Short-term and Residual Effects of Laccase Application on Creeping Bentgrass Thatch Layer

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Abstract. Organic layer formation in the form of thatch is a major problem in managed turfgrass systems. Biweekly application of laccase enzyme has been well-documented to facilitate the degradation of thatch and reduce the accumulation rate of organic matter in ‘Crenshaw’ creeping bentgrass (Agrostis stolonifera L.). A field experiment involving creeping bentgrass was conducted to evaluate the residual effects on thatch accumulation after ceasing laccase applications. A significant reduction in thatch layer thickness was observed at 6, 12, and 18 months after treatment initiation when laccase was applied at different rates and frequencies. Residual effects of laccase application were observed for thatch layer thickness, but no additional accumulation of thatch was observed 6 months after treatment cessation. At 18 months after treatment initiation, a significant increase in the thatch layer was observed where treatments had been ceased for 12 months, but no thatch accumulation was observed for laccase treatment for a second 6-month period during the second year. This information is critical to turf practitioners when developing laccase application protocols. Limiting laccase applications for a period of 6 months during 1 year was shown to be effective for thatch control.

Lignin is a plant cell wall constituent that acts as a protective matrix and limits the availability of readily biodegradable plant materials, such as cellulose and hemi-celluloses, for microbial degradation (Ledeboer and Skogly, 1967). Lignin is formed in plants by oxidative coupling of monolignols of three primary hydroxycinnamyl alcohol: p-coumaryl, coniferyl, and sinapyl alcohols (Wong, 2009). Lignin is extremely recalcitrant to degradation due to its complex structure without a regular pattern, which is derived from random oxidative coupling of lignin monomers and cross-linking of polymers via radical mechanisms; this process is known as lignification (Ledeboer and Skogly, 1967). A lignin macromolecule contains monolignols randomly bonded by C-O-C and C-C linkages including β-O-4, β-5, β-β, 5-5, 4-O-5, and β-1 bonds (Alder, 1977; Del Rio et al., 2007; Ralph et al., 2004). Several models of the lignin molecular structure have been proposed, but these models do not imply any particular sequence of monomeric units in the lignin macromolecule (Chen and Sarkaney, 2003; Davin and Lewis, 2003). Other researchers have indicated a homogeneous structure of lignin based on studies suggesting lignin formation by repetitive units (Banoub and Delmas, 2003). The formation of a thatch-mat layer at home lawn and recreational turfgrass sites, especially golf greens, is accelerated when organic matter production exceeds the degradation rate (Beard, 1973). Thatch, a layer of highly organic matter that accumulates between the soil and green turfgrass, consists of dead and living stolon, rhizome, root, crown, leaf sheath, and blade tissues (Engel, 1954; Roberts and Bredakis, 1960). A mat layer is generally below the thatch layer, where soil or sand is intermingled with thatch as a result of earthworm activity or cultural practices, such as core aeration and topdressing (McCarty, 2005). A thatch layer is often desirable to increase resilience and wear tolerance of the turfgrass surface, reduce surface hardness, and moderate soil temperature extremes (Beard, 1973). However, an excessive thatch or mat layer is undesirable in turfgrass because it leads to decreased saturated hydraulic conductivity (SHC), decreased movement of oxygen through the thatch or mat zone, low oxygen levels within the thatch/mat layer during wet periods, and increased water retention (Carroll, 2003; Hartwiger, 2004; McCarty et al., 2007).

Cultural or mechanical control practices of core aeration, vertical mowing, grooming, and topdressing are often effective for reducing thatch, but they are known to adversely impact turf quality (Landreth et al., 2008; McCarty et al., 2007). Additionally, these practices have intensive requirements for labor, equipment, and energy (Barton et al., 2009; Landreth et al., 2008; McCarty et al., 2007), and they have shown contrasting results regarding reducing the organic matter content in the thatch layer (Barton et al., 2009; Carrow et al., 1987; Dunn et al., 1981; McCarty et al., 2005; McWhirter and Ward, 1976; Weston and Dunn, 1985; White and Dickens, 1984). Nondestructive biological and chemical attempts to enhance organic matter degradation in the thatch layer have included the usage of glucose, cellulase solutions (Ledeboer and Skogly, 1967), and commercial products containing mixtures of amino acids, microbial inocula, and fertilizers. These products target the degradation of cellulosic and hemi-cellulosic sugars in thatch biomass by improving conditions for microbial populations. However, the efficacy of these products may be inconsistent for reducing thatch in turfgrass (Lancaster et al., 1977; McCarty et al., 2005; Murdoch and Barr, 1976).

The rate of microbial decomposition is partially dependent on the lignin content of organic matter. Lignin degradation can act as the rate-limiting step in organic matter decomposition (Taylor et al., 1989; Sinsabaugh et al., 1993) conducted a plant litter decomposition study and reported a close relationship between lignocellulose-degrading enzymes and plant litter mass loss. Certain white-rot fungi are responsible for the natural degradation of lignin by producing extracellular lignolytic enzymes, thus exposing cellulosic materials to further bacterial degradation in the environment (Blanchette, 1984; Kirk et al., 1975, 1976; Mester et al., 2004; Oljen and Blanchette, 1987). Lignolytic enzymes such as tyrosinases, catechol oxidases, laccase, catechol dioxygenases, and monophenol monooxygenase that use oxygen as an electron acceptor to oxidize phenolic compounds are likely present in environmental samples and provide estimates of the sum of activity from all or some combinations of these enzymes. Therefore, in most published work, the collective activity of these enzymes is referred to as phenol oxidase, which represents the activity of enzymes that use oxygen and oxidize phenols. Phenol oxidase activity in the top 7.5 cm of soil samples of turfgrass systems has been reported to range from 0.7 to 2.8 mmol/kg soil/h (Yao et al., 2009, 2011).

Weight loss of bermudagrass pellets, Stenotaphrum secundatum (Walt.) Kuntze, and zoysiagrass (Zoysia japonica Stued., ‘Meyer’) stolons were observed when inoculated with different wood-decaying fungi under controlled greenhouse and laboratory conditions (Martin and Dale, 1980). In similar controlled studies, researchers
have reported reductions in cellulose content and total oxidizable organic matter of bermudagrass (Cynodon dactylon L.) and centipedegrass (Eremochloa ophiuroides) after inoculation with wood-decaying fungi (Sartain and Volk, 1984). However, field inoculation experiments involving bermudagrass showed no thatch degradation (Martin and Dale, 1980). Microbial inoculation under field conditions may be ineffective because it is difficult to maintain specific microbial activity for longer periods under turfgrass management systems (Yao et al., 2009, 2011).

