The Contribution of Earthworm Communities to Nitrogen Cycling in Agroecosystems of Québec

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January, 2007

A thesis submitted to McGill University in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

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ISBN: 978-0-494-32179-9
Our file Notre référence
ISBN: 978-0-494-32179-9

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PREFACE

This thesis is composed of 6 chapters, preceded by a general introduction, and followed by a summary and general conclusions. The first chapter is the literature review, which summarizes the work of other researchers and justifies the research questions. Chapters two to six are the experiments and results, which are presented in manuscript format according the guidelines of the Faculty of Graduate Studies. All manuscripts are co-authored by the candidate and Joann Whalen. The candidate designed and completed the experiments, data analysis and wrote the manuscripts. Joann Whalen provided financial support, advice about the experiments and editorial assistance with the manuscripts. The manuscripts are presented in the following order:

1. Eriksen-Hamel, N.S., Whalen, J.K. 2006. Growth rates of *Aporrectodea caliginosa* (Oligochaetae: Lumbricidae) as influenced by soil temperature and moisture in disturbed and undisturbed soil columns. Pedobiologia. 50, 207-215.

2. Eriksen-Hamel, N.S., Whalen, J.K. 2006. Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems. Agriculture, Ecosystems and the Environment. In press.

3. Eriksen-Hamel, N.S., Whalen, J.K. 2007. The "*Deduction*" Approach: A Non-Invasive Method for Estimating Secondary Production of Earthworm Communities. Oikos. In review.

4. Eriksen-Hamel, N.S., Whalen, J.K. 2007. Modeling the contribution of earthworm communities to nitrogen cycling in maize-soybean agroecosystems. Nutrient Cycling in Agroecosystems. In review.

5. Eriksen-Hamel, N.S., Whalen, J.K. 2007. Measuring the sensitivity of earthworm – nitrogen flux models. Proceedings of the 8th International Symposium on Earthworm Ecology. Krakow, Poland. European Journal of Soil Biology. In review.

ACKNOWLEDGEMENTS

Initial gratitude goes to my supervisor Dr. Joann Whalen for her guidance and support. I am very grateful to have had the opportunity to working with Joann on this project and I appreciate her dedication, encouragement and, timely, helpful responses to my questions.

I would like to acknowledge Jonathan Perreault, Alicia Speratti, Kevin Tiessen and all my other lab mates from the Soil Fertility Lab for their invaluable aid, interesting discussions and, most importantly, for occasionally distracting me from my work. Many thanks to Marie Kubecki, Hélène Lalande and Peter Kirby for valuable administrative and research assistance during my research.

Finally, to my mother and father I wish to express many thanks for their instruction and guidance throughout my education. I would not have come so far without you. To my girlfriend Tina, I would like to express my deepest thanks. For your love, patience, invaluable and infallible moral support I thank you. Once again I am sorry for bringing in the smell of cow manure every spring!

....and an honourable mention to all the earthworms who gracefully sacrificed their lives in the name of science. I am sorry, but thanks!

ABSTRACT

Earthworms have an important role in the decomposition of organic matter, mineralization of nutrients and physical mixing of soils. Despite a large number of laboratory and greenhouse-level studies investigating how earthworms modify soil properties and promote soil fertility, we lack reliable methods to scale-up and quantify earthworm contributions to nutrient cycling at the agroecosystem level. The objective of this thesis is to determine the influence of earthworm communities on nitrogen (N) transformations in soils and to quantify their contribution to nitrogen flux through soils for soybean and maize cropping systems of Québec. Laboratory growth rates were used to predict how earthworm growth responded to seasonal fluctuations in soil temperature and moisture. The relationships between earthworm populations, soil-N pools and annual crop production were evaluated in a field experiment. When favourable conditions occurred in 2004 (temperatures <20°C, and rainfall at least once a week), a positive relationship was found between earthworm numbers and the plant available-N, including soil mineral-N, microbial biomass-N and total-N removed in soybean grain. In 2005, soil conditions were unfavourable (temperatures > 20°C and little or no rainfall) to earthworm survival and growth, and no relationship was found between earthworm populations, soil N pools and corn production. These data permitted me to make assumptions about earthworm activity and life histories under field conditions, which were used to estimate N flux through earthworm communities with two models. The models were tested for their sensitivity to varying parameter values within the range reported in the scientific literature. During a crop growing period with favourable climate conditions, a large earthworm population (100 g fresh weight biomass m^{-2} or greater) is predicted to cycle as much as 120 kg N ha^{-1}. Model predictions were very sensitive to input parameters and did not correspond to the partial N budget calculated at the site. Accurate predictions of N mineralization by earthworms require more species- and site-specific parameter values. Further investigation using stable ^{15}N isotopes as tracers would help us to follow the N transformations and evaluate the N flux mediated by earthworms at the field scale.

RÉSUMÉ

Il est reconnu que les verres ont un rôle important dans la décomposition de matiere organique, minéralisation des nutriments et le mélange des sols. Malgré le grand nombre d'études recherchant comment les verres modifient les sols et ameliorent la fertilité des sols, nous manquons des méthodes fiables pour mettre a l'échelle du agroecosystem et quantifier la contribution des verres au cycles de nutriments a ce niveau. L'objectif de cette thèse est de déterminer l'influence des communautes de verres sur les tranformations d'azote dans les sols et quantifier leur contribution au flux d'azote dans les sols pour des systems de maïs et soya au Québec. Le taux de croissance obtenu en laboratoire ont été utiliser pour prévoir comment la croissance des verres répond au fluctuations saisonnier de température et humidité du sol. Les relations entres les populations de verres, l'azote du sol et la recolte des cultures ont été evalué dans une expérience au champ. Quand les conditions ont été favourable en 2004 (temperatures < 20°C et la précipitation au moins une fois par semaine), une relation positif a été decouvert entre les verres et l'azote disponible aux plantes, incluant l'azote minéral du sol, l'azote microbial et l'azote total dans le grain de soya. En 2005, les conditions du sol n'etaient pas favourable (temperature >20°C et peu de précipitation) au survie et croissance des verres, et aucun relation a été trouver entre les populations verres et les nutriements du sol et rendement de maïs. Ces donnés m'ont permet de faire des assomptions de l'activité et vie des verres sous des conditions du champ, qui ont été utliser pour estimer le flux d'azote dans les communautés de verres dans deux modèles. La sensibilité des modèles ont été tester en variant les valeurs des parametres entre la gamme trouver dans la literature scientifique. Durant la période de pousse avec des conditions favourable, une grande population de verres (100 g matiere frais m^{-2} ou plus) est prédit d'etre responsible pour un flux autant que 120 kg N ha^{-1}. Les prédictions de modèles sont très sensible au parametres d'entrée et n'ont pas modèles n'ont pas correspondu avec le budget partielle d'azote obtenu au champ. Des prédictions précis de la minéralisation d'azote par les verres exigent des valeurs de paramètres spécifique au espèces et du site. Plus de recherche utilisant d'isotop stable ^{15}N comme traceur pourrait aider a suivre les transformations d'azote et evaluer le flux d'azote par les verres au niveau du champ.

CONTRIBUTION TO KNOWLEDGE

It has been well established that earthworms play an important role in organic matter decomposition, mineralization of nutrients and physical mixing of soils. Despite an abundance of laboratory and greenhouse-level research on how earthworms affect soil properties and plant growth, few researchers have scaled up such observations to quantify the contribution of earthworms to N cycling in agroecosystems. The current estimates vary substantially, and the N flux through earthworm populations ranges from negligible to as much as 363 kg N ha^{-1} per year. This wide range of estimates arises from differences in climate, soil properties, cropping systems, as well as disperate assumptions implicit in the quantitative methods and models used by various research. Of these factors, climate has the greatest impact on earthworm activity and, hence, is a controlling factor determining the N flux through earthworm populations. In North America, the N flux through earthworms was estimated for populations found in row-cropped agroecosystems in Ohio and Georgia, however no studies have quantified the N flux through earthworms in cold and humid temperate agroecosystems, such as those found in Québec. My Ph.D. dissertation used laboratory growth rates to predict the dynamics of earthworm growth in reponse to seasonal fluctuations in soil temperature and moisture that occur in Québec agroecosystems. Earthworm population dynamics, including survival, growth and reproduction, were assessed in a field-level manipulation experiment designed to evaluate the relationships between earthworm populations, soil-N pools and annual crop production. These data permitted me to make assumptions about earthworm activity and life histories under field conditions, and to test the sensitivity of two models used to determine the contribution of earthworms to N cycling. The experiments conducted in this thesis were designed to address these aspects, and thus, provide the following major contributions to knowledge.

1. I determined that *Aporrectodea caliginosa* grew optimally at 20°C and -5 kPa water potential, and they lost weight when the soil water potential was less than -54 kPa and when the temperature was less than 5°C.

2. I determined that earthworm growth rates are strongly influenced by the number of earthworms in a container and by the shape of the container used to culture earthworms. This work proposes the development of standard methods for assessing growth rates, so that results from laboratory studies can be extrapolated to respresent earthworm growth rates in the field.

3. Field manipulations of earthworm communities showed that when favourable climate conditions occur, larger earthworm populations are associated with more mineral-N and microbial biomass-N in surface soils, and higher grain-N yield in soybeans.

4. I developed the new "*deduction*" approach to estimate earthworm secondary production in earthworm manipulation experiments. My estimates of the N flux through secondary production range from 0.9 – 4.6 g N m^{-2} per year, and are consistent with other published rates of secondary production.

5. I present the first estimates of the contribution of earthworms to nitrogen cycling in Québec agroecosystems. My model predictions show that during the crop growing period, under favourable climate conditions, high earthworm biomass of greater than 100 g fw m^{-2} is responsible for the cycling of up to 120 kg N ha^{-1} in arable fields. However, these models are very sensitive to input parameters and accurate predictions of N mineralization require more species and site- specific parameter values, as well as validation with field data.

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General Introduction

The excessive use of inorganic mineral fertilisers and pesticides in some agricultural production systems has led to significant environmental problems in surrounding ecosystems and waterways. Improved fertiliser use would reduce crop production costs and lessen the negative impacts of nutrients in the environment. Successful management of soil nutrient pools and fertilisers requires an excellent understanding of the highly diverse, delicate and heterogeneous properties of soils. Past soil nutrient management research has focused exclusively on soil chemical and physical properties, largely overlooking the importance of soil biology. Soil organisms play a crucial role in soil fertility functions such as the decomposition and comminution of organic matter (OM), mineralisation of nutrients, and physical mixing of soils (Wardle and Lavelle, 1997; Lavelle et al., 1998; Lavelle and Spain, 2001). Amongst temperate soil fauna, earthworms are considered to have the most significant impact on macro-properties of soils, and as such are called "ecosystem engineers" (Jones et al., 1994; Lavelle et al., 1997). Their impact on soil formation was first recorded by Darwin (1881) and since then their regulation of fundamental soil processes such as nutrient cycling, OM decomposition, soil structure and biological community structure has been well established (Syers and Springett, 1984; Makeschin, 1997; Edwards, 1998).

Quantifying the contribution that earthworm communities make to nutrient transformations and fluxes in an agroecosystem is fundamental to developing better on-farm nutrient management. However, further research is needed to better understand the temporal variation in earthworm population dynamics, and to scale up laboratory data to the farm scale. The majority of research investigating nutrient fluxes through earthworm communities has been determined in laboratory microcosms, greenhouse pot experiments or small-scale field manipulations. Scaling up results from laboratory microcosms to the field level, and developing mechanistic and nutrient budget models at larger spatial and temporal scales has been identified as an essential research priority (Bohlen et al., 1995). The direct and indirect influence of earthworm communities on the flux of nutrients in soils have been quantified using a variety of methods (Christensen, 1988; Marinissen and de Ruiter, 1993; Bouché et al., 1997; Whalen and Parmelee, 2000). These estimates of N mineralization are largely based on laboratory measurements and can vary from negligible to as much as 363 kg N ha^{-1} per year due to variability in soil type, food availability, and climatic conditions as well as uncertainty in model parameters (Marinissen and deRuiter, 1993). Furthermore, many of the models lack validation with field data, and this is proposed as an important step to improving field level estimates of nutrients through earthworm communities (Whalen et al., 2000; Bouché et al., 1997).

For cold and humid temperate agroecosystems such as those found in Québec, a lack of research exists for (1) properly integrating the laboratory-based studies on earthworms into farm-scale nutrient budgets, and (2) measuring the contribution that earthworm communities have to soil nitrogen pools and plant nutrition. The purpose of this thesis project will be to determine the influence of earthworm communities on nitrogen transformations in soils and to quantify their contribution to nitrogen flux through soils for maize and soybean cropping systems of Québec.

CHAPTER 1.

Literature Review

1.1 Earthworms of Québec: Life cycle and ecological classes

Earthworms belong to the class Oligochaeta within the Annelida phylum and are divided into about 12 families largely based on geographic areas (Edwards and Bohlen, 1996). The majority of earthworm research has been conducted in Europe and North America, where the Lumbricidae family is dominant. As such the majority of research has focussed on species of this family. In southern Québec, fifteen lumbricid species of earthworms are known to exist (Reynolds, 1977; Tomlin and Fox, 2003). The most common species found in row-cropped, pasture and hayfield agroecosystems in Québec are *Lumbricus terrestris, Aporrectodea longa, Aporrectodea rosea*, and the *Aporrectodea caliginosa* complex of *Aporrectodea tuberculata* and *Aporrectodea turgida* (Whalen, 2004).

Born from cocoons, and maturing under field conditions at about 20 – 52 weeks (Wilcke, 1952; Gerard, 1967), the life span of mature lumbricid earthworms is probably no longer than a year (Satchell, 1967). A review of the literature shows that for earthworm species found in agroecosystems of Québec life history parameters have been reported for the following species: *L. terrestris, A. longa, A.caliginosa, A. tuberculata, Lumbricus rubellus, Lumbricus castaneus, Aporrectodea / Allolobophora chlorotica,* and *Octolasion cyaneum* (Lofs-Holmin, 1982; Andersen, 1987; Butt, 1993; Butt, 1997; Butt, 1998; Whalen and Parmelee, 1999).

Earthworms are commonly classified into anecic, endogeic and epigeic ecological functional groups based on their feeding and burrowing habits, and life history parameters (Bouché, 1977; Edwards and Bohlen, 1996). Anecic earthworms are large earthworms characterised as having slow growth, low reproduction rates, and considered K-strategists. They build semi-permanent vertical burrows and come to the surface to feed on litter and mate. Endogeic earthworms generally form horizontal desultory burrows in the mineral layers of the soil. They consume more mineral rich soil, are medium in size, and have high reproductive rates. The epigeic earthworms generally are smallest in size, grow rapidly, have high reproductive rates, and are considered r-strategists. They feed primarily on rich organic substrates and live in organic-rich litter layers, compost and manure piles. As such epigeic earthworms are rare in row-cropped agroecosystems, where anecic and endogeic earthworms dominate.

1.2 Earthworm growth rates

Growth rates are an important biological parameter that can be used to determine population turnover, organic matter consumption, nutrient assimilation and excretion from earthworms. Growth rates are affected by environmental conditions, food availability and food palatability. Soil moisture and soil temperature are the most important environmental parameters that influence earthworm growth rates and activity. Unlike other environmental parameters that have a significant impact on growth rates (i.e., pH, OM, texture), moisture and temperature may fluctuate significantly on short temporal scales (hours to days). For this reason, it is necessary to calculate growth rates of earthworms using the range of soil moisture and temperature conditions encountered in the field.

The life cycle and growth rates of *L. terrestris* have been well described by K. Butt and co-workers (Butt el al., 1992; Butt, 1993; Butt et al., 1994a; Butt et al., 1994b; Lowe and Butt, 2003). However, most of this research focuses on the effect of food type and palatability on growth rates and the effect of temperature on cocoon incubation times and hatchling growth. Whalen and Parmelee (1999) determined the growth rates of both *L. terrestris* and *A. tuberculata* at two soil moistures and three temperatures in laboratory cultures, and during the spring and fall in field mesocosms. Growth rates from the laboratory and field were very similar for both species. Although not a direct measurement of growth, Daniel (1991) determined food consumption by *L. terrestris* over a wide range of temperatures and moistures, and found higher consumption at temperatures of around 22°C and a matric potential greater than -20 kPa. The growth rates of *A. caliginosa* have been described for individuals consuming various food sources (Lofs-Holmin, 1982; Bostrom and Lofs-Holmin, 1986), and under different soil water potentials (Holmstrup, 2001). Doube and Styan (1996) measured the distribution, but not growth, of *A. rosea* and *A. trapezoides* to a moisture gradient in three soils with different texture and found that earthworms of both species avoided soils with a matric potential of less than -20 kPa. Although the growth of the different earthworm species have been described under these different food types and soil moistures, few studies have published growth rates for the important endogeic earthworm *A. caliginosa* under a wide range of both soil moistures and temperatures. Furthermore, in many studies soil moistures were not reported as matric potential, which makes the transferability of reported data much more difficult and specific only to the soil type tested.

1.3 Earthworm population dynamics

The temporal heterogeneity of earthworm communities is an important yet under researched topic. Since earthworms are highly responsive to small changes in soil moisture and temperature, climatic conditions control earthworm community dynamics. The length of the growing season varies in temperate regions, and studies have established that earthworm populations fluctuate throughout the year in the agroecosystems of these regions (Hendrix et al., 1992; Marinissen, 1992). However, the reasons for temporal variation and the shape of population curves are still not fully understood. Earthworms are more numerous during and just after peak precipitation periods (late spring/summer), while the fewest earthworms are collected in the driest periods of the cropping season (late summer/early autumn) (Callaham and Hendrix, 1997). Within the frost-free period of the year, a wide range of ratios of minimum : maximum populations have been recorded in a variety of ecosystems. Ratios as high as 1:16 in corn-soya agroecosystems in north-eastern USA (Werner and Dindal, 1989), and 1:10 in Slovakian meadows (Zajonc, 1970; Zajonc, 1982) have been recorded, while ratios as low as 1:2 to 1:4 have been found in temperate European grasslands (Ryl, 1984; Daniel, 1992; Spurgeon and Hopkin, 1999).

The diversity of earthworms species found in agroecosystems is surprisingly low. Most earthworm communities contain around 3 – 6 species, with a remarkable degree of consistency among different habitats and geographic regions (Edwards and Bohlen, 1996). Furthermore, the earthworm communities are often characterised by associations of specific species living together. Earthworm species *L. terrestris*, *A. longa*, *A. caliginosa*, and *A. rosea* are often found in association with one another in a variety of agroecosystems (Baker, 1983; Falco et al., 1995). The common occurrence of species together in the same community may be explained by some characteristic of the habitat or by niche overlap of the different species (Falco et al., 1995; Edwards and Bohlen, 1996).

The seasonal pattern of earthworm populations in temperate climates is very different from those of other climatic zones. In temperate climates, cocoon production tends to be greater in spring and early summer; however, due to an accumulation of cocoons during the colder months (autumn to spring), many cocoons hatch in spring, producing a large cohort of juveniles (Christensen and Mather, 1990). Juveniles surviving to late summer mature into adults and produce cocoons. Many of these individuals then die during the winter due to frost or lack of food (Daniel, 1992; Marinissen, 1992). Yet cocoons are protected from these perturbations, tending to over-winter safely and hatch in the spring to start the cycle again. This life cycle of a large juvenile dominated population in spring, and a smaller, more evenly distributed population in late summer is commonly observed (Scheu, 1992; Tomlin et al., 1992; Wyss and Glasstetter, 1992). However, some exceptions are found. In some long season grasslands in Europe (Zajonc, 1970; Ryl, 1984), and in Kansas (James, 1992), larger earthworm populations were found in autumn than in spring and summer. In both organic and conventional farms in Pennsylvania, earthworm populations were 4 – 10 times greater in autumn than in late spring/early summer (Werner and Dindal, 1989). Cocoons laid in spring may hatch during favourable humid conditions in mid-summer and may be responsible for the second cohort of juveniles in autumn (Bostrom and Lofs, 1996).

Extreme climatic events, such as drought or prolonged surface freezing, may also influence the populations. Whalen et al. (1998) found that earthworm populations, initially large in spring and autumn, were reduced significantly following a drought period in late summer and autumn, and populations did not recover for another year. Similarly, farm management activities such as tillage and fertilization may also influence the population dynamics significantly. Bostrom (1995) showed that rotary cultivation and ploughing of a grassland caused a reduction of earthworm populations by up to 77%, however a year later, earthworm numbers increased to pre-ploughing levels. This shows that earthworm populations reduced by adverse weather (drought) or physical disturbance (tillage) can recover within one season, provided food and soil conditions are favourable.

Since earthworm population dynamics can vary quite significantly between ecosystems and between climatic zones, any assessment of population dynamics needs to be determined on a climate- and ecosystem-specific basis. The majority of published reports on earthworms in Québec are surveys in forest ecosystems (Lesage and Schwert, 1978; Garceau and Coderre, 1991; Coderre et al., 1995), however there are a few reports of earthworm populations in arable agricultural systems of Quebec (Estevez et al., 1996; Whalen, 2004).

1.4 Earthworm community dynamics

Competitive and mutualistic relationships between earthworm species may significantly affect the community structure of earthworm populations. Many studies have shown that high populations and biomasses in single- and multi-species laboratory pots can have negative feedbacks on earthworms, reducing growth and fecundity (Hartenstein and Amico, 1983; Butt et al., 1994b; Dalby et al., 1998; Baker et al., 2002). Slower earthworm growth will reduce earthworm activity since earthworms are consuming less food to increase their body mass, which may consequently decrease organic matter decomposition and nutrient mineralization rates. This suggests that the results obtained in pot experiments may not quite represent the field situation if earthworm populations in pots are too high. In a recent review describing the optimal levels of abiotic and biotic factors for successful laboratory cultures of soil dwelling earthworms, population density was identified as a potentially limiting factor for earthworm growth and production (Lowe and Butt, 2005). However, greater amounts of food and improved food quality may compensate for these negative effects and allow more earthworms to be reared in cultures (Butt et al., 1994a). A better understanding of how earthworms of the endogeic and anecic functional groups coexist may help to determine how nutrient sources are partitioned and cycled through the ecosystem.

Cocoon production, hatchling growth and overall reproductive success of earthworms under different inter- and intra-species interactions have been well documented by Butt and co-workers (Butt et al., 1994b; Butt, 1998; Lowe and Butt, 2002). Cocoon production by large species such as *L. terrestris* decreases significantly as the population size increases. Similarly, reproductive effort and growth of new hatchlings of *L. terrestris* seems to be affected negatively in the presence of other species. However, this trend does not hold for all species. Some smaller species such as *Octolasion cyaneum* and *Dendrobaena veneta* were not affected or showed slight increases in cocoon production and hatchling growth (Butt, 1998). With a few exceptions, Garvin et al. (2002) found negative effects on cocoon production and growth of *Hormogaster elisae*, *A. rosea* and *A. caliginosa* when grown in the presence of the other species. However, interactions between *H. elisae* and *A. caliginosa* were not very clear. Only the growth of *H. elisae* and cocoon production of *A. caliginosa* were negatively affected by inter-species interactions. In general, reproductive success of most species is negatively affected by interactions with other species.

Selective competition for a shared food or habitat resource by competing earthworm species is hard to determine in the field (Dalby et al., 1998). In laboratory cultures, *A. caliginosa* was more strongly affected by inter-species competition with the larger sized *A. longa* than intra-species competition, while both inter- and intra-species competition are equally strong for *A. longa* (Dalby et al., 1998; Baker et al., 2002). Intra-species interactions amongst *L. terrrestris*, *A. longa*, *A. chlorotica* and *L. rubellus* in laboratory cultures caused a decrease in growth rates and lower cocoon production compared to mixed species cultures and monocultures (Lowe and Butt, 2002; Lowe and Butt, 2003). Dalby et al. (1998) showed that predation of cocoons of *Microscolex dubius* by *A. longa* could occur theoretically, suggesting it as a form of selective competition between the competing peregrine and endemic species found in Australian pastures.

Although the evidence of a decline in earthworm reproductive success due to interactions is compelling, it is not known whether competition for food or habitat occurs between the earthworm species found in Quebec agroecosystems. It is suggested that the influence of inter- and intra-species interactions on growth rates of both *A. caliginosa* and *L. terrestris* under increasing population be examined to determine competition for food resources and the possible carrying capacity of certain soils.

