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COMMUNAUTE FRANCAISE DE BELGIQUEE

ACADEMIE UNIVERSITAIRE WALLONIE-EUROPE

UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH

EFFECTS OF AGRICULTURAL PRACTICES AND HEAVY METAL CONTAMINATION ON THE COMMUNITY DYNAMICS OF EARTHWORMS IN RELATION TO SOIL PHYSICAL AND CHEMICAL FACTORS IN AGRICULTURAL FIELDS (BELGIUM)

LEMTIRI Aboulkacem

A thesis submitted for

PhD degree in Agronomic Sciences and Biological Engineering

Department of AgroBioChem, Functional and Evolutionary Entomology
and
Department of Biosystems Engineering, Water - Soil - Plant Exchanges

Supervisors: Frédéric Francis and Gilles Colinet

Academic Year: 2014-2015

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ABSTRACT

We investigated the effect of different agricultural practices on the abundances, biomass, and species diversity of earthworms. Specifically, we aimed to identify the relationship between certain soil physico-chemical properties and earthworm communities in agricultural soils. Two tillage systems and crop residue management were investigated. After conducting the study over four years, we found that the abundance, biomass, and diversity of earthworms were negatively affected by tillage application and the removal of crop residues.

All ecological groups were negatively affected by conventional tillage system and crop residues exportation. However, crop residues removal had a greater impact than the conventional tillage system. In this study area, the earthworm community was dominated by the endogeic species *A. c. caliginosa* (64%), while few epigeic and anecic species were observed (5%). Endogeic and epi-anecic (*L. terrestris*) species appeared to be highly influenced by tillage and of crop residues exportation. When crop residues were exported from the field, the concentrations of chemical elements were low, particularly P and K nutrients. Earthworm activity contributed to nutrient dynamics and soil structure after four years of incorporating of crop residues to the fields and reduced tillage application. No consistent relationship was detected between soil and earthworm variables, even though different soil properties responded differently with respect to the tillage systems, crop residues removal and the presence of certain earthworm species. The number of years that our field was managed might have also affected our results.

On the basis of the primarily research focused on understanding how earthworms participate and contribute towards improving soil quality (structure, nutrient dynamics and fertility), we subsequently focused on investigating how two plants (*Vicia faba* and *Zea mays*) and the *Eisenia fetida* earthworm contribute to uptake of different metals: Cd, Zn, Pb and Cu from the land surrounding of a former Zn-Pb ore treatment plant. Specifically, we tested whether the earthworm *Eisenia fetida* could act as a catalyzor to enhance phytoremediation efficiency. After 42 days of exposure, our results showed that certain earthworm life-cycle traits are affected by metal contamination and by the addition of plants. Specifically, the concentrations of metals in earthworm tissues decreased in the presence of plants. Our findings demonstrate that earthworm activities modify the availability of metals in soils, enhancing metal uptake by plants. This innovative system offers new investigation possibilities by considering earthworm-plant-soil interaction. In conclusion, this work confirmed that earthworms are important catalyzor optimizing the phytoremediation processes of polluted soils.

Keywords: Conventional tillage, reduced tillage, crop residue management, soil properties, metal contaminated soils, bioavailability, accumulation, *Eisenia fetida*, *Vicia faba*, *Zea mays*.

RESUMÉ

Alors que les techniques culturales sans labour ou utilisant un labour réduit sont de plus en plus utilisées par les agriculteurs, leurs effets à court et à moyen terme sur les propriétés physiques, chimiques et biologiques du sol restent encore mal documentés en particulier lorsqu'elles sont associées à une exportation de résidus de culture. L'objectif de ce travail était d'évaluer l'effet de différentes pratiques agricoles sur l'abondance, la biomasse et la diversité de la communauté lombricienne. Aussi, la relation entre les propriétés physico-chimiques du sol et les paramètres lombriciens a été étudiée en contexte agricole.

Ce travail repose sur un dispositif expérimental mis en place à la ferme de la faculté de Gembloux Agro-Bio Tech. Il combine deux modalités contrastées de travail du sol (labour conventionnel – travail du sol réduit) et deux niveaux de restitution des résidus de culture (exportation complète ou non des résidus). Quatre années suivant l'essai, les résultats indiquent que l'abondance, la biomasse et la diversité de la communauté lombricienne ont été affectées par le labour conventionnel et par l'exportation des résidus de culture. Toutes les catégories écologiques de vers de terre ont été influencées par l'application du labour et la gestion des résidus de culture. Le peuplement lombricien observé sur le site d'étude est dominé par les espèces endogées, particulièrement *A. c. caliginosa* (64%), alors que les espèces épigées et anéciques ne représentent que 5 % du peuplement. Les espèces endogées et épi-anéciques (*L. terrestris*) apparaissent sensibles au labour et à l'exportation des résidus de culture. Les résultats démontrent que les systèmes de labour (conventionnel et réduit) modifient considérablement la résistance à la pénétration dans le sol, d'où une compaction plus importante en labour réduit comparé au labour conventionnel. Les modifications de la structure du sol sous l'influence des pratiques agricoles pourraient être liées à l'activité de certaines espèces lombriciennes présentes.

Le travail du sol et l'exportation des résidus de culture modifient les propriétés physico-chimiques du sol. En effet, sur les dix premiers centimètres du sol, les résultats démontrent que l'exportation des résidus de culture diminue les concentrations en éléments chimiques, particulièrement les concentrations en potassium et en phosphore. Cependant, il n'y a pas de relation entre les paramètres lombriciens et les propriétés physico-chimiques du sol. Ceci s'explique par la courte durée de l'expérimentation et par les conditions pédo-climatiques des sites d'échantillonnage.

Ce résultat obtenu lors de la première phase de ce travail a permis la mise en évidence de l'impact négatif que peuvent avoir le labour et l'exportation des résidus de culture sur la communauté lombricienne, ainsi de rendre compte du rôle bénéfique de la communauté lombricienne dans l'amélioration de la qualité et de la fertilité des sols (structure, dynamique des nutriments). L'objectif de cette deuxième partie de la thèse est de déterminer les mécanismes d'accumulation de certains éléments traces métalliques (Cd, Zn, Pb et Cu) chez deux plantes : *Vicia faba* et *Zea mays* et chez le ver de terre *Eisenia fetida*. Le but est d'utiliser le ver de terre *Eisenia fetida* comme un catalyseur dans le processus de phytoremédiation. Après une exposition de 42 jours, certains traits d'histoire de vie d'*Eisenia fetida* ont été affectés par la contamination métallique et par la présence de plantes. L'addition des plantes induit une diminution des concentrations des métaux dans les tissus d'*Eisenia fetida*. Nos résultats démontrent que l'activité biologique du ver de terre *Eisenia fetida* peut modifier la disponibilité des éléments traces métalliques et peut aider à leur accumulation dans les plantes. L'association *Eisenia fetida*-*Vicia faba* ou *Eisenia fetida*-*Zea mays* a un potentiel considérable pour la décontamination de sols pollués par des éléments traces métalliques.

Mots clés : Travail conventionnel, travail réduit du sol, gestion des résidus de culture, propriétés du sol, sols contaminés, éléments traces métalliques, biodisponibilité, *Vicia faba*, *Zea mays*.

Dedication

THIS THESIS IS DEDICATED TO MY MOM AND DAD, WHO HAVE ALWAYS ENCOURAGED ME TO FOLLOW MY HEART AND CHASE MY DREAMS.

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PARTICIPATION TO CONFERENCES AND WORKSHOPS

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ABBREVIATIONS

BD	Bulk Density
C	Carbon
Ca	Calcium
CT	Conventional Tillage
HWC	Hot Water Carbon
K	Potassium
Mg	Magnesium
MTE	Metallic Trace Elements
Na	Sodium
OM	Organic Matter
P	Phosphorus
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	PolyChlorinated Biphenyls
p, p’-DDE	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene)
RT	Reduced Tillage
SOC	Soil Organic Carbon
SOM	Soil Organic Matter

GENERAL INTRODUCTION

I- Soil functions

Soil is a vital and non-renewable resource that is largely threatened but also overlooked by policy. Recently, the European Commission (EC) adopted a Thematic Strategy on the Protection of Soil (EC 2006). The aim of this strategy is to provide guidelines for a holistic approach to soil protection at the European Union level; the soil is a habitat for a vast, complex and interactive community of soil organisms, whose activities largely determine the physical and chemical characteristics of the soil (Lee and Pankhurst, 1992). It may be described as a multicomponent and multifunctional system, with definable operating limits and characteristic spatial configuration. The agricultural soil system is a subsystem of the agrosystems, and the majority of its internal functions interact in a variety of ways across a range of spatial and temporal scales. Soil is a living system and as such distinguishes from weathered rock (regolith) mainly by its biological features. Nonetheless, it should be emphasized that these functions operate by complex interaction with the abiotic physical and chemical environment of soil. Agricultural soils are the habitat for many different organisms, which collectively contribute to a variety of soil-based goods and ecosystem services (Wall, 2004). Previous research describe and categorize ecosystem services, identify methods for services, assess threats and estimates economic values (Daily, 1997; Millenium Ecosystem Assessement, 2005), but does not quantify the underlying role of biodiversity in providing services (Kremen and Ostfeld, 2005). The services include the natural processes that support the production of food, the regulation of water quantity and quality, and the emission of greenhouse gases. According to Kibblewhite et al. (2008) ecosystems services are under the regulation of several key ecosystem functions: (i) organic carbon decomposition, (ii) nutrient dynamics, (iii) soil structure and maintenance, and (iv) biological population regulation.

Agricultural biodiversity encompasses the variety and variability of organisms, which are necessary for sustained and secure food production. Biodiversity can be characterized by (i) species richness, which determine the number of species within an area by giving equal weight to each species, together with (ii) species diversity, which counts the species in area by adjusting for sampling effect and species abundance,

(iii) taxon diversity, which measures the taxonomic dispersion of species and (iv) functional diversity, which assess the richness of functional features and interrelations in a area by identifying food webs, keystone species, and guilds. The biological processes that contribute to soil functions, carbon transformation, organic matter (OM) decomposition, and nutrient cycling, are provided by assemblages of interacting organisms, sometimes termed « key functional groups » (Lavelle, 1997; Swift et al., 2004), which are subsets of the full soil community. A wide range of studies have shown the importance of soil organisms in regulating rate of OM decomposition through the release of nutrients and by stimulating microbial population turnover through their feeding activity (Coleman et al, 1983). These processes are supported by a larger detritivorous fauna, such as earthworms, termites and other microarthropods, which consume both OM and microorganisms, often together with soil. The participation of soil functions by soil organisms can be regulated by climate (soil moisture and temperature), soil conditions (pH, habitat structure) and resource quantity and quality (crop residues, content of nutrients, lignin and polyphenols) (Swift et al., 1979; Lavelle et al., 1997). A substantial proportion of the organisms participating in soil functions and soil structure maintenance are also primary or secondary agents of decomposition; while earthworms and termites can clearly be identified as major « ecosystem engineers » (Jones et al., 1994).

II- Earthworms and agricultural management systems

Earthworms belong to the group of macro-invertebrates that live in the soil and play a major role in ecosystem functioning. They are soil ecosystem engineers with physical, chemical and biological effects on plants and the environment (Lavelle et al., 2006). In agricultural ecosystems they enhance the turnover of organic residues (Amador and Görres, 2005; Bohlen et al., 1997; Bohlen et al., 1999; Lee, 1985), increase the microbial activity (Bohlen and Edwards, 1995; Ernst et al., 2008; Tiunov et al., 2001; Wolters and Joergensen, 1992) and therefore contribute to an enhanced mineralization and nutrient availability in soil. The effect of earthworms on soil properties differs between earthworm species, functional groups (Ernst et al., 2009; Haynes et al., 2003; Schütz et al., 2008) and varying levels of biodiversity (Marhan and Scheu, 2006). Different species and ecological categories of earthworms differ in their ability to digest various organic residues and assimilate nutrients from ingested organic matter (Lattaud et al. 1998, 1999; Suárez et al., 2006).

A change in the land use of natural ecosystems can cause land degradation in physical, chemical and biological properties. In the latter cases, a significant reduction in the biodiversity of earthworm species tends to occur (Decaëns et al., 2001). Earthworms are sensitive to changes in the soil, climate and management systems and induced changes in microclimatic conditions (Hendrix and Bohlen, 2002; Jiménez and Decaëns, 2004). The activities and communities of earthworms are intimately linked to the type of applied agricultural practices, such as the type of tillage, residue management, crop rotation, fertiliser type and overall management. Furthermore, the magnitude of the effect, whether positive or negative, also depends on the interactions with the soil type and soil properties. Understanding the relative importance of such key variable operating at both regional and local scales is essential for predicting the effects of land use and field management on earthworm populations. Such predictions are becoming increasingly valuable as cultivation practices that rely on soil biological processes (e.g., no-till, reduced tillage, and organic farming) are expanding their popularity (Holland, 2004). Using earthworms as soil quality indicators similarly requires a conception of how earthworms respond to the inherent variability of soil properties.

Sustainable farming systems that maximize the positive and synergistic effects of soil biota in a holistic manner are increasingly important in order to enhance inherent soil fertility (Brussaard et al., 2010).
In this context, reduced tillage practices have become a promising yet challenging option across Europe (Mäder and Berner, 2012). Yet, reported benefits of reduced tillage can be significant and include decreased soil degradation by erosion and run-off, reduced energy inputs and costs (Holland, 2004), and enhanced effects of soil biota (Van Capelle et al., 2012). In terms of earthworms, Chan (2001) has reviewed the influence of tillage on earthworm parameters in different agro-ecosystems and has reported a controversial trend for the results. Some authors argue that tillage in arable soils reduces the number of earthworms due to the disturbance and loss of physical quality of the soil, as compared to permanent pasture or reduced tillage, whereas other researchers have demonstrated that the earthworm population is maintained or increased after tillage operations. The damages of the earthworms under tillage operations depend on the frequency, the depth of the tillage and the incorporation of crop residues into the soil. Some of the negative effects of tillage on earthworms can be reduced in soils by incorporating crop residues into the soil. The input of organic material and crop diversification provides both biotic and abiotic conditions that are favourable for earthworm populations and an increase of the organic substrate (Osler et al., 2008). The quality of the OM is a factor that

affects both the population and the biomass of earthworms (Leroy et al., 2007). While many studies evaluating the impact of tillage practices on earthworm communities and have been performed in conventionally arable systems, few have looked at the effects of such practices in systems using different tillage operations associated with crop residues management. Thus there is still need to investigate and assess the effects of reduced tillage and crop residues incorporation into the soil on earthworm communities for agricultural fields, in order to resolve the new practices and agricultural systems which can ne adopted by farmers. In addition, data on earthworm communities are lacking for the study region (Wallonia, Belgium) and local scale on which a reduction in tillage intensity remains a challenge for the development of sustainable agricultural practices.

III- Earthworms and heavy metals in contaminated soils

Expanding human activities have generated a mounting stream of waste, which is sometimes released into ecosystems at concentrations considered toxic for living organisms. These activities can drastically alter the soil environment and influence soil biota by physical disturbances and by altering the physico-chemical environment and the food supply. Some of the main ways in which activities such as mining, deforestation and afforestation, grassland management, and pesticide use may influence soil organism communities. As a result, populations of plants and animals are frequently exposed to toxic risks derived from industrial wastes, pesticides, heavy metals and and other organic and inorganic compounds that are released into the environment (Kappeler, 1979). Many soil organisms, including beneficial soil fauna, are thus routinely exposed to high levels of pollution. Organic and inorganic pollutants poisoning and other disturbances in the natural habitat of soil organisms can lead to an ecological imbalance (Laird and Kroger, 1981; Luo et al., 1999; Weber et al., 2008). These pollutants typically end up in soils, where potential toxic compounds come into direct contact with clays and organic material, which have a high capacity for binding to chemical compounds and substances (Bollag, 1992). The management of polluted areas by anthropogenic activities is of major concern in areas with a long history of industrial activity as it is the case of Wallonia in Belgium. Contamination of soil is an important issue as these toxic elements could be transported in terrestrial ecosystem posing potential risk on food quality, soil health, and the environment (Gray et al., 2003). Among the criteria allowing the evaluation of the soil quality, the integrity of the habitat function of soils is of major importance. Indeed, soil functioning is highly dependent on soil organisms.

"Heavy metal" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4000 kg m^{-3}, or 5 times more than water (Garbarino et al., 1995), especially the transition metals, such as Pb, Cd and Hg, that can cause toxicity problems (Duffus, 2002; Kemp, 1998). However, "heavy metal" is difficult to define precisely and some experts consider it inappropriate (Duffus, 2002). Although several alternative group names, including "toxic metals" and "trace metals" have been put forward, there are also problems with their use. In our thesis, the "metallic trace elements" (MTE) will be used. Although some of them act as essential micronutrients for living beings, at higher concentrations they can lead to severe poisoning (Lenntech, 2004). In the environment, the heavy metals are generally more persistent than organic contaminants such as pesticides. They can become mobile in soils depending on soil pH and their speciation. The accumulation of heavy metals in the environment can affect the health of humans and animals. It is, therefore, important to make an accurate assessment of the availability and toxicity of heavy metals in each polluted soil. In the terrestrial ecosystems invertebrates and microorganisms drive a diverse array of biological and biochemical processes and play important roles in soil functioning. The study of the toxic effects that MTE have on living organisms, especially in the populations and communities of particular ecosystems, is the aim of the multidisciplinary field of ecotoxicology. Most studies of soils are based on invertebrates and focus on earthworms, collembolans, and enchytraeids as bioindicators. The use of these groups has become standard because they are widely distributed. They play important ecological roles, live in permanent contact with soils, reproduce quickly, and are easily maintained in laboratories (Edwards, 1989; Edwards et al., 1995; Römbke et al., 1996). At microscale, MTE also have an adverse effect on earthworm population, which in turn affects the global functioning of ecosystems. Changes in earthworm communities may reduce their ability to maintain soil fertility in the long term. Beneficial roles of earthworms in many soil processes are known to be highly dependent on their activities (creation of burrows, and burial of organic matter). Changes in earthworm activity may influence the transfer properties of soils concerning air, water and solutes (Bastardie et al., 2003; Monestiez and Kretzchmar, 1992) but also the fraction distribution and the bioavailability of various metals (Cheng and Wong, 2002; Ma et al., 2002; Wen et al., 2004). Earthworms can provide important information about environmental risks and could serve as useful biological indicators of contamination because of the fairly consistent correlation between the concentration of some contaminants in their tissues and in soils (Nannoni et al., 2011). Abundance and biomass of earthworms establish them as major factors in soil biology (Coleman et al., 2004). Earthworm abundance and biomass were found to be more sensitive to pollution in comparison with other indicator taxa (Spurgeon et al., 1994). In general short-term microcosm experiments in controlled conditions were performed with artificially contaminated soils (Nahmani et al., 2007a) and with a single metal element. However in reality, field pollution often concerns a mixture of contaminants (Dumat et al., 2006; Pascaud et al., 2014). The main studies on the effects of metals on earthworms measured mortality, weight loss and fertility (Nahmani et al., 2007b) or were focused on ecotoxicity tests (Reinecke et al., 2001; Scheffczyk et al., 2014; van Coller-Myburgh et al., 2014). A number of studies have shown that species richness and diversity of earthworms decreased along a gradient of metal pollution (Pizl and Josens, 1995; Nahmani and Lavelle, 2002; Lukkari et al., 2004). A generally observed pattern is that the earthworm communities lose their diversity with increasing metal pollution. This is due to the fact that abundance of most species decreases, although some species may survive even in the most polluted sites (Vandecasteele et al., 2004). Understanding the effects of MTE exposure on earthworm communities is particularly important, since earthworms are efficient decomposers and contribute considerably to nutrient mineralization and soil structure (Edwards and Bohlen 1996; Spurgeon et al., 2003; Lavelle et al., 2006). Furthermore, the response of earthworms to metal contamination in terms of abundance, biomass and loss or persistence of species may be evident after significant contamination of the environment (Spurgeon et al., 2005).

IV- Bio-remediation of heavy metal contaminated soils

Soil contamination with MTE may cause changes in the composition of soil organisms community, adversely affecting soil characteristics (Giller et al., 1998; Kozdrój and van Elsas, 2001). High concentrations of metals in soil, particularly Cd and Zn can negatively affect crop growth, as these metals interfere with metabolic functions in plants, including physiological and biochemical processes, inhibition of photosynthesis, and respiration and degeneration of main cell organelles, even leading to death of plants (Garbisu and Alkorta, 2001; Schmidt, 2003).

Although researches involving soil quality are facing an important technologic challenge with several actions being taken in order to assess and reduce the potential risks of contaminants in the soil, standardized monitoring methods combined with remediation strategies are still needed. However, the treatment of polluted sites and the reduction of MTE exposure by conventional procedures could be expensive (CarrilloGonzález and González-Chávez, 2006). Thus, several researches aiming to remediate the effects of the soil contaminants have been carried out worldwide. Remediation of a contaminated area involves the application of one or more techniques aiming to remove or contain harmful substances in order to allow the reuse of the area with acceptable risk limits for human and environmental health. For this purpose, an ideal remediation process must remove all the contaminants of the soil or, at least, reduce the percentage of contamination of the environment to acceptable limits; should also avoid the migration of contaminants to other areas. Several techniques can be used to remediate polluted soil (Scullion, 2006). For example, immobilisation techniques focus on reducing the availability and activity of MTE but not on removing the pollutant from the soil. These techniques are based on the addition of suitable amendments to accelerate natural soil processes (sorption, precipitation, and complexation reactions), with the aim of decreasing mobility and bioavailability of toxic elements (Bolan et al., 2003). The remediation of contaminated soils is therefore receiving increasing attention from governments, legislators, industries, researchers and general population. For soils contaminated with inorganic polluants like heavy metals, the amendments include, clay minerals, zeolites, lime or composts (Mench et al., 2003; Vangronsveld et al., 2009).

The application of this kind of *in situ* amendments does not remove the contaminants from the soil, so their success is based on a reduction in the bioavailability and/or mobility of the contaminants considered (in question). Keeping the sustainability issues and environmental ethics in mind, the technologies encompassing natural chemistry, bioremediation, and biosorption are recommended to be adapted in appropriate cases.

In general, the availability of heavy metals in soils is considered an important parameter for the effectiveness of the uptake and accumulation of heavy metals by organism plants and/or terrestrial invertebrates). Since the primary aim of the extraction technique is to remove metals from polluted soils by concentrating them in the harvestable parts of plants or terrestrial invertebrates, the efficiency depends on amount and the bioavailability of metals. Little is known about how the relationships and the association between plants and soil fauna affect plant growth and metal uptake. Only a few studies have been carried out into soil fauna-assisted metal extraction by plants. Earthworms are important components of the rhizosphere ecosystem and can significantly increase plant production by improving soil fertility and nutrient cycling (Brown et al., 2004). Some earthworms (for example *Lumbricus terrestris*, *Lumbricus rubellus*, or *Aporrectodea caliginosa*) can survive in soils polluted with heavy metals and can even accumulate heavy metals such as Cd, Pb, Cu and Zn (Morgan and Morgan, 1999; Kizilkaya, 2004, 2005). They can also increase metal availability in soil by burrowing and casting and can, therefore, modify the efficiency of phytoremediation (Ma et al., 2002). Earthworms can survive in heavy-metal contaminated soils, can accumulate efficiently and can tolerate high tissue metal concentrations using a variety of sequestration mechanisms (Peijnenburg, 2002; Andre et al., 2009). To date, reported data about the use of earthworms for the enhancement of plant uptake are very scarce. The relationship between plants, earthworms and heavy metal-contaminated soils shall be explored and considered in order to better understand the added value of the application of organisms in the the improvment of the remediation efficiency.

The disturbances caused by human activities have had a detrimental impact on the different functions of soils, which has indirectly affected the health and fertility of agricultural soils. These soils are altered through agricultural practices and atmospheric fallouts of dusts enriched in metals. Protection, sustainability and remediation of agricultural soils are necessary (imperative) to maintain a multiple services delivered by these soils. The major challenge within sustainable soil management is to conserve ecosystem services delivery while optimising agricultural yield. It is proposed that soil quality and fertility are dependant on the maintenance of soil biodiversity and functions.

AIMS AND OBJECTIVES OF THE THESIS

The major challenge within sustainable soil management is to conserve ecosystem service delivery while optimising agricultural yield. It is proposed that soil quality and fertility are dependant on the maintenance of soil biodiversity and functions.

The purpose of this study is to highlight the role and the contribution of earthworm communities, in agricultural fields, in restoration of ecosystem processes after anthropogenic disturbances.

The study will comprise five inter-related chapters:

Chapter 1 addresses and discusses the current state of knowledge on the earthworm's contribution on soil dynamics. The emphasis of this chapter is given to an important role of earthworms on soil biology (soil structure, decomposition activities, nutrient mineralization, bioturbation…) and to an effect of agricultural management practices on earthworm community parameters in agricultural fields.

Chapter 2 explores and quantifies the effects of different levels of soil perturbation (depth tillage and exportation of crop residues) on earthworm communities and physico-chemical properties in different arable systems, in terms of soil, climate and crop conditions. A further part of this chapter involves relationships between the groups of physico-chemical and biological variables.

Chapter 3 attempts to investigate changes in the earthworm communities at metal polluted natural soils originating from a gradient of pollution, taking into account changing soil physico-chemical properties. This section also helps to improve our understanding on the combined effects of metals on earthworms and provide more information on the combined ecological risk of metals mixture in soil ecosystems.

Chapter 4 considers the beneficial effects of earthworms on soil metal availability, their use for enhancement of plant metal uptake and their role in the phytoremediation process. This part of research focuses on the uptake of the mixture of metals from agricultural and natural soils polluted by atmospheric dusts using a combination of plants and the earthworms. This allows a greater understanding of the relationships «contaminated soils-earthworms-plants».

Chapter 5 provides a synthesis of the preceding four separate chapters, which have been published as independent papers in peer-reviewed journals. The answers to the research questions are discussed in this last part of the thesis.

