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REGULAR ARTICLE

Plant growth regulator and nitrogen fertilizer effects on soil organic carbon sequestration in creeping bentgrass fairway turf

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Abstract The objective of this study was to determine the effects of plant growth regulator (PGR) (no PGR, trinexapac-ethyl, and paclobutrazol) and N fertilizer (zero N, an average of 37 kg N ha^{-1} $month^{-1}$, 6 and 12 kg N ha^{-1} $week^{-1}$) on soil organic C (SOC) and soil N in creeping bentgrass (*Agrostis stolonifera* L.) fairway turf. After 4 years of field experiments soil samples were obtained from soil depths of 0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–15, 15–20, and 20–30 cm. Soil bulk density, SOC, total N, NO_3^-–N, and NH_4^+–N concentrations were determined. Paclobutrazol and trinexapac-ethyl application increased SOC. The 37 kg N ha^{-1} $month^{-1}$ application increased SOC at the 0–2.5 cm depth with both PGRs. When paclobutrazol was used, N fertilizer always increased SOC; however, the greatest increase was observed with the 12 kg N ha^{-1} $week^{-1}$ application when compared to other rates, inversely related to the NH_4^+–N concentration. Nitrogen application increased soil total N and NO_3^-–N in the upper three depths. The application of PGRs and N fertilizer to creeping bentgrass fairway turf is an effective strategy for promoting C sequestration.

Keywords Turfgrass · Creeping bentgrass · Plant growth regulator · Soil organic carbon · Carbon sequestration · Nitrogen

Abbreviations

SOC	Soil organic carbon
PGR	Plant growth regulator

Introduction

There are approximately 32,000 golf courses worldwide (Smith 2009). Although the United States represents the largest golf market in the world with around 16,000 courses (National Golf Foundation 2006), Europe has a strong presence, with more than 6,000 golf courses. Considering, roughly, that all of the courses have 18 holes and that the average area is 50 ha, golf courses would encompass a total worldwide area of 16,000 km^2. This would be equivalent to slightly more than half the area of Belgium. Obviously, as an

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ecosystem, this area is of little particular importance on global scale. However, its social relevance is important because of courses proximity to cities and the millions of people who practice this sport, interacting with the ecosystem. It is likely, for this reason, that the environmental focus on golf course management is becoming increasingly relevant. Environmental concern over excessive CO_2 emissions into the atmosphere is yet another issue for course management. Thus, turfgrass areas in general, and golf courses in particular, cannot be disregarded in this current environmental context. Moreover, superintendents should know how the cultural practices affect this issue, yet virtually no information is available to them. When comparing a golf course with an agricultural crop, the main difference is that there is no export of fixed C by the golf course. In spite of this lesser production of biomass, its role in SOC should not be ignored. According to Qian and Follett (2002), SOC sequestration in turf soils occurred at a significant rate, because of the input of organic C from the roots and the turnover of root and litter biomass. Therefore, research data on biomass input and turnover is needed to identify specific management options for increasing C sequestration. Understanding SOC dynamics is a useful criterion in identifying recommended management practices (Bandaranayake et al. 2003).

Plant growth regulators are widely used on golf courses for specific turfgrass species. The use of a PGR is often, determined by the type of turfgrass area and the level of maintenance. Commonly used PGRs include trinexapac-ethyl and paclobutrazol which inhibit gibberellin biosynthesis. These PGRs are applied repeatedly to highly managed turfgrasses to reduce clipping production (i.e. mowing frequency), increase canopy density (uniformity), enhance turfgrass tolerance to environmental factors (e.g. shade, disease, and drought stress), and darken canopy color (Fagerness et al. 2000; Fagerness and Yelverton, 2000; Ervin and Koski 2001; Steinke and Stier 2003; McCarty et al. 2004; Ervin and Zhang 2007). Therefore, mowing frequency reduction by PGR contributes to reduced fuel use and greenhouse gas fluxes (Bandaranayake et al. 2003). No information exists regarding the effects of the use of PGRs on SOC sequestration; however, previous works have demonstrated the existence of an increase in the production of root biomass. According to Koski (1997), autumn and spring applications of paclobutrazol enhance root growth in a creeping bentgrass fairway. Han and Fermanian (1995) observed that trinexapac-ethyl increased both root growth and root carbohydrate levels. McCullough et al. (2006a, b) reported that dwarf-type bermudagrass treated with trinexapac-ethyl had greater root biomass and enhanced root length than untreated bermudagrass. These data and reports of post-inhibition shoot growth enhancement (Fagerness and Yelverton 2000; Lickfeldt et al. 2001) support the hypothesis that total non-structural carbohydrates may either accumulate in root tissue or be used to fuel other developmental events such as increased tillering, stem growth, or rooting (Ervin and Zhang 2007). Therefore, it would seem logical to think that the application of PGRs—as a result of their effect on root biomass—could also affect SOC sequestration.

