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Species and Cultural Management of Earthworms on Golf Course Turf in Arkansas and Oklahoma

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Species and Cultural Management of Earthworms on Golf Course Turf in Arkansas and Oklahoma

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Abstract

As earthworms feed, they can egest soil and nutrient-rich aggregates (casts) on the soil surface. In low-cut turfgrass systems, such as golf course greens, tees, and fairways, surface casting can result in a muddy playing surface, ball roll issues, weed and pest invasion, reduced aesthetics, surface softening, and reduced photosynthesis. Because the use of pesticides for earthworm control is illegal in the U.S., earthworm casting must be managed through cultural practices. Sand topdressing is one method of earthworm control studied for use on golf courses, with the supposition being that the abrasive sand particles will deter the soft-bodied earthworms from remaining in the system; however, effects have been varied. Because treatment effects may vary depending on earthworm species composition, earthworm identification may be a critical step in establishing a casting control plan.

The objectives of this study were to 1) test the effect of heavy (2.54 cm yr^{-1}) or light (0.64 cm yr^{-1}) sand topdressing treatments and the effect of native soil (Captina silt loam; fine-silty, siliceous, active, mesic Typic Fragiudults) and sand-capped rootzones on casting activity of earthworms in ‘Patriot’ bermudagrass (*Cynodon* spp.), 2) to assess the relationships between soil moisture content and soil temperature on earthworm casting activity, and 3) determine earthworm species composition on golf course turf in Arkansas and Oklahoma.

Results indicate that light topdressing on a native soil rootzone may reduce casting activity, while topdressing rate in a sand-capped rootzone may not significantly impact casting activity. Soil moisture was not a significant predictor of earthworm casting activity. Soil temperature was a significant predictor of earthworm casting activity and explained 10-34% of the variation in casting activity between the four treatment combinations.

Diplocardia spp. were predominant across three sampling locations, and *Amynthas* spp. were present across all five sampling locations. The dominance of *Diplocardia* spp. in the turfgrass systems in this study is counter to expectations, as previous reports on earthworm composition in turfgrass systems have primarily reported non-native European and Asian earthworm species.

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Literature Review

Earthworm Activities in the Soil

The volume of soil that earthworms inhabit and influence is referred to as the drilosphere. Because of their ability to alter soil properties through burrowing, incorporation and breakdown of organic material, and mixing of soils, earthworms are considered ecosystem or soil engineers (Jouquet et al., 2006). Generally, earthworm activity in the drilosphere is considered beneficial, as burrowing creates macropores for air and water transport and root growth, moves organic matter into the soil profile, can improve decomposition of organic matter and thatch layers, and increases the availability of nutrients for plant uptake (Bityutskii et al., 2012; Brown, 1995; Kiyasudeen et al., 2016a; Lavelle, 1988). As earthworms burrow and ingest soil and organic material, they expel soil- and nutrient-rich aggregates known as casts, which are deposited either within the soil profile or on the soil surface (Brady and Weil, 2002; Hamilton and Sillman, 1989; Lee, 1985; Potter, et al., 2011a, 2011b). Cast properties are greatly influenced by soil type as well as earthworm species (Clause et al., 2014). Casts occur in three forms: spherical granular pellets of various sizes (typically 1-12 mm in diameter; Fig. 1), paste-like globular slurries with rounded, irregular shapes (Fig. 2), or tall heaps or columns with a range of shapes and sizes (Edwards and Bohlen, 1996). Generally, granular casts are easily removed by rainfall, while globular casts can persist in the system (Lavelle, 1988).

Casting stimulates microbial activity and releases beneficial exudates and plant-available nutrients into the soil (Backman, 1999; Brady and Weil, 2002; Hamilton and Sillman,1989; Jefferson, 1958; Kiyasudeen et al., 2016b; Potter, 1991; Potter et al., 1990; Slater and Hopp, 1948). Basker et al. (1994), Pommeresche et al. (2009), and Vos et al. (2014) observed increased P, K, Mg, Na, Ca, total-C and total-N in casts compared to the bulk soil. The extent of the increase in nutrients is dependent on both earthworm species and soil properties. Lunt and Jacobson (1944) measured significantly higher nitrogen, organic carbon, and calcium in earthworm casts compared to the surrounding forest topsoils. Basker et al. (1994) determined that, although casts from *Lumbricus rubellus* Hoffmeister, 1843 and *Aporrectodea caliginosa* (Savigny, 1826) contained higher exchangeable K compared to the control Raumai soil (Typic Haplaquept sandy, mixed, mesic; high non-exchangeable K), exchangeable K in casts from the two earthworm species was lower than the control Milson soil (Typic Fragiaqualf silty, mixed, mesic; low non-exchangeable K). Further, the casts of *L. rubellus* had more exchangeable K than those of *Ap. caliginosa* in the Raumai soil (Basker et al., 1994).

Casting on Golf Course Turfgrass

In low-cut turfgrass systems, earthworm presence and surface casting can have many negative effects. Casting on golf course greens, tees, and fairways can result in a muddy playing surface, water retention in the canopy layer, weed and pest invasion, reduced aesthetics, surface softening, reduced photosynthesis, issues with ball roll and playability, equipment damage, and can affect height of cut when casts build up on mower reels (Backman, 1999; Backman et al., 2001; Escritt and Arthur, 1948; Grant, 1983; Landschoot, 2017). Earthworms were reported to be one of the most common and widespread pests on golf course greens in the U.K. and Ireland in 2001 (Mann and Newell, 2005). In 2010, earthworms were listed in the top ten topics for pest and disease queries by the Turfgrass Protection department of the Sports Turf Research Institute (STRI, 2011), indicating that earthworm casting is a prevalent issue in low cut turfgrass situations. Casting can be affected by earthworm population size and composition; soil moisture, temperature, pH and texture; shade level; and cultural practices (Jefferson, 1956; Kirby and

Baker, 1995; Roy, 1957; Scullion and Ramshaw, 1988; Thomson and Davies, 1973; Tomlin et al., 1995).

Earthworm Habitat

Generally, earthworms are rare in coarse textured soils due to the abrasiveness of the sand particles and drought-prone conditions (Edwards and Bohlen, 1996; Lee, 1985). Earthworms are also infrequently found in high clay soils where oxygen gas concentrations can become deficient (Edwards and Bohlen, 1996; Lee, 1985). Lee (1985) suggests that earthworms that normally cast in subsurface burrows may cast at or near the surface in compacted soils. Baker and Binns (1998) determined through surveys of golf courses in the U.K., that on fairways with a sandy soil texture, less casting activity was observed compared to fairways with clay or clay loam soil texture; however, casting was not significantly correlated to soil texture on golf course tees under the same study.

Lavelle (1988) states that temperature is the most limiting factor for earthworm activity in temperate and cold climates. Evans and Guild (1948) determined that the number of earthworms was significantly correlated to the temperature of a grass field soil. Optimal temperature for temperate earthworms is between 10 and 20 °C (Berry and Jordan, 2001; Lee, 1985). James (1990) states that different earthworms have different temperature tolerances, and suggests that earthworm species of the Megasolecidae family native to the Americas, such as *Diplocardia* spp., have higher temperature tolerances than exotic earthworms in the Lumbricidae family.

Temperatures above 15 °C led to “fatigue” of *Lumbricus terrestris* Linnaeus, 1758, which Butt (1991) suggested could be directly related to temperature stress or potentially related to reproductive exhaustion. Temperatures above 20 °C in the same study by Butt (1991) led to clitellate loss, mass loss, and eventually mortality of *L. terrestris*. Temperatures above 35 °C can lead to moisture stress and in turn, earthworm desiccation or death due to an inability to supply adequate oxygen levels to earthworm tissues, a result of the increased metabolic rate that occurs at higher temperatures (Lee, 1985).

Along with temperature, soil moisture content is important for earthworm growth. Earthworm body tissue is maintained between 82-85% water (Grant, 1955), and earthworms need a moist environment so the external mucus layer will not desiccate. This mucus layer is necessary because earthworms breathe via diffusion of air through the mucus layer. Evans and Guild (1947) determined that soil moisture was a highly significant predictor of number and weight of earthworm casts. Berry and Jordan (2001) determined that the optimum gravimetric soil water content for *L. terrestris* is between 25 and 30% in a silty clay loam or loam soil in Iowa. Grant (1955) suggests that the optimum gravimetric moisture content for *Allolobophora caliginosa* (since reclassified as *Aporrectodea caliginosa* Savigny, 1826) and *Pheretima hupeiensis* (Michaelsen, 1985) is between 20 and 30%. Soil pH can also affect earthworms. Earthworms in temperate climates are generally found within a pH range of 5.0 to 7.0, and are rarely found in soil below a pH of 3.5 to 4, though ranges are variable among species (Curry, 1998; Jefferson, 1956; Lee, 1985).

General Classification of Earthworms

Earthworms can be loosely classified by their burrowing and feeding habits. Epigeic earthworms are generally pigmented and live near the soil surface where they feed on the organic material found in the litter layer (Brady and Weil, 2002; Doube and Brown, 1998; Hendrix and Bohlen, 2002). These species generally have high mortality and reproductive rates and are highly active when environmental conditions are favorable (Lavelle, 1988). Endogeic earthworms create shallow horizontal burrows, which provide more stable environmental conditions as they consume the surrounding soil and organic matter (Brady and Weil, 2002; Doube and Brown, 1998; Lavelle, 1988). Anecic earthworms burrow vertically and can create permanent burrows several meters long which open to the soil surface (Brady and Weil, 2002; Doube and Brown, 1998). Anecic earthworms feed partly on litter which they pull down into their permanent burrows (Lavelle, 1988). Classification is species dependent, and a species can be classified into multiple groups (Hendrix and Bohlen, 2002). Epi-anecic species consume plant litter and create permanent burrows in which they cast (Jouquet et al., 2006). Geophagous (soil-ingesting) species move within the soil to find optimum environmental conditions and are thought to be more dependent on environmental conditions than epi-anecic species (Jouquet et al., 2006). Generally, horizontally burrowing earthworms deposit casts within their burrows, while vertically burrowing earthworms deposit their casts on the soil surface (Edwards and Bohlen, 1996; Lee, 1985).

Morphological Earthworm Identification

Earthworm response to control methods is likely species-dependent, which could account for the variation in responses seen in previous research; as such, it is important to identify species in research focusing on earthworm control. This could help better target control methods and help increase efficiency in casting control. Earthworm taxonomy, however, is a very controversial field. Inconsistencies and redundancies in nomenclature make earthworm identification difficult for many species.

According to Schwert (1990), all well-preserved, clitillated adult earthworms of the family Lumbricidae can be identified using external characteristics. These include, but are not limited to, pigmentation; prostomium (extension over the mouth) shape; and clitellum (swollen band around the earthworm associated with cocoon production) placement, shape, size, and markings; among other features. James (1990) states that *Diplocardia* spp., several of which are known to be common in Arkansas and Oklahoma (Causey, 1952; Reynolds, 2008, 2010) cannot be identified using only external features, and that dissection of the dorsal side of the earthworm must be done to examine internal characteristics for identification. *Amynthas* spp. also require dissection for identification (Reynolds, 1978). In addition to the external features listed previously, internal features that assist in identification of *Diplocardia* and *Amynthas* spp. include spermathecae number; placement, and shape, position of the intestine; and heart placement, among other features (James, 1990; Reynolds, 1978).

A major issue that arises with morphological earthworm identification is that identification, either using internal or external features, relies on sexual characteristics, which are absent in juvenile earthworms (Richard et al., 2010; Schwert, 1990). This lack of a defining characteristic makes identification of sexually immature specimens difficult, if not impossible, and can affect subsequent research conclusions on earthworm composition, diversity, and ecosystem services (Boyer and Wratten, 2010; Klarica et al., 2012; Richard et al., 2010). As alluded to above, another concern with morphological earthworm identification is the redundancy in species names in the literature. In earthworm taxonomy, this synonymy can even extend to the genus level (Briones et al., 2009) Within the species-level classification, subspecies, forms (same species with different morphological characteristics), clades (phylogenetically related), and cryptic species (morphologically similar, but different phylogenetically) have been recognized, some of which are also potentially repetitively referring to a single species. This is, in part, due to the great variation and potential overlap of the “taxonomically important” identification features (Briones et al., 2009; Dayrat, 2005; Richard et al., 2010). Alternatively, there are instances where specimens are collected and do not correspond to any morphological description, indicating that there are species yet to be described in the literature (Boyer and Wratten, 2010; Buckley et al., 2011; James, 1990).

One example of the complex issues that taxonomists face with morphological identification was addressed by Pérez-Losada et al. (2009) when reviewing the identification of *Aporrectodea caliginosa.* This earthworm is the most abundant earthworm in agricultural systems across the world and in Paleartic grasslands, but the taxonomic status of the *Ap. caliginosa* complex has been debated for over a century (Pérez-Losada et al., 2009). The complex included three species (*Ap. caliginosa s.s., Ap. trapezoides,* and *Ap. nocturna*) and one subspecies (*Ap. tuberculata*; Pérez-Losada et al., 2009). These specimens are difficult to differentiate due to similar clitellum placement and morphological similarity; however, other identifying characteristics, including pigmentation and tuberculata pubertatis position and shape, can vary within species and between species, which makes identification and taxonomic differentiation difficult (Pérez-Losada et al., 2009). Pérez-Losada et al. (2009) used molecular techniques to determine species boundaries within the *Ap. caliginosa* complex, and determined that there were five valid species making up this complex, with the potential for additional unrecognized species and subspecies.

Molecular Earthworm Identification

Because of the issues that arise with morphological identification, more recent research has begun to explore molecular methods of earthworm identification. The goal with molecular identification is to identify the diversity in the earthworm community and to differentiate earthworms with ambiguous morphology. An important benefit of molecular identification is the potential ability to identify juvenile earthworms and partial specimens, which is difficult and sometimes impossible to do when relying solely on morphological cues (Klarica et al., 2012).

The mitochondrial cytochrome-*c* oxidase subunit I (COI) gene is the most popular sequence for species identification in animals (Klarika et al., 2012), and is targeted because this gene regularly shows greater than 2% species divergence (Hebert et al., 2003). Briones et al. (2009) determined through review that the 16S rDNA and COI genes can help discriminate differences at the species level. Klarica et al. (2012) had success obtaining sequences from the 12S, 16S, and COI sequences, but less success obtaining genes from the COII sequences for some of the earthworm species analyzed. Huang et al. (2007) also had success identifying earthworm species using a COI-based approach. According to Klarica et al. (2012), the 12S and 16S genes have lower genetic distances compared to the protein coding genes; however, the GenBank system currently has three times more lumbricid COI sequences than COII, 12S, and 16S sequences combined. Klarica et al. (2012) states that the COI gene is the best choice for addressing earthworm lineages, while the two rRNA coding genes provide enough variability to discern congeneric species. Novo et al. (2011) determined that the 18S rRNA gene was the least variable, while COI and 16S genes were the most variable in uncorrected pairwise divergence when analyzing DNA from hormogastrid earthworms in the Mediterranean basin. Richard et al. (2010) used COI genes to identify lumbricid adult and juvenile earthworms, and discovered the presence of possible cryptic species within *Lumbricus terrestris* Linnaeus, 1758, *L. castaneus* (Savigny, 1826), and *L. rubellus* Hoffmeister, 1843.

Although molecular sequencing does provide benefits for earthworm identification, there are still issues that arise. Earthworm relatedness can vary depending on the gene used, and sometimes, genes do not agree on identification (Pop et al., 2007). Earthworms can still be misidentified or labeled as synonymous with other species (Chang et al., 2009; Pérez-Losada, 2012), which can lead to faulty interpretations in taxonomy and identification.

Earthworms in Turfgrass

While some research has been conducted to investigate earthworms in turf, relatively little is known about earthworm species composition in turfgrass systems, and this information is especially lacking for turfgrass systems in the transition zone of the U.S. Redmond et al. (2014) surveyed six golf courses in central Kentucky and found seven species of earthworms, with *Aporrectodea trapezoides* (Dugès, 1828), an endogeic species (Hendrix and Bohlen, 2002; Pathma and Sakthivel, 2012), dominating the earthworm community structure. The same study also determined that soil parameters such as pH, plant available micronutrients, and percent of sand, silt, clay, and organic matter were not strong predictors of earthworm community composition.

Potter et al. (1994) studied the effects of pesticides on earthworms in Kentucky bluegrass turfgrass near Lexington, KY. The earthworm community was primarily comprised of *Aporrectodea turgida* (Eisen, 1873), with some *Ap. trapezoides* (Dugès, 1828), *Lumbricus terrestris* Linnaeus, 1758, and *Eisenia* spp. (Potter et al., 1994). *Aporrectodea turgida* is listed as an endogeic species (Kernecker et al., 2014), *Ap. trapezoides* is an endogeic species (Bartlett et al., 2006; Brown, 1995; Hendrix and Bohlen, 2002; Pathma and Sakthivel, 2012), and *L. terrestris* is an anecic species (Bartlett et al., 2006; Brown, 1995; Hendrix and Bohlen, 2002; Pathma and Sakthivel, 2012; Pérès et al., 2010). While studying the effect of fungicides and insecticides on earthworms, Tu et al. (2011) observed that *Diplocardia* spp. dominated the earthworm populations, though some *L. rubellus* Hoffmeister, 1843 specimens were observed as well. *Lumbricus rubellus* is alternately listed as endogeic, epigeic, or epi-anecic (Bartlett et al., 2006; Brown, 1995; Pérès et al., 2010).

In the U.K., Bartlett et al. (2008) surveyed five golf courses and determined that of the seven species collected, *Aporrectodea rosea* (Savigny, 1826), *Lumbricus rubellus* Hoffmeister, 1843, *Ap. longa* (Ude, 1885), and *L. terrestris* Linnaeus, 1758 dominated the earthworm communities. *Aporrectodea rosea* is alternately listed as anecic or endogeic (Bartlett et al., 2006; Brown, 1995), while *Ap. longa* is considered an anecic species (Pérès et al., 2010). Jefferson (1955) found 27 species of earthworms on a turfgrass research site in the U.K., dominated by *Allolobophora terrestris* (Savigny) f. *longa* (Ude), *A. caliginosa* (since reclassified as *Aporrectodea caliginosa* Savigny, 1826), and *Lumbricus* spp. *Allolobophora caliginosa* is listed as endogeic by Pérès et al. (2010). Binns et al. (1999) surveyed 32 golf courses in the U.K. and determined that species composition was dominated by *Aporrectodea longa*, *L. terrestris*, and *Ap. caliginosa* (Savigny, 1826), an endogeic species (Brown, 1995; Hendrix and Bohlen, 2002).