Under greenhouse conditions, decreases in the rate of thatch layer build-up and accumulations of total organic matter in the top 2.5 cm of creeping bentgrass were reported in response to the direct application of laccase, an extracellular lignolytic enzyme produced from white-rot fungi Trametes versicolor (Sidhu et al., 2012). However, a net accumulation of organic matter in the thatch layer treated with laccase was observed over time with all treatments (Sidhu et al., 2012). A biweekly application of laccase enzymes on the thatch layer of dead creeping bentgrass verified the effectiveness of laccase for facilitating organic matter decomposition and the loss of the total sugar content of the thatch biomass. These results suggested that laccase application exposed cellulose and semi-cellulosic sugars to microbial degradation by opening the biomass structure (Sidhu et al., 2013a).

Field studies conducted using creeping bentgrass, ultra-dwarf bermudagrass, and zoysiagrass verified the effectiveness of laccase for thatch management on different turfgrass species (Sidhu et al., 2013b, 2014). In other experiments, creeping bentgrass treated biweekly with laccase, core aeration, and sand topdressing had significant reductions in thatch accumulation (Sidhu et al., 2014). In previous studies, organic matter degradation in response to enzyme treatment was determined during and at the end of the application period (Sidhu et al., 2012, 2013a, 2013b, 2014)

The fate of naturally occurring laccase enzymes depends on the interaction with soil, which is composed of mineral constituents and organic matter. These soil constituents can adsorb extracellular enzymes and provide surfaces for enzymatic reactions. Adsorption of enzymes to soil constituents can immobilize laccase enzymes (Giaveno et al., 2010), change their efficacy (Ahn et al., 2007; Gianfreda and Bollag, 1994; Zimmerman et al., 2004), and change their stability and denaturation (Rao et al., 2000; Yan et al., 2010). Gianfreda and Bollag (1994) observed that montmorillonite and Kollinite adsorbed 71% and 64% of laccase, respectively. However, compared with free enzymes, the kinetic parameters of laccase were improved when immobilized on montmorillonite. Wu et al. (2014) also observed similar results when laccase was adsorbed on iron and aluminum minerals for 18 h. However, the long-term residual impacts of laccase under field conditions, particularly the presence of thatch and mat layers, requires investigation.

The current study was designed to expand the results of previous studies by investigating the residual effects of laccase application on organic matter degradation. Knowledge of any residual effects would lead to turf management and economic implications. Therefore, the major objectives of this study were to determine the residual effects of laccase application on physical and chemical properties of the thatch layer of creeping bentgrass and to compare the residual effects of laccase with and without repeated applications.

Materials and Methods

Experimental design

A field experiment was conducted on ‘Cresenhaw’ creeping bentgrass (Engelke et al., 1995) Agrostis stolonifera L. at the University of Georgia, Griffin Campus (Griffin, GA), as an 18-month study from July 2010 to Jan. 2012. The bentgrass green was established as a sand-based putting green on 90:10 sand and organic matter mix (Michigan Peat) per the recommendations of the United States Golf Association (USGA Golf Association Green Section Staff, 1973). Fertilizer applications for 2010 and 2011 consisted of 50 kg ha⁻¹ granular fertilizer 24–4–10 (N-P₂O₅-K₂O) (Lesco Inc., Strongsville, OH) during the third weeks of March, September, and October, and 2 kg ha⁻¹ soluble 20–20–20 fertilizer (JR Peters Inc., Allentown, PA) every 2 weeks starting the third week of April through September. Bentgrass plots were mowed three times per week with a Toro Greensmaster 3100 (The Toro Company, Bloomington, MN) and maintained at a height of 4.2 mm.

The field study was conducted on plots (0.305 × 0.61 m) with 12 treatments replicated four times in a completely randomized block design. A priori comparisons of the rate of application, frequency of application, influence of cultural management practices (core aeration and topdressing), and sources of laccase was used for the evaluations (Table 1). Laccase was applied for 6 months from July 2010 to Dec. 2010 for treatments T1 to T12. Laccase applications were repeated only for treatment T11 from July 2011 to Dec. 2011 (Table 1). All plots were sampled at either 6, 12, and 18 months after treatment initiation or 0, 6, and 12 months after the end of the initial treatment application period to observe the potential residual effects of laccase application. Laccase treatments were sprayed using a flat fan nozzle and portable CO₂ sprayer system as 410 mL of solution in a 2-L bottle. Laccase enzymes from Trametes versicolor, a white-rot fungus, were purchased from Sigma-Aldrich (product 53739; Sigma Aldrich Inc., St. Louis, MO) and applied at activity levels of 0 (control), 0.5, 1.0, 2.0, and 4.0 units·cm⁻² every 2 weeks and at a laccase activity level of 2.0 units·cm⁻² every 2, 4, 8, and 12 weeks to optimize the rate and frequency of laccase application. Plots receiving cultural management treatments were core-aerated and sand-topdressed twice yearly in April and September. Core aeration was accomplished using a Ryan Greensaire 24 Aerator (Ryan Inc., Johnson Creek, WI) fitted with 1.27-cm tines with a spacing of 5.0 × 5.0 cm and adjusted to penetrate to a depth of 6.25 cm. Immediately following core aeration, sand topdressing with 1134 g of sand (Quikrete Premium Play Sand, Atlanta, GA) per plot was accomplished using a Scotts Precision Green Spreader (Scotts Miracle-Gro, Marysville, OH). Laccase was applied at 2.0 units·cm⁻² every 4 weeks on plots core-aerated and sand-topdressed twice per year to observe the effectiveness of laccase in combination with the cultural management practice. Hereafter, rate and frequency treatments are presented as the rate of the laccase activity level followed by the frequency of application in parentheses; for example, “2.0 (4)” denotes treatments involving laccase with an activity of 2.0 units·cm⁻² applied at 4 weeks (Table 1).

