Casting activity of Lumbricid earthworms from temperate agroecosystems.

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Abstract:

Earthworms are well known for their casting and burrowing activities which improve soil structure and soil fertility. However, earthworm populations in temperate regions exhibit patchy distribution in space and time. This makes it difficult to predict how earthworm activities may influence paedogenesis and nutrient cycling processes at the ecosystem level. The main objective of this study was to determine the spatiotemporal dynamic of surface cast production of two earthworm species, *Aporrectodea turgida* and *Lumbricus terrestris*, on the row-interrow scale in a temperate soybean agroecosystem. Our observations demonstrated that casting activity was synchronous with plant growth. More casts were also found in the row intercepts compared to the interrow ones. Both the spatial and temporal variations are thought to be caused by the microclimate found under the plant canopy, verifying results from controlled laboratory studies that show casting activity to be controlled by soil temperature and moisture. These results suggest a possible mutualism between earthworm and plants in cultivated temperate soybean agroecosystems, but this remained to be confirmed.

Résumé:

Les vers de terre sont bien connus pour leur création de tunnels et turricules qui améliorent les caractéristiques physiques et la fertilité des sols. Par contre, la sporadicité spatiotemporelle des populations de vers de terre limite notre compréhension de leur influence sur la pédogenèse et le cycle des nutriments à l'échelle écosystémique. L'objectif principal de cette recherche fut de déterminer la dynamique spatiotemporelle de la production de turricules de surface chez deux espèces de vers de têrre, *Aporrectodea turgida* et *Lumbricus terrestris,* à l'échelle des rangées de culture dans une plantation de soya en climat tempéré. Nos observations démontrèrent que la période de production de turricules de surface correspondait à la période de croissance des plants de soya. Il fut aussi observé q'une plus grande quantité de turricules fut déposée sous les rangées qu'entre les rangées de la plantation. La cause probable des variations spatiotemporelles observées est le microclimat créé par le couvert végétal, ce qui confirme nos résultats obtenu en laboratoire démontrant que la production de turricules de surface est contrôlée par la température et l'humidité du sol. Nos résultats suggèrent un possible mutualisme entre les vers de terre et les plantes cultivés en milieu tempéré, ce qui reste toutefois à confirmé.

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Introduction

Considered as keystone organisms and ecosystem engineers, earthworms have a great influence on the soil environment. Earthworm burrowing activity is well known for improving water and air infiltration into the ground as well as creating habitats for other soil invertebrates. Their underground casting activity is known to contribute to paedogenesis, while surface casting transforms the soil profile development and structure. Earthworm casts have also been shown to improve soil physical quality and stability since they are more stable and have a higher tensile strength than soil aggregates. Chemically speaking, casts also contain a greater amount of plant available nutrients such as N, P, K, and Mg, as well as higher concentrations of organic carbon than the bulk soil. Earthworms also play an important role in nutrient cycling by promoting the decomposition of plant litter by incorporating and mixing it within the soil layers.

However, earthworm activity is strongly influenced by soil temperature and moisture as well as food sources. The objective of the first two experiments was to investigate the influence of these three factors, through controlled laboratory conditions, on earthworm burrowing and casting activity as well as cast chemical characteristics.

Also, since little is known about the spatiotemporal dynamics of earthworm populations, especially in temperate regions, it is difficult to judge the extent of their impacts on soil ecosystems. The third experiment was designed with the main objective of determining earthworm spatiotemporal surface casting activity in order to improve our understanding of earthworm interactions with plants and soils of temperate agroecosystems.

Literature review

Earthworms are members of the Oligochaeta subclass of the class Clitellata within the Annelida phylum (Bohlen and Hendrix 2002). Of the approximately 5000 species of earthworms inhabiting the Earth, 147 species in 12 families, excluding Enchytraeidae, are known to live in North America north of Mexico (Reynolds 1995). Of those species, 31 or 45 percent are known to be introduced species, belonging mostly to the Lumbricidae and Megascolecidae families (Reynolds 1995). However, since Canada was completely covered by ice during the Wisconsinan glaciation, and because earthworm migration is very slow, there were no Lumbricids or Megascolecids in the regions where European explorers first reached North America. (Hendrix 1995, Edwards and Bohlen 1996) In fact, all of the species of earthworms now present in Canada east of the Pacific Northwest are alien species which are all peregrine and have been spread by man (Edwards and Bohlen 1996, Bohlen and Hendrix 2002). Due to the nature of their activity there is no doubt that earthworms have many effects on the North American ecosystems. Even though earthworms are the most well-known of soil organisms (Bohlen and Hendrix 2002), our knowledge is mainly limited to a few dozen species (Hendrix 1995). In fact, when trying to understand earthworm ecology, population dynamics, their impacts on different ecosystems, etc. we often face a lack of information (Hendrix 1995, Giller *et al.* 1997, Whalen and Costa 2003). This literature review will look at earthworms' biology and ecology in general as well as in perspective of the experiments performed during my Masters program.

Earthworms as ecosystem engineers:

Because their activities cause direct physical changes to the environment they inhabit, earthworms are known as ecosystem engineers (Lawton 1994, Lavelle *et al.* 1997, Kladivko 2001). Earthworms are good tillers of the soil (Hartenstein and Amico 1983) and by casting, burrowing and mixing the soil they significantly transform their milieu in many ways (Lawton 1994). One of the most obvious impacts of earthworms' activity is that they increase air and water infiltration into the ground. However, a wide variety of more subtle, but very important impacts are also due to earthworm activity (Lavelle *et al.* 1997). For example, earthworm casts have been observed to be a safe haven promoting the germination of *Trifolium* seeds. Meanwhile the earthworms were also observed to selectively bury the seeds of certain competing plant species too deep for them to germinate. This demonstrates how earthworms can selectively impact the plant diversity of particular habitat (Lawton 1994). More precisely, earthworms are allogenic engineers, meaning that they change an ecosystem due to their activity in contrast with autogenic engineers that change the environment due to their sole presence, such as coral reefs (Lawton 1994).

Earthworms: Beneficial or deleterious?

Generally seen as beneficial organisms for the soil, earthworms definitely do deserve a favorable definition from anyone who examines their contribution to primary production (Schmidt 2001). Earthworms are well known for increasing water and air infiltration into the ground with their burrows and improving soil aggregation with their casts. (Edwards and Bohlen 1996, Bohlen and Hendrix 2002) Earthworms also improve nutrient cycling by increasing the rate of decomposition of plant litter, promoting the growth of beneficial microorganisms, and improving solute transport, allowing faster nutrient uptake by plants (Lawton 1994, Bohlen and Hendrix 2002). Earthworm casts are also rich in nutrients essential to plants such as N, P, K, Ca and Mg (Sharpley and Syers 1976, Lawton 1994). Earthworm burrows also create new habitats for other species (Lawton 1994). With all the beneficial direct and indirect impacts coming from earthworms' activity it would be very difficult to put a price on the earthworm contribution to our activities, but earthworms are economically beneficial. Examples of direct economic gains from earthworms include over 50 million dollars in sales of fish bait annually in Canada alone (Tomlin 1983) as well as the marketing of their casts which are sold as fertilizer.

As for every introduced species however, earthworms also have negative impacts on the environment. Most foreign species invasion where earthworms are already present do not seem to take place by competitive exclusion, but rather by the invasive species taking advantage of an ecological disturbance to fill in the empty niche created by the disturbance, thus excluding the native species (Bohlen and Hendrix 2002). For the many regions of North America where earthworms are not present, an introduction is all that is required to establish a worm population which will transform the ecosystem (Bohlen and Hendrix 2002). In a forest of Minnesota for example, the earthworm population went from 0 to 592 per square meter in 14 years. This new population decreased by almost 85 percent the forest floor thickness and weight. The introduced earthworms also completely transformed the soil horizons, creating new horizons while destroying others (Alban and Berry 1994).

Only in rare occasions have earthworms been found to be crop pests. Some earthworms have been observed killing plants by pulling their leaves in their burrows for later consumptions. Other species have also been observed to produce enough cast to make agriculture impossible unless bulldozers were used to destroy the casts. However, earthworms should not be considered as crop pests since they always prefer to eat dead plant tissues and very few species placed under certain conditions will produce enough casts to interfere with agriculture (Edwards and Bohlen 1996).

Earthworms can also be vectors of plant and animal diseases. *Lumbricus terrestris* is now known to be able to carry the pathogenic agent of foot and mouth disease (Edwards and Bohlen 1996, Bohlen and Hendrix 2002). Many animal parasites also have a facultative or obligate stage in an earthworm before reaching their final host (Edwards and Bohlen 1996). Earthworms are also known to be vectors of plant fungal disease pathogens like *Fusarium* and *Pythium* (Edwards and Bohlen 1996, Bohlen and Hendrix 2002). However, some species of earthworms have been observed to transport biocontrol agents to the infected roots of plants. For example, *Aporrectodea trapezoides* can bring a biocontrol bacterium down to 9 centimeters and increase the colonization of infected roots by this bacterium (Stephens *et al.* 1993).

Aesthetically and practically earthworms' casts can be a problem in damaging the appearance of lawn and interfering with outdoor activity such as golf (Edwards and Bohlen 1996).

Earthworm ecological groups:

Bouché (1971, 1977), in a practical classification, divided earthworms in three artificial groups according to species differences in burrowing and feeding activities as well as their stratification in the soil profile.

A. Epigeics: The earthworms of this group are usually found on the soil surface or in the uppermost part of the mineral soil. They reproduce and grow rapidly.
B. Anecic: This group is composed of species that forms vertical burrows in the mineral soil. Their burrows can be either permanent or temporary. The earthworms of this group generally feed at the soil surface on dead plant tissues and decomposing organic matter.
C. Endogeic: This group is composed of species that live within the mineral horizons. Compared to the other groups these earthworms consume larger amount of soil from which they metabolize more humified material. They are sometimes seen feeding at the surface under the litter layer.

Many species adopt behaviors somewhere in between any two of these categories, therefore many intermediate categories are also present (Bouché 1977). Earthworms feeding behavior falls under two categories. The geophagous earthworms ingest large quantities of soil from which they digest the organic fraction while the detrivorous feed directly on organic matter and ingest soil mostly when digging their burrows (Lee 1985).

Moisture and temperature preferences and impacts on earthworms:

As poikilotherms, earthworms cannot control their own body temperature (Lagerspetz 1980). They also most likely lack a physiological water retention mechanism making them incapable of controlling their water content apart from behavioral adaptations (Edwards and Bohlen 1996). Since earthworms are unable to control their temperature and water content, soil temperature and moisture level are primordial factors determining earthworm survival, activity, growth, embryonic development, sexual maturation, reproductive success, longevity and behavior (Lee 1985, Daugbjerg 1988, Whalen and Parmelee 1999, Wever *et al.* 2001). In addition, all those factors influence earthworm population dynamics (Whalen and Parmelee 1999).

Although temperature and moisture conditions are so crucial for earthworms, only a few studies have examined the combined influence of temperature and moisture on earthworm biology and ecology (Wever *et al.* 2001). Some studies which have looked at the impact of temperature and moisture on earthworms were performed with a practical goal for vermiculture (Fayolle *et al.* 1996) or vermicomposting (Viljoen and Reinecke 1992), while others have tried to understand earthworm responses under field conditions through laboratory experiments (Daugbjerg 1988, Daniel *et al.* 1996, Wever *et al.* 2001), field experiments (Cortez 1997) or both (Whalen and Parmelee 1999). The limited amount of research conducted and the variety of objectives for which they were designed make their results difficult to interpret and compare (Daugbjerg 1988).

However, some general trends are evident from the scientific literature. The temperature preferred by *L. terrestris* was found by Daugbjerg (1988) to be of 10° C, which also corresponds to the maximum mass gain temperature found by Whalen and Parmelee (1999). However, Daniel (1996) found the maximum mass gain of *L. terrestris* to be in the range of 15 and 17.5°C. Daniel (1996) however noticed a difference in the field versus laboratory maturation time of *L. terrestris*. Whalen and Parmelee (1999) also noticed differences in field versus laboratory conditions for *L. terrestris* and suggested that the differences were caused by the experimental settings being inappropriate for the observation of anecic species such as *L. terrestris*. For *Aporrectodea spp.* similar reactions to different temperatures were also found. Whalen and Parmelee (1999) found *Aporrectodea tuberculata* to have a maximum mass gain at 18°C while Wever *et al.* (2001) found the same species to increase in mass up to a temperature of 20-°C. Whalen and Parmelee (1999) also found that their results were similar to their field results, probably due to the fact that field conditions for epigeic earthworms are easier to replicate. Other *Aporrectodea* species, *A. caliginosa* and *A. longa*, were investigated by Daugbjerg (1988) and were found to prefer temperatures of 10 and 15°C. Wever *et al.* (2001) suggested that temperature was the determinant factor for earthworm activity and could outweigh the effect of moisture. For example, low temperatures were found to change the impact of inadequate soil moisture by reducing the earthworms' respiration (Wever *et al.* 2001). However, moisture had the most impact on earthworm survival. It is also important to note that the temperatures and moistures of highest activity are not necessarily the one of highest mass gain or preference of the earthworms (Edwards and Bohlen 1996). This phenomenon can in part explain the lack of agreement according to the influence of temperature and moisture on earthworms because of the different factors used to measure activity (casting, burrowing) and the variation of the factors to which activity is compared to, mass gain or preference.

Earthworm casts:

Casts are formed of the soil and digested organic matter that is excreted after passing through the gut of earthworms (Lee 1985, Edwards and Bohlen 1996). Casts have a very different composition than the bulk soil in which they are deposited and can be produced in large enough quantities to change the soil's characteristics (Lee 1985, Edwards and Bohlen 1996, Schrader and Zhang 1997, Binet and Le Bayon 1999). Earthworms produce cast both within the soil profile and on the soil surface- their underground casts participating in paedogenesis, while their surface casts transform the soil profile development and structure (Lee 1985). Five different cast shapes are recognized: spherical, ellipsoidal, cylindrical, platy, and threadlike (Lee 1985, McKenzie and Dexter 1987, Edwards and Bohlen 1996). With the exception of some particular situations (Sharpley and Syers 1976, Schrader and Zhang 1997), earthworm casts usually improve soil quality and stability, making earthworms and their casts useful for soil rehabilitation (McKenzie and Dexter 1987, Scullion and Ramshaw 1988, Schrader and Zhang 1997).

Earthworm casts are generally more stable than soil aggregates and their stability is determined mostly by age, organic matter content, fungal colonization and microbial activity (Lee 1985, McKenzie and Dexter 1987, Schrader and Zhang 1996, Binet and Le Bayon 1998). The casts produced by earthworms have a higher tensile strength than natural soil aggregates, making them more resistant to compaction (McKenzie and Dexter 1987, Schrader and Zhang 1996). On the other hand, their water stability is less than that of soil aggregates (Schrader and Zhang 1996). Cast characteristics vary greatly between different species and among different soil types (Schrader and Zhang 1996, Whalen *et al.* 2003). Food sources are also responsible for creating different physical and chemical cast characteristics (Flegel et al 1998, Flegel and Schrader 2000). Due to this high variability in the determinants of cast stability, more research is required to understand cast characteristics (Springett and Syers 1984).

Cast chemical composition is also significantly different than the one of soil aggregates. The organic carbon content of casts is higher than that of the bulk soil (McKenzie and Dexter 1987, Schrader and Zhang 1996) and casts also contain a higher concentration of plant available nutrients such as N, P K and Mg. (Tomati *et al.* 1994, Chaoui *et al.* 2003). However, casts can also be associated with nutrient loss through leaching and run-off water if their stability is low (Sharpley and Syers 1976, Binet and Le Bayon 1998). Casts also have a biological impact on the environment. When grown in their presence, the dry mass of plants has been shown to increase. Casts have also been observed to increase N, P, K and Mg plant uptake among others (Tomati 1994, Chaoui 2003). Although casts characteristics have been well studied, cast production rates are not known for many lumbricid earthworm species. If we hope to quantify the ecosystem level effects of earthworms, we need to know how much cast production occurs.

Effect of food on earthworms with a focus on genetically modified organisms:

Earthworms are known for being capable of feeding on a broad diversity of organic materials. When faced with a shortage of food, earthworms can survive by eating microorganisms present in the soil (Edwards and Bohlen 1996). The type and amount of food present is also responsible for determining both the species that will inhabit a region and their growth and fecundity (Edwards and Bohlen 1996). Cast production and food consumption rate have also been observed to be strongly influenced by food sources (Flegel *et al.* 1998). Preference of food sources is observed in earthworms and the complete refusal of a plant, bromegrass, was observed even in the absence of any other food sources (Shipitalo *et al.* 1988). In the same experiment, foods of preference to earthworms were also the ones that caused the highest mass gain (Shipitalo *et al.* 1988).

Of the 54 approved genetically modified crop plants and plants variety, only one, Bt corn, has been studied according to its possible impacts on earthworms (Saxena and Stotzky 2001, AgBios 2003, Zwhalen *et al.* 2003). The Bt toxin produce by the corn plant is known to reach the soil in three different ways, by pollen deposition, root exudates as well as plant litter (Zwhalen *et al.* 2003). To this date, only three research endeavors have looked at the possible impact of Bt corn, two short term laboratory experiments (Saxena and Stotzky 2001) and one long term laboratory and field experiment (Zwhalen *et al.* 2003). Both short term studies (Saxena and Stotzky 2001) did not discover any toxin related impacts. The long term experiment (Zwhalen *et al.* 2003) found a significant mass loss for earthworms being fed Bt corn litter in the field and a small mass loss for the same treatment in the laboratory. With only one genetically modified plant vaguely studied, one is faced with an extreme lack of information about the impacts of GMOs on earthworms.

