RESEARCH

Abundance of Bacteria, Fungi, and *Sclerotinia homoeocarpa* in the Thatch and Soil of Golf Courses

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ABSTRACT

Thatch management in turfgrass has been recommended as part of integrated pest management; however, there is limited understanding of the microbial community in thatch. Previous studies on the turfgrass phytobiome mostly focused on the soil; however, culture-based studies have suggested that the thatch layer of golf courses contains higher bacterial and fungal abundances than the soil. In our study, quantitative PCR was used to investigate total abundance of bacteria, fungi, and the turfgrass pathogen, *Sclerotinia homoeocarpa* (causal agent of dollar spot) in the thatch and soil of three golf courses on two sampling dates. Additionally, we compared the abundance of these organisms among roughs, fairways, and putting greens, which are under different management intensities. Our results demonstrate bacterial abundance was higher in May than in September,

but not consistently higher in the thatch or soil among the three golf courses or management areas. Fungi, and specifically *S. homoeocarpa*, are more abundant in the thatch than in the soil. These results show the necessity for future turfgrass phytobiome studies to analyze both thatch and soil to obtain a complete picture of bacterial and fungal microbial community structure and dynamics on golf courses. Despite the differences in fungicide usage and management inputs, there were no differences in *S. homoeocarpa* abundance among the three management areas in the soil. *S. homoeocarpa* abundance was higher in the thatch on the conventional golf course fairway in September. These results may have important practical implications for development of integrated disease management strategies and for understanding the epidemiology of *S. homoeocarpa* on golf courses.

Studies on turfgrass microbes, especially pathogens, are critical for understanding pathogen epidemiology and biocontrol potential to reduce disease pressure on golf courses. Perennial turfgrass ecosystems, such as golf courses, consist of three distinct layers: the canopy or grass blades, the thatch, and the underlying soil. The thatch is the layer of decaying grass leaf blades residing between the actively growing canopy and the soil, which would be classified as the organic horizon in soil profiles. Most studies on the turfgrass phytobiome have focused on soil or rhizosphere communities, but studies using culture-based approaches have reported that the thatch has higher amounts of bacteria and fungi than the soil (Dell et al.

2012; Liu et al. 1995; Mancino et al. 1993). Typically, the thatch has a higher moisture content and more nutrients, due to decomposition of cellulose and hemicellulose, which may be responsible for the increase in microbial biomass (Mancino et al. 1993). The presence and abundance of turfgrass pathogens in the thatch and soil should be assessed for better evaluation of inoculum abundance and better understanding of where the organisms are located to improve cultural and chemical disease management approaches.

Sclerotinia homoeocarpa F.T. Bennett is a sterile ascomycete fungus that causes dollar spot disease of turfgrass worldwide. It is the most economically damaging disease of turfgrass in the northern United States, and fungicides for its control account for the majority of chemical budgets on golf courses (Bennett 1937; Walsh et al. 1999). It can infect all species in the family Poaceae, including the important golf course species: *Agrostis stolonifera* Linnaeus (creeping bentgrass), *A. capillaris* Linnaeus (colonial bentgrass), *Poa annua* Linnaeus (annual bluegrass), and *Poa pratensis* Linnaeus (Kentucky bluegrass) (Latin 2011). Better understanding of *S. homoeocarpa* epidemiology is essential for disease control as it can aid in developing chemical, cultural, and biological control practices. Fenstermacher (1980) determined that *S. homoeocarpa*

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overwinters in turfgrass as mycelia in decaying plant matter. Halisky et al. (1981) determined that increased thatch led to an increase in dollar spot disease. More recently, Lee et al. (2015) examined plant canopy, thatch, and soil for the presence of *S. homoeocarpa* at different distances from dollar spot infection centers to soil. They were able to culture *S. homoeocarpa* from 100% of the plant canopy and 87% of the thatch samples near the infection center using a toothpick-baiting mediated culture method; however, *S. homoeocarpa* was never detected from the soil samples even under the infection center.