RESEARCH HYPOTHESES

Considering the role of earthworms in soil functions, and due to their large number, high species diversity and their sensitivity to environmental disturbances, earthworms were chosen and could serve as useful biological indicators of sustainable management practices and for assessment of environmental risks. The major hypotheses posed for this research are:

1- In experimental agricultural plots, tillage systems and exportation of wheat crop residues affect negatively earthworm community parameters and modify soil physic-chemical properties. Biological parameters were expected to be correlated with soil physical and chemical properties.

2- In agricultural fields affected by atmospheric contamination, different earthworm descriptors such as abundance, biomass, species diversity and ecological and specific structure were negatively affected by contamination along a gradient of metal pollution (Cd, Pb, Zn and Cu).

3- In heavy-metal polluted soils, the *E. fetida* earthworms was expected to decrease the availability of metals and to increase the *V. faba* and *Z. mays* uptake of metals. The presence of *E. fetida* could contribute to remediate contaminated soils.

CHAPTER 1

LITTERATURE REVIEW

a- Importance of earthworms as «ecosystem engineers»

Earthworms are an important biotic element of agricultural soils and contribute significantly to the physico-chemical and microbiological formation of the soil environment. Although not numerically dominant, their large size makes them one of the major contributors to invertebrate biomass in soils. Earthworms and termites are the major and most studied soil engineers, due to their dominant abundance and biomass in temperate and tropical soils. They modify resource availability to other soil organisms through the creation of biopores and biogenic aggregates. They are involved in most key soil functions, such as the decomposition of organic residues at the soil surface, the regulation of OM breakdown, nutrient cycling, water infiltration, soil erosion and plant growth (Lavelle and Spain, 2001).

Earthworms affect the soil properties as they dig burrows, deposit casts on the soil surface and within, mix horizons and bury above ground-litter (Lee, 1985). Darwin (1881) began the modern era of earthworm research relating their activities to soil properties. Darwin was among the first to include biota in the list of factors responsible for soil formation process. In his work «The formation of Vegetable Mould through the Action of Worms», Darwin brought attention to the extreme importance of earthworms in the breakdown of dead plants and animal matter that reaches soils and the continued turnover and maintenance of soil structure, aeration, drainage and fertility. Earthworm activity creates structure, casts and galleries that modify soil aggregation, porosity and the connection among pores. Depending on their ecological groups, earthworms have a leading role in transport of OM through soil profile. Earthworm excretion intensely consists of clay and silt particles and various organic compounds with the size of 210 – 500 µm giving a strong binding characteristic to the excretion and hence increasing aggregates stability (Chan and Heenan, 1995). In addition, in temperate environments, earthworms have been suggested to significantly contribute to the regeneration of compacted soils because of their burrowing and casting activity.

b- Ecology of earthworms

Organisms are usually differentiated into functional groups according to their influence on specific ecological functions. These classifications can be based on both trophic and functional criteria. Earthworms belong to the order Oligochaeta, which includes more than 8000 species from about 800 genera. Earthworms are present in most areas of the world, except those with extreme climates, such as deserts and areas that under constant snow and ice. Some genera and species of earthworms, particularly those belonging to the Lumbricidae, are extremely widely distributed and are termed *peregrine*; often where these species are introduced to new areas, they become dominant over the endemic species (Edwards, 2004). Population of earthworms vary greatly in terms of numbers or biomass and diversity. Populations range from only a few individuals to more than 1000 per square meter (Lee, 1985; Edwards and Bohlen, 1996; Lavelle et al., 1998).

The size of species depends on a wide range of factors, including pH_{water}, moisture-holding capacity of the soil, rainfall and temperatures, but most importantly, on the availability of OM. Earthworms appear to attain their highest populations in pasture, and deciduous woodland of Europe and temperate regions of Asia. The species found most commonly in temperate agro-ecosystems are members of the family Lumbricidae, a group with 16 genera and about 300 species worldwide (Lee, 1985). In acid soil, earthworms are absent for $pH < 3.5$ and sparse for $pH < 4.5$ (Muys and Granval, 1987; Curry, 2004). Depending on the organic resources availability, some species may be preferred to others, which require well-established plants (for the provision of litter) or organic amendments (Butt, 1999; Langmaack et al., 2002). The earthworm biomass in most soils exceeds the biomass of all other soil inhabiting invertebrates. The diversity of species of earthworms varies greatly between sites and habitats, and there often tend to be species associations in different soil type and habitats. The activity of earthworms differs also between seasons in temperate regions, where earthworms are active mainly in the spring and autumn. During the winter, they penetrate deeper into soils, where they are much more protected from the adverse winter and cold temperature. In dry summer periods, they also burrow deeper into soil and sometimes construct cells lined with mucus in which they estivate in a coiled position until environmental conditions become favourable again (Edwards, 2004).

c- Taxonomy of earthworms

Earthworms like termites (Eggleton and Tayasu, 2001), have been assigned a categorization of species into functional groups, known by the names given to them by Bouché, 1977: epigeic, anecic and endogeic groups. They are based on morphological and behaviour traits correlated with ecology (Lee, 1959; Bouché, 1977).

- Epigeic (1 – 2.5 mm in diameter): live and feed on nearly pure OM substrate, such as thick forest floor leaf packs, fallen treetrunks, epiphyte mats, suspended arboreal soils, and ferns. They are usually small bodied, darkly pigmented, have the ability to move rapidly, have minimal development of intestinal surface area and have high reproduction rates and short life spans.

- Anecic (4 – 8 mm in diameter): live in permanent, vertical burrows that may extended three meters depending on soil texture. The burrows are open at the soil surface and surrounded by middens, a mixture of plant residues and casts, serving as protection and food resource. Anecic species pull dead leaves and other decaying organic materials down into their burrows; where they are rapidly colonized by microorganisms and consumed at later time. They usually are large bodied, have dark anterior pigmentation, and have lower reproductive rates.

- Endogeic (2 – 4.5 mm in diameter): live and feed in the mineral soil layer, are usually lightly pigmented or unpigmented, range in size from small to very large, do not move quickly, and have lower reproductive rates. They don't create permanent burrows; rather randomly burrow through the upper layer of the mineral soil. Further division of endogeic species has been made based on the degree of decomposition of humification of the OM consumed, from less humified to more: polyhumic, mesohumic, and oligohumic (Lavelle, 1983).

The three ecological groups interact with each other, with other soil organisms and with other functional domains in soil such as the rhizosphere and the porosphere. The functional domain relating earthworm activity to soil processes is reffered to as the drilosphere, and has been described by Lavelle (1988) and Brown et al. (2000). Drilosphere is a burrow linings, which is directly and indirectly modified by earthworms (Bouché, 1975). This hot spot contain plant nutrients, high amount of polysaccharides, and has higher enzyme activities, which increases soil aggregate stability. The concept of drilosphere was later expanded to include earthworm itself, its gut, soil that is in contact with the earthworm, casts, middens, and burrows (Lavelle, 1988). The drilosphere effect for each functional group is dynamic, changing constantly in space and time. Functional groups exert their influence on soil processes in different ways.

d- Earthworms and organic matter

Earthworms participate in soil functions through the drilosphere system, which involves earthworms, casts, burrows and the whole microbial and invertebrate community that inhabits these structures. They are particularly important agents of soil organic matter (SOM) decomposition in most terrestrial ecosystems, accelerating rates of comminution and dispersal through feeding activities, although whether their activity is a net sink or source of carbon (Oyedele et al., 2006; Don et al., 2008). The effects of earthworms on the dynamics of OM are likely to vary between earthworm species and functional groups (Bouché, 1977; Edwards and Bohlen, 1996). The activity of endogeic species accelerates initial SOM turnover through indirect effects on soil carbon as determinants of microbial activity.

As a result of earthworm digestion processes and creation of soil structure, the composition, structure, and relative importance of the drilosphere system to soils is clearly determined by climate, soil parameters, and the quality of microbial communities. They are known to choose the organic and mineral soil components that they ingest. As a result, their casts often have much higher contents of SOM and nutrients than the surrounding soil (Lee, 1985). Some earthworm species (e.g. *Pontoscolex corethrurus*) were able to select both large organic debris and small mineral particles, depending on soil types. A wide range of experimental studies have shown the importance of earthworms and other soil organisms in regulating the rate of OM decomposition through the release of nutrients and by stimulating microbial population turnover through their feeding activity (Coleman and Hendrix, 2000). The rate of decomposition is shown as being regulated by climate and resource quality (Lavelle et al., 1997). Earthworms may participate in the accumulation of OM through (i) an overall increase of amounts of OM produced in an ecosystem and (ii) the protection of SOM in structures of the earthworm drilosphere (burrow system) (Martin, 1991; Edwards, 2004).

e- Earthworms and microorganisms interaction

In agricultural landscapes, earthworms provide a range of beneficial roles, including increased mineralization of OM, generation and stabilisation of soil structure, stimulation of microbial activity (Reddell and Spain, 1991; Zarea et al., 2009). Although earthworms habitats and their beneficial impacts on soil structure have long been reported, little is knwon of how earthworms create microhabitats for microorganisms and stimulate microbial activities (Gange, 1993; Zarea, 2009). Microorganisms play a key role in OM decomposition, nutrient cycling and other chemical transformation in soil (Abbot and Murphy, 2007). They immobilize significant amounts of carbon and other nutrients within their cells. A major part of the beneficial effects of earthworm activity on soil properties is contributed to interactions with soil microorganisms.

Microorganisms themselves may be a source of food for earthworms but the amounts consumed and the ability of earthworms to digest and assimilate microbial biomass varies with earthworm species (Brown and Doube, 2004). Lavelle and Gilot (1994) showed that several temperate earthworm species had a mutualistic digestive system in which the mixture of soluble organic carbon in the form of low molecular weight mucus with ingested OM, together with the moist conditions and neutral pH in the foregut, promoted the development of a microbial community that could digest cellulose and other substances that earthworms can not typically digest. The earthworm gut can act like a bioreactor where microbial activity and biomass are increased due to favourable conditions with readily available carbon of mucus and water.

Earthworms can converte organic waste into simple products and thereby improve the nutritive quality of the soil (Buckerfield and Kretzschmar, 1992; Joy et al., 1992). During such a process, different soil organisms penetrate into the earthworm gut. Many of them multiply well in the gut while a few are fully digested (Parthasarathi et al., 1997). All other microorganisms that escape the gut digestion proliferate well and return to the soil in the earthworm casts. However, these interactions are still poorly understood, including the effect of gut passage on the community structure of soil microorganisms (Egert et al., 2004). Molecular and isotopic techniques are increasingly being used to identify microbial communities involved in labelled-source degradation. It open new possibilities for understanding the role of earthworms in microbial community structure and function. At a macroscale (e.g., that of field), few data are available, but earthworm-induced changes in microbial function have the potential to influence broader scale processes such as soil structure, microbial diversity, patterns of plant abundance, soil productivity, and crop yields. Earthworm activity has also been shown to influence the distribution and activity of bacteria, fungi, and protozoa in soils. Bacteria and fungi are the organisms that can process any organic material present in soils. Microorganisms usually operate via chain processing, with different generalist or specialist groups progressively breaking down even the most complex organic molecules while releasing metabolites that are then available to all the other soil organisms. Earthworms that have developed mutualist digestion systems in association with free-soil bacteria, as hypothesized by Lavelle et al. (1995). When geophagous species ingest soil, they add an equivalent volume of water in their anterior gut plus 5 to 40% of the soil dry weight as intestinal mucus, a highly energetic product of the anterior gut wall (Martin et al. 1987; Barois et al. 1999).

f- Factors affecting earthworms communities

Land management practices have considerable impact on the size and dynamics of earthworm communities. Intensification of agriculture has focused on the use of chemical and mechanical inputs, often at the expense of biologically mediated processes. Both organic and inorganic sources of fertilizer have residue effects in the field. These effects are a vital component of sustainability because they smooth season-to season variations in soil fertility and crop productivity, but they are difficult to asses quantitatively.

Agricultural practices such as tillage, pesticides, industrial contamination and fertilization applications have a deep impact on earthworm activities. Due to their relatively large size, fragile body, their mode of life and their spatial mobility, earthworms are susceptible to many environmental factors that affect their living conditions and their habitats in soils. For example, tillage affects earthworm populations building their galleries and burrows in deeper soil layers. In addition, intensive and frequent tillage activities were found to reduce earthworm populations more severly, while no-till management systems promoted their increase (Edwards and Lofty, 1982; Edwards and Bohlen, 1996; Chan, 2001; Johnson-Maynard et al., 2007). Tillage however, doesn't always result in lower abundance and biomass, but also has a potential to induce a shift in ecological groups and species diversity. Moroever, reduced tillage was shown to positively affect anecic earthworms, whereas endogeic species may benefit from conventional tillage practices such as ploughing (Ernest and Emmerling, 2009; Simonsen et al., 2011). Higher earthworm numbers and biomass in no-tillage agroecosystems have been attributed to more beneficial soil conditions, including the presence of surface litter, accumulation of SOM, favourable climate and a lack of disturbance. Yet, in some cases, earthworm abundance and biomass may be no different or slightly lower in no-tillage than conventional tillage agrocecosystems (Kladivko et al., 1997). One reason for this inconsistency is that tillage often occurs in conjunction with the incorporation of crop residues and duration of tillage application. In the short term, the effect of tillage on earthworm ecological groups may be more variable. A clear effect of ploughing has been demonstrated for anecic and epigeic species, as these two groups require the presence of litter on soil surface and can not tolerate regular disturbance of their habitat. The endogeic species living in superficial layers seem to be less sensitive to soil inversion, although conflicting results have been obtained on this point. The effects of tillage on earthworm diversity are variable and seem to be influenced by the intensity and frequency of tillage operation and amount of residues.

Earthworms ingest large amount of soil and are therefore exposed to chemical substances through their intestine as well as through the skin, therefore concentrating toxics from the soil in their body (Morgan and Morgan, 1999). Earthworm may serve as bioindicators of soil contaminated with pesticides, polychlorinated biphenyls, polycyclic hydrocarbons (Saint-Denis et al., 1999), and heavy metals (Spurgeon and Hopkins, 1999). Metal contamination can seriously impede the rehabilitation of mine spoil adversely affecting revegetation, litter decomposition processes and soil organisms. This could impact organism physiological parameters, including reproduction rate.

The direct impacts of metal toxicity on earthworm populations can have important detrimental effects of their population dynamics, by influencing basic reproduction and survival parameters, and thereby modifying the size, sex ratio, and stability of populations (Nahmani et al., 2007a). In general, the indirect effects are more difficult to evaluate and are less well studied than the direct effects. The indirect effect can be due to a contamination of food supply, involve a modification on the functions of earthworms. Thus, metal contamination can influence soil functioning at all trophic levels, altering individual organisms, populations, or communities, and at different spatio-temporal scales. In agricultural and polluted soils, the abundance, biomass, diversity and behaviour of earthworm communities are determined by interactions between numerous factors. These factors determine the physico-chemical parameters of soil environment, the type of vegetation than can be supported, the quantity, quality of the crop residues produced, and the applications of toxic compounds. Various forms of human activities will influence earthworm populations, often adversely, when the intervention is disruptive, as in mining and mechanical cultivation. Features common to all kinds of soil degradation are significant decrease in organic reserves, degradation of soil structure, and depletion in earthworm populations. The relationships between earthworm populations and changes in soil properties; and the role of earthworm in detoxification of contaminated soil are not thoroughly understood.

g- Earthworms and environmental detoxification

Van Hook (1974) suggested that earthworms could serve as useful biological indicators of contamination because of the fairly consistent relationships among the concentrations of certain pollutants in earthworms. They can also play a key role in terrestrial ecotoxicological risk assessment (Sheppard et al., 1998; Weeks et al., 2004). They play a valuable role for assessing biological risks from toxic components in terrestrial environments (Morgan, 1982) and they are vulnerable to chemical and physical impacts on soils. Since, earthworms are easy to cultivate and to handle for experiments, they have become part of standard test organisms in ecotoxicology (OECD, 1984; 2004). The use of earthworms as tools in the detection of chemical contamination includes bioassays at contaminated sites in the field or in the laboratory using soil taken from the contaminated site in microcosms. Contaminated sites are often characterized by combining effects of complex pollutants mixture rather than single pollutant. Determination of causal relationships between specific pollutants and observed effects is difficult because of the complexity and variability of biological responses. There is currently considerable renewed interest in studies of the mesocosm, semifield, and model ecosystem type (Edwards, 2002). The effects of toxicants are assessed in systems of various complexities, simulating field conditions as closely as possible. Field tests can also provide valuable data on the indirect effects of chemicals on earthworms through effects on food supply and soil cover. Some authors found that earthworms are less sensitive to contaminants than other soil organisms and can tolerate high concentrations of certain contaminants (Contreras-Ramos et al., 2006; Fitzpatrick et al., 1992; Langdon et al., 2005; Safawat and Weaver, 2002; Tejada et al., 2011). The concentration of pollutants in earthworms is sometimes a useful indicator of whether the pollutant is near toxic levels in the environment. The proximity of earthworms to the soil contaminants makes them useful monitoring organisms for the soil environment. The presence of toxic amounts of heavy metals in earthworms poses a serious risk of secondary poisoning of vertebrate predators. It is important not only to know what the earthworm body burdens of any particle toxic metals are, but also to understand the various pathways of metabolism and detoxification. Taking into account the indicator role of earthworms in contaminated environments is a topic considerable interest but of limited practicality.

Their uses are limited because of variety of environmental influences could be responsible for the observed condition of the earthworm communities, making the establishment of causal relationships between the earthworm community densities and the degree of metal pollution very difficult.

The positive effect of earthworms on the removal of contaminants, such as oil, polycyclic aromatic hydrocarbons (PAHs), Polychlorinated biphenyls (PCBs), pesticides and MTE have been reported by several studies (Binet et al., 2006; Contreras-Ramos et al., 2008; Eijsackerset al., 2001; Geissen et al., 2008; Tejada and Masciandaro, 2011). Earthworms are able to change soil physico-chemical properties by (i) increasing available carbon and nitrogen in the soil with urine and mucus secreted; (ii) ingesting and mixing soil particles with organic material during their gut passage; (iii) modifying the soil structure through their burrowing and casting activities; and (iv) changing the soil bacterial and fungal communities (Curry and Schmidt, 2007; Drake and Horn, 2007; Jayasinghe and Parkinson, 2009). This effect is variable, dependent on soil physico-chemical properties and earthworm species.

Earthworms can affect important soil properties such as porosity, pH and OM content; therefore, they have an indirect effect on pollutants decontamination (Bennett, Hiebert et al., 2000). In addition, a number of chemicals secreted by other microorganisms (fungi, bacteria) can influence desorption and the removal of metals from the soil matrix (Mulligan and Kamali, 2003). Furthermore, some studies found that earthworm cast adsorb a contaminant, such as Atrazine by hydrophobic interaction between the contaminant and SOM, thereby delaying the removal of the pollutant (Alekseeva et al., 2006; Binet et al., 2006; Shan et al., 2011).

Several studies have reported on the removal contaminants when earthworms were added to a contaminated soils (Hickmanand and Reid, 2008; Sinha et al., 2008a,b; Tejada and Masciandaro, 2011). They found that earthworms accelerate the removal of several organic contaminants. The majority of studies showed that plant biomass, extractable metals, pore water-concentrations and metal uptake by plants are increased by earthworm activity. The presence of earthworms associated with plants can modify metal extraction process. Peters et al. (2007) reported that phytoextraction of organochlorine compound p, p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) with *Cucurbita pepo* ssp. pepo increased at least 25% in the presence of the earthworms *E. fetida*, *L. terrestris*, or *A. caliginosa*. The authors observed a decrease in hydrophobicity of humic acids in soil in which both *C. pepo* and *E. fetida* were present. In the case of phytoremediation, the link between the services and soil biodiversity is indirect compared to microbial mediated bioremediation because soil biodiversity plays a role in regulating plant abundance and distribution. The application of bioremediation using either organisms (animals and plants) is feasible and relatively less-cost. Setting a bioremediation protocol in contaminated sites requires excellent knowledge of the nature and distribution of the pollution as the local soil organisms and plants. Numerous studies have investigated the influence of earthworms on heavy metals mobility in soil through their ingestion, burrowing and casting activities (e.g., Vijver et al., 2003; Lukkari et al., 2006; Sizmur et al., 2011; Qui et al., 2011). Sizmur et al. (2011) showed that *Lumbricus terrestris* species decreased water soluble Cu and arsenic As but increased the solubility of Pb and Zn in soil, and Natal-da-Luz et al. (2011) did not observed an influence of *Dendrobaena veneta* on the solubility of Cr, Cu, Ni, and Zn in soil. The bioavailability of heavy metals in soil is determined by their chemical speciation (Babich and Stotzky, 1980) and their interactions with OM and mineral soil particles (Li and Li, 2000), which can be influenced by soil organisms. The beneficial role of earthworms in soils, influencing a range of chemical, physical and biological processes, is beyond dispute (Scheu, 1987; Edwards and Bohlen, 1992; McCredie and Parker, 1992; Curry and Baker, 1998). Because of the capacity to accumulate and concentrate large quantities of organic and inorganic pollutants, earthworm species are widely recognized as suitable organisms for biomonitoring the effects of heavy metals and chemical in contaminated soils (Reddy and Rao, 2008; Peijnenburg and Vijver, 2009).

Impacts of earthworms on soil components and dynamics. A review

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Earthworm populations are important decomposers contributing to aggregate formation and nutrient cycling processes involving nitrogen cycles, phosphorus and carbon. They are known to influence soil fertility by participating to important processes in soil such as soil structure regulation and OM dynamics. Earthworms also modify the microbial communities through digestion, stimulation and dispersion in casts. Consequently, changes in the activities of earthworm communities, as a result of soil management practices, can also be used as indicators of soil fertility and quality. It is therefore important to understand how earthworm communities affect soil dynamics. This review adresses the current state of knowledge on earthworm's impacts on soil structure and soil OM (carbon, nitrogen, and phosphorus) dynamics, with special emphasis on the effects of land management practices on earthworm communities.

Keywords. Earthworms, *Oligochaeta*, nutrient cycling in ecosystems, microbial communities, soil organic matter dynamics, soil fertility, agricultural practices.

Impacts des vers de terre sur les composants et la dynamique du sol (synthèse bibliographique). Les vers de terre sont des décomposeurs importants contribuant à la formation d'aggrégats et aux différents cycles d'éléments nutritifs tels que l'azote, le phosphore et le carbone. Ils sont connus pour leur influence sur la fertilité du sol en participant à la régulation de la structure du sol et à la dynamique de la matière organique. En ingérant d'importantes quantités de sol, les vers de terre modifient la communauté microbienne lors du passage à travers leur tube digestif. Par conséquent, les changements dans les activités de la communauté lombricienne, à la suite de pratiques de gestion des sols, peuvent également être utilisés comme indicateurs de la fertilité et de la qualité des sols. Il est important de comprendre comment les communautés lombriciennes affectent la dynamique des sols. Cette synthèse bibliographique porte sur l'état actuel des connaissances sur les impacts de vers de terre sur la structure des sols et la dynamique de la matière organique, en mettant l'accent sur les impacts des pratiques agricoles sur les communautés lombriciennes.

Mots-clés. Ver de terre, *Oligochaeta,* cycles nutriments dans écosystèmes, flore microbienne, matière organique du sol, fertilité du sol, pratique agricole.

1. Introduction

Soil forms a narrow interface between the atmosphere and the lithosphere. The structure of cultivated soil results from climatic, anthropogenic, and biological processes, but the precise roles of each of these processes is difficult to assess. The impact of earthworms activity on soil structure was underlined long ago, and these organisms are now recognized as major biological drivers in temperate agrosystems. Soil characteristics (pH, organic matter, nitrogen, granulometry, etc.) are influenced by earthworms because they participate in the construction and destruction of the soil particles, as well as in organic matter transfer. The soil ingested by earthworms undergoes chemical and microbial changes when it passes throught the gut. Organic matter is digested and both the pH and the microbial activity of the gut contents increase (Edwards et al., 1996; Lukkari et al., 2006). Earthworms accelerate nitrogen mineralization from organic matter, but the effect depends on the species and their interaction with soil characteristics, organic matter location and soil biota (Butenschoen et al., 2009).

Soil biodiversity has been widely studied since the soil itself is the base for farming (Stockdale et al., 2006). The conservation of biodiversity is necessary to maintain the sustainable functioning of soil. In 1881, Darwin was one of the first scientists who noted that the topsoil consisted mostly of earthworm castings, thus highlighting the importance of earthworms in pedogenesis processes (soil organo-mineral complex). For example, the earthworm population builds galleries and ingests large quantities of organic and mineral matter, thus modifying the porosity and aggregation of the soil. This earthworm bioturbation may subsequently be reflected in soil profiles (Zhang et al., 1995), for example: soil profile disturbance, soil structure modification, and vertical and horizontal redistribution of soil and organic matter (OM). This redistribution of OM depends on the earthworm ecological groups. Endogeic earthworms keep moving inside the soil to feed on soil organic matter (SOM) while anecic ones feed on plant litter and organic residues at the soil surface and tend to stay in the same burrow (Lavelle et al., 1997). Epigeic species, which consume considerable amounts of raw OM have a broad range of enzymatic capacities, probably mainly originating from ingested microflora (Curry et al., 2007). As discussed by Lavelle (1997), the soil biogenic structure (mixture of casts, burrows, OM, etc.) created by earthworms is commonly termed the "drilosphere" (Brown et al., 2000).

In agrosystems, the intensification of human activities (tillage, use of mineral fertilizers, etc.) has led to deterioration in structural and biological soil characteristics (Edwards, 1984; Lee, 1985). Soil degradation is often associated with decreases in biodiversity and the abundances of earthworms and other invertebrate communities (Lee et al., 1991; Lavelle, 1997). However, there is a perceived lack of information to characterize adequately their functional role in soil ecosystem processes such as soil carbon sequestration and loss, decomposition of organic residues, and the maintenance of soil structure.