Techniques to assess earthworm activity under field conditions:

Because of the patchy nature of earthworm populations and the behavioral differences between species, no single sampling method is adequate for every species or situation (Baker and Lee 2000). Three methods exist for extracting earthworms from the soil: physical, behavioral and indirect methods (Baker and Lee 2000). Physical methods include hand sorting and washing and sieving. An advantage of the hand sorting method is the ability to determine vertical distribution by sampling successive layers one at a time. The washing and sieving method is most likely the most effective method for collecting every size of earthworms as well as their cocoons. Although more demanding than most other methods, much of the effort of this method has been eliminated with the development of automatic washing and sieving machines (Baker and Lee 2000). Behavioral techniques include the use of chemical repellants or electrical currents in order to force earthworms to crawl out of the ground. Chemicals utilized are various irritants, typically, formaldehyde, potassium permanganate or mercuric chloride. The chemical technique is very effective for the sampling of anecic species, but is mostly inefficient for the collection of species forming horizontal burrows (Baker and Lee 2000, Chan and Munro 2001). Even if used in very small concentration, all the chemicals previously mentioned are harmful to the environment, however, one environmentally friendly alternative does exist (Lawrence and Bowers 2001, Chan and Munro 2001). Allyl isothiocyanate, the active ingredient of mustard, was shown by Zaborski (2003) to be as effective as formaldehyde while Chan and Munro (2001) and Lawrence and Bowers (2002) found up to 67 percent more earthworms in extractions with allyl isothiocyanate than formaldehyde. However, allyl isothiocyanate should be use at concentrations of around 100 mg per liter in order to avoid a decrease of juvenile earthworm response to higher concentration treatments (Zaborski 2003). The electrical method for sampling consists of placing electrodes in the soil and passing an electrical current between the electrodes. This method was shown to be able to collect up to 87% of the earthworms that could have been collected by hand sorting (Baker and Lee 2000). A variation of the electrical method, Thielemann's octet method, was demonstrated by Schmidt (2001) to be more effective than formalin. Schmidt (2001) concluded that the electrical octet method was a reliable alternative for earthworm sampling as well as being the least destructive of the sampling methods Pit fall and baited trap have also been used to sample earthworms that are active at the surface of the soil. This method is effective to estimate earthworm number and activity. It can also give useful information on age, species and daily and seasonal changes in earthworms active at the surface. Casts number on the soil surface could also be used to determine earthworm population. However, one must be careful when interpreting the results of this sampling technique since casting activity is influenced by many environmental factors (Baker and Lee 2000).

Conclusion and future research:

Even though earthworms are abundant and their activity creates significant direct and indirect impacts on the environment as well as our economy we are still lacking important information about their biology and ecology, which is why many researchers have stressed the need for more research (Hendrix 1995, Giller *et al.* 1997, Whalen and Costa 2003). Because earthworms are ecosystem engineers (Lawton 1994, Lavelle *et al.* 1997, Kladivko 2001), knowing more about them could prove extremely valuable to improve the extent of their beneficial impacts and to use them at their full potential by finding new tasks which they could help us to perform. With more knowledge, earthworms could become an effective tool for many applications saving us time, effort and money.

Our newly gained knowledge could also prove to be important in protecting natural ecosystems.

This study had three main objectives: To determine the influence of temperature and moisture on *Aporrectodea turgida* and *Lumbricus terrestris* mass gain, burrowing and surface casting activity, to compare the influence of genetically and non-genetically modified food sources on mass gain, casting activity and casts characteristics of the same two species and to determine the temporal and spatial variation in surface cast production of *A. turgida* and *L. terrestris* on the row-interrow scale in a temperate soybean agroecosystem.

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Earthworm casting and burrowing activities as influenced by soil temperature and moisture.

Introduction

As allogenic ecosystem engineers, earthworms have important effects on the environment (Lawton 1994, Lavelle *et al.* 1997, Kladivko 2001). Earthworms are known to improve nutrient cycling by increasing the rate of decomposition of plant litter, promoting the growth of beneficial microorganisms, and improving solute transport, allowing faster uptake by plants (Lawton 1994, Bohlen and Hendrix 2002). Earthworms also exert an important influence on soil structure through their burrowing and casting activities (Lawton 1994). Earthworms' burrows increase water and air infiltration into the soil and create habitats for other species (Lawton 1994, Edwards and Bohlen 1996, Bohlen and Hendrix 2002). The casts produced as earthworms burrow through the soil transform the soil profile development and structure (Lee 1985, Edwards and Bohlen 1996, Bohlen and Hendrix 2002). Because of their selective feeding on organic matter and burrowing activity, earthworms also mix and disperse organic matter throughout the soil profile (Potter *et al.* 1990). Earthworm casts are also richer than the bulk soil in nutrients essential to plants such as N, P, K, Ca and Mg (Sharpley and Syers 1976, Lawton 1994).

As poikilothermic organisms, earthworms are directly influenced by the abiotic conditions of their environment like temperature and moisture and generally adapt themselves through their behavior (Lagerspetz 1980, Daugbjerg 1988, Edwards and Bohlen 1996). Moisture and temperature are known to influence earthworm survival, growth, embryonic development, sexual maturation, reproductive success, longevity, behavior and of course activity (Lee 1985, Daugbjerg 1988, Whalen and Parmelee 1999, Wever *et al.* 2001). Earthworms are active when environmental conditions are favorable and sufficient food resources are available. Lumbricid earthworms generally prefer soil temperatures from 10°C to 15 °C and a soil moisture between 18 to 22%

(Daugbjerg 1988). However, greater food consumption rates, mass gain and activity are observed with increasing temperature, usually up to the maximum of 20°C (Daugbjerg 1988, Daniel 1991). Fluctuations in soil temperatures and moisture greatly affects earthworm activity, however, only a few studies have tried to understand the combined influence of temperature and moisture on earthworm activity (Wever *et al.* 2001).

It is important to note that the temperatures and moistures conditions leading to the greatest activity are not necessarily the ones preferred by earthworms (Daugbjerg 1988, Edwards and Bohlen 1996). It is also essential to understand the specific ecological characteristics of the earthworm species of interest, since these affect earthworm activities. For example, the activity level of *L. terrestris* might be better estimated while looking at its surface casting activity than its burrowing activity due to its anecic nature. On the contrary, endogeic species such as *A. turgida* are most likely better studied for their activity while looking at their burrowing activity rather than their surface casting because of their underground lifestyle. However, important constraints would be faced if we wanted to estimate the activity of earthworms in the field by using burrow length or total casting as indicators of their activity. This experiment was in part designed to help in investing earthworms' activity in the field through surface evidence. With such data, it is possible to estimate the extent of a burrow system using the surface casting activity found in an environment with similar physical characteristics.

The objective of the present study was to determine the impact of moisture and temperature on the burrowing and surface casting activity of two earthworm species, *Lumbricus terrestris* L. and *Aporrectodea turgida* (Eisen). We hypothesized that because earthworms are poikilotherms, their burrowing and casting activity would increase as temperature increases. An increase in activity is considered as longer burrows and a higher surface casts area. In terms of moisture, our hypothesis consists in a reduction in activity at the highest field capacity due to an increased difficulty to travel through the soil and a reduction in available oxygen.

Materials and Methods

- Earthworm, soil and litter collection

Earthworms, soil and plants were collected in August and September 2003 from the Macdonald Research Farm, McGill University, Sainte-Anne-de-Bellevue, Québec, Canada (45 ° 28' N, 73 ° 45' W). We collected *Lumbricus terrestris* L. from an alfalfa (*Medicago sativa* L.) field by pouring a dilute formalin solution (0.5% formalin) on the soil surface, then the earthworms were rinsed well with tap water. Individuals of *Aporrectodea turgida* (Eisen) were collected by hand sorting soil (0-15 cm depth) from a soybean (*Glycine max* (L.) Merrill) field. The earthworms were placed in 37 L plastic containers with field-moist soil and litter, and reared in the laboratory (average temperature = 20°C). We used the same soil and litter for earthworm cultures and the experimental chambers (see below). The soil was a fine-silty, mixed, frigid Typic Endoaquent containing 320 g sand kg^{-1}, 580 g silt kg^{-1}, and 100g clay kg^{-1}, with 29.8 g total C kg^{-1}, and pH = 6.0. The litter included oven dried, ground (<1mm mesh) soybean plants (stems and leaves) collected at pod stage and oven-dry, ground (<1mm mesh) composted cattle manure containing about 20.7 g total N kg^{-1} (Carefoot and Whalen 2003)

- Experimental chambers

The clear plexiglass chambers used in this study allowed easy monitoring of earthworm activities, namely casting and burrowing. For a description of the chambers dimensions and construction, the reader is referred to Whalen *et al.* (2004) and Fig. 1. Briefly, the chambers were prepared by placing a 3 cm piece of cardboard at the bottom for support, and then adding soil to a height of 19 cm from the top of the chambers. The two side panels of the chambers were separated by 3 mm for A. *turgida* and 4.5 mm for *L. terrestris*. The space left from 19 cm to 4 cm from the top was filled with of a mixture of soil and food (6.5g compost and 4.5g ground soybean kg^{-1} soil). The area covered by soil and soil/food mixture in the chambers was 0.14 m^2.The soil and soil/litter mixture were previously moistened to 75% or 85% of field capacity and evenly spread on one plexiglass panel before the chamber was constructed by placing the other panel on top. Then the chambers were incubated in the dark at 20°C for one week before adding the earthworms.

- Surface casting and burrowing activity

Prior to their introduction to the chambers, juveniles of *A. turgida* and *L. terrestris* were placed in plastic jars on damp paper towels to void their guts for 24 hours. The earthworms were then patted dry and weighted (gut free fresh weight). The average mass of *A. turgida* was 0.50 S.E. ±0.007 grams n=40 while *L. terrestris* averaged 1.81 S.E. ±0.03 grams n=40. A single earthworm was added to each chamber, which was then placed in a dark incubator set at one of the four experimental temperatures (5, 10, 15 and 20°C). There were five replicates per treatment. Once in the chambers, the earthworms were left undisturbed for 3 days. Recordings of the surface casting and burrowing activity started on the fourth day, and were made every 24 hours for the next four days. New burrows and surface casts were marked distinctly with colored markers every day. The length of the burrows was calculated by running a string along the newly marked burrows and measuring the length of string. The outlines of surface casts were traced on transparencies, which were then scanned and surface casts area was calculated using the software Winrhizo Pro 5.0a (Regent Instruments Inc., Ste-Foy, Québec, Canada). At the end of the four day monitoring period, the earthworms were taken out of the chambers and weighed after voiding their gut for 24 h. The average final mass of earthworms in this study 0.53 S.E. ±0.015 grams n=40 for *A. turgida* and 1.99 S.E. ±0.037 grams n=38 for *L. terrestris.*

- Statistical analysis

Earthworm cast production data were log transformed (log +1) to equalize variance before analysis. An analysis of variance (ANOVA) was conducted with the PROC GLM function of SAS 6.12 for windows (SAS Institute Inc., Cary, NC). The effects of temperature and moisture on earthworm activity (mass gain, surface casting, burrow length and maximum burrow depth) were evaluated using two-factor ANOVA. Variables that significantly affected earthworm activity ($P<0.05$) were adjusted for multiple comparisons and analyzed using a LSD test at the 95% confidence level. Regression analysis of relationships between temperature and earthworm activity, for each moisture level, was conducted with line-fitting function in Excel XP (Microsoft Corporation, Redmont, WA, USA).

Results and Discussion

Influence of Temperature and Moisture on Earthworm Mass:

We found that juveniles of *A. turgida* tended to lose mass at 5 and 10°C, but gained mass at 15 and 20°C (Fig. 2). The mass gain or mass loss at each temperature was not affected by the soil moisture (Fig. 2). In terms of moisture, these results are similar to those reported by Daugbjerg (1988) for other *Aporrectodea* species. However, the species studied by Daugbjerg (1988) preferred temperatures of 10 and 15°C rather than 15 and 20°C that we observed to stimulate mass gain in *A. turgida*.

Chambers incubated at 5°C with soil moisture at 75% of field capacity was the only treatment where *L. terrestris* was found to lose mass (Fig. 3). Although a small mass gain was observed at 5°C and 85% of field capacity it was statistically equivalent to the treatment at 5°C and 75% of field capacity mass loss. Of all the temperature treatments 15°C was the only one observed were mass gain was significantly influenced by moisture with *L. terrestris* gaining an average of 6.3 times more mass at 85% than 75% of field capacity (Fig. 3). At

85% of field capacity the mass gains observed at all temperatures, with the exception of 5°C, were equivalent (Fig. 3). This tends to show that both moisture and temperature have a significant effect for mass gain in *L. terrestris*. The presence of only few significant mass gain differences in terms of both temperature and moisture is most likely due to the highly resilient nature of *L. terrestris*, as well as the presence of only two moisture treatments both close to the soil moisture conditions preferred by *L. terrestris* (Daugbjerg 1988). This might also partly explain why there appears to be a discrepancy between authors according to the optimal temperature for mass gain in *L. terrestris*. As moisture changes the observed optimal mass gain temperature is shifted. Daniel *et al.* (1996a) found, with a moisture level of 40% w/w, the optimal temperature for *L. terrestris* activity to be between 15-17.5°C while Berry and Jordan (2001) identified 20°C as the optimal temperature for mass gain at 30% humidity.

It is interesting to note that at 75% of field capacity, both species produced longer burrows, but fewer surface casts than at 85% of field capacity. The lowest surface casting found at that moisture level can be considered as a sign of lower food consumption while the longest burrows are a direct sign of a higher level of activity (Shipitalo *et al.* 1988). This combination of low consumption and high activity is thought to be part of the explanation for the lower mass gains found at 75% than 85% of field capacity. Further investigation is needed since subsurface casting was not monitored in the present study. However, a similar experiment performed by Whalen *et al.* (2004) indicated that subsurface casting of *L. terrestris* and *Aporrectodea* species was lower than the surface casting and diminished with time.

Temperature and Moisture Influence on Surface Cast Area, *A. turgida:*

Endogeic earthworm species are not generally recognized to cast on the soil surface, but an increase in surface cast production is often observed in the first few days after earthworms are added to undisturbed soil (Bolton and Phillipson 1976, Scheu 1987, Edwards and Bohlen 1996, Whalen *et al.* 2004).

The surface cast area produced by *A. turgida* was significantly ($p<0.05$) greater at 85% than 75% of field capacity for the temperature range studied (Fig. 4). The largest difference found in casting under the same temperature was at 5°C where the total average amount of casts produced at 85% of field capacity was 51.4 times larger than what was observed at 75% (Fig. 4). The last three temperatures had differences respectively of 4.6, 3.7 and 5.0 times more casts at 85% of field capacity (Fig. 4). The influence of temperature on the surface casting activity of *A. turgida* was only recorded when comparing 5°C to all other temperatures (Fig. 4). Within each moisture treatments, all temperature treatments from 10 to 20°C yielded equivalent surface casts areas (Fig. 4). The surface cast area was significantly influenced by all possible factors i.e. temperature, moisture as well as the interaction between temperature and moisture. Similar findings were found by Whalen *et al.* (2004) on other *Aporrectodea* species and by Daniel *et al.* (1996b) in a field study looking at *Aporrectodea nocturna.*

The temporal variation analysis of surface casting was performed using the data collected at 75% of field capacity. In the last three days of experimentation, after an initial elevated activity level, the surface cast area was observed to decrease in all temperature treatments (Table 1). The 5°C treatment was the only one observed to produce more surface cast in the second half of the experiment than in the first one (Table 1). On the sixth day only, earthworms at 5°C were observed to produce surface casts and by the seventh day surface casting activity had completely stopped in all treatments (Table 1). The higher surface casting activity observed at 5°C can be explained by the production of shorter burrows, keeping the earthworms close to the surface throughout the whole experiment.

Trend lines (Fig. 5) were generated from the results expressed in Fig. 4 in order to obtain equations to predict the surface casting activity within the tested field capacities. The surface cast production in relation to temperature was found to be described by the two following equations: $y = 0.669x + 0.157$ when at 75% of field capacity and $y = 1.5504x + 5.27$ for 85% of field capacity (Fig. 5). The trend lines from which those equations are derived have R^2 values of respectively 0.5622 and 0.5398 (Fig. 5).

Temperature and Moisture Influence on Surface Cast Area, *L. terrestris:*

The surface casting of *L. terrestris* showed the same trend as the one of *A. turgida* with higher surface area found at the 85% of field capacity level, but only under the two highest temperatures (15 and 20°C) (Figs. 6 & 7). At 15°C the total casting area was found to be 3.8 times larger at 85% of field capacity while at 20°C the highest humidity promoted the deposition of 6.4 times more cast (Fig. 6). For the two lowest temperatures, the surface cast areas under both humidity levels were statistically equivalent for each temperature (Fig. 6). The absence of a moisture effect at the lowest temperatures could be explained by the fact that *L. terrestris* was shown to have the ability to tolerate low soil moisture content (Daugbjerg, 1988). *L. terrestris* surface casting was also influenced by all possible experimental factors.

For the temporal analysis of the surface casting behavior the data recorded at 75% field capacity were used. In the last three days of experimentation each temperature treatment generated a different surface casting pattern in *L. terrestris* (Table 1). However, a general trend was found where the lowest and highest temperatures decreased surface casting to almost nil by the seventh day and the two intermediate temperatures yielded slightly higher surface casts area on the seventh day (Table 1). While the surface casting activity at 15°C was highest in the second half of the experiment, there was more surface casts deposited in the first half at 5, 10 and 20°C (Table 1). In *L. terrestris* the difference between the initial and final activity rate was much less pronounced that in *A. turgida*. This difference was also observed by Whalen *et al.* (2004) when comparing *L. terrestris* to other *Aporrectodea* species. The distinct reaction to each temperature, and the temporal reduction in surface casting only at the two extremes, can be explained by the fact that *L. terrestris* might have spent more time at the surface at to 10°C and 15°C, two temperatures known to be in its preference range (Daugbjerg 1988, Daniel *et al.* 1996a).

The data found in Fig. 6 were used to calculate trend lines (Fig. 7). At 75 % of field capacity the surface casting activity of *L. terrestris* can be described by the following equation: $y = -0.3848x + 4.984$. The trend line from which this equation was taken showed an R^2 value of 0.1374. When exposed to 85% of field capacity the increase in activity yields the following equation $y = 5.5392x - 2.716$ with an R^2 equal to 0.9897 (Fig. 7).

