THESIS

IMPLICATIONS FOR THE INTRODUCTION OF EARTHWORMS IN A BIOSOLIDS AMENDED AGROECOSYSTEM

Submitted by

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ABSTRACT

IMPLICATIONS FOR THE INTRODUCTION OF EARTHWORMS IN A BIOSOLIDS AMENDED AGROECOSYSTEM

The earthworms of Colorado are non-native lumbricid species that are widely distributed across the state, but they are generally absent in eastern Colorado dryland agroecosystems. Recently, interest has grown in using earthworms in no-till, dryland soils to promote nutrient availability to plants, alter soil structure and porosity to change solute flow patterns, change soil water retention, and enhance microbial activities related to nutrient cycling. However, very little is known about the ecology of earthworms and their influences on soil properties in Colorado. A series of laboratory studies were conducted to investigate the effects of *Aporrectodea caliginosa* in a soil from eastern Colorado, in the presence or absence of added organic matter (in the form of biosolids). The studies included a laboratory incubation study, a drought stress study, and a soil physical property study.

SURVIVAL OF *APORRECTODEA CALIGINOSA* AND ITS EFFECTS ON NUTRIENT AVAILABILITY IN BIOSOLIDS AMENDED OR UNAMENDED SOIL:

A 12-week laboratory incubation study was established to study the effects of a common endogeic earthworm (*Aporrectodea caliginosa*) on soil microbial biomass and soil nutrient availability. The study's design was a factorial design with the treatments of *A. caliginosa*, biosolids addition, soil sterilization, and time. The samples were destructively sampled at one, two, four, eight, and twelve weeks. The sterilization process had a large impact on the nutrient

content of the soil and the availability of metals; therefore, no statistical comparisons were made between the sterile and non-sterilized soil.

During the 12-week study, all of the worms in the non-sterile soil survived, and the survival of the earthworms was not significantly affected by the addition of biosolids. However, the addition of biosolids did significantly reduce the gain in mass of the earthworms. Presence of *A. caliginosa* significantly increased soil N mineralization and nitrification activities, but did not affect microbial biomass C. At the onset of increased nitrification activity (8 weeks into the incubation), we measured a corresponding reduction in soil pH, an increase in total soil C, and increased availability of several plant micronutrients. We concluded that it is possible for *A. caliginosa* to survive in a low-organic matter Colorado soil under optimal moisture content and that *A. caliginosa* can enhance inorganic N and perhaps micronutrient availability to plants through their interaction with the native microbial community.

In the sterilized soil, the observed effects of the earthworms were different from that of earthworm-free non-sterile soil. The addition of biosolids did not aid in the survival of the earthworms. The soil C and N were significantly affected by the earthworms. The soil nitrate-nitrogen (NO_3-N) and ammonium-nitrogen (NH_4-N) were also significantly affected by earthworms. There was a greater increase in the soil NH_4-N level with the earthworms due to earthworms releasing ammonium through their biological processes. The earthworms had a significant effect on many of the plant available micronutrients; however, the ability to determine the cause of the affects was complicated by the changes due to the sterilization process.

SURVIVABILITY OF *APORRECTODEA CALIGINOSA* IN RESPONSE TO DROUGHT STRESS:

The distribution of earthworms is limited in many areas in the semi-arid western United States by the availability of water. However, earthworms have been shown to adapt to periods of low soil moisture by making small chambers and entering diapause to protect the earthworm from the declining soil moisture. The objective of this part of the study was to investigate the effects of varying lengths of drought stress on the survival of earthworms in a low organic matter soil from eastern Colorado (Adena, Ustic Paleargid). The earthworms were exposed to constant water content or a one, two, or three-week, drought stress period. The pots were destructively sampled at 22, 43, and 64 days. At sampling, the earthworms were either classified as active, in diapause, or dead. The percent change in mass of the earthworms was also measured. The results of the study showed that there was a significant effect of drought period on the number of earthworms in diapause after two weeks of drought. The longer drought periods also significantly increased the mortality rate. A mortality rate of 14% was observed following cycles of three-week drought period, with all other periods having a negligible rate. The result of the study indicated that earthworms have the potential to survive up to three-week drought periods in Colorado soil.

EFFECTS OF *APORRECTODEA CALIGINOSA* ON SOIL HYDRAULIC PROPERTIES UNDER LABORATORY CONDITIONS:

Earthworms have historically been absent from agricultural fields in eastern Colorado. Recently there have been a growing number of earthworms seen in no-till fields, and as a result, there is a growing interest in the effect of earthworms on soil nutrient availability and physical properties. The objectives of this study was to determine the effect of *Aporrectodea caliginosa* on solute leaching, water retention, and soil texture on soil from eastern Colorado (Adena, Ustic Paleargid) that was amended with biosolids as a source of organic matter and plant-available nutrients. To help understand *A. caliginosa*'s effect, a laboratory column study was established to investigate the impacts of the earthworms on the solute breakthrough curves (BTC) of column sections with and without earthworms. After 16 weeks of worm interactions with the soil, the columns were divided into three depth increments (0-15 cm, 15-50 cm, and 30-45 cm). Saturated and unsaturated BTC were developed on each section using a non-absorbing dye. The BTC data was analyzed using Hydrus 1-D to determine the solute dispersivity of the dye for each column section. A transient model both with and without dual porosity (mobile and immobile water) was used for the development of the BTC.

The effects of the earthworms were only seen in the top and middle sections of the columns (0-30cm). This is the depth range where *A. caliginosa* was the most active. Under saturated flow conditions, earthworms resulted in an 80-fold increase in the dispersivity of the dye in the 0-30 cm depth increments. Under unsaturated conditions, the dispersivity in the 0-30 depth increments was approximately 10 times greater in the presence of earthworms than without earthworms. When the dual porosity portion of the model was added, there was still an increase in dye dispersivity of approximately 35 times with the addition of earthworms under saturated

flow conditions. Under unsaturated flow conditions, the earthworms had an effect but it was not limited to the top and middle sections. The addition of earthworms resulted in an increase in dispersivity in all the sections that had an average increase of approximately 4 times.

The water retention curves were also affected by *A. caliginosa* in the sections of the columns where the earthworms were the most active. The addition of earthworms altered the soil so that it drained at a lower tension, drained slower over a lager range of tensions, and have higher residual water content. These results could be explained by the creation of burrows and cast by earthworms. Burrows are large macropores, which would allow the soil to begin to drain sooner. Earthworm burrows and casts would also have increased the variety of the pore sizes in soil, resulting in the soil draining over a larger, range of tensions. The ability of the soil to slow the loss of water in the presence of *A. caliginosa* earthworms may help to increase crop production by due to greater water retention in the soil profile.

There was not an effect seen on the soil texture as a result of the addition of earthworms, it had been expected that there would be increased clay and silt due to earthworms concentrating clay and silt in their casts. This may have been due to the earthworms creating larger more stable soil aggregates that were not broken up during analysis resulting in the small changes not being seen.

The ability of earthworms to survive in the soil from eastern Colorado and change the availability of the nutrients and the flow and availability of water indicates that earthworms may become more important to the management decisions and practices in no-till dryland agricultural practices.

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CHAPTER 1: INTRODUCTION OF *APORRECTODEA CALIGINOSA* IN A DRY LAND NO TILL AGROECOSYSTEM

In much of the United States no native earthworms are present due to the last ice age (Smith, *et al.*, 1990). Several theories have developed on how earthworms have been able to repopulate much of North America. These include earthworms from the southern part of the United States moving northward following the recession of the glaciers (Gates, 1970, Delcourt & Delcourt, 1984), earthworms surviving on land that was not covered by ice (McKey-Fender & Fender, 1982, Smith, *et al.*, 1990), and human movement of earthworms (Gates, 1966, Lee & Foster, 1991, Tomlin & Fox, 2003, Reynolds, 2011). The movement of soil by humans would have a large impact since earthworms only expand their range by 5.5 - 7 m $year^{-1}$ (Marinissen & Vandenbosch, 1992). The rate of expansion depends on the soil conditions because *Aporrectodea caliginosa* will enter diapause, reducing their movement and also reproduction for that year (Marinissen & Vandenbosch, 1992). Many of the earthworms that are found in North America are the same species that are found in Europe, and one theory is that soil and rocks that were used as ballast in ships during the colonial period was then deposited on the shores in North America (Tomlin & Fox, 2003). The ballast would contain adult and juvenile earthworms and cocoons.

The distribution of earthworms can be separated into passive and active categories. Passive distribution of earthworms is when the earthworms are moved with soil or water movement (Marinissen & Vandenbosch, 1992). Active distribution is defined by the earthworms moving under their own power, usually in the search for food (Martin, 1982). There are a few species, including *A. caliginosa* that will also move by traveling on the soil surface (Bouche, 1976).

The presence and distribution of earthworms in Colorado has been studied for over 100 years and the interest in earthworms continues to grow. In 1942, Gates presented a review of earthworm surveys in Colorado that showed four species of earthworms (Gates, 1942). Twenty five years later Gates (1967) reported that additional species were present in Colorado and reported the total number of identified species as 12. Fender (1985) repeated the work of Gates and confirmed the species found and did not discover any new species. Reynolds (2011) conducted a comprehensive survey of the earthworms of Colorado with the help of local volunteers and reported a total of 17 earthworm species residing in Colorado. Reynolds (2011) reported that the most common species of earthworms found belonged to the *Lumbricidae* family. Within this family, there are three species: *Aporrectodea trapezoides*, *Aporrectodea tuberculata* , and *Aporrectodea trugida*, that appear to be the most widely distributed endogeic, soil feeding earthworms in Colorado. In Europe, these three species are combined into *Aporrectodea caliginosa* complex, and for this paper *A. caliginosa* will be used to reference these species due to the difficulty of species identification.

In Colorado *A. caliginosa* are found in 46 of the 63 counties (Reynolds, 2011) as shown in Figure 1.1. Many of the earthworms that were reported in the survey were found near bodies of water or in areas that receive large amount of water. The abundance of earthworms decreased with increasing distance from the water source and no earthworms were present 3 to 15 meters away (Reynolds, 2011). Earthworms are susceptible to injury or death as a result of soil becoming too dry as shown in the areas of North Dakota that average less than 40 cm year^{-1} of precipitation (Merrill, *et al.*, 1996).

The limitations for earthworm presence and expansion in Colorado may not be only the low moisture content of soil but also by the low level of organic matter in the soil. *A. caliginosa* feeds on the organic material in the soil and needs a source of high quality organic matter with high levels of nitrogen (Bostrom, 1988) to be able to survive and thrive. There have been studies that have investigated the addition of an organic amendment in an attempt to increase the suitability for earthworms. The first study conducted by Baker *et al.* (2002) investigated the effects of the addition of biosolids on the abundance of earthworms. They found that the addition of approximately 2.7 Mg ha^{-1} of biosolids increased the abundance of earthworms. The concentrations of copper (Cu), zinc (Zn), nickel (Ni), and cadmium (Cd) residing in the biosolids could have a potential negative impact on the earthworms, but Baker *et al.* (2002) did not observe an effect of the biosolids on earthworm survival. The second study was conducted in Colorado where raw and composted dairy manure were applied to pasture and bare soil (Hurisso, *et al.*, 2011). This study found that the addition of manure increased the number of earthworms over the control. They found that the earthworms might be sensitive to the increase in soil salinity from the addition of raw manure since they observed a larger increase in earthworm numbers with the composted manure. The other proposed explanation given for greater increase in earthworms with composted manure was that earthworms might prefer the compost due to higher quality organic matter (low carbon/nitrogen ratio).

In addition to applications of biosolids or compost to increase soil organic matter the use of tillage practices that leave residue on the soil surface also increases soil organic matter. Many locations around the world have seen a transition from conventional tillage (CT) to reduced tillage and no-tillage (NT) over the last several decades and the semi-arid western United States has largely embraced the practice. The use of limited tillage and NT practices helps to reduce the negative impacts of CT on erosion (Shipitalo & Butt, 1999), and the loss of organic matter (Juo & Lal, 1979, Hargrove, *et al.*, 1982, Blevins, *et al.*, 1983, Stinner, *et al.*, 1983), and moisture (House & All, 1981). The changes in the soil properties that benefit crop production are changes that improve the soil suitability for earthworms. Recently, agricultural producers in Colorado have seen earthworms in fields that are NT. The implementation of NT has been shown in several studies to increase the earthworm populations in agricultural fields (Edwards & Lofty, 1982, House & Parmelee, 1985, VandenBygaart, *et al.*, 1999).

However, there have also been several studies (Edwards & Lofty, 1982, Rosas-Medina, *et al.*, 2010) that reported conventional tillage practices were beneficial to some earthworm species, especially *A. caliginosa*. The reasons for this increase may be due to the incorporation of organic matter deeper into the soil profile and the reduction of competition between different species of earthworms (De Oliveira, *et al.*, 2012). The effect of tillage has shown negative impacts on the survival of anecic species (De Oliveira, *et al.*, 2012) since these species create large permanent burrows that reach the soil surface. The tillage operation would destroy the burrow and reduce the ability of the earthworms to survive.

In Colorado, many of the soils have a low organic carbon concentration and the use of NT has a large impact on the organic matter content of the soil. The addition of biosolids to the soil as a nutrient source will add organic matter to the soil which may help increase the survival of earthworms. In CT the use of tillage implements helps to distribute the organic matter that is on the soil surface deeper into the profile (Edwards & Lofty, 1982). Edwards and Lofty (1982) found that the addition of earthworms were able to increase the distribution of organic matter within the profile. The use of the combination of NT and the establishment of earthworm populations may produce a similar result as CT on the distribution of organic matter over time.

The ability of earthworms to mix the soil and to distribute organic matter throughout the depths of their burrows, typically the top 25 cm for endogeic species (Simonsen, *et al.*, 2010), has also increased the favorability to have earthworms in production agricultural fields.

During the transition from CT to NT there can be negative effects on the production of crops due to changes in soil properties. The porosity of soil in NT is about half of CT (Shipitalo & Protz, 1987) but the porosity can be greater in NT if earthworms are present (VandenBygaart, *et al.*, 1999). The ability of earthworms to improve soil porosity (De Oliveira, *et al.*, 2012), along with other soil physical properties such as infiltration (Bouma, *et al.*, 1982) and aggregate stability (Dutt, 1948) has increased the interest in management practices that will help to increase the earthworm populations in Colorado agricultural fields.

The ability to manage earthworm populations to improve vegetative production and soil quality became an interest after World War II (Barrett, 1949). Edwards (1992) introduced earthworms into a field and observed an improvement in soil structure, a reduction in compaction, a reduction of the surface organic matter layer, and an increase in productivity. Activity of earthworms also increased the cycling of nutrients in soil (Eriksen-Hamel & Whalen, 2007), which plays a large role in the increase in soil productivity. Earthworms also benefit crop productivity when plant roots grow into burrows of earthworms (Pitkanen & Nuutinen, 1997). Burrows created by earthworms have a higher concentration of available nutrients than the bulk soil (Edwards & Lofty, 1980) creating a move favorable environment for root growth and increased fertility for the plants. Pitkanen and Nuutinen (1997) showed that *A. caliginosa* creates the majority of its burrows in the upper 20 cm of the soil profile and that 60% of the burrows in the top 15 cm of soil had roots growing in them. The exact depth that earthworms will be found depends on the soil temperature and water content (Baker, *et al.*, 1992).

Changes in management practices to include NT appears to improve the environment for earthworms by increasing soil water content, keeping burrows intact and reducing death of earthworms by the damage from tillage operation or by being brought to the soil surface and eaten by birds (Tomlin & Miller, 1988). Reduced tillage also results in higher organic matter and cooler soil temperatures, which are preferred by earthworms (Baker, *et al.*, 1992, Shipitalo & Butt, 1999).

Changes in land management practices in Colorado that may lead to an increase in the population of earthworms, especially *A. caliginosa,* have raised questions about the impact of earthworms in Colorado agricultural production. The ability for earthworm populations to be aided by the addition of manure or biosolids and the question of the effect of biosolids on earthworms in Colorado were investigated in this study. I hypothesized that because biosolids are a source of organic matter and nutrients, biosolids amendment would improve the survivability of *A. caliginosa* in a low organic matter Colorado soil. I also hypothesized that because of alteration in soil structure and porosity, *A. caliginosa* would increase the dispersivity of a solute under saturated condition, and reduce the tension need for the soil water to begin to drain because of the addition of macropores. My specific predictions/expected findings for the project were:

1.) *A. caliginosa* can survive in the soil from eastern Colorado.

2.) The addition of biosolids will increase the survival of *A. caliginosa* due to the addition of organic matter to the soil, providing another food source for the earthworms.

3.) The introduction of *A. caliginosa* will increase the availability of nitrate-nitrogen and ammonium-nitrogen and will decrease the soil organic carbon.

4.) The addition of *A. caliginosa* will not affect other plant available nutrients.

5.) The effects of *A. caliginosa* will be greater in the soil with reduced microbial populations.

6.) The survival of *A. caliginosa* would be unaffected by drought periods two weeks or less in length but any longer time period would impact the survival of the earthworms.

7.) When soil is saturated, the dispersivity of the solute will increase with the addition of earthworms. The effects will be limited to the upper two depths where the *A. caliginosa* will be the most active.

8.) When the water content of the soil is less than saturation, the effects of the *A. caliginosa* will not be seen due to the larger pores created by the earthworms not being filled with water.

9.) The *A. caliginosa* will change the pore size distribution and therefore affect the rate that water drains from the soil, and the earthworms will increase the residual water content.

10.) *A. caliginosa* affect the soil texture by accumulating clay and silt in the depths that they are the most active.

To address these hypotheses and predictions, a series of laboratory studies were conducted to investigate each of the effects under controlled conditions and in soils that earthworms are currently not found. Each of the studies had its own objectives, but each was designed to provide information towards addressing the overarching goals about the influence of earthworms in Colorado agricultural fields. The first experiment was a laboratory incubation study with the objective of investigating the effects of *A. caliginosa* and biosolids on nutrient availability in a low organic matter soil from eastern Colorado. This study was conducted under ideal conditions to determine biosolids effects on earthworm survival and subsequent effects of earthworms on nutrient availability when the earthworms were not under stress. Following the completion of the incubation study, a second experiment was designed to investigate the effects of varying lengths of drought stress on the survival of *A. caliginosa* in the same soil. The intent of this study was to determine how long earthworms could survive and be able to continue having an effect. The final portion of the study was to determine the effect of *A. caliginosa* on soil physical properties.

The design and a discussion of the results of the above studies are reported the following chapters of this thesis. Following the results of the three studies, a final chapter will discuss the interactions between the different studies and the implication of the research as a whole on the potential effects of earthworms in eastern Colorado.

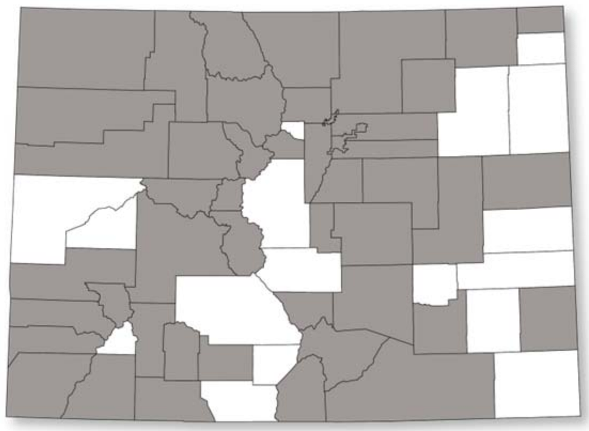

Figure 1.1. The distribution of *Aporrectodea caliginosa* in the state of Colorado, as indicated by the shaded counties (modified from Reynolds (2011)).

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CHAPTER 2: SURVIVAL OF *APORRECTODEA CALIGINOSA* AND ITS EFFECTS ON NUTRIENT AVAILABILITY IN BIOSOLIDS AMENDED OR UNAMENDED SOIL

Introduction:

The study of the effect of earthworms on soil was first documented by Darwin (1886). Farmers have believed for many years that the presence of earthworms in their fields was an indication of good soil quality (Roming, *et al.*, 1996). Over the years many scientists have also began to believe the same following research into the farmers' hypothesis (Doran & Safley, 1997, Yeates, *et al.*, 1998). One of the main effects earthworms have on soil quality is the ability to change nutrient availability. Earthworms primarily affect the availability of nitrogen (N) in the soil, but they can also affect the availability of other nutrients. As earthworms enter agricultural fields in Colorado, it will become important to understand the effect of earthworms on soil fertility to be able to make the correct management decisions.

Earthworms can have a large effect on the concentration of plant available N through the mineralization of soil organic matter (Edwards & Lofty, 1977). This process may be more important when the fertility of the soil is dependent on the mineralization of organic matter (Lubbers, *et al.*, 2011). When organic nutrient sources such as manure or biosolids are used for fertilization, the majority of the N present is in an organic form and not immediately available to the plants. The organic amendment acts as a slow release fertilizer source due to the reliance on mineralization by microorganism. Earthworms aid not only by direct mineralization of organic matter but also by stimulating microbial activity in soil (Curry & Schmidt, 2007). Earthworms primarily affect N availability by increasing the concentration of ammonium-nitrogen (NH_4-N) in the soil due to their digestion and the release of mucus (Whalen, *et al.*, 2000).

Once NH_4-N becomes available in the soil it can be taken up by plants, transformed back to an organic form, or transformed to nitrate-nitrogen (NO_3-N). The fate of NH_4-N is determined by in large part the environmental conditions of the soil that affect biological activity. If a plant is currently growing, the NH_4-N may be taken up by the plant. In soil that contains a high carbon/nitrogen ratio (C/N) immobilization by microorganisms will take place and reduce the amount of available NH_4-N. The NH_4-N could also be transformed into NO_3-N by nitrifying bacteria. The transformation of NH_4-N to NO_3-N is an oxidation process and the burrowing activities of earthworms creates conditions that are favorable to nitrification (Parkin & Berry, 1999) by increasing the oxygen concentration deeper in the soil profile (Costello & Lamberti, 2008).

The use of biosolids as a source of N fertilizer has been regulated in Colorado since 1973 (Colorado Department of Public Health, 2010). The application of biosolids to agricultural land is regulated and requires the application rate to be based on the N need for the crop being produced (Colorado Department of Public Health, 1993). Since biosolids contain very little inorganic N, the application rate is based on the expected mineralization rate (Barbarick & Ippolito, 2000). Due to earthworms' ability to increase the mineralization of organic matter, it may be necessary to adjust the expected mineralization rate if earthworms are present. The potential for a change in the management practices would only matter if earthworms are present; therefore it is necessary to investigate if the application of biosolids will affect the survival of earthworms.

Biosolids have been studied extensively as a soil amendment (for a review, see (Haynes, *et al.*, 2009), and Colorado, a main focus area of research has been to study the accumulation of metals in soil with repeated biosolids application (Ippolito & Barbarick, 2008). Studies have been conducted to investigate the interaction between the application of biosolids and earthworm effects on metal availability. It was discovered that walls of earthworm burrows and cast material have increased soluble metal concentration when compared to the bulk soil (Protz, *et al.*, 1993, Tomlin, *et al.*, 1993). However both of these studies focused on the effects of *Lumbricus terrestris* which is an aneic earthworms species. Anecic earthworms create permanent vertical burrows and move organic matter from the soil surface deeper into the soil profile. These earthworms may have a larger effect on nutrient availability due to the potential to redistribute surface-applied biosolids down into the burrows. The species of interest in this study was *Aporrectodea caliginosa* which appears to be the most common species in Colorado (Reynolds, 2011) and also has the greatest potential for colonization of agricultural fields. *A. caliginosa* is an endogeic species and has a different burrowing and feeding strategy then *L. terrestris* so the effect on metal availability may not be the same. Endogeic earthworms create temporary horizontal burrows, feed on the soil organic matter, and do not feed on surface organic matter. It has been shown that earthworms in the *Aporrectodea* genus can increase the availability of metals in soil (Stephens, *et al.*, 1994, Lukkari, *et al.*, 2006), but it has also been shown that earthworms will decrease metal availability (Zorn, *et al.*, 2005, Lukkari, *et al.*, 2006) in soil without biosolids. The differences in effect tend to be related to the initial conditions and the metals that were being studied.

Currently there are few earthworms present in agricultural production fields in Colorado but the transition to no-tillage (NT) practices is improving the favorability of conditions for earthworms (Edwards & Lofty, 1982, House & Parmelee, 1985, VandenBygaart, *et al.*, 1999). One of the reasons for the limitations may be due to the low organic matter in the soil where many earthworms feed (Bostrom, 1988). After the conversion to NT the amount of soil organic matter will also increase over time, however, it takes time for the soil organic matter to build up. 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 (Baker, *et al.*, 2002, Hurisso, *et al.*, 2011).

The objective of this study was to investigate the effects of *A. caliginosa* and biosolids on nutrient availability in a low organic matter soil from eastern Colorado, under laboratory conditions. The study addresses the following questions:

1.) Can *A. caliginosa* survive under ideal environmental conditions in a soil from eastern Colorado?

2.) Does the addition of biosolids affect the ability of *A. caliginosa* to survive?

3.) What is the effect of *A. caliginosa* on soil nitrogen and carbon?

4.) What is the effect of *A. caliginosa* on the availability of plant available micronutrients?

5.) Are the same effects seen in a soil with a reduced microbial population?

The study hypothesis was that because biosolids are a source of organic matter and nutrients, biosolids amendment would improve the survivability of *A. caliginosa* in a low organic matter Colorado soil. The specific predictions and expectations for this study were:

1.) *A. caliginosa* can survive in the soil from eastern Colorado.

2.) The addition of biosolids will increase the survival of *A. caliginosa* due to the addition of organic matter to the soil, providing another food source for the earthworms.

3.) The introduction of *A. caliginosa* will increase the availability of NO_3-N and NH_4-N and will decrease the soil organic C.

4.) The addition of *A. caliginosa* will not affect other plant available nutrients.

5.) The effects of *A. caliginosa* will be greater in the soil with reduced microbial populations.

Methods and Materials:

The sandy loam (56% sand, 30% silt, 14% clay; Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex (representing approximately 896197 ha in the western United States (Soil Survey Staff)) soil utilized in the study was obtained from the top 10 cm of a dryland NT 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 1000.0 g of oven dry soil was added to plastic containers (13.5 cm × 11.0 cm × 9.5 cm), and packed to a bulk density of 1.0 Mg m^{-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 the worms from escaping. A plastic lid was then placed on each container.

In order to determine the direct contributions of earthworms, as well as the contribution of biosolids-born microbes, to N mineralization, a sterile soil was included. For sterilization, 72 of the filled containers were exposed to a minimum of 21.2 kGy of gamma radiation by Synergy Health (Denver, CO). The sterilization treatment did not result in complete sterilization of soil microorganisms (discussed below), so this treatment is referred to as the “reduced microbial population” treatment.

Water was added to the containers of soil to adjust the gravimetric water content to approximately 0.14 gravimetric water content (approximately 70% of field capacity). The containers were then placed in an incubator at 17^{o} 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. Gamma-irradiated soils were moistened with sterile distilled water, whereas the rest of the soils were moistened with non-sterile distilled water. The sterile distilled water was sterilized by autoclaving.

Adult *A. caliginosa* were used for this study, 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 Byers research site. The worms were allowed to equilibrate in the new environment 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.

A factorial design was used for this study with the main effects of: biosolids application, earthworms, time, and reduced microbial population. There were four replicates of each treatment. It was believed 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 (1, 2, 4, 8, and 12 weeks).

The biosolids used in the experiment were obtained from the Littleton/Englewood Wastewater Treatment Plant. The biosolids were aerobically digested and then dried on a sand bed before they were applied. The biosolids were mixed with soil for each pot individually before filling the pots at rate equivalent to 11.2 Mg ha^{-1} and incorporated to a depth equivalent to 20 cm.

Soil containers were destructively sampled at the end of each time period and individually homogenized in a large beaker. Two soil samples from each container were taken and either air-dried (for chemical analyses) or refrigerated at 4°C (for NH_4-N and NO_3-N and microbial biomass determinations). If the container contained earthworms, the earthworms were removed before the soil samples were collected and placed in petri dishes with wet filter paper. The worms were placed in the incubator overnight and an evacuated, and fresh weight was obtained for each pair of worms.

Soil NO_3-N and NH_4-N samples were extracted two days after sampling, following the Mulvaney (1996) procedure, on soil that had been stored in the refrigerator. The equivalent of 10.0 g of oven dry soil was used. Fifty mL of 2.0 M KCl was added to each sample, the flask was capped with a rubber stopper and shaken on a reciprocating shaker for one hour. The solution was then filtered using Whatman #1 filter paper that had been previously rinsed with 20 mL of 2.0 M potassium chloride (KCl) to remove any NO_3-N and NH_4-N that may be on the filter paper. The filtrate of the soil solution was collected in 20 mL scintillation vials. The samples were then frozen until analysis of NO_3-N and NH_4-N on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station, Texas).

The microbial biomass was analyzed using a chloroform fumigation extraction method (Brookes, *et al.*, 1985, Vance, *et al.*, 1987) two days after destructive sampling on the soil that had been stored in the refrigerator. Ten g of oven dry equivalent soil from each sample were placed in a 50 mL beaker for fumigation and a second 10.0 g equivalent sample was placed into a 250 mL Erlenmeyer flask (non-fumigated control). Fifty mL of 0.5 M potassium sulfate (K_2SO_4) was added to each non-fumigated sample, which was then shaken for one hour on a reciprocating shaker, uncovered. The samples were then filtered through Whatman #1 filter paper into 20 mL scintillation vials. The vials were immediately placed in the freezer until analysis. Meanwhile, the 50 mL beakers were placed into a vacuum desiccator with a beaker containing approximately 50 mL of HPLC grade, ethanol-free chloroform ($CHCl_3$) and several boiling chips to facilitate a more even boil. A vacuum was placed on the desiccators to allow the $CHCl_3$ to boil for two minutes. The vacuum was released, and this process was repeated for three, two-minute boils. On the third boil, the vacuum was maintained and the samples were fumigated under the $CHCl_3$ vapor. After six days, the vacuum was released, and the $CHCl_3$ that remained in the beaker was removed. A vacuum was then pulled four times for two minutes, with the vacuum being released each time to remove the $CHCl_3$ from the soil samples. The soil was then quantitatively transferred into 250 mL Erlenmeyer flask using the 50.0 mL of K_2SO_4 that was then used to extract microbial biomass C and N. The extraction followed that same procedure used with the non-fumigated samples. Prior to analysis on a total organic carbon analyzer (Shimadzu Total Organic Carbon Anayzer (model TOC-Vcpn+TNM-1, Columbia Maryland)), a 10-fold dilution was performed on the samples.

Plant available nutrients were determined using an AB-DTPA extraction (Barbarick & Workman, 1987). Ten g of air-dried soil that had been passed through a 2 mm sieve was placed in 250 mL Erlenmeyer flask. The extraction solution was 0.005 M DTPA made in 1.0M NH_4HCO_3. Twenty mL of the extraction solution was added to each flask. The flasks were then shaken, uncovered, on a reciprocating shaker, for one hour. The samples were filtered through Whatman #1 filter paper into glass scintillation vials and refrigerated until analyzes using inductively coupled plasma-atomic emission spectrometry (ICP-AES) (IRISADVANTAGE Radial ICP, TJA Solutions, West Palm Beach, Florida).

Soil pH (Thomas, 1996) and electrical conductivity (EC) (Rhoades, 1996) analyses were performed on air-dried soil, which was previously screened using a 2-mm sieve, using the saturated paste method (Rhoades, 1996). The saturated paste was allowed to equilibrate overnight before analysis. Soil pH was determined with a pH probe (Model 420A Orion, Boston, Massachusetts). The water was then separated from the soil using a Buchner funnel, Fisher Brand #5 filter paper, and vacuum filtration. The filtrate was then analyzed for EC using a handheld EC meter (Waterproof ECTestr High+, Oakton Instruments, Vernon Hills, Illinois). Total soil C and N was determined by sample combustion using a LECO CHN-100 Autoanalyser® (St. Joseph, Michigan). Soil that had been passed through a 2-mm sieve was then fine ground using a roller table. Approximately 0.20 g of soil was placed in tin foil and then analyzed. Inorganic C was analyzed using the pressure transducer method (Sherrod, *et al.*, 2002). One g of fine ground soil was placed in a 20 mL Wheaton serum vial. A glass auto-sampler vial filled with 2.0 mL of 6.0 M HCl with $FeCl_2$. The vial was then capped with a butyl rubber stopper and crimped with an aluminum tear-cap seal. After capping the vials were vigorously shaken to ensure the $CaCO_3$ in the soil reacted completely with HCl. The samples sat for a minimum of two hours to ensure the reaction had completed before being analyzed using a pressure transducer.

Data analysis was performed with SAS version 9.2 (SAS Institute, 2008) using Proc Glimmix. An alpha value of 0.1 was used for determining statistical significance. The data was checked for normality and homogeneity of variance and when necessary a long transformation was used. The data presented in the results are the non-transformed data.

Samples where two earthworms were not located at the end of the study were not included in the statistical analysis. These samples were put in the data set as a missing data point. Since the effect of the earthworms would not be accurately represented in these samples as the exact time of the earthworm loss was unknown. In addition, there would be less interaction of the earthworms with the soil and biosolids, resulting in a bias in the data.

In the reduced microbial population, by the end of the study there were no biosolids-treated samples that still contained both earthworms. Therefore, the 12 weeks data for the reduced microbial population soil was not included in the data to avoid a bias.

Gamma irradiation of soil resulted in significant changes to the availability of trace elements in the soil (Table 2.1). The sterilization process killed microorganisms. However, this also resulted in many of the metals being reduced due to the addition of free electrons, changing their availability. Due to the changes that took place with the sterilization of the soil, the data were analyzed separately from the control soil. Comparisons were made between the effects of reduced microbial population and the control soil based on trends only.

The results that are discussed below are only the results where the earthworms had a significant effect. All other statistically significant results (due to biosolids or sampling event) can be found in Appendix A.

Results and Discussion:

Non-Sterilized Soil:

The soil that was used for this study has low organic matter content (1.35%), and the earthworms that were used feed on soil organic matter. In order to determine if there was enough organic matter for the earthworms to survive, the change in mass was studied, as well as the survival of the earthworms. The change in the mass of *A. caliginosa* on a percent of starting mass basis was affected by the addition of biosolids (P=0.038). Both with and without the addition of biosolids, the mass of the earthworms increased over incubation time but there was a larger increase without biosolids (17.7%) than with biosolids (7.65%). There was not a significant time effect (P=0.185) indicating biosolids do not have an increasing negative effect on earthworms over time.

The gain in mass of the earthworms was about double without the addition of biosolids to the soil. It has been shown in other studies that the addition of biosolids is beneficial to earthworm populations (Baker, *et al.*, 2002). The results seen in their study indicate that biosolids amendment added significant organic matter to their soil, which aided in the survival of earthworms. Baker *et al.* (2002) showed that the benefits of biosolids was related to the application rate and that at higher rates there is no longer a benefit. The application rate that was used in our study was 11 Mg ha^{-1}, which is higher than the typical application rate for the production of many crops.

The addition of biosolids caused a significant time by biosolids interaction (P=<0.001) on the soil EC that resulted in an increase from 0.59 dS m^{-1} at that start of the study to 2.74 dS m^{-1} at week 12 with biosolids. This increase of approximately 2 dS m^{-1} may have resulted in stress on the earthworms. A soil EC of 1.58 dS m^{-1} has been shown to have a negative effect on earthworms mass if they are exposed to the soil for more than 30 days (Jun, *et al.*, 2012). The application of the biosolids used in this study to dryland field plots has not shown any increase in the soil EC (unpublished data). In the incubation study the allow the soil to be free draining. Consequently, the salts were unable to move deeper into the profile, thus allowing for an increase in the EC of the soil. In a field setting, it would not be expected that the addition of biosolids would result in an increase in the EC, and therefore there may not be a negative effect on the earthworms.

The application rate that was used is the typical rate of application for the production of irrigated corn and was used because one of the objectives was to see if the addition of biosolids would aid in the survival of earthworms due to the low soil organic matter content of the soil. The belief was that a higher application rate might be needed to improve the low organic matter. Barbarick and Ippolito (2000) have recommend a rate of 4.4 Mg ha^{-1} of dry biosolids for the production of winter wheat in Colorado.

One of the reasons it was believed that earthworms were not present in eastern Colorado was due to the low organic matter of the soil not providing the high quality organic matter that *A. caliginosa* uses as it food source (Bostrom, 1988). The results showed that the when the soil is kept at an optimal temperature and water content the earthworms are able to survive and gain mass.

A log transformation was performed on the NO_3-N and NH_4-N data to correct for heterogeneity of variance. The addition of the earthworms influenced both NO_3-N (P=0.082) and NH_4-N (P=0.084). The NO_3-N levels increased 4% with the addition of earthworms to 53.7 mg kg^{-1} NO_3-N compared to 51.3 mg kg^{-1} NO_3-N without earthworms. The NH_4-N levels had an increase 24% with earthworm of 2.51 mg kg^{-1} NH_4-N compared to 1.91 mg kg^{-1} NH_4-N without earthworms.

The earthworms produce a mucous coat to reduce the loss of moisture, facilitate respiration, and to act as a lubricant as the earthworm moves through the soil (Whalen, *et al.*, 2000). The mucus contains mucoproteins and it is high in N, accounting for approximately one-half of the N loss from earthworms each day (Needham, 1957). The remainder of the N loss from earthworms is from urine, which contains primarily NH_4 and urea (Edwards & Bohlen, 1996) and from casts that contains most of the NH_4 that is released from earthworms (Tillinghast, 1967). The increase in the NH_4-N concentration that was seen is in large part a result of the direct release of NH_4 by the earthworms.

There was also an interaction between sampling time and biosolids, which resulted in an increase in the NH_4-N concentration (Figure 2.1). In the absence of biosolids (average over the presence or absence of earthworms), there was a slight increase in the NH_4-N concentration. This would be related to the effects of earthworms also due to nitrification of organic matter present in the soil by microorganisms. When biosolids were present, there was a large increase from time 0 to 1 week. This increase may be due to initially a large amount of activity by the microbial populations in the soil immediately after the addition of biosolids that then slowed when the most available food sources were depleted. The application of biosolids also added NH_4-N due to it being present in the biosolids (Table 2.2).

All of the forms of N that are released by earthworms are either organic or NH_4 based and the majority of the N is in an NH_4 based form. When the soil was sampled, there was an increase in the concentration of NO_3-N, indicating that nitrification was taking place. The increase in NO_3-N was largely controlled by an interaction between time and biosolids application (Figure 2.2). Biosolids contain a large amount of organic N and requires the ability of microorganism to perform mineralization and nitrification. The effect of earthworms on the increase in NO_3-N was significant (P=0.0817). One way that earthworms would aid in nitrification is by supplying NH_4. Earthworms have been shown to influence the microbial populations that are responsible for nitrification (Blair, 1995).

During the incubation period, the concentration of NO_3-N in soil without biosolids increased threefold, but with the addition of biosolids, there was a six-fold increase. The factorial design of the experiment should have allowed for separation of earthworm and biosolids effects but due to the magnitude of the biosolids, earthworm effects were overshadowed. The results indicate that when decisions are being made on management of lands, the addition of organic fertilizer has a larger effect on NO_3-N than the presence or absence of earthworms.

The earthworms did not have a significant impact on the microbial biomass C (P=0.963), but there was a time by earthworm impact on the microbial biomass N (P=0.099) (Figure 2.3). At week 1, there was increase in the microbial biomass C and N. This effect may have been due to the initial conditions of the soil at the start of the experiment. When the soil was collected, it had low moisture content, and it also had been subjected to higher temperature since it was collected at the end of the summer. The transition to a cooler, moister environment would be more favorable for microbial activity. At week 1, the addition of *A. caliginosa* resulted in a higher concentration of microbial biomass N than without earthworms. The earthworms did not have an effect again until week 12, where there was a decrease in microbial biomass N.

In this study *A. caliginosa* did not have an effect on pH, EC, or many of the plant available micronutrients of interests. The soil pH and EC both had a time by biosolids interaction, but these data will not be presented (see Appendix A for details). The only plant available nutrient affected by the addition of earthworms was chromium (Cr) with a time by earthworm interaction (P=0.051) (Figure 2.4). There was an increase in Cr at week 2 and week 4 that was greater without earthworms than with earthworms. By week 8 the concentration had returned to a level that was same as week 0 and 1. The results may reflect changes in the redox of the soil overtime as Cr availability is susceptible to redox conditions (Essington, 2004).

Reduced Microbial Population Soil:

The survival of *A. caliginosa* was not affected by biosolids or incubation time in the gamma-irradiated soil. Gamma-irradiation, however, appeared to negatively affect earthworms because it was observed that when first added to sterilized soil, earthworms avoided burrowing into the soil and attempted to escape the containers. This avoidance may have been due to changes in the soil properties such as the availability of micronutrients and metals. The earthworms may have been able to detect the reduction of microbial communities and therefore a lower concentration of food. When the change in mass on a percent basis was compared, biosolids addition (P=0.857) or time (P=0.376) did not have an effect. When the mass of the earthworms was averaged over all weeks and treatments there was an average increase in mass of 10.5%.

The main effect of earthworm addition, as well as its interaction with biosolids, time, and biosolids × time, were significant for many of the soil characteristics of interest. Below are discussed the earthworm's effects on nutrient availability and the concentrations of soil C and N.

Over the length of the study there was a time by biosolids interaction (P=0.079) on the total percent of C present in the soil (Figure 2.5). There was not a significant change in the total C without the addition of biosolids, nor the addition of earthworms. The addition of earthworms resulted in a significant (P=0.004) increase in total soil C from 1.77% without earthworms to 1.85%. This increase indicates that there was an addition of C to the system with the addition of earthworms but the reason for the cause is unknown.

There was a three-way interaction between biosolids, earthworms, and time for inorganic C (P=0.009). The reduced microbial soil alone remained constant over the course of the study except for a small increase at week 1. All of the other treatments increased the inorganic C during week 1 and week 2, before returning to the level without biosolids or *A. caliginosa*. The increase followed the trend for increased microbial biomass C at week 1. The formation of $CaCO_3$ in the soil is a result of microbial and plant respiration. In the treatments with biosolids, or with earthworms, there was increased respiration taking place in the soil. After week 1, there was a reduction in microbial biomass carbon, but there was a lag period before the reduction in inorganic carbon.

An interaction between biosolids and earthworms resulted in a significant (P=0.002) impact on soil NO_3-N concentrations (Figure 2.6; after a log transformation to correct for heterogeneity of variance). The application of biosolids increased the concentration of soil NO_3-N, but the addition of earthworms did not cause a significant difference. However, the addition of earthworms in the absence of biosolids resulted in a significant increase in the concentration of NO_3-N. The initial concentration of NO_3-N in the reduced microbial soil was lower than the non-sterilized soil. The addition of biosolids also resulted in an increasing concentration of NO_3-N with time, to a doubling of the NO_3-N concentration by week 8 (Figure 2.7). The biosolids that were added to the soil contained very little NO_3-N (Table 2.2), therefore the increase would be due to mineralization and then nitrification taking place. The biosolids that were added were not sterilized before their addition to the containers, and the results indicate the biosolids contain microorganisms that facilitate mineralization. The introduction of nitrifying microorganisms by the biosolids is also indicated by the NO_3-N concentration remaining constant in the soil without biosolids. The increase in NO_3-N with the application of biosolids could have also been affected by the biosolids adding approximately 13.3 g NH_4-N kg^{-1}.

When earthworms were added to the soil, an effect on NO_3-N was seen in the absence biosolids. Blair (1995) indicated that earthworms aid in the formation of populations of microorganism that mineralize N in the soil. When biosolids were not applied, earthworms resulted in an increase of NO_3-N by about 3 mg kg^{-1}. The earthworms would produce NH_4 (Needham, 1957) that would have to be transformed to NO_3 by nitrifying bacteria.

The microbial biomass C was affected by an interaction between the addition of biosolids and earthworms (P=0.063) (Figure 2.8). The amount of microbial biomass C only changed with the addition of biosolids and earthworms. Both of these additions added an amendment to the soil that was not sterilized and the combination of the two had a large effect. This treatment resulted in the microbial biomass C reaching a level that was about the same as the non-sterile soil. In addition, the digestion of the soil the earthworms also added microorganisms to the soil (originating from earthworm intestines) and the addition of earthworms and biosolids together had a larger impact than either individual effect.

There was also a significant three-way interaction among time, biosolids, and earthworms (P=0.004) for the concentration of NH_4-N (Figure 2.9). A log transformation was required to correct for heterogeneity of variance. The addition of biosolids resulted in a large increase in the NH_4-N level and there was not a significantly greater effect seen by the addition of *A. caliginosa*. The increase was due to the NH_4-N that was added from the biosolids (Table 2.2). The addition of earthworms to the soil without biosolids increased the NH_4-N level about as much as the biosolids. The increase in NH_4-N concentration by the earthworms is due to the release of NH_4 compounds during digestion and also the excretion of mucus (Scheu, 1991). In the non-sterile soil there was a much smaller increase seen in the concentration of NH_4-N due to the presence of microorganisms to transform the NH_4-N to NO_3-N. At the latter sampling points, especially with biosolids present, an ammonia gas odor was detected in some samples. In the absence of biosolids, the earthworms produced approximately 20 mg kg^{-1} more NH_4-N. Since this portion of the study was conducted with reduced microbial populations, it gives a more accurate representation of the amount of NH_4-N that is produced directly by earthworms under ideal moisture and temperature conditions in a soil from eastern Colorado.

The nitrification taking place also resulted in a reduction in the soil pH. The interaction between biosolids and earthworms was significant (P=0.063) and showed an inverse effect to changes in NO_3-N concentration (Figure 2.10). When NH_4-N is transformed to NO_3-N, hydrogen ions are released, causing the pH of the soil to be reduced. The reduction in the pH of the soil may increase the availability of some micronutrients.

The EC of the soil was also affected by an interaction between the addition of biosolids and earthworms (P=0.035) (Figure 2.11). Electrical conductivity increased in the biosolids treatment without earthworm treatment as well as the earthworm without biosolids treatment. The biosolids applied contain soluble salts and NH_4, and earthworms release ammonium, which will increase the EC due to the formation of soluble salts. However, the increase in the EC of the soil was relatively small and should not lead to impacts on plant productivity due to osmotic effects. The EC with the addition of biosolids resulted in the EC being elevated from approximately 0.75 to 1.3 dS m^{-1}. This value is still below the EC level where a soil is considered to have a salinity problem.

The addition of earthworms also affected the availability of some plant available nutrients in the soil with reduced microbial populations. However, the effects that were seen were complicated by the effects of the sterilization of the availability of micronutrients (Table 2.1) and many of the effects that were seen may have been an artifact of sterilization. This would be especially true with the concentration of metals. Many of the metals that changed were chemically reduced in the sterilization process and the effects of the sterilization process cannot be easily separated from the treatment effect. Therefore, the effects on micronutrients will not be discussed in this paper, and data are presented in Appendix A instead.

Conclusions:

The results of this study confirmed that *A. caliginosa* could survive in a soil from eastern Colorado. However, my hypothesis, that the addition of biosolids will increase the survival of *A. caliginosa,* was not supported. The biosolids addition did not result in the earthworms losing mass, but there was a reduction in the mass gained on a percent of starting mass basis. The addition of biosolids may lead to secondary toxicity effects that reduce the ability of the earthworms to reproduce, gain weight, or develop. A longer-term study would need to be performed to definitively determine if the overall effect is negative or not. The addition of earthworms increased the availability of both NO_3-N and NH_4-N but did not affect organic C levels. *A. caliginosa* also increased the concentration of Cr, whereas none of the other micronutrients were affected, as predicted.

When the soil had reduced microbial populations, the effects of *A. caliginosa* were greater. *A. caliginosa* was able to survive in this soil, but the earthworms did gain less mass than in the non-sterilized soil. The addition of biosolids did not affect the survival, disproving my hypothesis. Soil C, N, NH_4-N, and NO_3-N levels increased in response to earthworm addition. There was a larger effect seen in the availability of plant available nutrients in the reduced microbial population soil than in the control soil.

It has long been stated that the addition of earthworms improves the fertility of the soil (Yeates, *et al.*, 1998), and this study showed that, in both the non-sterilized soil and the reduced microbial population soil, there was increased availability of NO_3-N and NH_4-N. This increase could aid in improving plant fertility and productivity, but there could also be negative impacts if the NO_3-N is leached below the root zone. To determine if there is an increased potential for leaching, the physical changes of the soil resulting from of the burrowing of *A. caliginosa* would need to be investigated.

Table 2.1. Chemical and microbial biomass properties of soil used in laboratory incubation study. A sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) was used for this study and half of the containers of soil were irradiated with gamma radiation to sterilize the soil. The radiation did not completely sterilize the soil; therefore, the sterilized soil is referred to as reduced microbial population. (Metal and other elemental concentrations are AB-DTPA extractable concentration).

Parameter, units	**Non-Sterile soil**	**Reduced Microbial Population**	**P Value**
Total N, %	0.165	0.166	0.884
Total C, %	1.82	1.72	0.034
Inorganic C, %	0.468	0.453	0.235
Organic C, %	1.35	1.27	0.091
NO_3-N, mg kg^{-1}	24.1	15.6	0.003
NH_4-N, mg kg^{-1}	0.00	13.7	<0.001
Microbial biomass C, mg kg^{-1}	350	49.8	0.002
Microbial biomass N, mg kg^{-1}	58.5	11.1	0.008
pH	7.46	7.59	0.060
Electrical conductivity, dS m^{-1}	0.585	0.663	0.389
Ba, mg kg^{-1}	0.548	0.538	0.190
Ca, mg kg^{-1}	253	231	<0.001
Cd, mg kg^{-1}	0.073	0.0700	0.705
Cr, mg kg^{-1}	0.000	0.000	--
Cu, mg kg^{-1}	6.54	5.18	0.013
Fe, mg kg^{-1}	6.37	4.86	0.026
K, mg kg^{-1}	448	399	0.001
Mn, mg kg^{-1}	1.15	46.4	<0.001
Mo, mg kg^{-1}	0.013	0.0575	0.147
Na, mg kg^{-1}	0.913	0.210	0.156
Ni, mg kg^{-1}	0.753	0.963	0.002
P, mg kg^{-1}	39.5	38.6	0.724
Pb, mg kg^{-1}	1.22	1.25	0.617
Si, mg kg^{-1}	1.66	1.41	0.001
Sr, mg kg^{-1}	0.086	0.753	<0.001
Ti, mg kg^{-1}	0.000	0.000	--
V, mg kg^{-1}	0.260	0.313	0.007
Zn, mg kg^{-1}	4.17	4.58	0.742

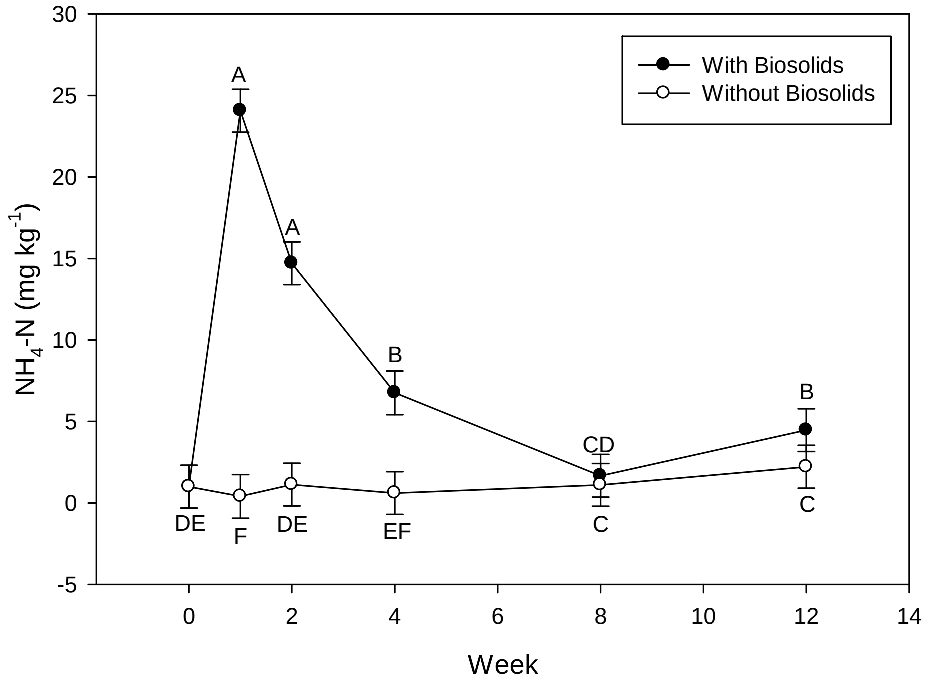

Figure 2.1. Ammonium-nitrogen concentrations over time. Under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids and time had a significant interaction on the log transformed soil NH_4-N concentration. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table 2.2. Chemical properties of biosolids. The biosolids used in a laboratory incubation study to investigate the effects of *A. caliginosa* on nutrient availability were from the Littleton/Englewood Wastewater Treatment Plant.