This paper addresses the current state of knowledge on earthworms' impacts on soil structure and SOM (carbon, nitrogen, and phosphorus) dynamics, with special emphasis on the effects of land management practices on earthworm communities.

2. The role of earthworms in organic matter decomposition and nutrient dynamics

2.1. Decomposition of organic matter

Organic matter (OM) is mainly present in the top 20 – 30 cm of most soil profiles and is essentially an array of organic macromolecules consisting principally of combinations of carbon (C), oxygen (O) hydrogen (H), nitrogen (N), phosphorus (P) and sulfur (S). Almost all OM in soil is directly or indirectly derived from plants *via* photosynthesis. Specifically, atmospheric carbon dioxide is transformed by reduction into simple and complex organic carbon (OC) compounds, which in combination with key nutrients enable the plant to function and grow. Soil organic matter (SOM) provides food and substrates for soil organisms, ranging from macroinvertebrates to heterotrophic bacteria (Lavelle et al., 2001). This is of great importance, given that the soil biota is increasingly recognized as playing a major role in soil functions. In cultivated soils, earthworm communities could play an important role in SOM dynamics through regulation of the mineralization and humification processes (Lavelle et al., 1992). On the basis of the results of current literature, it appears that there are some differences among studies regarding the effects of earthworms on soil organic carbon content. Lachnicht et al. (1997) and Desjardins et al. (2003) found a negative effect of earthworms addition on soil carbon content, whereas Gilot (1997) found an opposite effect. In the experiment of Desjardins et al. (2003), the maize crop was grown under no tillage, and this factor can explain the weak loss of carbon in the non-inoculated plots. The decrease by 28% of the total carbon content in the earthworm-inoculated plots indicates that the endogeic tropical earthworm *Pontoscolex corethrurus* affects the SOM dynamics dramatically. The observed losses of SOM in continuously cropped fields are often attributed to a rapid mineralization of SOM following cultivation. Earthworms caused a decrease in SOM and carbon mineralization by mobilizing recalcitrant forms of OM. Earthworms enhance mineralization by fragmenting SOM and by mixing SOM, mineral particles and microorganisms, thus creating new contact surfaces between the SOM and microorganisms (Parmelee et al., 1998). Since earthworms of different ecological groups prefer different food resources, they likely affect nutrient mineralization. Anecic earthworms incorporate litter material into the mineral soil thereby making it available for the soil food web (Bossuyt et al., 2006). Endogeic earthworm species, in contrasts, primarily consume soil and associated humified OM in the upper layer of the mineral soil.

Soil microorganisms, mainly fungi and bacteria, are primarily responsible for the transformation of organic molecules in soil, and their activity is thus a key factor in SOM dynamics (Coq et al., 2007). Aira et al. (2008) characterized changes in fungal populations, bacterivore nematodes communities and the biochemical properties of an organic substrate over a short (72 h) exposure to four densities of the epigeic earthworm *Eisenia fetida*. Calcium and N-mineralization increased with increasing earthworms density, as did microbial metabolic activity. In addition, Coq et al. (2007) showed that casts of endogeic species *Pontoscolex corethrurus* were slightly enriched in C and showed significantly higher mineralization than the non-ingested soil. The higher mineralization in casts might indicate a higher concentration of labile compounds (soluble carbon, lignin, etc.), and probably a higher microbial activity. Earthworms have indirect effects on soil organic carbon as determinants of microbial activity. In addition, mucus production associated with water excretion in the earthwom gut is known to enhance the microbial activity (Barois, 1986).

In soil, earthworms control biomass, diversity and activity of soil microorganisms (Doube et al., 1998). However, microorganisms may constitute an important part of the diet of earthworms, which can feed on them selectively (Moody et al., 1995; Edwards, 2004). In most natural and managed ecosystems, up to half of the OC added to soil on an annual basis by plant detritus and root exudates is rapidly consumed by microorganisms, and released as carbon dioxide (Hopkins et al., 2005; Wolf et al., 2005). The remainder of the added OM, together with organic compounds synthesized by soil organisms during decomposition and which is released mainly as detritus, persist in the soil for an extended period. The importance of soil fauna in the decomposition of OM is well known. However, the complex interactions between earthworm and soil microorganisms are less understood. While soil invertebrates yield about 15% of the C and 30% of the N in some ecosystems (Anderson, 1995), their indirect effects through activation of microflora are likely to be much greater.

2.2. Consumption and humification

Epigeic earthworm species may feed directly on microorganisms or litter material and inhabit the organic layer of soil. They have been shown to strongly affect decomposition processes (Sampedro et al., 2008) and modify the fungal composition of forest soils (McLean et al., 2000). Generally, effects of earthworms on microbial biomass and activity depend on soil conditions (Shaw et al., 1986; Wolters et al., 1992).

Aira et al. (2006) showed that microbial biomass and activity in pig slurry were significantly decreased by transit through the gut of the epigeic species *Eudrilus eugeniae*. It appears that *E. eugeniae* is able to digest microorganisms present in pig slurry (Aira et al., 2006). The effects of earthworms on microorganisms depend on the kind of food source and availability and the species of earthworms involved (Flegel et al., 2000; Tiunov et al., 2000). McLean et al. (2006) found that invasive earthworms decreased microbial biomass in surface soils with a high organic carbon content and increased microbial biomass in the underlying mineral soils. Zhang et al. (2000) found that large numbers of the anecic earthworm *Metaphire guillelmi* decreased microbial biomass C, N and P after 24 h, thereby concluding that earthworms used microorganisms as a secondary food source.

An attempt to distinguish between nutrient-enrichment processes associated with the OM incorporation and gut-associated processes associated with the passage of soil and OM through the gut of *Lumbricus terrestris* was made by Devliegher et al. (1997). They concluded that nutrient-enrichment processes but not gut associated processes were responsible for the increased microbial biomass and activity reported in the presence of *L. terrestris*. Meanwhile, endogeic earthworms can transport fresh organic detritus from the soil surface into burrows while mixing it with mineral soil. In the case of tropical endogeic species, it has been demonstrated that the addition of water and readily assimilable intestinal mucus to the ingested soil rapidly stimulates microbial activity. In the second half of the earthworm gut, the mucus will have been almost entirely metabolized and the microorganisms start to degrade the SOM into assimilable OM. This form of OM is then used by both the worms and the microorganisms. Furthermore, the interactions between earthworms and microorganisms occur at several spatial scales in the drilosphere (Brown et al., 2004). The drilosphere concept (**Figure 1**) was developed by Bouché (1972), originally to describe the 2-mm-thick zone around the earthworm burrow walls. Lavelle (1997) completed the meaning of drilosphere by including earthworm communities, the digestive tract content, and all microbial and invertebrate populations. Up to 60% of the C losses from earthworms during their life span can be in the form of mucus secretion, and this soluble organic carbon is an important microbial stimulant in the drilosphere (Brown et al., 2004).

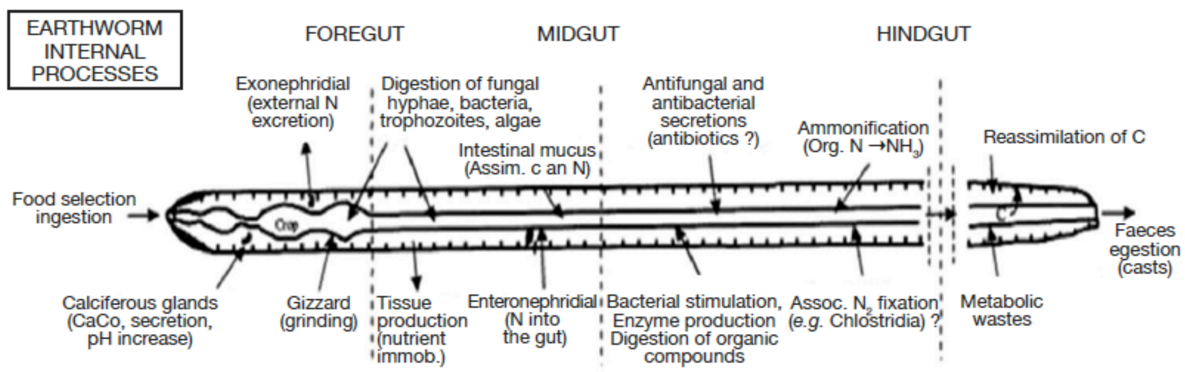

Figure 1. Digrammmatic illustration of different internal components of the drilosphere, from ingestion to excretion in earthworms (Brown et al., 2000).

Different species differ in their ability to digest organic residues and assimilate nutrients (Lattaud et al., 1998). *Aporrectodea caliginosa* earthworms consume a mixture of soil and OM, often choosing to feed in patches of soil that are relatively rich in OM, or in microsites since they are enriched with bacteria and fungi (Wolters and Sheu, 1999). Lavelle et al. (1994) showed that several temperate earthworm species have a mutualistic digestive system. The mixture of soluble OC, in the form of low-molecular-weight mucus with ingested OM, together with the moist conditions and neutral pH in the foregut, promoted the development of a microbial community that could digest cellulose and other substances that earthworms typically cannot digest. Essentially, the earthworm gut can act like a bioreactor where microbial activity and biomass are increased due to favorable conditions, with readily available C, from mucus, and water. Hence, earthworm casts (EC) may contain large amounts of OM that has not been assimilated, but that has been modified both physically and chemically during passage through the earthworm gut. The EC are usually rich in ammonium-nitrogen and partially digested OM, providing a good substrate for growth of microorganisms. It has been established that there are larger populations of fungi, bacteria, and actinomycetes (Shaw et al., 1986), and higher enzymatic activities in EC than in bulk soil (**Figure 1**).

Earthworms produce a huge amount of intestinal mucus composed of gluco-proteins and small glucosidic and proteic molecules (Morris, 1985). The microorganisms entering the worm guts consume these nitrogenous compounds in mucus (Zhang et al., 2000), which largely increases their activity. The biological decomposition of OM is mediated by a variety of biochemical processes in which enzymes play a key role (Garcia et al., 1992). The major constituents of OM, like cellulose, hemicellulose, lignin, and proteins, are degraded by specific enzymes. Earthworms fragment the substrate in the process of feeding and thereby increase the surface area for further microbial colonization. The enhanced microbial activity accelerates the decomposition process leading to humification, thus oxidizing unstable OM into more stable forms. Humification processes are accelerated and enhanced not only by the fragmentation and size reduction of the OM, but also by the greatly increased microbial activities within the intestines of the earthworms and by the aeration and turnover of the OM through earthworm movement and feeding.

2.3. Nutrient inputs, mineralization

Earthworms are known to be important regulators of major soil processes and functions such as soil structure, OM decomposition, nutrient cycling, microbial decomposition and activity, and plant production. Cortez et al. (2000) reported that the presence of earthworms whatever the ecological category, increased the quantity of inorganic N in the soil. This was caused by enhanced mineralization of N forms, both of a ^{15}N-labelled residue and that of the soil organic matter. Earthworms can impact plant growth by promoting N-availability (Li et al., 2002; Ortiz-Ceballos et al., 2007). Several factors may contribute to the mineral weathering mediated by earthworms, such as low pH and a bacteria-rich microenvironment in the gut of earthworms. However, the presence of earthworms may have an effect on the production of greenhouse gases such as nitrous oxide (N_2O). Research by Rizhiya et al. (2007) indicated that earthworms increased N_2O fluxes when grass residue was applied to the soil. The formation and production of N_2O in soils is determined by microbial processes: nitrification, denitrification, and nitrifier denitrification (Wrage et al., 2001). The earthworm gut provides ideal conditions for N_2O producing microorganisms by providing abundant substrate, an anaerobic environment, suitable pH and a high moisture content (Horn et al., 2003; Drake et al., 2007). The powerful mechanical grinding action of the gut is caused by the peristaltic actions used to move food along the gut, and the action of ligands originating from earthworms and their gut microorganisms (Carpenter et al., 2007). Earthworm guts are, consequently, enriched in microorganisms, with concentrations much higher than in the surrounding environment (Carpenter et al., 2007). High numbers of other organisms that are capable of producing N_2O (*i.e.*, nitrate-dissimilating and nitrifying bacteria) are also present in the *A. caliginosa* earthworm gut (Ihssen et al., 2003). Production of N_2O by nitrate-dissimilating bacteria is favored in systems that contain high levels of organic carbon, like the rumen or the gastrointestinal tracts of animals. Some nitrifiers are able to use nitrate or nitrite as electron acceptors and, by using this nitrifier denitrification system, can produce N_2O and/or N_2 under oxygen-limited conditions (Freitag et al., 1987). The *in situ* conditions of the gut are ideal for activation of dormant bacteria and bacterial spores that might be present in soil. Many endospore-forming bacilli that are abundant in soil (Felske et al., 1998) have been detected in the gut of *A. caliginosa* species and can reduce nitrate or nitrite to N_2O (Ihssen et al., 2003).

The increased total nitrogen may be due to the release of nitrogenous metabolic products through *E. eugeniae* earthworm excreta, urine, and mucoproteins (Padmavathiamma et al., 2008). Indeed, Dash et al. (1977; 1979) reported higher levels of N in casts of *Lampito mauritii* than in the surrounding soil. In the gut of earthworms, it is possible that the mucus secreted from the gut epithelium provides an energy source that stimulates biological N-fixation (Lee, 1985).

To determine the role of earthworms in agrosystem sustainability, it may be necessary to focus on processes by which earthworms increase or decrease the storage or loss of nutrients, and how they influence productivity and nutrient uptake by crops. As shown in **figure 2**, the presence of earthworms can change the sizes of various nutrients pools, and the fluxes of C and N, significantly (Bohlen et al., 2004a; **figure 2**).

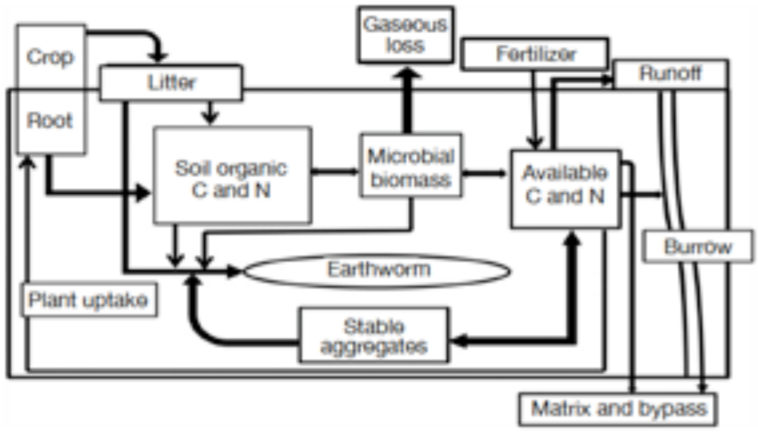

Figure 2. Ecosystem budget model to examine pools and fluxes of C and N in the presence of earthworms (Bohlen et al., 2004a).

This model emphasizes the major pathways by which earthworms change the retention and loss of C and N, incorporating the effects of earthworms on soil biological, physical and chemical processes. Through interactions of earthworms with the microbial community and by processing OM, earthworms can increase the system flux of CO_2 (gaseous C loss). These same interactions, coupled with earthworm excretion, can also lead to increased availability of N.

2.4. Nutrient dynamics

Earthworms are important decomposers contributing to nutrient cycling processes involving nitrogen (Lavelle et al., 1992), phosphorus (Chapuis-Lardy et al., 1998) and carbon (Lee, 1985; Lavelle et al., 1992; Zhang et al., 1995; Curry et al., 2007). They ingest organic matter with relatively wide C:N ratios and convert it to earthworm tissues of lower C:N ratios (Syers et al., 1984). This accelerates the cycling of nutrients in soil, particularly N. Some field studies indicate that earthworms feed on organic materials with low C:N ratio, thereby leaving behind a pool of organic materials with a higher C:N ratio (Bohlen et al., 1997; Ketterings et al., 1997).

Field studies have shown variable effects of earthworm invasion on soil N dynamics. Invasion of maple sugar forests in New York by *Lumbricus* spp. increased leaching of NO_3 in a historically plowed site.

However, at another site that had never been plowed, the effects have not been observed, which could be attributed to the greater potential for N immobilization in the more C-rich unplowed site (Frelich et al., 2006).

Total soil N was not significantly changed by earthworm invasion (Bohlen et al., 2004b). During earthworm feeding, the nutrients, phosphorus (P) and potassium (K), are converted into an available form for plants. Lavelle et al. (1992) highlight the importance of earthworm feeding behaviors, which may contribute to the long-term effects of earthworms on nutrient cycling processes. Suarez et al. (2004) found an increase in P leaching and decrease in P availability on plots in a New York sugar maple forest dominated by *Lumbricus rubellus*. Sugar maple forests invaded by several species including *L. rubellus* had lower P availability than control parcels without those earthworm species (Hale et al., 2005). Loss of P with earthworm invasion can be associated with maple decline. The magnitude of earthworm invasion impacts on nutrient cycling depends on the species assemblage of earthworms that invade as well as land-use history. In order to have systems of sustainable agriculture, it is important to maintain a global balance of nutrients to ensure that the outputs and loss of nutrients are offset by nutrient inputs (Giller, 2001). Potassium is one of the major nutrients for plant growth that can significantly affect the growth and production of crops, along with N and P (Amtmann et al., 2007; Sugumaran et al., 2007; Chen et al., 2008). However K, in the form of silicates, can hardly be used by plants (Liu et al., 2006). Earthworms can help in releasing K from silicate minerals. For instance, Basker et al. (1994) reported that exchangeable K-content increased significantly in soils populated by earthworms compared with soils devoid of earthworms (Basker et al., 1992). They concluded that the increase was due to the release of K, from the non-exchangeable K-pool, as soil material passed through the worm gut. Some microorganisms in the earthworm gut can enhance the weathering of minerals by lowering pH or by producing ion-complexing organic ligands (Sanz-Montero et al., 2009). EC are usually found to have greater exchangeable K, calcium (Ca), and magnesium (Mg) contents than bulk soil (Edwards et al., 1996; Mariani et al., 2007). This was also confirmed by Teng et al. (2012) who examined the physical, chemical and biological properties of casts produced by endogeic species *Metaphire tschiliensis tschiliensis* in clay soil incubated in the dark for two weeks. The findings suggested improved nutrient content in EC as compared to WWS and BS (**Table 1**). This was shown by higher content of macronutrients (N, Ca) in EC than in both WWS and BS. This is probably due to the intimate mixing of OM through the earthworm gut which can further enhance mineralization and humification processes (Lavelle, 1988; Blanchart et al., 1999).

Table 1. Mineral elements in worm-worked soil (WWS), earthworm casts (EC) and bulk soil (BS) (Teng et al., 2012).

Elements (mg·kg^{-1} dry soil)	WWS	EC	BS
N	2.34	3.78	3.39
P	1.11	1.42	1.24
K	2.42	2.54	2.48
Ca	3.67	5.00	3.92
Mg	1.14	1.42	1.16

Improved Ca-content in EC was probably due to the presence of an active calciferous gland in earthworms that actively secretes mucus rich in calcium carbonates into the esophagus (Drake et al., 2007). This leads to the elimination of excess Ca ions *via* casting activity, and greatly increases Ca availability in soil.

3. Earthworms and microorganisms

The impact of earthworms on soil OM breakdown has been studied before. However, despite the fact that importance of soil fauna in OM-turnover is well known, the complex interactions between soil fauna and microorganisms, and the indirect effects on microbial communities, are less understood. The biochemical decomposition of OM is primarily accomplished by microorganisms, but earthworms are crucial drivers of the process as they may affect microbial decomposer activity by grazing directly on microorganisms (Monroy et al., 2008; Aira et al., 2009; Gómez-Brandón et al., 2011), and by increasing the surface area available for microbial attack after comminution of OM (Domínguez et al., 2010). Some microorganisms may be a source of food for earthworms, but the amounts consumed and the ability of earthworms to digest and assimilate microbial biomass vary with earthworm species, its ecologogical category, food substrate, and the environmental conditions in which the earthworms are living (Brown et al., 2004). Earthworms affect directly the decomposition of soil through gut-associated processes, *via* the effects of ingestion, digestion, and stimulation of the OM breakdown and microorganisms (Monroy et al., 2008; Aira et al., 2009). After passage of microorganisms through the earthworm gut (mainly fungal and protozoan spores and some resistant bacteria), they provide inocula for microbial colonization of newly formed EC (Brown, 1995). Some bacteria are activated during passage through the gut, whereas others remain unaffected, and yet others are digested in the intestinal tract and thus decrease in number (Pedersen et al., 1993; Drake et al., 2007). The microbial composition of the earthworm intestine contents has been considered to reflect that of the soil ingested (Brown, 1995). Furthermore, the numbers, biomass, and activity of microbial communities in the earthworm gut have also been shown to be different from that in uningested soil (Schönholzer et al., 1999). Singleton et al. (2003) studied bacteria associated with the intestine and casts of earthworms and found *Pseudomonas*, *Paenibacillus*, *Azoarcus*, *Burkholderia*, *Spiroplasm* and *Actinobacterium*. Some of these bacteria, such as *Pseudomonas alcaligenes* and *Acidobacterium*, are known to degrade hydrocarbons (Johnsen et al., 2005). Monroy et al. (2008) observed a reduction in the density of total coliforms by 98%, after the passage of pig slurry through the gut of the epigeic earthworm *E. fetida*. Accordingly, Pedersen et al. (1993) reported a selective reduction in the coliform *Escherichia coli* BJ 18 in cattle dung during passage through the gut of several species of earthworms of one genus *Lumbricus*. The selective effects on ingested microorganisms through the earthworm gut may be caused by competitive interactions between those ingested and the endosymbiotic microrganisms that reside in the gut (Brown et al., 1981). Indeed, Byzov et al. (2007) found that the mid-gut fluid of earthworms possess a selective suppressive activity while stimulating certain soil microorganisms. Meanwhile, Thakuira et al. (2010) found that food resource type can cause shifts in the gut wall-associated bacterial community, but that the magnitude of these shifts did not obscure the delineation between ecological group specificity. For instance, spores of some fungi that survived in the mid-gut environment (*Alternaria alternata*) started to germinate and grew actively in fresh excrement. The fate of microorganisms passing through the digestive tract of earthworms is an important factor in the formation of the soil microbial community and the degradation of OM. Recently, Rudi et al. (2009) observed a rapid and homogenous change in the microbiota in gut selective effects on the presence and abundance of ingested microorganisms. These selective effects may alter the decomposition pathways, probably by modifying the composition of microbial communities involved in decomposition. Previous studies were mostly aimed to evaluate the effect of gut transit on the microbial population, biomass and enzyme activities of different organic residues (Devliegher et al., 1995; Zhang et al., 2000; Scheu et al., 2002; Aira et al., 2006). But recently Aira et al. (2007a; 2007b) showed that earthworms can modify the microbial community physiology and trigger enzyme activities during vermicomposting of pig slurry. Several enzymes isolated from earthworm guts allowed to digest some bacteria, fungi and microinvertebrates (*e.g.*, protozoa, nematodes) (Brown et al., 2000). Studies using 6 earthworm species and more than 10 soil and litter fungal species (Moody et al., 1995; Bonkowski et al., 2000) have shown that earthworms prefer, and digest, the rapid-growing fungi species typically associated with the early successional stages of decomposition.

4. Effects of earthworms on soil structure

The beneficial effects of SOM on soil productivity through the supply of plant nutrients, enhancement of cation exchange capacities, and improvements in soil and water retention are well established (Woomer et al., 1994). In addition, SOM supports various soil biological processes by acting as a substrate for decomposer organisms and ecosystem engineers, such as earthworms. They play a role in both acceleration of decomposition and mineralization processes (C loss) and in carbon storage or protection from decomposition (C accumulation) in stable aggregates (Brown et al., 2000). Aggregate stability is a key factor for physical soil fertility and it also affects SOM dynamics (Abiven et al., 2009). Aggregates are formed through the combination of clay, silt, and sand, with organic and inorganic compounds. Their stability is used as an indicator of soil structure (Six et al., 2000). The size, quantity, and stability of soil aggregates reflect a balance between factors such as organic amendments, soil microorganisms, fauna, and disrupting factors as bioturbation and culture (Six et al., 2002) (**Figure 3**).

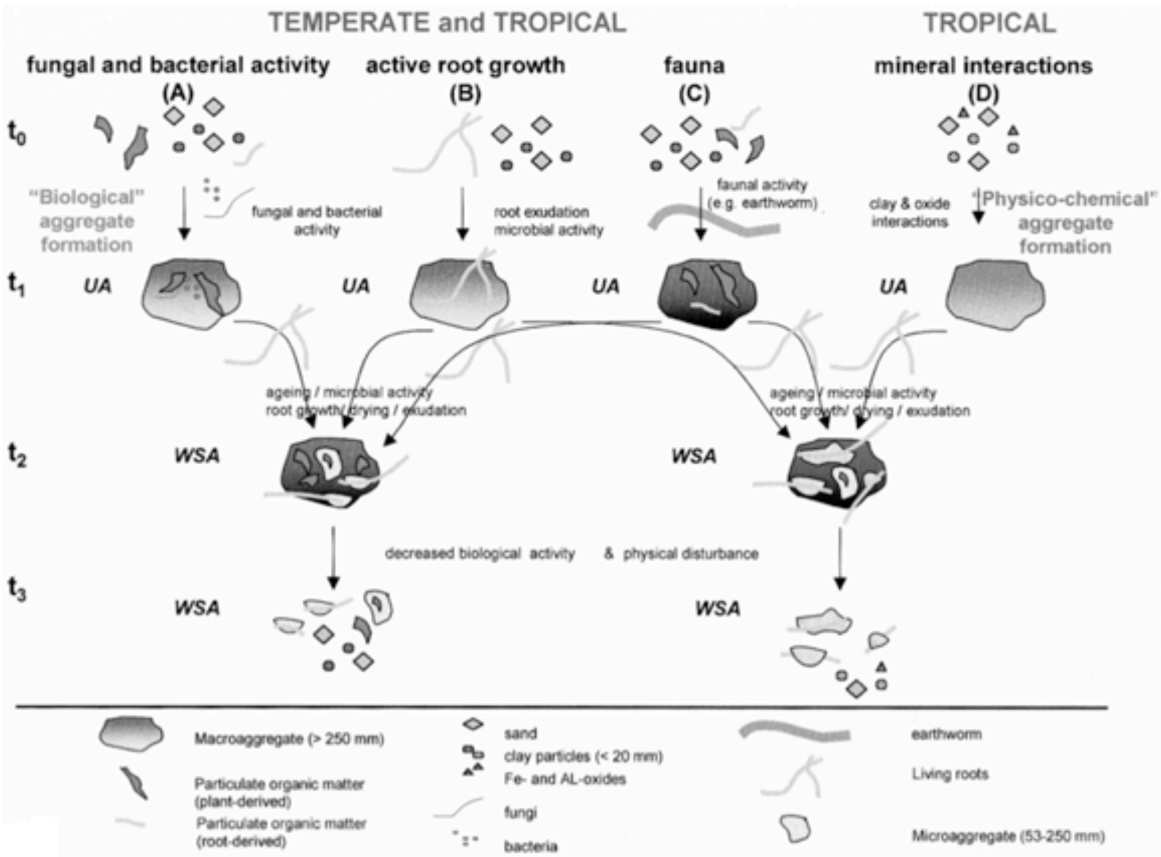

Figure 3. Aggregate formation and degradation mechanisms in temperate and tropical soils (Six et al., 2002).