The greater surface casting activity found by our experiment at higher moisture level had also been observed by other authors on related lumbricid species (Hindell *et al.* 1994, Daniel *et al.* 1996b). Our results are consistent with Whalen *et al.* (2004) observations that surface casts production is elevated beyond 15°C. However, the high cast production at 10°C observed in both species contradicts the results found by Bolton and Phillipson (1976) and Daniel *et al.* (1996b). The increase in casting activity with higher temperature and moisture might in part be explained by an increase in food consumption as temperature and moisture rise, as demonstrated by Daniel (1991). The physiological state of the earthworms might have also contributed to the low level of surface casting at low temperatures, since they lost mass under those conditions. The monitoring of surface casting activity by surface cast area measurement was used for the first time in this experiment. Therefore, it is difficult to directly compare our results to other published studies.

Temperature and Moisture Influence on Burrow Length, *A. turgida:*

With more burrows produced at 75% of field capacity, burrow length in both *A. turgida* and *L. terrestris* showed the inverse trend than the surface cast area (Figs. 8 & 9). Chambers containing *A. turgida* had burrows that were significantly longer when the earthworms were exposed to 75% of field capacity compared to 85% at both 15 and 20°C (Fig. 8). At 5 and 10°C the burrows lengths were statistically equivalent between the two moisture levels, but distinct in terms of temperatures with longer burrows at 10°C (Fig. 8). At 15 and 20°C the burrows were 1.7 and 2.5 times longer respectively at 75% compare to 85% of field capacity (Fig. 8). At 75% of field capacity the burrows increased in length from 5 to 20°C with each temperature yielding significantly longer burrows (Fig. 8 & 10). At 85% of field capacity only the 5°C treatment was different from the other temperatures (Fig. 8). Our experiment found that the burrowing activity of *A. turgida* was influenced by both temperature and the interaction between temperature and moisture. This observation can once again be explained by the fact that earthworms consume more food as the temperature rises, which also increases their burrowing activity since endogeic species feed by eating their way through the soil (Daniel 1991, Edwards and Bohlen 1996). The general reduction in burrowing activity found at 85% of field capacity could be due to the factors defined in our hypothesis; oxygen deprivation and more difficulty for earthworms to travel through the soil, but further investigation is needed to confirm these assumptions.

Following the initial increase in burrowing activity after their introduction, the earthworms in all temperatures at 75 % of field capacity showed an increase in activity from day 5 to day 6 (Figs. 11, Table 2). In the last 24 hours from day 6 to 7 the burrowing activity decreased in all temperature treatments with the only exception of an increase found at 20°C (Table 2). When exposed to any of the experimental temperatures we observed that most of the burrowing activity had been performed in the first half of the study. Bolton and Phillipson (1976) as well as Scheu (1987) had already observed this phenomenon following the introduction of earthworms into a new environment.

In Fig. 10, trend lines derived from Fig. 8 show the relationship between burrowing and temperature in *A. turgida*. When submitted to 75% of field capacity, the surface casting activity of *A. turgida* was as follow $y = 24.43x - 20.85$ with an R^2 value of 0.9665. When found in a soil at 85% of field capacity, the earthworms responded in a way described by the following equation $y = 6.87x + 7.16$ with an R^2 of 0.9129 (Fig. 10).

Temperature and Moisture Influence on Burrow Length, *L. terrestris:*

For *L. terrestris*, the burrow length was always significantly longer under every temperature in the 75% field capacity treatment (Fig. 9). Burrow length also increased with increasing temperatures with the longest burrows found at 20°C (Figs. 9 & 11). At 85% of field capacity only the burrows produced at 5°C were significantly different from the other observations (Fig. 9). At 5°C the burrows at 75% of field capacity were 13.9 times longer that what was found at 85%. All burrows at 10°C, 15°C and 20°C were also found to be longer (2.3 to 3.3 times longer) at 75% compared to 85% of field capacity (Fig. 9). The length of the burrows produced by *L. terrestris* was influenced by both temperature and moisture, but not by their interaction. Although *L. terrestris* is known to feed at the soil surface, the litter in our experiment was mixed into the soil. This characteristic of our experiment could in part explain the highest burrowing found with higher temperatures since food consumption is known to increase with temperature and moisture levels in *L. terrestris*, (Daniel 1991). However, the inverse trend was observed for moisture levels where shorter burrows were found at higher moisture. In this case, our hypothesis of an increased difficulty to travel through the soil and a reduction in available oxygen at higher humidity could explain this observation.

Under 75% of field capacity and the three highest temperature treatments most of the burrowing activity was performed in the first half of the experimentation period (Table 2). In the last tree days, the burrowing activity observed at those temperatures was only a fraction of the initial activity and stayed low and somewhat constant throughout the last tree days (Table 2). At 5°C more burrowing activity was observed in the second half of the seven days of observation (Table 2). However, this activity sharply declined and became similar to the other temperature results by the last day of observation (Table 2). Since *L. terrestris* builds semi-permanent burrows, its burrowing activity declines once its burrow system is created as it was observed in our experiment (Edwards and Bohlen 1996). The delayed reduction in burrowing activity at 5°C is most likely due to a longer acclimation period and/or a reduced capacity to perform burrowing at low temperature (Table 2).

From this experiment we found that the burrowing activity of *L. terrestris* when placed in a soil at 75% of field capacity is in relation to the equation: y = 32.418x - 16.27 with an R^2 value of 0.9643 (Fig. 11). The reduced burrowing activity observed at 85% of field capacity was found to be determined by: y = 11.986x - 9.1 R^2 = 0.9759 (Fig. 11).

Temperature and Moisture Influence on Maximum Burrow Depth:

In *A. turgida* it is only at 15°C that the difference between the two soil moisture treatments had a significant influence on burrow depth (Fig.12). Within each moisture treatment, all maximum depth measured in the three highest temperatures were found to be statistically equivalent to one another. At 75% of field capacity, the depth reached at 20 and 15°C was equivalent to what was found for 10°C, but significantly different from the one at 5°C. In the lowest humidity, the average depth at which the earthworms burrowed at 5°C was equivalent only to the one measured at 10°C at the same moisture, but similar to all depths of all temperatures recorded at 85% of field capacity. At the highest moisture treatment, the depths reached at 5 and 20°C were statistically distinct, but both were equivalent to the depths found at 10 and 15°C. It was therefore observed that the depth reached by earthworm burrows increases as temperature is increased, but that moisture only had a limited influence for one temperature. Statistically, *A. turgida* burrow depth was significantly influenced by temperature and moisture independently. The maximum depth observed in our experiment (0.22m) was also found to be preferred by another endogeic species, *Aporrectodea rosea,* in a field experiment with environmental conditions similar to the one we created in the laboratory (Lavelle 1997).

L. terrestris burrow depth showed no significant difference for both soil moisture treatments at 10, 15 and 20 °C (Fig.13). Only at 5°C were the two observations significantly different. At this temperature, while the measured depth at 85% of field capacity showed no relation to any other measurements, the one at 75% of field capacity was equivalent to the depth reached at both humidity levels at 10°C. All temperatures from 10 to 20°C were observed to produce burrows of statistically similar depths. Consequently, burrow depth was once again higher at higher temperature and the influence of moisture limited to one temperature treatment. Statistically, the depth of the burrows made by *L. terrestris* was influenced by all factors; temperature, moisture, and temperature moisture interaction.

Conclusion

In all recorded observations the highest temperatures were found to yield the highest results. We can therefore conclude that earthworm activity increases as temperature increases. The effect of moisture was more variable and had, depending on the activity (casting or burrowing), a positive or a negative influence.

When considered in the context of the present experiment, we believe the formulated hypotheses can explain the reactions observed in *L. terrestris and A. turgida* behaviors. Further experimentation should be performed with the same design with higher temperatures since no hyperthermia was noted in the present experiment leaving us unaware of the maximum temperature until which the earthworms would increase their activity. Moisture levels above field capacity should also be investigated to have a complete picture of the effect of moisture. Since the thin design of the experimental chambers used in this experiment could influence the diffusion of oxygen and the compaction of the soil, the field moisture value that would trigger the hypothesized reactions might be significantly different from the one tested. Therefore an extension of this experiment to the field would be of interest.

The biomass variations of the earthworms were recorded only as an indication of the physiological states of the individuals in order to interpret the observed differences in activity levels found under each experimental treatment.

It is important to keep in mind that the temperatures of preference, fastest growth, and highest activity are not necessarily related to one another due to the confounding effects of different moisture conditions, soil types, food sources and other factors in laboratory experiments (Edwards and Bohlen 1996, Berry and Jordan 2001). In order to avoid this problem and allow for easier comparison between studies, a standard protocol for the measurement of earthworm activity should be developed.

A standard procedure for the measurement of soil moisture would also be of great convenience for the study of the earthworm activity. In order to better understand earthworm behavior, this procedure should as much as possible reflect the ecological characteristics of the soil. The authors would like to recommend field capacity as the standard technique for expressing soil moisture in studies of soil organisms.

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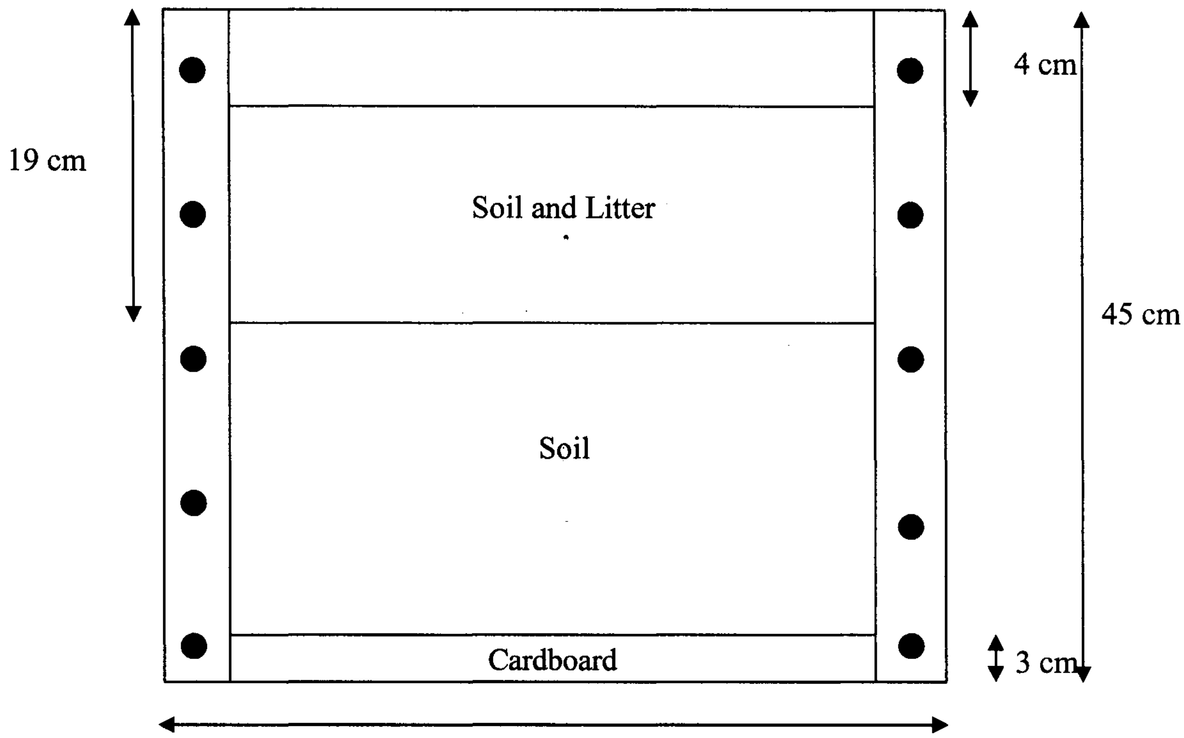

Figure 1: Plexiglass observation chamber design.

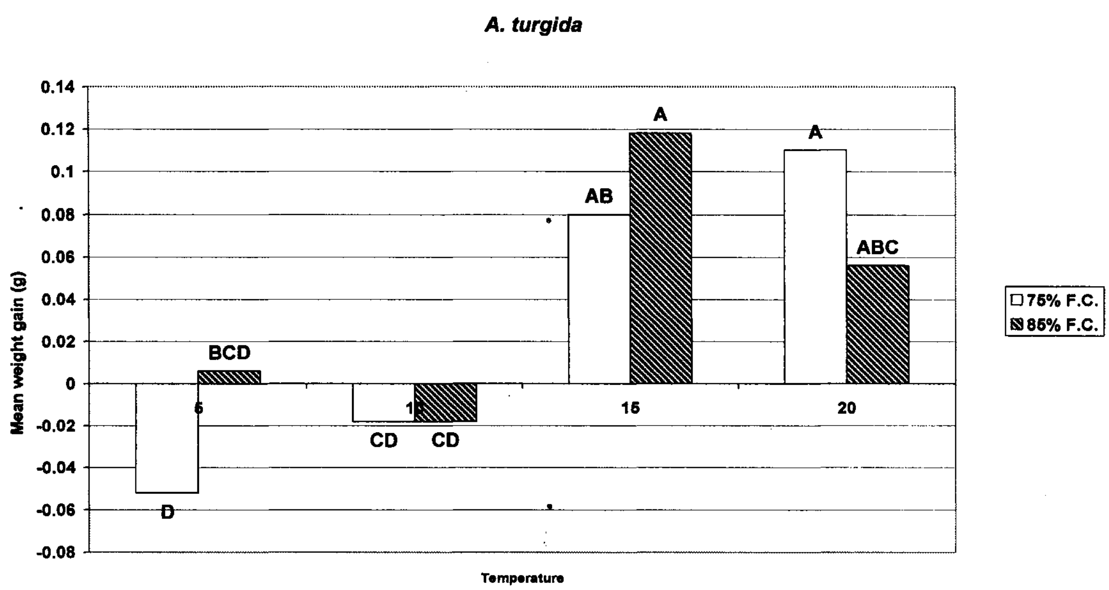

Figure 2: Mean mass gain or loss (g, fresh weight) of *Aporrectodea turgida* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other. F.C. Field Capacity

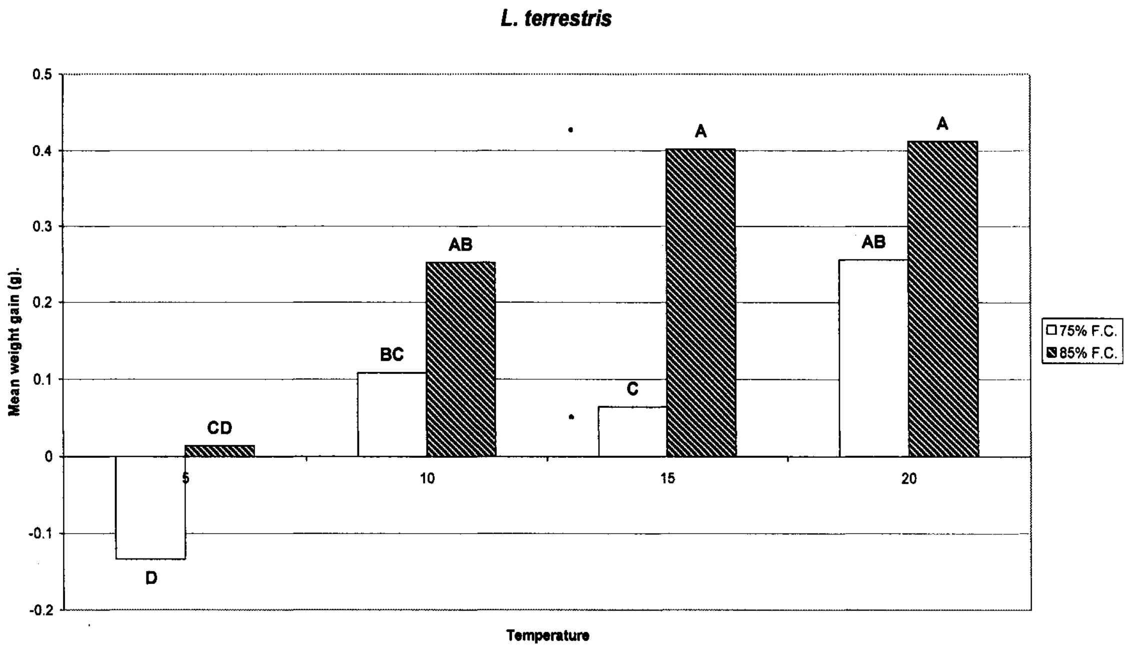

Figure 3: Mean mass gain or loss (g, fresh weight) of *Lumbricus terrestris* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different ($P<0.05$ LSD test) from each other.

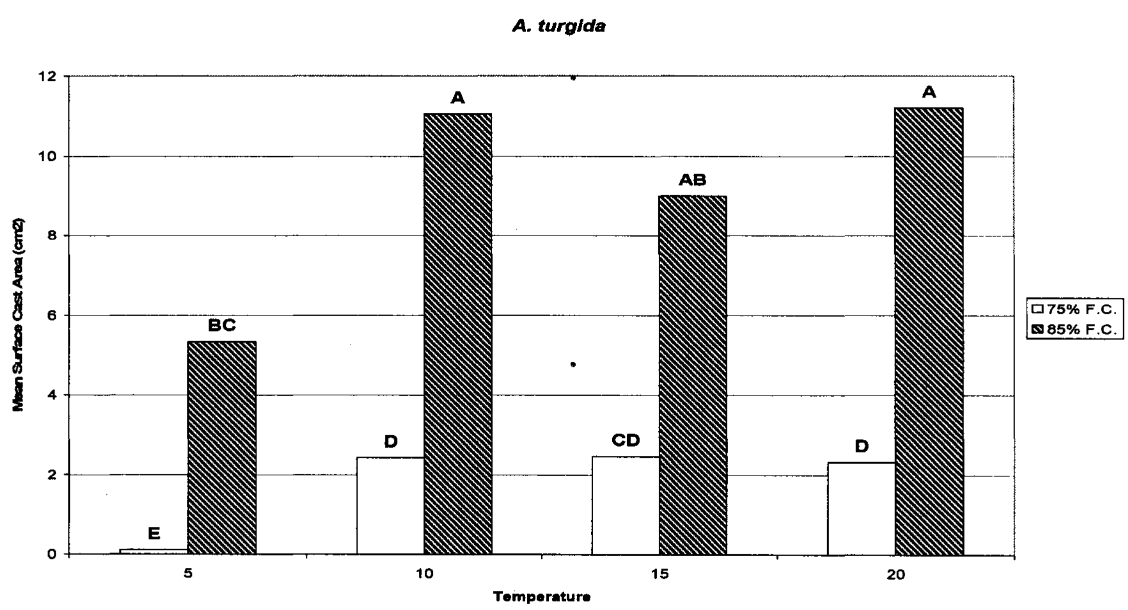

Figure 4: Mean total surface cast area (cm^2) of *Aporrectodea turgida* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different ($P<0.05$ LSD test) from each other.

