Zoysiagrass (Zoysia japonica Steud.) has relatively good tolerance to most turfgrass diseases compared with other turf species, with the exception of large patch, which is caused by Rhizoctonia solani Kühn AG 2-2 LP (Green et al., 1993). The infected yellow to brown patches could vary from <0.3 to >6 m in diameter. Without control, affected zoysiagrass starts to thin out and eventually dies, leaving voids that are often infested by weeds and typically taking most of the growing season for the turf to recover. Environmental conditions that favor large patch occurrence are relatively wet.
and cool weather with thatch layer temperatures between 15 and 25°C (Green et al., 1993). Other conditions that promote disease severity include compacted and poorly drained soils and excessive and prolonged leaf wetness (Green et al., 1993). In the transition zone, large patch symptoms are most common in spring and again in mid- to late fall, when temperatures are cool.

Management practices, such as fertilization or adjusting mowing height, show limited effects on R. solani suppression (Green et al., 1994). Other cultural practices, such as aerification or topdressing, can effectively reduce thatch (Dunn et al., 1995), and excessive thatch accumulation has been linked to a wide range of turf problems including increased soilborne diseases (McCarty et al., 2005). Although direct evidence has yet to be developed, it is likely that frequent cultural practices, such as topdressing or aerification, may contribute to R. solani control on zoysiagrass.

As a soilborne pathogen, R. solani tends to reoccur and affect zoysiagrass on the same site each year. It is believed that the initial infection occurs in fall, prior to zoysiagrass entering winter dormancy (Smith and Walker, 1972). Effective disease control requires regular applications of fungicides, such as azoxystrobin, flutolanil, or tebuconazole, with a typical split application first in the fall and again the following spring (Vincelli and Munshaw, 2014). Repeated fungicide application for control of R. solani is considerably costly and increases the possibility for the development of fungicide resistance (Van Bruggen and Arneson, 1984).

Alternatively, soil organic amendments have demonstrated suppressive effects on various soilborne pathogens, including Pythium spp., Phytophthora spp., Fusarium spp., and R. solani (Hoitink and Boehm, 1999). For instance, El-Sharouny (2015) reported that addition of Indian mustard [Brassica juncea (L.) Czern.] seed meal to soil resulted in a 77-fold increase of Pseudomonas spp. population. As a ubiquitous bacterium in agricultural soils, Pseudomonas spp. are known for their bio suppressive characteristics against soilborne pathogens (Weller, 2007). The increase of Pseudomonas spp. likely contributed to the 100% control of apple (Malus domestica Borkh.) root rot caused by R. solani AG-5 observed in this experiment (El-Sharouny, 2015). Additionally, Indian mustard plant, like other species in the Brassicaceae family, contains secondary metabolites called glucosinolates (GSLs) (Brown and Morra, 1997). In disrupted tissues, GSLs can be hydrolyzed by the enzyme myrosinase to form biologically active compounds, including isothiocyanates (ITCs), oxazolidinethiones, nitriles, and thiocynates (Vaughn et al., 2006). These compounds, especially ITCs, are highly toxic to a variety of organisms such as plant seeds, insects, bacteria, fungi, and nematodes (Brown and Morra, 1997). Similarly, You and Sivasithamparam (1995) found that incorporation of organic mulch (oat [Avena sativa L.] straw and chicken manure mixture) increased the population of soil actinomycete, a plant growth promoter and a known biocontrol agent against soilborne pathogens (El-Tarabily and Sivasithamparam, 2006). The increase of actinomycetes was associated with the suppression of Phytophthora root rot on avocado (Persea americana Mill.) caused by Phytophthora cinnamomi (You and Sivasithamparam, 1995).

Although not fully understood, proposed mechanisms by which organic amendments suppress soilborne pathogens include competition, antibiosis, parasitism and predation, and systemic induced resistance (Lockwood, 1988).

Research investigating the interaction of cultural practices and organic amendments in relation to zoysiagrass large patch and the soil microbial community is limited. Although not fully understood, proposed mechanisms by which organic amendments suppress soilborne pathogens include competition, antibiosis, parasitism and predation, and systemic induced resistance (Lockwood, 1988).

