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# Root Zone Organic Matter Effects on Creeping Bentgrass Growth in Controlled Environments

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*Abstract.* **Turfgrass growth resulting from soil organic matter (SOM) mineralization in putting green root zones has been explored sparingly. Glasshouse and laboratory studies were conducted in Knoxville, TN, USA, to discern if SOM content would result in differential mineralization affecting creeping bentgrass (CBG) (*Agrostis stolonifera* L.) growth. Cores (10.8-cm diameter by 10-cm depth) were collected from CBG putting greens varying in SOM content: low (6.4 to 12.6 g·kg$^{-1}$), medium (12.7 to 19.4 g·kg$^{-1}$), and high (19.5 to 25.6 g·kg$^{-1}$). One putting green from each SOM group was selected for further investigation during Aug to Oct 2023. After 8 weeks in the glasshouse, SOM decreased 5 g·kg$^{-1}$ within cores originating from putting greens in the high and medium SOM groups, compared to only 1 g·kg$^{-1}$ for those in the low SOM group. Inorganic N within cores from putting greens in the high and medium SOM groups increased more than those from the low SOM group, suggesting a greater percentage of inorganic N was allocated for CBG growth in the glasshouse. When incubated at 25 °C under dark conditions in the laboratory, inorganic N increased 36 mg·kg$^{-1}$ in cores from the high SOM group, indicating the potential for as much as 50 kg·ha$^{-1}$ of N to become available within root zones of CBG putting greens. If unaccounted for when making synthetic fertilizer applications, available N from SOM in CBG putting greens could lead to excessive growth under field conditions requiring additional maintenance practices. Future research to confirm this response under field conditions is needed to develop sustainable fertilizer programs supplying N to CBG putting greens.**

Putting greens are the most intensively managed area of a golf course (Shaddox et al. 2022). Putting greens are often constructed on root zones of sand that conforms to particle size distribution specifications outlined by the United States Golf Association (USGA 2018) and surfaced with CBG (*Agrostis stolonifera* L.) or ultradwarf bermudagrass (UDBG) [*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Davy] in the transition zone (Brosnan et al. 2022). Organic matter accumulates within root zones of both CBG and UDBG putting greens after establishment, particularly within the uppermost (0 to 2 cm) layer of the profile (Kahiu et al. 2024). Although there have been several reports of SOM accumulation in putting greens (Linde et al. 2022; Schmid et al. 2014), recent efforts have been made to analyze total organic material (TOM) within the profile (Gaussoin et al. 2024; Kahiu et al. 2024). Assessments of TOM quantify all the organic material in an undisturbed core including thatch, mat, and verdure near the surface that affects playability and is targeted via regular maintenance practices (Glasgow et al. 2005; Kauffman et al. 2011). In Tennessee, CBG and UDBG putting greens of varying age contained 58 and 101 g·kg$^{-1}$ TOM in the uppermost 2-cm of the root zone, respectively (Kahiu et al. 2024). These values aligned with national averages for CBG and UDBG putting greens across the United States (USGA Green Section, personal communication).

Organic matter in the root zone provides a source of inorganic nitrogen (N) made available via mineralization. Nitrogen mineralization is a biological process that converts organic N to ammonium ($NH_4^+$) and nitrate ($NO_3^-$) (Powlson 1993; Schimel and Bennett 2004). Organic matter mineralization increases as biomass production, soil temperature, soil moisture content, and quantities of organic N increase (Anton et al. 1994; Brown and Huggins 2012; Gilmour 1998; Janzen and Kucey 1988; Lee et al. 2001). In laboratory incubation studies, the initial amount of organic matter in a soil influenced $NH_4$-N and $NO_3$-N concentrations following mineralization (Janzen and Kucey 1988). At the end of a 33-d laboratory incubation experiment across temperatures of 5 to 35 °C, a calcareous Chernozem soil initially containing 3.6% organic matter mineralized more $NH_4$-N and $NO_3$-N compared with a brown forest soil containing 1.5% SOM (Anton et al. 1994). When evaluating seasonal soil microbial activity, net mineralization trended lower in warm-season turfgrass despite these soils containing higher microbial biomass (Yao et al. 2011), a response that could be attributed to N fertilizer applications used in turfgrass management (Higby and Bell 1999). Availability of N in the soil can have a higher influence on soil microbial biomass and activity than seasonal climatic variations (Yao et al. 2011).

