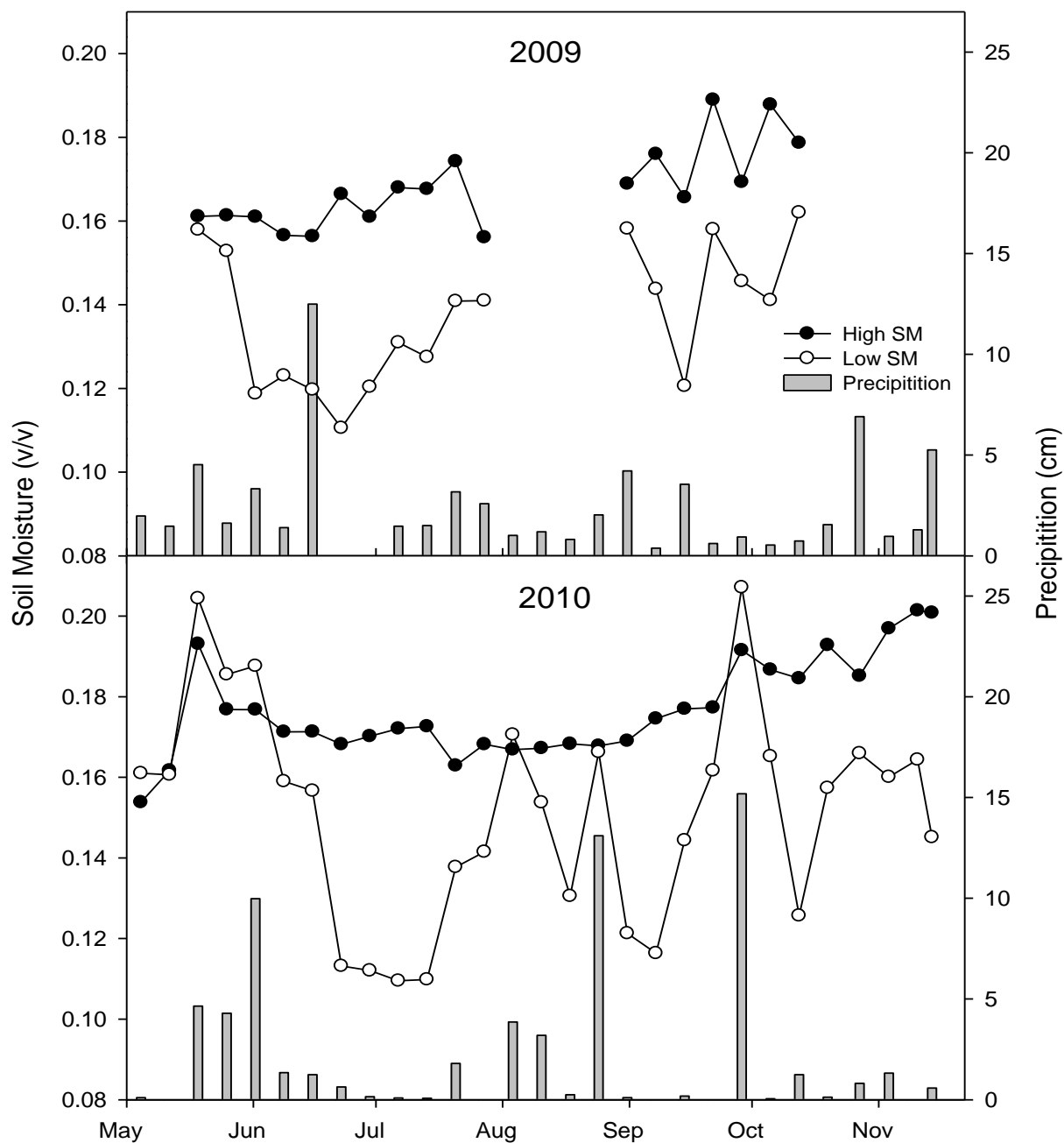


Figure 3. Weekly average precipitation and volumetric soil moisture (v/v) under high and low soil moisture conditions recorded May to November 2009 and 2010. Soil moisture was measured every 5 minutes at a 5 cm depth by Toro Turf Guard® Wireless Soil Sensors.

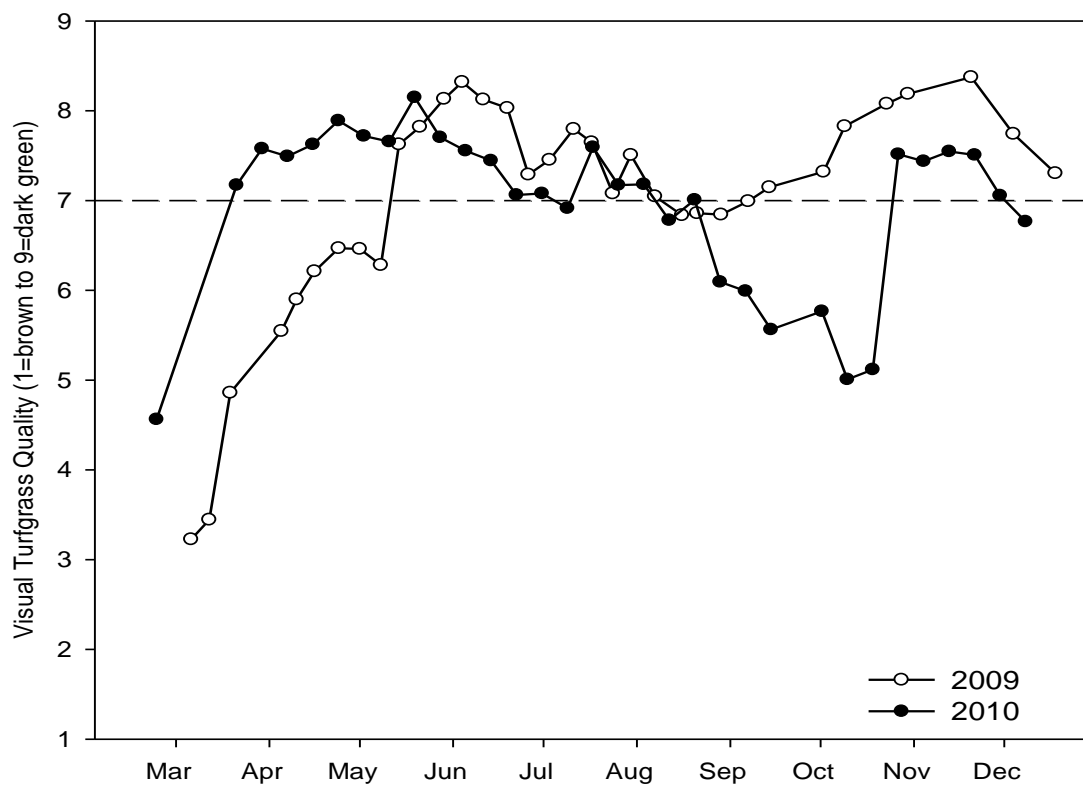


Figure 4. Weekly visual turfgrass quality ratings averaged across all treatments from March through mid-December in 2009 and 2010.

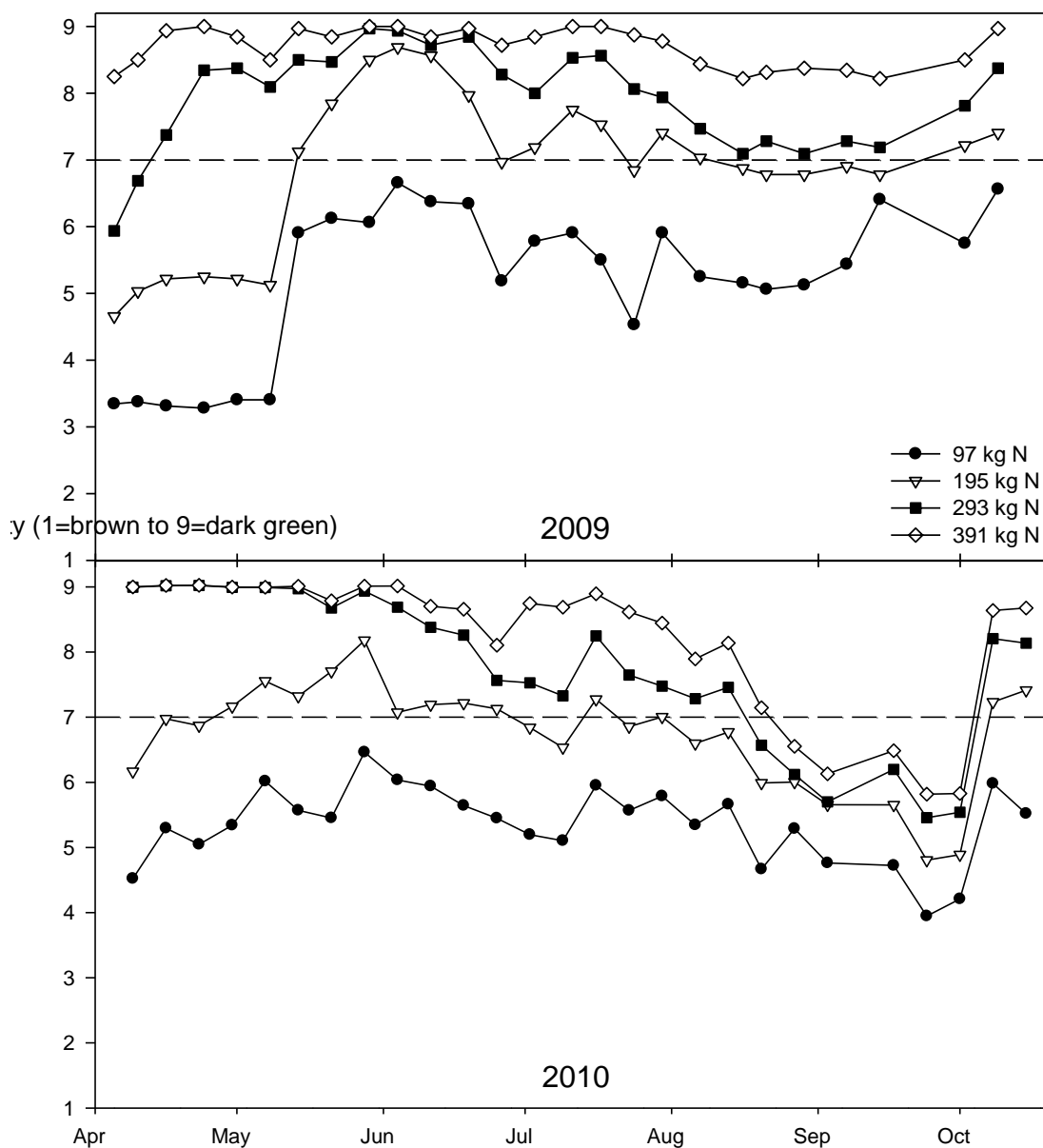


Figure 5. Weekly visual turfgrass quality ratings for turfgrass that received 97, 195, 293 and 391 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Horizontal line qualifies acceptable turfgrass quality.