1.5 Earthworm contribution to soil nutrient pools and plant nutrition – pot studies

Although the general belief is that earthworms are beneficial for plant growth, the evidence for this in the scientific literature is not convincing. The effect of earthworms on plant growth and nutrition is not consistent and seems to be highly dependent on plant species, soil type, and earthworm species involved (Doube et al., 1997; Callaham et al., 2001; Scheu, 2003). The difficulty and inability of observing the movement and behaviour of earthworms and other soil invertebrates within the medium they reside in is one of the major obstacles of soil invertebrate ecology (Villani and Wright, 1990). Hence, the majority of studies have focused on greenhouse pot studies where environmental variables, populations and soil conditions can be controlled.

In a pot experiment with populations of *A. rosea* and *A. trapezoides* at levels equivalent to about 460 individuals m^{-2}, the biomass, grain weight and N content of wheat was greater than the control, but above-ground biomass of clover was only significantly greater than the control in pots with *A. trapezoides*, but not *A. rosea* (Baker et al., 1997). In microcosms involving the same two species, a significant increase in oat (*Avena fatua*) grain was recorded under treatments with both species separately and combined, however the yield of lupin (*Lupinus angustifolius*) was not affected by the addition of earthworms (Baker et al., 2003). Increasing the number of *A. rosea* and *A. trapezoides* had a significant increase on shoot weight and foliar nitrogen content of wheat in a pot experiment using a sandy loam soil (Stephens et al., 1994a). The addition of *L. rubellus* earthworms to pots at levels equivalent to about 500 individuals m^{-2} to pots did not increase maize shoot yield compared to lower populations of 0 and 250 individuals m^{-2} (Mackay and Kladivko, 1985). Doube et al. (1997) found similar significant increases in wheat and barley grown in sandy loam soils with increasing number of *A. trapezoides* but no effect on the growth of faba beans (*Vicia faba*). Ryegrass grown in a pot experiment with a high number (1040 ind. m^{-2}) of *A. caliginosa* showed significant increases in yield over controls with no earthworms (McColl et al., 1982). This contradicts results by James and Seastedt (1986) which show that the yield of big bluestem tall grass (*Andropogon gerardii*) was not affected by either Lumbricid earthworms, *Aporrectodea turgida*, or native Acanthodrilidae earthworms of the genus *Diplocardia spp.*

The majority of pot experiments suggest that yield improvements due to earthworms may benefit cereals and grasses greater than legumes. This is possibly due to the independent nitrogen uptake associated with leguminous plants. Conversely, the burrowing activity of earthworms in known to increase the vertical transport of microflora, which may benefit leguminous plants if the dispersion of symbiotic flora is enhanced (Madsen and Alexander, 1982; Thorpe et al., 1996). Root nodulation of subterranean clover by *Rhizobium leguminosarium* was enhanced in the presence of *A. trapezoides* (Doube et al., 1994). Similarly, *Rhizobium meliloti* was found on roots of alfalfa in greater numbers and at greater depths as the number of *A. trapezoides* in pots was increased (Stephens et al., 1994b).

Doube et al. (1997) found that there was no universal rule predicting the effect of earthworms on plant growth, and that the effects are highly dependent on soil type. They found that wheat and barley plants showed significantly better results due to earthworm addition when grown in sandy loam soils, but that the effect of the addition of earthworms was less in loamy and clay soils, with the barley yield in clay soils lower when earthworms were added. Callaham et al. (2001) reported that the influence of native *Diplocardia* spp.and exotic *Octolasion tyrtaeum* on soil microbial biomass and plant N uptake in tall grass prairie soils differed significantly between earthworm species. As well as soil type and earthworm species, plant species (Kreuzer et al., 2004; Wurst et al., 2005), and fertility treatments (Blair et al., 1997) are also major factors that affect the relationship between earthworms, soil-N pools, plant nutrient uptake and yield.

An important argument against the scaling up of results from pot studies to the farm-scale are the high populations often used. Populations in pot experiments that range as high as

630 ind. m^{-2} (Doube et al., 1997) and 1040 ind. m^{-2} (McColl et al., 1982) are much higher than field populations. Another argument is the high mortality rate of introduced earthworms, ranging from 10 – 46 % (McColl et al., 1982; Doube et al., 1997; Baker et al., 2003), which may contribute a significant amount of nutrients through the decomposition of earthworm tissues. Determining earthworm nutrient contribution to plants from pot studies may lead to large differences from actual values due to the high populations and high mortality rates that occur in pot studies.

To improve the reliability of estimates it is necessary to increase the size of the "pot" so as to include more natural soil structure, weather conditions and realistic populations. The migration habits of earthworm species can be used in developing a method to enclose them so as to better study their effects on soil and plants. Horizontal movement by endogeic earthworms typically occurs in the upper 20cm of the soil while anecic species typically travel on the soil surface (Bouché, 1977; Francis et al., 2001; Bastardie et al., 2003). Therefore, a barrier dug to depths of 35 – 50 cm and protruding above the ground by 10 cm should, in theory, retain most of the earthworms in the "pot". Field studies with buried enclosures may be the best method of estimating field level contributions of earthworms to soils and plants.

1.6 Earthworm contribution to soil nutrient pools and plant nutrition – field studies

Enclosure studies have provided a unique way of studying the effects of earthworm communities on soils and plants *in situ*. Field enclosures studies involving earthworms are relatively recent and very few studies have been conducted. Therefore, efficient methods to manipulate earthworm communities *in situ* are still under development. The size of field enclosures have varied from 30 cm diameter cylinders (Baker et al., 1996) to large 6.1 x 6.1 m enclosures (Subler et al., 1997) with most other experiments using rectangular enclosures ranging from 1 – 20 m^2 (Bohlen et al., 1995; Zaller and Arnone, 1999). The manipulation of earthworm communities *in situ* requires new methods and unique field techniques to be developed. Electro-shocking has been used to reduce earthworm populations in arable soils (Bohlen et al., 1995). Removing top soil monoliths during periods when earthworms are absent from the topsoil was shown to be a successful method to reduce resident populations of earthworms (Baker et al. 1996). The addition of earthworms to soils with very low or no resident earthworm community, such as mine spoils, landfills, peat lands or volcanic ash soils (andisols), has been useful to study the effects of added earthworms separately from any naturally occurring population without the specific need for enclosures (Curry and Boyle, 1987; Boyer et al. 1999; Emmerling and Pausch, 2001; Butt et al., 2004).

The manipulation of earthworm communities in field enclosures has had varied success. In enclosure experiments in Ohio, high mortality among added earthworms was suspected since populations showed either moderate or no growth in the added earthworm treatments (Bohlen et al., 1995; Subler et al., 1997). Similar low to moderate survival rates of introduced earthworms, and invasion of moderate numbers of non-introduced species have been recorded in enclosure experiments in Australia and Reunion Island (Baker et al., 1996; Baker et al., 1999; Boyer et al., 1999; Baker et al., 2002).

However, successful manipulations of earthworm communities have been recorded in German and Swiss enclosures studies where populations increased between 1.5 – 5 fold in earthworm addition treatments (Zaller and Arnone, 1999; Emmerling and Pausch, 2001). In all of these studies, the success of earthworm manipulations into field enclosures was not consistent for any particular species or functional group.

Due to the limited number of earthworm enclosure studies and the varied success of earthworm community manipulations the effects of earthworms on soil nutrient pools are inconsistent and show mixed results. In a maize-based enclosure study in Ohio, the addition of earthworms increased the incorporation of surface litter and an increase in the C:N ratio of surface litter (Bohlen et al., 1997). Furthermore, earthworm additions increased soil NO_3-N concentration over a two-year period in inorganically fertilized plots but not in manure or legume fertilized plots (Blair et al. 1997). In another enclosure study in Ohio, Subler et al. (1998) reported a greater increase in soil-N pools with earthworm addition treatments to inorganically fertilized plots but not in legume or manure fertilized plots. Earthworm additions also influence the depth stratification of available nitrogen either through the incorporation of litter, mineralization of the soil OM or increased nutrient flow (Bohlen et al, 1997; Shuster et al., 2002). In enclosure studies in Ohio, earthworm additions increased soil NO_3-N concentration at lower depths (15 – 45 cm) in two consecutive growing seasons and in the 0 – 15 cm depth in only one of the two growing seasons (Blair et al., 1997). In contrast, a mesocosm experiment by Bohlen and Edwards (1995) demonstrated that earthworms increased the amount of NO_3-N at the 0 – 5 cm depth but had no effect at the 5 – 15 cm depth. In another enclosure study in

Ohio, the addition of earthworms did not increase mineral-N in maize-soybean or maize-soybean-wheat systems in the 0 – 45 cm depths but did increase pools of organic N (MBN and DON) (Subler et al. 1997). There have been many conflicting reports on how earthworms affect MBN in the field, which may be due to differences in soil organic matter, earthworm species, nutrient inputs and climate (Blair et al., 1995; Bohlen and Edwards, 1995; Blair et al., 1997; Subler et al., 1997; Callaham and Hendrix, 1998; Aruajo et al., 2004).

The effects of earthworm manipulations on plant growth in field enclosure studies also show mixed results. In a field enclosure study in Ohio, increasing earthworm populations did not affect biomass, tissue-N concentration or total-N yield of maize. In fact, maize yield was higher in plots with reduced earthworm populations, and this was partially explained by less weed and pest pressure in the reduced earthworm treatments (Stinner et al., 1997). In field enclosures on Reunion Island, maize yield was greater in earthworm addition treatments but only when a trefoil cover crop was present (Boyer et al., 1999). The trefoil probably provided food for the earthworms, who accelerated decomposition of the cover crop, releasing available nutrients for the maize plants (Boyer et al., 1999). Overall, there have been very few studies reporting the effects of earthworm manipulations on plant growth in enclosure studies. This justifies further field enclosure studies to improve our knowledge of nutrient flows from earthworm communities to crops.

1.7 Calculation of nutrient fluxes through earthworm communities – Comparison of different models

Estimates of the direct and indirect nitrogen flux through earthworm communities range from 7 to 363 kg N ha^{-1} per year (Satchell, 1963; Syers and Springett, 1984; Christensen, 1987; Parmelee and Crossley, 1988; Marinissen and de Ruiter, 1993; Curry et al., 1995; Whalen et al., 2000). The wide range of estimates is primarily caused by differences in the methods and value of parameters used to make estimates, and by differences in earthworm biomasses in different agroecosystems. Estimates made by different methods for the same field can vary as much as 7-fold (Marinissen and de Ruiter, 1993). While estimates using the same model but with small differences in the value of parameters can vary as much as 4-fold (Curry et al. 1995). It is therefore important to compare estimates of N flux among different methods and with varying parameter values.

Secondary production is an energetics approach to determine the production of earthworm biomass and turnover of earthworm populations. It has been used to estimate N flux through earthworm populations ranging from 15 – 55 kg N ha^{-1} per year (Bostrom, 1988; Parmelee and Crossley, 1988; Curry et al., 1995; Whalen and Parmelee, 2000). The "*food web*" model is a static model that considers a mean earthworm biomass over the season. It derives N mineralization for the mean earthworm biomass from feeding rates and the partitioning of nutrients between the consumer (earthworm), the food source (detritus or microbes), and the environment (soil) (Hunt et al., 1987; de Ruiter et al., 1994). Estimates of N mineralization of 11 – 51 kg N ha^{-1} per year have been reported using the "*food web*" model for agroecosystems in the Netherlands but no estimates for other agroecosystems have been reported in the literature (Marinissen and de Ruiter, 1993; Didden et al., 1994;). The summation of all direct N excretions from earthworm casts, urine, mucus and dead biomass provides another method to calculate N flux from earthworm communities. Estimates of direct N excretions range from 7 – 74 kg N ha^{-1} per year, but these estimates are very sensitive to small differences in parameter values (Christensen, 1987; Christensen, 1988; Parmelee and Crossley, 1988; Marinissen and de Ruiter, 1993; Curry et al., 1995).

The "*food web*" model may also be used to estimate the stimulatory effect of earthworms grazing on microbial populations, and the subsequent microbial N mineralization. Estimates of N mineralization from the stimulated microbes is estimated to be 5 – 10 fold higher than estimates of direct contributions without microbial grazing (Marinissen and de Ruiter, 1993; de Ruiter et al., 1994). However, estimates of the indirect N mineralization from earthworms are very sensitive to parameter values and were shown to vary between 5 – 70 kg N ha^{-1} per year for small differences in parameter values (Marinissen and de Ruiter, 1993).

The majority of parameters used in these model predictions are taken from literature values and may be applicable to only certain agroecosystems. As I have discussed in the previous sections, growth rates and other life history parameters, community level interactions, and the effects of earthworms on soil and plant nutrient pools are earthworm species-, soil-, and climate-specific. Therefore, there is a need to obtain as many of these parameters from similar studies in order to make valid and accurate predictions of the contribution of earthworms to nitrogen cycling. With the exception of Whalen et al. (1999) who showed good correlation between observed growth of *A. tuberculata* individuals and model predictions based on nutrient flow through earthworm bodies, few studies have shown field scale validation of model-based nutrient flux predictions (Bouche et al., 1997). This justifies the need to further investigate the different types of models, the sensitivity of models to parameter values, and validate model predictions with field level data.

1.8 Research questions

The objectives of this research project are 1) to obtain earthworm growth rates for soil conditions and earthworms specific to Québec, 2) to measure the influence of earthworm communities on soil nutrient pools in Québec agroecosystems, and 3) develop a model that can be used in other agricultural fields in Québec to predict the nitrogen flux through earthworm communities. To this end the following research questions will be answered.

Research question 1) How are earthworm activity and growth rates affected by environmental conditions and community interactions?

Research question 2) Are earthworm growth rates affected by the size of the experimental container, and are earthworm growth rates obtained in the laboratory equivalent to growth rates in the field?

Research question 3) What contribution do earthworm communities make to soil nutrient pools, plant nutrition and yield in soybean and maize agroecosystems in Québec?

Research question 4) How much variability is there in current earthworm nitrogen mineralization models, and can field data be used to validate model predictions of nitrogen flux through earthworm communities?

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CHAPTER 2.

Growth rates of *Aporrectodea caliginosa* (Oligochaetae: Lumbricidae) as influenced by soil temperature and moisture in disturbed and undisturbed soil columns.

2.1 Abstract

Earthworm growth is affected by fluctuations in soil temperature and moisture and hence, may be used as an indicator of earthworm activity under field conditions. There is no standard methodology for measuring earthworm growth and results obtained in the laboratory with a variety of food sources, soil quantities and container shapes cannot easily be compared or used to estimate earthworm growth in the field. The objective of this experiment was to determine growth rates of the endogeic earthworm *Aporrectodea caliginosa* (Savigny) over a range of temperatures (5-20°C) and soil water potentials (-5 to-54 kPa) in disturbed and undisturbed soil columns in the laboratory. We used PVC cores (6 cm diameter, 15 cm height) containing undisturbed and disturbed soil, and1-l cylindrical pots (11 cm diameter, 14 cm height) with disturbed soil. All containers contained about 500 g of moist soil. The growth rates of juvenile *A. caliginosa* were determined after 14 to 28 days. The instantaneous growth rate (IGR) was affected significantly by soil moisture, temperature, and the temperature × moisture interaction, ranging from -0.092-0.037 d^{-1}. Optimum growth conditions for *A. caliginosa* were at 20°C and -5 kPa water potential, and they lost weight when the soil water potential was -54 kPa for all temperatures and also when the temperature was 5°C for all water

potentials. Growth rates were significantly greater in pots than in cores, but the growth rates of earthworms in cores with undisturbed or disturbed soil did not differ significantly. The feeding and burrowing habits of earthworms should be considered when choosing the container for growth experiments in order to improve our ability to extrapolate earthworm growth rates from the laboratory to the field.

2.2 Introduction

Earthworms are known to accelerate nutrient mineralization and improve soil fertility in temperate agroecosystems (Lee, 1985; Edwards and Bohlen, 1996). The contribution of various earthworm species to nutrient mineralization is affected by their feeding habits and life-history strategies, because individuals from different ecological groups are active in different parts of the soil profile when environmental conditions are favourable (Bouché, 1977; Brown et al., 2004). Furthermore, earthworm mediated nutrient mineralization may be related to their activity and growth (Marinissen and de Ruiter, 1993). Earthworm growth rates are very responsive to fluctuations in soil temperature and moisture, and may be used to estimate activity and dynamics of earthworm populations (Buckerfield et al., 1997). In temperate agricultural soils, earthworm growth is fastest at soil temperatures from 15–20°C when the soil moisture is close to field capacity (Daniel et al., 1996; Holmstrup, 2001; Wever et al., 2001; Baker and Whitby, 2003). However, soil temperatures range from about 0-25°C and there may be periodic flooding and drought during the crop growing season. Researchers wishing to estimate nutrient mineralization from earthworms require detailed information on how earthworm growth rates fluctuate with changing soil temperature and moisture conditions.

There is no standard methodology for measuring earthworm growth rates. A review of the literature reveals that growth rates for the major lumbricid earthworm species have been determined using a variety of food sources, amounts of soil and containers (Butt, 1997; Fayolle et al., 1997; Whalen and Parmelee, 1999; Booth et al., 2000). When provided with abundant organic matter with a high N content, earthworms grow faster than when they receive a restricted amount of food or one with a low N content (Boström and Lofs-Holmin, 1986; Boström 1988; Daniel, 1991). Many earthworms grow faster when they consume finely-ground than coarsely ground organic substrates (Boström and Lofs-Holmin, 1986; Lowe and Butt, 2003). Little is known of the relationships between the amounts of soil or the shape of the culture vessel may have on earthworm growth rates. Growth rates have been measured commonly in the laboratory in 40 g to 2000 g of soil in containers with volumes ranging from 0.12 l to 2.2 l (Butt et al., 1994; Whalen and Parmelee, 1999; Baker and Whitby, 2003). In these studies, loose soil was packed or placed into the container before earthworms were added.

We hypothesize that earthworm growth rates will differ when earthworms are grown in disturbed soil than in undisturbed soil. An undisturbed soil core obtained from the field will likely contain some burrows and macropores that facilitate earthworm movement and reduce their energy expenditure in moving through soil, thereby increasing growth rates. Containers may constrain earthworm movement, reducing the energy used to burrow and increasing the energy allocated for growth. Whalen and Parmelee (1999) reported that growth rates of *A. tuberculata* (Eisen) were similar in 0.12 l laboratory pots and 7.9 l field cores, but juvenile *L. terrestris* L. had slower growth rates in field cores than in laboratory cultures. The amount of soil and shape of the culture vessel used in laboratory studies should provide growth data that is representative of earthworm activity under field conditions.

The objectives of our experiment were: (1) to determine how growth rates of *A. caliginosa* were influenced by soil temperature and moisture; and (2) to determine whether earthworm growth rates were influenced by soil disturbance and culture vessel shape.

2.3 Materials and Methods

2.3.1 Collection of earthworms and soils

Juvenile individuals of *A. caliginosa* were collected by hand-sorting in September 2003 from fields under alfalfa (*Medicago sativa* L.) and soybean (*Glycine max* (L.) Merrill) production at the Macdonald Campus Farm of McGill University, Ste-Anne-de-Bellevue, Québec, Canada. Earthworms were reared for about 6 weeks at room temperature (20°C) in soil from the field site, moistened to near field capacity. Newly emerged earthworms (<0.25 g) and pre-clitellite earthworms (>0.70 g) were excluded from the analysis as their growth rates may not be truly representative of juvenile earthworms. In total less than

20% of selected earthworms were excluded from the analysis for being outside of the desired size range (0.25 - 0.70 g).

The soil was a sandy-loam mixed, frigid Typic Endoquent of the Chicot series taken from a field under soybean production. It had a pH (H_2O) of 6.3, a C content of 30.2 g C kg^{-1}, and contained 580 g kg^{-1} sand, 300 g kg^{-1} silt and 120 g kg^{-1} clay. Soils were air-dried to about 10% gravimetric moisture content (-200 kPa matric potential) before use. The earthworm food was composted cattle manure containing about 383 g C kg^{-1} and 19.9 g N kg^{-1} (Carlo Erba Flash NC Soils Analyzer, Milan, Italy).

2.3.2 Calculation of soil moisture content

Four soil gravimetric moisture contents (15%, 20%, 25%, and 30%) were used in the experiment to test a range of moisture conditions. Since matric potential is a more meaningful way to express biological water availability, the matric potential was calculated for each gravimetric moisture content using the Rosetta software program (Schaap, 2000). A SSCBD (texture and bulk density) pedotransfer function was used to predict the parameters necessary for calculating matric potential using the van Genuchten function for water retention (van Genuchten, 1980; Schaap et al., 1998). The calculated matric potentials are -5, -11, -23, -54 kPa, corresponding to 30%, 25%, 20%, 15% gravimetric moisture content, respectively.

2.3.3 Pot experiment

This experiment involved a completely randomised factorial design with four temperatures (5°C, 10°C, 15°C and 20°C), and four soil water potentials (-5, -11, -23, and -54 kPa), for a total of 16 factorial treatments. Each treatment was replicated 10 times. Each replicate pot was a 1-l cylindrical plastic pot (11 cm diameter, 14 cm height) with a perforated lid containing 400–480 g of air dry soil (sieved < 10 mm mesh, 500 g of moist soil), and 3 g (dry matter basis) of manure (sieved < 4 mm mesh). The manure was mixed into the top 5cm of the soil where endogeic earthworms typically consume their food. The food and soil mixture was incubated for 2–5 days before adding the earthworm.

Juvenile earthworms with a mean mass of 0.35 ± 0.11 g (S.D.) (n=1028) were washed and placed on moistened paper to void their guts for 24 h. The next day the earthworms were washed, gently blotted dry with paper towels and weighed (gut-free fresh weight). One earthworm was added to each pot which was then sprayed with approximately 3 ml water to remoisten the earthworm and soil surface. Pots were placed into controlled climate incubators at four temperatures in darkness for the duration of the experiment.

Earthworms were reared in pots for 8 weeks and were removed every 13–15 days for weight measurements. At each weighing, earthworms were washed, placed on a moistened paper to void their guts for 24 h, weighed gut-free fresh weight and then returned to the same pot for 13–15 days. Washing and keeping the earthworms on a moistened paper for 24 h ensures that the earthworms from different soil moisture treatments have equal hydration status when weighing them gut-free. Before returning earthworms to the pots, about 1 g (dry matter basis) of manure was added to the soil surface, pots were weighed and tap water was added to replace moisture lost through evaporation. When dead earthworms were found, they were removed and a replacement earthworm of similar weight and age class was added to the pot. The growth rates for replacement earthworms were considered to be missing values in the statistical analysis.

2.3.4 Core experiment

The experiment was designed as a completely randomised factorial design with three temperatures (10, 15 and 20°C), three soil water potentials (-5, -11, and -23 kPa), and two soil disturbance treatments (undisturbed and disturbed) with 8 replicates of each treatment. Each replicate core was soil in a PVC plastic tube with an internal diameter of 6 cm, a height of 15 cm and a volume of 0.425 l. Disturbed soil cores contained sieved (<10 mm mesh) soil that was packed to a bulk density of 1.23 ± 0.01 g cm^{-3} (S.E.) (n=72), equivalent to the bulk density found in the undisturbed cores. This was achieved by gently pounding the core on the lab bench until the desired bulk density was achieved. Undisturbed soil cores, taken from the same field site, were obtained by hammering the PVC tube into the ground above a visible earthworm burrow and digging out the core. Fine plastic mesh (1.5 mm) was secured with elastic bands on both ends of the core to prevent soil losses. Undisturbed soil cores were kept in a cold room at 0°C for 6 weeks to kill any earthworms that may have been collected in the core. Each core contained between 300–425 g of air dry soil (400-600 g of moist soil after adding different amounts of tap water based on the moisture treatments).

Juvenile earthworms were washed and placed on moistened paper to void their guts for 24 h, then removed, washed, gently blotted dry with paper towels and weighed (gut-free fresh weight). Earthworms added to the undisturbed and disturbed soil cores had a mean gut-free fresh biomass of 0.43 ± 0.14 g (S.D.) (n=59), and 0.38 ± 0.11 g (S.D.) (n=61), respectively. One earthworm was added per core, and 5 g dry matter of manure was placed on the soil surface. The surface of the soil in each core was sprayed with approximately 3 ml water to remoisten the earthworm and soil surface. Cores were placed in controlled climate incubators in darkness for 28 days, then earthworms were removed from each core, placed on a moistened paper to void their guts for 24 h, and their gut-free fresh weights determined. Replicates with dead earthworms were excluded from the statistical analysis.