Chemical Control of Earthworms: Pesticides

Several methods have been tested to control earthworm populations and casting activity on golf course systems. Until the 1950s, substances such as mercuric chloride, copper sulfate, derris dust, potassium permanganate, and lead arsenate were used to control earthworms (Dawson, 1930; Escritt and Arthur, 1948). These methods mainly fell out of use, however, due to high cost, damage to the turf, incidents of increased earthworm activity, lack of supply, potential hazard to the users, or restrictions and bans on use (Dawson, 1930; Escritt and Arthur, 1948; Kirby and Baker, 1995). Additionally, other than lead arsenate, none of these methods provided significant long-term control (Dawson, 1930; Escritt and Arthur, 1948; Leach, 1928). A survey by Baker and Binns (1998) showed that golf courses in the U.K. were using carbendazim, gamma-HCH + thiophanate-methyl, and carbaryl for earthworm suppression; however, of the 297 responding courses, 70% stated that suppression was only effective for three months or less. A study conducted by Schread (1952) indicated that parathion, chlordane, aldrin, lindane, and dieldrin caused mortality of the Asian earthworm *Pheretima hupeiensis* (Michaelsen, 1985), although repeat application of the pesticides caused some turf damage and aldrin initially caused an increase in casting activity before mortality occurred several weeks post-application. Pesticides such as chlordane were extremely effective and provided long-term earthworm control, but were banned in the U.S. and the U.K. in the early 1980s and 1990s, respectively, due to their persistence in the environment and the associated health and environmental risks (Baker and Binns, 1998; Bartlett, 2008; Kirby and Baker, 1995; National Pesticide Information Center [NPIC], 2001; Potter et al., 2011a, 2011b). Insecticides such as diazinon, bendiocarb and carbaryl are no longer used for earthworm control, due to replacement by more target-pest-specific chemicals (Potter et al., 2011a).

Some relatively recent research has revisited the idea of chemical control using insecticides and fungicides such as thiophanate-methyl, imidacloprid, isofenphos, trichlorfon, chlorpyrifos, and carbaryl, with some short-term but little long-term success, and in many cases, repeated applications were required (Mostert et al., 2002; Potter, 1991; Tu et al., 2011; Williamson and Hong, 2005). Potter et al. (1994) tested 23 pesticides and growth regulators, and determined that only benomyl, ethoprop, thiophanate-methyl, and fonofos were effective at reducing earthworm abundance and biomass three weeks after application. The entomopathogenic nematode *Steinernema carpocapsae* reduced earthworm biomass but not abundance when applied in the fall (Potter et al., 1994). Research conducted by Larson et al. (2011) indicated that a joint clothianidin-bifenthrin application and an application of carbaryl to turfgrass reduced earthworm abundance, biomass, and casting for up to five weeks. An application of clothianidin reduced earthworm abundance and biomass after one week and reduced casting activity compared to the untreated control (Larson et al., 2011). The use of the anthranilic diamide chlorantraniliprole did not adversely affect the number or biomass of earthworms nor the number of earthworm casts when compared to the untreated control (Larson et al., 2011). Regardless of efficacy, there are currently no pesticides labeled for use on earthworms in the U.S., and any use of pesticides specifically to kill or control earthworms would therefore be illegal (Potter et al., 2011a). It should also be noted that repeated off-label use of pesticides for earthworm control can potentially lead to resistance issues of the target pest, and should be avoided.

Chemical Control of Earthworms: Expellants

In addition to pesticides, chemical expellants have been used for earthworm population control. Chemical solutions containing compounds that cause earthworm cuticle irritation are applied to the soil surface; to escape the chemical, the earthworms move to the soil surface, where they can then be removed or left to dry and mowed into the turf canopy (Escritt and Arthur, 1948; Potter et al., 2011a, 2011b). Mustard seed has been researched as a possible earthworm expellant. Ground mustard seed or mustard flour solutions contain allyl isothiocyanate, which is a product of the enzymatic degradation of glucosinolates (sulfur-containing compounds) in the mustard flour (Zaborski, 2003). Research has shown that mustard meal extraction is somewhat effective on anecic species, adults, and large specimens, but not as effective on endogeic earthworm species, juveniles, or small species (Bartlett et al., 2006). Bartlett et al. (2006) suggest that endogeic species are more likely to move laterally to avoid the irritant rather than move to the soil surface.

Mowrah meal, a byproduct of extracting oil from *Bassia latifolia* seeds, has historically been used as a chemical earthworm expellant (Dawson, 1930; Escritt and Arthur, 1948). The active ingredient in mowrah meal, mowrin, was effective at bringing earthworms quickly to the surface; however, there was some phytotoxicity effect on the turf (Escritt and Arthur, 1948). Occasionally, earthworm activity increased after application of mowrah meal, as the organic matter in the mowrah meal provided a food source for the earthworms after the decomposition of the mowrin (Escritt and Arthur, 1948). Mowrah meal is no longer sold in the U.S. and is not labeled for earthworm control (Potter et al., 2009).

Tea seed meal extract, a product of *Camellia oleifera,* has recently been researched as a potential expellant. Potter et al. (2009; 2011a; 2011b) has shown that the natural saponins in tea seed meal can expel up to 200 earthworms in 0.93 m^2, mostly *Aporrectodea* spp. The tea seed pellets were successful in reducing casting activity 4-5 weeks after application, and required less water than mowrah meal to apply (Potter et al., 2009). Additionally, no phytotoxicity was observed on the turfgrass, though the authors do note that if labeled for earthworm use, restrictions would need to specify buffer zone use to reduce risk to aquatic organisms on golf courses (Potter et al., 2009). Kowalewski and McDonald (2016) observed that organic fertilizers such as Early Bird™ (Ocean Organics, Ann Arbor, MI) and TourTurf® TAG (E. Marker A/S, Padborg, Denmark) act as earthworm expellants and have shown some promise in reducing earthworm casting activity by *Lumbricus terrestris* Linnaeus, 1758. Seamans et al. (2015) also showed reductions in numbers of *Lumbricus* spp. and *Aporrectodea* spp. with applications of Early Bird™ fertilizer.

The use of expellants declined in the 1940s and 1950s with increased use of pesticides (Potter et al., 2011a). Since there are no longer pesticides labeled for earthworm control, recent research has revisited the idea of chemical expellants; however, several issues still arise. The use of expellants is only effective at times of the year when earthworms are near the soil surface, so the expellants can come in contact with the earthworms. Additionally, expellants do not necessarily work on all earthworms. There has been success with expelling *Aporrectodea* spp., but less success on *Amynthas* spp, with little known about the effects on other species (Potter, 2009). Once expelled, there is also the need to clear expelled earthworms from the turf surface, which can lead to potential odor issues associated with the dead earthworms and potential for phytotoxicity issues on the turfgrass (Escritt and Arthur, 1948; Kirby and Baker, 1995; Potter et al., 2011b). While biodegradation of saponins occurs quickly, saponins are toxic to fish, and precautions would need to be taken to prevent toxic levels in golf course waterways (Potter et al., 2011a; 2011b). Furthermore, no expellants are currently labeled or marketed for use in earthworm control (Potter et al., 2009; 2011a; 2011b), so application of these products to intentionally kill earthworms is also illegal.

Cultural Control of Earthworms: Soil Modification

In addition to chemical control measures, various turfgrass cultural management practices have been researched for earthworm casting control. One control method that has been attempted in turfgrass systems is soil pH modification. While Backman et al. (2001) determined that neither acidifying fertilizers (ammonium sulfate or ferrous sulfate) nor liming of the soil influenced cast count numbers significantly, Baker et al. (1996), Baker and Owen (2004), and Escritt and Arthur (1948) did see significant positive correlation between soil pH and casting count and significant reductions in casting by undocumented earthworm species through the use of acidifying fertilizers such as sulfur and aluminum sulfur. Baker and Binns (1998) had inconclusive results with differences in soil pH and earthworm casting by undocumented earthworm species. It has been suggested that the effect of acidifying fertilizers may be earthworm species dependent, and that special care must be taken to balance casting control with the health and quality of the turf when using acidifying fertilizers, as high rates of sulfur fertilizer can cause phytotoxicity (Baker and Binns, 1998; Baker and Owen, 2004).

Other soil modification techniques that have been investigated for earthworm casting control are hollow tine cultivation and water injection cultivation. Hollow tine cultivation increased casting activity, while water injection cultivation was shown to decrease earthworm casting activity (Karcher et al., 2001).

Cultural Control of Earthworms: Removal of the Food Supply

Modification of the food supply has also been tested as an earthworm control method. Abbott and Parker (1981) and Martin (1982) suggest that earthworm casting activity may increase as food supply decreases, as the earthworms ingest more soil to obtain the food and nutrient supply they need. Removal of this food supply through processes such as thatch degradation or residue removal may therefore lead earthworms to increase burrowing and soil ingestion and egestion in the search of food (Binet and Le Bayon, 1999; Martin, 1982); however, the effects of residue removal on earthworm casting have been varied. Baker and Binns (1998) saw no significant difference in casting activity on tees where clippings were removed compared to tees where clippings were rarely or never removed, while Baker et al. (2000) determined that continuous removal of grass clippings throughout the year led to reduced casting activity.

Cultural Control of Earthworms: Sand Topdressing

Another cultural method with potential for earthworm casting control is the use of sand topdressing. Sand topdressing is a common practice on golf courses, and is used to smooth the playing surface, enhance turfgrass recovery, modify the soil properties, and promote organic matter dilution in the profile (McCarty, 2011). Sand has been shown to deter burrowing by *Lumbricus terrestris* Linnaeus, 1758 in wastewater treatment systems (Hawkins et al., 2008), and it has been suggested that sand topdressing may lead to reduced moisture content, abrasiveness, and reduced organic matter, which might repel earthworms from the system (Mann, 2004). Additionally, it has been suggested that repeated applications of topdressing sand may provide an area for earthworms to disperse their casts within the soil profile, instead of at the soil surface (Mann, 2004). Alternatively, sand topdressing may introduce earthworms into the turfgrass system via cocoons (Redmond et al., 2014).

Like pH and residue removal, incorporation of sand into the turfgrass canopy for earthworm control has had varied results. Bartlett et al. (2008) saw no significant reduction in earthworm populations on sand-capped tees and Baker et al. (2005) saw no significant reduction in casting on sand-topdressed fairways, regardless of sand type or size used. A preliminary study by Henderson et al. (2011) determined that high rates (1.0 and 1.5 cm in year one and 1.4 and 2.1 cm in year two) of sand topdressing resulted in significantly lower rates of earthworm casting than the low-rate (0.5 cm in year one and 0.7 cm in year two) and non-topdressed control plots. Backman et al. (2002) saw similar results in heavily-topdressed (3.8 cm yr^{-1}) plots compared to lightly-topdressed (1.9 cm yr^{-1}) and control plots. Williamson and Hong (2005) determined that topdressing with the use of angular soil aggregates such as Black Jack (coal slag) or Zeolite (angular mineral) had some suppression of casting activity. The effects of these angular soil aggregates did not last throughout the growing season, potentially due to the aggregates being incorporated gradually into the turfgrass canopy, and because of this, the authors suggested that multiple applications during the growing season could be necessary to suppress earthworm casting activity (Williamson and Hong, 2005).

Pesticide use to control earthworms is illegal in the U.S., so cultural practices must be used to reduce earthworm casting activity. Research exploring cultural management of earthworm casting have had inconsistent results. As management is likely earthworm species-specific, and information regarding earthworm species composition on turfgrass systems in the U.S. is lacking, further research is needed to identify earthworm species on golf course turf, and to assess the effect of different cultural practices on different earthworm species’ casting activity.

Figure Legend

Figure 1	Granular earthworm cast on ‘Patriot’ bermudagrass (*Cynodon* spp.). 29 August 2016, Fayetteville, AR.
Figure 2	Globular earthworm cast on ‘Patriot’ bermudagrass (*Cynodon* spp.). 19 February 2016, Fayetteville, AR.

Figure 1

Figure 2

Table 1. Earthworm species that have been identified in turfgrass systems. Earthworm species are referred to by the name assigned in the cited literature, although it is recognized that in some cases, some species may have been renamed or reassigned.

Reference	Location	Turfgrass Species, if known	Earthworm Species
Backman, 1999	Pacific Northwest United States	*Poa annua, Agrostis stolonifera,* and *Lolium perenne* mixture	*Lumbricus terrestris*
Backman et al., 2001	Pacific Northwest United States	*Poa annua* and *Agrostis stolonifera* mixture	*Lumbricus terrestris*
Bartlett et al., 2006	Bedfordshire, UK	Pasture, unspecified species	*Allolobophora chlorotica* *Aporrectodea rosea* *Aporrectodea caliginosa* *Lumbricus castaneus* *Lumbricus festivus* *Lumbricus rubellus* *Lumbricus terrestris*
Bartlett et al., 2008	5 golf courses in Bedfordshire and Buckinghamshire, UK	Unlisted species; fairway turfgrass	*Allolobophora chlorotica* *Aporrectodea caliginosa* *Aporrectodea longa* *Aporrectodea rosea* *Lumbricus festivus* *Lumbricus rubellus* *Lumbricus terrestris*
Binns et al., 1999	32 UK golf courses	Unlisted species; fairway turfgrass	*Allolobophora chlorotica* *Aporrectodea caliginosa* *Aporrectodea icterica* *Aporrectodea longa* *Aporrectodea rosea* *Dendrodrilus rubidus* *Lumbricus castaneus* *Lumbricus festivus* *Lumbricus rubellus* *Lumbricus terrestris* *Octolasion cyaneum* *Octolasion tyrtaeum tyrtaeum*
Jefferson, 1955	West Yorkshire, UK	*Agrostis tenuis*, *Festuca rubra*, *Festuca tenuifolia* mixture	*Allolobophora caliginosa* *Allolobophora chlorotica* *Allolobophora terrestris* f. *longa*
Kowalewski and McDonald, 2016	Oregon	*Agrostis stolonifera* and *Agrostis capillaris* mixture	*Lumbricus terrestris*
Potter et al., 1994	Kentucky	*Poa pratensis*	*Aporrectodea turgida* *Aporrectodea trapezoides* *Lumbricus terrestris* *Eisenia* spp.
Redmond et al., 2014	6 golf courses in central Kentucky	*Zoysia* spp.	*Allolobophora chlorotica* *Aporrectodea trapezoides* *Diplocardia singularis*
		Agrostis stolonifera	*Allolobophora chlorotica* *Amynthas* spp. *Aporrectodea rosea* *Aporrectodea trapezoides* *Diplocardia singularis*
		Lolium perenne	*Allolobophora chlorotica* *Amynthas* spp. *Aporrectodea rosea* *Aporrectodea trapezoides* *Diplocardia singularis* *Lumbricus rubellus*
Tu et al., 2011	North Carolina	*Cynodon* spp.	*Diplocardia* spp. *Lumbricus rubellus* *Lumbricus terrestris*
Williamson and Hong, 2005	Wisconsin	*Agrostis palustris* and *Poa annua* mixture	*Lumbricus terrestris*

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Effect of sand topdressing rate and soil rootzone texture on earthworm casting activity in golf course turf

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Abstract

As earthworms feed, they can egest soil and nutrient-rich aggregates (casts) on the soil surface. In low-cut turfgrass systems, such as golf course greens, tees, and fairways, surface casting can result in a muddy playing surface, ball roll issues, weed and pest invasion, reduced aesthetics, surface softening, and reduced photosynthesis. As the use of pesticides for earthworm control is illegal in the U.S., earthworms must be managed through cultural practices. Sand topdressing is one method of earthworm control studied for use on golf courses, with the supposition being that the abrasive sand particles will deter the soft-bodied earthworms from remaining in the system; however, effects have been varied.

The objectives of this study were to 1) test the effect of heavy (2.54 cm yr^{-1}) or light (0.64 cm yr^{-1}) sand topdressing treatments and the effect of native soil (Captina silt loam; fine-silty, siliceous, active, mesic Typic Fragiudults) and sand-capped rootzones on casting activity of earthworms in 'Patriot' bermudagrass (*Cynodon* spp.) and 2) to assess the relationships between soil moisture content and soil temperature on earthworm casting activity.

Results indicate that light topdressing on a native soil rootzone may reduce casting activity, while topdressing rate in a sand-capped rootzone may not significantly impact casting activity. Soil moisture was not a significant predictor of earthworm casting activity. Soil temperature was a significant predictor of earthworm casting activity and explained 10-34% of the variation in casting activity between the four treatment combinations.

Earthworm abundance was greatest in the light topdressing soil rootzone plots, which displayed the least amount of casting activity. These results are counter to expectations, and may be due to earthworm species composition. *Diplocardia* spp. were present across all treatments. *Amynthas* spp. were present in the sand treatments and the light topdressing soil treatment, while

some non-native species were present in the soil treatments and the light topdressing sand treatment. Sand topdressing may impact surface casting, though the results varied over time and effects may be earthworm species specific.

Introduction

Because of their ability to alter soil properties through burrowing, incorporation and breakdown of organic material, and mixing of soils, earthworms are considered ecosystem or soil engineers (Jouquet et al., 2006). Generally, earthworm activity is considered beneficial, as burrowing creates macropores for air and water transport and root growth, moves organic matter into the soil profile, can improve decomposition of organic matter and thatch layers, and increases the availability of nutrients for plant uptake (Bityutskii et al., 2012; Brown, 1995; Kiyasudeen et al., 2016; Lavelle, 1988). As earthworms burrow and ingest soil and organic material, they expel soil- and nutrient-rich aggregates known as casts, which are deposited either within the soil profile or on the soil surface (Brady and Weil, 2002; Hamilton and Sillman, 1989; Lee, 1985; Potter, et al., 2011a, 2011b). Cast properties are greatly influenced by soil type as well as earthworm species (Clause et al., 2014).

In low-cut turfgrass systems, earthworm presence and surface casting can have many negative effects. Casting on golf course greens, tees, and fairways can result in a muddy playing surface, water retention in the canopy layer, weed and pest invasion, reduced aesthetics, surface softening, reduced photosynthesis, and issues with ball roll and playability (Backman, 1999; Backman et al., 2001; Escritt and Arthur, 1948; Grant, 1983). Earthworms were reported to be one of the most common and widespread pests on golf course greens in the U.K. and Ireland in 2001 (Mann and Newell, 2005). In 2010, earthworms were listed in the top ten topics for pest and disease queries by the Turfgrass Protection department of the Sports Turf Research Institute (STRI, 2011), indicating that earthworm casting is a prevalent issue in low cut turfgrass situations. Casting can be affected by earthworm population size and composition; soil moisture, temperature, pH and texture; shade level; and cultural practices (Jefferson, 1956; Kirby and

Baker, 1995; Roy, 1957; Scullion and Ramshaw, 1988; Thomson and Davies, 1973; Tomlin et al., 1995).

Earthworms can be loosely classified ecologically, e.g. by their burrowing and feeding habits. Epigeic earthworms live near the soil surface where they feed on the organic material found in the litter layer (Brady and Weil, 2002; Doube and Brown, 1998; Hendrix and Bohlen, 2002). Endogeic earthworms create shallow horizontal burrows as they consume soil and organic matter; these burrows provide more stable environmental conditions for the earthworms (Brady and Weil, 2002; Doube and Brown, 1998; Lavelle, 1988). Anecic earthworms burrow vertically and can create permanent burrows several meters long which open to the soil surface (Brady and Weil, 2002; Doube and Brown, 1998). Ecological classification is species dependent, and a species can be classified into multiple groups. Generally, horizontally burrowing earthworms deposit casts within their burrows, while vertically burrowing earthworms deposit their casts on the soil surface (Edwards and Bohlen, 1996; Lee, 1985).

