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Composted biosolids for golf course turfgrass management: Impacts on the soil microbiome and nutrient cycling

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ABSTRACT

Land application of biosolids is a primary means of recycling human waste products globally; however, because biosolids are also heavily enriched with nutrients and pollutants, it is necessary to consider the environmental impacts of land applications in various agro-ecosystems. To reduce costs and divert waste, biosolids from Joint Base Lewis-McChord, a military installation south of Tacoma, WA, USA, are composted with organic materials derived from the base itself. The potential for turfgrass management using the composted biosolids was tested in a field study at the military recreation facility, Eagles Pride golf course. The composted biosolids were surface-amended to golf course fairways (n = 3) at a rate of 46 Mg ha^{-1}, in split applications, fall and spring, over two years, 2015 and 2016. Soil bacterial and fungal community responses were evaluated in amended and un-amended soils from each fairway, along with soil chemical properties, including soil pH, total carbon (C), total nitrogen (N), and the C:N ratio. Soil microbial community analysis on soils sampled pre- and post-amendment did not demonstrate changes in community structure as a result of the composted biosolids application. Differences observed in soil microbial community structure appears to reflect edaphic and vegetative differences between fairways. Generally, correlations between soil properties and bacterial community OTUs were stronger than those with fungal OTUs, and predicted bacterial community functional analysis revealed several KO term groups were significantly correlated with soil TC and TN. Turfgrass biomass was unaffected, while leaf tissue N was increased by 4.5 and 10.6% in 2015 and 2016, respectively. These results suggest that amendment with composted biosolids does not strongly impact microbial communities across two years; however, long-term amendment could have affects that are not detected during this short-term study.

1. Introduction

As a byproduct of the wastewater treatment process, in 2007 biosolids production in the U.S. was estimated at 7.18 MT year^{-1} (Beech et al., 2007), and has increased steadily since that time. Land application is the internationally preferred biosolids disposal method based primarily on the potential to recycle plant nutrients and improve soil physical properties (European Commission, 2016; Sharma et al., 2017; USEPA, 2009). However, the potential for environmental degradation is a major concern, as environmental contamination, and surface or groundwater pollution can result from improper land application from a variety of organic and inorganic pollutants present in municipal biosolids (Clarke and Smith, 2011; Edwards et al., 2009; Topp et al., 2008). Therefore, biosolids land application is well-studied and international regulatory bodies have determined local application limits and standards (Al-Rajab et al., 2015; Fytili and Zabaniotou, 2008; Lloret et al., 2016; Veeresh et al., 2003). But, due to food safety concerns, use of biosolids in agroecosystems remains limited (Latare et al., 2014; Udeigwe et al., 2015). It has been proposed that combining biosolids with organic amendments may mitigate some environmental impacts of biosolids land applications (Paramashivam et al., 2017), and could create a suitable soil and nutrient management product for use in non-food specialty crops, such as golf course turfgrass.

In 2002, there were 15,827 golf courses in operation across the United States alone (Haydu et al., 2008). An 18-hole golf course occupies a median of 60.7 ha, of which 38.4 ha is actively maintained turfgrass (GCSAA, 2017), though different playing areas (e.g., fairways, greens, or roughs) are each subject to varying management intensities (Throssell et al., 2009; USEPA, 2013). The production of high-quality turfgrass, driven by aesthetics, playing conditions, and plant health,

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typically includes intensive use of irrigation, fertilization, and pesticides (Beard, 2002; Fry et al., 2013; Gillette et al., 2016; Hejl et al., 2016; King et al., 2001; Lyman et al., 2007; Mangiafico and Guillard, 2007; Slavens and Petrovic, 2012; Wong and Haith, 2013).

The composition and function of the soil microbial community largely controls organic matter decomposition, carbon (C) sequestration, soil aggregation, and many other aspects of soil health and ecosystem function (Rillig and Mummey, 2006; Six et al., 2006; Zhou et al., 2011). In particular, the rate of nitrogen (N) cycling and the size of N pools are heavily moderated by soil microorganisms (Elser et al., 2007; Hayatsu et al., 2008), which consequently moderate plant productivity in agro-ecosystems including turfgrass systems (Gai et al., 2016; Sabagh et al., 2017). Many microbial taxa and the related enzymatic systems responsible for key transformations in the N cycle are well studied (Levy-Booth et al., 2014; Regan et al., 2017); however examination of the microbial community in its entirety (structure, composition, and diversity comprising the "microbiome") is often a better predictor of overall soil function than any individual enzyme or one specific taxon (Kaiser et al., 2014). In fact, shifts in the microbiome have been linked to global scale ecosystem processes (Wagg et al., 2014) and their inclusion in biogeochemical modeling enhances predictive power dramatically (Powell et al., 2015).

Previous research has shown that land-use change (conversion from a forest to a turfgrass system) can be a dominant force shaping soil microbial communities in turfgrass systems (Bartlett et al., 2007; Yao et al., 2006). Yet, very little is known concerning what occurs in the turfgrass microbial community as a result of the varying and highly intensive management regimes of golf course turfgrass systems, particularly with the addition of composted biosolids (CB).

In this study, next-generation sequencing (NGS) was used to investigate bacterial and fungal soil community responses to CB amendments in a golf course turfgrass system located in the high-rainfall region of the Pacific Northwest (PNW). Further, these data were used to examine bacterial community functional response as a potential predictor of nutrient management strategies in response to CB additions. Surface applications of CB to a golf course fairway were hypothesized to result in altered soil microbial community structure and predicted C and N cycling functions.

2. Materials and methods

2.1. Study site and design

The study was conducted at Eagle's Pride golf course (47.0841° N, 122.6692° W), owned by Joint Base Lewis-McChord (JBLM), but civilian-operated. The golf course is a 27-hole, public facility in the southern Puget Sound region, near Dupont, WA, where average annual precipitation is 1056 mm and mean annual temperature is 11 °C. Three golf course fairways (sites) were selected to receive amendment applications by developing a geostatistical approach. Briefly, soil physical and chemical data were collected from geo-referenced sampling points which, were used to create a geographic information system (GIS) paired with a novel algorithm developed in R (version 3.3.3; R Development Core Team) that ordered, ranked, and compared potential study sites. Soil texture in the 0–20 cm depth ranged from 88.4 to 89.9% (w/w) sand, 6.8 to 8.8% silt (w/w), and 2.2 to 3.3% (w/w) clay. Turfgrass fairways were a mixed sward of annual bluegrass (*Poa annua* L.) and creeping bentgrass (*Agrostis stolonifera* L.), both considered cool-season species, and managed under normal golf course conditions for the area (i.e. mowing twice each week, clippings returned, irrigated as necessary).