Laccase from two different sources was compared for its effectiveness on thatch management. Laccase from the Pyecoporus genus was procured from Jiangnan University, China [CHU (2)], and from a commercial industrial wholesale supplier in China [CHI (2)]; it was applied at an activity level of 2.0 units·cm⁻² every 2 weeks (Table 1). The CHU (2) treatment (i.e., T12) was applied from July 2010 to Dec. 2010 and from July 2011 to Dec. 2011 to compare the effects of continued application of laccase every year for 6 months with the residual effects of one 6-month period of laccase application (Table 1).

Measurements

The residual effects of laccase application on the physical and chemical properties of the thatch layer were determined at 6, 12, and 18 months after the initiation of treatment application. Variables measured included the total organic matter content at a depth of 0 to 2.5 cm (OM₀), a depth of 2.5 to 5.0 cm (OM₁), a depth of 0 to 5.0 cm (OM₂), thatch layer thickness (TLT), and SHC. Similarly, the extractive-free acid-soluble lignin (L₅) and acid-insoluble lignin (L₄) contents were determined to observe the impact of treatment applications on the chemical composition properties of the thatch layer biomass. The total lignin content (L₇) was calculated after the addition of the L₅ and L₄ contents.

Laccase activity assay. The activity of laccase was quantified by a colorimetric assay using a Beckman DU 640B spectrophotometer (Beckman Instruments Inc., Fullerton, CA). One activity unit of laccase corresponded to the amount of enzymes causing a 0.001 absorbance change at 468 nm at a rate of 1.0 unit·min⁻¹ in 3.4 mL of 1 mM 2, 6-dimethoxyphenol, which is a specific substrate for laccase, in citrate-phosphate buffer at pH 3.8 (Park et al., 1999).

Total organic matter content. The total organic matter content was determined by the
Table 1. Description of laccase treatments applied on creeping bentgrass. Treatments are presented as laccase source followed by activity levels (units·cm⁻²) followed by application frequency (weeks) in parenthesis.

| Treatment | Volume of solution (mL) | Laccase activity level (units·cm⁻²) | Application frequency (wk) | Cultural management practice | Source of laccase | Designation | Application timeframe |
|-----------|------------------------|--------------------------------------|-----------------------------|-----------------------------|-------------------|-------------|----------------------|
| T1: Control | 410 mL | 0 | 2 | No | NA | Control | 0–6 mo., 12–18 mo. |
| T2: SA² 0.5 (2) | 410 mL | 0.5 | 2 | No | Sigma Aldrich | 0.5 (2) | 0–6 mo. |
| T3: SA 1.0 (2) | 410 mL | 1.0 | 2 | No | Sigma Aldrich | 1.0 (2) | 0–6 mo. |
| T4: SA 2.0 (2) | 410 mL | 2.0 | 2 | No | Sigma Aldrich | 2.0 (2) | 0–6 mo. |
| T5: SA 4.0 (2) | 410 mL | 4.0 | 2 | No | Sigma Aldrich | 4.0 (2) | 0–6 mo. |
| Application frequency | | | | | | | |
| T6: SA 2.0 (4) | 410 mL | 2.0 | 4 | No | Sigma Aldrich | 2.0 (4) | 0–6 mo. |
| T7: SA 2.0 (8) | 410 mL | 2.0 | 8 | No | Sigma Aldrich | 2.0 (8) | 0–6 mo. |
| T8: SA 2.0 (12) | 410 mL | 2.0 | 12 | No | Sigma Aldrich | 2.0 (12) | 0–6 mo. |
| Cultural management | | | | | | | |
| T9: CMC³ | 410 mL | 0 | 0 | Yes | NA | CMC | |
| T10: CMC³+SA 2.0 (4) | 410 mL | 2.0 | 4 | Yes | Sigma Aldrich | CMC+2.0 (4) | 0–6 mo. |
| Laccase sources | | | | | | | |
| T11: CHI 2.0 (2) | 410 mL | 2.0 | 2 | No | Industrial-China | CHI (2) | 0–6 mo. |
| T12: CHU 2.0 (2) | 410 mL | 2.0 | 2 | No | University-China | CHU (2) | 0–6 mo., 12–18 mo. |

²SA denotes laccase procured from Sigma Aldrich; CHU denotes laccase procured from China (Jiangnan University); CHI denotes laccase procured from China (industrial supplier).
³CMC denotes cultural management control.

Method described by Carrow et al. (1987). Two soil cores (diameter, 2.0 cm) were obtained at two depths from each plot: 0 to 2.5 cm (OM₁) and 2.5 to 5.0 cm (OM₂). The cores were dried in an oven at 100 ± 5 °C for 24 h, weighed to determine the moisture content, ashed in a muffle furnace at 450 ± 10 °C for 24 h, and weighed again. The difference in the two readings was used to calculate the total organic matter content.

Thatch layer thickness. The TLT was measured by two replaceable wedge-shaped turf profiles (width, 8.9 cm; thickness, 2.5 cm) using the AMS Turf Profiler (AMS Inc., American Falls, ID). The TLT was measured from four points across the width of each profile and averaged. A clear visible distinction between the thatch layer and the sand layer below was considered for the measurement.

Saturated hydraulic conductivity. The SHC was measured by a constant hydraulic head method using a Marriott tube apparatus (McCarthy, 1934). An intact core (diameter, 4.7 cm; length, 7.7 cm) was obtained from each plot in a brass cylinder using a soil corer (model 0200 soil sampler; Soilmoisture Equip. Corp., Santa Barbara, CA). The bottom of the core was covered with a double layer of cheesecloth held in place with a rubber band and saturated overnight in a 0.05 N CaCl₂ solution. Steady-state flow was measured by flowing 0.05 N CaCl₂ through the core for 0.5 cm (OM₁) and 2.5 to 5.0 cm (OM₂). The cores were dried in an oven at 100 ± 5 °C for 24 h, weighed to determine the moisture content, ashed in a muffle furnace at 450 ± 10 °C for 24 h, and weighed again. The difference in the two readings was used to calculate the total organic matter content.