Briones et al. (2009) determined through review that the 16S rDNA and mitochondrial cytochrome-*c* oxidase subunit I (COI) genes can help discriminate differences at the species level. Huang et al. (2007) also had success identifying earthworm species using a COI-based approach. Richard et al. (2010) used COI genes to identify *Lumbricus* adult and juvenile earthworms, and discovered the presence of possible cryptic species within *L. terrestris* Linnaeus, 1758, *L. castaneus* (Savigny, 1826), and *L. rubellus* Hoffmeister, 1843. Molecular identification has been used to determine the possible existence of unrecognized species within clades of *Aporrectodea longa* (Ude, 1885; Pérez-Losada et al., 2009). The GenBank system currently has three times more lumbricid COI sequences than COII, 12S, and 16S sequences combined (Klarica et al., 2012). An important benefit of molecular identification is the potential ability to identify juvenile earthworms and partial specimens, which is difficult and sometimes impossible to do when relying solely on morphological cues (Klarica et al., 2012).

Currently, there are no pesticides labeled for use for earthworm control in the U.S. Because of this, turfgrass managers must rely on cultural practices to manage issues with earthworm casting. One cultural method with potential for earthworm casting control is the use of sand topdressing. Sand topdressing is a common practice on golf courses, and is used to smooth the playing surface, enhance turfgrass recovery, modify the soil properties, and promote thatch decomposition (McCarty, 2011). Sand has been shown to deter burrowing by *Lumbricus terrestris* in wastewater treatment systems (Hawkins et al., 2008), and it has been suggested that sand topdressing may lead to reduced moisture content, abrasiveness, and reduced organic matter, which might repel earthworms from the system (Mann, 2004). Additionally, repeated applications of topdressing sand may provide an area for earthworms to disperse their casts instead of at the soil surface (Mann, 2004). Alternatively, sand topdressing may introduce earthworms into the turfgrass system via cocoons (Redmond et al., 2014).

Like many of the cultural practice methods researched for earthworm control, sand topdressing has had varied results. Bartlett et al. (2008) saw no significant reduction in earthworm populations on sand-capped tees and Baker et al. (2005) saw no significant reduction in casting on sand-topdressed fairways, regardless of sand type or size used. A preliminary study by Henderson et al. (2011) determined that high rates (1.0 and 1.5 cm in year one and 1.4 and 2.1 cm in year two) of sand topdressing resulted in significantly lower rates of earthworm casting than the low rate (0.5 cm in year one and 0.7 cm in year two) and non-topdressed control plots. Backman et al. (2002) saw similar results in heavily-topdressed (3.8 cm yr^{-1}) plots compared to lightly-topdressed (1.9 cm yr^{-1}) and control plots. Williamson and Hong (2005) determined that topdressing with the use of angular soil aggregates such as Black Jack (coal slag) or Zeolite (angular mineral) had some suppression of casting activity. The effects of these angular soil aggregates did not last throughout the growing season, potentially due to the aggregates being incorporated gradually into the turfgrass canopy; because of this, the authors suggested that multiple applications during the growing season could be necessary to suppress earthworm casting activity (Williamson and Hong, 2005). Preliminary earthworm casting counts completed by Anderson (2012) on simulated bermudagrass tee box plots in Arkansas showed that heavy topdressing (2.54 cm yr^{-1}) on a sand-capped rootzone resulted in the greatest amount of casting activity, while a lighter topdressing rate (0.64 cm yr^{-1}) on a native soil rootzone resulted in the least amount of casting activity. In general, the native soil rootzone had lower casting activity than the sand capped rootzone for each topdressing treatment (Anderson, 2012).

Results assessing the effect of topdressing on casting activity have been inconsistent and inconclusive thus far. Therefore, the objectives of this research were to 1) determine the effect of sand topdressing rate and rootzone soil texture on soil temperature and soil volumetric moisture content, 2) determine the effect of sand topdressing rate and rootzone soil texture and the relationships between soil temperature and soil volumetric soil moisture content on earthworm casting activity, and 3) determine if sand topdressing rate or rootzone texture impacts earthworm species diversity or composition in turfgrass systems in the Arkansas-Oklahoma region.

Materials and Methods

Study Area

The study was conducted between November 2015 and October 2017 at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas (36.100229°N, -94.168845°W) on an experimental area established in 2010 for a previous study (Anderson, 2012). The area consisted of two rootzones – a native soil rootzone (Captina silt loam; fine-silty, siliceous, active, mesic Typic Fragiudults with an average pH of 6.2) and a sand-capped rootzone containing a 12.5 cm depth of a sub-rounded, medium size sand that meets the United States Golf Association (USGA) particle size specifications for putting green construction (USGA, 1993). Details regarding the construction of the rootzones were provided previously (Anderson, 2012). The rootzones were split into 1.5 x 4.9 m strips of light (0.64 cm yr^{-1}) and heavy (2.54 cm yr^{-1}) topdressing rates. Several cultivation treatments had been imposed over the rootzones continually since establishment of the plot area in 2010. The plots used in the current study were established to hybrid bermudagrass (*Cynodon dactylon* (L.) Pers. x *C. transvaalensis* Burtt-Davy cv. Patriot) at the beginning of the previous trial (Anderson, 2012).

The experimental design was a two-factor (rootzone and topdressing) randomized complete block. Sixteen 1.5 x 4.9 m (7.4 m^2) plots were used for the current study and were comprised of four replications of each rootzone and topdressing treatment combination. Maintenance of these plots was consistent across all rootzone and topdressing treatments, with a mowing height of 15 mm (clippings returned), 4.9 g N m^{-2} fertilization as urea per active growing month (typically May-August), and deep, infrequent irrigation in the absence of rainfall. Large sampling areas were used in this study due to the clustered distribution common in earthworm populations (Hendrix and Bohlen, 2002; Roy, 1957; Satchell, 1955) and to reduce variance of cast counts through the increase in sampling unit area as suggested by Rossi and Nuutinen (2004).

Sand Topdressing Treatments

Sand topdressing treatments were continued at either 0.64 cm of sub-rounded sand applied once (light treatment) or four times during the growing season (typically May-August) for a total of 2.54 cm per year (heavy treatment), with four replications of each topdressing treatment per rootzone. These topdressing rates are within ranges listed for heavy and light topdressing rates (1.0-3.8 cm yr^{-1} and 0.5-1.9 cm yr^{-1}, respectively) used in previous earthworm control research (Backman et al., 2002; Henderson et al., 2011).

Data Collection

Each discreet, visible cast above or within the turfgrass canopy, regardless of size, was counted. A tally counter was used to track cast counts throughout the plot, and a 2.25 m^2 grid was used to help guide counting. After cast counts were completed, dry earthworm casts were brushed into the canopy with a cocoa drag mat or, during the growing season, dry earthworm casts were mowed into the canopy after counting. Removing earthworm casts was necessary to prevent double counting of casts during the subsequent counting event. Cast counts were conducted at least twice per month and reported as monthly averages.

After cast counts were completed, average (n=5) soil temperature was measured at a 10.2 cm depth for each plot using a hand-held temperature probe (Hanna Instruments, Woonsocket, RI). Average (n=5) volumetric soil moisture content was measured for each plot using a FieldScout 300 Time Domain Reflectometer probe with 7.6 cm rod length (Spectrum Technologies, Aurora, IL). Data were combined and reported as monthly averages.

Cast and Soil Analysis

To determine where in the profile the earthworms were feeding, earthworm casts were collected from each plot in April 2017 using a mini shop vacuum (Shop-Vac Corporation, Williamsport, PA) with an attachment that allowed vacuuming of individual casts. Casts were dried at 105 °C for 24 h then ground and sieved (2 mm). Fifty g of oven-dry, sieved casting material were used to determine particle size distribution using the 12-h hydrometer method of particle size analysis (Gee and Orr, 2002). Cast material (10 g) was transferred to a muffle furnace at 425 °C for 8 h to determine percent organic matter by loss on ignition. Additionally, soil from each research plot to a 20 cm depth was collected, dried at 105 °C for 24 h, ground, sieved, and analyzed for particle size distribution and percent organic matter, as outlined above, as well as Melich-3 extractable analytes (P, K, Ca, Mg, Na, S, Fe, Mn, Zn, Cu, B) and pH.

Data Analysis

Cast count, soil moisture, and soil temperature data were analyzed as monthly averages, and month was included as a factor in a repeated measures analysis of variance model in PROC MIXED (SAS v. 9.4, SAS Institute, Inc., Cary, NC). Non-linear Gaussian regression was used to assess the relationship between soil temperature or soil moisture with casting activity (SigmaPlot, Systat Software, Inc., San Jose, CA). The 95% confidence intervals for critical temperature for maximum casting activity were calculated using GraphPad Prism (GraphPad Software Inc., La Jolla, CA). A Tukey's HSD (JMP Pro 13.0, SAS Institute Inc., Cary, NC) was used to determine significant differences between average organic matter and particle size distribution of the soil samples and cast samples between the four rootzone and topdressing treatment combinations.

Earthworm Collection

Samples were collected 20 Dec 2016 by the dig-and-sort method from a 0.3 x 0.3 m subplot to a depth of 0.2 m (0.018 m^3) and stored at 10-20 °C until processing. Sampling included four replications each of heavily-topdressed (2.54 cm yr^{-1}) and lightly-topdressed (0.64 cm yr^{-1}) sand-capped and native silt loam soil rootzones. Earthworms were manually collected and washed with DI water to remove soil particles. Earthworms were counted, boiled to kill, then separated by general morphology based on pigmentation, length, and the presence or absence of a clitellum (a defining sexual characteristic to differentiate juveniles from adults). Earthworm samples were stored in 95% ethanol solution at -80 °C for preservation.

Earthworm DNA Extraction and Purification

At least two earthworms specimens per morpho-group from each sample were selected for DNA barcoding, when possible. A DNeasy® Blood & Tissue kit (QIAGEN, Germantown, MD) was used for DNA extraction and purification. No more than 25 mg of sample tissue was used for lysing to ensure quality of purified DNA. Due to the small size of many of the earthworm specimens, when it was not possible to collect a section of skin tissue, the entire earthworm was lysed. An ethanol precipitation step was included to increase DNA purity and remove possible co-purified contaminants. Cleaned samples were stored at -20 °C.

PCR Amplification and Sequencing of Earthworm DNA

A 710-bp gene fragment of the COI gene was targeted for amplification using primers LCO1490 [GGT CAA CAA ATC ATA AAG ATA TTG G] and HCO2198 [TAA ACT TCA GGG TGA CCA AAA AAT CA] (Folmer et al., 1994). Information on reagent concentrations and amplification parameters has been detailed by Boyle (2018). Amplification was confirmed by gel electrophoresis in 1.5% agarose gel, and digitally visualized by ethidium bromide fluorescence. Samples were purified using a Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI) and DNA was quantified using nanodrop spectrophotometry (ND-1000; Thermo Fisher Scientific Inc., Waltham, MA.). Purified PCR products were sent to Eurofins Genomics (Louisville, KY) for sequencing in both the forward and reverse directions.

Sequence Alignment, Adjustment, and Tree Building

Sequencher software (Gene Codes Corporation, Ann Arbor, MI) was used to trim, align, and edit sequences and to generate consensus sequences. Molecular Evolutionary Genetics

Analysis ([MEGA7]; Kumar et al., 2015) was used to build neighbor-joining, maximum-parsimony, and maximum-likelihood trees, which included 62 sequences from this study as well as 45 samples collected for a golf course survey of earthworms in Arkansas and Oklahoma (Boyle, 2018). Originally, 134 sequences from the National Center for Biotechnology Information ([NCBI], Bethesda, MD) were included in the phylogenetic analysis. Redundant or uninformative sequences were removed for clarity in visualizing phylogenetic trees.

Results and Discussion

Casting Activity

There was a three-way interaction between rootzone, topdressing, and month on earthworm casting activity (Table 1). Within the light topdressing treatment, the sand rootzone resulted in significantly greater casting activity compared to the native soil rootzone in the fall and spring of both years and in the winter of year two (Fig. 1). These results are counter to those seen by Baker and Binns (1998), who observed less casting activity on fairways with a sandy soil texture compared to fairways with a clay or clay loam soil texture; however, casting was not significantly correlated to soil texture on tees under the same study. There was generally very little casting activity in the light topdressing soil rootzone treatment throughout the two years of this study (Fig. 1). In the heavy topdressing treatment, rootzone generally had little effect on casting activity (Fig. 1).

Within the soil rootzone, heavy topdressing resulted in significantly greater casting activity in the fall of year one and the spring of both years (Fig. 2). In the sand rootzone, there was no significant difference in casting activity between topdressing treatments in year one; however, in year two, light topdressing in the sand rootzone resulted in significantly greater casting activity compared to the heavy topdressing treatment (Fig. 2). The inconclusive results of topdressing on earthworm casting activity (Backman et al., 2002; Baker et al., 2005; Bartlett et al., 2008; Henderson et al., 2011; Williamson and Hong, 2005), including the present study, may be due to the differences in angularity of the topdressing materials used. Future studies should review the effects of angularity of topdressing material on casting activity.

Soil Temperature

There was a significant two-way interaction between rootzone and month on soil temperature (Table 1), which resulted in significant differences in temperatures between rootzones, with the sand rootzone and soil rootzone having significantly greater temperatures at different points in time (Fig. 3). There was also a significant two-way interaction between topdressing and month on soil temperature (Table 1). Soil temperature was significantly greater under heavy topdressing in the spring of 2016, winter of 2016-2017, and October 2017 (Fig. 3).

A Gaussian regression was used to examine the relationship between soil temperature and casting activity for each of the rootzone and topdressing treatment combinations (Fig. 4). The regressions were significant and explained 10-34% of the variation in earthworm casting activity (Fig. 4). These results tentatively support those of Lavelle (1988), who stated that temperature is the most limiting factor affecting earthworm activity in temperate environments. Butt (1991) stated that temperatures above 15 °C led to “fatigue” of *Lumbricus terrestris* Linnaeus, 1758, and that temperatures over 20 °C led to clitellate loss, mass loss, and mortality of *L. terrestris*; however, in the current study, casting activity was still observed above 20 °C (Fig. 4). This could be due to the differences in earthworm species composition between the two studies. *Diplocardia* spp., which dominated the species composition at this location (Boyle, 2018), are thought to be more active at a wider range of temperatures than some of the non-native earthworm species (James, 1991).

The regressions also indicated the critical temperature at which casting was maximized within each treatment combination. The critical temperatures for peak casting activity in the light topdressing and heavy topdressing soil rootzone were 11.1 °C and 14.5 °C, respectively (Fig. 4). Critical temperatures in the sand rootzone were similar, at 13.1 °C and 13.4 °C for the heavy and light topdressing treatments, respectively (Fig. 4). When 95% confidence intervals were placed around the critical temperatures associated with casting activity, those temperatures were not significantly different between topdressing and rootzone treatment combinations (data not shown). The critical temperatures for peak casting activity under all four treatment combinations fell within the previously published range of optimum temperatures (10-20 °C) for temperate earthworm activity (Berry and Jordan, 2001; Lee, 1985).

Soil Moisture Content

There was a significant three-way interaction of rootzone, topdressing treatment, and month on soil volumetric moisture content (Table 1). Within the light topdressing treatment, the soil rootzone generally resulted in significantly greater soil moisture content except for August 2016 and the spring and fall of 2017, when soil moisture content was not significantly different between rootzones (Fig. 5). Within the heavy topdressing treatment, the soil rootzone generally resulted in significantly greater soil moisture content, except for February and August 2016 and February, March, and September 2017, when soil moisture content was not significantly different between rootzones (Fig. 5). Within both rootzones, the light topdressing treatment resulted in significantly greater soil moisture content across the two years of the study (Fig. 6).

The Gaussian regression analysis examining the relationship between soil moisture content and casting activity was not significant (data not shown), indicating that soil moisture content is not a significant predictor of earthworm casting activity. These results are counter to

Evans and Guild's (1947) results, which determined that soil moisture is a highly significant predictor of earthworm casting activity. The differences between the two studies could be due to differences in moisture replacement; Evans and Guild (1947) relied on precipitation events and experiences several drought spells, while the current study utilized irrigation to avoid drought stress. Differences could also be due to differences in earthworm species composition, as Evans and Guild (1947) primarily researched temperature and moisture effects on casting activity of *Allolobophora nocturna*- and *A. longa*-dominated systems, while the current study's earthworm composition was primarily made up of *Diplocardia* spp. (Boyle, 2018).

Soil and Cast Analysis

There were no significant differences in sand content between topdressing treatments in either rootzone; however, the heavy topdressing sand rootzone treatment had significantly greater sand content than either topdressing treatment in the soil rootzone (Table 2). There was significantly greater silt in the light topdressing soil rootzone (Table 2), as would be expected under the less intensive topdressing treatment. Percent clay was not significantly different between any of the topdressing and rootzone treatment combinations (Table 2).

It has been hypothesized that reducing organic matter in the rootzone may increase casting activity by causing earthworms to forage more to obtain organic matter (Abbott and Parker, 1981; Martin, 1982); however, in this study, organic matter in the rootzone was not significantly different between rootzones under light topdressing (Table 2), even though casting activity was significantly greater in the sand rootzone compared to native soil under light topdressing (Fig. 1). Under heavy topdressing, there was no significant difference in organic matter or casting activity between rootzones (Fig. 1, Table 2). Additionally, in the soil rootzone, heavy topdressing resulted in increased casting activity, while in the sand rootzone, heavy topdressing resulted in no difference or reduced casting activity (Fig. 2), despite reduced organic matter in heavy topdressing treatments under both rootzones (Table 2). This suggests that there was not a correlation between rootzone organic matter content and earthworm casting activity in 'Patriot' bermudagrass tee boxes under the present study. Although percent sand was not significantly different between topdressing treatments within each rootzone, the differences between the heavy topdressing sand rootzone and light topdressing soil rootzone suggest that there may be some effect of increased sand content on reduced organic matter (Table 2). This is to be expected, as sand topdressing is a cultural practice commonly used by golf course superintendents to dilute organic matter within the turfgrass rootzone. The effects of soil particle size distribution and organic matter on earthworm casting activity should be researched further.

Not enough cast material was collected form the light topdressing soil rootzone plots to obtain the minimum critical mass needed for particle size analysis (50 g oven-dry material). In the sand rootzone, sand content was significantly greater in casts under heavy topdressing compared to the light topdressing (Table 3). There was no significant difference between sand content of casts collected from either of the sand rootzone treatments compared to the heavy topdressing soil rootzone treatment (Table 3).

The cast organic matter was significantly greater in the light topdressing sand rootzone treatment compared to the light topdressing soil rootzone (Table 3), suggesting that earthworms feeding in the sand rootzone may be more efficient at foraging for organic matter compared to those feeding in the soil rootzone under the same topdressing treatment, as the soil organic matter was not significantly different between these two treatments (Table 2). This trend does not extend to the heavy topdressing treatment, however, where neither cast nor soil organic matter was significantly different between rootzones (Tables 2, 3). Within each rootzone, organic matter content was greatest in casts collected from the light topdressing treatment compared to the heavy topdressing rate in the corresponding rootzone (Table 3), possibly due to the increased organic matter content in the soil samples under the same conditions (Table 2). James (1991) determined that organic matter in the casts of *Diplocardia* spp. was significantly greater than that of non-native Lumbricidae earthworm species, so it is possible that species composition could affect cast organic matter content.