The study was a two factor (i.e., site (n = 3) and treatment), randomized complete block design with three replications installed on each of three fairways. The treatment factor consisted of two levels: CB amended or unamended plots which, were each 4 × 10 m (n = 3 plots of each treatment, per fairway site). CB was applied at approximately 46 Mg ha^{-1} to meet a target depth of 0.635 cm per application and an annual depth of 1.27 cm. Amendments were applied in July and September of 2015, as well as April and September of 2016, and samples were collected 28 days following amendment. CB were surface applied using a pull-behind Dakota turf tender 410 (Grand Forks, ND) and immediately brushed into the turfgrass canopy after each application using a GreensGroomer topdressing drag brush (Indianapolis, IN).

Table 1
Listed are chemical properties for as-received (AR) and dry weight (DW) composted biosolids used in 2013 and 2016, along with methods of analysis.

Characteristics	Year				Methods[a]
	2013		2016		
	AR	DW	AR	DW	
Moisture (%)	53	–	44	–	03.09A (70 C)
Solids (%)	47	–	56	–	03.09A (70 C)
pH	6.3	–	5.8	–	04.11A (1:5 w:w)
E.C. (dS m^{-1})	0.087	0.186	0.189	0.335	04.10A (1:5 w:w)
Organic C (%)	12.2	26.1	17.9	31.8	04.01A
Organic matter (%)	22.9	49.1	29.5	52.4	05.07A
Ash (%)	23.7	50.9	26.8	47.6	03.02
C:N ratio	–	14	–	14	[b]
Total N (%)	0.87	1.87	1.28	2.28	04.02D
Ammonium-N (mg kg^{-1})	222	476	47	83	05.02C
Nitrate-N (mg kg^{-1})	193	414	539	957	04.02B
Chloride (mg kg^{-1})	605	1297	337	598	04.12D
Sulfate-S (mg kg^{-1})	114	244	568	1008	04.12D
B (mg kg^{-1})	8.1	17.3	8	14	04.12B/04.14A
Zn (mg kg^{-1})	192	412	103	183	04.12B/04.14A
Cu (mg kg^{-1})	106	227	60	107	04.12B/04.14A
Fe (mg kg^{-1})	6236	13,365	5026	8923	04.12B/04.14A
As (mg kg^{-1})	–	3.9	–	6.1	04.12B/04.14A
Cd (mg kg^{-1})	–	1.6	–	0.8	04.12B/04.14A
Cr (mg kg^{-1})	–	29.6	–	4.2	04.12B/04.14A
Co (mg kg^{-1})	–	4.3	–	17.7	04.12B/04.14A
Pb (mg kg^{-1})	–	144	–	23	04.12B/04.14A
Hg (mg kg^{-1})	–	0.71	–	0.68	04.12B/04.14A
Mo (mg kg^{-1})	–	8.5	–	7.4	04.12B/04.14A
Ni (mg kg^{-1})	–	19	–	11.2	04.12B/04.14A
Se (mg kg^{-1})	–	0.8	–	–	04.12B/04.14A

[a] Method codes refer to Test Methods for the Examination of Composting and Compost (TMECC) (Leege et al., 2002), unless otherwise noted.
[b] Total nitrogen and organic carbon, combustion method (Gavlak et al., 2005).

The CB for the project were generated at JBLM's composting facility (Earthworks, Joint Base Lewis-McChord, WA) which uses base- and yard-debris (e.g., grass clippings and ground woody plant material), pre-consumer food waste, and biosolids from the on-base waste-water treatment facility as feedstocks. The chemical characteristics for the CB produced in 2013 and utilized in this study are given in Table 1. The CB were stored outdoors, beneath covers, and used for all applications in the study.

2.2. Turfgrass sample collection

In order to determine correspondence of the microbiome response with turfgrass response, shoot biomass and tissue N concentration were measured in Spring and Fall of 2016, at 7, 14, and 21 days following amendment. Using hand-shears and a randomly-placed 0.2 m^2 steel frame (2 × plot^{-1}), turfgrass clippings were collected from each plot, combined in the field, dried at 58 °C for 72 h in a forced-air convection oven (Gilson Company, INC., Lewis Center, OH) and weighed so that dry yield could be evaluated per unit area (2 × 0.2 m^2). To analyze total N concentrations, clippings were sub-sampled after drying, ground to pass a 20 mesh sieve using a Wiley mill grinder (Thomas-Wiley Mill Co., Philadelphia, PA), and dry combusted using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI) (McGeehan and Naylor, 1988).

2.3. Soil sample collection

Soils were collected prior to applications, for a baseline measurement in June 2015 (Bl), and post-application for the April (Sp) and September 2016 (Fa) dates, but, an unintended fertilizer application was made prior to the Bl collections and so represents conventional turfgrass management (i.e., fertilizer application). Soil samples were collected from control and CB amended plots in each of three replicated plots from three fairway sites. Ten soil cores (5 cm diameter) were each collected to a depth of 15 cm. Verdure (green tissue) and thatch-mat material (decaying shoots and roots) were removed, discarded, and the remaining mineral soil (0–5 cm) was composited by plot, homogenized in the field and stored on ice and in the dark until transportation to the lab. Composited samples were aseptically sieved to 2 mm and sub samples were aliquoted either into sterile 2 mL Snaplock Microtubes (Thermo Fischer Scientific, Waltham, MA) and stored at −80 °C until DNA extraction, or allowed to air dry and subsequently analyzed for soil chemical properties.

2.4. Soil chemical properties

Sub samples from the soil cores listed above were air-dried and sieved to 2 mm, pH was determined on a 1:1 soil: water (w:v) slurry which was mixed, allowed to stand for 1 h, then agitated for 30 s, and analyzed for pH using a custom-made robot outfitted with an Atlas Scientific pH chip (Brooklyn, NY) (McLean, 1982). Total C and N (TC and TN, respectively) were determined by dry combustion using a LECO Tru-Spec CN analyzer (Leco Corp., St. Joseph, MI) (Gavlak et al., 2005).