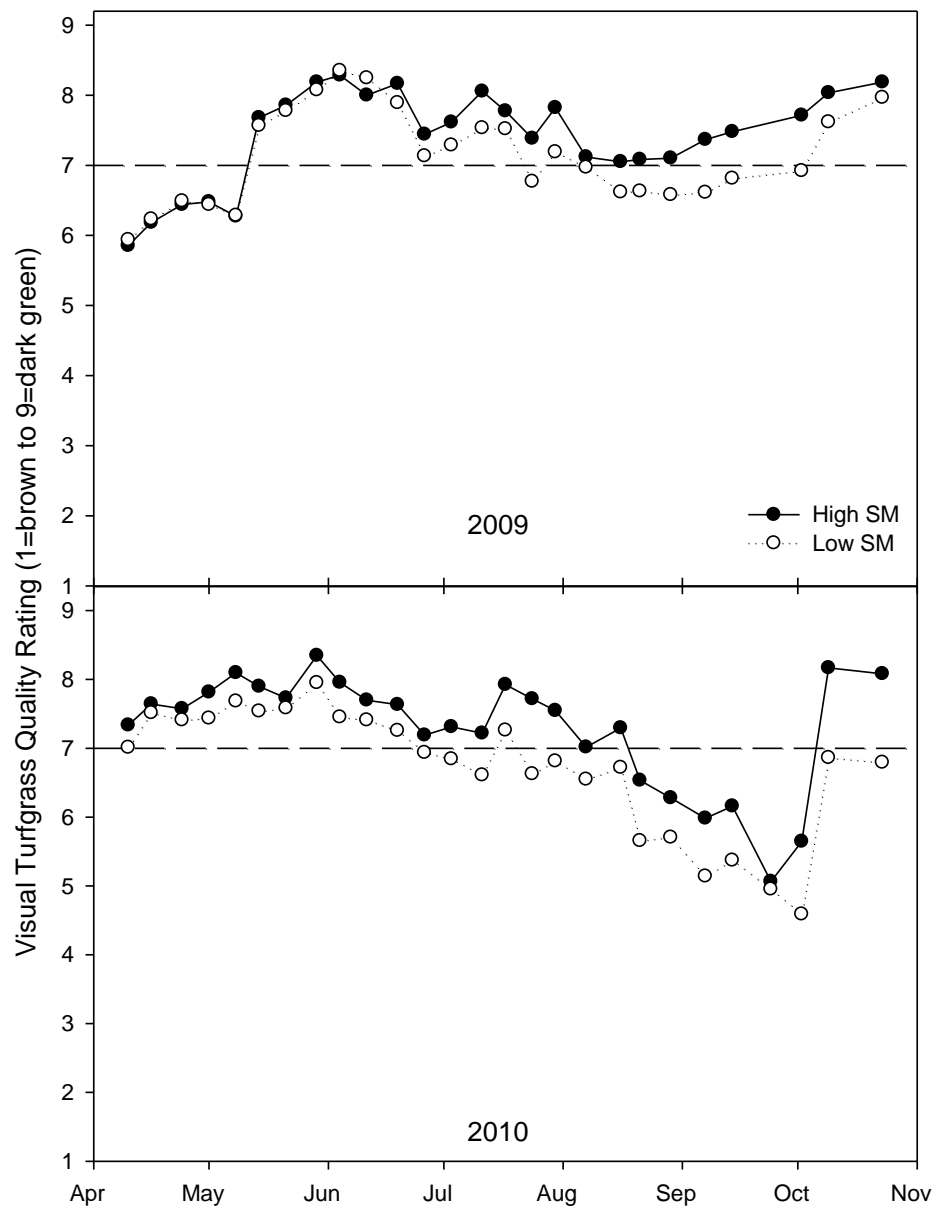


Figure 6. Weekly visual turfgrass quality ratings for turfgrass in response to high and low soil moisture. Horizontal line qualifies acceptable turfgrass quality.

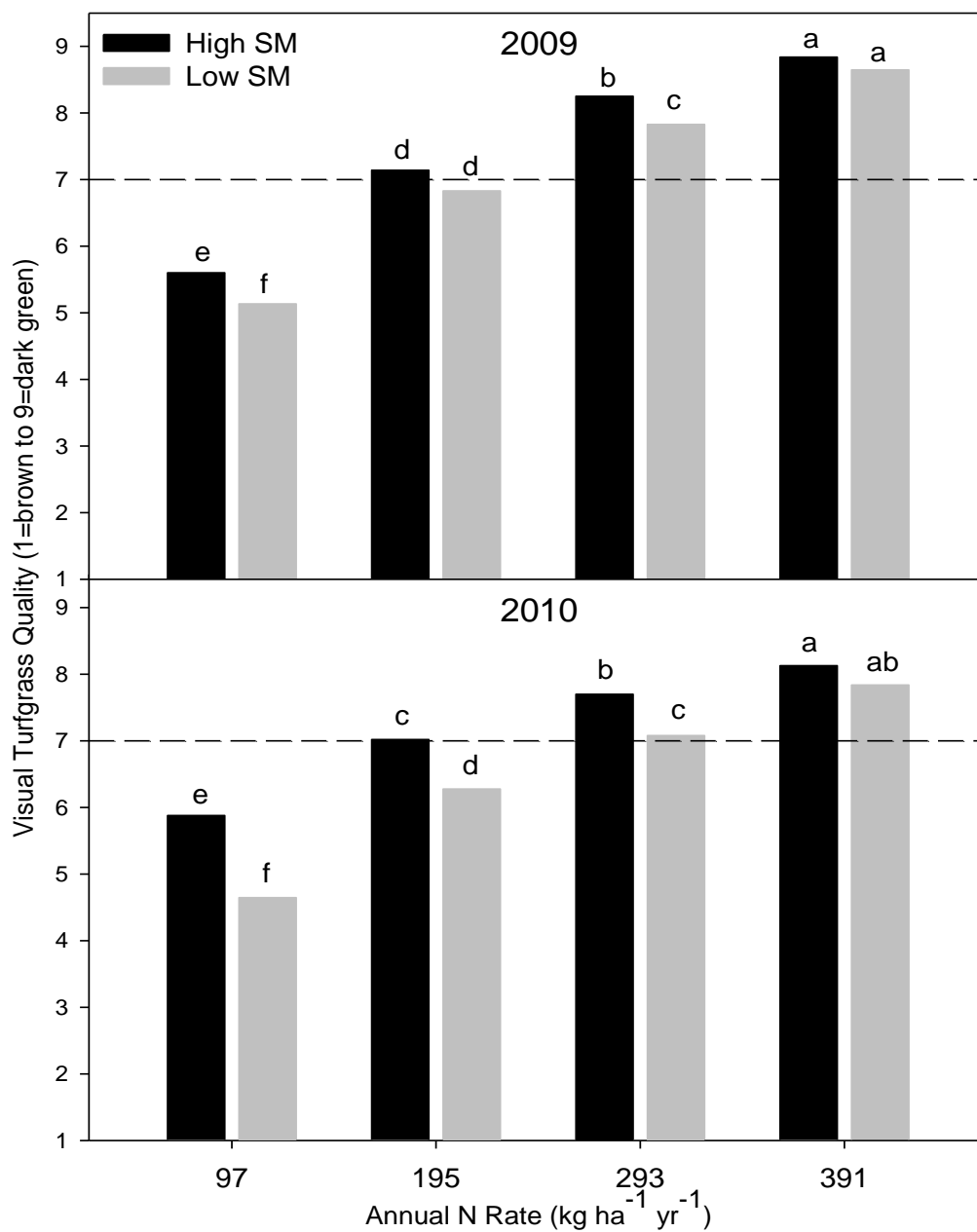


Figure 7. Visual turfgrass quality ratings in response to four N rates (97, 195, 293 and 391 kg ha<sup>-1</sup>yr<sup>-1</sup>) under high and low soil moisture (SM) averaged June through October in 2009 and 2010. Horizontal line qualifies acceptable turfgrass quality. Differences in letters indicate statistical differences across N rates according to Fisher's protected LSD<sub>0.01</sub>.

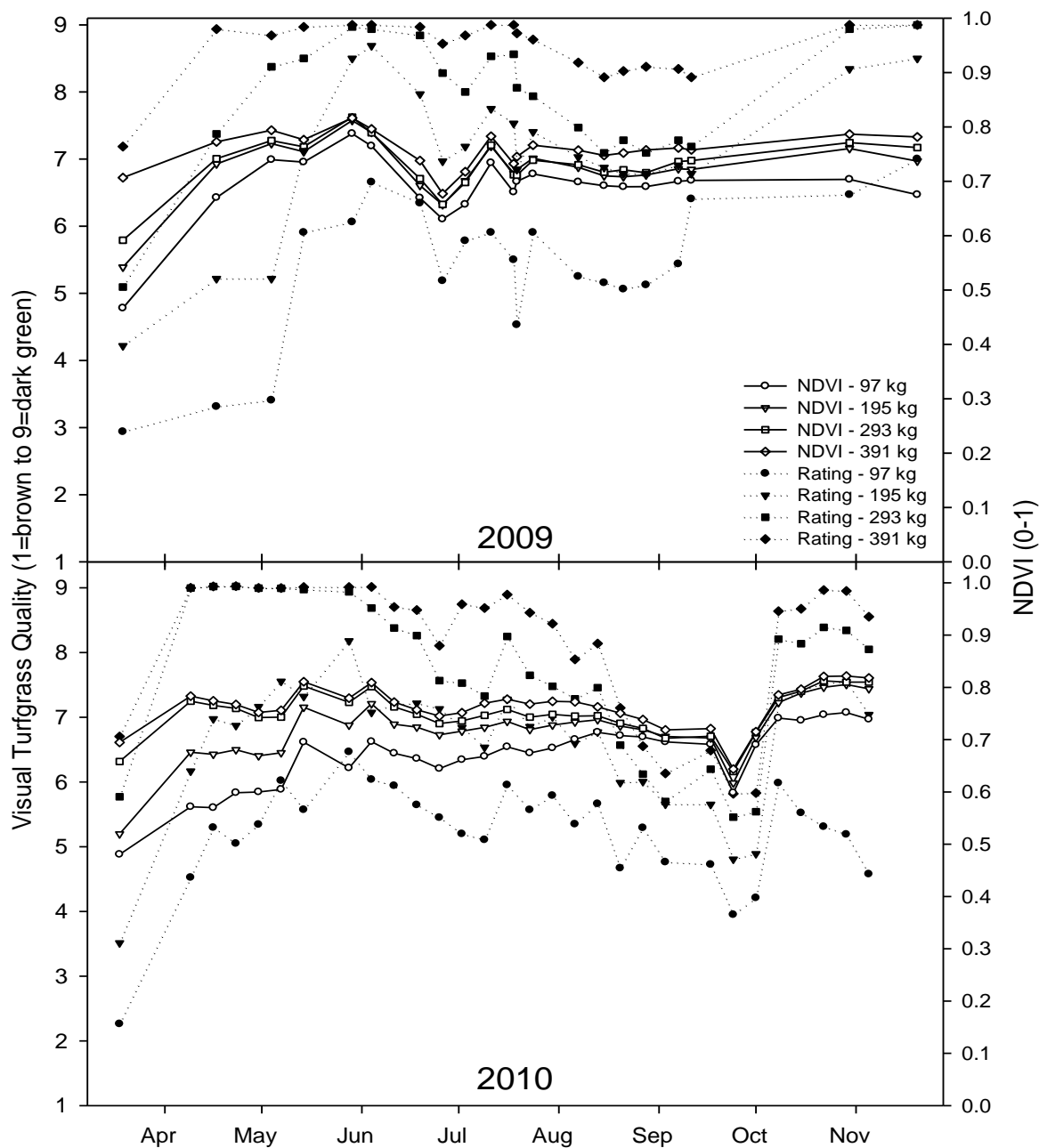


Figure 8. Weekly NDVI and visual turf quality ratings, March – December, for turfgrass that received 97, 195, 293 and 391 kg N ha<sup>-1</sup> yr<sup>-1</sup>. Correlation coefficients were 0.406, 0.568, 0.703 and 0.451 in 2009, and 0.753, 0.875, 0.738 and 0.779 in 2010, respectively. Correlation coefficients across all treatments were 0.628 in 2009 and 0.783 in 2010.