Earthworm Community

While some research has been conducted to investigate earthworms in turf, relatively little is known about earthworm species composition in turfgrass systems, and this information is especially lacking for turfgrass systems in the transition zone of the U.S. Redmond et al. (2014) surveyed six golf courses in central Kentucky and found seven species of earthworms, with *Aporrectodea trapezoides* (Dugès, 1828), an endogeic species (Hendrix and Bohlen, 2002; Pathma and Sakthivel, 2012), dominating the earthworm community structure. Potter et al. (1994) studied the effects of pesticides on earthworms in Kentucky bluegrass turfgrass near Lexington, KY. The earthworm community was primarily comprised of *Aporrectodea turgida* (Eisen, 1873), with some *Ap. trapezoides* (Dugès, 1828), *Lumbricus terrestris* Linnaeus, 1758, and *Eisenia* spp. (Potter et al., 1994). *Aporrectodea turgida* is listed as an endogeic species (Kernecker et al., 2014), *Ap. trapezoides* is an endogeic species (Bartlett et al., 2006; Brown, 1995; Hendrix and Bohlen, 2002; Pathma and Sakthivel, 2012), and *L. terrestris* is an anecic species (Bartlett et al., 2006; Brown, 1995; Hendrix and Bohlen, 2002; Pathma and Sakthivel, 2012; Pérès et al., 2010). While studying the effect of fungicides and insecticides on earthworms, Tu et al. (2011) observed that *Diplocardia* spp. dominated the earthworm populations, though some *L. rubellus* Hoffmeister, 1843 specimens were observed as well. *Lumbricus rubellus* is alternately listed as endogeic, epigeic, or epi-anecic (Bartlett et al., 2006; Brown, 1995; Pérès et al., 2010).

In this study, average abundance of adults, juveniles, or partial/unknown specimens collected December 2016 was not significantly different between treatments (Table 4). When comparing the heavy topdressing soil rootzone and the heavy topdressing sand rootzone, the two treatment combinations were not significantly different in casting activity (Fig. 1); however, though not statistically significant, the sand rootzone averaged 1.5 times as many earthworms as the soil rootzone (Table 4). This disparity between earthworm abundance and casting activity could be due to differences in species composition. Both treatments had *Diplocardia* spp. present; however, the heavy topdressing sand rootzone species included specimens that grouped with *Amynthas* spp., while the heavy topdressing soil rootzone included some specimens that grouped with *Eisenia* and *Dendrobaena* spp. (Boyle, 2018). Though different species were present, differences in species abundance may have led to similar casting activity between the two treatments.

Though not significant, the light topdressing soil rootzone treatment had the greatest abundance of earthworms (Table 4). This is counter to what was expected, as this treatment had the least amount of casting activity (Fig. 1). Species composition in this treatment was comprised of *Diplocarida*, *Amynthas*, and some European spp. (Boyle, 2018), and was similar to that of the light topdressing sand rootzone treatment, which had significantly greater casting activity (Fig. 1). One possible explanation for the difference in casting activity could be potential differences in abundance of each of the species, as well as the ecological classification of those species. Additionally, casting rates may be different for juveniles and adults, and the high abundance of juveniles in the soil rootzone under the light topdressing treatment could explain the differences in casting activity. Resources may have been favorable in this treatment, leading to energy expenditures being focused on reproduction instead of foraging and biomass production. A majority of the casts in this study were large compared to the size of the majority of the earthworms found (personal observation). This could indicate increased activity of larger species, despite lower abundances of those species. Since more specific species classification was not possible in this study, more research needs to be done to assess the species-level designation and differences in casting activity between the earthworms observed in this study. Earthworm identification is highly controversial, and although molecular sequencing does provide benefits for earthworm identification, there are still issues that arise. Earthworm relatedness can vary depending on the gene used, and sometimes, genes do not agree on identification (Pop et al., 2007). Earthworms can still be misidentified or labeled as synonymous with other species (Chang et al., 2009; Pérez-Losada, 2012), which can lead to ambiguous identifications, as is the case in this study.

Sand topdressing may impact casting activity, but the extent of the impact differs between rootzone and topdressing rate, and changes over time. While the effect of sand topdressing on surface casting activity of different earthworm species is still unclear, it has been previously determined that *Diplocardia* spp. are more active surface casters than *Aporrectodea caliginosa* and *Octalasion cyaneum*, despite the greater biomass of the two European spp. (James, 1991). This indicates that species composition can greatly affect surface casting activity. Future research should focus on identification of earthworm species in turfgrass systems, as well as species-specific cultural management of earthworm casting on golf course turf.

Figure Legend

Figure 1	Effect of rootzone within sand topdressing treatment on casting activity between November 2015 and October 2017 on ‘Patriot’ bermudagrass tee boxes in Fayetteville, Arkansas. Heavy topdressing = 2.54 cm per growing season and light topdressing = 0.64 cm per growing season. Bar represents the least significant difference for comparing means within a date.
Figure 2	Effect of sand topdressing rate within rootzone on casting activity between November 2015 and October 2017 on ‘Patriot’ bermudagrass tee boxes in Fayetteville, Arkansas. Heavy topdressing = 2.54 cm per growing season and light topdressing = 0.64 cm per growing season. Bar represents the least significant difference for comparing means within a date.
Figure 3	Interactions between rootzone and month (top) and sand topdressing rate (bottom) on soil temperature between November 2015 and October 2017 on ‘Patriot’ bermudagrass tee boxes in Fayetteville, Arkansas. Heavy topdressing = 2.54 cm per growing season and light topdressing = 0.64 cm per growing season. * indicates a significant difference within a date.
Figure 4	Gaussian regression showing the relationship between average soil temperature and casting activity on ‘Patriot’ bermudagrass tee boxes in Fayetteville, Arkansas. X_o indicates the critical temperature at which casting was maximized under each sand topdressing rate and rootzone combination. Heavy topdressing = 2.54 cm per growing season and light topdressing = 0.64 cm per growing season.
Figure 5	Effect of rootzone within sand topdressing treatment on soil volumetric water content between November 2015 and October 2017 on ‘Patriot’ bermudagrass tee boxes in Fayetteville, Arkansas. Heavy topdressing = 2.54 cm per growing season and light topdressing = 0.64 cm per growing season. Bar represents the least significant difference for comparing means within a date.
Figure 6	Effect of sand topdressing rate within rootzone on soil volumetric water content between November 2015 and October 2017 on ‘Patriot’ bermudagrass tee boxes in Fayetteville, Arkansas. Heavy topdressing = 2.54 cm per growing season and light topdressing = 0.64 cm per growing season. Bar represents the least significant difference for comparing means within a date.

Figure 1

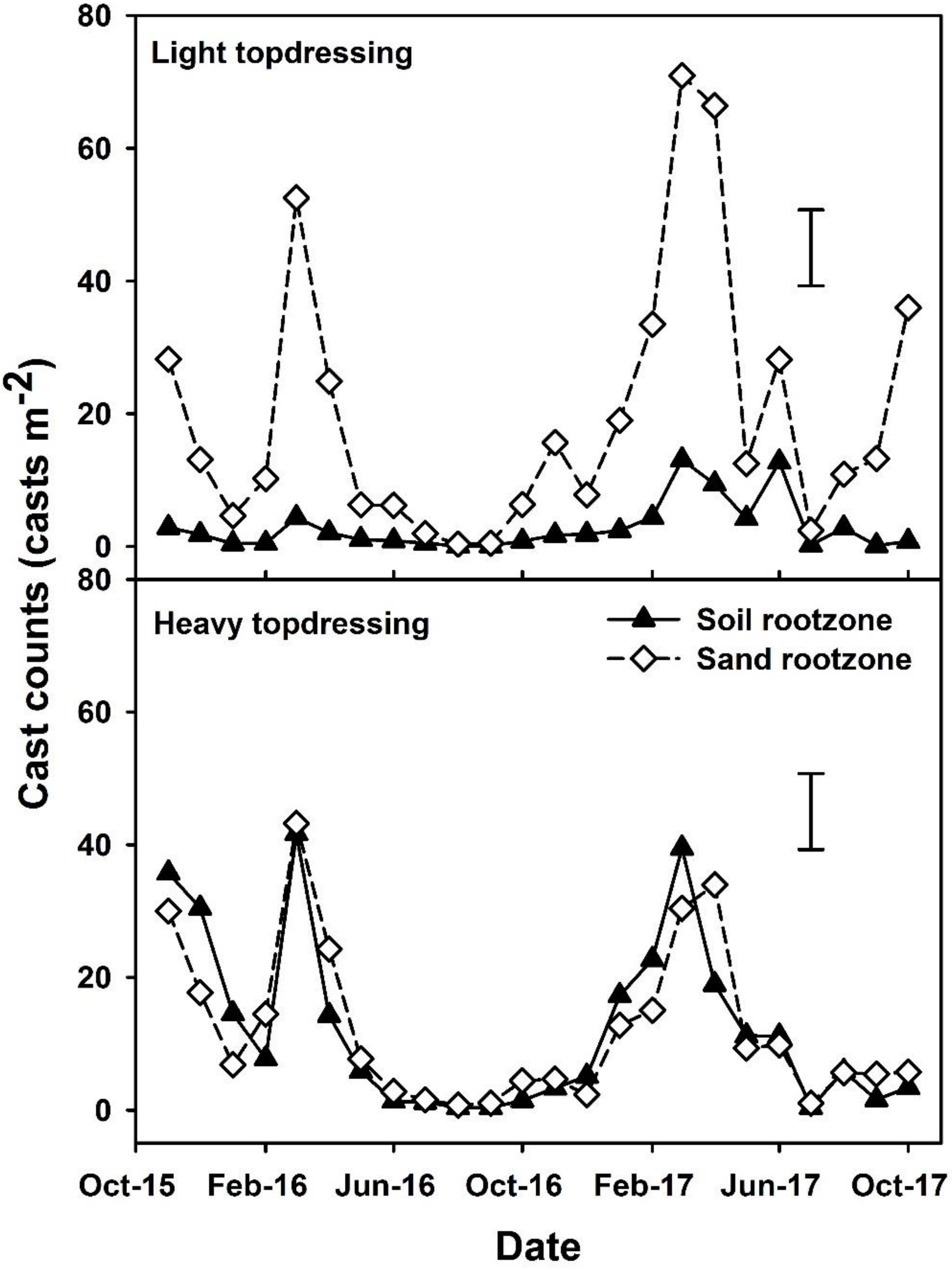
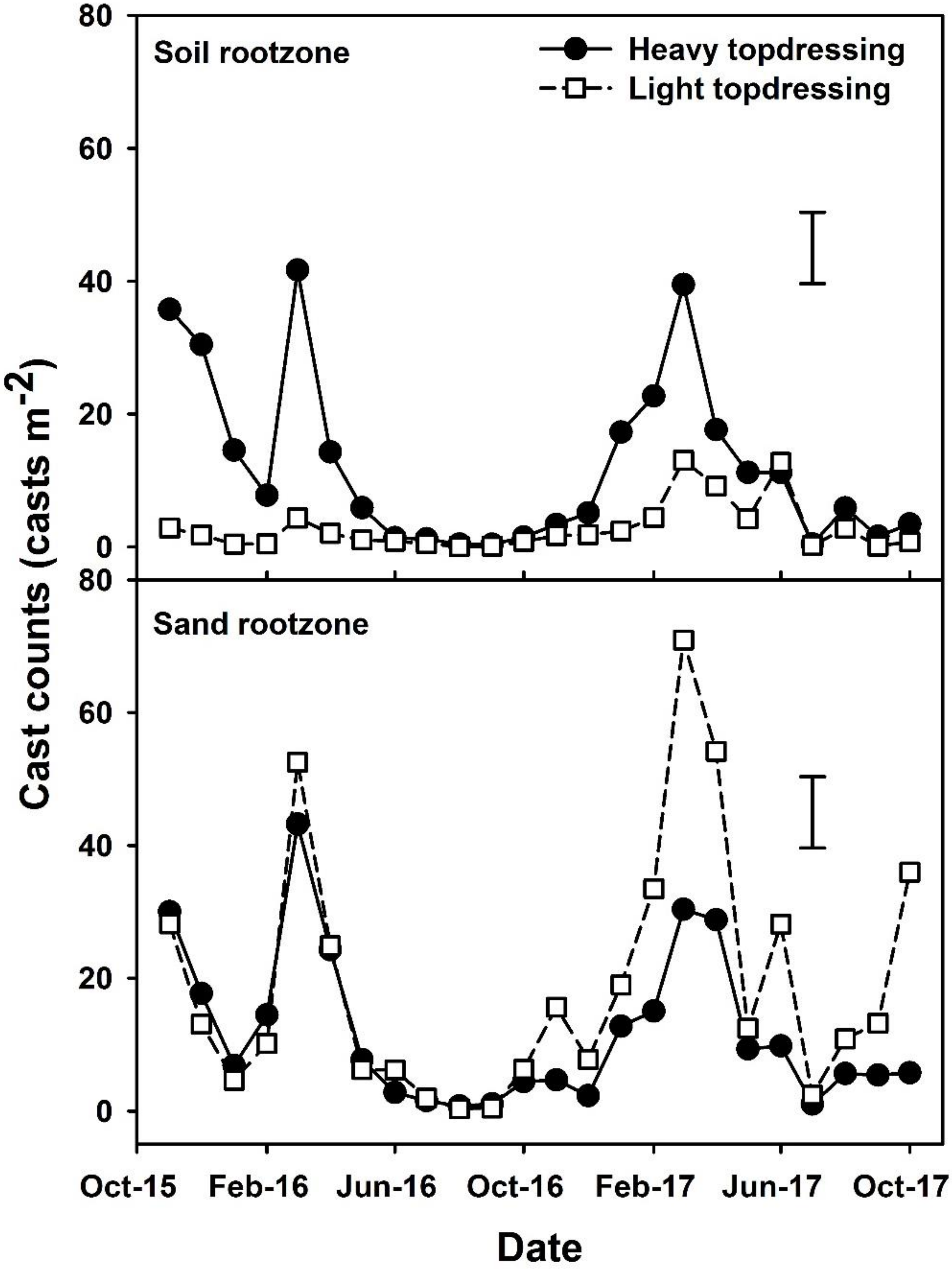

Light topdressing
Heavy topdressing
Soil rootzone
Sand rootzone
Cast counts (casts m^{-2})
80
60
40
20
0
80
60
40
20
0
Oct-15
Feb-16
Jun-16
Oct-16
Feb-17
Jun-17
Oct-17
Date

Figure 2



Soil rootzone
Heavy topdressing
Light topdressing
Sand rootzone
Cast counts (casts m-2)
80
60
40
20
0
Oct-15
Feb-16
Jun-16
Oct-16
Feb-17
Jun-17
Oct-17
Date

Figure 3

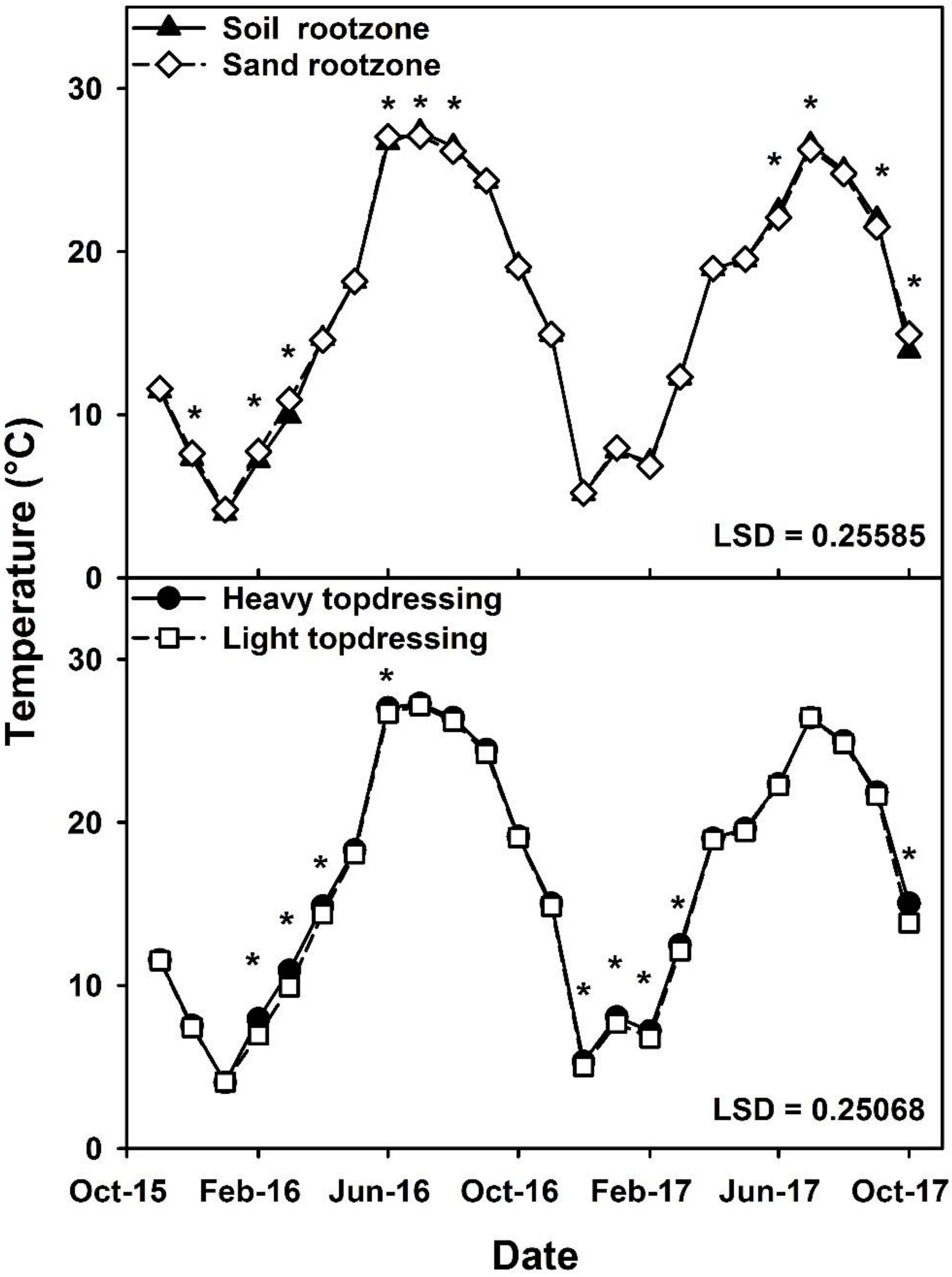

Soil rootzone
Sand rootzone
Heavy topdressing
Light topdressing
Temperature (°C)
0
10
20
30
LSD = 0.25585
LSD = 0.25068
Oct-15
Feb-16
Jun-16
Oct-16
Feb-17
Jun-17
Oct-17
Date