2.5. Soil genomic DNA extraction

Total soil genomic DNA (gDNA) was extracted from 0.25 g of each composite soil sample using the PowerSoil DNA Isolation Kit (Mo Bio, Carlsbad, CA) according to the manufacturer's protocol. Soil community DNA quality and quantity were assessed with the NanoDrop 2000 UV–Vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Genomic DNA extracts were diluted with nuclease-free water to 2 ng μL^{-1} prior to sequencing.

2.6. Soil community sequencing and sequence processing

Using the DNA extracts described above, the 16S and ITS rRNA genes were sequenced (16S = V3–V4 region, ITS = ITS1f–ITS2, paired end, 2 × 150 bp) by Molecular Research (MRDNA, Shallowater, TX, USA) via Illumina sequencing technology (MiSeq) with an average of 20k reads per sample. The primer set used for 16S amplicon generation included the forward primer: CCTACGGGNGGCWGCAG and reverse primer: GACTACHVGGGTATCTAATCC (Klindworth et al., 2013), while the primer set used for ITS amplicon generation included the forward primer: CTTGGTCATTTAGAGGAAGTAA and reverse primer: GCTGCG TTCTTCATCGATGC.

Data analyses were performed according to Lewis et al. (2018). Briefly, paired-end sequences were processed using MICCA (MICrobial Community Analysis, version 1.6, MICCA development team) (Albanese et al., 2015) and sequences with an error rate > 0.5 or length < 250 nucleotides (nts) and > 0.5 or length < 200 nt were removed and truncated to 250 and 200 nts for 16S and ITS sequences, respectively. After rarefying sequences to an even depth, operational taxonomic units (OTUs) were assigned using an open-reference approach following two separate methods for bacterial and fungal sequences as follows: (1) bacterial sequences were assigned with the Greengenes reference database (version 13.8; ftp://greengenes.microbio.me/greengenes_release/, clustered at 97%) (McDonald et al., 2012b) or clustered de novo and, OTUs were classified with the Greengenes taxonomic reference database (97%), (2) fungal sequences were assigned with the Unite/INSDC reference database (QIIME version 7.2, clustered at 97%) (Koljalg et al., 2013), or clustered de novo and, OTUs were classified with the Unite taxonomic reference database (97%); both communities were classified after removing chimeric sequences. Predicted functional responses of bacterial communities were assessed using the rarefied OTU table (after closed-reference OTU picking using the Greengenes database) and PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States, version 1.1.0) analysis (Langille et al., 2013). Raw sequence data have been submitted to the Sequence Read Archive (SRA) and have been assigned the following BioProject accession number: PRJNA549740.

2.7. Statistical analysis

Statistical analysis was conducted in the R statistical computing environment (R Development Core Team, 2017), and, for microbial community analysis, a biom file (McDonald et al., 2012a) was generated by MICCA and then assessed using the Phyloseq, Vegan, and ggplot2 packages (Dixon, 2003; McMurdie and Holmes, 2013; Wickham, 2010). For the turfgrass shoot biomass and tissue N concentrations analysis, turfgrass responses were evaluated in a linear mixed model which treated site as a fixed effect and repetition, and all interactions with repetition, as random. Included in the model were the main effects of site, treatment (i.e., amended or unamended), day, and all possible interactions. Assumptions of normality were assessed by inspecting residual plots and, because the responses were modeled across time, repeated measures analysis was used to model temporal correlation for each individual plot. Three correlation structures were tested for each model, and the lowest AICc value dictated final model selection. Soil environmental variables were analyzed using ANOVA which tested for treatment and site effects. When appropriate, means were separated using Tukey's HSD test ($\alpha = 0.05$).

To examine bacterial and fungal community structure, Bray-Curtis distances were calculated using relativized OTU tables, and ordinations were plotted using non-metric multidimensional scaling (NMDS) analyses. Environmental vectors were fit to NMDS plots using the envfit function in Vegan (Dixon, 2003), with tests for significance based on a permutation test of 999 iterations and alpha = 0.05. To examine the effect of treatment, site, and sampling period on bacterial community structure, NMDS plots were generated including Bl samples by site, where Bl samples represented conventional management (i.e., fertilizer application). Treatment and sampling period effects were examined at each site using pairwise PERMANOVA analysis (Kelly et al., 2015). Factors with a significant effect on community composition as determined via NMDS and envfit were regressed against the relative frequency of KEGG orthology (KO) term groups at “Level 3”. Additionally, relativized OTU abundances regressed against environmental factors to identify taxa responsive to TC, TN, C:N, and pH. Reported data were filtered by *p*-value (≤ 0.05), by variance explained (r^2), and environmental significance.

3. Results and discussion

3.1. Soil chemistry and turf response to biosolids application

In each of three sampling periods and for the soil environmental variables tested, no significant differences were observed between CB amended and non-amended plots (Table 2). Rather, differences in TC, TN, C:N, and to a lesser extent, pH, were attributed to the specific characteristics of a given site (e.g. lower values for Site 2 were observed in each sampling period) except for C:N values in the Bl period and one pH difference in the Fa period. As soil pH is known to vary in turfgrass systems (Lu et al., 2015), this one small detectable difference could be considered an unremarkable outlier. For TC and TN, similar results have been reported in other highly-managed turfgrass systems. In 2012, Guertal and Green observed no difference in soil TC and TN following organic fertilizer amendment which, the authors noted, may have been

Table 2
Soil properties (pH, TC, TN, C:N) used as best correlating environmental variables in the non-metric multidimensional scaling (NMDS) ordination plots and mean values for composted biosolid (CB) and control (Con) from three sites, pre-application (Bl) and post-application (Sp and Fa).

June 2015 (Bl)			April 2016 (Sp)			Fall 2016 (Fa)		
Site[a]	CB	Con	Site	CB	Con	Site	CB	Con
pH								
1	5.5	5.7	1	5.5	5.4	1ab	5.6	5.6
2	5.8	6.0	2	5.6	5.4	2a	5.8	5.6
3	5.7	5.5	3	5.4	5.3	3b	5.5	5.5
TC (%)								
1a	5.02	6.11	1a	4.94	4.65	1a	5.29	4.13
2b	1.04	2.26	2b	1.97	1.55	2b	1.63	1.82
3c	4.57	3.72	3a	4.49	4.42	3a	4.48	4.64
TN (%)								
1a	0.40	0.47	1a	0.45	0.42	1a	0.48	0.37
2b	0.1	0.21	2b	0.2	0.16	2b	0.18	0.20
3a	0.38	0.32	3a	0.41	0.41	3a	0.42	0.43
C:N								
1a	12.7	13.1	1a	11.0	11.1	1a	10.9	11.2
2b	11.3	11.0	2b	10.1	10.0	2b	8.88	8.61
3a	12.0	11.6	3a	11.0	10.9	3a	10.8	10.8

[a] Sites, within a collection period (Bl, Sp, Fa) and environmental variable, followed by the same lowercase letter are not significantly different ($p < 0.05$).

due to the short term nature of the research (i.e., one-year duration studies). Additionally, in the Guertal and Green (2012) work, as well as in this study, the thatch layer, which is a matrix of decaying shoots and roots, was removed prior to soil analysis. This was a likely exportation of measurable C and N additions, but standard practice in turfgrass analyses studies.