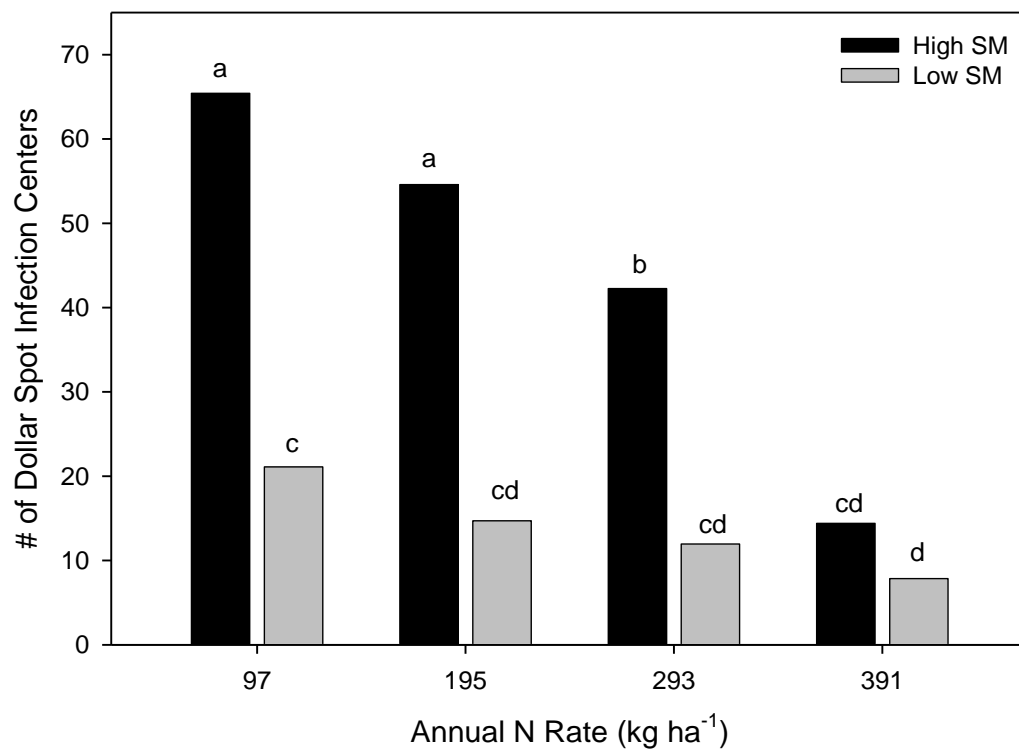


Figure 9. Dollar spot incidence in response to four N rates (97, 195, 293 and 391 kg ha<sup>-1</sup>yr<sup>-1</sup>) N under high and low soil moisture (SM) averaged June through October in 2009. Differences in letters indicate statistical differences across N rates and soil moisture treatments according to Fisher's LSD<sub>0.05</sub>.

## CHAPTER 2

### **Influence of Cultural Practices on Creeping Bentgrass Greens: Organic Matter Concentration, Water Infiltration, Soil O<sub>2</sub> and CO<sub>2</sub> and Microbial Biomass**

Soil properties beneath creeping bentgrass (*Agrostis stolonifera* L.) putting greens are in constant flux. Changes in soil properties are largely reflective of cultural management strategies and can influence overall turfgrass health. The objectives of this study were to detail the impacts of nitrogen (N) fertility, soil moisture content, and hollow- and solid-tine cultivation on organic matter accumulation, water infiltration rate, soil O<sub>2</sub> and CO<sub>2</sub>, and microbial populations. A two year study was initiated in September 2008 in Raleigh, NC. Cultural treatments included four N rates (97, 195, 293 and 391 kg ha<sup>-1</sup> yr<sup>-1</sup>), four hollow-tine cultivation programs (6.4 mm diameter tines two times yr<sup>-1</sup>, 9.5 mm diameter tines two and three times yr<sup>-1</sup> and a control that received no core cultivation), two soil moisture levels (low and high), and summer solid-tine cultivation (spiked or not spiked). At the conclusion of the two year study, non-cultivated turf plots accumulated 24.8 g kg<sup>-1</sup> of organic matter in the top 2.5 cm, and cultivating three times per year with 9.5 mm diameter tines removed 1.5 g kg<sup>-1</sup> of organic matter. Bentgrass accumulated the most organic matter, 20.4 g kg<sup>-1</sup>, when fertilized with 391 kg ha<sup>-1</sup> N yr<sup>-1</sup>. A net reduction of 2.2 g kg<sup>-1</sup> of organic matter was observed when 97 kg ha<sup>-1</sup> N was applied. Water infiltration rate was highest in turf plots cultivated with 9.5 mm diameter tines three times per year, and lowest in non-cultivated plots. Water infiltration was an average of 16.6 cm h<sup>-1</sup> slower in turf plots under high soil moisture than low soil moisture. When applied, summer spiking increased water infiltration compared to no spiking. High soil

moisture and non-core cultivated treatments had the lowest soil O<sub>2</sub> and highest soil CO<sub>2</sub> concentrations. Oxygen levels remained above 17.5%. Soil CO<sub>2</sub> was correlated with soil moisture,  $r = 0.50$  ( $p < 0.0001$ ), with high soil moisture leading to increased soil CO<sub>2</sub>. Microbial biomass C and N were 6.5 times greater in the surface 2.5 cm of the soil profile than 2.5 – 7.5 cm. At the 0- 2.5 cm depth, high intensity cultivation resulted in less biomass C and N. Results from this study indicate cultural management programs can influence certain soil properties that have been shown to affect turfgrass health.

### Introduction

Creeping bentgrass is a cool-season turfgrass characterized by fine leaf texture, vigorous lateral growth, ability to form a very dense stand, and tolerance to close mowing (Salaiz et al., 1995). The aforementioned traits contribute to its popularity as a putting surface. Newer varieties of creeping bentgrass such as the 'Penn A'-series, form a much denser canopy than older varieties. As a result, the newer varieties are becoming more popular due to the improvements they provide to putting surface quality (Fraser, 1998). However, research has found they accumulate more organic matter compared to older cultivars, and require a more intense cultivation program (Stier and Hollman, 2003).

Thatch is the accumulation of living and dead grass leaves, stems, roots, and organic debris between the soil surface and the green vegetation in a turf, while organic matter includes any carbon containing material in the entire soil matrix (Beard, 1973). Organic matter production is directly related to plant growth rate and accumulation occurs when the rate at which it is produced exceeds decomposition (Beard, 1973). Organic matter in the soil provides benefits to a turfgrass stand as it increases soil cation exchange capacity, buffering capacity, and water retention. (McCoy, 1992; Murphy et al., 1993; Carrow et al., 2001; Carrow, 2003). Accumulation of excessive surface organic matter can be detrimental to a putting green, as it may lead to increased disease and insect activity, proneness to mower scalping and footprinting, decreased in ball roll, saturated hydraulic conductivity and gas exchange rates (Turgeon 2005).

Organic matter can be removed from bentgrass greens via cultivation practices. If conducted with adequate frequency and aggressiveness, verticutting and hollow-tine core

cultivation reduced and/or prevent net accumulation of thatch/mat and organic matter (Eggens, 1980; Murphy et al., 1993a; Callahan et al., 1998; McCarty et al., 2007; Landreth et al., 2008; Fu et al., 2009). Several studies on creeping bentgrass putting greens have documented thatch/organic matter removal strategies. Murphy et al. (1993b) reported hollow-tine aerification three times yearly reduced organic matter content by  $29 \text{ g kg}^{-1}$ , compared to no cultivation. Callahan et al. (1998) reported vertical mowing plus core aeration was the most effective program, reducing thatch depth by 2 mm when compared to no cultivation applications. Verticutting 4 – 8 times annually and core cultivation 4 times annually reduced thatch depth by ~1.5 and 0.6 mm, respectively. Contrary to these studies, Sorokovsky et al. (2006) found uncultivated systems had similar organic matter content to greens core-cultivated twice per year. The author contributes the lack of organic matter reduction in cultivated systems to relatively low (< 5%) surface area impact.

Organic matter is also removed from the soil through decomposition by microorganisms. The primary food source for microbes comes from root exudates and organic matter in the soil (Mueller and Kussow, 2005; Yao et al., 2006). Soil C and putting green age have been found to have a direct, positive correlation with microbial population and diversity (Mancino et al., 1993; Kerek et al., 2002; Yao et al., 2006). Mancino et al. (1993) and Raturi et al. (2004) observed that the thatch layer of creeping bentgrass putting greens contained >100 times greater microbial populations than the underlying sand-based rootzone.

Microbial metabolism increases with temperature (Lee et al., 2001). Microbial activity would be expected to be at peak levels during the warmer summer months, however

Mueller and Kussow (2005) found a decline in microbe populations in creeping bentgrass putting greens as summer progressed. Likely, SBD attributed to decreased root activity and organic matter production, which limited food sources for soil microorganisms (Mueller and Kussow, 2005).

Organic matter content is greatest in the surface 5 cm of the soil profile, where the majority of stolons and roots reside (Carrow, 1996; Carrow, 2003). Increased density of organic matter can transform a layer of the original, medium to coarse-textured rootzone, to a much finer texture. The new formed layer retains excess water and percolation near the surface is reduced. Water retention near the surface leads to spongy greens that are prone to scalping, foot-printing, and increased wear stress. Carrow (2003), McCarty et al. (2007), and Lewis et al. (2010) found putting greens with higher organic matter also had low infiltration rates.

Hollow- and solid-tine cultivation are common practices effective in increasing infiltration rate. Creating an open channel through the layer of most resistance can allow water to more easily percolate through the soil profile (Murphy et al., 1993b; Carrow 1998; Green et al., 2001; McCarty, 2007; Sorokovsky, 2007). Core cultivation is only recommended in the spring and fall when turfgrass is actively growing (Carrow, 2003; Fu et al., 2009). However, cultivating during these times alone is not enough to maintain high infiltration rates over an extended time period. Carrow (2003) and Sorokovsky et al. (2007) reported significant decreases in water infiltration rates 5 – 8 weeks after core cultivation. Cultivating with solid-tines remains possible during the hot summer months because it is less disruptive and damaging, resulting in less recovery time (Turgeon, 2005). Both Green et al.

(2001) and Carrow (2003) observed increased water infiltration rates from spiking bentgrass greens every 2 – 3 weeks. However, Murphy et al. (1993b) found no advantage in cultivating putting greens to improve infiltration.

The O<sub>2</sub> and CO<sub>2</sub> concentrations in the soil fluxes, with an increase in soil CO<sub>2</sub> coinciding with a decrease in O<sub>2</sub> concentration (Bremmer and Blackmer, 1982). The soil atmosphere, particularly O<sub>2</sub> and CO<sub>2</sub> concentrations, is influenced by soil organic matter content, moisture, texture and temperature (Carrow, 1998; Huang et al., 1998a and 1998b; Chong et al., 2003; Bunnell et al., 2004; Rodriguez et al., 2005). Roots require O<sub>2</sub> for respiration, and root tips have an especially high O<sub>2</sub> demand. CO<sub>2</sub> is a product of cellular respiration. As respiration rates increase during the summer O<sub>2</sub> demand increases as does CO<sub>2</sub> production.

Under hot humid conditions, Carrow (1998) reported critical oxygen diffusion rates ( $< 0.20 \mu\text{g O}_2 \text{ cm}^{-2} \text{ min}^{-1}$ ) in bentgrass greens. The author observed the lowest rates in non-cultivated greens that had the greatest organic matter content. Huang et al. (1998a and 1998b) found the combination of high temperatures (35/25°C, day/night) and low oxygen diffusion rates ( $< 0.20 \mu\text{g O}_2 \text{ cm}^{-2} \text{ min}^{-1}$ ) had the most severe adverse effects on turf quality, leaf chlorophyll content, photosynthesis and respiration. However, Ervin and Corwin (1999) observed acceptable bentgrass quality despite low soil O<sub>2</sub> levels (3.3%). Research conducted by Green et al. (2001) suggest no cultivation practices on sand-based putting greens are necessary to maintain healthy oxygen diffusion rates,  $> 0.40 \mu\text{g O}_2 \text{ cm}^{-2} \text{ min}^{-1}$  and soil porosity.

Rodriguez et al. (2005), Bunnell et al. (2002), and Chong et al. (2004) have shown that elevated soil CO<sub>2</sub> levels occur concurrent with reductions in creeping bentgrass quality. Bunnell et al. (2002) observed a decrease in root mass and depth at soil CO<sub>2</sub> concentrations  $\geq 2.5\%$ , and turf quality decreased at 10% soil CO<sub>2</sub>. In a field experiment, Chong et al. (2004) reported unacceptable bentgrass quality when  $\geq 5\%$  soil CO<sub>2</sub> was detected. Rodriguez et al. (2005) observed a decrease in photosynthesis and increase in respiration at higher soil CO<sub>2</sub> concentrations. Also, soil CO<sub>2</sub> levels tend to be highest in the midst of summer resulting from increased root and microbial respiration (Wolfenden and Diggle, 1995; Bunnell et al., 2002; Chong et al., 2004). Rodriguez et al. (2005) reported increasing day/night temperatures from 26.5/21 to 32/26.5C further reduced photosynthetic rates and root dry weights. Contrary to these studies, Ervin and Corwin (1999) did not observe a decline turfgrass quality when soil CO<sub>2</sub> concentrations consistently exceeded 10%.