Turfgrass growth from use of N derived via organic matter mineralization has been explored sparingly. High foliar N concentrations were reported in nonfertilized Kentucky bluegrass (*Poa pratensis* L.) lawn plots and attributed to organic matter mineralization (Grégoire et al. 2022). In an experiment on a perennial ryegrass (*Lolium perenne* L.) fairway, soils from nonfertilized plots had higher amounts of inorganic N in April and May compared with plots fertilized with granular urea (Miltner et al. 2001). There is limited literature quantifying turfgrass growth as a result of organic matter mineralization in putting green root zones. Considering that putting greens currently receive 117 to 268 kg·ha$^{-1}$ N per year across the United States (Shaddox et al. 2023), improved understanding of organic matter mineralization on growth could allow golf course superintendents to avoid applications of N fertilizer that may be unnecessary and have potential negative environmental effects (Bock and Easton 2020). We hypothesize that differences in root zone SOM content will result in differential mineralization affecting CBG growth. This paper presents results of glasshouse and laboratory experiments designed to explore this hypothesis in detail.

## Material and Methods

*Sample collection.* A survey conducted in Feb 2023 delineated differences in root zone organic matter content among sand-based putting greens in Tennessee (Kahiu et al. 2024). In that survey, soil organic matter content (SOM throughout a 10-cm depth) ranged from 6.4 to 25.6 g·kg$^{-1}$. Standard deviations of the overall soil organic matter content mean (17 g·kg$^{-1}$) were used to separate putting greens into three soil organic matter content groups: low (6.4 to 12.6 g·kg$^{-1}$), medium (12.7 to 19.4 g·kg$^{-1}$), and high (19.5 to 25.6 g·kg$^{-1}$). One putting green from each

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group was selected for inclusion in glasshouse and laboratory research conducted at the University of Tennessee (Knoxville, TN, USA) during Aug 2023. Composite samples (10-cm depth) were collected and analyzed for SOM content (via loss on ignition testing at 360 °C) before initiating experiments to confirm each putting green selected was representative of its SOM grouping. Putting greens selected to represent low, medium, and high SOM groups contained 10, 16, and 20 $g \cdot kg^{-1}$, respectively. The soil pH ranged from 6.7 to 6.9.

After confirmation, cores for experimentation were selected from one putting green within previously described low, medium, and high SOM groups. A total of seven cores (10.8-cm diameter × 10-cm depth) were collected from each of three putting greens on 22 Aug 2023. All putting greens were surfaced with CBG maintained at 3.2-mm height of cut. Six of these cores were used for glasshouse experiments with the remaining core used for our laboratory studies.

*Glasshouse experiment.* Experimentation was conducted in a glasshouse research complex at the University of Tennessee, Knoxville, TN, USA (35.944°N, −83.936°W). Cores were established in greenhouse pots (10.7 cm × 10.7 cm × 12.5 cm) containing sand that conformed to USGA specifications for putting green construction (USGA 2018). CBG atop all cores was maintained at a 3.2-mm height of cut using scissors (Maker Industries, Australia, https://www.makerindustries.com.au/). Cores were allowed to acclimate to the glasshouse environment for 6 d before experiments began on 28 Aug 2023. On the same day, a preventive fungicide application of chlorothalonil (Daconil Weatherstik®; Syngenta Crop Protection, Greensboro, NC, USA) was delivered at 261 $g \cdot ha^{-1}$. In addition, chlorothalonil (261 $g \cdot ha^{-1}$) was applied in a mixture with mancozeb (Dithane Rainshield 75DF®; Corteva Agriscience, Indianapolis, IN, USA) at 13,700 $g \cdot ha^{-1}$ on 15 Sep and 2 Oct 2023. Fungicides were applied in an enclosed spray chamber (Generation III Track Sprayer; DeVries Manufacturing, Hollandale, MN, USA) using a water carrier volume of 281 $L \cdot ha^{-1}$ and delivered from 8004 EVS nozzles (TeeJet®, Wheaton, IL, USA).