Aggregation is a complex procedure that includes environmental factors, soil management factors, plant influences, and soil properties such as mineral composition, texture, SOC-concentration, pedogenic processes, microbial activities, exchangeable ions, and moisture availability (Kay, 1998). For several decades, the role of soil organisms in soil structure has been recognized by farmers, but the impact of soil organisms on the formation of aggregates was conceptualized in the hierarchical model of soil aggregates only in the last 25 years (Tisdall et al., 1982). This model shows that the activity of fungi, bacteria, plant roots, and macrofauna (*e.g.* earthworms) lead to the formation of biological macroaggregates (Six et al., 2002). The breakdown of soil macroaggregates increases over time because the action of binding agents is gradually disrupted. However, despite the disruptive forces, the microaggregates remain stable and become blocks during the formation of new soil macroaggregates. A study conducted by Bossuyt et al. (2006) showed that there was a significant influence of earthworm activity and residue application on stable aggregate formation. Soil aggregates were 4.3 times greater than the control (no earthworms) when *A. caliginosa* was present in residues-incorporated soils. Further, in the presence of *L. rubellus*, soil aggregates were three times greater than the control.

Nunan et al. (2003) reported that bacteria are not randomly distributed throughout the soil; there are variations in biomass and differential colonization among different sizes of aggregates. Using molecular methods, Mummey et al. (2006) examined the bacterial communities associated with different aggregate size-fractions in earthworm-worked soil relative to soil receiving only plant litter. Earthworms altered the bacterial community composition in all soil fractions that were analyzed. When earthworms ingest the soil, the soil particles are broken down and the soil is compacted during passage through the gut prior to excretion. Barré et al. (2009) reported that earthworms were shown to bring initially loose or compacted soil to an intermediate mechanical state that is more favorable for structural stability and root growth. In addition, soil size-distribution is significantly affected by earthworms in the 0 – 2 cm layer of soil (Snyder et al., 2009). Earthworm presence shifted soil aggregate-size to the > 2,000 µm fraction from smaller fractions by reducing the amount of soil in the 200 – 250 and 250 – 253 µm fractions.

5. Effects of agricultural practices on the dynamics of earthworm communities

Agricultural practices such as tillage, drainage, irrigation, lime application, pesticide use, fertilization and crop rotation, can influence significantly earthworm biomass and activity (Edwards et al., 1996). In a review of several studies exploring the effects of tillage on earthworms, it was concluded that deep ploughing and intensive tilling reduced earthworm populations in clay loam soils. In sandy loams tillage effects were variable and dependent upon several factors including the earthworm species present in the soil (Chan, 2001). No-till management systems promoted earthworm abundance (Edwards et al., 1996; Johnson-Maynard et al., 2007). However, populations tend to recover within one year from less-severe forms of cultivation, provided the disturbance is not repeated. When performed once a year, the effect of tillage on earthworm populations was even found to be less destructive than that of birds feeding on earthworms. Larger, anecic species such as

L. terrestris and *Aporrectodea longa* which require a supply of surface litter and inhabit relatively permanent burrows, are the species most adversely affected by repeated soil disturbance; smaller endogeic species such as *Allolobophora chlorotica* and *A. caliginosa* are less affected and can benefit from plowed-in crop residues (Lofs-Holmin, 1983; Edwards, 1984). Eriksen-Hamel et al. (2009) investigated the effects of tillage on the earthworm *Aporrectodea turgida* and suggested that in cool, humid agrosystems, tillage-induced disturbance probably has a greater impact on earthworm populations and biomass than food availability. Mechanical weeding was found to be responsible for habitat disturbance, physical damage to earthworms, and disturbance in reproduction functions among other factors (Ernst et al., 2009; Peigné et al., 2009).

Agricultural systems are characterized by high levels of inputs. Biological activity in agricultural soils is driven by organic C inputs. Inputs of organic materials from crop residue, cover crops, manure applications or organic fertilizers have a strong positive effect on the composition, size and activity of the soil biological community (Kirchner et al., 1993). Use of solid materials and organic fertilizers obtained from plants and animal origins were reported to increase earthworm populations (Leroy et al., 2007; Leroy et al., 2008; Reinecke et al., 2008). However, most chemical fertilizers influence earthworms indirectly through an increase in plant yield and consequently an increase in plant residues that remain in the field after harvest.

Earthworms play an important role in surface residue decomposition rate, distribution of OM throughout the soil profile, and soil physical property modification. Lowe et al. (2002) found that OM management is important in the development of sustainable earthworm populations and their role in soil amelioration at restored sites. Also the conversion of grassland to arable land can affect the SOM and also decrease earthworm populations. Indeed, Van Eekeren et al. (2008) found a strong decrease in earthworm abundance after conversion of grassland to arable land. On the contrary, conversion of arable land to grassland stimulated the species richness and abundance, even in the second year after conversion (Van Eekeren et al., 2008).

6. Conclusion

Earthworms are important biological factors in soil ecosystems. They are sensitive to cultivation techniques and consequently may be used as bioindicators of soil health. Earthworms have been suggested as potential indicators of the sustainability of agricultural practices that farmers could use, thereby optimizing different farming systems. Nevertheless, further research regarding the impact of cultivation techniques, crop rotations, and crop residue management on earthworm populations within Europe is required. Also, it will be important to explore the potential role of earthworms in soil fertility and agricultural sustainability.

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CHAPTER 2

EARTHWORMS DYNAMICS UNDER DIFFERENT AGRICULTURAL PRACTICES IN BELGIUM

Short-term effects of tillage practices and crop residue exportation on earthworm communities and soil physico-chemical properties in silt loam arable soil (Belgium)

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Abstract

Earthworms are known to play integral roles in soils, and are often referred to as vital soil and ecosystem engineers. They have the ability to influence a wide range of chemical, physical, and biological properties of soil environment. In the present study, earthworm and soil samples were collected from wheat cultivated fields in Gembloux, Belgium under four agricultural practices: (1) conventional tillage with crop residues incorporated into the soil (CT/IN); (2) conventional tillage with crop residues exported from the field (CT/OUT); (3) reduced tillage with crop residues incorporated into the soil (RT/IN); and (4) reduced tillage with crop residues exported from the field (RT/OUT). The different agricultural practices were applied on luvisol soil for four consecutive years prior to the initiation of the current study. The purpose of this study was to research the influence of agricultural practices on earthworms with considering species and their interactions with soil properties.

Results indicated that agricultural practices affected soil properties and earthworm communities. For each depth, measures of soil physico-chemical properties showed significant differences among treatments. The penetration resistance (PR) measured to a depth of 50 cm increased with increasing soil depth in all treatments. PR was significantly higher in RT compared with CT. Soil moisture was measured before PR determination. Soil samples showed higher P and K concentrations in 0 – 10 cm depth compared with other depths. The main reason for the large K and P accumulation near the soil surface is the incorporation of crop residues.

Significant differences were not detected between residue incorporation depth treatments, where results showed mean earthworm abundance was respectively 182 and 180 individuals m-2 in CT and RT. Mean earthworm biomass was similarly not significantly different between CT and RT, where results were respectively 48.5 and 57.3 g.m-2. However, a significant difference was observed between IN and OUT treatments, suggesting the exportation of crop residues will limit earthworm abundance and biomass, and will mask the effect of tillage. The endogeic species *Apporectodea caliginosa* strongly dominated the earthworm community (64%), whereas epigeic and anecic species remained < 3% and 5% of all earthworms. Findings indicate that endogeic and epi-anecic groups appears to be highly affected by tillage practice and the exportation of crop residues. Consequently, it seems that the effect of residue exportation was stronger than tillage effect.

In compacted soils, *L. terrestris*, *L. castaneus* and *A. caliginosa* species showed an increased abundance. The obtained results were attributable to earthworm activity and crop residues, suggesting earthworms contributed to nutrient dynamics and soil structure, particularly at increased soil depths. Overall, the results emphasise the influence of crop residues exportation on earthworm community and also, the important influence of earthworm activity on soil physico-chemical properties change, processes which are closely linked.

Keywords: Tillage systems; Crop residue management; Earthworm ecological groups; Soil physico-chemical properties; Soil compaction; Soil nutrient dynamics.

1. Introduction

From an ecological perspective, soil represents an interactive system consisting of different components: the soil's physical, chemical properties, organic matter and biological activities (Coleman and Odum., 1992). Earthworms are an important component of soil fauna in numerous soils (Lee, 1985). They are soil ecosystem engineers with physical, chemical and biological effects on plants and the environment (Lavelle et al., 2006). The effect of earthworms on soil properties differs between earthworm species. Observed differences are often attributed to variation in their feeding and burrowing behavior (Lattaud et al., 1997; Suárez et al., 2004). Earthworms are typically classified in three ecological categories: (i) epigeic (feed on surface litter and live in the litter layer and top centimetres of soil); (ii) endogeic (feed on soil and associated organic matter and live in non-permanent burrows deeper in the soil); and (iii) anecic species (feed on surface litter and make permanent vertical burrows) (Bouché, 1977). However, some species show characteristics belonging to multiple groups and can, therefore, not be fully classified within one functional group. For instance, the behavior of *Lumbricus terrestris*, an earthworm that used to be classified as anecic, is recently more often described as epi-anecic.

Tillage practices can affect soil biota through changes in habitat (Van Capelle et al., 2012), loss of organic matter (Hendrix et al., 1992), moisture and temperature dynamics (Curry, 2004) and mechanical damage (Lee, 1985). It is generally acknowledge, that different types of soil tillage can modify the density and community structure of earthworms. Tillage however, does not always result in lower abundance and biomass, but also has a potential to induce a shift in ecological groups and species diversity. Moreover, reduced tillage was shown to positively affect anecic earthworms, whereas endogeic species may benefit from conventional tillage practices such as ploughing (Ersnt and Emmerling, 2009; Simonsen et al., 2011). A clear effect of ploughing has been demonstrated for anecic and epigeic species, as these two groups require the presence of litter on soil surface and can not tolerate regular disturbance of their habitat. The endogeic species living in superficial layers (the top of 30 cm of soil) seem to be less sensitive to soil inversion, altough conflicting results have been obtained on this point. Ploughing was found to decrease the abundance of endogeic earthworms in the study of Berner et al. (2008). These authors compared ploughing with various reduced tillage systems and found that the numbers of adults endogeic earthworms were 70% greater for the reduced tillage. However, the total biomass of the endogeic adult earthworms in the reduced tillage systems was divided by two in the ploughed system, and individual biomass under reduced tillage was only one third that under ploughing. The authors argued that food conditions were more favorable for endogeic earthworms in the ploughed plots than in plots subjected to reduced tillage. One reason for this inconsistency is that tillage often occurs in conjunction with the incorporation of crop residues, which are food source for earthworms. Thus, depending on the quality and quantity of the residues incorporated versus that exported from the fields, tillage may inhibit or enhance earthworm populations (Siegrist et al., 1998; Carpenter-Boggs et al., 2000; Chan, 2001; Zaller and Kopke, 2004). The spatial pattern of earthworms is controlled by biotic and abiotic conditions, but some species may dramatically affect the physical structure of their habitat, potentially affecting the distribution of other species.

Soil management practices involving crop residues, including soil incorporation can alter soil environment for organisms involoved in organic matter decomposition and nutrient dynamics (Clapperton et al., 1999). Numerous researchers have demonstrated that conservation tillage systems (reduced tillage and no-tillage) are effective in improving soil physical and chemical properties, crop yield, soil water storage and soil protection against wind and water erosion (Peixoto et al., 2006; Thomas et al., 2007; Madejon et al., 2009; De Vita et al., 2007; Naudin et al., 2010). Organic matter and nutrient accumulation near the surface under reduced tillage results in beneficial effects on soil physical, chemical and biological properties (Beare et al., 1992; Tebbrüge and Düring, 1999).

The relationships between earthworm activity, soil structure and soil organic matter (SOM) dynamics has long been recognised (Pulleman et al., 2003). Earthworm population are reported to change with soil management practices, and they have frequently been recognised and proposed as soil quality indicators (Huerta, 2009; Lemtiri et al., 2012). Soil compaction from tillage and short-term practices such as system conversion can be detrimental to earthworms when its limits their burrowing activity (Langmaack et al., 1999; Postma-Blaauw et al., 2010; Capowiez et al., 2012).

Recent studies have investigated arable soil tillage effects on earthworms (Capowiez et al., 2009a; Ernst and Emmerling, 2009; Peigné et al., 2009; De Oliveira et al., 2012). However, an extensive litterature revealed few studies that have assessed the effects of tillage systems on earthworm populations in relation with soil physical and chemical properties. Additionnaly, it remains unclear how earthworm communities and soil properties vary based on agricultural practices. Therefore, earthworm community responses to agricultural practices and crop residues exportation in relationship to physical and chemical soil properties must be examined.

The objective of this study was to quantify the effects of four different agricultural practices on earthworm communities (abundance, biomass, and species diversity), and soil physical (penetration resistance, bulk density) and chemical (electrical conductivity, residual humidity, pH, organic and nutrient status) properties. It was hypothesized that tillage systems and exportation of crop residues affect negatively earthworm community parameters and modify soil properties. Likewise, species diversity of earthworms were expected to be positively correlated with nutrient contents but negatively correlated to soil compaction.

2. Materials and methods

2.1. Field trial design

The experimental site was at Gembloux, approximately 45 km southeast of Brussels in Belgium (50°33’49’’N. 4°42’45’’E). The experiment was established in 2008 to study the impact of agricultural practices. The site was integrated into the SOLRESIDUS project (Gembloux Agro-Bio-Tech, University of Liege, Belgium). We studied two management regimes. The first was “crop residues management” as follows: harvestable straw was either exported from the field (OUT), or incorporated into the soil (IN). Stubble and chaff were left in the soil in both cases. The second regime was “residue incorporation depth”, where two depths were studied: 0 – 10 cm (Reduced Tillage-RT) and 0 – 25 cm (Conventional Tillage-CT). Four treatments were compared: RT/IN; RT/OUT; CT/IN; and CT/OUT. The experimental design was based on four Latin squares. The site was composed of 16 plots, each 15 * 40 m wide and separated by buffer zones (3 m).

2.2. Crop operations and climate

From September 2008 to October 2012, crop rotation systems were conducted over four growing seasons (rapeseed Brassica napus, RS-2009; winter wheat Triticum aestivum, WW-2010; winter wheat, WW-2011; and winter wheat, WW-2012), and three inter-crop periods (IC-2009; IC-2010; and IC-2011). Stubble and and volunteer plants of rapeseed or cereals form previous crops were broken down twice by stubble cultivations at a depth of 10 cm. This tillage was applied to all 16 plots. Ploughing was carried out on 8 plots (P01, P02, P07, P08, P09, P10, P15, P16) on september 2008, october 2009, november 2010, and october 2011 for RS-2009, WW-2010, WW-2011 and WW-2012, respectively. The objectives were to destroy weeds, to incorporate residues into the soil and to decompress the soil. The upper layer of the soil was turned over at a depth of 25 cm. This operation was realized just before the sowing. During the sowing, seedbed preparation was conducted with a rotary harrow at a depth of 8 cm, and grains were sown at 2 cm depth. To quantify the residues over the soil surface after harvest, an area of 2*0.5 m was delimited for each of the 16 plots. All residues were collected. The quantity of crop residues for different agricultural practices is: CT/IN= 5.73 ± 2.25 t ha-1; CT/OUT= 2.76 ± 0.58 t ha-1; RT/IN= 4.47 ± 0.97 t ha-1; RT/OUT= 2.38 ± 0.57 t ha-1. All post-sowing fertilizer, herbicide, fungicide, and pesticide applications were identical on CT and RT treatments. Pesticides were classified as non-toxic to earthworms (EFSA, European Food Safety Authority).

Average annual air temperature for 2009, 2010, and 2011 was higher than local averages (12.2°C *vs*. 9.1°C) (measured at Ernage over 30 years from 1971 to 2000, The Royal Meteorological Institute of Belgium, 2004 – 2009). Recorded annual rainfall (2009, 2010, and 2011) was lower than the local averages (538 mm vs. 772 mm). Weather during the 2012 was measured by the CRA-W (Walloon Agricultural Research Centre) at the Ernage-Gembloux station (Fig. 1), which is located 3 km from our experimental site.

2. 3. Soil characteristics

2.3.1. Soil type and morphological characteristics

Description and characterization of augerings and soil profiles were performed in April 2012 to classify soil type, and verify soil homogeneity within the experimental field. We dug one 2 x 2 x 2 m pit for each treatment, and one face/aspect of the pit was described according to standardized procedures (Delecour and Kindermans 1980). Samples were collected from each soil horizon for laboratory determinations. Ten soil sub-samples in each plot were taken at several depths using an auger. The sub-samples were mixed to produce a composite sample for each treatment, layer and replication and sieved through a 2 mm sieve before analysis. Soil bulk density (BD) was estimated from soil cylinders (100 cm3). Additional observations and sampling were performed in every plot. Texture was measured with an automated Robinson pipette; pH was measured in 1N KCl after equilibration for 2 h (2: 5 w: v ratio); and total organic carbon (TOC) was determined following the Walkley-Black method (Nelson and Sommers, 1996). Organic carbon was oxidized by adding 1 g of soil to $K_2Cr_2O_7$ and H_2SO_4. Excess $Cr_2O_7^{2-}$ was titrated with ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2*6H_2O$].

World Reference Base classification (WRB, 2014) indicated the trial field soil is a Luvisol (Hypereutric, Siltic), locally presenting albeluvic tonguing. Prior to experimentation, tilling depth reached between 25 cm and 30 cm. Consequently, an E horizon cannot be observed under the plough layer. Textural B-horizons contained 5 to 10% more clay than the A horizon, with a blocky, sub-angular (30 mm size clods), friable structure, which is porous (primarily small sized pores) but compact. Variation in soil characteristics occurred primarily in the A soil surface horizon. Three sub-layers were observed. The first two sub-layers were very similar under conventional tillage, while the 0 – 10 cm sub-layer was less dense, and richer in organic matter under reduced tillage. Numerous clods were platy under systems without residue restitution, while biogenic clods (granular and subangular blocky) were more important under the other systems. Regardless of differentiation in the surface sub-layers, soil type in the trial field was considered homogeneous. Selected physical and chemical characteristics of the soil profile studied are reported in Table 1.

Table 1. Primary soil properties in the field trial according to agricultural practices: CT / IN: conventional tillage with crop residues incorporated into the soil; CT / OUT: conventional tillage with crop residues exported from the field; RT / IN: reduced tillage with crop residues incorporated into the soil; and RT / OUT: reduced tillage with crop residues exported from the field.

Agricultural practices	Horizon	Depth	Bulk Density	Structure / Compaction / Coherence	pH	TOC	Clay	Silt	Sand
		cm	mg. m^{-3}		KCl	g. kg^{-1}		%	
CT / IN	Ap1	0-6	1.41	Gr + BS / C0 / F0	6.7	11.0	14.8	79.1	6.1
	Ap2	6-25		BS + Gr / C2 / F1	6.7	10.8	14.9	79.3	5.8
	Ap3	25-35		Pl / C3 / F2	6.8	10.8	15.5	79.2	5.3
	Bt1	35-45	1.60	BS / C2-C3 / F2	6.6	4.7	19.4	76.1	4.5
	Bt2	45-85	1.58	BS - Ma/ C2 / F2	6.5	2.0	26.7	69.5	3.8
	Bt3	85-120	-		6.5	1.6	23.8	73.7	2.5
	C1	120-135	1.61		6.5	1.3	21.6	74.5	3.9
	C2	135-175	-		6.4	1.0	21.3	74.3	4.5
	C3	>175	-		6.2	0.7	21.9	75.6	2.4
	IIC	>250	-		6.2	0.4	19.1	76.5	4.4
RT / IN	Ap1	0-5	1.34	Gr + Pl / C0 / F0	6.6	12.8	14.0	79.8	6.1
	Ap2	5-12	-	Pl + Gr / C1 / F0	6.7	13.5	13.9	80.3	5.8
	Ap3	12-35	1.56	BA / C2 / F0-F1	6.8	11.1	13.5	81.5	4.9
	Bt1	35-44	1.51	BA / C2-C3 / F0-F1	6.7	4.1	16.0	79.0	5.0
	Bt2	44-75	1.55	BS / C1-C2 / F0-F1	6.6	2.7	20.4	75.2	4.4
	Bt3	75-122	-		6.5	1.1	22.7	73.3	4.0
	C1	122-185	1.55	BS - Ma / C1 / F1	6.4	1.2	21.3	74.0	4.7
	C2	185-220	-		6.3	0.6	20.7	75.7	3.6
	IIC1	> 220	-		6.3	0.7	19.4	74.2	6.4
	IIC2	> 250	-		6.3	0.5	30.8	65.7	3.5
CT / OUT	Ap1	0-10	1.38	Gr + Pl / C0 / F0	6.5	10.8	14.8	79.7	5.5
	Ap2	10-15		Gr + BA / C2 / F2	6.6	11.5	14.5	80.9	4.7
	Ap3	15-25		Gr + BA / C1 / F1	6.6	11.7	14.8	79.7	5.5
	A4	25-35	1.53		6.7	11.4	14.4	80.3	5.4
	Bt1	35-50	-	BA / C2-C3 / F2	6.5	4.3	19.0	77.3	3.8
	Bt2	50-110	1.66		6.2	1.6	25.0	71.8	3.2
	Bt3	110-140	1.61		6.2	0.7	22.0	74.1	3.9
	C	140-180	-	Ma / C1 / F1	6.1	0.7	21.3	72.4	6.3
	IIC	>180	-		6.0	0.4	19.7	76.8	3.5
RT / OUT	Ap1	0-12	1.26	Gr + BS / C1 / F0	6.5	13.6	15.8	78.1	6.1
	Ap2	12-20	1.50	Pl / C1-C2 / F1	7.0	11.8	16.0	77.1	6.9
	Ap3	20-30	1.44	Pl / C2 / F1	7.2	12.0	16.1	76.4	7.5
	Bt1	30-55	1.56	BA / C1-C2 / F1-F2	6.6	3.3	23.3	72.3	4.5
	Bt2	55-80	1.53		6.4	2.1	24.3	71.8	3.8
	Bt3	80-100	-	Ma / C1 / F1	6.4	1.2	22.8	73.0	4.3
	C	100-160	1.56		6.4	0.8	21.2	72.8	6.0
	C/IIC	160-200	-		6.3	0.6	20.7	76.2	3.2
	IIC	>200	-	Ma / C1 / F0	6.3	0.4	19.2	74.0	6.8

Gr: Granular	C0: Loose	F0: Very friable
BS: Blocky subangular	C1: Weakly compact	F1: Friable
BA: Blocky angular	C2: Compact	F2: Weakly friable
Pl: Platty	C3: Very compact	F3: Hardly friable
Ma: Massive		

* Texture measured with automated Robinson pipette; pH: 2:5 w:v ratio. 0.1N KCl. 2h contact;

TOC: Total Organic Carbon after Walkley-Black; Bulk density in 100cm^3-cylinders.

Organic matter (OM) equal TOC * 2.

2.3.2. Physico-chemical indicators

In April 2012, soil samples and earthworms were collected from the 16 plots. Soil samples were composites of 10 individual cores, and three sample depths (0 – 10 cm; 10 – 20 cm and 20 – 30 cm) were collected. Samples were maintained at 4 °C until laboratory analyses. Hot Water Carbon (HWC) was determined as described by Ghani et al. (2003). The hot-water extraction method is a procedure that can be used to estimate a pool of labile decomposable SOM (Keeney and Bremner, 1966). The HWC is frequently used as a measure for potentially bioavailable soil organic carbon and as a useful index of soil quality (Liang et al., 2012). Six g of moist soil were shaken in 60 ml of water at 80 °C for 16 hours, and subsequently filtered through a 0.45 cellulose membrane filter. A 50 ml aliquot was oxidised by excess $K_2Cr_2O_7$ under acid conditions, and the remaining oxidant was titrated with $Fe(NH_4)_2(SO_4)_2*6H_2O$. Soluble P, Mg, K, Ca, Na, and electrical conductivity (EC) were measured following water extraction at 20 °C after shaking 10 g of soil in 50 ml of water for 30 min (w :v ratio = 1 : 5). EC was measured with a conductivity meter and soluble elements were measured by flame absorption (Ca, Mg), emission (Na, K), or colorimetry (P). Soil pH was measured in distilled water (pH_{water}) and 1N KCl (pH_{KCl}) (2: 5 w: v ratio) using air-dried soil. Soil penetration resistance (PR) indication of soil compaction was measured in situ at each of the 16 sampling points to 50 cm depth at intervals of 1 cm, using a fully automated penetrometer (30° angle cone with a base area of 10 mm²); mounted on a small vehicle. The insertion speed was 2 cm s^{-1}. The water content of soil samples taken from each parcels was measured (not presented here), and the average water content was 23 – 25%

(volumetric) at 0 – 30 cm soil depth. PR mean values were calculated in the soil layers of 0 – 10, 10 – 20, 20 – 30, and 30 – 50 cm, and used for statistical analyses. PR measurements used for analyses were taken every 1 cm beginning at 5 cm. The first few soil centimetres were not included to avoid bias due to the tillage in soil surface.