MATERIALS AND METHODS

Experimental Site and Maintenance

A 2-yr (2013–2014) field experiment was conducted on a zoysiagrass (Z. japonica ‘Meyer’) fairway at Columbia Country Club in Columbia, MO. The soil was silt loam, Keswick-Urban land complex (fine, smectitic, mesic Aquertic Chromic Hapludalf), with 5% organic matter (OM) and a pH of 6. The fairway was maintained at 1.9-cm mowing height and fertilized with 37 kg N ha⁻¹ mo⁻¹ during the growing season. Supplemental irrigation, ~0.6 cm per each irrigation event, was supplied as needed, mainly in summer months to prevent turf from wilting. Sporadic large patch occurrences were observed in the experimental site historically and have been managed by application of tebuconazole (Torque, Nufarm Americas) one or two times per year on the basis of disease pressure in the past year. Weather data, including daily average temperature and precipitation during this experiment, were recorded by a weather station located 2.9 km away from the experimental site.

Inocula Preparation

Prior to initiation of the field experiment, six isolates of R. solani AG 2-2 LP were obtained from infected zoysiagrass in fall 2012 from the Columbia Country Club and the University South Farm in Columbia, as described by Green et al. (1993). Zoysiagrass at both sites were previously exposed to demethylation-inhibitor (DMI) fungicides. Confirmation of fungal isolates as R. solani AG 2-2 LP was performed by polymerase chain reaction (PCR, data not shown) using R. solani AG 2-2 LP-specific primers as described in Toda et al. (2004). The inocula were prepared from the mixture of the isolates using organic wheat (Triticum aestivum L.), following the procedure described by Carling and Sumner (1992), and stored at 4°C prior to use.

Field Inoculation

Field plots measuring 1.5 × 2.4 m individually with a 0.3-m border between plots were inoculated with 25 g inocula for
of two inoculation foci on 9 Oct. 2012. The inoculation foci were situated in the middle of the plots and evenly spaced. The inocula were placed 1 cm below the soil surface using a spatula for all plots including control plots. In June 2013, large patch symptoms developed in ~90% of the inoculation centers, with patches ranging from 10 to 20 cm in diameter prior to the initial treatment application.

Treatment Description

Organic amendments included an animal waste-based product (AW; chicken manure, Back to Nature), a sewage-based organic N fertilizer (ON, Milorganite), and a plant-based byproduct (PB; oriental mustard seed meal, Wisconsin Spice). Treatments also included a synthetic N fertilizer (SN; UMAXX 47–0–0 [N–P–K], Koch Agronomic Services) and a synthetic fungicide (SF; Heritage, a.i. azoxystrobin, Syngenta Crop Protection), in addition to a nontreated control (NC).

Applications of PB were performed at 1500 kg ha⁻¹ on the basis of a preliminary experiment (data not shown). According to labels, all organic amendments contain 5% N; therefore, the same rate was used for AW and ON. The SN was applied at 75 kg N ha⁻¹ per each application, which led to a total annual N fertilization of 150 kg ha⁻¹. The application of SF followed the manufacturer recommended rate of 0.6 kg a.i. ha⁻¹. The concentrations of GSLs in PB were determined by high-performance liquid chromatography following the procedure described by Charron et al. (2005). The primary GSL in PB was identified to be sinigrin, accounting for 96% of the total 122 μmol g⁻¹ GSLs.

All amendments were applied as aeration, followed by topdressing (aeration + topdressing) or topdressing alone. For aeration, plots were first aerified in two passes using hollow tines measuring 1.3 cm in diameter and 7.6 cm apart to a depth of 5.1 cm. Soil cores were then broken down and incorporated into the soil by dragging a metal mat multiple times. Topdressing was performed by mixing organic amendment or synthetic N fertilizer at a predetermined rate with topdressing sand that met the United States Golf Association (USGA) specifications (USGA, 2007) and applying to a depth of 0.6 cm. The mixture was prepared using a concrete mixer prior to the application, and after the application, the mixtures were incorporated into the turf canopy using brooms. Fungicide-treated plots and the NC plots received topdressing with sand only. After topdressing, designated plots were sprayed with azoxystrobin using a CO₂–pressurized backpack sprayer equipped with TeeJet 8006 flat fan nozzles (Spraying Systems) that were calibrated at 275 kPa to deliver 561 L ha⁻¹. Immediately after treatment application, all plots were hand-watered to provide ~2 cm of water, except the fungicide-treated plots, which were irrigated 24 h later. During this 2-yr experiment, treatments were applied twice per year, with one in spring (11 June 2013, 21 May 2014) and one in fall (1 Oct. 2013, 17 Sept. 2014).