2.3.5 Calculation of earthworm growth rates

Earthworm growth rates are commonly reported as either average growth rates or relative growth rates, and while these measurements may be useful for laboratory experiments in which the growth of an age-specific cohort is followed to maturity, they assume that earthworm growth through time is a continuous linear function (Whalen, 1998). It has been well established that earthworm growth through time follows a logistic curve (Daniel et al., 1996; Phillipson and Bolton, 1977). As an earthworm approaches maturity, a greater proportion of the energy from food resources is likely used in the formation of sexual organs and reproduction rather than the formation of new tissues (Daniel et al., 1996). Instantaneous growth rates (IGR, d^{-1}), which assume that growth proceeds logistically rather than linearly, are better able to account for these factors by calculating the change in an individual's growth during an infinitely short time interval (Diehl and Audo, 1995; Pertrusewicz and Macfayden, 1970). The IGR was calculated using equation (1).

$$IGR = \ln (W_f / W_i)/?t \qquad (1)$$

where W_i and W_f are initial and final earthworm mass (g), respectively, and ? t is the growth interval measured in days (Brafield and Llewellyn, 1982). The IGR was calculated for 14 and 28 day growth intervals in the pot study, and for a 28 day interval in the core study. The effects of container shape on earthworm growth were assessed using the IGR calculated for a 28 day growth interval.

2.3.6 Statistical analysis

The effect of temperature, moisture, container type, sampling time and the temperature×moisture interaction on earthworm growth rates from the pot and core study were evaluated using the PROC MIXED function of SAS software (SAS Institute, 2001). The MIXED procedure uses generalized least squares to estimate and test for fixed effects in the model, which is superior to the ordinary least squares used by the GLM procedure, and is the preferred method for analysis of animal growth experiments with repeated measures data since it can handle missing data in an unbalanced design (Wang and Goonewardene, 2004; Spilke et al., 2005). The difference between least square means of significant treatment effects were evaluated at the 95% confidence level using the LSMEANS statement in SAS. Regression lines were fitted using the PROC REG function of SAS.

2.4 Results

2.4.1 Mortality

Earthworm mortality in the pot study was generally less than 8%, although in soils at -54 kPa water potential there was up to 26% mortality. In the core study, mortality ranged from 0–28.5%, and was not different in the intact and packed cores.

2.4.2 Temperature and moisture effects on earthworm growth

In the pot study, soil temperature (F=26.1, P<0.0001), moisture (F= 23.8, P<0.0001) and the interactions between temperature and moisture (F=4.1, P<0.0001). were all significant factors affecting growth. Growth rates were significantly affected (F=4.8, P<0.003) by the repeated weight measurements on the same individual. This indicates a change in growth rate as the individual earthworm grows. The change in growth rates as an individual changes in weight is a common relationship in many earthworm and animal growth studies (Wange and Goonewardene, 2004; Mir et al., 1998; McElroy et al., 1997). All earthworms lost weight when placed in soil with a water potential of -54 kPa, so the growth data for this treatment were excluded from Fig. 1. Growth was negative (indicating weight loss) at 5°C, regardless of the moisture content, and at 10°C when the soil water potential was -11 and -23 kPa (Fig 1). The IGR was greatest at -5 and -11 kPa water potential.

2.4.3 Effects of container on growth

In the core study, soil moisture (F=63.0, P<0.0001) was the most significant factor affecting growth, followed by soil temperature (F=34.3, P<0.0001), the interactions between temperature and moisture (F=10.7, P<0.0001) and container type (F=4.9, P<0.008). A paired means comparison test showed that growth rates in the pot study were greater than in disturbed soil cores (P = 0.017) and undisturbed soil cores (P=0.006). However, the growth rates obtained from undisturbed and disturbed soil cores were not significantly different.

In soils at 10°C, earthworm growth rates were positive at water potentials greater than -11 kPa (Fig. 2A). In soils at 15°C and 20°C, positive growth rates were observed at dryer conditions in pots (-23 kPa) than cores (-11 to -15 kPa) (Fig. 2B & C). Logistic growth describes best earthworm growth in pots at all three temperatures, whereas earthworm growth in disturbed and undisturbed cores were described best by linear equations at 10°C, and both linear and logistic equations at 15°C and 20°C (Table 1).

2.5 Discussion

The rates of growth of *A. caliginosa* were influenced by interactions between soil temperature and moisture. Growth rates increased logistically with rising water potential when the soil temperature was 10 to 20°C, but growth remained negative at 5°C for all water potentials. Growth rates were significantly greater at -5 kPa than at -11 kPa when the soil temperature was 10 to 20°C, but were not different between water potentials of -11 and -23 kPa for temperatures between 5 and 15°C. In other experiments soil temperature and moisture interacted significantly to influence the growth of *A. tuberculata* (Wever et al., 2001) and *L. terrestris* (Berry and Jordan, 2001). They found that earthworm growth rates were influenced more by soil moisture at higher temperatures (20°C or higher) than at lower temperatures. In our study, earthworms lost weight when the soil water potential was lower than -11 kPa at 10°C, and -23 kPa at 15°C and 20°C, suggesting that there may be critical moisture levels for earthworm growth. Holmstrup (2001) reported a significant reduction in weight of adult and juvenile *A. caliginosa* when the water potential was lower than -12 and -19 kPa, respectively. At water potentials lower than -19 kPa, all juveniles entered diapause and lost weight. Similar results were obtained for other species in laboratory studies. *A. trapezoides* avoided soil with a water potential less than -15 kPa in sandy loam and -25 kPa in loam (Doube and Styan, 1996), and *A. longa* lost weight at water potentials lower than -40 kPa (Kretzchmar and Bruchou, 1991).

The earthworm growth rates in this experiment ranged from -0.092-0.037 d^{-1}, and were slightly slower than those reported elsewhere (Whalen and Parmelee, 1999; Booth et al., 2000). The growth rates for *A. tuberculata* (Whalen and Parmelee, 1999) were 2 to 3 times faster (0.0108–0.0167 d^{-1}) than those in this experiment at 10°C and water potentials of -5 kPa to -23 kPa. The growth rates for *A. tuberculata* (Wever et al., 1999) ranged from -0.05 – 0.05 d^{-1} at 20°C and -0.007–0.015 d^{-1} at 15°C in soils with moisture contents of 10%–25%. These results agree with our values obtained at similar moisture contents (water potentials of -11 to -23 kPa). Booth et al. (2000) measured growth rates for *A. caliginosa* over the same range of gravimetric moistures (15–30%) and temperatures (5–20°C) as we did, but with more variability in their experiment. In their experiment, optimal conditions for earthworm growth were at 10–15°C in soils with 25-30% moisture content, and the IGR ranged from 0.026–0.063 d^{-1}. Earthworms lost weight when the soil moisture was 15%, regardless of temperature (Booth et al., 2000). Mazantseva (1982) reported that the IGR of *Nicodrilus caliginosus* (a variant name for *A. caliginosa*, Reynolds (1977)) was 0.019–0.028 d^{-1} at 15–20°C and optimal soil moisture, while earthworms lost weight at temperatures below 12°C, similar to our findings.

Differences in the IGR of *A. caliginosa* in these studies may be explained by the initial body mass of the earthworm. Earthworm growth rates are related inversely to their initial body masses, where rates of weight gain decrease as the initial body masses of earthworms increase (Daniel et al., 1996; Whalen and Parmelee, 1999). Mazantseva (1982) showed that the IGR was 50% less for 20-30 day old earthworms than for newly-emerged earthworms. The earthworms used in many previous studies were smaller than those used in this study, which may explain why they reported faster growth rates for *A. caliginosa*.

Other factors that may affect growth rates are the quantity of soil, shape of the container and fluctuating temperature regimes. Some researchers kept earthworms in 40 g (Whalen and Parmelee, 1999) and 100 g of soil (Wever et al., 2001), which is 10–25 times less than the quantity used in other experiments (Booth et al., 2000). We demonstrated that growth rates of earthworms in pots were greater than those of earthworms in soil cores. It is important to consider the behaviour of earthworms when selecting a container for measuring earthworm growth rates. The soil cores had half the diameter of the pots, which may have forced the earthworms to burrow vertically, contrary to the natural habits of this endogeic species to build temporary, shallow horizontal burrows (Francis et al., 2001; Jégou et al. 2001). Uvarov (1995) showed that earthworms kept in cultures at a constant temperature (15°C) lost more weight than those kept in cultures at a fluctuating temperature regime (10-20°C). However, the effects of different fluctuating temperature regimes on weight loss were not significant until after 4 months in culture (Uvarov, 1995). Since our earthworms were kept for only 8 weeks in controlled climate incubators, we assume that there was no effect of a constant temperature regime on growth rates.

The treatment effects of container type are not entirely due to the shape of the container only. To maintain an undisturbed soil it was not possible to mix the food into the top 5 cm of the soil as in the pot study. Therefore, the pot and cores have different shapes and placement of food. However, since endogeic earthworms typically consume more humified organic matter in the mineral horizons of the soil (Edwards and Bohlen, 1996), the placement of fresh organic matter on the surface would most likely have had little effect on available food resources. The volume of soil in each container was small compared to how much soil an earthworm could burrow through, therefore regardless of where the food was placed it was still easily accessible to the earthworm. Visual observations confirmed that earthworms were active throughout the containers and came into contact with the surface applied food. We assume that the different placement of food in the two container types could be a considered a minor source of error.

Soil disturbance did not affect the growth of *A. caliginosa* because the IGR did not differ between disturbed and undisturbed soil cores. Since the amounts of soil were similar in both pot and core studies, we suggest that the container shape influenced earthworm growth more than soil disturbance. It appears that the presence of intact earthworm burrows and other macropores in undisturbed soil cores did not increase *A. caliginosa* growth. Capowiez and Belzunces (2001) reported that earthworm burrow systems are individual structures, rarely used by other earthworms. They suggest that abandoned burrows may be recolonised only by earthworms from the same ecological class. The undisturbed soil cores were obtained above a surface burrow, most likely created by an anecic earthworm, and were probably not used by the endogeic *A. caliginosa* species introduced into the core.

Our study confirms that temperature and moisture strongly influence earthworm growth rates and activity. Optimum environmental conditions for growth of *A. caliginosa* were

20°C and a water potential of -5 kPa. Higher temperatures were not tested, but the upper limit for survival of many lumbricid species is around 25°C, because many life history parameters, such as growth rates, cocoon production, and time to reach sexual maturity, decrease at temperatures above 20°C (Butt, 1991; Daniel et al., 1996; Berry and Jordan, 2001; Baker and Whitby, 2003). Furthermore, we showed that earthworm growth rates were influenced by the shape of the container used. Further work is needed to establish standard experimental parameters (i.e., food source, growth interval, quantity of soil and shape of container) that ensure laboratory measurements of earthworm growth rates are representative of those in the field.

2.6 References

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Table 1. Regression equations describing the instantaneous growth rate (IGR) for *A. caliginosa* as a function of soil water potential (?) for each container type and soil temperature conditions presented in Fig. 2. Lines were fitted through the average IGR values at each water potential.

	10°C	
Pot	$IGR = -0.0042Ln(\psi) + 0.011$	$R^2 = 0.986$
Disturbed Core	$IGR = -0.0002*\psi + 0.0029$	$R^2 = 0.989$
Undisturbed Core	$IGR = -0.0004*\psi + 0.004$	$R^2 = 0.940$
	15°C	
Pot	$IGR = -0.0034Ln(\psi) + 0.0118$	$R^2 = 0.991$
Disturbed Core	$IGR = -0.0001*\psi + 0.0024$	$R^2 = 0.953$
Undisturbed Core	$IGR = -0.0073Ln(\psi) + 0.0195$	$R^2 = 0.958$
	20°C	
Pot	$IGR = -0.007Ln(\psi) + 0.022$	$R^2 = 0.967$
Disturbed Core	$IGR = -0.0079Ln(\psi) + 0.0218$	$R^2 = 0.997$
Undisturbed Core	$IGR = -0.0007*\psi + 0.0123$	$R^2 = 0.985$

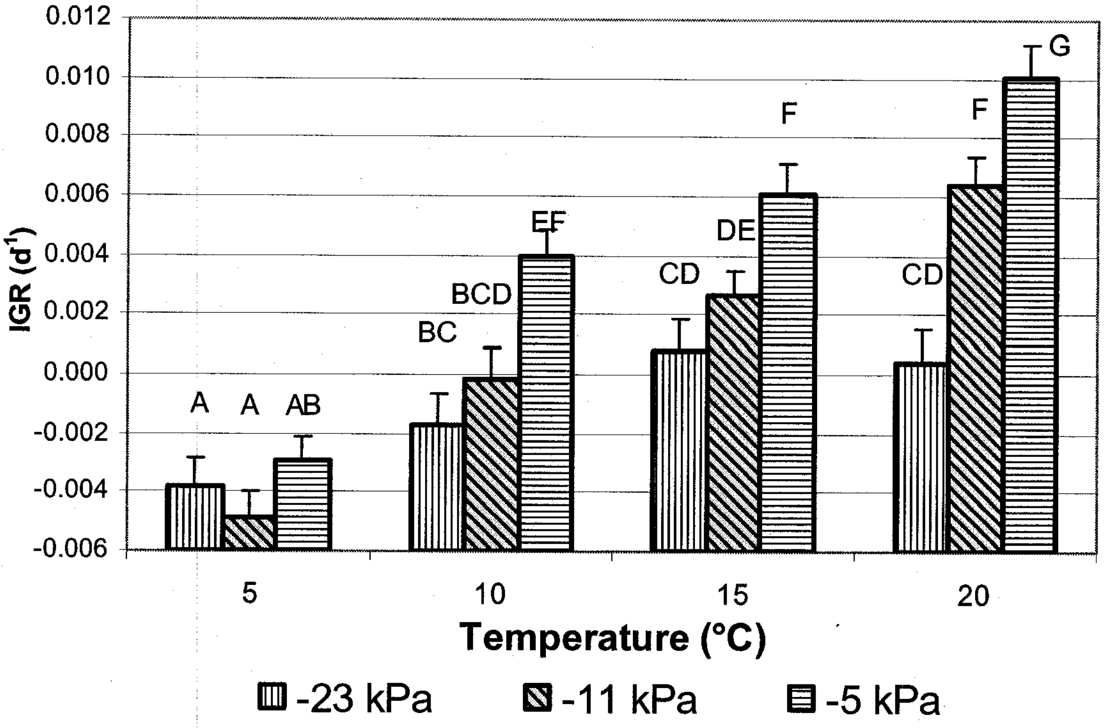

Figure 1. Influences of soil temperature and moisture on the instantaneous growth rate (IGR) of *A. caliginosa* in pots. Values are shown as mean ± S.E. (n=10). Columns with the same letters did not differ significantly ($P < 0.05$).

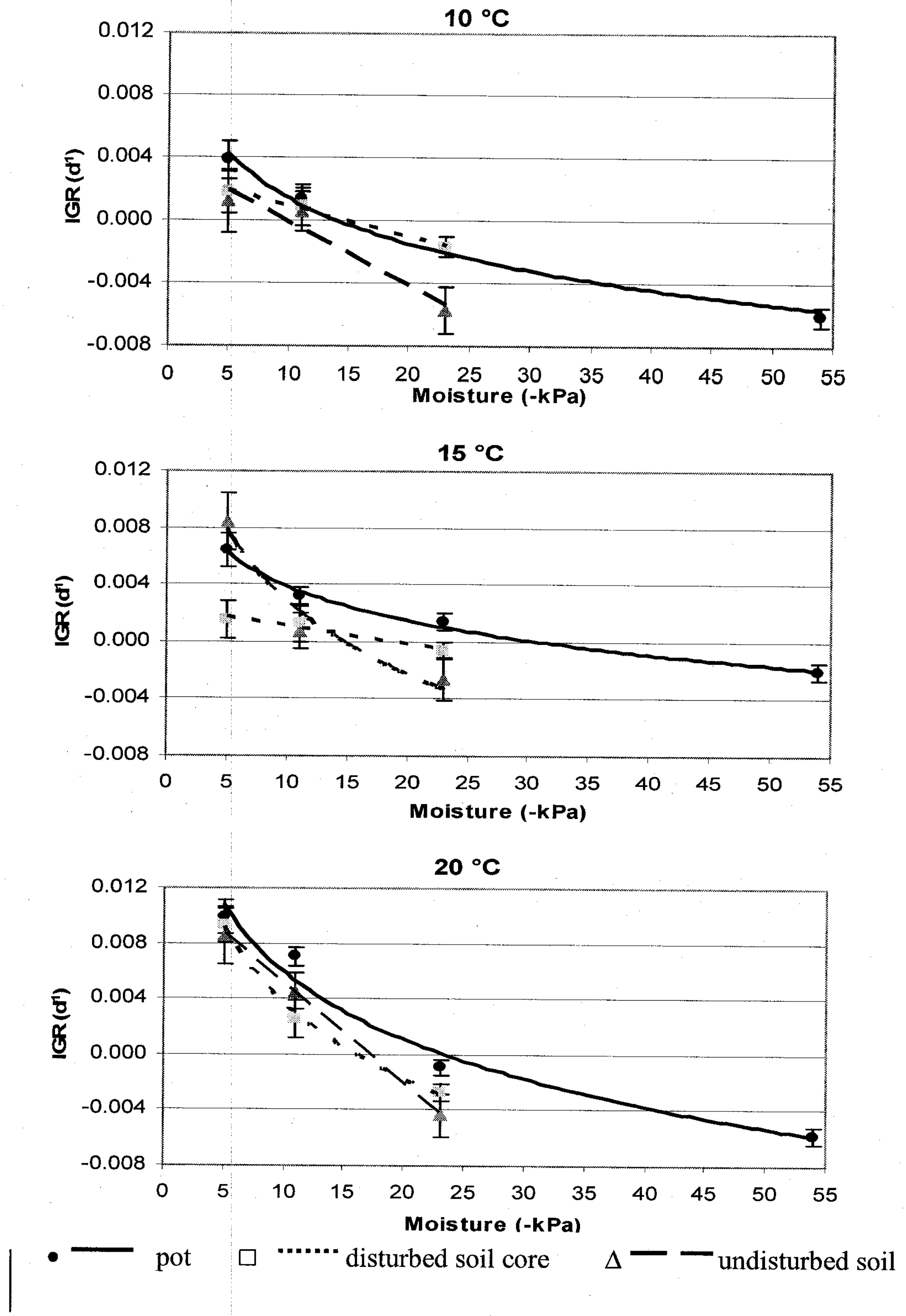

Figure 2. Effects of container type, soil temperature and soil water potential on the instantaneous growth rate (IGR) of *A. caliginosa*. Values are shown as mean ± S.E.

FOREWORD TO CHAPTER 3

In the previous chapter we obtained growth rate data for *Aporrectodea caliginosa* earthworms grown individually in containers across a wide range of soil temperatures and soil moistures that are specific to Québec agroecosystems. I demonstrated that earthworm growth rates are sensitive to the shape of container used for culturing the earthworms. Therefore there may be bias if the relationship between earthworms, soils and plants are studied in pot experiments in the greenhouse. Field enclosures will provide a much larger "pot" within which to study to effects of earthworms on soil nutrient cycles. The field experiment will also provide more realistic climate conditions than a climate-controlled greenhouse. The growth rate and mortality data obtained in the previous chapter will be useful in Chapter 4 when I combine the data from the laboratory and field experiments and begin to build a model to make predictions about N mineralization from earthworms.

CHAPTER 3.

Impacts of earthworms on soil nutrients and plant growth in soybean and maize agroecosystems.

3.1 Abstract

The objective of this experiment was to determine the effects of earthworms on soil N pools and plant growth in soybean and maize agroecosystems. The species and number of individuals in earthworm communities were manipulated in plot-scale field enclosures (2.4 x 1.2 m) by first reducing earthworm populations within enclosures with carbaryl pesticide, and then adding earthworm treatments to the enclosures. Soybean was grown in the enclosures in the first year and stover maize in the second year.

The success of earthworm manipulations in field enclosures was affected by climate conditions and available food resources. The endogeic earthworm species *Aporrectodea caliginosa* was dominant in all enclosures, while introduced anecic *Lumbricus terrestris* earthworms had poor survival. In the first season, when climate conditions were favourable for earthworm survival and growth, there was a significant ($P < 0.05$) linear increase in soil mineral-N and microbial biomass N concentrations in the 0 – 15 cm depth of enclosures with more earthworms. Similarly, soybean grain and grain-N yield was significantly ($P < 0.05$) greater in enclosures with the largest earthworm populations than the control which had no earthworms added. In the second season, when climate

conditions were less favourable, there was no effect of earthworms on soil N pools or maize plants, probably due to poor survival of introduced earthworms.

3.2 Introduction

Earthworms are commonly referred to as ecosystem engineers for their ability to modify soils and plant communities (Lavelle et al., 1997; Hale et al., 2005). Through the incorporation of surface litter, casting, burrowing and other activities, earthworms can significantly alter soil physical properties (Edwards and Shipitalo, 1998), soil nutrients (Edwards and Bohlen, 1996), soil biological communities (Doube and Brown, 1998), and above-ground plant communities (Piearce et al., 1994; Wurst et al., 2005).

The functional relationships between earthworms, soils and plants have been extensively studied in microcosm and laboratory experiments. However, extrapolating these results to the ecosystem-level is difficult. Earthworm activities may be overstated in small-scale experiments due to the control of environmental variables like temperature, soil moisture and food availability or because an unrealistic number of earthworms are added to small containers or mesocosms. The challenge is to quantify the influence of realistic earthworm communities at the field-level (Bohlen et al., 2004), which is often done by manipulating earthworm populations and communities in large-scale field enclosures (see Bohlen et al., 1995; Baker et al., 1996; Subler et al., 1997). However, there is considerable variation in the success of earthworm manipulations in field enclosures, depending on the methods used, climate and soil conditions (Bohlen et al., 1995; Baker et al., 1996; Zaller and Arnone, 1999; Emmerling and Pausch, 2001).

Therefore, the objectives of this experiment were, (1) to determine the effects of an earthworm community, dominated by *A. caliginosa* and *L. terrestris*, on soil nutrient dynamics and plant growth in soybean and maize agroecosystems, and (2) determine the success of manipulating earthworm communities by reducing population with pesticide and adding earthworms belonging to different functional groups.

3.3 Materials and Methods

The study was conducted from May to September in 2004 and 2005 on the Research Farm of Macdonald Campus of McGill University, Quebec, Canada (45°25' N, 73°56' W). The field was used for soybean and maize production in the two years prior to this experiment and before that was a turfgrass sports field. The soil was a mixed, frigid Typic Endoquent, classified as a Chicot series sandy loam. It had a pH (H_2O) of 5.9, an organic C content of 24.5 g C kg^{-1}, and contained 580 g kg^{-1} sand, 300 g kg^{-1} silt, and 120 g kg^{-1} clay. A field survey in May, 2003 found an earthworm community with an average of 50 individuals m^{-2} of *A. caliginosa* and 15 individuals m^{-2} of *L. terrestris*, and age class ratios of juveniles to adults of 4:1 and 3:1, respectively.

Field enclosures were installed in April, 2004. These rectangular sheet metal enclosures measured 2.4 x 1.2 m (2.9 m^2) and were buried to a depth of 0.30 – 0.40 m. The corners and top edges of the enclosures were bent at right angles to ensure a tight fit between pieces and minimize earthworms escaping from the enclosures. The enclosures remained in place for the 2004 and 2005 seasons.

At the beginning of each season, carbaryl pesticide (Sevin®) was applied to reduce earthworm populations in the enclosures. Beginning on April 28th, 2004, carbaryl was applied five times during a 25 day period, giving a total load of about 0.02 kg a.i. m^{-2}. The next year, we began on April 16th, 2005, and applied carbaryl four times during a 35 day period for a total load of about 0.04 kg a.i. m^{-2}. In both years, the last application of carbaryl was made 10 days before adding earthworms to the enclosures.