Nitrogen fertilizer application is a fundamental practice in golf course management because it promotes vigor, visual quality, recovery from damage, and overall health (Carrow et al. 2001; Hull and Liu 2005; Liu et al. 2008). Starting from the fact that N fertilizer increases biomass production in turfgrasses, it is logical to think that it could also have an effect on SOC sequestration. Nonetheless, this subject has recently led to debate as a result of the publication of a paper titled “The Myth of Nitrogen Fertilization for Soil Carbon Sequestration” written by Khan et al. (2007), cited 28 times to date. This paper stated that N fertilizer did not increase SOC sequestration in agricultural crops due to the fact that it promoted the decomposition of crop residues and soil organic matter. However, although this may also occur in turfgrasses, it should not be forgotten that there is no export of C from the system, unlike agricultural systems. Just as with PGRs, no information exists on how N fertilizer affects SOC sequestration.

The absence of information on SOC in golf courses has been reflected in the introduction. Therefore, the objective of this study was to determine the effects of PGR and N fertilizer, two very common practices, on SOC sequestration and soil N levels in creeping bentgrass fairway turf.

Methods

Site and experimental design

A field study was completed at the Ohio State University Turfgrass Research Center (Columbus, OH). Soil samples were obtained from a mature stand of creeping bentgrass (cv. López) established in 1997 and maintained at fairway conditions. Soil type was a Crosby silt loam (mesic Aeric Epiaqualfs). Field research plots for this study were established in spring 2004. The experimental design was a randomized complete block with treatments in a strip-plot arrangement in each of the three blocks, where the horizontal-strip plot factor was PGR application, and the vertical-strip plot factor was N fertilizer rate. Plant growth regulator treatments were: no PGR, trinexapac-ethyl [4-(cyclopropyl-ahydroxy-methylene)-3,5-dioxocyclohexanecarboxylic acid ethyl ester] applied monthly at a rate of 0.1 kg active ingredient (a.i.) ha^{-1}, and paclobutrazol [(2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)pentan-3-ol] applied monthly at a rate of 0.42 kg a.i. ha^{-1}. All N fertilization treatments were applied from late May to September each year. The types and doses of N fertilizer selected were chosen because they are common in fairways in the region. Nitrogen fertilizer rate, timing, and type were: zero N fertilizer application (control); 49 kg N ha^{-1} in late May and September and 24 kg N ha^{-1} in July and August of Turf Rally 16–4–8 (Grigg Bross., ID), denominated in the manuscript as 37 kg N ha^{-1} month^{-1} (average) for simplification purposes, 6 kg N ha^{-1} week^{-1} of Bulldog NPK (28–8–18)(Grigg Bross., ID), and 12 kg N ha^{-1} week^{-1} of Bulldog NPK (28–8–18). Turf Rally is a granular fertilizer consisting of 2% ammoniacal nitrogen, 11% water soluble organic N, and 3% of water insoluble organic N and micronutrients. Bulldog NPK (28–8–18) is water soluble powder consisting of a 4.8% NO_3^-–N and 22.2% urea N with 0.2% chelated iron and other micronutrients. Plot size was 1.82 by 0.91 m.

Turf management

Plots were mowed three times a week (Monday, Wednesday, and Friday), using a Toro 3100 Triplex mower (The Toro Company, Bloomington, MN) with a bench setting of 13 mm (fairway golf course conditions). Irrigation was provided on a regular basis to prevent wilting, while no fungicides, insecticides, or other pesticides were applied. The experiment was core cultivated at the end of each season, i.e. late October-early November. Cores were verticut and returned by dragging.