Two experimental runs were conducted in separate glasshouse bays with similar environmental conditions. Average air temperatures within these glasshouse bays were 24.1 °C and 24.8 °C. A mist irrigation system in both bays irrigated cores with 5 mm of water per day. Glasshouse bays received both natural and supplemental light (PKB, Arize Element L1000 Next-Gen; Current Lighting Solutions, LLC, Cleveland, OH, USA). Light conditions in the glasshouses were recorded using quantum sensors (SQ-500; Apogee Instruments, Inc., Logan, UT, USA) and averaged between 42 and 43 $mol \cdot m^{-2}$ per day. Treatments (i.e., core type – low, medium, or high SOM) in each experiment were arranged in a completely randomized design with three replications. Cores were re-randomized every week using a random number generator function in Microsoft Excel (version 1997–2004; Microsoft Corporation, Redmond, WA, USA).

Data collection occurred from 28 Aug through 24 Oct 2023. During this timeframe, clipping yield was quantified every 7 d using scissors. Fresh clippings were then stored in yellow envelope bags (ULINE, Pleasant Prairie, WI, USA) and placed in a −20 °C freezer (American Biotech Supply, Salem, NH, USA) for the duration of the study. At the end of the 8-week data collection period, clippings harvested from each core were combined, dried at 60 °C overnight, weighed, and analyzed for mineral nutrient content in a commercial laboratory (Brookside Laboratories, New Bremen, OH, USA). Harvest dates were combined to provide enough tissue for mineral nutrient analysis via nitric acid and hydrogen peroxide digestion (Miller et al. 2013). Inorganic N was extracted from clippings using acetic acid (2%) as described by Miller et al. (2013).

Destructive harvesting was conducted at the end of the study to analyze SOM and nutrient content within the root zone of each core. Both analyses were conducted in a commercial laboratory (Brookside Laboratories). SOM content was determined (by weight) via loss on ignition testing at 360 °C (Schulte and Hopkins 1996). Root zone nutrients were extracted using the Mehlich-3 extraction procedure (Mehlich 1984), with soil inorganic N ($NO_3^-$ and $NH_4^+$) quantified using the potassium chloride (KCl) cadmium reduction method, similar to Dahnke and Johnson (1990).

Data were expressed as the overall change in SOM and mineral nutrient content over the 8-week data collection period before being subjected to analysis of variance (ANOVA) using PROC MIXED in SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). No significant treatment-by-experimental run interactions were detected; therefore, data from both runs were pooled for analysis. Treatment means for significant effects were separated using Fisher's protected least significant difference test at $\alpha = 0.05$.

*Laboratory mineralization.* A mineralization study was conducted at the University of Tennessee (Knoxville, TN, USA) during Summer 2023. Cores from the same sites outlined in the glasshouse study were used in this experiment. Sample preparation involved removing verdure, thatch, mat, and other living plant material before passing mineral material through a 2.0-mm sieve (Sieve number 10; Dual Manufacturing Co., Chicago, IL, USA). Contents that remained on the sieve were discarded and those passing through the sieve were dried for 2 h in a forced air oven (The Grieve Corporation, Round Lake, IL, USA) at 105 °C. Sieved material was divided into 20-g aliquots for experimentation. Each core produced 25 aliquots: 24 of these were used for experimentation and one was used to quantify initial inorganic N content upon study initiation via methods of Dahnke and Johnson (1990). The remaining 24 aliquots were added to scintillation vials (Thermo Fisher Scientific, Waltham, MA, USA) and adjusted to a gravimetric moisture content of 25%. Scintillation vials were then placed in 1-L Mason jars (Ball Mason jars, Westminster, CO, USA) similar to Yao et al. (2011).

Treatments (i.e., core type – low, medium, or high SOM) were arranged in a completely randomized design with three replications. Concurrent experiments were conducted in separate incubators: a Conviron G1000 (Controlled Environments INC, Pembina, ND, USA) and a 3956 Forma Scientific (Thermo Fisher Scientific). Both incubators were configured to provide a constant temperature of 25 °C under complete darkness. Mason jars were capped and placed in incubators to begin the experiment. Every 14 d, one vial was extracted from the Mason jar and stored in a −20 °C freezer (American Biotech Supply, Salem, NH, USA); this process was repeated four times during this experiment. During extraction, Mason jars were re-randomized and left open to allow for a 30-min aeration (Calderón et al. 2005; Yao et al. 2011). Mineralization from each sample was determined for 8 weeks via assessments of inorganic N content using the potassium chloride (KCl) cadmium reduction method, similar to Dahnke and Johnson (1990).