Soil microorganisms play an important role in organic matter stabilization and decomposition, nutrient transformations, and soil structure (Mueller and Kussow, 2005; Yao et al., 2006). Soil C and putting green age have been found to have a direct, positive correlation with microbial population and diversity (Mancino et al., 1993; Kerek et al., 2002; Yao et al., 2006). Despite high sand content, rootzones of creeping bentgrass putting greens have microbial populations similar to some finer texture native soils (Mancino et al., 1993).

The objectives of the study were to quantify the effects of N fertility, soil moisture, and hollow- and solid-tine cultivation on organic matter accumulation, microbial population, field water infiltration rate and soil O<sub>2</sub> and CO<sub>2</sub> levels.



### Materials and Methods

The study was conducted on two, 2-year old creeping bentgrass putting greens at the Lake Wheeler Turf Field Laboratory at North Carolina State University in Raleigh, NC. The greens were built to United States Golf Association (USGA) specifications (USGA Greens Section Staff, 2004). The soil was a 90:10 (sand:peat) mixture by volume with a pH of 6.1. The greens were seeded in November 2006 with 'Penn A-1' creeping bentgrass and were fully grown-in before treatments were initiated in fall 2008. Putting greens were mowed five times per week, June through September at 4 mm. The remainder of the year, the greens were mowed at 3.5 mm three to five times per week.

The experiment was arranged in a complex strip-strip block design. Each green measured 25.9 x 25.9 m, and were separated into four equal quadrants. Quadrants were separated by a plastic barrier installed through the depth of the rootzone, which allowed for isolated irrigation and drainage. Individual plots within each quadrant measured 2.4 by 2.7 m.

Zoned irrigation and drainage by quadrant enabled the creation of two moisture levels. Soil moisture treatments were maintained mid-May through October via scheduled irrigation applications. An irrigation audit was performed to ensure the irrigation system had acceptable distribution uniformity. Distribution Uniformity (lower quarter) test showed 0.60 uniformity, and Christiansen's Uniformity test resulted in a uniformity distribution of 0.76. Soil moisture treatments will be referred to as *high soil moisture* and *low soil moisture* from this point forward. High soil moisture was maintained via daily irrigation equivalent to 80% of daily evapotranspiration (ET) based on a thirty year historical average. The low soil

moisture treatment received irrigation every four to five days equal to 40% historic ET. Actual irrigation amounts ranged from 3.2 - 4.6 mm. In the event of rainfall, scheduled irrigation was withheld. 'Hotspots' and localized dry spots were lightly hand watered to prevent turf mortality. Otherwise, both greens were lightly syringed as needed to alleviate noticeable signs of wilt ('foot-printing' and/or purplish tint).

Each quadrant was equipped with a Toro Turf Guard Wireless Sensor (The Toro Company Riverside, CA) providing real-time monitoring of soil moisture, temperature and salinity. Soil sensors were installed parallel to the surface, 5 cm deep. Due to equipment failure, soil temperature and moisture data were unavailable late-July to late-August 2009. Soil moisture data were pooled by week. There were 17 weeks of available sensor data in 2009 and 21 weeks in 2010. Air temperature and precipitation data were obtained from a nearby (<1200 m) weather station maintained by the State Climate Office of North Carolina.

Four nitrogen-based fertilizer treatments were applied in strips within each quadrant, running west-east. The fertilizer programs were based of annual N rates of 97, 195, 293, or 391 kg ha<sup>-1</sup>. Both foliar and granular fertilizers were used. Contec DG 18N-9P-18K (Andersons Golf Products, Maumee, OH) greens-grade granular fertilizer was applied immediately following hollow-tine cultivation. Fertilizer treatments were pre-weighed for individual plots and applied using shaker bottles. A 0-52-34 and 0-0-30 fertilizer was applied to turfgrass plots receiving a lesser rate of the 18-9-18 fertilizer so all plots received equal amounts of the P and K. Foliar fertilizer applications were made using a CO<sub>2</sub> powered boom sprayer calibrated to apply 374 L ha<sup>-1</sup>. Green Flo 30-0-0 (John Deere Landscapes, Troy, MI) was used for foliar applications that were applied on the first or fifteenth day of each month,

+/- 4 days due to weather adjustments. In both the foliar and granular fertilizers, half of the total N was quick-release urea-N and the other half was slow-release urea-Triazone™-N.

Table 1 outlines the schedule and rate of fertilizer applications.

Four hollow-tine cultivation programs were also stripped within each quadrant, running north-south. Two of the cultivation treatments used 9.5 mm diameter tines two and three times yearly (9.5 mm x 2 and 9.5 mm x 3). A second cultivation treatment used 6.4 mm diameter tines twice per year (6.4 mm x 2). The last treatment was a control that received no coring. The control treatment was initiated 18 September 2009. Prior to this date, bentgrass was cultivated following the 9.5 mm x 2 schedule. The control was only applied in four of the eight quadrants. A Toro ProCore 648 (The Toro Company, Bloomington, MN) was set to remove cores 8.9 cm deep on a 5.1 cm by 5.1 cm spacing. Cores were harvested and removed from the site. Greens were immediately topdressed with straight quartz sand and sand was brushed in until all holes were completely filled. Greens were aerified on 8 October in 2008, 19 March and 18 September in 2009, and 26 March and 15 September in 2010. The lone treatment receiving three aerification applications per year was cored on 15 May in 2009 and 14 May in 2010.

The final cultural program involved solid-tine cultivation, or spiking. From June through mid-September, the two southern quadrants of each green were spiked twice per month and the northern half was not spiked. A Toro ProCore 648 aerator was used and equipped with bayonet tines. Tines were spaced 5.1 cm by 7.6 cm and penetrated the soil to an 8.9 cm depth.

In 2009, curative fungicide applications of chlorothalonil were tank-mixed with either iprodione or boscalid were made for dollar spot control on 9 April, 5 May, 29 June, 17 and 31 July, 4 and 14 September, and 20 October. On 12 June only boscalid was applied and 18 August iprodione only was applied. Fungicide treatments were effective at reducing dollar spot infections to minimal levels. One curative application of propiconazole was made for brown patch on 17 July.

In 2010, algae were the most prominent pest problem and chlorothalonil was curatively applied on 30 April, 14 and 27 May, 7 and 28 June and 6 July. Dollar spot was curatively sprayed with chlorothalonil and iprodione on 14 May, 28 June and 12 August. Brown patch was curatively sprayed on 7 June, 2 July and 18 August.

### *Organic Matter*

Soil organic matter was measured by loss-on-ignition. Three soil cores 2.5-cm in diameter and 7.6-cm deep were removed from each plot area. Green verdure was cut off with a knife and the remaining core was divided into 0 – 2.5 cm and 2.5 – 7.6 cm sections. Sub-samples were stored in separate plastic bags and transported to the laboratory. Each sectioned soil core was oven-dried at 105°C in crucibles for 24 h, weighed, ashed in a muffle furnace at 550°C for 12 h, and weighed again. Organic matter content was calculated by dividing the sample organic matter mass by the total sample mass. Soil organic matter was measured on 3 June, 8 August and 23 October in 2009 and 1 June, 27 July and 11 October in 2010.

### *Water Infiltration*

The double-ring falling head method (Wu et al., 1997; ASTM, 2003; Gregory et al., 2005) was used to determine water infiltration rates into the soil profile. The inner ring measured 15 cm in diameter and the outer 30 cm. Both were 10 cm in height. Prior to measurement, 2.5 cm of irrigation was applied to initially saturate the entire green. Rings were hammered 5 cm into the turfgrass, and both inner and outer rings were completely filled to the top with water. The outer ring was continuously filled to the top, throughout the entire procedure. After the initial filling, the inner ring was left to completely infiltrate into the soil. This assured complete saturation of the inner column of soil. When no water remained, the inner ring was again filled to the top. Time was recorded for 2.5 cm of water to move into the soil profile, from 3.7 cm to 1.2 cm above the surface of the turfgrass. Infiltration rates were measured on 23 June, 25 August and 11 October in 2009, and 14 June, 6 August and 15 October in 2010.

### *Soil Gas*

A portable soil gas analyzer (RKI Industries, Hayward, CA) was used to measure soil oxygen and carbon dioxide levels. A 2.5 cm diameter core was removed and the probe was quickly inserted to a 7.5 cm depth. When left in the ground, readings from the gas analyzer continually drifted towards that of atmospheric concentrations. As a result, the minimum oxygen level and the maximum carbon dioxide readings were recorded. Two readings were taken from each turfgrass plot area. Soil gas was measured between 0700 and 0900 HR. A time domain reflectometry device (Field Scout TDR 300, Spectrum Technologies, Inc., East

Plainfield, Illinois) measured volumetric soil moisture in the surface 7.6 cm. Soil gas readings were taken 2 July, 29 July, 10 September and 23 October in 2009, and 16 May, 23 June, 22 July, 27 August, 14 October in 2010.

### *Microbial Biomass*

The chloroform-fumigation extraction method was used to estimate microbial biomass (Brooks et al., 1985; Vance et al., 1987). For economic reasons, only turf plots under high soil moisture were sampled across all levels of hollow- and solid-tine cultivation, and N fertility treatments. Sub-samples were taken from plots under low soil moisture conditions that received 97 and 391 kg N ha<sup>-1</sup> and were core cultivated with 6.4 mm twice yearly and 9.5 mm three times yearly. Another set of sub-samples were taken from bentgrass plots under high soil moisture, that received 391 kg N ha<sup>-1</sup> and core cultivated with 6.4 mm twice yearly and 9.5 mm three times yearly. The latter set was sectioned according to profile depth, 0 – 2.5 cm (thatch included) and 2.5 – 7.6 cm.

Three soil cores 2.5-cm in diameter and 7.6-cm deep were taken from each plot area. Green plant material and thatch were removed with a knife from the cores. The cores were placed in a plastic bag and kept in a cooler on ice until transferred to a refrigerator maintained at 4°C. Soil was sieved to <4 mm and forceps were used to remove any remaining visible plant material. Three 15-g sub-samples were taken; one was to determine volumetric soil moisture, one underwent chloroform-fumigation while the third was an unfumigated control. Organic C and N were extracted from both fumigated and unfumigated sub-samples with 0.5 M K<sub>2</sub>SO<sub>4</sub>. Extracts were analyzed for organic C and N. Microbial

biomass C and N was calculated by dividing the difference of each respective fumigated and unfumigated samples by the conversion factors 0.45 for biomass C and 0.54 for biomass N (Brookes et al., 1985; Vance et al., 1987). Microbial biomass was measured on 10 June, 14 August and 23 October in 2009, and 1 June, 4 August and 31 October in 2010.

Treatment and interaction effects were determined using PROC MIXED of the Statistical Analysis System (SAS Institute, Cary, NC). Means were separated using the LSMEANS statement and were subject to Fisher's protected least significance test ( $p < 0.05$  or  $0.01$ ). Analysis revealed significant differences among treatments and years. Therefore data for each year are presented separately.

### Results and Discussion

#### *Organic Matter*

At the 0 – 2.5 cm depth, organic matter was influenced by hollow-tine cultivation ( $p < 0.0001$ ) and N fertility ( $p < 0.0001$ ), and no cultural input affected organic matter content at the 2.5 - 7.5 cm depth (Table 2). Mean organic matter concentration at the 0 – 2.5 and 2.5 – 7.5 cm depths were  $50.0 \text{ g kg}^{-1}$  and  $14.8 \text{ g kg}^{-1}$ , respectively. Differences among soil depth may be age related. In young greens, Murphy (1993a) and Goodman (2009) reported relatively low organic matter concentrations deeper in the soil profile, compared to concentrations near the surface. Initially, organic matter accumulation near the surface is very rapid. This is a result of high stolon and root production (McCarty et al., 2007). Deeper in the soil organic matter accumulates in a more linear fashion (Goodman, 2009). In two-year old greens we would expect to find little organic matter accumulation deeper in the soil profile.