Measurements

Phytotoxicity, for assessing potential injury caused by treatment applied, was evaluated by visual assessment on a scale of 1 to 9, where 1 represents dead grass, 9 indicates no phytotoxicity, and 6 means minimally acceptable phytotoxicity (Xiong et al., 2015). The evaluation was performed weekly in spring and fall during the growing season until any injury symptoms disappeared. Large patch development was assessed visually every other week and recorded as percentage large patch cover. The progression of large patch over the 2-yr period was summarized as area under disease progress curve (AUDPC; Campbell and Madden, 1990), calculated using Eq. [1]:

\[
\text{AUDPC} = \sum_{i=1}^{n-1} \left[ \left( X_i + X_{i+1} \right) / 2 \right] \times \left( t_{i+1} - t_i \right)
\]

where \( X_i \) = percentage large patch cover at the \( i \)th observation, \( t_i \) = days after initial treatment at the \( i \)th observation, and \( n \) = number of total observations.

Percentage green turf cover was monitored in spring and fall by digital image analysis. Digital images were captured by a Canon PowerShot SX20 IS camera on auto setting at 2, 4, 6, 8, and 19 wk for Year 1 or 20 wk for Year 2 after the initial treatment application. The images were taken at 2.5 m above the canopy using a ladder for capturing the entire plot (1.5 × 2.4 m) and analyzed by SigmaScan Pro 5 software package (Systat Software, 2007) to determine percentage green cover (Richardson et al., 2001).

At 1 and 2 yr after the initial treatment application (YAIT) in May 2014 and April 2015, respectively, five arbitrary soils cores were taken from each plot using a cup-cutter with 7.5-cm diameter to a depth of 10 cm right before the spring treatment application. The depth of thatch layers from each soil core were measured onsite using a ruler. The soil cores were immediately separated from the thatch, passed through a 2-mm sieve, mixed thoroughly, and stored at −20°C for further analysis. Soil OM was determined by the Soil and Plant Testing Laboratory at University of Missouri (Jones, 2001).

Soil samples collected at 1 and 2 YAIT were further analyzed for treatment effects on microbial community structure changes using the high throughput soil phospholipid fatty acid (PLFA) analysis described by Buyer and Sasser (2012). Briefly, soil samples between 2 and 2.5 g were added to Teflon-lined, screw-cap culture tubes and freeze dried before PLFAs were extracted, purified, and esterified into fatty acid methyl esters. Dried extracts were dissolved in hexane for identification and quantification via gas chromatography using Agilent Chemstation software (Agilent Technologies, 2004). The Sherlock (MIDI Corporation) microbial identification system was used to assign PLFA biomarkers to taxonomic microbial groups including fungi, bacteria, Gram-positive bacteria, Gram-negative bacteria, actinomycetes, and mycorrhizae. Individual microbial groups were expressed as a proportion of the total PLFA (mol %, percentage of a component in the unit of mole in a mixture). Community change indicators, including ratios of fungi to bacteria (F/B) and Gram–positive to Gram–negative bacteria (G+/G–), and stress indicators, including ratios of cyclopropyl 17 to monoenic precursors (Cy17/pre) and saturated to monounsaturated fatty acids (sat/mono), were calculated as well (Supplemental Table 1). Phospholipid fatty acid analysis was also performed for the three organic amendments used in this study. The most abundant fatty acids in three known Pseudomonas fluorescens isolates were used as markers to indicate the presence of P. fluorescens in organic amendments as well.
Treatment Effects on Large Patch Severity