2.4. Earthworm sampling method

In april 2012, earthworm populations were sampled from all 16 plots, during the maximum activity period (Bouché, 1972; Edwards and Bohlen, 1996). Earthworms were sampled using formaldehyde solution coupled with hand sorting method (Bouché, 1977; Cluzeau et al., 1999) within 1 m x 1 m quadrats. Specifically, three applications of a formaldehyde solution in water (10 L per application, 30 L/quadrat in total, formaldehyde solution concentrations: 0.25%; 0.25%; 0.4%) were administered to the soil surface at 15 min intervals. All earthworms that surfaced were sampled. This step was followed by hand sorting method to allow for data correction, and earthworms were collected (25 cm x 25 cm, 25 cm depth). Four replicates were performed for each treatment. Collected earthworms were placed in plastic bags that contained 4% formaldehyde solution, taken to the laboratory, counted, individually weighed, and identified to the lowest taxonomic level possible (family, genus, species) using the identification keys and descriptions of Bouché (1972). Earthworms are classified in four functional groups: epigeic, epi-anecic, anecic and endogeic earthworms. Some species were common and easily distinguished by the naked eye (e.g. *Lumbricus terrestris* and *Allolobophora rosea rosea*, among others). Some juvenile earthworm species were not clearly identified, and classified as unidentified juveniles. Earthworms are considered adult if they are clitellate, and juvenile if tubercula pubertatis or clitellum were absent. Earthworms are very sensitive to soil disturbance, therefore expulsions were not conducted on traffic lines to eliminate any impacts of soil compaction (Pizl, 1993; Sochtig and Larink, 1992).

2.5. Data analysis

Prior to other analysis, Shapiro-Wilk's and Bartlett's tests were used to test data normality and homoscedasticity. In most cases, parametric tests were permitted, but in others equivalent non-parametric tests were indicated. The a priori significance level for all tests was fixed at $P < 0.05$.

Agricultural practices effects on functional groups abundances, total earthworm abundance (adults and juveniles), total earthworm biomass and species richness, were investigated using linear mixed effects model. Residue incorporation depth and straw management were considered as crossed fixed factors while line and column were considered as crossed random factors. Earthworm species abundances and total biomass were averaged per plot before statistical analysis. This model was chosen to test the effects of factors "residue incorporation depth" and "crop residue management" on changes in earthworm abundance, biomass, functional groups, and diversity species. Soil properties were examined by residue incorporation depth, crop residue management, and depth. Average values of all sampling points of each plot and depth were used to detect significant differences in soil properties. Means comparisons were conducted using a post-hoc Tukey test. When assumption of parameters model where not met, a Kruskal-Walis test was used to detect effects of factors. Additionally, multivariate analyses were performed (Principal Component Analysis) using ADE4 (Thioulouse et al., 2001) did not indicate clear associations between the groups of physico-chemical and biological variables. Comparisons of biological, chemical, and physical variables in experimental practices were performed using R software (R Development Core Team, 2008).

3. Results

3.1. Effects of agricultural practices on soil physico-chemical properties

Soil physico-chemical properties results from all samples sites are shown in Tables 1 and 2. pH values ranged from 6.2 to 7.0, with no significant differences among the four treatments ($P > 0.05$). Interactions between crop residue management and residue incorporation depth factors, which facilitated soil property comparisons across residue amounts and incorporation treatments, were not detected. The mean volumetric soil moisture (%) for different agricultural practices is: CT/IN= 26.9%; CT/OUT= 25.9%; RT/IN= 25.5%; RT/OUT= 24.5%. The average water content at 0 – 30 cm depth was not significantly different between agricultural practices.

The mean BD values ranged from 1.41 to 1.61 mg m-3 under CT/IN, from 1.38 to 1.66 mg m-3 under CT/OUT, from 1.34 to 1.56 mg m-3 under RT/IN and from 1.26 to 1.56 mg m-3 under RT/OUT. With increased depth, BD values increased. In all soil layers, there was a slight tendency to lower BD for RT compared with CT treatments. Our results showed soils under RT treatment had higher BD than soils under CT in the 10 – 20 cm layer. However, significant differences were not observed between treatments ($P > 0.05$).

The soil PR after tillage practices applied for wheat production, which were plotted against soil depth for different tillage practices, is illustrated in Fig. 2. Average PR values of 0 – 10, 10 – 20, 20 – 30 and 30 – 50 cm soil depths for different treatments were given. The results showed that PR increased with soil depth at all agricultural practices. The difference between CT and RT treatments for 10 – 20 cm and 20 – 30 cm was greater than that observed for the depth 30 – 50 cm. When the effect of treatments on PR was examined, results indicated soil compaction was significantly higher in RT compared with CT.

Soil property comparisons among agricultural practices were analysed for each depth. The concentrations of HWC showed no significant differences among the treatments at all depths. However, the concentration of labile carbon fractions in all treatments tended to decrease with depth. Soil physico-chemical analysis (EC, RH, P, K, Mg, and Ca) revealed significant differences among the four treatments. In the first centimetres of soil 0 – 10 cm, total P and K concentrations were higher in RT compared with CT. 10 – 20 and 20 – 30 cm depths exhibited inverse results, where K and P amounts were lower in RT. Although soil K and P concentrations declined with soil depth under different treatments, significant differences among the four treatments were observed. Also, Ca concentrations were higher when residues were incorporated into the soil, regardless of treatment, but no significative difference was observed. Surface layers were the most informative in assessing management impact on nutrient status, because surface soils were modified directly by cultivation. The depth factor suggested an effect on residual humidity (RH). RH values were significantly ($P < 0.05$) higher at depth 20 – 30 cm under CT compared with RT treatment.

Table 2. Mean of soil physical and chemical properties among the four agricultural practices: CT / IN: conventional tillage with crop residues incorporated into the soil; CT / OUT: conventional tillage with crop residues exported from the field; RT / IN: reduced tillage with crop residues incorporated into the soil; and RT / OUT: reduced tillage with crop residues exported from the field, at various depths (0 – 10 cm; 10 – 20 cm; and 20 – 30 cm). Different letters indicate a significant difference according to Tuckey's-test at $P \leq 0.05$.

Depth	EC	RH	HWC	P	K	Mg	Na	Ca
0 – 10 cm	S m^{-1}	%			mg kg^{-1}			
CT/IN	81.5	16.3	411	4.2 a	16.5 ac	3.7	26.2	54.0
CT/OUT	79.2	16.3	448	4.2 a	14.5 a	3.7	32.3	52.3
RT/IN	76.5	17.3	463	5.8 b	21.7 b	3.6	22.4	48.8
RT/OUT	73.7	16.8	443	4.9 ab	18.5 bc	3.4	22.6	47.3
10 – 20 cm								
CT/IN	68.8	17.7	434	3.8 a	13.2 a	3.4	24.5	50.3
CT/OUT	69.1	17.6	403	3.8 a	12.1 a	3.3	22.3	47.8
RT/IN	71.0	17.4	388	3.9 a	11.6 b	3.3	21.5	52.1
RT/OUT	66.7	17.0	397	3.7 b	9.1 b	3.1	20.8	45.8
20 – 30 cm								
CT/IN	68.5 a	18.2 a	384	4.0 a	14.9 a	3.4 ab	23.5	50.2 ab
CT/OUT	67.6 a	18.2 a	378	4.1 a	14.8 a	3.1 a	23.0	44.3 a
RT/IN	84.5 b	17.1 ab	380	3.3 b	14.1 ab	4.1 b	21.7	58.5 b
RT/OUT	77.4 b	17.0 b	382	3.2 b	12.3 b	3.7 ab	21.5	52.9 ab

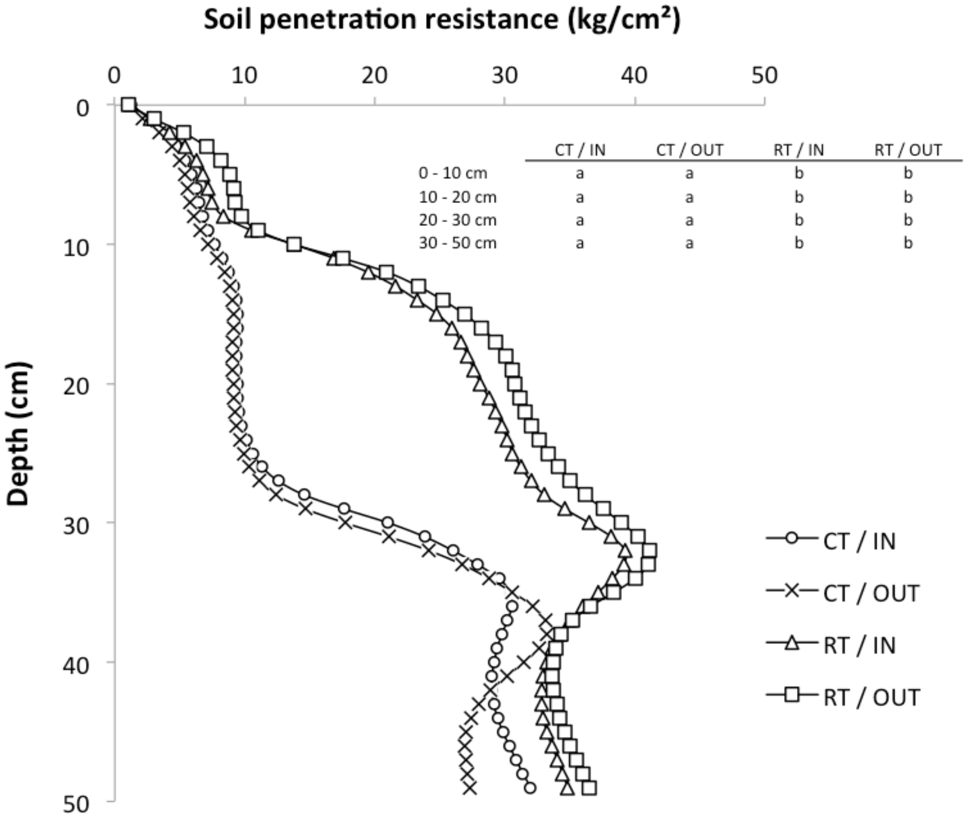

Fig. 2. Penetration resistance determined in the field at 0 – 50 cm depth for the four agricultural practices: CT / IN: conventional tillage with crop residues incorporated into the soil; CT / OUT: conventional tillage with crop residues exported from the field; RT / IN: reduced tillage with crop residues incorporated into the soil; and RT / OUT: reduced tillage with crop residues exported from the field.

3.2. Effects of agricultural practices on earthworm abundance and biomass

Earthworm communities were variable throughout the four experimental systems. A total of eight species were recovered from the samples (all plots combined); 1458 total individuals were from CT plots, and 1440 individuals were collected from RT plots. When the effect of treatments on earthworm total abundance was examined, the “crop residue management” factor had a significant effect ($P < 0.01$).

Residue incorporation depth and the interaction between the two factors were not significant ($P > 0.05$). Despite the high degree of observed earthworm variability, earthworm abundance (180 individuals m^{-2}, 182 individuals m^{-2}) was considered equivalent between CT and RT treatment (Fig. 3A). Total earthworm community biomass exhibited a non-significant trend towards increased biomass in RT *vs*. CT fields ($P > 0.05$). However, a significant effect of crop residue management was observed in CT and RT ($P < 0.01$); greater biomass was found in CT and RT when crop residues were incorporated into the soil (Fig. 3B).

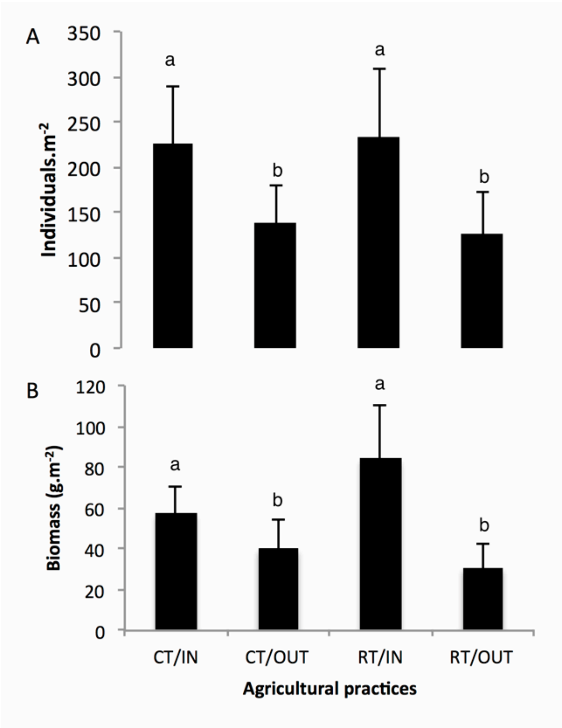

Figure 3. Earthworm abundance in numbers m^{-2} and biomass in g m^{-2} (±Standard deviation) measured in CT / IN: conventional tillage with crop residues incorporated into the soil; CT / OUT: conventional tillage with crop residues exported from the field; RT / IN: reduced tillage with crop residues incorporated into the soil; and RT / OUT: reduced tillage with crop residues exported from the field.

3.3. Effects of agricultural practices on earthworm diversity

A total of 2898 individuals were collected; 68% were adults, and 32% were juveniles. Eight species were recovered from the experimental trials (Table 3). In the four treatments, we analysed earthworm abundance based on functional groups (Fig.4). Results showed the following species diversity: epigeic species 3%; epi-anecic species 28%; anecic species 5%; and endogeic species 64%. *Aporrectodea caliginosa* was the dominant adult endogeic species collected in all agricultural systems. This species dominance remained relatively unchanged in RT for IN and OUT (64% and 57%, respectively). *Lumbricus rubellus castanes*, *Lumbricus terrestris*, *Allolobophora rosea rosea* and *Aporrectodea caliginosa* individuals were identified in each experimental treatment, and the species abundance varied among agricultural practices. The only species observed in CT was the endogeic taxon *Allolobophora chlorotica chlorotica typica*. In CT, the proportion of *A. caliginosa* species was higher in CT/OUT (64.5%) compared with CT/IN (30.5%).

Octolasium cyaneum, *Aporrectodea caliginosa meridionalis,* and *Dendrobaena mammalis* species were less abundant, and were only observed in CT/IN fields. The *Aporrectodea* juvenile earthworms were only recovered in CT plots, when crop residues were incorporated into the soil, while *Lumbricus* juveniles were collected in all plots. Total densities of all earthworm species were higher in CT compared with RT.

Table 3. Total abundance of earthworm species and functional groups recorded in the four agricultural practices: CT / IN: conventional tillage with crop residues incorporated into the soil; CT / OUT: conventional tillage with crop residues exported from the field; RT / IN: reduced tillage with crop residues incorporated into the soil; and RT / OUT: reduced tillage with crop residues exported from the field.

Earthworm species	Functional group	CT/IN	CT/OUT	RT/IN	RT/OUT
Dendrobaena mammalis	Epigeic	16			
Lumbricus rubellus castaneus		1	2	29	29
Lumbricus terrestris	Epi-anecic	189	142	276	187
Aporrectodea caliginosa meridionalis	Anecic	184			
Aporrectodea caliginosa caliginosa	Endogeic	390	354	599	284
Allolobophora chlorotica chlorotica typica		48	17		
Allolobophora rosea rosea		78	35	33	3
Octolasium cyaneum		2			

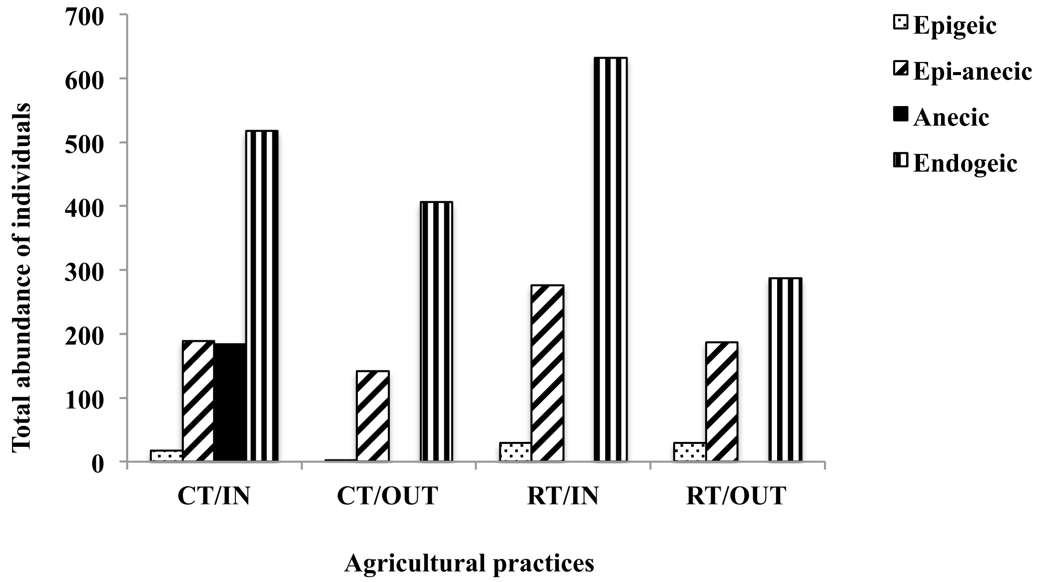

Figure 4. Total abundance of earthworm functional groups. CT / IN: conventional tillage with crop residues incorporated into the soil; CT / OUT: conventional tillage with crop residues exported from the field; RT / IN: reduced tillage with crop residues incorporated into the soil; and RT / OUT: reduced tillage with crop residues exported from the field.

4. Discussion

4.1. Effects of agricultural practices on soil physico-chemical variables

We conducted BD measurements in several soil layers at all plots (Table 1); we have found that BD in all treatments was lowest near the soil surface while at deeper layers the opposite occurred. In all soil layers, there was a slight tendency to lower BD for RT compared with CT because it was minimally impacted by tillage operations over the years. The values were assumed to represent BD under natural conditions. BD is variable due to wheel compaction, tillage management and biological activity (Heuscher et al., 2005). Børresen (1999) reported no significant effects of straw on BD, even following eight years of straw incorporation, consistent with our results. BD increased slightly at 10 – 25 cm, and can be attributed to low OM content, soil compaction, crop operations, and agricultural practices, including weed control and crop exportation. PR is a useful parameter for evaluation of soil physical quality. PR and the water content of a soil are interrelated, and both are affected by the soil's texture, structure, aggregation and bulk density (Glinski and Lipiec, 1990). PR of soils increased with depth at all agricultural practices. The results indicated residue incorporation depth had a significant influence on soil PR ($P < 0.01$). However, incorporation of crop residues did not affect PR. We observed as water content increases, PR decreases. Similar results from Laboski et al. (1998) showed an increase of PR in corn fields when water content decrease. The observed compaction in RT in the present study might be attributed to natural soil compaction resulting from continued RT application since 2008. The result is consistent with the findings Çelik (2011) who found that RT treatment provided higher PR values according to CT. Lower PR values in soil under CT could be associated with the increase in the intensity of soil loosening due to tillage. Already, Osunbitan et al. (2005), Topa et al. (2011) and Yavuzcan et al. (2002) also expressed that tillage creates loosening in the affected soil layer, and concluded that tillage practice decreased the soil strength due to greatest loosening.

Whatever treatment, labile carbon fraction represented with HWC ranged from 378 mg kg^{-1} to 463 mg kg^{-1}. In the Chen et al. (2009) research, the average HWC values in the arable soil ranged from 375 mg kg^{-1} to 554 mg kg^{-1} in the conventional tillage with residue removal and shallow tillage with residue cover, respectively. HWC showed higher values in 0 – 10 cm and 10 – 20 cm depths, compared with 20 – 30 cm depth. The explanation for this increase emerges from the different quantity of crop residues incorporated into the soil. Residues might enter to the labile carbon pools, provide substrate for soil organisms, and contribute to accumulation of labile carbon. An increase in labile carbon fractions leads to improvement of soil fertility through increase of labile source of nutrients. Soil EC mainly depends on soil clay content, soil water content, soil temperature, and indirectly, on soil compaction due to changes in soil water content (Corwin and Lesch, 2005; Friedman, 2005). EC increased slightly when crop residues were incorporated and significant difference between treatments was only observed in the 20 – 30 cm layers. EC varies between 67.6 S m^{-1} and 84.5 S m^{-1}, which is relatively small range of variations for agricultural fields (Brosten et al., 2011).

K and P concentrations were significantly higher at the 0 – 10 cm depth, particularly under RT treatment.

Mixing soil at a depth of 25 cm under CT makes organic matter more available. These higher values can be attributed to K and P quantities released from harvest residues incorporated into the first 10 centimetres of soil under RT. Jansa et al. (2003) and Lopèz-Fando et al. (2007) showed that under RT, P and K concentrations were higher in the surface soil than in lower soil horizons because previous fertilizer applications released P and K, which remained in plant residues at the soil surface. Crop residues restore an important part of K (Girard et al., 2005). Inconsistent with other elements, Ca concentrations were higher in RT and CT when residues were incorporated into the soil. Ca element exhibits reduced soil mobility compared with K and Mg (Guo and Sims, 2001), reflected by maintenance of high soil Ca concentration under CT/IN and RT/IN treatments. Regardless of residue incorporation depth, K and Ca showed increased concentrations, which is again linked to the concentration of crop residues.

4.2. Effects of agricultural practices on earthworm abundance and biomass

Earthworm abundance in CT plots (66 – 180 individuals m^{-2}) may be considered normal. Metzke et al. (2007) found 0 – 84 earthworms m^{-2} in ploughed organic systems in similar soil types, and Pfiffner and Luka (2007) found average of 210 individuals m^{-2} in organic cereal fields. We did not observe negative effects based on the depth of residue incorporation influencing earthworm abundance and biomass. This suggested CT had no substantial impacts on earthworms, or the species that comprise earthworm communities are tolerant to these forms of disturbance. However, previous studies reported long-term intensive tillage significantly reduced earthworm abundance (Edwards and Bohlen, 1996; Gaston et al., 2003). Tillage systems applied in this region of Belgium remain at acceptable levels of intensification, compatible with rather high earthworm activities. The absence of significant differences in earthworm abundance and biomass might result from the following observations: increased earthworm mobility, and/or increased tolerance to tillage. In a study conducted in Switzerland, Berner et al. (2008) measured the effects of chisel compared to mouldboard ploughing, which was applied as a RT system on earthworm populations. After three years, total earthworm biomass exhibited no significant changes in total earthworm biomass or abundance.

Earthworm communities showed significant variability based on crop residue management. Crops under no-tillage and RT have higher earthworm populations than those submitted to CT, primarily due to the negative effects of extensive and frequent soil disturbance (Brown et al., 2008; Sautter et al., 2007), and the associated reduction in soil OM content. SOM and decomposing plant residues are the primary food source for earthworms (Brown et al., 2000), and an increase in RT systems generally leads to an increase in earthworm abundance (Brown et al., 2004; Hendrix et al., 1992). Crop rotation and cover crops are also vital to earthworm populations, because these practices determine richness and food quality available (Franchini et al., 2004). Earthworm abundance and biomass varied based on crop residue incorporation or exportation.

Regardless of the agricultural practices, when crop residues were incorporated into the soil, a significant difference was detected between earthworm abundance and biomass than when crop residues were exported from fields, i.e. significantly higher earthworm abundance and biomass was recorded ($P < 0.05$). Specific microsite surface factors can serve to explain the results between IN and OUT treatments, i.e. higher carbon content (Brown et al., 2002), food supply, wetter climate, and grass cover. Although the presence of organic materials provides a benefit to earthworms (Kladivko et al., 1997; Schmidt et al., 2003), OM availability depends on soil residue stratification, and feeding behaviour of resident earthworm species and functional groups. Earthworm activity can be stimulated by reduced soil disturbance and/or crop residue incorporation and as such be an important determinant of soil structural characteristics under different crop management systems (Pulleman et al., 2003).

4.3. Effects of agricultural practices on earthworm diversity

The earthworm community found in our study was similar to others in north western Europe (Ernst and Emmerling, 2009; Valckx et al., 2009; Nieminen et al., 2011; De Oliveira et al., 2012). Endogeic earthworms (64.5%), particularly *A. caliginosa* (Savigny, 1826) dominated the community, which might be explained by a re-building capability of the earthworm community (Marinissen, 1992). *A. caliginosa* species reportedly exhibits high adult survival and cocoon production rates, and appeared to have become firmly established. The predominance of *A. caliginosa* in cultivated fields has been reported in several studies (Boström, 1995; Capowiez et al., 2009a; Riley et al., 2008). Indeed, *A. caliginosa* has been described as a species with a low sensitivity to the environmental disturbance caused by tillage (Rosas-Medina et al., 2010). We assume common earthworm species in northern Belgium cultivated soils are endogeic, and feed predominantly on decomposing OM, already incorporated into the mineral soil layer (Ernst and Emmerling, 2009). *A. caliginosa* and *A. rosea* presence under the four treatments indicated the species were sympatric in the field at the observed species abundance. These observations suggested co-existence or habitat/microsite factors facilitate these species to inhabit this soil with limited resource availability (Jiménez et al., 2006). Our results confirm that endogeic earthworms may adapt themselves to the disturbance caused by tillage practices and crop residue exportation. These findings are consistent with those of most other studies on the effect of tillage (e.g. Chan, 2001; Peigné et al., 2009; Pelosi et al., 2009; Riley et al., 2008).

