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Evaluation of Humic Fertilizers on a Sand-Based Creeping Bentgrass Putting Green

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Abbreviations: BG, black gypsum; CEC, cation exchange capacity; DGCI, dark green color index; HCU, humic-coated urea; HDG, humic dispersing granules; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PMC, potentially mineralizable carbon; PMN, potentially mineralizable nitrogen; WAIT, weeks after initial treatment.

ABSTRACT

Turfgrass with sand-based root zones, such as golf course putting greens, are highly important economically and require intense management. However, problems are often associated with sand-based root zones such as low nutrient retention and limited microbial activity. Soil additives may increase soil biological activity, improve nitrogen (N) cycling efficiency, and thus reduce fertilizer N inputs. A two-year experiment was conducted on a sand-based creeping bentgrass (*Agrostis stolonifera* L.) putting green to investigate whether humic products could increase soil biological activity and improve turfgrass quality. Treatments included humic-coated urea (HCU; 2/3 rate and full rate), HCU + humic

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dispersing granules (HDG), HCU + black gypsum (BG), urea, HDG, and a nontreated control. Minimal differences were seen in microbial biomass and activity besides HCU + BG. The HCU + BG had 60% greater potentially net N mineralization (PMN) relative to the HDG and the nontreated control. Overall, incorporating humic substances with N fertilizer did not increase turfgrass quality, cover, and clipping biomass compared to N fertilizer alone. However, the addition of BG to N fertilizer enhanced microbial activity (i.e., PMN).

INTRODUCTION

Sand-based root zones are often used for highly important economic areas such as sports fields and golf course putting greens. Sand is the primary component of the root zone mixture because it eliminates surface water ponding and has a high degree of particle stability (Bigelow & Soldat, 2013). However, there are disadvantages of sand-based root zones, which include low nutrient and water retention, and decreased microbial activity (Bigelow, Bowman, & Cassel, 2001, 2004; Shi, Bowman, & Rufty, 2007). Furthermore, extensive applications of fertilizer and water are crucial for maintenance of high-quality turfgrass (Gomez-Armayones, Kvalvein, Aamlid, & Knox, 2018; Wheeler & Nauright, 2006). However, excessive fertilizer inputs, especially nitrogen (N) and phosphorus, can lead to impaired ground and surface water quality (Bigelow et al., 2001; Gomez-Armayones et al., 2018; Soldat & Petrovic, 2008). Managing these turf systems to more efficiently use nutrients like N is key for both economic and environmental sustainability, and enhancing turf soil health is crucial to obtaining these goals (Singh, 2018).

Soil health or quality can be defined as the capacity of living soil (within natural or managed systems) to function, sustain productivity, maintain or enhance water and air quality, and promote biological health (Doran, 2002). Soil health incorporates physical, chemical, and biological indicators and properties (Karlen et al., 1997). Physical indicators include soil moisture, compaction, texture, bulk density, and porosity (Cardoso et al., 2013; Mann, Lynch, Fillmore, & Mills, 2019). Chemical indicators include soil nutrient analysis, including carbon (C) and N, cation exchange capacity (CEC), pH, and organic matter (Cardoso et al., 2013; Mann et al., 2019). Biological indicators include microbial biomass [microbial biomass C (MBC) and microbial biomass N (MBN)], soil respiration, and N mineralization (Bunemann et al., 2018; Cardoso et al., 2013; Mann et al., 2019).

Soil respiration or potentially mineralizable C (PMC) can be measured by the flush of CO_2 during a soil incubation (Franzluebbers & Pershing, 2018). Soil respiration can be used as an integrated indicator of the pool of labile soil organic matter, soil microbial activity, and can be correlated to the N needs of crops (Franzluebbers, 2018; Franzluebbers, Haney, Honeycutt, Schomberg, & Hons, 2000; Franzluebbers, Pershing, Crozier, Osmond, & Schroeder-Moreno, 2018). Potentially mineralizable N (PMN) is the inorganic N released during the incubation (Franzluebbers et al., 2018; McDaniel & Grandy, 2016). Both PMC and PMN can be an indicator of soil N-supplying power, as has been shown with maize and turfgrass N response studies in the United States (Franzluebbers et al., 2018; McDaniel et al., 2020; Moore, Guillard, Morris, & Brinton, 2019a, 2019b; Yost et al., 2018).

Sand-based root zones are often amended with peat moss, manures, compost, and humic substances to improve soil properties (i.e., structure, and water and nutrient retention) and soil health (i.e., microbial activity) (Bigelow & Soldat, 2013). Humic substances are complex and heterogeneous mixtures of materials formed by humification, which is the chemical and biochemical reactions during the transformation and decay of microbial and plant remains (IHSS, 2020). Humic fertilizers or soil amendments are often derived from leonardite by alkaline extraction and then separated into different fraction by acidification (Schmidt, Ervin, & Zhang, 2003). Humic substances can contain humin, humic acid, fulvic acid, and humic acid precursor (Pope, Eichenberg, & Birthisel, 2013). Humic products have been reported to positively affect root growth and branching, respiration and photosynthesis, nutrient uptake and nutrient use-efficiency, and abiotic stress tolerance (Canellas et al., 2015; Nardi, Pizzeghello, Muscolo, & Vianello, 2002; Trevisan, Francioso, Quaggiotti, & Nardi, 2010). The mode-of-action of humic substances has been speculated as a bio-stimulant effect and hormone-like or auxin-like activity (Canellas et al., 2015; Nardi et al., 2002; Trevisan et al., 2010).