No obvious trends were observed in turfgrass shoot biomass and tissue N concentrations following CB amendment and in comparisons with the unamended control (Figs. 1, 2). Turfgrass shoot biomass was significantly affected by site in each of two collections Sp and Fa, by day in the Fa period, and by amendment which, was only detected in interactions with day during the Sp collection (Fig. 1A–C). In contrast and for tissue N concentrations, significant increases of 4.5 and 10.6%, were observed in comparisons between CB amended and unamended plots in post-amendment collections, Sp and Fa, respectively (Fig. 2A), while a significant day interaction was observed in the Fa collection only which, revealed a significant decrease in tissue N concentration from day 7 to day 21 (Fig. 2B).

When CB was amended to highly managed turfgrass golf course fairways, we observed increases in turfgrass tissue N concentrations (Fig. 2A) but did not see comparable increases in turfgrass biomass yield, except during one collection period and only revealed by an interaction with day (Fig. 1C). This suggests that CB amendment at this rate supplied adequate N to sustain turfgrass growth, but that other limiting factors besides N availability inhibited turfgrass production. At similar rates (50 Mg ha^{-1}) of application, Zhang et al. (2010) observed comparable increases in turfgrass N concentrations following a CB amendment which, were related to significant increases in turfgrass biomass as well. In our study, the CB amendment was surface applied, which may have resulted in a physical shading, impeding the turfgrass growth. We did detect significant effects related to collection time (i.e., days after amendment) and though the effects were inconsistent, turfgrass growth and tissue N concentration appear to be at least somewhat temporally variable in both amended and unamended soils (Figs. 1B, 2B). Therefore, cumulative analysis (e.g., total yield) may be more appropriate in detecting amendment differences in highly managed turfgrass systems. In addition, other researchers have observed similar inconsistencies between turfgrass growth and tissue N concentration, demonstrating that ample or elevated tissue N does not necessarily

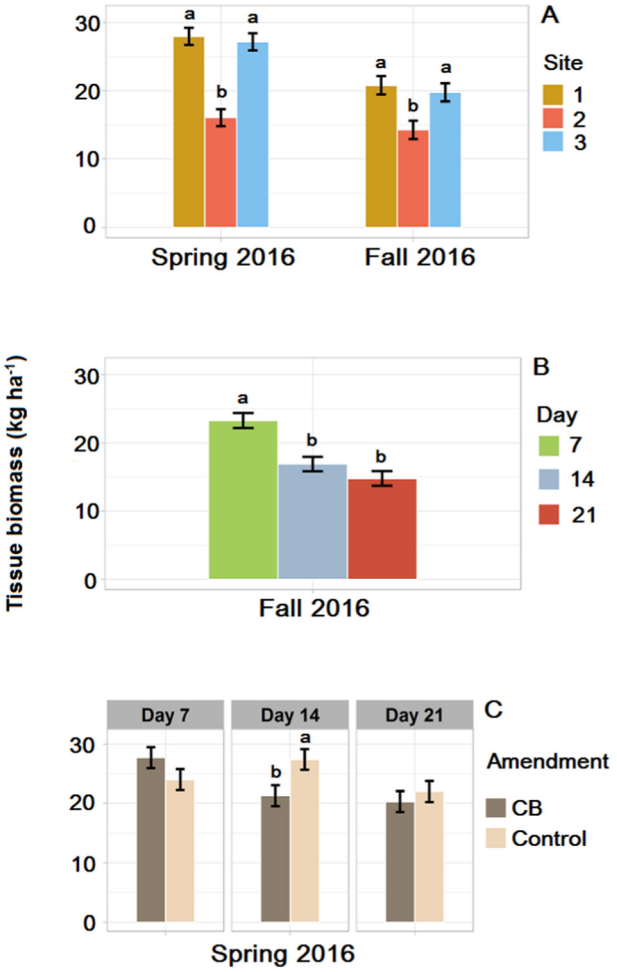

Fig. 1. Mean values for turfgrass tissue biomass depicting the effects of: (A) site during the Spring and Fall collection, (B) day during the Fall period, and (C) the amendment by day interaction during the Spring period. Different letters above bars indicate significant differences among treatments at $p \leq 0.05$ following Tukey's HSD test.

reflect shoot biomass production (Garling and Boehm, 2001; Zhang et al., 2010). And, it has been noted that due to variations between climate, management practices, and turfgrass species, calibrations between turfgrass tissue N concentration and growth are needed (Mangiafico and Guillard, 2007). Significant reductions in turfgrass biomass were detected at site 2 in both the Sp and Fa collection period (Fig. 1A). Though it is unclear precisely the mechanism for this reduction, the differences observed in soil chemical properties inherent to site 2 (Table 2) could easily be contributing to the reduced turfgrass vigor.