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Extractive-free lignin content. The thatch biomass was collected from the top 2.5 cm of each core after sampling for SHC. Thatch samples were first air-dried, ground, washed by adding water in a Mason jar and shaking using a rotary shaker at 200 rpm, and then passed through a series of sieves with an 841-μm sieve at the top and a 177-μm sieve at the bottom. The material retained by the 177-μm sieve was used for analysis. The thatch biomass was extracted for 24 h using the Soxhlet method for water- and alcohol-soluble impurities using de-ionized water and 16.26 M (95% USP grade) ethyl alcohol, respectively. The Lₕ and Lₗ contents in the thatch layer were determined using a two-step acid-hydrolysis procedure according to the laboratory analytical procedure developed by the National Renewable Energy Laboratory (2008). Acid-soluble lignin is primarily low-molecular-mass phenolic compounds. During the first step, extractive-free thatch samples were hydrolyzed for 60 min with 72% H₂SO₄ at 30 °C. During the second step, H₂SO₄ was diluted to 4%; the samples were autoclaved at 121 °C for 1 h and then vacuum-filtered. The solids remaining after acid hydrolysis were dried in an oven at 105 ± 5 °C for 24 h, weighed, ashed in a muffle furnace at 600 ± 10 °C for 24 h, and weighed again to calculate the acid-insoluble lignin content using the weight difference. Acid-soluble lignin was determined using this hydrolysis liquid at a wavelength of 240 nm in a Beckman DU-640B spectrophotometer (Beckman Instruments Inc., Fullerton, CA).

Statistical analysis

A repeated-measures design was used to analyze the full model for laccase residual effects; this consisted of 11 treatments, three levels of treatment duration, and four replications. The CHU treatment (i.e., T12) was repeated from July to Dec. 2011, and it was not considered in the full model. Treatments were combined to form the following: a rate effect (different rates of application significantly affected (P < 0.001) (Table 2). Strong duration (time after treatment applications was initiated) effects (P < 0.001) were observed for Oₜ, CMC, and all lignin content measurements (Table 2), indicating the residual effects of laccase applications on these parameters. No duration effects were observed for CHU and Oₜ. Interaction effects of duration × treatment (P < 0.001) were observed for Lₕ and Lₗ, indicating that different treatments had different effects on extractive-free acid-insoluble lignin and total lignin.

Rate of application. The rate of laccase application significantly affected (P < 0.001) TLT, Lₕ, and Lₗ (Table 2). Strong duration effects were observed for Oₜ (P < 0.001), SHC (P < 0.001), Lₕ (P < 0.001), and Lₗ (P < 0.01) (Table 2). In interaction effects (P < 0.001) of duration × treatment were observed for Lₕ and Lₗ contents. After 6 months of treatment, no differences were observed for Oₜ, Oₜ, or Oₜ with any of the treatments. Samples obtained at 12 months after treatment initiation showed that Oₜ at a laccase activity level of 4.0 units·cm⁻² decreased by 21.5 mg·g⁻¹ when compared with control (Table 3). No differences were observed for Oₜ and Oₜ in the 12-month sampling.
Table 2. Analysis of variance (ANOVA) table showing the effects of laccase treatments, treatment duration, and duration × treatment interactions on creeping bentgrass.

| Source of variation       | df | Mean square value |
|---------------------------|----|-------------------|
| Full model                |    |                   |
| Repetition                | 3  | 1,429             |
| Treatment                 | 10 | 2,583**           |
| Error A (repetition × treatment) | 30 | 699***            |
| Duration                  | 2  | 7,934***          |
| Duration × treatment      | 20 | 316               |
| Error                     | 66 | 221               |
| Rate of application       |    |                   |
| Repetition                | 3  | 4,105             |
| Treatment                 | 4  | 400               |
| Error A (repetition × treatment) | 12 | 313              |
| Duration                  | 2  | 2,900***          |
| Duration × treatment      | 8  | 225               |
| Error                     | 30 | 249               |
| Application frequency     |    |                   |
| Repetition                | 3  | 1,333             |
| Treatment                 | 4  | 322               |
| Error A (repetition × treatment) | 12 | 663***            |
| Duration                  | 2  | 4,108***          |
| Duration × treatment      | 8  | 496               |
| Error                     | 30 | 249               |
| Cultural management       |    |                   |
| Repetition                | 3  | 194               |
| Treatment                 | 3  | 4,865*            |
| Error A (repetition × treatment) | 9  | 906***            |
| Duration                  | 2  | 1,343***          |
| Duration × treatment      | 6  | 275               |
| Error                     | 24 | 225               |
| Laccase sources           |    |                   |
| Rep                       | 3  | 531               |
| Treatment                 | 2  | 1,088*            |
| Error A (repetition × treatment) | 6  | 337              |
| Duration                  | 1  | 34                |
| Duration × treatment      | 2  | 37                |
| Error                     | 9  | 193               |
| 2-Year application        |    |                   |
| Rep                       | 3  | 862               |
| Treatment                 | 1  | 4,150**           |
| Error A (repetition × treatment) | 3  | 120              |
| Duration                  | 2  | 3,337*            |
| Duration × treatment      | 2  | 97                |
| Error                     | 12 | 526               |

* Significant at P = 0.05, ** at P = 0.01, and *** at P = 0.001, respectively.
Table 3. Total organic matter content at three depths of 0–2.5 cm (OMU), 2.5–5.0 cm (OML), and 0–5.0 cm (OM) at 6, 12 and 18 mo. after initiation of different laccase treatments applied on creeping bentgrass. Treatments are presented as laccase source followed by activity levels (units-cm$^{-2}$) followed by application frequency (weeks) in parenthesis.