Therefore, we investigated the total abundance of bacteria, fungi, and specifically *S. homoeocarpa* in the thatch and soil of golf courses using quantitative PCR (qPCR). This technique can detect low amounts of DNA from a single organism and may be more sensitive to the presence of small amounts of *S. homoeocarpa* mycelia (Rioux et al. 2014) than the culture methods employed by Lee et al. (2015). We hypothesized there would be a greater abundance of bacteria, fungi, and *S. homoeocarpa* in the thatch than in the soil. We also hypothesized that fungal and *S. homoeocarpa* abundance would be negatively affected by increased management intensities and therefore we would find a lower abundance of these organisms on the putting greens in spring and fall seasons. We expected to find higher abundances of *S. homoeocarpa* on the fairways compared with the roughs and putting greens. Disease may be more severe on fairways than the roughs due to frequent mowing that creates wound sites that can be readily colonized by the fungi (Latin 2011), but not frequent enough to reduce soil moisture and inhibit fungal growth, as well as fewer fungicide applications on the fairways than the putting greens due to financial constraints and lower expectations. The results of this study will provide important recommendations for sampling procedures for future turfgrass phytobiome studies. The results will add further insight on microbial colonization of thatch and soil on roughs, fairways, and putting greens, and more specifically provide a greater understanding of the life cycle of *S. homoeocarpa*. Since important microbes reside in the thatch, this area is important to include in turfgrass phytobiome research as it will provide a more complete understanding of turfgrass pathogens, beneficial microbes, and the interrelationship of these organisms with plants to develop better disease management strategies.

MATERIALS AND METHODS

Thatch and soil samples were collected on 21 May and 8 September 2014 on three golf courses in Martha's Vineyard,

MA located within 10 km of one another. The golf courses were managed conventionally (with synthetic pesticides, plant growth regulators, and fertilizers), organically (no synthetic pesticides, plant growth regulators, or fertilizers), and the third was managed conventionally on putting greens but with reduced inputs on the fairways and roughs that we have referred to as "hybrid". Products used on the conventional course included succinate dehydrogenase inhibitor fungicides, demethylation inhibitor fungicides, quinone outside inhibitor fungicides, dithiocarbamate fungicides, indoxacarb insecticide, and carfentrazone herbicide. These same products were used on the hybrid course putting greens along with polyoxin fungicides, carbamate fungicides, chlorothalonil fungicide, and bifenthrin insecticide. No synthetic pesticides were used on the hybrid fairways, except for one application of fluzinam fungicide in 18 years. The biological control agent *Pseudomonas aureofaciens* TX-1 was applied through the irrigation lines on the hybrid fairways. The organic course applied the biological control agents *Bacillus subtilis* (Rhapsody, Bayer CropScience, NC) and *B. licheniformis* (Serenade, Bayer CropScience, NC), and the petroleum byproduct CivitasONE (Suncor Energy, Inc., Alberta, Canada) for fungal diseases, and the hot water-detergent weed control system Waipuna (Waipuna Systems LTD, IL). Samples were taken from roughs, fairways, and putting greens of three holes (representing the play area from tee box to putting green) as biological replicates per course. Samples were taken with a 2.5-cm-diameter tubular soil sampler to a depth of 10 cm below the thatch layer, as described in Allan-Perkins et al. (2017), with the sampler wiped clean between sampling locations. Sampling transects were established based on GPS coordinates and irrigation heads along each fairway, rough, and putting green. Four transects, equidistant along the entire length and width of each fairway, were set up and extended into the rough. Three samples were taken along each fairway transect ($n = 12$) and one on each rough transect ($n = 4$) per hole. The putting green transects were two perpendicular lines bisecting the putting green, and samples ($n = 4$ per transect, total $n = 8$ per hole) were taken along those lines at 1.5 and 4.6 m from the edge of the putting green. Soil texture was analyzed as described in Allan-Perkins et al. (2017), and showed the conventional roughs and fairways were loamy fine sand and all other samples were fine sand. The thatch was removed from each core, and the thatch samples were pooled for each management area on each hole and placed in a resealable zipper storage bag resulting in one fairway, one rough, and one putting green sample per hole ($n = 9$ thatch samples per course, $n = 27$ samples per sampling time). The soil portion of the core samples after thatch removal were

TABLE 1
P values generated by repeated measures analysis of variance for bacterial abundance measured as 16S copies

Sample	16S copies per milliliter of starting material			16S copies per gram of starting material		
	Management area	Season	Management area $\times$ season	Management area	Season	Management area $\times$ season
Soil						
Conventional	<0.0001	<0.0001	0.0019	0.0009	0.0042	NS ^a
Hybrid	0.0421	<0.0001	NS	0.0048	NS	NS
Organic	NS	<0.0001	NS	0.0063	NS	NS
Thatch						
Conventional	0.0050	NS	NS	0.0035	NS	NS
Hybrid	NS	NS	0.0266	NS	NS	0.0388
Organic	NS	NS	NS	0.0463	NS	NS

^a NS indicates no significant results ($P > 0.05$).

not pooled, resulting in a total of 24 samples per hole per course per sampling time ($n = 72$ soil samples per course, $n = 216$ samples per sampling time) for use in additional experiments. All samples were transported back to the laboratory on ice and frozen upon arrival.