Humic products claim numerous benefits to turfgrass plant and soil health, including enhanced microbial activity, increased nutrient availability and uptake efficiency, improved stress tolerances, increased fertilizer effectiveness and efficiency, and improved soil structure and water holding capacity (Liu & Cooper, 2000; Pettit, 2004). However, these claims are largely unfounded, with some exceptions (Table 1), and need further investigation or field validation. We hypothesize that incorporating humic substances with N fertilizer will allow for reducing the N rates while maintaining turfgrass quality by enhancing turfgrass N use efficiency. This may be due to the benefits seen in Table 1, such as improved root growth or photosynthesis efficiency, and/or efficient N cycling by microbial stimulation from humic products (Cheng, Wang, Wang, Chang, & Wang, 2017; Magill & Aber, 2000). Finally, we hypothesize that adding humic substances, which contains C, to fertilizers will increase microbial biomass and activity. The additions of N fertilizer without C have been shown to decrease microbial biomass and activity (Grandy et al., 2013; Ramirez, Craine, & Fierer, 2010; Wang, Liu, & Bai, 2018). The objectives of this field-based experiment were to 1) determine if incorporating humic substances to fertilizers allows for a reduction in N rate while maintain turfgrass quality on a sand-based root zone, and 2) determine if the addition of humic substances to fertilizers increases turfgrass soil microbial biomass and activity.

MATERIALS AND METHODS

Experimental site and plot maintenance

A two-year (Apr. 2019-Nov. 2020) field experiment was conducted on a mature (10+ year-old) 'Penncross' creeping bentgrass (*Agrostis stolonifera* L.) putting green at the Iowa State University Horticulture Research Station (Ames, IA, USA). The 50 yr mean annual temperature and precipitation for the region is 9.5 °C and 889.3 mm, respectively (IEM, 2021). The putting green root zone is a sand-based root zone that meets United States Golf Association (USGA) specifications (USGA, 2018). Turfgrass was maintained at a 0.36 cm height of cut using a reel-mower (model 2500B, John Deere, Moline, IL, USA) six times per week. Clippings were collected and removed after each mowing. Irrigation was applied daily, unless rainfall was sufficient, to apply 25.4 mm of water per week throughout the growing season. Sand topdressing was conducted bi-weekly from throughout the majority of the growing season (May to Sept.). Chlorothalonil (tetrachloroisophthalonitrile; Pegasus 6L, Phoenix Environmental Care, LLC, Valdosta, GA, USA) was applied at 8.2 kg ha^{-1} in May (two applications) and at 5.1 kg ha^{-1} in June (two applications), and mefenoxam {(R)-2-[(2,6-dimethylphenyl)-methoxyacetylamino] propionic acid methyl ester; Mefenoxam 2 AQ, Quali-Pro, Control Solutions Inc., Pasadena, TX, USA} was applied at 0.8 kg ha^{-1} in June (two applications) and in Aug. (one application) as preventative fungicides to minimize turfgrass stress and damage.

Treatments

Treatments were organized in a randomized complete block design with three replications. Treatments included: humic-coated urea (HCU; 2/3 rate and full rate; The Andersons, Inc., Maumee, OH, USA), HCU + humic dispersing granules (HDG; The Andersons, Inc., Maumee, OH, USA), HCU + black gypsum (BG; The Andersons, Inc., Maumee, OH, USA), urea (The Andersons, Inc., Maumee, OH, USA), HDG, and a nontreated control (Table 2). The humic substances were derived from leonardite and contained humic acid, fulvic acid, humin, and humic acid precursor. Applications were made with a wooden box (1 x 1 m) with three sets of offsetting wire mesh to ensure uniform treatment distribution across the plots (1 x 2 m). Irrigation was applied and mowing was withheld for 48 hrs after treatment application. Urea (102.5 kg N ha^{-1} yr^{-1}), HCU (102.5 kg N ha^{-1} yr^{-1}), and HCU (2/3 rate; 68.4 kg N ha^{-1} yr^{-1}) were applied every two wks from Apr. to Oct., HDG (222.8 kg HDG ha^{-1} yr^{-1}) was applied in Apr., May, Sept., and BG (439.5 kg BG ha^{-1} yr^{-1}) was applied in Apr., July, and Oct., in 2019 and 2020. Treatments were made to the same experimental units throughout the duration of the field experiment.

Data Collection

Visual quality ratings of the turfgrass were graded biweekly using a 1-9 scale (1 = poor, 9 = ideal, 6 = minimally acceptable) (Morris & Shearman, 1998). Following techniques by Thoms, Sorochan, Brosnan, and Samples (2011), digital images were collected biweekly. Digital images were subjected to digital image analysis (DIA) to calculate percent green cover (Richardson, Karcher, & Purcell, 2001). Threshold settings of hue 71 to 176, saturation 10 to 100, and brightness 0 to 100 were used for DIA. Clippings were collected monthly from one mowing event using a reel-mower, dried at 80 °C for 3 d, and weighed (Lindsey, Thoms, & Christians, 2020). Sand was removed from clippings before being weighed. To ensure enough tissue could be collected, mowing was delayed three d prior to clipping collections. Soil moisture data was measured monthly on three locations per plot using a time domain reflectance (TDR) sensor with 7.6 cm tines (Field Scout 350, Spectrum Technologies Inc., Aurora, IL, USA). Soil compaction measurements (determined as the maximum resistance) were collected monthly on three locations per plot using a digital soil penetrometer to a depth of 22.9 cm (Turf-Tec International, Tallahassee, FL, USA). Soil moisture and compaction were measured on the same day each month.

