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Survival of *Aporrectodea caliginosa* and its effects on nutrient availability in biosolids amended soil

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ABSTRACT

Few earthworms are present in production agricultural fields in the semi-arid plains of Colorado, where earthworm populations may be constrained by limited water and/or organic matter resources. We conducted a 12-week laboratory incubation study to determine the potential of a non-native endogeic earthworm (*Aporrectodea caliginosa*) to survive in a low-organic matter Colorado soil (1.4% organic C content), supplemented with or without biosolids, and to determine the effects of *A. caliginosa* on soil microbial biomass and soil nutrient availability. A factorial design with three main effects of *A. caliginosa*, biosolids addition, and time was used. Data was collected through destructively sampling at one, two, four, eight, and twelve weeks. During the 12-week study, 97.5% of the worms in the soil survived, and the survival of the earthworms was not significantly affected by the addition of biosolids. The addition of biosolids, however, did significantly reduce the gain in mass of the earthworms (8% mass gain compared to 18% in soil without biosolids). The presence of *A. caliginosa* significantly increased soil NH_4-N, and NO_3-N concentrations by 31% and 4%, respectively, which was less than the six fold increases in both soil NH_4-N, and NO_3-N concentrations supplied from biosolids. Microbial biomass carbon was not affected by *A. caliginosa*, but microbial biomass N was affected by an earthworm × biosolids interaction at week 1 and 12. We concluded that *A. caliginosa* can survive in a low-organic matter Colorado soil under optimal moisture content and that once established, *A. caliginosa* can provide modest increases in inorganic N availability to crops Colorado agroecosystems.

1. Introduction

The effects of earthworms on soil were first documented by Darwin (1886), and for many years, farmers and soil scientist have associated the presence of earthworms as an indication of good soil quality (Doran and Safley, 1997; Roming et al., 1996; Yeates et al., 1998). This is namely due to the effects of earthworms on plant nutrient availability, particularly on increasing the availability of N in the soil. Earthworms can also improve soil quality through their effects on soil physical properties, including mixing and reorganizing soil (Darwin, 1886; Martin and Marinissen, 1993; Oades, 1993; Schrader and Zhang, 1997), creating macropores (Tisdall, 1978, 1985), and changing and improving water and gas flow (Lee and Foster, 1991).

Currently there are few earthworms present in production agricultural fields in eastern Colorado, but the transition to no-tillage (NT) practices could improve conditions for earthworms by reducing physical disturbance, increasing water holding capacity, and/or increasing organic matter content (Edwards and Lofty, 1982; House and Parmelee, 1985; VandenBygaart et al., 1999). Besides the conversion from conventional tillage to no-tillage, another way to increase the soil organic matter would be the addition of an organic amendment such as manure or biosolids. The addition of organic matter has been shown to increase earthworm populations in irrigated, forage agroecosystems in Colorado (Hurisso et al., 2011).

As earthworms expand into agricultural fields in Colorado, it will become important to understand the effect of earthworms on soil fertility to make correct nutrient management decisions. Earthworms can dramatically affect the concentration of plant available N through the mineralization of soil organic matter and excretion of nitrogenous wastes (Edwards and Lofty, 1977). This process is important when the fertility of the soil is dependent on the mineralization of organic materials such as biosolids or manure (Lubbers et al., 2011). Earthworms aid not only by direct mineralization of organic matter but also by stimulating microbial activity in soil (Curry and Schmidt, 2007). Earthworms primarily affect N availability by increasing the concentration of ammonium-nitrogen (NH_4-N) in the soil due to digestion and excretions of wastes, as

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well as their release of mucus (Whalen et al., 2000). The NH_4-N may be oxidized to nitrate-nitrogen (NO_3-N) by nitrifying bacteria, which are stimulated by earthworm burrowing activities (Parkin and Berry, 1999) that increase the oxygen concentration deeper in the soil profile (Costello and Lamberti, 2008).

Biosolids have been studied extensively as a soil amendment (for a review, see Haynes et al., 2009), and in Colorado, a main focus area of research has been to study the accumulation of metals in soil with repeated biosolids application (Ippolito and Barbarick, 2008). Outside of Colorado, studies have been conducted to investigate heavy metal availability due to biosolids and earthworm interactions (Protz et al., 1993; Tomlin et al., 1993), but have not focused on the potential of biosolids to prove organic matter to support earthworm populations. Historically, earthworms have been employed for toxicity tests of biosolids materials (Artuso et al., 2011; Moreira et al., 2008; Natal-da-Luz et al., 2009). Many of these studies utilized anecic or epigeic species, such as *Lumbricus terrestris* and *Eisenia andrei*, respectively. Anecic earthworms create permanent vertical burrows and move organic matter from the soil surface deeper into the soil profile, whereas epigeic species live on the surface and feed on organic residues. These earthworms may have a larger effect on nutrient availability due to the potential to redistribute surface-applied biosolids down into the burrows.

In contrast, *Aporrectodea caliginosa* appears to be the most common species in Colorado (Reynolds, 2011) and has the greatest potential for colonization of agricultural fields. *A. caliginosa* is an endogeic species that primarily creates temporary horizontal burrows and feeds on soil organic matter rather than surface organic matter. Because *A. caliginosa* feeds on soil organic matter, its expansion in agricultural soils of eastern Colorado may be constrained by low quantities of organic matter, relative to less arid regions of the world.