DNA was extracted from three soil samples and three thatch samples from each management area and hole combination ($n = 27$ soil extracts per course, $n = 81$ soil extracts per sampling date). The soil samples used for DNA extraction were chosen from three different transects spaced apart along the fairways and roughs and from three equidistant points on the putting greens and the same sampling sites were used for May and September to ensure repeated measures. Three subsamples were taken from each pooled thatch sample and used for DNA extraction ($n = 27$ thatch extracts per course, $n = 81$ thatch extracts per sampling date). DNA was extracted from 0.25 g of each thatch and soil sample using the PowerClean Soil DNA Extraction Kit (Mo Bio Laboratories, Carlsbad, CA), without modifications. DNA concentration was checked by Nanodrop ND-1000 spectrophotometer.

qPCR was performed for 16S ribosomal DNA (rDNA) for bacterial abundance, 18S rDNA for fungal abundance, and elongation factor alpha one gene (EF1 α) for *S. homoeocarpa* abundance. Primers for 16S abundance were 27F and 338R targeting the V1 to V3 region (Lane et al. 1985; Marchesi et al. 1998). The 18S primers were nu-SSU0817 and nu-SSU1196 designed by Borneman and Hartin (2000). Although these 18S primers have

been reported to amplify rDNA from invertebrates, it is considered optimal for amplifying all four major families of fungi (Anderson et al. 2003). The EF1 α primers were EF1 α -Nest-F and EF1 α -Nest-R developed by Abd-Elmagid et al. (2013) and Rioux et al. (2014) to be specific for *S. homoeocarpa*. We confirmed the specificity of these primers again using NCBI PrimerBlast 3, to which we only found matches to the EF1 α gene within *S. homoeocarpa*.

All reactions were performed on a Rotor-Gene Q thermocycler (Qiagen, Germantown, MD) using ThermoFisher Maxima Syber-Green Master Mix (final concentration 1 $\times$) with primers at a final concentration of 0.5 μ M in a final reaction volume of 30 μ l. For the total bacteria (16S) and fungi (18S) qPCR reactions, 2 μ l of DNA extract (20 $\times$ dilution) was added to each well; and for the *S. homoeocarpa* qPCR, 2 μ l of undiluted DNA extract was used. DNA extractions yielded average concentrations of 30 ng/ μ l; therefore, approximately 60 ng of DNA was used for *S. homoeocarpa* qPCRs and 3 ng of DNA for bacteria and fungi qPCRs. Nucleotide-free water was used as a negative control. DNA standards were created by cloning the genes of interest (16S from *Pseudomonas putida* Trevisan KT2440, 18S from *Aspergillus niger* Tieghem, and EF1 α from *S. homoeocarpa*) into pCR2.1 vector using the Invitrogen TA cloning kit (Thermo Fisher Scientific Inc., Carlsbad, CA), transforming into competent *Escherichia coli* cells, and purifying the product with the QiaPrep Spin Miniprep Kit (Qiagen, Hilden, Germany). The pure cultures of *Pseudomonas*

TABLE 2
P values generated by repeated measures analysis of variance for fungal abundance measured as 18S copies

Sample	18S copies per milliliter of starting material			18S copies per gram of starting material		
	Management area	Season	Management area $\times$ season	Management area	Season	Management area $\times$ season
Soil						
Conventional	<0.0001	<0.0001	0.0024	<0.0001	0.0002	NS ^a
Hybrid	<0.0001	0.0025	NS	<0.0001	0.0030	NS
Organic	<0.0001	0.0045	NS	0.0003	0.0384	NS
Thatch						
Conventional	0.0295	NS	NS	0.0223	NS	NS
Hybrid	0.0425	NS	NS	NS	NS	NS
Organic	0.004	NS	0.0277	0.0302	NS	0.0257

^a NS indicates no significant results ($P > 0.05$).