Figure 4

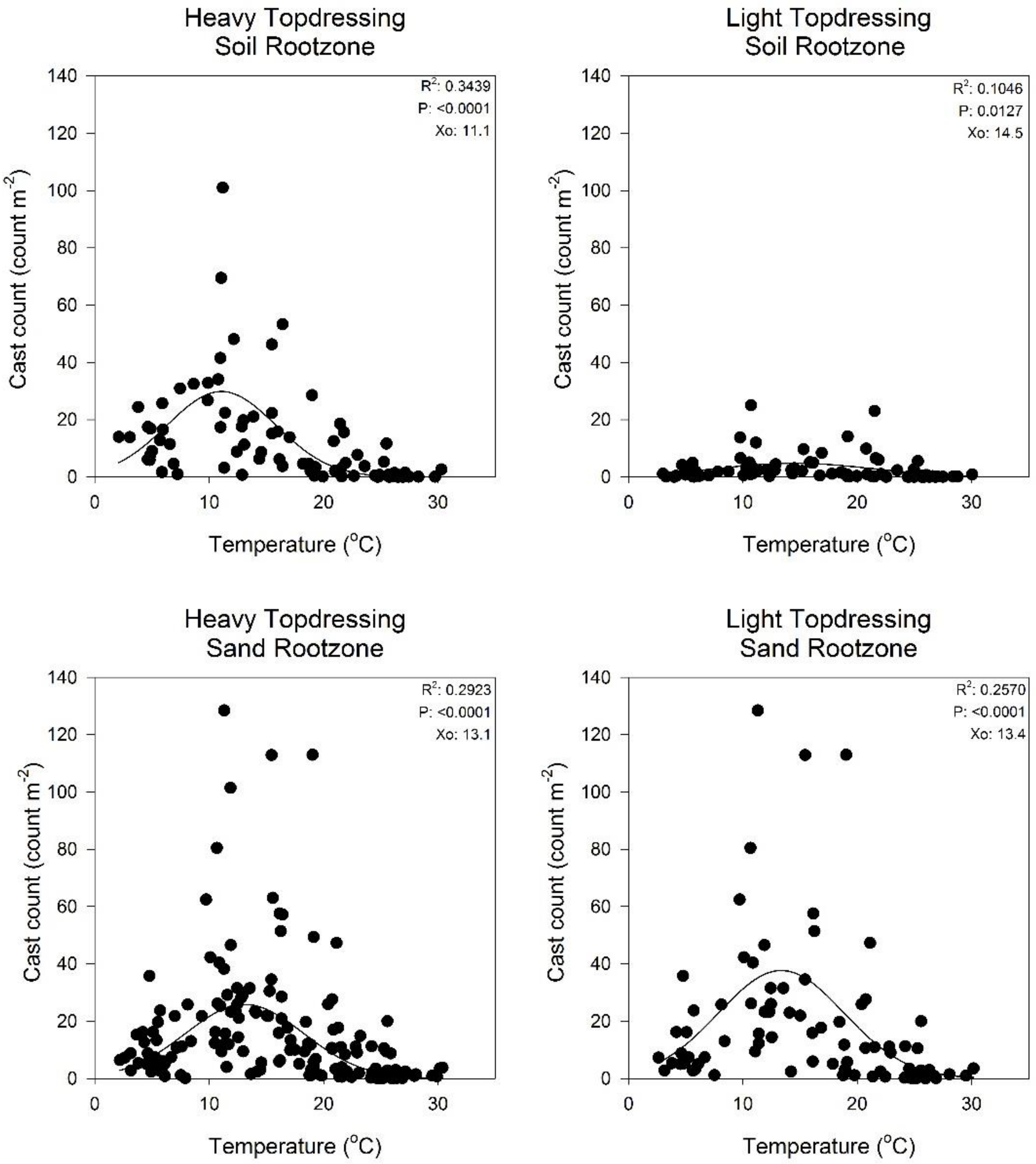

Heavy Topdressing
Soil Rootzone
R^2: 0.3439
P: <0.0001
Xo: 11.1
Light Topdressing
Soil Rootzone
R^2: 0.1046
P: 0.0127
Xo: 14.5
Heavy Topdressing
Sand Rootzone
R^2: 0.2923
P: <0.0001
Xo: 13.1
Light Topdressing
Sand Rootzone
R^2: 0.2570
P: <0.0001
Xo: 13.4
Cast count (count m^{-2})
0
20
40
60
80
100
120
140
Temperature (°C)
0
10
20
30

Figure 5

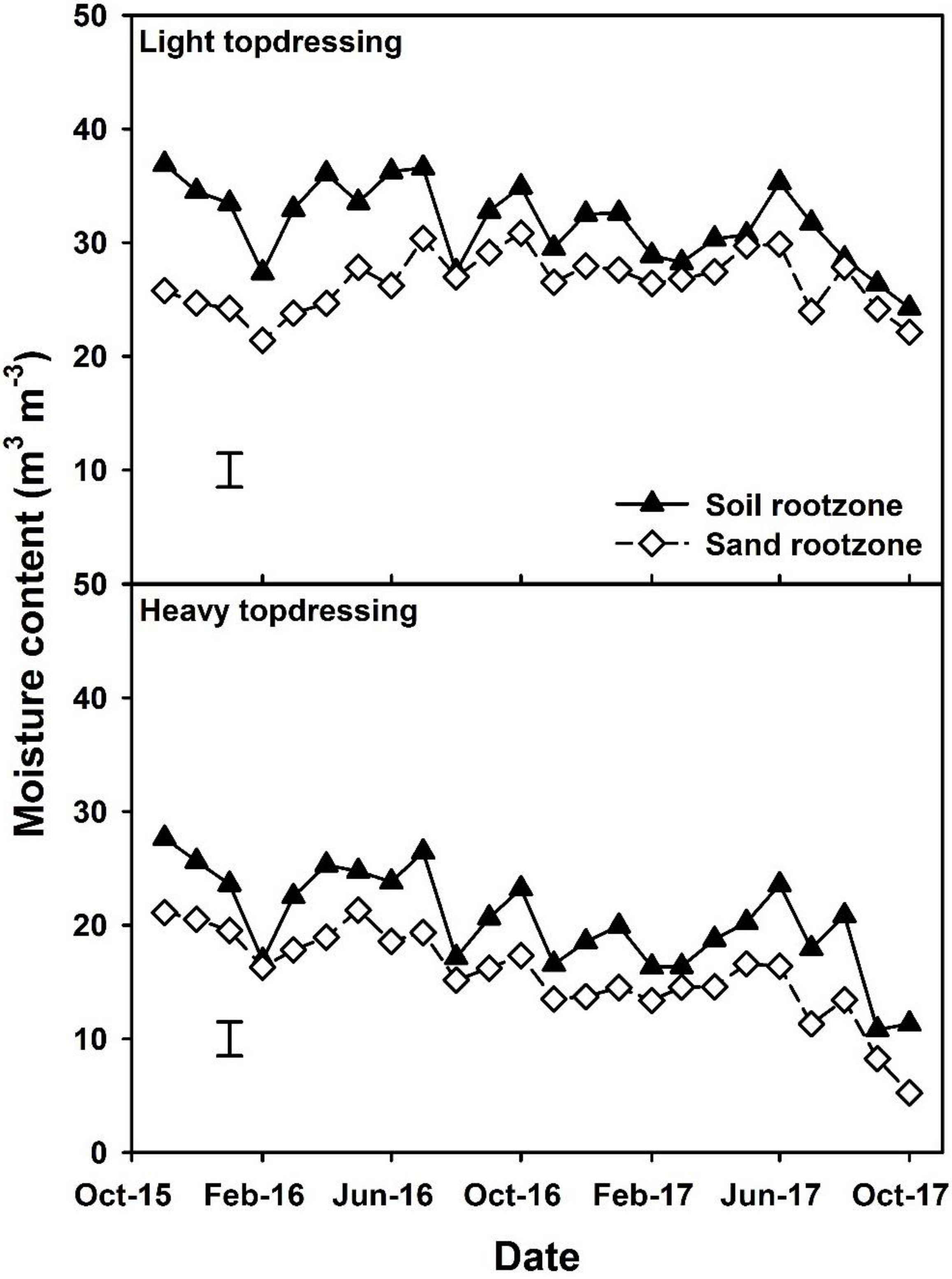

Light topdressing
Heavy topdressing
Soil rootzone
Sand rootzone
Moisture content (m^3 m^{-3})
50
40
30
20
10
50
40
30
20
10
0
Oct-15
Feb-16
Jun-16
Oct-16
Feb-17
Jun-17
Oct-17
Date

Figure 6

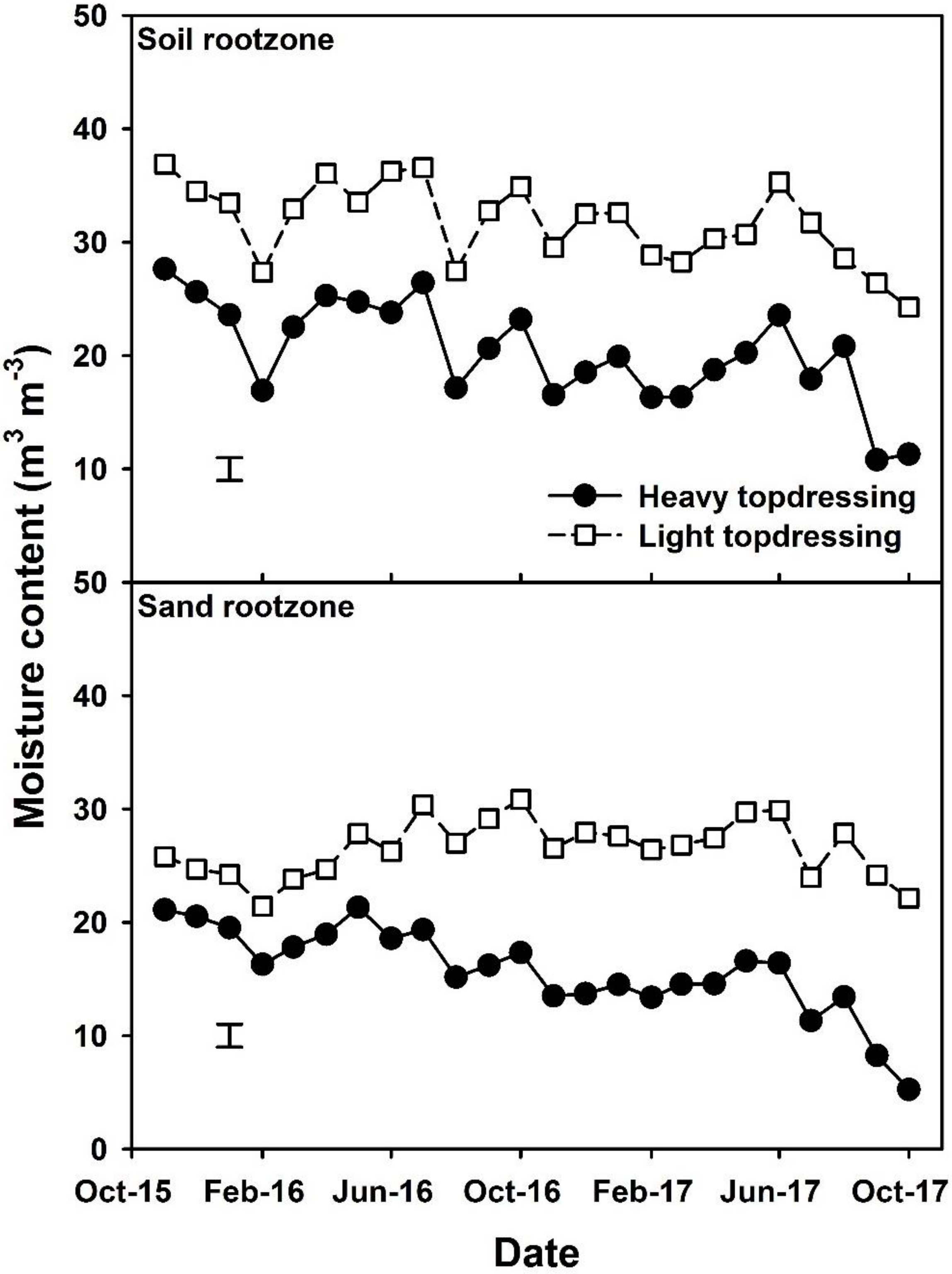

Soil rootzone
Sand rootzone
Heavy topdressing
Light topdressing
Moisture content (m^3 m^{-3})
Date
0
10
20
30
40
50
Oct-15
Feb-16
Jun-16
Oct-16
Feb-17
Jun-17
Oct-17

Table 1. Analysis of variance showing main effects and interactions of rootzone†, sand topdressing rate‡, and month on casting activity, soil temperature, and volumetric soil moisture content (α = 0.05) at the University of Arkansas Agricultural Research and Extension Center, Fayetteville, AR.

	Casting activity	Soil temperature	Soil moisture
Effect		Prob>F	
Block	0.3213	0.0276	0.1143
Rootzone	0.0575	0.2509	0.0263
Topdressing	0.5431	0.0001	<0.0001
Rootzone*Topdressing	0.0027	0.2680	0.3050
Month	<0.0001	<0.0001	<0.0001
Rootzone*Month	<0.0001	<0.0001	<0.0001
Topdressing*Month	<0.0001	<0.0001	<0.0001
Rootzone*Topdressing*Month	<0.0001	0.9335	<0.0001

† Rootzones were a native silt loam soil or a sand-capped rootzone built above the native soil.

‡ Topdressing treatments were heavy (2.54 cm per growing season) or light (0.64 cm per growing season).

Table 2. Average particle size distribution and percent organic matter for composite soil samples (0-20 cm) collected 20 December 2016 from ‘Patriot’ bermudagrass tee boxes under different rootzone and sand topdressing rate combination treatments in Fayetteville, AR.

Rootzone†	Topdressing‡	Sand		Silt		Clay		Organic matter	
		---------------------------------			%			--------------------------------------	
Sand	Heavy	95.0	a §	4.9	c	0.06	a	2.07	c
	Light	85.0	ab	13.5	c	1.58	a	3.21	ab
Soil	Heavy	69.1	bc	27.2	ab	3.70	a	2.86	bc
	Light	60.6	c	36.4	a	3.03	a	3.83	a

† Rootzones were a native silt loam soil or a sand-capped rootzone built above the native soil.

‡ Topdressing treatments were heavy (2.54 cm per growing season) or light (0.64 cm per growing season).

§ Means within a column not sharing the same letter are significantly different according to Tukey’s HSD (P<0.05).

Table 3. Average particle size distribution and percent organic matter for earthworm casts collected 1 April 2017 from ‘Patriot’ bermudagrass tee boxes under different rootzone and sand topdressing rate combination treatments in Fayetteville, AR.

Rootzone†	Topdressing‡	Sand		Silt		Clay		Organic matter	
		%							
Sand	Heavy	94.4	a§	5.5	b	0.25	a	4.09	c
	Light	89.5	b	10.3	a	0.25	a	8.37	a
Soil	Heavy	92.3	b	7.5	b	0.25	a	2.90	c
	Light	nd		nd		nd		5.78	b

† Rootzones were a native silt loam soil or a sand-capped rootzone built above the native soil.

‡ Topdressing treatments were heavy (2.54 cm per growing season) or light (0.64 cm per growing season).

§ Means within a column not sharing the same letter are significantly different according to Tukey’s HSD (P<0.05)

Table 4. Average (n=4) earthworm abundance (earthworms m^{-2}) in ‘Patriot’ bermudagrass tee boxes under different rootzone and sand topdressing rate combination treatments in Fayetteville, AR.

Rootzone†	Topdressing‡	Juvenile	Adult	Unknown	Total
		----------------- abundance m^{-2}			--------------------
Sand	Heavy	31	86	31	148
	Light	117	108	28	253
Soil	Heavy	44	42	8	94
	Light	339	72	144	556
	Prob > F	0.278	0.370	0.508	0.375

† Rootzones were a native silt loam soil or a sand-capped rootzone built above the native soil.
‡ Topdressing treatments were heavy (2.54 cm per growing season) or light (0.64 cm per growing season).

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Identification of earthworm species on golf course turfgrass systems in Arkansas and Oklahoma

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Abstract

Very little is known about earthworm species composition across Arkansas and Oklahoma, and even less is known about species composition in U.S. turfgrass systems. The objective of this research was to identify species in golf course turf in Arkansas and Oklahoma. Earthworms from five golf turf locations across Arkansas and Oklahoma were collected for identification. Morphological identification was conducted on formalin-preserved earthworms collected from two sampling locations. A 710-bp gene fragment of the cytochrome-*c* oxidase subunit I (COI) gene was targeted for amplification and sequencing of earthworm DNA from three sampling locations. 107 sequences from this study were combined with 134 sequences from GenBank for neighbor-joining, maximum-likelihood, and maximum-parsimony analysis. The analyses indicate that *Diplocardia* spp. are predominant at three sampling locations, and *Amynthas* spp. were present across all five sampling locations. The dominance of *Diplocardia* spp. in the turfgrass systems in this study is counter to expectations, as previous reports on earthworm composition in turfgrass systems have primarily reported non-native European and Asian earthworm species.

Introduction

Because of their ability to alter soil properties through burrowing, incorporation and breakdown of organic material, and mixing of soils, earthworms are considered ecosystem or soil engineers (Jouquet et al., 2006). Generally, earthworm activity is considered beneficial, as burrowing creates macropores for air and water transport and root growth, moves organic matter into the soil profile, can improve decomposition of organic matter and thatch layers, and increases the availability of nutrients for plant uptake (Bityutskii et al., 2012; Brown, 1995; Kiyasudeen et al., 2016; Lavelle, 1988).

Earthworms can be loosely classified ecologically, e.g. by their burrowing and feeding habits. Epigeic earthworms live near the soil surface where they feed on the organic material found in the litter layer (Brady and Weil, 2002; Doube and Brown, 1998; Hendrix and Bohlen, 2002). Endogeic earthworms create shallow horizontal burrows as they consume soil and organic matter; these burrows provide more stable environmental conditions for these earthworms (Brady and Weil, 2002; Doube and Brown, 1998; Lavelle, 1988). Anecic earthworms burrow vertically and can create permanent burrows several meters long which open to the soil surface (Brady and Weil, 2002; Doube and Brown, 1998). Ecological classification is species dependent, and a species can be classified into multiple groups.

Earthworm taxonomy is a highly controversial field, and the inconsistencies and redundancies in nomenclature, lack of meaningful distinguishing features, and the variability among characteristics in the environment make morphological earthworm identification difficult, if not impossible, for many species. A major issue that arises with morphological earthworm identification is that identification, using either internal or external features, relies on sexual characteristics, which are absent in juvenile earthworms (Richard et al., 2010; Schwert, 1990).

This lack of defining characteristics makes identification of sexually immature specimens problematic and can affect subsequent research conclusions on earthworm composition, diversity, and ecosystem services (Boyer and Wratten, 2010; Klarica et al., 2012; Richard et al., 2010). Earthworms without morphologically distinguishing characteristics indicate that there are species yet to be described in the literature (Boyer and Wratten, 2010; Buckley et al., 2011; James, 1990).