Organic matter in the surface 2.5 cm was directly related to the intensity of the hollow-tine cultivation regime in place (Table 3). The non-cultivated turfgrass plots consistently contained the greatest organic matter, and the most intensely cultivated plots had the least. At the conclusion of this two year study, turfgrass that received core cultivation three times per year with 9.5 mm tines was the only program that reduced organic matter in the surface 2.5 cm, though it was only by  $1.5 \text{ g kg}^{-1}$  (a 3.5% reduction). Bentgrass that received no core-cultivation accumulated  $10.6 \text{ g kg}^{-1}$  of organic matter (a 24.8% increase).

Nitrogen fertility consistently influenced organic matter concentration (Table 3). Turf plots fertilized with  $97 \text{ kg ha}^{-1}$  N had the least organic matter throughout the study, and those that received  $391 \text{ kg ha}^{-1}$  N had the most. At the conclusion of this two year study, organic matter was reduced by  $2.2 \text{ g kg}^{-1}$  (a 5.2% reduction) when bentgrass received  $97 \text{ kg ha}^{-1}$  N. Turfgrass plots fertilized with 195, 293 and  $391 \text{ kg ha}^{-1}$  N had accumulated 0.6, 7.3, and  $11.4 \text{ g kg}^{-1}$  of organic matter (a 10.3, 16.4 and 20.3% increase), respectively. Increased N application likely promoted increased shoot, root, and stolon growth and developed the organic layer more rapidly.

In both years, large increases in organic matter in the top 2.5 cm were observed between the October and June. This was followed by reduced concentrations between June and August. In 2009, organic matter increased from August to October, but remained relatively consistent during that period in 2010. Patterns in the change of organic matter concentration may be related to environmental conditions and overall plant growth. Fall and spring-type weather, combined with increased N applications promoted maximum plant growth, and consequently maximum organic matter production. Organic matter is removed



from a turfgrass system through cultivation practices or biological decomposition by microorganisms. Though no seasonal differences in microbial biomass C or N were detected in this study, it is generally accepted that microbial activity is directly related to temperature (Lee et al., 2001; Shi, 2006). Cooler temperatures from October to June decrease microbial activity and organic matter degradation rate. Core cultivation applications made in March and May (if applicable) of both years, did not prevent net organic matter accumulation from October to June. However, organic matter decreased from June to August, when no aerification was applied. This may suggest the relationship between slowed plant growth and increased microbial activity is more effective in removing organic matter than hollow-tine cultivation. Fu et al. (2009) observed similar seasonal trends in the thatch layer of a creeping bentgrass putting green, but with larger differences. The authors reported organic concentration increased  $26.7 - 119 \text{ g kg}^{-1}$  during the fall and spring and decreased  $17.2 - 33.6 \text{ g kg}^{-1}$  throughout the summer.

Mean differences in organic matter between the most and least intensely cultivated bentgrass plots doubled from 2009 to 2010 ( $5.7 \text{ g kg}^{-1}$  and  $11.8 \text{ g kg}^{-1}$ , respectively). This further underscores the effectiveness of a cultural practice on organic matter accumulation over time. A particular practice may promote significant accumulation in the long run, but its impacts may not be immediately evident. As a result it is important for golf course superintendents to avoid complacency. Reducing core aerification applications may not show significant increases in soil organic matter initially, but overtime, organic matter accumulation will likely become problematic (Carrow, 1996; Carrow, 2003).

### *Water Infiltration*

Water infiltration rate was also affected by hollow-tine cultivation programs ( $p < 0.0001$ ). Differences were observed in water infiltration rate between all hollow-tine cultivation treatments at every measurement date (Table 4). Field infiltration rates were directly related to the intensity of the aerification program. In 2010, bentgrass aerified with 9.5 mm tines three times per year possessed infiltration rates twice that of non-cultivated turf plots. Turf plots cultivated with 6.4 mm diameter tines two times yearly and the non-cultivated treatment were mostly unchanged from June through October in 2010. Despite differences between cultivation practices, infiltration rates amongst all treatments remained above the minimum  $15 \text{ cm h}^{-1}$  suggested by USGA Greens Section Staff (2004). Carrow (2003), McCarty et al. (2007) and Sorokovsky (2007) all found bentgrass that was core aerified provided higher water infiltration rates compared to non-cored greens.

Water infiltration rate has been shown to be related to soil organic matter content (Waddington et al., 1974; McCoy, 1992; Murphy et al. 1993b). Organic matter accumulation can clog macropores and impede water's path through the profile. Findings from this study were consistent with this idea. Core cultivation programs that promoted the least amount of organic matter also had the fastest infiltration rates. A weak negative correlation existed ( $p < 0.0001$ ) between organic matter concentration at the 0 – 2.5 cm depth and infiltration rate,  $r = -0.20298$ .

During the two year study, mean overall water infiltration rate was reduced by  $30.9 \text{ cm h}^{-1}$  (Table 4). Infiltration rates were affected by soil moisture ( $p = 0.0019$ ). High soil moisture conditions produced lower infiltration rate at each assessment date. In 2009, both

moisture treatments decreased water infiltration by  $\sim 24.2 \text{ cm h}^{-1}$  between June and October. In the same period in 2010, water infiltration rates decreased  $12.5 \text{ cm h}^{-1}$  under high soil moisture conditions. Bentgrass exposed to low soil moisture showed a small increase in infiltration rate from June to October in 2010.

Since soil moisture did not directly influence organic matter concentrations this response was not expected. Rooting was not measured, but one explanation is that lower soil moisture may have promoted a more expansive root system. Numerous roots provide vertical channels which water can easily move through the soil profile (Bennett and Doss, 1964; Jordan et al., 2003; Fu and Dernoeden, 2009). Fu and Dernoeden (2009) observed greater total root surface area at 0 – 6 cm depths in bentgrass that was irrigated deep and infrequent compared to a light, frequent irrigation program. Similarly, Jordan et al. (2003) observed greater root length density at 1 – 7.5 cm depths in bentgrass that was irrigated on four day intervals compared to those irrigated more frequently. Infiltration rates may have also decreased under high soil moisture due to the presence of algae, where in low soil moisture algae was not as prevalent. Algae are much denser than the turf canopy and can further reduce infiltration rates (Nus, 1994; Carrow, 1996).

Spiking bentgrass greens from June to September influenced infiltration rates during that time (Table 4). Non-spiked turf plots demonstrated slower infiltration rates than spike plots in both 2009 and 2010 ( $p = 0.0051$ ) at each assessment date. Spiking putting greens increased water infiltration by an average of  $6.1 \text{ cm h}^{-1}$ . Similar to our study, Green et al. (2001) found summer solid-tine cultivation increased field infiltration rates by an average of

5.2 cm h<sup>-1</sup>. However, Murphy et al. (1993b) did not find any differences in infiltration rates when solid-tine cultivation was applied.

Considering N fertility influenced organic matter content and organic matter content influenced infiltration rate, we might expect N fertility to affect infiltration rate. However, this relation was not evident (data not shown). An explanation for this may be that the density which the organic layer is formed near the surface is similar across N fertility rates, but the rate at which it builds in depth is slower when less N is applied. As a result the depth of the organic layer is shallower in bentgrass that received less N. Infiltration rate of the soil profile is regulated by the infiltration rate of its slowest layer (Jury et al; 1991). In a sand-based soil medium, the density of the surface organic layer likely determines the water infiltration rate. Consequently, bentgrass that received less annual N may have accumulated less total organic matter at the 0 – 2.5 cm depth than more heavily fertilized turf, but did not differ in water infiltration rate. Another factor to consider is the presence of algae. Algae were only found in bentgrass fertilized with 97 and 195 kg ha<sup>-1</sup> N. Where we might expect lower N rates have increased infiltration rates due to less surface organic matter, the presence of algae may have been the factor causing decreased infiltration rates.

### *Soil Gas*

Measured soil O<sub>2</sub> concentrations ranged from 17.6 to 20.9% (Table 5), well above the 5 to 15% range needed for optimum plant growth (Luxmoore et al., 1970; Barden et al., 1987). Regardless of cultural program, soil O<sub>2</sub> levels were more than adequate for optimal

turf growth. Contrary to our findings, Carrow (2003) observed periods of critically low O<sub>2</sub> levels under hot summer conditions.

Soil O<sub>2</sub> levels were different among soil moisture levels ( $p < 0.0001$ ), and hollow-tine cultivation programs ( $p < 0.0001$ ). Though differences existed, they were small relative to the amount of O<sub>2</sub> present in the soil. Turf plots under the high soil moisture regime maintained an average of 19.6% soil O<sub>2</sub>, and 19.8% soil O<sub>2</sub> in those exposed to low soil moisture. Soil O<sub>2</sub> was directly related to the intensity of the applied hollow-tine cultivation program. The greatest soil O<sub>2</sub> concentrations, 19.8%, were observed in turf plots subject to the most intense core cultivation program (9.5 mm x 3), while the lowest O<sub>2</sub> concentrations, 19.4%, were found in non-cultivated bentgrass. Soil O<sub>2</sub> was not affected by N fertility or summer spiking.

Soil moisture ( $p = 0.0053$ ) and core cultivation ( $p < 0.0001$ ) had opposite affects on soil CO<sub>2</sub> compared to soil O<sub>2</sub> (Table 6). Measured soil CO<sub>2</sub> levels were consistently >10 times atmospheric CO<sub>2</sub> concentrations (~0.03%). Volumetric soil moisture and CO<sub>2</sub> were correlated,  $r = 0.50$  ( $p < 0.0001$ ). Turf plots under the high soil moisture regime maintained an average of 0.57% soil CO<sub>2</sub>, as compared to 0.39% soil CO<sub>2</sub> in those exposed to low soil moisture. Soil CO<sub>2</sub> was directly related to the intensity of the applied hollow-tine cultivation program. The lowest soil CO<sub>2</sub> concentrations, 0.41%, were observed in turf plots subject to the most intense core cultivation program (9.5 mm x 3), while the highest CO<sub>2</sub> concentrations, 0.58%, were found in non-cultivated bentgrass. The 9.5 mm x 3 program decreased soil CO<sub>2</sub> 29.0% compared to no cultivation. Soil CO<sub>2</sub> was not affected by N fertility or summer spiking. Regardless of cultural input, CO<sub>2</sub> concentrations remained low and based on previous research did not influence turfgrass health.

There was an interaction between soil moisture and hollow-tine cultivation programs that influenced soil CO<sub>2</sub> ( $p = 0.0171$ ) (Figure 2). Uncultivated turf plots exposed to high soil moisture possessed the greatest soil CO<sub>2</sub> concentration, while the least CO<sub>2</sub> was observed in bentgrass cultivated three times per year with 9.5 mm diameter tines under low soil moisture. Water retention in the soil displaces air from macropores, and is exemplified in soil with high organic matter. When the total air volume is reduced, so is its ability to buffer concentration changes. Ongoing root respiration removes O<sub>2</sub> from the soil air and adds CO<sub>2</sub>. CO<sub>2</sub> occupies only 0.03% of atmospheric air, and small additions can lead to a proportionally larger concentration. If a dense organic matter layer is present near the soil surface, diffusion of soil gases into the atmosphere may be reduced, and CO<sub>2</sub> may become trapped in the soil.

#### *Microbial Biomass*

The only differences detected in microbial biomass came from soil sub-sampled according to profile depth (Figure 3). Average microbial biomass C and N in the 0 – 2.5 cm depth were about 6.5 times greater than those found 2.5 – 7.5 cm below the surface. Within the 0 – 2.5 cm depth, turf plots cultivated with 6.4 mm tines two times yearly contained greater microbial biomass C and N than those aerified with 9.5 mm tines three times yearly. No seasonal fluxes were observed in microbial biomass. Results suggest differences in microbial populations are driven by organic matter content in the soil, and are consistent with those found by Mancino et al. (1993) and Raturi et al. (2004).