Large patch development, monitored as percentage large patch cover in the NC plots, was averaged and plotted over the 2-yr period (Fig. 1). After inoculation in the previous fall, the initial disease breakout was first observed in early June 2013. During the summer months, disease symptoms disappeared, and no symptoms were observed for the remainder of 2013. In 2014, disease activity reoccurred in late May in the control plots. However, the initial disease severity in 2014 was up to 55% greater than in the previous year (Fig. 1B) and remained relatively high, with symptoms observed until mid-August and reoccurring in early October. Studies in the literature reported that diseases caused by R. solani were promoted by increasing precipitation (Yang et al., 1990). In this study, precipitation in 2014 was 55% higher than 2013 (Fig. 1A), which likely contributed to the elevated disease severity.

Neither cultural practices of aerification + topdressing nor topdressing alone significantly affected R. solani over the 2-yr study period. A similar finding was reported by Obasa et al. (2013), who found that topdressing or aerification once per year did not affect large patch occurrence or severity. Although aerification + topdressing generally reduced thatch accumulation compared with topdressing (Table 1), it apparently did not lead to fungal suppression in our study. Soil amendments, however, significantly influenced AUDPC. At 1 YAIT, AUDPC in ON-treated plots was only 64% of NC (Fig. 2A). This result is different from that reported by Green et al. (1994), who found ON at 148 kg N ha⁻¹ yr⁻¹ did not affect large patch size. This disparity could be attributed to the time of application. In the study reported by Green et al. (1994), ON was applied in summer, when zoysiagrass growth was optimum and the plants had recovered from disease activity that occurred in spring; in our study, ON was applied in spring and fall, when the pathogen was active and symptoms were developing.

Without amendment application, large patch symptoms progressed in the NC plots, and the resulting AUDPC at 2 YAIT reached 14 times that observed at 1 YAIT (Fig. 2A and 2B). At 2 YAIT, all treated plots appeared to suppress disease development relative to NC, but significant disease reductions were only observed in SF- and AW-treated plots.

### Table 1. Thatch depth as influenced by the interaction of cultural practice and amendment applied over the 2-yr period. Soil samples were collected at 1 and 2 yr after the initial treatment application. There were no three-way interactions detected with year of sampling; hence, data were pooled over year. Cultural practices included aerification followed by topdressing (aerification + topdressing) and topdressing. Amendments applied included organic amendments of an animal waste-based product (AW), a sewage-based organic N fertilizer (ON), and a plant byproduct (PB), in addition to a synthetic N fertilizer (SN), a synthetic fungicide (SF), and a nontreated control (NC).

<table>
<thead>
<tr>
<th>Cultural practice</th>
<th>Thatch depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AW</td>
</tr>
<tr>
<td>Aerification + topdressing</td>
<td>8.5bA†</td>
</tr>
<tr>
<td>Topdressing</td>
<td>10.2aA</td>
</tr>
</tbody>
</table>

† Means followed by the same lowercase letter within the same column were not significantly different based on Fisher’s Protected LSD (p > 0.05). Means followed by the same capital letters in the same row were not significantly different based on Fisher’s Protected LSD (p > 0.05).
Treatment Influence on Phytotoxicity and Percent Green Cover

During this 2-yr period, phytotoxicity was only observed from plots receiving PB, which is attributed to the GSLs it contains (Vaughn et al., 2006). Injuries observed in this study included necrosis and stunted growth, which resulted in unacceptable phytotoxicity ratings (<6) in two evaluations. The injury symptoms, however, were transient and the affected zoysiagrass plants were fully recovered in 2 wk after treatment. Cultural practices influenced the degree of injury caused by PB. In Year 1, injury symptoms were minimal or absent when PB was incorporated into the rootzone soil through aerification + topdressing, compared with plots receiving topdressing only.