Parameter, units	
Solids, g kg^{-1}	796
pH	6.60
EC, ds m^{-1}	15.7
Organic N, g kg^{-1}	24.4
NH_4-N, g kg^{-1}	13.3
NO_3-N, mg kg^{-1}	1.51
K, g kg^{-1}	1.92
P, g kg^{-1}	23.5
Al, g kg^{-1}	7.50
Fe, g kg^{-1}	14.3
Cu, mg kg^{-1}	708
Zn, mg kg^{-1}	665
Ni, mg kg^{-1}	10.6
Mo, mg kg^{-1}	11.6
Cd, mg kg^{-1}	1.45
Cr, mg kg^{-1}	17.9
Pb, mg kg^{-1}	15.4
As, mg kg^{-1}	5.36
Se, mg kg^{-1}	6.48
Hg, mg kg^{-1}	1.15
Be, mg kg^{-1}	0.02
Ag, mg kg^{-1}	1.53
Mn, mg kg^{-1}	279
Ba, mg kg^{-1}	27.9

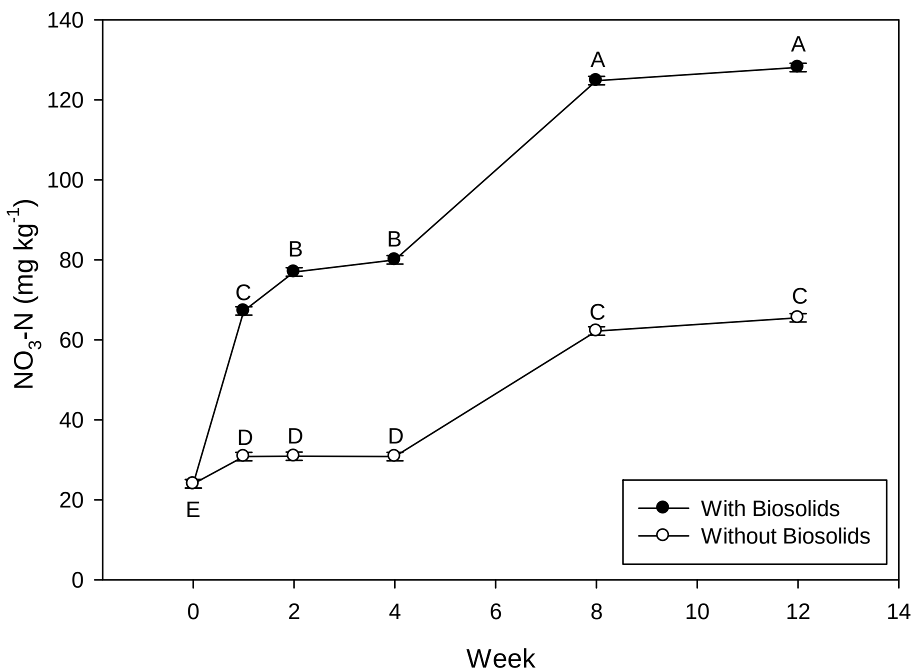

Figure 2.2. Nitrate-nitrogen concentrations over time. Under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids and time had a significant interaction on the log transformed soil NO_3-N concentration. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

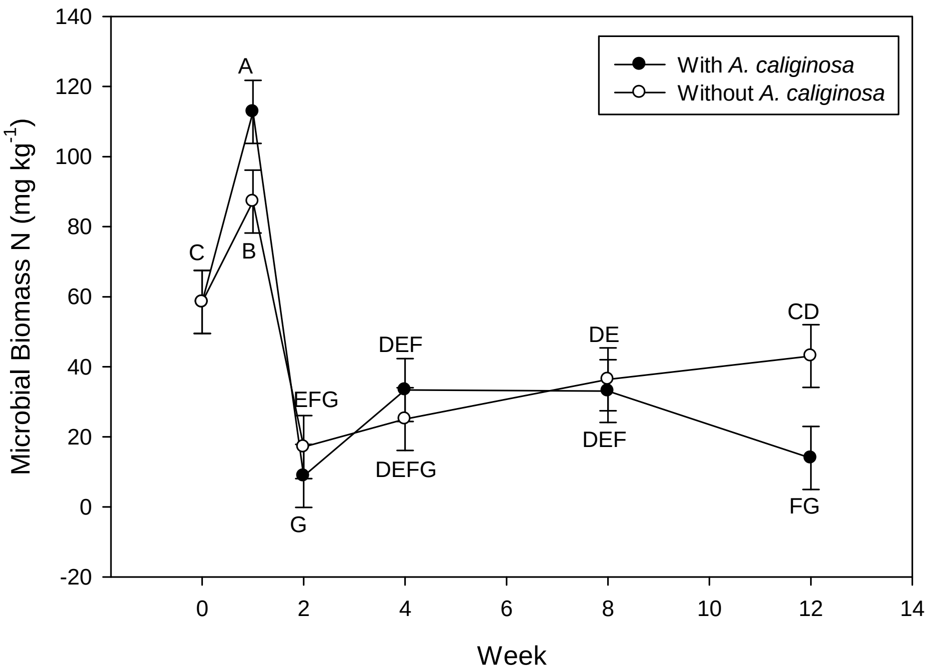

Figure 2.3. Microbial biomass N over time. Under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of *A. caliginosa* and time had a significant interaction on soil microbial biomass N concentration. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

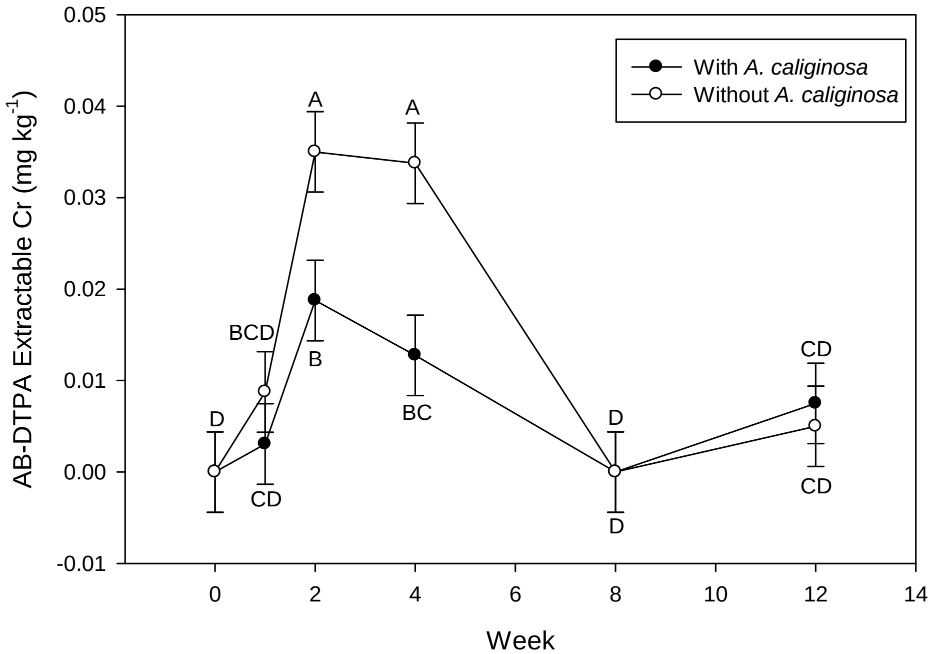

Figure 2.4. AB-DTPA extractable Cr over time, with and without *A. caliginosa*. Under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of *A. caliginosa* and time had a significant interaction on soil AB-DTPA extractable Cr concentration. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

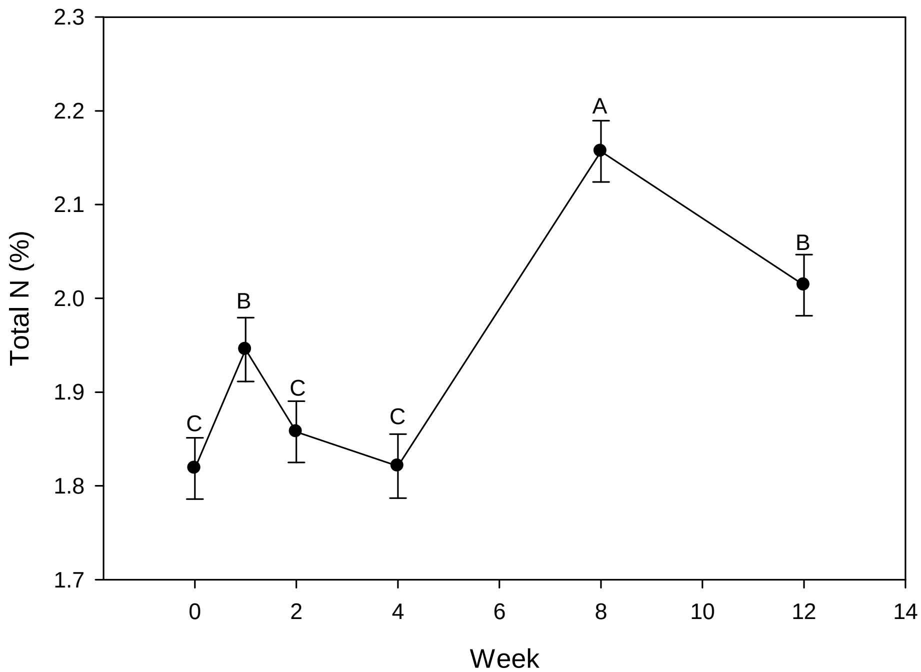

Figure 2.5. Total soil N concentration over time. Under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time, when averaged over the addition of biosolids and *A. caliginosa*, had a significant effect on total soil N percent. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

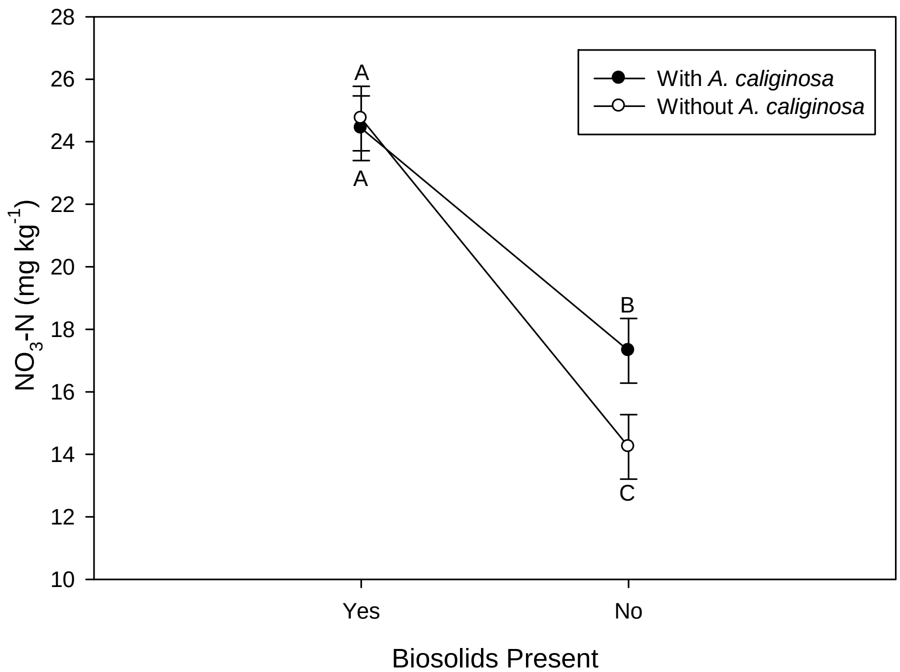

Figure 2.6. Soil NO_3-N in a reduced microbial population soil. Under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of *A. caliginosa* and biosolids when averaged over time on the log transformed soil NO_3-N concentrations. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

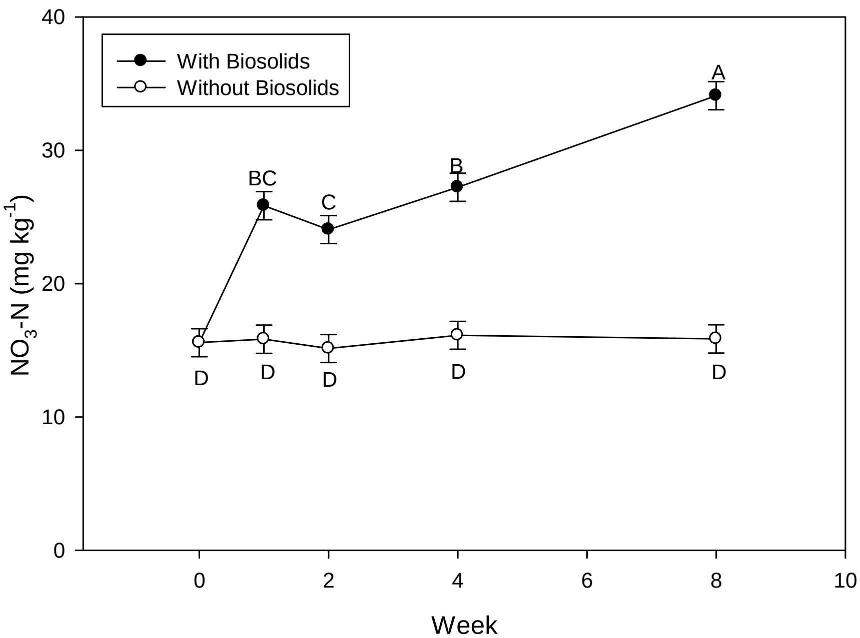

Figure 2.7. Effect of biosolids addition on NO_3-N concentration in a soil with reduced microbial population. Under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and time when averaged over the presence of *A. caliginosa* on the log transformed soil NO_3-N concentrations. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

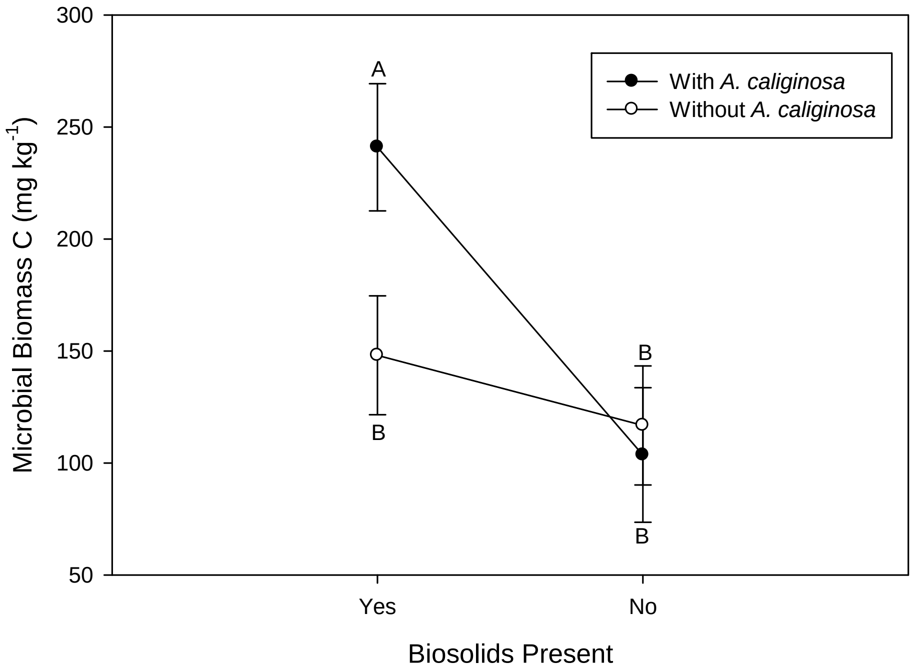

Figure 2.8. The effect of *A. caliginosa* and biosolids on the concentration of microbial biomass carbon in soil with a reduced microbial population. Under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of *A. caliginosa* and biosolids when averaged over time on the soil microbial biomass C concentrations. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

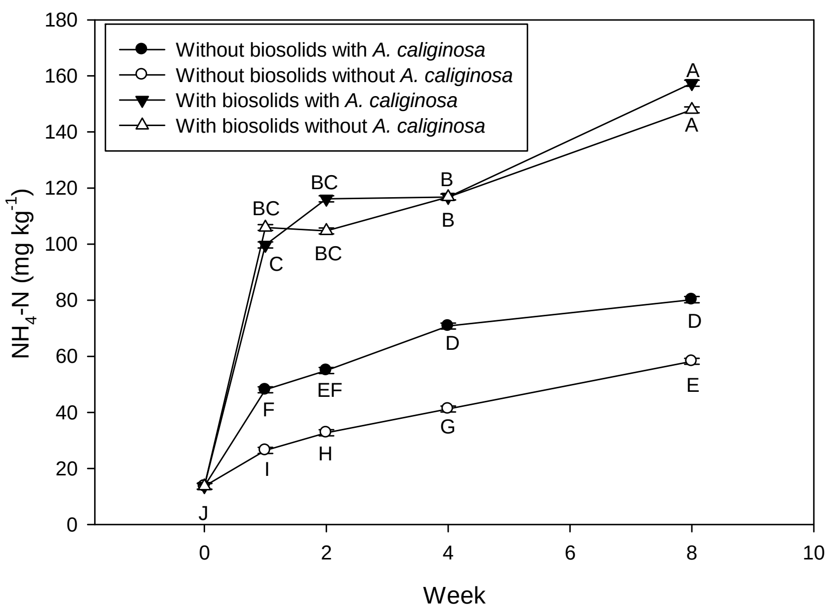

Figure 2.9. Ammonium nitrogen concentrations in a soil with reduced microbial populations. Under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant three-way interaction between the addition of *A. caliginosa*, biosolids, and week on the log transformed soil NH_4-N concentrations. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

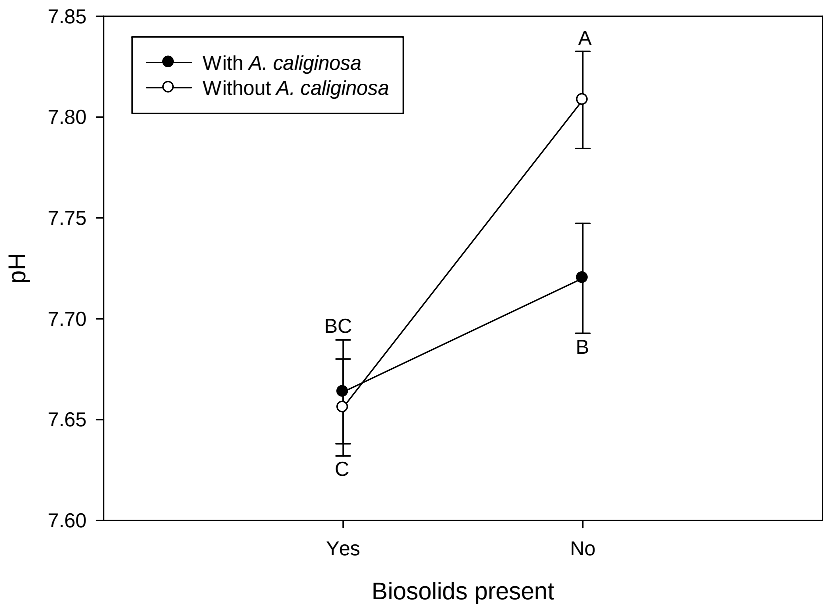

Figure 2.10. The effect of biosolids and *A. caliginosa* addition on the soil pH in a soil with reduced microbial population. Under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of *A. caliginosa* and biosolids when averaged over time on the soil pH. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

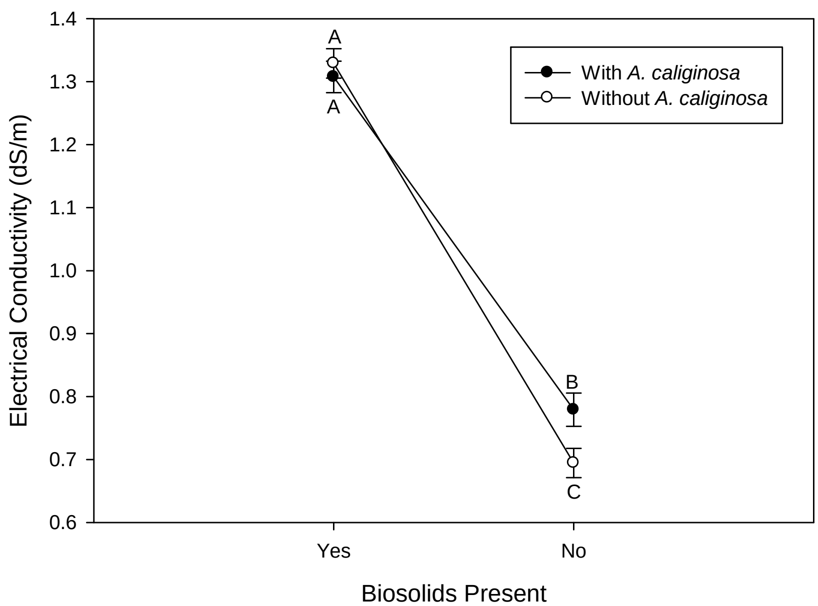

Figure 2.11. Electrical conductivity with the addition of biosolids and *A. caliginosa* in a reduced microbial population soil. Under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of *A. caliginosa* and biosolids when averaged over time on the soil EC. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

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CHAPTER 3: SURVIVABILITY OF *APORRECTODEA CALIGINOSA* IN RESPONSE TO DROUGHT STRESS

Introduction:

There is a growing interest in the use of earthworms in agriculture for their ability to improve soil physical properties (Dutt, 1948, Bouma*, et al.*, 1982, De Oliveira*, et al.*, 2012) and nutrient cycling (Eriksen-Hamel & Whalen, 2007). The distribution and survival of earthworms is controlled and greatly limited by the presence of water in the soil (Lee, 1985). Earthworms have little ability to control the release of moisture from their bodies (Carley, 1978). Earthworms require water to keep their bodies turgid, and water is needed to create the mucus that coats their body and helps them move through the soil and for respiration (Ramsay, 1949, Whalen*, et al.*, 2000).

Aporrectodea caliginosa is very sensitive to changes in soil water content (Bayley*, et al.*, 2010). Changes in soil moisture on a water content basis does not have as much of an impact on the activity of earthworms as the matrix potential of the soil (Collisgeorge, 1959, Gerard, 1967, Kretzschmar & Bruchou, 1991). As the matrix potential declines, moisture escapes from earthworms through a semi-permeable membrane. Studies have shown that the activity of earthworms is the greatest at matrix potentials not less than -0.1 to -0.2 MPa (Gerard, 1967, Nordstrom, 1975, Rundgren, 1975, Kretzschmar & Bruchou, 1991, Baker*, et al.*, 1992).

One of the reasons for lack of earthworms in many areas of Colorado may be due to the low matrix potential of the soil during the summer. The soils may have higher water content deeper into the profile and the earthworms could move down into the profile to find conditions that are more favorable. During the summer months in the semi-arid western United States, the soil becomes very dry on the soil surface and the drying front then move downs into the profile. Roundy and Abbott (1997) investigated the rate of surface soil drying following summer rain events in Arizona and found that it takes between approximately 1 and 7 days for the top 3 cm of soil to dry to a matrix potential of -1.5 MPa. The variation was due to differences in the amount of precipitation and the water held deeper (1-15 cm) in the soil profile. Once the surface of soil had dried, the drying front moved deeper into the profile at an average rate of 3.58 cm day^{-1}. The results of their study may show a faster drying of soil than would be seen in Colorado due to differences in climate. Roundy and Abbott (1997) also found that the use of mulch on the soil surface slowed the rate of soil drying. One of the main places in Colorado that the interest in the use of earthworms is in no-till agriculture where there would be crop residue on the soil surface.

Aporrectodea caliginosa have some means to cope with dry conditions by the formation of estivation chambers (Holmstrup, 2001, Friis, *et al.*, 2004) to reduce water loss allowing the earthworm to better survive drought periods. *A. caliginosa* will form a sealed chamber in the soil that is coated on the inside with their mucus (Lee, 1985, Holmstrup, 2001, Friis, *et al.*, 2004). They then twist their bodies up into small balls so that they have less surface area exposed. The earthworms will then stay in the estivation chambers until the moisture conditions of the soil improve, when they will re-emerge from the chamber.

In Colorado, the distribution of earthworms is limited to mostly areas that receive higher precipitation or irrigation. Even though Colorado has a semiarid climate, *A. caliginosa* is common in Colorado (Reynolds, 2011) but mostly around sources of water. The question is whether earthworms are not present in fields in eastern Colorado because they have not yet invaded or because soils are too dry for them to survive. If the earthworms are able to survive in the soil there may be a desire to add the earthworms to no-till fields to improve the soil structure and help to mix nutrients within the soil profile. Therefore, a laboratory incubation study was conducted to determine if *A. caliginosa* has the ability to survive in a Colorado soil exposed to different lengths of drought. If *A. caliginosa* can survive extended periods in dry soil, then it may be possible that these worms can be successfully established in NT dryland agricultural soils of eastern Colorado.

Methods and Materials:

Initially this study was established in a greenhouse to have temperatures and light conditions that would be closer field conditions. By the first sampling time, however, all of the earthworms were found to have been killed. This is because the use of the small black pots in a greenhouse setting resulted in lethal temperatures because sunlight was able to reach all sides of the pot and not just the soil surface. The ability of the soil to maintain lower temperatures at lower depths was lost and the earthworms did not have the ability to move deeper into the soil to locate a zone of more optimal temperature. The study was re-established with new soil, biosolids, and earthworms and conducted on a bench in the laboratory and not the greenhouse to be able to reduce the heating of the soil in the pots. The plots were randomly placed on the bench and were randomly rearranged near daily to reduce the effects of pots being on the edge.

A laboratory study with a factorial design was established to investigate the effect of drought stress on the survival of adult *A. caliginosa* in a biosolids-amended soil from eastern Colorado. A randomized complete block design was used for the study. There were four different levels of drought stress: constant water content and 1, 2, or 3 weeks of drought stress where no water was added. Pots were destructively sampled at three time points: 21, 42, and 63 days, and there were six replications for each treatment, at each sampling time point.

A sandy loam (56% sand, 30% silt, and 14% clay; Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) soil was collected from a dryland wheat-corn-fallow rotation field near Byers, Colorado latitude 39.7631921, longitude 103.7973089). The top 10 cm of soil was collected and passed through a 1.0 cm sieve and homogenized before being used. The soil was packed into square pots (10.2 cm wide × 25.4 cm high) at a bulk density of 1.1 Mg m^{-3} to a volume of approximately 1.8 L. The bottoms of the pots were sealed with tape to prevent water loss due to evaporation from the bottom.

Anaerobically digested biosolids obtained from the Littleton/Englewood Wastewater Treatment Plant were surface applied and not incorporated to each pot at a rate equivalent to 11 Mg of dry biosolids ha^{-1}. The biosolids had been dried to approximately 80% solids before being applied. Characteristics of the biosolids used can be found in Table 3.1.

Adult *A. caliginosa* were collected by hand sorting from the buffer strip beside an irrigated alfalfa field near Fort Collins, Colorado, in June 2010. The earthworms were then placed in soil from Byers for four days to acclimate to the new soil before the start of the study. The earthworms were randomly separated into 72 groups of four and placed on wet filter paper in a Petri dish overnight for gut evacuation. The earthworms were rinsed with deionized distilled water (DDI), gently blotted dry, and then weighed as a group to obtaining a starting fresh weight (FW). One group of earthworms was placed on the soil surface of each pot which was covered with a piece of fiberglass screen to keep the earthworms in but not to stop airflow or evaporation.

The four drought stress treatments were maintained by the use of near daily weighing and the addition of DDI was necessary. All of the pots were initially wetted to approximately 70% of field capacity (0.17 volumetric water content or approximately -0.12 MPa). When a pot was watered, as required by treatment, it was brought back to the original water content by applying water to the soil surface.

Water retention curves were developed for the soil that was used for this study. The gravimetric water content was converted to a matrix potential using the Brooks-Corey equation. This method has some errors due to the hysteresis of the rewetting of the soil and drying for each of the drought cycles. However, the pots did not dry evenly due to the evaporation being concentrated on the surface and the unknown location of the earthworms. The use of the mass of the pots to determine the water content provided an average water content, which may better represent the actual water content of the pot.

At each sampling times, the mass of the pots were determined before pots were destructively sampled by hand sorting of soil to locate the earthworms. As the earthworms were found, they were classified as active, in diapause, or dead. If the earthworms were found in an evacuated sphere of soil and coiled into a ball they were classified as in diapause, any other live earthworms were classified as alive. Earthworms that were found as dead or not found were classified as dead. For each pot the alive and diapause earthworms that were found were placed on wet filter paper in a petri dish overnight to evacuate their gut, before being rinsed with DDI, blotted dry, and weighed.

Once the earthworms were collected, all the soil from each pot was homogenized, and a sample was removed. The sample was stored in a sealed plastic bag at 3^{o} C until analysis for soil nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), and dissolved organic carbon (DOC). Soil NO_3-N and NH_4-N was determined using the wet soil at an equivalent mass 10.00g of oven-dry soil and a 2M KCl extract (Mulvaney, 1996) and analyzed on an Alpkem Flow Solution IV Automated wet chemistry system (O.I. Analytical, College Station, Texas). The total organic C was also analyzed using wet soil at an equivalent mass of 10.00 g oven-dry soil and using modified methods of Zsolnay (2003). The soil was placed in a 50 mL centrifuge tube with 40 mL of DDI. It was shaken for 1 hour before being centrifuged for 15 minutes. The supernatant was then filtered with 0.45-µm polycarbonate filter membrane. The sample was then frozen until analysis on a Shimadzu Total Organic Carbon Analyzer (model TOC-Vcpn+TNM-1, Columbia, Maryland).

Statistical analysis was performed on the data using Proc Glimmix in SAS (SAS Institute, 2008). An alpha value of 0.05 was used for the determination of significant differences.

Results and Discussion:

This section will focus only on the significant results; all other results can be found in Appendix B.

A change in mass of the earthworms as a result of the diapause was expected but was not seen. The mass of each group of four earthworms was recorded prior to the study and was converted to a per worm mass. This methodology was used so that if an earthworm died during the study, the other earthworms that were in that pot could be analyzed. This mass was then compared to the per worm mass at the end and there was not a significant difference ($P=0.147$). However, the method that was used to obtain the final mass of the earthworms, involved placing the earthworms on wet filter paper overnight. During this time, the earthworms would be able to rehydrate and effects on the mass resulting from water loss would not be shown. This does show though that after the earthworms experience a period of drought and are rehydrated their mass is unchanged, indicating the ability to recover from a three-week drought period. Friss (2004) also found that the earthworms are able to recover even from severe dehydration if they are rehydrated.

The activity and survival of the earthworms were affected by the different drought stress treatments. There was a significant treatment effect (P=<0.001) on the number of earthworms that entered a state of diapause. When looking at the individual treatments there was a trend for an increasing number of diapause earthworms with increasing length of drought stress (Table 3.2). The formation of estivation cells and twisting into knots indicated the earthworm was in diapause (Friis*, et al.*, 2004).

At the end of the each drought period the average matrix potential was -0.12 MPa for constantly moist soil, -0.19 MPa for a one-week drought stress period, -0.33 MPa for two-week drought stress period, and -0.57 MPa for a three-week drought stress period (Table 3.3). There was not a statistical difference in the number of earthworms in diapause between constant water content and a one-week drought stress period. The ending matrix potential between these two treatments were similar and others have shown that earthworms begin to be affected by matrix potential when it falls below -0.1 to -0.2 MPa (Gerard, 1967, Nordstrom, 1975, Rundgren, 1975, Kretzschmar & Bruchou, 1991, Baker*, et al.*, 1992). When the soil was exposed to longer drought stress periods, the matrix potential was reduced below the range where the earthworms are not affected and the results of the study show that Colorado earthworms are also affected similarly to the populations in Europe.

Sampling day significantly affected earthworm condition (P=<0.001). The number of earthworms in diapause was averaged across drought treatments because there was not an interaction. The same numbers of earthworms were in diapause at the first two sampling times, but fewer at the third (Table 3.4). At the first two sampling times the average matrix potential of all samples was -0.28 MPa and -0.27 MPa respectively. The third sampling time had a matrix potential of -0.32 MPa. At the third sampling point, the matrix potential was lower, so it would have been expected that more of the earthworms would be in diapause. However, earthworms may be able to adjust better to changes in the water content of the soil if it takes place more slowly. Holmstrup (2001) has speculated that when drought stress is imposed on earthworms in laboratory settings the stress is worse because the change of environment may exaggerate the impact of the drought. It may be that by the third sampling on day 64 the earthworms have coped with the stress of being moved to the laboratory, and now the true level of impact of drought stress is seen. Frund *et al.* (2010) has recommended that earthworms be allowed at least one week to adjust to the new conditions of an experiment prior to beginning the study. The earthworms that were used in our study were only allowed four days to adjust to the new soil before the beginning of the study. The adjustment period is most often used as a time for the earthworms to clear their gut of the soil they were living in and replace it with the soil of the new study, and this process should be completed for *A. caliginosa* within 96 hours (Pokarzhevskii, *et al.*, 2000). Even though the earthworm has replaced all of the soil in their gut in 96 hours to 1 week, they may not have adjusted to the new environment due to other factors than clearing their gut, to a level that they are no longer stressed.

Drought stress treatments affected the number of earthworms that died (P=<0.001). A three-week drought stress period resulted in a significantly higher death rate of earthworms than any of the other treatments (Table 3.2). All of the earthworms survived the constant water content and cycles of one-week drought stress. There were some earthworms that did not survive cycles of two-week drought stress, but it was not significantly different than the constant water content or a one-week drought stress treatments. On average 0.56 earthworms or 14% of the earthworms died per pot with cycles of three-week drought stress.

One of the largest restrictions on the expansion of earthworms is the water content of the soil (Lee, 1985). The long-term survival ability of earthworms under these conditions may be limited. Not only was there an 86% survival rate but also a large number of earthworms entered diapause even with a shorter drought stress period. The ability of earthworms to reproduce and to expand their range is also limited by diapause because it would limit the amount of time that they were active over the course of a year. The potential combination of the reduction in reproduction rate and the increased mortality rate during a three-week drought stress period may limit earthworm survival and expansion in dryland soils of eastern Colorado.

The soil NO_3-N level was significantly affected by the length of the drought period ($P=0.015$). The concentration of NO_3-N increased as the length of the drought period decreased. There was also a significant effect of the sampling day ($P=0.035$). The latter sampling periods resulted in a higher concentration (Figure 3.1). These effects are mostly due to an increase in microbial mineralization due to the higher soil water content. The laboratory incubation study (Chapter 2) had shown a trend for increased NO_3-N with earthworms, but since there were not any pots that did not contain earthworms the effects of the earthworms and the microbial populations could not be separated.

The soil NH_4-N level was unaffected by both treatment (P=0.677) and sampling day (P=0.124). The laboratory incubation study (Chapter 2) had shown that the earthworms have a significant effect on soil NH_4-N levels. The effect was of a larger magnitude when microbial populations were reduced. In the current study, the soil microbial populations were not reduced and the conditions were favorable for nitrification with the repeated wetting and drying cycles.

The DOC was not affected by the length of drought stress. However there was an effect of the sampling day (P=0.010) (Figure 3.2). The first sampling time and the last sampling time were the same, but the second time was significantly higher. This is probably due to the effects of soil microbial populations. The first and the last sampling periods were in the middle of the two-week drought stress period. This sampling method resulted in there being effectively 12 samples that were at the end of a one-week drought stress and no samples at the end of a two-week drought stress period. The 42 day sampling point had all of the treatments represented at the end for each treatment and therefore there was more of a distribution of water contents.

Conclusion:

A. caliginosa may survive periods of moderate drought stress in an eastern Colorado soil. The earthworms were not affected significantly by the drought stress periods up to two weeks but cycles of three-week drought stress did have negative effects on the earthworms and resulted in a 14% mortality rate over the 63 day period. More earthworms were expected to enter diapause but it was not expected that the earthworms would experience mortality at this rate.

The ability of the earthworms to enter diapause and not to lose mass after being rehydrated shows that *A. caliginosa* has the ability to survive the drought periods that occur in a semi-arid environment such as Colorado. The ability of *A. caliginosa* to tolerate drought appears to vary depending on the population of earthworms that was used in the study (Holmstrup, 2001) suggesting that *A. caliginosa* has the ability to adapt to different environmental conditions. All of the earthworms that were used in this study were collected from the edge of an irrigated alfalfa field and the earthworms may have adapted to this moisture regime. The mortality of the earthworms that was seen with the longer drought periods may change with time if the earthworms were collected from an area that had a lower average soil water content. The earthworms that survived the drought stress periods could also be earthworms that are able to survive harsher environments, and if they were introduced into an environment that was simulated in this study the population of earthworms would stabilize and then start to increase. The results that were seen for survival in this study were the result of a laboratory study that had controlled conditions and the drought stress may not have been to the degree that would be seen in the field.

Table 3.1. Chemical properties of biosolids. The biosolids used in a laboratory study on the effects of drought stress on *A. caliginosa* were from the Littleton/Englewood Wastewater Treatment Plant.

Parameter, units	
Solids, g kg^{-1}	796
pH	6.60
EC, ds m^{-1}	15.7
Organic N, g kg^{-1}	24.4
NH_4-N, g kg^{-1}	13.3
NO_3-N, mg kg^{-1}	1.51
K, g kg^{-1}	1.92
P, g kg^{-1}	23.5
Al, g kg^{-1}	7.5
Fe, g kg^{-1}	14.3
Cu, mg kg^{-1}	708
Zn, mg kg^{-1}	665
Ni, mg kg^{-1}	10.6
Mo, mg kg^{-1}	11.6
Cd, mg kg^{-1}	1.45
Cr, mg kg^{-1}	17.9
Pb, mg kg^{-1}	15.4
As, mg kg^{-1}	5.36
Se, mg kg^{-1}	6.48
Hg, mg kg^{-1}	1.15
Be, mg kg^{-1}	0.02
Ag, mg kg^{-1}	1.53
Mn, mg kg^{-1}	279
Ba, mg kg^{-1}	27.9

Table 3.2. Effects on earthworm's condition because of varying lengths of drought stress periods. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The data in the table below was averaged over sampling day. Treatments with different letters are significantly different at an alpha value of 0.05. Comparisons can only be made between the treatments within the same effect.

Treatment	**Average Number of Earthworms in Diapause**	**Average Number of Dead Earthworms**	**Average Percent Change in Earthworm Mass (%)**
Constant water content	0.44c	0.00b	-0.397
1 week drought stress	0.89bc	0.00b	-6.08
2 week drought stress	1.33b	0.06b	-2.24
3 week drought stress	2.89a	0.56a	-7.24
P Value	<0.001	<0.001	0.147

Table 3.3. Ending volumetric water content from a laboratory drought stress study of *A. caliginosa*. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The data in the table below was averaged over sampling day. Treatments with different letters are significantly different at an alpha value of 0.05.

Treatment	**Average Ending Volumetric Water Content**
Constant water content	0.185a
1 week drought stress	0.175b
2 week drought stress	0.161c
3 week drought stress	0.144d
P Value	<0.001

Table 3.4. Average number of earthworms in diapause per pot at each of the sampling points. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The data in the table below was averaged across drought treatments. The sampling days with differing letters are significantly different at an alpha value of 0.05.

Sampling Day	**Number of Diapause Earthworms Per Pot**
21 Day	1.71a
42 Day	1.71a
63 Day	0.750b
P Value	<0.001

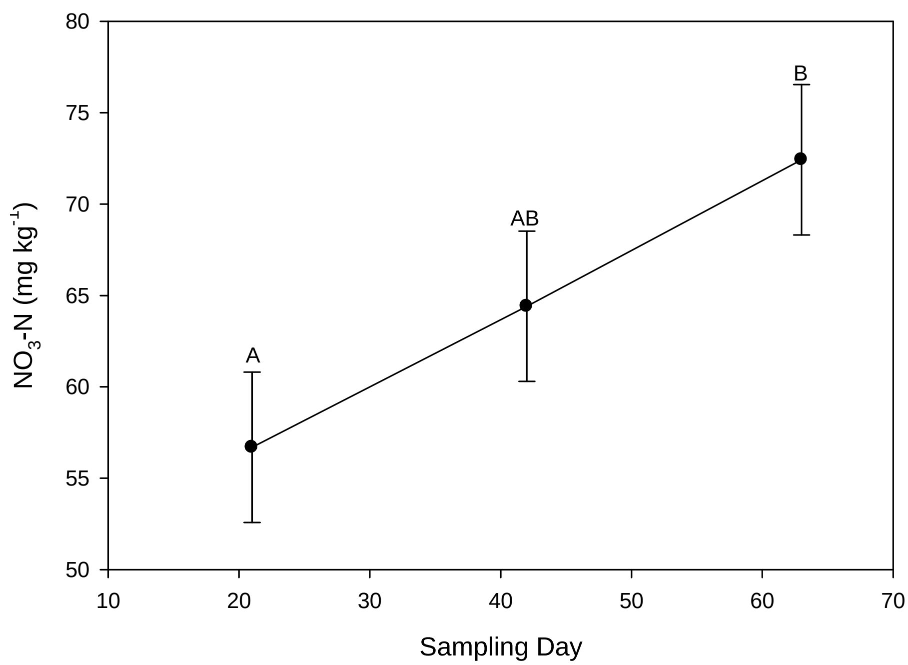

Figure 3.1. Soil NO_3-N concentration across time in a laboratory drought stress study on *A. caliginosa*. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

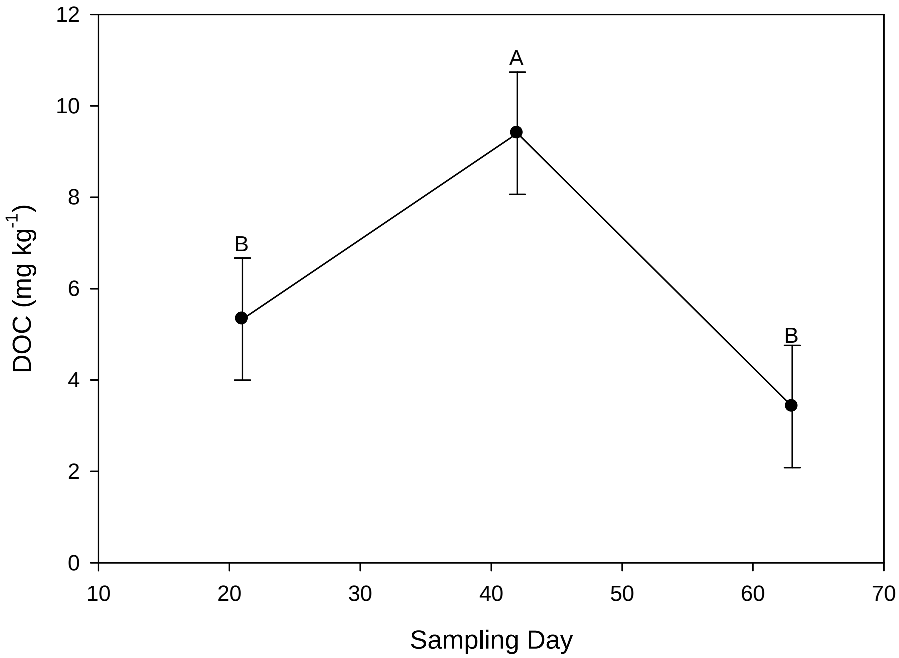

Figure 3.2. Concentration of soil DOC across time in a laboratory drought stress study on *A. caliginosa*. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Table 3.5. Mean soil NO_3-N and NH_4-N concentrations from a laboratory drought stress study on *A. caliginosa* soil. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The effects on a test value can be compared within a test value but not across. Treatments with different letters are significantly different at an alpha value of 0.05.

Treatment	**Average Soil NO_3-N (mg kg^{-1})**	**Average soil NH_4-N (mg kg^{-1})**
Constant water content	75.7a	5.63
1 week drought stress	66.2ab	3.78
2 week drought stress	63.2ab	1.87
3 week drought stress	52.9b	4.54
P Value	0.015	0.677

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CHAPTER 4: EFFECTS OF *APORRECTODEA CALIGINOSA* ON SOIL HYDRAULIC PROPERTIES UNDER LABORATORY CONDITIONS

Introduction:

Earthworms have long been viewed as soil engineers due to their ability to mix and reorganize soil (Darwin, 1886, Martin & Marinissen, 1993, Oades, 1993, Schrader & Zhang, 1997). The ability of earthworms to change and improve the movement of water and gasses (Lee & Foster, 1991) can have a large impact on soil quality. As cropland is transitioned from conventional tillage (CT) to no-tillage (NT), there can be negative changes to the soil physical properties, such as reduced soil porosity (Shipitalo & Protz, 1987). However, these changes are also improving the suitability of the soil for earthworms by reducing the loss of organic matter (OM) (Juo & Lal, 1979, Hargrove*, et al.*, 1982, Blevins*, et al.*, 1983, Stinner*, et al.*, 1983) and moisture from the soil (House & All, 1981).

The effect of earthworm burrows on the physical properties of the soil depends on the feeding functional group to which the earthworms belong. Epigeic earthworms will have the least effect because they live in the litter layer of the soil feeding on above ground OM. Anecic earthworms will have the largest impact on the deep movement of water and gases due to the permanent burrows they create that are open to the surface and tend to be larger in diameter. Endogenic species may overall have the largest total impact on the movement of solutes and gas exchange and are more common in agricultural fields. Endogenic species make impermanent burrows that create a large network of random, interconnected burrows with fewer connection to the soil surface (Lee & Foster, 1991).

Earthworm burrows have a large impact on the amount of macropores in the soil (Tisdall, 1978, Tisdall, 1985) which can subsequently affect infiltration and movement of water into the soil (Lee, 1985) due to the size of the burrows (Smettem & Collisgeorge, 1985). The pores created by earthworms have a large diameter, so they will drain quickly and only play a role in water flow at or near saturated conditions. When the soil is not saturated, the majority of the earthworm burrows will be air filled and will not contribute to the movement of water or solutes. Due to the ability of earthworms' burrows to facilitate the faster flow of solutes, the effect of earthworms on the potential for leaching of chemicals and fertilizer is of interest.

As the earthworms burrow, they ingest soil and re-deposit it as cast material. The cast contains higher levels of small particles due to the earthworms selectively ingesting the smaller soil particles (clay and silt) (Lavelle, 1988). Edwards and Lofty (1977) indicated that earthworms can reduce the particle size of soil ingested due to the grinding of the material that takes place in their gizzard. However, this is not likely to have a large impact on the soil texture due to the relatively small amount of pressure that would be exerted on the soil particles. Should earthworms have an impact on the texture of soil, it is more likely that the impact would be from the concentration of the smaller soil particles due to the selective feeding.

When earthworms burrow through the soil, they make burrows by one of two methods depending on the level of compaction in the soil. If the soil is loose enough they will move by expanding and contacting the segments of their bodies to push their way through the soil (Lee & Foster, 1991). The earthworms use the mucous coating on the outside of their bodies to help lubricate their movement (Lee, 1985). If the soil is too compact to push their way through, they ingest the soil that is in front of them to allow for their movement. Both of these methods result in burrows being created that will increase the macropore space in the soil. When the soil is moved by pressing the soil out of the way the soil around the moved soil is compacted to create the void space. This type of action should not reduce the bulk density of the soil due to the compaction around the void space leading to no change in the overall soil bulk density.

The creation of the void space and the compacted zones in the soil should have an effect on the water retention curves of the soil. The creation of the larger voids will lead to the water draining faster, but the compaction around the burrows will lead to a larger variety in the pore sizes in the soil.

In Colorado, there is a growing interest in earthworms due to their ability to improve soil porosity (De Oliveira, *et al.*, 2012), along with other soil physical properties such as infiltration (Bouma, *et al.*, 1982) and aggregate stability (Dutt, 1948). The objectives of these studies were to investigate the effects of *A. caliginosa* on the soil physical properties. There were three questions that the studies addressed:

1.) What is the effect of *A. caliginosa* burrowing activity on solute dispersivity (is a measure of the spread of a compound of interest in the soil) in saturated and unsaturated soil under laboratory conditions?

2.) How is the water retention curve affected by the presence of *A. caliginosa* under laboratory conditions?

3.) Does the burrowing of *A. caliginosa* change the soil texture in the areas where burrowing is taking place?

The study hypotheses was that because of alteration in soil structure and porosity, *A. caliginosa* would increase the dispersivity of a solute under saturated condition, reduce the tension at which the soil water would begin to drain because of the addition of macropores.

The specific predictions for the study were:

1a.) When the soil was saturated, the dispersivity of the solute will increase with the addition of earthworms. The effects will be limited to the upper two depths where the *A. caliginosa* will be the most active.

1b.) When the water content of the soil is less than saturation, the effects of the *A. caliginosa* will not be seen due to the lack of water filling the larger.

2.) *Apporrectodea caliginosa* will change the pore size distribution and therefore affect the rate that water drains from the soil, and the earthworms will increase the residual water content.

3.) *Apporrectodea caliginosa* will affect the soil texture by accumulating clay and silt in the depths where they are the most active.

Methods and Materials:

Breakthrough Curve Study:

Repacked soil columns were used as microcosms to produce soil to which *A. caliginosa* were added. The columns were 15 cm diameter PVC pipe and the earthworms were allowed 16 weeks to create burrows. Each column consisted of three 15-cm tall sections and a 5 cm buffer section on the bottom, to reduce the boundary effect of the bottom of the column. The columns were filled with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) from a dryland wheat-corn-fallow field near Byers, Colorado (latitude 39.761921, longitude 103.7973089). The soil was packed to a bulk density of 1.14 Mg m^{-3}. Anaerobically digested biosolids from Littleton-Englewood Wastewater Treatment Plant were surface applied at rate equivalent to 11.2 Mg dry matter ha^{-1} and not incorporated. The soil was then wetted to an average gravimetric water content of 0.17 (approximately 70% of field capacity). Fifteen adult *A. caliginosa* were added to each of four columns and allowed to burrow into the soil. Four additional columns, without earthworms, were included in the study as a control. After the earthworms had burrowed into the soil, a piece of fiberglass screen was placed on top of the soil surface to reduce the disturbance to the soil with the addition of water to the surface to maintain the soil water content during the length of the study. The columns also had a piece of screen placed over the top of the column that was secured with a rubber band to ensure the earthworms did not escape from the columns.

Initially the columns were placed in the greenhouse, but when samples from another study were sampled after 1 week, it was discovered that all the earthworms had died due to the high heat. The temperature in the greenhouse should not have caused this effect but due to the size of the columns, the soil was being heated from all sides and not just the top, reducing the ability of the soil to insulate the deeper depths. The columns were dismantled and set up again as described above in the laboratory where they were stored for the duration of the experiment. The columns were weighed weekly and distilled water was added as needed to maintain the desired water content. After 16 weeks, the columns were moved to the cold room (4^{o} C) to reduce the activity of the earthworms until analyses were performed. The columns were also covered with plastic while in the cold room to reduce soils drying and pulling away from the sides of the containers. During storage, the weekly watering continued for the columns.

The design of this experiment was a repeated measurements model with two treatments: with and without *A. caliginosa*. There were four replications of each treatment. The columns were separated into the 15 cm sections for the development of the breakthrough curves. The individual sections had a 2.5 cm section of 15 cm diameter PVC added to the top of each section prior to analysis to allow for the formation of a pond on top of the soil for the saturated breakthrough curve. The column sections were then attached to a 0.05 MPa bubbling pressure ceramic plate to allow negative pressure to be pulled on the lower boundary of the column section. The ceramic plate was flushed with a dilute hydrochloric acid (HCl) and then calcium chloride ($CaCl_2$) in the opposite direction as the leachate flowed through the plate between each of the sections to help reduce the effects the plate clogging. The column was placed on top of an evacuated chamber with a fraction collector in it to collect approximately 12 mL fractions of the leachate. A nonabsorbent dye was added to the top of each section at a rate of 0.33 g m^{-2}. A tensiometer was placed in the middle of each of the column sections through the side of the PVC to measure the degree of saturation.

A breakthrough curve was determined for both the unsaturated and saturated flow on each section. The soil was leached with 5.0 mM $CaCl_2$ to maintain soil structure during the length of the study. For the saturated flow, the soil was saturated by wetting the soil from the bottom of the column until a 1-cm pond was established on top. The soil was wetted from the bottom to ensure that there were not air pockets in the soil that would interfere with the flow of the leachate. The lower boundary of the column was closed, by placing a clamp on the outflow tube of the ceramic plate, and the pond was removed by the use of suction. The dye was then evenly applied as drips over the soil surface with a pipettor. After three minutes, the pond was reestablished and maintained with the use of a mariotte bottle.

The unsaturated flow breakthrough curve was measured following the saturated breakthrough curve. The analysis was performed in this order because the larger macropores may have become damaged during the wetting and draining of the soil and the macropores were more important to the saturated water flow. The column section was leached with $CaCl_2$ to ensure all of the dye had been removed. The soil was then drained and the tension on the bottom of the column and the rate of $CaCl_2$ additions were adjusted to achieve near constant water content. The $CaCl_2$ was added to the soil by the use of a needle drip head connected to a syringe pump. The needle head used 21 gauge needles and had a density of 0.3 needles cm^{-2}. The unsaturated flow was maintained by small adjustments to the dosing rate of the syringe pump or the negative pressure on the lower boundary of the column as necessary.

The leachate was collected to a total volume of approximately 4.5 liters in approximately 12 mL fractions for each column section for both saturated and unsaturated flow. The exact volume of each fraction was determined by rate of recovery of the dye from the leachate. The concentration for the dye in each of the fractions was measured using a 96 well micro well plate reader (BIO-RAD, Model 680, Hercules, California) at a wavelength of 840 nm.

The data from each breakthrough curve were analyzed separately using Hydrus 1-D (Šimůnek, *et al.*, 2008) to determine the dispersivity of the dye. When the data were modeled for both saturated and unsaturated water flow, a unit gradient approach for the pressure within the column was used. The value for the saturated hydraulic conductivity was calculated but the value was not realistic due to the effects of the plate. The data were modeled initially as a steady state model with the plate as a second layer, however, other models were then used to improve the fit. When the experiment was conducted, it was expected that the column sections would remain under steady state, but this was not true. The data and also observations during the experiment showed that a transient flow model would better fit the data. The transient flow was most likely the result of the ceramic plate becoming clogged as more leachate traveled through the column and the plate, reducing the flow rate. As the flow rate was reduced, adjustments were made to both the rate of addition of $CaCl_2$ and the tension on the bottom of the plate to attempt to maintain constant water content in the column.

The column sections were modeled with transient flow to improve the Hydrus 1-D model's representation of the observed data. The transient flow model allowed for the soil under saturated conditions, to potentially drain at times, to less than saturation, but it is not believed that this took place. When the transient model was used, the water retention properties that were measured on the soil from the respective section were averaged and used as inputs for the model to better represent the rate of the water draining.

It was expected that the earthworms would create dead end pore space; therefore, a dual porosity model (physical nonequilibrium model) with mobile and immobile water content with transient flow model were also fitted. This model has two additional parameters: theta immobile (immobile water content), and a term that represents the rate of solute movement from the mobile to the immobile water fractions. The sum of the immobile water content and the mobile water content is equal to the total water content of the soil.

The dispersivity of the dye was compared between treatments using Proc GLIMMIX in SAS (SAS Institute, 2008). Comparisons cannot be made between saturated and unsaturated flow or between the models. The data were checked for homogeneity of variance, and when necessary, a log transformation was used. The data that are presented are the non-transformed data.