### Conclusions

Results from this research indicate organic matter content, field water infiltration rates and soil CO<sub>2</sub> can be influenced through traditional cultural practices. These factors were influenced by hollow-tine cultivation practices. Increased tine size and/or frequency of hollow-tine cultivation applications followed by sand topdressing ultimately created open channels into the soil profile, which promoted faster water infiltration, higher soil O<sub>2</sub> and lower CO<sub>2</sub>.

Organic matter concentration also increased as annual N rates increased from 97 to 391 kg ha<sup>-1</sup>. Organic matter accumulated fastest in the surface 2.5 cm in minimally and non-cultivated bentgrass, and played a role in decreased water infiltration and air-soil gas exchange. Regardless of organic matter content, creating an opening through the turf canopy allowed water to more easily percolate into and through the soil profile. Excessive irrigation or high soil moisture also proved to reduce infiltration rate and increase soil CO<sub>2</sub>.

This study was only conducted over two growing seasons. Implementing these cultural programs for several consecutive years may intensify results found in this study. Most golf course putting greens are greater than two years old. Poor cultural management by a golf course superintendent for an extended number of years may lead to excessive organic matter accumulation, inadequate water infiltration, and soil O<sub>2</sub> deficiency and CO<sub>2</sub> toxicity. In the short-term, this study demonstrated cultural practices influenced organic matter content, water infiltration and soil CO<sub>2</sub>, but infiltration rates and soil gas levels remained above acceptable levels. A long-term study is needed to determine the upper limit effect of cultural practices.

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Table 1. Yearly schedule of granular and foliar N fertilizer applications. Monthly foliar rates are split between two separate application made on the 1<sup>st</sup> and 15<sup>th</sup> of each month (+/- 4 days).

Month	Total N applied (kg ha <sup>-1</sup> yr <sup>-1</sup> )							
	97		195		293		391	
	Foliar	Granular	Foliar	Granular	Foliar	Granular	Foliar	Granular
	Monthly N Rate (kg ha <sup>-1</sup> )							
Feb			6.1†		9.8†			24.4
Mar	4.9†	12.2	12.2	24.4	19.5	36.6	24.4	36.6
Apr	9.8		12.2		19.5		24.4	
May	9.8		12.2	24.4	12.2	36.6	24.4	36.6
June	9.8		12.2		12.2		24.4	
July	9.8		12.2		12.2		24.4	
Aug	9.8		12.2		12.2		24.4	
Sept	9.8	12.2	12.2	36.6	19.5	36.6	24.4	36.6
Oct	9.8		12.2		19.5		24.4	
Nov			6.1†		9.8†	36.6	24.4	36.6

† Indicates only one scheduled application in that month

Table 2. Mean squares from combined analyses of variance for soil organic matter content 0 - 2.5 cm below the surface, water infiltration rate and soil O<sub>2</sub> and CO<sub>2</sub>.

Source of Variation†	df	Mean Square			
		Organic Matter	Infiltration	Soil O <sub>2</sub>	Soil CO <sub>2</sub>
Spiking, S	1	0.307	483.8*	0.03	0.012
Moisture, M	1	0.003	7298.1**	13.01**	6.493**
S x M	1	5.369	261.0	1.78	0.054
Quad(S x M)	4	1.749	232.9	0.61**	0.853**
Fertility, F	3	18.391**	50.1	0.21	0.122
F x S	3	0.740	50.6	0.01	0.030
F x M	3	0.519	29.6	0.01	0.055
F x S x M	3	0.410	16.4	0.07	0.012
F x Quad(S x M)	12	0.474	32.9*	0.05	0.179**
Cultivation, C	3	28.009**	2669.7**	4.64**	1.974**
C x S	3	0.110	4.6	0.17*	0.008
C x M	3	0.476	33.7	0.23**	0.180*
C x S x M	3	0.422	4.7	0.13	0.025
C x Quad(S x M)	8	0.638	30.1*	0.02	0.095*
C x F	9	0.265	12.3	0.05	0.038
C x F x S	9	0.287	11.7	0.06	0.032
C x F x M	9	0.590	3.3	0.01	0.011
C x F x S x M	9	0.532	4.8	0.02	0.032
C x F x Quad(S x M)	24	0.346	9.8	0.01	0.091
CV %		20.0	28.4	3.6	37.5
Mean‡		3.139	591.6	1.11	0.542

\*, \*\* Indicates significance at 0.05 and 0.01 level, respectively.

† S = summer solid-tine cultivation, M = soil moisture, F = N fertility program and C = hollow-tine cultivation, Quad = quadrant

‡ Units = Organic Matter, g kg<sup>-1</sup>; Infiltration, cm h<sup>-1</sup>; Soil O<sub>2</sub> and CO<sub>2</sub>, %



Table 3. Organic matter concentration in the surface 2.5 cm of the soil in response to core cultivation and annual N fertility on a 'Penn A-1' creeping bentgrass putting green, 2008-2010.

Treatments	2008		2009		2010			Reduction or increase <sup>†</sup>
	13 Oct	3 June	6 Aug	23 Oct	1 June	26 July	11 Oct	
Organic Matter								
Core cultivation <sup>†‡</sup>	g kg <sup>-1</sup>							%
control	42.7	n/a	n/a	47.8 a	66.6 a	53.7 a	53.3 a	+ 24.8
6.4 mm x 2	42.7	52.3 a§	44.6 a	48.7 a	61.2 b	51.9 a	51.0 a	+ 19.4
9.5 mm x 2	42.7	49.4 ab	40.7 b	43.5 b	58.1 bc	45.2 b	43.3 b	+ 1.4
9.5 mm x 3	42.7	48.3 b	40.0 b	40.2 c	56.6 c	40.4 c	41.2 b	- 3.5
N fertility (kg ha <sup>-1</sup> yr <sup>-1</sup> )								
97	42.7	49.8 a	39.0 b	40.4 b	51.3 c	41.5 c	40.5 b	- 5.2
195	42.7	50.9 a	41.5 ab	47.3 a	56.5 b	47.2 b	47.1 a	+ 10.3
293	42.7	50.3 a	41.9 ab	47.0 a	66.4 a	52.0 a	49.7 a	+ 16.4
391	42.7	48.6 a	44.7 a	45.5 a	68.2 a	50.2 ab	51.4 a	+ 20.4

<sup>†</sup> Core cultivation abbreviations: control = no cultivation, 6.4 mm x 2 = two yearly applications using 6.4 mm diameter tines, 9.5 mm x 2 = two yearly applications using 9.5 mm diameter tines, 9.5 mm x 3 = three yearly applications using 9.5 mm diameter tines.

## Table 3. Continued

‡ Cultivation was applied on 8 October 2008, 19 March and 18 September in 2009, and 26 March and 15 September in 2010. The third additional cultivation was applied on 15 May 2009 and 14 May 2010. The control treatment was not established until the 18 September 2009 application date. Tines were spaced 5.1 cm by 5.1 cm.

§ Means in a column followed by the same letter within treatment type are not different based on Fisher's protected  $LSD_{0.05}$  test.

¶ Difference in organic matter from 13 October 2008 to 11 October 2010.

Table 4. Field water infiltration rates in response to core cultivation, soil moisture and summer spiking on a 'Penn A-1' creeping bentgrass putting green, 2008-2010.

Treatments	2009			2010		
	23 June	25 Aug	11 Oct	14 June	6 Aug	15 Oct
	Infiltration Rate					
	cm h <sup>-1</sup>					
<u>Core Cultivation†‡</u>						
control	n/a	n/a	28.3 d	20.5 d	27.7 d	22.0 c
6.4 mm x 2	55.5 c§	42.8 c	40.0 c	27.9 c	35.2 c	28.5 c
9.5 mm x 2	64.5 b	49.8 b	47.0 b	42.3 b	43.6 b	36.6 b
9.5 mm x 3	70.9 a	58.0 a	51.5 a	55.0 a	52.3 a	43.7 a
<u>Soil Moisture¶</u>						
High	55.2 b	43.5 b	31.8 b	31.7 b	32.4 b	19.2 b
Low	72.0 a	56.9 a	47.0 a	41.1 a	47.0 a	46.2 a
<u>Summer Spiking#</u>						
Spiked	65.4 a	57.3 a	n/a	38.8 a	43.6 a	n/a
Not spiked	61.8 a	43.1 b	n/a	34.1 b	35.8 b	n/a

† Core cultivation program abbreviations: control = no cultivation, 6.4 mm x 2 = two yearly applications using 6.4 mm diameter tines, 9.5 mm x 2 = two yearly applications using 9.5 mm diameter tines, 9.5 mm x 3 = three yearly applications using 9.5 mm diameter tines.

‡ Core cultivation was applied on 8 October 2008, 19 March and 18 September in 2009, and 26 March and 15 September in 2010. The third additional cultivation was applied on 15 May 2009 and 14 May 2010. The control treatment was initiated 18 September 2009. Tines were spaced 5.1 cm by 5.1 cm.

§ Means in a column followed by the same letter within treatment type are not different based on Fisher's protected LSD<sub>0.05</sub> test.

Table 4. Continued

¶ Maintained soil moisture regimes: low = irrigation applied every four to five days equal to 40% historic ET, high = daily irrigation applied equivalent to 80% of daily historic ET.

# Summer spiking applications were made twice monthly June through mid-September in 2009 and 2010.

Table 5. Soil O<sub>2</sub> concentration in response to core cultivation and soil moisture on a ‘Penn A-1’ creeping bentgrass putting green, 2008-2010.

Treatments	2009				2010				
	2 July	29 July	10 Sept	23 Oct	16 May	23 June	22 July	27 Aug	14 Oct
	Soil O <sub>2</sub> concentration								
Core Cultivation†‡	%								
control	n/a	n/a	20.82 a	19.82 a	19.36 c	19.60 c	19.12 c	18.95 a	19.44 b
6.4 mm x 2	19.71 c§	20.33 b	20.68 a	19.82 a	19.76 b	19.76 b	19.24 bc	18.97 a	19.56 b
9.5 mm x 2	19.85 b	20.37 b	20.71 a	19.83 a	20.28 a	19.94 a	19.34 ab	19.07 a	19.72 a
9.5 mm x 3	20.02 a	20.45 a	20.72 a	19.84 a	20.22 a	20.03 a	19.39 a	19.09 a	19.70 a
Soil Moisture¶									
High	19.60 b	20.27 b	20.78 a	19.81 a	19.94 a	19.56 b	19.31 a	18.86 b	19.26 b
Low	20.11 a	20.50 a	20.68 a	19.84 a	19.87 a	20.11 a	19.24 a	19.17 a	19.94 a

† Core cultivation program abbreviations: control = no cultivation, 6.4 mm x 2 = two yearly applications using 6.4 mm diameter tines, 9.5 mm x 2 = two yearly applications using 9.5 mm diameter tines, 9.5 mm x 3 = three yearly applications using 9.5 mm diameter tines.

‡ Cultivation was applied on 8 October 2008, 19 March and 18 September in 2009, and 26 March and 15 September in 2010. The third additional cultivation was applied on 15 May 2009 and 14 May 2010. The control treatment was initiated 18 September 2009. Tines were spaced 5.1 cm by 5.1 cm.

Table 5. Continued

§ Means in a column followed by the same letter within treatment type are not different based on Fisher's protected  $LSD_{0.01}$  test.

¶ Maintained soil moisture regimes: low = irrigation applied every four to five days equal to 40% historic ET, high = daily irrigation applied equivalent to 80% of daily historic ET.

Table 6. Soil CO<sub>2</sub> concentration in response to core cultivation and soil moisture on a ‘Penn A-1’ creeping bentgrass putting green, 2008-2010.