Soil measurements

Soil samples were obtained on the 20th±2 d of April 2008. Before sampling all verdure and thatch were removed from the soil. Thatch thickness was measured but no significant differences were found between the treatments. Two soil cores (2.22 cm in diameter and 30 cm deep) were taken per plot. Soil samples were divided into 0–2.5, 2.5–5, 5–7.5, 7.5–10, 10–15, 15–20, and 20–30 cm depths. A 5/8″ threaded, 1 1/8″ × 24″ stainless steel soil probe with a plastic tube liner (AMS Inc., ID) was used for this purpose. Plastic tube liners were cut into segments according to the corresponding increments prior to taking the samples. Upon extraction of the samples, the increments of plastic tube liner were separated and cut with nylon fishing thread. This procedure was used to reduce any possible soil core compaction which may slide out under normal conditions. Bulk density was determined by the core method using a soil core for each plot and depth (Grossman and Reinsch 2002). Total soil C and N concentrations were determined by a dry combustion method (Nelson and Sommers 1996) in a Vario Max CN analyzer (Elementar Instrument, Hanau, Germany). Soil was analyzed for inorganic C using a modified pressure-calcimeter method (Sherrod et al. 2002). The SOC concentration was calculated by subtracting inorganic C from total C. The SOC stock (Mg ha^{-1}) was calculated using SOC and the bulk density. Soil extraction with 2M KCl was performed before soil NO_3^-–N and NH_4^+–N content analyses. Soil NO_3^-–N and NH_4^+–N were determined by a colorimetric method: Griess–Illosvay modified by Markus et al. (1985) and Kempers (1974), respectively. A continuous flow colorimeter was used for this purpose (QUAATRO, Bran-Luebbe, Norderstedt, Germany).

Statistical analysis

Treatment effects were evaluated using analysis of variance for each depth within the Statistix v. 8.1 software package (Analytical Software 2005). Means separation was performed using Fisher's protected least significant difference (LSD) test with alpha = 0.05. Means separation was also performed using LSD.

Results

Soil organic carbon

Application of PGR had a significant effect on SOC concentration for almost all depths, as well as the SOC stock of the total profile. Furthermore, N fertilizer and PGR × N fertilizer interaction also had a significant effect on SOC for the 0–2.5 cm depth (Table 1). The SOC concentration was greater with paclobutrazol application in comparison with no PGR for all depths between 0 and 15 cm. There were no differences in SOC concentration below the 15 cm, indicating the root system penetration depth. The application of trinexapac-ethyl had less of an effect than paclobutrazol, but more of an effect than the control (Fig. 1). Application of both PGRs increased total profile SOC stock, which was 64 Mg C ha^{-1} for paclobutrazol, 56 Mg C ha^{-1} for trinexapac-ethyl and 48 Mg C ha^{-1} for no PGR (Fig. 1).

Application of nitrogenous fertilizer increased SOC concentration in comparison with the control, but only for the 0–2.5 cm depth: SOC concentration was 65.4 g C kg^{-1} for 37 kg N ha^{-1} $month^{-1}$, 63.0 g C kg^{-1} for 6 kg N ha^{-1} $week^{-1}$, 62.9 g C kg^{-1} for 12 kg N ha^{-1} $week^{-1}$ and 56.3 g C kg^{-1} for the control. Using a bulk density value, the maximum C contribution of N fertilizer to the total profile SOC stock, in comparison with the control, was 1.6 Mg C ha^{-1}, which does not represent an important quantity of soil C sequestration with respect to total profile stock (Fig. 1). Plant growth regulator × N fertilizer interaction showed that N fertilizer application increased SOC at the 0–2.5 cm depth when

Table 1 Significant effects of plant growth regulator (PGR) and N fertilizer (N) at 0–30 cm depth on soil organic carbon, total soil N, C:N ratio, soil NO_3^-–N, and soil NH_4^+–N after 4 years of treatment at Columbus, OH