In earthworm taxonomy, nomenclature synonymy can even extend to the genus level (Briones et al., 2009). Within the species-level classification, subspecies, forms (same species with different morphological characteristics), clades (phylogenetically related), and cryptic species (morphologically similar, but different phylogenetically) have been recognized, some of which are also potentially repetitively referring to a single species. An example of this is the synonymy between *Lumbricus terrestris* Linnaeus, 1758 and *L. herculeus*, two species which have been erroneously classified under *L. terrestris* due to a lack of distinguishing morphological characteristics (James et al., 2010). This synonomy within earthworm taxonomy is, in part, due to the great variation of the taxonomically important morphological identification features (Briones et al., 2009; Dayrat, 2005; Richard et al., 2010). These include, but are not limited to, the placement, shape, and size of the prostomium and clitellum, among other features. Pigmentation and body size, parameters once thought to be irrelevant to taxonomic determination, are once again being used to aid in identification, due to recent research that suggests they are more useful measurements than previously thought (Decaëns et al., 2013). Another important drawback of morphological identification is the potential inability to identify partial specimens (Klarica et al., 2012).

Earthworms of the genus *Diplocardia*, native to the U.S., several species of which are known to be common in Arkansas and Oklahoma (Causey, 1952; Reynolds, 2008; 2010; 2011; Thomason et al., 2017), cannot be identified using only external features, and dissection of the dorsal side of the earthworm by an experienced earthworm taxonomist must be done to examine internal characteristics for identification purposes (James, 1990). *Amynthas* spp. also require dissection for identification (Reynolds, 1978). In addition to the external features listed previously, internal features that assist in identification of *Diplocardia* and *Amynthas* spp. include spermathecae number; placement, and shape, position of the intestine; and heart placement, among other features (James, 1990; Reynolds, 1978). Many of these earthworms, especially *Diplocardia* spp., are so small that dissection can be very difficult, even for experienced taxonomists.

Molecular identification can help address some of the issues with ambiguous morphology and inexperience with dissection, and allows for the identification of juveniles and partial specimens. The mitochondrial cytochrome-*c* oxidase subunit I (COI) gene is the most popular sequence for species identification in animals (Klarika et al., 2012), and is targeted because this gene regularly shows greater than 2% species divergence (Hebert et al., 2003). Briones et al. (2009) determined through review that the 16S rDNA and COI genes can help discriminate differences at the species level. Klarica et al. (2012) had success obtaining sequences from the 12S, 16S, and COI sequences, but less success obtaining genes from the COII sequences for some of the earthworm species analyzed. Huang et al. (2007) also had success identifying earthworm species using a COI-based approach. According to Klarica et al. (2012), the 12S and 16S genes have lower genetic distances compared to the protein coding genes; however, the GenBank system currently has three times more lumbricid COI sequences than COII, 12S, and

16S sequences combined. Klarica et al. (2012) states that the COI gene is the best choice for addressing earthworm lineages, while the two rRNA coding genes provide enough variability to discern congeneric species. Novo et al. (2011) determined that the 18S rRNA gene was the least variable, while COI and 16S genes were the most variable in uncorrected pairwise divergence. Richard et al. (2010) used COI genes to identify lumbricid adult and juvenile earthworms, and discovered the presence of possible cryptic species within *Lumbricus terrestris* Linnaeus, 1758, *L. castaneus* (Savigny, 1826), and *L. rubellus* Hoffmeister, 1843.

Relatively little is known about earthworm community composition in turfgrass systems in general, and this information is especially lacking for turfgrass systems in the U.S. Those studies that have been done have primarily focused on non-native Asian and European species. Although earthworms have been identified from Arkansas and Oklahoma (Reynolds, 2008; 2010; Thomason et al., 2017), to the authors' knowledge, only a few studies exist on earthworm identification in these states, and so distributions and identifications are incomplete. None have been sampled exclusively from turf fields in this region. Preliminary examination of earthworms at the University of Arkansas Agricultural Research and Extension Center suggested the earthworm community was comprised mostly of small earthworms, likely belonging to the native *Diplocardia* genus. (Dr. Mary Savin, personal communication). Therefore, the objective of this study was to determine earthworm species present on golf courses in Arkansas and Oklahoma.

Materials and Methods

Earthworm Collection

The study was conducted between November 2015 and December 2017. A simulated tee-box system at the University of Arkansas Agricultural Research and Extension Center (UA) as well as four golf courses in the transition zone of Arkansas and Oklahoma were surveyed to determine the earthworm species composition present in the region (Table 1). On the golf course collection sites, with guidance from golf course staff, three sampling locations were selected on or near fairways, tees, greens, and roughs for dig-and-sort sampling. Samples from Lew Wentz and Jimmie Austin golf courses measured 0.2 x 0.2 m to a depth of 0.3 m (0.012 m^3). Samples from Chenal and Meadowbrook measured 0.3 x 0.3 m to a depth of 0.2 m (0.018 m^3). Collections encompassed both native soil and sand-capped greens, tees, fairways, and roughs. Samples were transported from the golf course back to the laboratory (Fayetteville, AR) and were stored at 10-20 °C until processing. 16 samples from UA were collected by the dig-and-sort method from 0.3 x 0.3 m to a depth of 0.2 m (0.018 m^3) subplots and stored at 10-20 °C until processing. Sampling locations at this site included four replications each of heavily-topdressed (2.54 cm yr^{-1}) and lightly-topdressed (0.64 cm yr^{-1}) sand-capped and native silt loam soil rootzones.

Earthworms from all samples were manually collected and washed with DI water to remove soil particles. Earthworms were counted, boiled to kill, then separated by general morphology based on pigmentation, length, and the presence or absence of a clitellum (defining sexual characteristic). Earthworm samples from Lew Wentz Memorial Golf Course and Jimmie Austin Golf Club were stored in 5% formalin solution at room temperature, and samples from UA, Chenal Country Club and Meadowbrook Country Club were stored in 95% ethanol solution at -80 °C for preservation.

Soil Analysis

Soil from each sample was dried at 105 °C for 24 hours, ground, sieved, and analyzed for particle size distribution using the 12-hour hydrometer method. Soil samples were also analyzed for Mehlich-3 extractable analytes ((P, K, Ca, Mg, Na, S, Fe, Mn, Zn, Cu, B), pH, and organic matter content by loss on ignition at 425 °C for 8 h.

Earthworm DNA Extraction and Purification

At least two replications per morpho-group from each sample were selected for DNA barcoding, when possible. A DNeasy® Blood & Tissue kit (QIAGEN, Germantown, MD) was used for DNA extraction and purification. For specimens stored in formalin, samples were double washed in a sterile phosphate buffer solution before lysing and extraction, per DNeasy kit instructions. When possible, a piece of cutaneous tissue from the caudal end was collected and rinsed with DI water. No more than 25 mg of sample tissue was used for lysing to ensure quality of purified DNA. Due to the small size of many of the earthworm specimens, when it was not possible to collect a section of skin tissue, the entire earthworm was lysed.

An ethanol precipitation step was included to increase DNA purity and remove possible co-purified contaminants. Briefly, 50 µL eluted DNA was mixed with 5 µL sodium acetate and 100 µL ice cold 95% ethanol. Samples were stored overnight at -20 °C. Samples were then centrifuged for 30 minutes at 14,000 x g at 0 °C. Supernatant was removed and discarded and 100 µL of 70% ethanol was added. DNA was resuspended and the sample was centrifuged at 14,000 x g at 4 °C for 30 minutes. Supernatant was removed and discarded, and the sample was stored at room temperature for 15 minutes with the vials open to evaporate the ethanol. DNA was redissolved in 50 µL PCR-H_2O. Cleaned samples were stored at -20 °C.

PCR Amplification and Sequencing of Earthworm DNA

A 710-bp gene fragment of the COI gene was targeted for amplification using primers LCO1490 [GGT CAA CAA ATC ATA AAG ATA TTG G] and HCO2198 [TAA ACT TCA GGG TGA CCA AAA AAT CA] (Folmer et al., 1994). Reactions (20 µl) contained a final concentration of 1X PCR buffer, 3.0 mM $MgCl_2$, 200µM each dNTP, 400 ng μL^{-1} BSA, 1.0 µM of each primer, 1.5 units Hot Start Taq DNA polymerase, and 2 µL of a 1:10 dilution sample of template DNA. A Peltier Thermal Cycler 200 (Bio-Rad Laboratories, Inc., Hercules, California) was used to carry out PCR reactions. Optimum target amplification conditions were determined experimentally and are as follows: initial denaturation at 96 °C for 10 minutes, 40 cycles of 95 °C for 30 seconds, 50 °C for 45 seconds, and 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. Amplification was confirmed by gel electrophoresis in 1.5% agarose gel, and digitally visualized by ethidium bromide fluorescence. Samples were purified using a Wizard® SV Gel and PCR Clean-up System (Promega, Madison, WI) and DNA concentration was quantified using nanodrop spectrophotometry (ND-1000; Thermo Fisher Scientific Inc., Waltham, MA.). Purified PCR products were prepared according to submission guidelines with a reduction in the amount of final eluate used to increase final DNA concentration. Purified samples were sent to Eurofins Genomics (Louisville, KY) for sequencing in both the forward and reverse directions.

Sequence Alignment, Adjustment, and Tree Building

Sequencher software (Gene Codes Corporation, Ann Arbor, MI) was used to trim, align, and edit sequences and to generate consensus sequences. Molecular Evolutionary Genetics Analysis ([MEGA7]; Kumar et al., 2015) was used to build neighbor-joining, maximum parsimony, and maximum-likelihood trees, which included 107 samples from this study and 134 sequences from the National Center for Biotechnology Information ([NCBI], Bethesda, MD). Short (<500 bp) or ambiguous sequences from the current study were not included in tree-building analysis. An Ocnerodrilidae sp. was used as an outgroup to root the trees. All trees were calculated based on 1000 bootstrap replications. After analysis of the original trees, reduced trees were generated with 50 of the 134 known sequences from NCBI that grouped with the individuals from this study. Database sequences that did form clusters with the individuals from the current study were removed.

Results and Discussion

Total abundance ranged between 94 and 556 individuals m^{-2} at the UA Research and Extension Station, 250-700 individuals m^{-2} at Lew Wentz, 100-275 individuals m^{-2} at Jimmie Austin, 411-1688 individuals m^{-2} at Chenal, and 0-211 individuals m^{-2} at Meadowbrook (Table 2). No juveniles were collected from the No. 12 tee at Jimmie Austin Golf Club, the No. 15 rough at Meadowbrook Country Club, or any of the Lew Wentz samples (Table 2). No adults were collected from the No. 5 practice tee at Jimmie Austin Golf Club, the Chenal green, or any of the Meadowbrook samples (Table 2).

Extraction of DNA from formalin-fixed earthworm specimens from Lew Wentz and Jimmie Austin golf courses did not provide DNA sequences, so these specimens could not be identified using molecular techniques. Morphological identification indicated that the Lew Wentz adult specimens were comprised of *Aporrectodea* and *Amynthas* spp. Morphological identification designated some *Amynthas* spp. as well as some unidentified adult and juvenile specimens at Jimmie Austin Golf Course. Because no adults were collected from the No. 5 practice tee at Jimmie Austin Golf Course (Table 2), no morphological identification was possible from this sample. No juveniles were collected from Lew Wentz Memorial Golf Course. This could be due to temporal differences in hatching times of species located at this site. No earthworms were collected from one of the Meadowbrook sites (Table 2). This sampling location was under a tree where there was no turfgrass cover and no observable root mass.

Although there were no clear monophyletic assemblages in the original phylogenetic analyses, many specimens collected from golf course turf in Arkansas and Oklahoma grouped with the North-American native *Diplocardia* spp. (Fig. 1). This information was confirmed by comparison with original neighbor-joining and maximum-parsiomony analysis (data not shown). This grouping included specimens from UA, Meadowbrook Country Club, and a majority of the specimens from Chenal Country Club. *Diplocardia* spp. were present across a variety of turfgrass species, soil textures, pH levels, and organic matter and nutrient contents (Tables 3-4).

Several species of *Diplocardia* have previously been reported in Arkansas and Oklahoma (Reynolds, 2008, 2010; Thomason et al., 2017). James (1991) reported a decline in *Diplocardia* spp. after invasion by non-native *Aporrectodea caliginosa* and *Octalasion cyaneum*. James (1991) indicated a reduced ability of the smaller *Diplocardia* spp. to compete with the larger non-native earthworms; however, the current study's results suggest this may not always be the case. James (1991) also determined that *Diplocardia* spp. process more soil and thus, contribute more to nutrient cycling than the non-native species. Furthermore, *Diplocardia* also demonstrated increased casting activity (James 1991). An increased presence of *Diplocardia* spp. could be important on golf courses, where organic matter reduction is desired to prevent soft surfaces and disease pressure and where surface casting can lead to issues with weed, disease, and pest invasion, equipment damage, reduced aesthetics, and issues with ball roll and playability.

Several individuals from UA and Meadowbrook grouped with *Amynthas* and *Metaphire* spp. (Fig. 1, 2). These two genera were once classified under a single genus, *Pheretima*, which has since been divided into eight genera (Edwards and Bohlen, 1996). Several species of *Amynthas* have been previously reported in Arkansas and Oklahoma (Reynolds, 2008, 2010), and one species of *Metaphire* has been reported in Arkansas, though not in the counties sampled in the current study (Reynolds, 2008). Four UA samples (UA Light Soil 84, UA Light Soil 85, UA Light Soil 21, UA Light Sand 73, and UA Light Sand 32) grouped loosely with Lumbricidae species under neighbor-joining analysis (Fig. 1), but this grouping was not confirmed by the maximum-likelihood analysis (Fig. 2).

Previous earthworm identification in U.S. turfgrass has shown species composition to be comprised primarily of non-native species (Backman, 1999; Backman et al., 2001; Kowalewski and McDonald, 2016; Potter et al., 1994; Redmond et al., 2014; Tu et al., 2011; Williamson and Hong, 2005). The results of the current study are similar to those of Tu et al. (2011), where *Diplocardia* spp. dominated the earthworm community in a bermudagrass tee box in North Carolina. Redmond et al., (2014) observed some *Diplocardia singularis* on golf course turfgrass in Kentucky, but this species was not predominant. The current study is the only other known study where *Diplocardia* spp. dominated the earthworm species composition of a golf course turfgrass system.

Although molecular sequencing does provide benefits for earthworm identification, there are still issues that arise. Earthworm relatedness can vary depending on the gene used, and sometimes, genes do not agree on identification (Pop et al., 2007). Earthworms can still be misidentified or labeled as synonymous with other species (Chang et al., 2009; Pérez-Losada, 2012). Pérez-Losada et al. (2012) suggest that there may be taxonomic misidentifications or barcode mislabels within the database that hinder identification efforts. The authors also state that *Aporrectodea, Eisenia, Metaphire, Amynthas,* and *Diplocardia* are not valid genera and require revision and rearrangement (Pérez-Losada et al., 2012). This again highlights the difficulties faced by earthworm taxonomists, as ambiguities and discrepancies within the genus-level designation are not uncommon and misidentification of earthworms within the GenBank system can even lead to discrepancies with molecular identification. Further research should focus on establishment of clearly designated genera and species classifications. Additionally, further research is needed to determine earthworm species composition in golf course turfgrass systems in the U.S.

Figure Legend

Figure 1	COI Neighbor-Joining tree of earthworm species. Bootstrap proportions >50% are indicated. Sequences generated in this study are labeled as follows: UA = University of Arkansas, Fayetteville, AR; Chenal = Chenal Country Club, Little Rock, AR; Meadowbrook = Meadowbrook Country Club, Tulsa, OK.
Figure 2	COI Maximum-Likelihood tree of earthworm species. Bootstrap proportions >50% are indicated. Sequences generated in this study are labeled as follows: UA = University of Arkansas, Fayetteville, AR; Chenal = Chenal Country Club, Little Rock, AR; Meadowbrook = Meadowbrook Country Club, Tulsa, OK.
Figure 3	COI Maximum-Parsimony tree of earthworm species. Bootstrap proportions >50% are indicated. Sequences generated in this study are labeled as follows: UA = University of Arkansas, Fayetteville, AR; Chenal = Chenal Country Club, Little Rock, AR; Meadowbrook = Meadowbrook Country Club, Tulsa, OK.