On May 28th, 2004, a single row of 100 soybeans (*Glycine max* (L.) cv. Merril) was sown by hand lengthwise, in the centre of each enclosure (equivalent to a planting density of 350,000 plants ha^{-1}). Germination and seedling establishment was even across all treatments, except in one enclosure. Here we planted 30 additional seeds and thinned to a similar density as the other enclosures within three weeks of the original sowing date. On June 1st, 2005, a single row of 15 silage maize (*Zea mays* (L.) cv. Mycogene 2K350) seeds were sown by hand lengthwise, in the centre of each enclosure (equivalent to a planting density of 52,000 plants ha^{-1}). Germination and seedling establishment was uneven and additional seeds were planted seven days later. After two weeks, we thinned to 12 plants per enclosure. No fertiliser or pesticide was added to either crop. Weeds were removed by hand as required throughout the season.

3.3.1 Experimental Design

The experiment was a randomised complete block design with seven earthworm population treatments and four blocks. The seven earthworm population treatments were three combinations of earthworms as *A. caliginosa* only (A), *L. terrestris* only (L), and a combined *A. caliginosa* and *L. terrestris* treatment (AL), at either a background population level (1x) or double the background population level (2x), and a control treatment with a reduced earthworm population. In the one to four weeks before the experiment began, earthworms were collected from around the field site and nearby arable fields by hand-sorting and formalin extraction (Raw, 1959). The earthworms were sorted by species and age-class and kept in laboratory cultures (38 l plastic bins) containing soil from the field site, regularly watered and fed with composted cattle manure. The mean fresh weight biomass of earthworms added to enclosures was similar in both years. In 2004 the fresh weight biomass of adult and juvenile *A. caliginosa* was 0.48 ± 0.19 g and 0.31 ± 0.11 g, respectively, and 4.79 ± 1.07 g and 1.53 ± 0.87 g for adult and juvenile *L. terrestris*, respectively. In 2005 the fresh weight biomass of adult and juvenile *A. caliginosa* was 0.59 ± 0.27g and 0.24 ± 0.13 g, respectively, and 4.72 ± 0.86 g and 1.87 ± 0.99 g for adult and juvenile *L. terrestris*, respectively. The ratio of juvenile to adult earthworms added to enclosures in both years was 1.5 for *A. caliginosa* and 3.9 for *L. terrestris*. In both years, we attempted to add earthworms to the enclosures on a cloudy overcast day; June 1st in 2004, and June 6th in 2005. Earthworms were transported to the field in one litre pots, each containing 10 – 30 earthworms in about 100 g of moist field soil. The earthworms in each pot were spread evenly in two trenches (5 –

10 cm deep), dug lengthwise in the enclosures. The earthworms were then lightly covered with soil and about seven litres of water was poured evenly along the trenches. Straw was lightly placed above the trenches to provide additional protection from direct sunlight and predators. The straw was removed three days later. The number and biomass of earthworms added to each treatment in June and collected in October in 2004 and 2005 are presented in Tables 1 and 2. We considered the results from each season independently since carbaryl was applied to reduce the earthworm populations in both years, before treatments were applied. To avoid the confounding effect of previous earthworm manipulation during the 2005 season, the earthworm treatments within each block were re-randomised in April, 2005.

3.3.2 Plant, Earthworm and Soil Analysis

In 2004, five soybean plants from each enclosure were carefully uprooted six, ten and fourteen weeks after sowing. In week six and ten only, plant roots were washed and the root dry weight and number of *Rhizobium* nodules recorded. On Sept 29th, about 18 weeks after sowing, the shoots of 20 soybean plants were cut at the soil surface from each enclosure. Shoot dry weights were determined in all weeks, and grain dry weights recorded in week 18 only. On August 2nd, 2005, about nine weeks after sowing, six maize leaves per enclosure were taken for nutrient analysis. On September 26th, all 12 maize plants were harvested from each enclosure by cutting shoots at the soil surface and the shoot, cob and grain dry weights were determined. In both years, sub-samples were taken from all shoot, leaf and grain samples and ground with a Wiley mill (< 1mm mesh).

Plant shoot, leaf and grain sub-samples were digested with H_2SO_4/H_2O_2 (Parkinson and Allen, 1975) and digests were analysed colorimetrically for N and P using a Lachat Quick Chem autoanalyzer (Lachat Instruments, Milwaukee, WI, USA), and for K using atomic absorption spectrometry. N-yield of soybean grain and maize plants were determined on a plant specific basis by multiplying the grain or tissue N concentration by the grain or total weight for soybean and maize plants, respectively.

In both years, earthworm populations were sampled from a soil pit (50 x 30 cm to a depth of 20 cm) dug in the middle of each enclosure one to five days after plant harvest. The removed soil was hand sorted for surface-dwelling earthworms, and formalin extraction (Raw, 1959) was used to collect earthworms from lower depths beneath the pit. Earthworm numbers, age classes, and fresh biomasses of earthworms were later recorded in the lab. Sexually mature individuals were identified to the species level using the key provided by Reynolds (1977). In May 2005, earthworm populations were sampled using the same method described above from a soil pit (15 x 15 cm to a depth of 20 cm) dug in the middle of each enclosure one week prior to adding earthworms.

In both years, soils from each enclosure were sampled two to three days after plant harvest. Four soil cores were taken diagonally across each enclosure from two depths (0 – 15 cm and 15 – 30 cm) with a soil auger (2 cm internal diameter) and composited into one sample per depth per enclosure. Soil samples were kept at 4°C until laboratory analysis. Mineral nitrogen (NO_3-N + NH_4-N) was determined by extracting 5 g field-moist soil with 50 ml of 2 M KCl (Maynard and Kalra, 1993). After shaking for one hour and filtering, the extract was analysed by colorimetry for NO_3-N and NH_4-N on a Lachat Quick Chem auto-analyser (Lachat Instruments, Milwaukee, WI, USA). Microbial biomass nitrogen (MBN) in soil samples was analysed using the chloroform fumigation-direct extraction method followed by persulfate digestion and calculated as: [(total extractable N after fumigation – total extractable N before fumigation)/0.54] (Brookes et al., 1985; Joergensen and Mueller, 1996). Dissolved organic nitrogen was calculated as the difference between the NO_3-N and NH_4-N concentrations in a persulfate digested soil extract and the original undigested soil extract (Cabrera and Beare, 1993). Available P and K were determined by extracting 2.5 g air-dry soil with 25 ml Mehlich-III solution (Tran and Simard, 1993). The P concentration in extracts was analysed colorimetrically on a Lachat Quick Chem auto-analyser (Lachat Insturments, Milwaukee, WI, USA) and K concentration was measured using atomic absorption spectrophotometry.

3.3.3 Statistical Analysis

The effects of earthworm treatment on soil properties, soybean nodulation, plant nutrients and yields were evaluated by one-way analysis of variance using the PROC GLM function of SAS software (SAS Institute, 2001). The differences between least square means of significant treatment effects were evaluated using the Tukey-Kramer HSD test ($P = 0.05$). Regression lines were fitted using the PROC REG function of SAS software (SAS Institute, 2001).

3.4 Results

Daily temperature fluctuations and weekly rainfall patterns in 2004 were similar to the 30-year mean for the region (Environment Canada, 2005). More extreme temperature and precipitation events were observed in 2005. In 2005, above normal temperatures began in early June and continued for the next 12 weeks. There were eight days in June and nine days in July, 2005 with a maximum temperature of over 30°C. In contrast, the 30-year mean indicated 1.6 days in June and four days in July with a maximum temperature above 30°C. In 2004, temperatures were cooler than normal, with no days in June and only one day in July with a maximum temperature of over 30°C. The total precipitation during the experiment was similar in 2004 (305 mm) and 2005 (404 mm) to the 30-year mean (395 mm). However, the frequency of rainfall events was low in 2005 but the average rainfall per event was greater. Weekly rainfall tended to be lower than long term averages in June and July of 2005, except for three above-average rainfall events totalling 178 mm. In the week following earthworm introductions in 2004, temperatures ranged from 9°C to 25°C with a mean temperature of 15°C, and total precipitation was 36 mm in the week following earthworm introduction. In 2005, temperatures ranged from 14°C to 33°C with a mean temperature of 23°C, and total precipitation was only 6 mm in the week after earthworms were placed in enclosures.

In 2004 and 2005 the manipulation of species and abundance of earthworms in each treatment was not successful. Although significant differences in earthworm population ($P < 0.01$) and biomass ($P < 0.03$) recovered in October were found between treatments in 2004 (Table 1), there were no significant differences in the number of earthworms of each species recovered in October between treatments in 2004 or 2005 (data not shown).

In October 2004, the earthworm community in all enclosures was dominated by *A. caliginosa* (59 – 100 %) with a few *L. terrestris* (0 – 33%) and *Aporrectodea longa* (0 – 23%), and in October 2005 it was dominated by *A. caliginosa* (56 – 100 %) with a few *L. terrestris* (0 – 44%) and *A. longa* (0 – 8%).

In May, 2005, after three applications of pesticide and before the addition of earthworms, the populations within enclosures were reduced to 32 individuals m^{-2} with a fresh weight biomass of 3 g m^{-2}, on average. There was no difference between enclosures applied the previous year suggesting that the re-randomising of treatments was not biased by the earthworm treatments.

Even though the manipulation of the abundance of different earthworm species was not successful, the manipulations did achieve a wide range of population and biomass across all enclosures. In 2004, the earthworm populations in enclosures ranged from 53 – 553 individuals m^{-2} and biomass ranged from 11 – 159 g fw m^{-2}, and in 2005 the populations ranged from 33 – 347 individuals m^{-2} and biomass ranged from 9 – 104 g fw m^{-2}.

In 2004, a significant increasing linear relationship was found between earthworm numbers and NO_3-N ($P = 0.01$), NH_4-N ($P = 0.03$), and MBN ($P < 0.001$) concentrations, and between earthworm fresh-weight biomass and MBN ($P = 0.008$) in the 0 – 15 cm soil depth. A decreasing linear relationship was found between earthworm numbers and the DON ($P = 0.017$) concentration in the 0 – 15 cm soil depth. No significant relationships were found between earthworm numbers, biomass and soil nutrients in the 15 – 30 cm depth. The relationships between earthworm numbers and the mineral-N (NO_3-N + NH_4-N), and MBN concentrations in the 0 – 15 cm depth are presented in Figures 1 and 2. In 2005, there were no significant relationships between earthworm numbers or biomass and soil nutrient concentrations at both depths.

In 2004, soybean grain yield ranged from 15.7 – 28.8 g plant^{-1}, and total harvested yield ranged from 44.2 – 74.8 g plant^{-1}. Significant logistic relationships were found between earthworm numbers and total grain-N per plant ($P = 0.002$) (Fig. 3) and grain yield ($P = 0.036$), and earthworm fresh-weight biomass and total grain-N per plant ($P= 0.004$), grain yield ($P = 0.016$), and total yield ($P = 0.03$). No relationships were found between earthworm numbers or biomass and the number of nodules per plant at six and ten weeks, and nutrient concentrations in grain at harvest.

In 2005, maize grain yield ranged from 62.5 – 184 g plant^{-1}, and total silage yield ranged from 184 – 384 g plant^{-1}. However, no relationships were found between earthworm numbers or biomass and nutrient concentrations in stover and grain, total N yield per plant at harvest, and grain, stover and total silage yield.

3.5 Discussion

The manipulation of earthworm functional groups was not successful in both years. *A. caliginosa* earthworms were most numerous in all treatments indicating that earthworms or cocoons of this species survived better after carbaryl application than those of other species. The average number of *L. terrestris* in all treatments did not differ significantly in both years (24 vs 29 individuals m^{-2} in 2004 and 2005, respectively) and were similar to the average number found in background samples (17 and 30 individuals m^{-2} in 2004 and 2005, respectively). Moreover, in both years, treatments with introduced *L. terrestris* had the same number as those with no introduced *L. terrestris*. This may indicate high mortality of introduced *L. terrestris* and persistence of the initial *L. terrestris* population and cocoons, even after several applications of carbaryl pesticide. The number of *A. longa* earthworms in enclosures decreased from an average of 19 to 2 individuals m^{-2} between the first and second years, indicating that it may take longer than two years to eliminate non-introduced species from enclosures with carbaryl pesticide.

The introduction of earthworms, regardless of functional group, was more successful in 2004 than in 2005, even though earthworms were added on almost the same day each year. A combination of higher temperatures, lower precipitation and lower food availability may have led to greater mortality when earthworms were introduced in a 2005 than 2004.

The effectiveness of carbaryl applications at reducing the naturally-occurring earthworm populations was not very consistent. Carbaryl applications did not completely eliminate earthworms from the enclosures, and the earthworms that persisted were probably active and capable of growing and reproducing as introduced earthworms. However, the control treatments were similar in both years and had the fewest number of earthworms, from 77 – 93 individuals m^{-2}, and lowest biomasses, ranging from 23 – 25 g fw $m^{-2}$of all enclosures. Furthermore, the earthworm populations within control enclosures were similar to populations at locations adjacent to the enclosures (background population) in 2004 but not 2005 (Tables 1 & 2).In sandy loam soils, such as at our field site, carbaryl has a half-life of about four to seven days (Venkateswarlu et al., 1980). Nonetheless, carbaryl can reduce earthworm numbers and biomass by up to 90 % and these low numbers persist for up to three weeks after application (Potter et al., 1990; Vangestel, 1992).

In other field studies, earthworm community manipulations have had varied success. In pastures of south-eastern Australia, the introduction of earthworms into 30 cm diameter cores has generally shown moderate survival of introduced species (50 – 80 %), a high number of non-introduced species (25 – 200 individuals m^{-2}) and varied population growth ranging from 0.5 – 3 times the introduced population (Baker et al., 1996; 1999a,b; 2002; Chan et al., 2004). In Ohio, USA, earthworms introduced over a three year period into large 4.5 x 4.5 m field enclosures had high mortality since populations grew by only 12 – 22 % even though the total population added over three years was three times greater than the final population (Bohlen et al., 1995). In another earthworm manipulation study in Ohio, earthworm populations did not differ between increased and unmodified population treatments five months after earthworms were introduced into 6.1 x 6.1 m enclosures (Subler et al., 1997). Similar low survival rates were reported by Boyer et al. (1999) on Reunion Island, where only about 10 % of earthworms added to 6.0 x 1.5 m enclosures survived seven months after introduction. Emmerling and Pausch (2001) had better success in Germany where earthworm populations introduced into 1.4 x 0.9 m enclosures increased between two to five fold over two years. Similarly, two years after adding earthworms to 1 x 1 m enclosures in a Swiss grassland, populations were about 50% greater than ambient populations, and ambient populations were about twice the size of reduced populations (Zaller and Arnone, 1999). In all of these studies, the success of earthworm manipulations into field enclosures was not consistent for any particular species or functional group.

The effect of earthworms on soils differed in each year. In 2004, our results indicate that the size of the earthworm population was related positively to the total mineral-N (NO_3-N + NH_4-N) and MBN concentrations after harvest (September, 2004). The relationship described in Figure 1 suggests that an individual earthworm can increase the soil mineral-N pool by 0.02 kg N m^{-2}. Expressed in more tangible terms, a field with a high earthworm population (300 individuals m^{-2}) could have 14 kg N ha^{-1} more in the 0 – 15 cm soil depth than a field with a low population (30 individuals m^{-2}). We assume that most of this mineral-N was generated by the activities of the endogeic *A. caliginosa* since they were the dominant species at our site. The greater amounts of mineral-N in soils suggest that high earthworm populations in the autumn may increase the risk of N leaching from soils after harvest. However, the increase in MBN also suggests that some of the increase in available-N was being captured in the microbial biomass.

In 2005, a wide range of earthworm populations and biomass was found across all enclosures as a result of earthworm manipulations, yet there were no relationships between earthworm population, biomass and soil nutrients. This important result suggests that the effect of earthworms on soil N dynamics cannot be predicted by earthworm population or biomass alone.

Previous studies investigating the effect of earthworm additions on soil nitrogen dynamics gave mixed results. In a maize-based enclosure study in Ohio, Blair et al. (1997) found that the addition of earthworms increased the soil NO_3-N concentration over a two year period in inorganically fertilized plots but not in manure or legume fertilized plots. Furthermore, they found that earthworm addition had increased soil NO_3-N concentration at lower depths (15 – 45 cm) in two consecutive growing seasons and in the 0 – 15 cm depth in only one of the two growing seasons. In contrast, results from a mesocosm experiment by Bohlen and Edwards (1995) show that earthworms increased the amount of NO_3-N at the 0 – 5 cm depth but had no effect at the 5 – 15 cm depth. In another enclosure study in Ohio, the addition of earthworms did not increase mineral-N concentrations in maize-soybean or maize-soybean-wheat systems in the 0 – 45 cm depths but did increase pools of organic N (MBN and DON) (Subler et al. 1997). This is consistent with our results of greater MBN concentrations in the 0 – 15 cm soil depth with increasing earthworm population, however DON concentrations decreased as earthworm population size increased.

Soybean and silage maize responded differently to earthworm populations. When weather conditions were more favourable for earthworm activity, as under soybean production in 2004, there were more noticeable effects of earthworms on plant growth and nutrient uptake. Regression analysis shows that soybean grain yield could be 25 % greater and the total N removed in soybean grain (g N per plant) could be 40 % greater in fields with high earthworm populations (> 400 individuals m^{-2}) than in fields with low earthworm populations (< 50 individuals m^{-2}) (Figure 3).

A lack of response by maize to earthworm activity has been found in other field and greenhouse studies (Mackay and Kladivko, 1985; Stinner et al., 1997; Boyer et al., 1999). The differences between the effects of earthworms on soybean and maize growth may be partially related to the N requirements and rooting pattern of each plant. The recommended N fertiliser requirements of silage maize (120 – 170 kg N ha^{-1}) are much greater than soybeans (0 – 30 kg N ha^{-1}) due to the N fixation ability of soybeans (CRAAQ, 2003). Therefore, any contribution of nitrogen from earthworms will supply a much greater proportion of the recommended N for soybeans than for maize. Furthermore, soybean plants have three times greater proportion of thin roots (< 0.12 cm) than maize plants in the 0 – 10 cm soil depth and the root dry matter density per soil unit volume is greater for maize than soybean plants at lower depths (Venzke et al., 2004). The greater proportion of thin roots of soybean plants in the 0 – 10 cm depth provides greater contact with the soil matrix, enabling greater nutrient and water uptake. Since the effects of earthworms on soil nutrients in our experiment occurred in the 0 – 15 cm depth, the shallow rooted soybeans would probably benefit more from earthworm activities that increase soil-N pools than the deep rooted maize.

3.6 Conclusions

Our research suggests that in some agroecosystems earthworms contribute positively to plant growth only when populations are high (> 300 ind. m^{-2}) and when favourable weather conditions exist. Although we did not detect significant differences between the endogeic and anecic earthworm treatments, a higher earthworm population, principally consisting of the endogeic species *A. caliginosa*, was associated with greater mineral-N and MBN concentrations in surface (0 – 15 cm) soils, and higher grain N yield in soybeans. A combination of poor survival of introduced earthworms and low activity of the surviving earthworms may explain the lack of effects of earthworms on maize plants.

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Table 1. Earthworm populations and biomass added in June, 2004 and collected in October, 2004 from enclosures under soybean production[†]

Earthworm Treatment	Population (individuals m^{-2} ± S.E.)		Biomass (g fresh weight m^{-2} ± S.E.)	
	June	October	June	October
Control	0	77 ± 12 b	0	25 ± 7.5 b
A1x	50	190 ± 56 ab	21	56 ± 15 ab
A2x	100	330 ± 87 a	42	86 ± 22 ab
L1x	15	170 ± 24 ab	34	55 ± 9.2 ab
L2x	30	220 ± 46 ab	67	77 ± 12 ab
AL1x	65	180 ± 62 ab	55	86 ± 25 ab
AL2x	130	380 ± 47 a	109	92 ± 8.7 a
Background[††]		123 ± 30		59 ± 4.5
	ANOVA treatment effects			
Treatment		$P = 0.01$		$P = 0.03$

† Values in each column for each treament followed by similar letters are not significantly different by Tukey's HSD test ($P = 0.05$).

†† Background samples were the average of two pits and therefore were not included in the statistical analysis

Table 2. Earthworm populations and biomass added in June, 2005 and collected in October, 2005 from enclosures under silage maize production.

Earthworm Treatment	Population (individuals m^{-2} ± S.E.)		Biomass (g fresh weight m^{-2} ± S.E.)	
	June	October	June	October
Control	0	93 ± 18	0	23 ± 5.4
A1x	50	132 ± 23	24	34 ± 7.1
A2x	100	135 ± 33	47	25 ± 8.4
L1x	15	147 ± 67	37	44 ± 19
L2x	30	117 ± 45	75	33 ± 10
AL1x	65	153 ± 24	61	43 ± 11
AL2x	130	95 ± 10	123	44 ± 20
Background†		233 ± 15		118 ± 3
	ANOVA treatment effects			
Earthworm		n.s.		n.s.

† Background samples were the average of two pits and therefore were not included in the statistical analysis

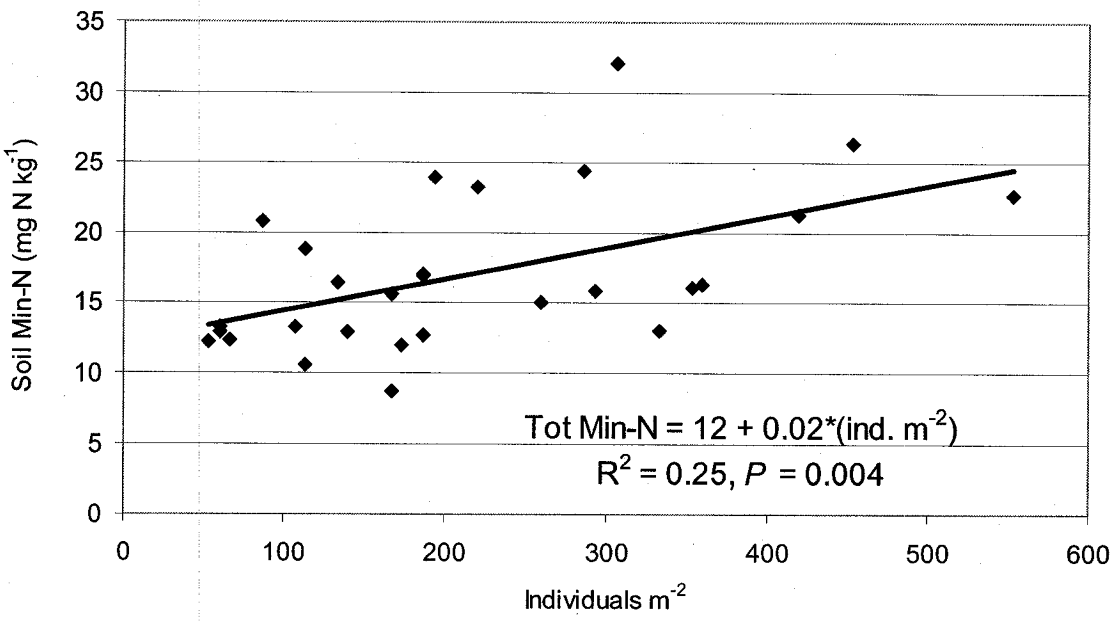

Figure 1. Relationship between soil mineral-N concentration (mg N kg^{-1}) in the 0 – 15 cm depth and earthworm population under soybean production in 2004 (n = 28).