Parameter and source	Depth (cm)							Stock (0–30 cm) (Mg ha^{-1})
	0–2.5	2.5–5	5–7.5	7.5–10	10–15	15–20	20–30	
Soil organic carbon (g kg^{-1})								
PGR	*	*	*	*	*	ns	ns	**
N	*	ns	ns	ns	ns	ns	ns	ns
PGR × N	*	ns	ns	ns	ns	ns	ns	ns
Total soil N (g kg^{-1})								
PGR	ns	ns	ns	ns	ns	ns	ns	ns
N	*	**	*	ns	ns	ns	ns	*
PGR × N	ns	ns	ns	ns	ns	ns	ns	ns
C:N ratio								
PGR	ns	ns	*	*	ns	ns	ns	–
N	ns	*	ns	ns	ns	ns	ns	–
PGR × N	ns	ns	ns	ns	ns	ns	ns	–
Soil NO_3^-–N (mg kg^{-1})								
PGR	ns	ns	ns	ns	ns	ns	ns	ns
N	**	*	*	ns	ns	ns	ns	ns
PGR × N	ns	ns	ns	ns	ns	ns	ns	ns
Soil NH_4^+–N (mg kg^{-1})								
PGR	ns	ns	ns	ns	ns	ns	ns	ns
N	*	ns	ns	ns	ns	ns	ns	ns
PGR × N	*	ns	ns	ns	ns	ns	ns	ns

* Significant at the 0.05 probability level

** Significant at the 0.01 probability level

*** Significant at the 0.001 probability level

ns not significant

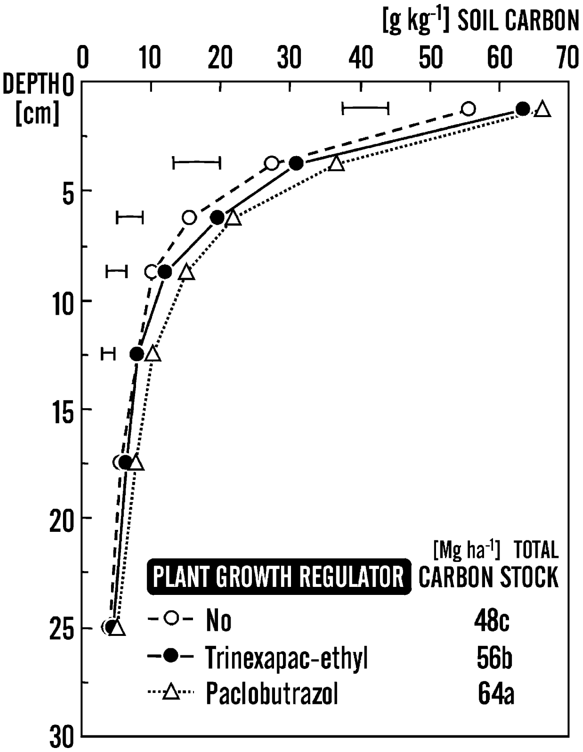

Fig. 1 Soil organic carbon by depth and total profile C stock affected by plant growth regulator after 4 years of treatment in creeping bentgrass fairway turf. *Horizontal bar* within a soil depth represents LSD at $p<0.05$. For C stock, different *letters* indicate significant differences at $p<0.05$ according to LSD

compared with the control for all PGR treatments (Fig. 2). However, only SOC concentrations for some N fertilizer treatments were different from the controls with each PGR: SOC concentration for 37 kg N ha^{-1} month^{-1} was greater than no PGR and trinexapac-ethyl; with paclobutrazol application, all N fertilizer rates had a greater SOC concentration than the control, but the highest concentration was obtained for 12 kg N ha^{-1} week^{-1}. At 6 kg N ha^{-1} week^{-1}, trinexapac-ethyl increased SOC compared with the control. For 12 kg N ha^{-1} week^{-1}, PGR application increased SOC concentration, which was the highest concentration recorded with paclobutrazol application (Fig. 2).

Soil nitrogen

The rate of N fertilizer had a significant effect on total soil N for the top three depths (Table 1). For the 0–2.5 and 5–7.5 cm depths, total soil N concentration was greater when N fertilizer was applied, with no significant differences among rates of N application (Fig. 3). For the 2.5–5 cm depth, total soil N concentration was lower with the zero N rate and there was a significant difference between 37 kg N ha^{-1} month^{-1} and 12 kg N ha^{-1} week^{-1} applications (Fig. 3). Total N stock in the profile increased with the application of N fertilizer (Fig. 3).