Soil samples (3.8 diameter x 5.1 cm depth) were collected from three locations per plot on 13 May and 31 Oct. in 2019 and 2020 (after two treatment applications and end of growing season, respectively). Soil tests were conducted to obtain soil nutrient concentrations, pH, CEC, and organic matter (Solum, Inc., Ames, IA, USA; SureTech Laboratories Indianapolis, IN, USA). Soil concentrations of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), sulfur (S), and zinc (Zn) were determined using the Mehlich 3 extraction method (Mehlich 1984). The Mehlich 3 extraction reagent was composed of 0.2M glacial acetic acid, 0.015 M ammonium fluoride, 0.25 M ammonium nitrate, 0.013 M nitric acid, and 0.001 M ethylenediaminetetraacetic acid (EDTA) and extractions were analyzed using an inductively coupled plasma (ICP) spectrometer. The pH was measured in a 1:2 $CaCl_2$ suspension, using 0.01M $CaCl_2$, and correlated back to 1:1 water. The soil CEC was determined by the standard summation of $cmol_c$ of Ca^{2+} + Mg^{2+} + K^+ + acid portion (H^+, Al_3^+, and NH_4^+). Soil organic matter was measured using the loss-on-ignition method (Nelson & Sommers, 1996).

Soil cores (3.8 diameter x 5.1 cm depth) were collected from three locations per plot on 29 Apr. and 17 Oct. in 2019 and 2020 (after one treatment application and end of growing season, respectively). Field moist soil cores were homogenized and sieved to 2 mm diameter particles before being used for soil analysis. Field moist soil was used to determine soil microbial biomass and air-dried soil was used to determine PMC and PMN.

Soil microbial biomass (MBC and MBN) were measured using the modified chloroform-fumigation extraction method (McDaniel, Grandy, Tiemann, & Weintraub, 2014; Vance, Brookes, & Jenkinson, 1987). One subset of a soil sample (5 g) was placed in a 50 ml beaker (fumigated sample) and another subset (5 g) was placed in a 50 ml centrifuge tube and capped (non-fumigated sample). The 50 ml beakers were placed in a desiccator with 1 ml of chloroform for 24 h. Both subsets sat for 24 h before extracting the soils with 25 ml of 0.5 M K_2SO_4. Soil extracts, the fumigated and non-fumigated, were analyzed using a total organic carbon-total nitrogen (TOC-TN) analyzer (TOC-V-CPN, Shimadzu Scientific Instruments Inc., Columbia, MD, USA) to determine the TOC and TN. The MBC and MBN were corrected using extraction efficiencies of 0.45 and 0.54 respectively (Brookes, Landman, Pruden, & Jenkinson, 1985; Joergensen, 1996).

The PMC was determined using techniques described by McDaniel et al. (2014) and McDaniel and Grandy (2016). Soil samples (5 g) were placed in centrifuge tubes and brought up to 50% water-holding capacity, which is near optimal water content for microbial respiration, and incubated for 14 d (Grandy & Robertson, 2007). Water-holding capacity was determined by measuring the gravimetric water content of the soil after it was brought to saturation and allowed to drain for 6 h (McDaniel & Grandy, 2016). During the 14 d incubation, CO_2 production was measured 1, 3, 5, 7, 10, and 14 d after the start of the incubation using a LI-830 CO_2 analyzer (LI-COR, Lincoln, NE, USA). The PMC was calculated by the cumulative CO_2 produced over the 14 d incubation. The 14 d length of the incubation is long enough to capture most of the change in CO_2 and a good measure of PMN (McDaniel et al., 2020).

The PMN was measured on the same incubated soils for the PMC, using methods described by De et al. (2020). The PMN was determined by extracting the total inorganic N (ammonium and nitrate) before and after the 14 d incubation using 2 M KCl. Soil extract ammonium (NH_4^+-N) concentration was measured colorimetrically using salicylate and ammonia cyanurate reagent packets (Hach Company, Loveland, CO, USA) at a 595 nm wavelength. Soil extract nitrate (NO_3^--N) was measured colorimetrically using the single reagent method (vanadium [III], sulfanilamide, and N-[1-naphthyl]-ethylenediamine dihydrochloride) at a 540 nm wavelength. Both, ammonium and nitrate, were analyzed using a Synerg HTX Multi-Mode Microplate Reader (BioTek Instruments, Inc., Winooski, VT, USA). The PMN was calculated by subtracting the initial total inorganic N from the total inorganic N after the 14 d incubation.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) with repeated measures using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA). A significant year-by-treatment interaction was present for turfgrass visual quality and microbial biomass (MBC and MBN), and thus data is described by year. A significant rating date-by-treatment interaction was present for turfgrass visual quality, percent green cover, and DGCI, and thus data is presented by rating date. Treatment means for soil moisture, compaction, nutrient concentrations, pH, CEC, organic matter, PMC, PMN, C:N ratio, and clipping collections have been combined across years and data collection dates due to a lack of a significant interaction with treatment effect. Treatment mean comparisons were separated using Fisher's protected least significant difference (LSD) at the $p \leq 0.05$ level.

RESULTS AND DISCUSSION

Turfgrass plant response

Treatments had a significant effect on visual quality ratings within years and across years (Table 3). In 2019, all treatments that received N, including HCU (2/3 rate), had similar visual quality ratings to each other and had greater ratings compared to HDG and the nontreated. The results were similar in 2020, but the treatments that had full N rates outperformed HCU (2/3 rate). However, on average across 2019 and 2020 all treatments that received N maintained acceptable visual quality ratings (six or above). Treatments that received a full N rate had a cumulative effect on visual quality ratings from 2019 to 2020. In

2020, HCU, HCU + HDG, HCU + BG, and urea had greater visual quality ratings compared to 2019. All other treatments performed equally from year to year.