Change in inorganic N was determined by subtracting final inorganic N content values from initial assessments. These change values were subjected to ANOVA using PROC MIXED in SAS. No significant treatment-by-experimental run interactions were detected; therefore, data from both runs were pooled for analysis. Treatment means for significant effects were separated using Fisher's protected least significant difference test at $\alpha = 0.05$. Means were also plotted over time using GraphPad Prism (v.10.0.2, La Jolla, CA, USA).

## Results and Discussion

*Glasshouse experiment.* Significant changes in SOM content were detected due to core type (Table 1). Over the 8-week assessment period, SOM decreased 5 $g \cdot kg^{-1}$ within cores originating from putting greens in the high and medium soil organic matter groups. Comparatively, SOM only decreased 1 $g \cdot kg^{-1}$ within cores harvested from the low soil organic matter group. Similarly, inorganic N within cores originating from putting greens in the high (25 $mg \cdot kg^{-1}$) and medium (29 $mg \cdot kg^{-1}$) soil organic matter groups increased to a greater degree than those from putting greens in the low (19 $mg \cdot kg^{-1}$) organic matter group. This response aligns with reports of Janzen and Kucey (1988) who observed that initial root zone organic matter content influenced inorganic N content after a 12-week incubation when samples (50% surface soil and 50% silica sand) were treated with supplemental N via residues of wheat (*Triticum aestivum* L.), lentil (*Lens culinaris* Medik), and oil seed rape (*Brassoca napus* L.). Considering that no supplemental N was applied during the 8-week assessment period in the current study, reduced values likely indicate a greater percentage of inorganic N within cores harvested from the low soil organic matter group (<12.6 $g \cdot kg^{-1}$) was allocated for CBG growth.

Table 1. Changes in soil nutrient and organic matter content during an 8-week glasshouse study conducted at the University of Tennessee (Knoxville, TN, USA; 35.944°N, −83.936°W) during Aug to Oct 2023.

| Glasshouse study[i] | Changes in soil during 8-week study | | Turfgrass clipping characteristics after 8-week study | |
|---|---|---|---|---|
| Core type[ii] | SOM $g \cdot kg^{-1}$ | Soil inorganic nitrogen $mg \cdot kg^{-1}$ | Clipping dry weight $g \cdot m^{-2}$ per d | Clipping nitrogen content % |
| High SOM | −5 a[iii] | +25 b | 3.77 a | 1.98 a |
| Medium SOM | −5 a | +29 a | 3.64 a | 1.96 a |
| Low SOM | −1 b | +19 c | 3.26 a | 1.61 a |

[i] The experiment was conducted from 28 Aug to 24 Oct 2023. Temperature in the glasshouses ranged between 24.1 ° and 24.8 °C, whereas average light was 42 and 43 $mol \cdot m^{-2}$ per day during the trial period.
[ii] Measurements made on cores selected from creeping bentgrass (*Agrostis stolonifera* L.) putting green root zones falling within three distinct soil organic matter groups: low soil organic matter (SOM; 6.4 to 12.6 $g \cdot kg^{-1}$), medium SOM (12.7 to 19.4 $g \cdot kg^{-1}$), and high SOM (19.5 to 25.6 $g \cdot kg^{-1}$).
[iii] Means in a column followed by the same letter are not significantly different according to Fisher's protected least significant difference test at $\alpha = 0.05$.
SOM = soil organic matter.

Although not statistically significant, there was a trend for clipping yields on cores originating from putting greens within the high and medium SOM groups to be greater than those from the low SOM group (Table 1). A similar trend was observed among cores in foliar N content at the end of the study as well. Overall, these trends align with previous reports of unfertilized bermudagrass (*C. dactylon* Rich) fairway plots having increased clipping yields due to SOM mineralization (Lee et al. 2003). Given that supplemental N was not applied, the trend of increasing clipping yield with greater SOM in the current study indicates that root zone organic matter content contributes to foliar growth of CBG. If not addressed when developing a fertilizer program for CBG putting greens, N inputs could lead to excessive growth that compromises performance and requires additional maintenance (e.g., aeration, topdressing) to be remedied. Increased turfgrass growth can negatively influence ball roll distance if mowing practices are not adjusted (Salaiz et al. 1995). To reduce clipping production, turfgrass managers may need to apply plant growth regulators more intensively (Pornaro et al. 2017). Accumulation of SOM and TOM in putting greens requires cultivation practices such as grooming, hollow tine aeration, and topdressing to be managed optimally (Atkinson et al. 2012; Espevig et al. 2012; Rowland et al. 2009). Lastly, turfgrass equipment used when conducting these additional maintenance practices exposes these surfaces to increased vehicular traffic (Samaranayake et al. 2008).