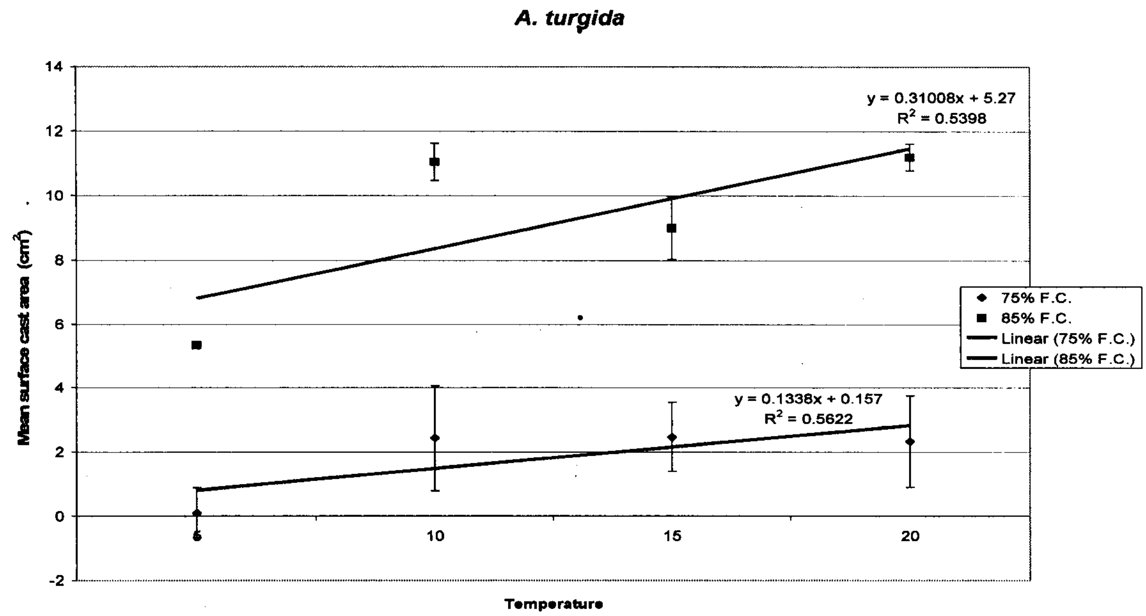

Figure 5: Relationship between soil temperature and the mean surface cast area produced by *A. turgida* at 75% and 85% of field capacity. Trend lines were fitted through points (± standard errors, n=5) using linear regression analysis.

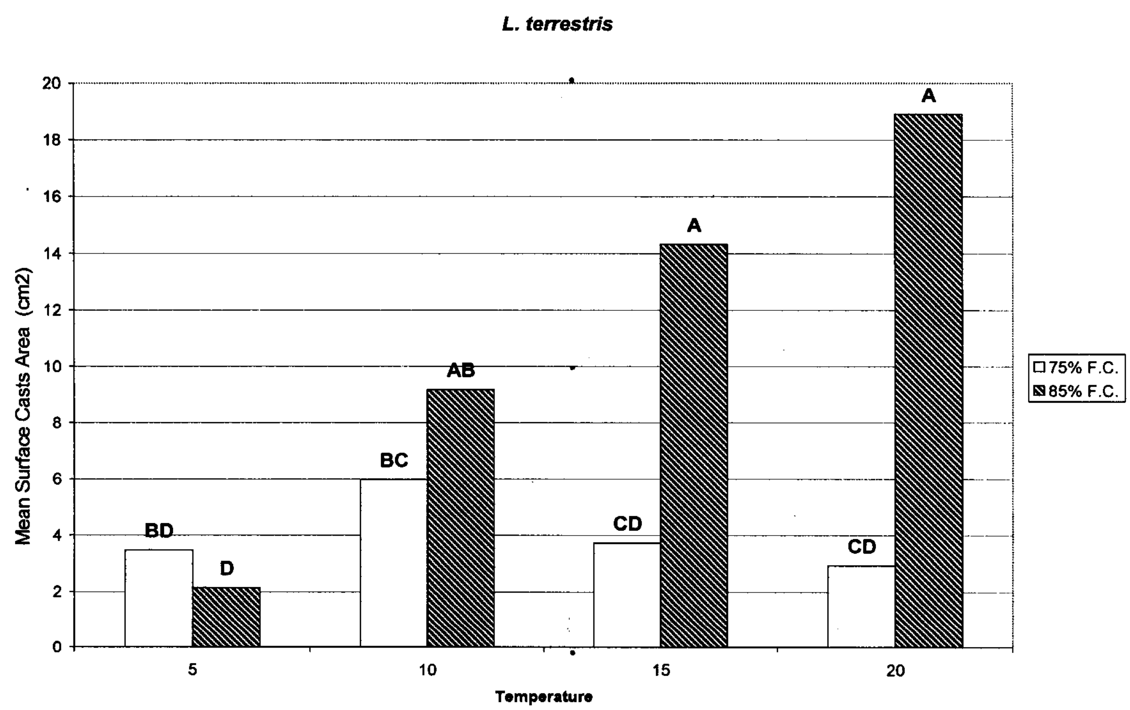

Figure 6: Mean total surface cast area (cm^2) of *Lumbricus terrestris* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other.

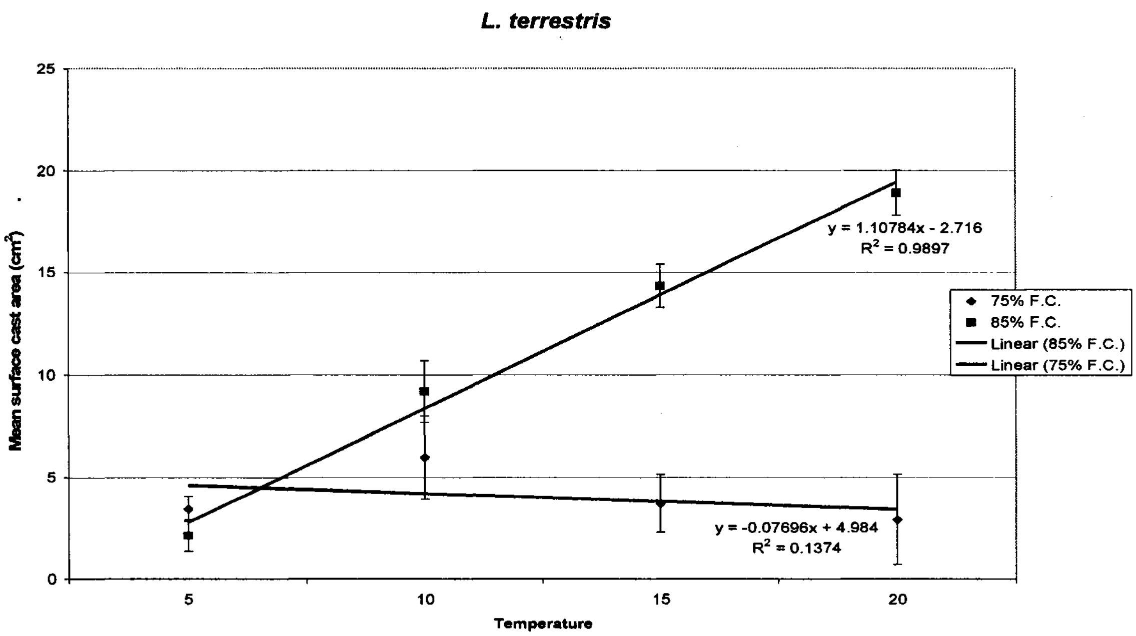

Figure 7: Relationship between soil temperature and the mean surface cast area produced by *Lumbricus terrestris* at 75% and 85% of field capacity. Trend lines were fitted through points (± standard errors, n=5) using linear regression analysis.

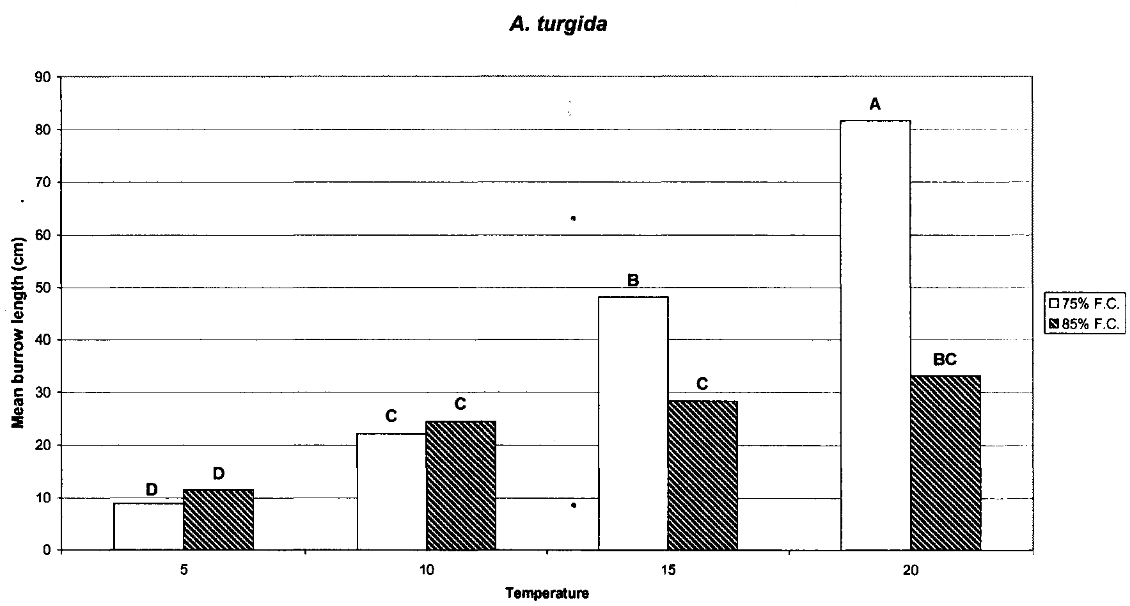

Figure 8: Mean total burrow length (cm) of *Aporrectodea turgida* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different ($P<0.05$ LSD test) from each other.

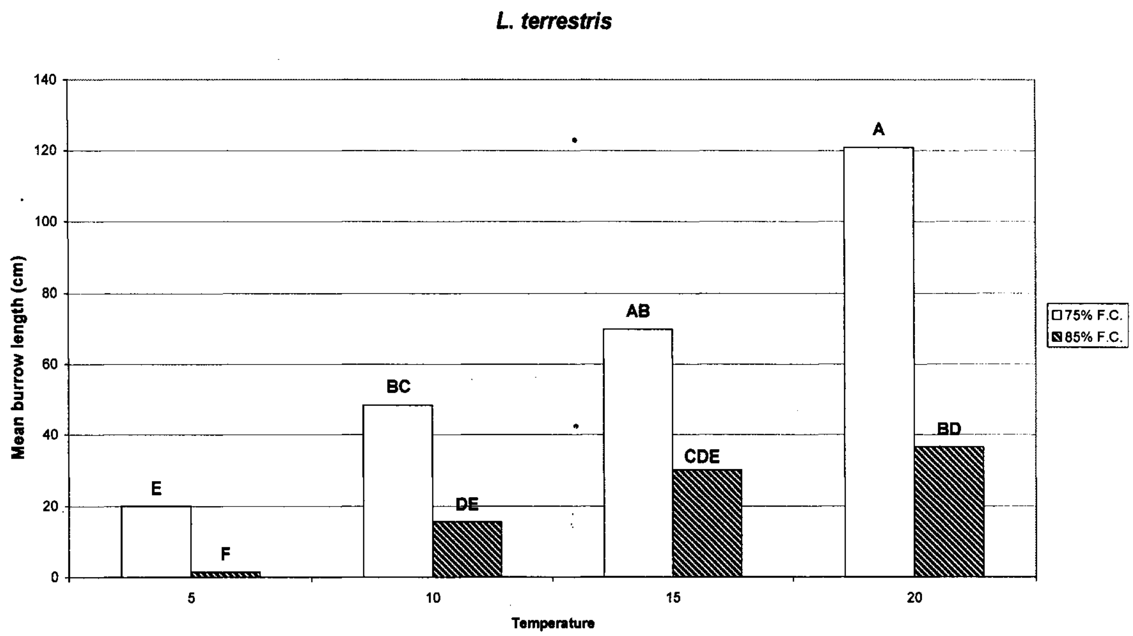

Figure 9: Mean total burrow length (cm) of *Lumbricus terrestris* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other.

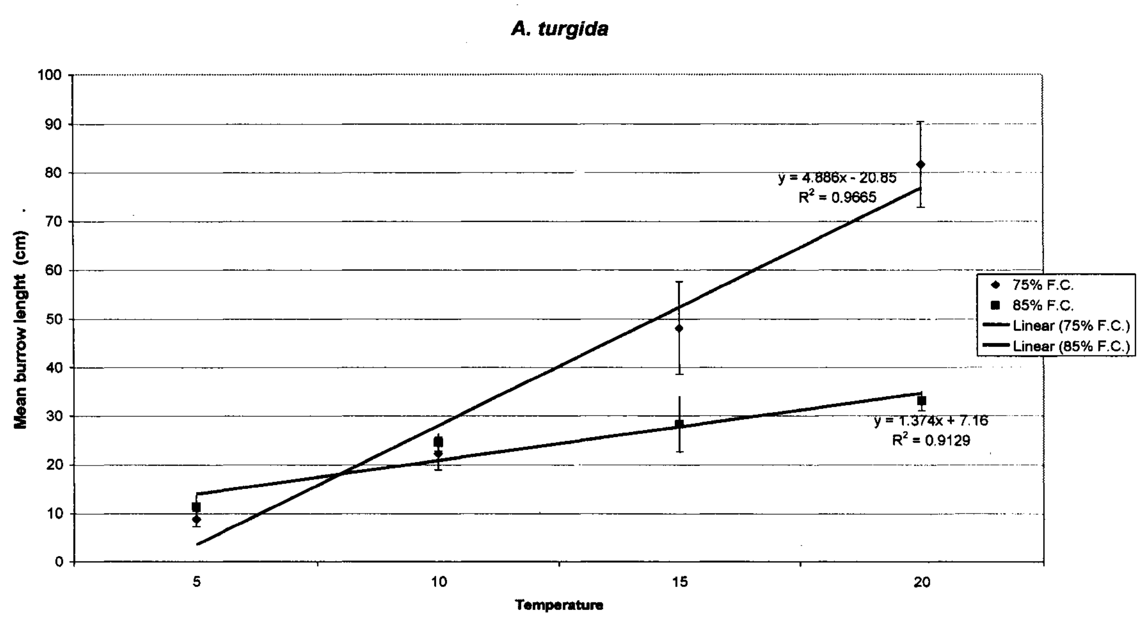

Figure 10: Relationship between soil temperature and the mean total burrow length produced by *A. turgida* at 75% and 85% of field capacity. Trend lines were fitted through points (± standard errors, n=5) using linear regression analysis.

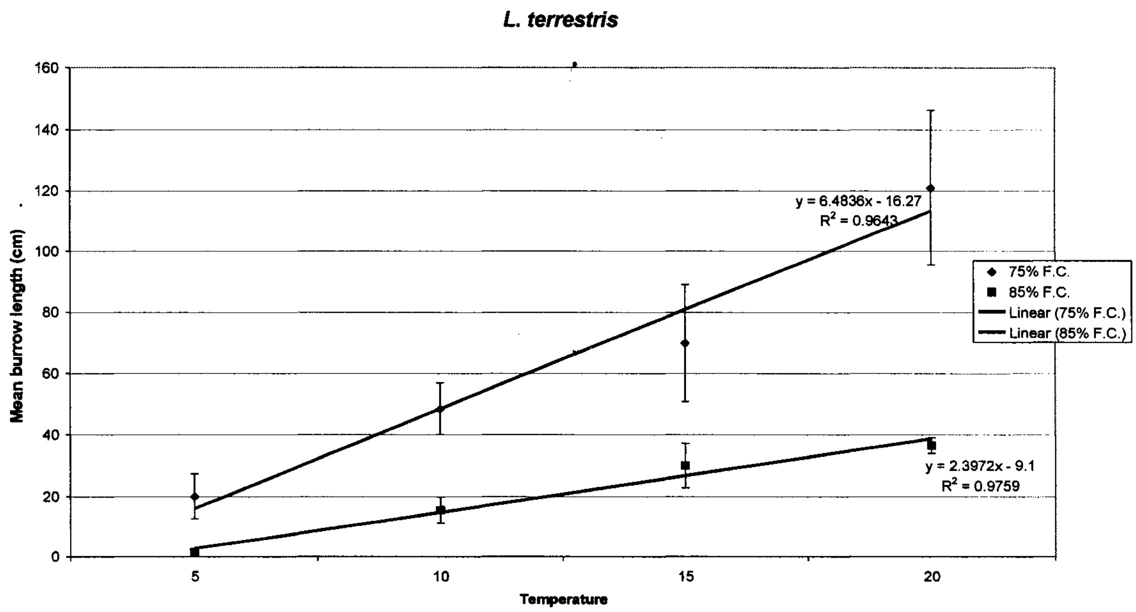

Figure 11: Relationship between soil temperature and the mean total burrow length produced by *Lumbricus terrestris* at 75% and 85% of field capacity. Trend lines were fitted through points (± standard errors, n=5) using linear regression analysis.