Treatments had a significant effect on visual quality ratings on all rating dates (Figure 1). The treatments HCU, HCU + HDG, HCU + BG, and urea maintained acceptable visual quality ratings (six or above) on all rating dates within the study. The nontreated and HDG only had two rating dates, 12 weeks after initial treatment (WAIT) and 14 WAIT, with visual quality ratings above six. Reducing the N rate [HCU (2/3 rate)] resulted in visual quality ratings below the acceptable standards on two rating dates, 4 WAIT and 28 WAIT. However, all treatments had a reduction in turfgrass quality on 28 WAIT, which was likely due to the low temperatures at the end of the growing season. On average across years, HCU (2/3 rate) outperformed HDG and the nontreated and performed similarly to treatments that received full N rates (HCU, HCU + HDG, HCU + BG, and urea) in terms of visual quality. The similar performance of HCU (2/3 rate) to the full rate of urea could be due to the lack of a 2/3 rate of urea for comparison. It is unclear if this result is due to the N alone or the combination of N and humic substances. Overall, the addition of humic substances to fertilizers did not improve turfgrass quality compared to fertilizer alone.

Treatments had a significant effect on percent green cover on six of the 15 rating dates (Figure 2). There were no treatment differences in percent green cover until six WAIT. From six WAIT to 26 WAIT, all treatments that received N applications maintained 95% or greater percent green cover. The nontreated and HDG had one rating and three rating, respectively, below 95% green cover from six WAIT to 26 WAIT. On 28 WAIT, treatments with N had a greater percent green cover compared to HDG and the nontreated. In general, HCU (2/3 rate) performed similarly, in terms of percent green cover, to treatments with full N rates and outperformed HDG and the nontreated. The similar performance of HCU (2/3 rate) to the full rate of urea could be due to the lack of a 2/3 rate of urea for comparison and it is unclear if this result is due to the combination of N and humic substances or N alone. Furthermore, the addition of humic substances to fertilizers did not increase turfgrass percent green cover relative to fertilizer alone.

In general, treatments that received N had improved turfgrass quality and cover compared to treatments with no N (humic alone and the nontreated). Humic products incorporated with N fertilizer did not increase turfgrass quality and cover relative to N fertilizer alone. Ervin, Zhang, Goatley, and Askew (2004) reported that humic acid applications did not affect turfgrass quality or cover, which is consistent with the results in this paper. In contrast, Gao and Li (2012) and Zhang and Ervin (2004) found that humic acid applications alone and in combination with fertilizers improved turfgrass quality. Humic substances applied with reduced rates of N fertilizer maintained turfgrass quality and cover similar to full N rates. However, it is unclear if the response is due to the N alone or the combination of N and humic substances.

There were no treatment differences in terms of clipping biomass (data not shown in figure or table). On average throughout the duration of the study, the clipping biomass ranged from 1.8 g to 2.5 g m^{-2} among treatments. Receiving N fertilizer and/or humic substances did not affect clipping accumulation. This is consistent with Clapp et al. (2008) and Zhang and Ervin (2004), which found that applications of humic acid did not affect shoot growth compared to the control. Conversely, it has reported that clipping yields were reduced with the addition of humic substances to fertilizer tank mixes, which was not a result seen in this experiment (Gao and Li, 2012). Overall, the addition of humic substances to fertilizers did not increase turfgrass quality, cover, and clipping biomass relative to fertilizers alone. Additionally, incorporating humic substances with fertilizers at reduced N rates provided acceptable turfgrass quality and cover and did not affect clipping accumulation, thus it may have the potential to be incorporated into an environmentally conservative and sustainable fertilizer program. However, the response could be due to the N alone or the combination of N and humic substances.

Turfgrass soil health

Treatments did not have an effect on soil moisture, compaction, P, K, Mg, Ca, and Zn concentrations, pH, and CEC (Table S1). On average, the soil moisture and compaction ranged from 36.0 to 37.0 percent v/v and 1769.3 to 1879.1 kPa, respectively. In contrast, Van Dyke, Johnson, & Grossl (2008, 2009) found that applications of humic acid decreased soil moisture and shortened the time between irrigation. Soil nutrient concentrations ranged from 10.4 to 12.2 mg kg^{-1}, 45.8 to 56.7 mg kg^{-1}, 206.3 to 243.8 mg kg^{-1}, 1894.5 to 2205.9 mg kg^{-1}, and 3.9 to 5.2 mg kg^{-1} for P, K, Mg, Ca, and Zn, respectively. Hunter and Bulter (2005) reported that humic acid application did not affect soil nutrient concentrations, which is consistent with the results in this paper. Soil pH and CEC varied from 7.3 to 7.4 and 11.0 to 12.1, respectively. Conversely, it has been reported that soil pH decreases following N fertilizers and humic acid applications (Liu, Dell, Yao, Rufty, & Shi, 2011; Zhu and Li, 2018). The inconsistent responses in soil pH could be due to differences in fertilizer/humic substances type (i.e., liquid vs. granular) and/or soil type and age (i.e., fairway vs. putting green and newly established vs. mature putting green). In general, it is difficult to observe changes in physical and chemical soil properties over short-term management changes (Bunemann et al., 2018; Cardoso et al., 2013).

Treatments had a significant effect on soil S concentration and organic matter (Table S1). However, differences in soil organic matter were minimal. The HCU (2/3 rate) and HCU + HDG treatments had greater soil organic matter compared to the nontreated, but the difference was only 0.7% and 0.4%, respectively. All other treatments were similar to the nontreated in terms of soil organic matter. Zhu and Li (2018) reported that humic acid treatments did not affect soil organic matter. The HCU + BG (8.8 mg kg^{-1}) resulted in the greatest soil S concentration relative to all other treatments (7.2-7.7 mg kg^{-1}). Plant-available S, extracted by the Mehlich 3 reagent, was 18% greater for HCU + BG compared to all other treatments. To explain this result, HCU + BG was the only treatment that contained gypsum ($CaSO_4 \cdot 2H_2O$) and applications of gypsum can increase soil S concentrations (Prakash, Dhumgond, Shruthi, & Ashrit, 2020). Thus, soil S concentrations were increased with HCU + BG likely due to the application of gypsum and not humic substances. Overall, treatments had little impact on soil physical and chemical properties besides soil S concentration and organic matter. Long-term studies should be conducted to determine if applications of humic fertilizers could affect soil physical and chemical properties.