3.2. Bacterial community composition

Across all sites, the most significant factors controlling bacterial community composition included TC, TN, C:N ratio and soil pH (Fig. 3A). The greatest correlation between soil factors and bacterial community composition was observed in soil TC ($r^2 = 0.88$) followed by soil TN ($r^2 = 0.85$) and soil C:N ($r^2 = 0.65$). Soil pH had the least explanatory power ($r^2 = 0.37$) and was related primarily to the specific characteristics of Site 2 (Fig. 3A). Soil TC, TN, C:N and pH correlations with bacterial community composition are often expected in soil microbiome studies. Researchers have linked microbial community structural shifts to various C compounds, C:N ratios, TN and soil pH (Barth et al., 2018; Carson et al., 2010; Cleveland et al., 2007; Fierer

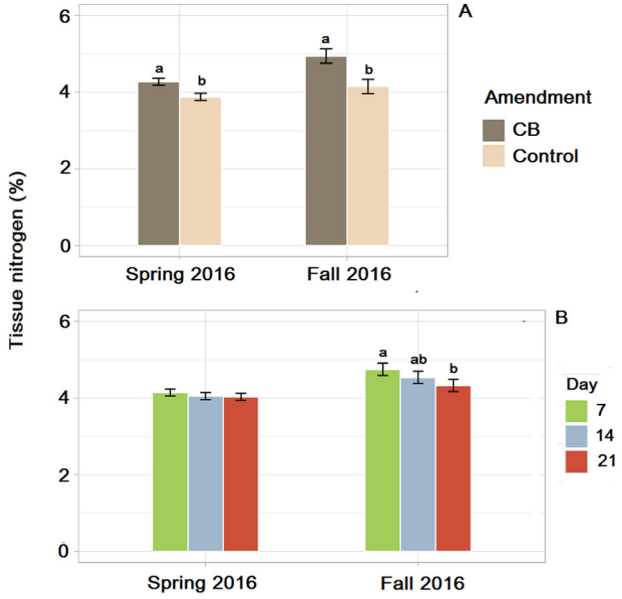

Fig. 2. Mean values for tissue nitrogen concentrations during the Spring and Fall periods depicting the effects of: (A) amendment, and (B) day. Different letters above bars indicate significant differences among treatments at $p \leq 0.05$ following Tukey's HSD test.

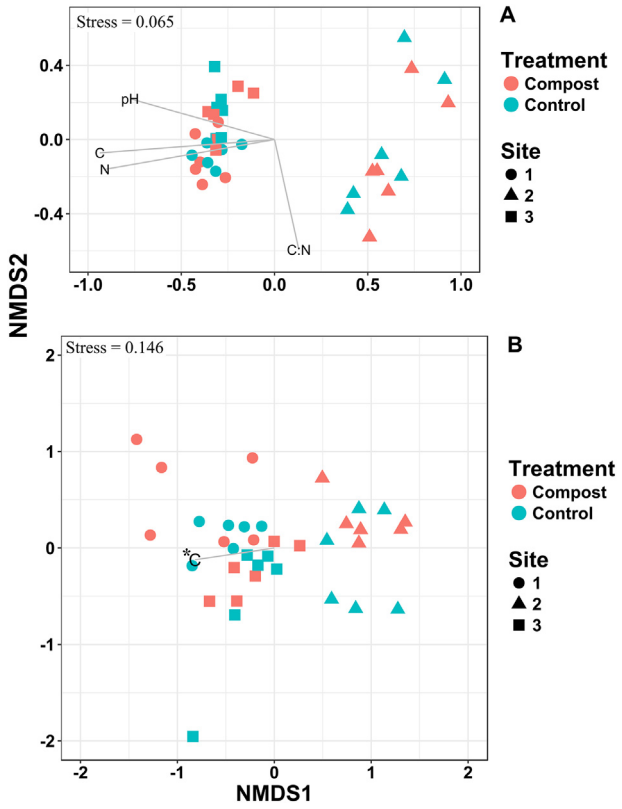

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities of bacterial (A) and fungal (B) community in response to site and treatment (control and composted biosolid). Included in the plots are best correlating environmental variables (TC (%), TN (%), C:N, pH). The asterisks in (B) indicates that TN and C:N fell along this same vector but TC had the highest r^2 value. OTU tables were relativized prior to ordination.

et al., 2007; Lewis et al., 2018; Song et al., 2015; Zhao et al., 2014) for many years, and the results of the this study agree with previous observations. The quality of substrate C is known to have a measurable

Table 3
p-Values for treatment (control, composted biosolids (CB), and baseline (Bl)) and sampling period (June 2015 (Bl), April 2016 (Sp), and September 2016 (Fa)) effects on bacterial community structure separated by site.

Treatment

Site 1			Site 2			Site 3		
	Bl[a]	CB		Bl	CB		Bl	CB
Control	0.006**	0.620	Control	0.690	0.800	Control	0.024*	0.403
CB	0.012*	–	CB	0.480	–	CB	0.018*	–

Sampling period

Site 1			Site 2			Site 3		
	Fa	Sp		Fa	Sp		Fa	Sp
Bl	0.004**	0.004**	Bl	0.220	0.560	Bl	0.009**	0.027*
Sp	0.004**		Sp	0.560***	–	Sp	0.182	–

[a] Baseline samples include a fertilizer application prior to sampling.
* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

effect on community structure (Eilers et al., 2010; Paterson et al., 2007) and while measurements of TC are quantitative, providing no information on the quality or composition of the substrate (Nelson and Sommers, 1996), the explanatory power of TC in bacterial community structure is well within reason. In addition, highly managed turfgrass systems are unique in that turfgrass plants provide cover year-round, with little, if any, tissue removal. These types of turfgrass management strategies are far less deleterious than conventional tillage in many typical agro-ecosystems, and researchers have shown that turfgrass systems act as carbon sinks, essentially accumulating both C and N with age of the golf course (Bandaranayake et al., 2003; Shi et al., 2006).

Significant groupings in bacterial community structure were not observed as a result of CB application ($p = 0.92$), or sampling period ($p = 0.76$). However, a clear and significant separation in bacterial community composition was observed by Site ($p < 0.001$) (Fig. 3A) leading to further statistical investigations into the effect of Site. According to pairwise PERMANOVA analysis, at Sites 1 and 3, significant impacts of CB were observed, but these differences were only observed in comparisons with the Bl community structure, not with those in control plots (Table 3, Fig. 4A). At Site 1, significant community changes were observed among each of the three sampling periods ($p = 0.005$) (Table 3).