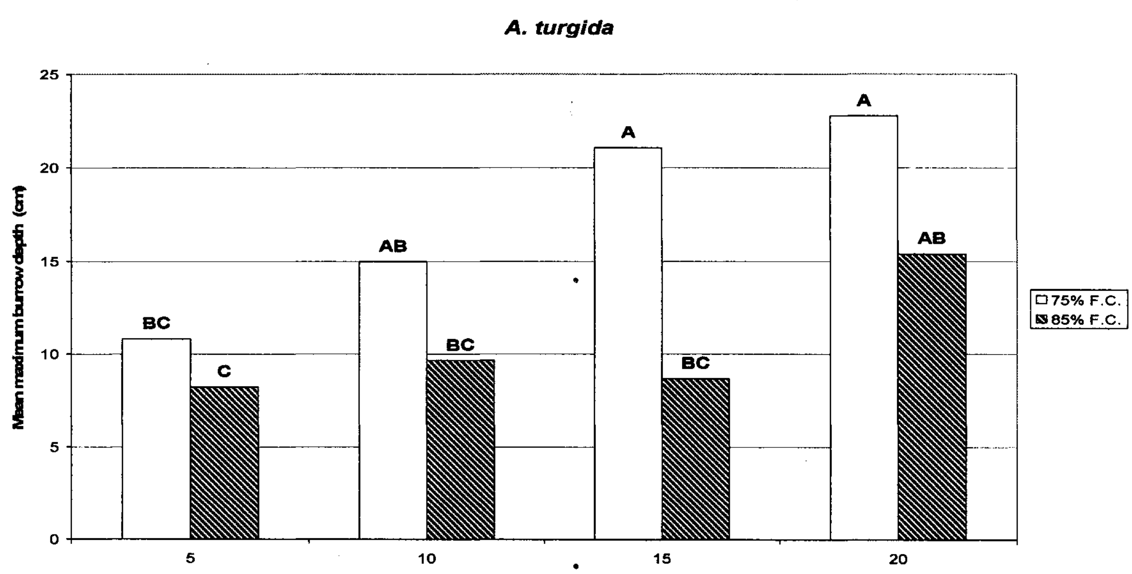

Figure 12: Mean maximum burrow depth (cm) of *Aporrectodea turgida* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different ($P<0.05$ LSD test) from each other.

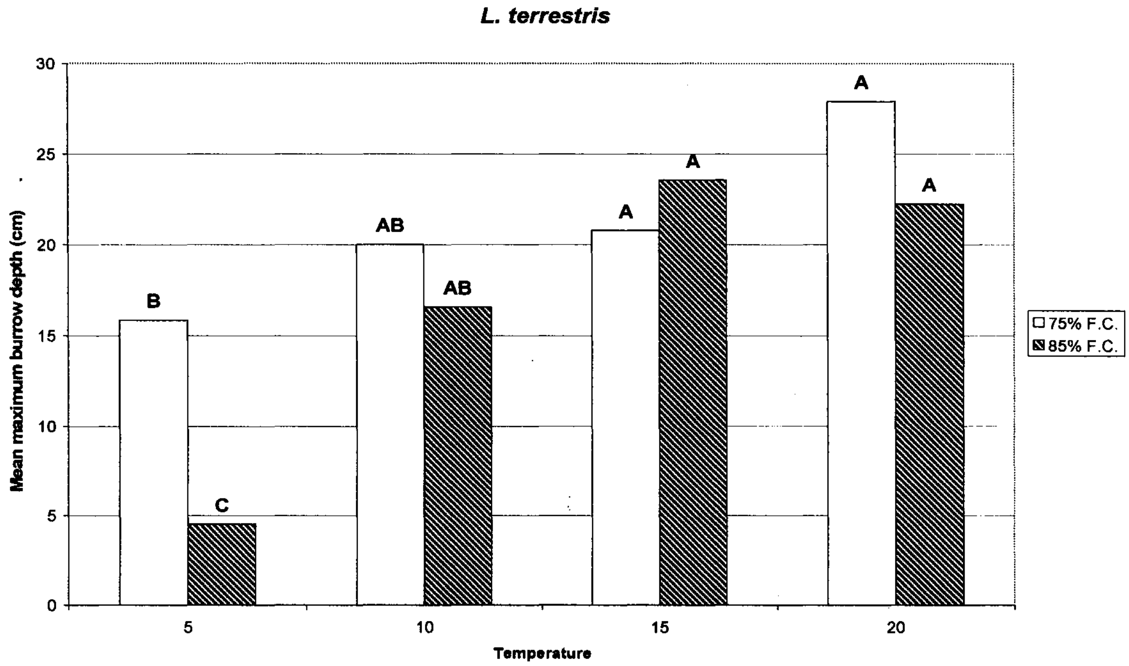

Figure 13: Mean maximum burrow depth (cm) of *Lumbricus terrestris* (n=5) for all temperature and moisture treatments. Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other.

Table 1: Percentage of surface cast area produced on days 5, 6 and 7 of the experiment, relative to the first four days of casting. Casting was measured at 75% of field capacity (n=5) for each temperature and species.

Species	Temperature	% Surface cast area (cm^2)		
		Day 5	Day 6	Day 7
A. turgida	5	72	36	0
	10	4.8	0	0
	15	3.2	0	0
	20	0	0	0
L. terrestris	5	32.9	40.5	4.1
	10	13.6	13.0	15.3
	15	41.7	27.4	44.2
	20	40.6	29.4	0

Table 2: Percentage of burrow length produced on days 5, 6 and 7 of the experiment, relative to the first four days of burrowing. Burrowing was measured at 75% of field capacity (n=5) for each temperature and species.

Species	Temperature	Days		
		Day 5	Day 6	Day 7
A. turgida	5	5.15	11.92	3.25
	10	15.20	22.59	20.03
	15	24.51	26.39	16.18
	20	17.29	26.67	31.70
L. terrestris	5	59.31	54.25	15.86
	10	9.92	17.99	11.71
	15	10.83	11.37	1.85
	20	18.17	6.98	11.41

Connecting Paragraph:

The previous chapter demonstrated the influence of temperature and moisture on earthworm activity, i.e. mass gain, casting, and burrowing. In the next chapter, the influence of food sources on earthworm mass gain and casting activity will be investigated. A special interest was given to genetically modified food sources, and how they might influence earthworm growth and casting activities.

Food Influence on Casting Activity and Cast Nutrient Content with a Focus on Genetically Modified Plants.

Introduction

Casts are well known to modify soil structure and formation. Specifically, earthworm casting activity is known to lead to soil paedogenesis when casts are deposited below the surface, while surface casts transform the soil profile development and structure (Lee 1985). However, casting activity and cast characteristics vary greatly between different species and among different soil types (Schrader and Zhang 1996, Whalen *et al.* 2004). Casting qualities are also directly influenced by food sources (Shipitalo *et al.* 1988, Flegel et al 1998, Flegel and Schrader 2000). Earthworm casts are physically more stable than soil aggregates and they also have a higher tensile strength than natural soil aggregates, making them more resistant to compaction (Lee 1985, McKenzie and Dexter 1987, Schrader and Zhang 1996, Binet and Le Bayon 1998). This improved stability is due to the high organic carbon content found in the cast which is directly related to the C/N ratio of the earthworm's food sources (Guggenberger *et al.* 1996, Flegel *et al.* 1998, Buck *et al.* 1999). However, their water stability is less than that of soil aggregates (Schrader and Zhang 1996). Chemically, casts are also significantly different than the soil in which they are produced (Sharpley and Syers 1976, Lawton 1994). The organic carbon content of casts is higher than the one found in bulk soil (McKenzie and Dexter 1987, Schrader and Zhang 1996) and casts also contain a higher concentration of plant available nutrients such as N, P K and Mg. (Tomati *et al.* 1994, Chaoui *et al.* 2003). These differences are directly linked to the earthworm's trophic resources content of those nutrients as well as their transformation to available forms by enzymatic activity (Basker *et al.* 1992, Buck *et al.* 1999). Those physical and chemical characteristics of casts improve soil quality and stability and have had earthworms to be used in soil rehabilitation (McKenzie and Dexter 1987, Scullion and Ramshaw 1988, Schrader and Zhang 1997).

The number of hectares farmed with genetically modified organisms, mostly Bt corn, Roundup Ready soybean and Roundup Ready canola, has increased 40 fold since 1996 reaching a global area of 67.7 million hectares in 2003. However, virtually no research has been performed on the impact of those genetically modified organisms on earthworms (Biotech Knowledge Center, 2004). So far, only three studies are known to have looked at the possible impact of a genetically modified plant, Bt corn, on earthworms, two short term laboratory experiments (Saxena and Stotzky 2001) and one long term laboratory and field experiment (Zwhalen *et al.* 2003). Both short term studies did not discover any toxin related impacts while the long term experiment found a significant mass loss for earthworms being fed Bt corn litter in the field and a small mass loss for the same treatment in the laboratory (Saxena and Stotzky 2001, Zwhalen *et al.* 2003).

Knowing that earthworms are influenced by the nature of their food, this experiment was designed with the objective to investigate the influence of genetically modified plants on cast chemical properties and casting activity in *Lumbricus terrestris* L. and *Aporrectodea turgida* (Eisen) by comparing soybean (*Glycine max* L.) and corn (*Zea mays* L.) to their genetically modified equivalent, Roundup Ready soybean and Bt corn (Shipitalo *et al.* 1988, Flegel et al 1998, Flegel and Schrader 2000). Cast production and food consumption rate are strongly influenced by food sources (Flegel *et al.* 1998). The preference of food sources is also observed in earthworms and casting activity is known to increase with higher food consumption rates (Shipitalo *et al.* 1988, Flegel *et al.* 1998).

Since the traits added to corn and soybean by the Bt and Roundup Ready genetic manipulations are not designed to target earthworms, and because only long term effects have been reported in previous studies, our hypothesis is that no differences in the casting activity or casts characteristics of both *Lumbricus terrestris* and *Aporrectodea turgida* would be observed when comparing genetically modified to non-genetically modified corn and soybean.

Materials and Methods

- Earthworm, soil and litter collection

Earthworms, soil and plants were collected in August and September 2003 from the Macdonald Research Farm, McGill University, Sainte-Anne-de-Bellevue, Québec, Canada (45 ° 28' N, 73 ° 45' W). We collected *Lumbricus terrestris* from an alfalfa (*Medicago sativa* L.) field by pouring a dilute formalin solution (0.5% formalin) on the soil surface, then rinsed the earthworms well with tap water. Individuals of *Aporrectodea turgida* were collected by hand sorting soil (0-15 cm depth) from a soybean (*Glycine max* (L.) Merrill) field. The earthworms were then placed in 37 L plastic containers with field-moist soil and litter, and reared in the laboratory (average temperature = 20°C). We used the same soil and litter for earthworm cultures and the experimental chambers. The soil was a fine-silty, mixed, frigid Typic Endoaquent containing 320 g sand kg^{-1}, 580 g silt kg^{-1}, and 100g clay kg^{-1}, with 29.8 g total C kg^{-1}, and pH = 6.0. The litter included oven dried, ground (< 1mm mesh) soybean plants (stems and leaves) collected at pod stage and oven-dry, ground (< 1mm mesh) composted cattle manure containing about 20.7 g total N kg^{-1} (Carefoot and Whalen 2003)

- Food sources production and preparation:

Soybean (OAC Champion, BELCAN), corn (Pioneer 38A24), Roundup Ready soybean and Bt Corn (Mycogen 2K350) were all grown in nine inches diameter pot in a greenhouse. Soil was amended with N, P and K fertilizers as required for plant growth to avoid nutrient deficiencies. Corn and Bt corn were both harvested at the onset of the tasseling stage. Soybean and Roundup Ready soybean were collected at the pod stage before pod filling started. All plants were oven dried at 60°C for 48 hours before being finely ground (<0.5 mm). Characteristics of the plant litter are described in Table 1.

- Experimental design:

To test the influence of the different food sources the earthworms were placed in 500 ml plastic containers filled with 200g (dry weight) of 0.5 mm sieved soil remoistened to 85% of field capacity and mixed with 4.75g kg^{-1} of plant litter from each treatment. An additional 0.2 grams of plant litter was also placed on the soil surface. Six treatments per earthworm species were applied with 5 replicates each. The treatments included:

-A soil control where no earthworm or litter were introduced.

-A worm control where earthworms were added but not litter.

-Corn litter

-Bt corn litter

-Soybean litter

-Roundup Ready soybean litter

Prior to their introduction to the containers, juveniles of *A. turgida* and *L. terrestris* were placed on damp paper towels to void their guts for 24 hours. The earthworms were then patted dry and weighted (gut free fresh weight). *A. turgida* average mass was of 0.41 S.E. ±0.01 grams n=25 while *L. terrestris* averaged 1.25 S.E. ±0.03 grams n=25. A single earthworm was added to each container. Once the earthworms were introduced, the containers were placed in a 20°C Conviron incubator at a constant air humidity of 80% in total darkness for 7 days. The average mass of *A. turgida* remaining at the end of the incubation period was 0.47 S.E. ±0.02 grams n=16, while the *L. terrestris* remaining weighed 1.47 S.E. ±0.06 grams n=15.

- Casts collection and analysis:

At the end of the experiment all surface casts were hand picked and oven dried (60°C for 48 hours). The subsurface casts were collected by oven drying the soil (60°C for 48 hours) and sieving it through stacked sieves of 2mm, 1mm, and 0.5mm and collecting the casts left on the sieves. Before being stored in

Petri dishes, all surface and subsurface casts were weighted to obtain the total mass of cast per container. Those totals were later used to calculate the mass of cast deposited per grams of initial earthworm gut free fresh weight (iw).

The total carbon and nitrogen content in casts and bulk soil were analyzed using a NC soil analyzer (Flash EA 1112 Series, Thermo-Finnigan Carlo Erba). Plant available P and K in earthworm casts and bulk soil were measured in Melich-3 extracts (1:10 soil: extractant). The Melich-3 P concentration was determined by the molybdenum blue method (Murphy and Riley, 1962) on an autoanalyzer (Lachat Instruments, Milwaukee, WI) while the Melich-3 K concentration was determined by atomic absorption spectrometry (Perkin-Elmer, Boston, MA).

- Statistical analysis

The effects of food sources on earthworm activity (mass gain, surface and subsurface casting) as well as cast nutrient content (total C, total N, Melich-3 P and Melich-3 K) were evaluated using one-factor ANOVA. The analysis of variance (ANOVA) was conducted with the PROC GLM function of SAS 6.12 for windows (SAS Institute Inc., Cary, NC). Variables that significantly affected earthworm activity ($P<0.05$) were adjusted for multiple comparisons and analyzed using a LSD test at the 95% confidence level.

Results and Discussion

Mortality and Mass Gain:

There was significant mortality in some treatments during this study, although earthworms were placed in a controlled environment that should have been favorable for their survival (Table 2). However, casting activity did not appear to be significantly influenced by mortality since most mortalities occurred on day 6 or 7 of the experiment (Figs. 1 & 2). Earthworms with only the cephalic half of their body still alive were considered as mortality. With the exception of the worm control treatment, the mortalities observed in this experiment are not thought to be treatment specific. The earthworms' pre experimental physiological state induced by incubation conditions and pre weighting starvation or standard experimental conditions such as temperature are probably to blame. The non-lethality of Bt corn and the B.t. toxin on earthworms had already been demonstrated with B.t. corn litter as well as B.t. formulations (Addison and Holmes 1995, Saxena and Stotzky 2001, Zwhalen *et al.* 2003). No data on earthworms fed Roundup Ready soy are known to exist but it appeared that there was no negative effect of this litter on earthworm survival.

In both species, mass loss was only observed in the worm control treatment (Table 2). The mass gains of *A. turgida* in all treatments were found to be equivalent (Table 2). In *L. terrestris* all food treatments but corn were found to produce similar mass gains. The corn treatment was influenced by mortality since the only earthworm that survived did not change mass (Table 2).

Casting Activity:

The amount of casts deposited on the soil surface by *A. turgida* ranged from 7.97 g g^{-1}iw to 11.79 g g^{-1}iw. The subsurface casting activity ranged from 22.48 g g^{-1}iw to 26.46 g g^{-1}iw (Fig. 1). For this species, both surface and subsurface casting activities were not influenced by the food treatments since all treatments, including the worm control, yielded statistically similar total amounts of casts (data not shown). However, significantly more subsurface than surface casting activity took place under all treatments (Fig. 1). The absence of food preferences, the geophagous feeding strategy and the endogeic nature of *A. turgida* explain these observations (Bouché 1972, Edwards 2004).

L. terrestris surface casting activity ranged from 1.28 g g^{-1}iw to 6.66 g g^{-1} iw. Its lowest subsurface casting activity was of 4.34 g g^{-1} iw and the maximum of 8.98 g g^{-1} iw (Fig. 2). The surface casting activity of *L. terrestris* showed equivalent amounts of casts to have been egested in the soybean, Roundup

Ready soybean and Bt corn treatments. Corn yielded a lower amount of surface cast equivalent to what was observed in Roundup Ready soybean as well as the control. In the subsurface area, all four food treatments were observed to yield similar quantities of casts. All treatments but Roundup Ready soybean were statistically different from the worm control measurements. Unlike *A. turgida,* all treatments except corn had equivalent amounts of casts deposited on the surface than within the soil (Fig. 2). More subsurface than surface casts were deposited in the pots receiving corn litter. The absence of differences in the total casting activity (data not shown) indicates that *L. terrestris* also expressed no food preferences (Shipitalo *et al.* 1988). The production of less cast per gram of initial body mass, when compared to *A. turgida*, and the absence of difference between the amounts of surface and subsurface casts can be explained by the detrivorous feeding habit as well as the anecic nature of *L. terrestris* (Buck *et al.* 1999, Whalen *et al.* 2004).

Nutrient Content:

Available Phosphorus

Many studies have demonstrated that earthworms increase the availability of phosphorus and that their egesta contain a higher concentration of available phosphorus than soil (Satchell and Martin 1984, Scheu 1987, Tiwari 1989, Basker *et al.* 1992, Hendrix 1995). It has been established that the activity of phosphatases, both from the earthworms` gut as well as from microbes, are responsible for this enhanced phosphorus concentration in earthworm casts (Satchell and Martin 1984).