Treatments	2009				2010				
	2 July	29 July	10 Sept	23 Oct	16 May	23 June	22 July	27 Aug	14 Oct
	Soil CO <sub>2</sub> concentration								
Core Cultivation†‡	%								
control	n/a	n/a	0.42 a	0.29 a	0.84 a	0.68 a	0.56 a	0.62 a	0.67 a
6.4 mm x 2	0.78 a§	0.55 a	0.36 b	0.25 b	0.70 b	0.54 b	0.48 b	0.57 a	0.51 b
9.5 mm x 2	0.70 b	0.51 b	0.36 b	0.24 b	0.51 c	0.43 c	0.41 c	0.47 b	0.35 c
9.5 mm x 3	0.62 c	0.47 c	0.36 b	0.23 b	0.52 c	0.38 d	0.36 d	0.44 b	0.32 c
Soil Moisture¶									
High	0.84 a	0.58 a	0.41 a	0.28 a	0.64 a	0.67 a	0.48 a	0.63 a	0.75 a
Low	0.55 b	0.44 b	0.33 b	0.22 a	0.64 a	0.33 b	0.41 b	0.41 b	0.17 b

† Core cultivation program abbreviations: control = no cultivation, 6.4 mm x 2 = two yearly applications with 6.4 mm diameter tines, 9.5 mm x 2 = two yearly applications with 9.5 mm diameter tines, 9.5 mm x 3 = three yearly applications with 9.5 mm diameter tines.

‡ Cultivation was applied on 8 October 2008, 19 March and 18 September in 2009, and 26 March and 15 September in 2010. The third additional cultivation was applied on 15 May 2009 and 14 May 2010. The control treatment was initiated 18 September 2009. Tines were spaced 5.1 cm by 5.1 cm.

Table 6. Continued

§ Means in a column followed by the same letter within treatment type are not different based on Fisher's protected  $LSD_{0.05}$  test.

¶ Maintained soil moisture regimes: low = irrigation applied every four to five days equal to 40% historic ET, high = daily irrigation applied equivalent to 80% of daily historic ET.



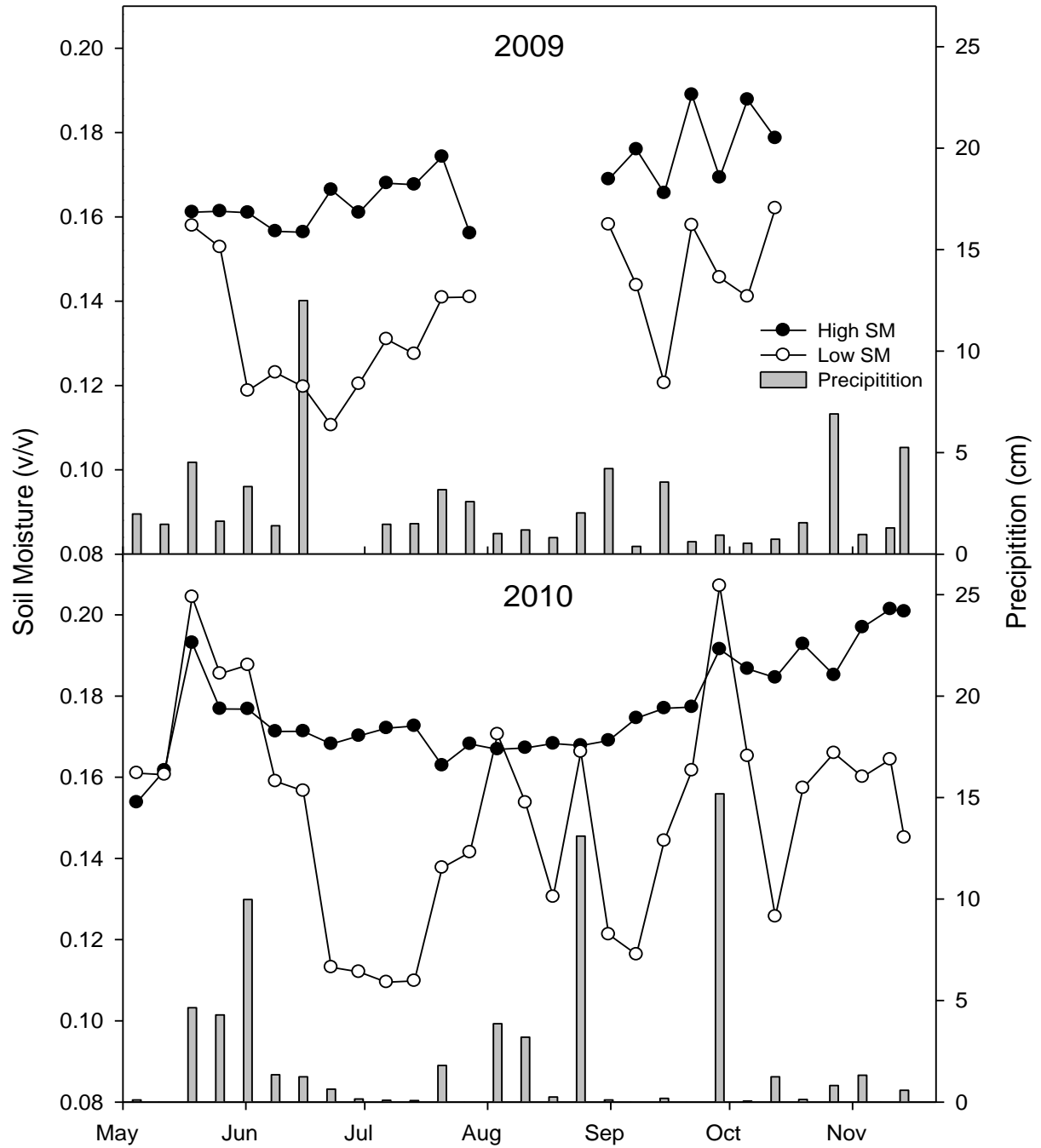


Figure 1. Weekly average precipitation and volumetric soil moisture (%) under high and low soil moisture conditions recorded May to November 2009 and 2010. Soil moisture was measured every 5 minutes at a 5 cm depth by Toro Turf Guard® Wireless Soil Sensors.

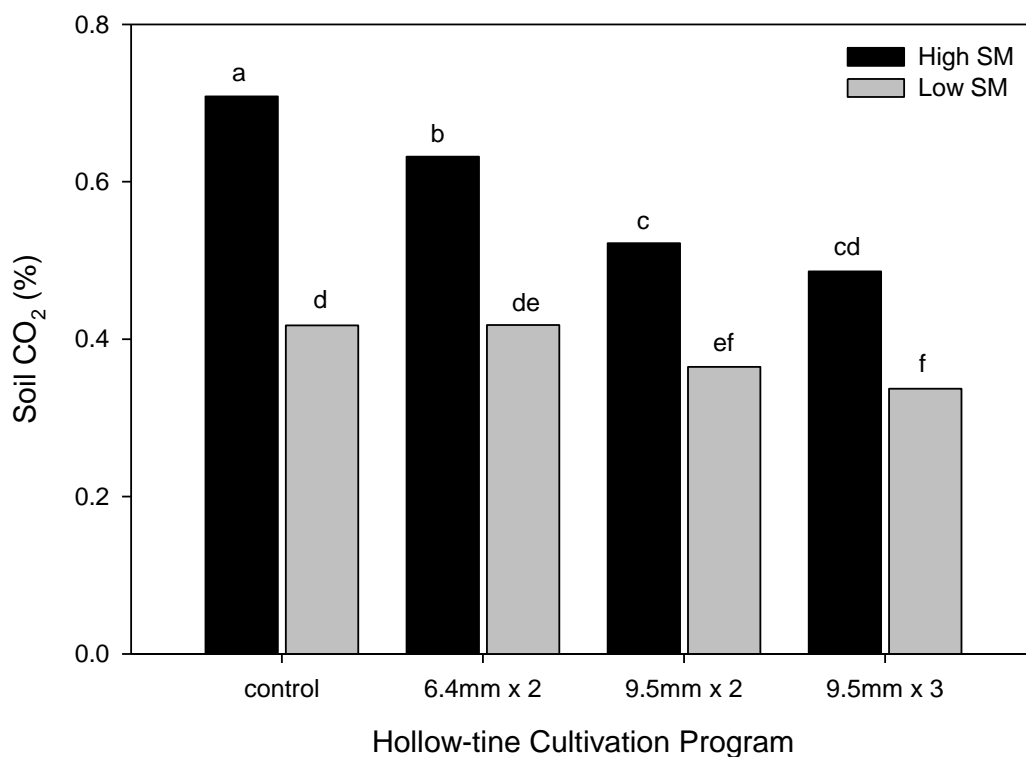
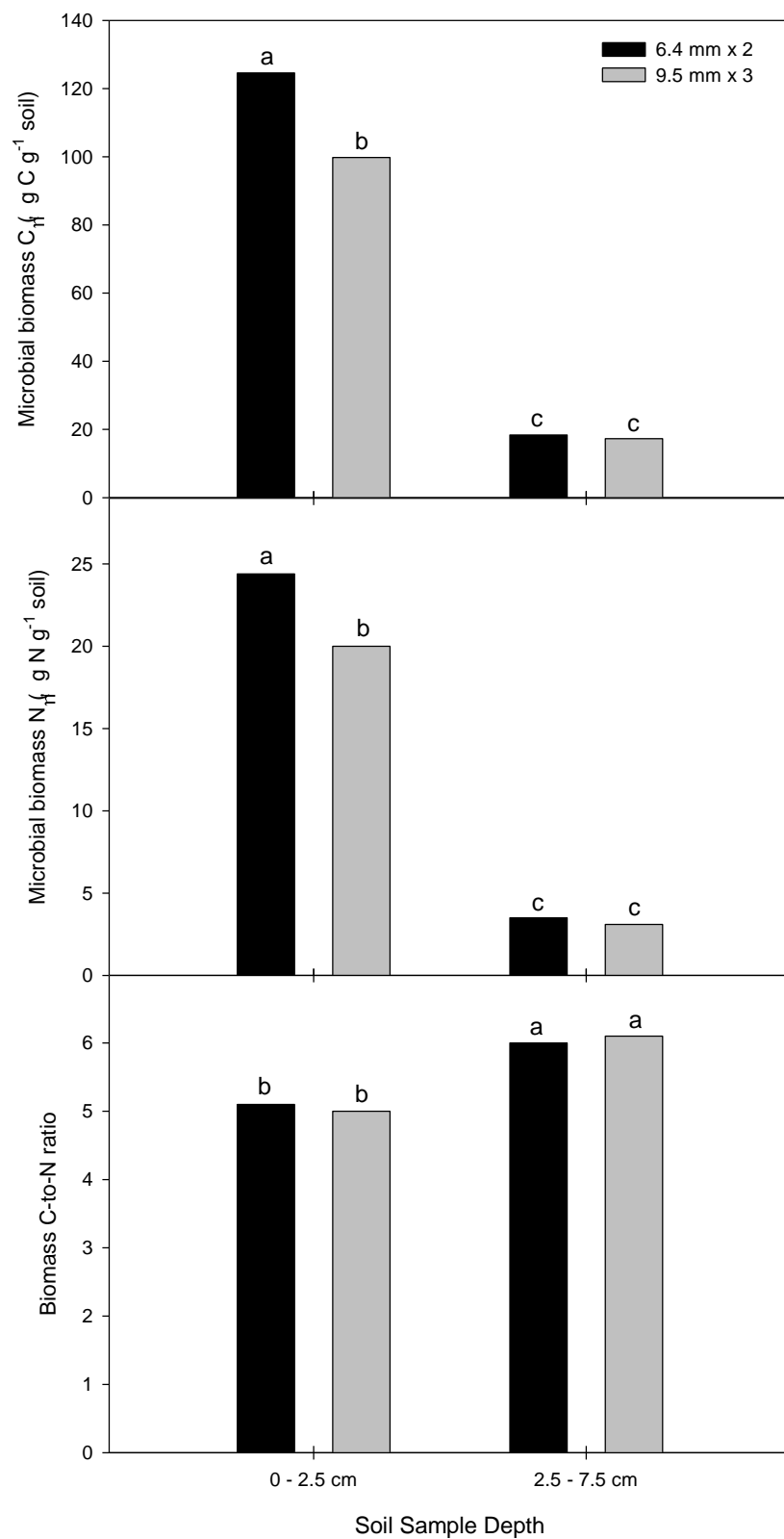


Figure 2. Soil CO<sub>2</sub> as influenced by four hollow-tine cultivation programs (non-cultivated, cultivated 6.4 mm diameter tines twice yearly, and 9.5 mm tines two and three times yearly) and two soil moisture regimes (high and low). Data represents mean of four measurement dates in 2009 and five dates in 2010. Differences in letters indicate statistical differences according to Fisher's LSD<sub>0.05</sub>.