Soil disturbances have been correlated with changes in community structure through both direct and indirect mechanisms (via changes in the environment) (Griffiths and Philippot, 2013; Shade et al., 2012). In this study, the addition of a CB, which was hypothesized as a disturbance, did not consistently alter bacterial community structure. The differences in bacterial community structure were related to site conditions, which suggests that the physico-chemical properties related to site may have exerted greater control on structuring the microbial community in the soil layers below the thatch. While only a few soil physico-chemical properties were measured in the current study, the differences observed at Site 2, do help support this (Table 2). On the other hand, golf course fairways receive non-uniform supplementary irrigation which, in combination with site specific soil conditions, influences soil moisture (Miller et al., 2014). Considering the importance soil moisture has on community structure and function (Brockett et al., 2012), the differences observed could have been influenced by the distribution of non-uniform overhead irrigation. Additionally, prior to soil sampling, the thatch layer, which is a barrier between the surface and bulk soil, was removed. This may have contributed to the lack of

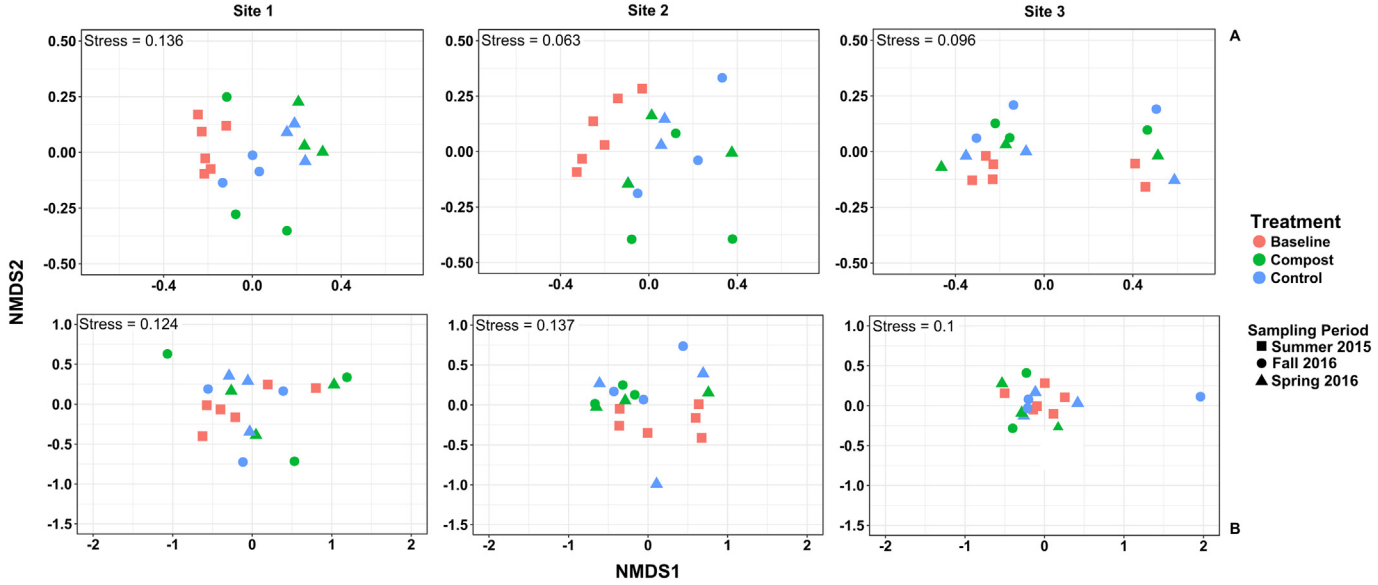

Fig. 4. Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarities in both bacterial (A, top row) and fungal (B, bottom row) communities according to the effects of treatment and sampling period using pairwise PERMANOVA analysis. OTU tables were relativized prior to ordination.

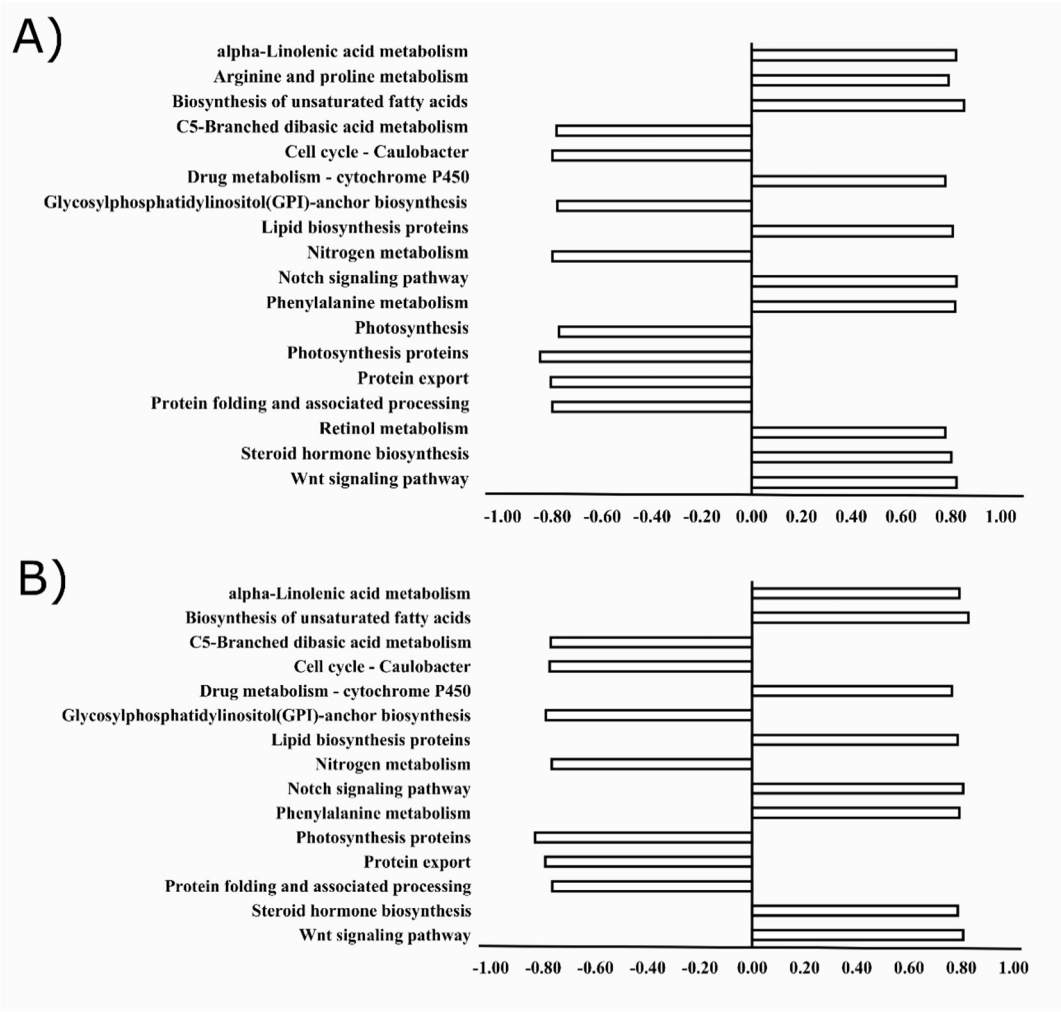

Fig. 5. KO term groups having significant correlations with TC (A) and TN (B). Pearson's correlation coefficients are shown on the x axis. Reported correlations are limited to those with an $r^2 \geq 0.6$ and a p-value ≤ 0.05. KO term groups were also filtered for environmental significance. Relative frequencies of the KO term groups were used for the analysis.