In *A. turgida* the surface casts of both the soybean and Roundup Ready soybean treatments showed a higher concentration of available phosphorus than the soil control (Table 3). The subsurface casts found under the soybean treatment had an equivalent amount of phosphorus than the surface ones and were also different from the soil control. In contrast, the Roundup Ready soybean treatment yielded subsurface casts with a significantly lower amount of available phosphorus than the surface casts. Those subsurface casts were equivalent to the soil control in their phosphorus concentration.

In *L. terrestris* (Table 4) the surface and subsurface casts of each soybean treatments had equivalent available phosphorus concentrations within their respective treatment. Soybean surface cast phosphorus content was the only one found to be significantly different from the soil control.

For both corn and Bt corn, the only casts produced by *A. turgida* (Table 3) found to have an amount of available phosphorus higher than the soil control were the surface casts of the corn treatment. The subsurface casts of the corn treatment had a significantly lower amount of phosphorus than the surface one, but were equivalent to both surface and subsurface Bt corn casts.

All casts of *L. terrestris* (Table 4) of the corn and Bt corn treatments were found to have an equivalent amount of available phosphorus than the soil control. All casts from all treatments and positions were equivalent to each other in their phosphorus content except for the Bt corn subsurface casts when compared to the corn surface casts.

Like many of the food treatments tested, both *A. turgida* and *L. terrestris* worm control did not produce higher amounts of available phosphorus than the soil control treatments. This might indicate that the experimental conditions were not adequate for the study of phosphorus since the availability of this nutrient is well known to be increased in earthworm casts (Satchell and Martin 1984, Scheu 1987, Tiwari, 1989, Basker *et al.* 1992, Hendrix 1995).

Available Potassium

Potassium is another nutrient which earthworms are known to increase the availability in their casts (Tiwari *et. al.* 1989, Basker *et al.* 1992). Like phosphorus, this higher availability of potassium in earthworms' casts is caused by microbial and enzymatic activity (Basker *et al.* 1992). Earthworms modify the equilibrium between the different forms of potassium by increasing the quantity of the more available forms (Basker *et al.* 1992).

All casts deposited by *A. turgida* in the soybean and Roundup Ready soybean treatments had significantly higher levels of available potassium than the soil control (Table 3). The surface and subsurface cast potassium content of the soybean treatment were statistically similar to one another as well as to both surface and subsurface casts of the Roundup Ready soybean treatment. The surface and subsurface casts of Roundup Ready soybean were however dissimilar, with the subsurface casts having significantly less available potassium.

L. terrestris, under all treatments, also produced casts with a higher concentration of available potassium than the soil control (Table 4). In the soybean treatment a significant difference was found between the surface and subsurface casts potassium content where less potassium was present in the subsurface casts. The Roundup Ready soybean treatment did not produce any such difference between the surface and subsurface casts.

In both corn and Bt corn treatments all casts of *A. turgida were* found to hold more available potassium than the soil control (Table 3). Significantly more potassium was found in the surface compared to the subsurface casts in both treatments. The potassium content of the subsurface casts of the corn treatment is statistically similar to both observations of the Bt corn treatment. The surface casts egested in the corn treatment had the highest content of available potassium and were not found to be similar to any other treatment.

All *L. terrestris* casts under both corn and Bt corn treatments were also found to contain more potassium than the soil control (Table 4). In the corn treatment the surface and subsurface casts were statistically equivalent where in Bt corn the subsurface casts had an inferior concentration of available potassium when compared to the surface casts of the same treatment. There is in fact no difference between those two treatments since the surface casts in both treatments share a similar amount of potassium and the same trend is found in the subsurface casts.

The general similarity in the results observed for casts available potassium content shows that there was no distinct treatment effect. It is therefore concluded that genetically modified Bt corn has no impact on the quantity of available potassium found in earthworm surface and subsurface casts.

The worm control of both species produced casts that had available potassium content similar only to the soil control treatment. Consequently we can infer that in the present experiment the available potassium was derived from the food sources of each treatment. The increased amount of available potassium found in all food treatment is in accordance with the results of Basker *et al.* (1992) and Tiwari *et al.* (1989). When looking at the comparison of soybean and genetically modified soybean and corn and genetically modified corn there is no evidence that the genetically modified plants had either a positive or negative impact on the amounts of available potassium found in earthworm casts.

Total Carbon

Many authors have observed that earthworm casts contain a different quantity and form ratio of carbon than soil (Lee 1985, Hendrix 1995). The higher organic carbon content of casts has been linked with the selective feeding behaviour of earthworms on organically rich material (Tiwari *et al.* 1989, Hendrix 1995, Flegel *et al.* 1998). In the present study, only total carbon was measured.

In *A. turgida all* treatment observations within the two plant categories were statistically equivalent to one another as well as to the soil control total carbon measurements (Table 3). This lack of difference in the total carbon content found in the casts of A. *turgida* can be explained by the endogeic nature of this species. With their geophagous feeding strategy, endogeic earthworms are less selective toward organically rich substrates and tend to ingest more soil than other species, resulting in less carbon in their egesta (Scheu 1987).

Only the surface casts of *L. terrestris* (Table 4) found in the Roundup Ready soybean treatment were observed to hold more carbon than the control soil. In both treatments the surface casts contained an equivalent amount of carbon than the subsurface ones.

The surface casts of *L. terrestris* in the corn treatment had significantly higher carbon content than all other food treatments and were also different from the subsurface casts of the same treatment (Table 4). The subsurface casts of the corn treatment contained a similar amount of carbon than the surface and subsurface casts of the Bt corn treatment. All casts, with the exception of the surface casts of the corn treatment, were not found to be enriched in total carbon when compared to the soil control.

The analysis of the casts for total carbon content did not demonstrate the presence of a diet influence on carbon content. Therefore, we find no indication that the genetically modified corn and soybean used in this experiment have an impact on total carbon content of casts. In both species, both the surface and subsurface casts of the worm control treatment were found to be similar to the soil control. This further demonstrates the absence of a diet effect on casts total carbon in this experiment.

Since earthworms cannot modify the total amount of nutrients found in their milieu and only augment the total carbon content of their casts while feeding selectively on organic matter rich substrates, a separate analysis for organic carbon should be performed if this experiment is replicated (Scheu 1987, Basker *et al.* 1992).

Total Nitrogen

Many studies have demonstrated the presence of an increased quantity of total nitrogen in earthworm casts when compared to soil (Syers *et al.* 1979, Lee 1985, Svensson *et al.* 1986, Tiwari *et al.* 1989). This increase in nitrogen is believed to be the result of the mixing of plant residues, microbial excretions and soil in the gut of earthworms (Tiwari *et al.* 1989). Earthworms can also increase nitrogen availability in their casts by increasing the mineralization of organic nitrogen by promoting an elevated microbial population and enzymatic activity

(Syers *et al.* 1979, Basker 1992). However, a non selective geophagous diet can produce casts with a similar amount of nitrogen than what is found in the soil (Scheu 1987).

As it was found for total carbon, there was no diet effect on the total nitrogen content of *A. turgida* casts (Table 3). All surface and subsurface total nitrogen levels found in the casts were equivalent between soybean and Roundup Ready soybean as well as between corn and Bt corn. All casts nitrogen measurements were also statistically equivalent to the soil control. This can once again be explained by the absence of selectivity in the feeding strategy of *A. turgida* as it was observed by Scheu (1987) on another *Aporrectodea* species.

There were no observed differences between the surface and subsurface casts that were egested in the soybean treatment by *L. terrestris* (Table 4). Those same casts were also found to be similar in their nitrogen content to the soil control as well as to the nitrogen content of the surface and subsurface cast of the Roundup Ready treatment. The total nitrogen content of the subsurface cast of the Roundup ready treatment was the only one observed to be different from the soil control. The surface casts of the earthworms fed Roundup Ready soybean were also found to be significantly richer in total nitrogen than their subsurface counterpart.

None of the nitrogen concentrations found in the casts of *L. terrestris* (Table 4) produced under the corn and Bt corn treatment were observed to be significantly different from the one found in the soil control. The nitrogen content of both the surface and subsurface casts of the Bt corn fed earthworms were equivalent to one another as well as to the surface and subsurface casts of the corn treatment. In the corn fed earthworms, the surface casts were found to hold a lower amount of total nitrogen than the subsurface ones.

The lower amounts of total nitrogen found in the surface casts of the worm control, the corn and the Roundup Ready soybean treatments are thought to be due to an increased production and subsequent loss of more labile forms of nitrogen by the microbial activity in the surface casts (Edwards, 2004).

No evidence of a diet impact was observed when comparing the soybean to the Roundup Ready soybean treatment as well as the corn to the Bt corn treatment. Therefore, the genetically modified soybean and corn were not found to influence the total nitrogen content of earthworm casts. In a replication of this experiment the organic and inorganic nitrogen content should be analyzed in order to get a clearer picture of nitrogen dynamics in cast as well as a possible influence of the genetically modified plants on the ratio of organic and inorganic nitrogen.

The factors that could have influenced casts nutrient content include the litters nutrient content (Table 1) as well as a reduced possibility of selective feeding behaviour in *L. terrestris* due to the experimental design (Scheu 1987). We believe that no other research has so far compared the surface and subsurface casts in terms of nutrient content. In this experiment we found that subsurface casts are poorer in some nutrients than the surface cast. We suggest that subsurface casts are less microbiologically active than the surface ones, leading to a lower nutrient content. Further research should be performed to confirm our observations and hypothesis. If this trend appears to be the norm, estimates of earthworms contributions to nutrient cycling would have to be corrected since subsurface casts amounts and characteristics are generally estimated from surface casts.

Conclusion

The results of the present experiment confirm our hypothesis that Bt corn and Roundup Ready soybean do not produce any difference in the casting activity as well as the casts nutrients content of *L. terrestris* and *A. turgida* when respectively compared to regular corn and soybean plants. For both species the equivalence in the amounts of total casts egested in all food treatments demonstrated that the earthworms expressed no food source preference (Shipitalo *et al.* 1988). The lack of difference in the total carbon and total nitrogen content of the earthworm casts when comparing a genetically modified food source to its natural counterpart shows the absence of food treatment influence in this experiment. However, in order to have a clearer picture, experimental conditions that would favour selective feeding behaviour (e.g. Shipitalo *et al.* 1988) and the analysis of the different forms of carbon and nitrogen would be recommended. The similarity in the measurements of cast available potassium and phosphorus within the two food sources group also demonstrates the absence of a food treatment impact. It has been well established that the availability of the nutrients found in casts is influenced by the enzymes contained within the casts as well as their microbial population (Lee 1985, Tiwari *et al.* 1989, Basker *et al.* 1992). Therefore, the lack of difference in available potassium and phosphorus can be in part explained by the observation of Saxena and Stotzky (2001) that the Bt toxin has no effect on soil bacteria and earthworms. As far as we know, this was the first attempt to determine the influence of Roundup Ready soybean on earthworm. This short term laboratory experiment demonstrates that Bt corn and Roundup Ready soybean have no influence on cast qualities. More research will be needed to understand in more details the possible effects of genetically modified organisms on earthworms and therefore soil quality.

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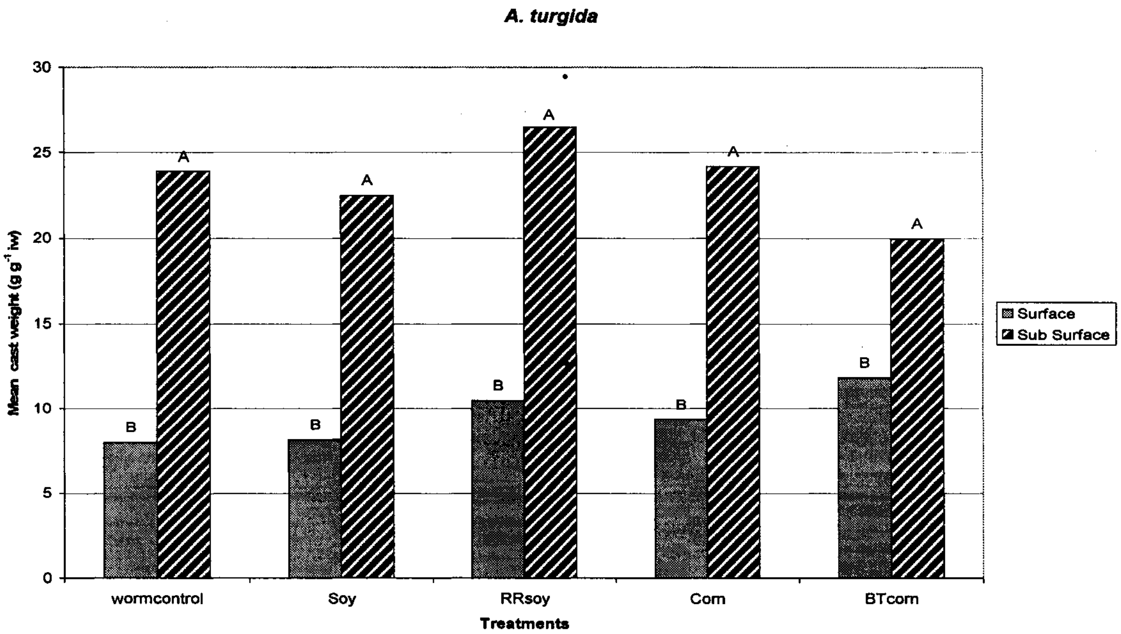

Figure 1: Mean total cast mass (g g^{-1} iw) of *Aporrectodea turgida* (n=5) for all food treatments. Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other.

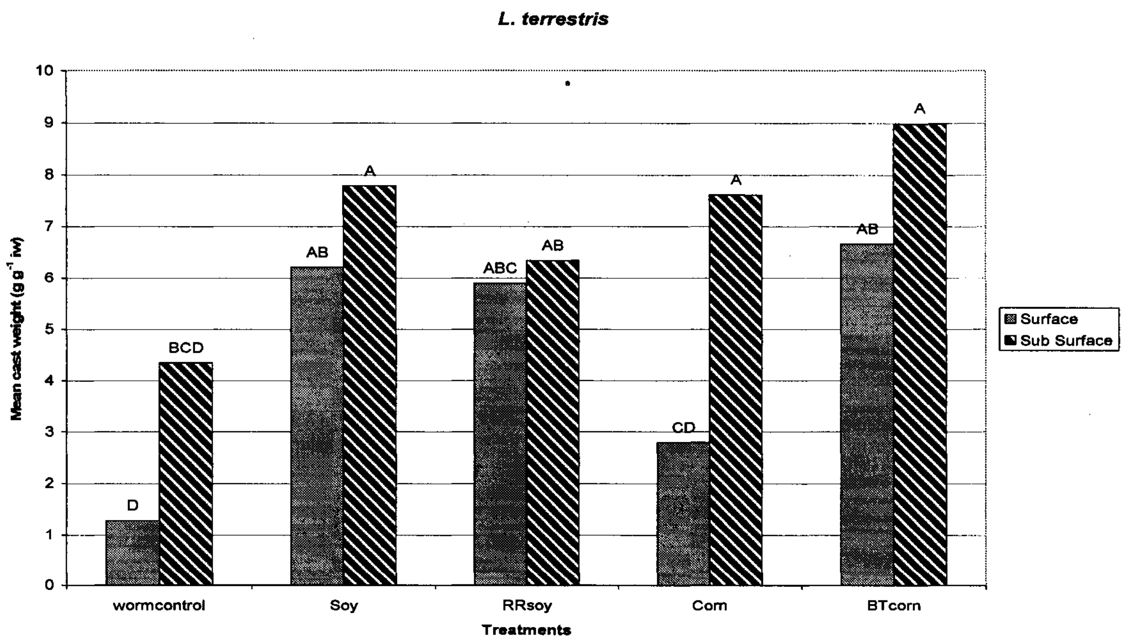

Figure 2: Mean total cast mass (g g^{-1} iw) of *Lumbricus terrestris* (n=5) for all food treatments. Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other.

Table 1: Plant tissue nutrient analysis (g/Kg) of litter used in this study.

	Nutrient Content (g/kg)			
	C[a] g/Kg	N[a] g/Kg	P[b] g/Kg	K[c] g/Kg
Corn	435.1	17.4	3.2	25.3
Bt Corn	458.9	14.8	2.9	23.4
Soybean	443.3	22.7	5.1	13.9
Roundup Ready Soybean	427.8	17.7	2.7	30.4

[a] Total C and N were analyzed with a NC analyzer (Flash EA 1112 Series, Thermo Finnigan Carlo Erba)

[b] Digestion with H_2SO_4/H_2O_2 (Parkinson and Allen 1975), followed by analysis on a Lachat Quick-Chem AE flow injection autoanalyser (Lachat Instruments, Milwaukee, WI) using the molybdenum blue method (Murphy and Riley, 1962).

[c] Digestion with H_2SO_4/H_2O_2 (Parkinson and Allen 1975), analysed with absorption spectrometry.

Table 2: Mortality and mass gain of earthworms during the study. There were 5 replicates earthworms in each treatment. Letters represent treatments that are significantly different (LSD P<0.05) from each other

Treatments	*A. turgida*		*L. terrestris*	
	Mortality	Mass Gain	Mortality	Mass Gain
Worm Control	2	-0.07 [b] ±0.04	3	-0.08 [c] ±0.13
Soybean	1	0.08 [a] ±0.02	0	0.43 [a] ±0.07
Roundup Ready Soybean	1	0.08 [a] ±0.03	2	0.25 [ab] ±0.1
Corn	2	0.13 [a] ±0.02	4	0 [bc]
Bt Corn	3	0.14[a] ±0.04	1	0.24 [ab] ±0.08

Table 3: Mean surface and subsurface casts nutrient content of *Aporrectodea turgida* (n=5). †* indicate treatments that are similar (LSD P<0.05) to each other within each column.

	Nutrient Content							
Treatment	C (%)		N (%)		P (mg/L)		K (mg/L)	
	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface
Soil Control	2.98 †*	2.98 †*	0.31 †*	0.31 †*	9.29 *	9.29 *	4.14	4.14
Worm Control	2.67	2.74	0.31	0.27	9.35	9.49	4.16	3.74
Soybean	2.72 †	2.86 †	0.33 †	0.33 †	10.3	10.1	7.83	7.76
RR Soybean	2.84 †	2.84 †	0.36 †	0.33 †	10.1	9.13 †	8.48	6.88
Corn	3.02 *	2.96 *	0.32 *	0.34 *	10.1	8.69 *	12.7	8.60
BT Corn	3.02 *	3.07 *	0.30 *	0.35 *	8.89 *	8.74 *	9.64	7.48

Table 4: Mean surface and subsurface casts nutrient content of *Lumbricus terrestris* (n=5). †* indicate treatments that are similar (LSD P<0.05) to each other within each column.