TABLE 3
P values generated by repeated measures analysis of variance for *Sclerotinia homoeocarpa* abundance measured as EF1 α copies

Sample	EF1 α copies per milliliter of starting material			EF1 α copies per gram of starting material		
	Management area	Season	Management area $\times$ season	Management area	Season	Management area $\times$ season
Soil						
Conventional	NS ^a	NS	NS	NS	NS	NS
Hybrid	NS	NS	NS	NS	NS	NS
Organic	NS	NS	NS	NS	NS	NS
Thatch						
Conventional	0.0005	0.0023	0.0316	<0.0001	0.0002	0.0154
Hybrid	NS	NS	NS	NS	NS	NS
Organic	NS	NS	NS	NS	NS	NS

^a NS indicates no significant results ($P > 0.05$).

putida and *A. niger* were obtained from the USDA-ARS Laboratory in Fort Collins, CO and the *S. homoeocarpa* culture was field isolate HRS10 (Hulvey et al. 2012; Popko et al. 2012). The concentration of each plasmid was estimated using the Nanodrop ND-1000

Fig. 1. Average abundance of bacteria (based on 16S rDNA) in soil and thatch on the conventional, hybrid, and organic courses across two time points (May and September 2014). Bars represent average abundance for three technical and three biological replicates among all management areas and both sampling dates ($n = 486$). Lowercase letters represent statistically significant differences ($P < 0.05$) within a golf course as determined by repeated measures analysis of variance and mean separation by Tukey's honest significant difference.

Fig. 2. Average abundance of fungi (based on 18S rDNA) in soil and thatch on the conventional, hybrid, and organic courses across two time points (May and September 2014). Bars represent average abundance for three technical and three biological replicates among all management areas and both sampling dates ($n = 486$). Lowercase letters represent statistically significant differences ($P < 0.05$) within a golf course as determined by repeated measures analysis of variance and mean separation by Tukey's honest significant difference. A lack of letters for the hybrid course represents no significant differences.

spectrophotometer and diluted to make the standards. The bacteria and fungi standards were at 1,000, 100, 10, 1, and 0 pg/ μ l and the *S. homoeocarpa* standards were 100, 10, 5, 1, 0.5, and 0 pg/ μ l. Samples and standards were run in triplicate using the following qPCR parameters for 16S, 18S, and EF1 α on the Qiagen Rotor-Gene: hold of 95°C for 3 min, then 35 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 60 s, followed by a final annealing at 72°C for 5 min and final melting of 72°C to 95°C at 1°C intervals with 5 s between each step. Melting curves were examined to confirm primer specificity with qPCR parameters (data not shown).

DNA was quantified against the standards using the critical threshold (Ct) value. Standard curves were generated for each qPCR by linear regression of the known concentrations of the DNA standards to the Ct value for each standard. Standard curve R^2 values below 0.95 were considered poor and the qPCR was rerun. The concentration of DNA for the unknown samples was estimated from the standard curves and converted to gene copies per gram of starting material using the equation of Frost et al. (2011) with the modification of correcting for plasmid volume and dilution factor (20 $\times$) for 16S and 18S and (1 $\times$) for *S. homoeocarpa*. Although equal weights of thatch and soil were used for the DNA extractions, the large difference in density of material resulted in different volumes of starting material being used. Therefore, we measured the density of each soil sample and thatch sample using the water displacement method. We converted the grams of starting material to milliliters of starting material using sample density to obtain gene copies for milliliter of starting material. All values were logarithmically transformed to meet assumptions of normality.

Significant differences among bacterial (16S), fungal (18S), and *S. homoeocarpa* (EF1 α) abundance were analyzed using repeated measures analysis of variance (ANOVA) first for gene copy per gram of starting material and then again for gene copy per milliliter of starting material. The repeated measures ANOVA was analyzed among management areas within each golf course using a split-plot

Fig. 3. Average abundance of *Sclerotinia homoeocarpa* (based on EF1 α DNA) in soil and thatch on the conventional, hybrid, and organic courses across two time points (May and September 2014). Bars represent average abundance for three technical and three biological replicates among all management areas and both sampling dates ($n = 486$). Lowercase letters represent statistically significant differences ($P < 0.05$) within a golf course as determined by repeated measures analysis of variance and mean separation by Tukey's honest significant difference.

with management area and sampling depth (soil versus thatch) as the main effects, season as the split plot, and hole as a random effect using PROC GLIMMIX in SAS v. 9.4 (SAS Institute, Cary, NC). Where sampling depth was significantly different, additional repeated measures ANOVA analyses were run for each sampling depth separately for each golf course with season as the split plot, hole as the random effect, and management area as the main effect. Means were separated using Tukey's honest significant difference (HSD) and the analyses were done using PROC GLIMMIX.