The effect of PGR application was significant on the C:N ratio for the 5–7.5 and 7.5–10 cm depths (Table 1). The C:N ratios for the 5–7.5 cm depth were: 19.2 for paclobutrazol, 16.4 for trinexapac-ethyl and 12.7 for no PGR, with significantly lower values for no PGR application. For the 7.5–10 cm depth C:N ratios were: 18.5 for paclobutrazol, 15.4 for trinexapac-ethyl and 12.3 for the control, with significant differences between paclobutrazol and the control. Nitrogen fertilizer had a significant effect on

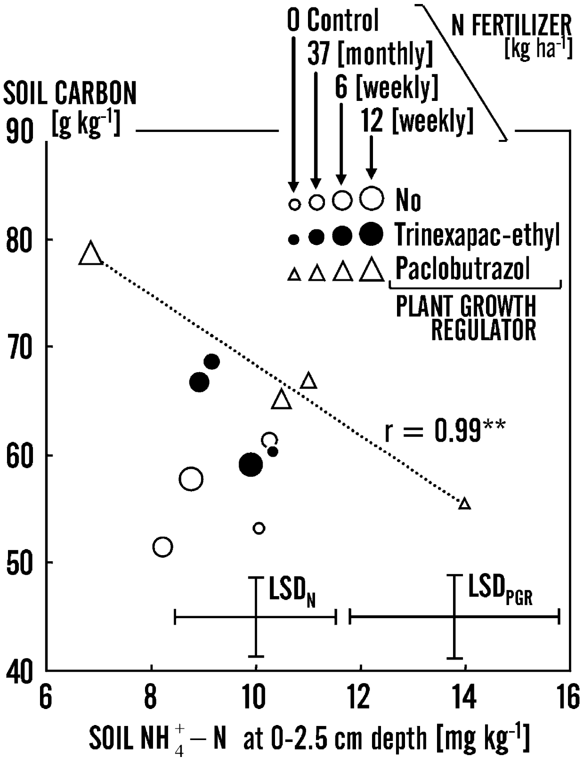

Fig. 2 Soil organic carbon and soil NH_4^+–N at 0–2.5 cm depth affected by plant growth regulator × N fertilizer interaction after 4 years of treatment in creeping bentgrass fairway turf. *Cross* represents LSD ($P<0.05$) for comparisons between treatments within vertical and horizontal measures: LSD_N compares N fertilizer rates for the same level of plant growth regulator; LSD_{PGR} compares plant growth regulators for the same or different level of N fertilizer rate. ** significant at the 0.01 probability level

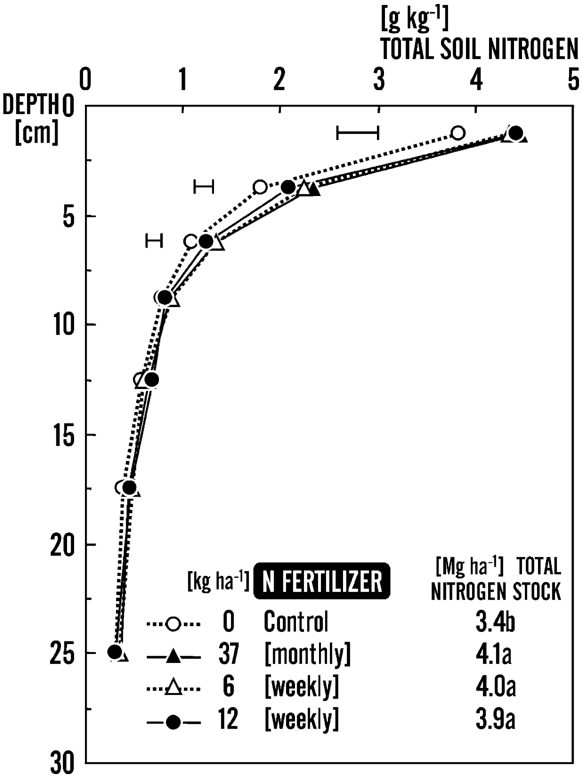

Fig. 3 Total soil N by depth and total profile N stock affected by N fertilizer after 4 years of treatment in creeping bentgrass fairway turf. *Horizontal bar* within a soil depth represents LSD at $p<0.05$. For N stock, different letters indicate significant differences at $p<0.05$ according to LSD

the C:N ratio for the 2.5–5 cm depth (Table 1). The C:N ratios were: 17.0 for the control, 16.1 for 12 kg N ha^{-1} week^{-1}, 14.7 for 6 kg N ha^{-1} week^{-1}, and 14.1 for 37 kg N ha^{-1} month^{-1}.