Table 4
p-Values for treatment (control, composted biosolids (CB), and baseline (Bl)) and sampling period (June 2015 (Bl), April 2016 (Sp), and September 2016 (Fa)) effects on fungal community structure separated by site.

Treatment								
Site 1			Site 2			Site 3		
	Bl	CB		Bl	CB		Bl	CB
Control	0.26	0.29	Control	0.25	0.19	Control	0.34	0.61
CB	0.26	–	CB	0.19	–	CB	0.19	–

Sampling period								
Site 1			Site 2			Site 3		
	Fa	Sp		Fa	Sp		Fa	Sp
Bl	0.26	0.26	Bl	0.17	0.46	Bl	0.20	0.41
Sp	0.42	–	Sp	0.68	–	Sp	0.85	–

treatment driven differences in soil microbial communities given that thatch layers are known to harbor microbial communities in much greater numbers than the bulk soil (Mancino et al., 1993; Raturi et al., 2005). Finally, the effect of seasonal fluctuations (sampling period) on microbial community structure is also widely reported (Beirn et al., 2017; Jangid et al., 2008; Siles et al., 2017; Yan et al., 2017) as was observed here. But, it is important to recognize that seasonal effects on the microbial community structure may be the result of abiotic temperature differences or changes in the above ground plant communities as well (Legay et al., 2014). Furthermore, because an unintended fertilizer application was made prior to sampling (Bl), the observed differences between the Bl, and the Sp and Fa periods, may instead reflect microbial community changes resulting from that application, as opposed to changes in season.

Abundance of several bacterial OTUs had significant correlations with TC, TN, and C:N (Tables S1, S2, and S3). *Verrucomicrobia* abundance was the most highly correlated with C:N, TC, and TN, specifically the ELLin515 family, which is a family found in the *Pedosphaerales* order (Tables S1, S2, and S3). Several taxa belonging to the *Deltaproteobacteria* class and the *Chloroflexi* phylum showed strong negative correlations with soil C:N, TC, and TN (Tables S1, S2, and S3). *Verrucomicrobia*, *Deltaproteobacteria*, and *Chloroflexi* were previously found to show strong negative correlations with N amendments across several soil microbiomes (Yan et al., 2017). Additionally, these authors report consistent decreases in the abundance of *Planctomycetes* with N amendment, and at least one member of this phylum had a strong negative correlation with TN (Table S3). Both *Planctomycetes* and *Chloroflexi* (particularly *Anaerolineae*) are known to have a diverse set of carbohydrate hydrolytic genes (Baker et al., 2015) and members of these taxonomic groups were negatively correlated with TC, TN, and C:N. Several members of the *Acidobacteria* phylum were negatively correlated with C:N, TC, and TN (Tables S1, S2, and S3). This phylum appears to be fairly ubiquitous in soils, but others have found *Acidobacteria* were positively correlated with C:N in woodland soils compared with crop soils (Pershina et al., 2015). The observed strong negative correlations between TN and several bacterial OTU abundances is not surprising given that the persistent suppressive effects of nitrogen amendments on soil microbial activity and biomass have been known for some time (Söderström et al., 1983).

3.3. Bacterial community predicted function

Predicted bacterial community functional analysis revealed several KO term groupings were significantly correlated with soil TC and TN (Fig. 5). Many pathways associated with various aspects of metabolism showed similar responses to TC and TN. For instance, N metabolism, C5-branched dibasic acid metabolism, glycosylphosphatidylinositol-anchor biosynthesis, photosynthesis proteins, protein export, and protein folding, and associated processes were all negatively correlated with both TC and TN (Fig. 5). Positive correlations with TC and TN were observed for alpha-Linolenic acid metabolism, biosynthesis of unsaturated fatty acids, drug metabolism-cytochrome P450, phenylalanine metabolism, lipid biosynthesis, and steroid hormone biosynthesis. Arginine and proline metabolism and retinol metabolism had a strong positive correlation with TC but not TN (Fig. 5). Notch and wnt signaling pathways showed positive correlations with TC and TN.

3.4. Fungal community composition

Three soil properties, TC, TN, and C:N were significantly associated with fungal community composition ($p < 0.001$ for each soil property, Fig. 3B). Unlike the soil bacterial community, the fungal community did not demonstrate a significant response to soil pH. For fungal community structure, explanatory power was greatest in soil TC ($r^2 = 0.67$) followed by soil TN and soil C:N ($r^2 = 0.64$ and 0.45, respectively). Each of the three soil properties clearly grouped at Sites 1 and 3, apart from Site 2 (Fig. 3B).

Fungal community clustering was significantly observed by site ($p < 0.001$), but not as a result of CB additions or season ($p = 0.38$ and 0.70, respectively). PERMANOVA analysis of treatment and seasonal effects revealed no significant effects on fungal community composition (Fig. 4B, Table 4).

The linkage between soil TC, TN, and C:N and fungal community structure is not unexpected, as many previous studies have shown correlations with fungal communities and edaphic properties (Lauber et al., 2008; LeBlanc et al., 2015; Peay et al., 2016). A divergent pH effect between bacterial and fungal communities was observed, which has been corroborated by several researchers at different scales and in various systems (Lauber et al., 2008; Rousk et al., 2010) but here, soil pH exhibited a small difference within a very narrow range, and therefore, causal interpretation may be unwarranted.

As with the bacterial community analysis, the shifts in fungal community composition in the soil below the thatch were not statistically associated with CB amendment. Instead changes in community structure were associated with a specific site. Contrary to the bacterial community, when site effects were further investigated, neither the treatment nor the sampling period were responsible for significant shifts in the fungal community (Table 4). While seasonal changes in microbial community structure are often expected (Feng and Simpson, 2009), recent work suggests that the fungal community may be less responsive to temperature variation (Koyama et al., 2018), which may explain the differences observed here. Golf course managers tend to discourage diverse turfgrass species composition, preferring a uniform playing surface, but here, the turfgrass sward was a mix of two grass species. In grassland environments, fungal community structure is known to vary according to the diversity in plant species (LeBlanc et al., 2015; Sayer et al., 2013), the differences observed here may reflect a variation in turfgrass species composition at Site 2.