RESULTS

The statistical differences were the same for gene copy number per gram or per milliliter of starting material, with a few exceptions (Tables 1, 2, and 3). The conventional course had approximately 1.5 times more bacteria in the thatch than in the soil ($P < 0.0001$). However, the hybrid and organic course had about 1.5 times more

bacteria in the soil than in the thatch ($P = 0.0007$ for milliliter of starting material and $P < 0.0001$ for grams of starting material, Fig. 1). Fungi were significantly greater in abundance in the thatch than in the soil on all three courses ($P = 0.0007$ for the conventional course and $P < 0.0001$ for the hybrid and organic courses). There was approximately 176 times more fungi in the thatch than in the soil on the conventional course and approximately 70 times in the thatch on the soil on the hybrid and organic courses (Fig. 2). *S. homoeocarpa* was about 7 times more abundant in the thatch on the hybrid ($P > 0.05$, not significant) course and about 14 times greater in the thatch on the conventional ($P < 0.0001$) and organic ($P = 0.0028$ for milliliter of starting material and $P = 0.0021$ for grams of starting material) courses compared with the soil on all sampling dates (Fig. 3).

Within soil samples, there were more bacteria in May than in September for all three golf courses (Table 1; Fig. 4B, D, and F). There were significant differences among management areas for the

Fig. 4. Average abundance of bacteria (based on 16S rDNA abundance) from the soil samples for **A**, conventional course among management areas and sampling dates ($n = 27$), **B**, conventional course among sampling dates ($n = 81$), **C**, hybrid course among management areas and sampling dates ($n = 27$), **D**, hybrid course among sampling dates ($n = 81$), **E**, organic course among management areas and sampling dates ($n = 27$), and **F**, organic course among sampling dates ($n = 81$). **A**, **C**, and **E**, Lowercase letters represent statistically significant differences ($P < 0.05$) among management areas for May and x, y, and z among management areas for September) as determined by repeated measures analysis of variance (ANOVA) and mean separation by Tukey's honest significant difference. **B**, **D**, and **F**, Lowercase letters represent statistically significant differences ($P < 0.05$) among sampling dates as determined by repeated measures ANOVA. A lack of letters represents no significant differences.

conventional and hybrid golf courses, but not the organic course (Table 1; Fig. 4A, C, and E). On the conventional golf course, there was a significant interaction of management area and season (Table 1). The roughs had higher bacterial abundances per milliliter of soil on the conventional course and the putting greens were lowest in May only (Fig. 4A). The hybrid course tended to have more bacteria on the putting greens; however, the mean separation was not significant when analyzed by Tukey's honest significant differences (Fig. 4C).

In the thatch, bacterial abundance was only significantly different among management areas for the conventional course and the interaction of management area and season for the hybrid course (Table 1). On the conventional course, the fairways had the greatest bacterial abundance per milliliter of thatch (Fig. 5A). On the hybrid course, the fairways had a higher bacterial abundance than the roughs, with the putting greens having similar abundance to both areas in September (Fig. 5C).

Fungal abundance in soil was significantly different among management areas and seasons on all three golf courses (Table 2). All three courses had higher total fungal abundances in May than in September (Fig. 6B, D, and F). The roughs had the greatest fungal abundances on all courses in both seasons (Fig. 6A, C, and E).

In the thatch, fungal abundance was significantly different among management areas for all courses and for the interaction of management area and season for the organic course (Table 3). Although there was no significant interaction of management area and season for the conventional course, least mean square separation determined the fairways were greatest in fungal abundance in September (Fig. 7A). The fairways and putting greens had higher abundances of fungi than the roughs on the hybrid course; however, mean differences were not significant when analyzed by Tukey's HSD (Fig. 7C). The roughs on the organic course had greater fungal abundance in May only (Fig. 7E).