| Treatment group | Total organic matter (0–2.5 cm) OMg | Total organic matter (2.5–5.0 cm) OMg | Total organic matter (0–5.0 cm) OMg |
|-----------------|-------------------------------------|--------------------------------------|-------------------------------------|
|                 | 6 Mo. | 12 Mo. | 18 Mo. | 6 Mo. | 12 Mo. | 18 Mo. | 6 Mo. | 12 Mo. | 18 Mo. |
| Control         | 132.0 a A | 138.6 a A | 140.1 a A | 63.9 a A | 62.8 a A | 61.5 ab A | 90.4 a A | 89.7 a A | 91.1 b A |
| SA 0.5 (2)      | 135.8 b B | 139.6 a AB | 155.3 a A | 69.9 a A | 69.0 a A | 60.6 ab A | 95.8 a A | 95.2 a A | 93.8 ab A |
| SA 1.0 (2)      | 131.5 a A | 133.9 b B | 165.4 a A | 64.9 a A | 65.5 a A | 67.6 a A | 90.6 a A | 91.6 a A | 101.3 a A |
| SA 2.0 (2)      | 125.2 a A | 127.7 ab A | 156.9 a A | 66.4 a A | 59.8 b A | 60.2 b bB | 90.0 a A | 85.5 a A | 94.4 ab A |
| SA 4.0 (2)      | 129.9 a AB | 117.1 b B | 142.2 a A | 63.1 a A | 56.1 a A | 55.1 b bB | 88.6 a A | 80.1 a B | 85.8 AB |
| Frequency of application | | | | | | | | | |
| Control         | 132.0 a A | 138.6 a A | 140.1 a B | 63.9 a A | 62.8 a A | 61.5 b bB | 90.4 a A | 89.7 a A | 91.1 c A |
| SA 2.0 (2)      | 125.2 a A | 127.7 a A | 156.9 ab A | 66.4 a A | 59.0 b A | 60.2 b bB | 90.0 a A | 85.5 a B | 94.4 bc B |
| SA 2.0 (4)      | 134.7 a A | 141.7 a A | 160.9 a A | 67.7 a A | 73.2 a AB | 79.9 a A | 93.8 a B | 99.8 b A | 109.8 A |
| SA 2.0 (8)      | 134.8 a A | 139.7 a A | 148.8 a A | 64.4 a A | 65.0 a A | 68.6 a A | 91.7 a B | 93.0 b B | 97.2 abc A |
| SA 2.0 (12)     | 131.6 a A | 129.0 a B | 183.9 a A | 62.6 a A | 94.5 a A | 70.6 a A | 89.8 a A | 106.7 a A | 107.9 a A |
| Cultural management | | | | | | | | | |
| Control         | 132.0 ab A | 138.6 a A | 140.1 a B | 63.9 a A | 62.8 a A | 61.5 b bB | 90.4 a A | 89.7 a A | 91.1 b A |
| CMC             | 107.1 b B | 88.6 b B | 118.4 b A | 66.7 a A | 60.5 a A | 70.8 a aB | 83.6 a AB | 72.4 b B | 90.5 b A |
| SA 2.0 (4)      | 134.7 a A | 141.7 a A | 160.9 a A | 67.7 a A | 73.2 a AB | 79.9 a A | 93.8 a B | 99.8 B bB | 109.8 A |
| CMC+SA 2.0 (4)  | 113.0 ab AB | 97.9 b B | 118.4 b A | 71.1 a A | 63.9 a aB | 71.1 ab aB | 89.1 a AB | 77.7 b B | 89.8 b A |
| Laccase sources | | | | | | | | | |
| SA 2.0 (2)      | 125.2 b B | 127.7 a A | 156.9 a A | 66.4 a A | 59.0 b A | 60.2 b bB | 90.0 a A | 85.5 a A | 94.4 b A |
| SA 2.0 (8)      | 136.8 ab AB | 132.6 a A | 163.9 a A | 69.1 a A | 65.6 a A | 76.6 a A | 95.5 a AB | 91.5 a B | 108.9 a A |
| CHU 2.0 (2)     | 152.2 a A | 146.7 a A | 189.8 a A | 69.5 a A | 68.5 a A | 81.8 a aB | 101.1 a AB | 98.1 B bB | 118.4 a A |
| Continuous application | | | | | | | | | |
| SA 2.0 (2)      | 125.2 a A | 127.7 a A | 156.9 a A | 66.4 a A | 59.0 b A | 60.2 b bB | 90.0 a A | 85.5 a A | 94.4 a A |
| CHU 2.0 (2)     | 152.2 a A | 146.7 a A | 189.8 a A | 69.5 a A | 68.5 a A | 81.8 a aB | 101.1 a AB | 98.1 B bB | 118.4 a A |

$^a$Means within a column in a treatment group (treatment effect) followed by the same lowercase letter are not significantly different according to the least significant difference (LSD) at $\alpha = 0.05$.

$^b$Means within a row in a treatment group (duration effect) followed by the same uppercase letter are not significantly different according to the LSD at $\alpha = 0.05$.

$^c$Denotes laccase procured from Sigma Aldrich; CHU denotes laccase procured from China (Jiangnan University); CHI denotes laccase procured from China (industrial supplier); CMC denotes cultural management control.

Samples obtained at 18 months after the start of the experiment showed a 10.4-mg g$^{-1}$ increase in the organic matter content for the 1.0 (2) treatment compared with control. The organic matter content (0–2.5 cm) increased by 19.5 mg g$^{-1}$ at 0.5 units cm$^{-2}$ when sampled between 6 and 18 months after treatment initiation. Significant reductions of 6.2 and 8.0 mg g$^{-1}$ in the OMg contents from 6 to 18 months for treatments 2.0 (2) and 4.0 (2), respectively, were observed, and a reduction in organic matter (8.5 mg g$^{-1}$) from 6 to 12 months was observed for treatment 4.0 (2), suggesting the residual effects of laccase.

Laccase treatments at different activity levels were equally effective; after 6 months of treatment application, TLT was lowered by 3.8 to 4.8 mm compared with control (Fig. 1A). Twelve months after the start of treatment, TLT was lowered by all activity levels of laccase. However, treatments with laccase at activity levels of 2.0 and 4.0 units cm$^{-2}$ showed significant reductions in TLT values compared with nontreatment and treatments involving 0.5 and 1.0 units cm$^{-2}$ of laccase activity. A reduction in TLT was observed for all treatments compared with control when sampled 18 months after the initiation of treatment. Applications of laccase at 0.5, 1.0, and 2.0 units cm$^{-2}$ were effective for maintaining the TLT up to 6 months after treatment completion; however, with 4.0 units cm$^{-2}$ laccase treatment, TLT was lowered from 14.5 mm at 6 months after treatment initiation to 13.3 mm at 12 months after treatment initiation. A significant increase in TLT occurred when laccase was applied at 0.5 and 1.0 units cm$^{-2}$ over the course of the three sampling dates.

Laccase activity levels had no effect on SHC at the time of any of the sampling dates (Table 4). After 6 months of treatment, laccase applications up to 2.0 units cm$^{-2}$ lowered Ls by 7.8 to 8.9 mg g$^{-1}$ compared with control (Table 4). Acid-soluble lignin with treatment involving 4.0 cm$^{-2}$ of laccase activity was reduced by 12.2 mg g$^{-1}$ when compared with control at the end of treatment (6 months) (Table 4). No differences in Ls were observed at the sampling times of 12 and 18 months after treatment initiation.