Water Retention:

Following the completion of the breakthrough curve study, soil was extracted to measure soil water retention. The soil samples were extracted intact from two positions within each of the sections (Figure 4.1). The top 3 cm of soil was removed and three brass rings (approximately 5 cm in diameter) were driven into the soil to collect three intact soil samples from the 3-to-6 cm depth increment (corresponding to 3-6 cm in the top column section, 18-21 cm from the middle column section, and 33-36 cm from the bottom column section). The rings were then extracted and the exact mass and volume of soil for each ring was determined. Soil was removed from each column section to a depth of 9 cm before three more rings were filled from the 9-to-12 cm depth increment (corresponding to 9-12 cm in the top column section, 24-27 cm in the middle column section, and 39-42 cm in the bottom column section). The bottom 3 cm of soil from each column section was not used.

The soil samples were placed in a plastic tub of 5 mM $CaCl_2$ overnight to completely saturate the soil sample. The samples were then weighed to determine the saturated water content. The $CaCl_2$ level was then reduced to midpoint of the samples and allowed to drain for four hours before it was reweighed.

The samples were placed on a ceramic plate with a 0.1 MPa bubbling pressure. The plate were soaked overnight in water and had a thin pond of water on it when the plate was placed in the 0.1 MPa extraction chamber. A hanging water table was used for the collection of data points near saturation to increase the accuracy of these measurements. The height of the water table was based on the midpoint of the samples and measurements were taken with the water table at -5 (-4.9×10^{-4} MPa), -10 (-9.8×10^{-4} MPa), -20 (-2.0×10^{-3} MPa), and -30 (-2.9×10^{-3} MPa) cm of tension. For each of these points the samples were allowed three days to equilibrate before the mass of the samples was recorded. For the readings of -60 (-5.9×10^{-3} MPa), -102 (-1.0×10^{-2} MPa), -200 (2.0×10^{-2} MPa), -340 (-3.3×10^{-2} MPa), and -600 (-5.9×10^{-2} MPa) cm of tension, air pressure was used and these samples were allowed three days to equilibrate.

Following the -600 cm (-5.9×10^{-2} MPa) tension reading, the samples were moved to a ceramic plate with a 1.5 MPa bubbling pressure and were placed in a 1.5 MPa extraction chamber. With the use of air pressure, measurements were taken at 0.15 MPa, 0.5 MPa, and 1.5 MPa. These samples were allowed to equilibrate for one week. Following the 1.5 MPa reading the samples were oven dried.

Subsamples from the soil water retention samples, from two columns with earthworms and two columns without were removed before the remaining samples were oven dried. These subsamples were further analyzed using a Decagon Devices WP4 dew point potentiometer (Pullman, Washington). This instrument allows for the collection of data on the relationship between soil water content and matrix potential of the soil sample as it approaches air-dryness. Soil samples were measured immediately after the 1.5 MPa reading. The reading from the meter was recorded and the mass of the sample was measured on an analytical balance. The samples were then left open for between 15 minutes and 45 minutes to allow the samples to dry slightly before the next measurement. The variation in the time was due to the rate that samples dried as affected by temperature and the humidity in the laboratory. It was not necessary for each sample to be measured at the same potential because the data points collected were used to improve the fit of a curve. The soil samples only needed to slowly dry so that several readings could be taken. The process of the drying and measuring was repeated until the samples reached air-dryness.

Following the collection of the complete data set, each of the matrix potentials was paired with its corresponding volumetric water content. The data from saturation through 1.5 MPa for each sample were modeled in Hydrus-1D to determine the terms for the Brooks-Corey equation. The inverse function of Hydrus-1D was used to estimate the residual water content, alpha term, and n term. The saturated water content was fixed and the measured value for residual water content was used.

The Brooks-Corey equation:

$$\frac{\theta - \theta_r}{\theta_s - \theta_r} = S_e = \begin{cases} |\alpha h|^{-n} & h < -\dfrac{1}{\alpha} \\ 1 & h \geq -\dfrac{1}{\alpha} \end{cases}$$

was used to describe the water retention curve, where S_e is the effective saturation, Θ_r is the residual water content, Θ_s is the saturated water content, h is the tension in cm, Θ is the water content at a given h, the inverse of alpha is the air entry value, and n is the pore size distribution index.

Data were analyzed using SAS Proc Glimmix (SAS Institute, 2008) for treatment effects on the terms for the Brooks-Corey equation. An alpha value of 0.05 was used for all analysis. A repeated measurement design was used for the analysis because each column was separated into 15-cm sections which were individually analyzed but are associated with each other. The treatments, with and without earthworms were repeated four times. There were also three sections in each column. There were two positions within each section and three replications within each position. Since soil rings were filled from two positions within a section, the position term was nested in section. The nested design allowed the position term to be only associated with the section that it was sampled from and for pooling if there was not a position within section effect.

The Brooks-Corey equation has a term that is interpreted to represent the residual water content of the soil. However, when a curve is fitted to the data this term is viewed as only a curve fitting parameter. In some of the fits, the value for residual water approached zero, and this is not a realistic value because the soil will still have water films on the soil surface when air-dried. A value of -245 MPa of tension was used as the point of air-dryness for this study. This was based on the readings from the dew point potentiometer. The soil water content had very little change beyond -196 to -245 MPa of tension. The value of -245 MPa of tension was fitted to each of water retention curves to produce more realistic residual water contents.

Soil Texture:

The soil samples that were used in the water retention curves were taken and broken up using a mortar and pestle. Thirty-five g of soil were placed into a 500 mL plastic bottle with 100 mL of 50.0g/L sodium hexametaphosphate $(NaPO_3)_6$ and 250 mL of deionized distilled water. The samples were placed on a horizontal shaker for 16 hours. The samples were removed and allowed to sit on the bench for several hours to return to room temperature (the temperature of the samples was elevated due to the heat given off by the shaker). The samples were qualitatively transferred to a 1000 mL glass cylinder. The sample was brought to a final volume of 1000 mL with distilled water. The sample was shaken for 1 minute and then a reading was taken with the hydrometer at 30, 40, 50, and 60 seconds. If there was foam on the surface of the sample, several drops of amyl alcohol were added to the surface to break up the foam. The hydrometer was then removed, and then the process of shaking and hydrometer readings was repeated a total of three times. A reading with the hydrometer was then taken at 2 and 7.5 hours. A blank reading of the hydrometer was taken in a solution containing sodium hexametaphosphate and distilled water at each of the time points. The temperate of the samples were also recorded for each reading.

The particle size that settled out at each of the time points was calculated using the sedimentation equation and used to determine the percent of sand, silt, and clay in each of samples. Corrections were made for the density and the viscosity changes of the water due to changes in temperature. A linear regression was fitted to the measurements taken from 30 to 60 seconds and the exact point where all sand sized particles (>0.05 mm) had settled out. It was then assumed that all silt-sized particles (<0.05 mm and >0.002 mm) had settled out by 7.5 hours.

Results and Discussion:

Breakthrough Curves:

Saturated Flow:

The presence of *A. caliginosa* in the soil under saturated conditions affected solute flow as indicated by the dispersivity of the dye. Under saturated flow conditions, averaged over the addition of earthworms, the average dye recovery was 91.9%. When the transient model was used, the dispersivity required a log transformation, and there was an interaction between the column section and the presence of earthworms (P=0.004). The top section and the middle section with earthworms were statistically the same and had the highest average dispersivity at 65.0 cm. All other sections (the bottom with earthworms and all sections without earthworms) were the same and were different from the top two with an average dispersivity of 0.93 cm (Table 4.1).

The top and the middle sections were where the majority of the earthworms were recovered (Table 4.2 and 4.3), and these two sections covered the typical burrowing depth for *A. caliginosa* (Lee & Foster, 1991, Francis, *et al.*, 2001). Overall 73.3% of the adult earthworms that were added at the start of the study were recovered. This value is not an accurate representation of the number of earthworms that were alive at the end of study since some earthworms were present in the soil rings used to determine the water retention curves and these were not recovered. Of the adult earthworms that were recovered at the end of the study, 72.6% of the earthworms were found in the middle section and the remaining earthworms were in the top section. The zones of activity in the soil columns were very similar to what has been reported for field settings for the typical depth of activity for *A. caliginosa* (Pitkanen & Nuutinen, 1997).

Larger dispersivity values indicate that the solute had a more tortuous path to follow due primarily to large variation in pore sizes and connectivity. The larger value will also result in the pulse of the solute being spread over a much larger range of depths as it travels through the soil. Visual inspection of the saturated breakthrough curves with earthworms shows an initial pulse that came out of the soil column (Figure 4.2) due to large macropores created by the earthworms that allowed for the dye and the leachate to quickly travel through the soil profile. The rapid downward movement of the dye under saturated conditions in the top and middle sections indicates that many of the burrows in this section were interconnected, with some connections to the soil surface. In studies of the burrowing patterns of *A. caliginosa* it has been found that even though *A. caliginosa* are endogenic earthworms they still make burrows that have some connectivity to the soil surface (Lee & Foster, 1991).

When the breakthrough curves were developed on each of the column sections under saturated conditions, the columns were kept at saturation but the flow rate out of the bottom of the column sections was not consistent between all of the sections. In order to be able to compare the breakthrough curves of the columns, pore volumes of leachate were used instead of time.

When the average saturated breakthrough curves are compared visually (Figure 4.2) it is noticed that the earthworms resulted in the dye having a large initial pulse followed by a longer right tail. The effects on the breakthrough curves are only seen in the top and middle sections. Under saturated conditions, the ponded water will continue to move through the soil primarily by the large macropores and will have little flow through the rest of the soil matrix. When the dye was added to the column, it was applied to the saturated soil without a pond, resulting in the dye spreading across the entire soil surface and not limited to only flowing into the macropores. Preferential flow through the macropores when the soil is saturated will result in these areas having the dye removed quickly and then allowing for larger amounts of leachate to travel through the column without removing any of the dye. The less effective leaching through majority of the soil matrix leads to the larger right tail and the dye being distributed over a larger range of depths in the soil profile.

Even though the top and middle sections were affected by earthworms, the data showed a trend for a greater impact on the top section than the middle. The middle section with earthworms also had a more rapid downward movement of the dye than the middle section of the control column or the bottom section of a column with worms suggesting that some of the earthworm burrows did extend all the way through this section. However, when the middle section was separated from the bottom section and mounted on the ceramic plate there were not any visible channels at the bottom of the section. This, combined with the larger number of pore volumes for the initial pulse of dye to efflux tends to indicate that at least some of the burrows were open at the surface of the middle section but terminated before the bottom of this section. This scenario would result in the dye quickly moving through the middle column section via macropores, but then traveling through the smaller pores at the bottom. This would explain the initial peak being shifted to the right when compared to the top section.

The flow patterns that were observed in the top and middle sections would produce a leading edge of the solute pulse that is deep in the profile but the tailing edge would stay close to the surface and would slowly travel through the profile causing the solute to be distributed over a large area. The change in the dispersivity in the top and middle sections with earthworms compared to the other sections can have management implications.

The dye that was used was a nonabsorbing dye that had a negative charge; therefore, it did not bind with the soil or organic matter demonstrating the fastest rate of downward movement for a chemical. The earlier appearance of dye at the bottom of the sections would also be associated with a lower number of pore volumes of water being needed to move some of the dye deeper into the profile. Under saturated conditions, the more rapid movement of a solute in the presence of *A. caliginosa* may result in the movement of some anionic chemicals or fertilizers deeper into the profile, although the depth would be restricted to the burrowing depth of the earthworm.

However, there was also the longer right tail due to the dye moving into dead end pores or into pores that were not directly connected to the macropores, leaving the dye in areas that are not easily connected to the main flow of leachate through the column. The dye in these areas had to diffuse before it could be removed from the profile. For the column sections without earthworms, the breakthrough curves do not have a long right tail (Figure 4.2). The tails of the breakthrough curves under saturated conditions without earthworms were more evenly distributed around the main peak. Earthworms created pores that were of varying sizes and varying levels of connectivity since *A. caliginosa* backfills about 80% of their burrows (Francis, *et al.*, 2001). This burrowing behavior leaves the soil with many more tortuous paths through the soil that the solute has to follow.

The right tail contains a larger portion of the mass of the dye that was applied to the column. The mass in the right tail is the mass that was left in the soil profile following the initial pulse and was slower to leach out. When the number of pore volumes required to remove each quartile of the dye was compared between with and without *A. caliginosa,* a larger number of pores volumes was required to remove the dye with earthworms present (Table 4.4). The ability of the earthworms to increase the number of pore volumes needed to remove all of the dye would allow for the dye, or in a field setting the chemical of interest, to be more available in a zone that would increase the time of interaction with plants.

The higher amount of solute required to remove a chemical from the rooting zone may have both positive and negative impacts on the management of cropland. If the need is to have a fertilizer remain in the root zone for plants to be able to use it, then the effect is positive. However, if the soil has a salinity problem that is being mitigated, then the increased amount of water needed to remove the salts would have a negative effect due to an increase in the amount of clean water needed to remove the salts.

The effect of the change in dispersion under saturated conditions when applied to a field setting is not easily inferred from this data alone. In a field setting the distribution of earthworms would not be evenly distributed across the field (Holmstrup, *et al.*, 2011). There would be areas in the field where the solute would quickly travel deeper into the profile due to the earthworm burrows and other areas of the field where the solute would stay near the surface initially. There would also be locations that would have the solute moving as a much narrower band due to the limited earthworm activity. All of the effects that were seen in the laboratory under saturated flow may not have significant direct impact to Colorado field soils due to the very limited amount of time that field soil is saturated. However, the effects would be seen near the surface during large rainfall events and with the use of irrigation.

The type of flow pattern that was observed (the dye reaching the bottom of the column very quickly) could also be observed if there was sidewall flow, however it is not believe that this occurred. Before each of breakthrough curves were developed the side of each of the column sections was checked to determine if the soil had pulled away from the sides of the column during storage. If this was found, fine ground dry soil was put into any space between the soil and the side of the column. It was then wetted and packed in place along the edge to reduce the potential for edge effects and preferential flow down the sides of the column. In addition, if sidewall flow took place a similar flow pattern would have been observed in the control treatment. When columns are used to study the effect of earthworms on water flow, earthworms preferentially burrow on the sides of the PVC pipe (Francis & Fraser, 1998). Preferentially burrowing did take place to small extent, and was observed when the soil was removed from the PVC pipe. However, there were relatively few burrows on the sides of the pipe compared to the total number of burrows. In addition, a relatively large-diameter column (15 cm) was used to help reduce the effects of any burrowing on the sides of the column and reduce the percentage of the column affected by edge effects.

The dispersivity values for the saturated water flow with earthworms, as determined by the transient model, were approximately 80 times greater than values for no-earthworm controls. These values show that the earthworms had a large impact on the movement of solute, although the scale of the effect may be larger than what would be seen in the field. This would reduce the effects that were seen on the dispersivity of the solute but there would still be some effect. The analysis of intact soil columns from field plots with and without earthworms would need to be analyzed to determine the dispersivity in a field setting. This study did show though that the earthworms have an effect, but the extent of the effect in a field setting is unclear.

The dual porosity model with transient flow model was used to better explain the tail effect under saturated flow and to quantify these factors. The dispersion of the dye needed a log transformation, and it had the same interactions of treatment and column section (P=0.023) as the transient model. Again, with this model the top and middle sections with earthworms were different from all other sections and had a higher dispersivity (9.93 cm). The remainder of the column sections had an average dispersivity of 0.28 cm, and they were statistically all the same (Table 4.5). Earthworms should increase the amount of immobile water due to the partially backfilled pores (Francis, *et al.*, 2001) in the columns, but there was not a significant difference in the amount of immobile water (theta immobile) (P=0.612) or the rate of movement between mobile and immobile water (P=0.402).

Unsaturated Flow:

When the breakthrough curves were developed for the unsaturated soil it was expected that there would be no earthworm effect, but this was not the case. The transient model had an earthworm by section interaction (P=0.019) for the log transformed dispersivity values. An average of 92.6% of the dye was recovered in the unsaturated flow breakthrough curves. The results of the unsaturated breakthrough curves followed the same trends as the saturated, the top and middle sections with earthworms were the same but significantly different from all others (Figure 4.3). The top and middle sections with earthworms had an average dispersivity of 5.82 cm and the remainder of the sections had an average dispersivity of 0.59 cm. The dispersivity for the unsaturated flow was about an eleven-fold decrease than the saturated. In the unsaturated soil, the larger pores will not be water filled and therefore there will be fewer pores for the dye to travel through. In addition, the large macropores that went all the way through the top section of the column would not be transporting the solvent as much as in the saturated flow due to the pores not being water filled. Even without the macropores being involved with the solute transport the dispersivity was still about ten times higher with *A. caliginosa* than without.

These results indicate that the effects of earthworms on solute movement still exist even with their burrows are not water filled. One possible explanation is that earthworms changed the pore size distribution and the amount of dead end pore space, changing the flow patterns in the soil. An increase in variation of pore sizes would result in a more tortuous path for the solute to follow in order to leave the profile. In addition, many authors have shown that earthworms improve the aggregation of the soil (Lee, 1985). This would affect the movement of a solute through the soil. The improved aggregation in the soil can help the solute move faster through the soil.

With the transient dual porosity model there was only an effect on the log transformed values for dispersivity due to the presence or absence of earthworms (P=0.001). The presence of earthworms had a dispersivity of 0.94 cm, compared to 0.25 cm without earthworms. The effect of the earthworms was not limited to the top and middle sections. There was no effect on the amount of immobile water (P=0.299). While there is no direct method for measuring the amount of dead end pore space, it was assumed that if there was more dead end pore space there would be an increase in the amount of immobile water. This suggests that the earthworms did not affect the amount of dead end pore space. It is most likely that the results that were seen were due to the earthworms creating pores that were more effective for water movement and leading to areas that had limited flow. This is similar to what was seen under saturated conditions but to a lesser extent due to the pores involved being smaller.

The dispersivity values are lower for the transient model with dual porosity than the transient model alone for both saturated and unsaturated water flow. When the model attempted to fit the data in the transient model, the only variable that the model was able to adjust was dispersivity. When the dual porosity portion was added, there were two additional terms that the model could use to help explain the data. When theta immobile is increased, the amount of water that is in the mobile fraction will decrease and cause the curve to shift to the left. The total water content of the soil can be separated into immobile water (theta immobile) and mobile water (theta mobile). The term for the diffusion of the dye between the mobile and immobile fractions will affect the length of the tail of the curve. When the model had the ability to use these additional factors to explain the shape of the curve, the dispersivity was reduced, because the variability in the data could be explained by more parameters.

Water Retention Curves:

The introduction of *A. caliginosa* to the soil columns did not have an effect on the saturated water holding capacity (P=0.260). However, the residual water content was increased by the addition of *A. caliginosa*. There was an interaction between earthworms and the sections of the columns (P=<0.001). The effect of *A. caliginosa* was only seen in the top and middle sections, all of the other sections, both with and without earthworms were the same. The top section with earthworms had a residual volumetric water content of 0.154, the middle section with earthworms was 0.135, and all of the other sections averaged 0.074. When the columns of soil were separated for analysis, there were a large number of burrows visible within the top and middle sections, or the 0-30 cm depth increment, which corresponds with the primary zone of activity for *A. caliginosa* (Lee & Foster, 1991, Pitkanen & Nuutinen, 1997). In addition, when the soil was removed from the soil columns, the earthworms were primarily found in these two sections and very few were found in the bottom section (Table 4.2 and 4.3). The increase in the residual water holding capacity may be due to the earthworm casts. Smagin and Prusak (2008) have shown that the cast material has a higher water retention than the bulk soil. The casts tend to have a higher concentration of fine soil particles since the earthworms preferentially ingest clay and silt and a coat them with a hydrophilic surface layer. The hydrophilic coating will increase the amount of water that held by the soil when the capillary water has drained and only the water films are left. The ability for the soil to hold more water at the drier end of the curve may aid in the soil having water available for more of the year for plants to use.

The top and middle sections with *A. caligionsa* exhibited an interaction between treatment and section (P=0.030) for the alpha term (Table 4.6) of the Brooks-Corey equation. The negative inverse of the alpha term is the air entry value, the tension at which air will continuously be present in the soil. The top and middle sections with earthworms were similar to each other and different from all other column sections. These sections with earthworms had an average alpha value of 0.407 or an air entry value of -2.46 cm ($-2.4X10^{-4}$ MPa). The remainder of the sections had an average alpha value of 0.1185 or an air entry value of -8.44 cm ($-8.3X10^{-4}$ MPa). The air entry value is affected by capillary pressure of the pore space in the soil. When the pore size increases, the capillary pressure will decrease, allowing air to enter the soil at a lower tension. An approximation of the pore size that will drain at a given tension is $0.15cm^2/h$ where cm equals the radius of the pore. When earthworms were present the pores that drained initially had a radius of 0.009 cm and in the absence of earthworms, the radius was 0.003 cm. *A. caligionsa* backfills most of their burrow but the ones that are left open will be much larger than soil pore space (Francis*, et al.*, 2001) and will drain with less tension. The faster draining in the presence of earthworms may affect the availability of water if there are long periods between rainfall events.

The addition of *A. caliginosa* to the soil also had an effect on the slope of the water retention curve. The slope of the line is a representation of the pore size distribution of the soil and in the Brooks-Corey equation, the n term explains the pore size distribution. In the control soil, the n term was 0.253 and the earthworms reduced it to 0.152. When the pores are of similar size, the n term will be larger and will result in a steeper slope. If there is a large distribution of pore sizes, the soil will drain slower and over a larger range of tensions, represented as a flatter slope of the water retention curve. As the earthworms burrowed in the soil and backfilled some of their burrows there would be many pores created of varying sizes. There would also be the formation of dead end pore spaces, pores that are not connected to the main flow of water through the soil. The digestion of the soil by the earthworms creating the hydrophilic coating on their cast material (Smagin & Prusak, 2008) may have resulted in an increase in the capillary pressure needed to drain the smaller pores and increasing the water content at the soil at higher tensions, thus flattening the curve.

The earthworms created large macropores in the soil, and it would be expected that these pores will result in the soil drying faster, which was confirmed by the lower air entry value. However, the data on the pore size distribution showed that the presence of earthworms resulted in the soil drying slower and the residual water holding capacity was higher. The data suggest that presence of earthworms will result in the soil draining faster initially but it will hold water longer. This can help the soil absorb more water before runoff would start. Earthworms are known to increase the infiltration of water (Lee, 1985, Smettem & Collisgeorge, 1985), and it was believed that this was due to the burrows that were open to the surface. The data from this study indicates that more water may be able to enter the profile due to the water moving downward more rapidly due to the larger pores and the lower air entry value.

Slightly different results were obtained when soil sections were analyzed with the Decagon WP4 dew point potentiometer. This meter allowed for more data points to be collected at the drier end of the water retention curve, helping to better represent the residual water content. When these points were included in the development of the water retention curves, the data from the 1.5 MPa plate did not match the curve very well. It shows that the soil had not reached equilibrium at this tension within the one-week time period. With the additional points, the effects on saturated water content were the same as without the additional points. The top and middle section with earthworms still had the highest residual water content but they were also statistically similar as the top and middle sections without earthworms (Table 4.7). There was still a large difference in mean values between the top two sections with earthworms and the other sections, but there was a large amount of variation between the samples. If the surfaces of the soil were affected by the earthworms' digestion of the soil, the results would be affected by the amount of soil in the sample that has been ingested by the earthworms. The soil sample size was much smaller with the dew point potentiometer than the sample sizes used on the plate.

The alpha term did not show an influence by earthworms or sections, as it did without the drier points. When Hydrus-1D fitted a curve to the data it used the values for the alpha, n, and residual water content as a curve fitting parameter. If the curve has to fit many points that are at a higher tension than between saturation and the start of draining, there is a greater chance for the term being fitted to lose the relevance to the soil property it is intending to describe and become solely a curve fitting parameter. When the Brooks-Corey model was developed, researchers used data points out to approximately 0.015 MPa of tension (Brooks & Corey, 1964). Therefore, the data points collected above 1.5 MPa are well above the range the model design and the alpha term would have more application relevance if it only included these data points that were closer to saturation.

The n term with the dew point readings was significantly different for the interaction of treatment by position within section (Table 4.8). The lowest n term was the same for the position B (Figure 4.1) and C, and position C was the same as position A. Positions B and C were the zone where the earthworms would be the most active based on their burrowing characteristics (Lee & Foster, 1991, Pitkanen & Nuutinen, 1997). It was also observed when the soil columns were sampled that these zones had a large amount of earthworm activity. Position A exhibited limited burrowing. The earthworms were applied to the soil surface and would have to burrow though this depth to reach the deeper depths. The addition of the data points at the drier end would aid in improving the accuracy of this term. The data points at the drier end of the curve increase the accuracy of the residual water content and the slope of the line, which is represented by the n term.

Soil Texture:

It was predicted that the earthworms would result in an increase in the silt and clay accumulations in sections where they were active; however, the data did not support this prediction. The earthworm activity did not resulted in a change in the percentage of any of the fractions. Earthworms have been shown by many authors to increase aggregate stability (Lee, 1985), so it is possible that the method that was used to disperse the soil was not effective in breaking up the aggregates created by the earthworms. If the aggregates were still intact they would settle as larger particles, resulting in an over estimation of sand and possibly silt and an underestimation of clay. The changes that were expected to take place were small and it is possible that the method used did not allow for enough resolution to see the changes. The effects would be more likely seen if comparisons were made between the bulk soil and cast material.

Conclusion:

The results of the breakthrough curve study showed that earthworms affect solute dispersivity under both saturated and unsaturated water conditions, confirming the hypothesis that the earthworms would affect the dispersivity under saturated conditions; however, the affect was also seen in the unsaturated samples, contrary to our predictions. This indicated that the *A. caliginosa* were making other structural changes to the soil in addition to pores they created. The results from the water retention curves showed that the earthworms changed the pore size distribution, and thus altered flow paths in the soil. The increase in variety of pore sizes would lead to a more tortuous flow through the soil, thus increasing the dispersivity even under unsaturated conditions.

Earthworms increased residual water content and the rate that water was lost, as predicted. There was some variation in the results if the drier points were included or not. These differences can be explained by looking at the intended interpretation of the parameter and how the parameter was developed for the equation. The earthworms did not have an effect on the saturated water content but they did increase the residual water content. As the soil moves from saturation to dry, the addition of worms causes the soil to start drying faster in the zones that had the highest amounts of earthworm burrowing. However, once drying began, the control soil dried at a faster rate than the soil with earthworms. By the time the soil was air-dried, the soil with earthworms still had higher residual water content.

In semiarid environments such as Colorado, that ability to increase the soil's capacity to hold water has great importance and value to producers. The burrowing activity of the earthworms also changed the way that the water flowed as shown by the breakthrough curves and the combination of these may change the effect on the movement of not only water downward but also the upward movement of water due to evaporation. If under field conditions the earthworms are creating a large number of pore spaces that are not connected to each other, it may have a similar effect as having a course layer in the profile. When a course-textured layer of soil is on top of a fine textured layer, the ability of water to move upward by capillary forces is reduced, keeping more of the deeper moisture in the root zone.

The effects that were seen on the soil took place only in the portions of the profile that had earthworm activity. Each of the sections was separated into small sections and they were analyzed in thin subsections. However, these conditions are not what would be seen in the field; each of the sections would be one continuous profile. Some of the effects observed in the soil subsections may not be the same as the effects to the soil profile as a whole. There would need to be further study with columns that represent a larger range of depths, or modeling would need to be done to combine the effects of the individual profile sections into one continuous profile.

The exact implications of the effects that the earthworms will have in the field have yet to be determined. However, this study demonstrated the potential for earthworms to increase solute dispersivity, which might impact management decisions regarding the application of fertilizer and other chemicals when earthworms are present in the soil. Currently in Colorado, earthworms are primarily found in irrigated fields, but a few are being found in no-till dryland fields. As the presence of earthworms in agricultural fields in Colorado increase, the effects of earthworms may warrant further study at a field plot level.

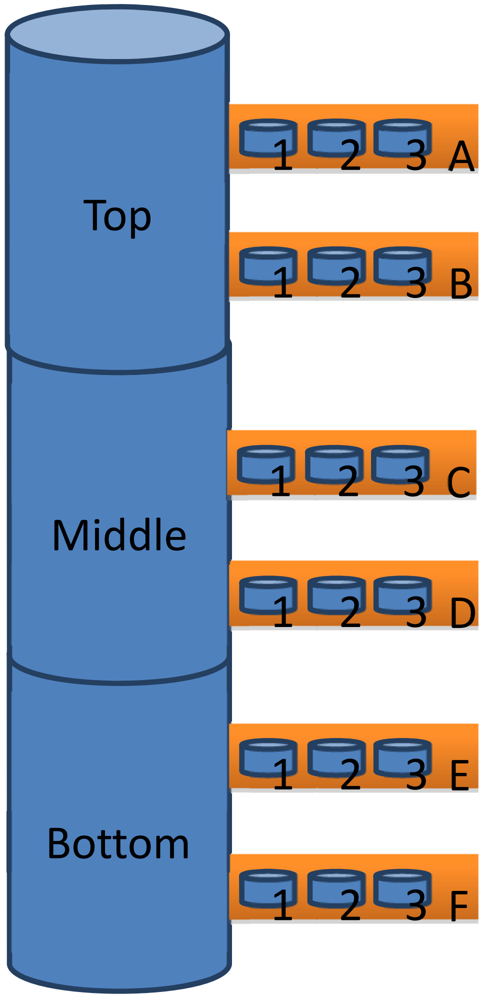

Figure 4.1. Sampling pattern used for the soil rings for the development of water retention curves. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Table 4.1. The dispersivity under saturated flow conditions with the transient model. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken and modeled with Hydrus-1D.

Column Section	*A. caliginosa* Present	Dispersivity (cm)
Top	No	1.22b
Middle	No	0.93b
Bottom	No	0.61b
Top	Yes	126a
Middle	Yes	33.7a
Bottom	Yes	1.10b

Table 4.2. The number of earthworms recovered from each of the columns with earthworms by section. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before the earthworms were recovered by hand sorting.

Replication	Section	Number of Adults	Number of Juveniles
1	Top	2	-
1	Middle	9	10
1	Bottom	-	4
2	Top	4	4
2	Middle	7	6
2	Bottom	-	-
3	Top	3	-
3	Middle	7	9
3	Bottom	-	-
4	Top	3	3
4	Middle	9	8
4	Bottom	-	-

Table 4.3. The percentage of recovered adult *A. caliginosa* by column section. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before the earthworms were recovered by hand sorting.

Section	Percentage
Top	27.4%
Middle	72.6%
Bottom	0.00%

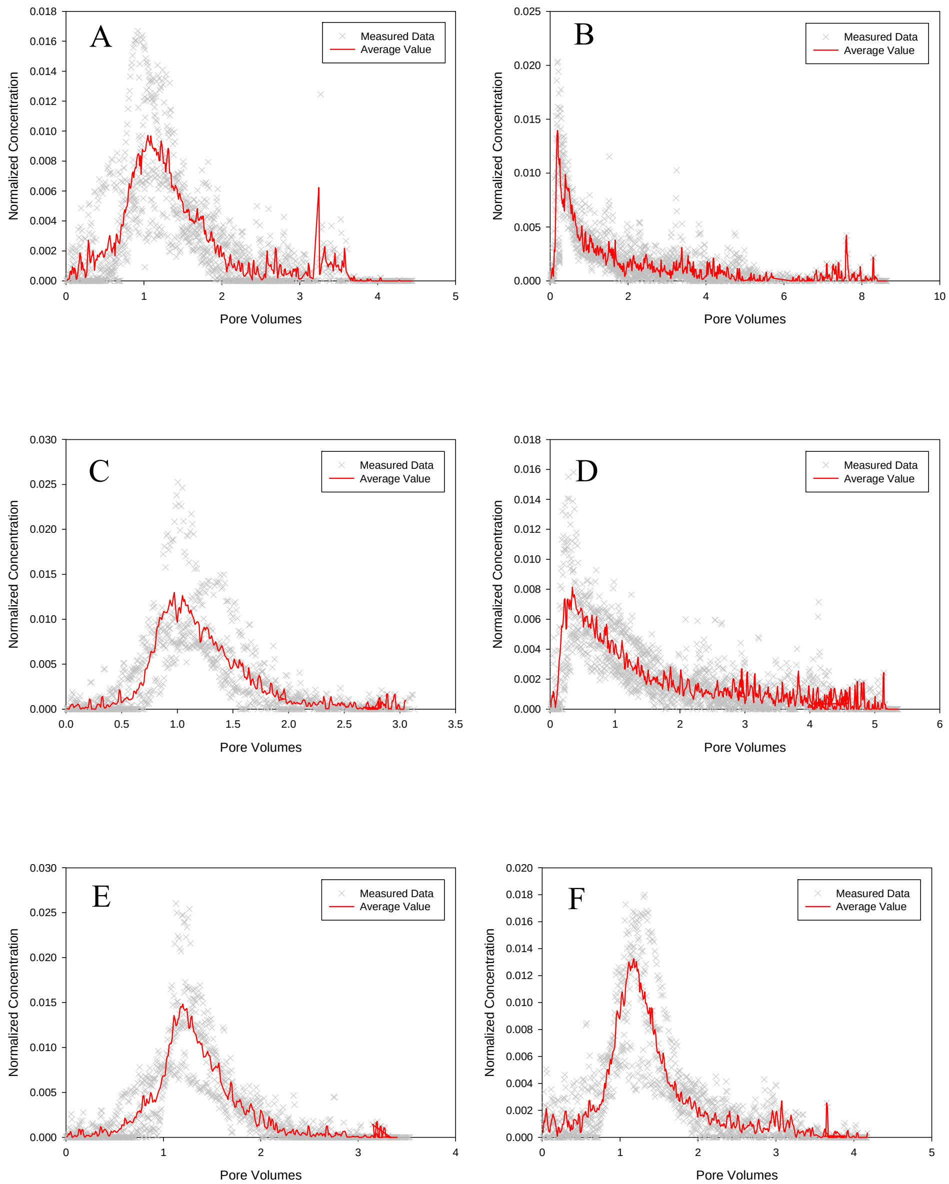

Figure 4.2. Average breakthrough curves for saturated water flow. A. top section without earthworms, B. top section with earthworms, C. middle section without earthworms, D. middle section with earthworms, E. bottom section without earthworms, F. bottom section with earthworms. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken and modeled with Hydrus-1D.

Table 4.4. The cumulative pore volumes required to remove the dye from the column sections with saturated flow. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. The letters are the comparisons between with and without *A. caliginosa* at a given quartile, and comparisons are not made between the quartiles.

Quartile	**With *A. caliginosa***	**Without *A. caliginosa***
1	1.14a	0.781b
2	2.30a	1.56b
3	3.46a	2.38b
4	4.58a	3.15b

Table 4.5. The dispersivity under saturated flow conditions with the transient and dual porosity model. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken and modeled with Hydrus-1D.

Column Section	***A. caliginosa* Present**	**Dispersivity (cm)**
Top	No	0.345b
Middle	No	0.152b
Bottom	No	0.289b
Top	Yes	12.4a
Middle	Yes	7.97a
Bottom	Yes	0.457b

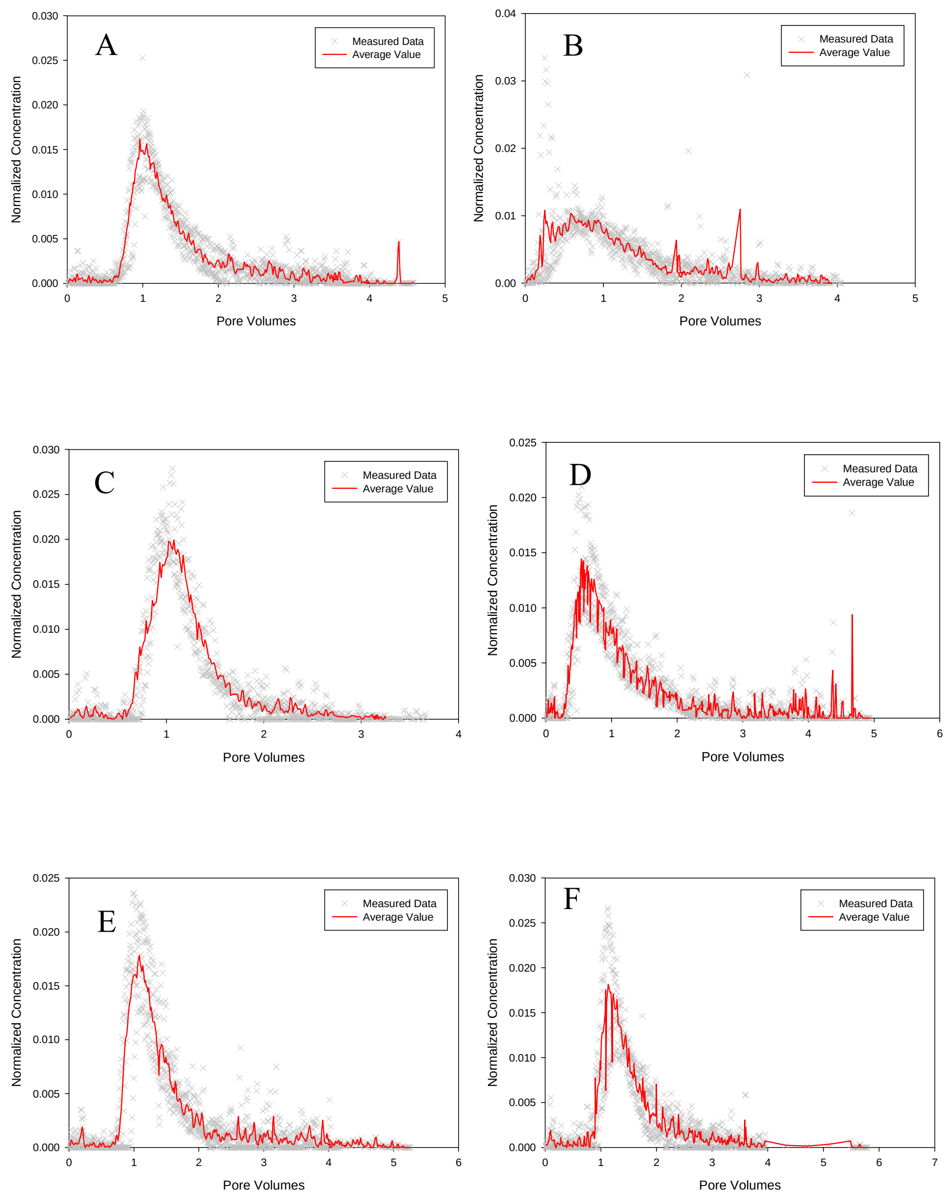

Figure 4.3. Average breakthrough curves for unsaturated flow. A. top section without earthworms, B. top section with earthworms, C. middle section without earthworms, D. middle section with earthworms, E. bottom section without earthworms, F. bottom section with earthworms. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken and modeled with Hydrus-1D.

Table 4.6. The effect of the addition of earthworms on the alpha term of the Brooks-Corey equation. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. Average values labeled with different letters are significantly different.

Treatment	**Section**	**Average**
With earthworms	Top	0.361a
With earthworms	Middle	0.453a
With earthworms	Bottom	0.156b
Without earthworms	Top	0.105b
Without earthworms	Middle	0.129b
Without earthworms	Bottom	0.0846b

Table 4.7.Average residual water content at 245 MPa of tension for the data with the dew point potentiometer readings. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. Average values labeled with different letters are significantly different at an alpha value of 0.05.

Treatment	**Section**	**Residual water content average**
With earthworms	Top	0.0771a
With earthworms	Middle	0.0751a
With earthworms	Bottom	0.0496b
Without earthworms	Top	0.0577ab
Without earthworms	Middle	0.0526ab
Without earthworms	Bottom	0.0575b

Table 4.8. Average n terms from the Brooks-Corey equation with the data points from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. The n term is the pore size distribution of the soil. Average values labeled with different letters are significantly different at an alpha value of 0.05.

Treatment	Section	Position	N term average
With earthworms	Top	A	0.164cd
With earthworms	Top	B	0.142e
With earthworms	Middle	C	0.152de
With earthworms	Middle	D	0.169bc
With earthworms	Bottom	E	0.188ab
With earthworms	Bottom	F	0.189ab
Without earthworms	Top	A	0.186abc
Without earthworms	Top	B	0.185abc
Without earthworms	Middle	C	0.196a
Without earthworms	Middle	D	0.188abc
Without earthworms	Bottom	E	0.194ab
Without earthworms	Bottom	F	0.184abc

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CHAPTER 5: CONCLUSIONS FOR THE IMPLICATIONS FOR THE INTRODUCTION OF EARTHWORMS IN A BIOSOLIDS AMENDED AGROECOSYSTEM

The presence of earthworms in agricultural fields in eastern Colorado has the potential affect soil physical, chemical, and biological quality. The effects may be seen if the earthworms move to these areas on their own or if they are introduced to the agroecosystem and then management practices are used to promote their survival and expansion. The change to no-till has several potential negative impacts to the soil due to higher compaction of the soil that reduces air and water movement, and less incorporation of organic matter. The results of these studies showed that earthworms have the potential to lessen these effects in eastern Colorado soils.

The results of our study showed that earthworms are able to survive in the soil under optimal moisture as well as cyclical periods of moderate drought stress. The level of drought stress that was seen in this study may not be the extent of what would be seen in the field, especially on the drier end of the spectrum. However, the lab setting does not allow for the use of containers that are large enough to provide the earthworms the ability to move deeper into the soil to find soil with more moisture and cooler temperatures which should increase survival. The use of field studies to determine the survivability of earthworms under drought stress are needed to fully understand the tolerance to drought.

The water retention curves also indicated another level of complexity to the ability of earthworms to survive drought since the earthworms changed the soil water retention curves. When the drought stress study was conducted, the earthworms were placed in the pots and the drought stress started almost immediately. However, the water retention curves were measured on soil that had active earthworms for 16 weeks under optimal conditions. One factor that may have an impact on the ability of the earthworms to survive is the length of time that they have in the soil before the drought stress begins. If they have time to create burrows in the soil and affect the water retention curves the survival rates may be different from what was observed. However, another possibility has to be considered as well; the increase in the larger macropores in the soil would allow for not only faster solute movement but also for increased gas flow. The increased gas flow could also lead to the soil drying faster and a higher mortality rate of earthworms.

In the field, earthworms showed a potential to change the mineralization rate of organic matter and increase nitrogen availability. Here, only one species of earthworm was studied, and when field sampling of earthworms across Colorado takes place there are usually several species found together (Reynolds, 2011), and they could have different feeding strategies. *A. caliginosa* was used in these studies, and it feeds on organic matter and does not bring vegetation from the surface into the soil to eat as anecic earthworms do. If both anecic and endogenic are in the same fields, there will be some competition between the two groups of earthworms but there could also be a benefit for the endogenic species.

In eastern Colorado, agricultural soils have relatively low organic matter content (~1%), but the content seems adequate for *A. caliginosa* because the addition of an organic amendment such as biosolids did not enhance the survival of the earthworms. However, the downward movement of plant residue and other surface organic matter by anecic species may prove to be beneficial to endogenic species as the organic matter decomposes.

Following the completion of the laboratory incubation study, questions were raised about what the implications of increased NO_3-N and NH_4-N and the changes in the soil structure and water flow. The results were not exactly as expected. The earthworms caused the soil to have a quick initial pulse of solute that moved through the profile under saturated conditions. After the initial pulse, more leachate was required to move the solute in soil with earthworms than without. It is not very likely that the soil will be under saturated conditions under dryland production, therefore the results of the unsaturated flow have more application to a field setting, and the results showed that there was no difference in the movement of solute and the amount of leachate required to move the solute. These results indicate that even though earthworms have a large initial impact on solute movement, they may not effect solute movement or to slow the movement depending on the level of saturation of the soil.

This series of studies showed that there can be large impacts of earthworms on soil and that the activities of the earthworms have implications for more than just one property under study. It will be necessary to look at the effects of earthworms in a field setting and to approach the impacts of earthworms in dryland agriculture from an agroecosytem level. There are many effects of the earthworms that are interrelated and failing to understand these relationships may lead to the true impacts being missed.

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APPENDIX A: SURVIVAL OF *APORRECTODEA CALIGINOSA* AND ITS EFFECTS ON NUTRIENT AVAILABILITY IN BIOSOLIDS AMENDED OR UNAMENDED SOIL

Table A .1. ANOVA table of the effects on the percent change in earthworm. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on the percent change in earthworm mass.

Parameter	Source of Variation	F Value	P Value
Percent Change in Earthworm Mass	Time	1.68	0.185
	Biosolids	4.81	0.038
	Time x Biosolids	1.40	0.264
	Replication	2.86	0.057

Table A.2. ANOVA table for soil total C and N, inorganic C, and organic C, from the non-sterilized soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Total N	Time	23.1	<0.001
	Biosolids	11.5	0.001
	Time x Biosolids	1.36	0.245
	Earthworms	1.28	0.263
	Time x Earthworms	0.94	0.461
	Biosolids x Earthworms	1.34	0.251
	Time x Biosolids x Earthworms	0.21	0.957
	Earthworms x Replication	0.43	0.729
	Replication	1.23	0.306
Total C	Time	16.3	<0.001
	Biosolids	6.25	0.015
	Time x Biosolids	0.58	0.714
	Earthworms	0.01	0.923
	Time x Earthworms	0.49	0.784
	Biosolids x Earthworms	0.09	0.765
	Time x Biosolids x Earthworms	0.75	0.589
	Earthworms x Replication	0.32	0.808
	Replication	2.52	0.066
Inorganic C	Time	34.2	<0.001
	Biosolids	1.58	0.213
	Time x Biosolids	0.26	0.932
	Earthworms	1.14	0.289
	Time x Earthworms	0.35	0.878
	Biosolids x Earthworms	0.26	0.613
	Time x Biosolids x Earthworms	0.11	0.990
	Earthworms x Replication	0.64	0.589
	Replication	0.64	0.814
Organic C	Time	20.9	<0.001
	Biosolids	7.05	0.010
	Time x Biosolids	0.52	0.800
	Earthworms	0.06	0.840
	Time x Earthworms	0.41	0.725
	Biosolids x Earthworms	0.13	0.663
	Time x Biosolids x Earthworms	0.65	0.855
	Earthworms x Replication	0.26	0.855
	Replication	2.55	0.064

Table A.3. ANOVA table for soil NO_3-N and NH_4-N, and microbial biomass C and N for the non-sterilized soil after earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
NO_3-N	Time	180	<0.001
	Biosolids	481	<0.001
	Time x Biosolids	21.9	<0.001
	Earthworms	3.13	0.082
	Time x Earthworms	0.36	0.873
	Biosolids x Earthworms	0.19	0.666
	Time x Biosolids x Earthworms	0.58	0.717
	Earthworms x Replication	0.36	0.779
	Replication	0.46	0.711
NH_4-N	Time	8.09	<0.001
	Biosolids	114	<0.001
	Time x Biosolids	16.4	<0.001
	Earthworms	3.09	0.084
	Time x Earthworms	1.74	0.139
	Biosolids x Earthworms	0.29	0.590
	Time x Biosolids x Earthworms	1.45	0.220
	Earthworms x Replication	1.72	0.171
	Replication	0.33	0.803
Microbial Biomass C	Time	20.0	<0.001
	Biosolids	5.69	0.020
	Time x Biosolids	1.41	0.232
	Earthworms	0.00	0.963
	Time x Earthworms	0.50	0.777
	Biosolids x Earthworms	0.03	0.864
	Time x Biosolids x Earthworms	0.72	0.609
	Earthworms x Replication	0.09	0.967
	Replication	5.90	0.001
Microbial Biomass N	Time	22.6	<0.001
	Biosolids	0.50	0.483
	Time x Biosolids	4.99	0.001
	Earthworms	0.05	0.832
	Time x Earthworms	1.95	0.099
	Biosolids x Earthworms	0.40	0.531
	Time x Biosolids x Earthworms	0.97	0.444
	Earthworms x Replication	0.36	0.784
	Replication	3.53	0.020

Table A.4. ANOVA table for soil pH and electrical conductivity for the non-sterilized soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
pH	Time	16.3	<0.001
	Biosolids	32.1	<0.001
	Time x Biosolids	2.08	0.079
	Earthworms	0.04	0.844
	Time x Earthworms	0.09	0.994
	Biosolids x Earthworms	0.26	0.613
	Time x Biosolids x Earthworms	0.11	0.990
	Earthworms x Replication	0.05	0.985
	Replication	5.43	0.002
EC	Time	84.1	<0.001
	Biosolids	477	<0.001
	Time x Biosolids	20.4	<0.001
	Earthworms	1.59	0.212
	Time x Earthworms	0.29	0.917
	Biosolids x Earthworms	1.27	0.265
	Time x Biosolids x Earthworms	0.68	0.642
	Earthworms x Replication	0.21	0.890
	Replication	0.49	0.687

Table A.5. ANOVA table for AB-DTPA extractable Ba, Cd, Cr, and Cu for the non-sterilized soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Ba	Time	31.1	<0.001
	Biosolids	82.1	<0.001
	Time x Biosolids	7.50	<0.001
	Earthworms	0.89	0.349
	Time x Earthworms	0.11	0.990
	Biosolids x Earthworms	0.94	0.336
	Time x Biosolids x Earthworms	0.45	0.815
	Earthworms x Replication	1.09	0.362
	Replication	0.60	0.615
Cd	Time	5.38	<0.001
	Biosolids	0.21	0.648
	Time x Biosolids	2.49	0.034
	Earthworms	0.01	0.933
	Time x Earthworms	0.11	0.990
	Biosolids x Earthworms	0.07	0.800
	Time x Biosolids x Earthworms	0.26	0.931
	Earthworms x Replication	0.73	0.540
	Replication	1.63	0.191
Cr	Time	14.0	<0.001
	Biosolids	0.05	0.825
	Time x Biosolids	0.23	0.946
	Earthworms	6.85	0.011
	Time x Earthworms	2.35	0.051
	Biosolids x Earthworms	0.18	0.673
	Time x Biosolids x Earthworms	0.11	0.991
	Earthworms x Replication	0.33	0.8045
	Replication	0.94	0.429
Cu	Time	26.2	<0.001
	Biosolids	1.49	0.226
	Time x Biosolids	1.17	0.332
	Earthworms	0.32	0.571
	Time x Earthworms	0.18	0.968
	Biosolids x Earthworms	0.00	0.999
	Time x Biosolids x Earthworms	0.08	0.995
	Earthworms x Replication	0.23	0.877
	Replication	1.96	0.130

Table A.6. ANOVA table for AB-DTPA extractable Fe, K, Mn, and Mo for the non-sterile soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Fe	Time	29.9	<0.001
	Biosolids	2.64	0.109
	Time x Biosolids	0.32	0.900
	Earthworms	1.06	0.306
	Time x Earthworms	0.75	0.592
	Biosolids x Earthworms	0.02	0.875
	Time x Biosolids x Earthworms	0.19	0.965
	Earthworms x Replication	0.26	0.857
	Replication	7.30	<0.001
K	Time	50.0	<0.001
	Biosolids	0.14	0.712
	Time x Biosolids	0.69	0.633
	Earthworms	0.01	0.928
	Time x Earthworms	0.11	0.990
	Biosolids x Earthworms	0.03	0.853
	Time x Biosolids x Earthworms	0.18	0.967
	Earthworms x Replication	0.25	0.864
	Replication	2.07	0.113
Mn	Time	69.4	<0.001
	Biosolids	0.03	0.865
	Time x Biosolids	0.27	0.9278
	Earthworms	0.33	0.571
	Time x Earthworms	1.07	0.387
	Biosolids x Earthworms	1.84	0.180
	Time x Biosolids x Earthworms	0.63	0.676
	Earthworms x Replication	1.94	0.132
	Replication	4.28	0.008
Mo	Time	15.2	<0.001
	Biosolids	0.14	0.709
	Time x Biosolids	1.68	0.152
	Earthworms	0.06	0.811
	Time x Earthworms	0.51	0.764
	Biosolids x Earthworms	0.26	0.614
	Time x Biosolids x Earthworms	0.78	0.568
	Earthworms x Replication	0.63	0.601
	Replication	3.92	0.012

Table A.7. ANOVA table for AB-DTPA extractable Na, Ni, P, and Pb for the non-sterile soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Na	Time	119	<0.001
	Biosolids	29.1	<0.001
	Time x Biosolids	1.49	0.207
	Earthworms	2.37	0.129
	Time x Earthworms	0.80	0.555
	Biosolids x Earthworms	0.25	0.617
	Time x Biosolids x Earthworms	1.78	0.129
	Earthworms x Replication	1.05	0.378
	Replication	1.42	0.246
Ni	Time	59.5	<0.001
	Biosolids	0.17	0.679
	Week x Biosolids	0.99	0.428
	Earthworms	0.42	0.518
	Time x Earthworms	0.49	0.783
	Biosolids x Earthworms	0.48	0.491
	Time x Biosolids x Earthworms	0.48	0.787
	Earthworms x Replication	1.17	0.328
	Replication	2.16	0.102
P	Time	27.3	<0.001
	Biosolids	0.89	0.349
	Time x Biosolids	0.35	0.882
	Earthworms	0.16	0.694
	Time x Earthworms	0.19	0.964
	Biosolids x Earthworms	1.18	0.282
	Time x Biosolids x Earthworms	0.20	0.963
	Earthworms x Replication	0.19	0.903
	Replication	6.31	0.001
Pb	Time	66.8	<0.001
	Biosolids	0.20	0.655
	Time x Biosolids	1.27	0.290
	Earthworms	0.18	0.675
	Time x Earthworms	0.30	0.909
	Biosolids x Earthworms	0.48	0.490
	Time x Biosolids x Earthworms	0.39	0.851
	Earthworms x Replication	1.92	0.135
	Replication	6.43	0.001

Table A.8. ANOVA table for AB-DTPA extractable Si, Sr, Ti, and V for the non-sterile soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Si	Time	32.4	<0.001
	Biosolids	0.75	0.391
	Time x Biosolids	0.66	0.658
	Earthworms	0.41	0.525
	Time x Earthworms	0.62	0.688
	Biosolids x Earthworms	0.01	0.944
	Time x Biosolids x Earthworms	0.64	0.670
	Earthworms x Replication	0.68	0.566
	Replication	2.07	0.113
Sr	Time	13.2	<0.001
	Biosolids	1.48	0.228
	Time x Biosolids	1.91	0.104
	Earthworms	1.46	0.231
	Time x Earthworms	0.38	0.860
	Biosolids x Earthworms	0.19	0.663
	Time x Biosolids x Earthworms	0.41	0.841
	Earthworms x Replication	0.06	0.979
	Replication	0.92	0.437
Ti	Time	370	<0.001
	Biosolids	0.72	0.398
	Time x Biosolids	0.35	0.880
	Earthworms	0.88	0.352
	Time x Earthworms	1.08	0.378
	Biosolids x Earthworms	0.02	0.885
	Time x Biosolids x Earthworms	0.23	0.948
	Earthworms x Replication	0.91	0.442
	Replication	2.23	0.094
V	Time	362	<0.001
	Biosolids	0.69	0.409
	Time x Biosolids	0.55	0.739
	Earthworms	0.05	0.817
	Time x Earthworms	0.27	0.926
	Biosolids x Earthworms	1.05	0.310
	Time x Biosolids x Earthworms	0.17	0.974
	Earthworms x Replication	0.47	0.706
	Replication	2.68	0.054

Table A.9. ANOVA table for AB-DTPA extractable Zn for the non-sterilized soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on AB-DTPA extractable Zn.