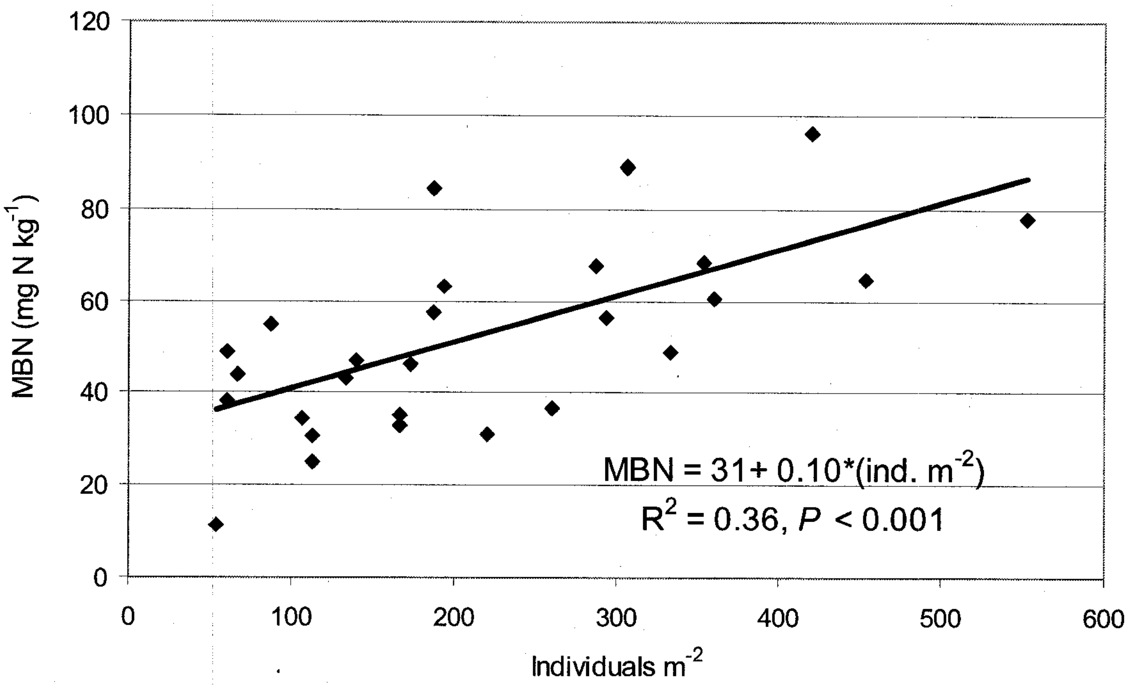

Figure 2. Relationship between MBN concentration (mg N kg^{-1}) in the 0 – 15 cm depth and earthworm population under soybean production in 2004 (n = 28).

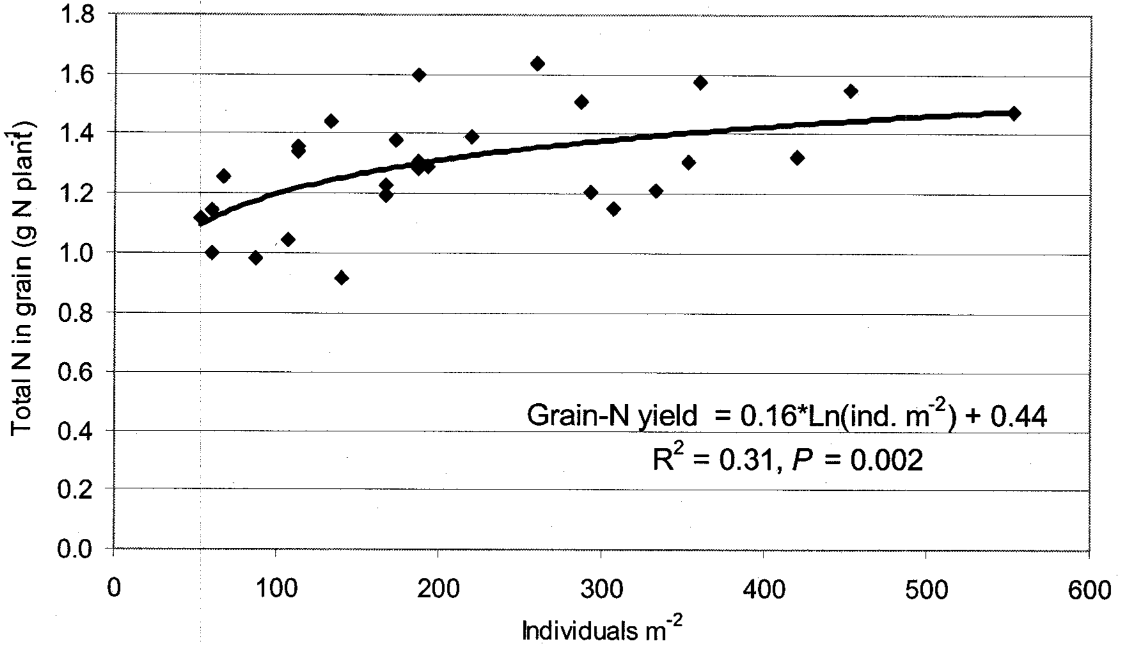

Figure 3. Relationship between total grain-N per soybean plant (g N plant^{-1}) and earthworm population in 2004 (n = 28).

FOREWORD TO CHAPTER 4

The previous chapter determined how the manipulation of earthworm communities in field enclosures affected soil nitrogen pools, and soybean and maize growth. The influence of weather patterns was significant on the success of earthworm introductions and N mineralization by earthworms. A modelling approach will be used to extrapolate this information and predict N mineralization from earthworm communities in other agroecosystems. Laboratory growth rate data from Chapter 2 and field data from Chapter 3 will be used to develop a model to make estimates of N flux through earthworm communities. A sub-set of this model involves the calculation of earthworm secondary production. The N in earthworm biomass can represent a significant amount of the N cycled by earthworms and therefore an accurate estimate of earthworm secondary production is required. In the following chapter we present the "*deduction*" approach as a means of estimating earthworm secondary production without repeatedly sampling earthworms from the enclosures.

CHAPTER 4.

The "*Deduction*" Approach: A Non-Invasive Method for Estimating Secondary Production of Earthworm Communities

4.1 Abstract

Secondary production is an important parameter for the study of population dynamics and energy flow through animal communities. Secondary production of earthworm communities is commonly determined by using the size-frequency or instantaneous growth rate methods whereby earthworm populations are repeatedly sampled at regular intervals and the change in earthworm biomass determined between sampling dates. The major disadvantage with these methods is the physical disturbance of soils and removal of individuals from the community. The "*deduction*" approach is a theoretical model which divides earthworms into specific "pools" and makes assumptions about the growth, recruitment and mortality of the different pools in order to estimate secondary production of the earthworm community. In 2004 and 2005, the size of earthworm communities were manipulated in field enclosures and the "*deduction*" approach was used to estimate secondary production of these earthworm communities during the crop growing period (17 – 18 weeks) in each year. Secondary production estimates made by the "*deduction*" approach were similar to estimates made by other direct sampling methods in other ecosystems. This method may be used for the estimation of secondary production of other organisms in manipulation studies where repeated removal of individuals or physical disturbance could bias results.

4.2 Introduction

Secondary production describes the growth, mortality and recruitment of organisms in a community and is an important parameter for the study of population dynamics and the analysis of energy flow through animal communities. Both direct and indirect methods of estimating secondary production have been developed. Calculations of secondary production using direct methods such as the cohort method (Crisp, 1971), size-frequency method (Hynes, 1961), and the instantaneous growth rate method (IGR) (Romanovsky and Polishchuck, 1982) generally yield comparable results (Sardá et al., 2000; Medernach and Gremare, 1999). Indirect methods for estimating secondary production are based on empirical relationships between body size and production. They provide similar estimates of secondary production only when a large number of populations and a broad range of environmental conditions are used to develop the relationships (Sardá et al. 2000).

Secondary production of fish and aquatic invertebrates is monitored routinely (Benke, 1984), but is also relevant to terrestrial organisms such as earthworms. Due to their key influence on soil organic matter decomposition, nutrient cycling and primary production (Fragoso et al., 1997; Lavelle et al., 1997), many researchers have attempted to quantify energy and nitrogen flux through earthworm communities from secondary production estimates (Parmelee and Crossley, 1988; Whalen and Parmelee, 2000). Secondary production represents about 8 – 18% of the N cycled through earthworm communities, which is estimated at between 7 and 363 kg N ha^{-1} year^{-1} (Parmelee and Crossley, 1988; Marinissen and de Ruiter, 1993; Curry et al., 1995; Whalen and Parmelee, 2000). The contribution of earthworms in N cycling can be important considering that the N demand of field crops (wheat, maize, etc) is often greater than 100 kg N ha^{-1}.

Secondary production of earthworm communities can be determined by repeatedly collecting earthworms at regular intervals throughout the frost-free periods of the year and inferring the change in earthworm biomass between sampling dates (Satchell, 1963; Boström, 1988; Parmelee and Crossley, 1988). It is challenging to estimate earthworm secondary production due to difficulties in gauging the age of individuals, the lack of cohort-specific developmental phases and the heterogeneous distribution of naturally-occurring communities (Rossi et al., 1997; Whalen and Parmelee, 2000). Earthworm manipulation experiments permit researchers to add individuals of known age and biomass to a designated area (enclosure), and determine their impact on soil properties and plant growth during a period of time (Eriksen-Hamel and Whalen, 2007). It becomes impossible to estimate their secondary production using routine methods because repeated earthworm collection would disturb the earthworm community in an enclosure. We developed the "*deduction*" approach which would permit researchers to estimate secondary production of earthworms or other terrestrial animals in laboratory mesocosms or field enclosures without repeated invasive sampling.

The objective of this paper was to use the "*deduction*" approach to estimate secondary production of earthworm communities in field enclosures. This was based on assumptions about growth, recruitment and mortality of an introduced earthworm community during a 16 week period (June – October) in soybean and maize agroecosystems.

4.3 Materials and methods

4.3.1 Description of Field Site and Experiment Design

A field experiment was designed to evaluate the effects of controlled earthworm populations on soil properties and crop yield in field enclosures. It was conducted from May 2004 to September 2005 on the Macdonald Campus Research Farm, McGill University, Quebec, Canada (45°25' N, 73°56' W). In May 2004, rectangular sheet metal field enclosures, measuring 2.4 m x 1.2 m (2.9 m^2), were buried to a depth of 0.30 – 0.40 m. Seven enclosures were arranged in four blocks, for a total of 28 experimental units. During the months of May 2004 and May 2005, carbaryl pesticide (Sevin®) was applied 4 – 5 times to each enclosure (total application of 220 kg a.i. ha^{-1} $year^{-1}$) to reduce earthworm populations. The pesticide was applied according to recommended applications rates made by Potter et al. (1990). Native earthworms collected from the surrounding field were added to the enclosures on June 1, 2004 and on June 6, 2005, and remained in enclosures for about 17 – 18 weeks. The seven treatments included three earthworm populations as *Aporrectodea caliginosa* only (Ac), *Lumbricus terrestris* only (Lt), and a combined *A. caliginosa* and *L. terrestris* treatment (AcLt), at either the background population level (1x) or double the background population level (2x), and a control treatment (Control). Earthworms were sampled from a soil pit (50 x 30 cm to a depth of 20 cm) dug in the middle of each enclosure on September 30, 2004 and September 28, 2005. Formalin extraction (Raw, 1959) was used to collect earthworms from lower depths beneath the pit. Earthworm numbers, age classes, formalin-preserved biomass and ash-free dry weight (AFDW) of collected earthworms were later recorded in the lab. Ash-free dry weights were determined by placing dried (90°C for 24 hours) earthworms in a muffle furnace at 500°C for 4 hours. Sexually mature individuals were identified to the species level using the key provided by Reynolds (1977). The number and biomass of earthworms added in each treatment in the spring and collected in the autumn are provided in Table 1. Further details of the experimental design, description of soils, and methods of plant and soil sampling and analysis are described in Eriksen-Hamel and Whalen (2007).

4.3.2 Assumptions about Earthworm Activity

The number of days that earthworms are active throughout the year significantly impacts the estimates of secondary production. Based on weekly soil temperature and moisture measurements from each enclosure (Fig. 1), growth rate data for *A. caliginosa* determined in soils from the same site (Eriksen-Hamel and Whalen, 2006) and casting activity of earthworms in enclosures (Perreault et al., 2007), we determined that earthworm growth and activity ceased when soil temperatures exceeded 22°C and soil moisture was below 20% WFPS. Thus, earthworm activity was positive (+) at soil temperature < 22°C and soil moisture > 20% WFPS, while neutral (0) activity occurred at these values and negative (–) activity occurred when the critical temperature and moisture levels were exceeded. Although earthworms were expected to be inactive on days with negative activity, we assumed that earthworms could possibly be active on days with neutral activity. We estimated the number of days with positive earthworm activity was was 77 (± 14) in 2004 and 49 (± 14) in 2005, where the uncertainty associated with these values is the number of days with neutral activity (14 d) in each year (Fig 1, Table 2).

4.3.3 Assumptions about Earthworm Growth and Mortality to Estimate Earthworm Secondary Production

Earthworm numbers and biomass in each enclosure changed between the date of earthworm addition (early June) and the final population assessment following crop harvest. Most lateral movement by earthworms occurs in the 0 – 20 cm depth (Francis et al., 2001; Bastardie et al., 2003) and since the enclosures extended 30 – 40 cm deep, we assumed that there was no immigration or emigration from the enclosures. We also assumed that earthworm removal by predation (eg. birds) was negligible in all enclosures. Bird flocks were observed often on neighbouring alfalfa fields, which typically have large earthworm populations, and we presume that foraging success was greater elsewhere than at our field site. Removal of surface residues in the spring and regular weeding probably made field enclosures unattractive for other earthworm predators, as we saw no evidence of voles or snakes in the vicinity of our study site. Therefore, the fluctuations in earthworm populations and biomass were due to recruitment of hatchlings from cocoons, growth (secondary production) and biomass lost via mortality.

The "*deduction*" approach involves separating the earthworms into different pools and deducing the fate of each pool with assumptions about growth, reproduction and mortality (Fig. 2). The earthworm biomass in the control treatment at the end of the season ($\text{Control}_{\text{final}}$) was from earthworms that survived pesticide application (S_f), earthworms that hatched from a cocoon deposited prior to pesticide application (C_f) and the offspring of these earthworms (R1). To differentiate these three earthworm pools (S_f, C_f and R1), we determined the maximum biomass that an earthworm could achieve if it emerged from a cocoon the day after the last pesticide application. This was based on the number of days with soil temperature and moisture conditions conducive for earthworm growth (77 d in 2004, 49 d in 2005) and growth curves for each earthworm species obtained from the literature (Table 3). The maximum fresh weight biomass that newly emerged earthworms could grow to was 0.4 g for the three earthworm species found in the control treatment in 2004 and 0.3 g for these species in 2005 (Table 3). This corresponds to instantaneous growth rates (IGR) of approximately 0.01 – 0.03 d^{-1} for all species, which are comparable to IGRs calculated in other studies (Mazantseva, 1982; Whalen and Parmelee, 1999; Booth et al., 2000; Wever et al., 2001). Thus, earthworms in the control treatment with a biomass greater than these maximum limits were considered to have survived pesticide application (S_f).

The biomass of earthworms belonging to the C_f and R1 pools was calculated by subtracting the pesticide survivors (S_f), and adding mortality (M_S, M_C, M_{R1}) from the earthworms found in the control treatment at the end of the season ($\text{Control}_{\text{final}}$):

$$C_f + R1 = \text{Control}_{\text{final}} - S_f + (M_S + M_C + M_{R1}), \qquad (2)$$

where the mortality ($M_S + M_C + M_{R1}$) of earthworms in the control treatment was estimated to be 35% in 2004 and 50% in 2005 of the final earthworm biomass ($Control_{final}$), based on the number of active days and the mortality rates that occur under high temperatures (>20°C) and low soil moisture conditions (< -30 kPa) in laboratory cultures and field experiments (Table 2).

The earthworm community in the control treatment ($Control_{final}$) was assumed to represent the background earthworm community in all enclosures, thus, the earthworm community in treatment enclosures ($Treatment_{final}$) was calculated from equations 3 and 4. Any *Aporrectodea longa* found in enclosures were considered to be part of the background community ($Control_{final}$) since no *A. longa* were added to enclosures.

$$Treatment_{final} = Control_{final} + Treatment_{added}, \tag{3}$$

$$Treatment_{added} = A_f + R2 - (M_A + M_{R2}), \tag{4}$$

where A_f is the final biomass of earthworms added to the enclosures, R2 are the offspring of A_i, and M_A and M_{R2} is the biomass lost through mortality. Hence, the biomass in the $Treatment_{added}$ pool comes from earthworms added to the enclosures only.

The biomass of earthworms in the A_f pool was determined using a biomass criterion similar to that in the control treatment. The initial fresh weight biomass (B_i) of *A. caliginosa* juveniles added to enclosures (A_i) were 0.31 g ± 0.01 (S.E.) in 2004 and 0.23 g ± 0.01 in 2005, while *L. terrestris* juveniles weighed 1.5 g ± 0.10 in 2004 and 1.9 g ± 0.11 in 2005. Based on field IGR values from Whalen and Parmelee (1999) and the number of active days in each season (Table 2), we calculated the final fresh weight biomass (B_f) for each earthworm species using equation 5.

$$B_f \text{ (g fw)} = B_i \times IGR \times \text{active days (d y}^{-1}\text{)}, \qquad (5)$$

We calculated that *A. caliginosa* would grow to a minimum biomass of 0.45 g in 2004 and 0.35 g in 2005, while *L. terrestris* were expected to grow to 1.6 g in 2004 and 2.0 g in 2005. Earthworms weighing more than the minimum biomass were considered in the A_f pool, while earthworms weighing less than this critical level were considered as being recruited from the S (R1) and A (R2) pools. Mortality of added earthworms (M_A) was estimated to be 50% of the A_f pool (Table 2). The mortality of added earthworms was assumed to be higher than the mortality of naturally occurring earthworms (M_S, M_C, M_{R1}) because of the stress involved in handling and adding earthworms to the enclosures. The mortality of the recruited earthworms (M_{R2}) was considered similar to the mortality (35 - 50%) of naturally occurring earthworms (M_S, M_C, M_{R1}). Overall, earthworm mortality (M_t) from each enclosure was the sum of mortality from all pools.

$$M_t \text{ (g fw m}^{-2}\text{)} = M_S + M_A + M_C + M_{R1} + M_{R2}, \qquad (6)$$

Earthworm secondary production (P) was biomass accumulated from all pools between earthworm addition and final biomass measurement.

$$P \text{ (g AFDW m}^{-2}\text{)} = \Delta S + \Delta A + C_f + R1 + R2, \qquad (7)$$

$$\Delta S \text{ (g AFDW m}^{-2}\text{)} = S_f - S_i = S_f \times G_{SA}/(1 + G_{SA}) \qquad (8)$$

$$\Delta A \text{ (g AFDW m}^{-2}\text{)} = A_f - A_i = A_f \times G_{SA}/(1 + G_{SA}) \qquad (9)$$

where ΔS and ΔA is the biomass accumulated in the S and A pools, G_{SA} is the growth of earthworms in the S and A pools during the growing season. Estimates of growth were based on growth rates obtained from laboratory and field core experiments (Whalen and Parmelee, 1999; Zwhalen et al., 2003; Eriksen-Hamel and Whalen, 2006) and the number of active days determined in each year (Table 2). We estimated the increase in earthworm biomass (G_{SA}) from the S_i to S_f pool, and A_i to A_f pools to be 40% for *A. caliginosa* and 10% for *L. terrestris* in 2004 and 30% for *A. caliginosa* and 5% for *L. terrestris* in 2005 (Table 2).

4.3.4 Calculation of Mean Earthworm Biomass During the Season

The mean biomass (B_{mean}) of active earthworms in the enclosures during the growing season was calculated by equations 10 – 12:

$$B_{mean} \text{ in control enclosures (g AFDW m}^{-2}) = (S_i + \text{Control}_{final}) / 2, \quad (10)$$

$$B_{mean} \text{ in treatment enclosures (g AFDW m}^{-2}) = (S_i + A_i + \text{Treatment}_{final}) / 2, \quad (11)$$

$$S_i \text{ (g AFDW m}^{-2}) = S_f / (1 + G_{SA}), \quad (12)$$

where A_i is the initial biomass of earthworms added to each treatment at the beginning of the experiment (Table 1), S_i and S_f are the initial and final biomass of earthworms surviving pesticide application, and G_{SA} is the percentage increase in biomass of the S pool during the growing season (Table 2).

4.3.5 Statistical Analysis

Regression lines of the model estimates were fitted using the PROC REG function and Pearson's correlation coefficients were obtained using the PROC CORR function of SAS software (SAS Institute, 2001).

4.4 Results

Only three earthworm species, *A. caliginosa*, *L. terrestris* and *A. longa*, were found in the enclosures. The naturally occurring population of *A. longa* earthworms were about 9% of the total population in 2004 and 2% in 2005. Manipulation of earthworm species was not successful and the proportion of *A. caliginosa* and *L. terrestris* did not differ between treatments (data not shown). However, we were able to obtain a wide range of earthworm biomass across all enclosures. The final earthworm biomass of the different treatments ranged from 25 – 92 g fw m^{-2} in 2004 and 23 – 44 g fw m^{-2} in 2005, suggesting greater survival and growth of earthworms introduced to enclosures during 2004 than 2005 (Table 1).

4.4.1 Secondary Production Estimates

The relationships between secondary production and earthworm biomass were significant in 2004, but not in 2005 (Fig. 3). Secondary production of Lumbricid earthworms measured in different temperate ecosystems by direct methods have a high correlation ($r = 0.89$, $P < 0.001$) with mean earthworm biomass (Fig. 4). Similarly when secondary production estimates made by the "*deduction*" approach in 2004 and 2005 were extrapolated for a 35 week growing season a strong correlation ($r = 0.80$, $P < 0.001$) exists between secondary production and mean earthworm biomass when results from this study were combined with other published data (Fig. 4).

4.5 Discussion

4.5.1 Secondary Production Estimates

The estimates of secondary production during the 17 week period determined by the "*deduction*" approach ranged from 3 – 13 g AFDW m^{-2} in 2004 and from 4 – 8 g AFDW m^{-2} in 2005. If we assume that secondary production during the growing season could be extrapolated for the frost-free period of the year from April to November (35 weeks) and presented on an annual basis, then our estimates (6 – 27 g AFDW m^{-2} $year^{-1}$) are similar to the estimates of 4 – 32 g AFDW m^{-2} $year^{-1}$ reported in other cold temperate arable agroecosystems and calculated using direct methods (Fig. 4) (Boström, 1988; Curry et al., 1995; Whalen and Parmelee, 2000). The only exception is the high secondary production (47 g AFDW m^{-2}) recorded in the study of Parmelee and Crossley (1988) which probably arises from the longer growing season (February – November) in Georgia, USA compared to the other studies from cold temperate climates (Ohio, USA, Sweden and Ireland). We assumed no cocoon production during this study because peak cocoon production occurs in early spring and late fall (Whalen et al., 1998). The secondary production through cocoons was calculated to be about 4 – 8 % of total tissue production (Parmelee and Crossley, 1988; Curry et al., 1995). Therefore, the secondary production determined by the "*deduction*" approach could be underestimated by about 0.5 – 2 g AFDW m^{-2} $year^{-1}$.

Secondary production has been measured for the naturally-occurring earthworm community in forests and agroecosystems of the UK and Ireland (Satchell, 1963; Boström, 1988; Curry et al., 1995), the USA (Parmelee and Crossley, 1988; Whalen and Parmelee, 2000), and in India (Senapti et al., 1991; Senapti et al., 1992). Comparison of secondary production estimates between these studies is difficult due to differences in earthworm biomass, species, ecosystem and methods used. Furthermore, none of these studies specifically address the relationship between secondary production and the size of the earthworm community. This can be overcome by presenting secondary production versus mean earthworm biomass (Fig. 4). Also, there is little difference in secondary production estimates for earthworm communities when the size-frequency and IGR methods are used (Whalen and Parmelee, 2000). Therefore, we assume that secondary production estimates from these studies can be compared.

The relationship between secondary production (P) and mean biomass (B) indicates the biomass turnover rate of populations, while the reciprocal of the P/B ratio indicates the time required to replace biomass in populations (Benke, 1984). The high correlation between secondary production and mean earthworm biomass across all studies suggests that population turnover of earthworm communities is related to population size and climate, and not necessarily available resources. The average population turnover was shorter in 2004 (P/B = 2.1) than in 2005 (P/B = 1.7) due to the longer active period in 2004. In a field study in Ohio, the biomass and secondary production of earthworm communities was greater in manure fertilized plots than inorganic fertilized plots, however no significant differences were found between the population turnover in both systems (P/B was 2.5 in manure plots vs 2.2 in inorganic plots) (Whalen and Parmelee, 2000). Similarly, the biomass and secondary production of earthworm communities was greater in a lucerne field than in a meadow, yet no differences were found between the population turnover in both fields (P/B was 1.4 in lucerne vs 1.2 in the meadow) (Boström, 1988).