The extractive-free L I content was lower compared with control when treated with laccase up to 2.0 and 1.0 units cm$^{-2}$ at 6 and 12 months after initiating treatment, respectively (Table 4). At the end of the treatment application, L I was higher with 4.0 units cm$^{-2}$ of treatment compared with control. Similarly, in samples obtained 12 months after treatment initiation, the L I content was higher than that of the control when treated with 2.0 and 4.0 units cm$^{-2}$ of laccase activity, suggesting the residual effects of laccase. Laccase treatments showed higher L I content compared with control at 18 months after treatment initiation (Table 4). An increase in the L I content was observed with all treatments (Table 4). Variations in L I content followed trends that were similar to those for L I content with different laccase activity levels (Fig. 2A).
The cultural management and laccase treatments increased extractive-free lignin and LT contents. Duration × treatment interaction effects ($P \leq 0.001$) were observed for LS and LT when compared with control (Table 2). Significant duration effects were observed for treatments including cultural management practices (Fig. 1C).

After 6 months of application, treatment involving core aeration and sand topdressing showed an increase of 13.5 cm h$^{-1}$ in SHC compared to nontreatment (Table 4). Laccase treatment with or without cultural management resulted in no differences in SHC (Table 4). No changes in SHC were observed during a comparison with the control for other sampling durations. No duration effects were recorded for SHC in this group of treatments (Table 4). Reductions of 3.6, 7.8, and 3.8 mg g$^{-1}$ in the LS contents were recorded for the CMC, 2.0 (4), and CMC+2.0 (4) treatments, respectively, when compared with control at the 6-month sampling. However, no differences in the LS contents were observed at the time of sampling at 12 and 18 months after treatment initiation. A slight but significant duration effect was observed for the LS content with the 2.0 (4) treatment (Table 4). Extractive-free LS and LT contents 6 months after treatment application were lower with laccase treatment alone and increased with the CMC and CMC+2.0 (4) treatments when compared with control (Table 4, Fig. 2C). A similar trend was recorded for LS at the time of sampling conducted at 12 months after treatment initiation. Acid-insoluble contents of all treatments increased compared to the control at 18 months after treatment initiation. Significant...

**Fig. 1.** Thatch layer thickness (TLT; in mm) at 6, 12, and 18 months after treatment initiation on creeping bentgrass. (A) Laccase application rates. (B) Frequency of application of laccase. (C) Cultural management and laccase treatments. (D) Laccase sources. The stacked bars in (C) represent the depths of the thatch layer and sand deposition on plots after topdressing. Treatments are presented as the laccase source followed by the activity level (units cm$^{-2}$) and the application frequency (weeks) in parentheses. Values are the means of four replicates. The same letter within the bars (6 months = lowercase bold; 18 months = lowercase italics) and the same letter on top of the bars (duration effect = uppercase bold) are not considered statistically different according to Fisher’s protected least significant difference (LSD) at $\alpha = 0.05$. CMC, cultural management control; SA, laccase procured from Sigma Aldrich; CHU, laccase procured from China (Jiangnan University); CHI, laccase procured from China (industrial supplier).

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duration effects were observed for Ls and LT, with increases in the lignin content occurring with all treatments over time (Table 4, Fig. 2C).

Sources of laccase enzyme. Laccase enzymes procured from different sources were similarly effective for organic matter content (OMs, OM, and OM) (Table 3), TLT (Fig. 1D), and SHC (Table 4). Slight differences in LS and LI contents were observed for treatments involving laccase from different sources (Table 4). Six months after treatment initiation, TLT was slightly lower than it was after 6 months. However, TLT with the 2.0 (2) treatment remained lower than the control after 18 months (Figs. 3 and 4). The baseline measurement of TLT was 17.2 mm. The TLT of the control continued to increase with time, whereas for the 2.0 (2) and CHU (2) treatments, TLT was significantly reduced after 6 months. At 12 months after treatment initiation, TLT was slightly lower than it was after 6 months. However, TLT with the 2.0 (2) treatment was 3.3 mm higher than it was with CHU (2) after 18 months (Figs. 3 and 4).

Discussion

Nondestructive methods used to manage thatch are desired, but they are often ineffective. Commercial microbial inoculums, such as Biodethatch, Thatch-Away, and Earth Anew, on bermudagrass, creeping bentgrass, and annual bluegrass have been reported to be ineffective for reducing the thatch layer depth (Gibbault et al., 1976; Lancaster et al., 1977; Murdoch and Barr, 1976). Similarly, applications of the biological granular supplement Thatch-X (Lebanon Seaboard Corporation, Lebanon, PA) on creeping bentgrass (McCarty et al., 2007) and of wetting agents Aqua-Gro (Aquatrols Corp of America, Paulsboro, NJ), Milorganite (Milorganite, Milwaukee, WI), and acti- vated sewage sludge on Kentucky bluegrass (Murray and Juska, 1977) were ineffective for decreasing the organic matter content of the thatch layer. One of the possible reasons for the inconsistent results of organic matter decomposition was the emphasis on the degradation of cellulose and hemi-cellulose sugars instead of lignin. Lignin protective matrix must be removed to open the biomass structure and increase access to readily decomposable structural carbohydrates (Ledeboer and Skogly, 1967).

Direct microbial inoculation of turfgrass systems have been ineffective for managing thatch (Martin and Dale, 1980). Different microbial populations require a specific microclimate with particular moisture and temperature regimes for growth. The inability to maintain a microbe-specific preferred microenvironment for prolonged durations under turfgrass management systems may lower the possibility of maintaining specific microbial activities over time.