Treatments had significant effects on microbial biomass (Table 4). However, there were no differences in microbial C:N ratio, which ranged from 7.3 to 8.6. In 2019, HCU and HCU (2/3 rate) had 26% greater MBC compared to HCU + BG. In 2020, HCU + BG had a 30% increase in MBC relative to HDG and HCU. However, HCU + BG was not different from urea alone in terms of MBC. There were no differences in MBN in 2019. In 2020, HCU + BG had 40% greater MBN compared to HDG and the nontreated. Once again, there was no difference in MBN between HCU + BG and urea alone. Zhu and Li (2018) reported that MBC increased by 17% after humic acid applications. This is in contrast to other studies that found N fertilizer decreased or had no effect on MBC and MBN (Liu et al., 2011; Treseder, 2008). The inconsistent responses on MBC and MBN could be due to differences in fertilizer type (i.e., liquid vs. granular and nitrogen type), soil type (i.e., putting green vs. agriculture, forest, desert, and grassland), and/or soil age (newly established vs. mature putting green). In general, HCU + BG resulted in the greatest microbial biomass in year two, however, it was not different from urea alone.

Treatments did not have an effect on PMC (Table 5). Humic substances incorporated with fertilizers appears to have no effect on PMC. The PMC ranged from 119 to 163 mg CO_2-C kg^{-1}. Liu et al. (2011) reported that N fertilizer did not increase or decrease PMC, which is similar to the results in this paper. In contrast, other studies found that N fertilizer reduced PMC (Lu, Bowman, Rufty, & Shi, 2015; Treseder, 2008; Yao, Bowman, Rufty, & Shi, 2009). Furthermore, Moore et al. (2019a) reported that PMC increased with increasing N rates. The varying results in PMC response to N fertilizer may be a result of N rates, in which higher N (100-257 kg N ha^{-1}) amounts decrease PMC, while small N (12.5-50 kg N ha^{-1}) additions may increase PMC (Grandy et al., 2013; Moore et al., 2019b). There was a nonsignificant trend for treatments with higher PMC to exhibit better turfgrass quality. Moore et al. (2019b) reported that greater PMC was correlated with increased turfgrass quality, which is similar to the results in this paper.

Treatments had a significant effect on PMN (Table 5).The only treatment that had a greater PMN compared to HDG and the nontreated was HCU + BG. The HCU + BG had 54% greater PMN compared to HDG and the nontreated. All other treatments were similar in terms of PMN. Fertilizer alone did not improve PMN, which is similar to the result reported by Liu et al. (2011). Similar results to PMC, there are conflicting results to the response of PMN to N fertilizer. Moore et al. (2019a) found that increasing N rates corresponded with increased PMN or biological active N. Conversely, Yao et al. (2009) reported that PMN decreased with N fertilizer. These contrasting results may be due to soil characteristics like total soil organic matter, pH, and texture. It appears that the addition of BG to fertilizer treatments increases the PMN by 17% and 38% compared to HCU and urea, respectively. Future studies should incorporate BG with reduced N rates to determine if soil health can be improved or maintained while still providing acceptable turfgrass quality.

Overall, incorporating humic substances with N fertilizer had minimal effect on soil physical and chemical properties besides soil S concentration. Minimal differences were seen in soil organic matter and HCU + BG was the only treatment to contain gypsum, which has been shown to increase soil S concentrations. Humic substances incorporated with reduced N fertilizers provided similar turfgrass quality and cover, without reducing clipping biomass compared to full N rates. However, the similar performance of HCU (2/3 rate) to the full rate of urea could be due to the lack of a 2/3 rate of urea for comparison. Future research is needed to determine if this response is due to the N alone or the combination of N and humic substances. The addition of humic substances to fertilizers did not increase turfgrass quality, cover, and clipping biomass compared to fertilizers alone. However, the addition of BG to N fertilizer resulted in the greatest microbial biomass in year two. Furthermore, N fertilizer with BG had the greatest microbial activity in terms of PMN. In conclusion, humic products incorporated with N fertilizer did not improve turfgrass quality, cover, and clipping biomass relative to N fertilizer alone. Humic substances, especially BG, incorporated with N fertilizer has the potential to improve microbial biomass and microbial activity. Environmentally conservative and sustainable turfgrass fertilizer programs could incorporate the application of humic substances with N fertilizers.

AUTHOR CONTRIBUTIONS

Conceptualization, A.J.L., A.W.T., M.D.M. and N.E.C.; methodology, A.J.L., A.W.T., M.D.M. and N.E.C.; software, A.J.L. and M.D.M.; validation, A.J.L., A.W.T., M.D.M. and N.E.C.; formal analysis, A.J.L.; investigation, A.J.L. and A.W.T.; resources, A.J.L., A.W.T., and M.D.M.; data curation, A.J.L.; writing—original draft preparation, A.J.L.; writing—review and editing, A.J.L., A.W.T., M.D.M., and N.E.C.; visualization, A.J.L.; supervision, A.W.T.; project administration, A.J.L. and A.W.T.; funding acquisition, A.W.T. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

In accordance with Taylor & Francis policy and our ethical obligation as researchers, we are reporting that we received funding from a company that may be affected by the research reported in the enclosed paper. We have disclosed those interests fully to Taylor & Francis, and we have in place an approved plan for managing any potential conflicts arising from that involvement.