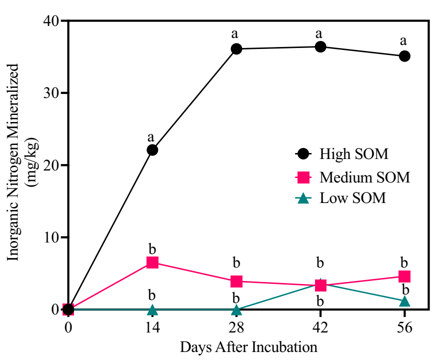


Fig. 1. Inorganic nitrogen mineralized ($mg \cdot kg^{-1}$) during an 8-week incubation conducted under dark conditions at 25 °C at the University of Tennessee (Knoxville, TN, USA) from 23 Aug to 17 Oct 2023. Measurements made on cores selected from creeping bentgrass (*Agrostis stolonifera* L.) putting green root zones from three distinct soil organic matter groups: low soil organic matter (SOM; 6.4 to 12.6 $g \cdot kg^{-1}$), medium SOM (12.7 to 19.4 $g \cdot kg^{-1}$), and high SOM (19.5 to 25.6 $g \cdot kg^{-1}$). Plotted means indicated by the same letter at each day after incubation are not significantly different according to Fisher's protected least significant difference test at $\alpha = 0.05$.

An analysis of nutrient content in the soil and turfgrass clippings at the end of the study revealed inconsistent differences in P, K, Ca, S, and Mg content among core types (data not shown). This may be attributed to bundling of turfgrass clippings by treatment across the 8 weeks of experimentation to produce enough sampling material for analysis. This may also be attributed to various management practices at different golf courses before sample collection. A field study evaluating similar factors could help deduce potential differences further.

*Laboratory mineralization.* Mineralization of inorganic N was greatest within samples harvested from putting greens in the high SOM group (Fig. 1). These samples mineralized 22 to 36 $mg \cdot kg^{-1}$ inorganic N over the 8-week study. Changes in mineralized inorganic N were greatest in the initial 28 d of the experiment and plateaued thereafter. Comparatively, samples originating from putting greens in the medium and low SOM groupings mineralized $\leq 6.5$ $mg \cdot kg^{-1}$ during the same 8-week period. Similar to findings of Grégoire et al. (2022) and Miltner et al. (2001), this response indicates that significant amounts of inorganic N may become available via mineralization within putting greens with $>19.4$ $g \cdot kg^{-1}$ SOM when soil temperatures are 25 °C. Over the past three seasons in Knoxville, TN, USA (2022–24), soil temperatures (5 cm depth) were $\geq 25$ °C during an average of 60 of the 100 d from 1 Jun to 8 Sep.

Given that 36 $mg \cdot kg^{-1}$ mineralized after 28 d translates to ~50 $kg \cdot ha^{-1}$ N, there could be an oversupply of available N within root zones of CBG putting greens in Tennessee, especially with the addition of synthetic fertilizers during the growing season. As previously discussed, excessive N causes a host of negative issues on putting greens such as excess foliar growth that may reduce firmness to the extent that ball mark scaring is more severe (Nemitz et al. 2008). Furthermore, to avoid higher mowing frequency, increased foliar growth may necessitate a more intensive use of plant growth regulators (Chabon et al. 2017). An excess of N in putting green root zones could also potentially increase leaching to unintended sinks (Espevig and Aamlid 2012; Mancino and Troll 1990).

It should be noted that samples were prepared using a 10.8-cm diameter by 10-cm depth core with living plant material removed. Recent reports highlight that removing living plant material eliminates 38% of the organic material within a putting green sample (Kahiu et al. 2024). Future research exploring mineralization of undisturbed cores is warranted to fully understand the potential for available N to be made available to CBG putting greens during the growing season.

Overall, these two studies indicate that mineralization occurs in putting green root zones, potentially supplying as much as 50 $kg \cdot ha^{-1}$ N in CBG putting greens with soil organic matter content $\geq 19.4$ $g \cdot kg^{-1}$ at temperatures of 25 °C. Nitrogen mineralized to become plant available from SOM should be considered or factored into N fertilizer inputs; future field research is needed to confirm this to optimize fertilizer programs supplying N to CBG putting greens.

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