Correlations among abundance of fungal OTUs and environmental factors were weaker compared with bacterial OTU abundance and no obvious trends were observed in phylum level comparisons with environmental conditions (Table 5). The greatest correlations with soil C:N ratios ($r^2 = 0.6$, Table 5) were negative and observed in the phylum *Ascomycota*, while two members of the *Ascomycota* phylum also showed relatively strong negative correlations with TC and TN (Table 5). Members of the phylum *Ascomycota* have been observed as the dominate fungal group in grassland leaf litter (Mouginot et al., 2014), in grasslands across drastic precipitation gradients (Chen et al., 2017) and in highly maintained turfgrass systems (Chen et al., 2019) and therefore, our observations are in line with these reports. Two members of the *Mortierellaceae* family were strongly negatively

Table 5
Fungal OTUs having significant correlations with C:N, TC, and TN Measurements. PCC is Pearson's Correlation Coefficients and reported correlations are limited to those with an $r^2 \geq 0.3$ and a *p*-value ≤ 0.05. Relative OTU abundances were used in the analysis.

Phylum	Class	Order	Family	PCC	r^2
Soil C:N					
Ascomycota	*Arthoniomycetes*	Unidentified	Unidentified	−0.67	0.5
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.63	0.5
Blastocladiomycota	*Blastocladiomycetes*	*Blastocladiales*	*Catenariaceae*	−0.65	0.4
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.64	0.4
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.63	0.4
Ascomycota	*Pezizomycetes*	*Pezizales*	*Pyronemataceae*	−0.63	0.4
Basidiomycota	*Agaricomycetes*	*Boletales*	*Suillaceae*	−0.61	0.4
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.61	0.4
Basidiomycota	*Agaricomycetes*	*Agaricales*	*Inocybaceae*	−0.61	0.4
Unidentified	Unidentified	Unidentified	Unidentified	−0.61	0.4
Ascomycota	*Arthoniomycetes*	Unidentified	Unidentified	−0.61	0.4
Basidiomycota	*Tremellomycetes*	*Filobasidiales*	*Piskurozymaceae*	−0.60	0.4
Unidentified	Unidentified	Unidentified	Unidentified	−0.59	0.4
Chytridiomycota	*Rhizophydiomycetes*	*Rhizophydiales*	*Rhizophydiales*	−0.57	0.3
Basidiomycota	*Agaricomycetes*	*Agaricales*	*Inocybaceae*	−0.55	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.56	0.3
Ascomycota	*Dothideomycetes*	*Dothideales*	*Dothioraceae*	−0.55	0.3
Basidiomycota	*Agaricomycetes*	*Polyporales*	Unidentified	−0.54	0.3
Ascomycota	*Eurotiomycetes*	*Eurotiales*	*Trichocomaceae*	−0.52	0.3
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.52	0.3
Soil total carbon					
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.78	0.6
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.75	0.6
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.73	0.5
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.66	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.62	0.4
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.62	0.4
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.60	0.4
Ascomycota	*Pezizomycetes*	*Pezizales*	*Pyronemataceae*	−0.59	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.59	0.3
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.59	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.58	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.58	0.3
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.57	0.3
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.56	0.3
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Hypocreales_fam_Incertae_sedis*	0.56	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.55	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.55	0.3
Ascomycota	*Sordariomycetes*	Unidentified	Unidentified	0.55	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.54	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.53	0.3
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Clavicipitaceae*	0.52	0.3
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	0.51	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.51	0.3
Soil total nitrogen					
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.79	0.6
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.76	0.6
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.74	0.6
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.66	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.64	0.4
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.62	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.62	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.61	0.4
Ascomycota	*Pezizomycetes*	*Pezizales*	*Pyronemataceae*	−0.61	0.4
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.60	0.4
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.60	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.59	0.4
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.57	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.57	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.57	0.3
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Hypocreales_fam_Incertae_sedis*	0.56	0.3
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.56	0.3
Ascomycota	*Sordariomycetes*	Unidentified	Unidentified	0.55	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.54	0.3
Glomeromycota	*Glomeromycetes*	*Archaeosporales*	Unidentified	−0.54	0.3
Ascomycota	*Leotiomycetes*	*Helotiales*	Unidentified	−0.53	0.3
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Clavicipitaceae*	0.52	0.3
Ascomycota	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	0.51	0.3
Mortierellomycota	*Mortierellomycotina_cls_Incertae_sedis*	*Mortierellales*	*Mortierellaceae*	−0.79	0.6

correlated with TC and TN (r^2 = 0.6; Table 5) and because members of the *Mortierellales* order are abundant in soils and are known to exhibit diverse capacities for carbon metabolism (Hanson et al., 2008), it is not surprising that TC and TN responses were observed in this study. In addition, a recent report linked N flux (i.e., the ratio of reactive N loss to soil microbial biomass N) with the fungal phylum *Glomeromycota* (among others) (Chen et al., 2019) which, is a group we observed to have a strong negative correlation with TN (r^2 = 0.6, Table 5). Though the authors of that study did not define a functional mechanism for this link, their observations along with our report, do suggest that further investigations into turfgrass N cycling and fungal community composition are necessary.

4. Conclusion

The primary objective of this research was to test CB additions to golf course fairways and resultant N dynamics and soil microbial community response. According to NMDS plots and including all sites, both bacterial and fungal community shifts were not directly related to CB amendment. The soil microbiome after CB additions still reflected site conditions, most likely the above-ground species composition. It was not possible to definitively determine the mechanism responsible for this difference, whether the community shifts are a function of turfgrass management (i.e., irrigation), turfgrass species distribution, or other edaphic properties requires further testing. The results do suggest CB may be added to turfgrass management regimes without unfavorably influencing soil microbial communities or turfgrass biomass and N content, likely maintaining playability and aesthetics. In the current study, however, data were collected over a short-term period and continued application of biosolids may have negative impacts that are not clear after only 2 years of application. Future work should address the varied constituents in the biosolids, both organic and inorganic contaminants, the microbiome of the thatch as well as the soil below the thatch, and long-term impacts of repeated applications. Additionally, as turfgrass microbiome research is in its infancy, the bacterial and fungal OTUs along with predicted functional responses identified here should provide a point of comparison as the field moves forward.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2019.06.006.

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