Earthworm communities in arable land are often dominated by endogeic species with low amount of anecics, especially when under intensive tillage systems (Ernst and Emmerling, 2009; De Oliveira et al., 2012). Endogeic and epi-anecic groups decreased in the tillage systems when crop residues were exported. We observed that when the abundance of earthworms was higher, then effect of residue exportation was stronger. Epigeic species *L. castaneus* was more abundant in RT compared with CT. According to Ivask et al. (2007), *L. castaneus* species is sensitive to ecological factors such water content of soil, and its presence indicates favourable agricultural soil conditions under RT. Epigeic species are sensitive to tillage; therefore the CT treatments had negative effects on these species. In addition, residue incorporation at a 25 cm depth associated with wheat culture (tillage, soil cover) might be enough to affect epigeic species. By contrast, epi-anecic earthworm *L. terrestris* were less affected by CT and crop residue exportation. Surprisingly, *L. terrestris* species, which is known to be sensitive to tillage, were present in CT treatment. In our study fields, *L. terrestris* was found in large numbers and their abundance are positively increased by decreasing the intensity of tillage (Capowiez et al., 2009a; Simonsen et al., 2011). *L. terrestris* such as *A. caliginosa* appear to be sensitive to crop residue exportation. *L. terrestris* is one of the predominant species found in this study that produces spring hatching cocoons. This explains the large number of juveniles collected at the sampling sites.

In this study, eight species were collected and identified. The species diversity observed in our fields is consistent with the observations of Peigné et al. (2009), who found seven species at each of their differently tilled farming sites, and those of Binet *et al*. (1993) for conventional arable systems in western France. Despite tillage, earthworm species diversity was higher when crop residues were incorporated in the soil. This can be attributed to compensation from soil disturbing effects (tillage) due to the presence of crop residues. In addition, the increased number of endogeic species, including. *A. caliginosa*, *A. rosea,* and *A. c. c. typica* under CT compared with RT demonstrated these species were markedly less sensitive to soil disturbance, and their adaptation capability to the conditions of tilled soil and exportation of crop residues (Paoletti, 2001; Tischer, 2005). These results are congruent with several reports (Ivask et al., 2007; Capowiez et al., 2009a). Also, the presence of *A. c. c. typica* could be enhanced by crop residues incorporated into the soil. The low earthworm species composition under RT was probably attributable to other soil properties, such as compaction, exportation of crop residues and rainfall decreasing during the three months (february, march and april) before earthworm sampling.

4.4. Earthworm-soil properties relationships

The measured soil pH range was optimal for most earthworm species. Some epigeic species (e.g. *Dendrobaena octaedra*, *Lumbricus rubellus*) are more tolerant of low soil pH (Tiunov et al., 2006) whereas endogeic (Aporrectodea spp.) and epi-anecic (*L. terrestris*) species may be more drought tolerant (Bohlen et al., 1995). In our study, correlation analyses were performed, and no relationships was found between soil properties and earthworm communities under agricultural practices. In the condition of the experiment, soil PR were highly significantly impacted by residue incorporation depth treatment. Comparing earthworm species of different agricultural practices, *L. terrestris*, *L. castaneus* and *A. caliginosa* showed a general increase in compacted soils. Epi-anecic *L. terrestris* inhabits permanent burrow systems. As in an experiment of Langmaack et al. (1999), this species' burrowing activities appear to be widely unaffected by soil compaction. *A. caliginosa* is the dominant earthworm species in compact soils (Boström, 1986). Our results, although from only one experiment show that increased PR does not necessarily reduce earthworm communities. In temperate environments, Valckx et al. (2009) found that the spatial variability of soil properties was not linked to the spatial distribution of several earthworm species, among which *L. terrestris*, *A. caliginosa* and *A. rosea*. Earthworm communities were composed of the association of decompacting and compacting species known to be the main feature in natural populations (Rossi, 2003) and regulating soil structure (Blanchart et al., 1997). Earthworms are able to partly counteract detrimental effects caused by soil compaction with time. Recently, Capowiez et al. (2009b) found evidence from field experiments conducted directly on wheel tracks and plough pans that earthworms can contribute significantly to the regeneration of those compacted zones.

HWC decreased as earthworm abundance showed a decrease. Increased of HWC were more pronounced in plots with incorporation of crop residues. In the 0 – 10 cm depth, HWC provides a measure of the labile C fraction in soil available to organisms (Ghani et al., 2003). The total HWC content might be an indicator of earthworm dynamics and general soil fertility as there is more HWC in the soil with the higher OM content, due to the processes of intensive transformation of crop residues.

Nutrients in the first centimetres of soil (0 – 10 cm) under RT treatment can be improved by maintaining crop residues at the soil surface layer. Coppens et al. (2006) showed that mixing crop residues with soil particles by mouldboard plough practices can lead to an acceleration of the residues decomposition and hence to a faster release of nutrients. Under RT/IN treatment, P, K and HWC values were higher in 0 – 10 cm depth. These results can be associated to the high biomass of earthworms. Earthworm impacts on soil P dynamics and availability might depend on the specific soil properties, the organic P source, and the specific earthworm species burrowing behaviour and food preferences (Bünemann et al., 2011). The trend in nutrient concentrations observed for the 0 – 10 cm depth can be attributed to the depth of residues placement in which incorporated residues to accelerated decomposition caused by mechanical mixing of the soil (Hernanz et al., 2009) and earthworm activity. Earthworm activity can affect P concentration in the soil surface, particularly by endogeic species living in superficial soil layers. The study conducted by Sharpley et al. (2011) reported that earthworm activity promoted the incorporation of P from the soil surface into the soil profile. In addition, it has been previously shown that endogeic earthworms, by enhancing P availability in their casts (Le Bayon and Binet, 2006) are able to influence P dynamics in soil. Some microbial populations are stimulated in earthworm casts and it can increase available P (Richardson and Simpson, 2011). Following ingestion, the casts produced from soil have increased Ca, Mg, K, and P amounts than the original soil (Sharpley et al., 2011). The higher concentration of Ca when crop residues were incorporated into the soil is probably due to the intimating mixing of OM through the earthworm gut which can further enhance mineralization and humification processes (Lavelle, 1988; Blanchart et al., 1999). Schrader and Zhang (1993) showed that casts and burrows of *A. caliginosa* had a particularly high content of $CaCO_3$, and Canti and Piearce (2003) showed that *L. terrestris* had calciferous glands that produced calcium carbonate. Greater nutrient amounts in CT/IN and RT/IN treatments suggest increased earthworm abundance, biomass, and diversity. Earthworm effects on soil nutrient dynamics cannot be predicted by earthworm population biomass and diversity alone. Decaëns et al. (2003) reported soil type was a primary contributing factor in the spatial distribution of earthworm community structure and diversity at the landscape scale, while at the field scale, agricultural practices had the most impact. The present study emphasises the importance of locally obtained data that evaluate abundance, biomass, and diversity of common, native earthworm species, governed by various geographical, edaphic, and climatic factors, as well as crop residue management. In general, the relationship between earthworm populations and soil nutrient availability is complex.

Although in this study we try to interpret relationships between earthworm communities, soil properties and nutrients as resulting from the effects of earthworms under different agricultural practices, long term experiment will be continue for causal evidence of our hypothesis.

5. Conclusion

This study clearly demonstrated that earthworm abundance and biomass are significantly affected by the exportation of wheat crop residues. After four years of agricultural practices application, exportation of wheat residues effect was strong than tillage effect. It is often considered that reduced tillage has a greatest positive effect on earthworm abundance and biomass, but this could not be confirmed in our study. Earthworm functional groups appeared tolerant to tillage but decreased when residues were exported from soil.

Although different soil properties reacted differently to tillage systems, exportation of crop residues and presence of some earthworm species, no consistent relationship between soil properties and earthworm community was observed. The number of years that our field was managed under conventional tillage and exportation of crop residues might have contributed to the lack of tillage effect. Finally, the overall results of our study showed earthworms stimulate nutrient dynamics by stimulating OM content from crop residues. This preliminary study emphasises the need to consider soil physico-chemical properties and earthworm population dynamics when trying to elucidate the effects of cultivation techniques on soil productivity potential.

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CHAPTER 3

CHANGE OF EARTHWORM COMMUNITIES UNDER DIFFERENT TRACE METAL CONTAMINATED SOILS

Effects of heavy metal contamination from a surroundings of a former Zn-Pb ore treatment on earthworm communities

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Abstract

The effect of soil contamination by heavy metals on earthworm communities was studied in agricultural fields, in Sclaigneaux, one of the most polluted regions of Walloon Region in Belgium. In spring 2014, earthworms were collected and soil samples taken in agricultural soils along transects at four different distances (1295, 1295, 2800 and 2910 m) from historically Zn-Pb ore ore exploitation. Earthworms were collected using the formaldehyde method. All collected earthworms were counted and identified. Basic physico-chemical properties of soil and concentrations of Cu, Cd, Zn and Pb were analysed from soil from each sampling point. Earthworm abundance and biomass did not significantly differ between distances, although there was a trend of increase in the number of earthworms with decreasing distance. The earthworm diversity found in our fields was low. The endogeic species (*Aporrectodea chlorotica* and *Aporrectodea rosea*) and epi-anecic species (*Lumbricus terrestris*) dominate in contaminated field. Epigeic species were absent in all fields. There were no significant correlations between soil heavy metal contents and earthworm parameters. Other environmental factors (soil texture, pH and organic carbon content) are suggested as the determining factors for the occurrence of different earthworm species. One reason of the no-significant correlations between earthworm parameters and soils properties can be related to a single sampling.

Keywords: Soil pollution, agricultural fields, earthworm communities, soil properties.

1. Introduction

Soil health is defined as the continued capacity of soil to sustain its biological productivity, maintain the quality of the surrounding air and water environments, and promote plant, animal, and human health (Doran et al., 1996). Soil health is threatened by various materials derived from human activity, which include industrial pollutants, pesticides, and mine drainage (Thornton, 1983; Alloway, 1990; Yeo and Kim, 1997; Kim et al., 2002). Among these, contamination of soil, especially by heavy metals is one of the most important causes of soil quality decrease, as excess amounts of heavy metals are detrimental to both human health and plants (Järup, 2003; Li and Yang, 2003). Since they are produced by a variety of sources, reach toxic concentrations over wide and usually densely populated areas, cannot be degraded, and accumulate in soil and trophic chains for a long time, these elements pose a serious threat to ecosystems and human health (Caussy et al., 2003). Among heavy metals Pb, Cd, Zn, Pb and Cd in soil receiving extensive attention because of acute and chronic toxicological effects on plants and soil organisms (Cheng and Wang, 2002; Li et al., 2009). If the metals are originating from a point source, their concentrations in polluted soils decrease with the distance from the pollution source. In contaminated soils with heavy metals, the activities, density and species composition of a faunal community containing nematodes, protozoa, and earthworms were reduced (Mhatre and Pankhurst, 1997).

Earthworms are currently considered good environmental indicator since (i) they are well represented in the soil system in terms of abundance (density), (ii) they respond to variety of environmental and ecological factors such as changes in soil chemistry soil disturbances, and (iii) they can be considered as an indicator of soil health and quality, due to their impact on soil (Bouché, 1981; Lavelle and Spain, 2001; Paoletti, 1999; Tondoh et al., 2007; Pérès et al., 2008; Lemtiri et al., 2012). Earthworm descriptors such as density, biomass, species richness and ecological structure (abundance and ecological groups) are indicators of fertilization and pesticides treatments (Cluzeau et al., 2009). A number of studies have shown that species richness and diversity of earthworms decreased along a gradient of metal pollution (Pizl and Josens, 1995; Nahmani and Lavelle, 2002; Lukkari et al., 2004) and most laboratory ecotoxicity tests or field studies have shown negative effects of metals on survival, growth, feeding activity and reproduction of earthworms (Cikutovic et al., 1993). A generally observed pattern is that the earthworm communities lose their diversity with increasing metal pollution. This is due to the fact that abundance of most species decreases, although some species may survive even in the most polluted sites (Vandecasteele et al., 2004).

Various abiotic soil characteristics can be used for evaluating soil health. It is known that texture, humidity, humus content, and soil acidity are important soil properties (Lee, 1985; Edwards and Bohlen, 1996; Römbke et al., 2005; Ivask et al., 2006) and can be used as the basis for the interpretation of earthworm composition and abundance (Tischer, 2005). Metal bioavailability is controlled by various physico-chemical and biological factors such as soil reaction, organic matter and clay mineral particles, oxygen status or the activity of soil living organisms (Ernst, 1996). The large number of these factors and their considerable spatial and temporal variability in field conditions makes it difficult to predict metal ecotoxicity and complicates risk assessment.

Because of their influence in soil food-webs and their sensitivity to environmental disturbances, the composition of the earthworm community has been suggested to be useful indicator in contaminated environments. Therefore, the present study was to examine the impact of heavy metals on earthworm community structure, especially abundance, biomass, taxal and species composition along a pollution gradient in polluted agricultural soils. We hypothesized that measured earthworm parameters are affected by soil heavy metals. Moreover, the soil toxicity is correlated with total and available pollutant levels and other environmental factors. For this, we selected four agricultural sites, offering contrasting soil textures, in order to examine the applicability of the functional trait approach.

2. Materials and Methods

2.1. Sampling sites

The study was carried out in the environs of Sclaigneaux, one of the most polluted regions of Walloon Region in Belgium. This area is delineated within 3 km radius circle surrounding stacks of the former zinc-ore treatment plant. The study was conducted along heavy metal pollution gradients in four agricultural fields adjacent to historically Zn-Pb ore ore exploitation (Liénard et al., 2011). Four plots of about 20 – 25 m^2 in size were selected for the study. During the study period, agricultural soils were densely covered with corn grass (*Zea mays*). The study sites were as follows: Cs4 (most polluted) 810 m from the Zn-Pb ore treatment plant, Cs3 1295 m from the Zn-Pb ore treatment plant, Cs2 2800 m from the Zn-Pb ore treatment plant, Cs1 2910 m from the Zn-Pb ore treatment plant.

2. 2. Soil sampling and analysis

In april 2014, Luvisol soil was collected from the upper 10 cm layer from all locations, mixed and sieved (2 mm); ten soil sub-samples in each plot were taken using an auger. The sub-samples were mixed and homogenised to produce a composite sample for each site. The particle size distribution (sand, silt and clay fractions) was measured with an automated Robinson pipette. pH was measured in 1N KCl after equilibration for 2 h (2: 5 w: v ratio). Total organic carbon (TOC) was determined following the Walkley-Black method (Nelson and Sommers, 1996). Organic carbon was oxidized by adding 1 g of soil to $K_2Cr_2O_7$ and H_2SO_4. Excess $Cr_2O_7^{2-}$ was titrated with ferrous ammonium sulfate [$Fe(NH_4)_2(SO_4)_2*6H_2O$]. Total nitrogen (N) was estimated by modified Kjeldahl method using a Kjeltec 2300 (Nelson et al., 1996). Content of K, Ca, Mg, Cu, Cd, Pb and Zn in soil (termed in this paper as "total") was determined after aqua regia digestion following ISO 11466. Soil samples were digested in a mixture of 15 ml of nitric acid (HNO_3, 65%) and 15 ml of perchloric acid ($HClO_4$, 70%) during 16 h. After complete evaporation, 5 ml of HCl (10%) was added and samples were led in a 25 ml volume with distilled water. Solutions were stored at 4 °C in polyethylene tubes before analysis. Available major (K, Ca and Mg) and trace (Cu, Cd, Pb and Zn) elements were determined after extraction with CH_3COONH_4 (0.5 M) and EDTA (0.02 M) at pH 4.65 (w:v 1:5 ratio) and agitation for 30 min (referred to as available metal concentration) (Lakanen and Erviö, 1971). Element concentrations in the samples were measured by flame atomic absorption spectrometry (VARIAN 220, Agilent Technologies, Santa Clara, CA, USA). The detection limits for Cu, Zn, Pb and Cd were 1.00, 0.33, 3.33 and 0.67 mg l^{-1} respectively.

2. 3. Earthworm extraction method

In april 2013, earthworm populations were sampled from the four agricultural fields, during the maximum activity period (Bouché, 1972; Edwards and Bohlen, 1996). Earthworms were sampled using formaldehyde solution coupled with hand sorting method (Bouché, 1977; Cluzeau et al., 1999) within 1 m x 1 m quadrats. Specifically, three applications of a formaldehyde solution in water (10 L per application, 30 L/quadrat in total, formaldehyde solution concentrations: 0.25%; 0.25%; 0.4%) were administered to the soil surface at 15 min intervals. All earthworms that surfaced were sampled. This step was followed by hand sorting method to allow for data correction, and earthworms were collected (25 cm x 25 cm, 25 cm depth). Four replicates were performed for each treatment. Collected earthworms were placed in plastic bags that contained 4% formaldehyde solution, taken to the laboratory, counted, individually weighed, and identified to the lowest taxonomic level possible (family, genus, species) using the identification keys and descriptions of Bouché (1972). Earthworms are classified in four functional groups: epigeic, epi-anecic, anecic and endogeic eatrthworms. Some species were common and easily distinguished by the naked eye (e.g. *Lumbricus terrestris* and *Allolobophora rosea rosea,* among others). Some juvenile earthworm species were not clearly identified, and classified as unidentified juveniles. Earthworms are considered adult if they are clitellate, and juvenile if tubercula pubertatis or clitellum were absent. Earthworms are very sensitive to soil disturbance, therefore expulsions were not conducted on traffic lines to eliminate any impacts of soil compaction (Pizl, 1993; Sochtig and Larink, 1992).

2.4. Statistical analysis

Prior to other analysis, Shapiro-Wilk's and Bartlett's tests were used to test data normality and homoscedasticity. In most cases, parametric tests were permitted, but in others equivalent non-parametric tests were indicated. The *a priori* significance level for all tests was fixed at $P < 0.05$.

The data were subjected to a repeated measures analysis of variance (ANOVA) to evaluate the effect of "treatment" (contaminated soil) on various ecological indices for earthworm community structure. Metal concentrations effects on functional groups abundances, total earthworm abundance, total earthworm biomass and species richness, were investigated using linear mixed effects model. Earthworm species abundances and total biomass were averaged per plot before statistical analysis. This model was chosen to test the effects of metal concentrations gradient on changes in earthworm abundance, biomass, functional groups, and diversity species. Average values of all sampling points of each plot were used to detect significant differences in soil physicochemical characteristics. Means comparisons were conducted using a post-hoc Tukey test. When assumption of parameters model where not met, a Kruskal-Walis test was used to detect effects of factors. Additionally, multivariate analyses were performed (Principal Component Analysis) using ADE4 (Thioulouse *et al*., 2001); Comparisons of biological, chemical, and physical variables were performed using R software (R Development Core Team, 2008).

3. Results and discussion

The study site differed in humus layer heavy metals concentrations and some soil physicochemical properties (Table 1). The studied Cs2 have lower soil pH value and sand content compared with other contaminated soils.

Assessments of soil fauna on long-term monitoring sites are often restricted to earthworms as the only taxon. Their abundance and biomass are important parameters for soil evaluation (Krück et al., 2006). However, they vary over a wide range (Edwards and Bohlen, 1996; Whalen and Costa, 2003).

In our study, results showed earthworm abundance increased with increasing metal contamination, ranging from 9 to 294 individuals m^{-2} (Fig. 1) and no significant difference was observed (P = 0.21). The biomass of earthworms found was similar at all contaminated sites (Fig. 2); the difference was insignificant (P = 0.27). The litearture on the impact of industrial pollution on earthworms in the field reports decreased density in the vicinity of pollution sources (Bengtsson et al., 1983; Bouché, 1992). The highest value of total earthworm numbers was found in the Cs4, the most contaminated soil. The pollution level of heavy metals is not related to the abundance and biomass of earthworms. Possible explanations for the lack of negative effect of heavy metals on earthworm abundance and biomass are: a low metal bioavailability in the soils, or developement of resistance to metal pollution (Rozen, 2006). Relatively high pH as well as higher clay fractions can limit metal mobilisation, and hence reduce metal toxicity.

Four species of earthworms were recorded, the epi-anecic *Lumbricus terrestris* and the endogeic *Allolobophora chlorotica* being highly predominant (Fig. 3). The abundance of *Allolobophora chlorotica* was higher in the most contaminated soils (Cs3 and Cs4). However, the proportion of earthworms increased with increasing contamination level. Endogeic species were absent in Cs2; this can be due to the pH value (6.15). In soils with low pH, deep-burrowing species are unimportant and are replaced by surface dwellers (epi-anecic and anecic species). Epigeic species are not present in agricultural soils. Our results contradict findings from a works of Spurgeon and Hopkin, 1999 and Lukkari et al. (2004) which demontrated that epigeic species are often the predominat species of metal contaminated soils. The high dominace of species like *Aporrectodea rosea rosea* and *Allolobophora chlorotica* seems to indicate their tolerance towards intensive soil tillage and soil pollution. The endogeic, small, short-living species with a higher reproduction rate can better survive the disturbances than anecic species because they produce more cocoons for reproduction (Paoletti, 2001). The structure of earthworm community is sensitive to particular physico-chemical soil properties (Decaëns et al., 2003). Many authors refer to the influence of pH-value (Whalen and Costa, 2003).

There were no significant correlations between soil properties and earthworm parameters. This could be explained by the presence of other environmental factors, which could mitigate the often shown toxicity of metals for earthworms (Brown et al., 2004; Contreras-Ramos et al., 2006). Hobbeln et al. (2006) did not found direct effects of metal pollution on earthworms, in contaminated floodplain area in the Netherlands, though metal concentrations in soils were very high. Possible explanation for these results are an adaptation of detritivores to metal pollution, or the presence of other more important factors overruling toxicity effects.

Table 1. Total and available metal concentrations (mg kg^{-1}), available major elements, and other chemical parameters of former Zn-Pb ore contaminated soil (Cs), measured in the four contaminated agricultural areas (0 – 10 cm) along a pollution gradient. Cs4 – most polluted site, Cs1 – control site.

Variable	Cs1	Cs2	Cs3	Cs4
Soil physicochemical property				
Sand (%)	1.23	0.36	1.32	0.87
Silt (%)	46.63	46.18	44.41	42.576
Clay (%)	18.88	17.93	17.38	20.90
pH $_{KCl}$	7.61	6.15	7.39	7.08
Total C (%)	1.20	1.27	1.52	1.49
Total N (%)	0.12	0.13	0.15	0.15
Available K (mg 100g^{-1})	23.55	28.06	41.05	30.51
Available Ca (mg 100g^{-1})	345.00	194.52	335.83	338.48
Available Mg (mg 100g^{-1})	20.30	17.88	12.34	10.45
Available Cu (mg kg^{-1})	2.94	3.02	7.49	9.84
Available Cd (mg kg^{-1})	0.61	1.29	7.76	10.93
Available Zn (mg kg^{-1})	14.85	32.01	162.41	699.55
Available Pb (mg kg^{-1})	15.07	24.95	239.10	268.20
Total K (mg 100g^{-1})	525.76	396.21	340.22	413.62
Total Ca (mg 100g^{-1})	558.68	282.26	522.03	515.50
Total Mg (mg 100g^{-1})	416.15	350.13	293.41	398.21
Total Cu (mg kg^{-1})	14.83	14.35	20.98	29.74
Total Cd (mg kg^{-1})	0.93	1.50	9.56	14.37
Total Zn (mg kg^{-1})	104.14	152.81	893.67	1214.41
Total Pb (mg kg^{-1})	29.03	53.02	300.76	389.77

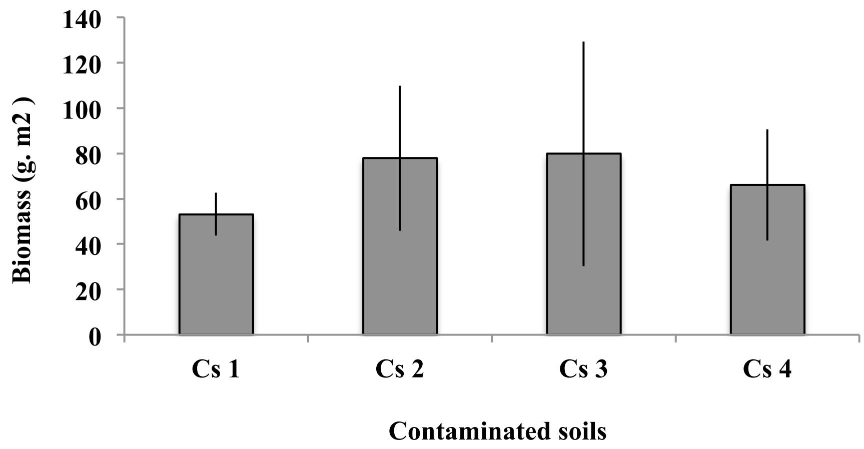

Figure 1. Average value of earthworm biomass in g m^{-2} (±Standard deviation) in different contaminated soils.

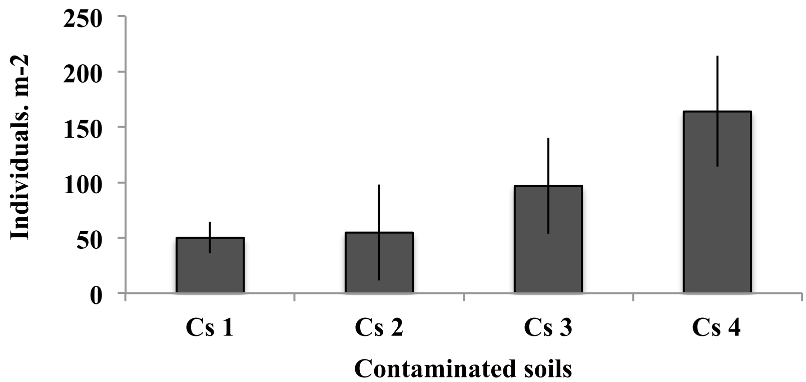

Figure 2. Average value of earthworm in numbers m^{-2} (±Standard deviation) in different contaminated soils.