Figure 1

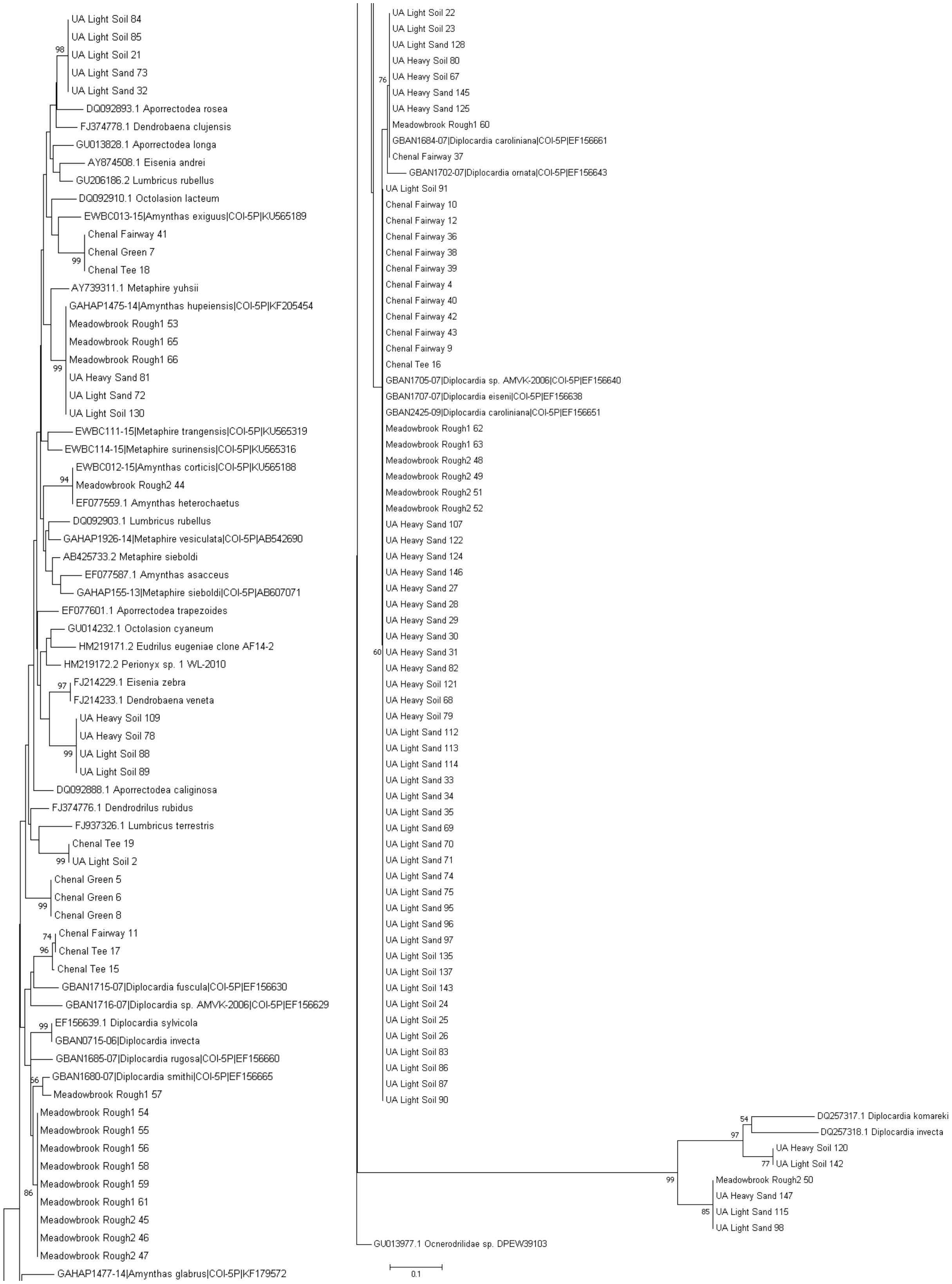
UA Light Soil 84
UA Light Soil 85
98
UA Light Soil 21
UA Light Sand 73
UA Light Sand 32
DQ092893.1 Aporrectodea rosea
FJ374778.1 Dendrobaena clujensis
GU013828.1 Aporrectodea longa
AY874508.1 Eisenia andrei
GU206186.2 Lumbricus rubellus
DQ092910.1 Octolasion lacteum
EWBC013-15|Amynthas exiguus|COI-5P|KU565189
Chenal Fairway 41
Chenal Green 7
99
Chenal Tee 18
AY739311.1 Metaphire yuhsii
GAHAP1475-14|Amynthas hupeiensis|COI-5P|KF205454
Meadowbrook Rough1 53
Meadowbrook Rough1 65
Meadowbrook Rough1 66
99
UA Heavy Sand 81
UA Light Sand 72
UA Light Soil 130
EWBC111-15|Metaphire trangensis|COI-5P|KU565319
EWBC114-15|Metaphire surinensis|COI-5P|KU565316
EWBC012-15|Amynthas corticis|COI-5P|KU565188
94
Meadowbrook Rough2 44
EF077559.1 Amynthas heterochaetus
DQ092903.1 Lumbricus rubellus
GAHAP1926-14|Metaphire vesiculata|COI-5P|AB542690
AB425733.2 Metaphire sieboldi
EF077587.1 Amynthas asacceus
GAHAP155-13|Metaphire sieboldi|COI-5P|AB607071
EF077601.1 Aporrectodea trapezoides
GU014232.1 Octolasion cyaneum
HM219171.2 Eudrilus eugeniae clone AF14-2
HM219172.2 Perionyx sp. 1 WL-2010
97
FJ214229.1 Eisenia zebra
FJ214233.1 Dendrobaena veneta
UA Heavy Soil 109
UA Heavy Soil 78
99
UA Light Soil 88
UA Light Soil 89
DQ092888.1 Aporrectodea caliginosa
FJ374776.1 Dendrodrilus rubidus
FJ937326.1 Lumbricus terrestris
Chenal Tee 19
99
UA Light Soil 2
Chenal Green 5
Chenal Green 6
99
Chenal Green 8
74
Chenal Fairway 11
96
Chenal Tee 17
Chenal Tee 15
GBAN1715-07|Diplocardia fuscula|COI-5P|EF156630
GBAN1716-07|Diplocardia sp. AMVK-2006|COI-5P|EF156629
99
EF156639.1 Diplocardia sylvicola
GBAN0715-06|Diplocardia invecta
GBAN1685-07|Diplocardia rugosa|COI-5P|EF156660
56
GBAN1680-07|Diplocardia smithi|COI-5P|EF156665
Meadowbrook Rough1 57
Meadowbrook Rough1 54
Meadowbrook Rough1 55
Meadowbrook Rough1 56
Meadowbrook Rough1 58
Meadowbrook Rough1 59
86
Meadowbrook Rough1 61
Meadowbrook Rough2 45
Meadowbrook Rough2 46
Meadowbrook Rough2 47
GAHAP1477-14|Amynthas glabrus|COI-5P|KF179572
UA Light Soil 22
UA Light Soil 23
UA Light Sand 128
UA Heavy Soil 80
76
UA Heavy Soil 67
UA Heavy Sand 145
UA Heavy Sand 125
Meadowbrook Rough1 60
GBAN1684-07|Diplocardia caroliniana|COI-5P|EF156661
Chenal Fairway 37
GBAN1702-07|Diplocardia ornata|COI-5P|EF156643
UA Light Soil 91
Chenal Fairway 10
Chenal Fairway 12
Chenal Fairway 36
Chenal Fairway 38
Chenal Fairway 39
Chenal Fairway 4
Chenal Fairway 40
Chenal Fairway 42
Chenal Fairway 43
Chenal Fairway 9
Chenal Tee 16
GBAN1705-07|Diplocardia sp. AMVK-2006|COI-5P|EF156640
GBAN1707-07|Diplocardia eiseni|COI-5P|EF156638
GBAN2425-09|Diplocardia caroliniana|COI-5P|EF156651
Meadowbrook Rough1 62
Meadowbrook Rough1 63
Meadowbrook Rough2 48
Meadowbrook Rough2 49
Meadowbrook Rough2 51
Meadowbrook Rough2 52
UA Heavy Sand 107
UA Heavy Sand 122
UA Heavy Sand 124
UA Heavy Sand 146
UA Heavy Sand 27
UA Heavy Sand 28
UA Heavy Sand 29
UA Heavy Sand 30
60
UA Heavy Sand 31
UA Heavy Sand 82
UA Heavy Soil 121
UA Heavy Soil 68
UA Heavy Soil 79
UA Light Sand 112
UA Light Sand 113
UA Light Sand 114
UA Light Sand 33
UA Light Sand 34
UA Light Sand 35
UA Light Sand 69
UA Light Sand 70
UA Light Sand 71
UA Light Sand 74
UA Light Sand 75
UA Light Sand 95
UA Light Sand 96
UA Light Sand 97
UA Light Soil 135
UA Light Soil 137
UA Light Soil 143
UA Light Soil 24
UA Light Soil 25
UA Light Soil 26
UA Light Soil 83
UA Light Soil 86
UA Light Soil 87
UA Light Soil 90
54
DQ257317.1 Diplocardia komareki
97
DQ257318.1 Diplocardia invecta
UA Heavy Soil 120
77
UA Light Soil 142
99
Meadowbrook Rough2 50
UA Heavy Sand 147
85
UA Light Sand 115
UA Light Sand 98
GU013977.1 Ocnerodrilidae sp. DPEW39103
0.1

Figure 2


UA Light Soil 87
UA Light Soil 90
UA Light Soil 86
UA Light Soil 83
UA Light Soil 26
UA Light Soil 25
UA Light Soil 24
UA Light Soil 143
UA Light Soil 137
UA Light Soil 135
UA Light Sand 97
UA Light Sand 96
UA Light Sand 95
UA Light Sand 75
UA Light Sand 74
UA Light Sand 71
UA Light Sand 70
UA Light Sand 69
UA Light Sand 35
UA Light Sand 34
UA Light Sand 33
UA Light Sand 114
UA Light Sand 113
UA Light Sand 112
UA Heavy Soil 79
UA Heavy Soil 68
UA Heavy Soil 121
UA Heavy Sand 82
82
UA Heavy Sand 31
UA Heavy Sand 30
UA Heavy Sand 29
UA Heavy Sand 28
UA Heavy Sand 27
UA Heavy Sand 146
UA Heavy Sand 124
UA Heavy Sand 122
UA Heavy Sand 107
Meadowbrook Rough2 52
Meadowbrook Rough2 51
Meadowbrook Rough2 49
Meadowbrook Rough2 48
Meadowbrook Rough1 63
Meadowbrook Rough1 62
GBAN2425-09|Diplocardia caroliniana|COI-5P|EF156651
GBAN1707-07|Diplocardia eiseni|COI-5P|EF156638
GBAN1705-07|Diplocardia sp. AMVK-2006|COI-5P|EF156640
Chenal Tee 16
Chenal Fairway 9
Chenal Fairway 43
Chenal Fairway 42
Chenal Fairway 40
Chenal Fairway 4
Chenal Fairway 39
Chenal Fairway 38
Chenal Fairway 36
Chenal Fairway 12
Chenal Fairway 10
UA Light Soil 91
GAHAP1477-14|Amynthas glabrus|COI-5P|KF179572
Chenal Fairway 37
GBAN1684-07|Diplocardia caroliniana|COI-5P|EF156661
Meadowbrook Rough1 60
UA Heavy Sand 125
UA Heavy Sand 145
UA Heavy Soil 67
UA Heavy Soil 80
UA Light Sand 128
UA Light Soil 22
UA Light Soil 23
GBAN1702-07|Diplocardia ornata|COI-5P|EF156643
DQ257317.1 Diplocardia komareki
91
DQ257318.1 Diplocardia invecta
UA Heavy Soil 120
99
UA Light Soil 142
99
UA Heavy Sand 147
Meadowbrook Rough2 50
99
UA Light Sand 115
UA Light Sand 98
GBAN1716-07|Diplocardia sp. AMVK-2006|COI-5P|EF156629
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Chenal Tee 17
Chenal Fairway 11
94
Chenal Tee 15
67
GBAN1680-07|Diplocardia smithi|COI-5P|EF156665
Meadowbrook Rough1 57
99
EF156639.1 Diplocardia sylvicola
GBAN0715-06|Diplocardia invecta
GBAN1685-07|Diplocardia rugosa|COI-5P|EF156660
Meadowbrook Rough1 54
Meadowbrook Rough1 55
Meadowbrook Rough1 56
Meadowbrook Rough1 58
Meadowbrook Rough1 59
92
Meadowbrook Rough1 61
Meadowbrook Rough2 45
Meadowbrook Rough2 46
Meadowbrook Rough2 47
DQ092888.1 Aporrectodea caliginosa
Chenal Green 8
Chenal Green 5
99
Chenal Green 6
99
Chenal Tee 19
UA Light Soil 2
FJ374776.1 Dendrodrilus rubidus
FJ937326.1 Lumbricus terrestris
EF077601.1 Aporrectodea trapezoides
EF077587.1 Amynthas asacceus
GAHAP155-13|Metaphire sieboldi|COI-5P|AB607071
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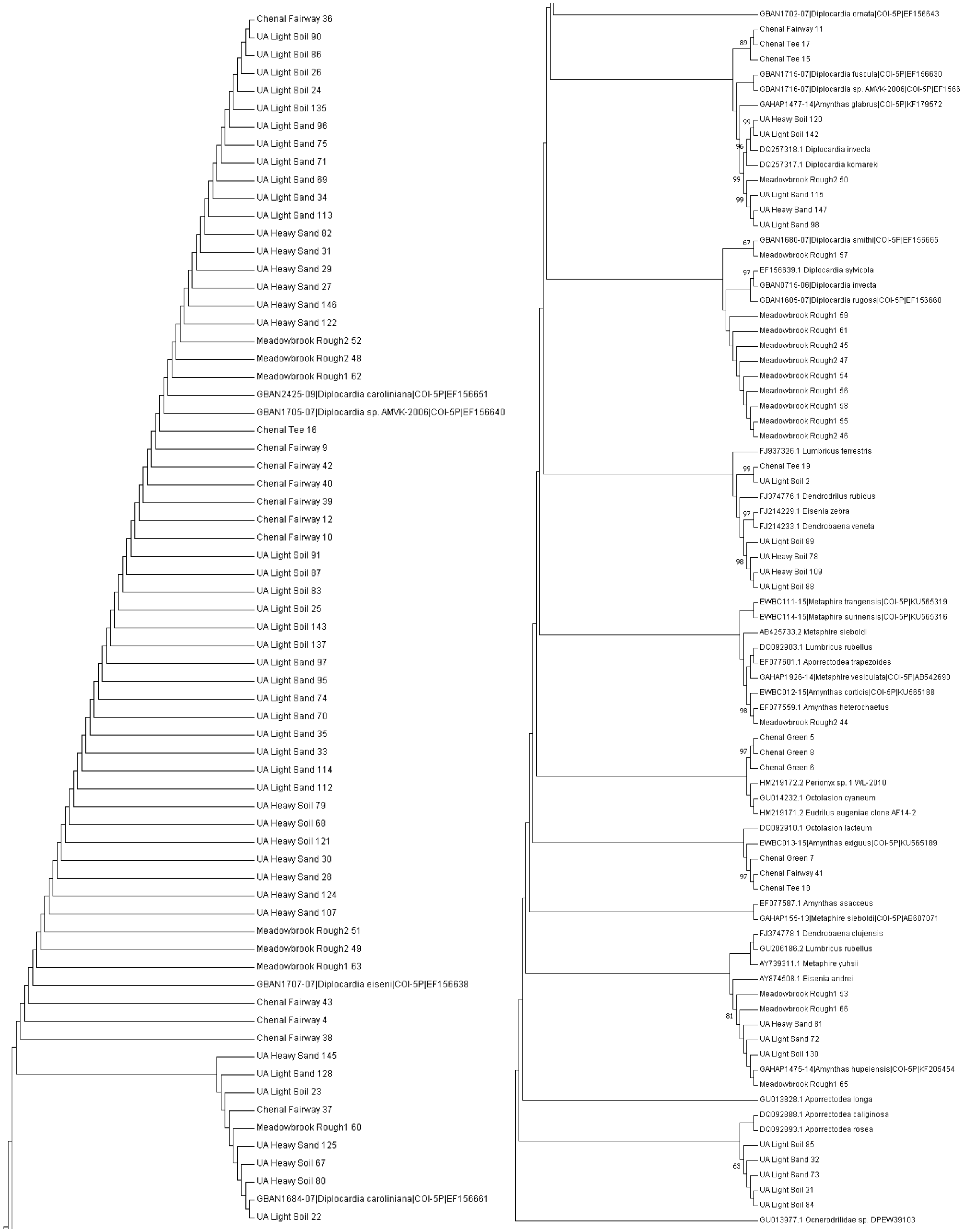
DQ092903.1 Lumbricus rubellus
EF077559.1 Amynthas heterochaetus
EWBC012-15|Amynthas corticis|COI-5P|KU565188
98
Meadowbrook Rough2 44
GAHAP1926-14|Metaphire vesiculata|COI-5P|AB542690
EWBC111-15|Metaphire trangensis|COI-5P|KU565319
EWBC114-15|Metaphire surinensis|COI-5P|KU565316
GU013828.1 Aporrectodea longa
AY874508.1 Eisenia andrei
Chenal Fairway 41
99
Chenal Green 7
Chenal Tee 18
EWBC013-15|Amynthas exiguus|COI-5P|KU565189
DQ092910.1 Octolasion lacteum
GU206186.2 Lumbricus rubellus
UA Light Sand 32
UA Light Sand 73
97
UA Light Soil 21
UA Light Soil 84
UA Light Soil 85
DQ092893.1 Aporrectodea rosea
FJ374778.1 Dendrobaena clujensis
AY739311.1 Metaphire yuhsii
GAHAP1475-14|Amynthas hupeiensis|COI-5P|KF205454
Meadowbrook Rough1 53
Meadowbrook Rough1 65
Meadowbrook Rough1 66
98
UA Heavy Sand 81
UA Light Sand 72
UA Light Soil 130
HM219172.2 Perionyx sp. 1 WL-2010
GU014232.1 Octolasion cyaneum
HM219171.2 Eudrilus eugeniae clone AF14-2
98
FJ214229.1 Eisenia zebra
FJ214233.1 Dendrobaena veneta
UA Heavy Soil 109
UA Heavy Soil 78
99
UA Light Soil 88
UA Light Soil 89
GU013977.1 Ocnerodrilidae sp. DPEW39103
0.1

Figure 3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UA Light Soil 86
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UA Light Soil 24
UA Light Soil 135
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UA Light Sand 75
UA Light Sand 71
UA Light Sand 69
UA Light Sand 34
UA Light Sand 113
UA Heavy Sand 82
UA Heavy Sand 31
UA Heavy Sand 29
UA Heavy Sand 27
UA Heavy Sand 146
UA Heavy Sand 122
Meadowbrook Rough2 52
Meadowbrook Rough2 48
Meadowbrook Rough1 62
GBAN2425-09|Diplocardia caroliniana|COI-5P|EF156651
GBAN1705-07|Diplocardia sp. AMVK-2006|COI-5P|EF156640
Chenal Tee 16
Chenal Fairway 9
Chenal Fairway 42
Chenal Fairway 40
Chenal Fairway 39
Chenal Fairway 12
Chenal Fairway 10
UA Light Soil 91
UA Light Soil 87
UA Light Soil 83
UA Light Soil 25
UA Light Soil 143
UA Light Soil 137
UA Light Sand 97
UA Light Sand 95
UA Light Sand 74
UA Light Sand 70
UA Light Sand 35
UA Light Sand 33
UA Light Sand 114
UA Light Sand 112
UA Heavy Soil 79
UA Heavy Soil 68
UA Heavy Soil 121
UA Heavy Sand 30
UA Heavy Sand 28
UA Heavy Sand 124
UA Heavy Sand 107
Meadowbrook Rough2 51
Meadowbrook Rough2 49
Meadowbrook Rough1 63
GBAN1707-07|Diplocardia eiseni|COI-5P|EF156638
Chenal Fairway 43
Chenal Fairway 4
Chenal Fairway 38
UA Heavy Sand 145
UA Light Sand 128
UA Light Soil 23
Chenal Fairway 37
Meadowbrook Rough1 60
UA Heavy Sand 125
UA Heavy Soil 67
UA Heavy Soil 80
GBAN1684-07|Diplocardia caroliniana|COI-5P|EF156661
UA Light Soil 22
GBAN1702-07|Diplocardia ornata|COI-5P|EF156643
Chenal Fairway 11
89
Chenal Tee 17
Chenal Tee 15
GBAN1715-07|Diplocardia fuscula|COI-5P|EF156630
GBAN1716-07|Diplocardia sp. AMVK-2006|COI-5P|EF156629
GAHAP1477-14|Amynthas glabrus|COI-5P|KF179572
99
UA Heavy Soil 120
UA Light Soil 142
96
DQ257318.1 Diplocardia invecta
DQ257317.1 Diplocardia komareki
99
Meadowbrook Rough2 50
99
UA Light Sand 115
UA Heavy Sand 147
UA Light Sand 98
67
GBAN1680-07|Diplocardia smithi|COI-5P|EF156665
Meadowbrook Rough1 57
97
EF156639.1 Diplocardia sylvicola
GBAN0715-06|Diplocardia invecta
GBAN1685-07|Diplocardia rugosa|COI-5P|EF156660
Meadowbrook Rough1 59
Meadowbrook Rough1 61
Meadowbrook Rough2 45
Meadowbrook Rough2 47
Meadowbrook Rough1 54
Meadowbrook Rough1 56
Meadowbrook Rough1 58
Meadowbrook Rough1 55
Meadowbrook Rough2 46
FJ937326.1 Lumbricus terrestris
99
Chenal Tee 19
UA Light Soil 2
FJ374776.1 Dendrodrilus rubidus
97
FJ214229.1 Eisenia zebra
FJ214233.1 Dendrobaena veneta
UA Light Soil 89
98
UA Heavy Soil 78
UA Heavy Soil 109
UA Light Soil 88
EWBC111-15|Metaphire trangensis|COI-5P|KU565319
EWBC114-15|Metaphire surinensis|COI-5P|KU565316
AB425733.2 Metaphire sieboldi
DQ092903.1 Lumbricus rubellus
EF077601.1 Aporrectodea trapezoides
GAHAP1926-14|Metaphire vesiculata|COI-5P|AB542690
EWBC012-15|Amynthas corticis|COI-5P|KU565188
98
EF077559.1 Amynthas heterochaetus
Meadowbrook Rough2 44
Chenal Green 5
97
Chenal Green 8
Chenal Green 6
HM219172.2 Perionyx sp. 1 WL-2010
GU014232.1 Octolasion cyaneum
HM219171.2 Eudrilus eugeniae clone AF14-2
DQ092910.1 Octolasion lacteum
EWBC013-15|Amynthas exiguus|COI-5P|KU565189
Chenal Green 7
97
Chenal Fairway 41
Chenal Tee 18
EF077587.1 Amynthas asacceus
GAHAP155-13|Metaphire sieboldi|COI-5P|AB607071
FJ374778.1 Dendrobaena clujensis
GU206186.2 Lumbricus rubellus
AY739311.1 Metaphire yuhsii
AY874508.1 Eisenia andrei
Meadowbrook Rough1 53
Meadowbrook Rough1 66
81
UA Heavy Sand 81
UA Light Sand 72
UA Light Soil 130
GAHAP1475-14|Amynthas hupeiensis|COI-5P|KF205454
Meadowbrook Rough1 65
GU013828.1 Aporrectodea longa
DQ092888.1 Aporrectodea caliginosa
DQ092893.1 Aporrectodea rosea
UA Light Soil 85
63
UA Light Sand 32
UA Light Sand 73
UA Light Soil 21
UA Light Soil 84
GU013977.1 Ocnerodrilidae sp. DPEW39103

Table 1. Collection sites in Arkansas and Oklahoma sampled for identification of earthworm species. Number in parentheses indicates the number of samples collected from each sampling location.