The rate of N fertilizer had a significant effect on soil NO_3^-–N concentration for the top three depths (Table 1). The highest NO_3^-–N concentration was observed for the rate of 12 kg N ha^{-1} week^{-1} for the 0–2.5 and 5–7.5 cm depths. For the 0–2.5 cm depth, the NO_3^-–N concentration was higher with 12 kg N ha^{-1} week^{-1} than with all other treatments; no difference was observed between the other two N fertilizer rates. For the 2.5–5 cm depth, the NO_3^-–N concentration differed only in comparison with the control, and NO_3^-–N concentration. At the 2.5–5 cm depth, only the NO_3^-–N concentration for zero N fertilizer was lower than the concentration for other rates (Fig. 4).

The PGR × N fertilizer interaction had a significant effect on soil NH_4^+–N concentration at the 0–2.5 cm depth (Table 1). Application of N fertilizer decreased soil NH_4^+–N concentration only when paclobutrazol was applied (Fig. 2). For 0 kg N ha^{-1}, application of paclobutrazol increased soil NH_4^+–N in comparison with no PGR and trinexapac-ethyl. Application of N fertilizer had no effect on N soil concentration (total and inorganic) below a depth of 7.5 cm, indicating that losses of NO_3^-–N are not significant beneath this depth (Table 1).

Discussion

There are no published data on the effects of PGR on SOC stock. Therefore, our results must be discussed with previous studies based on the effects of PGR on root biomass. However, these studies were conducted under putting green conditions, and where the management stress is completely different. Therefore, such a comparison may not be valid. Similar to the data of present study, Koski (1997) reported that

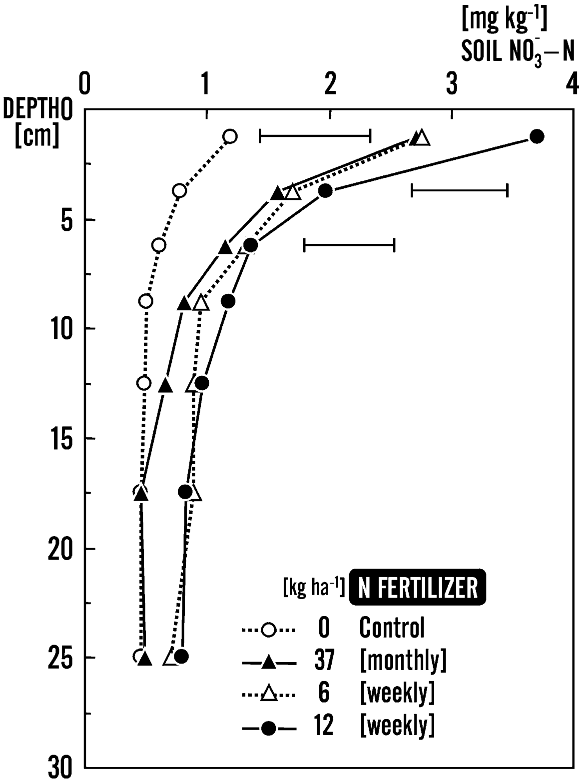

Fig. 4 Soil NO_3^-–N by depth affected by N fertilizer after 4 years of treatment in creeping bentgrass fairway turf. *Horizontal bar* within a soil depth represents LSD at $p<0.05$

application of paclobutrazol enhanced root growth in a creeping bentgrass fairway. On the other hand, Han and Fermanian (1995) reported that application of trinexapac-ethyl increased root growth. Several other studies conducted with dwarf-type bermudagrass have shown that trinexapac-ethyl application increased root biomass (McCullough et al. 2004, 2005, 2006a, b). The hypothesis that total non-structural carbohydrates may accumulate during the period of trinexapac-ethyl inhibition or may be used to develop a greater root system seems to be quite logical (Fagerness and Yelverton 2000; Lickfeldt et al. 2001; Ervin and Zhang 2007). Moreover, these results have been obtained with other species, and this hypothesis is valid for the results of Koski (1997) and Han and Fermanian (1995).