Laccase enzymes are stable over wide pH and temperature ranges (Baldrian, 2006; Munoz et al., 1997; Stoilova et al., 2010; Thurston, 1994). Laccase, a multico- pper oxidase, is an extracellular enzyme known to oxidize a wide range of phenolic compounds using oxygen as an electron acceptor (Baldrian, 2006). Lignin phenolic components are oxidized due to laccase-mediated cleavage of different covalent bonds formed within lignin macromolecules and between lignin and structural sugars (Wong, 2009). This opens the biomass structure, leading to increased availability of easily degradable sugars by microbes. By using laccase enzymes, turfgrass managers could effectively manage thatch over wide ranges of

Table 4. Saturated hydraulic conductivity (SHC), extractive-free acid-soluble lignin (Ls), and extractive-free acid-insoluble lignin (LT) at 6, 12, and 18 mo. after initiation of different laccase treatments applied on creeping bentgrass. Treatments are presented as laccase source followed by activity levels (units-cm⁻²) followed by application frequency (weeks) in parenthesis.

| Treatment group | Saturated hydraulic conductivity SHC | Extractive-Free Acid-soluble lignin Ls | Extractive-Free Acid-insoluble lignin LT |
|-----------------|-----------------------------------|----------------------------------------|------------------------------------------|
|                 | 6 Mo. | 12 Mo. | 18 Mo. | 6 Mo. | 12 Mo. | 18 Mo. | 6 Mo. | 12 Mo. | 18 Mo. |
| Rate of application | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ |
| Control         | 3.3 ab | 4.2 a | 6.9 a | 82.5 a | 76.2 a | 82.9 a | 279.6 b | 316.6 b | 326.8 d | a |
| SA 0.5 (2)      | 2.3 b | 5.9 a | 4.7 a | 74.1 AB | 69.5 b | 80.8 a | 274.5 c | 297.6 c | 346.2 c | b |
| SA 1.0 (2)      | 2.5 b | 3.8 a | 6.5 a | 74.7 b | 72.2 a | 82.6 a | 257.6 d | 306.7 b | 359.1 b | a |
| SA 2.0 (2)      | 2.5 b | 5.5 a | 9.9 a | 73.6 bc | 72.9 a | 83.4 a | 250.0 b | 366.4 a | 365.7 b | a |
| SA 4.0 (2)      | 5.3 a | 3.5 a | 4.9 a | 70.3 c | 75.6 b | 84.0 a | 291.2 a | 355.1 b | 379.3 a | a |
| Frequency of application | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ |
| Control         | 3.3 ab | 4.2 a | 6.9 a | 82.5 a | 76.2 a | 82.9 a | 279.6 b | 316.6 b | 326.8 d | a |
| SA 2.0 (2)      | 2.5 b | 5.5 a | 9.9 a | 73.6 c | 72.9 a | 83.4 a | 250.0 b | 366.4 a | 365.7 a | c |
| SA 2.0 (4)      | 1.6 b | 3.6 a | 4.0 a | 74.7 b | 75.7 a | 83.0 a | 264.1 c | 306.2 b | 338.7 c | a |
| SA 2.0 (8)      | 4.5 a | 4.2 a | 4.5 a | 77.7 b | 65.4 a | 85.0 a | 280.8 b | 348.9 b | 371.0 a | b |
| SA 2.0 (12)     | 3.1 ab | 4.3 a | 4.3 a | 76.7 b | 67.7 a | 80.3 a | 285.4 a | 338.4 b | 356.1 b | a |
| Cultural management | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ |
| Control         | 3.3 b | 4.2 a | 6.9 b | 82.5 a | 76.2 a | 82.9 a | 279.6 c | 316.6 b | 326.8 c | a |
| SA 2.0 (2)      | 2.5 a | 5.5 a | 9.9 a | 73.6 c | 72.9 a | 83.4 a | 250.0 b | 366.4 a | 365.7 a | c |
| SA 2.0 (4)      | 1.6 b | 3.6 a | 4.0 a | 74.7 b | 75.7 a | 85.0 a | 264.1 c | 306.2 b | 338.7 c | a |
| SA 2.0 (8)      | 4.5 a | 4.2 a | 4.5 a | 77.7 b | 65.4 a | 85.0 a | 280.8 b | 348.9 b | 371.0 a | b |
| SA 2.0 (12)     | 3.1 ab | 4.3 a | 4.3 a | 76.7 b | 67.7 a | 80.3 a | 285.4 a | 338.4 b | 356.1 b | a |
| Laccase sources | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ |
| SA 2.0 (2)      | 2.5 a | 5.5 a | 9.9 a | 73.6 b | 72.9 a | 83.4 a | 250.0 b | 366.4 a | 365.7 ab | a |
| CHI 2.0 (2)     | 2.5 a | 7.3 a | 4.3 a | 78.5 a | 73.1 b | 84.5 a | 275.9 a | 359.6 b | 355.1 ab | a |
| CHU 2.0 (2)     | 3.4 a | 8.0 a | 4.3 a | 79.0 a | 73.9 a | 80.3 a | 248.6 b | 313.4 b | 373.4 a | b |
| Continuous application | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ | cm h⁻¹ | mg g⁻¹ |
| SA 2.0 (2)      | 2.5 a | 5.5 a | 9.9 a | 73.6 a | 72.9 a | 83.4 a | 250.0 a | 366.4 a | 365.7 a | a |
| CHU 2.0 (2)     | 3.4 a | 8.0 a | 4.3 a | 79.0 a | 73.9 a | 80.3 a | 248.6 a | 313.4 b | 373.4 a | b |
environmental conditions and improve their ability to use existing populations of soil microbes for the decomposition of organic matter. In previous studies, reductions in thatch/mat layers with laccase treatment were demonstrated during greenhouse and field research (Sidhu et al., 2012, 2014). However, the question of residual effects was not addressed.

**Laccase rate and frequency.** In this study, laccase treatments at different rates and frequencies of application were ineffective for reducing OMU, OMU, and OM contents after 6 months of application (Table 3). However, when laccase was applied at 0.5 units cm⁻² every 2 weeks, a significant reduction in OMU occurred at 12 months after treatment initiation (Table 3). This observation indicated that laccase application for 6 months continued to slow the accumulation of OMU over the next 6 months. However, a significant increase in the thatch layer was observed again for 6 months during year 2, a significant reduction in the thatch layer was observed after treatment cessation (Fig. 1A). Although laccase treatments did decrease TLT, there were only minor differences in SHC, and no apparent trend (Table 4).

During the sub-study involving laccase application for 6 months during year 1 and again for 6 months during year 2, a significant reduction in the thatch layer was observed when comparing laccase applications for 6 months during year 1 and samples at 18 months after treatment initiation (Fig. 3). After treatment cessation, residual effects of laccase reduced thatch layer build-up during the second year. However, no thatch build-up was observed when laccase was applied during the second year, suggesting that annual applications of laccase for 6 months during the summer was effective for reducing or stabilizing thatch accumulation. No further reduction in the thatch layer was observed during the second year, even with the application of laccase. This suggested a threshold level for thatch layer reduction with the application of laccase. Increased OMU was observed where laccase was applied for 6 months during the second year when compared with laccase applied for 6 months during the first year (Table 3). This may be attributed to the tight stacking of thatch biomass due to removal of lignin bonds and the reduction of structural sugars leading to weak thatch biomass.