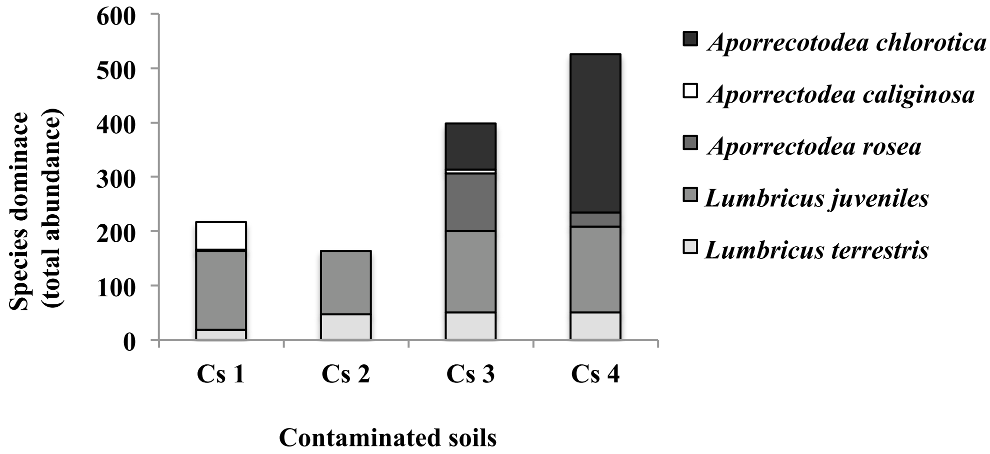

Figure 3. Abundance of individual species of earthworms in contaminated soils with decreasing distance from contamination source.

4. Conclusion

In this study, results showed that heavy metal pollution of soils did not affect earthworm community parameters in terms of abundance, biomass and species diversity, which are more related to above-mentioned soil properties. The soil properties (pH, carbon organic content, soil texture,…) and the type of land use are suggested as the determining factors for the occurrence of different earthworm species. The present work aims at promoting the potentially of a trait-based approach in elucidating invertebrate responses to environmental constraints in complex media such as soils.

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CHAPTER 4

REMEDIATION OF METAL-CONTAMINATED SOIL BY THE COMBINATION OF *VICIA FABA* AND *ZEA MAYS* PLANTS, AND *EISENIA FETIDA* EARTHWORM

Effects of earthworms (*Eisenia fetida*) on the uptake of heavy metals from polluted soils by *Vicia faba* and *Zea mays*

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Abstract

Earthworms increase the availability of heavy metals and aid in maintaining the structure and quality of soil. The introduction of earthworms into metal-contaminated soils has been suggested as an aid for phytoremediation processes. In Wallonia, Belgium, a century of industrial metallurgic activities has led to the substantial pollution of soils by heavy metals, including copper (Cu), zinc (Zn), lead (Pb) and cadmium (Cd), due to atmospheric dusts. Two plant species, *Vicia faba* and *Zea mays*, and earthworm (*E. fetida*) (Savigny, 1826) were exposed to different concentrations of long-term-contaminated soils for 42 days. The soils which were collected from the land surrounding of a former Zn-Pb ore-treatment plant, exhibited different levels of heavy metals. Our aim was to evaluate the role of earthworms *E. fetida* on the availability of metals in soils and their effects on metal uptake by *V. faba* and *Z. mays* plants at different soil concentrations.

The results suggest that earthworms and plants modified the availability of metals in contaminated soils after 42 days of exposure. Earthworm life-cycle parameters were affected by metal contamination and/or the addition of plants; cocoon production and weight were more sensitive to adverse conditions than earthworm survival or weight change. The concentrations of Pb and Cd in earthworms decreased in the presence of plants. Results showed that metal accumulation in plants depended on the metal element considered and the presence of earthworms. In particular, *V. faba* accumulated higher concentrations of Cu and Zn compared with *Z. mays*, which accumulated higher concentrations of Cd. These findings have revealed that earthworm activities can modify the availability of heavy metals for uptake by plants in contaminated soils. Moreover, the study results show that the ecological context of phytoremediation should be broadened by considering earthworm-plant-soil interactions, which influence both the health of the plant and the absorption of heavy metals.

Keywords: Heavy metals-contaminated soils; *Eisenia fetida*; *Vicia faba*; *Zea mays*; Bioavailability; Bioaccumulation; Phytoextraction.

1. Introduction

Heavy metals are continuously being added to soils through anthropogenic activities such as industrialization, mining, smelting, and land application of sewage sludge (Harlavan et al., 2010). In Wallonia (South region of Belgium) during the two last centuries, processing of metal-bearing ore has emitted metal-bearing particulates laden with Cd, Pb and Zn (Graitson, 2005). The fallouts in the vicinity of plants have led to significant contamination of topsoil (Liénard et al. 2014). Among the heavy metals, copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) levels in soil are receiving extensive attention because of their acute and chronic toxicological effects on plants and animals (Cheng and Wong, 2002; Li et al., 2009). When these four elements coexist in the soil, their combined effects are very complex. Mixture toxicity is difficult to study because, metals can interact at various levels (Dickson et al., 1994) such as the exposure level, the uptake level, the target level (Rüdiger and Ralf-Rainer, 2010), and the internal pathway of detoxification (Vijver et al., 2011). Cleaning up soils contaminated with heavy metals using traditional techniques can be destructive to the soil. Remediation of contaminated soils using earthworms and plants appears to be cost-effective and environmentally friendly technology. Most of accumulating plants are characterized by slow growth, low biomass yield and undefined growth requirements. Moreover, they are often incapable of accumulating high concentration of potentially phytotoxic metals, such as Cu, Zn, Pb, and Cd, in their above ground biomass (Komarek et al., 2007; Kumar et al., 1995). Plants vary in response to metals, in mechanism of uptake and of avoiding damage. Metal tolerant plants such as *Thlaspi caerulesens* (Papoyan et al., 2007; Salt et al., 1998) and *Brassica juncea* (Brunet et al., 2009) have been used for phytoextraction of lead from contaminated sites. A plant which can be used for phytoextraction will be able grow rapidly, produce large biomass and be able to accumulate high concentrations of metals. Bioavailability of metals in soil has been reported to be dependent on soil type, metal species, metal kinetics and age of metal contamination in the soil (Beeby, 1993; van Gestel et al., 1995). The bioavailability of heavy metals in soil is determined by their chemical speciation (Babich and Stotzky, 1980) and their interactions with organic matter and mineral soil particles (Li and Li, 2000), which can be influenced by soil organisms. There have been many study concerned with the combined effects of heavy metals on earthwoms and plants (Morgan, 1988; Brokbartold, 2012; Hao Qui et al., 2011; Israr et al., 2011).

Earthworms improve soil structure, contribute to organic matter decomposition and nutrient cycling (Lemtiri et al., 2014) and play a key role in terrestrial ecotoxicological risk assessment (Sheppard et al., 1997; Weeks et al., 2004). They are therefore important terrestrial model organisms and require toxicity testing. The beneficial role of earthworms in soil, influencing a range of chemical, physical and biological processes, is beyond dispute (Scheu, 1987; Edwards and Bohlen, 1996; McCredie and Parker, 1992; Curry and Baker, 1998). Since, they are, in addition, easy to cultivate and handle for experiments, earthworms have become part of standard test organisms in ecotoxicology (Organisation for Economic Co-operation and Development (OECD), 1984, 2004). On the other hand, it is well recognized that the accumulation of heavy metals in earthworms depends strongly on the metal that is bioavailable for uptake rather than the total. Dai et al. (2004); Spurgeon and Hopkin, (1996) and Hobbelen et al. (2006) observed the significant correlations between the concentrations of heavy metal accumulated in earthworms and bioavailable metal concentrations of field soils. Because of their capacity to accumulate and concentrate large quantities of organic and inorganic pollutants, earthworm species are widely recognized as suitable organisms for biomonitoring the effects of heavy metals in contaminated soils (Reddy and Rao, 2008; Peijnenburg and Vijver, 2009). Numerous studies concern the effects of metals on earthworms in terms of mortality (Neuhauser et al., 1985; Fitzpatrick et al., 1996; Spurgeon et al., 1994, 2000), loss of weight (e.g. Khalil et al., 1996; Spurgeon and Hopkin, 1996; Maboeta et al., 2004), cocoon production (e.g. Ma, 1988; Spurgeon and Hopkin, 1996; Spurgon et al., 2000), cocoon viability (e.g. van Gestel et al., 1992; Spurgeon and Hopkin, 1996) and growth (e.g. van Gestel et al., 1991; Khalil et al., 1996). Most of these studies are short-term experiments (14 or 21 days), performed in artificial soils or soil artificially contaminated by the addition of metal in solution (Nahmani et al., 2007). Few concern field-contaminated soils with multiple contaminants (Weltje, 1998; Conder and Lanno, 2000; Feisthauer et al., 2006). Despite a large body of literature on the impact of earthworms on the availability of metals in soils, only a few studies have been carried out into earthworm-assisted metal extraction by plants.

The original aspect of this experimental study consists in the utilization of historically polluted soil from industrial sites in Belgium using a combination of plants and earthworms. In this way, a mixture of metals could be studied. In order to gain better understanding of the metal uptake by plants in contaminated soils, in the presence of earthworms, the aims of the present study are to: (1) investigate the change in metal availability in the soil given the presence of earthworms and plants; (2) evaluate the effects of a metal concentrations on survival, body weight, cocoon production and cocoon weight of earthworms *E. fetida* and (3) assess the effect of earthworms on metal uptake by plants. Earthworm and plant metal bioaccumulation studies were performed to better understand the relationships between bioavailability of metals in soil and their bioaccumulation in organisms.

2. Materials and methods

2.1. Study area and characterization of experimental soils

The study area consists of a 3 km radius circle surrounding the Sclaigneaux calaminary site. The origin of calaminary sites appellation comes from “Calamine” a mining expression to describe zinc-ores such as zinc-silicate, zinc-carbonate or the assemblage of hemimorphite, smithsonite, hydrozincite and willemite (Dejonghe, 1998). This area is known for its historical soil contamination by a former Zn-Pb ore treatment plant (Liénard et al., 2014). The origin of Cu, Cd, Pb and Zn is the same, this is the old atmospheric fallouts of dusts enriched in these four metals. The sampled soils for the experimentation are part of one of the three major soils types present on the study area (Liénard et al., 2011). It is a Cambisols (Siltic) (WRB, 2014) with a gravels load of the old terraces of Meuse river. Three soils are collected on contaminated fields and a control is sampled in an uncontaminated field. Sampling points were located according to distance from contaminant source. Four plots of about 20-25 m2 in size were selected for the study. The study sites were as follows: C3 (most polluted) 1 km from the former Zn-Pb ore treatment plant, C2 2.3 km from the former Zn-Pb ore treatment plant, C1 3.5 km from the former Zn-Pb ore treatment plant. C0 (the control) site has properties comparable to other contaminated soils (C1, C2 and C3). For analysis of the physicochemical characteristics of the soil (including heavy metal concentrations), soil samples of approximately 1 kg each, were taken in April 2014 from four places in each plot and mixed together. Four core samples randomly taken from each plot to a depth of 20 cm were then combined to form a composite sample for each plot. The soil samples were dried under shade conditions for two weeks and then sieved through a 8 mm to remove the gravel load. They were then stored at 4 °C until undergoing physicochemical analysis. The 200 μm fraction was used for the pot experiment. Particle size distribution (clay, silt and sand fractions) was determined by sedimentation using the pipette method (Van Ranst et al., 1999). Soil pH was measured by creating a slurry in distilled water (pHwater) and 1N KCl (pHKCl) (w:v 2:5 ratio). Total organic carbon (TOC) was determined following the Walkley and Black method (Walkey and Black, 1934) and total nitrogen (N) was estimated by modified Kjeldahl method (Nelson and Sommers, 1996). Effective cation exchange capacity (CEC) was determined by using a hexamminecobalt trichloride solution following ISO 23470. Pseudo-total trace element (Cu, Cd, Pb, Zn) concentrations were determined after aqua regia digestion following ISO 11466 (referred to as ARmetal concentration). Available major (Ca, Mg, K, P) and trace (Cu, Cd, Pb, Zn) elements were determined after extraction with CH_3COONH_4 (0.5 M) and EDTA (0.02 M) at pH 4.65 (w:v 1:5 ratio) and agitation for 30 min (referred to as available metal concentration) (Lakanen and Erviö, 1971). The concentration of P in the resulting extract was determined by colorimetry at 430 nm. The concentrations of the other elements in the solution were measured by flame atomic absorption spectrometry (VARIAN 220, Agilent Technologies, Santa Clara, CA, USA). The detection limits for AR/available metals were, respectively, 0.66/0.10 mg kg^{-1} (Cd), 0.99/0.15 mg kg^{-1} (Cu), 3.33/0.50 mg kg^{-1} (Pb) and 0.33/0.05 mg kg^{-1} (Zn). To assess the available (soluble) fraction of polluants in the soil, $CaCl_2$ extraction was used (at both the start and end of the experiment). Extractions with 0.01 M $CaCl_2$ have been widely used for assessing the mobility and bioavailability of heavy metals in soils. As part of the quality control program for the study, a standard reference material was used and analyzed with each set of samples. Selected physicochemical soil characteristics and metal concentrations in the soils are presented in Table 1.

Table 1. AR, available and soluble-$CaCl_2$ metal concentrations (mg kg^{-1}), available major elements, other chemical parameters measured in the four experimental soils and local background values for agricultural soils (Ministère de la Région Wallonne, 2008).

Parameters	C0	C1	C2	C3	Background values
pHwater	8.20	7.45	7.11	8.08	N.V
pHKCl	7.64	6.78	6.61	7.75	N.V
TOC (%)	2.04	1.74	1.67	1.50	N.V
N (%)	0.19	0.16	0.18	0.15	N.V
CEC (meq $100g^{-1}$)	15.2	11.8	10.7	10.5	N.V
Clay (%)	21.0	12.3	13.3	14.6	N.V
Silt (%)	62.5	72.4	59.4	46.1	N.V
Sand (%)	16.5	15.3	27.2	39.3	N.V
AR					
Cd	0.71	2.48	4.27	9.28	0.20
Cu	15.0	11.7	21.7	19.3	14.0
Pb	29.0	69.0	129	255	25
Zn	103	208	384	743	67
Available					
Cd	0.55	1.95	2.89	6.07	N.V
Cu	3.59	13.34	4.89	6.97	N.V
Pb	12.4	41.1	71.2	168.8	N.V
Zn	15.8	61.9	65.6	172.3	N.V
Ca (mg $100g^{-1}$)	479	191	221	554	N.V
Mg (mg $100g^{-1}$)	23.2	22.2	11.2	10.6	N.V
K (mg $100g^{-1}$)	23.4	37.8	20.4	27.7	N.V
P (mg $100g^{-1}$)	13.5	16.1	14.7	16.2	N.V
Soluble					
Cd	0.01	0.05	0.10	0.06	N.V
Cu	0.14	0.12	0.11	0.14	N.V
Pb	0.00	0.00	0.00	0.00	N.V
Zn	0.02	0.37	0.69	0.24	N.V

N.V indicates no value is applicable

2.2. Experimental procedure

The experimental design takes into account four factors: (i) metal concentration represented by four different soils with an increasing range of contamination (C_0, C_1, C_2 and C_3), (ii) plants (*Z. mays*, *V. faba*), (iii) earthworms *E. fetida*, and (iv) food for earthworms (Table 2a). Ten treatments were prepared from the soil samples to create a set of samples with different combinations of (the two) plants, earthworms and food present and each treatment was replicated four times, except for the control sample with food only ($CP_0E_0F_1$) which was replicated once (Table 2b). The experiment was conducted under controlled conditions (i.e. 16 h light and 8 h dark at 20 ±1 °C) (Reneicke and Kriel, 1981) during 42 days. Pots (16 cm diameter x 20 cm high) were prepared with 2.25 kg of drying soil. At the beginning of the experiment, soil moisture was measured and adjusted to 18%, corresponding to 65% of the soil's water holding capacity, then checked regularly and adjusted to the desired value by adding deionized water.

For each treatment with earthworms, 20 specimens *E. fetida* were introduced by being deposited on the surface of the soil in which they can dig. According to the OECD methods (OECD, 2004), earthworms were acclimatized for at least 48 h prior to exposure, in the experiment conditions (temperature, obscurity, soil moisture, etc.). Healthy earthworms weighing between 200 and 600 mg each, and well-developed clitellum were introduced seven days after plant seeding. To ensure the earthworms survival during the experimentation period, they were fed weekly with a dried mixture of horse manure (75%) and oat flakes (25%) to provide 0.5 g per earthworm. The test containers were covered with a perforated lid to limit water loss due to evaporation, to allow aeration and prevent the earthworms from escaping. Earthworms were introduced 7 days after plant seeding.

Z. mays and *V. faba* were the plant species used during this experiment. *V. faba* is not known as a metal tolerant plant. To the contrary, its sensitivity to pollutants justifies its use in ecotoxicity tests (Cordova Rosa et al., 2003; Ünyayar et al., 2006) as it seems to be one of the most metal sensitive (Rahoui et al., 2008). Ten seeds were seeded in pots according to experimental design. Plant growth was held under controlled conditions: 20 ±1°C and 18 ±1°C day and night temperatures respectively, with 60% ± 5 % relative humidity. Germination was determined by visual seedling emergence (Gong et al., 2001). The plants were watered with deionized water. The distribution of the microcosms in the chamber was randomized and changed biweekly.

Table 2. Experimental design for ecotoxicity test: (a) Presentation of different factors and treatments, (b) Description of treatments with differents combinations of factors.

(a)

Factors	Treatments
Concentration	C_0, C_1, C_2, C_3
Plants	No plant (P_0)
	Vicia faba (P_1)
	Zea mays (P_2)
Earthworms	Presence (E_1)
	Absence (E_0)
Food	Presence (F_1)
	Absence (F_0)

(b)

Treatment	Description	n
1	$C_{0,1,2\&3}$ P_0 E_0 F_1	4
2	$C_{0,1,2\&3}$ P_0 E_0 F_0	16
3	$C_{0,1,2\&3}$ P_0 E_1 F_0	16
4	$C_{0,1,2\&3}$ P_0 E_1 F_1	16
5	$C_{0,1,2\&3}$ P_1 E_0 F_0	16
6	$C_{0,1,2\&3}$ P_1 E_0 F_1	16
7	$C_{0,1,2\&3}$ P_1 E_1 F_1	16
8	$C_{0,1,2\&3}$ P_2 E_0 F_0	16
9	$C_{0,1,2\&3}$ P_2 E_0 F_1	16
10	$C_{0,1,2\&3}$ P_2 E_1 F_1	16

2.3 Acquisition of organismal data

After 42 days of exposure, earthworm mortality was checked, and the living adults were hand-collected. Earthworms were classified as dead when they did not respond to gentle mechanical stimulus at the anterior end. The earthworms and cocoons were counted using the procedure described in the OECD method (OECD, 2004). The numbers and weights of cocoons per container were recorded as an individual replicate. After 48 h cleaning out of gut content on moist filter paper, the earthworms were then weighed and checked for any morphological symptoms. The filter paper was changed three times per day to prevent coprophagy. All earthworms in each container were weighed as a group and recorded as a datum. The relative growth rate of earthworms was calculated according to the following equation: Relative growth rate = $(W_t - W_0)/(W_0) \times 100\%$, where W_0 is the initial average weight of earthworms, and W_t is the average weight at checking day.

With regard to the plants, after 42 days of exposure, the shoots were removed by cutting the *V. faba* and *Z. mays* plants close to the soil surface and weighed. The roots were recovered and added to the shoots. They were washed thoroughly in deionized water to remove soil particles. They were then put in paper bags and dried at 40 °C for one week. Similarly, and following depuration during 48 h, the earthworms were placed into an oven at 40 °C over night; the dry earthworms were then weighed.

Samples of dried plant and earthworm tissues were digested in a mixture of 15 ml of nitric acid (HNO_3, 65%) and 15 ml of perchloric acid ($HClO_4$, 70%) during 16 h. After complete evaporation, 5 ml of HCl (10%) was added and the samples were led in a 25 ml volume with distilled water. These solutions were stored at 4 °C in polyethylene tubes before analysis. Metal concentrations in the samples were measured by flame atomic absorption spectrometry (VARIAN 220, Agilent Technologies, Santa Clara, CA, USA). The detection limits for Cu, Zn, Pb and Cd were 0.6, 0.2, 2 and 0.4 mg kg^{-1} respectively.

2.4. Statistical analysis

Data normality and homoscedasticity were verified with Shapiro-Wilk's and Bartlett's tests. In most cases, parametric tests were permitted, but in others equivalent non-parametric tests were indicated.

Firstly, the effects of soils' pollutant concentration ($C_{0,1,2\&3}$), plants (P_0, P_1, P_2), earthworms (E_0,E_1), food (F_0, F_1), time (T_0, T_1) and bloc repartition (random factor) on metal bioavailability were evaluated by a variance analysis ANOVA within a general linear model in Minitab 16 software (Minitab Inc., State College, PA, USA). Secondly, the effects of soils concentration ($C_{0,1,2\&3}$), plants (P_0, P_1, P_2), food (F_0, F_1) and bloc repartition (random factor) on earthworm mortality, earthworm weight, earthworm reproduction (numbers of cocoons and weight of cocoons) and the concentration of metals in earthworms were evaluated. Finally, the effects of soils' pollutant concentration ($C_{0,1,2\&3}$), earthworms (E_0,E_1), food (F_0, F_1) and bloc repartition (random factor) on the uptake of metals by plants (P_1, P_2) were investigated. Differences were considered significant at $P \leq 0.05$.

When the difference was significant, a comparison of means was conducted by a post-hoc Tukey test. If the hypothesis of parameters of that model was not satisfied, a Kruskal-Wallis test was used instead to detect the effects of the various factors.

3. Results

3.1. Effect of earthworm and plant activities on the availability of metals in soil

Soil samples taken from the sample treatments (control, soil with earthworms, soil with plants, and soil with earthworms and plants combination) were analyzed to compare pHwater and the availability of metal fraction after extraction with 0.01 M$CaCl_2$ (McGrath and Cegarra, 1992). Table 3 gives a pH values and a calculated percentage of contaminants remaining (negative value) and losing (positive value) in the soil after 42 days of exposure, in the presence or absence of organisms (plants or earthworms), according to the equation: relative contaminant in soil = $(V_0 - V_F)/(V_0) \times 100\%$, where V_0 is the initial fraction of metal in the soil before experiment, and V_F is the fraction of metal at the end of experiment. This could help investigate the mechanisms of how earthworms, plants and their combination could affect the availability of heavy metals in soils.

The soil pH values varied from 6.3 to 7.2. No statistically difference was observed among the six treatments ($P > 0.05$). The introduction of earthworms, or plants, or the comibination between earthworms and plants did not affect the soil pH values.

On the other hand, the introduction of *V. faba* decreased the availability of Cd in soil ($P = 0.02$), but no significant differences on the availability of metals were observed for the other treatments.

Table 3. pH_{water} and bioavailable fractions of metals in soil (%) of six experimental treatments, with Tukey indices for pH_{water}. Different letters indicate a significant difference according to Tukey's test at $P \leq 0.05$.

Soil parameters	Soil	Treatments					
		$P_0 E_0 F_0$	$P_0 E_1 F_1$	$P_1 E_0 F_0$	$P_2 E_0 F_0$	$P_1 E_1 F_1$	$P_2 E_1 F_1$
pH	C0	7.2 ± 0.17	7.2 ± 0.16	7.2 ± 0.18	7.1 ± 0.18	7.1 ± 0.14	7.1 ± 0.21
	C1	6.4 ± 0.23	6.3 ± 0.19	6.4 ± 0.12	6.4 ± 0.11	4.9 ± 0.15	6.6 ± 0.04
	C2	6.4 ± 0.05	6.4 ± 0.16	6.3 ± 0.18	6.4 ± 0.12	6.4 ± 0.11	6.6 ± 0.19
	C3	7.1 ± 0.13	7.1 ± 0.13	7.1 ± 0.18	7.1 ± 0.15	7.0 ± 0.15	7.0 ± 0.15
Cd	C0	-65.8	-88.0	0.9	0.9	-32.4	0.9
	C1	-66.0	-66.0	-52.5	-42.9	-60.2	-144.8
	C2	-32.0 a	-63.0 ab	-61.0 b	-42.0 ab	-55.0 ab	-49.0 ab
	C3	-60.4	-54.0	-73.1	-41.3	-88.9	-88.9
Cu	C0	6.5	14.2	0.1	-14.0	14.2	-91.4
	C1	-2.2	-2.2	3.0	11.6	11.6	-3.9
	C2	-4.5	10.6	-5.4	10.6	-67.7	-210.2
	C3	14.2	14.2	14.2	14.2	14.2	-49.2
Pb	C0	0.0	0.0	0.0	0.0	0.0	0.0
	C1	0.0	0.0	0.0	0.0	0.0	0.0
	C2	0.0	0.0	0.0	0.0	0.0	0.0
	C3	0.0	0.0	0.0	0.0	0.0	0.0
Zn	C0	0.0	0.0	0.0	0.0	0.0	0.0
	C1	23.8	-88.6	-81.7	-66.9	-49.9	-45.7
	C2	-14.3	-38.7	-84.6	-24.2	-21.7	-25.5
	C3	-138.2	-67.9	-132.8	-87.4	-141.1	-211.3

3.2. Earthworm traits

3.2.1. Earthworm mortality

An analysis of variance was performed on the results of earthworm life-cycle parameters in order to evaluate whether there was a change in mortality after 42 days of exposure. Throughout the experimental period, no earthworms died in the control soils (C0). Thus, this observation suggests that the experimental conditions were valid in terms of providing suitable media for earthworms survival.

After 42 days of exposure, no significant differences in mortality rates were found between the contaminated soils. In addition, regardless of the concentration of polluted soil in any sample, earthworm mortality was not affected by the addition of plants *V. faba* or *Z. mays*. Only the "food" factor significantly affected the mortality of *E. fetida* ($P = 0.036$).

3.2.2. Earthworm body weight

Twenty worms were weighted at the beginning of the experiment and the remaining number of earthworms after 42 days was > 17 for all samples. The questions investigated were: was there a general change in earthworm weight or did the change vary according to worm feeding, soil contamination or the presence of a plants?