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FIGURES AND TABLES

Table 1. Summary of literature on the benefits of humic products on turfgrass plant and soil health.

Turfgrass	Type of study	Humic product	Average increase (%) compared to nontreated control	Reference
Creeping bentgrass (*Agrostis stolonifera* L.)	Greenhouse (G)	Leonardite humic acid (LHA)	15% maximum root length	Cooper, Liu, & Fisher, 1998
Creeping bentgrass	G	Humic acid (HA)	11% photosynthetic rate, 51% root dehydrogenase activity, 8% root mass	Liu, Cooper, & Bowmans, 1998
Kentucky bluegrass (*Poa pratensis* L.)	G	LHA	19% shoot mass, 60% root mass, 38% α-tocopherol (antioxidant), 25% ascorbic acid (antioxidant)	Zhang & Schmidt, 1999
Creeping bentgrass	G	LHA	41% root mass, 117% α-tocopherol, 37% ascorbic acid	Zhang & Schmidt, 2000
Tall Fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.; syn. *Festuca arundinacea* Scherb.]	G	LHA	57% root mass, 78% α-tocopherol, 39% ascorbic acid	Zhang & Schmidt, 2000
Creeping bentgrass	Field (F) & G	LHA	55% superoxide dismutase activity (antioxidant), 6% photochemical activity, 115% root mass, 7% leaf color	Zhang, Schmidt, Ervin, & Doak, 2002
Creeping bentgrass	F	LHA	114% superoxide dismutase activity, 15% photochemical activity	Zhang, Ervin, & Schmidt 2003a
Kentucky bluegrass	F	HA	14% photochemical efficiency, 36% heat tolerance, 22% root strength	Zhang, Ervin, & Schmidt 2003b
Tall Fescue	F	LHA	9% photochemical efficiency, 5% heat tolerance, 21% root	Zhang, Ervin, & Schmidt 2003c

			strength	
Creeping bentgrass	F	HA + seaweed extract	47% superoxide dismutase activity, 5% photochemical efficiency	Ervin, Zhang, Goatley, & Askew, 2004
Creeping bentgrass	G	HA	11% turfgrass quality, 38% root mass, 67% α-tocopherol	Zhang & Ervin, 2004
Creeping bentgrass	Growth chamber	LHA and fulvic acid (FA)	16% root mass	Clapp et al., 2008
Kentucky bluegrass	F	LHA and peat HA	54% root mass	Ervin, Zhang, & Roberts, 2008
Creeping bentgrass	G	LHA	21% root length	Van Dyke, Johnson, & Grossl, 2009
Perennial rye (*Lolium perenne* L.)	Field container (C)	LHA	8% total chlorophyll, 9% root length	Nikbakht, Pessarakli, Daneshvar-Hakimi-Maibodi, & Kafi,, 2014
Perennial rye	C	LHA	4% turfgrass quality, 10% shoot length	Daneshvar-Hakimi-Maibodi, Kafi, Nikbakht, & Rejali, 2015
Kentucky bluegrass	F	HA and FA	21% turfgrass quality, 16% normalized vegetation index (NDVI), 11% microbial biomass carbon	Zhu & Li, 2018
Kentucky bluegrass	G	Leonardite humic substances (LHS) [a]	87% total root length, 91% root surface area, 94% root volume	Lindsey, Thoms, & Christians, 2020
Bermudagrass [*Cynodon dactylon* (L.) Pers.]	G	LHS	87% longest root	Lindsey et al., 2020

[a] Humic substances contained humic acid, fulvic acid, humin, and humic acid precursor.

Table 2. Treatments, nutrient analysis, and application rates.

Treatment [a]	Nutrient analysis	Application rate (ha^{-1})	Yearly application rate (ha^{-1} yr^{-1})
Humic-coated urea (HCU)	44N-0P-0K, 2% (w/w) humic acid (HA)	7.3 kg N [b]	102.5 kg N
HCU (2/3 rate)	44N-0P-0K, 2% HA	4.9 kg N	68.4 kg N
HCU + humic dispersing granules (HDG)	44N-0P-0K, 2% HA + 1N-0.6P-0.3K,70% (w/w) HA	7.3 kg N + 55.7 kg HDG	102.5 kg N + 222.8 kg HDG
HCU + black gypsum (BG) [c]	44N-0P-0K, 2% HA + 0.3N-0P-0K, 48% (w/w) gypsum, 21% (w/w) HA	7.3 kg N + 146.5 kg BG	102.5 kg N + 439.5 kg BG
Urea	46N-0P-0K	7.3 kg N	102.5 kg N
HDG	70% HA	55.7 kg HDG	222.8 kg HDG
Nontreated		-	

[a] Urea, HCU, and HCU (2/3 rate) were applied every two wks from Apr.-Oct., HDG was applied in Apr., May, Sept., and Oct., and BG was applied in Apr., July, and Oct., in 2019 and 2020.

[b] The full nitrogen rate was 7.3 kg N ha^{-1} and the reduced nitrogen rate was 4.9 kg N ha^{-1}.

[c] Black gypsum is a combination of humic substances and gypsum.

Table 3. Effects of various treatments on visual quality ratings across years (1-9 scale where 1 = poor and 9 = ideal, 6 or above being acceptable) of a sand-based 'Penncross' creeping bentgrass (*Agrostis stolonifera* L.) putting green in Ames, IA, USA in 2019 and 2020.