Parameter	**Source of Variation**	**F Value**	**P Value**
Zn	Time	20.7	<0.001
	Biosolids	13.3	0.001
	Time x Biosolids	0.99	0.434
	Earthworms	0.18	0.674
	Time x Earthworms	0.19	0.967
	Biosolids x Earthworms	0.08	0.772
	Time x Biosolids x Earthworms	0.16	0.976
	Earthworms x Replication	0.08	0.968
	Replication	1.31	0.279

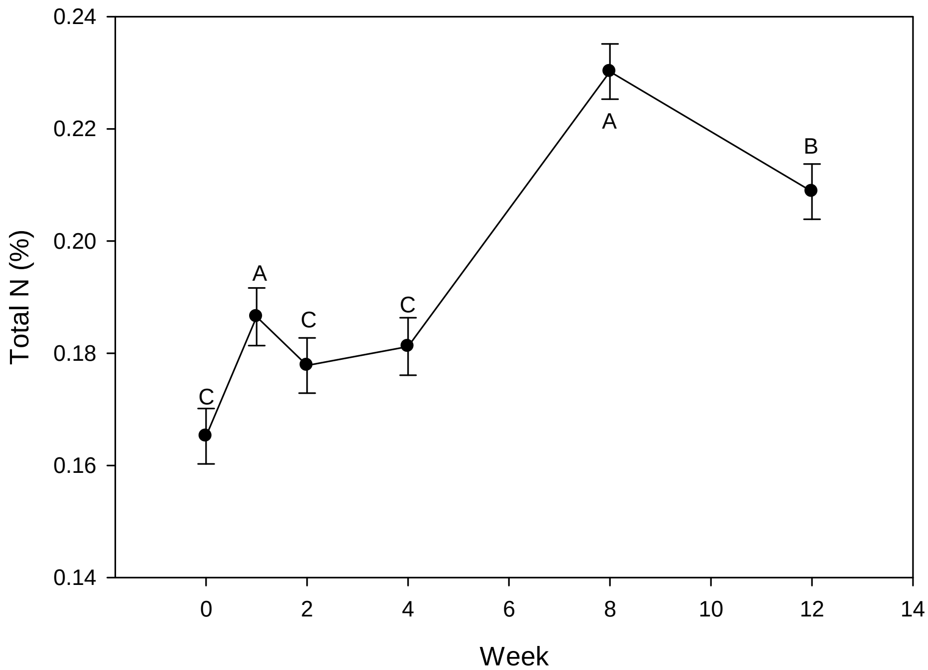

Figure A.1. Total percent N in the soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on percent total soil N when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.10. Total percent of N in the soil with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on percent total soil N when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average Total % N	**Standard Error**
With Biosolids	0.196a	0.00288
Without Biosolids	0.185b	0.00288

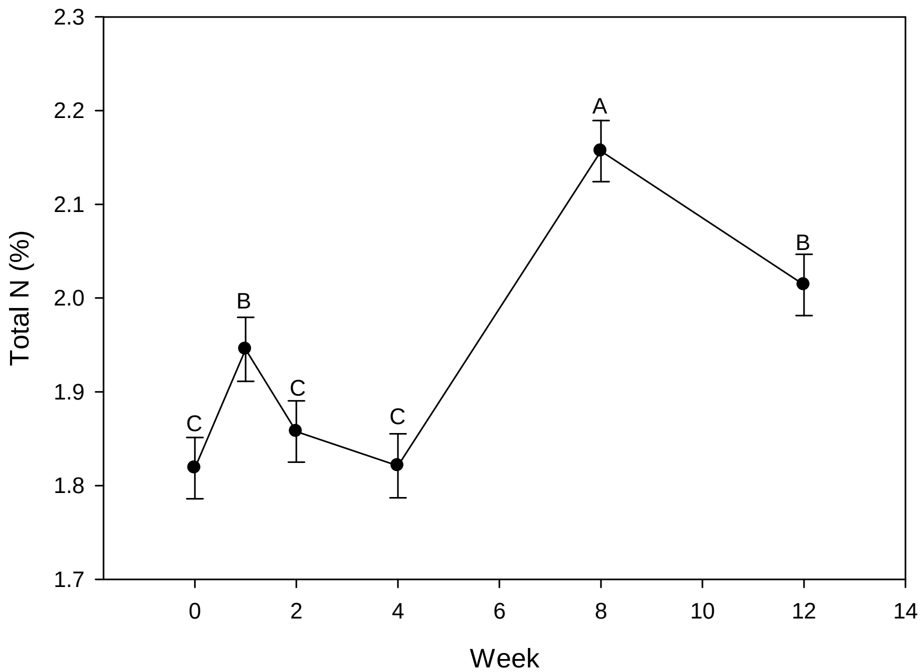

Figure A.2. Total soil N averaged over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on percent total soil N when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.11. Total percent of N in the soil with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on percent total soil N when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average Total % N	**Standard Error**
With Biosolids	1.97a	0.0192
Without Biosolids	1.90b	0.0192

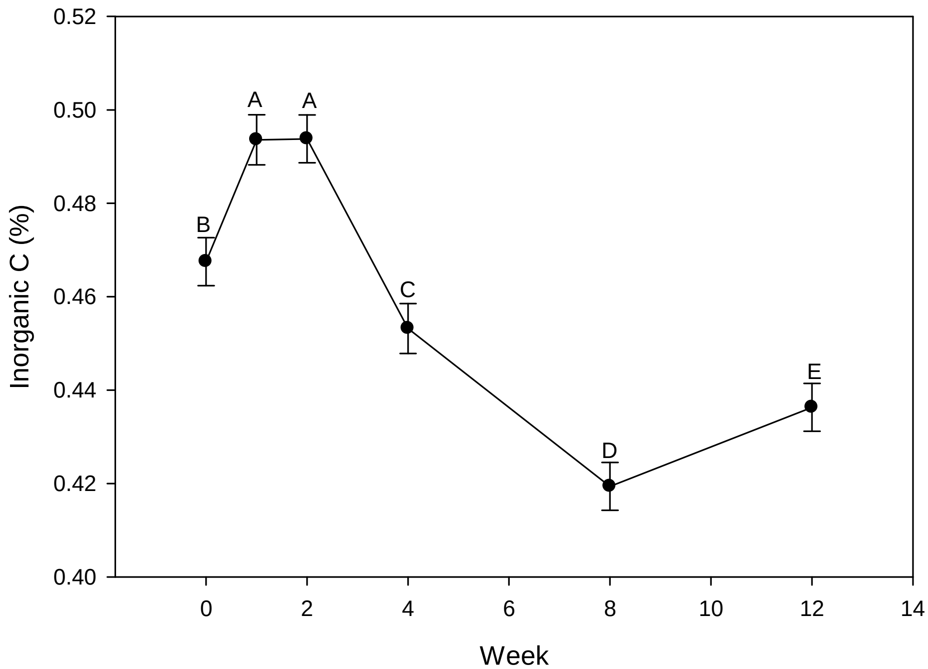

Figure A.3. Percent inorganic C in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on percent inorganic C when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

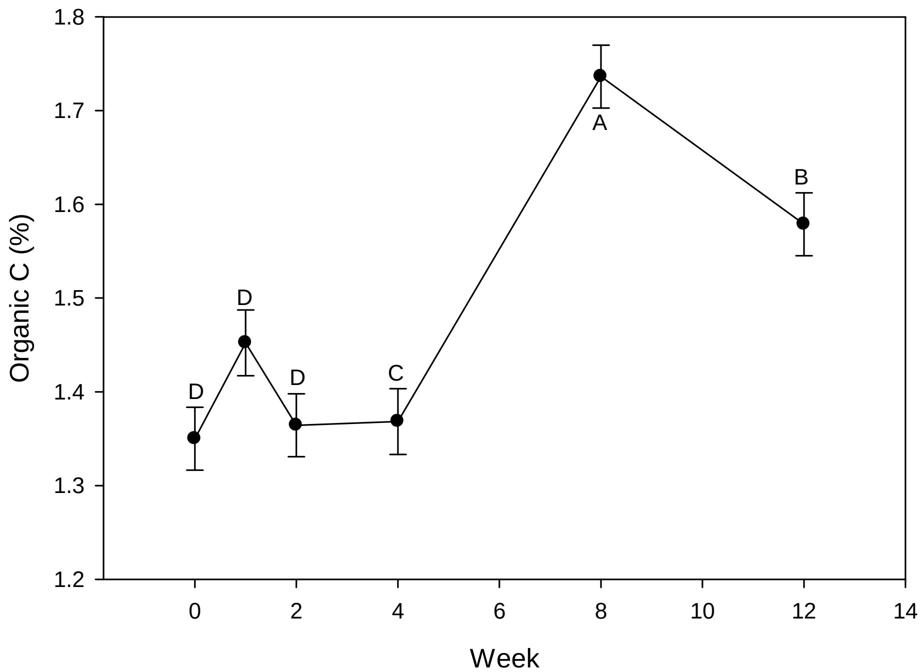

Figure A.4. Soil organic C in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on percent organic soil C when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.12. Organic C in the soil with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on percent organic soil C when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average % Organic C	**Standard Error**
With Biosolids	1.51a	0.0197
Without Biosolids	1.44b	0.0197

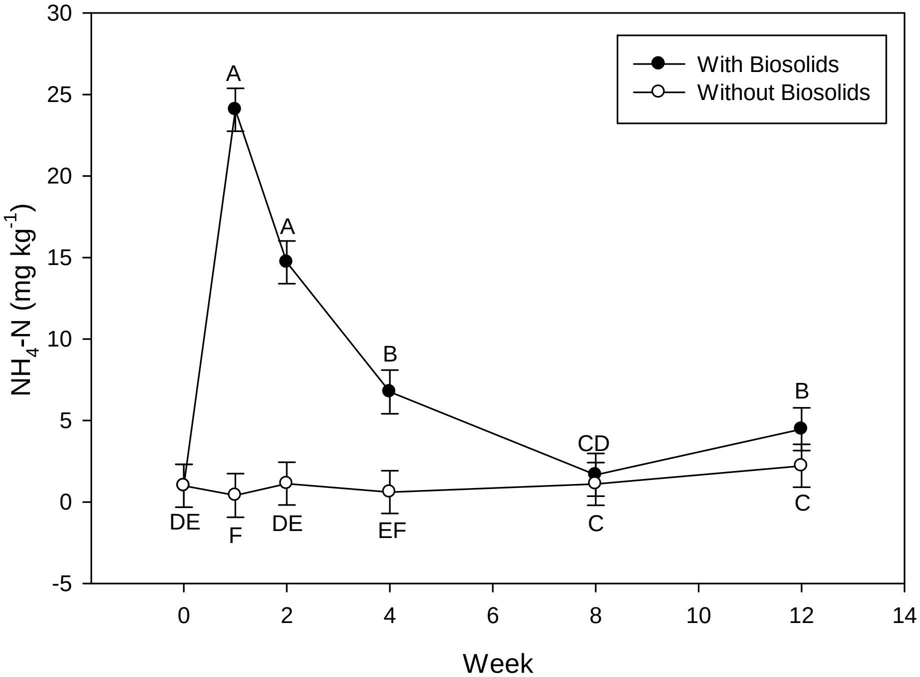

Figure A.5. Ammonium-nitrogen concentration effected over time in the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time and biosolids addition had a significant effect on log transformed soil NH_4-N when averaged over the addition of *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

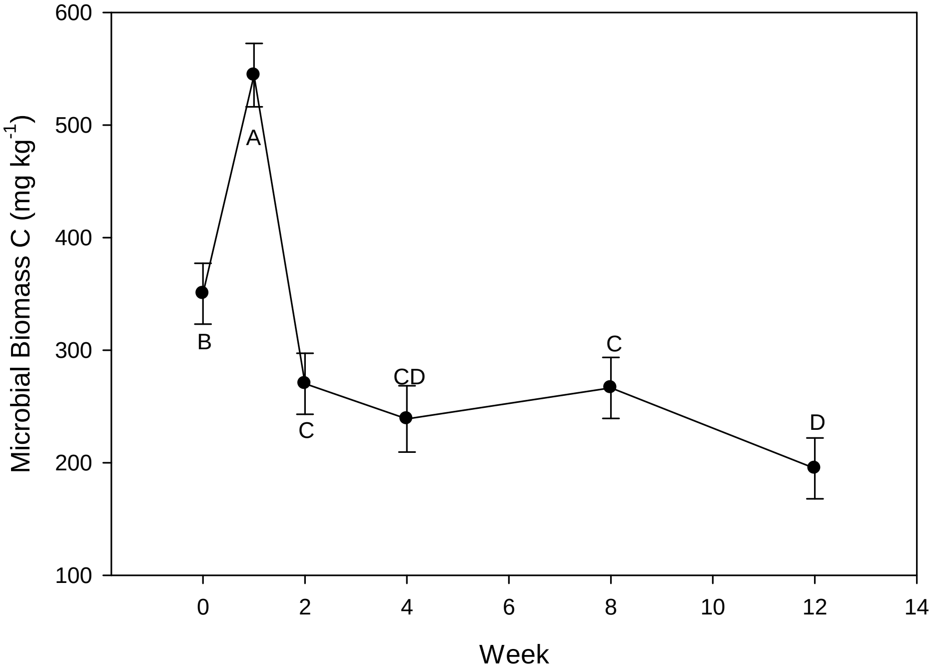

Figure A.6. Microbial biomass C averaged over time in the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on microbial biomass C when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.13. Microbial biomass C in the non-sterilized soil with the addition of biosolids, in the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on percent organic soil C when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average Microbial Biomass C (mg kg^{-1})	**Standard Error**
With Biosolids	338a	15.8
Without Biosolids	284b	16.1

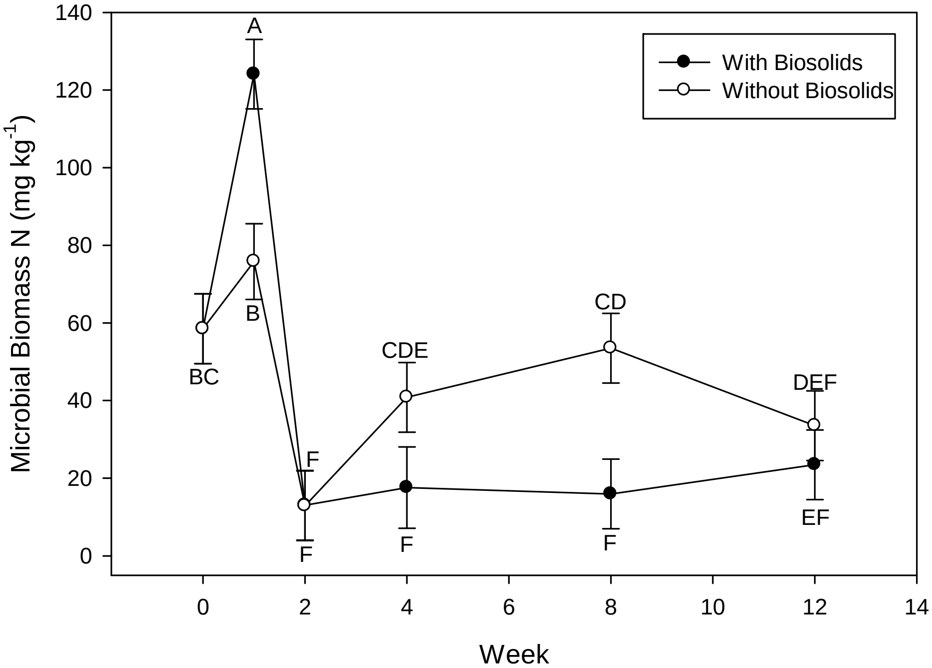

Figure A.7. Microbial biomass N concentration over time in the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time and biosolids had a significant effect on microbial biomass N when averaged over the addition of *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

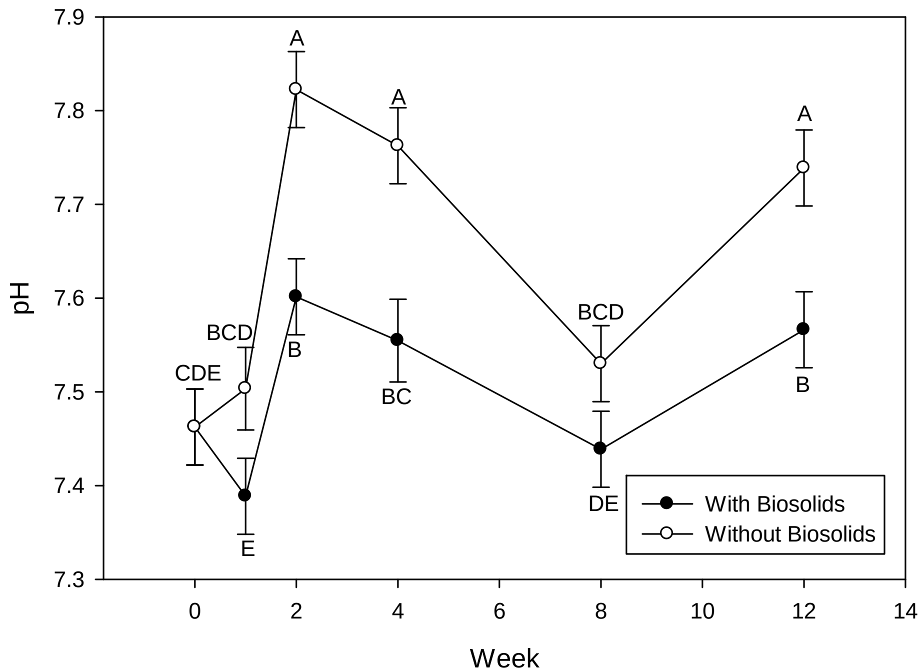

Figure A.8. Soil pH over time in the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time and biosolids had a significant effect on soil pH when averaged over addition of *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

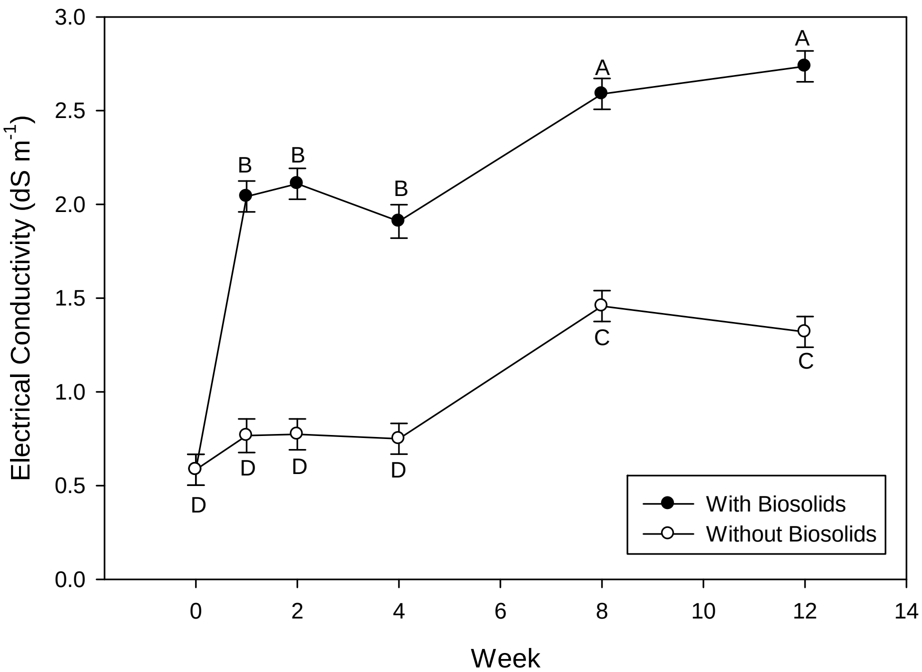

Figure A.9. Interaction of biosolids addition and time on the non-sterilized soil electrical conductivity. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on soil EC when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

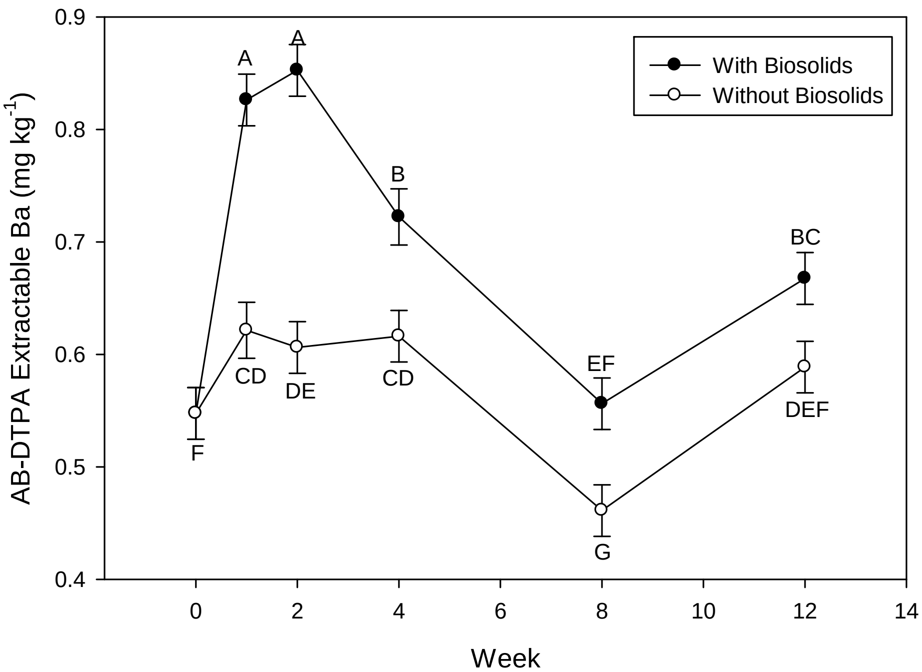

Figure A.10. Interaction between biosolids addition and time on AB-DTPA extractable Ba in the non-sterilized soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time and biosolids had a significant effect on AB-DTPA extractable Ba when averaged over the addition of *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

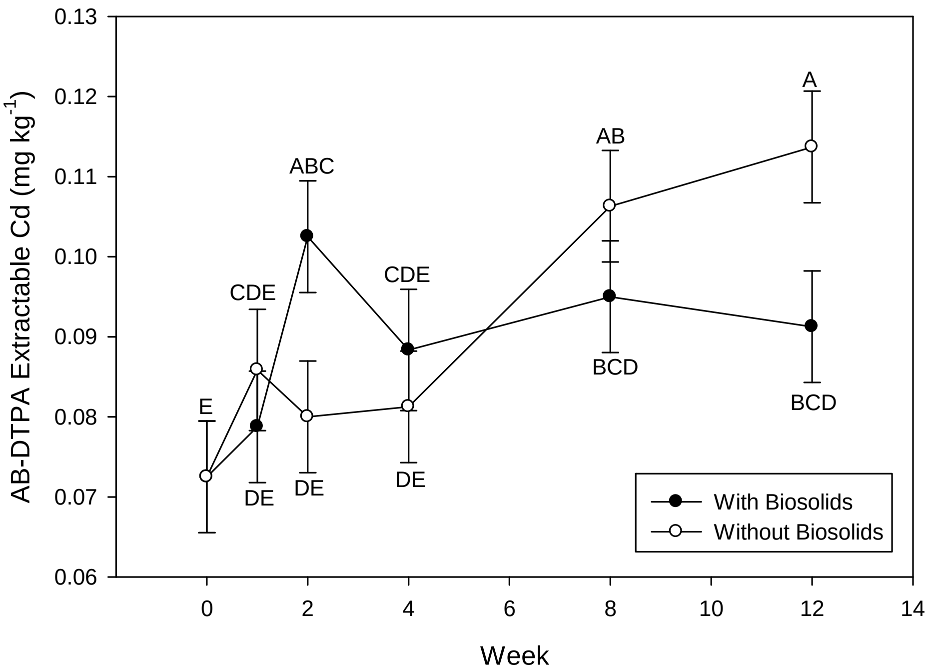

Figure A.11. Interaction between biosolids addition and time on AB-DTPA extractable Cd in the non-sterilized soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time and biosolids had a significant effect on AB-DTPA extractable Cd when averaged over the addition of *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

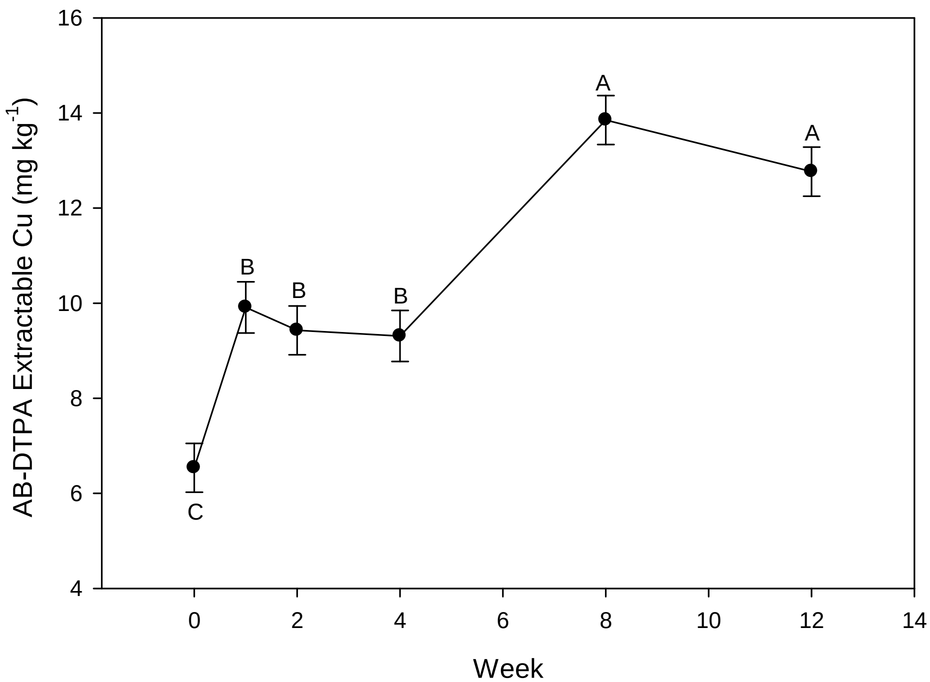

Figure A.12. Concentration of AB-DTPA extractable Cu over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Cu when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

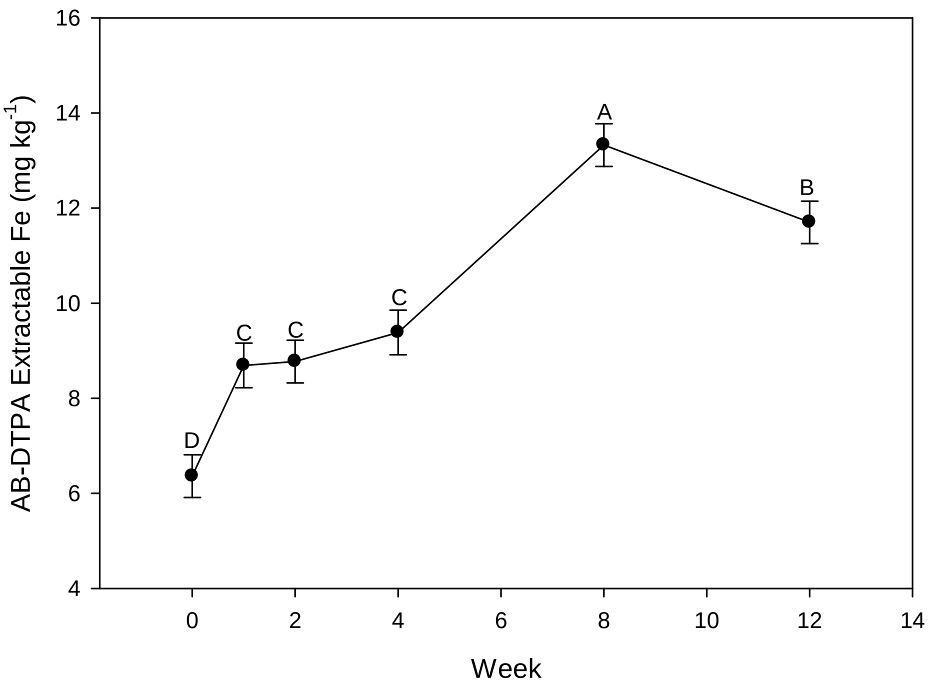

Figure A.13. Concentration of AB-DTPA extractable Fe over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Fe when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

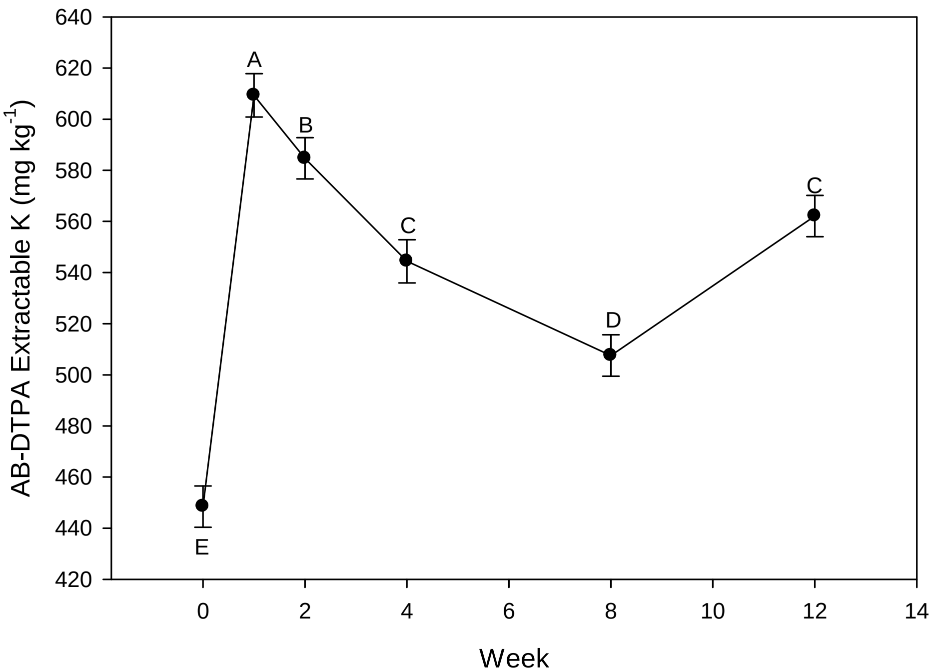

Figure A.14. Concentration of AB-DTPA extractable K in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable K when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

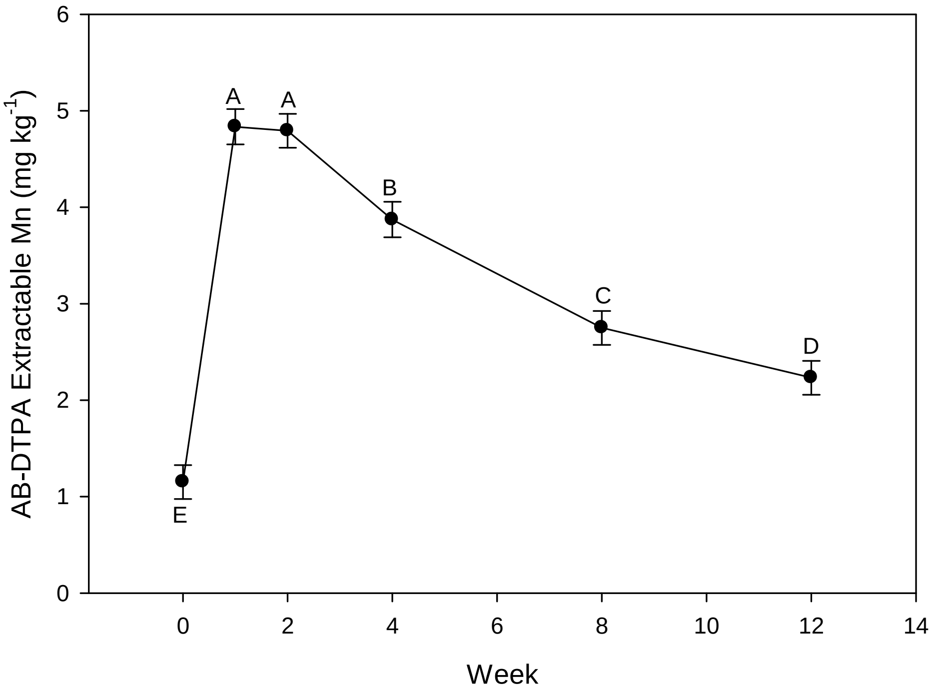

Figure A.15. Concentration of AB-DTPA extractable Mn in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Mn when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

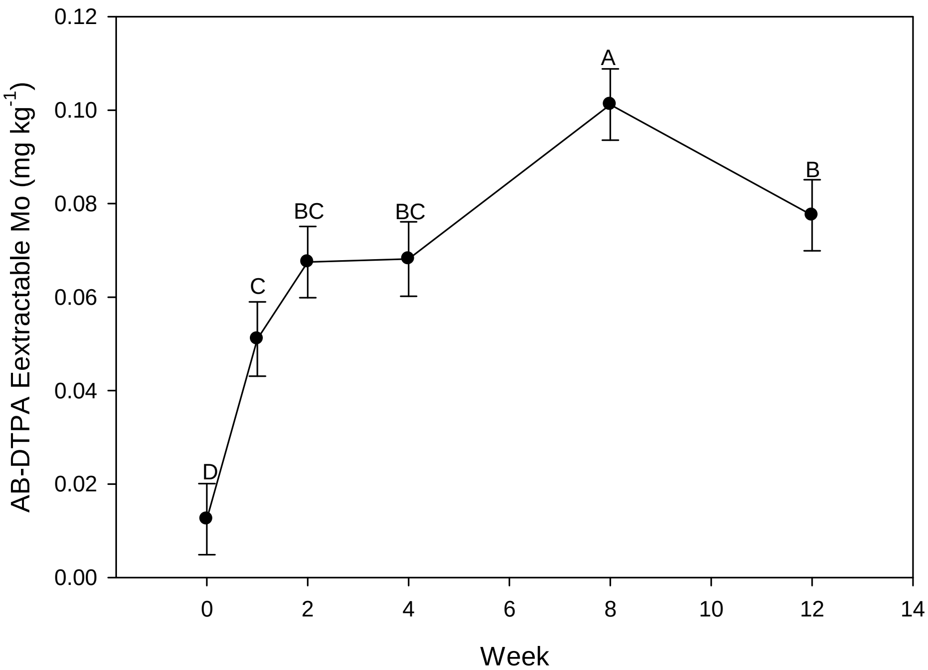

Figure A.16. Concentration of AB-DTPA extractable Mo in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Mo when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

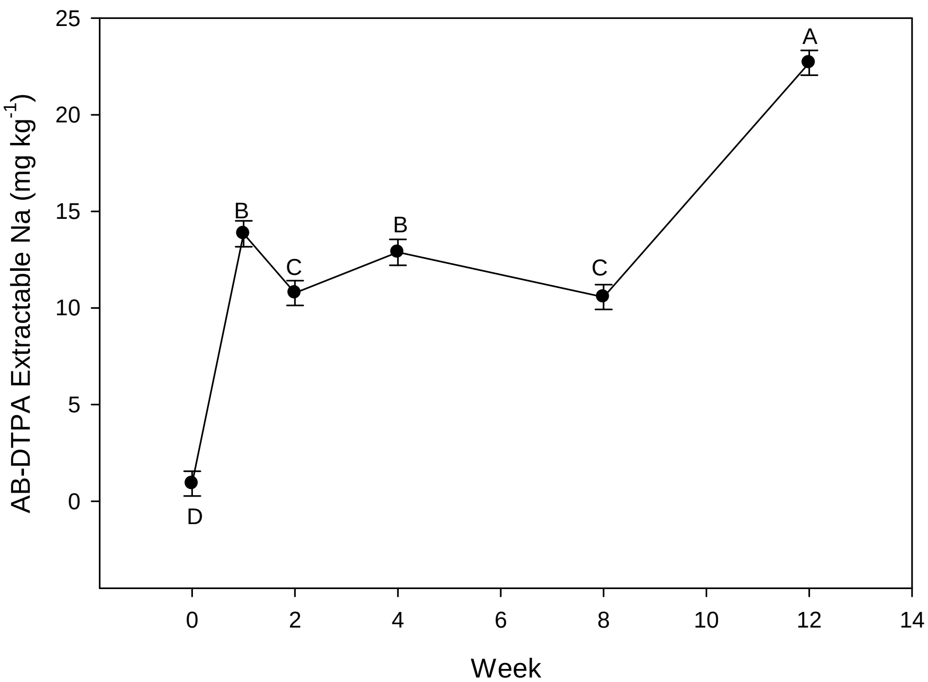

Figure A.17. Concentration of AB-DTPA extractable Na in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Na when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.14. AB-DTPA extractable Na in the non-sterilized soil with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on AB-DTPA extractable Na when averaged over the addition of *A. caliginosa* and time, following a 12 incubation study. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average AB-DTPA Extractable Na (mg kg^{-1})	**Standard Error**
With Biosolids	13.4a	0.375
Without Biosolids	10.5b	0.375

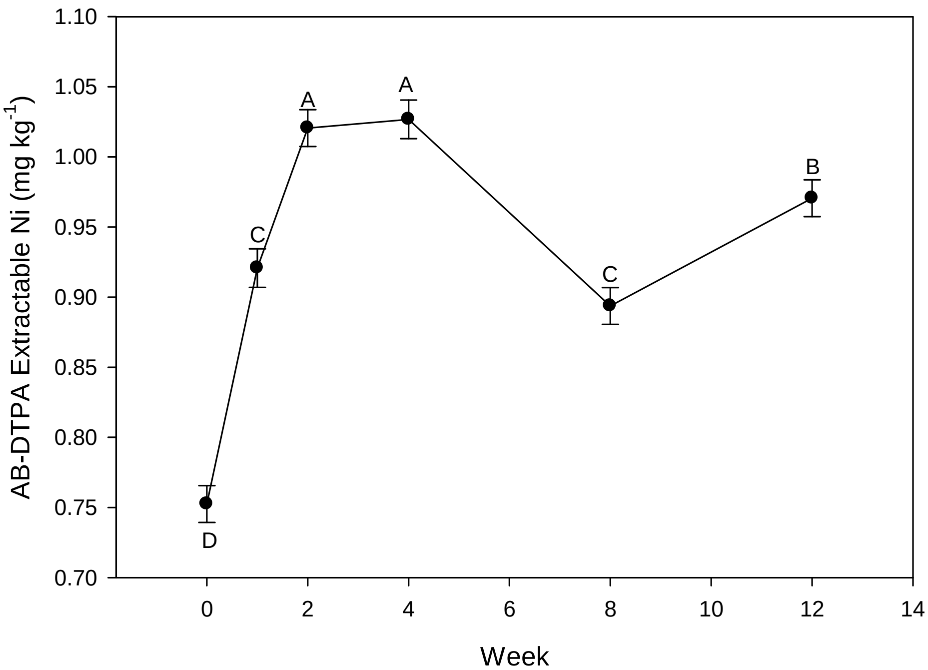

Figure A.18. Concentration of AB-DTPA extractable Ni in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Ni when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

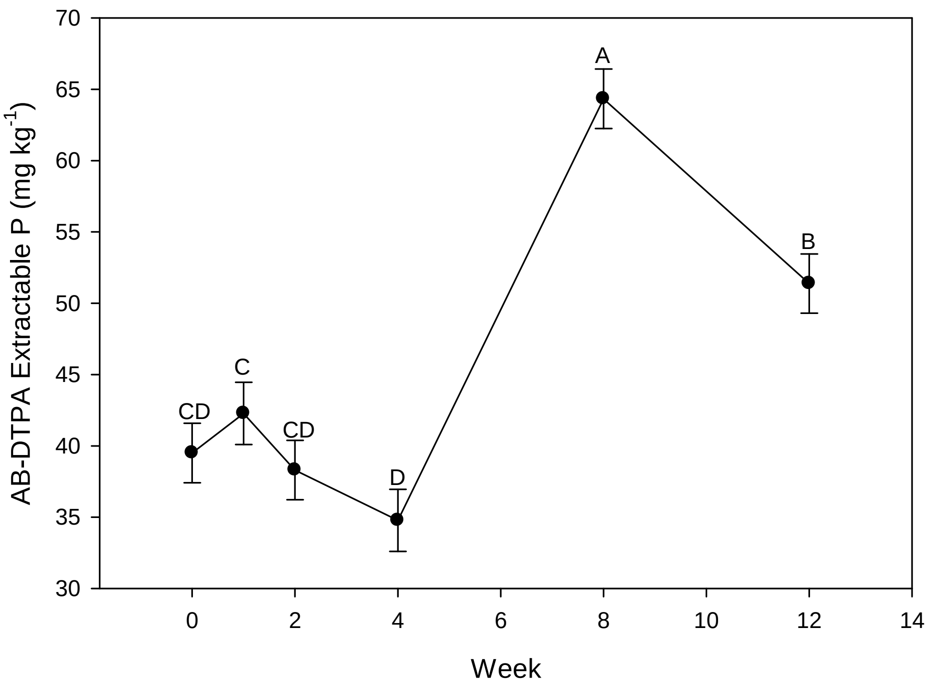

Figure A.19. Concentration of AB-DTPA extractable P in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable P when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

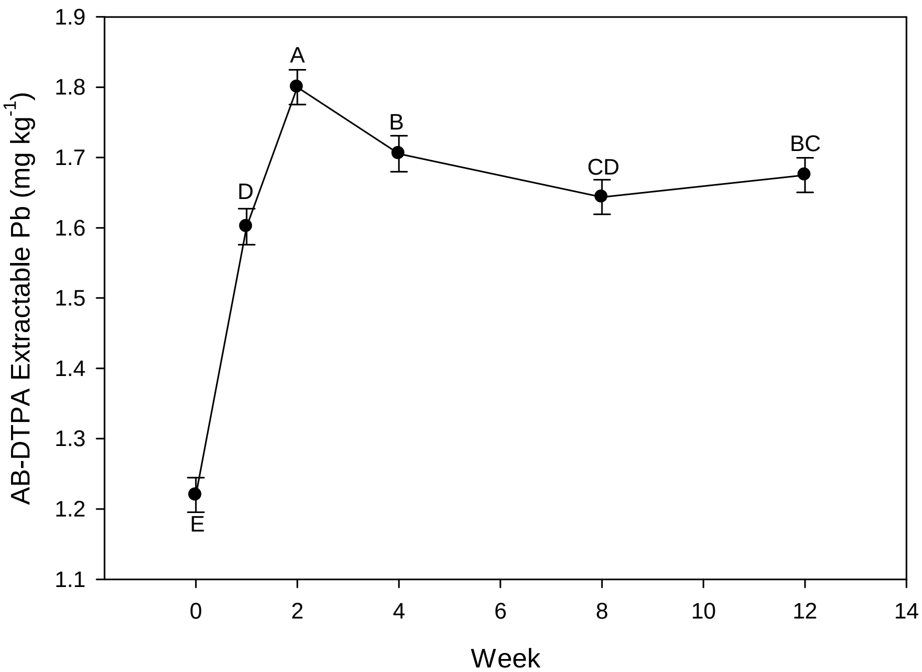

Figure A.20. Concentration of AB-DTPA extractable Pb in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Pb when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

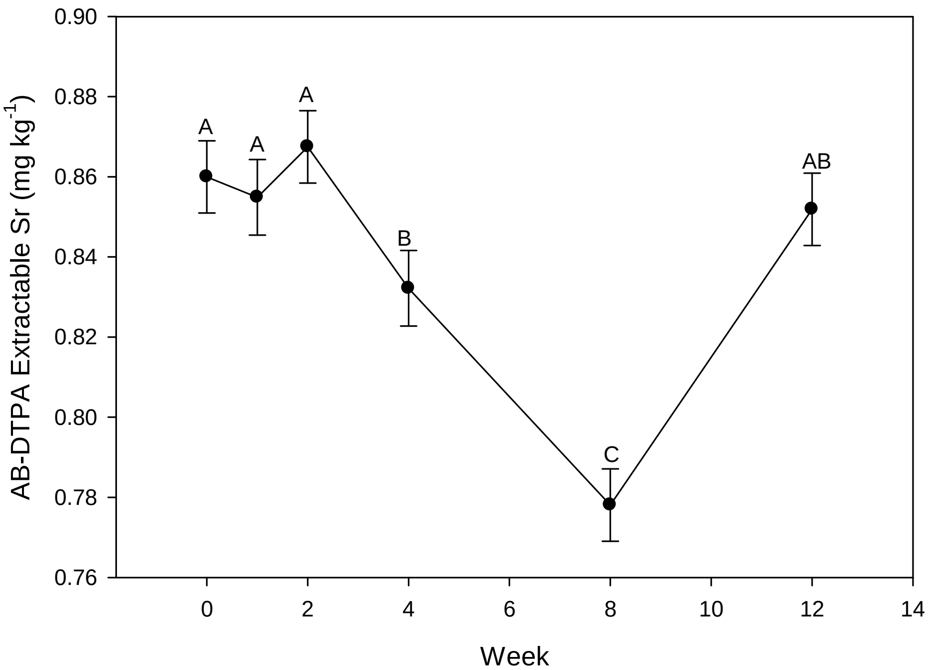

Figure A.21. Concentration of AB-DTPA extractable Sr in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Sr when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

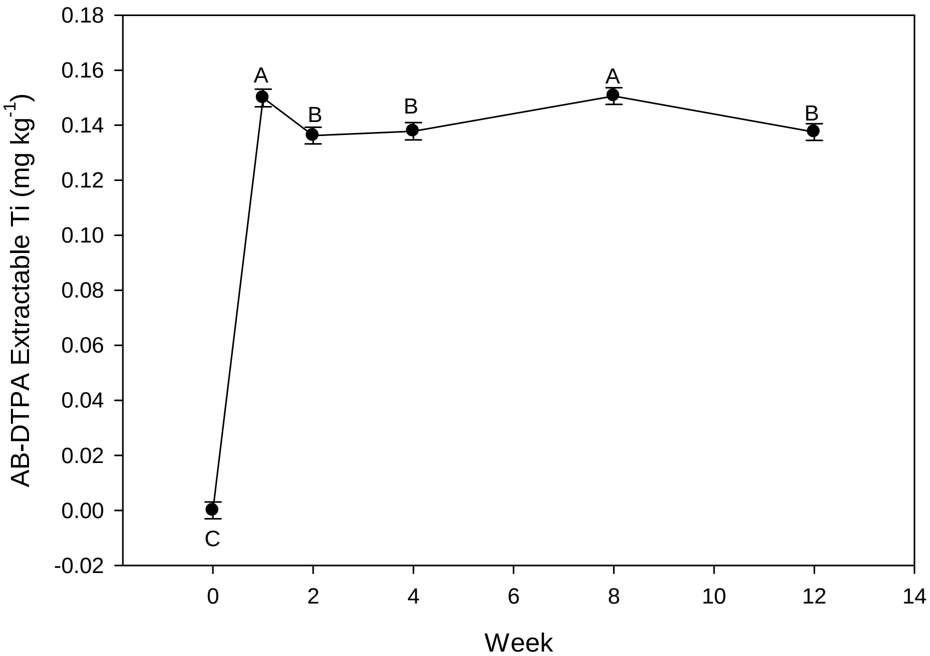

Figure A.22. Concentration of AB-DTPA extractable Ti in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Ti when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

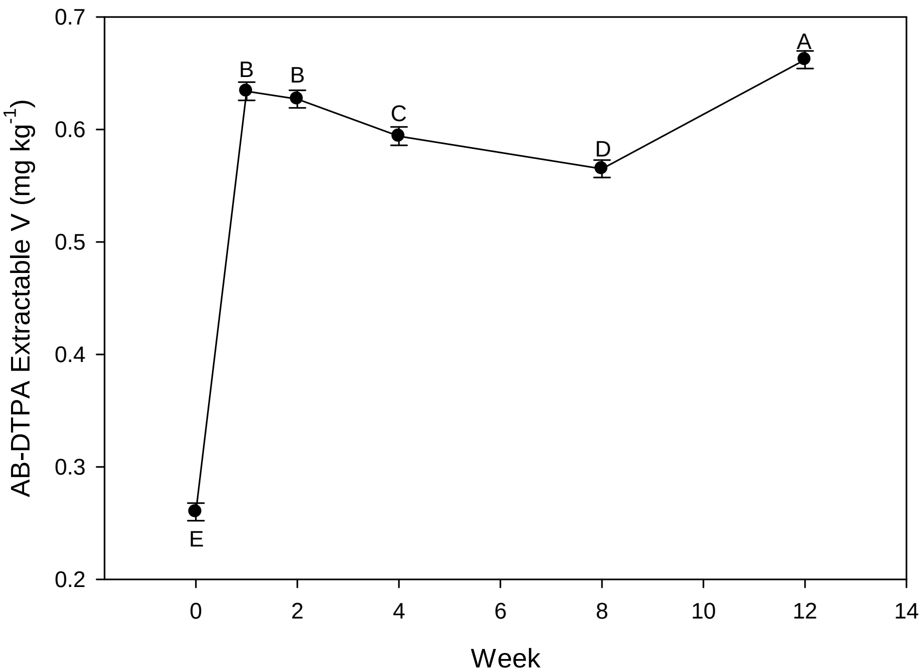

Figure A.23. Concentration of AB-DTPA extractable V in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable V when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

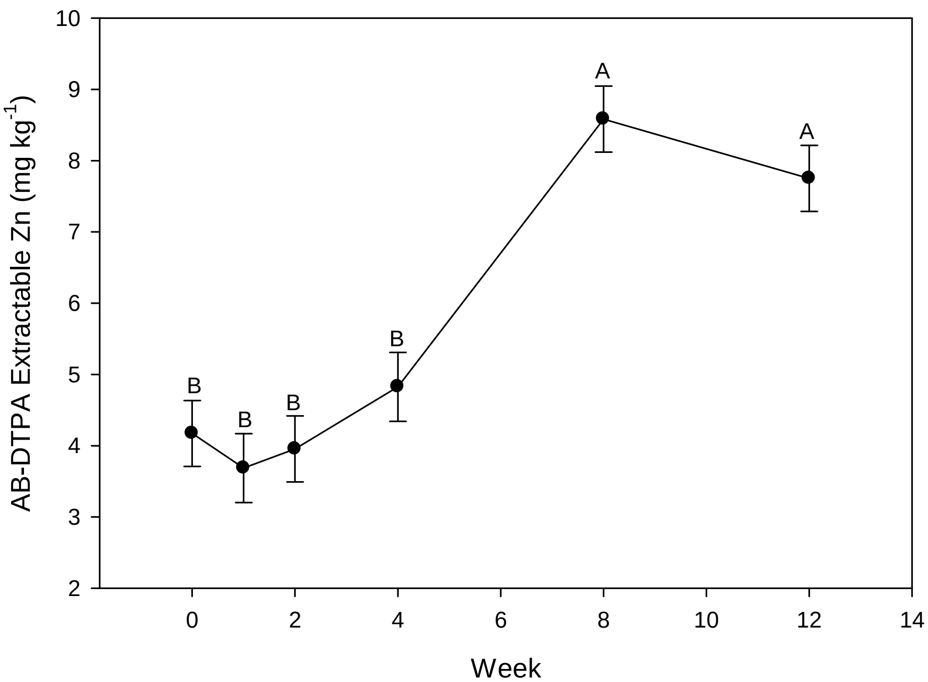

Figure A.24. Concentration of AB-DTPA extractable Zn in the non-sterilized soil over time. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on AB-DTPA extractable Zn when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.15. AB-DTPA extractable Zn in the non-sterilized soil with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on AB-DTPA extractable Zn when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average AB-DTPA Extractable Zn (mg kg^{-1})	**Standard Error**
With Biosolids	6.19a	0.271
Without Biosolids	4.80b	0.271

Table A.16. ANOVA table for percent change in earthworm mass in the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions with a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on the percent change in earthworm mass.