4.5.2 Limitations and Constraints of the "*Deduction*" Approach

The "*deduction*" approach is a reasonable method that can be used if the experimental design prevents repeated sampling or physical disturbance of the experimental plots. The major conditions for using the "*deduction*" approach are the use of manipulation experiments where individuals are added to a contained area (eg: laboratory mesocosms or field enclosures), and the inclusion of a control treatment without any added individuals. In addition, accurate estimates of growth rates, duration of growth, and mortality must be known for the organism in question. We acknowledge that these conditions may limit the wide-spread use of this method, however there is growing interest in conducting plot-level manipulation experiments to determine the role of different species, functional groups and abundance of soil organisms in ecosystems (Blair et al., 1995).

A drawback of our experiment was that the earthworm manipulations were conducted during the summer (June – September) when soil temperatures were high (>20°C) and soil moistures were highly variable. Visual observations of casting activity show that earthworms were most active in the early spring (April and May) and autumn (late September and October) when soils were cooler (10 – 15°C) and moister. Clearly we have missed peaks periods of secondary production and therefore would expect greater biomass production if the experiment had included the entire frost-free period of our temperate agro-ecosystem. Nonetheless, the extrapolated estimates made by the "*deduction*" approach are within the range of secondary production estimates made by other direct methods (Fig. 4). Yet, this method still requires validation and future field work should compare this approach with other direct methods of assessing secondary production such as the IGR and size-frequency methods.

4.6 Conclusions

The "*deduction*" approach to estimating secondary production can be used in studies where frequent and repeated measurement of earthworm biomass is not possible. The accuracy of these estimates are based on the accuracy of estimates of the duration of earthworm activity, field growth rates and mortality that are used in the model. Our estimates of secondary production using the "*deduction*" approach are very similar to those obtained in other studies using direct methods of assessing secondary production. Nonetheless, further field work is required to validate the estimates made by the "*deduction*" approach and compare them with other methods of calculating earthworm secondary production such as the IGR and size-frequency methods. At a broader scale the "*deduction*" approach may be useful to determine secondary production for other organisms in manipulation studies where repeated removal of individuals or disturbance could bias experimental results. This approach increases the set of tools available to ecologists to assess secondary production of organisms and may help to better understand the energy flow and nutrient fluxes through communities in different ecosystems.

4.7 References

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	2004				2005			
Earthworm Treatment	Earthworms Added (A_i)		$\text{Treatment}_{\text{final}}$	B_{mean}	Earthworms Added (A_i)		$\text{Treatment}_{\text{final}}$	B_{mean}
	Ind. m^{-2}	g fw m^{-2}	g fw m^{-2} (± S.E.)		Ind. m^{-2}	g fw m^{-2}	g fw m^{-2} (± S.E.)	
1: Control	0	0	25 ± 7.5	20	0	0	23 ± 5.4	19
2: Ac1	50	21	56 ± 15	53	50	24	34 ± 7.1	42
3: Ac2	100	42	86 ± 22	75	100	47	25 ± 8.4	46
4: Lt1	15	34	55 ± 9.2	56	15	37	44 ± 19	55
5: Lt2	30	67	77 ± 12	85	30	75	33 ± 10	66
6: AcLt1	65	55	86 ± 25	97	65	61	43 ± 11	65
7: AcLt2	130	109	92 ± 8.7	127	130	123	44 ± 20	93

Table 2. Parameter values used in the "*deduction*" approach for estimating secondary production of earthworm communities in 2004 and 2005.

Parameter	Estimate	References
Active period (Non-aestivation)	2004: 77 days 2005: 49 days	Fig. 1; Eriksen-Hamel and Whalen , 2007
Mortality over season $(M_S + M_C + M_{R1})$ & (M_{R2})	2004: 35 % 2005: 50 %	Experimental observations; Al-Yousef and Shoreit, 1992; Wever et al., 2001; Eriksen-Hamel and Whalen , 2007
Mortality of added earthworms (M_A)	50 %	Subler et al., 1997; Boyer et al., 1999; Eriksen-Hamel and Whalen, 2006, 2007
Growth of added and surviving earthworms (G_{SA})	*A. c.* 2004: 40 % 2005: 30 % *L. t. & A. l.* 2004: 10 % 2005: 5 %	Whalen and Parmlee, 1999; Zwhalen et al., 2003; Eriksen-Hamel and Whalen, 2006
g fw: g AFDW	7.5	Experimental observations; Boström, 1988; Curry et al., 1995

Table 3. Maximum biomass (g fw) attained by each earthworm species surviving pesticide application in the control treatments. Literature values from laboratory and field studies were considered when choosing the value used for the model.

Earthworm Species		2004 (77 days)	2005 (49 days)	References
A. caliginosa	Literature	Lab: 0.30 - 0.80 Field: 0.30	Lab: 0.20 - 0.50 Field: 0.20	Lofs-Holmin, 1982; Boström and Lofs-Holmin, 1986; Whalen and Parmelee, 1999
	Model	0.4	0.3	
L. terrestris	Literature	Lab: 0.40 - 1.2 Field: 0.25	Lab: 0.30 - 0.6 Field: 0.20	Butt, 1991; Whalen and Parmelee, 1999; Lowe and Butt, 2003
	Model	0.4	0.3	
A. longa	Literature	Lab: 0.4 – 1.3	Lab: 0.30 – 0.8	Butt, 1998; Lowe and Butt, 2002; Baker and Whitby, 2003
	Model	0.4	0.3	

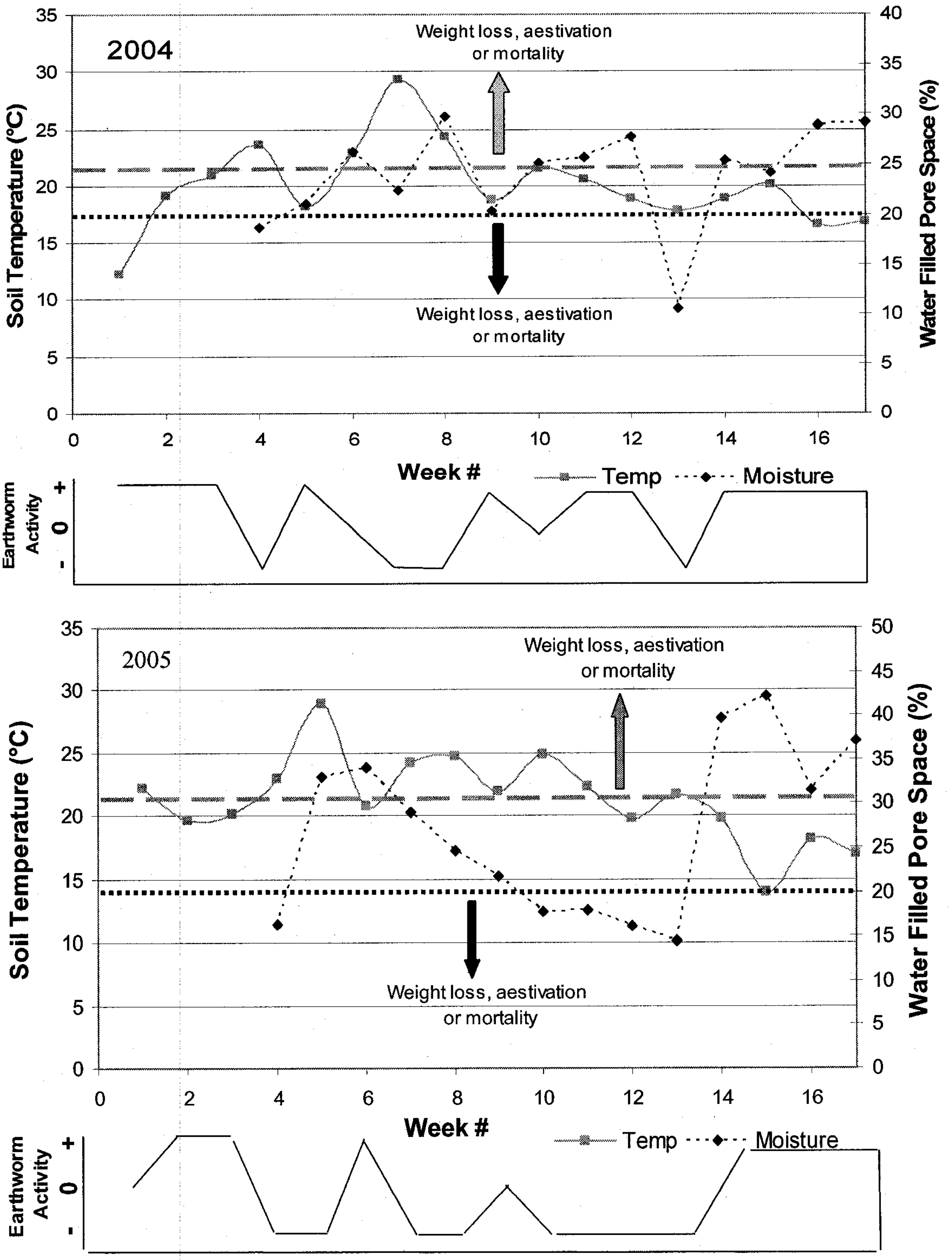

Figure 1. Mean weekly soil temperature (°C) (grey and solid line) and water-filled pore space (%) (black and dashed line) in enclosures in 2004 and 2005. The grey long dashed line at 22°C and black dotted line at 20% indicate the limits of earthworm growth. Periods of positive and negative earthworm activity are shown for each week.

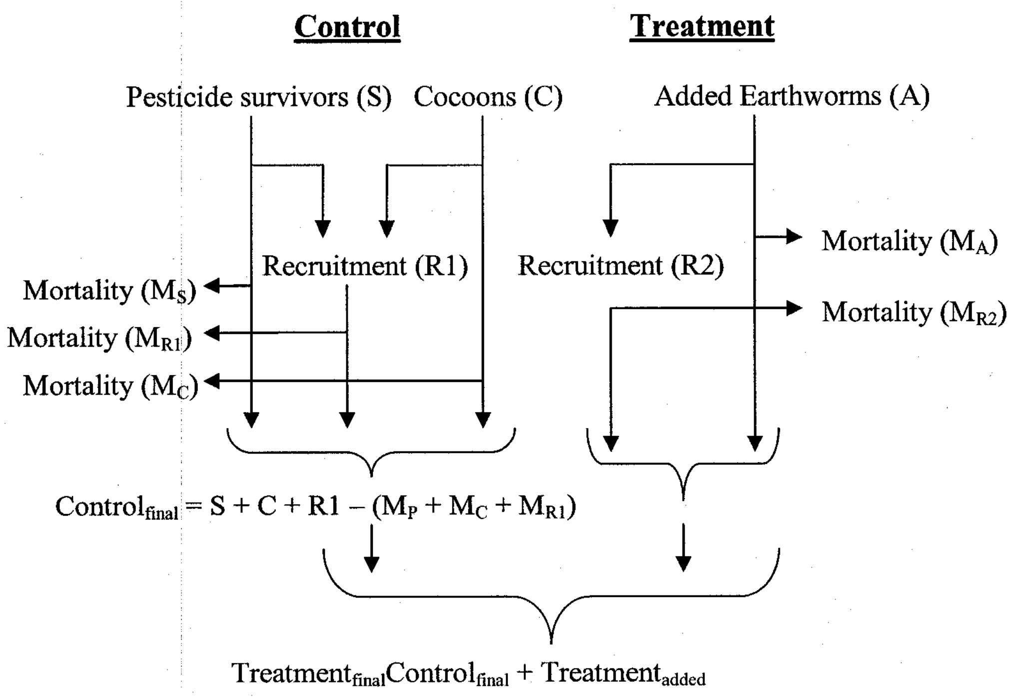

Figure 2. Flowchart of the earthworm population dynamics in control and treatment enclosures used to infer growth and mortality rates for the calculation of secondary production using the "*deduction*" approach.

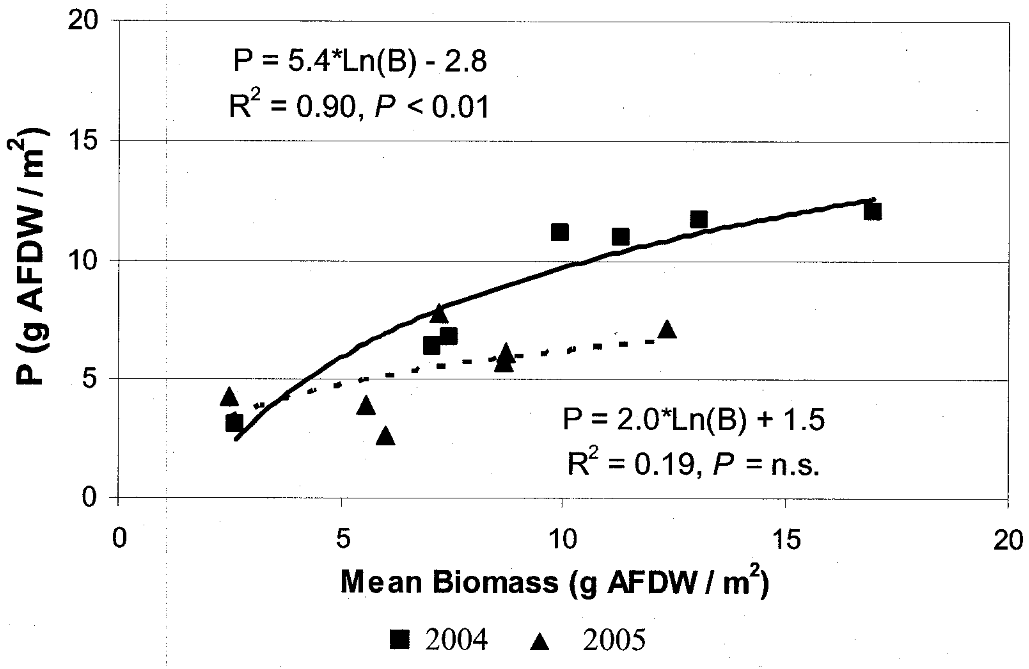

Figure 3. Relationships between secondary production (P) (g AFDW m^{-2}) from the "*deduction*" approach and mean earthworm biomass (B) (g AFDW m^{-2}) during the period June - October in 2004 (■) and 2005 (▲).

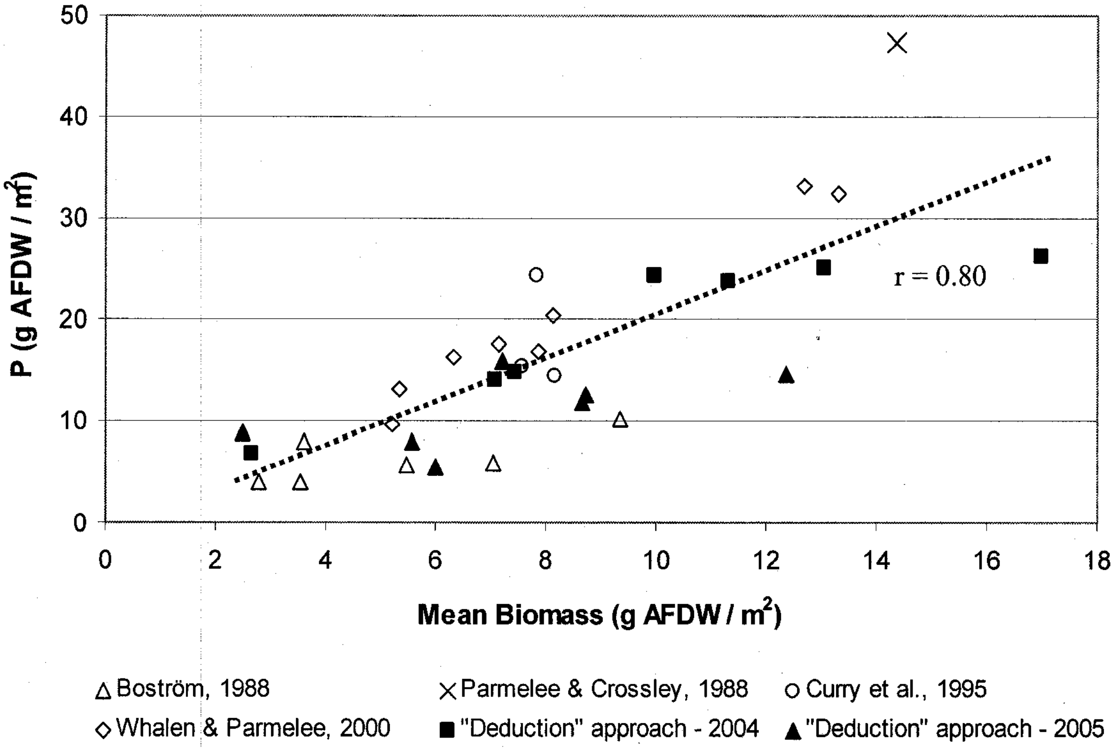

Figure 4. The relationship between secondary production (P) (g AFDW m^{-2}) and mean earthworm biomass (B) (g AFDW m^{-2}) from different studies. Data from this study were extrapolated to a 35 week period, to be consistent with other studies. Pearson's correlation coefficient across all studies is r = 0.80, $P < 0.001$.

FOREWORD TO CHAPTER 5

In the previous chapter we presented the "*deduction*" approach as a non-invasive method to estimate secondary production of earthworm communities in field enclosures. We will build off this initial modeling work to develop and compare two models, the "*growth and output*" and "*food web*" models, that estimate N flux through earthworm communites. Manipulation of earthworm communities in field enclosures allowed us to evaluate how the spatial variability in earthworm populations may affect N flux estimates, while differences in climate in the two study years allowed us to evaluate the effect of the duration of earthworm activity on N flux estimates. Model parameters in both the "*growth and output*" and "*food web*" models will be varied between minimum and maximum literature values to evaluate the robustness and variance in model estimates.

CHAPTER 5.

Modelling the contribution of earthworm communities to nitrogen cycling in temperate maize-soybean agroecosystems

5.1 Abstract

In temperate agroecosystems, the nitrogen (N) cycled from earthworms is estimated at between 7 and 363 kg N ha^{-1} $year^{-1}$. The wide range of estimates is partly explained by differences in climate and agricultural practices across temperate agroecosystems, which affects earthworm population dynamics and activity. However, there is also uncertainty associated with the methods and parameters used to estimate N flux for earthworms. The objectives of this paper were to compare the N flux estimates for earthworms using the "*growth and output*" and "*food web*" models, and to determine the range of N flux estimates obtained when model parameters were set to the minimum, mean and maximum values reported in the literature. The "*growth and output*" model includes the total-N leaving the active earthworm biomass in cast, urine and mucus excretions, the N released in dead earthworm biomass, and the N used for secondary production. The "*food web*" model calculates N mineralization by earthworms as a function of feeding rates. The N flux predictions from both models varied considerably (10 – 50 fold) when model parameters were set to the minimum and maximum values. In particular, the "*food web*" model made either unrealistically high or negative estimates of N mineralization when

certain model parameters were set to minimum and maximum values. This indicates that the "*growth and output*" model is more robust than the *"food web"* model since a wide range of parameter values can be used without causing unrealistic estimates. The N flux from earthworms is strongly affected by soil temperature and moisture conditions, indicating that frequent or continuous monitoring is necessary to accurately gauge the level of earthworm activity in the field. More accurate data for weather dependent parameters will permit us to better quantify N flux from earthworms.

5.2 Introduction

Among soil fauna, earthworms are the most important organism contributing to soil N mineralization and cycling in temperate ecosystems (Lee, 1982). Earthworms release considerable quantities of N from their bodies through urine and mucus excretions, in casts and in dead biomass (Blair et al. 1995; Whalen et al., 1999a). Furthermore, earthworm interactions with soil microbial communities through litter comminution, casting, and burrowing also increase N mineralization and cycling rates (Wolters, 1991; Blair et al. 1995). Therefore, earthworm communities may have a significant impact on soil N cycling and primary production in agricultural systems.

Earthworm communities are estimated to cycle between 7 and 363 kg N ha^{-1} per year (Satchell, 1963; Syers and Springett, 1984; Christensen, 1987; Parmelee and Crossley, 1988; Marinissen and deRuiter, 1993; Curry et al., 1995; Whalen et al., 2000). Such a wide estimate leads to a high degree of uncertainty about the role of earthworms in N cycling. Some of the variation arises from the differences in cropping systems, agrochemicals, tillage and residue management among the temperate agroecosystems where earthworm populations and communities have been studied. Yet, there is also considerable uncertainty associated with the methods used to estimate the contribution of earthworms to N cycling.

One way to determine the contribution to N cycling is to sum all N excretions from earthworms, first described by Satchell (1963). This method determines the total-N flux, in organic and inorganic forms, leaving the active earthworm biomass in casts, urine and mucus excretions, as well as the total-N released in dead earthworm biomass. Although this method has been used frequently to estimate total-N flux through earthworm communities (Satchell, 1963; Christensen, 1987; Christensen, 1988; Marinissen and de Ruiter, 1993), it does not provide a complete estimate of total-N flux because it is based on the mean earthworm biomass present in an agroecosystem during the year. The fluctuation in biomass due to growth, reproduction and mortality, also known as secondary production, is not considered. A more complete "*growth and output*" model includes the N excreted from earthworms and the N used for secondary production. Secondary production of earthworm communities is commonly estimated by sampling earthworm populations at regular intervals throughout the frost-free periods of the year and inferring the change in earthworm biomass determined between sampling dates. The N used for secondary production is then determined by multiplying the N concentration of earthworm biomass by the secondary production. Estimates of total-N flux through earthworms using variations of the combined "*growth and output*" model range from 3 –

74 kg N ha^{-1} (Böström, 1988; Parmelee and Crossley, 1988; Curry et al., 1995; Whalen and Parmelee, 2000; Whalen et al., 2000). The total-N flux from earthworm populations, as estimated by the "*growth and output*" model, includes organic and inorganic forms of N. While inorganic N is considered to be plant-available, organic forms of N may be immobilised, which makes it difficult to determine how much the total-N flux contributes to crop production in agroecosystems.

Some researchers have used the *"food web"* model, originally developed by Hunt et al. (1987), to determine the contribution of earthworm communities to N mineralization in arable ecosystems (Marinissen and de Ruiter, 1993; de Ruiter et al., 1994; Didden et al., 1994; Zwart et al., 1994). The *"food web"* model uses an energetics approach to calculate the flux of inorganic-N from earthworms based on feeding rates and the partitioning of nutrients between the consumer (earthworm), the food source (detritus or microbes), and the environment (soil) (de Ruiter et al., 1994). The model can estimate the direct N mineralization due to earthworm consumption of organic substrates and the indirect N mineralization from micro-organisms stimulated by earthworm feeding activities. Estimates of direct N mineralization due to earthworms range from 1 – 97 kg N ha^{-1} (Marinissen and de Ruiter, 1993; de Ruiter et al., 1994; Didden et al., 1994), while indirect N mineralization was from 11 – 267 kg N ha^{-1} (Marinissen and de Ruiter, 1993).

The uncertainty in model parameters partly explains the wide range in N flux estimates made by both models. Curry et al. (1995) reported that estimates of N turnover from earthworms increased about 4-fold, from 5.1 - 7.4 g N m^{-2} $year^{-1}$ to 21 - 26 g N m^{-2} $year^{-1}$, depending on which N excretion rates were selected from the literature. The majority of growth, excretion and cast production data come from laboratory studies, partly because of the difficulty of quantifying these parameters in the field. However, it is not clear that all laboratory values can be extrapolated directly to field conditions. Furthermore, few studies have evaluated how using laboratory-based parameters may affect N flux estimates (Marinissen and de Ruiter, 1993). For example, both the "*growth and output*" and "*food web*" models include physiological parameters (i.e: excretion rates, growth rates and mortality) that are strongly related to soil temperature and moisture conditions. Weather conditions lead to seasonal fluctuations in soil temperature and moisture conditions, which affect the intensity and duration of earthworm activity during the growing season. Despite this, many previous studies have not explicitly considered the variation in soil temperature and moisture conditions when estimating the contribution of earthworms to N cycling (Böström, 1988; Parmelee and Crossley, 1988; Marinissen and de Ruiter, 1993; de Ruiter et al., 1994; Curry et al., 1995; Whalen and Parmelee, 2000). Furthermore, earthworm communities show high spatial variation in population and biomass (Whalen, 2004). This implies that some areas within an agroecosystem will be "hotspots" for N cycling due to earthworm activities. As far as we know, previous modelling work has not explicitly considered how spatial variation in earthworm numbers and biomass could influence the estimates of total-N flux or N mineralization from earthworms in agroecosystems.