The extractive-free L₃ content initially decreased with the application of laccase, as was evident from sampling conducted after the conclusion of treatments at 6 months (Table 4). The extent of reduction at 6 months was dependent on the amount of laccase applied and the rate and frequency of applications. Applications of laccase up to 2.0 units cm⁻² decreased L₃ in the thatch biomass, but applications of laccase at 4.0 units cm⁻² showed an increase in the L₃ content expressed as a proportion of the sample based on dry weight when compared with the control (Table 4).

The increased L₃ content could have been attributed to the loss of excessive structural sugars with laccase treatment at 4.0 units cm⁻² (Sidhu et al., 2014). Three major components of plant biomass are cellulosic sugars, hemi-cellulosic sugars, and lignin. Therefore, with the application of laccase,
lignin bonds are broken, which leads to the opening of the biomass structure and makes sugars more available for microbial decomposition. As the sugar content is decreased, the lignin content is increased; this is because it is expressed as the proportion of the total dry weight. Decreased structural carbohydrate (cellulosic and hemicellulosic sugars) content in thatch biomass after laccase treatment was previously reported by Sidhu et al. (2013a, 2014).

A decrease in the structural sugar content was observed as the rate of laccase activity increased, indicating more availability of sugars for microbial degradation (Sidhu et al., 2014). Residual effects of laccase applications were observed between the 6- and 18-month sampling dates in the proportion of lignin present in thatch biomass. Lignin continued to accumulate during this period, suggesting the continued loss of sugar content from the biomass, even after laccase treatments had ceased (Table 4). L<sub>T</sub> is the major component of L<sub>T</sub>, and a similar trend in L<sub>T</sub> was observed with increasing laccase application rates (Fig. 2A). Maximum reductions in L<sub>OM</sub>, L<sub>T</sub>, and L<sub>T</sub> were observed at 6 months when laccase was applied every 2 weeks. The extent of this reduction decreased with the decreased laccase application frequency (Table 4, Fig. 2B), suggesting that the extent of lignin reduction was dependent on the amount of laccase applied.

Cultural management and laccase. Previous research related to the use of several different management techniques as a means to reduce the TLT and the accumulation of organic matter has shown contrasting results. Carrow et al. (1987) reported a decreased thatch layer depth of 44% to 62% with one or two applications of topdressing annually. Sand topdressing four times per year was reported to be effective for reducing the thatch layer when compared with a single application (White and Dickens, 1984). Barton et al. (2009) reported a significant reduction in organic matter content with sand topdressing twice per year on Kikuyu turfgrass. It was also noted that core aeration combined with sand topdressing was equally effective for reducing the thatch layer depth and organic matter content. However, Engel and Alderfer (1967), McCarty et al. (2007), and Rieke (1994) observed no reduction in the thatch layer by topdressing alone. It has been suggested that the application of sand topdressing improves the microenvironment for microbial growth (Ledeboer and Skogly, 1967). However, some researchers believe that the dilution of organic matter in the thatch layer is the primary influence on sand topdressing (Couillard et al., 1997; Rieke, 1994). Topdressing alone had no effect on water infiltration rates (McCarty et al., 2007).

A 10% reduction in the TLT was reported after core aeration four times annually on creeping bentgrass (McCarty et al., 2007) and three to six times per year on Tifgreen bermudagrass (McWhirter and Ward, 1976). Carrow et al. (1987) noted no effects of core aeration applied once or twice per year on the thatch-mat depth of Tifway bermudagrass, although a reduction in sand density was observed. Several studies have reported an increase in water infiltration in turfgrass fields after core aeration due to the formation of water channels and porous profiles (Bunnell et al., 2001; Canaway et al., 1986; McCarty et al., 2005).

During this study, organic matter content in the top 2.5 cm was lower at 12 months with cultural management practices when compared with the control. This may be attributed to the dilution effect created by sand topdressing on the surface layer because sand topdressing showed no effect on organic matter at a depth of 2.5 to 5.0 cm (Table 3). The increase in OM<sub>OM</sub> (18 months) and OM (12 and 18 months) for laccase-treated thatch may be related to a more dense thatch biomass occurring due to laccase activity in cellulose and hemicellulosic sugars resulting in higher L<sub>T</sub> contents, as seen at 18 months (Fig. 2C). As raw organic matter decomposes, such as in composting situations, lignin content and density in the resulting material increases.

Lignin dynamics were also apparent in TLT results; the application of laccase along with cultural management effectively decreased TLT at 6 and 18 months and increased L<sub>T</sub> content at all three sampling times (Figs. 1C and 2C). The increase in L<sub>T</sub> may be attributed to the change in the thatch biomass.
structure caused by laccase, making structural sugars more available for decomposition and a better microclimate for microbial growth due to core aeration and sand top-dressing. Increased losses of structural sugars from the thatch biomass may be responsible for the increased levels of lignin in the remaining thatch material. A significant loss in structural sugars of creeping bentgrass thatch biomass was also observed with the application of laccase during greenhouse and field studies (Sidhu et al., 2013a, 2014).

SHC was higher at 6 months with CMC treatment (Table 4). At 18 months, SHC was higher when laccase was applied in combination with CMC when compared with the control and only laccase treatments. The increase in SHC may be attributed to core aeration, which creates channels for rapid water movement. The laccase and CMC data illustrated that the 2.0 (4) treatment of laccase was effective for reducing TLT at 6, 12, and 18 months when applied alone or with CMC. Laccase alone did not influence SHC; however, in combination with CMC, SHC increased relative to the control. Laccase from different sources was equally effective for organic matter decomposition and thatch layer reduction. Laccase applications for 6 months during the second year were effective for reducing thatch layer buildup. The results of this study indicated that laccase, when applied at an optimum rate and/or frequency for 6 months (July to December) during 1 year, is effective for reducing the organic matter content and TLT. For golf course superintendents managing bentgrass putting greens, the application of laccase for 6 months each year could be an effective means of preventing thatch layer accumulation.

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