Parameter	Source of Variation	F Value	P Value
Percent Change in Earthworm Mass	Time	1.10	0.376
	Biosolids	0.03	0.850
	Time x Biosolids	0.78	0.524
	Replication	0.22	0.883

Table A.17. ANOVA table for soil total C and N, inorganic C, and organic C, from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Total N	Time	6.46	<0.001
	Biosolids	13.5	0.006
	Time x Biosolids	1.48	0.224
	Earthworms	1.17	0.284
	Time x Earthworms	0.14	0.966
	Biosolids x Earthworms	0.92	0.343
	Time x Biosolids x Earthworms	0.73	0.575
	Earthworms x Replication	0.29	0.836
	Replication	0.77	0.518
Total C	Time	4.45	0.004
	Biosolids	16.9	<0.001
	Time x Biosolids	2.23	0.079
	Earthworms	9.31	0.004
	Time x Earthworms	0.86	0.497
	Biosolids x Earthworms	2.42	0.127
	Time x Biosolids x Earthworms	0.32	0.864
	Earthworms x Replication	0.11	0.951
	Replication	0.86	0.467
Inorganic C	Time	23.4	<0.001
	Biosolids	0.70	0.408
	Time x Biosolids	3.12	0.023
	Earthworms	8.39	0.006
	Time x Earthworms	1.91	0.124
	Biosolids x Earthworms	8.99	0.004
	Time x Biosolids x Earthworms	3.82	0.009
	Earthworms x Replication	0.47	0.705
	Replication	8.87	<0.001
Organic C	Time	5.09	0.002
	Biosolids	14.1	0.001
	Time x Biosolids	2.26	0.076
	Earthworms	6.61	0.013
	Time x Earthworms	0.63	0.642
	Biosolids x Earthworms	1.30	0.259
	Time x Biosolids x Earthworms	0.20	0.935
	Earthworms x Replication	0.14	0.934
	Replication	1.36	0.265

Table A.18. ANOVA table for soil NO_3-N and NH_4-N, and microbial biomass C and N from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
NO_3-N	Time	16.6	<0.001
	Biosolids	191	<0.001
	Time x Biosolids	14.7	<0.001
	Earthworms	7.89	0.007
	Time x Earthworms	1.18	0.323
	Biosolids x Earthworms	10.3	0.002
	Time x Biosolids x Earthworms	1.17	0.336
	Earthworms x Replication	0.28	0.841
	Replication	3.75	0.017
NH_4-N	Time	683	<0.001
	Biosolids	666	<0.001
	Time x Biosolids	48.4	<0.001
	Earthworms	55.9	<0.001
	Time x Earthworms	4.35	0.004
	Biosolids x Earthworms	45.3	<0.001
	Time x Biosolids x Earthworms	4.43	0.004
	Earthworms x Replication	0.48	0.700
	Replication	2.63	0.060
Microbial Biomass C	Time	16.8	<0.001
	Biosolids	9.14	0.004
	Time x Biosolids	1.60	0.189
	Earthworms	2.03	0.161
	Time x Earthworms	1.00	0.417
	Biosolids x Earthworms	3.61	0.063
	Time x Biosolids x Earthworms	1.39	0.252
	Earthworms x Replication	1.12	0.348
	Replication	0.75	0.528
Microbial Biomass N	Time	2.73	0.034
	Biosolids	4.34	0.043
	Time x Biosolids	1.15	0.344
	Earthworms	1.74	0.194
	Time x Earthworms	0.68	0.611
	Biosolids x Earthworms	1.29	0.261
	Time x Biosolids x Earthworms	0.89	0.478
	Earthworms x Replication	0.89	0.455
	Replication	0.74	0.535

Table A.19. ANOVA table for soil pH, and EC from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
pH	Time	17.9	<0.001
	Biosolids	17.1	<0.001
	Time x Biosolids	1.80	0.144
	Earthworms	2.53	0.118
	Time x Earthworms	0.31	0.868
	Biosolids x Earthworms	3.61	0.063
	Time x Biosolids x Earthworms	0.78	0.542
	Earthworms x Replication	0.76	0.523
	Replication	0.20	0.898
EC	Time	62.3	<0.001
	Biosolids	566	<0.001
	Time x Biosolids	39.0	<0.001
	Earthworms	1.67	0.203
	Time x Earthworms	0.72	0.582
	Biosolids x Earthworms	4.70	0.035
	Time x Biosolids x Earthworms	1.65	0.1756
	Earthworms x Replication	1.10	0.357
	Replication	4.45	0.008

Table A.20. ANOVA table for AB-DTPA extractable Ba, Cd, Cr, and Cu from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Ba	Time	19.55	<0.001
	Biosolids	17.08	<0.001
	Time x Biosolids	3.36	0.017
	Earthworms	2.34	0.132
	Time x Earthworms	0.58	0.678
	Biosolids x Earthworms	0.80	0.377
	Time x Biosolids x Earthworms	0.15	0.963
	Earthworms x Replication	0.67	0.573
	Replication	0.86	0.466
Cd	Time	10.3	<0.001
	Biosolids	0.22	0.641
	Time x Biosolids	2.28	0.074
	Earthworms	0.01	0.906
	Time x Earthworms	1.05	0.392
	Biosolids x Earthworms	0.22	0.641
	Time x Biosolids x Earthworms	0.18	0.947
	Earthworms x Replication	1.08	0.368
	Replication	0.41	0.744
Cr	Time	9.36	<0.001
	Biosolids	0.66	0.422
	Time x Biosolids	1.26	0.298
	Earthworms	0.27	0.603
	Time x Earthworms	0.48	0.753
	Biosolids x Earthworms	2.54	0.118
	Time x Biosolids x Earthworms	2.38	0.065
	Earthworms x Replication	1.11	0.355
	Replication	3.00	0.039
Cu	Time	19.4	<0.001
	Biosolids	23.7	<0.001
	Time x Biosolids	2.39	0.064
	Earthworms	5.56	0.023
	Time x Earthworms	0.50	0.733
	Biosolids x Earthworms	0.94	0.337
	Time x Biosolids x Earthworms	0.39	0.818
	Earthworms x Replication	0.12	0.945
	Replication	2.63	0.061

Table A.21. ANOVA table for AB-DTPA extractable Fe, K, Mn, and Mo from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Fe	Time	29.14	<0.001
	Biosolids	46.89	<0.001
	Time x Biosolids	6.62	<0.001
	Earthworms	11.7	0.001
	Time x Earthworms	2.20	0.083
	Biosolids x Earthworms	0.00	1.00
	Time x Biosolids x Earthworms	0.64	0.640
	Earthworms x Replication	0.11	0.955
	Replication	0.37	0.774
K	Time	108	<0.001
	Biosolids	15.8	<0.001
	Time x Biosolids	1.41	0.245
	Earthworms	13.4	0.001
	Time x Earthworms	1.94	0.118
	Biosolids x Earthworms	12.9	0.001
	Time x Biosolids x Earthworms	1.28	0.290
	Earthworms x Replication	0.27	0.845
	Replication	0.43	0.730
Mn	Time	32.9	<0.001
	Biosolids	0.81	0.374
	Time x Biosolids	1.80	0.143
	Earthworms	2.96	0.092
	Time x Earthworms	1.86	0.132
	Biosolids x Earthworms	5.37	0.025
	Time x Biosolids x Earthworms	0.80	0.533
	Earthworms x Replication	0.77	0.516
	Replication	0.88	0.456
Mo	Time	5.41	0.001
	Biosolids	5.90	0.019
	Time x Biosolids	1.46	0.228
	Earthworms	0.10	0.756
	Time x Earthworms	0.47	0.754
	Biosolids x Earthworms	0.04	0.842
	Time x Biosolids x Earthworms	0.33	0.856
	Earthworms x Replication	0.12	0.950
	Replication	2.86	0.047

Table A.22. ANOVA table for AB-DTPA extractable Na, Ni, P, and Pb from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Na	Time	109	<0.001
	Biosolids	39.9	<0.001
	Time x Biosolids	5.85	0.001
	Earthworms	0.73	0.396
	Time x Earthworms	0.22	0.929
	Biosolids x Earthworms	0.00	0.947
	Time x Biosolids x Earthworms	0.79	0.540
	Earthworms x Replication	0.89	0.451
	Replication	0.98	0.409
Ni	Time	106	<0.001
	Biosolids	0.25	0.619
	Time x Biosolids	1.16	0.339
	Earthworms	4.36	0.042
	Time x Earthworms	1.19	0.328
	Biosolids x Earthworms	0.92	0.343
	Time x Biosolids x Earthworms	1.36	0.260
	Earthworms x Replication	3.32	0.027
	Replication	6.92	0.001
P	Time	39.6	<0.001
	Biosolids	5.15	0.028
	Time x Biosolids	11.2	<0.001
	Earthworms	8.30	0.006
	Time x Earthworms	2.63	0.046
	Biosolids x Earthworms	2.18	0.146
	Time x Biosolids x Earthworms	5.03	0.002
	Earthworms x Replication	1.33	0.276
	Replication	3.27	0.029
Pb	Time	124	<0.001
	Biosolids	2.31	0.135
	Time x Biosolids	1.35	0.265
	Earthworms	0.12	0.727
	Time x Earthworms	0.59	0.673
	Biosolids x Earthworms	0.19	0.661
	Time x Biosolids x Earthworms	1.15	0.346
	Earthworms x Replication	0.04	0.988
	Replication	1.70	0.180

Table A.23. ANOVA table for AB-DTPA extractable Si, Sr, Ti, and V from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of variation on various soil parameters.

Parameter	Source of Variation	F Value	P Value
Si	Time	45.73	<0.001
	Biosolids	5.05	0.030
	Time x Biosolids	1.76	0.151
	Earthworms	2.34	0.133
	Time x Earthworms	1.39	0.254
	Biosolids x Earthworms	2.47	0.123
	Time x Biosolids x Earthworms	0.60	0.668
	Earthworms x Replication	0.93	0.436
	Replication	0.84	0.479
Sr	Time	20.49	<0.001
	Biosolids	0.68	0.413
	Time x Biosolids	0.73	0.575
	Earthworms	2.69	0.108
	Time x Earthworms	0.96	0.438
	Biosolids x Earthworms	1.16	0.287
	Time x Biosolids x Earthworms	0.26	0.903
	Earthworms x Replication	0.69	0.565
	Replication	0.82	0.488
Ti	Time	882.47	<0.001
	Biosolids	25.90	<0.001
	Time x Biosolids	5.52	0.001
	Earthworms	1.73	0.194
	Time x Earthworms	0.66	0.624
	Biosolids x Earthworms	0.10	0.756
	Time x Biosolids x Earthworms	0.15	0.963
	Earthworms x Replication	0.66	0.583
	Replication	2.66	0.058
V	Time	467	<0.001
	Biosolids	0.36	0.553
	Time x Biosolids	0.67	0.614
	Earthworms	1.61	0.211
	Time x Earthworms	0.47	0.756
	Biosolids x Earthworms	1.72	0.196
	Time x Biosolids x Earthworms	0.82	0.517
	Earthworms x Replication	0.60	0.621
	Replication	1.27	0.296

Table A.24. ANOVA table for AB-DTPA extractable Zn from the reduced microbial population soil after the earthworm incubation. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the table below shows the sources of AB-DTPA extractable Zn.

Parameter	Source of Variation	F Value	P Value
Zn	Time	3.44	0.015
	Biosolids	10.7	0.002
	Time x Biosolids	3.01	0.027
	Earthworms	0.09	0.764
	Time x Earthworms	0.81	0.524
	Biosolids x Earthworms	3.20	0.080
	Time x Biosolids x Earthworms	0.49	0.744
	Earthworms x Replication	0.36	0.781
	Replication	1.27	0.295

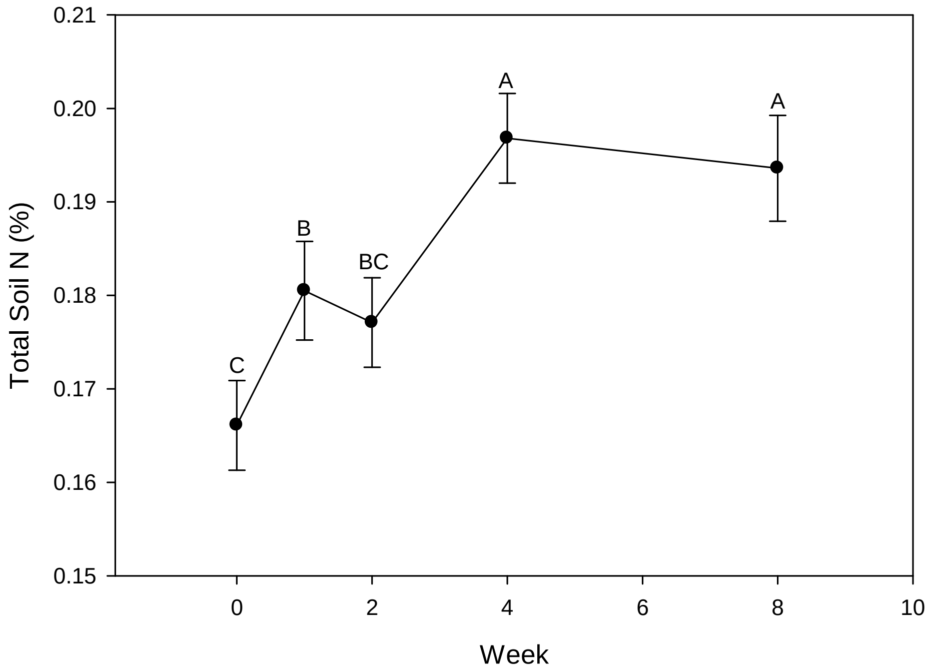

Figure A.25. Total soil N in a soil with a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a reduced microbial population sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), time had a significant effect on percent total soil N when averaged over the addition of biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.25. Total soil N in the soil with reduced microbial population with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on percent total soil N when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average Total Soil N (%)	**Standard Error**
With Biosolids	0.191a	0.00324
Without Biosolids	0.175b	0.00314

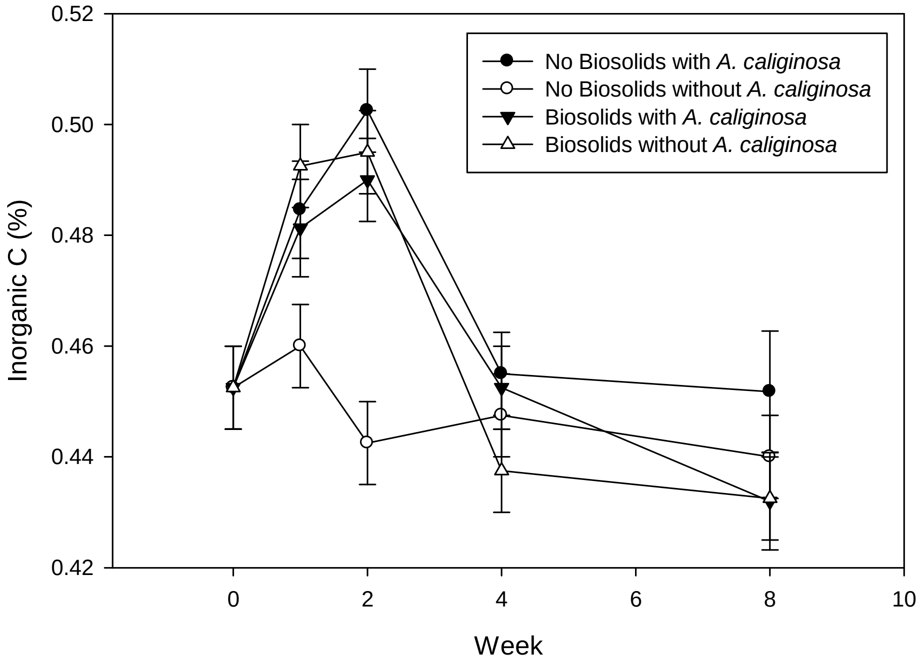

Figure A.26. Soil inorganic C in a soil with reduced microbial populations. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant three-way interaction between the addition of *A. caliginosa*, biosolids, and week on the percent inorganic soil C. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

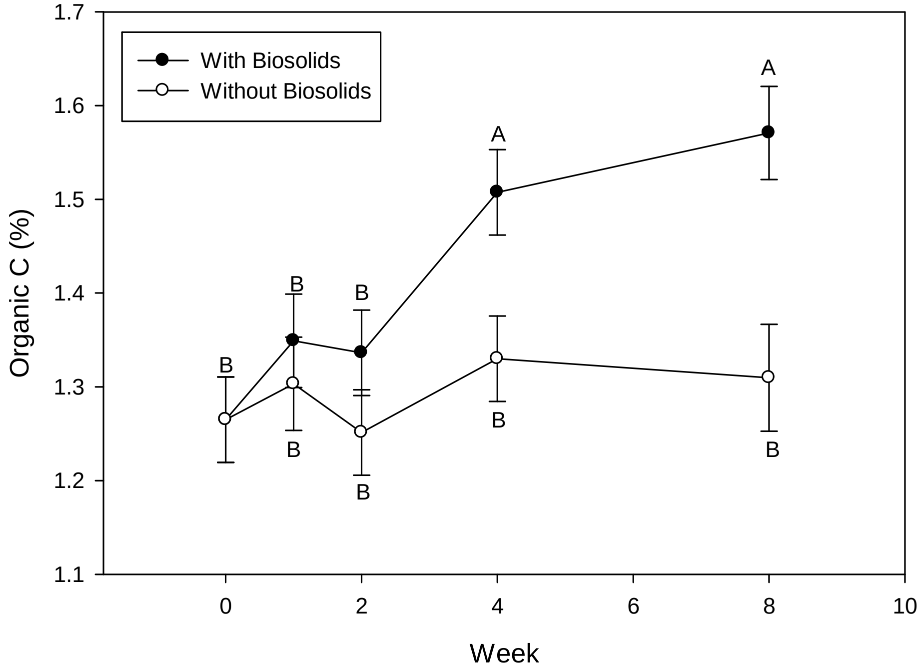

Figure A.27. Soil organic C in a soil with reduced microbial populations. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the percent organic soil C. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.26. Percent organic C in the reduced microbial population soil with the addition of *A. caliginosa*. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of *A. caliginosa* had a significant effect on percent organic soil C when averaged over the addition of biosolids and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Average % Organic C	**Standard Error**
With *A. caliginosa*	1.31a	0.0204
Without *A. caliginosa*	1.39b	0.0225

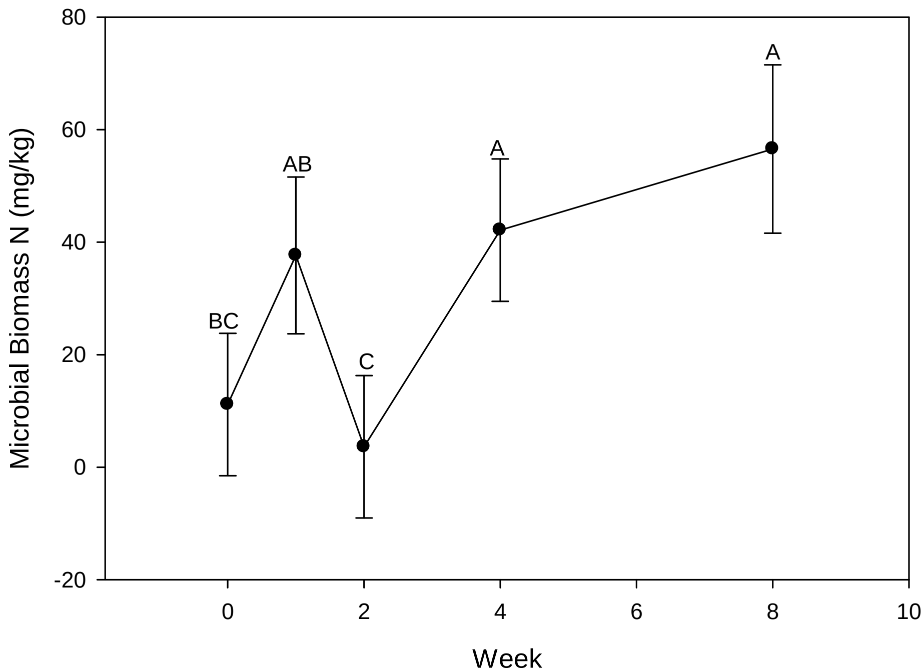

Figure A.28. Microbial biomass N concentration over time in a reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on microbial biomass N when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.27. Microbial biomass N concentration in the soil with reduced microbial population with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on microbial biomass N concentration when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	Microbial Biomass N (mg kg^{-1})	**Standard Error**
With biosolids	42.6a	8.55
Without biosolids	17.8b	8.29

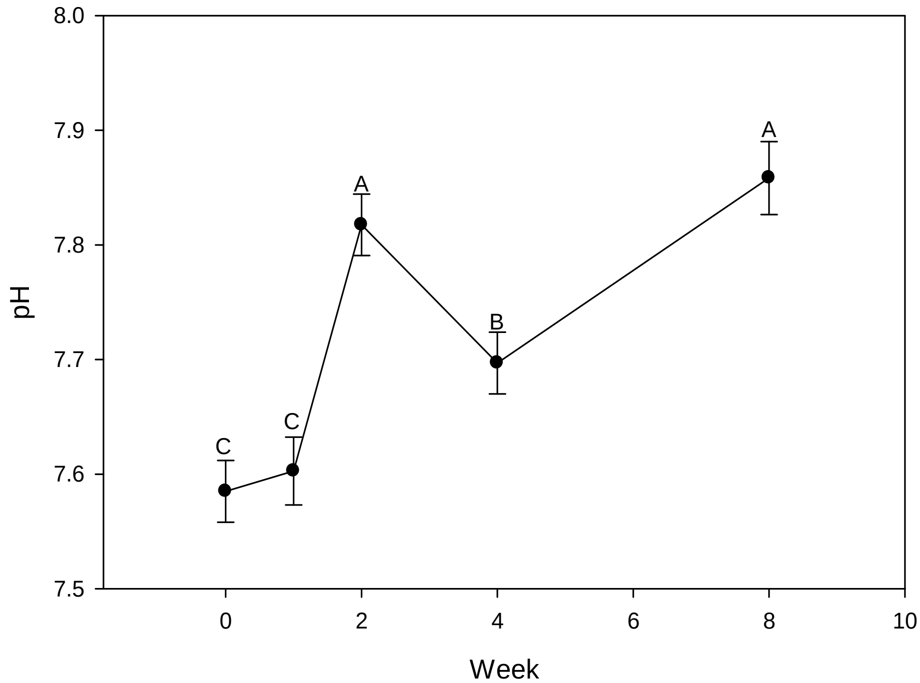

Figure A.29. Soil pH in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on soil pH when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

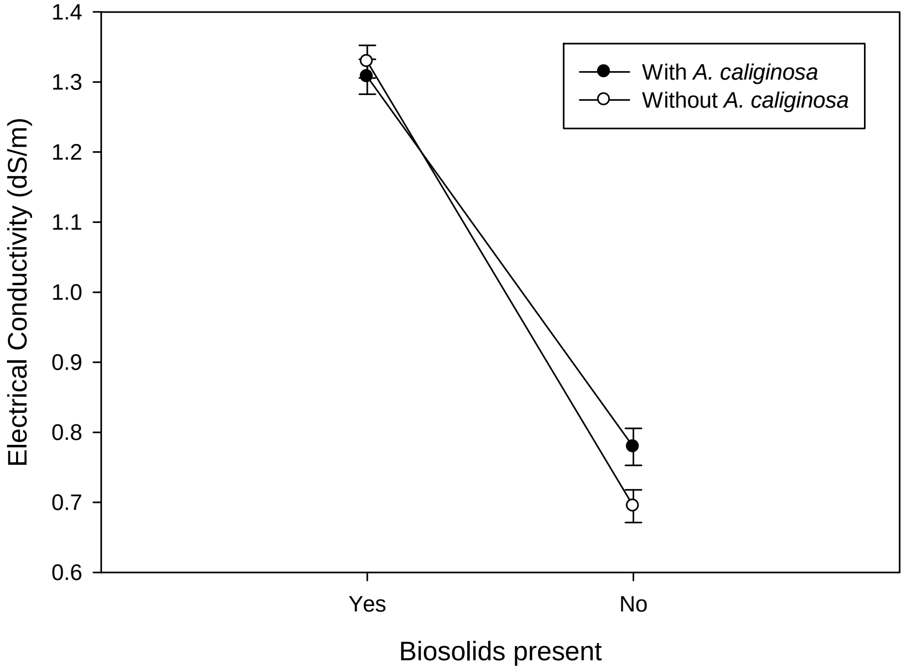

Figure A.30. Soil EC with the addition of biosolids and *A. caliginosa* in a reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) the addition of biosolids and *A. caliginosa* had a significant effect on soil EC when averaged over time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

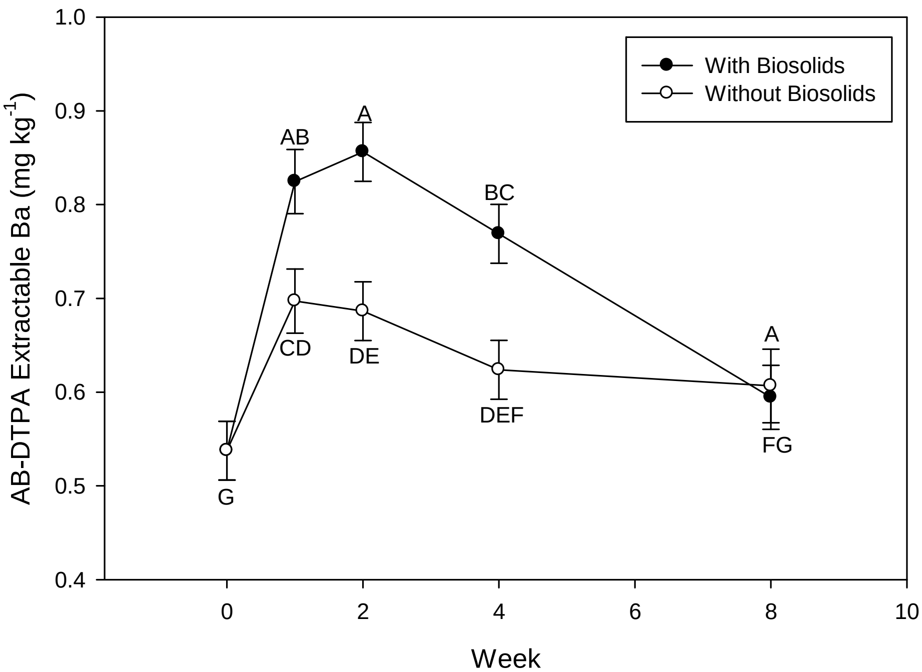

Figure A.31. Concentration of AB-DTPA extractable Ba averaged in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the concentration of AB-DTPA extractable Ba. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

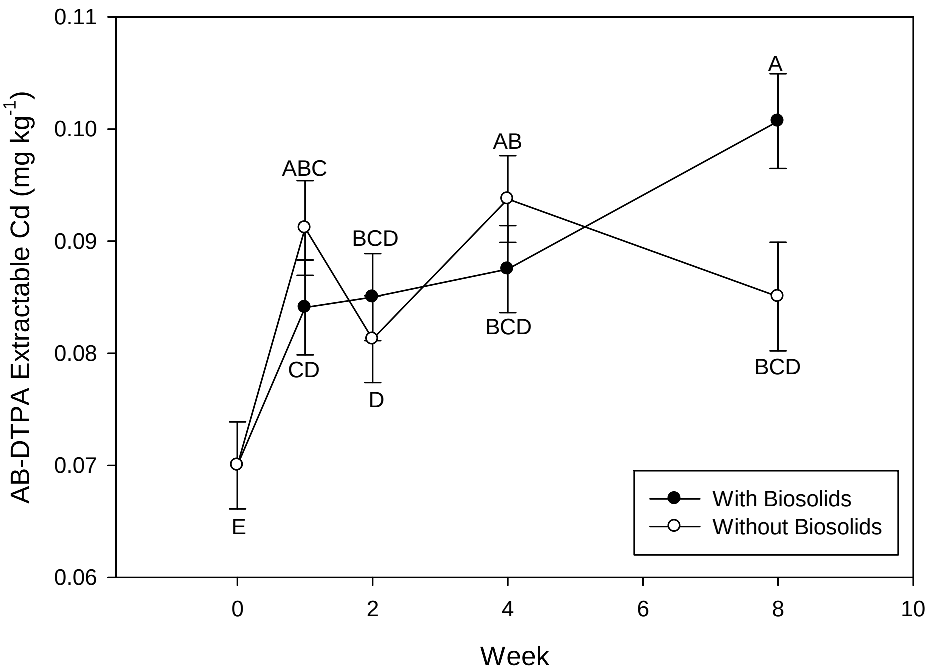

Figure A.32. Concentration of AB-DTPA extractable soil Cd in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the concentration of AB-DTPA extractable Cd. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

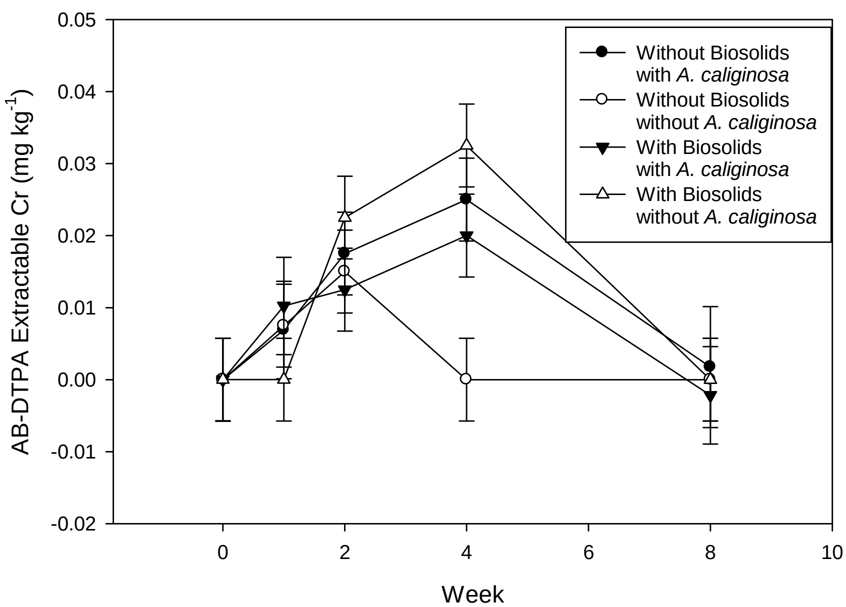

Figure A.33. Concentration of AB-DTPA extractable soil Cr in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant three-way interaction between the addition of *A. caliginosa*, biosolids, and week on the concentration of AB-DTPA extractable soil Cr. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

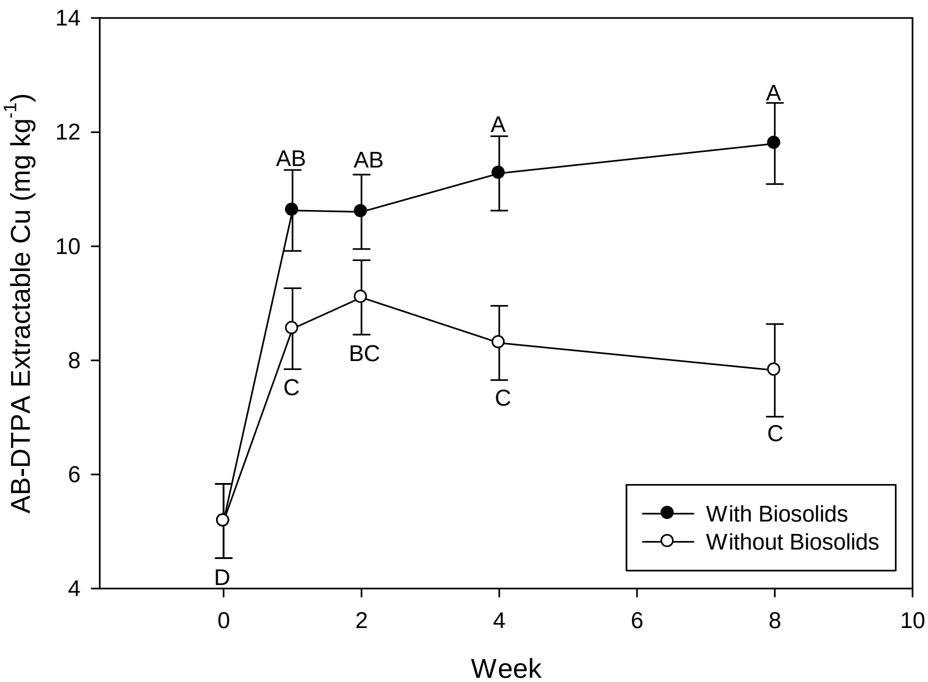

Figure A.34. Concentration of AB-DTPA extractable soil Cu in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the concentration of AB-DTPA extractable Cu. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.28. Concentration of AB-DTPA extractable Cu in the soil with reduced microbial population with the addition of *A. caliginosa*. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of *A. caliginosa* had a significant effect on AB-DTPA extractable Cu when averaged over the addition of biosolids and time.

	AB-DTPA Extractable Cu (mg kg^{-1})	**Standard Error**
With *A. caliginosa*	9.36a	0.321
Without *A. caliginosa*	8.33b	0.291

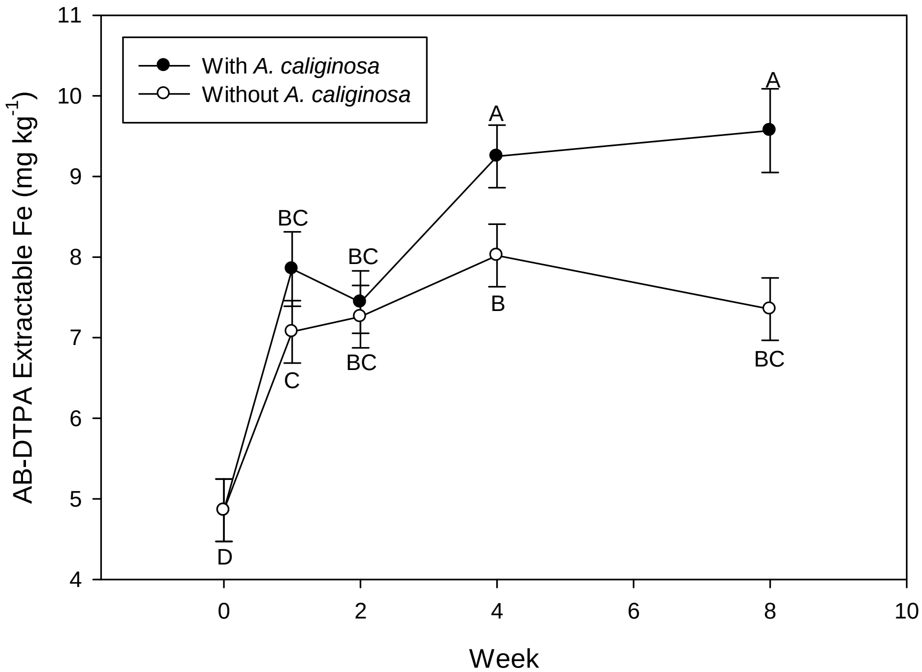

Figure A.35. Concentration of AB-DTPA extractable soil Fe in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of *A. caliginosa* and week on the concentration of AB-DTPA extractable Fe. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

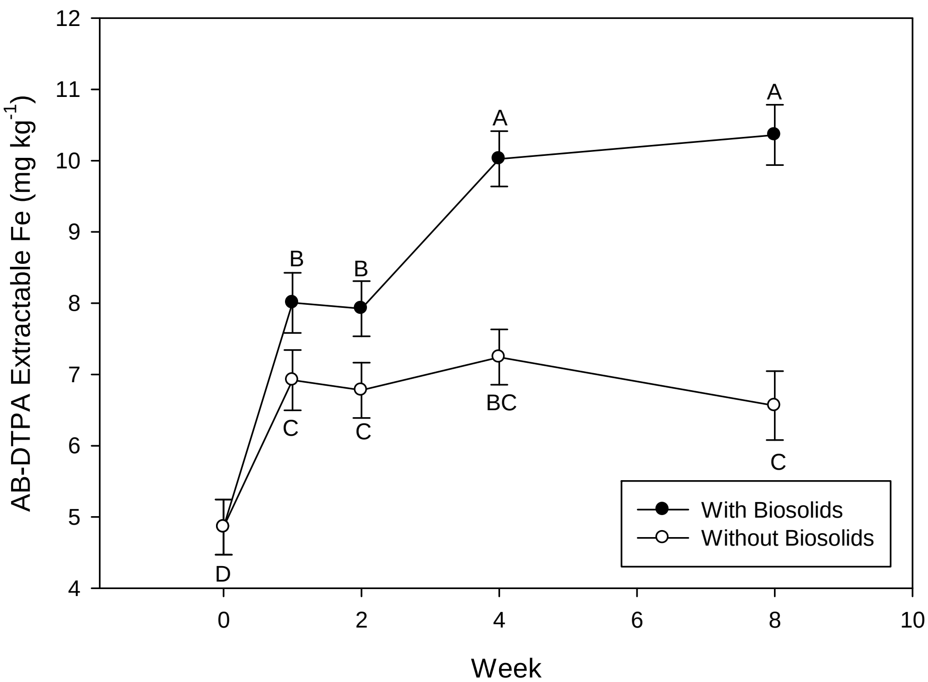

Figure A.36. Concentration of AB-DTPA extractable soil Fe in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the concentration of AB-DTPA extractable Fe. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

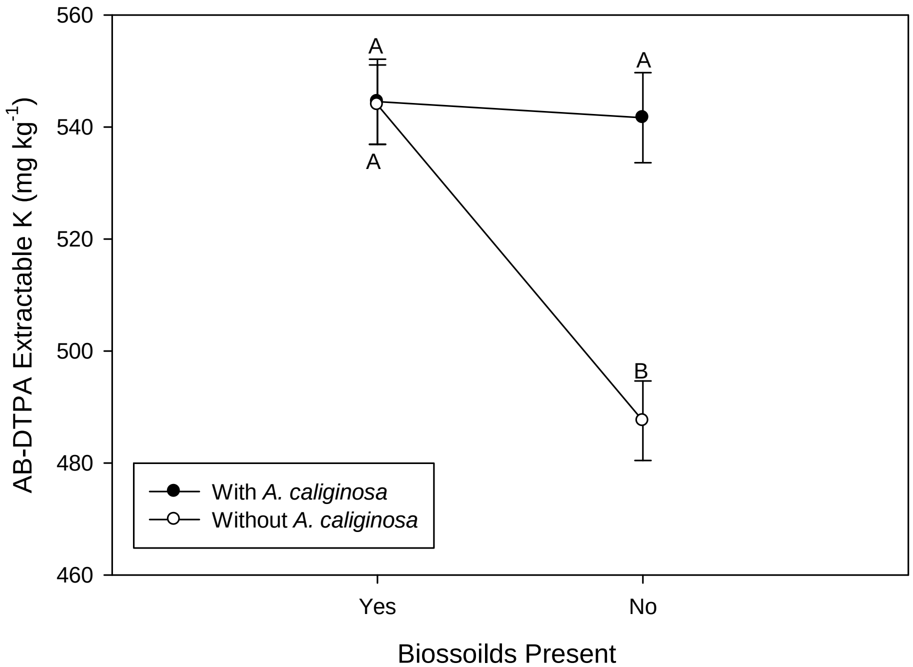

Figure A.37. Concentration of AB-DTPA extractable K with the addition of biosolids and *A. caliginosa* in a reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) the addition of biosolids and *A. caliginosa* had a significant effect on the concentration of AB-DTPA extractable K when averaged over time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

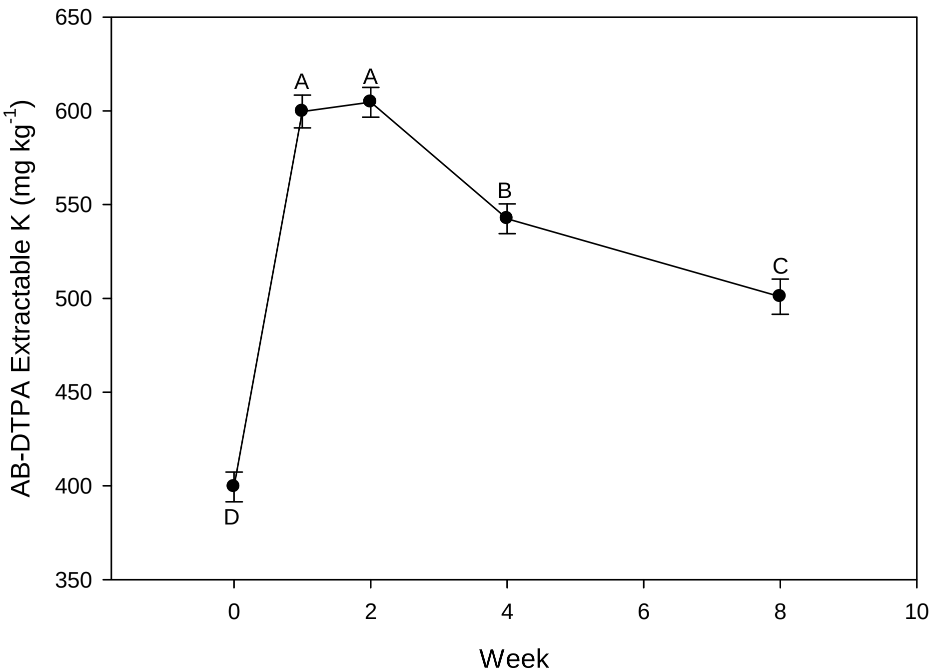

Figure A.38. Concentration of AB-DTPA extractable K concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable K when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

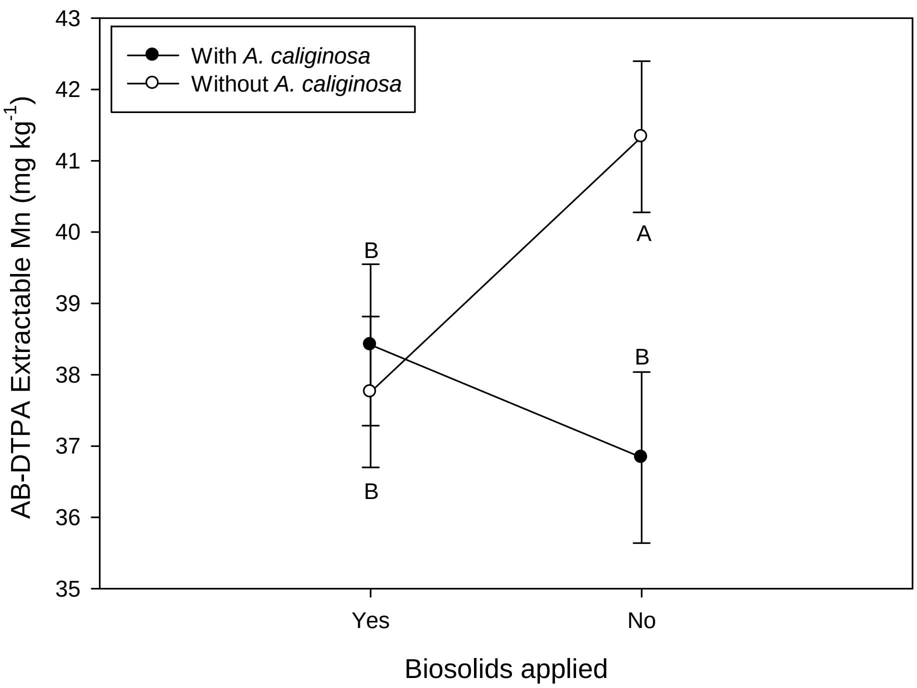

Figure A.39. Concentration of AB-DTPA extractable Mn with the addition of biosolids and *A. caliginosa* in a reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) the addition of biosolids and *A. caliginosa* had a significant effect on the concentration of AB-DTPA extractable Mn when averaged over time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

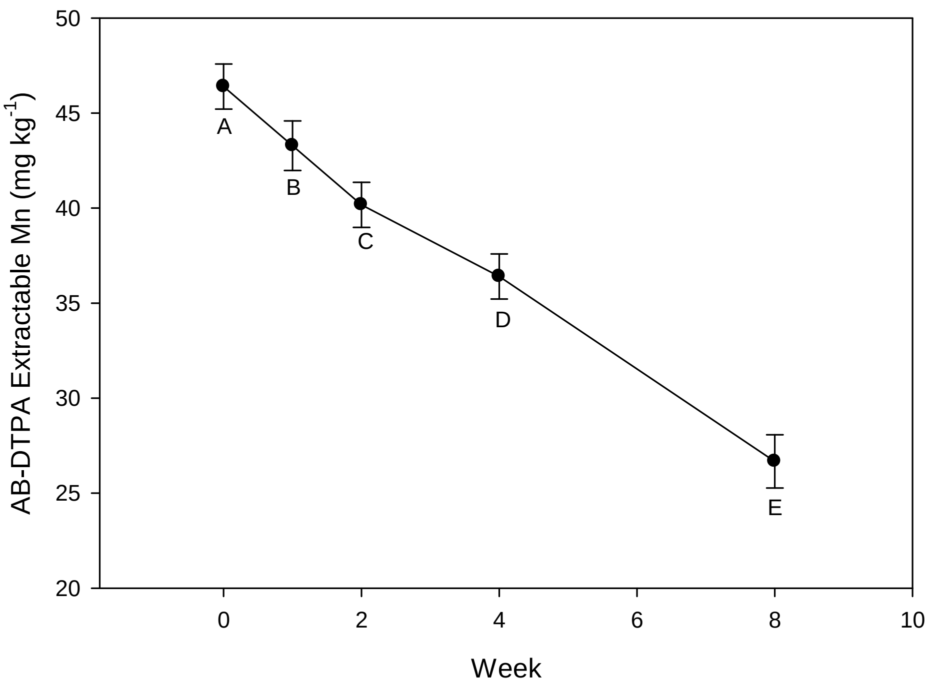

Figure A.40. Concentration of AB-DTPA extractable Mn concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable Mn when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

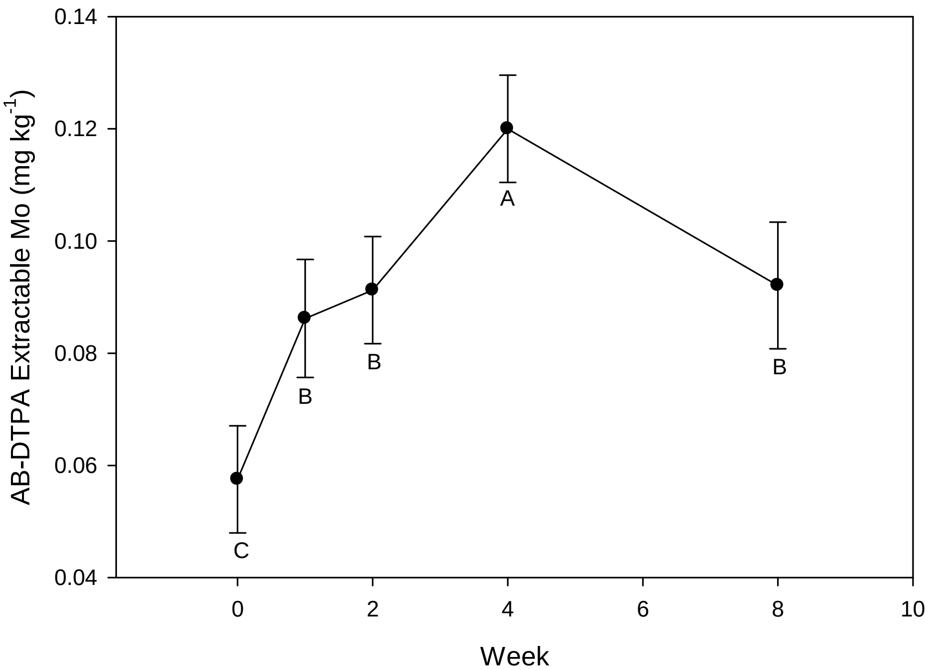

Figure A.41. Concentration of AB-DTPA extractable Mo concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable Mo when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.29. Concentration of AB-DTPA extractable Mo in the soil with reduced microbial population with the addition of biosolids. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex), the addition of biosolids had a significant effect on the concentration of AB-DTPA extractable Mo when averaged over the addition of *A. caliginosa* and time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

	AB-DTPA Extractable Mo (mg kg^{-1})	**Standard Error**
With Biosolids	0.100a	0.00625
Without Biosolids	0.0785b	0.00645

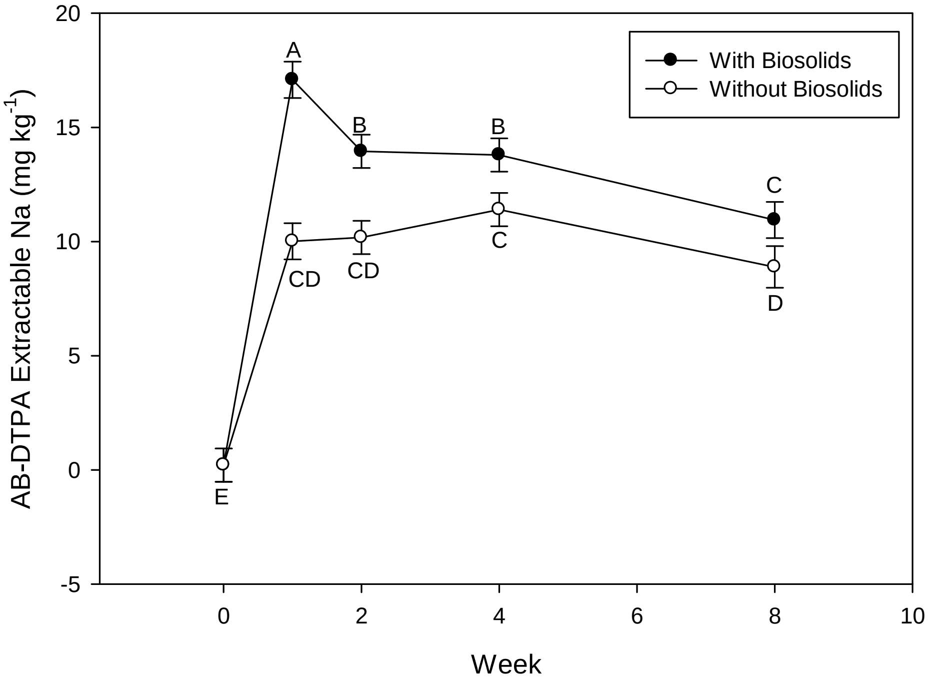

Figure A.42. Concentration of AB-DTPA extractable soil Na in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the concentration of AB-DTPA extractable Na. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

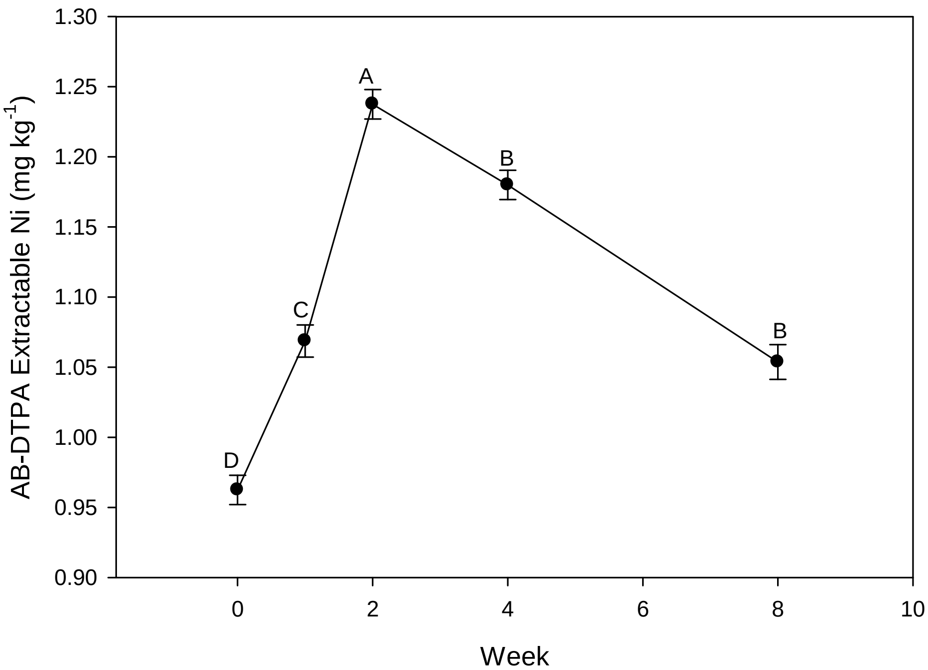

Figure A.43. Concentration of AB-DTPA extractable Ni concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable Ni when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

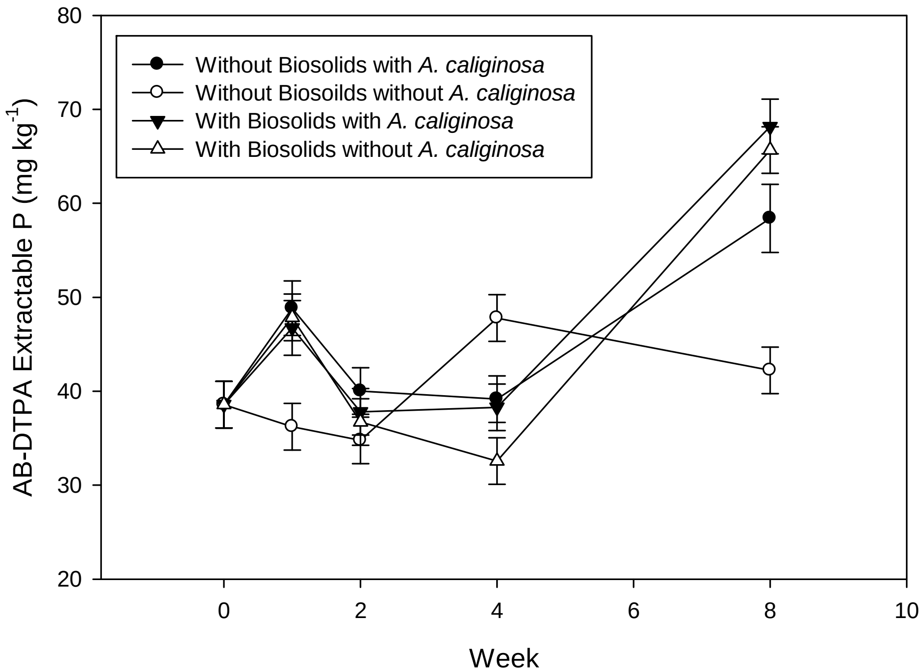

Figure A.44. Concentration of AB-DTPA extractable soil P in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant three-way interaction between the addition of *A. caliginosa*, biosolids, and week on the concentration of AB-DTPA extractable soil P. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