Percentage green cover showed significant interactions between year and other factors; hence, data were analyzed separately by year (Table 2). Cultural practices did not significantly influence percentage green cover in either year. In Year 1, the only amendment that significantly affected percentage green cover relative to NC was PB, showing a 5% reduction. This again likely reflected a combined effect of phytotoxicity and large patch severity in PB-treated plots. In Year 2, none of the amendments affected percentage green cover significantly. Percentage green cover varied considerably along with seasonal changes, with generally higher values in the middle of the growing season (mid-summer) and reduced values toward end of the season (late fall). The dominant effect of seasonal changes on zoysiagrass turf color might have compromised the detection of

Application of fungicide resulted in 86% reduction in large patch development compared with NC. This result is in agreement with those reported by Vincelli and Munshaw (2014), who found that azoxystrobin provided excellent R. solani control. Plots treated with AW resulted in 49% reduction of AUDPC over the 2-yr period, which is likely attributed to the shift in the soil microbial community (You and Sivasithamparam, 1995).

**Fig. 1.** (A) Daily precipitation (mm) and average air temperature (°C), and (B) percentage large patch (%) at each evaluation time for the nontreated control during the experimental period from May 2013 to November 2014.

**Fig. 2.** Area under disease progress curve (AUDPC) influenced by amendments applied at (A) 1 or (B) 2 yr after the initial treatment application. Bars labeled with the same letters were not significantly different based on Fisher’s Protected LSD \( (p = 0.05) \). Amendments applied included an animal waste-based product (AW), a sewage-based organic N fertilizer (ON), and a plant byproduct (PB), in addition to a synthetic N fertilizer (SN), a synthetic fungicide (SF), and a nontreated control (NC).
Table 2. Percentage green cover influenced by aerification, followed by topdressing (aerification + topdressing) or topdressing, amendments applied, and weeks after the initial treatment application (WAIT) in Years 1 and 2 of this experiment. Amendments applied included organic amendments of an animal waste-based product (AW), a sewage-based organic N fertilizer (ON), and a plant byproduct (PB), in addition to a synthetic N fertilizer (SN), a synthetic fungicide (SF), and a nontreated control (NC).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultural practice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerification + topdressing</td>
<td>87†</td>
<td>82</td>
</tr>
<tr>
<td>Topdressing</td>
<td>88</td>
<td>81</td>
</tr>
<tr>
<td>Amendment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AW</td>
<td>87a‡</td>
<td>80</td>
</tr>
<tr>
<td>ON</td>
<td>89a</td>
<td>82</td>
</tr>
<tr>
<td>PB</td>
<td>83b</td>
<td>81</td>
</tr>
<tr>
<td>SN</td>
<td>89a</td>
<td>80</td>
</tr>
<tr>
<td>SF</td>
<td>89a</td>
<td>84</td>
</tr>
<tr>
<td>NC</td>
<td>87a</td>
<td>80</td>
</tr>
<tr>
<td>WAIT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>92b</td>
<td>92ab</td>
</tr>
<tr>
<td>4</td>
<td>91b</td>
<td>83c</td>
</tr>
<tr>
<td>6</td>
<td>97a</td>
<td>95a</td>
</tr>
<tr>
<td>8</td>
<td>99a</td>
<td>89b</td>
</tr>
<tr>
<td>19§</td>
<td>58c</td>
<td>47c</td>
</tr>
</tbody>
</table>

† Mean separation was not performed when ANOVA indicated a nonsignificant effect at 0.05 probability level.
‡ Means followed by the same letters within the same column influenced by the same factor were not significantly different based on Fisher’s Protected LSD (p = 0.05).
§ In Year 2, percentage green cover was collected at 20 WAIT.

Large patch development influenced by treatment applied. In comparison, data summarized in AUDPC factored in disease development over time, which collectively reflected the overall treatment effect.