	Nutrient Content							
Treatment	C (%)		N (%)		P (mg/L)		K (mg/L)	
	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface	Surface	Subsurface
Soil Control	2.98 †*	2.98 †*	0.31 †*	0.31†*	8.48 †*	8.48 †*	3.52	3.52
Worm Control	2.86	2.76	0.21	0.32	9.26	9.21	4.20	3.50
Soybean	3.22 †	3.00 †	0.33 †	0.37 †	10.6	9.47 †	10.3	7.46
RR Soybean	3.32	3.19 †	0.31 †	0.39	9.04 †	9.05 †	9.48	8.88
Corn	3.64	3.08 *	0.30*	0.36 *	9.43 *	8.14 *	10.7	9.63
BT Corn	3.15 *	3.08 *	0.33*	0.36 *	8.68 *	7.30 *	11.6	8.83

Connecting Paragraph:

In the last two chapters, the influence of moisture, temperature and food resources on earthworm casting activity were investigated under controlled laboratory conditions. It is expected that these factors will influence the earthworms casting activity under field conditions. This final chapter investigates the spatial and temporal distribution of surface casting activity from controlled populations of the same two species (*Aporrectodea turgida and Lumbricus terrestris*) in a soybean agroecosystem during the 2004 field season.

<u>Temporal and Spatial Dynamics of Earthworm Surface Casting in a Soybean Agroecosystem</u>

Introduction

As ecosystem engineers, earthworms generate important changes in the soil environment. Since many of these changes are seen as beneficial, earthworms are considered valuable soil organisms and have also proved to be useful in soils rehabilitation (Lawton 1994, Lavelle *et al.* 1997, Kladivko 2001, Langmaack *et al.* 2002). The burrowing activity of earthworms is well known to improve water and air infiltration into the ground (Edwards and Bohlen 1996, Lavelle *et al.* 1997). Earthworm casting activity has been shown to improve soil quality and stability due to the formation of better soil aggregates (McKenzie and Dexter 1987, Scullion and Ramshaw 1988, Schrader and Zhang 1997). Casts also contain a greater amount of organic carbon as well as higher concentrations of plant available nutrients such as N, P K and Mg than the bulk soil, which suggest that casts could have a positive influence on plant growth and nutrients dynamic (Sharpley and Syers 1976, McKenzie and Dexter 1987, Lawton 1994, Tomati *et al.* 1994, Schrader and Zhang 1996, Chaoui *et al.* 2003). Earthworms also play a role in the improvement of nutrient cycling by increasing the rate of decomposition of plant litter, promoting the growth of beneficial soil microorganisms, and improving solute transport. These processes are expected to facilitate uptake of nutrients by plants (Lawton 1994, Bohlen and Hendrix 2002).

The patchy nature of earthworm populations, however, limits our understanding of the impacts of earthworm activity on natural systems (Hendrix 1995, Baker and Lee 2000, Whalen 2004). As suggested by Hendrix (1995), a better understanding of earthworm spatiotemporal distribution would help in assessing the influence of earthworms on soil structure and nutrient availability. In temperate regions, much remains to be discovered about the spatial distribution as well as the temporal modification in the distribution of earthworm populations (Whalen 2004). As it was demonstrated by Le Bayon *et al.* (2002) in a corn agroecosystem, earthworm activity is synchronized with the plant growth. Therefore, plants could have a beneficial effect on earthworm activity and earthworms, by their activity, could benefit to the plants. A better understanding of the spatial and temporal distribution of earthworms would therefore be important to improve our comprehension of their influence on ecological systems.

Only two studies are known to have been performed to determine the spatiotemporal activity of earthworms on the row/interrow scale of a temperate agroecosystem (Binet *et al.* 1997, Binet and Le Bayon 1999). Both those studies, performed in corn fields, found links to earthworm spatiotemporal distribution with soil compaction and the proximity to plant rows. A higher number of earthworms, earthworm biomass and surface casts were found within the row than in the interrow region (Binet *et al.* 1997, Binet and Le Bayon 1999). This difference, however, was not found in compacted soils (Binet *et al.* 1997, Binet and Le Bayon 1999).

This field experiment was designed with the objective of determining the temporal and spatial variation in surface cast production of two earthworm species, *Aporrectodea turgida* (Eisen) and *Lumbricus terrestris* L. on the row-interrow scale in a temperate soybean agroecosystem. The nutrient casts characteristics were also determine in order to understand the influence of earthworm casting on soil nutrient content.

With our present understanding of earthworm ecology we hypothesize that more earthworms, and therefore more casts, will be present in the row compared to the interrow area due to the more favorable microclimate found under the plants. The design of this experiment let us also suggest that more casts would be found on the north side of the plant row due to an increase amount of shade. The quantity of earthworms added is also believed to increase the total casting activity with more casts found under the 2x treatments.

Materials and Methods

- Site description

The study site was located on the Macdonald Campus Research Farm, Ste. Anne de Bellevue, Québec, Canada (45 ° 28' N, 73 ° 45 ° W). The average daily temperature during the months of this study was of 18.67°C and the average amount of precipitation of 0.42 mm d^{-1} (Environment Canada, unpublished data). The soil, a silty-loam Humic Gleysol (fine-silty, mixed, frigid Typic Endoaquent), contained 320 g kg^{-1} of sand, 580 g kg^{-1} of silt and 100 g kg^{-1} of clay with 24.5 g C kg^{-1}, 1.98 g N kg^{-1} and pH 6.0.

Earthworm populations in this field were assessed at 8 randomly selected sampling locations within a 30m * 30m grid on June 18, 2003. Populations were dominated numerically by *Aporrectodea* spp. (*Aporrectodea turgida* (Eisen) and *Aporrectodea trapezoides* (Dugés)), and a few individuals of *L. terrestris* L. were found. Earthworm populations ranged from 28 to 161 individuals m^{-2} (mean = 66 m^{-2}, mean biomass = 40.341 g fresh weight m^{-2}), with approximately 50 individuals m^{-2} of *Aporrectodea* spp. and 15 individuals m^{-2} of *L. terrestris* L.

- Experimental design

In April 2004, earthworm enclosures were established using 0.1 cm thick steel sheets. Each of the 28 enclosures measured 2.5 m in length, 1.25 m in width and 0.6 m in height of which 0.4 m was buried underground. The area within each enclosure was sprayed 5 times from April 28 to May 21 2004, with Sevin XLR Plus (Bayer Group) for a total application of 27.39 kg active ingredient (carbaryl) ha^{-1} to reduce natural earthworm population. This rate of application is over three times higher than the carbaryl rate applied by Potter *et al.* (1990) to turfgrass, which reduced earthworm population by 90%. On May 28 a row of soybean (*Glycine max* L. (Merrill) (OAC Champion, BELCAN) was planted by hand along the middle of the enclosure at a rate corresponding to 400, 000 seeds ha^{-1}, roughly 100 seeds per enclosures. Earthworms were collected in August and September 2003 from the Macdonald Research Farm. We collected *Lumbricus terrestris* from an alfalfa (*Medicago sativa* L.) field by pouring a dilute formalin solution (0.5% formalin) on the soil surface, then rinsed the earthworms well with tap water. Individuals of *Aporrectodea turgida* were collected by hand sorting soil (0-15 cm depth) from a soybean (*Glycine max* (L.) Merrill) field. The earthworms were then placed in 37 L plastic containers with field-moist soil and litter, and reared in the laboratory (average temperature = 20°C). The earthworms were introduced to the enclosures on 1st June and covered with straw to avoid predation and dehydration.

On June 1 2004, earthworms were introduced into the enclosures and covered with straw to minimize predation and desiccation. These earthworms were collected in August and September 2003 and reared in the laboratory over the winter to provide the requisite number of adults and juveniles of *A. turgida* and *L. terrestris* for this experiment. Further details of rearing earthworms in the laboratory are provided in Chapter 2 and 3 of this thesis. The straw cover was removed on June 4th after the earthworms had all gone underground. There was no ploughing, fertilization or herbicide treatments applied to the field before or after the enclosures were established.

The experiment was designed as a randomized complete block with seven earthworm treatments, replicated four times. The seven earthworm treatments are described in Table 1. While the control received no earthworms, the other enclosures received single species (*A. turgida* or *L. terrestris)* or mixed earthworm communities at rates approximating the natural population level (1x) or double the natural population (2x) in this field.

- Surface cast collection

Each enclosure was divided in twenty 0.1x1m transects (north-south direction) running perpendicular to the soybean row. Surface casts were collected from one randomly selected transect every 7 to 8 days from June 21 to

September 21, for a total of 14 collection dates. The randomization of the transect to be sampled was performed to avoid the presence of a confounding effect in the repeated measures. A week before collecting casts from a transect, all surface casts from the transect to be observed were removed by racking. In order to determine the spatial variation in casting activity, the transects were divided in 5 intercepts of 0.2m each and the casts were collected and grouped according to the intercept in which they were found. Intercept 1 corresponded to the southern most section, the soybean plants were rooted in intercept 3 and intercept 5 was at the northern most part of the transect. The number of casts in each intercept as well as their dry mass was measured. Using a thermocouple, soil temperature was recorded at 0.1 m below ground in one randomly chosen treatment per block every week. Soil moisture was determined by oven drying (60°C for 48h) one soil sample taken at 0.1m below the surface for each of 2 randomly chosen treatments every week.

- Cast and soil nutrient analysis

At two occasions during the growing season, casts and soil were collected for nutrient analysis. Microbial biomass nitrogen was also determined. Total carbon and nitrogen were analyzed using a NC soil analyzer (Flash EA 1112 Series, Thermo-Finnigan Carlo Erba). Mineral N (NH_4-N and NO_3-N) was determined in 0.5M K_2SO_4 soil extracts (1:5 soil: extractant) and analysed colorimetrically using the cadmium reduction-diazotization and salicylate methods (Lachat Instruments 2000) with a Lachat Quick-Chem AE flow injection autoanalyzer (Lachat Instruments, Milwaukee, WI). Dissolved organic N was determined by the difference between the NO_3–N concentration in the oxidized sample and the mineral N (NH_4–N, NO_3–N) concentration in the unoxidized one. Microbial biomass N (MBN) was determined using the chloroform fumigation direct extraction procedure followed by persulfate digestion and calculated as: [(total extractable N after fumigation - total extractable N before fumigation)/K_{en}] where K_{en} value of 0.54 was used to correct for extraction efficiency (Voroney *et* *al.* 1993, Joergensen and Mueller 1996). The dissolved organic C concentration in the persulfate extract was measured by wet combustion with a Shimadzu TOC-V carbon analyzer (Shimadzu Corporation, Kyoto, Japan). Phosphate concentrations in Mehlich-3 extracts were evaluated by the molybdenum blue reaction (Murphy and Riley 1962).

- Statistical analysis

The effect of earthworm treatments on surface casting activity, as well as the effect of the distance of the plant row on earthworm surface casting activity were each evaluated using one-factor ANOVA. An analysis of variance (ANOVA) was conducted with the PROC GLM function of SAS 6.12 for windows (SAS Institute Inc., Cary, NC). Variables that significantly affected earthworm casting activity ($P<0.05$) were adjusted for multiple comparisons and analyzed using a LSD test at the 95% confidence level. Equations and R^2 values were fitted with Excel XP (Microsoft Corporation, Redmont, WA, USA).

Results and Discussion

Surface Cast Production.

During this experiment, a total of 3528 surface casts were collected over the 392 transect observations performed. The total mass of the casts was 2573.995 grams. The average daily production of casts ranged from 4.33 g (d.w.) m^{-2} d^{-1} in the control enclosures to 11.75 g (d.w.) m^{-2} d^{-1} in the Lt 2x treatment. If weeks 1 to 7 were removed from analysis, because of their low casting activity, the averages rise up to 8.22 g (d.w.) m^{-2} d^{-1} in the control enclosures to 22.77 g (d.w.) m^{-2} d^{-1} in the Lt 2x treatment (Table 2). Averages of casting activity vary greatly from authors to authors. The daily average casting activity found in our study are greater than what was found by Binet and Le Bayon (1999) (8.6 g m^{-2} d^{-1} (d.w.)) in a temperate corn agroecosystem, but much less than what Graff (1971, in Lee 1985) (70.5 g m^{-2} d^{-1} (d.w.)) observed in a temperate pasture. All 2x treatments were found to contain a higher total surface casts mass than the 1x treatments, however this difference was only significant for a treatment pair when comparing Ap 1x vs Ap 2x (Fig. 1). All 2x treatments were similar to one another, and so were all 1x treatments. Ap 1x was the only 1x treatment to be significantly different from all 2x treatments. The control treatment was found to have a fewer total casts than any other treatments, demonstrating that the carbaryl treatment was effective in reducing earthworm populations at the field site and that many of the earthworms added must have survived (Fig. 1). The casts found in the control can be explained by the presence of earthworms that hatched from cocoons after pesticide degradation (Potter *et al.* 1990). The higher cast mass found in all 2x treatments as well as the difference between the control and the other treatments can be simply explained by the greater number of earthworms added to each treatment, consistent with observation made by Scullion and Ramshaw (1988).

Moisture and Temperature Effect on Surface Cast Production

The influence of moisture on earthworm casting activity was found to be similar to other reports that surface casting increases as soil moisture increases (Sharpley and Syers 1977, Hindell *et al.* 1994, Binet and Le Bayon 1999) (Fig. 2). The highest surface casts mass were found at 16 % moisture, the highest soil moisture recorded. Soil temperature was found to influence surface casting activity in the opposite way of moisture were the lowest recorded temperatures triggered the highest surface cast mass (Fig. 3). The opposite trend is usually observed in field experiments, however, for the reason that this experiment took place in the summer, it is important to note that the lowest temperatures were in fact average temperatures for earthworms (Sharpley and Syers, 1977). A sharp decline in surface casts mass was observed as the field temperatures increased. This can not only be explained by the fact that earthworms avoid high temperatures, but also because soil moisture is reduced under high temperatures due to an increase in evapotranspiration.

Temporal Variations in Surface Casting

The quantity of surface casts found in the first half of the experiment was consistently low with the first significant increase in casting activity observed on week eight (Fig 4.). Although different factors could explain the low initial casting activity, the low food supply is most likely the determinant factor that restrained earthworm activity (Sharpley and Syers 1977, Shipitalo 1988). The presence of organic matter within the enclosures stayed low until the soybean plants were at least six weeks old. Also, the raking of the soil surface to remove the cast for the next observation artificially reduced the amount of organic matter present in the transect to be observed. The reduction in casting activity observed from week ten to eleven is explained by a very dry period where soil moistures were found to be as low as 5.86%. Throughout the whole field season, with the sole exception of week seven, the control treatment was found to contain fewer casts than any other treatments (Fig. 4). This demonstrates not only the presence of a general treatment effect, but also that the treatment effect was sustained during the field season.

Spatial Variations in Surface Casting

The spatial distribution of the casts among the different intercepts showed that earthworms had a clear preference for the row space, intercepts 2, 3 and 4, compared to the interrow intercepts 1 and 5 (Fig. 5). Both interrow intercepts were observed to have significantly less casts deposited inside them than the intercepts immediately beside them, 2 and 4. The amounts of casts deposited within the three row intercepts were statistically equivalent to one another. However, the amount of casts found in intercept 3, were the soybeans were planted, was also equivalent to the one found in the interrow intercepts (Fig. 5).

This lower amount, although similar to the other row intercepts, could be explained by the dense root network of the soybean plants which could have skewed the preference of the earthworms toward intercepts 2 and 4.

The spatial preference of earthworms for the row space was also observed by Binet *et al.* (1997) as well as Binet and Le Bayon (1999) in temperate corn fields. The possible explanations of this phenomenon are a higher reproduction and survival rate, a preference for the lower soil density found in the row, an increase amount of organic material to feed on as well as the creation of a microclimate favourable to earthworms (Binet *et al.* 1997). Schmidt *et al.* (2003) also suggested that living plants could provide the same advantages to earthworms than mulch, i.e. a protection against light and predation, lower variations of extreme temperatures, a higher microbial activity and a decrease in soil moisture loss. Once the soybean plants were mature, intercepts 2, 3 and 4 add more foliage over them and therefore received more shade and protection than intercept 1 and 5. It is suggested that plant cover had the same benefits to earthworms as mulch.

Our observations on the spatial distribution of casts confirm our hypothesis that more casts would be produced in the row rather than in the interrow space. However, no difference in surface casting was observed between the intercepts found on the south side and the ones found on the north side of the planted row.

Nutrient Content in Surface Casts and Bulk Soil

The chemical analysis of the casts collected in the field in comparison to the soil showed their total carbon and nitrogen content to be similar (Table 3). Total carbon and nitrogen content of casts is dependent on diet and is usually found to be higher in casts than soil or, under some circumstances, as similar to soil (Syers *et al.* 1979, Svensson 1986, Scheu 1987). As previously observed by Syers *et al.* (1979) and Parle (1963) the concentrations of NH_4-N were found to be lower than the concentrations of NO_3-N in the surface casts of both species. NH_4-N concentrations were found to be lower in the casts of both species than the soil. Dissolved organic carbon amounts were lower in the casts of both species than in soil. However, the dissolved organic nitrogen of casts was greater than what was found in soil. The microbial nitrogen content of casts was found to be superior to the soil content in the casts of *A. turgida* but lower in *L. terrestris* (Table 3). On the other hand, the microbial biomass carbon (MBC) of *A. turgida* was equivalent to the soil (MBC) while *L. terrestris* (MBC) was slightly lower (Table 3). This is contradictory to what is usually found in the literature (Aira *et al.* 2004).