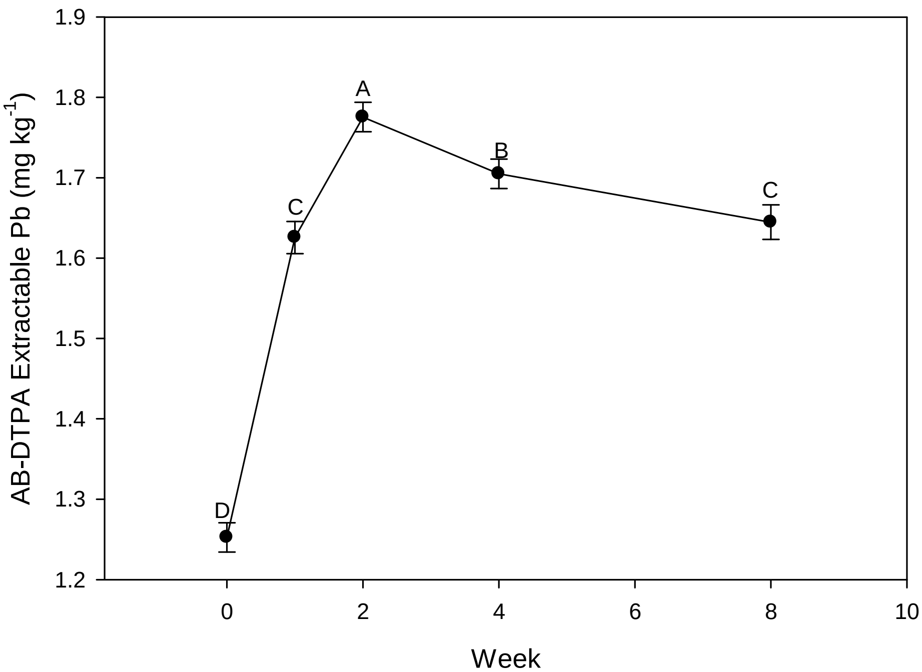

Figure A.45. Concentration of AB-DTPA extractable Pb concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable Pb when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

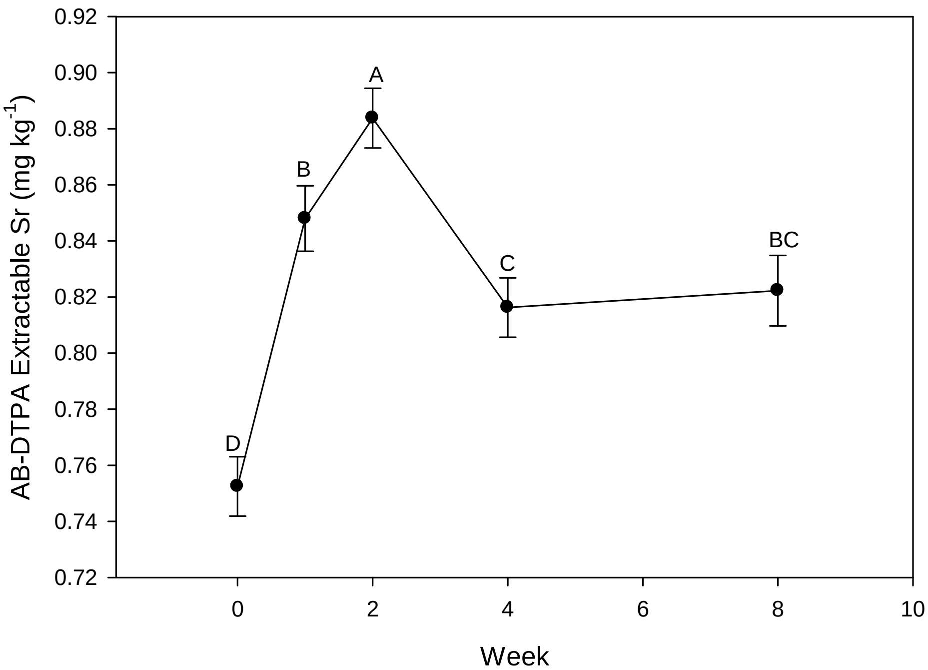

Figure A.46. Concentration of AB-DTPA extractable Sr concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable Sr when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

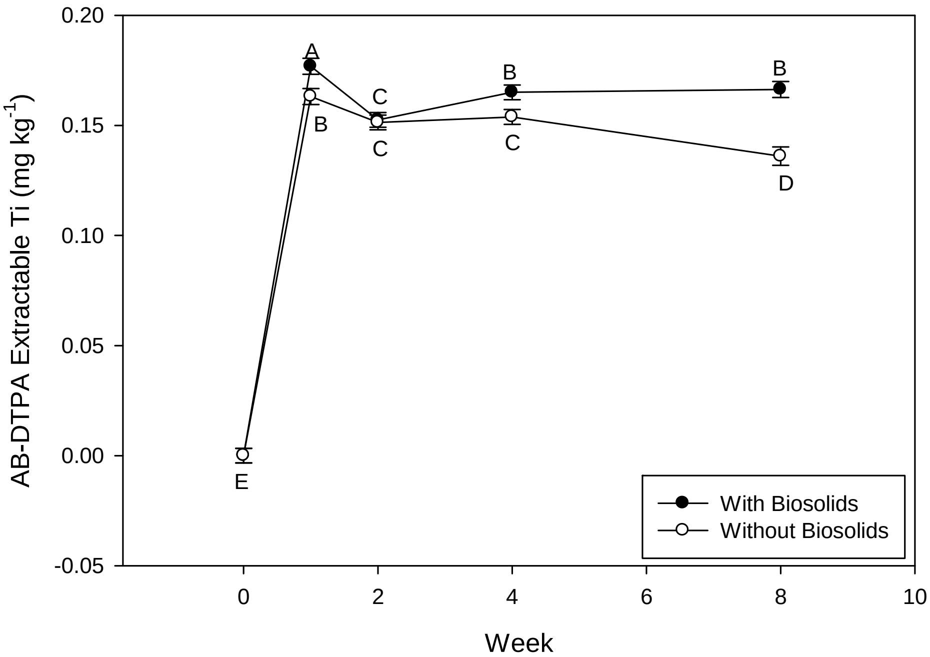

Figure A.47. Concentration of AB-DTPA extractable soil Ti in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and week on the concentration of AB-DTPA extractable Ti. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

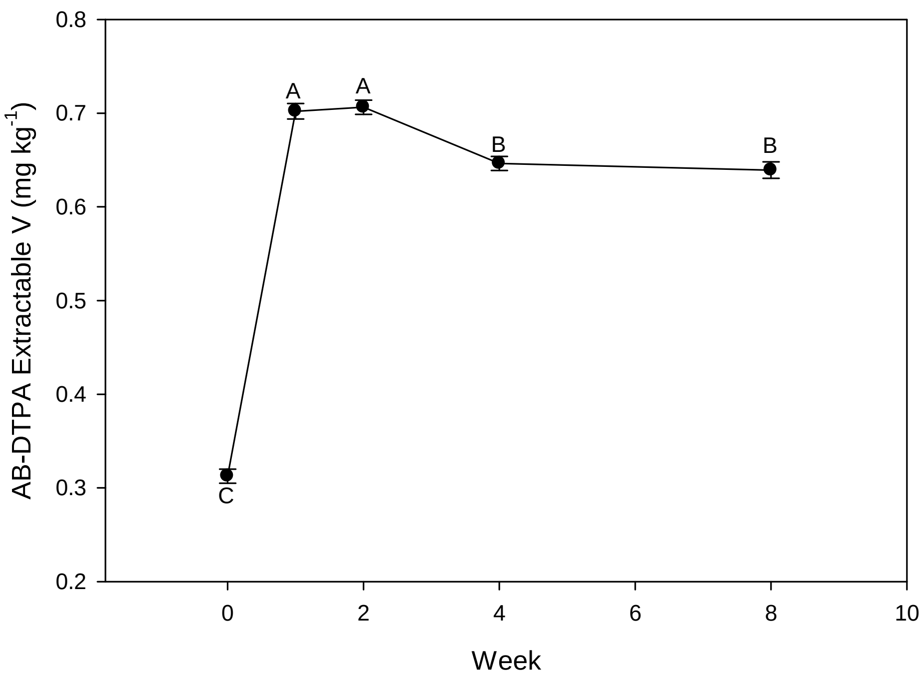

Figure A.48. Concentration of AB-DTPA extractable V concentration in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) time had a significant time effect on the concentration of AB-DTPA extractable V when averaged over the addition biosolids and *A. caliginosa*. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

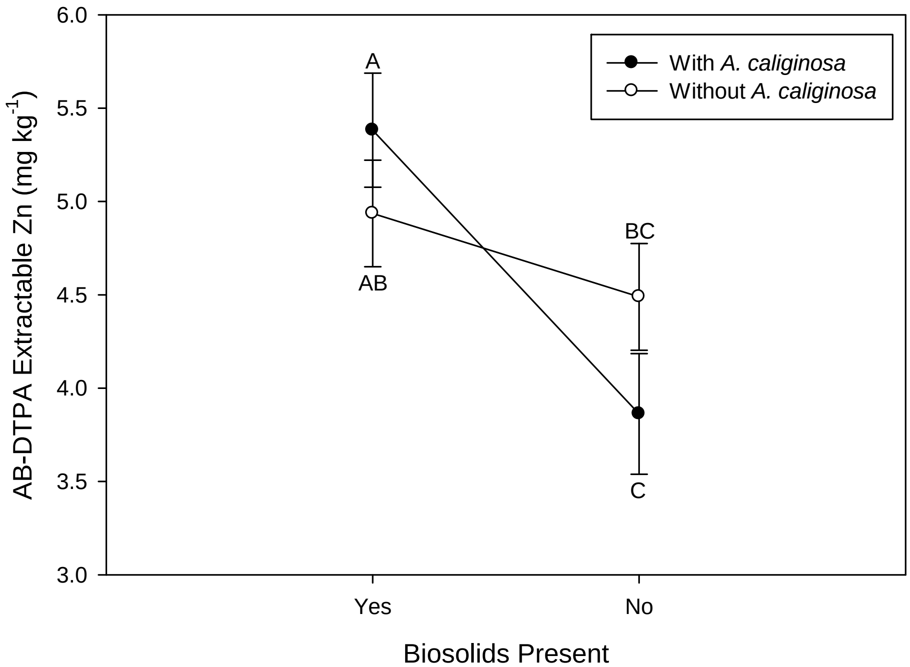

Figure A.49. Concentration of AB-DTPA extractable Zn with the addition of biosolids and *A. caliginosa* in a reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) the addition of biosolids and *A. caliginosa* had a significant effect on the concentration of AB-DTPA extractable Zn when averaged over time. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

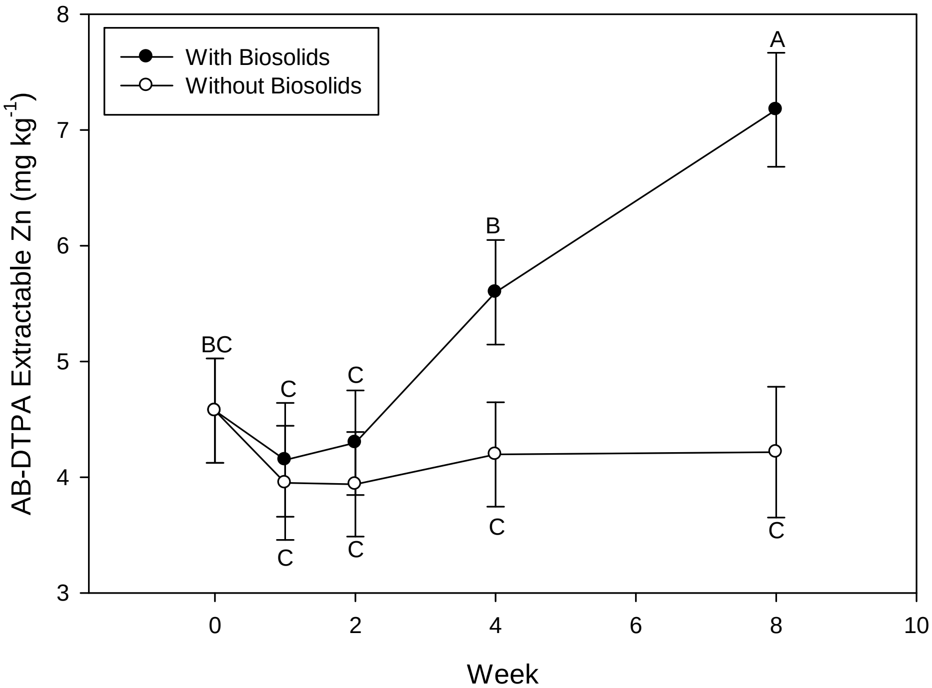

Figure A.50. Concentration of AB-DTPA extractable soil Zn in a reduced microbial population soil over time. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) there was a significant interaction between the addition of biosolids and time on the concentration of AB-DTPA extractable Zn. Values labeled with the same letter are not significantly different at an alpha value of 0.10.

Table A.30. Total soil C from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	1.82	0.0648	1.76	1.90
0	No	Yes	1.82	0.0648	1.76	1.90
0	Yes	No	1.82	0.0648	1.76	1.90
0	Yes	Yes	1.82	0.0648	1.76	1.90
1	No	No	1.84	0.0637	1.77	1.93
1	No	Yes	1.91	0.0693	1.83	1.95
1	Yes	No	2.03	0.259	1.77	2.30
1	Yes	Yes	2.00	0.134	1.84	2.16
2	No	No	1.85	0.0218	1.83	1.87
2	No	Yes	1.82	0.0291	1.79	1.86
2	Yes	No	1.88	0.0415	1.85	1.94
2	Yes	Yes	1.87	0.0258	1.85	1.91
4	No	No	1.76	0.214	1.48	2.00
4	No	Yes	1.85	0.137	1.74	2.05
4	Yes	No	1.90	0.117	1.79	2.04
4	Yes	Yes	1.80	0.0540	1.75	1.86
8	No	No	2.10	0.228	1.87	2.37
8	No	Yes	2.11	0.0743	2.02	2.20
8	Yes	No	2.15	0.155	1.99	2.33
8	Yes	Yes	2.26	0.264	1.89	2.51
12	No	No	2.03	0.101	1.96	2.18
12	No	Yes	1.91	0.102	1.81	2.03
12	Yes	No	2.08	0.187	1.88	2.29
12	Yes	Yes	2.04	0.112	1.92	2.19

Table A.31. Total soil N from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	0.165	0.006	0.158	0.174
0	No	Yes	0.165	0.006	0.158	0.174
0	Yes	No	0.165	0.006	0.158	0.174
0	Yes	Yes	0.165	0.006	0.158	0.174
1	No	No	0.170	0.005	0.167	0.178
1	No	Yes	0.171	0.009	0.162	0.178
1	Yes	No	0.208	0.034	0.178	0.252
1	Yes	Yes	0.197	0.020	0.176	0.217
2	No	No	0.172	0.001	0.170	0.173
2	No	Yes	0.170	0.006	0.166	0.179
2	Yes	No	0.189	0.005	0.182	0.195
2	Yes	Yes	0.180	0.010	0.169	0.191
4	No	No	0.181	0.023	0.164	0.216
4	No	Yes	0.175	0.023	0.149	0.204
4	Yes	No	0.201	0.019	0.172	0.210
4	Yes	Yes	0.172	0.010	0.163	0.184
8	No	No	0.218	0.041	0.169	0.261
8	No	Yes	0.234	0.012	0.221	0.248
8	Yes	No	0.231	0.011	0.220	0.243
8	Yes	Yes	0.237	0.046	0.177	0.282
12	No	No	0.201	0.003	0.199	0.204
12	No	Yes	0.193	0.018	0.173	0.217
12	Yes	No	0.225	0.028	0.185	0.248
12	Yes	Yes	0.216	0.015	0.195	0.227

Table A.32. Soil inorganic C from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	0.47	0.02	0.44	0.49
0	No	Yes	0.47	0.02	0.44	0.49
0	Yes	No	0.47	0.02	0.44	0.49
0	Yes	Yes	0.47	0.02	0.44	0.49
1	No	No	0.49	0.03	0.45	0.51
1	No	Yes	0.50	0.00	0.50	0.50
1	Yes	No	0.49	0.02	0.47	0.50
1	Yes	Yes	0.50	0.01	0.49	0.51
2	No	No	0.50	0.01	0.49	0.51
2	No	Yes	0.50	0.02	0.48	0.51
2	Yes	No	0.49	0.02	0.47	0.51
2	Yes	Yes	0.49	0.02	0.47	0.51
4	No	No	0.46	0.03	0.41	0.48
4	No	Yes	0.46	0.03	0.42	0.50
4	Yes	No	0.45	0.02	0.43	0.47
4	Yes	Yes	0.45	0.04	0.42	0.49
8	No	No	0.42	0.02	0.40	0.44
8	No	Yes	0.42	0.02	0.39	0.45
8	Yes	No	0.41	0.01	0.40	0.42
8	Yes	Yes	0.43	0.01	0.42	0.44
12	No	No	0.44	0.02	0.42	0.46
12	No	Yes	0.44	0.02	0.42	0.47
12	Yes	No	0.43	0.01	0.42	0.45
12	Yes	Yes	0.44	0.02	0.42	0.46

Table A.33. Soil organic C from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	1.35	0.08	1.28	1.46
0	No	Yes	1.35	0.08	1.28	1.46
0	Yes	No	1.35	0.08	1.28	1.46
0	Yes	Yes	1.35	0.08	1.28	1.46
1	No	No	1.35	0.04	1.32	1.41
1	No	Yes	1.41	0.07	1.33	1.46
1	Yes	No	1.55	0.24	1.30	1.80
1	Yes	Yes	1.50	0.12	1.35	1.64
2	No	No	1.35	0.03	1.31	1.38
2	No	Yes	1.33	0.02	1.31	1.35
2	Yes	No	1.40	0.02	1.38	1.43
2	Yes	Yes	1.39	0.04	1.37	1.44
4	No	No	1.30	0.24	1.01	1.59
4	No	Yes	1.38	0.16	1.28	1.62
4	Yes	No	1.45	0.14	1.32	1.61
4	Yes	Yes	1.36	0.07	1.31	1.44
8	No	No	1.68	0.24	1.45	1.95
8	No	Yes	1.69	0.09	1.60	1.81
8	Yes	No	1.74	0.16	1.57	1.92
8	Yes	Yes	1.84	0.27	1.46	2.09
12	No	No	1.59	0.10	1.53	1.74
12	No	Yes	1.48	0.09	1.38	1.57
12	Yes	No	1.65	0.18	1.45	1.84
12	Yes	Yes	1.61	0.11	1.49	1.75

Table A.34. Soil NO_3-N from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	24.1	3.27	21.0	28.6
0	No	Yes	24.1	3.27	21.0	28.6
0	Yes	No	24.1	3.27	21.0	28.6
0	Yes	Yes	24.1	3.27	21.0	28.6
1	No	No	30.9	3.72	25.6	33.7
1	No	Yes	31.1	4.96	25.4	34.6
1	Yes	No	64.4	8.49	58.4	76.8
1	Yes	Yes	70.2	7.01	62.1	79.2
2	No	No	31.4	3.25	28.1	34.8
2	No	Yes	30.7	4.12	24.6	33.6
2	Yes	No	75.3	16.3	58.9	97.4
2	Yes	Yes	79.8	9.17	73.5	93.4
4	No	No	29.7	8.51	22.8	41.4
4	No	Yes	33.3	5.96	27.0	41.3
4	Yes	No	80.0	11.2	69.8	95.8
4	Yes	Yes	82.1	11.7	75.2	95.63
8	No	No	56.0	10.4	46.9	67.0
8	No	Yes	69.5	6.96	62.6	77.2
8	Yes	No	122	9.33	112	134
8	Yes	Yes	128	17.3	109	145
12	No	No	63.2	4.47	58.6	67.9
12	No	Yes	68.2	5.99	60.9	73.8
12	Yes	No	128	24.5	107	163
12	Yes	Yes	131	11.6	118	142

Table A.35. Soil NH_4-N from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.00	0.00	0.00	0.00
0	No	Yes	0.00	0.00	0.00	0.00
0	Yes	No	0.00	0.00	0.00	0.00
0	Yes	Yes	0.00	0.00	0.00	0.00
1	No	No	0.22	0.27	0.00	0.54
1	No	Yes	0.11	0.13	0.00	0.25
1	Yes	No	22.7	7.11	14.4	30.5
1	Yes	Yes	30.8	18.1	12.1	54.3
2	No	No	0.00	0.00	0.00	0.00
2	No	Yes	1.36	1.66	0.00	3.76
2	Yes	No	20.0	13.8	11.9	40.6
2	Yes	Yes	16.8	17.0	7.22	42.2
4	No	No	0.06	0.11	0.00	0.23
4	No	Yes	0.75	0.85	0.00	1.51
4	Yes	No	4.83	3.36	0.97	7.87
4	Yes	Yes	11.5	1.72	9.53	12.7
8	No	No	0.24	0.33	0.00	0.71
8	No	Yes	1.81	1.40	0.00	2.97
8	Yes	No	2.36	2.21	0.50	5.03
8	Yes	Yes	2.21	1.56	0.88	4.46
12	No	No	1.29	0.18	1.10	1.48
12	No	Yes	4.10	1.45	2.07	5.17
12	Yes	No	5.69	6.55	0.37	15.2
12	Yes	Yes	7.38	2.78	4.16	9.74

Table A.36. Soil microbial biomass C from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	350	94.58	274	479
0	No	Yes	350	94.58	274	479
0	Yes	No	350	94.58	274	479
0	Yes	Yes	350	94.58	274	479
1	No	No	474	167	228	592
1	No	Yes	439	153	263	537
1	Yes	No	556	237	207	721
1	Yes	Yes	701	341	208	951
2	No	No	191	129	0.00	277
2	No	Yes	251	36.6	217	302
2	Yes	No	347	63.2	294	439
2	Yes	Yes	291	81.7	173	360
4	No	No	244	32.8	215	291
4	No	Yes	221	63.9	157	310
4	Yes	No	247	66.1	193	321
4	Yes	Yes	229	5.75	224	235
8	No	No	257	54.4	227	338
8	No	Yes	266	31.0	233	304
8	Yes	No	2670	29.2	247	310
8	Yes	Yes	274	67.2	208	356
12	No	No	202	79.5	124	299
12	No	Yes	154	71.5	108	259
12	Yes	No	251	61.6	193	323
12	Yes	Yes	173	49.4	130	244

Table A.37. Soil microbial biomass N from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	58.5	21.9	39.7	88.5
0	No	Yes	58.5	21.9	39.7	88.5
0	Yes	No	58.5	21.9	39.7	88.5
0	Yes	Yes	58.5	21.9	39.7	88.5
1	No	No	75.5	15.9	55.1	90.7
1	No	Yes	73.8	18.0	54.7	90.6
1	Yes	No	98.8	38.7	43.9	131
1	Yes	Yes	149	74.0	40.6	206
2	No	No	14.5	11.3	0.00	27.1
2	No	Yes	11.3	5.70	3.39	16.5
2	Yes	No	19.6	29.5	0.00	62.5
2	Yes	Yes	6.44	12.9	0.00	25.8
4	No	No	42.1	19.7	24.6	69.8
4	No	Yes	39.6	23.0	27.6	74.0
4	Yes	No	6.47	11.2	0.00	19.4
4	Yes	Yes	23.6	13.2	10.7	37.1
8	No	No	55.5	12.6	42.7	72.3
8	No	Yes	51.5	11.1	35.0	57.8
8	Yes	No	17.3	12.0	0.00	26.7
8	Yes	Yes	14.6	11.5	0.00	27.1
12	No	No	42.4	23.9	16.8	65.3
12	No	Yes	24.7	13.0	13.0	43.0
12	Yes	No	43.7	54.9	4.96	124
12	Yes	Yes	3.21	4.88	0.00	10.4

Table A.38. Soil pH from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean	Standard Deviation	Min.	Max.
0	No	No	7.46	0.07	7.37	7.53
0	No	Yes	7.46	0.07	7.37	7.53
0	Yes	No	7.46	0.07	7.37	7.53
0	Yes	Yes	7.46	0.07	7.37	7.53
1	No	No	7.52	0.28	7.15	7.77
1	No	Yes	7.51	0.23	7.28	7.74
1	Yes	No	7.39	0.18	7.16	7.54
1	Yes	Yes	7.39	0.14	7.21	7.52
2	No	No	7.82	0.03	7.78	7.84
2	No	Yes	7.83	0.03	7.80	7.86
2	Yes	No	7.61	0.05	7.54	7.65
2	Yes	Yes	7.59	0.04	7.56	7.63
4	No	No	7.78	0.10	7.64	7.88
4	No	Yes	7.75	0.09	7.65	7.84
4	Yes	No	7.52	0.07	7.46	7.62
4	Yes	Yes	7.57	0.07	7.49	7.62
8	No	No	7.54	0.25	7.17	7.72
8	No	Yes	7.52	0.16	7.30	7.66
8	Yes	No	7.43	0.13	7.25	7.54
8	Yes	Yes	7.45	0.15	7.28	7.64
12	No	No	7.76	0.05	7.69	7.79
12	No	Yes	7.72	0.04	7.66	7.75
12	Yes	No	7.58	0.03	7.55	7.61
12	Yes	Yes	7.56	0.06	7.50	7.62

Table A.39. Summary of soil electrical conductivity from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (ds m^{-1})	Standard Deviation	Min. (ds m^{-1})	Max. (ds m^{-1})
0	No	No	0.59	0.16	0.43	0.76
0	No	Yes	0.59	0.16	0.43	0.76
0	Yes	No	0.59	0.16	0.43	0.76
0	Yes	Yes	0.59	0.16	0.43	0.76
1	No	No	0.75	0.09	0.65	0.86
1	No	Yes	0.78	0.07	0.72	0.86
1	Yes	No	1.96	0.10	1.84	2.08
1	Yes	Yes	2.13	0.19	1.85	2.28
2	No	No	0.73	0.12	0.62	0.84
2	No	Yes	0.82	0.09	0.75	0.94
2	Yes	No	2.11	0.48	1.71	2.80
2	Yes	Yes	2.11	0.23	1.87	2.41
4	No	No	0.71	0.15	0.58	0.93
4	No	Yes	0.79	0.12	0.71	0.97
4	Yes	No	1.90	0.28	1.48	2.05
4	Yes	Yes	1.93	0.23	1.74	2.18
8	No	No	1.28	0.38	0.92	1.64
8	No	Yes	1.64	0.44	1.22	2.20
8	Yes	No	2.61	0.19	2.41	2.83
8	Yes	Yes	2.57	0.12	2.45	2.69
12	No	No	1.26	0.03	1.22	1.28
12	No	Yes	1.39	0.10	1.25	1.49
12	Yes	No	2.80	0.25	2.55	3.09
12	Yes	Yes	2.67	0.35	2.19	3.01

Table A.40. Concentration of AB-DTPA extractable Ba from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.55	0.01	0.53	0.56
0	No	Yes	0.55	0.01	0.53	0.56
0	Yes	No	0.55	0.01	0.53	0.56
0	Yes	Yes	0.55	0.01	0.53	0.56
1	No	No	0.62	0.01	0.60	0.63
1	No	Yes	0.63	0.03	0.61	0.66
1	Yes	No	0.81	0.03	0.77	0.85
1	Yes	Yes	0.85	0.06	0.78	0.93
2	No	No	0.61	0.04	0.57	0.66
2	No	Yes	0.60	0.01	0.59	0.62
2	Yes	No	0.82	0.10	0.75	0.97
2	Yes	Yes	0.89	0.16	0.74	1.12
4	No	No	0.63	0.05	0.55	0.67
4	No	Yes	0.60	0.04	0.54	0.63
4	Yes	No	0.70	0.08	0.61	0.80
4	Yes	Yes	0.74	0.10	0.63	0.82
8	No	No	0.46	0.07	0.39	0.53
8	No	Yes	0.47	0.05	0.40	0.52
8	Yes	No	0.56	0.12	0.46	0.71
8	Yes	Yes	0.55	0.05	0.50	0.62
12	No	No	0.58	0.05	0.53	0.63
12	No	Yes	0.60	0.02	0.57	0.62
12	Yes	No	0.67	0.06	0.59	0.74
12	Yes	Yes	0.67	0.02	0.65	0.69

Table A.41. Concentration of AB-DTPA extractable Cd from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.07	0.01	0.06	0.08
0	No	Yes	0.07	0.01	0.06	0.08
0	Yes	No	0.07	0.01	0.06	0.08
0	Yes	Yes	0.07	0.01	0.06	0.08
1	No	No	0.09	0.03	0.08	0.13
1	No	Yes	0.08	0.00	0.08	0.08
1	Yes	No	0.08	0.01	0.07	0.09
1	Yes	Yes	0.08	0.00	0.08	0.08
2	No	No	0.08	0.01	0.07	0.09
2	No	Yes	0.08	0.01	0.07	0.09
2	Yes	No	0.10	0.04	0.08	0.16
2	Yes	Yes	0.10	0.05	0.08	0.17
4	No	No	0.08	0.01	0.07	0.09
4	No	Yes	0.08	0.01	0.08	0.09
4	Yes	No	0.09	0.01	0.08	0.10
4	Yes	Yes	0.09	0.02	0.07	0.10
8	No	No	0.11	0.02	0.08	0.13
8	No	Yes	0.11	0.02	0.09	0.13
8	Yes	No	0.10	0.01	0.08	0.11
8	Yes	Yes	0.09	0.01	0.08	0.10
12	No	No	0.11	0.02	0.09	0.13
12	No	Yes	0.12	0.05	0.09	0.19
12	Yes	No	0.09	0.02	0.08	0.11
12	Yes	Yes	0.09	0.01	0.07	0.10

Table A.42. Concentration of AB-DTPA extractable Cr from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.00	0.00	0.00	0.00
0	No	Yes	0.00	0.00	0.00	0.00
0	Yes	No	0.00	0.00	0.00	0.00
0	Yes	Yes	0.00	0.00	0.00	0.00
1	No	No	0.01	0.02	0.00	0.03
1	No	Yes	0.00	0.00	0.00	0.00
1	Yes	No	0.01	0.02	0.00	0.04
1	Yes	Yes	0.01	0.01	0.00	0.02
2	No	No	0.04	0.01	0.03	0.05
2	No	Yes	0.02	0.01	0.00	0.03
2	Yes	No	0.04	0.01	0.03	0.04
2	Yes	Yes	0.02	0.02	0.00	0.04
4	No	No	0.04	0.01	0.03	0.04
4	No	Yes	0.02	0.02	0.00	0.03
4	Yes	No	0.03	0.03	0.00	0.06
4	Yes	Yes	0.01	0.02	0.00	0.03
8	No	No	0.00	0.00	0.00	0.00
8	No	Yes	0.00	0.00	0.00	0.00
8	Yes	No	0.00	0.00	0.00	0.00
8	Yes	Yes	0.00	0.00	0.00	0.00
12	No	No	0.00	0.00	0.00	0.00
12	No	Yes	0.01	0.02	0.00	0.03
12	Yes	No	0.01	0.02	0.00	0.04
12	Yes	Yes	0.01	0.02	0.00	0.03

Table A.43. Concnetration of AB-DTPA extractable Cu from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	6.54	0.77	5.86	7.28
0	No	Yes	6.54	0.77	5.86	7.28
0	Yes	No	6.54	0.77	5.86	7.28
0	Yes	Yes	6.54	0.77	5.86	7.28
1	No	No	8.91	0.79	8.41	10.1
1	No	Yes	9.05	0.93	8.00	9.77
1	Yes	No	10.3	1.20	9.31	11.7
1	Yes	Yes	11.3	0.29	10.9	11.6
2	No	No	8.49	0.29	8.14	8.78
2	No	Yes	8.72	0.53	7.95	9.08
2	Yes	No	9.83	1.28	8.70	11.5
2	Yes	Yes	10.7	0.98	9.44	11.8
4	No	No	8.88	2.42	7.09	12.5
4	No	Yes	8.72	2.63	7.12	12.6
4	Yes	No	10.3	1.66	8.17	12.0
4	Yes	Yes	9.61	2.35	7.81	12.3
8	No	No	14.1	4.49	9.48	18.0
8	No	Yes	14.6	3.57	11.2	19.1
8	Yes	No	13.4	3.89	8.91	17.3
8	Yes	Yes	13.4	2.67	9.50	15.7
12	No	No	12.6	3.05	10.3	17.1
12	No	Yes	13.3	1.46	11.7	14.7
12	Yes	No	12.3	1.32	10.4	13.3
12	Yes	Yes	12.9	2.01	11.0	15.5

Table A.44. Concentration of AB-DTPA extractable Fe from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	6.37	0.94	5.57	7.59
0	No	Yes	6.37	0.94	5.57	7.59
0	Yes	No	6.37	0.94	5.57	7.59
0	Yes	Yes	6.37	0.94	5.57	7.59
1	No	No	8.17	1.14	6.76	9.38
1	No	Yes	8.35	0.94	7.37	9.24
1	Yes	No	8.84	2.04	6.69	11.4
1	Yes	Yes	9.41	1.28	8.51	11.3
2	No	No	8.14	0.85	7.38	9.21
2	No	Yes	8.45	0.71	7.45	9.11
2	Yes	No	8.93	0.37	8.42	9.27
2	Yes	Yes	9.57	1.13	8.37	10.9
4	No	No	8.77	1.27	7.48	10.3
4	No	Yes	8.69	1.40	7.26	10.5
4	Yes	No	10.4	0.99	9.80	11.9
4	Yes	Yes	9.98	2.38	8.10	12.7
8	No	No	12.8	4.22	7.88	16.7
8	No	Yes	13.5	3.37	10.3	17.2
8	Yes	No	13.8	3.47	10.8	17.3
8	Yes	Yes	13.2	2.79	9.17	15.3
12	No	No	10.9	3.44	8.47	15.9
12	No	Yes	12.4	2.22	9.50	14.7
12	Yes	No	10.7	1.96	8.40	12.7
12	Yes	Yes	12.9	1.22	11.7	14.6

Table A.45. Concentration of AB-DTPA extractable K from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	448	13.2	434	466
0	No	Yes	448	13.2	434	466
0	Yes	No	448	13.2	434	466
0	Yes	Yes	448	13.2	434	466
1	No	No	625	64.5	539	694
1	No	Yes	610	48.7	556	651
1	Yes	No	600	39.1	553	635
1	Yes	Yes	599	33.4	559	634
2	No	No	578	40.0	529	611
2	No	Yes	572	43.3	533	615
2	Yes	No	585	38.6	530	619
2	Yes	Yes	604	55.1	528	658
4	No	No	537	13.5	524	550
4	No	Yes	537	17.0	523	561
4	Yes	No	557	24.0	526	582
4	Yes	Yes	550	20.4	531	572
8	No	No	507	21.0	481	527
8	No	Yes	511	24.8	482	543
8	Yes	No	504	28.3	467	535
8	Yes	Yes	508	29.0	468	538
12	No	No	555	31.7	529	601
12	No	Yes	566	23.2	540	588
12	Yes	No	564	33.3	531	594
12	Yes	Yes	564	31.4	535	603

Table A.46. Concentration of AB-DTPA extractable Mn from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	1.15	0.17	0.99	1.32
0	No	Yes	1.15	0.17	0.99	1.32
0	Yes	No	1.15	0.17	0.99	1.32
0	Yes	Yes	1.15	0.17	0.99	1.32
1	No	No	4.95	1.28	3.27	6.12
1	No	Yes	4.79	0.52	4.22	5.23
1	Yes	No	4.14	2.13	1.41	6.08
1	Yes	Yes	5.41	0.66	4.87	6.28
2	No	No	4.97	0.26	4.72	5.31
2	No	Yes	4.39	0.56	3.87	5.10
2	Yes	No	4.81	0.77	4.36	5.96
2	Yes	Yes	5.01	1.09	4.18	6.56
4	No	No	3.84	0.21	3.55	4.05
4	No	Yes	3.63	0.59	3.11	4.15
4	Yes	No	4.13	0.12	4.02	4.26
4	Yes	Yes	3.95	0.51	3.45	4.46
8	No	No	2.94	1.07	1.51	4.10
8	No	Yes	2.63	0.58	1.82	3.15
8	Yes	No	2.75	0.96	1.88	3.83
8	Yes	Yes	2.68	0.74	2.09	3.66
12	No	No	2.05	0.44	1.66	2.64
12	No	Yes	2.59	0.77	1.91	3.55
12	Yes	No	1.89	0.40	1.35	2.22
12	Yes	Yes	2.40	0.45	1.79	2.82

Table A.47. Concentration of AB-DTPA extractable Mo from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.01	0.03	0.00	0.05
0	No	Yes	0.01	0.03	0.00	0.05
0	Yes	No	0.01	0.03	0.00	0.05
0	Yes	Yes	0.01	0.03	0.00	0.05
1	No	No	0.07	0.03	0.03	0.10
1	No	Yes	0.04	0.01	0.03	0.05
1	Yes	No	0.05	0.04	0.00	0.09
1	Yes	Yes	0.06	0.03	0.03	0.09
2	No	No	0.06	0.02	0.04	0.07
2	No	Yes	0.04	0.01	0.03	0.06
2	Yes	No	0.08	0.04	0.04	0.13
2	Yes	Yes	0.10	0.03	0.07	0.14
4	No	No	0.07	0.03	0.04	0.11
4	No	Yes	0.06	0.02	0.05	0.09
4	Yes	No	0.08	0.01	0.07	0.09
4	Yes	Yes	0.06	0.01	0.05	0.07
8	No	No	0.11	0.06	0.03	0.16
8	No	Yes	0.12	0.06	0.06	0.20
8	Yes	No	0.10	0.05	0.03	0.15
8	Yes	Yes	0.08	0.03	0.05	0.11
12	No	No	0.08	0.02	0.06	0.10
12	No	Yes	0.09	0.04	0.06	0.15
12	Yes	No	0.06	0.02	0.03	0.07
12	Yes	Yes	0.08	0.02	0.06	0.10

Table A.48. Concentration of AB-DTPA extractable Na from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.91	0.76	0.00	1.78
0	No	Yes	0.91	0.76	0.00	1.78
0	Yes	No	0.91	0.76	0.00	1.78
0	Yes	Yes	0.91	0.76	0.00	1.78
1	No	No	11.3	2.04	9.36	14.2
1	No	Yes	13.2	1.47	11.9	14.8
1	Yes	No	16.2	2.61	12.6	18.5
1	Yes	Yes	14.9	1.97	12.7	16.7
2	No	No	8.92	0.95	7.49	9.46
2	No	Yes	7.97	1.91	5.18	9.3
2	Yes	No	11.7	3.40	7.58	15.8
2	Yes	Yes	14.5	1.83	12.3	16.7
4	No	No	11.3	4.27	5.59	15.6
4	No	Yes	11.0	2.88	7.22	14.0
4	Yes	No	12.0	2.20	9.96	14.8
4	Yes	Yes	17.3	3.76	12.9	19.9
8	No	No	9.33	1.96	7.28	11.5
8	No	Yes	8.65	2.69	5.50	12.0
8	Yes	No	12.4	2.02	10.4	15.0
8	Yes	Yes	11.9	1.09	10.8	13.3
12	No	No	19.6	5.59	11.3	23.3
12	No	Yes	23.2	4.03	19.0	28.0
12	Yes	No	23.8	1.31	22.7	25.6
12	Yes	Yes	24.1	3.46	19.1	26.8

Table A.49. Concentration of AB-DTPA extractable Ni from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	**Biosolids Present**	**Earthworms Present**	**Mean (mg kg^{-1})**	**Standard Deviation**	**Min. (mg kg^{-1})**	**Max. (mg kg^{-1})**
0	No	No	0.75	0.04	0.70	0.80
0	No	Yes	0.75	0.04	0.70	0.80
0	Yes	No	0.75	0.04	0.70	0.80
0	Yes	Yes	0.75	0.04	0.70	0.80
1	No	No	0.94	0.09	0.86	1.06
1	No	Yes	0.92	0.03	0.88	0.94
1	Yes	No	0.92	0.06	0.83	0.97
1	Yes	Yes	0.91	0.03	0.88	0.95
2	No	No	1.01	0.05	0.94	1.05
2	No	Yes	1.00	0.03	0.97	1.03
2	Yes	No	1.04	0.07	0.95	1.12
2	Yes	Yes	1.04	0.04	0.99	1.09
4	No	No	0.99	0.07	0.93	1.07
4	No	Yes	1.03	0.05	0.98	1.09
4	Yes	No	1.06	0.08	0.98	1.15
4	Yes	Yes	1.04	0.03	1.02	1.07
8	No	No	0.89	0.04	0.85	0.94
8	No	Yes	0.90	0.04	0.85	0.94
8	Yes	No	0.89	0.08	0.77	0.96
8	Yes	Yes	0.90	0.05	0.84	0.95
12	No	No	0.95	0.06	0.86	1.00
12	No	Yes	1.02	0.05	0.98	1.08
12	Yes	No	0.95	0.05	0.89	0.99
12	Yes	Yes	0.96	0.07	0.90	1.05

Table A.50. Concentration of AB-DTPA extractable P from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	39.5	4.53	35.1	44.4
0	No	Yes	39.5	4.53	35.1	44.4
0	Yes	No	39.5	4.53	35.1	44.4
0	Yes	Yes	39.5	4.53	35.1	44.4
1	No	No	43.3	4.54	38.1	49.1
1	No	Yes	42.8	0.92	42.0	43.8
1	Yes	No	41.5	5.01	35.6	47.0
1	Yes	Yes	41.0	4.35	36.4	45.1
2	No	No	36.1	8.98	22.8	42.4
2	No	Yes	38.3	5.76	30.5	44.0
2	Yes	No	41.5	1.49	39.3	42.7
2	Yes	Yes	37.3	5.21	33.0	44.0
4	No	No	36.2	2.92	31.9	38.1
4	No	Yes	38.4	1.90	36.0	40.4
4	Yes	No	33.0	10.4	20.4	42.7
4	Yes	Yes	33.6	5.81	26.9	37.3
8	No	No	62.3	4.95	56.5	68.6
8	No	Yes	68.2	5.11	63.1	74.7
8	Yes	No	65.3	8.35	54.9	74.6
8	Yes	Yes	61.6	3.92	57.7	65.5
12	No	No	54.5	15.0	32.2	65.0
12	No	Yes	51.3	20.4	31.6	70.0
12	Yes	No	52.5	15.6	36.1	68.6
12	Yes	Yes	47.3	21.9	19.4	66.8

Table A.51. Concentration of AB-DTPA extractable Pb from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	1.22	0.11	1.11	1.35
0	No	Yes	1.22	0.11	1.11	1.35
0	Yes	No	1.22	0.11	1.11	1.35
0	Yes	Yes	1.22	0.11	1.11	1.35
1	No	No	1.61	0.16	1.43	1.79
1	No	Yes	1.56	0.03	1.54	1.59
1	Yes	No	1.60	0.08	1.50	1.66
1	Yes	Yes	1.64	0.12	1.56	1.82
2	No	No	1.77	0.04	1.71	1.80
2	No	Yes	1.79	0.09	1.69	1.89
2	Yes	No	1.79	0.06	1.73	1.85
2	Yes	Yes	1.85	0.07	1.77	1.94
4	No	No	1.70	0.15	1.60	1.91
4	No	Yes	1.69	0.13	1.59	1.88
4	Yes	No	1.75	0.03	1.73	1.79
4	Yes	Yes	1.69	0.13	1.57	1.82
8	No	No	1.67	0.13	1.49	1.78
8	No	Yes	1.68	0.08	1.56	1.74
8	Yes	No	1.61	0.12	1.44	1.70
8	Yes	Yes	1.62	0.03	1.59	1.66
12	No	No	1.73	0.24	1.55	2.09
12	No	Yes	1.72	0.10	1.61	1.84
12	Yes	No	1.58	0.11	1.43	1.67
12	Yes	Yes	1.67	0.06	1.59	1.74

Table A.52. Concentration of AB-DTPA extractable Si from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	**Biosolids Present**	**Earthworms Present**	**Mean ($mg\ kg^{-1}$)**	**Standard Deviation**	**Min. ($mg\ kg^{-1}$)**	**Max. ($mg\ kg^{-1}$)**
0	No	No	1.66	0.04	1.62	1.70
0	No	Yes	1.66	0.04	1.62	1.70
0	Yes	No	1.66	0.04	1.62	1.70
0	Yes	Yes	1.66	0.04	1.62	1.70
1	No	No	4.04	1.04	2.92	5.17
1	No	Yes	4.50	0.23	4.35	4.77
1	Yes	No	4.36	1.70	2.39	6.00
1	Yes	Yes	4.10	1.00	3.40	5.58
2	No	No	3.60	0.55	3.03	4.11
2	No	Yes	3.55	0.21	3.31	3.77
2	Yes	No	3.87	0.36	3.37	4.20
2	Yes	Yes	4.70	1.59	3.65	7.07
4	No	No	4.33	1.11	3.43	5.91
4	No	Yes	3.64	0.43	3.34	4.27
4	Yes	No	3.93	0.81	3.04	4.74
4	Yes	Yes	3.75	0.39	3.43	4.18
8	No	No	2.90	0.31	2.57	3.20
8	No	Yes	3.41	0.58	2.73	3.91
8	Yes	No	3.54	0.67	2.99	4.51
8	Yes	Yes	3.37	0.71	2.37	4.06
12	No	No	3.68	0.09	3.60	3.80
12	No	Yes	3.99	1.18	3.17	5.67
12	Yes	No	3.61	0.75	2.92	4.60
12	Yes	Yes	4.10	1.02	3.41	5.61

Table A.53. Concentration of AB-DTPA extractable Sr from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.86	0.02	0.83	0.88
0	No	Yes	0.86	0.02	0.83	0.88
0	Yes	No	0.86	0.02	0.83	0.88
0	Yes	Yes	0.86	0.02	0.83	0.88
1	No	No	0.84	0.02	0.82	0.86
1	No	Yes	0.84	0.03	0.82	0.88
1	Yes	No	0.87	0.05	0.82	0.94
1	Yes	Yes	0.86	0.02	0.83	0.88
2	No	No	0.85	0.04	0.80	0.89
2	No	Yes	0.84	0.04	0.79	0.87
2	Yes	No	0.87	0.03	0.85	0.92
2	Yes	Yes	0.92	0.06	0.84	0.98
4	No	No	0.84	0.04	0.78	0.86
4	No	Yes	0.83	0.04	0.77	0.86
4	Yes	No	0.83	0.02	0.80	0.85
4	Yes	Yes	0.83	0.04	0.80	0.87
8	No	No	0.77	0.05	0.72	0.82
8	No	Yes	0.79	0.02	0.76	0.81
8	Yes	No	0.77	0.04	0.73	0.83
8	Yes	Yes	0.79	0.02	0.77	0.82
12	No	No	0.85	0.04	0.80	0.90
12	No	Yes	0.88	0.03	0.84	0.90
12	Yes	No	0.84	0.04	0.78	0.87
12	Yes	Yes	0.85	0.04	0.80	0.88

Table A.54. Concentration of AB-DTPA extractable Ti from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0	0	0	0
0	No	Yes	0	0	0	0
0	Yes	No	0	0	0	0
0	Yes	Yes	0	0	0	0
1	No	No	0.15	0.01	0.14	0.16
1	No	Yes	0.16	0.01	0.15	0.16
1	Yes	No	0.14	0.03	0.1	0.16
1	Yes	Yes	0.16	0.01	0.15	0.16
2	No	No	0.14	0.01	0.12	0.14
2	No	Yes	0.14	0.01	0.12	0.15
2	Yes	No	0.14	0.01	0.13	0.14
2	Yes	Yes	0.14	0.01	0.13	0.15
4	No	No	0.14	0.01	0.13	0.15
4	No	Yes	0.14	0.02	0.13	0.16
4	Yes	No	0.14	0	0.14	0.14
4	Yes	Yes	0.14	0.01	0.13	0.14
8	No	No	0.16	0.02	0.13	0.18
8	No	Yes	0.15	0.02	0.13	0.17
8	Yes	No	0.15	0.02	0.13	0.17
8	Yes	Yes	0.15	0.01	0.13	0.15
12	No	No	0.14	0.02	0.12	0.16
12	No	Yes	0.14	0.01	0.13	0.15
12	Yes	No	0.14	0.02	0.12	0.15
12	Yes	Yes	0.14	0.01	0.12	0.15

Table A.55. Concentration of AB-DTPA extractable V from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.26	0.01	0.25	0.27
0	No	Yes	0.26	0.01	0.25	0.27
0	Yes	No	0.26	0.01	0.25	0.27
0	Yes	Yes	0.26	0.01	0.25	0.27
1	No	No	0.64	0.08	0.56	0.72
1	No	Yes	0.64	0.04	0.59	0.67
1	Yes	No	0.63	0.03	0.60	0.66
1	Yes	Yes	0.63	0.03	0.58	0.65
2	No	No	0.62	0.02	0.60	0.65
2	No	Yes	0.63	0.03	0.59	0.67
2	Yes	No	0.62	0.03	0.58	0.65
2	Yes	Yes	0.63	0.04	0.58	0.67
4	No	No	0.60	0.03	0.57	0.65
4	No	Yes	0.60	0.04	0.57	0.66
4	Yes	No	0.60	0.04	0.57	0.65
4	Yes	Yes	0.57	0.03	0.55	0.60
8	No	No	0.56	0.04	0.50	0.58
8	No	Yes	0.56	0.02	0.54	0.58
8	Yes	No	0.58	0.04	0.52	0.62
8	Yes	Yes	0.57	0.01	0.56	0.58
12	No	No	0.67	0.03	0.62	0.69
12	No	Yes	0.68	0.03	0.63	0.70
12	Yes	No	0.66	0.02	0.64	0.69
12	Yes	Yes	0.65	0.01	0.63	0.66

Table A.56. Concentration of AB-DTPA extractable Zn from the non-sterile soil. Following a 12 week incubation study under laboratory conditions in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	4.17	2.30	2.17	6.38
0	No	Yes	4.17	2.30	2.17	6.38
0	Yes	No	4.17	2.30	2.17	6.38
0	Yes	Yes	4.17	2.30	2.17	6.38
1	No	No	3.02	0.51	2.50	3.71
1	No	Yes	3.01	0.13	2.86	3.09
1	Yes	No	4.49	0.96	3.55	5.77
1	Yes	Yes	4.33	0.30	3.91	4.59
2	No	No	3.05	0.13	2.94	3.24
2	No	Yes	3.11	0.16	2.93	3.29
2	Yes	No	4.52	1.03	3.42	5.91
2	Yes	Yes	5.14	0.25	4.87	5.47
4	No	No	3.52	1.69	2.50	6.03
4	No	Yes	3.43	1.62	2.48	5.85
4	Yes	No	7.05	1.97	5.49	9.77
4	Yes	Yes	5.38	1.97	3.78	7.58
8	No	No	8.17	3.01	5.18	11.1
8	No	Yes	8.27	2.43	6.21	11.4
8	Yes	No	9.11	3.55	5.89	13.5
8	Yes	Yes	8.79	1.09	7.20	9.68
12	No	No	6.99	2.03	4.87	9.24
12	No	Yes	6.74	0.78	5.73	7.57
12	Yes	No	8.65	2.22	6.92	11.7
12	Yes	Yes	8.62	1.96	6.59	11.0

Table A.57. Total soil N from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	0.17	0.01	0.156	0.180
0	No	Yes	0.17	0.01	0.156	0.180
0	Yes	No	0.17	0.01	0.156	0.180
0	Yes	Yes	0.17	0.01	0.156	0.180
1	No	No	0.16	0.00	0.159	0.168
1	No	Yes	0.18	0.00	0.180	0.185
1	Yes	No	0.19	0.01	0.173	0.203
1	Yes	Yes	0.19	0.02	0.164	0.201
2	No	No	0.17	0.01	0.164	0.183
2	No	Yes	0.17	0.00	0.167	0.176
2	Yes	No	0.18	0.01	0.172	0.19
2	Yes	Yes	0.19	0.02	0.175	0.208
4	No	No	0.18	0.01	0.173	0.197
4	No	Yes	0.19	0.03	0.163	0.227
4	Yes	No	0.21	0.05	0.165	0.256
4	Yes	Yes	0.21	0.03	0.180	0.254
8	No	No	0.17	0.01	0.159	0.175
8	No	Yes	0.19	0.02	0.174	0.202
8	Yes	No	0.22	0.02	0.198	0.238
8	Yes	Yes	0.20	0.03	0.187	0.233

Table A.58. Total soil C from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	1.72	0.04	1.69	1.77
0	No	Yes	1.72	0.04	1.69	1.77
0	Yes	No	1.72	0.04	1.69	1.77
0	Yes	Yes	1.72	0.04	1.69	1.77
1	No	No	1.71	0.06	1.67	1.81
1	No	Yes	1.83	0.07	1.77	1.90
1	Yes	No	1.83	0.06	1.77	1.92
1	Yes	Yes	1.83	0.06	1.76	1.88
2	No	No	1.62	0.04	1.59	1.67
2	No	Yes	1.83	0.04	1.77	1.86
2	Yes	No	1.81	0.02	1.78	1.83
2	Yes	Yes	1.85	0.14	1.68	2.01
4	No	No	1.70	0.04	1.67	1.75
4	No	Yes	1.86	0.14	1.74	2.05
4	Yes	No	1.94	0.28	1.71	2.30
4	Yes	Yes	1.96	0.24	1.73	2.30
8	No	No	1.67	0.01	1.66	1.69
8	No	Yes	1.84	0.01	1.83	1.85
8	Yes	No	1.93	0.11	1.81	2.05
8	Yes	Yes	2.07	0.25	1.86	2.35

Table A.59. Soil inorganic C from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	0.45	0.01	0.44	0.46
0	No	Yes	0.45	0.01	0.44	0.46
0	Yes	No	0.45	0.01	0.44	0.46
0	Yes	Yes	0.45	0.01	0.44	0.46
1	No	No	0.46	0.00	0.46	0.46
1	No	Yes	0.49	0.02	0.47	0.5
1	Yes	No	0.49	0.02	0.48	0.51
1	Yes	Yes	0.49	0.02	0.47	0.5
2	No	No	0.44	0.02	0.43	0.47
2	No	Yes	0.50	0.01	0.5	0.51
2	Yes	No	0.50	0.01	0.49	0.5
2	Yes	Yes	0.49	0.01	0.48	0.5
4	No	No	0.45	0.01	0.44	0.46
4	No	Yes	0.46	0.04	0.41	0.49
4	Yes	No	0.44	0.04	0.39	0.47
4	Yes	Yes	0.45	0.04	0.41	0.49
8	No	No	0.44	0.01	0.43	0.45
8	No	Yes	0.45	0.01	0.44	0.46
8	Yes	No	0.43	0.01	0.42	0.44
8	Yes	Yes	0.43	0.01	0.43	0.44