The objectives of this paper were: 1) to explicitly consider the temporal variation in the duration of earthworm activity when modelling physiological parameters in the "*growth* *and output*" and "*food web*" models, 2) to evaluate the relationship between the spatial variation in earthworm biomass and N flux, within the same agroecosystem, and 3) to determine the range of N flux estimates when model parameters are set to the minimum, mean and maximum values reported in the literature.

5.3 Materials and Methods

5.3.1 Description of Field Site and Experiment Design

The study was designed to evaluate the effects of controlled earthworm populations on soil properties and crop yield in field enclosures. It was conducted from May 2004 to September 2005 on the Macdonald Campus Research Farm, McGill University, Quebec, Canada (45°25' N, 73°56' W). In May 2004, rectangular sheet metal field enclosures, measuring 2.4 m x 1.2 m (2.9 m^2), were buried to a depth of 0.30 – 0.40 m. Seven enclosures were arranged in four blocks, for a total of 28 experimental units. During the months of May 2004 and May 2005, carbaryl pesticide (Sevin®) was applied 4 – 5 times to each enclosure (total application of 220 kg a.i. ha^{-1} $year^{-1}$) to reduce earthworm populations. Native earthworms collected from the surrounding field were added to the enclosures on June 1, 2004 and on June 6, 2005 and remained in enclosures for 17 – 18 weeks. The seven treatments included three earthworm populations as *Aporrectodea caliginosa* only (Ac), *Lumbricus terrestris* only (Lt), and a combined *A. caliginosa* and *L. terrestris* treatment (AcLt), at either the background population level (1x) or double the background population level (2x), and a control treatment (Control). On May 28th, 2004, a single row of 100 soybeans (*Glycine max* (L.) cv. Merril) was sown by hand lengthwise in the centre of each enclosure, and on June 1st, 2005, a single row of 15 silage maize seeds (*Zea mays* (L.) cv. Mycogene 2K350) was sown in a similar manner. Earthworms were sampled from a soil pit (50 x 30 cm to a depth of 20 cm) dug in the middle of each enclosure on September 30, 2004 and September 28, 2005. Formalin extraction (Raw, 1959) was used to collect earthworms from lower depths beneath the pit. Earthworm numbers, age classes, formalin-preserved biomass and ash-free dry weight (AFDW) of each earthworm collected were later recorded in the lab. Ash-free dry weights were determined by placing dried (90°C for 24 hours) earthworms in a muffle furnace at 500°C for 4 hours. Sexually mature individuals were identified to the species level using the key provided by Reynolds (1977). The number and biomass of earthworms added in each treatment are described in Chapter 3 & 4. Soil temperature and gravimetric moisture were measured weekly in the 0 – 10 cm depth of each enclosure in both years. Water filled pore space (WFPS) was calculated as: (gravimetric moisture x soil bulk density / total porosity). Soil bulk density was determined as the mean bulk density of two 400 cm^3 cylinders obtained from the 0 – 10 cm depth of each enclosure after harvest in 2005. Total porosity was determined as: 1 – (bulk density / particle density), where a particle density of 2.65 g cm^{-3} was assumed. Further details of the experimental design, description of soils, and methods of plant and soil sampling and analysis are described in Chapter 3.

5.3.2 Calculation of Total-N Flux using the "*Growth and Output*" Model

The *"growth and output"* model estimates the flux of total-N as the total amount of N released from earthworm excretion and casts (equation 1), and through secondary production (equation 2).

N-excreted (g N m^{-2}) = B_{mean} (g AFDW m^{-2}) x active days (d) x [urine & mucus-N excretion + cast-N x surface cast production x (1 + sub-surface : surface cast ratio)] (g N g $AFDW^{-1}$ d^{-1}), (1)

N-secondary production (g N m^{-2}) = P (g AFDW m^{-2}) x N_{ew} (g N g $AFDW^{-1}$) (2)

where B_{mean} (g AFDW m^{-2}) is the mean biomass of earthworms in the enclosures during the growing season and N_{ew} (g N g $AFDW^{-1}$) is the N concentration in earthworms. In 2004, surface cast production in each enclosure was measured (Perreault et al., 2007). We estimated surface cast production in 2005 and the N released in urine and mucus in both years from literature values and field observations (Table 1). Selected literature and experimental values were used to convert fresh weights (fw) into ash-free dry weight (AFDW) (Table 1). The number of active days, B_{mean} and secondary production (P) of earthworm biomass from the enclosures was determined using the "*deduction*" approach (see Chapter 4).

5.3.3 Calculation of Nitrogen Mineralization using the "F*ood Web*" Model

The *"food web"* model calculates the N mineralization (N_{min}) from earthworms based on a feeding rate (F) and the difference between the C:N ratios of the earthworm ($C:N_{ew}$) and food source ($C:N_{food}$) as shown in equations 3 and 4.

$$N_{min}\ (g\ N\ m^{-2}\ season^{-1}) = F \times E_{ass} \times ((1/C:N_{food}) - (E_{prod} / C:N_{ew})), \quad (3)$$

$$F\ (g\ C\ m^{-2}\ season^{-1}) = D \times B_{mean} \times E_{ass}^{-1} \times E_{prod}^{-1}, \quad (4)$$

where the assimilation efficiency of nutrients per unit consumed (E_{ass}), the production efficiency of nutrients per unit assimilated (E_{prod}), and C:N ratios of earthworm tissue and food are based on literature values (Table 1). The specific death rate (D) was calculated as the ratio of secondary production (P) to the mean biomass (B_{mean}) for each treatment (Eriksen-Hamel and Whalen, 2007b). Mean earthworm biomass (B_{mean}) was expressed as biomass-C using the ratio of biomass-C: ash free dry weight (AFDW) of earthworm biomass that was selected from literature values and experimental observations (Table 1). The proportion of earthworm diet that consists of detritus was varied between 20 – 100% and was initially set at 75% detritus and 25% microbes (Table 1).

5.3.4 Range in Model Estimates

The range of estimates calculated by the *"growth and output"* and *"food web"* models were evaluated by varying parameters between minimum and maximum values. The minimum and maximum values of parameters were selected from experimental observations and literature values (Table 1).

5.3.5 Statistical Analysis

Regression lines of the model estimates were fitted using the PROC REG function (SAS version 9.1, SAS Institute, 2001).

5.4 Results

Climate, soil conditions and earthworm communities in enclosures during the two study years have been reported in Chapter 4. The manipulation of earthworm communities in experimental enclosures allowed us to evaluate N flux from earthworm populations of varying size within the same agroecosystems. Furthermore, the drastic difference in climate in the two study years allowed us to evaluate N flux from earthworm populations with different durations of activity.

In 2004, the relationships between N flux estimates and earthworm biomass were significant for the "*growth and output*" and "*food web*" models, but was only significant for the "*growth and output*" model in 2005 (Fig. 1 & 2). Secondary production accounted for 10 – 12% of the N flux calculated by the "*growth and output*" model in 2004, and 8 – 18% in 2005. The ratio of maximum to minimum total-N flux estimates made by the "*growth and output*" model ranged between 10.1 – 11.4 in 2004 and 14.9 – 16.9 in 2005 (Fig. 1 & 2). In 2004, N mineralization estimates made using the *"food web"* model at minimum parameter values were negative because of the large difference between the minimum C:N ratio of earthworm tissue and maximum C:N ratio of detritus, therefore estimates are reported as zero. However, the ratio of maximum to mean values ranged between 41.4 – 49.5 in 2004, and the ratio of maximum to minimum values ranged between 27.0 – 32.4 in 2005 (Fig. 1 & 2).

5.5 Discussion

5.5.1 Uncertainty in Parameter Values and Model Constraints

The number of days for earthworm activity is a key driving parameter because when earthworms are inactive, they are expected to have slower growth, lower casting, excretion and consumption rates (Daniel, 1991; Holmstrup, 2001). Hence, their contribution to the N cycle would slow down or stop. Although we used soil conditions in the top soil (0 – 10 cm) as an indicator of earthworm activity (negative activity was assumed when soil temperature > 22°C and moisture < 20 % WFPS), we realize that earthworms can retreat to deeper depths to avoid desiccation and mortality. However, we assumed that growth and activity at lower depths would be negligible because the sub-surface soil contains fewer organic substrates than the surface layer, and we encounter a distinct sandy layer at 30 – 40 cm depth. The concept of "active days" helps us to predict the duration of general earthworm activity, although we realize that individuals differ in their tolerance to heat and soil moisture. For example, Wever et al. (2001) reported that up to 20% of *A. tuberculata* earthworm activity ceases, due to aestivation, when soils are at optimal temperatures of 15 – 20 °C and moistures of 20 – 25 % (w/w), and this increases to 60% of the population when soils are at temperatures of 20°C and moistures of <15 % (w/w).

A drawback of our experiment was that the earthworm manipulations were conducted during the summer (June – September) when soil temperatures were high (>20°C) and soil moistures were highly variable. Visual observations of casting activity show that earthworms were most active in the early spring (April and May) and fall (late September and October) when soils were cooler (10 – 15°C) and moister. Clearly, we have missed peaks periods of secondary production, cast production and mucus and urine excretions, and therefore would expect greater N flux estimates if the experiment had included the entire frost-free period of our temperate agroecosystem.

5.5.2 N flux estimates from each model

The mean estimates of total-N flux determined by the *"growth and output"* model during the 17 week growing season ranged from 3.9 – 12.2 g N m^{-2} in 2004 and 2.4 – 5.3 g N m^{-2} in 2005. If these results are extrapolated for the frost-free period of the year from April to November (35 weeks) and presented on an annual basis, then the N flux from earthworms in field enclosures (4.9 – 25.1 g N m^{-2} $year^{-1}$) would be greater than N fluxes reported in the literature of 0.3 – 7.4 g N m^{-2} $year^{-1}$ released from earthworm excretions, dead tissue and used in secondary production (Böström, 1988; Parmelee and Crossley, 1988; Curry et al., 1995; Whalen and Parmelee, 2000; Whalen et al., 2000). This is largely the result of uncertainty in mean values for N excretion in mucus and urine, cast-N and surface and sub-surface cast production. Our initial estimate (269 μg N g fw^{-1} d^{-1}) and maximum estimate (744 μg N g fw^{-1} d^{-1}) of N excreted in mucus and urine are much greater than the 30 – 88 μg N g fw^{-1} d^{-1} reported by Christensen (1987) and Needham

(1957). Similarly, our estimate for the cast-N concentration (3.1 mg N g dw^{-1}) was three times greater than the low estimate (0.9 mg N g dw^{-1}) reported by Flegel et al. (1998). James (1991) reported a sub-surface : surface cast production ratio of 50, which was determined by extrapolating a laboratory based model to field conditions. Since this value was 30 times greater than the next largest value, we did not include it in our parameter estimates. However, it shows that site-specific data is needed to improve our confidence in N flux estimates using the "*growth and output*" model. Although we have site-specific values for surface cast production and cast-N concentrations, it would be helpful to also have site-specific date for urine and mucus excretion, and sub-surface cast production.

The mean estimates of inorganic-N flux determined by the *"food web"* model during the 17 week growing season ranged from 0.2 – 0.6 g N m^{-2} in 2004, and from 0.1 – 0.4 g N m^{-2} in 2005. Extrapolated for the frost-free period of the year from April to November (35 weeks) and presented on an annual basis, these estimates (0.2 -1.2 g N m^{-2} year^{-1}) are lower than the N flux estimates of 1.1 – 5.1 g N m^{-2} year^{-1} reported using the *"food web"* model for agroecosystems in the Netherlands (Marinissen and de Ruiter, 1993; Didden et al., 1994). The maximum estimates made by the *"food web"* model, which were as large as 26.3 g N m^{-2} in 2004 may be unrealistically high. These estimates were obtained when we used the minimum value of 0.2 for the production efficiency (E_{prod}), the minimum value of 4 for the of C:N ratio of microbes and the maximum value of 80% for proportion of diet that is microbes (Table 1). The minimum estimates made by the *"food web"* model are negative because we used the maximum value of 40 for the C:N ratio of detritus and the maximum value of 100% for the proportion of diet that is detritus (Table 1). This confirms previously reported findings that N mineralization results from the *"food web"* model are sensitive to the production efficiency and food C:N ratio values (Marinissen and de Ruiter, 1993; de Ruiter et al. 1993). It also shows that the *"food web"* model is less robust than the "*growth and output*" model since the minimum and maximum literature values for certain parameters cause either unrealistically high or negative estimates of N mineralization.

It is expected that areas of fields with more earthworms will generate greater total-N flux. In 2004, when earthworm biomass was doubled, the "*growth and output*" model estimated an increase in total-N flux of 4.7 g N m^{-2}, and the "*food web*" model estimated an increase in inorganic-N flux of 0.2 g N m^{-2}. In 2005, the "*growth and output*" model estimated an increase in total-N flux of 1.9 g N m^{-2} when earthworm biomass was doubled; however no significant relationship between inorganic-N flux and earthworm biomass was estimated by the "*food web*" model. This suggests that as earthworm biomass increases, more organic-N than inorganic-N is excreted in the casts, urine and mucus. This is physiologically unlikely and is probably explained by increased burrowing and casting activity by earthworms which stimulate the growth and activity of microbial populations, and subsequently lead to an increase in extractable soil N concentrations, including inorganic-N and microbial biomass-N (Bohlen and Edwards, 1995; Edwards and Bohlen, 1996; Brown et al., 2000; Chaoui et al., 2003).

The *"food web"* model has been used to estimate the stimulating effect of earthworms on microbial N mineralization, but we did not have the necessary data on microbial populations to perform this analysis (Marinissen and de Ruiter, 1993; de Ruiter et al., 1994). However, Marinissen and de Ruiter (1993) showed that the indirect microbial N mineralization resulting from earthworms was between 2.5 – 11 fold greater than direct N mineralization from earthworms. This is partially validated in our field experiment, as we found a stronger positive linear relationship between microbial biomass-N, and earthworm population and than between soil-N concentration and earthworm population (see Chapter 3). Similarly, in arable farming systems in the Netherlands, soil microbes (bacteria and fungi) were responsible for 70% of the gross N mineralization and other soil fauna, primarily earthworms and nematodes, were responsible for 30% (Verhoef and Brussaard, 1990; de Ruiter et al. 1994). Further work is needed to quantify the influence of earthworms on microbially-mediated N mineralization in temperate agroecosystems.

Relating model estimates with plant-N and labile soil-N pools in the field is difficult because the fate of the N released from earthworms is poorly understood. The total-N flux estimates made by the "*growth and output*" model are likely greater than the quantity of N available to plants. During the growing season, N can be cycled through an earthworm community more than once, thereby being counted twice in model estimates. Up to 70% of the N that is excreted by earthworms or released from dead earthworm biomass can be immobilized in the microbial biomass or plant-N pool within 15 days (Whalen et al., 1999b; Hodge et al., 2000). If an earthworm dies early in the season, then the N estimated in its tissue could cycle through the microbial biomass and plant-N pools, and be consumed by another earthworm later in the season. This N would be reported twice, once as N excreted and again as the N used for secondary production. On the other hand, inorganic-N flux from the "*food web*" model is probably less than the labile-N pool since microbial mineralization of organic-N is not considered. This suggests that the actual quantity of N made available to plants from earthworms lies between these model estimates. Further research using improved nutrient budgets or isotope analysis will be necessary to validate these models and determine the contribution of earthworms to the plant-available N pool in agroecosystems.

5.6 Conclusions

Earthworm manipulation experiments provide a unique opportunity to deduce changes in earthworm biomass and determine N flux through earthworm communities. The wide range of estimates made by the "*growth and output*" and "*food web*" models arises from uncertainty in the model parameters. Site-specific soil temperature and moisture data, as well as excretion data, are required to make N flux estimates with greater certainty. The "*growth and output*" model appears to be more robust than the "*food web*" model, since adjusting some parameters to the minimum or maximum values reported in the literature generate unrealistically high or negative estimates of N mineralization. More complete nutrient budgets or isotope analysis are required to quantify the N flux through earthworms and predict their contribution to the plant-available N pool in temperate agroecosystems. Comparison of these models will help us advance our understanding of the temporal and spatial dimensions of earthworm involvement in N cycling and primary production.

5.7 References

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Table 1. Range of values for the parameters used in the models

Parameter	Units	Estimate	Minimum	Maximum	References
Active period (Non-aestivation)	days	2004: 77 2005: 49	2004: 63 2005: 35	2004: 91 2005: 63	Fig. 1, Eriksen-Hamel and Whalen, 2007b
g fw: g AFDW	ratio	7.5	5.0	8.3	Experimental observations; Böström, 1988; Curry et al., 1995
g N : g AFDW	ratio	0.1	0.08	0.13	Böström, 1988; Parmelee and Crossley, 1988; Whalen and Parmelee, 1999b; Curry et al., 1995
Urine & mucus- N excretions	μg N g fw^{-1} d^{-1}	269	30	744	Needham, 1957; Christensen, 1987; Whalen et al. 2000
Cast N content	mg tot-N g dw^{-1}	3.1	0.9	5.4	Syers et al., 1979; Lee, 1982; Flegel et al., 1998; Buck et al., 1999; Norgrove and Hauser, 1999; Jégou et al., 2001; Perreault et al., 2007
Cast production	g dw g fw^{-1} d^{-1}	0.09 – 0.28	0.09	0.4	Scheu, 1987; Shipitalo and Protz, 1989; Curry et al., 1995; Curry and Baker, 1998; Flegel et al., 1998; Whalen et al. 2004; Perreault et al., 2007
Sub-surface : surface cast production	ratio	1.25	1	1.5	Whalen et al., 2004; Perreault and Whalen, 2006
Assimilation efficiency (E_{ass})	ratio	0.2	0.1	0.4	Marinissen and de Ruiter, 1993; Whalen and Parmelee, 1999b;
Production efficiency (E_{prod})	ratio	0.4	0.2	0.45	de Ruiter et al., 1993; Marinissen and de Ruiter, 1993;
$\mathrm{C:N}_{\mathrm{ew}}$	ratio	5	4.5	8.0	Experimental observations; Whalen et al., 1999a
$\mathrm{C:N}_{\mathrm{detritus}}$	ratio	10	8	40	Marinissen and de Ruiter, 1993; Whalen et al., 1999a
$\mathrm{C:N}_{\mathrm{microbes}}$	ratio	6	4	8	Marinissen and de Ruiter, 1993; de Ruiter et al.,1994
g C : g AFDW	ratio	0.8	0.5	0.9	Experimental observations; Böström, 1988
Proportion of diet from detritus	%	75	20	100	Marinissen and de Ruiter, 1993; Didden et al., 1994

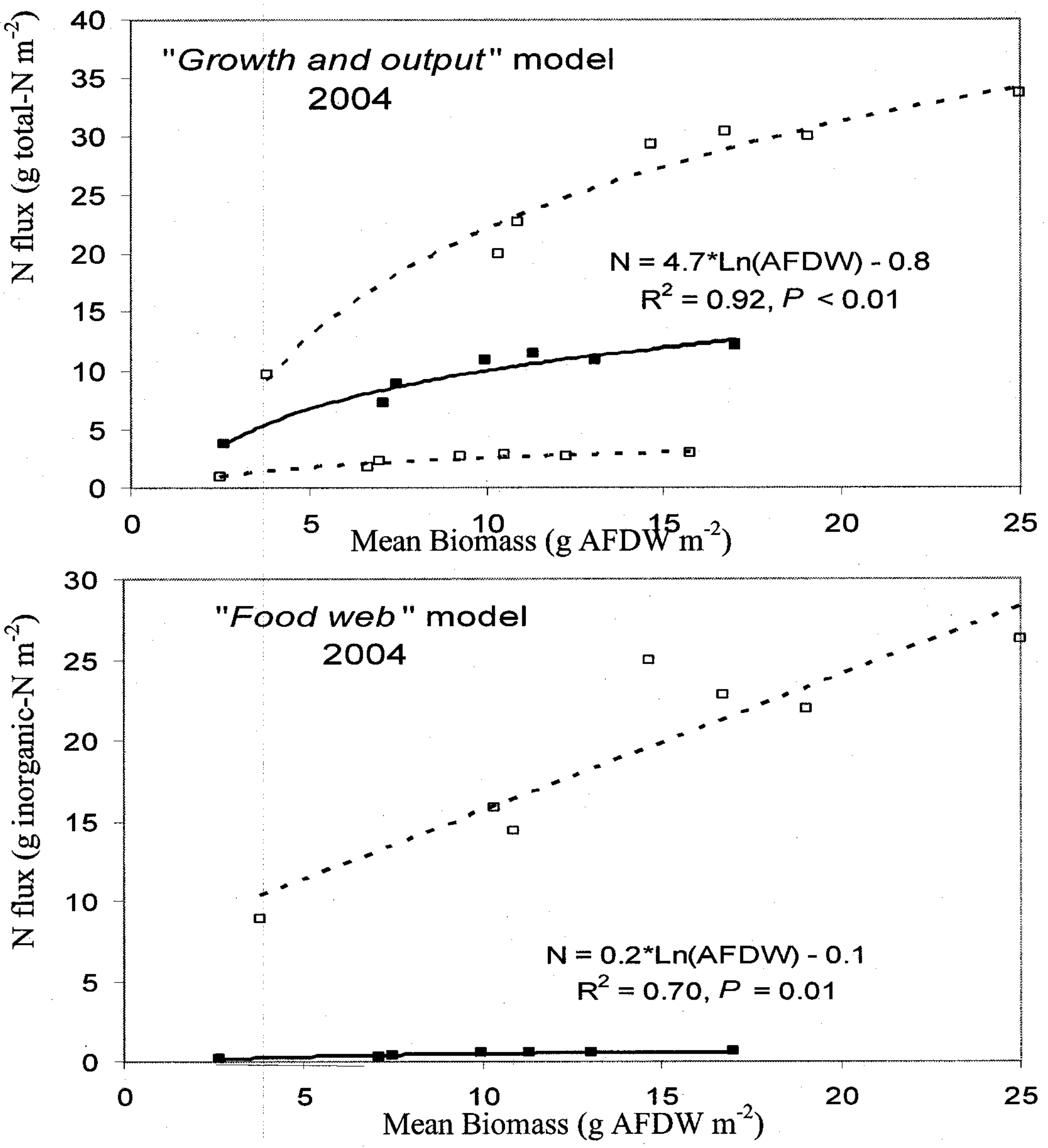

Figure 1. Relationships between earthworm ash-free dry weight biomass (g AFDW m^{-2}) in 2004 and N flux estimates (g N m^{-2}) made by the "*growth and output*" and "*food web*" models. Solid lines show the relationship at the initial parameter values and dotted lines show the relationships when parameters were set to the minimum and maximum values. The minimum estimates made by the "*food web*" model were negative and are not shown.