Fig. 5. Average abundance of bacteria (based on 16S rDNA abundance) from the thatch samples for **A**, conventional course among management areas and sampling dates ($n = 27$), **B**, conventional course among sampling dates ($n = 81$), **C**, hybrid course among management areas and sampling dates ($n = 27$), **D**, hybrid course among sampling dates ($n = 81$), **E**, organic course among management areas and sampling dates ($n = 27$), and **F**, organic course among sampling dates ($n = 81$). **A, C, and E**, Lowercase letters represent statistically significant differences ($P < 0.05$) among management areas within sampling dates (a, b, and c among management areas for May and x, y, and z among management areas for September) as determined by repeated measures analysis of variance (ANOVA) and mean separation by Tukey's honest significant difference. **B, D, and F**, Lowercase letters represent statistically significant differences ($P < 0.05$) among sampling dates as determined by repeated measures ANOVA. A lack of letters represents no significant differences.

There were no differences in *S. homoeocarpa* abundance in the soil among management areas or seasons for all three courses (Table 3; Fig. 8). In the thatch, there were significant differences for the conventional course only (Table 3). In September, there was significantly higher copies of EF1 α of *S. homoeocarpa* per milliliter of thatch on the fairways than on the roughs or putting greens, but no differences among management areas in May (Fig. 9A). There was an overall higher abundance of *S. homoeocarpa* in the thatch in September than in May for the conventional course, but not the other two courses (Fig. 9B, D, and F).

DISCUSSION

Our hypothesis that microbial abundance would be greater in thatch than in soil was only supported for the bacteria on the conventional course, not the hybrid or organic courses, whereas fungal abundance was consistently greater in the thatch. Mancino

et al. (1993) and Liu et al. (1995) reported increased bacterial abundance in the thatch compared with the soil using culture-based techniques. It is estimated that approximately only 10% of bacteria are identified by culture-based techniques (Hugenholtz 2002). Additionally, qPCR is considered to be a highly sensitive method, able to detect lower copy numbers than culture-based methods, assuming proper primer design (Lemmon and Gardner 2008). Therefore, it is likely that both the Mancino et al. (1993) and Liu et al. (1995) studies underestimated many bacterial species in the soil that we were able to detect with qPCR. The difference in bacterial abundance in the thatch and soil among the three courses could be due to different species on the courses. Potentially, the conventional course contains more bacterial species able to colonize the thatch, whereas the hybrid and organic courses contain more species better suited to the soil. Another possible explanation for the increased bacteria in the thatch of the conventional course could be that those bacterial species could metabolize the chemicals being

Fig. 6. Average abundance of fungi (based on 18S rDNA abundance) from the soil samples for **A**, conventional course among management areas and sampling dates ($n = 27$), **B**, conventional course among sampling dates ($n = 81$), **C**, hybrid course among management areas and sampling dates ($n = 27$), **D**, hybrid course among sampling dates ($n = 81$), **E**, organic course among management areas and sampling dates ($n = 27$), and **F**, organic course among sampling dates ($n = 81$). **A, C, and E**, Lowercase letters represent statistically significant differences ($P < 0.05$) among management areas within sampling dates (a, b, and c among management areas for May and x, y, and z among management areas for September) as determined by repeated measures analysis of variance (ANOVA) and mean separation by Tukey's honest significant difference. **B, D, and F**, Lowercase letters represent statistically significant differences ($P < 0.05$) among sampling dates as determined by repeated measures ANOVA.

applied on that course, allowing them to reproduce and increase in abundance (Sigler and Turco 2002). Future studies into the population growth of specific bacterial species after application of pesticides would better elicit this relationship. Since fungi are better able to degrade cellulose and hemicellulose, which are in high abundance in the thatch, we were not surprised that our results supported our hypothesis that fungi would have greater abundances in the thatch than in the soil. We found that the May sampling date had higher bacterial and fungal abundance than the September date. Since turfgrass represents a perennial system, there may have been more microbes in May due to increased soil moisture and decaying material left from the winter. Therefore, a targeted study on turfgrass phytobiome over a period of a season is needed.