Table A.60. Soil organic C from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	No	No	1.27	0.03	1.24	1.31
0	No	Yes	1.27	0.03	1.24	1.31
0	Yes	No	1.27	0.03	1.24	1.31
0	Yes	Yes	1.27	0.03	1.24	1.31
1	No	No	1.25	0.06	1.21	1.34
1	No	Yes	1.34	0.05	1.3	1.4
1	Yes	No	1.34	0.05	1.29	1.41
1	Yes	Yes	1.34	0.05	1.29	1.39
2	No	No	1.18	0.02	1.16	1.2
2	No	Yes	1.32	0.04	1.27	1.36
2	Yes	No	1.31	0.02	1.3	1.33
2	Yes	Yes	1.36	0.13	1.2	1.51
4	No	No	1.26	0.03	1.22	1.3
4	No	Yes	1.41	0.18	1.26	1.64
4	Yes	No	1.50	0.31	1.24	1.9
4	Yes	Yes	1.51	0.28	1.26	1.89
8	No	No	1.23	0.02	1.21	1.26
8	No	Yes	1.39	0.03	1.37	1.41
8	Yes	No	1.50	0.10	1.38	1.61
8	Yes	Yes	1.64	0.26	1.42	1.92

Table A.61. Soil NO_3-N from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	15.6	1.06	14.1	16.6
0	No	Yes	15.6	1.06	14.1	16.6
0	Yes	No	15.6	1.06	14.1	16.6
0	Yes	Yes	15.6	1.06	14.1	16.6
1	No	No	13.3	0.60	12.6	14.1
1	No	Yes	18.4	0.45	17.9	18.7
1	Yes	No	25.8	4.15	20.1	29.8
1	Yes	Yes	25.7	2.41	24.0	28.4
2	No	No	13.9	0.46	13.2	14.2
2	No	Yes	16.7	1.25	15.5	18.0
2	Yes	No	23.2	3.34	18.6	26.0
2	Yes	Yes	25.1	3.13	21.6	28.6
4	No	No	14.1	0.93	12.7	14.7
4	No	Yes	19.4	6.76	13.8	29.1
4	Yes	No	28.2	6.16	21.4	36.0
4	Yes	Yes	27.2	6.44	21.9	36.1
8	No	No	14.6	1.36	12.9	15.8
8	No	Yes	17.6	0.05	17.5	17.6
8	Yes	No	37.0	7.40	30.9	46.3
8	Yes	Yes	32.2	6.60	26.5	39.4

Table A.62. Soil NH_4-N from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	13.7	1.71	11.9	15.3
0	No	Yes	13.7	1.71	11.9	15.3
0	Yes	No	13.7	1.71	11.9	15.3
0	Yes	Yes	13.7	1.71	11.9	15.3
1	No	No	26.6	4.09	23.1	31.4
1	No	Yes	47.7	1.08	46.5	48.7
1	Yes	No	107	13.1	90.1	122
1	Yes	Yes	100	14.7	89.1	117
2	No	No	32.9	4.21	28.0	37.3
2	No	Yes	55.2	2.01	52.4	56.9
2	Yes	No	106	12.7	87.4	117
2	Yes	Yes	117	18.5	101	144
4	No	No	41.6	4.50	35.6	45.8
4	No	Yes	71.1	10.1	59.9	84.5
4	Yes	No	117	12.1	106	133
4	Yes	Yes	118	19.4	102	145
8	No	No	58.7	1.63	56.9	60.5
8	No	Yes	82.6	14.8	72.2	93.1
8	Yes	No	148	14.5	132	167
8	Yes	Yes	159	5.36	154	164

Table A.63. Soil microbial biomass C from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean ($mg\ kg^{-1}$)	Standard Deviation	Min. ($mg\ kg^{-1}$)	Max. ($mg\ kg^{-1}$)
0	No	No	49.8	57.6	0	104
0	No	Yes	49.8	57.6	0	104
0	Yes	No	49.8	57.6	0	104
0	Yes	Yes	49.8	57.6	0	104
1	No	No	340	49.2	294	409
1	No	Yes	216	196	0	384
1	Yes	No	365	137	216	482
1	Yes	Yes	548	237	353	812
2	No	No	0.00	0.00	0	0
2	No	Yes	4.26	8.52	0	17.0
2	Yes	No	100	165	0	346
2	Yes	Yes	68.2	46.4	39.8	137
4	No	No	35.0	40.4	0	70.6
4	No	Yes	64.6	106	0	224
4	Yes	No	80.6	61.5	24.5	156
4	Yes	Yes	315	313	124	783
8	No	No	159	79.5	95.1	269
8	No	Yes	158	35.0	133	183
8	Yes	No	145	40.1	111	203
8	Yes	Yes	232	85.5	165	328

Table A.64. Soil microbial biomass N from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	11.1	11.1	0	24.0
0	No	Yes	11.1	11.1	0	24.0
0	Yes	No	11.1	11.1	0	24.0
0	Yes	Yes	11.1	11.1	0	24.0
1	No	No	25.1	1.44	23.3	26.6
1	No	Yes	23.0	13.6	13.5	38.6
1	Yes	No	36.8	41.9	3.52	97.8
1	Yes	Yes	79.9	42.8	52.0	129
2	No	No	0.00	0.00	0	0
2	No	Yes	0.00	0.00	0	0
2	Yes	No	14.5	29.0	0	57.9
2	Yes	Yes	0.00	0.00	0	0
4	No	No	11.5	17.2	0	36.9
4	No	Yes	4.76	5.14	0	12.0
4	Yes	No	27.7	39.4	0	85.7
4	Yes	Yes	125	184	21.5	400
8	No	No	35.9	11.0	28.7	51.9
8	No	Yes	53.0	8.58	46.9	59.0
8	Yes	No	49.8	42.0	6.44	107
8	Yes	Yes	80.1	73.2	27.1	164

Table A.65. Soil pH from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean	Standard Deviation	Min.	Max.
0	No	No	7.59	0.08	7.51	7.66
0	No	Yes	7.59	0.08	7.51	7.66
0	Yes	No	7.59	0.08	7.51	7.66
0	Yes	Yes	7.59	0.08	7.51	7.66
1	No	No	7.78	0.01	7.77	7.80
1	No	Yes	7.58	0.17	7.42	7.75
1	Yes	No	7.50	0.23	7.18	7.72
1	Yes	Yes	7.55	0.17	7.36	7.68
2	No	No	7.93	0.02	7.90	7.94
2	No	Yes	7.84	0.02	7.81	7.86
2	Yes	No	7.76	0.12	7.58	7.85
2	Yes	Yes	7.74	0.05	7.69	7.80
4	No	No	7.79	0.02	7.77	7.81
4	No	Yes	7.66	0.17	7.41	7.79
4	Yes	No	7.66	0.11	7.51	7.75
4	Yes	Yes	7.68	0.11	7.58	7.79
8	No	No	7.96	0.03	7.93	8.00
8	No	Yes	7.93	0.06	7.89	7.97
8	Yes	No	7.77	0.08	7.70	7.88
8	Yes	Yes	7.76	0.10	7.68	7.87

Table A.66. Soil electrical conductivity from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean ($dS\ m^{-1}$)	Standard Deviation	Min. ($dS\ m^{-1}$)	Max. ($dS\ m^{-1}$)
0	No	No	0.66	0.06	0.61	0.74
0	No	Yes	0.66	0.06	0.61	0.74
0	Yes	No	0.66	0.06	0.61	0.74
0	Yes	Yes	0.66	0.06	0.61	0.74
1	No	No	0.72	0.05	0.66	0.76
1	No	Yes	0.78	0.03	0.76	0.81
1	Yes	No	1.52	0.08	1.43	1.61
1	Yes	Yes	1.57	0.04	1.52	1.6
2	No	No	0.67	0.05	0.62	0.71
2	No	Yes	0.79	0.05	0.74	0.86
2	Yes	No	1.35	0.06	1.28	1.41
2	Yes	Yes	1.36	0.13	1.24	1.5
4	No	No	0.69	0.03	0.65	0.71
4	No	Yes	0.85	0.22	0.72	1.18
4	Yes	No	1.56	0.22	1.43	1.88
4	Yes	Yes	1.39	0.27	1.2	1.78
8	No	No	0.74	0.03	0.71	0.77
8	No	Yes	0.83	0.07	0.78	0.88
8	Yes	No	1.56	0.08	1.48	1.64
8	Yes	Yes	1.55	0.07	1.47	1.6

Table A.67. Concentration of AB-DTPA extractable Ba from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.54	0.01	0.53	0.54
0	No	Yes	0.54	0.01	0.53	0.54
0	Yes	No	0.54	0.01	0.53	0.54
0	Yes	Yes	0.54	0.01	0.53	0.54
1	No	No	0.75	0.06	0.69	0.83
1	No	Yes	0.65	0.02	0.64	0.67
1	Yes	No	0.84	0.08	0.76	0.92
1	Yes	Yes	0.82	0.02	0.8	0.84
2	No	No	0.72	0.02	0.7	0.74
2	No	Yes	0.66	0.04	0.62	0.71
2	Yes	No	0.87	0.10	0.76	0.99
2	Yes	Yes	0.85	0.06	0.79	0.9
4	No	No	0.63	0.02	0.62	0.65
4	No	Yes	0.62	0.08	0.51	0.69
4	Yes	No	0.75	0.22	0.48	1
4	Yes	Yes	0.79	0.22	0.5	0.98
8	No	No	0.64	0.01	0.63	0.65
8	No	Yes	0.58	0.01	0.57	0.58
8	Yes	No	0.63	0.06	0.57	0.71
8	Yes	Yes	0.57	0.08	0.5	0.65

Table A.68. Concentration of AB-DTPA extractable Cd from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.07	0.01	0.06	0.08
0	No	Yes	0.07	0.01	0.06	0.08
0	Yes	No	0.07	0.01	0.06	0.08
0	Yes	Yes	0.07	0.01	0.06	0.08
1	No	No	0.09	0.02	0.08	0.12
1	No	Yes	0.09	0.00	0.09	0.09
1	Yes	No	0.09	0.01	0.08	0.09
1	Yes	Yes	0.08	0.01	0.08	0.09
2	No	No	0.08	0.01	0.07	0.09
2	No	Yes	0.08	0.01	0.08	0.09
2	Yes	No	0.08	0.01	0.07	0.09
2	Yes	Yes	0.09	0.00	0.09	0.09
4	No	No	0.10	0.02	0.09	0.13
4	No	Yes	0.09	0.02	0.07	0.11
4	Yes	No	0.09	0.01	0.08	0.11
4	Yes	Yes	0.09	0.01	0.07	0.1
8	No	No	0.08	0.01	0.08	0.09
8	No	Yes	0.09	0.01	0.08	0.09
8	Yes	No	0.10	0.01	0.09	0.11
8	Yes	Yes	0.10	0.01	0.09	0.11

Table A.69. Concentration of AB-DTPA extractable Cr from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.00	0.00	0	0
0	No	Yes	0.00	0.00	0	0
0	Yes	No	0.00	0.00	0	0
0	Yes	Yes	0.00	0.00	0	0
1	No	No	0.01	0.02	0	0.03
1	No	Yes	0.01	0.01	0	0.02
1	Yes	No	0.00	0.00	0	0
1	Yes	Yes	0.01	0.02	0	0.03
2	No	No	0.02	0.02	0	0.03
2	No	Yes	0.02	0.02	0	0.04
2	Yes	No	0.02	0.02	0	0.04
2	Yes	Yes	0.01	0.02	0	0.03
4	No	No	0.00	0.00	0	0
4	No	Yes	0.03	0.02	0	0.04
4	Yes	No	0.03	0.01	0.03	0.04
4	Yes	Yes	0.02	0.02	0	0.04
8	No	No	0.00	0.00	0	0
8	No	Yes	0.00	0.00	0	0
8	Yes	No	0.00	0.00	0	0
8	Yes	Yes	0.00	0.00	0	0

Table A.70. Concentration of AB-DTPA extractable Cu from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	5.18	0.15	4.99	5.35
0	No	Yes	5.18	0.15	4.99	5.35
0	Yes	No	5.18	0.15	4.99	5.35
0	Yes	Yes	5.18	0.15	4.99	5.35
1	No	No	7.76	0.32	7.46	8.17
1	No	Yes	8.98	1.79	7.74	11.0
1	Yes	No	10.2	0.76	9.44	10.9
1	Yes	Yes	10.7	0.81	9.85	11.5
2	No	No	8.64	0.28	8.38	9.03
2	No	Yes	9.56	0.57	8.84	10.2
2	Yes	No	10.3	1.11	9.61	12.0
2	Yes	Yes	10.9	0.54	10.2	11.5
4	No	No	6.82	0.27	6.53	7.1
4	No	Yes	9.79	2.97	7.16	13.4
4	Yes	No	11.2	5.37	7.46	18.9
4	Yes	Yes	11.4	4.25	7.66	17.2
8	No	No	6.96	0.43	6.51	7.43
8	No	Yes	8.80	0.27	8.61	8.99
8	Yes	No	11.0	1.01	10.2	12.4
8	Yes	Yes	12.6	1.91	11.5	14.8

Table A.71. Concentration of AB-DTPA extractable Fe from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	4.86	0.39	4.51	5.4
0	No	Yes	4.86	0.39	4.51	5.4
0	Yes	No	4.86	0.39	4.51	5.4
0	Yes	Yes	4.86	0.39	4.51	5.4
1	No	No	6.45	0.41	6	6.98
1	No	Yes	7.31	0.53	6.98	7.92
1	Yes	No	7.70	0.68	6.92	8.4
1	Yes	Yes	8.23	0.41	7.85	8.66
2	No	No	6.72	0.19	6.52	6.9
2	No	Yes	6.83	0.10	6.73	6.95
2	Yes	No	7.80	0.20	7.53	7.97
2	Yes	Yes	8.05	0.22	7.85	8.33
4	No	No	6.32	0.28	5.99	6.58
4	No	Yes	8.16	1.20	7.23	9.81
4	Yes	No	9.72	2.99	7.3	13.9
4	Yes	Yes	10.3	1.79	8.7	12.4
8	No	No	5.81	0.46	5.49	6.47
8	No	Yes	7.44	0.60	7.01	7.86
8	Yes	No	8.90	1.09	7.69	10.2
8	Yes	Yes	11.9	2.23	9.3	13.4

Table A.72. Concentration of AB-DTPA extractable K from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	399	9.43	394	414
0	No	Yes	399	9.43	394	414
0	Yes	No	399	9.43	394	414
0	Yes	Yes	399	9.43	394	414
1	No	No	524	3.26	522	529
1	No	Yes	628	18.3	607	641
1	Yes	No	613	51.1	569	659
1	Yes	Yes	626	50.8	567	657
2	No	No	570	10.9	557	582
2	No	Yes	619	60.5	535	679
2	Yes	No	622	52.2	545	661
2	Yes	Yes	608	34.3	559	632
4	No	No	481	10.7	467	493
4	No	Yes	558	24.9	533	586
4	Yes	No	566	37.1	531	600
4	Yes	Yes	564	35.3	534	609
8	No	No	463	8.49	451	469
8	No	Yes	502	46.4	469	535
8	Yes	No	519	17.9	502	542
8	Yes	Yes	523	9.16	517	534

Table A.73. Concentration of AB-DTPA extractable Mn from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	46.4	1.20	45.3	47.6
0	No	Yes	46.4	1.20	45.3	47.6
0	Yes	No	46.4	1.20	45.3	47.6
0	Yes	Yes	46.4	1.20	45.3	47.6
1	No	No	43.5	0.64	42.8	44.3
1	No	Yes	40.5	2.19	38.2	42.6
1	Yes	No	43.9	1.90	42.5	46.6
1	Yes	Yes	44.0	2.82	41.9	47.2
2	No	No	45.1	6.55	39.3	50.8
2	No	Yes	41.6	2.79	38.1	44.9
2	Yes	No	37.2	1.83	36.0	39.9
2	Yes	Yes	36.8	4.28	32.7	42.8
4	No	No	43.3	0.54	42.6	43.7
4	No	Yes	31.3	7.48	24.4	40.3
4	Yes	No	37.1	9.38	26.4	45.6
4	Yes	Yes	34.0	8.15	25.5	44.6
8	No	No	28.5	6.13	23.2	34.5
8	No	Yes	23.8	2.90	21.8	25.9
8	Yes	No	24.2	5.88	19.6	32.1
8	Yes	Yes	30.2	6.36	22.8	34.0

Table A.74. Concentration of AB-DTPA extractable Mo from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.06	0.05	0	0.11
0	No	Yes	0.06	0.05	0	0.11
0	Yes	No	0.06	0.05	0	0.11
0	Yes	Yes	0.06	0.05	0	0.11
1	No	No	0.08	0.04	0.05	0.13
1	No	Yes	0.08	0.02	0.06	0.09
1	Yes	No	0.10	0.02	0.08	0.12
1	Yes	Yes	0.08	0.01	0.07	0.09
2	No	No	0.10	0.01	0.1	0.11
2	No	Yes	0.08	0.02	0.06	0.1
2	Yes	No	0.09	0.02	0.08	0.11
2	Yes	Yes	0.10	0.01	0.08	0.11
4	No	No	0.09	0.03	0.07	0.14
4	No	Yes	0.10	0.04	0.06	0.14
4	Yes	No	0.15	0.08	0.08	0.26
4	Yes	Yes	0.14	0.08	0.08	0.25
8	No	No	0.06	0.00	0.06	0.06
8	No	Yes	0.07	0.00	0.07	0.07
8	Yes	No	0.10	0.02	0.08	0.12
8	Yes	Yes	0.13	0.04	0.1	0.17

Table A.75. Concentration of AB-DTPA extractable Na from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.21	0.42	0	0.84
0	No	Yes	0.21	0.42	0	0.84
0	Yes	No	0.21	0.42	0	0.84
0	Yes	Yes	0.21	0.42	0	0.84
1	No	No	11.1	2.42	7.95	13.7
1	No	Yes	8.76	1.46	7.11	9.91
1	Yes	No	16.6	1.82	14.1	18.3
1	Yes	Yes	17.4	3.18	14.7	20.9
2	No	No	10.1	1.06	9.14	11.1
2	No	Yes	10.2	4.11	5.07	14.2
2	Yes	No	13.8	2.63	11.7	17.6
2	Yes	Yes	14.1	2.76	11.1	17.7
4	No	No	11.4	1.28	9.66	12.8
4	No	Yes	11.4	2.72	7.87	14.4
4	Yes	No	14.8	0.56	14.3	15.3
4	Yes	Yes	12.8	2.35	9.94	15.6
8	No	No	9.02	2.24	6.64	11.3
8	No	Yes	9.34	2.43	7.62	11.1
8	Yes	No	11.6	1.98	9.25	13.9
8	Yes	Yes	10.8	0.52	10.2	11.2

Table A.76. Concentration of AB-DTPA extractable Ni from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.96	0.07	0.9	1.05
0	No	Yes	0.96	0.07	0.9	1.05
0	Yes	No	0.96	0.07	0.9	1.05
0	Yes	Yes	0.96	0.07	0.9	1.05
1	No	No	1.11	0.01	1.1	1.12
1	No	Yes	1.02	0.04	0.99	1.07
1	Yes	No	1.09	0.01	1.08	1.09
1	Yes	Yes	1.08	0.03	1.06	1.12
2	No	No	1.24	0.01	1.23	1.26
2	No	Yes	1.22	0.03	1.19	1.26
2	Yes	No	1.23	0.04	1.18	1.26
2	Yes	Yes	1.26	0.05	1.21	1.33
4	No	No	1.16	0.03	1.12	1.19
4	No	Yes	1.17	0.09	1.07	1.28
4	Yes	No	1.22	0.03	1.17	1.24
4	Yes	Yes	1.18	0.07	1.11	1.28
8	No	No	1.09	0.04	1.04	1.13
8	No	Yes	1.05	0.06	1.01	1.09
8	Yes	No	1.05	0.04	1.02	1.1
8	Yes	Yes	1.03	0.02	1.01	1.05

Table A.77. Concentration of AB-DTPA extractable P from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	38.6	2.15	36.4	41.4
0	No	Yes	38.6	2.15	36.4	41.4
0	Yes	No	38.6	2.15	36.4	41.4
0	Yes	Yes	38.6	2.15	36.4	41.4
1	No	No	36.2	1.66	34.3	38.3
1	No	Yes	49.2	1.45	48.0	50.8
1	Yes	No	47.9	2.66	44.5	50.2
1	Yes	Yes	47.1	3.71	43.1	50.4
2	No	No	34.8	3.70	29.3	37.4
2	No	Yes	40.0	2.30	36.6	41.5
2	Yes	No	36.7	15.2	14.1	46.0
2	Yes	Yes	37.8	4.74	30.7	40.4
4	No	No	47.8	3.06	45.4	52.0
4	No	Yes	39.2	3.47	34.5	42.4
4	Yes	No	32.6	9.78	19.4	40.8
4	Yes	Yes	38.3	4.83	31.2	42.1
8	No	No	42.2	4.18	36.3	46.1
8	No	Yes	58.6	1.10	57.8	59.4
8	Yes	No	65.7	6.98	58.5	74.8
8	Yes	Yes	68.4	2.59	65.9	71.1

Table A.78. Concentration of AB-DTPA extractable Pb from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	1.25	0.05	1.19	1.30
0	No	Yes	1.25	0.05	1.19	1.30
0	Yes	No	1.25	0.05	1.19	1.30
0	Yes	Yes	1.25	0.05	1.19	1.30
1	No	No	1.59	0.06	1.53	1.67
1	No	Yes	1.67	0.02	1.65	1.68
1	Yes	No	1.65	0.03	1.63	1.70
1	Yes	Yes	1.61	0.05	1.57	1.66
2	No	No	1.76	0.10	1.65	1.85
2	No	Yes	1.69	0.09	1.57	1.78
2	Yes	No	1.84	0.03	1.82	1.89
2	Yes	Yes	1.81	0.01	1.80	1.81
4	No	No	1.74	0.05	1.70	1.79
4	No	Yes	1.67	0.02	1.65	1.69
4	Yes	No	1.69	0.18	1.56	1.95
4	Yes	Yes	1.72	0.07	1.64	1.80
8	No	No	1.63	0.06	1.58	1.71
8	No	Yes	1.63	0.18	1.50	1.75
8	Yes	No	1.63	0.09	1.53	1.75
8	Yes	Yes	1.68	0.06	1.63	1.74

Table A.79. Concentration of AB-DTPA extractable Si from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	1.41	0.06	1.34	1.47
0	No	Yes	1.41	0.06	1.34	1.47
0	Yes	No	1.41	0.06	1.34	1.47
0	Yes	Yes	1.41	0.06	1.34	1.47
1	No	No	2.73	0.16	2.55	2.87
1	No	Yes	3.84	0.71	3.25	4.63
1	Yes	No	3.94	1.10	3.23	5.57
1	Yes	Yes	4.55	1.44	2.94	5.73
2	No	No	3.45	0.20	3.31	3.74
2	No	Yes	3.45	0.29	3.12	3.83
2	Yes	No	3.73	0.30	3.37	4.07
2	Yes	Yes	3.83	0.57	3.40	4.66
4	No	No	3.09	0.42	2.73	3.69
4	No	Yes	3.61	0.88	2.98	4.91
4	Yes	No	3.62	0.55	3.08	4.23
4	Yes	Yes	3.47	0.18	3.26	3.68
8	No	No	2.84	0.55	2.53	3.66
8	No	Yes	3.36	0.25	3.18	3.53
8	Yes	No	3.32	0.67	2.74	4.18
8	Yes	Yes	2.83	0.29	2.64	3.17

Table A.80. Concentration of AB-DTPA extractable Sr from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.75	0.01	0.74	0.77
0	No	Yes	0.75	0.01	0.74	0.77
0	Yes	No	0.75	0.01	0.74	0.77
0	Yes	Yes	0.75	0.01	0.74	0.77
1	No	No	0.87	0.05	0.81	0.92
1	No	Yes	0.81	0.03	0.79	0.84
1	Yes	No	0.86	0.04	0.82	0.90
1	Yes	Yes	0.86	0.05	0.81	0.90
2	No	No	0.90	0.03	0.87	0.93
2	No	Yes	0.85	0.06	0.78	0.92
2	Yes	No	0.91	0.08	0.83	1.01
2	Yes	Yes	0.89	0.03	0.87	0.93
4	No	No	0.81	0.02	0.79	0.82
4	No	Yes	0.81	0.06	0.74	0.86
4	Yes	No	0.82	0.07	0.74	0.88
4	Yes	Yes	0.84	0.07	0.75	0.90
8	No	No	0.85	0.02	0.82	0.87
8	No	Yes	0.82	0.01	0.81	0.83
8	Yes	No	0.83	0.03	0.80	0.86
8	Yes	Yes	0.80	0.05	0.76	0.86

Table A.81. Concentration of AB-DTPA extractable Ti from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.00	0.00	0.00	0.00
0	No	Yes	0.00	0.00	0.00	0.00
0	Yes	No	0.00	0.00	0.00	0.00
0	Yes	Yes	0.00	0.00	0.00	0.00
1	No	No	0.16	0.01	0.15	0.16
1	No	Yes	0.17	0.01	0.16	0.17
1	Yes	No	0.18	0.01	0.16	0.19
1	Yes	Yes	0.18	0.01	0.17	0.18
2	No	No	0.15	0.01	0.15	0.16
2	No	Yes	0.15	0.01	0.14	0.16
2	Yes	No	0.15	0.01	0.14	0.16
2	Yes	Yes	0.15	0.01	0.14	0.16
4	No	No	0.15	0.01	0.15	0.16
4	No	Yes	0.16	0.01	0.15	0.16
4	Yes	No	0.17	0.02	0.15	0.20
4	Yes	Yes	0.17	0.02	0.15	0.19
8	No	No	0.13	0.01	0.13	0.14
8	No	Yes	0.14	0.00	0.14	0.14
8	Yes	No	0.16	0.01	0.15	0.18
8	Yes	Yes	0.17	0.01	0.16	0.18

Table A.82. Concentration of AB-DTPA extractable V from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	Biosolids Present	Earthworms Present	Mean (mg kg^{-1})	Standard Deviation	Min. (mg kg^{-1})	Max. (mg kg^{-1})
0	No	No	0.31	0.03	0.28	0.34
0	No	Yes	0.31	0.03	0.28	0.34
0	Yes	No	0.31	0.03	0.28	0.34
0	Yes	Yes	0.31	0.03	0.28	0.34
1	No	No	0.68	0.02	0.66	0.71
1	No	Yes	0.71	0.01	0.70	0.72
1	Yes	No	0.72	0.05	0.68	0.80
1	Yes	Yes	0.70	0.02	0.68	0.71
2	No	No	0.69	0.01	0.68	0.71
2	No	Yes	0.73	0.06	0.66	0.78
2	Yes	No	0.71	0.06	0.63	0.77
2	Yes	Yes	0.70	0.01	0.68	0.71
4	No	No	0.63	0.01	0.61	0.64
4	No	Yes	0.65	0.01	0.64	0.66
4	Yes	No	0.64	0.02	0.62	0.65
4	Yes	Yes	0.67	0.02	0.65	0.70
8	No	No	0.65	0.03	0.63	0.69
8	No	Yes	0.65	0.04	0.62	0.67
8	Yes	No	0.64	0.03	0.61	0.68
8	Yes	Yes	0.63	0.02	0.62	0.65

Table A.83. Concentration of AB-DTPA extractable Zn from the reduced microbial population soil. Following a 12 week incubation study under laboratory conditions in a gamma-irradiated sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex).

Week	**Biosolids Present**	**Earthworms Present**	**Mean (mg kg^{-1})**	**Standard Deviation**	**Min. (mg kg^{-1})**	**Max. (mg kg^{-1})**
0	No	No	4.58	0.41	4.24	5.15
0	No	Yes	4.58	0.41	4.24	5.15
0	Yes	No	4.58	0.41	4.24	5.15
0	Yes	Yes	4.58	0.41	4.24	5.15
1	No	No	4.71	0.27	4.39	5.06
1	No	Yes	2.95	0.23	2.81	3.21
1	Yes	No	4.10	1.49	3.05	6.27
1	Yes	Yes	3.96	0.48	3.51	4.46
2	No	No	4.79	0.16	4.61	4.93
2	No	Yes	3.09	0.11	2.97	3.23
2	Yes	No	4.15	0.68	3.71	5.17
2	Yes	Yes	4.44	0.42	4.04	5.03
4	No	No	4.18	0.27	3.95	4.53
4	No	Yes	4.21	1.96	2.59	6.59
4	Yes	No	5.49	3.39	2.8	10.21
4	Yes	Yes	5.71	2.79	3.29	9.54
8	No	No	4.19	0.34	3.74	4.51
8	No	Yes	4.39	0.08	4.33	4.45
8	Yes	No	6.37	0.86	5.38	7.12
8	Yes	Yes	8.00	1.35	6.72	9.41

APPENDIX B: SURVIVABILITY OF *APORRECTODEA CALIGINOSA* IN RESPONSE TO DROUGHT STRESS

Table B.1. ANOVA table for the number of earthworms that had entered diapause. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Source of Variance	F Value	P Value
Treatment	27.2	<0.001
Sampling day	9.80	<0.001
Treatment x Sampling Day	1.33	0.268
Treatment x Replication	0.66	0.809
Replication	0.52	0.761

Table B.2. ANOVA table for the number of earthworms that died. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Source of Variance	F Value	P Value
Treatment	8.13	<0.001
Sampling day	0.60	0.552
Treatment x Sampling Day	0.72	0.637
Treatment x Replication	0.55	0.897
Replication	0.91	0.482

Table B.3. ANOVA table for the percent change in earthworm mass on a per worm basis. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Source of Variance	F Value	P Value
Treatment	1.89	0.147
Sampling day	0.83	0.444
Treatment x Sampling Day	0.59	0.738
Treatment x Replication	0.87	0.596
Replication	1.61	0.180

Table B.4. ANOVA table for the concentration of soil NO_3-N. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Source of Variance	F Value	P Value
Treatment	3.94	0.015
Sampling day	3.66	0.035
Treatment x Sampling Day	1.60	0.173
Treatment x Replication	1.22	0.296
Replication	0.84	0.532

Table B.5. ANOVA table for the concentration of soil NH_4-N. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Source of Variance	F Value	P Value
Treatment	0.51	0.677
Sampling day	2.20	0.124
Treatment x Sampling Day	1.04	0.414
Treatment x Replication	0.99	0.485
Replication	1.15	0.352

Table B.6. ANOVA table for the concentration of dissolved organic C. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Source of Variance	F Value	P Value
Treatment	0.42	0.741
Sampling day	5.21	0.010
Treatment x Sampling Day	0.96	0.465
Treatment x Replication	0.78	0.687
Replication	0.54	0.747

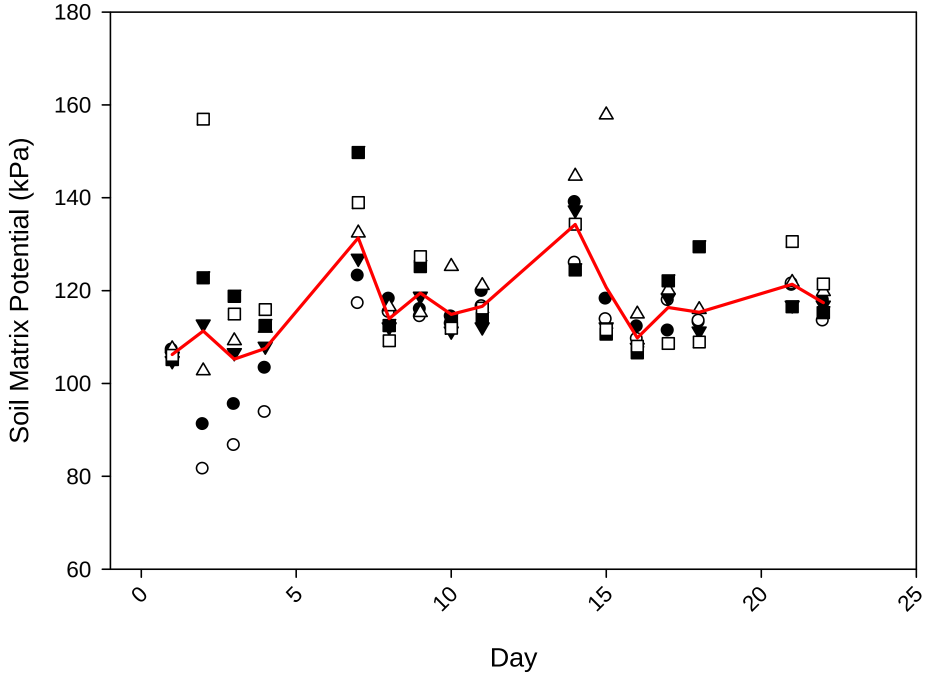

Figure B.1. Soil matrix potential for the constant treatments sampled at day 22. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

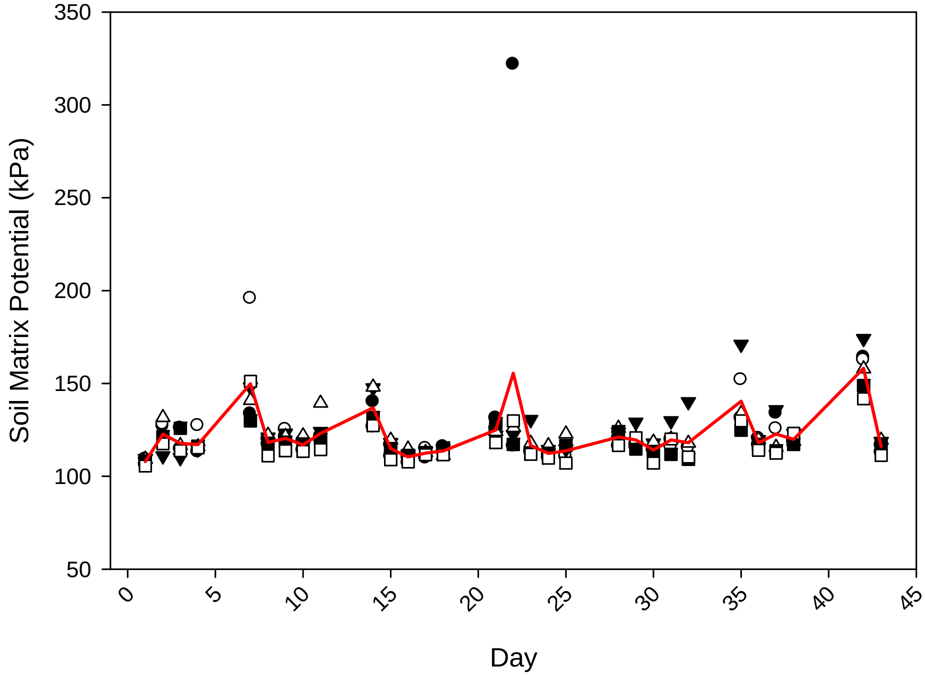

Figure B.2. Soil matrix potential for the constant treatments sampled at day 43. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

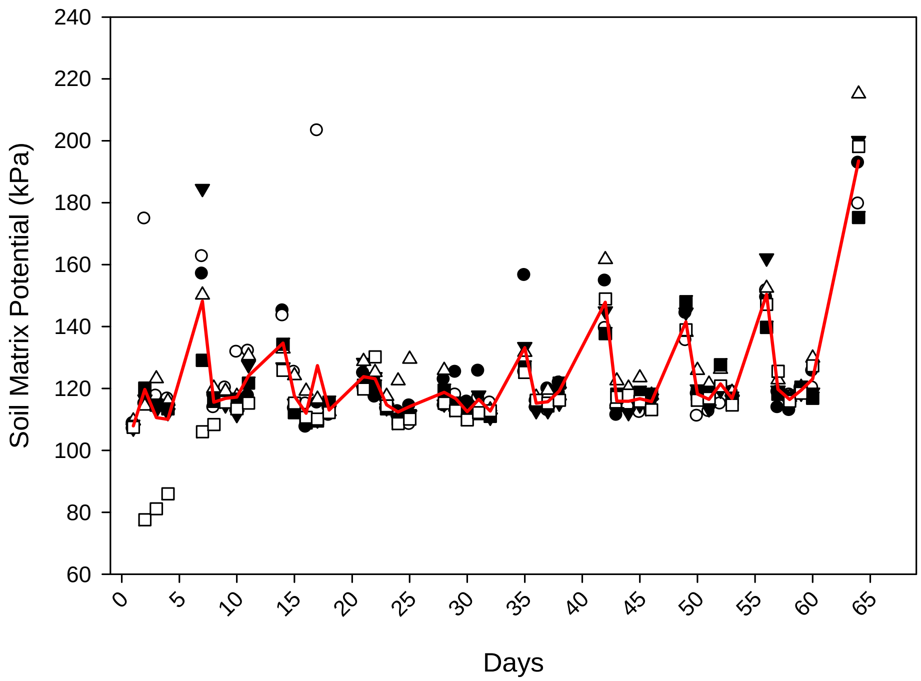

Figure B.3. Soil matrix potential for the constant treatments sampled at day 64. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

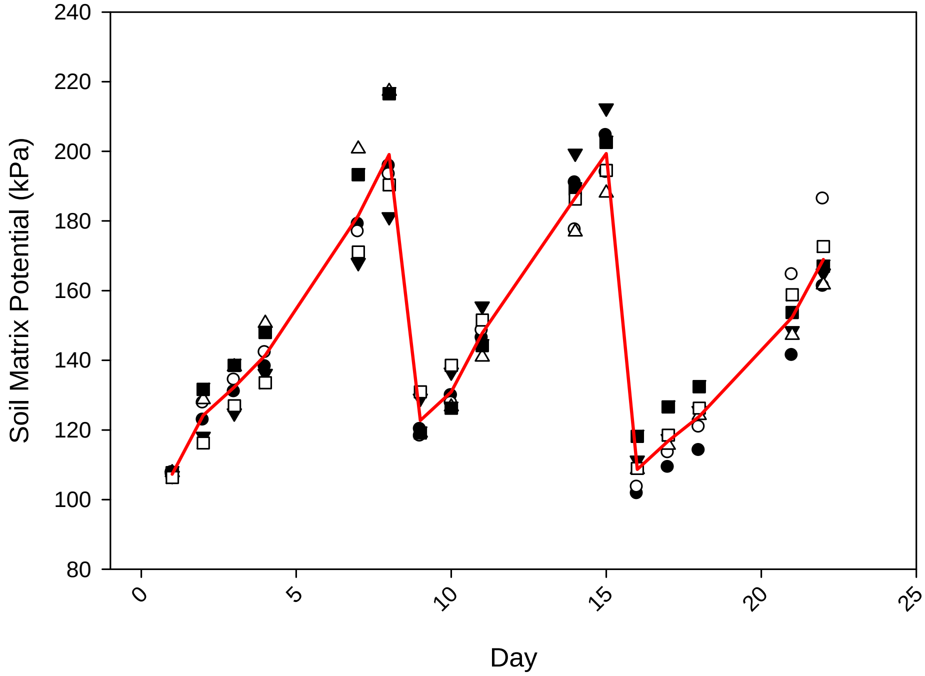

Figure B.4. Soil matrix potential for the one-week drought stress treatments sampled at day 22. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

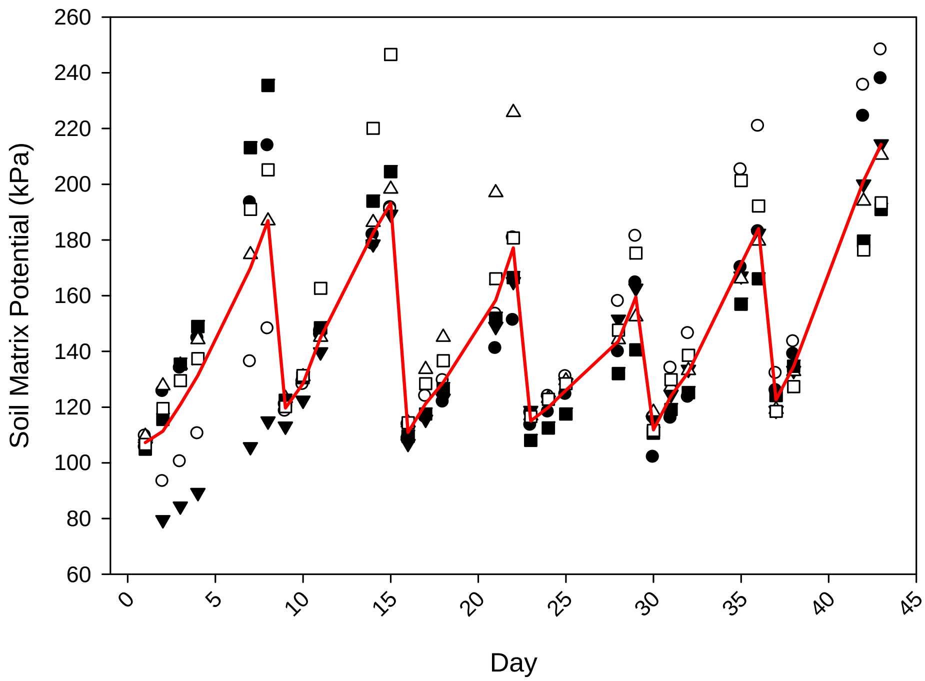

Figure B.5. Soil matrix potential for the one-week drought stress sampled at day 43. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

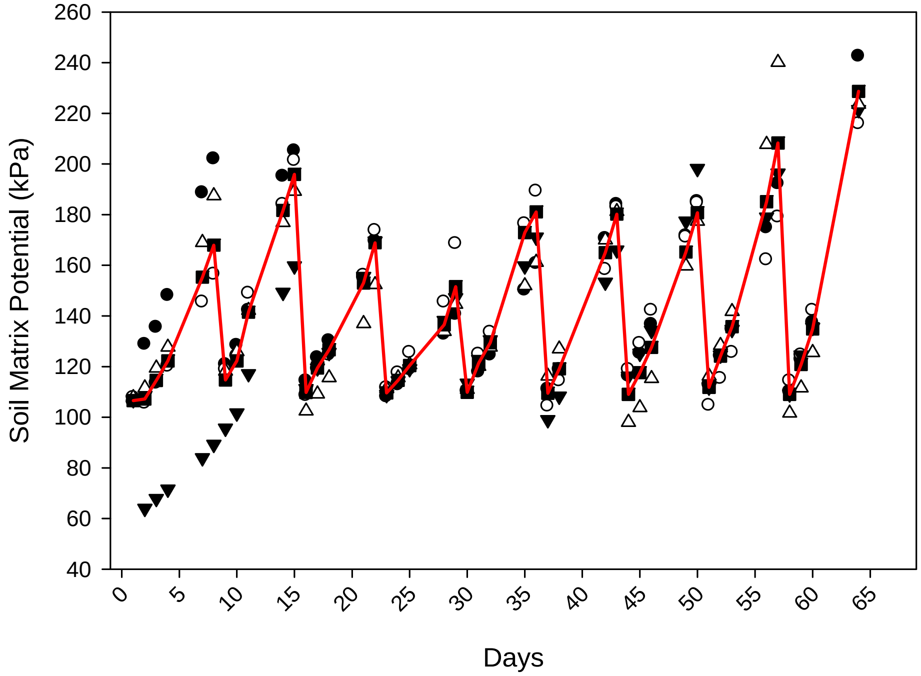

Figure B.6. Soil matrix potential for the one-week drought stress treatments sampled at day 64. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

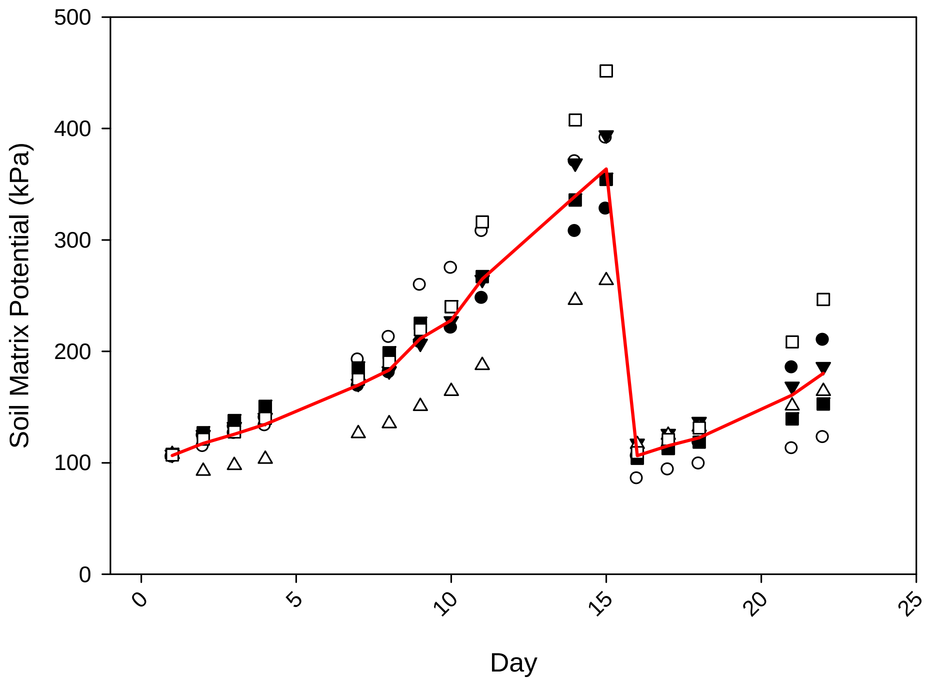

Figure B.7. Soil matrix potential for the two-week drought stress treatments sampled at day 22. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

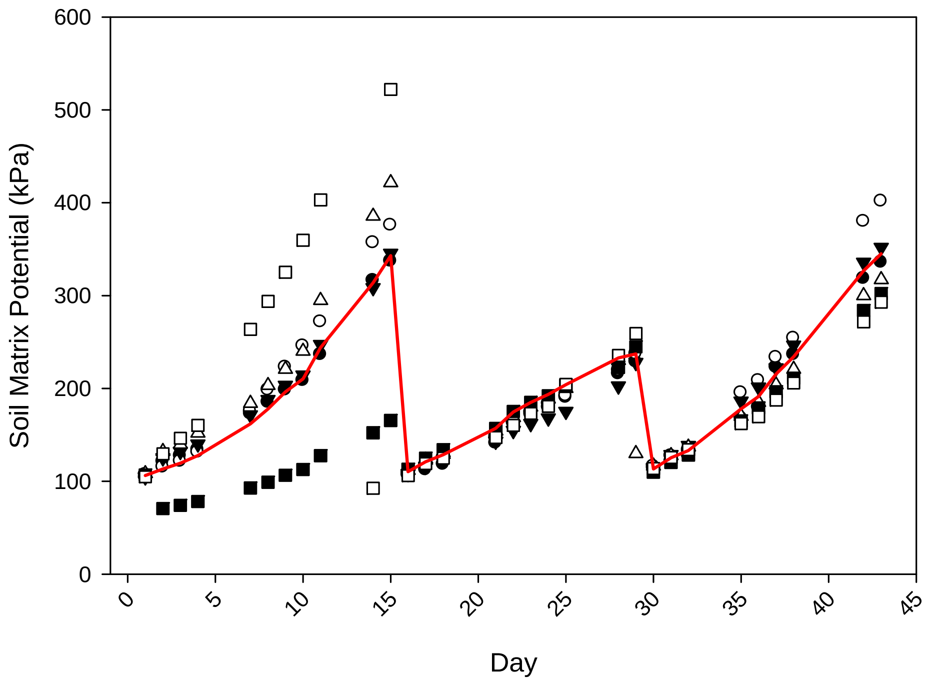

Figure B.8. Soil matrix potential for the two-week drought stress treatments sampled at day 43. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

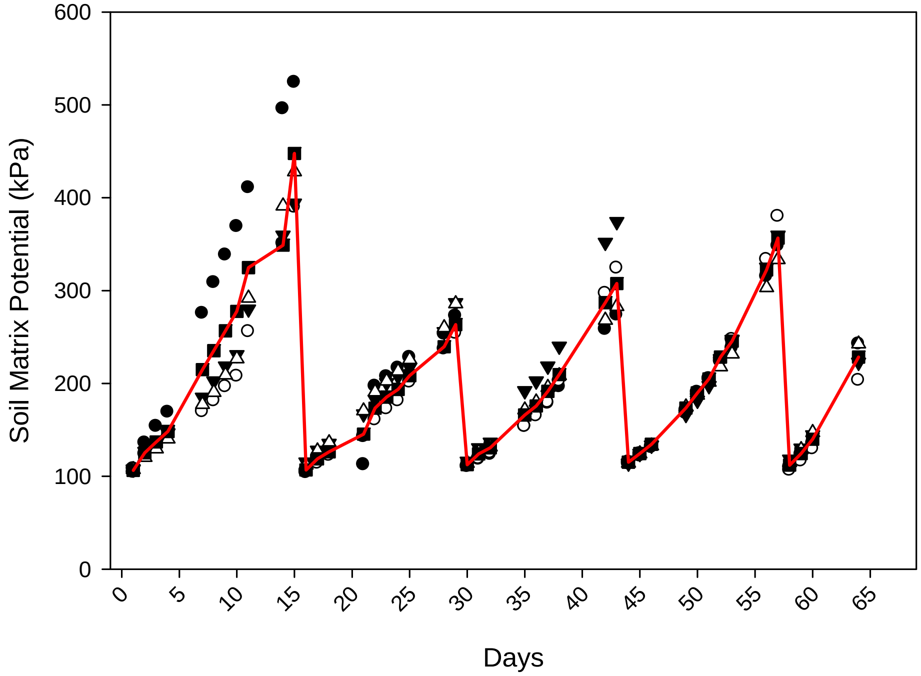

Figure B.9. Soil matrix potential for the two-week drought stress treatments sampled at day 64. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

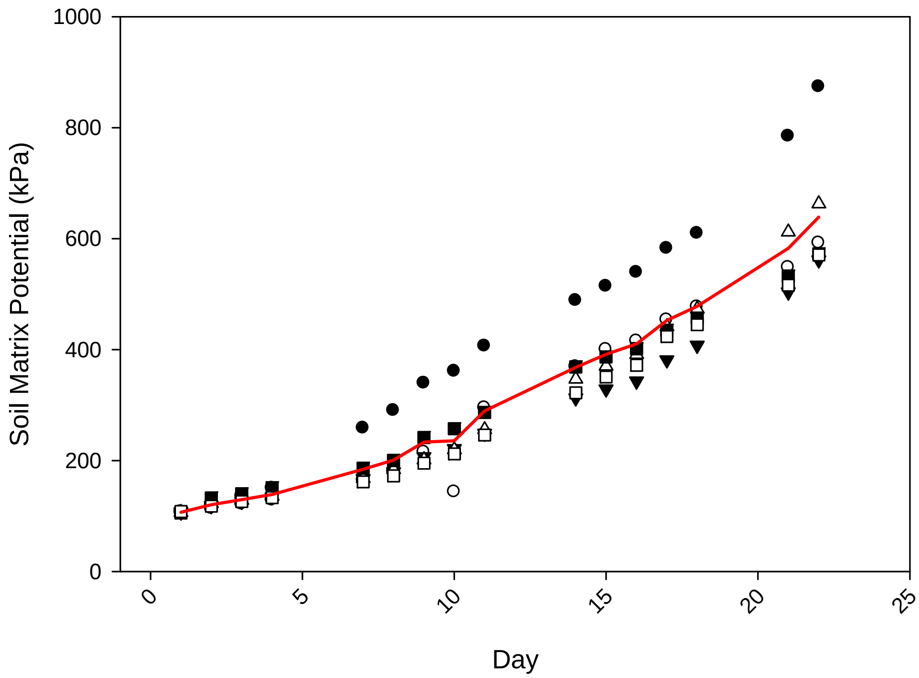

Figure B.10. Soil matrix potential for the three-week drought stress treatments sampled at day 22. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

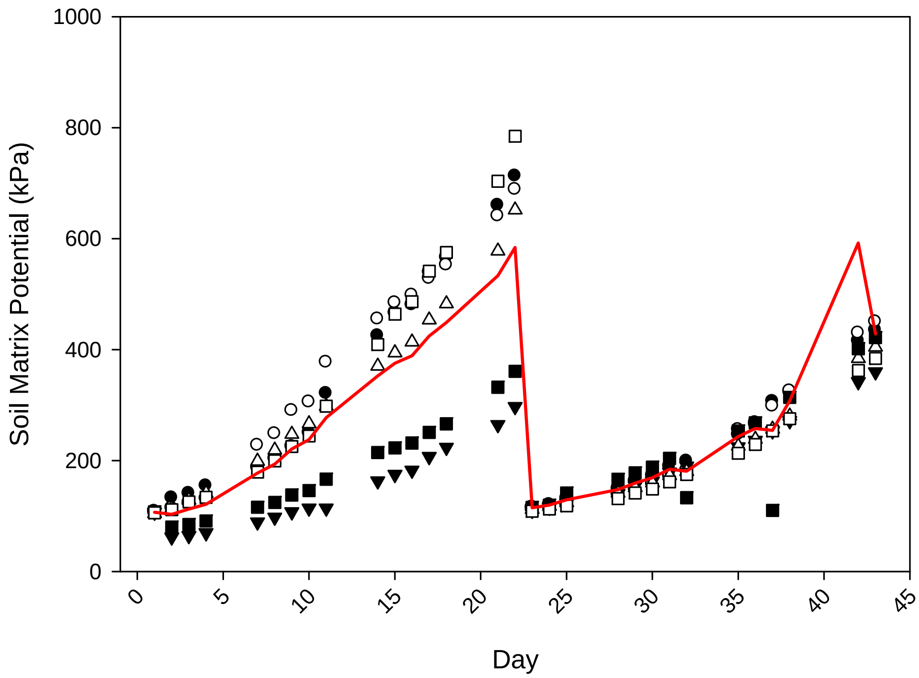

Figure B.11. Soil matrix potential for the three-week drought stress treatments sampled at day 43. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

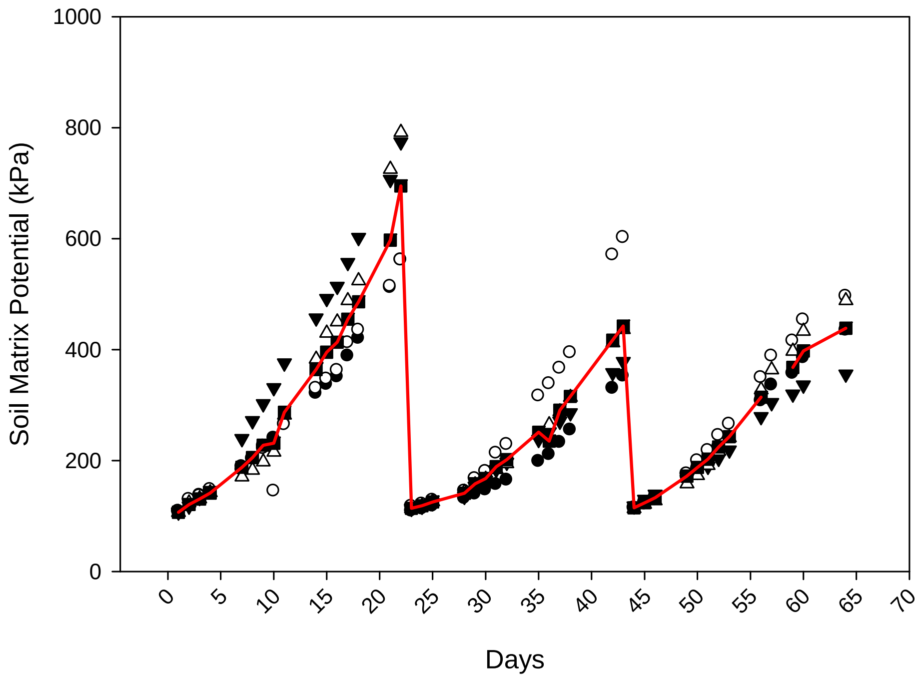

Figure B.12. Soil matrix potential for the three-week drought stress treatments sampled at day 64. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*. The red line is the average soil matrix potential.