The findings in Fig. 2 were quite striking. It is not easy to explain why a joint application of a high and frequent N dose (12 kg N ha^{-1} week^{-1}) and paclobutrazol increased SOC. There is no information in the literature regarding this interaction. However, some studies have pointed out the peculiarity of paclobutrazol in the soil in relation to the organic matter. Milfont et al. (2008) reported that its fate and toxicity is still unclear and poorly documented. According to these authors, paclobutrazol absorption is predominantly controlled by the organic matter, probably through hydrophobic bonding. Indeed, although slightly polar, this molecule is mainly composed of apolar groups, most likely responsible for its preferential affinity for soil organic matter. Their study thus confirmed that this PGR is likely to persist in soils in an adsorbed form, protected by organic matter. Research by Goncalves et al. (2009) is related to this study, although these authors went into greater depth regarding the effect of paclobutrazol on microbial respiration of the base soil than its effect on SOC dynamics. Obviously, changes in the structure of microbial communities may lead to changes in important functions such as organic matter decomposition. Goncalves et al. (2009) confirmed a negative effect of paclobutrazol on short-term soil respiration (30 days). Nevertheless, they emphasized in their conclusions that further field studies were required to examine and monitor the long-term effect of paclobutrazol application on soils. The role of the highest N dose is not clear in this interaction. However, it is obvious that the SOC concentration is stimulated as a consequence of both factors, while with other PGRs, excess N reduces SOC concentration through a change in mineralization dynamics. One factor which could be affecting SOC at that depth, with paclobutrazol application and the highest N dose, is the amount of soil NH_4^+–N, since this was the lowest (Fig. 2). In fact, this decrease in NH_4^+–N with the N dose when paclobutrazol was applied was inversely related to the SOC concentration (Fig. 2). These results must be studied further in greater depth to fully understand this occurrence.

Huh et al. (2008) reported that SOC sequestration in putting green was more propounded at the 10–25 cm depth than at the 0–10 cm depth. In the top 0–10 cm layer, little C sequestration occurred probably because of the intensive soil management disturbance at this depth. Huh and colleagues concluded that the SOC sequestration was mainly caused by the increasing humification of C in the undisturbed part of the soil (10–25 cm depth), as was also indicated by a significant decrease in the relative microbial activity. However, the results of the present study under fairway conditions show the opposite trend: 35 Mg C ha^{-1} were sequestered on average for the 0–10 cm depth compared with 21 Mg C ha^{-1} for the 10–30 cm depth (Fig. 1).

We did not find differences between the N fertilizers used, but the increase in SOC at the 0–2.5 cm depth was greater than that for the zero N rate, which could be due to the incorporation of organic matter from the thatch layer. These results are in contrast to the results provided by Khan et al. (2007) in herbaceous agricultural systems, who stated that N application did not increase SOC. Fagerness et al. (2004) reported that bermudagrass allocated approximately 50% more N to roots when treated with trinexapac-ethyl than with the control. However, we have found no effect of PGRs on soil N. Our results for the C:N ratio agreed with the results from Bandaranayake et al (2003), who also reported that regular use of N fertilizer reduced the C:N ratio. The change in SOC quality (i.e. C:N ratio) did not alter the interaction of microbial N transformations, possibly because its impact was offset by the change in microbial resource use efficiency (Shi et al. 2006).

Conclusions

Application of paclobutrazol and trinexapac-ethyl PGRs increased SOC sequestration in creeping bentgrass fairway turf under Ohio conditions, mainly at the 0–15 cm depth. Application of paclobutrazol increased SOC more than of trinexapac-ethyl application. The use of N fertilizer had a significant effect on SOC sequestration only at the 0–2.5 cm depth. Application of N fertilizer increased soil N (total and inorganic) for the 0–7.5 cm depth, indicating minimal loss of NO_3^-–N. The application of PGRs (paclobutrazol and trinexapac-ethyl) to creeping bentgrass fairway turf is an effective strategy to enhance C sequestration. Additional research is needed to understand the interaction when paclobutrazol is applied and high N fertilizer application decreases soil NH_4^+–N, increasing SOC sequestration for the 0–2.5 cm depth.

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