Treatment Effects on Soil Microbial Community Composition

Phospholipid fatty acid analysis for determining treatment effects on soil microbial structure changes at 1 and 2 YAIT were presented as interactions when detected, or as treatment main effects when appropriate. Among microbial groups detected, fungi and actinomycetes were affected significantly by cultural practices (Table 3). Aerification + topdressing resulted in lower fungi and higher actinomycetes, whereas topdressing provided the opposite effect. Fungal hyphae in the soil have been reported to be fragile and sensitive to physical disturbance (Kabir et al., 1999). Aerification, especially core aerification performed on turf, disturbs soil in a way that is similar to tillage on row crops (Haynes et al., 2013). A 1.5× reduction in fungal biomass was reported from conventional tillage on row crops compared with reduced-tillage (Frey et al., 1999) or no-tillage (Beare et al., 1997) systems. Actinomycetes are ubiquitous soil bacteria that possess biological control characteristics against soilborne fungal pathogens (Williams, 1976). These bacteria were reported to proliferate in soil with high contents of humus or litter (Lechevalier, 1981) but were less prevalent in waterlogged, anaerobic soils (Williams and Wellington, 1982). Therefore, aerification facilitated the incorporation of soil amendments into the rootzone that subsequently promoted actinomycetes, which, in turn, may suppress fungi through competition.

Although the overall bacterial proportion was not significantly influenced by cultural practices, the F/B ratios were ~2% lower in plots receiving aerification + topdressing than in plots receiving topdressing (Table 3). This was presumably due to the reduced fungal proportion in the plots receiving aerification + topdressing. Changes in relative abundance of the two microbial groups suggested a shift toward bacterial dominance after aerification, which resembles tilling, where F/B ratios can be up to 7% higher in a no-tillage soil system than conventional tillage (Zhang et al., 2012).

Cultural practices also influenced microbial stress indicators, ratios of Cy17/pre and sat/mono (Table 3). These indicators were developed to detect soil microbial shifts in response to unfavorable conditions, such as extreme temperatures and pH, suboptimal substrates and water, and toxin accumulations (Banks et al., 2014). Cyclopane fatty acids (CFAs), a group of fatty acids that contain a cyclopropane ring, are typically found in membrane phospholipids of Gram-negative bacteria (Zelles, 1999). Biosynthesis of CFAs is believed to be post-translationally modified in response to various stress conditions (Kaur et al., 2005). One of the cis monounsaturated CFAs, cy16:1ω7, is a metabolic precursor of cyclopropyl fatty acid cy17:0. The elevated transmethylation that converts cy16:1 to
cy17:0 was commonly observed in bacterial cultures when growth became stationary in response to nutrient deficiency (Bossio and Scow, 1998), lack of substrates (Kieft et al., 1997), or other environmental stresses such as heat (Trögl et al., 2015). Such a transition from unsaturated to saturated fatty acids is believed to alter membrane fluidity in response to cellular degradation under stresses (Kaur et al., 2005). In our study, ratios of Cy17/pre in plots receiving aerification + topdressing were ~5% lower than topdressed plots (Table 3), suggesting that aerification + topdressing likely created a less stressful condition than topdressing for Gram-negative bacteria in the rootzone soil.

Similar to Cy17/pre, aerification + topdressing led to a 3% lower sat/mono ratio than topdressing (Table 3). Earlier studies demonstrated that sat/mono ratios increased when bacteria were under substrate or nutrient limitations (Knivett and Cullen, 1965; Kieft et al., 1997). Hence, results from these microbial stress indicators collectively suggested that, under topdressing, bacterial community experienced greater stress than those under aerification + topdressing.

In addition to cultural practices, soil amendments also altered proportion of certain microbial groups in the total microbial biomass, in particular actinomycetes. The decreased proportion of actinomycetes was found in both PB- and SF-treated soils, compared with NC (Fig. 3A). This result is consistent with that reported by Bácimagá et al. (2015), who found that increasing rate of azoxygen (a.i. of SF) led to decreasing actinomycetes biodiversity in the soil microbial community. Additionally, You and Sivasithamparam (1995) suggested that elevated soil actinomycete could contribute to suppression of soilborne pathogens; however, results from our study did not support this observation.

Soil amendments also affected G+/G− ratios. Treatment with AW, SN, and SF resulted in a reduced ratio of G+/G− by 4, 3, and 3%, respectively, compared with NC (Fig. 3B). Lower G+/G− ratio in AW-treated soils compared with NC was previously reported by Larkin et al. (2006). Therefore, the shift to a greater proportion of Gram-negative bacteria in the AW-treated plots could be attributed, at least partially, to the higher amount of antifungal metabolites such as HCN, siderophore, protease, 2,4-diacyltrophoglucinol, fluorescent pigments, and pyrrolnitrin or by increasing chitinase and peroxidase activities (Nandakumar et al., 2001; Afsharmanesh et al., 2006). Therefore, the shift to a greater proportion of Gram-negative bacteria in the AW-treated plots could be attributed, at least partially, to the higher amount of P. fluorescens species that AW contains, which consequently led to the large patch suppression observed in our experiment.