Table B.7. Earthworms alive at the end of the study. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	Sampling Day	Mean	Standard Deviation	Min.	Max.
0	21	4.00	0.00	4.00	4.00
0	42	4.00	0.00	4.00	4.00
0	63	4.00	0.00	4.00	4.00
1	21	4.00	0.00	4.00	4.00
1	42	4.17	0.41	4.00	5.00
1	63	4.00	0.00	4.00	4.00
2	21	4.00	0.00	4.00	4.00
2	42	3.83	0.41	3.00	4.00
2	63	4.00	0.00	4.00	4.00
3	21	3.67	0.52	3.00	4.00
3	42	3.50	0.84	2.00	4.00
3	63	3.17	0.75	2.00	4.00

Table B.8. Number of earthworms dominate at the end of the study. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	**Sampling Day**	**Mean**	**Standard Deviation**	**Min.**	**Max.**
0	21	0.67	0.82	0.00	2.00
0	42	0.50	0.55	0.00	1.00
0	63	0.17	0.41	0.00	1.00
1	21	0.83	0.75	0.00	2.00
1	42	1.33	0.82	0.00	2.00
1	63	0.50	0.55	0.00	1.00
2	21	1.67	1.51	0.00	4.00
2	42	2.00	0.63	1.00	3.00
2	63	0.33	0.82	0.00	2.00
3	21	3.67	0.52	3.00	4.00
3	42	3.00	0.89	2.00	4.00
3	63	2.00	0.89	1.00	3.00

Table B.9. Number of earthworms dead in each pot. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	Sampling Day	Mean	Standard Deviation	Min.	Max.
0	21	0.00	0.00	0.00	0.00
0	42	0.00	0.00	0.00	0.00
0	63	0.00	0.00	0.00	0.00
1	21	0.00	0.00	0.00	0.00
1	42	0.00	0.00	0.00	0.00
1	63	0.00	0.00	0.00	0.00
2	21	0.00	0.00	0.00	0.00
2	42	0.17	0.41	0.00	1.00
2	63	0.00	0.00	0.00	0.00
3	21	0.33	0.52	0.00	1.00
3	42	0.50	0.84	0.00	2.00
3	63	0.83	0.75	0.00	2.00

Table B.10. Percent change in earthworm mass on a per worm basis. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	Sampling Day	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
0	21	-2.04	4.05	-8.99	1.47
0	42	-1.13	13.1	-16.2	19.5
0	63	1.98	2.45	-1.76	5.34
1	21	-3.45	4.87	-10.4	2.78
1	42	-5.10	14.3	-21.00	18.8
1	63	-9.69	8.88	-26.7	-3.74
2	21	0.68	15.7	-22.2	20.6
2	42	-4.65	10.3	-16.5	10.4
2	63	-2.77	8.06	-9.05	12.9
3	21	-3.62	4.93	-11.3	0.50
3	42	-12.3	14.3	-40.5	-2.09
3	63	-5.82	7.53	-13.5	6.61

Table B.11. Concentration of soil NO_3-N concentrations. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	**Sampling Day**	**Mean (mg kg^{-1})**	**Standard Deviation**	**Min. (mg kg^{-1})**	**Max. (mg kg^{-1})**
0	21	65.8	20.9	40.7	100
0	42	62.9	22.6	40.7	92.2
0	63	98.5	39.0	53.7	154
1	21	56.3	6.39	47.9	63.1
1	42	75.4	18.9	48.5	104
1	63	67.0	28.7	37.5	107
2	21	52.5	15.7	34.3	77.7
2	42	66.9	16.3	44.4	87.5
2	63	70.1	13.7	55.4	95.4
3	21	52.1	9.75	35.8	65.7
3	42	52.5	18.1	23.6	70.1
3	63	54.1	16.2	27.5	70.3

Table B.12. Concentration of soil NH_4-N concentration. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	**Sampling Day**	**Mean (mg kg^{-1})**	**Standard Deviation**	**Min. (mg kg^{-1})**	**Max. (mg kg^{-1})**
0	21	15.2	28.4	1.19	72.9
0	42	0.54	0.59	0.00	1.37
0	63	1.12	1.40	0.00	3.69
1	21	5.53	9.23	0.56	24.1
1	42	3.90	4.01	0.00	8.98
1	63	1.92	3.30	0.00	8.18
2	21	0.97	0.79	0.20	2.45
2	42	2.38	3.25	0.00	8.46
2	63	2.26	2.16	0.00	5.19
3	21	7.24	9.00	0.59	23.8
3	42	3.05	5.51	0.00	13.7
3	63	3.33	4.55	0.00	11.0

Table B.13. Concentration of soil dissolved organic C. A laboratory drought stress study was conducted with a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex) with four levels of drought stress to study the effects on *A. caliginosa*.

Drought Period Length (Weeks)	**Sampling Day**	**Mean (mg kg^{-1})**	**Standard Deviation**	**Min. (mg kg^{-1})**	**Max. (mg kg^{-1})**
0	21	6.43	2.03	4.28	9.83
0	42	4.35	1.59	2.30	6.26
0	63	4.14	4.87	0.95	13.9
1	21	4.66	1.65	3.17	7.67
1	42	12.8	12.4	2.74	35.1
1	63	1.78	0.87	1.09	3.43
2	21	4.17	1.50	2.46	6.58
2	42	10.1	10.5	1.66	30.2
2	63	2.37	1.61	0.95	4.98
3	21	6.07	1.58	3.43	7.78
3	42	10.3	9.40	3.10	25.2
3	63	5.39	8.72	0.76	23.1

APPENDIX C: EFFECTS OF *APORRECTODEA CALIGINOSA* ON SOIL HYDRAULIC PROPERTIES UNDER LABORATORY CONDITIONS

Table C.1. ANOVA table for the expected value, log transformed second moment, and log transformed variance in pore volumes for saturated flow. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Expect Value	Earthworms	0.70	0.416
	Section	1.06	0.370
	Earthworms x Section	0.33	0.725
Second Moment	Earthworms	6.67	0.019
	Section	1.03	0.377
	Earthworms x Section	0.87	0.438
Variance in Pore Volumes	Earthworms	22.3	<0.001
	Section	4.75	0.023
	Earthworms x Section	1.26	0.309

Table C.2. ANOVA table for the log transformed dispersion with transient flow under saturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Dispersivity	Earthworms	46.0	<0.001
	Section	13.9	<0.001
	Earthworms x Section	7.88	0.004

Table C.3. ANOVA table for the log transformed dispersion for transient flow with mobile immobile water, theta immobile, and alpha term under saturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Dispersivity	Earthworms	26.6	<0.001
	Section	3.83	0.042
	Earthworms x Section	4.78	0.023
Theta Immobile	Earthworms	0.27	0.612
	Section	0.11	0.898
	Earthworms x Section	0.04	0.963
Alpha Term	Earthworms	0.14	0.532
	Section	0.45	0.647
	Earthworms x Section	0.46	0.639

Table C.4. ANOVA table for the expected value, second moment, and log transformed variance in pore volumes for unsaturated flow. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Expect Value	Earthworms	0.91	0.352
	Section	7.31	0.005
	Earthworms x Section	3.34	0.058
Second Moment	Earthworms	0.01	0.919
	Section	4.31	0.033
	Earthworms x Section	2.23	0.137
Variance in Pore Volumes	Earthworms	0.02	0.901
	Section	0.15	0.861
	Earthworms x Section	0.17	0.846

Table C.5. ANOVA table for the log transformed dispersion with transient flow under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Dispersivity	Earthworms	31.8	<0.001
	Section	6.88	0.006
	Earthworms x Section	5.00	0.019

Table C.6. ANOVA table for the log transformed dispersion for transient flow with mobile immobile water, theta immobile, and alpha term under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Dispersivity	Earthworms	15.0	0.001
	Section	2.69	0.095
	Earthworms x Section	2.40	0.119
Theta Immobile	Earthworms	1.14	0.300
	Section	2.58	0.104
	Earthworms x Section	0.62	0.550
Alpha Term	Earthworms	2.21	0.154
	Section	0.14	0.868
	Earthworms x Section	0.60	0.561

Table C.7. ANOVA table for the quartile analysis of pore volumes under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
1st Quartile	Earthworms	6.22	0.047
	Section	2.58	0.117
	Earthworms x Section	1.71	0.223
2nd Quartile	Earthworms	7.07	0.038
	Section	3.35	0.070
	Earthworms x Section	2.42	0.131
3rd Quartile	Earthworms	6.23	0.047
	Section	3.47	0.065
	Earthworms x Section	2.71	0.107
4th Quartile	Earthworms	6.66	0.042
	Section	3.30	0.072
	Earthworms x Section	2.43	0.130

Table C.8. ANOVA table for the quartile analysis of pore volumes under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
1st Quartile	Earthworms	0.20	0.674
	Section	0.94	0.418
	Earthworms x Section	1.91	0.190
2nd Quartile	Earthworms	0.22	0.658
	Section	0.85	0.452
	Earthworms x Section	1.58	0.246
3rd Quartile	Earthworms	0.36	0.573
	Section	0.83	0.460
	Earthworms x Section	2.27	0.146
4th Quartile	Earthworms	0.07	0.798
	Section	0.91	0.429
	Earthworms x Section	2.35	0.137

Table C.9. ANOVA table for the comparison of saturated flow rate, saturated water content, and saturated pore volume size. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Flow Rate	Earthworms	0.04	0.847
	Section	2.41	0.132
	Earthworms x Section	2.02	0.175
Water Content	Earthworms	0.04	0.849
	Section	1.80	0.194
	Earthworms x Section	1.30	0.298
Pore Volume Size	Earthworms	5.51	0.256
	Section	3.21	0.367
	Earthworms x Section	3.06	0.375

Table C.10. ANOVA table for the comparison of unsaturated flow rate, unsaturated water content, and unsaturated pore volume size. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	**Source of Variation**	**F Value**	**P Value**
Flow Rate	Earthworms	3.75	0.101
	Section	12.1	0.001
	Earthworms x Section	5.97	0.016
Water Content	Earthworms	0.13	0.735
	Section	0.25	0.786
	Earthworms x Section	0.42	0.668
Pore Volume Size	Earthworms	0.33	0.573
	Section	0.60	0.559
	Earthworms x Section	0.10	0.905

Table C.11. ANOVA table for the comparison of flow rate, water content, and pore volume size between saturated and unsaturated water flow. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Parameter	Source of Variation	F Value	P Value
Flow Rate	Earthworms	0.12	0.743
	Section	3.38	0.048
	Earthworms x Section	3.22	0.054
	Flow	143	<0.001
	Earthworms x Flow	1.29	0.266
	Section x Flow	1.52	0.235
	Earthworms x Section x Flow	0.29	0.751
Water Content	Earthworms	0.00	0.968
	Section	1.38	0.278
	Earthworms x Section	1.25	0.310
	Flow	103	<0.001
	Earthworms x Flow	0.20	0.662
	Section x Flow	1.33	0.291
	Earthworms x Section x Flow	0.65	0.535
Pore Volume Size	Earthworms	2.35	0.143
	Section	1.61	0.227
	Earthworms x Section	1.17	0.337
	Flow	494	<0.001
	Earthworms x Flow	6.09	0.024
	Section x Flow	3.53	0.051
	Earthworms x Section x Flow	4.04	0.036

Table C.12. ANOVA table for the comparison of saturated water content from the Brooks-Corey water retention curve equations using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	1.35	0.260
Section	0.21	0.815
Earthworms x Section	0.45	0.645
Position(Section)	0.30	0.827
Earthworms x Position(Section)	1.14	0.335

Table C.13. ANOVA table for the comparison of residual water content from the Brooks-Corey water retention curve equations using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	0.00	0.960
Section	0.58	0.576
Earthworms x Section	1.76	0.214
Position(Section)	0.55	0.658
Earthworms x Position(Section)	1.14	0.360

Table C.14. ANOVA table for the comparison of the alpha term from the Brooks-Corey water retention curve equations using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	12.5	0.012
Section	8.48	0.005
Earthworms x Section	4.82	0.030
Position(Section)	2.06	0.141
Earthworms x Position(Section)	1.86	0.172

Table C.15. ANOVA table for the comparison of the N term from the Brooks-Corey water retention curve equations using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	6.99	0.038
Section	0.69	0.510
Earthworms x Section	0.30	0.742
Position(Section)	0.74	0.536
Earthworms x Position(Section)	1.18	0.332

Table C.16. ANOVA table the comparison of the fitted residual water content from the Brooks-Corey water retention curve equations using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	17.9	0.006
Section	16.9	<0.001
Earthworms x Section	18.6	<0.001
Position(Section)	1.24	0.299
Earthworms x Position(Section)	1.13	0.341

Table C.17. ANOVA table the comparison of the air entry value from the Brooks-Corey water retention curve equations using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	5.12	0.064
Section	12.33	<0.001
Earthworms x Section	0.22	0.807
Position(Section)	0.13	0.941
Earthworms x Position(Section)	2.52	0.076

Table C.18. ANOVA table for the comparison of saturated water content from the Brooks-Corey water retention curve equations using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	10.7	0.082
Section	1.88	0.265
Earthworms x Section	2.08	0.239
Position(Section)	0.72	0.546
Earthworms x Position(Section)	1.38	0.258

Table C.19. ANOVA table for the comparison of residual water content from the Brooks-Corey water retention curve equations using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	1.07	0.413
Section	1.88	0.162
Earthworms x Section	2.33	0.107
Position(Section)	0.39	0.763
Earthworms x Position(Section)	0.68	0.568

Table C.20. ANOVA table for the comparison of the alpha term from the Brooks-Corey water retention curve equations using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	1.55	0.339
Section	2.00	0.250
Earthworms x Section	1.82	0.274
Position(Section)	0.12	0.947
Earthworms x Position(Section)	1.35	0.269

Table C.21. ANOVA table for the comparison of the N term from the Brooks-Corey water retention curve equations using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	7.04	0.118
Section	6.37	0.057
Earthworms x Section	5.54	0.070
Position(Section)	4.59	0.006
Earthworms x Position(Section)	7.48	<0.001

Table C.22. ANOVA table the comparison of the fitted residual water content from the Brooks-Corey water retention curve equations using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	2.27	0.242
Section	5.54	0.006
Earthworms x Section	7.38	0.001
Position(Section)	0.30	0.827
Earthworms x Position(Section)	0.64	0.592

Table C.23. ANOVA table the comparison of the air entry value from the Brooks-Corey water retention curve equations using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	0.74	0.481
Section	3.82	0.117
Earthworms x Section	4.13	0.106
Position(Section)	0.15	0.929
Earthworms x Position(Section)	1.80	0.158

Table C.24. ANOVA table for the comparison of the alpha term from the Brooks-Corey water retention curve equations using only the data points to 0.1 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	5.19	0.063
Section	1.40	0.284
Earthworms x Section	1.10	0.365
Position(Section)	2.38	0.073
Earthworms x Position(Section)	2.23	0.089

Table C.25. ANOVA table the comparison of the air entry value from the Brooks-Corey water retention curve equations using only the data points to 0.1 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Source of Variation	F Value	P Value
Earthworms	4.15	0.088
Section	4.08	0.045
Earthworms x Section	0.19	0.832
Position(Section)	0.40	0.752
Earthworms x Position(Section)	1.77	0.157

Table C.26. ANOVA table for the percent sand, and percent silt and clay. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were collected to determine soil texture.

Parameter	Source of Variation	F Value	P Value
Percent Sand	Earthworms	0.32	0.592
	Section	0.25	0.783
	Earthworms x Section	1.16	0.347
	Position(Section)	0.37	0.776
	Earthworms x Position(Section)	0.91	0.456
Percent Silt	Earthworms	0.40	0.557
	Section	0.31	0.739
	Earthworms x Section	0.48	0.632
	Position(Section)	0.54	0.660
	Earthworms x Position(Section)	3.23	0.052
Percent Clay	Earthworms	0.31	0.600
	Section	1.29	0.319
	Earthworms x Section	0.84	0.460
	Position(Section)	1.62	0.226
	Earthworms x Position(Section)	1.00	0.418

Table C.27. Log transformation of second movement for the saturated breakthrough curve affected by *A. caliginosa*. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

	Second Movement	**Standard Error**
With *A. caliginosa*	0.397a	0.0411
Without *A. caliginosa*	0.250b	0.0390

Table C.28. Log transformation of variance in pore volumes for the saturated breakthrough curve affected by *A. caliginosa*. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

	Variance in Pore Volumes	**Standard Error**
With *A. caliginosa*	-0.163a	0.0850
Without *A. caliginosa*	-0.716b	0.0806

Table C.29. Log transformation of variance in pore volumes for the saturated breakthrough curve affected by section. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Section	**Variance in Pore Volumes**	**Standard Error**
Top	-0.239a	0.107
Middle	-0.402ab	0.987
Bottom	-0.678b	0.987

Table C.30. Expected value for the saturated breakthrough curve with the mobile immobile transient model affected by section. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Section	Expected Value	Standard Error
Top	1.26b	0.0750
Middle	1.12b	0.0750
Bottom	1.52a	0.0750

Table C.31. Second moment for the saturated breakthrough curve with the mobile immobile transient model affected by section. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Section	Second Moment	Standard Error
Top	2.13ab	0.278
Middle	1.72b	0.278
Bottom	2.83a	0.278

Table C.32. Interaction between the presences of *A. caliginosa* and section on flow rate under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Earthworms Present	Section	Flow Rate (mL min^{-1})	Standard Error
Yes	Top	0.347c	0.463
Yes	Middle	1.50b	0.463
Yes	Bottom	3.384a	0.463
No	Top	2.65ab	0.463
No	Middle	2.32ab	0.463
No	Bottom	3.12a	0.463

Table C.33. Flow rate affected by section for the comparison between saturated and unsaturated flow rate. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Section	**Flow Rate (cm min^{-1})**	**Standard Error**
Top	4.58b	0.604
Middle	6.00ab	0.604
Bottom	6.25a	0.604

Table C.34. Flow rate affected by the type of flow for the comparison between saturated and unsaturated flow rate. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

	Flow Rate (mL min^{-1})	**Standard Error**
Saturated Flow	8.99a	0.533
Unsaturated Flow	2.22b	0.533

Table C.35. Water content affected by type of flow for the comparison between saturated and unsaturated flow rate. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

	Water Content (θg)	**Standard Error**
Saturated Flow	0.445a	0.00941
Unsaturated Flow	0.332b	0.00941

Table C.36. Interaction between *A. caliginosa,* section, and flow type on pore volume size. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Earthworms Present	Section	Flow	Pore Volume Size (L)	Standard Error
Yes	Top	Unsaturated	0.888d	0.0499
Yes	Top	Saturated	1.06bc	0.0499
Yes	Middle	Unsaturated	0.934cd	0.0499
Yes	Middle	Saturated	1.28a	0.0499
Yes	Bottom	Unsaturated	0.880d	0.0499
Yes	Bottom	Saturated	1.19ab	0.0499
No	Top	Unsaturated	0.939cd	0.0499
No	Top	Saturated	1.29a	0.0499
No	Middle	Unsaturated	0.947cd	0.0499
No	Middle	Saturated	1.31a	0.0499
No	Bottom	Unsaturated	0.889d	0.0499
No	Bottom	Saturated	1.22a	0.0499

Table C.37. The N term of the Brooks-Corey equation with 1.5 MPa of tension affected by the presence of *A. caliginosa*. A laboratory column study was designed to investigate the effects of A. caliginosa on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

	N Term	Standard Error
With *A. caliginosa*	0.152b	0.0273
Without *A. caliginosa*	0.253a	0.0271

Table C.38. Interaction between the presence of *A. caliginosa* and section on the fitted residual water content to the Brooks-Corey equation out to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of A. caliginosa on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Earthworms Present	**Section**	**Fitted Residual Water Content (Θ_v)**	**Standard Error**
Yes	Top	0.154a	0.0102
Yes	Middle	0.135b	0.0102
Yes	Bottom	0.0826c	0.0103
No	Top	0.0736c	0.0102
No	Middle	0.0662c	0.0102
No	Bottom	0.0732c	0.0102

Table C.39. Air entry value of the Brooks-Corey equation out to 1.5 MPa of tension affected by section. A laboratory column study was designed to investigate the effects of A. caliginosa on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Section	**Air Entry Value (cm)**	**Standard Error**
Top	8.90b	1.64
Middle	6.35b	1.63
Bottom	14.37a	1.63

Table C.40. Air entry value of the Brooks-Corey equation out to 0.1 MPa of tension affected by section. A laboratory column study was designed to investigate the effects of A. caliginosa on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D. Values labeled with the same letter are not significantly different at an alpha value of 0.05.

Section	**Air Entry Value (cm)**	**Standard Error**
Top	7.53b	1.46
Middle	6.82b	1.46
Bottom	11.3a	1.46

Table C.41. Expected value under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	1.31	0.16	1.18	1.49
Yes	Middle	1.28	0.29	0.86	1.54
Yes	Bottom	1.35	0.12	1.18	1.44
No	Top	1.30	0.18	1.10	1.52
No	Middle	1.14	0.16	0.98	1.37
No	Bottom	1.32	0.01	1.31	1.34

Table C.42. Log transformed second moment under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean	Standard Deviation	Min.	Max.
Yes	Top	0.465	0.118	0.358	0.592
Yes	Middle	0.395	0.225	0.085	0.616
Yes	Bottom	0.329	0.103	0.186	0.429
No	Top	0.297	0.153	0.113	0.445
No	Middle	0.173	0.0946	0.0968	0.308
No	Bottom	0.281	0.0204	0.268	0.311

Table C.43. Log transformed variance in pore volumes under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	0.0887	0.136	-0.0459	0.227
Yes	Middle	-0.0472	0.239	-0.326	0.250
Yes	Bottom	-0.529	0.259	-0.829	-0.212
No	Top	-0.566	0.391	-1.02	-0.165
No	Middle	-0.756	0.278	-1.11	-0.436
No	Bottom	-0.826	0.276	-1.17	-0.466

Table C.44. Log transformed dispersion with transient flow under saturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (cm)	Standard Deviation	Min. (cm)	Max. (cm)
Yes	Top	2.10	0.0463	2.07	2.15
Yes	Middle	1.53	0.681	0.758	2.35
Yes	Bottom	0.0396	0.388	-0.314	0.582
No	Top	0.0859	0.513	-0.308	0.825
No	Middle	-0.0319	0.294	-0.393	0.305
No	Bottom	-0.215	0.419	-0.739	0.238

Table C.45. Log transformed dispersion with transient flow with dual porosity model under saturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (cm)	Standard Deviation	Min. (cm)	Max. (cm)
Yes	Top	1.09	0.329	0.713	1.31
Yes	Middle	0.902	0.898	-0.126	1.85
Yes	Bottom	-0.340	0.204	-0.593	-0.0949
No	Top	-0.462	0.342	-0.970	-0.230
No	Middle	-0.819	0.715	-1.68	0.0170
No	Bottom	-0.540	0.274	-0.940	-0.334

Table C.46. Theta immobile from the dual porosity model with transient flow under saturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (θv)	Standard Deviation	Min. (θv)	Max. (θv)
Yes	Top	0.241	0.0536	0.189	0.296
Yes	Middle	0.248	0.138	0.127	0.372
Yes	Bottom	0.216	0.176	0.0002	0.429
No	Top	0.173	0.188	0.0078	0.424
No	Middle	0.231	0.236	0.0176	0.440
No	Bottom	0.183	0.206	0.0042	0.383

Table C.47. Alpha term from the dual porosity model with transient flow under saturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean	Standard Deviation	Min.	Max.
Yes	Top	0.0068	0.0021	0.0054	0.0092
Yes	Middle	0.0074	0.0060	0.0021	0.0142
Yes	Bottom	0.0180	0.0131	0.0001	0.0314
No	Top	0.0092	0.0080	0.0017	0.0180
No	Middle	0.0225	0.0381	0.0005	0.0793
No	Bottom	0.0156	0.0167	0.0007	0.0309

Table C.48. Expected value under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	1.07	0.274	0.681	1.32
Yes	Middle	1.13	0.178	0.939	1.37
Yes	Bottom	1.58	0.314	1.30	1.95
No	Top	1.46	0.0665	1.40	1.54
No	Middle	1.11	0.175	0.862	1.25
No	Bottom	1.46	0.172	1.22	1.60

Table C.49. Second moment under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean	Standard Deviation	Min.	Max.
Yes	Top	1.63	0.711	0.669	2.36
Yes	Middle	1.94	0.738	1.11	2.90
Yes	Bottom	3.06	1.41	1.82	4.92
No	Top	2.62	0.193	2.40	2.87
No	Middle	1.50	0.300	1.16	1.79
No	Bottom	2.61	0.721	1.61	3.21

Table C.50. Log transformed variance in pore volumes under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	-0.391	0.208	-0.687	-0.212
Yes	Middle	-0.255	0.274	-0.637	0.0145
Yes	Bottom	-0.434	0.372	-0.849	0.0520
No	Top	-0.321	0.090	-0.418	-0.203
No	Middle	-0.397	0.694	-0.929	0.623
No	Bottom	-0.420	0.332	-0.880	-0.0944

Table C.51. Log transformed dispersion with transient flow under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (cm)	Standard Deviation	Min. (cm)	Max. (cm)
Yes	Top	0.806	0.538	0.492	1.61
Yes	Middle	0.724	0.216	0.528	1.02
Yes	Bottom	-0.147	0.416	-0.486	0.401
No	Top	-0.142	0.164	-0.313	0.0237
No	Middle	-0.331	0.190	-0.486	-0.0871
No	Bottom	-0.299	0.112	-0.449	-0.191

Table C.52. Log transformed dispersion with transient flow with the dual porosity model under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (cm)	Standard Deviation	Min. (cm)	Max. (cm)
Yes	Top	0.237	0.240	-0.121	0.390
Yes	Middle	0.183	0.194	0.0187	0.404
Yes	Bottom	-0.498	0.443	-1.12	-0.0911
No	Top	-0.618	0.582	-1.41	-0.0758
No	Middle	-0.570	0.211	-0.861	-0.357
No	Bottom	-0.617	0.347	-1.08	-0.312

Table C.53. Theta immobile from the dual porosity model with transient flow under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (ϴv)	Standard Deviation	Min. (ϴv)	Max. (ϴv)
Yes	Top	0.300	0.0266	0.261	0.320
Yes	Middle	0.132	0.0359	0.0906	0.176
Yes	Bottom	0.143	0.173	0.0006	0.353
No	Top	0.180	0.170	0.0044	0.330
No	Middle	0.0931	0.109	0.0212	0.256
No	Bottom	0.150	0.0934	0.0135	0.220

Table C.54. Alpha term from the dual porosity model with transient flow under unsaturated conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean	Standard Deviation	Min.	Max.
Yes	Top	0.0034	0.0025	0.0001	0.0062
Yes	Middle	0.0005	0.0001	0.0004	0.0006
Yes	Bottom	0.0082	0.0120	0.0004	0.0258
No	Top	0.0132	0.0138	0.0021	0.0333
No	Middle	0.0108	0.0183	0.0007	0.0382
No	Bottom	0.0079	0.0066	0.0024	0.0174

Table C.55. Number of pore volumes to remove the first 25% of the dye under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	1.39	0.603	0.772	2.21
Yes	Middle	1.13	0.217	0.828	1.32
Yes	Bottom	0.900	0.199	0.625	1.06
No	Top	0.853	0.176	0.696	1.08
No	Middle	0.688	0.114	0.524	0.777
No	Bottom	0.802	0.0721	0.714	0.870

Table C.56. Number of pore volumes to remove the 50% of the dye under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	2.83	1.09	1.78	4.36
Yes	Middle	2.29	0.436	1.70	2.68
Yes	Bottom	1.79	0.397	1.26	2.12
No	Top	1.69	0.359	1.35	2.15
No	Middle	1.39	0.232	1.06	1.57
No	Bottom	1.60	0.158	1.41	1.76

Table C.57. Number of pore volumes to remove the 75% of the dye under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	4.25	1.644	2.77	6.59
Yes	Middle	3.46	0.666	2.56	4.06
Yes	Bottom	2.66	0.569	1.90	3.15
No	Top	2.57	0.606	2.03	3.39
No	Middle	2.11	0.361	1.58	2.35
No	Bottom	2.47	0.230	2.13	2.65

Table C.58. Number of pore volumes to remove the 100% of the dye under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	5.60	2.13	3.71	8.64
Yes	Middle	4.57	0.891	3.37	5.34
Yes	Bottom	3.57	0.789	2.51	4.18
No	Top	3.40	0.777	2.72	4.45
No	Middle	2.79	0.469	2.11	3.13
No	Bottom	3.24	0.298	2.85	3.53

Table C.59. Number of pore volumes to remove the first 25% of the dye under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	0.788	0.263	0.438	1.01
Yes	Middle	0.964	0.257	0.634	1.24
Yes	Bottom	0.942	0.0812	0.842	1.04
No	Top	0.967	0.135	0.863	1.15
No	Middle	0.800	0.101	0.722	0.937
No	Bottom	1.034	0.206	0.810	1.30

Table C.60. Number of pore volumes to remove the 50% of the dye under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	1.60	0.497	0.941	2.03
Yes	Middle	1.89	0.612	1.07	2.48
Yes	Bottom	1.92	0.274	1.57	2.23
No	Top	2.00	0.230	1.77	2.31
No	Middle	1.61	0.245	1.40	1.95
No	Bottom	2.06	0.441	1.60	2.65

Table C.61. Number of pore volumes to remove the 75% of the dye under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	2.40	0.719	1.45	3.05
Yes	Middle	2.90	0.834	1.82	3.75
Yes	Bottom	2.88	0.371	2.37	3.25
No	Top	3.09	0.246	2.90	3.44
No	Middle	2.42	0.343	2.12	2.89
No	Bottom	3.13	0.681	2.40	4.04

Table C.62. Number of pore volumes to remove the 100% of the dye under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Section	Mean (PV)	Standard Deviation	Min. (PV)	Max. (PV)
Yes	Top	3.33	0.792	2.29	4.05
Yes	Middle	4.16	1.09	2.56	4.94
Yes	Bottom	4.20	1.13	3.11	5.79
No	Top	4.10	0.348	3.84	4.59
No	Middle	3.18	0.372	2.84	3.66
No	Bottom	4.13	0.872	3.14	5.26

Table C.63. The water flow rate under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Section**	**Mean ($mL\ min^{-1}$)**	**Standard Deviation**	**Min. ($mL\ min^{-1}$)**	**Max. ($mL\ min^{-1}$)**
Yes	Top	6.54	3.03	2.52	9.60
Yes	Middle	10.7	0.685	9.71	11.3
Yes	Bottom	10.3	1.24	9.56	12.1
No	Top	8.77	3.12	4.57	11.2
No	Middle	9.48	4.79	3.25	14.7
No	Bottom	8.24	3.02	4.79	11.6

Table C.64. Gravimetric water content under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Section**	**Mean (θg)**	**Standard Deviation**	**Min. (θg)**	**Max. (θg)**
Yes	Top	0.389	0.0345	0.339	0.414
Yes	Middle	0.466	0.0650	0.402	0.555
Yes	Bottom	0.473	0.108	0.377	0.627
No	Top	0.441	0.0302	0.400	0.473
No	Middle	0.464	0.0143	0.446	0.477
No	Bottom	0.436	0.0153	0.415	0.448

Table C.65. Pore volume size under saturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Section**	**Mean (mL)**	**Standard Deviation**	**Min. (mL)**	**Max. (mL)**
Yes	Top	10604	94.0	920	1116
Yes	Middle	1284	176	1109	1522
Yes	Bottom	1190	94.4	1050	1253
No	Top	1292	36.7	1254	1339
No	Middle	1305	59.3	1221	1351
No	Bottom	1217	56.7	1137	1266

Table C.66. Water flow rate under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Section**	**Mean (mL min^{-1})**	**Standard Deviation**	**Min. (mL min^{-1})**	**Max. (mL min^{-1})**
Yes	Top	0.347	0.153	0.138	0.506
Yes	Middle	1.50	0.817	0.338	2.13
Yes	Bottom	3.38	0.850	2.54	4.40
No	Top	2.65	0.553	2.19	3.46
No	Middle	2.32	0.733	1.74	3.37
No	Bottom	3.12	1.70	1.64	5.48

Table C.67. Gravimetric water content under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Section**	**Mean (ϴg)**	**Standard Deviation**	**Min. (ϴg)**	**Max. (ϴg)**
Yes	Top	0.324	0.0100	0.316	0.338
Yes	Middle	0.339	0.0722	0.302	0.447
Yes	Bottom	0.340	0.0273	0.304	0.363
No	Top	0.331	0.0055	0.325	0.338
No	Middle	0.337	0.0302	0.316	0.380
No	Bottom	0.318	0.0091	0.310	0.328

Table C.68. Pore volume size under unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Section**	**Mean (mL)**	**Standard Deviation**	**Min. (mL)**	**Max. (mL)**
Yes	Top	888	29.6	853	919
Yes	Middle	934	194	826	1225
Yes	Bottom	880	122	732	1010
No	Top	939	13.1	928	957
No	Middle	947	93.1	865	1076
No	Bottom	889	23.7	866	921

Table C.69. Water flow rate comparison between saturated and unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Flow	Section	Mean (mL min^{-1})	Standard Deviation	Min. (mL min^{-1})	Max. (mL min^{-1})
Yes	Saturated	Top	6.54	3.03	2.52	9.60
Yes	Saturated	Middle	10.7	0.69	9.71	11.3
Yes	Saturated	Bottom	10.3	1.24	9.56	12.1
Yes	Unsaturated	Top	0.35	0.15	0.14	0.51
Yes	Unsaturated	Middle	1.50	0.82	0.34	2.13
Yes	Unsaturated	Bottom	3.38	0.85	2.54	4.40
No	Saturated	Top	8.77	3.12	4.57	11.2
No	Saturated	Middle	9.48	4.79	3.25	14.7
No	Saturated	Bottom	8.24	3.02	4.79	11.6
No	Unsaturated	Top	2.65	0.55	2.19	3.46
No	Unsaturated	Middle	2.32	0.73	1.74	3.37
No	Unsaturated	Bottom	3.12	1.70	1.64	5.48

Table C.70. Gravimetric water content comparison between saturated and unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	**Flow**	**Section**	**Mean (θg)**	**Standard Deviation**	**Min. (θg)**	**Max. (θg)**
Yes	Saturated	Top	0.39	0.03	0.34	0.41
Yes	Saturated	Middle	0.47	0.07	0.40	0.56
Yes	Saturated	Bottom	0.47	0.11	0.38	0.63
Yes	Unsaturated	Top	0.32	0.01	0.32	0.34
Yes	Unsaturated	Middle	0.34	0.07	0.30	0.45
Yes	Unsaturated	Bottom	0.34	0.03	0.30	0.36
No	Saturated	Top	0.44	0.03	0.40	0.47
No	Saturated	Middle	0.46	0.01	0.45	0.48
No	Saturated	Bottom	0.44	0.02	0.41	0.45
No	Unsaturated	Top	0.33	0.01	0.32	0.34
No	Unsaturated	Middle	0.34	0.03	0.32	0.38
No	Unsaturated	Bottom	0.32	0.01	0.31	0.33

Table C.71. Pore volume size comparison between saturated and unsaturated flow conditions. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before measurements were taken.

Earthworms Present	Flow	Section	Mean (L)	Standard Deviation	Min. (L)	Max. (L)
Yes	Saturated	Top	1.06	0.094	0.920	1.12
Yes	Saturated	Middle	1.28	0.176	1.11	1.52
Yes	Saturated	Bottom	1.19	0.0944	0.105	1.25
Yes	Unsaturated	Top	888	0.0296	0.853	0.919
Yes	Unsaturated	Middle	934	0.194	0.826	1.23
Yes	Unsaturated	Bottom	880	0.122	0.732	1.01
No	Saturated	Top	1.29	0.0367	1.25	1.34
No	Saturated	Middle	1.31	0.0593	1.22	1.35
No	Saturated	Bottom	1.22	0.0567	1.14	1.27
No	Unsaturated	Top	0.939	0.0131	0.928	0.957
No	Unsaturated	Middle	0.947	0.0931	0.865	1.08
No	Unsaturated	Bottom	0.889	0.0237	0.866	0.921

Table C.72. Saturated volumetric water content from the Brooks-Corey water retention curve equation using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	**Section**	**Position**	**Mean (ϴv)**	**Standard Deviation**	**Min. (ϴv)**	**Max. (ϴv)**
Yes	Top	A	0.56	0.08	0.50	0.76
Yes	Top	B	0.55	0.14	0.34	0.84
Yes	Middle	C	0.53	0.05	0.46	0.61
Yes	Middle	D	0.57	0.06	0.50	0.74
Yes	Bottom	E	0.56	0.03	0.53	0.62
Yes	Bottom	F	0.55	0.03	0.51	0.62
No	Top	A	0.54	0.02	0.49	0.58
No	Top	B	0.54	0.03	0.50	0.58
No	Middle	C	0.56	0.03	0.52	0.62
No	Middle	D	0.55	0.02	0.50	0.59
No	Bottom	E	0.52	0.02	0.49	0.55
No	Bottom	F	0.52	0.02	0.49	0.58

Table C.73. Residual volumetric water content from the Brooks-Corey water retention curve equation using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	**Section**	**Position**	**Mean (ϴv)**	**Standard Deviation**	**Min. (ϴv)**	**Max. (ϴv)**
Yes	Top	A	0.04	0.08	0.00	0.23
Yes	Top	B	0.09	0.12	0.00	0.27
Yes	Middle	C	0.04	0.06	0.00	0.19
Yes	Middle	D	0.06	0.08	0.00	0.23
Yes	Bottom	E	0.01	0.03	0.00	0.12
Yes	Bottom	F	0.04	0.07	0.00	0.19
No	Top	A	0.05	0.06	0.00	0.14
No	Top	B	0.04	0.09	0.00	0.30
No	Middle	C	0.02	0.02	0.00	0.07
No	Middle	D	0.03	0.04	0.00	0.11
No	Bottom	E	0.07	0.06	0.00	0.18
No	Bottom	F	0.06	0.05	0.00	0.13

Table C.74. Alpha term from the Brooks-Corey water retention curve equation using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean	Standard Deviation	Min.	Max.
Yes	Top	A	0.27	0.16	0.07	0.57
Yes	Top	B	0.45	0.33	0.06	0.95
Yes	Middle	C	0.54	0.31	0.19	1.24
Yes	Middle	D	0.37	0.23	0.15	0.69
Yes	Bottom	E	0.18	0.12	0.04	0.40
Yes	Bottom	F	0.13	0.11	0.02	0.35
No	Top	A	0.10	0.05	0.03	0.18
No	Top	B	0.11	0.04	0.04	0.17
No	Middle	C	0.13	0.07	0.05	0.29
No	Middle	D	0.12	0.05	0.05	0.18
No	Bottom	E	0.08	0.05	0.02	0.18
No	Bottom	F	0.09	0.05	0.04	0.16

Table C.75. N term from the Brooks-Corey water retention curve equation using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean	Standard Deviation	Min.	Max.
Yes	Top	A	0.13	0.08	0.08	0.33
Yes	Top	B	0.14	0.09	0.02	0.31
Yes	Middle	C	0.10	0.03	0.06	0.17
Yes	Middle	D	0.16	0.09	0.07	0.38
Yes	Bottom	E	0.16	0.05	0.12	0.33
Yes	Bottom	F	0.23	0.16	0.11	0.65
No	Top	A	0.31	0.36	0.15	1.42
No	Top	B	0.22	0.18	0.12	0.77
No	Middle	C	0.19	0.03	0.14	0.24
No	Middle	D	0.28	0.32	0.12	1.28
No	Bottom	E	0.33	0.41	0.14	1.62
No	Bottom	F	0.19	0.05	0.13	0.26

Table C.76. Fitted residual volumetric water content from the Brooks-Corey water retention curve equation using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	**Section**	**Position**	**Mean (θv)**	**Standard Deviation**	**Min. (θv)**	**Max. (θv)**
Yes	Top	A	0.14	0.04	0.07	0.20
Yes	Top	B	0.16	0.09	0.01	0.30
Yes	Middle	C	0.15	0.04	0.09	0.21
Yes	Middle	D	0.12	0.04	0.07	0.19
Yes	Bottom	E	0.08	0.01	0.06	0.11
Yes	Bottom	F	0.08	0.02	0.05	0.12
No	Top	A	0.07	0.01	0.05	0.09
No	Top	B	0.08	0.02	0.05	0.12
No	Middle	C	0.06	0.01	0.04	0.09
No	Middle	D	0.07	0.02	0.04	0.12
No	Bottom	E	0.07	0.02	0.05	0.10
No	Bottom	F	0.08	0.02	0.06	0.11

Table C.77. Air entry value from the Brooks-Corey water retention curve equation using data to 1.5 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	**Section**	**Position**	**Mean (cm)**	**Standard Deviation**	**Min. (cm)**	**Max. (cm)**
Yes	Top	A	5.65	3.97	1.75	14.5
Yes	Top	B	5.05	5.29	1.05	16.3
Yes	Middle	C	2.53	1.49	0.81	5.32
Yes	Middle	D	3.90	2.18	1.45	6.66
Yes	Bottom	E	8.76	6.57	2.51	25.8
Yes	Bottom	F	15.2	14.5	2.84	50.6
No	Top	A	13.4	8.43	5.66	29.4
No	Top	B	11.4	5.87	5.73	25.2
No	Middle	C	9.41	5.04	3.42	21.5
No	Middle	D	9.57	4.71	5.43	19.5
No	Bottom	E	19.9	15.1	5.49	62.2
No	Bottom	F	13.6	6.85	6.09	23.0

Table C.78. Saturated volumetric water content from the Brooks-Corey water retention curve equation using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean (θv)	Standard Deviation	Min. (θv)	Max. (θv)
Yes	Top	A	0.60	0.02	0.57	0.64
Yes	Top	B	0.61	0.06	0.55	0.71
Yes	Middle	C	0.63	0.04	0.58	0.70
Yes	Middle	D	0.68	0.09	0.60	0.85
Yes	Bottom	E	0.65	0.03	0.61	0.69
Yes	Bottom	F	0.65	0.03	0.63	0.70
No	Top	A	0.55	0.02	0.53	0.58
No	Top	B	0.56	0.02	0.54	0.58
No	Middle	C	0.56	0.04	0.53	0.62
No	Middle	D	0.55	0.02	0.52	0.59
No	Bottom	E	0.57	0.09	0.51	0.75
No	Bottom	F	0.53	0.01	0.52	0.54

Table C.79. Residual volumetric water content from the Brooks-Corey water retention curve equation using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean	Standard Deviation	Min.	Max.
Yes	Top	A	1.62E-05	1.41E-05	2.22E-06	4.14E-05
Yes	Top	B	1.85E-05	1.88E-05	1.79E-07	5.33E-05
Yes	Middle	C	1.13E-05	5.69E-06	5.18E-06	1.87E-05
Yes	Middle	D	1.07E-05	9.19E-06	2.77E-06	2.83E-05
Yes	Bottom	E	1.56E-05	7.16E-06	3.33E-06	2.40E-05
Yes	Bottom	F	1.18E-05	1.16E-05	7.57E-07	2.63E-05
No	Top	A	1.31E-05	6.35E-06	5.23E-06	2.25E-05
No	Top	B	1.03E-05	6.02E-06	1.32E-06	1.70E-05
No	Middle	C	2.06E-05	2.53E-06	1.78E-05	2.39E-05
No	Middle	D	9.46E-06	3.71E-06	5.09E-06	1.60E-05
No	Bottom	E	2.60E-05	3.41E-05	2.69E-06	9.15E-05
No	Bottom	F	4.58E-05	6.39E-05	9.76E-06	1.74E-04

Table C.80. Alpha term from the Brooks-Corey water retention curve equation using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean	Standard Deviation	Min.	Max.
Yes	Top	A	0.07	0.03	0.05	0.13
Yes	Top	B	0.09	0.03	0.05	0.14
Yes	Middle	C	0.15	0.02	0.12	0.19
Yes	Middle	D	0.12	0.03	0.08	0.15
Yes	Bottom	E	0.13	0.05	0.06	0.19
Yes	Bottom	F	0.13	0.08	0.07	0.26
No	Top	A	0.09	0.02	0.06	0.11
No	Top	B	0.08	0.01	0.07	0.10
No	Middle	C	0.09	0.02	0.07	0.12
No	Middle	D	0.11	0.04	0.07	0.17
No	Bottom	E	0.07	0.03	0.04	0.11
No	Bottom	F	0.07	0.02	0.04	0.09

Table C.81. N term from the Brooks-Corey water retention curve equation using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean	Standard Deviation	Min.	Max.
Yes	Top	A	0.16	0.02	0.14	0.18
Yes	Top	B	0.14	0.01	0.14	0.15
Yes	Middle	C	0.15	0.01	0.15	0.16
Yes	Middle	D	0.17	0.01	0.15	0.18
Yes	Bottom	E	0.19	0.01	0.17	0.20
Yes	Bottom	F	0.19	0.02	0.18	0.22
No	Top	A	0.19	0.01	0.18	0.20
No	Top	B	0.18	0.01	0.17	0.20
No	Middle	C	0.19	0.01	0.18	0.21
No	Middle	D	0.19	0.01	0.18	0.20
No	Bottom	E	0.19	0.01	0.19	0.21
No	Bottom	F	0.18	0.01	0.17	0.19

Table C.82. Fitted residual volumetric water content from the Brooks-Corey water retention curve equation using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean (ϴv)	Standard Deviation	Min. (ϴv)	Max. (ϴv)
Yes	Top	A	0.08	0.01	0.06	0.09
Yes	Top	B	0.08	0.04	0.00	0.11
Yes	Middle	C	0.08	0.01	0.07	0.09
Yes	Middle	D	0.07	0.01	0.06	0.08
Yes	Bottom	E	0.05	0.01	0.04	0.06
Yes	Bottom	F	0.04	0.02	0.00	0.06
No	Top	A	0.06	0.01	0.05	0.06
No	Top	B	0.06	0.01	0.05	0.07
No	Middle	C	0.05	0.01	0.04	0.06
No	Middle	D	0.05	0.01	0.04	0.07
No	Bottom	E	0.06	0.01	0.05	0.06
No	Bottom	F	0.06	0.01	0.05	0.07

Table C.83. Air entry value from the Brooks-Corey water retention curve equation using the data from the dew point potentiometer. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean (cm)	Standard Deviation	Min. (cm)	Max. (cm)
Yes	Top	A	15.1	4.94	7.87	22.2
Yes	Top	B	12.6	4.20	7.35	20.0
Yes	Middle	C	6.88	1.04	5.29	8.13
Yes	Middle	D	8.48	2.25	6.49	12.8
Yes	Bottom	E	9.18	3.95	5.32	15.6
Yes	Bottom	F	9.98	4.18	3.82	14.6
No	Top	A	11.1	2.71	8.85	16.0
No	Top	B	12.9	2.05	9.73	14.8
No	Middle	C	11.6	2.68	8.58	14.5
No	Middle	D	10.6	3.87	5.79	14.8
No	Bottom	E	17.1	5.84	9.35	23.6
No	Bottom	F	16.5	4.69	11.7	22.4

Table C.84. Alpha value from the Brooks-Corey water retention curve equation using only the data points to 0.1 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean	Standard Deviation	Min.	Max.
Yes	Top	A	5.54	5.80	1.81	18.7
Yes	Top	B	3.98	4.85	1.07	17.8
Yes	Middle	C	6.54	12.4	1.04	45.6
Yes	Middle	D	3.60	2.09	0.87	6.46
Yes	Bottom	E	7.07	5.06	0.41	18.3
Yes	Bottom	F	11.0	8.25	2.51	26.9
No	Top	A	10.8	6.86	5.50	29.5
No	Top	B	9.79	3.73	6.03	16.8
No	Middle	C	8.12	2.76	3.59	14.6
No	Middle	D	9.00	4.90	5.84	21.3
No	Bottom	E	14.8	7.64	1.58	27.6
No	Bottom	F	12.2	6.23	3.06	21.0

Table C.85. Air entry value from the Brooks-Corey water retention curve equation using only the data points to 0.1 MPa of tension. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were taken to develop water retention curves that were then modeled with Hydrus-1D.

Earthworms Present	Section	Position	Mean (cm)	Standard Deviation	Min. (cm)	Max. (cm)
Yes	Top	A	0.32	0.17	0.05	0.55
Yes	Top	B	0.53	0.31	0.06	0.93
Yes	Middle	C	0.42	0.30	0.02	0.97
Yes	Middle	D	0.46	0.37	0.16	1.15
Yes	Bottom	E	0.42	0.67	0.05	2.42
Yes	Bottom	F	0.15	0.12	0.04	0.40
No	Top	A	0.12	0.04	0.03	0.18
No	Top	B	0.11	0.04	0.06	0.17
No	Middle	C	0.14	0.05	0.07	0.28
No	Middle	D	0.13	0.04	0.05	0.17
No	Bottom	E	0.12	0.16	0.04	0.63
No	Bottom	F	0.11	0.08	0.05	0.33

Table C.86. Percent sand. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were collected to determine soil texture.

Earthworms Present	Section	Position	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
Yes	Top	A	28.9	0.62	28.2	30.4
Yes	Top	B	28.9	0.65	27.8	30.2
Yes	Middle	C	28.8	1.02	27.5	30.3
Yes	Middle	D	28.9	0.89	27.5	30.0
Yes	Bottom	E	28.3	0.75	27.2	29.3
Yes	Bottom	F	28.4	0.51	27.7	29.3
No	Top	A	28.5	0.84	27.2	29.5
No	Top	B	28.6	0.85	27.1	30.0
No	Middle	C	29.0	0.37	28.2	29.5
No	Middle	D	29.0	0.47	28.2	29.8
No	Bottom	E	29.3	1.19	27.0	31.0
No	Bottom	F	29.0	0.77	27.8	30.0

Table C.87. Percent silt. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were collected to determine soil texture.

Earthworms Present	Section	Position	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
Yes	Top	A	0.38	0.02	0.36	0.42
Yes	Top	B	0.39	0.02	0.35	0.42
Yes	Middle	C	0.37	0.02	0.34	0.39
Yes	Middle	D	0.38	0.02	0.35	0.42
Yes	Bottom	E	0.36	0.04	0.30	0.41
Yes	Bottom	F	0.37	0.04	0.30	0.43
No	Top	A	0.38	0.02	0.35	0.41
No	Top	B	0.38	0.01	0.36	0.39
No	Middle	C	0.38	0.02	0.35	0.39
No	Middle	D	0.37	0.01	0.36	0.40
No	Bottom	E	0.39	0.02	0.36	0.43
No	Bottom	F	0.37	0.03	0.35	0.41

Table C.88. Percent clay. A laboratory column study was designed to investigate the effects of *A. caliginosa* on soil physical properties related to the flow and storage of water in a sandy loam soil (Adena (Ustic Paleargid)-Colby (Aridic Ustorthents) complex). The earthworms were allowed 16 weeks to interact with the soil before soil samples were collected to determine soil texture.

Earthworms Present	Section	Position	Mean (%)	Standard Deviation	Min. (%)	Max. (%)
Yes	Top	A	0.34	0.01	0.33	0.35
Yes	Top	B	0.34	0.01	0.32	0.36
Yes	Middle	C	0.35	0.02	0.33	0.38
Yes	Middle	D	0.34	0.02	0.31	0.37
Yes	Bottom	E	0.33	0.02	0.31	0.35
Yes	Bottom	F	0.32	0.02	0.30	0.34
No	Top	A	0.34	0.01	0.33	0.37
No	Top	B	0.34	0.01	0.32	0.35
No	Middle	C	0.35	0.02	0.33	0.37
No	Middle	D	0.34	0.02	0.33	0.37
No	Bottom	E	0.34	0.03	0.31	0.38
No	Bottom	F	0.34	0.02	0.32	0.37