Figure 3. Soil microbial biomass and its C-to-N ratio at 0 – 2.5 and 2.5 – 7.5 cm soil depth influenced by core cultivation two times yearly with 6.4 mm diameter tines and three times yearly with 9.5 mm tines. Data represent mean of two measurement dates in 2009 and three dates in 2010. Differences in letters indicate statistical differences according to Fisher's  $LSD_{0.05}$ .



**APPENDIX**

## Appendix A - SAS Programs for the GLM Procedure

```

title "1. analyze control not included";
title2 "all dates";
proc glm data= a; where year= ; where cult ne "control";
  class date cult fert spike moist quad;
  model y =
    spike
    moist
    spike*moist
    quad(spike*moist)
    fert
    fert*spike
    fert*moist
    fert*spike*moist
    fert*quad(spike*moist)
    cult
    cult*spike
    cult*moist
    cult*spike*moist
    cult*quad(spike*moist)
    cult*fert
    cult*fert*spike
    cult*fert*moist
    cult*fert*spike*moist
    fert*cult*quad(spike*moist)
    date
    date*spike
    date*moist
    date*spike*moist
    date*fert
    date*fert*spike
    date*fert*moist
    date*fert*spike*moist
    date*cult
    date*cult*spike
    date*cult*moist
    date*cult*spike*moist
    date*cult*fert
    date*cult*fert*spike
    date*cult*fert*moist
    date*cult*fert*spike*moist;
    random quad(spike*moist)
           fert*quad(spike*moist)
           cult*quad(spike*moist)
           fert*cult*quad(spike*moist)/test;
  output out=outglm student=StudentResid p=pred;
run;

*****;

title "2. analyze control included";
title2 "all dates";

```

```

proc glm data= a; /*insert actual dates < time of 'control' tmt
initiantion in 'where' statement*/

class date cult fert spike moist quad;
model y =
    spike
    moist
    spike*moist
    quad(spike*moist)
    fert
    fert*spike
    fert*moist
    fert*spike*moist
    fert*quad(spike*moist)
    cult
    cult*spike
    cult*moist
    cult*spike*moist
    cult*quad(spike*moist)
    cult*fert
    cult*fert*spike
    cult*fert*moist
    cult*fert*spike*moist
    fert*cult*quad(spike*moist)
    date
    date*spike
    date*moist
    date*spike*moist
    date*fert
    date*fert*spike
    date*fert*moist
    date*fert*spike*moist
    date*cult
    date*cult*spike
    date*cult*moist
    date*cult*spike*moist
    date*cult*fert
    date*cult*fert*spike
    date*cult*fert*moist
    date*cult*fert*spike*moist;
    random quad(spike*moist)
           fert*quad(spike*moist)
           cult*quad(spike*moist)
           fert*cult*quad(spike*moist)/test;
output out=outglm student=StudentResid p=pred;
run;

*****;

title "3. GLM analyze control included";
title2 "all dates both years";
proc GLM data= a;
class spike moist quad cult fert year date;

```

```

model y =
  spike
  moist
  fert      spike*moist
           fert*spike
           fert*moist
           fert*spike*moist
  cult      cult*spike
           cult*moist
           cult*spike*moist
           cult*fert
           cult*fert*spike
           cult*fert*moist
           cult*fert*spike*moist
  year      year*spike
           year*moist
           year*spike*moist
           year*fert
           year*fert*spike
           year*fert*moist
           year*fert*spike*moist
           year*cult
           year*cult*spike
           year*cult*moist
           year*cult*spike*moist
           year*cult*fert
           year*cult*fert*spike
           year*cult*fert*moist
           year*cult*fert*spike*moist
  date(year)
           date*spike(year)
           date*moist(year)
           date*spike*moist(year)
           date*fert(year)
           date*fert*spike(year)
           date*fert*moist(year)
           date*fert*spike*moist(year)
           date*cult(year)
           date*cult*spike(year)
           date*cult*moist(year)
           date*cult*spike*moist(year)
           date*cult*fert(year)
           date*cult*fert*spike(year)
           date*cult*fert*moist(year)
           date*cult*fert*spike*moist(year)
           quad(spike*moist)
  fert*quad(spike*moist)
  cult*quad(spike*moist)

```



```
    fert*cult*quad(spike*moist)
    year*quad(spike*moist*fert*cult) ;
random    quad(spike*moist)
    fert*quad(spike*moist)
    cult*quad(spike*moist)
    fert*cult*quad(spike*moist)
    year*quad(spike*moist*fert*cult) /test;
run;
```

## Appendix B - SAS Programs for the MIXED Procedure

```

title "1. analyze control not included";
title2 "all dates";
proc mixed data= a; where year=2009 and cult ne "control" ;
  class date cult fert spike moist quad;
  model y = spike
           moist
           spike*moist
           quad(spike*moist)
           fert
           fert*spike
           fert*moist
           fert*spike*moist
           fert*quad(spike*moist)
           cult
           cult*spike
           cult*moist
           cult*spike*moist
           cult*quad(spike*moist)
           cult*fert
           cult*fert*spike
           cult*fert*moist
           cult*fert*spike*moist
           fert*cult*quad(spike*moist)
           date
           date*spike
           date*moist
           date*spike*moist
           date*fert
           date*fert*spike
           date*fert*moist
           date*fert*spike*moist
           date*cult
           date*cult*spike
           date*cult*moist
           date*cult*spike*moist
           date*cult*fert
           date*cult*fert*spike
           date*cult*fert*moist
           date*cult*fert*spike*moist;
           random quad(spike*moist)
                  fert*quad(spike*moist)
                  cult*quad(spike*moist)
                  fert*cult*quad(spike*moist)/test;
  output out=outglm student=StudentResid p=pred;

lsmeans
    spike
    moist
    fert
    cult
    date /diff cl ;

```

```

lsmeans
    spike*moist
    fert*spike
    fert*moist
    fert*spike*moist
    cult*spike
    cult*moist
    cult*spike*moist
    cult*fert
    cult*fert*spike
    cult*fert*moist
    cult*fert*spike*moist
    date*spike
    date*moist
    date*spike*moist
    date*fert
    date*fert*spike
    date*fert*moist
    date*fert*spike*moist
    date*cult
    date*cult*spike
    date*cult*moist
    date*cult*spike*moist
    date*cult*fert
    date*cult*fert*spike
    date*cult*fert*moist
    date*cult*fert*spike*moist
    /slice=(spike moist fert cult date) diff cl;
ods output lsmeans =lsmnds diffs=diffds;
ods exclude diffs;
run;

data ppp;
  set diffds ; where effect= "x*x1" ;
run;
data mmm;
  set lsmnds; where effect="x*x1" ;
run;
%pdmix800(ppp,mmm,alpha=.01,sort=yes, slice= x )
quit;

*****;

title "2. MIXED analyze control included";
title2 "all dates";
proc mixed data= a; /*insert actual dates < time of 'control' tmt
initiantion in 'where' statement*/
  class date cult fert spike moist quad;
  model y =
    spike
    moist
    spike*moist
    quad(spike*moist)

```

```

fert
fert*spike
fert*moist
fert*spike*moist
fert*quad(spike*moist)
cult
cult*spike
cult*moist
cult*spike*moist
cult*quad(spike*moist)
cult*fert
cult*fert*spike
cult*fert*moist
cult*fert*spike*moist
fert*cult*quad(spike*moist)
date
date*spike
date*moist
date*spike*moist
date*fert
date*fert*spike
date*fert*moist
date*fert*spike*moist
date*cult
date*cult*spike
date*cult*moist
date*cult*spike*moist
date*cult*fert
date*cult*fert*spike
date*cult*fert*moist
date*cult*fert*spike*moist;
      random      quad(spike*moist)
              fert*quad(spike*moist)
              cult*quad(spike*moist)
              fert*cult*quad(spike*moist)/test;
      output out=outglm student=StudentResid p=pred;

lsmeans

spike
moist
fert
cult
date /diff cl ;

lsmeans

spike*moist
fert*spike
fert*moist
fert*spike*moist
cult*spike
cult*moist
cult*spike*moist
cult*fert
cult*fert*spike

```

```

cult*fert*moist
cult*fert*spike*moist
date*spike
date*moist
date*spike*moist
date*fert
date*fert*spike
date*fert*moist
date*fert*spike*moist
date*cult
date*cult*spike
date*cult*moist
date*cult*spike*moist
date*cult*fert
date*cult*fert*spike
date*cult*fert*moist
date*cult*fert*spike*moist
      /slice=(spike moist fert cult date) diff cl;

ods output lsmeans =lsmn ds diff s=diffds;
ods exclude diff s;
run;

data ppp;
  set diffds ; where effect= "x*x1" ;
run;
data mmm;
  set lsmn ds; where effect="x*x1" ;
run;
%pdmix800(ppp,mmm,alpha=.05,sort=yes, slice= x )
quit;

*****

title "3. MIXED analyze control included";
title2 "all dates both years";
proc mixed data= a;
  class spike moist quad cult fert year date ;
  model rate =
    spike
    moist
    fert
      spike*moist
      fert*spike
      fert*moist
      fert*spike*moist
    cult
      cult*spike
      cult*moist
      cult*spike*moist
      cult*fert
      cult*fert*spike

```

```

                                cult*fert*spike*moist
year
                                year*spike
                                year*moist
                                year*spike*moist
                                year*fert
                                year*fert*spike
                                year*fert*moist
                                year*fert*spike*moist
                                year*cult
                                year*cult*spike
                                year*cult*moist
                                year*cult*spike*moist
                                year*cult*fert
                                year*cult*fert*spike
                                year*cult*fert*moist
                                year*cult*fert*spike*moist

date (year)
                                date*spike (year)
                                date*moist (year)
                                date*spike*moist (year)
                                date*fert (year)
                                date*fert*spike (year)
                                date*fert*moist (year)
                                date*fert*spike*moist (year)
                                date*cult (year)
                                date*cult*spike (year)
                                date*cult*moist (year)
                                date*cult*spike*moist (year)
                                date*cult*fert (year)
                                date*cult*fert*spike (year)
                                date*cult*fert*moist (year)
                                date*cult*fert*spike*moist (year)
                                /outp=outmx residual ddfm=kr;;

random      quad (spike*moist)
            fert*quad (spike*moist)
            cult*quad (spike*moist)
            fert*cult*quad (spike*moist)
            year*quad (spike*moist*fert*cult) ;

lsmeans
            spike
            moist
            fert
            cult
            year /diff cl ;

lsmeans
            spike*moist
            fert*spike
            fert*moist
            fert*spike*moist

```

```

cult*spike
cult*moist
cult*spike*moist
cult*fert
cult*fert*spike
cult*fert*moist
cult*fert*spike*moist
year*spike
year*moist
year*spike*moist
year*fert
year*fert*spike
year*fert*moist
year*fert*spike*moist
year*cult
year*cult*spike
year*cult*moist
year*cult*spike*moist
year*cult*fert
year*cult*fert*spike
year*cult*fert*moist
year*cult*fert*spike*moist
date(year)

date*spike(year)
date*moist(year)
date*spike*moist(year)
date*fert(year)
date*fert*spike(year)
date*fert*moist(year)
date*fert*spike*moist(year)
date*cult(year)
date*cult*spike(year)
date*cult*moist(year)
date*cult*spike*moist(year)
date*cult*fert(year)
date*cult*fert*spike(year)
date*cult*fert*moist(year)
date*cult*fert*spike*moist(year)
/slice=(spike moist fert cult date) diff cl;
ods output lsmeans =lsmnds diffs=diffds;
ods exclude lsmeans diffs;
run;

```