Treatment [a]	2019	2020	$LSD_{0.05}$ [b]
Humic-coated urea (HCU)	7.0 [c]	7.6	0.2
HCU (2/3 rate)	6.8	7.0	NS [†]
HCU + humic dispersing granules (HDG)	7.0	7.6	0.2
HCU + black gypsum (BG)	6.9	7.7	0.2
Urea	6.8	7.5	0.2
HDG	5.3	5.3	NS
Nontreated	5.4	5.5	NS
$LSD_{0.05}$ [d]	0.2	0.4	

[a] Urea (7.3 kg N ha^{-1}), HCU (7.3 kg N ha^{-1}), and HCU (2/3 rate; 4.9 kg N ha^{-1}) were applied every two wks from Apr.-Oct., HDG (55.7 kg HDG ha^{-1}) was applied in Apr., May, Sept., and Oct., and BG (146.5 kg BG ha^{-1}) was applied in Apr., July, and Oct., in 2019 and 2020.

[b] Treatment mean comparisons within rows were separated using Fisher's protected least significant difference (LSD) at the $p \leq 0.05$ level.

[c] No year-by-rating date-by-treatment interaction. However, a significant year-by-treatment interaction was present; means are pooled over rating dates.

[d] Treatment mean comparisons within columns were separated using Fisher's protected least significant difference (LSD) at the $p \leq 0.05$ level.

[†] NS, nonsignificant at the 0.05 probability level.

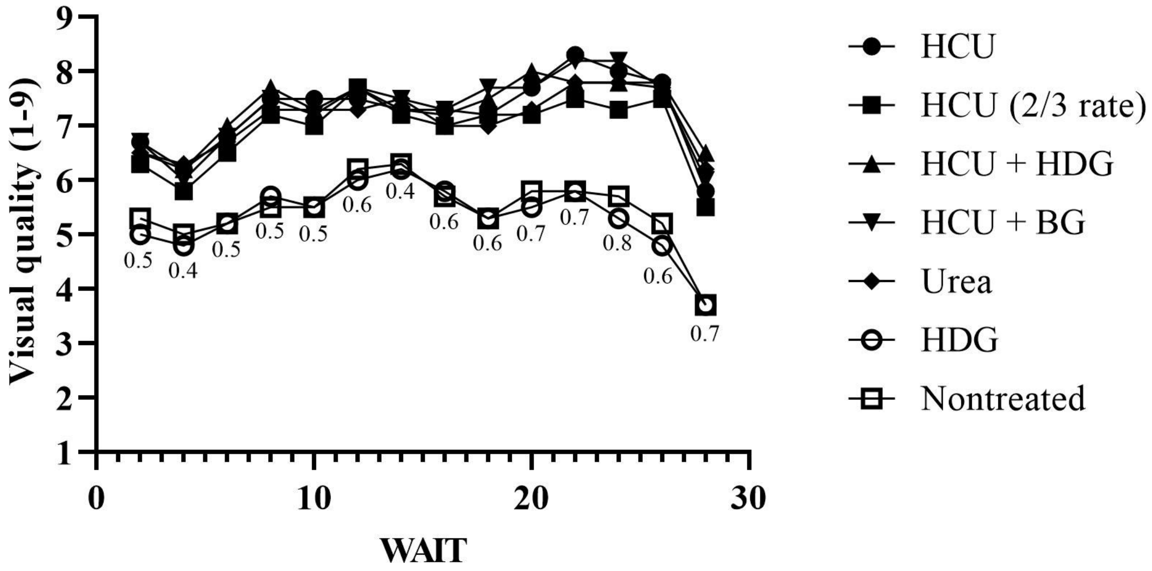

Figure 1. Effects of various treatments on visual quality ratings (1-9 scale where 1 = poor and 9 = ideal, 6 or above being acceptable) at rating dates (WAIT, weeks after initial treatment) of a sand-based ‘Penncross’ creeping bentgrass (*Agrostis stolonifera* L.) putting green in Ames, IA, USA in 2019 and 2020. Urea (7.3 kg N ha^{-1}), HCU (7.3 kg N ha^{-1}), and HCU (2/3 rate; 4.9 kg N ha^{-1}) were applied every two wks from Apr.-Oct., HDG (55.7 kg HDG ha^{-1}) was applied in Apr., May, Sept., and Oct., and BG (146.5 kg BG ha^{-1}) was applied in Apr., July, and Oct., in 2019 and 2020. No year-by-rating date-by-treatment interaction. However, a significant rating date-by-treatment interaction was present; means are pooled over years. Treatment mean comparisons were separated using Fisher’s protected least significant difference (LSD) at the $p \leq 0.05$ level. LSD and are placed under treatment means for each rating date.