Conclusion:

The significant differences in terms of total cast mass between every treatments and the control clearly determined the presence of a treatments effect for all earthworm introductions that was maintained during the growing season. This observation validates the use of such experimental techniques as pesticides application and earthworm introduction to manipulate earthworm populations in the field. The quantity of earthworms that were introduced positively influenced the quantity of casts deposited at the surface. However, of the three treatment pairs, Ap1x and Ap2x, Lt1x and Lt2x, and Ap+Lt1x and Ap+Lt2x, only Ap1x and Ap2x was found to have significantly more casts in the 2x treatment. Earthworm casting activity was negatively influenced by low moisture as well as high temperatures. A temporal effect was also observed where few cast were egested at the surface of the enclosures in the first seven weeks of observation. Food supply is thought to be the key factor in explaining this temporal variation since very low organic matter was found in the enclosures until plant maturation.

As hypothesized, more casts were found in the row intercepts compared to the interrow ones. Our observations were found to be similar to other experiments performed in temperate corn agroecosystems. The preference of the row space is thought to be caused by the presence of a plant cover that provided the earthworms with a microhabitat with physical and climatic protection as well as a higher food supply.

Since earthworm cast mass is positively correlated with earthworm activity, this experiment will help us to better understand the influence of earthworms on soil structure and nutrient availability by increasing our comprehension and predictive power of small scale earthworm spatiotemporal distribution. Also, the interpretation of this study have us to suggest that earthworms and certain plants can perform mutualism where earthworms obtain shelter and trophic resources and plants a better soil in terms of both physical and chemical characteristics.

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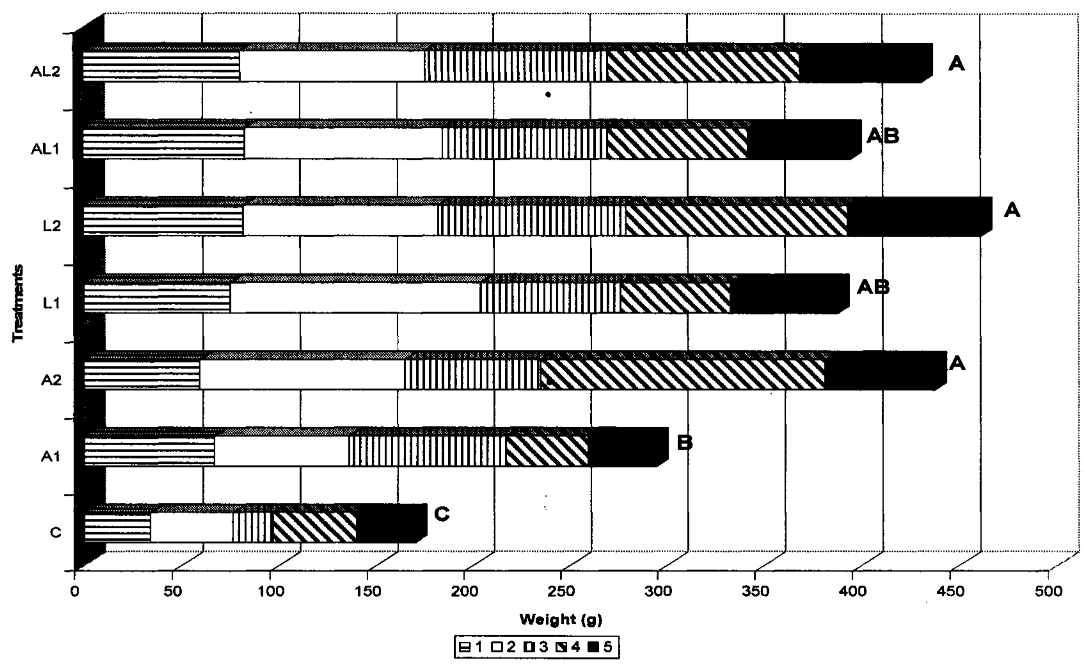

Figure 1: Total cast mass per treatment in a soybean agroecosystem during a 14 week period from June 21 to September 21 2004 (n=56). Bars with the same letter were not significantly different (P<0.05, LSD test).

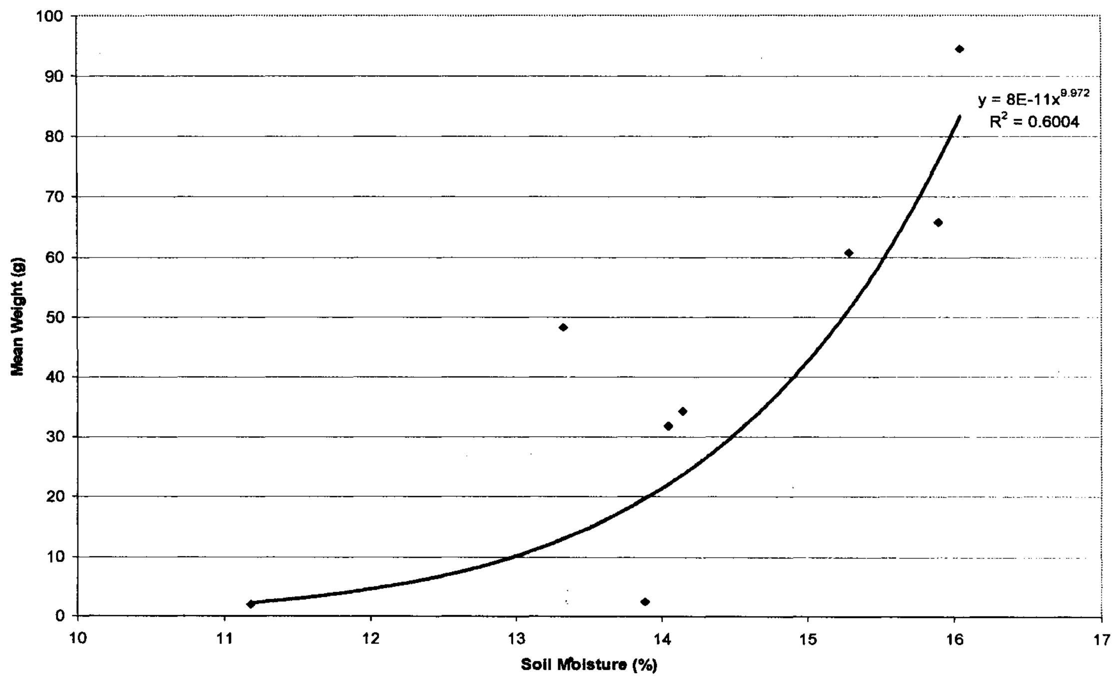

Figure 2: Relationship between soil moisture and mean total cast mass in soybean agroecosystems from week 6 to week 14, but excluding data from week 10 (n=16). With corresponding equation and R^2 value.

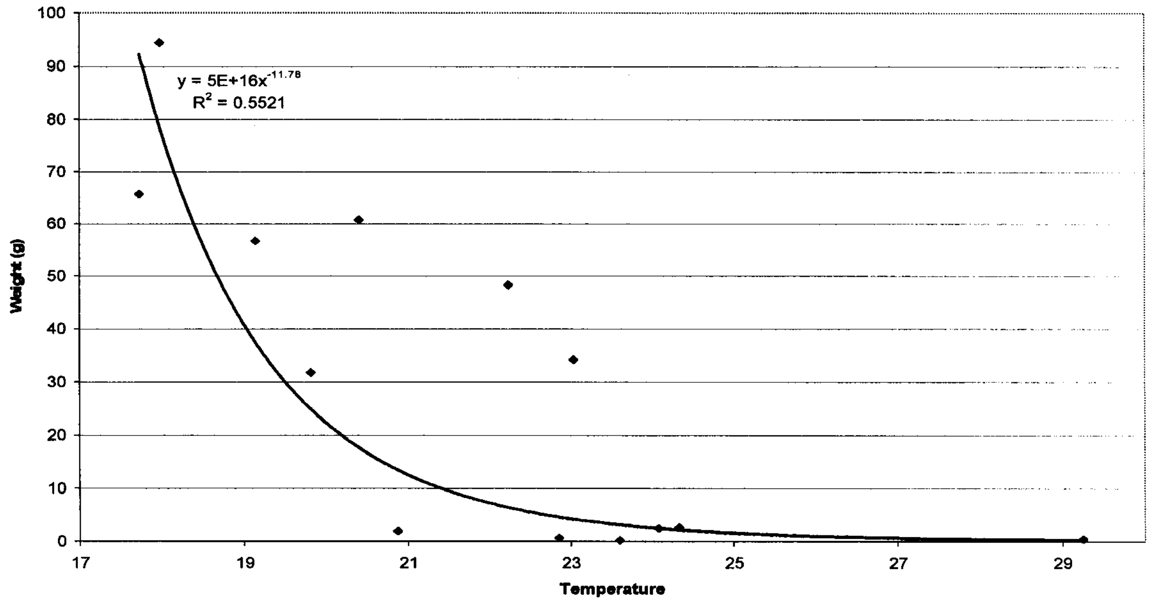

Figure 3: Relationship between soil temperature and mean total cast mass in soybean agroecosystems from week 1 to week 14, but excluding data from week 2 (n=26). With corresponding equation and R^2 value.

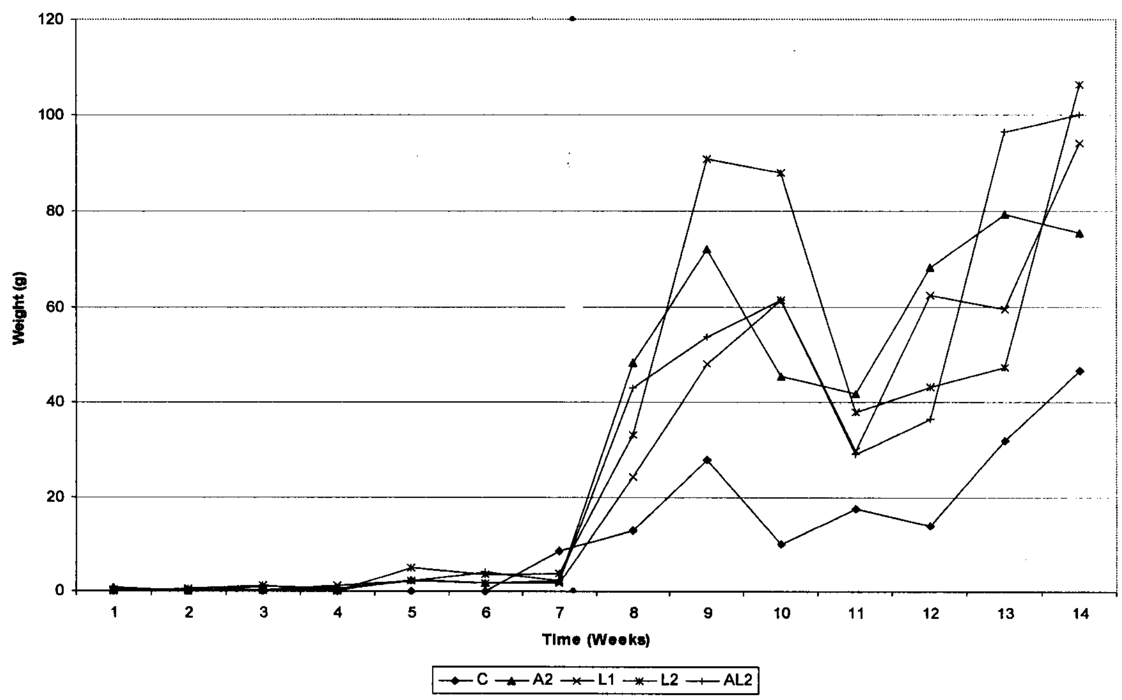

Figure 4: Fluctuations in cast total mass in selected treatments from soybean agroecosystem during a 14 week period (n=56).

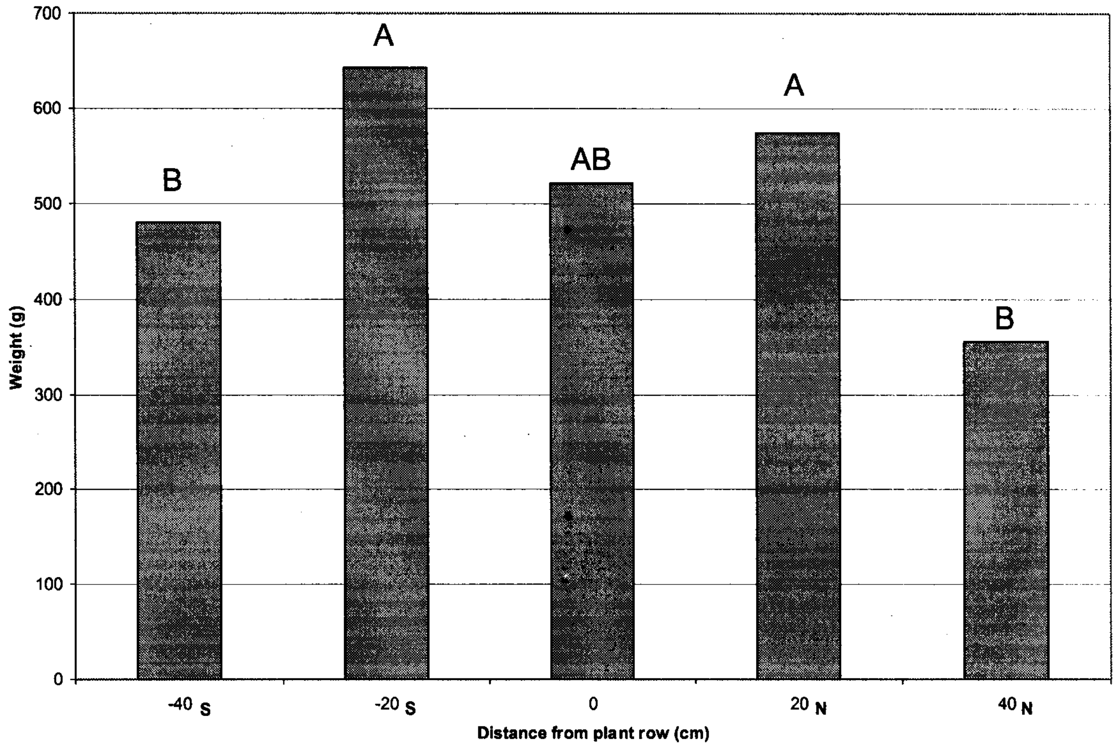

Figure 5: Total cast mass in a soybean agroecosystem as influenced by the distance from the planted row (n=56). Bars with different letters represent treatments that are significantly different (P<0.05 LSD test) from each other.

Table 1: Amounts of earthworms, *A. turgida* and *L. terrestris* added per enclosures corresponding to each treatment.

	A. turgida		*L. terrestris*	
Treatments	Adults	Juveniles	Adults	Juveniles
Control	0	0	0	0
Ap1x	96	48	0	0
Ap 2x	192	96	0	0
Lt 1x	0	0	9	35
Lt 2x	0	0	18	70
Ap + Lt 1x	96	48	192	96
Ap + Lt 2x	9	35	18	70

Table 2: Cast total mass (d.w.), and g (d.w.) m^{-2} d^{-1} with and without weeks of inactivity (1-7) (n=56).

Treatments	Total mass (d.w.) (g)	g (d.w.) m^{-2} d^{-1}	g (d.w.) m^{-2} d^{-1} (without week 1-7)
C	169.65	4.33	8.22
Ap 1x	294.33	7.51	14.62
Ap 2x	437.34	11.16	21.93
Lt 1x	387.61	9.89	19.38
Lt 2x	460.62	11.75	22.77
Ap + Lt1x	394.01	10.05	19.84
Ap +Lt 2x	430.44	10.98	21.45

Table 3: Nutrient content in bulk soil and surface casts produced by *A. turgida* and *L. terrestris* in a soybean agroecosystem.

Nutrients	Soil	Casts	
	Soil	*A. turgida*	*L. terrestris*
Total N %	0.31 ±0.02	0.37 ±0.02	0.31 ±0.04
Total C %	2.98 ±0.15	3.49 ±0.05	3.02 ±0.57
NH_4-N (mg Kg^{-1})	0.85 ±0.09	N.D.[1]	0.14 ±0.15
NO_3-N (mg Kg^{-1})	11.36 ±1.0	24.45 ±1.54	25.69 ±4.1
Microbial Biomass C	142,80 ±29.41	131,55 ±17.49	102,69 ±33.15
Dissolved Organic C	52.63 ±4.73	49.88 ±14.64	36.92 ±5.06
Microbial Biomass N	79.70 ±2.72	108.84 ±43.2	23.47 ±19.8
Dissolved Organic N	17.43 ±0.38	63.85 ±1.34	42.63 ±8.16

[1] N.D. = Non-detectable

Conclusion:

In the first laboratory experiment it was found that earthworm casting and burrowing activity increased as temperature increased. On the other hand, moisture had a variable influence on earthworm activity, demonstrating that temperature is the dominant factor affecting earthworm activity. Higher temperatures than the one tested in our first experiment should also be investigated in order to determine the maximum temperature for earthworm activity. The use of a standard method for the measurement of soil moisture would be a great improvement and permit researchers to easily compare the results from different studies. I would like to recommend the use of field capacity as the standard technique for adjusting soil moisture in studies involving earthworms.

No difference was found in the earthworm activity level and casts characteristics when they were fed genetically modified or non-genetically modified plant litter. Therefore, the earthworms expressed no food preference toward the natural or genetically modified food sources and cast qualities were not influenced by the presence of genetically manipulated litter. However, more long term and field studies should be performed in order to obtain a clearer picture of the possible influence of genetically modified plants on earthworms.

Our field experiment demonstrated both a temporal and a spatial variance in the surface casting activity of earthworms. Surface casting activity was synchronous with plant growth, which can be explained by a higher availability of trophic resources. Also, significantly more casts were deposited in the row than in the interrow space. The presence of a microclimate generated by the plant cover over the row likely provided physical and climatic protection to the earthworms. In addition, a larger food supply is thought to be available close to the planted row due to the presence of roots, litter and microorganisms that serve as a food source for earthworms. These factors (microclimate, food source) likely have an important influence on earthworm spatial distribution in soybean agroecosystem. The relationship between plants and earthworms that emerged in this study could be considered as mutualism, but further work is required to confirm if this relationship exists in other ecosystems.