In addition to investigating how microbial abundance was different between thatch and soil, we also investigated how management intensities, represented by management areas, would affect

microbial abundances. Although we did not expect to see differences in bacterial abundance among the three management areas in the soil, we did see a greater abundance of bacteria on the roughs of the conventional course and the putting greens of the hybrid course in May. In the thatch, bacterial abundance was greatest on the conventional fairway on both sampling dates and the hybrid fairway in September only. Potentially, the application of synthetic pesticides provided a food source for the bacteria on the thatch on the conventional course that accumulated over time (Sigler and Turco 2002). The hybrid course applied *Pseudomonas aureofaciens* to their fairways, which may have increased the bacterial population over the course of the season (Sigler and Turco 2002). We hypothesized that fungal abundance would be negatively affected by increased management intensities and therefore would be lowest on the putting greens. Although fungal copy number in the soil tended to be lowest on putting greens, there was no consistent differences

Fig. 7. Average abundance of fungi (based on 18S rDNA abundance) from the thatch samples for **A**, conventional course among management areas and sampling dates ($n = 27$), **B**, conventional course among sampling dates ($n = 81$), **C**, hybrid course among management areas and sampling dates ($n = 27$), **D**, hybrid course among sampling dates ($n = 81$), **E**, organic course among management areas and sampling dates ($n = 27$), and **F**, organic course among sampling dates ($n = 81$). **A**, **C**, and **E**, Lowercase letters represent statistically significant differences ($P < 0.05$) among management areas within sampling dates (a, b, and c among management areas for May and x, y, and z among management areas for September) as determined by repeated measures analysis of variance (ANOVA) and mean separation by Tukey's honest significant difference. **B**, **D**, and **F**, Lowercase letters represent statistically significant differences ($P < 0.05$) among sampling dates as determined by repeated measures ANOVA. A lack of letters represents no significant differences.

in fungal copy number in the thatch. Future studies using metagenomics may be able to better characterize the soil and thatch microbial species on these areas to understand how species composition and abundance change under different management intensities and practices and how this may affect overall bacterial and fungal abundance.

Our results represent 54 replicates for each management area (three golf courses, three holes, three samples per hole, and two seasons) and showed strong statistical evidence of high number of *S. homoeocarpa* in the thatch compared with the soil, and no differences in *S. homoeocarpa* abundance among management areas. Fenstermacher (1980) suggested *S. homoeocarpa* resided in decaying plant matter during the winter and Rioux et al. (2014) confirmed with qPCR that the fungus was found within symptomatic leaf tissues over the winter. Since the thatch layer represents an area of decaying leaf tissue, it is not surprising that our results

further show during the entire year *S. homoeocarpa* resides in the thatch layer and not in the soil. This confirms the work done by Lee et al. (2015) and also provides support for the conclusion of Halisky et al. (1981) that reducing the thatch would reduce *S. homoeocarpa* by decreasing its habitat.

The lack of differences in *S. homoeocarpa* abundance among management areas was unexpected, as we hypothesized it would be greatest on fairways and lowest on putting greens on all three courses. The putting greens receive more fungicide applications and have less dollar spot disease than the fairways. The use of fungicides should reduce the inoculum of *S. homoeocarpa*; however, the lack of differences in *S. homoeocarpa* abundance in the thatch of fairways, putting greens, and roughs suggest that the increased fungicide applications are not affecting inoculum levels. One explanation is the fungistatic nature of many of the chemicals used to control dollar spot leave low levels of inoculum throughout the

Fig. 8. Average abundance of *Sclerotinia homoeocarpa* (based on EF1 α DNA abundance) from the soil for **A**, conventional course among management areas and sampling dates ($n = 27$), **B**, conventional course among sampling dates ($n = 81$), **C**, hybrid course among management areas and sampling dates ($n = 27$), **D**, hybrid course among sampling dates ($n = 81$), **E**, organic course among management areas and sampling dates ($n = 27$), and **F**, organic course among sampling dates ($n = 81$). **A, C, and E**, Lowercase letters represent statistically significant differences ($P < 0.05$) among management areas within sampling dates (a, b, and c among management areas for May and x, y, and z among management areas for September) as determined by repeated measures analysis of variance (ANOVA) and mean separation by Tukey's honest significant difference. **B, D, and F**, Lowercase letters represent statistically significant differences ($P < 0.05$) among sampling dates as determined by repeated measures ANOVA. A lack of letters represents no significant differences.