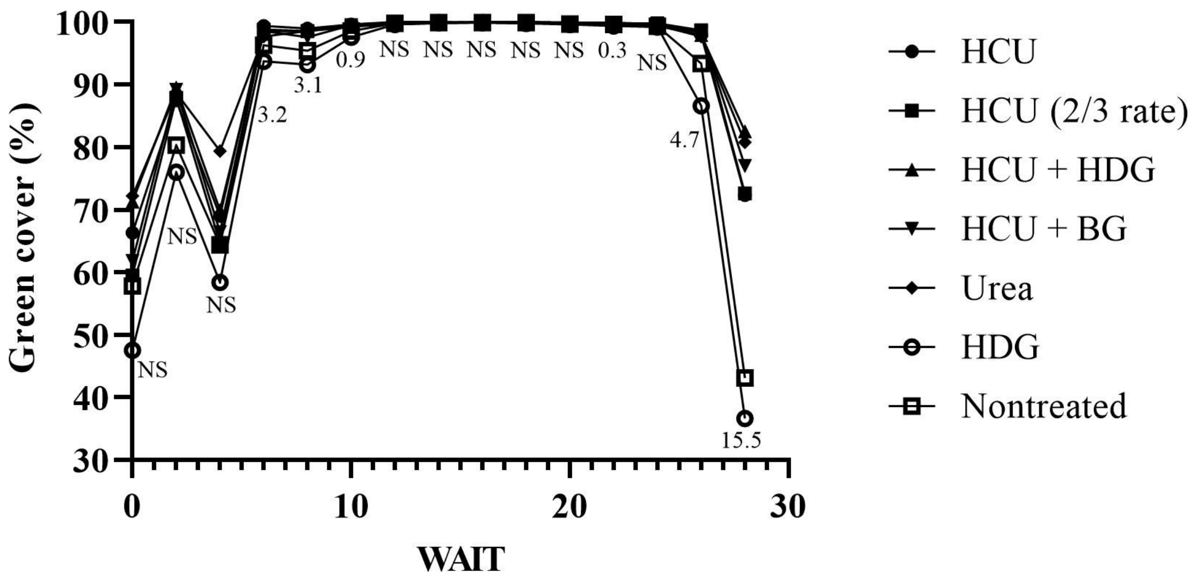

Figure 2. Effects of various treatments on percent green cover as determined by digital image analysis of a sand-based ‘Penncross’ creeping bentgrass (*Agrostis stolonifera* L.) putting green in Ames, IA, USA in 2019 and 2020. Urea (7.3 kg N ha^{-1}), HCU (7.3 kg N ha^{-1}), and HCU (2/3 rate; 4.9 kg N ha^{-1}) were applied every two wks from Apr.-Oct., HDG (55.7 kg HDG ha^{-1}) was applied in Apr., May, Sept., and Oct., and BG (146.5 kg BG ha^{-1}) was applied in Apr., July, and Oct., in 2019 and 2020. No year-by-rating date-by-treatment interaction. However, a significant rating date-by-treatment interaction was present; means are pooled over years. Treatment mean comparisons were separated using Fisher’s protected least significant difference (LSD) at the $p \leq 0.05$ level and were placed under each rating date. NS, nonsignificant at the 0.05 probability level.

Table 4. Effects of various treatments on soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass carbon to nitrogen ratio (C:N) of a sand-based 'Penncross' creeping bentgrass (*Agrostis stolonifera* L.) putting green in Ames, IA, USA in 2019 and 2020.

Treatment [a]	**MBC** [b]		**MBN**		**C:N**
	2019	**2020**	**2019**	**2020**	
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	
Humic-coated urea (HCU)	313 [c]	401	35	63	8.2 [d]
HCU (2/3 rate)	307	480	37	67	8.0
HCU + humic dispersing granules (HDG)	282	512	38	80	7.3
HCU + black gypsum (BG)	246	531	31	82	7.7
Urea	301	451	35	63	8.5
HDG	266	418	34	57	8.6
Nontreated	261	436	34	60	8.4
$LSD_{0.05}$ [e]	57	100	NS [†]	22	NS

[a] Urea (7.3 kg N ha^{-1}), HCU (7.3 kg N ha^{-1}), and HCU (2/3 rate; 4.9 kg N ha^{-1}) were applied every two wks from Apr.-Oct., HDG (55.7 kg HDG ha^{-1}) was applied in Apr., May, Sept., and Oct., and BG (146.5 kg BG ha^{-1}) was applied in Apr., July, and Oct., in 2019 and 2020. Soil cores were collected to a depth of 5.1 cm on 29 April and 17 Oct. in 2019 and 2020 (after one treatment application and end of field season, respectively).

[b] Microbial biomass was determined by the modified chloroform-fumigation extraction method.

[c] No year-by-rating date-by-treatment interaction. However, a significant year-by-treatment interaction was present; means are pooled over rating dates.

[d] No year-by-rating date-by-treatment interaction, means are pooled over years and rating dates.

[e] Treatment mean comparisons within columns were separated using Fisher's protected LSD at the $p \leq 0.05$ level.

[†] NS, nonsignificant at the 0.05 probability level.

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Table 5. Effects of various treatments on potentially mineralizable carbon (PMC), and potentially mineralizable net nitrogen (PMN) of a sand-based 'Penncross' creeping bentgrass (*Agrostis stolonifera* L.) putting green in Ames, IA, USA in 2019 and 2020.

Treatment [a]	PMC [b]	PMN [c]
	mg CO_2-C kg^{-1}	mg N kg^{-1}
Humic-coated urea (HCU)	152 [d]	6.5
HCU (2/3 rate)	156	6.3
HCU + humic dispersing granules (HDG)	163	6.6
HCU + black gypsum (BG)	156	7.6
Urea	151	5.5
HDG	119	5.0
Nontreated	126	4.9
$LSD_{0.05}$ [e]	NS [†]	2.1

[a] Urea (7.3 kg N ha^{-1}), HCU (7.3 kg N ha^{-1}), and HCU (2/3 rate; 4.9 kg N ha^{-1}) were applied every two wks from Apr.-Oct., HDG (55.7 kg HDG ha^{-1}) was applied in Apr., May, Sept., and Oct., and BG (146.5 kg BG ha^{-1}) was applied in Apr., July, and Oct., in 2019 and 2020. Soil cores were collected to a depth of 5.1 cm on 29 April and 17 Oct. in 2019 and 2020 (after one treatment application and end of field season, respectively).

[b] PMC was determined by measuring the CO_2 production during a 14 d soil incubation.

[c] PMN was calculated by subtracting the initial total inorganic nitrogen (N) from the total inorganic N after the 14 d incubation. Total inorganic N (ammonium and nitrate) was determined colorimetrically.

[d] No year-by-rating date-by-treatment interaction, means are pooled over years and rating dates.

[e] Treatment mean comparisons were separated using Fisher's protected least significant difference (LSD) at the $p \leq 0.05$ level.

[†] NS, nonsignificant at the 0.05 probability level.