Results of mycorrhizal proportion were found to be influenced by the interaction between amendments and YAIT. As beneficial fungi, mycorrhizae colonize plants roots and facilitate the uptake of immobile nutrients, especially P (Bolan, 1991). At 1 YAIT, mycorrhizal proportion in AW and SF were 2 and 6% higher than NC, respectively (Fig. 4). However, compared with the NC, no differences in mycorrhizal proportions were found at 2 YAIT. Previous studies reported that animal manure promoted up to 25% higher mycorrhizae in the soil (Tarkalson et al., 1998). A close examination of our results found that the interaction was attributed to treatment with...
SF, where mycorrhizal proportion reduced up to 5% at 2 YAIT compared with 1 YAIT (Fig. 4). This result suggested that repeated applications of SF for two consecutive years may lead to reduced mycorrhizal proportion, which might cause a negative impact on overall turf health, since mycorrhizae are considered beneficial fungi to plants.

CONCLUSION
Cultural practices and the addition of organic amendments to soil can lead to changes in soil microbial composition. Aerification + topdressing reduced fungi, stimulated bacterial community, and reduced F/B ratios compared with topdressing alone. Compared with topdressing, aerification reduced fungi but stimulated bacterial community and resulted in reduced fungi and F/B ratio and a less stressful environment for beneficial microbes, as indicated by two stress indicators. Over the 2-yr study period, AW, SN, and SF significantly lowered G+/G− ratios relative to the NC, with the pattern resembling the trend observed for AUDPC. Among the organic amendments included in this experiment, AW significantly suppressed \textit{R. solani} over the 2-yr period, with the effectiveness comparable with fungicide application. Collectively, our results highlight the potential for AW to be included in an integrated pest management plan for controlling \textit{R. solani} on zoysiagrass fairways.

Table 4. Proportion of bacteria, actinomycetes, and \textit{Pseudomonas fluorescens} in microbial community of organic amendments included in this experiment (i.e. an animal waste-based product, a sewage-based organic N fertilizer, and a plant byproduct).

<table>
<thead>
<tr>
<th>Organic amendment</th>
<th>Bacteria</th>
<th>Actinomycetes</th>
<th>\textit{P. fluorescens}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal waste-based product</td>
<td>45.3a†</td>
<td>8.1a</td>
<td>5.4a</td>
</tr>
<tr>
<td>Organic N fertilizer</td>
<td>24.3b</td>
<td>1.9b</td>
<td>3.1b</td>
</tr>
<tr>
<td>Plant byproduct</td>
<td>0.4c</td>
<td>0.0c</td>
<td>0.2c</td>
</tr>
</tbody>
</table>

† Means followed by the same letter for the same parameter were not significantly different based on Fisher’s Protected LSD ($p = 0.05$).

Fig. 4. Proportion of mycorrhizae (mol %) in the soil microbial community influenced by the interaction of amendments applied and years after the initial treatment application (YAIT). Soil samples were collected at 1 and 2 YAIT. Bars labeled with the same lowercase letters in the same year were not significantly different based on Fisher’s Protected LSD ($p = 0.05$). Bars labeled with the same capital letter for the same amendment were not significantly different based on Fisher’s Protected LSD ($p = 0.05$). Amendments applied included an animal waste-based product (AW), a sewage-based organic N fertilizer (ON), and a plant byproduct (PB), in addition to a synthetic N fertilizer (SN), a synthetic fungicide (SF), and a nontreated control (NC).

Conflict of Interest
The authors declare that there is no conflict of interest

Supplemental Material Available
Supplemental material for this article is available online.

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References


Systat Software. 2007. SigmaScan Pro software. Release 5.0. Systat Software, San Jose, CA.