growing season (Latin 2011). The DMI fungicides are fungistatic and were applied on the conventional and hybrid courses (Ok et al. 2011). Potentially, the high organic matter in the thatch may reduce the effectiveness of some of the chemicals, as was reported by Dell et al. (1994). If the fungicides are being bound up in organic matter or detoxified by microbes, they may be less effective at controlling *S. homoeocarpa* on the thatch. The ability of *S. homoeocarpa* to survive in the thatch of all three areas, being coupled with rarely showing disease symptoms on putting greens, poses important and complex questions into the biology of this fungus. The simplest explanation is that *S. homoeocarpa* is transferred readily among management areas through fractured mycelia either traveling on equipment, animals, or humans (Smiley et al. 2005). Another possibility is that *S. homoeocarpa*, which is known to have saprophytic capabilities, is extremely successful at living in the thatch (Orshinsky et al. 2012; Smiley et al. 2005). This may explain the

similar inoculum levels in all management areas of the golf course. The exception was for the thatch samples on the conventional course on the September sampling date when there was significantly more *S. homoeocarpa* in the fairways, which coincided with the observance of dollar spot infection centers on the conventional golf course fairway, but no obvious infection centers were observed on any other management areas or golf courses. Perhaps a certain amount of inoculum of *S. homoeocarpa* is always present in the thatch of golf courses, but lesion development is only observed when a certain threshold of inoculum is reached or when there is a favorable environment. Further research into inoculum densities prior and during active infection, as well as prior and during fungicide applications, may lead to important diagnostic tools for prediction of dollar spot development.

Future studies using RNA extracted from the thatch would provide insight into the percentage of inoculum that is active, while

Fig. 9. Average abundance of *Sclerotinia homoeocarpa* (based on EF1α DNA abundance) from the thatch for **A**, conventional course among management areas and sampling dates ($n = 27$), **B**, conventional course among sampling dates ($n = 81$), **C**, hybrid course among management areas and sampling dates ($n = 27$), **D**, hybrid course among sampling dates ($n = 81$), **E**, organic course among management areas and sampling dates ($n = 27$), and **F**, organic course among sampling dates ($n = 81$). **A, C, and E**, Lowercase letters represent statistically significant differences ($P < 0.05$) among management areas within sampling dates (a, b, and c among management areas for May and x, y, and z among management areas for September) as determined by repeated measures analysis of variance (ANOVA) and mean separation by Tukey's honest significant difference. **B, D, and F**, Lowercase letters represent statistically significant differences ($P < 0.05$) among sampling dates as determined by repeated measures ANOVA. A lack of letters represents no significant differences.

still providing higher sensitivity than culturing. If the pathogen were alive in the thatch, then understanding how it overcomes fungicide applications would be essential to better understand the biology of this organism and resistance development. Potentially anastomosis occurs in the thatch, which may have implications for fungicide resistance management. If the *S. homoeocarpa* detected in the thatch is actively growing, this could change our understanding of how its interacting with its hosts, nutrients, and other microbes.

The results of this study have important implications for future studies on microbial communities on golf courses, as well as broader phytobiome studies. Currently, studies on rhizosphere microbes have mostly been limited to the soil. In addition to golf course turfgrass studied here, other ecosystems with a substantial organic horizon layer, such as forage systems, biofuel grasses, and forest ecosystems, or agricultural fields with high crop residue may find an important contribution of microbes, nutrients, and insects in this layer to the phytobiome, as well as those in the soil. Studies on the organic horizon may lead to better optimization of biocontrol organisms, such as increasing their colonization, activity, or survival in the thatch over the soil. The thatch contains different levels of carbon and nitrogen than the soil (Raturi et al. 2005), so future phytobiome studies may investigate the role of this area in nutrient cycling and carbon sequestration. Additionally, turfgrass roots grow within the thatch area, therefore studies on the myriad of interactions among these plants with the thatch microbes and nutrients may lead to discoveries into signaling mechanisms and ways to improve overall plant health. A complete picture of the rhizosphere, the community directly interacting with plant roots, should include the thatch area as well as the soil to provide a more complete understanding of the rhizobiome on turfgrass system.

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In the article “Abundance of Bacteria, Fungi, and *Sclerotinia homoeocarpa* in the Thatch and Soil of Golf Courses” by E. Allan-Perkins, D. Manter, and G. Jung, the funding section was incomplete and has now been corrected to include the United States Golf Association (USGA 2013-14-475) and the New England Regional Turfgrass Foundation as partial funders.