We conducted a 12-week laboratory incubation study to determine the potential of *A. caliginosa* to survive in a low organic-matter agricultural soil from Colorado. We hypothesized that an amendment of incorporated biosolids would enhance earthworm survival and prevent weight loss by increasing organic matter availability to the earthworms. We also investigated the effects of *A. caliginosa*, in the presence or absence of biosolids amendments, on plant available nitrogen, and other nutrients, and microbial biomass.

2. Methods and materials

The soil selected for this study was a sandy loam (56% sand, 30% silt, 14% clay; 1.4% organic C content, 7.46 pH) Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex that represents approximately 900k ha in the western United States (Soil Survey Staff, 2012). The soil was obtained from the top 10 cm of a dryland no-till wheat-corn-fallow research plot near Byers, Colorado (latitude 39.7631921, longitude 103.7973089). The soil was passed through a 1.0-cm sieve and homogenized using a cement mixer. The equivalent of 1 kg of oven-dried soil was added to plastic containers (13.5 cm × 11.0 cm × 9.5 cm), and packed to a bulk density of 1 Mg m^{-3} (1 g cm^{-3}). Prior to adding the soil, a total of 16 holes approximately 0.65 cm in diameter were made in the sides of the containers and covered with fiberglass screen to provide air flow and prevent earthworms from escaping. A plastic lid was then placed on each container.

Water was added to the containers of soil to adjust the gravimetric water content to approximately 14% gravimetric water content (approximately 70% of field capacity). The containers were then placed in an incubator at 17 °C for four days prior to the start of the incubation study to allow the microbial communities to adjust to the new environment. The water content was held near constant conditions throughout the study with the use of weekly watering.

Table 1
Chemical properties of anaerobically digested biosolids utilized in this laboratory incubation study.

Parameter (units)	Biosolids utilized	The National Sewage Sludge Survey[a]			
		Median	Mean	Minimum	Maximum
Solids (mg kg^{-1})	796,000				
pH	6.60				
EC (dS m^{-1})	15.7				
Organic N (mg kg^{-1})	24,400				
NH_4-N (mg kg^{-1})	13,300				
NO_3-N (mg kg^{-1})	1.51				
Ag (mg kg^{-1})	1.53	13.6	31.6	1.94	856
Al (mg kg^{-1})	75,000	11,200	13,600	1400	57,300
As (mg kg^{-1})	5.36	4.96	7.09	1.18	49.2
Ba (mg kg^{-1})	27.9	426	567	76.9	2120
Be (mg kg^{-1})	0.02	0.278	0.391	0.04	2.34
Cd (mg kg^{-1})	1.45	1.76	2.67	0.208	11.8
Cr (mg kg^{-1})	17.9	32.7	81.5	6.74	1160
Cu (mg kg^{-1})	708	463	558	115	1720
Fe (mg kg^{-1})	14,300	15,700	27,700	1580	195,000
Hg (mg kg^{-1})	1.15	0.827	1.24	0.19	7.5
K (mg kg^{-1})	1920				
Mn (mg kg^{-1})	279	420	1220	34.8	14,900
Mo (mg kg^{-1})	11.6	11.5	16.3	2.51	86.4
Ni (mg kg^{-1})	10.6	23.5	48.9	7.61	
P (mg kg^{-1})	23,500	19,300	22,400	5720	77,100
Pb (mg kg^{-1})	15.4	48.2	76.6	5.81	350
Se (mg kg^{-1})	6.48	6.25	7.12	1.1	24.2
Zn (mg kg^{-1})	665	784	994	216	8550

[a] Robert Brobst, USEPA, personal communication.

Adult *A. caliginosa* were used for this study, which were collected by hand sorting in September 2009 from the edge of an irrigated alfalfa field at Colorado State University (CSU) Agricultural Research Development and Education Center (ARDEC). Earthworms were taken back to the laboratory and placed in a large plastic container of soil from the ARDEC site. The worms were allowed to equilibrate in field moist, ARDEC soil for four days. After four days, two worms were placed in each of 80 petri dishes on wet filter paper and placed back in the incubator overnight for gut evacuation of ARDEC soil. *A. caliginosa* were rinsed with deionized water and blotted dry before an evacuated, fresh weight was obtained on each pair of earthworms. Two earthworms were added to each container, which approximated 400 earthworms m^{-2}. While this density was higher than what has been reported in one Colorado study (Hurisso et al., 2011), it is lower than the density used in another laboratory incubation study which examined the effects of earthworms on nitrogen mineralization (Willems et al., 1996). Moreover, Hurisso et al. (2011) reported that the dry mass of earthworms was 2.3–13 g m^{-2} in the field, which was near the dry mass in the containers, determined at the end of this study to be 16.2 g m^{-2}. We believe that the earthworm density in this study is not an unreasonable representation of what could occur in Colorado field soil.

A factorial design was used for this study with the main effects of biosolids application, earthworms, and time. At each sampling time, there were four replicates of each treatment: the control, with biosolids, with earthworms, and with earthworms and biosolids. It was assumed that the size of the worms would be related to the worms' activity; therefore, the experiment was blocked by the starting mass of the earthworms. There were five time points that were designated for destructive sampling (one, two, four, eight, and 12 weeks).

Dry, anaerobically digested biosolids were obtained from the Littleton/Englewood Wastewater Treatment Plant in Englewood, CO. Biosolids chemical properties are shown in Table 1. Biosolids were mixed with soil for each pot individually before filling the pots at rate equivalent to 11.2 Mg ha^{-1} (based on 20 cm soil depth).