Collection site	City	Latitude / longitude	Date sampled	Turfgrass area sampled	Cultivar
Jimmie Austin Golf Club	Norman, OK	35.188541 N 97.427982 W	30 Nov 2015	No. 5 practice zoysiagrass tee (1)	Zeon
				No. 12 zoysiagrass tee (1)	Zeon
				No. 13 bermudagrass fairway (1)	Unknown
Lew Wentz Memorial Golf Course	Ponca City, OK	36.730351 N 97.024931 W	30 Nov 2015	No. 8 creeping bentgrass collar (3)	Unknown
Chenal Country Club	Little Rock, AR	34.778560 N 92.475937 W	26 Oct 2016	Founders no. 9 creeping bentgrass green (1)	A-1
				Founders no. 9 zoysiagrass fairway (1)	Meyer
				Bear Den no. 10 zoysiagrass tee (1)	Cavalier
University of Arkansas	Fayetteville, AR	36.100229N 94.168845W	20 Dec 2016	Simulated bermuadgrass tees (16)	Patriot
Meadowbrook Country Club	Tulsa, OK	36.042490 N 95.872778 W	12 Jun 2017	No. 14 bermudagrass rough (2)	
				No. 15 bermudagrass rough (1)	Unknown

Table 2. Earthworm abundances (earthworms m^{-2}) in golf course turfgrass systems in Arkansas and Oklahoma.

	Collection site															
	University of Arkansas				Lew Wentz Memorial Golf Course			Jimmie Austin Golf Club			Chenal Country Club			Meadowbrook Country Club		
	Turfgrass system†															
	Light TD sand tee‡	Heavy TD sand tee‡	Light TD soil tee‡	Heavy TD soil tee‡	C	C	C	Tee	Tee	FW	Green	FW	Tee	R	R	R
	Turfgrass species and cultivar (if known)§															
Earthworm abundance	‘Patriot’ BG	‘Patriot’ BG	‘Patriot’ BG	‘Patriot’ BG	CB	CB	CB	‘Zeon’ ZG	‘Zeon’ ZG	BG	‘A-1’ CB	‘Meyer’ ZG	‘Cavalier’ ZG	BG	BG	BG
Juvenile	117	31	339	44	0	0	0	100	0	125	289	1233	378	111	144	0
Adult	108	86	72	42	250	200	675	0	75	125	0	333	22	0	0	0
Unknown	28	31	144	8	0	50	25	0	25	25	0	122	11	100	67	0
Total	253	148	556	94	250	250	700	100	100	275	289	1688	411	211	211	0

† C = collar, FW = fairway, R = rough

‡ Light TD = 0.64 cm sand topdressing yr^{-1}, Heavy TD = 2.54 cm sand topdressing yr^{-1}; Average of four replications

§ BG = Bermudagrass, CB = Creeping bentgrass, ZG = Zoysiagrass

Table 3. Particle size analysis and percent organic matter of soil samples from earthworm collection sites in golf course turfgrass systems in Arkansas and Oklahoma.

Collection site	University of Arkansas				Lew Wentz Memorial Golf Course			Jimmie Austin Golf Club			Chenal Country Club			Meadowbrook Country Club		
Turfgrass system†	Light TD sand tee‡	Heavy TD sand tee‡	Light TD soil tee‡	Heavy TD soil tee‡	C	C	C	Tee	Tee	FW	Green	FW	Tee	R	R	R
Turfgrass species and cultivar (if known)§	'Patriot' BG	'Patriot' BG	'Patriot' BG	'Patriot' BG	CB	CB	CB	'Zeon' ZG	'Zeon' ZG	BG	'A-1' CB	'Meyer' ZG	'Cavalier' ZG	BG	BG	BG
Parameter (%)																
Sand	85.0	95.0	60.6	69.1	84.9	78.7	73.1	89.7	80.4	86.4	98.3	49.2	88.0	37.4	32.8	30.7
Silt	13.5	5.0	36.4	27.2	13.4	18.3	23.5	7.35	14.6	10.6	1.25	43.7	10.2	56.4	58.7	60.3
Clay	1.6	0.1	3.0	3.7	1.75	3.05	3.55	3.0	5.05	3.05	0.5	7.1	1.86	6.3	8.55	9.0
Organic matter	3.21	2.07	3.83	2.86	4.95	4.90	4.49	3.86	2.93	3.41	1.28	3.97	1.46	4.86	4.88	3.65

† C = collar, FW = fairway, R = rough

‡ Light TD = 0.64 cm sand topdressing yr^{-1}, Heavy TD = 2.54 cm sand topdressing yr^{-1}; Average of four replications

§ BG = Bermudagrass, CB = Creeping bentgrass, ZG = Zoysiagrass

Table 4. Nutrient content and pH of soil samples from earthworm collection sites in golf course turfgrass systems in Arkansas and Oklahoma.

	Collection site															
	University of Arkansas				Lew Wentz Memorial Golf Course			Jimmie Austin Golf Club			Chenal Country Club			Meadowbrook Country Club		
	Turfgrass system†															
	Light TD sand tee‡	Heavy TD sand tee‡	Light TD soil tee‡	Heavy TD soil tee‡	C	C	C	Tee	Tee	FW	Green	FW	Tee	R	R	R
	Turfgrass species and cultivar (if known)§															
Parameter	‘Patriot’ BG	‘Patriot’ BG	‘Patriot’ BG	‘Patriot’ BG	CB	CB	CB	‘Zeon’ ZG	‘Zeon’ ZG	BG	‘A-1’ CB	‘Meyer’ ZG	‘Cavalier’ ZG	BG	BG	BG
	mg kg^{-1}															
P	17.2	15.2	28.2	24.3	92.1	91.8	74.4	60.0	252.9	123.2	91.8	77.5	21.4	84.2	67.4	39.1
K	59.7	38.9	84.7	69.4	146.4	133.3	144.9	103.7	178.0	161.3	49.5	82.6	30.2	152.2	100.1	69.2
Ca	580.0	418.2	806.5	656.0	1387	1665	1553	1130	5541	1120	554	1104	287	1815	1012	1699
Mg	33.7	23.6	48.4	40.0	178.3	204.1	221.1	187.6	289.0	177.9	32.7	281.6	56.9	161.4	148.6	169.9
S	11.7	9.9	16.2	15.4	30.5	44.4	37.4	28.0	25.4	19.3	31.8	70.4	37.7	71.6	81.9	56.8
Na	14.0	11.3	15.4	13.1	22.7	34.0	30.7	82.5	34.6	45.9	11.6	37.5	12.7	31.7	31.5	64.8
Fe	130.3	106.0	154.9	174.0	208.3	293.9	219.8	124.8	95.6	156.3	123.5	211.9	216.2	339.7	240.6	213.1
Mn	41.2	19.7	67.9	57.2	59.3	67.1	71.2	66.0	48.0	36.7	125.2	103.3	55.3	34.9	59.5	39.4
Zn	1.4	1.4	1.8	1.7	8.09	9.09	6.40	1.80	6.24	3.32	21.42	2.75	2.99	3.56	2.69	3.80
Cu	0.6	0.4	1.0	0.9	1.76	0.75	2.54	1.03	1.08	0.82	1.87	1.01	0.89	0.20	0.27	0.59
B	0.2	0.2	0.3	0.3	0.98	1.06	0.93	1.41	1.76	1.54	0.26	0.59	0.40	0.80	0.47	0.60
pH	6.1	6.1	5.9	5.9	6.4	6.1	6.0	6.4	6.9	5.9	6.1	5.2	5.0	5.4	4.6	5.4

† C = collar, FW = fairway, R = rough

‡ Light TD = 0.64 cm sand topdressing yr^{-1}, Heavy TD = 2.54 cm sand topdressing yr^{-1}; Average of four replications

§ BG = Bermudagrass, CB = Creeping bentgrass, ZG = Zoysiagrass

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Conclusions

Earthworm Community

Total earthworm abundance ranged between 94 and 556 individuals m^{-2} at the UA Research and Extension Station, 250-700 individuals m^{-2} at Lew Wentz, 100-275 individuals m^{-2} at Jimmie Austin, 411-1688 individuals m^{-2} at Chenal, and 0-211 individuals m^{-2} at Meadowbrook. Morphological identification indicated that the Lew Wentz adult specimens were comprised of *Aporrectodea* and *Amynthas* spp. Morphological identification designated some *Amynthas* spp. as well as some unidentified adult and juvenile specimens at Jimmie Austin Golf Course. Although there were no clear monophyletic assemblages in the original phylogenetic analyses, many specimens collected from golf course turf in Arkansas and Oklahoma grouped with the North-American native *Diplocardia* spp. This grouping included specimens from UA, Meadowbrook Country Club, and a majority of the specimens from Chenal Country Club. *Diplocardia* spp. were present across a variety of turfgrass species, soil textures, pH levels, and organic matter and nutrient contents.

Several species of *Diplocardia* have previously been reported in Arkansas and Oklahoma (Reynolds, 2008, 2010; Thomason et al., 2017). James (1991) reported a decline in *Diplocardia* spp. after invasion by non-native *Aporrectodea caliginosa* and *Octalasion cyaneum* due to a reduced ability of the smaller *Diplocardia* spp. to compete with the larger non-native earthworms; however, the current study’s results suggest this may not always be the case. James (1991) also determined that *Diplocardia* spp. process more soil and thus, contribute more to nutrient cycling than the non-native species. Furthermore, *Diplocardia* also demonstrated increased casting activity (James 1991). An increased presence of *Diplocardia* spp. could be important on golf courses, where organic matter reduction is desired to prevent soft surfaces and disease pressure and where surface casting can lead to issues with weed, disease, and pest invasion, equipment damage, reduced aesthetics, and issues with ball roll and playability.

Several individuals from UA and Meadowbrook grouped phylogenetically with *Amynthas* and *Metaphire* spp. Several species of *Amynthas* have been previously reported in Arkansas and Oklahoma (Reynolds, 2008, 2010), and one species of *Metaphire* has been reported in Arkansas, though not in the counties sampled in the current study (Reynolds, 2008). Several UA samples grouped loosely with Lumbricidae species, but genera-level classification was not possible.

Previous earthworm identification in U.S. turfgrass has shown species composition to be comprised primarily of non-native species (Backman, 1999; Backman et al., 2001; Kowalewski and McDonald, 2016; Potter et al., 1994; Redmond et al., 2014; Tu et al., 2011; Williamson and Hong, 2005). The results of the current study are similar to those of Tu et al. (2011), where *Diplocardia* spp. dominated the earthworm community in a bermudagrass tee box in North Carolina. Redmond et al., (2014) observed some *Diplocardia singularis* on golf course turfgrass in Kentucky, but this species was not predominant. The current study is the only other known study where *Diplocardia* spp. dominated the earthworm species composition of a golf course turfgrass system. Further research is needed to determine earthworm species composition in golf course turfgrass systems in the U.S.

Since more specific species classification was not possible in this study, more research needs to be done to assess the species-level designation and differences in casting activity between the earthworms observed in this study. Earthworm identification is highly controversial, and although molecular sequencing does provide benefits for earthworm identification, there are still issues that arise. Earthworm relatedness can vary depending on the gene used, and sometimes, genes do not agree on identification (Pop et al., 2007). Earthworms can still be misidentified or labeled as synonymous with other species (Chang et al., 2009; Pérez-Losada, 2012), which can lead to ambiguous identifications, as is the case in this study.

Casting Activity

Under light topdressing (0.64 cm yr^{-1}), the sand rootzone generally resulted in significantly greater casting activity. These results are counter to those seen by Baker and Binns (1998), who observed less casting activity on fairways with a sandy soil texture compared to fairways with a clay or clay loam soil texture; however, casting was not significantly correlated to soil texture on tees under the same study. There was generally very little casting activity under a light topdressing soil rootzone treatment throughout the two years of this study. In the heavy topdressing treatment (2.54 cm yr^{-1}), rootzone generally had little effect on casting activity.

Within the soil rootzone, heavy topdressing generally resulted in significantly greater casting activity. In the sand rootzone, there was no significant difference in casting activity between topdressing treatments in year one; however, in year two, light topdressing in the sand rootzone resulted in significantly greater casting activity compared to the heavy topdressing treatment. The inconclusive results of topdressing on earthworm casting activity (Backman et al., 2002; Baker et al., 2005; Bartlett et al., 2008; Henderson et al., 2011; Williamson and Hong, 2005), including the present study, may be due to the differences in angularity of the topdressing materials used. Future studies should review the effects of angularity of topdressing material on casting activity.

Soil Temperature

Soil temperature was a significant predictor of casting activity and explained 10-34% of the variation in earthworm casting activity. These results tentatively support those of Lavelle (1988), who stated that temperature is the most limiting factor affecting earthworm activity in temperate environments. Butt (1991) stated that temperatures above 15 °C led to “fatigue” of *Lumbricus terrestris* Linnaeus, 1758, and that temperatures over 20 °C led to clitellate loss, mass loss, and mortality of *L. terrestris*; however, in the current study, casting activity was still observed above 20 °C. This could be due to the differences in earthworm species composition between the two studies. *Diplocardia* spp., which dominated the species composition at this location, are thought to be more active at a wider range of temperatures than some of the non-native earthworm species (James, 1991).

The critical temperatures for peak casting activity in the light topdressing and heavy topdressing soil rootzone were 11.1 °C and 14.5 °C, respectively. Critical temperatures in the sand rootzone were similar, at 13.1 °C and 13.4 °C for the heavy and light topdressing treatments, respectively. These temperatures were not significantly different between topdressing and rootzone treatment combinations. The critical temperatures for peak casting activity under all four treatment combinations fell within the previously published range of optimum temperatures (10-20 °C) for temperate earthworm activity (Berry and Jordan, 2001; Lee, 1985).

Soil Moisture Content

Soil moisture content was not a significant predictor of earthworm casting activity in this study. These results are counter to Evans and Guild’s (1947) results, which determined that soil moisture is a highly significant predictor of earthworm casting activity. The differences between the two studies could be due to differences in moisture replacement; Evans and Guild (1947) relied on precipitation events and experiences several drought spells, while the current study utilized irrigation to avoid drought stress. Differences could also be due to differences in earthworm species composition, as Evans and Guild (1947) primarily researched temperature and moisture effects on casting activity of *Allolobophora nocturna*- and *A. longa*-dominated systems, while the current study's earthworm composition was primarily made up of *Diplocardia* spp.

Soil and Cast Analysis

There were no significant differences in sand content between topdressing treatments in either rootzone; however, the heavy topdressing sand rootzone treatment had significantly greater sand content than either topdressing treatment in the soil rootzone. There was significantly greater silt in the light topdressing soil rootzone, as would be expected under the less intensive topdressing treatment. Percent clay was not significantly different between any of the topdressing and rootzone treatment combinations.

It has been hypothesized that reducing organic matter in the rootzone may increase casting activity by causing earthworms to forage more to obtain organic matter (Abbott and Parker, 1981; Martin, 1982); however, in this study, organic matter in the rootzone was not significantly different between rootzones under light topdressing, even though casting activity was significantly greater in the sand rootzone compared to native soil under light topdressing. Under heavy topdressing, there was no significant difference in organic matter or casting activity between rootzones. Additionally, in the soil rootzone, heavy topdressing resulted in increased casting activity, while in the sand rootzone, heavy topdressing resulted in no difference or reduced casting activity, despite reduced organic matter in heavy topdressing treatments under both rootzones. This suggests that there was not a correlation between rootzone organic matter content and earthworm casting activity in ‘Patriot’ bermudagrass tee boxes under the present study. Although percent sand was not significantly different between topdressing treatments within each rootzone, the differences between the heavy topdressing sand rootzone and light topdressing soil rootzone suggest that there may be some effect of increased sand content on reduced organic matter. This is to be expected, as sand topdressing is a cultural practice commonly used by golf course superintendents to dilute organic matter within the turfgrass rootzone. The effects of soil particle size distribution and organic matter on earthworm casting activity should be researched further.

The cast organic matter was significantly greater in the light topdressing sand rootzone treatment compared to the light topdressing soil rootzone, suggesting that earthworms feeding in the sand rootzone may be more efficient at foraging for organic matter compared to those feeding in the soil rootzone under the same topdressing treatment, as the soil organic matter was not significantly different between these two treatments. This trend does not extend to the heavy topdressing treatment, however, where neither cast nor soil organic matter was significantly different between rootzones. Within each rootzone, organic matter content was greatest in casts collected from the light topdressing treatment compared to the heavy topdressing rate in the corresponding rootzone, possibly due to the increased organic matter content in the soil samples under the same conditions. James (1991) determined that organic matter in the casts of *Diplocardia* spp. was significantly greater than that of non-native Lumbricidae earthworm species, so it is possible that species composition could affect cast organic matter content.

Sand topdressing may impact casting activity, but the extent of the impact differs between rootzone and topdressing rate, and changes over time. While the effect of sand topdressing on surface casting activity of different earthworm species is still unclear, it has been previously determined that *Diplocardia* spp. are more active surface casters than *Aporrectodea caliginosa* and *Octalasion cyaneum*, despite the greater biomass of the two European spp. (James, 1991). This indicates that species composition can greatly affect surface casting activity. Future research should focus on identification of earthworm species in turfgrass systems, as well as species-specific cultural management of earthworm casting on golf course turf.

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