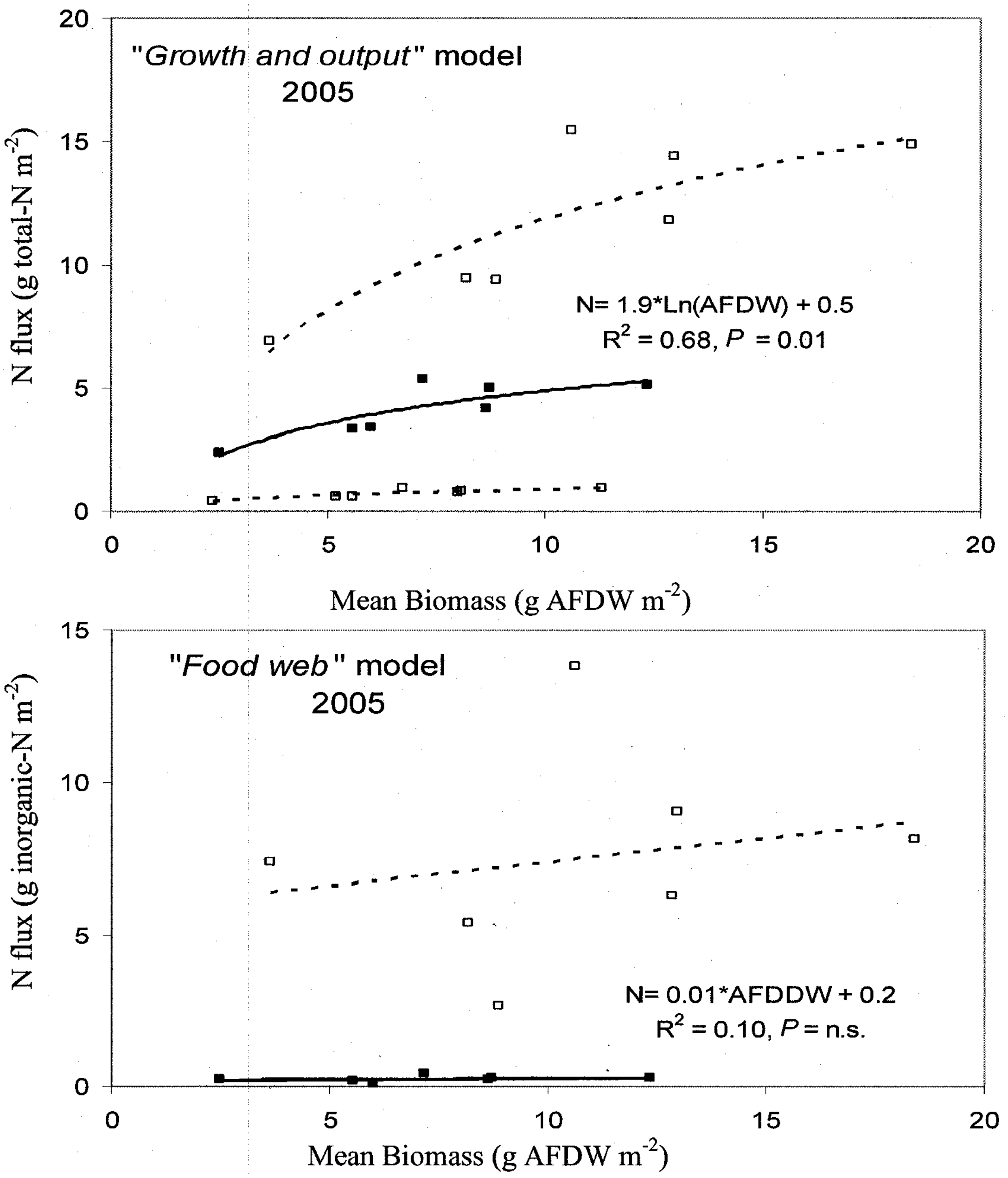

Figure 2. Relationships between earthworm ash-free dry weight biomass (g AFDW m^{-2}) in 2005 and N flux estimates (g N m^{-2}) made by the "*growth and output*" and "*food web*" models. Solid lines show the relationship at the initial parameter values and dotted lines show the relationships when parameters were set to the minimum and maximum values. The minimum estimates made by the *"food web"* model were negative and are not shown.

FOREWORD TO CHAPTER 6

In the previous chapters laboratory growth rates, results from the field enclosure experiment and literature data were used to model the contribution of earthworms to soil N cycling. Secondary production was estimated from field data using the "*deduction*" approach and used in the "*growth and output*" model while literature data was mostly used in the "*food web*"model. A wide range of estimates was found when parameter values were varied between minimum and maximum values.. The labour and financial resoureces required to obtain accurate estimates of all parameters for a given agro-ecosystem is simply too great. Therefore, it is important to determine the most important factors controlling N flux estimates in each model. In the following chapter a sensitivity analysis of each model will help to identify which parameters are most important. Future researchers will be able to focus on the identified parameters in order to improve the estimates of the contribution of earthworms to N cycling for other agroecosystems.

CHAPTER 6.

Measuring the sensitivity of earthworm – nitrogen flux models

6.1 Abstract

The objective of this paper was to conduct sensitivity analysis of the "*growth and output*" and "*food web*" models, which estimate the contribution to nitrogen (N) cycling by earthworms. Model parameters were incrementally varied between minimum and maximum values obtained experimentally and from the scientific literature. The duration of earthworm activity, cast production and cast N content were the most important factors controlling N flux estimates from the "*growth and output*" model. Site-specific data for these parameters would reduce uncertainty in N flux estimates made by this model. The production efficiency, C:N ratio of biomass, and biomass conversions were the most sensitive parameters in the "*food web*" model, suggesting that species-specific data are necessary to optimize N mineralization estimates from this model. Direct measurement of the most sensitive parameters we identified should permit us to more accurately model and quantify the N cycled by earthworms. Selecting literature values for other model parameters may be appropriate if monetary and human resources are not available to gather site- or species-specific data.

6.2 Introduction

Earthworm communities have a significant impact on soil N dynamics in temperate agroecosystems, and are thought to cycle between 7 and 363 kg N ha^{-1} per year (Satchell, 1963; Syers and Springett, 1984; Christensen, 1987; Parmelee and Crossley, 1988; Marinissen and deRuiter, 1993; Curry et al., 1995; Whalen et al., 2000). Some variation is expected due to differences in cropping systems, tillage and residue management in the temperate agroecosystems studied by these researchers, but such a wide estimate implies uncertainty about the role of earthworms in N cycling. Sensitivity analysis of the models used to estimate the contribution of earthworms to N cycles can provide insight into how model parameters contribute to the variation in N flux estimates.

One earthworm N flux model is the "*growth and output*" model described by Eriksen-Hamel and Whalen (2006a), based on a model first presented by Satchell (1963). This model accounts for the total-N leaving the active earthworm biomass in casts, urine and mucus excretions, the amount of N released in dead earthworm biomass, as well as the N used for secondary production. Variations of the "*growth and output*" model give estimates of earthworm N flux ranging from 3 – 74 kg N ha^{-1} (Böström, 1988; Parmelee and Crossley, 1988; Curry et al., 1995; Whalen and Parmelee, 2000; Whalen et al., 2000; Eriksen-Hamel and Whalen, 2006a). Small changes in some parameters of the "*growth and output*" model can greatly change the N flux estimates. Curry et al. (1995) reported that estimates of N turnover from earthworms increased about 4-fold, from 5.1 – 7.4 g N

m^{-2} $year^{-1}$ to 21 – 26 g N m^{-2} $year^{-1}$, depending on which N excretion rate was selected from the literature.

Another earthworm N flux model, the *"food web"* model, uses an energetics approach. Originally developed by Hunt et al. (1987) to analyze N flux through the detrital food web of a shortgrass prairie, the *"food web"* model was adapted to calculate N mineralization by earthworm communities in arable ecosystems (Marinissen and de Ruiter, 1993; de Ruiter et al., 1994; Didden et al., 1994; Zwart et al., 1994). The *"food web"* model estimates direct N mineralization due to earthworm consumption of organic substrates and the indirect N mineralization from micro-organisms stimulated by earthworm feeding activities. Estimates of direct N mineralization by earthworms range from 1 – 97 kg N ha^{-1} (Marinissen and de Ruiter, 1993; de Ruiter et al., 1994; Didden et al., 1994; Eriksen-Hamel and Whalen, 2006a), with an additional 11 – 267 kg N ha^{-1} released through indirect N mineralization (Marinissen and de Ruiter, 1993). Estimates from the *"food web"* model are sensitive to earthworm feeding rates and C:N ratios of organic substrates (de Ruiter et al., 1994).

The objective of this paper was to identify the parameters in the *"growth and output"* model and the *"food web"* model having the greatest impact on earthworm N flux estimates.

6.3 Materials and methods

6.3.1 Calculation of total-N flux using the "*growth and output*" model

The *"growth and output"* model estimates the total-N flux as the total amount of N released from earthworm excretion and casts (equation 1), dead tissue (equation 2) and secondary production (equation 3).

$$\text{N-excreted (g N m}^{-2}\text{)} = B_{mean} \text{ x } T_{active} \text{ x [urine \& mucus-N excretion +}$$
$$\text{surface cast production x } N_{cast} \text{ x (1 + sub-surface : surface cast ratio)],} \quad (1)$$

$$\text{N-dead tissue (g N m}^{-2}\text{)} = M_t \text{ x } N_{ew}, \quad (2)$$

$$\text{N-secondary production (g N m}^{-2}\text{)} = P \text{ x } N_{ew} \quad (3)$$

where B_{mean} is the mean biomass of active earthworms during the growing season; T_{active} is the number of days with positive earthworm activity; N_{cast} is the N concentration in earthworm casts; N_{ew} is the N concentration in earthworm biomass; M_t is the mortality of earthworms; and P is the secondary production of earthworm biomass.

The specific death rate (D), defined as the ratio of secondary production (P) to the mean biomass (B_{mean}) (Parmelee and Crossley, 1988), was used to determine earthworm mortality (M_t) (equation 4) and secondary production (P) (equation 5). The specific death rate, excretion rates and casting data were independent parameters selected from field observations and literature values (Table 1).

$$M_t \text{ (g AFDW m}^{-2}\text{)} = (D - 1)^* B_{mean} \quad (4)$$

$$P \text{ (g AFDW m}^{-2}\text{)} = D^* B_{mean} \quad (5)$$

6.3.2 Calculation of N mineralization using the "*food web*" model

The N mineralization from the *"food web"* model is calculated based on a feeding rate (F) and the difference between the C:N ratios of the earthworm ($C:N_{ew}$) and food source ($C:N_{food}$) as shown in equations 6 and 7.

$$F\ (g\ C\ m^{-2}\ season^{-1}) = D \times B_{mean} \times E_{ass}^{-1} \times E_{prod}^{-1}, \quad (6)$$

$$N_{min}\ (g\ N\ m^{-2}\ season^{-1}) = F \times E_{ass} \times [(1/C:N_{food}) - (E_{prod} / C:N_{ew})], \quad (7)$$

where D is the specific death rate of earthworms, E_{ass} is the assimilation efficiency of nutrients per unit consumed, and E_{prod} is the production efficiency of nutrients per unit assimilated (Table 1). The independent parameters for the C:N ratios of earthworm tissue and food are based on literature values (Table 1). The proportion of earthworm diet that consists of detritus was varied between 20 – 100% and was initially set at 75% detritus and 25% microbes (Table 1). Mean earthworm biomass (B_{mean}) was expressed as biomass-C using the ratio of biomass-C : ash free dry weight (AFDW) of earthworm biomass (Table 1).

6.3.3 Sensitivity of model predictions to variation in independent parameters

Both the "*growth and output*" and the "*food web*" models respond linearly to changes in the dependent field parameter B_{mean}, and the ratio of maximum to minimum estimates did not change when the B_{mean} varied. Therefore, N flux estimates were calculated for B_{mean} = 75 g fw m^{-2}, which was close to the background biomass of 60 – 120 g fw m^{-2} for a mixed *A. caliginosa* / *L. terrestris* community found in corn and soybean agroecosystems in

Québec (Eriksen-Hamel and Whalen, 2006b) (Table 2 & 3). Earthworm biomass was converted from fresh weight (fw) into ash-free dry weight (AFDW) and the N concentration (N_{ew}) in earthworm biomass was calculated on an AFDW basis using selected literature and experimental values (Table 1). The sensitivity of N flux estimates calculated by the *"growth and output"* and *"food web"* models were evaluated by varying the independent parameters one at a time, therefore interactions between parameters were not considered (Lenhart et al., 2002). The "*growth and output*" model is a linear model, and the change in total-N flux estimates was determined for every ± 1 % change from the initial value of the parameter, to the minimum or maximum value reported in Table 1. The "*food web*" model does not respond linearly to all parameters, therefore the sensitivity of N mineralization estimates was determined by varying the initial value of independent parameters by ± 10 % and ± 25 %.

6.3.4 Uncertainty associated with independent parameters

The uncertainty associated with each independent parameter in the models is reflected in the range of N flux estimates determined when minimum and maximum values were tested (Table 2 & 3). The ratio of these N flux estimates (maximum / minimum) indicates the maximum uncertainty in N flux estimates caused by varying each parameter.

6.4 Results and Discussion

The active period (T_{active}) had the greatest impact on total-N flux estimates from the "*growth and output*" model. There was a ± 0.8 % change in N flux estimates when the parameter varied by ± 1 % (Table 2). Cast production and cast N content are the next most important parameters, causing a ± 0.7 % change in N flux estimates with a ± 1 % change in parameter value. Varying other independent parameters in the "*growth and output*" model by ± 1 % caused minor (= ± 0.4 %) changes in N flux estimates. The max : min ratios of N flux estimates were greatest for the active period, cast production and cast N content.

The sensitivity analysis of the "*growth and output*" model showed that site-specific data on the period of earthworm activity, cast production and cast N content would greatly improve the reliability of model estimates. For the other parameters, changing the median values had little effect on model estimates, but it should be noted that N flux estimates were 20-60% greater at maximum than minimum values for these parameters (Table 2).

In the "*food web*" model, the production efficiency (E_{prod}) and C:N ratio of earthworm biomass ($C{:}N_{ew}$) had the greatest impact on N mineralisation estimates (Table 3). These independent parameters caused a change in N mineralization estimates that was greater than the change in parameter value (Table 3). The specific death rate (D) and biomass conversion parameters (fw to AFDW, AFDW to biomass-C) changed the N flux estimates proportionally to the change in parameter values (Table 3). Changes in the remaining parameters did not greatly change the N flux estimates (Table 3). The max : min ratios were greatest for the specific death rate, the production efficiency and C:N ratio of earthworm biomass, with N flux estimates 110 – 400 % greater at maximum than minimum values (Table 3). For all other parameters, the N flux estimates changed by 0 – 80 % when parameters varied between minimum and maximum values.

The sensitivity analysis of the "*food web*" model shows the importance of having species-specific physiological data such as the production efficiency, C:N ratio of earthworm biomass and accurate conversions for fresh weight, biomass-C and AFDW. Also of importance is site-specific information concerning the specific death rate of the earthworm community. Although the "*food web*" model is modelled around the composition of earthworm diet and the C:N ratio of detritus and microbes, our sensitivity analysis shows that these parameters do not cause significant changes in N flux estimates, contrary to findings by Marinissen and de Ruiter (1993). Selected literature values for these parameters may be used without the risk of increasing uncertainty in model estimates.

A high ratio of maximum to minimum values for an independent parameter may indicate high uncertainty regarding the initial value of this parameter. However, it does not necessarily indicate the impact that this parameter will have on N flux estimates. For example in the "*growth and output*" model, the extreme values (maximum and minimum) for mucus and urine-N excretions varied 25-fold (Table 1) yet the N flux estimates determined at these extreme values varied by only 1.4-fold (Table 2). Conversely, in the "*food web*" model, the extreme values (maximum and minimum) for the C:N ratio of earthworm biomass vary by 1.8 fold (Table 1), but N flux estimates determined at these extreme values vary by 2.1 fold (Table 3). Therefore, it is more important to have an accurate value for the C:N ratio of earthworm biomass than an accurate value for mucus and urine-N excretions. Choosing an inaccurate value for the C:N ratio of earthworm biomass within the range given by the literature (Table 1) will have a much greater effect on N flux estimates than choosing an inaccurate value for mucus and urine-N excretions. Fortunately, the ranking of important parameters by the sensitivity analysis closely follows the ranking made by the ratio of N flux estimates obtained at maximum and minimum literature values. The most sensitive parameters usually have the greatest ratio of maximum to minimum N flux estimates. Although not as precise as the sensitivity analysis, the ratio of N flux estimates obtained at maximum and minimum literature values could be used as a secondary indicator for determining the most important parameters in each model in the event that the sensitivity analysis shows equal ranking for two or more parameters.

6.5 Conclusions

Sensitivity analysis identified the parameters that have the greatest impact on N flux estimates from each model. In the "*growth and output*" model, site-specific information is required for the active period, cast production and cast N content. In the "*food web*" model, species-specific physiological information, such as the production efficiency, C:N ratio of biomass and biomass conversions are required. Our results suggest that most other parameters in earthworm N flux models can be adequately represented with literature values. Research to better quantify the sensitive parameters identified from our analysis could reduce the uncertainty associated with estimates from earthworm N flux models.

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Table 1. Range of values, and difference between maximum and minimum values (max./min), for the parameters used in the "*growth and output*" and "*food web*" models

Parameter	Units	Initial value	Minimum	Maximum	max./min.	References
Active period (T_{active})	days	80	50	110	2.2	Weather conditions in Québec for 2004 & 2005; Eriksen-Hamel and Whalen, 2006a
g fw: g AFDW	ratio	7.5	5.0	8.3	1.7	Experimental observations; Böström, 1988; Curry et al., 1995
g N : g AFDW	ratio	0.1	0.08	0.13	1.6	Böström, 1988; Parmelee and Crossley, 1988; Whalen and Parmelee, 1999; Curry et al., 1995
Urine & mucus-N excretions	μg N g fw^{-1} d^{-1}	269	30	744	25	Needham, 1957; Christensen, 1987; Whalen et al. 2000
Cast N content (N_{cast})	mg tot-N g dw^{-1}	3.1	0.9	5.4	6.0	Syers et al., 1979; Lee, 1982; Flegel et al., 1998; Buck et al., 1999; Norgrove and Hauser, 1999; Jégou et al., 2001; Perreault, 2005
Cast production	g dw g fw^{-1} d^{-1}	0.2	0.09	0.4	4.4	Scheu, 1987; Shipitalo and Protz, 1989; Curry et al., 1995; Curry and Baker, 1998; Flegel et al., 1998; Whalen et al. 2004; Perreault, 2005;
Sub-surface : surface cast production	ratio	1.25	1	1.5	1.5	Whalen et al., 2004; Perreault, 2005
Assimilation efficiency (E_{ass})	ratio	0.2	0.1	0.4	4.0	Marinissen and de Ruiter, 1993; Whalen and Parmelee, 1999;
Production efficiency (E_{prod})	ratio	0.4	0.2	0.45	2.2	de Ruiter et al., 1993; Marinissen and de Ruiter, 1993;
$C:N_{ew}$	ratio	5.0	4.5	8.0	1.8	Experimental observations; Whalen et al., 1999
$C:N_{detritus}$	ratio	10	8	40	5.0	Marinissen and de Ruiter, 1993; Whalen et al., 1999
$C:N_{microbes}$	ratio	6	4	8	2.0	Marinissen and de Ruiter, 1993; de Ruiter et al.,1994
g C : g AFDW	ratio	0.8	0.5	0.9	1.8	Experimental observations; Böström, 1988
Proportion of diet from detritus	%	75	20	100	5.0	Marinissen and de Ruiter, 1993; Didden et al., 1994
Specific Death rate (D)	ratio	2	1	4	4.0	Parmelee and Crossley, 1988; Curry et al., 1995; Whalen and Parmelee, 2000; Eriksen-Hamel and Whalen, 2006a

Table 2. Sensitivity of the "*growth and output*" model N flux estimates (g N m^{-2}) to incremental change in parameter values, and the uncertainty associated with each parameter (max. / min.).

Parameter	% change in N flux estimate when parameter value varied by ± 1%	N flux estimates (g N m^{-2})†			
		minimum	mean	maximum	max. / min.
Active period (T_{active})	± 0.8 %	8.0	13.0	16.7	2.1
Cast N content (N_{cast})	± 0.7 %	7.0	13.0	19.2	2.7
Cast production	± 0.7 %	8.4	13.0	21.4	2.5
Sub-surface : surface cast production	± 0.4 %	12.1	13.0	13.9	1.2
Specific Death rate (D)	± 0.3 %	11.0	13.0	17.0	1.5
g fw: g AFDW	± 0.2 %	12.7	13.0	14.5	1.1
g N : g AFDW (N_{ew})	± 0.2 %	12.4	13.0	13.9	1.1
Urine & mucus-N excretions	± 0.1 %	11.6	13.0	15.8	1.4

†: N flux estimates calculated for a B_{mean} = 75 g fw m^{-2}

Table 3. Sensitivity of the "*food web*" model N flux estimates (g N m^{-2}) to ± 10% and ± 25% change in parameter values, and the uncertainty associated with each parameter (max. / min.).

Parameter	% change in N flux estimate when parameter value varied by		N flux estimates (g N m^{-2}) †			
	± 10 %	± 25 %	minimum	mean	maximum	max. / min.
Production efficiency (E_{prod})	± 12 – 13 %	± 29 – 33 %	5.6	6.5	14.5	2.6
$C:N_{ew}$	± 12 – 13 %	± 29 – 33 %	3.5	6.5	7.4	2.1
g C : g AFDW	± 9 – 11 %	± 20 – 33 %	4.0	6.5	7.3	1.8
Specific Death rate (D)	± 10 %	± 25 %	3.2	6.5	13.0	4.0
g fw: g AFDW	± 10 %	± 25 %	5.9	6.5	9.7	1.7
$C:N_{detritus}$	± 3 – 4 %	± 8 – 12 %	5.9	6.5	7.5	1.3
Proportion of diet from detritus	± 2 %	±4 – 5 %	5.8	6.5	6.7	1.2
$C:N_{microbes}$	± 1 %	± 2 %	6.4	6.5	6.6	1.0
Assimilation efficiency (E_{ass})	0 %	0 %	6.5	6.5	6.5	1.0

†: N flux estimates calculated for a B_{mean} = 75 g fw m^{-2}

SUMMARY AND CONCLUSIONS

It is established that earthworms have an important role in the decomposition of organic matter, mineralization of nutrients and physical mixing of soils. Despite a large number of laboratory and greenhouse-level studies investigating how earthworms modify soil properties and promote soil fertility, we lack reliable methods to scale-up and quantify earthworm contributions to nutrient cycling at the agroecosystems level. My Ph.D. research used laboratory growth rates to predict the dynamics of earthworm growth in reponse to seasonal fluctuations in soil temperature and moisture that occur in Québec agroecosystems. Furthermore, the relationships between earthworm populations, soil-N pools and annual crop production were evaluated in a field-level manipulation experiment. These data permitted me to make assumptions about earthworm activity and life histories under field conditions, and to test the sensitivity of two models used to determine the contribution of earthworms to N cycling.

Earthworms contribute to N cycling primarily during periods when they are actively growing. The structures, such as burrows and casts, resulting from earthworm activity also provide enhanced microbial N mineralization. Therefore, in my first experiment, I developed growth curves for *A. caliginosa*, the most common earthworm in Québec agroecosystems, that considered the range of soil temperatures (5 to 20 °C) and soil water potentials (-5 to -54 kPa) normally encountered in field soils. The optimum growth conditions for *A. caliginosa* were at 20°C and -5 kPa water potential, and weight loss occurred when soil water potential was less than -54 kPa and when the temperature was less than 5°C. Earthworm growth rates were influenced by the shape of the container used to culture earthworms. Growth rates of *A. caliginosa* were reduced in small narrow cores compared to small pots. Further work is needed to develop standard methodologies so laboratory measurements are representative of earthworm growth rates in the field.

The enclosure experiment showed that varied success in manipulating earthworm populations in field enclosures was linked to climate conditions and possibly available food resources. In 2004, when favourable conditions (temperatures < 20°C, and rainfall at least once a week) occurred, earthworm manipulations were successful and a positive relationship was found between earthworm numbers and the plant available-N, including soil mineral-N, microbial biomass-N and total-N removed in soybean grain. In 2005, when unfavourable conditions (temperatures > 20°C and little or no rainfall) occurred in the weeks following earthworm additions, no relationship was found between earthworms and yield or plant and soil nutrients.

The fourth chapter combined the information from these experiments into a model to estimate the N flux through earthworm communities. My "*deduction*" approach to estimate earthworm secondary production gave estimates ranging from 0.9 – 4.6 g N m^{-2} per year, which were similar to other published rates of secondary production. The "*growth and output*" and "*food web*" models made significantly different estimates of N flux and had large variations due to the uncertainty in the minimum and maximum values of parameters. The "*growth and output*" model predicts that during the crop growing period, under favourable climate conditions, high earthworm biomass of greater than 100 g fw m^{-2} is responsible for the cycling of up to 120 kg N ha^{-1} in arable fields. Yet, under the same conditions, the "*food web*" model predicts that earthworms are responsible for cycling up to 15 kg N ha^{-1}. Both these models are very sensitive to input parameters. The relationships between earthworm biomass plant-N and labile soil-N at harvest obtained from the field were not validated by model predictions.

In the final chapter the model was tested for its sensitivity to parameter values. Sensitivity analysis identified the parameters that have the greatest impact on N flux estimates from each model. In the "*growth and output*" model, more accurate estimates of site-specific parameters are required while for the "*food web*" model, more accurate estimates of species-specific physiological parameters are required. Our results suggest that most other parameters in earthworm N flux models can be adequately represented with literature values. Improved estimates of the contribution of earthworms to N cycling can be obtained if accurate estimates are obtained for the sensitive parameters identified. More complete nutrient budgets or isotope analysis are also required to correctly evaluate the N flux through earthworms, predict their impact on N mineralization at the field scale and validate model N flux estimates.

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