Significant difference in body weight was found between earthworms in pots with food and earthworms in pots without food (Table 4) so we performed a separate analysis for pots with and without a food supply. In the pots without food, no plants were cropped. No significant interaction was found with time, while the factors "Soil" and "Time" were significant ($P = 0.021$) and highly significant ($P = 0.0001$), respectively. Regarding the effect of soil contamination, it seems that the differences were linked to the initial weight of the earthworms. The change in their body weight over time can be considered as equivalent for all the soil types, that is a mean decrease of 195 mg per earthworm after 6 weeks.

For pots which received food, significant interactions were found between "Plant and Time", "Bloc and Time" and "Plant and Soil". We found that the mean weight of earthworms at the beginning of the experiment was higher (40 mg) in Bloc 2 than in the other three. Separate analyses of variance were performed for the treatments without plants, with *Z. mays* or with *V. faba*. The first point to be noted is that there was an increase of worm weight for every pot without a plant, and a smaller increase for soil C0, C2 and C3 with *V. faba*. Regarding *Z. mays*, only the soil C1 showed an increase of the mean mass of the worms. We can conclude that the action of giving food to worms had a significant impact when no plant was cultivated, while the presence of a plant did facilitate the gain of mass.

Table 4. Mean weight of earthworms *E. fetida* earthworms at the beginning (T_0) and after 42 days (T_1) of the experiment with different combinations: in the presence (F_1) and absence (F_0) of food. (- : no value, ↘: decrease between T_0 and T_1, ↗ : increase between T_0 and T_1, ↗↗: high increase between T_0 and T_1). Different letters indicate a significant difference according to Tukey's-test at $P \leq 0.05$.

	F_0			F_1		
	T_0	T_1		T_0	T_1	
C_0 P_0	22.10 ± 4.65 ab	13.10 ± 3.20 ab	↘	21.85 ± 4.20 ab	25.45 ± 5.15 bc	↗↗
C_0 P_1				22.05 ± 4.30 ab	23.40 ± 4.95 ab	↗
C_0 P_2				21.55 ± 3.95 ab	20.60 ± 4.45 a	
C_1 P_0	21.60 ± 4.10 ab	12.45 ± 2.95 ab	↘	22.75 ± 4.45 ab	24.70 ± 6.35 bc	↗
C_1 P_1				21.95 ± 4.35 ab	22.00 ± 5.15 ab	
C_1 P_2				22.45 ± 4.50 ab	24.75 ± 5.40 bc	↗
C_2 P_0	21.35 ± 4.25 a	11.35 ± 3.00 a	↘	21.45 ± 4.15 ab	25.80 ± 6.45 bc	↗↗
C_2 P_1				21.50 ± 4.20 ab	22.45 ± 4.35 ab	↗
C_2 P_2				22.95 ± 4.15 ab	22.60 ± 5.95 ab	
C_3 P_0	23.30 ± 4.00 b	12.65 ± 3.05 b	↘	21.05 ± 3.85 a	27.15 ± 4.90 c	↗↗
C_3 P_1				23.10 ± 4.40 b	24.30 ± 5.10 bc	↗
C_3 P_2				22.75 ± 4.00 ab	21.95 ± 5.30 ab	

3.2.3. Earthworm reproduction

Cocoon production was calculated per surviving earthworm. Table 5 shows the effects of metals and the addition of plants on reproductive parameters of *E. fetida*, including cocoon production per earthworms and weight per cocoon in soils after 42 days of exposure. A significant ($P < 0.001$) reduction in cocoon production and cocoon weight was seen for the control and contaminated soils, when food was unavailable. With the addition of food, earthworms produced more cocoons (6.65 per worm) and with a greater mean weight (19 mg per cocoon) than in the absence of food (1.75 per worm; 12 mg per cocoon). However, no significant difference in earthworm reproduction was observed between earthworms kept in control soils and contaminated soils.

The presence of plants also enhanced the reproduction of *E. fetida*. Cocoon production in the treatments with plants was significantly higher than in the treatments without plants, suggesting that the reproduction response was influenced by the presence of plants in the soil. The presence of *V. faba* or *Z. mays* significantly increased the reproduction activity of *E. fetida* in control and contaminated soils. This suggests that reproduction and cocoon weight were sensitive to environmental changes such as the introduction of plants.

Table 5. Total number of cocoons, cocoon production rates (number of cocoons / surviving adult earthworms / month), and mean weight of cocoons collected after 42 days and produced by *E. fetida* exposed to soils collected from the three metal-contaminated sites and the uncontaminated control.

Treatments	Cocoons number	Cocoons production rate (Nb/Adult/Month)	Mean cocoon weight (mg)
C_0 P_0 E_1 F_0	151	1.35 ± 0.45	11.56 ± 1.84
C_1 P_0 E_1 F_0	187	1.67 ± 0.43	10.43 ± 2.45
C_2 P_0 E_1 F_0	97	0.87 ± 0.32	12.48 ± 3.38
C_3 P_0 E_1 F_0	123	1.10 ± 1.32	13.43 ± 3.00
C_0 P_0 E_1 F_1	451	4.03 ± 1.14	17.81 ± 2.78
C_1 P_0 E_1 F_1	436	3.89 ± 0.81	18.95 ± 1.92
C_2 P_0 E_1 F_1	457	4.08 ± 2.23	20.16 ± 1.53
C_3 P_0 E_1 F_1	536	4.78 ± 0.33	18.68 ± 1.91
C_0 P_1 E_1 F_1	539	4.81 ± 1.13	15.28 ± 2.70
C_1 P_1 E_1 F_1	560	5.00 ± 0.38	14.80 ± 1.11
C_2 P_1 E_1 F_1	571	5.10 ± 1.00	17.25 ± 4.55
C_3 P_1 E_1 F_1	608	5.43 ± 0.97	17.87 ± 4.25
C_0 P_2 E_1 F_1	500	4.46 ± 1.14	16.95 ± 3.95
C_1 P_2 E_1 F_1	547	4.88 ± 0.76	18.88 ± 2.88
C_2 P_2 E_1 F_1	505	4.51 ± 1.25	16.29 ± 1.33
C_3 P_2 E_1 F_1	594	5.30 ± 0.96	15.77 ± 1.27

3.3. Earthworm metal concentrations

The mean concentrations of Cu, Zn, Pb and Cd (expressed as mg kg^{-1} dry weight) in *E. fetida* after 42 days of exposure are reported in Figure 1. Differences were observed between the levels of elements found in the tissues of *E. fetida* for all the contaminated soils. The internal body Pb concentration clearly increased with exposure to raised soil Pb concentration ($P < 0.001$).

The addition of plants also affected earthworm metal concentrations. Internal concentrations of Pb and Cd decreased in *E. fetid*a incubated with *V. faba* or *Z. mays* for C2 and C3 samples. Internal concentration of Zn in *E. fetida* was higher in earthworms inoculated with plants.

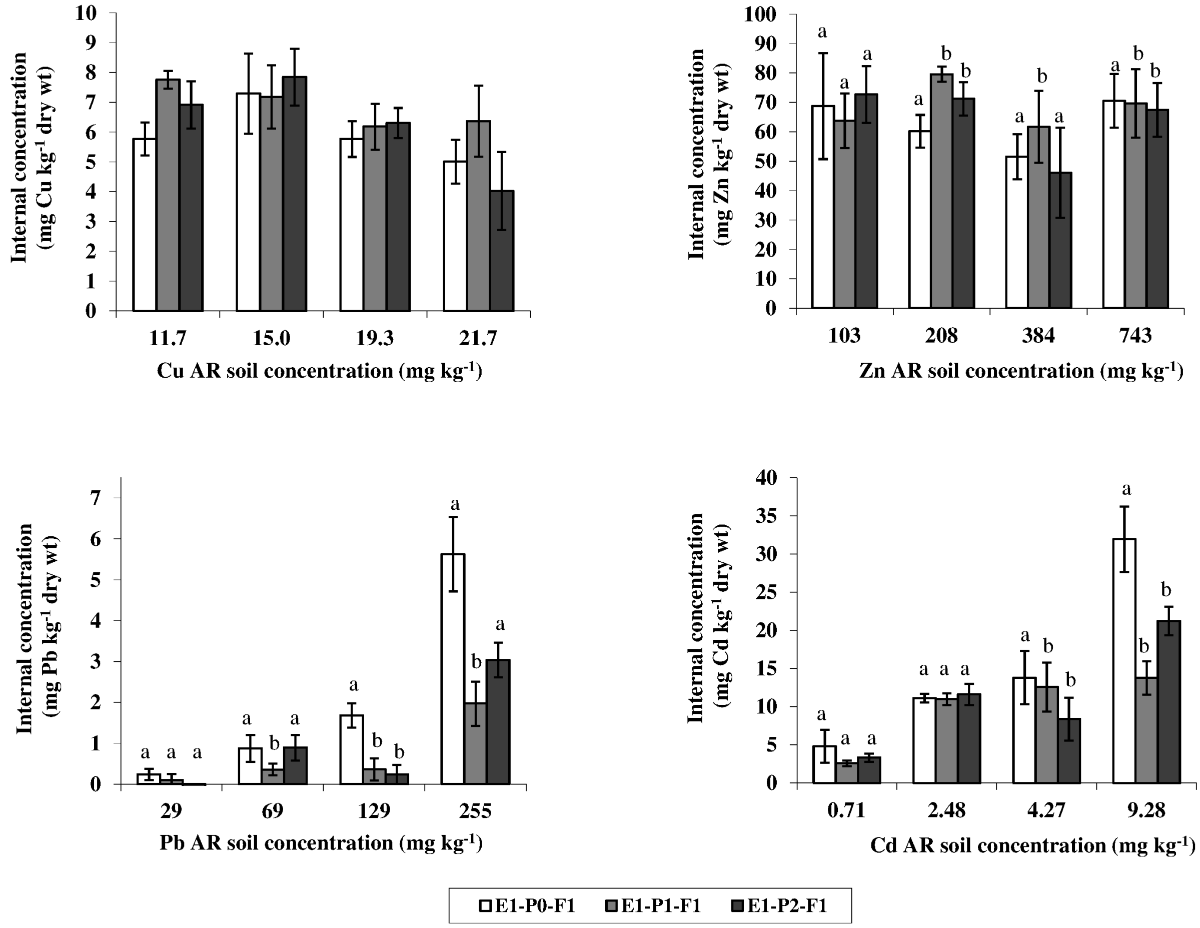

Fig. 1. Mean metal concentrations in *E. fetida* specimens after being exposed for 42 days to control and contaminated soils, in the presence/absence of *V. faba* (P1) and *Z. mays* (P2) plants. Bars represent standard error. Different letters indicate a significant difference according to Tukey's-test at $P \leq 0.05$.

3.4. Plant metal concentrations

The mean of the concentrations of heavy metals within *V. faba* and *Z. mays* plants, with and without the presence of *E. fetida*, are given in Figure 2. In contaminated soils, metal concentrations were significantly different between the two plants. Cu and Zn levels were lower in *Z. mays* compared with *V. faba*, whilst Cd concentration was higher in *Z. mays* in comparison with *V. faba*. The addition of earthworms to soils contaminated by Cu, Zn and Pb metals did not affect metal concentrations in plants; only for Cd did we observed an increased level in *Z. mays*. No significant differences were observed for the Pb metal concentration of *Z. mays* and *V. faba* as compared to the control. In *Z. mays* plants, as soil Cu, Zn and Cd levels increased, the Cu, Zn and Cd metal concentrations in plants also increased, while for *V. faba*, the plants' metal concentrations remained unchanged.

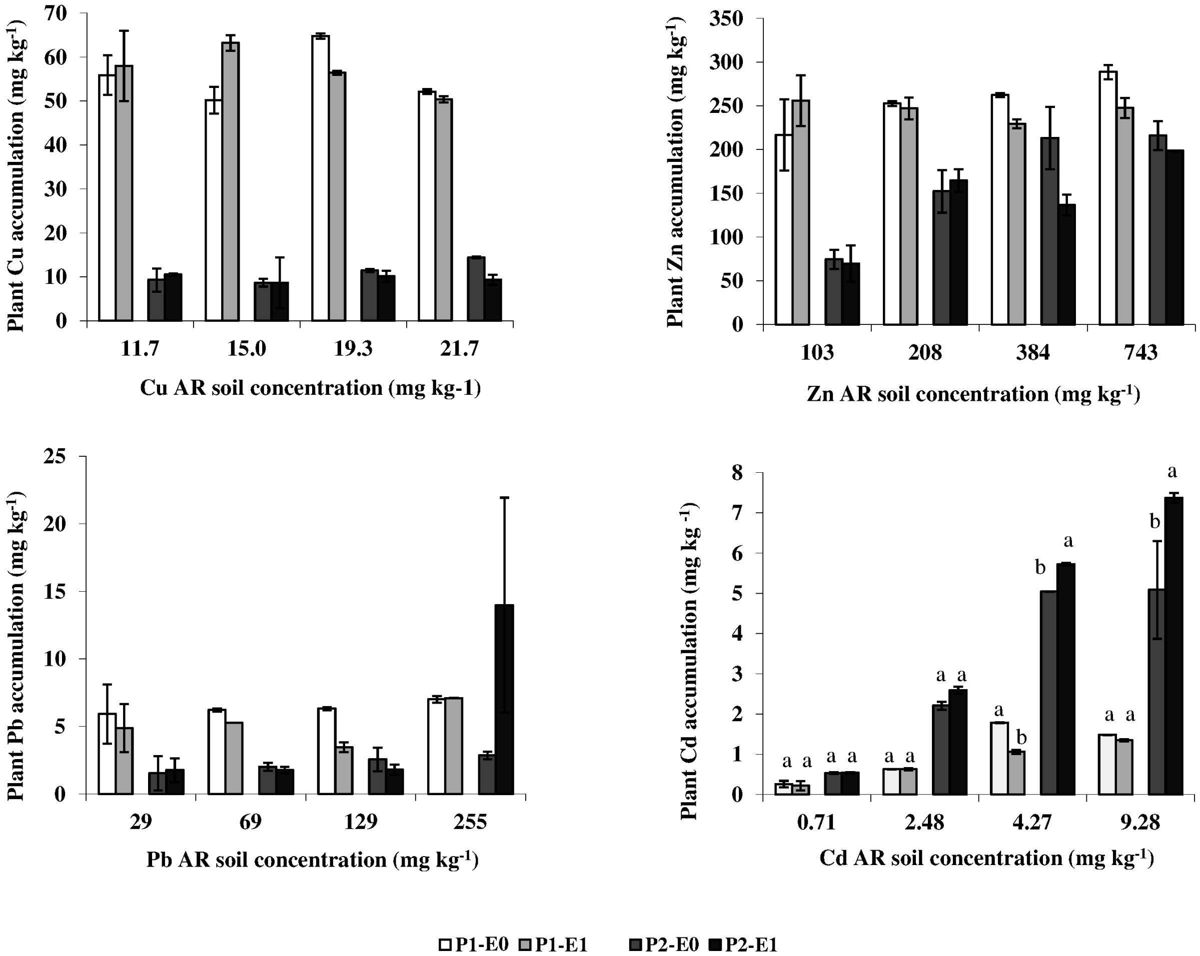

Fig. 2. Effects of *E. fetida* on the metal concentration in *V. faba* and *Z. mays* after 42 days of exposure. Bars represent standard errors. Different letters indicate a significant difference according to Tukey's-test at $P \leq 0.05$.

4. Discussion

4.1. Effects of earthworms and plants on the pH and the availability of metals in soil

It is weel established that soil pH is a key factor affecting the adsorption-desorption behaviors and hence bioavailability of heavy metals in soil. Therefore, it is important to determine the pH change due to plant and earthworm activities. In this study, it was found that the presence or absence of organisms (earthworms and plants) did not changed the pH values. The impact of earthworms on soil pH and metal availability is often variable, partly due to differences in earthworm species and soil conditions. The effect of earthworms on metal bioavailability in soils has not been extensively documented (Sizmur and Hodson, 2009). Our results are inconsistent with most previous studies, which have shown that, in contaminated soils, earthworm activities increase soil pH (Wen et al., 2006; Udovic and Lestan, 2007). The mechanisms by which *E. fetida* change pH value are still unclear (Sizmur and Hodson, 2009).

When the soil is contaminated with a mixture of metals, their combined effects have proven to be very complex. Despite the low concentration values found, some trends can be pointed out. After 42 days of exposure, following the addition of *E. fetida*, it can be seen that Cd and Zn metal fractions were less available than other metals. The significant decrease in the available fraction of Cd in soils suggested that *E. fetida* can either accumulate those metals in their tissues or that earthworms are capable of changing the chemical form and availability of those metal fractions in soils. The significant difference was observed for the C2 concentration, which represent the highest $CaCl_2$ Cd metal soil concentration. These findings are inconsistent with previous studies which suggest an increase in the metal availability fractions as a result of earthworm activities (Wen et al., 2004; Coeurdassier et al., 2007; Udovic and Lestan, 2007). Lukkari et al. (2006) reported that the residual Zn fractions increased in the presence of earthworms, concluding that earthworms tended to decrease the mobility of Zn in the soil. In some studies, the addition of earthworms has been shown to result in no clear effect on metal fractionation patterns with decreases or no change in metals bound to either organic matter or carbonates (Wen et al., 2004; Ruiz et al., 2011). The lack of observed differences in the availability of other metals (Cu, Pb, and Zn) between the control and the earthworm-inhabited soils may indicate that although passage through the earthworm gut has an impact on metal mobility, this is only a temporary effect (Lukkari et al., 2006). These conflicting results could be mainly due to differences in (i) soil characteristics, (ii) pollution mode, (iii) metal type, and (iv) even physiological and ecological differences among earthworm species. Our findings showed a significant correlation between extractable Zn and pH level. Changes in pH may affect the availability of Zn. This was a significantly negative correlation between the value of extractable Zn content and soil pH which is shown by the following multiple regression equation: $[Zn]_{av} = 3.76 + 0.000881\ [Zn]_T + 0.506\ OM - 0.678\ pH$, where $[Zn]_{av}$ is the available soil concentration and $[Zn]_T$ is the total soil concentration of zinc. The significance was lower than 0.001. Obviously, the values of $CaCl_2$-extractable Zn decreased with the increasing soil pH. Similar results were obtained by Mitsios et al. (2005), who found significant negative correlation between soil pH and extractable Zn. However, the significantly negative correlation of $CaCl_2$-extractable Zn content and soil pH was observed in this study, suggests a hypothesis that apart from soil pH, other soil parameters, at least organic matter content and link with other metals should be included to investigate the influence of soil properties on the availability of Zn fraction in soil.

The pH level, organic matter content and bioavailability of heavy metals are critical factors for the heavy metal accumulation by both plants and animals (Spurgeon and Hopkin, 1996; Oste et al., 2001). The presence of *V. faba* increased the availability of Cd and Zn metal fractions. Plants roots play a vital role in altering metal speciation in soils (Hashimoto et al., 2010). Rhizosphere processes may also modify the speciation and availability of metal. Another possibility for enhancing metal availability is the use of soil microorganisms and plant root-associated bacteria, which are stimulated by root exudates including a wide range of organic molecules (Kamnev and vander Lelie, 2000; vander Lelie, 1998).

4.2. Earthworm traits

4.2.1. Earthworm adult survival and growth

The high percentage survival rate observed for the earthworms in the metal contaminated soils after 42 days of exposure and the absence of visible damage on the plants, may be explained by the low range of metal contamination. Lethality effects were only observed in the soil without food. The absence of mortality could be explained by the different experimental conditions; experiment duration, and/or lower metal concentrations. Because of long-term chemical processes, metal bioavailability in field soils decreases over time (Lock and Janssen, 2003a,b). The stability and lack of mortality for biological organisms observed here were previously reported by Schreck et al. (2011) working with the same long term polluted soil.

The body weight of *E. fetida* was affected by food. In the treatments without food, the loss of body weight is most likely due to the fact that organic carbon available in these soils was insufficient for the earthworms to maintain their initial body weight. The results showed that metal mixtures did not affect earthworm body weight. This could be explained by the relatively lowest concentrations of metals or by their competition with other essential elements in the soils. These effects suggest that soil characteristics can decrease the bioavailability of metals and then modify their toxicity. As reported by Ernst et al. (2008), soil properties can modify metal bioavailability and their subsequent impacts on earthworm physiology and behavior. Some reports suggest that in some cases, organisms primarily respond to certain attributes rather than to the metal concentration (Chang et al., 1997). Soil pH and organic carbon have been claimed to be important factors for affecting metal bioavailability to ecological receptors (Spurgeon and Hopkin, 1996; Peijnenburg and Jager, 2003; Basta et al., 2005; Dayton et al., 2006). In our study, soil pH remained in the neutral to slightly alkaline range. Many other studies have reported the metal's toxicity to earthworms, tested both in artificial and field soil (Neuhauser et al., 1985; Spurgeon et al., 1994; Spurgeon and Hopkin 1995). The LC_{50} values of Cu, Zn, and Pb reported by Neuhauser et al. (1985) were 643 (549–753) mg kg^{-1}, 662 (574–674) mg kg^{-1}, and 5.941 (5.292–6.670) mg kg^{-1} in artificial soil, respectively. The Zn content in the our C3 contaminated soil (743.3 mg kg^{-1}) exceeded the LC_{50} value of Zn, quoted above but no mortality was observed in our study. This confirms that the toxic effects of metals are less severe in field soils (Spurgeon and Hopkin, 1995). It should be noted that simultaneous exposure to several metals can also lead to antagonistic, not necessarily to additive or synergistic effects, as observed by Khalil et al. (1996).

The addition of *V. faba* and *Z. mays* did not affect the earthworm weight after 42 days in all concentrations. We can suppose that metallic particles adhered quickly on the root surface and penetrated into plant tissues, which explains the inhibited development seen after 28 days for both plants which in turn would make metals less available (data not shown). A consequence of the presence of earthworms and plants in the same pots could change resource allocation and create a competition between the plants and the earthworms.

Nevertheless, earthworm weight did not respond in the same way following the addition of plants. Earthworm body weight was more sensitive with *Z. mays* than *V. faba*. The physiology of plants in contaminated soils with earthworms explains this result. The weight of earthworms increased in treatment C3 with *V. faba* and decreased with *Z. mays*; it can be assumed that *V. faba* participates with a nutrient stimulation mechanism which results in making nutrients more available for earthworms. This mechanism is not present with *Z. mays*, as earthworm weight decreased with metal concentration increases in the presence of *Z. mays*.

4.2.2. Cocoon production and cocoon weight

Several studies indicate that cocoon production is one of the most sensitive biological responses in toxicity tests (Ma, 1984; Spurgeon et al., 1994; Spurgeon and Hopkin, 1996; Spurgeon and Hopkin, 1999; Kula and Larink, 1997; Reinecke et al., 2001; Homa et al., 2003), with a strong decrease in cocoon production with increasing metal concentrations. In our study, only negative effects on cocoon weight, not production, were observed. The introduction of plants (*V. faba* or *Z. mays*) did not affect the cocoon production. This suggests that an important amount of energy has been allocated to the production of earthworm cocoons and the rest was allocated to cocoon development. Indeed, it is the availability of sufficient food that is of prime importance for maintaining a high cocoon production and weight (Reinecke and Viljoen, 1990) and this could explain the decrease in cocoon weight we observed. Spurgeon and Hopkin (1996) observed that *E. fetida* produced cocoons that increased in weight with increasing soil levels of a metal mixture, mainly Cu, Zn, Pb and Cd. In our study, the cocoon weight was independent of the metal mixture concentrations. Our findings disagree with several authors (Spurgeon et al., 2000; Ávila et al., 2009) who found negative effects on earthworm reproduction in metal contaminated soils. The direct relationship between *E. fetida* reproduction and soil metal concentration must be discussed with caution since earthworm reproduction depends on many different soil factors. In particular soil organic matter content plays an important role in mitigating the effects of metals (Ávila et al., 2009; van Gestel et al., 2011), while earthworm reproduction may also be reduced at high soil pH (van Gestel et al., 1992).

In spite of the absence of effects on survival *E. fetida*, cocoon production and cocoon weight parameters were differently affected by soil metal contamination and by the presence of plants. In fact, *E. fetida* cocoon production increased slightly in the presence of *V. faba* or *Z. mays*, even where lower cocoon weights were detected, particularly with *V. faba*. This suggests that plants such as *V. faba* and *Z. mays* may play an important role in *E. fetida* reproduction. The opposite response of the two endpoints for *E. fetida* suggests a trade-off of energy between cocoon production and cocoon weight. This pattern is very difficult to explain: it is possible that *E. fetida* living in a stressed environment is "forced to choose" on which process to spend its energy.

4.3. Metal accumulation in earthworms

Earthworms are considered to have two uptake pathways for heavy metal: dermal and intestinal. According to Lanno et al. (2004), earthworms can take up metals from soil either through direct dermal contact with them in soil solution or by ingestion of bulk soil or specific soil fractions. Distribution of metals among soil fractions is also considered to be important for their toxicity and bioavailability to earthworms (Becquer et al., 2005). The results we obtained showed that Zn and Cu metals were accumulated in *E. fetida* tissues without exceeding 80 mg kg^{-1} and 8 mg kg^{-1}, respectively. The ability to accumulate Zn and Cu metals could be explained by metal earthworm regulation. Despite the fact that Zn and Cu metal concentrations were accumulated in *E. fetida*, any lethality effects were observed. Numerous authors have suggested that *E. fetida* is able to regulate Zn by binding Zn in their chloragogenous tissue (Morgan, 1981; Morgan and Morris, 1982; Morgan and Winters, 1982; Cotter-Howells et al., 2005). Since the binding of Zn to metallothioneins is reversible, it is likely that Zn-thioneins may regulate the concentrations of metal in the body tissue by allowing rapid elimination of Zn (van Gestel et al., 1993; Marinussen et al., 1997). Indeed, Zn is an essential element and its internal level is regulated by earthworms; the efficiency of Zn accumulation probably relates to a necessity for a stored pool of available Zn in anticipation of future physiological demand (Nannoni et al., 2011). Our findings showed that Cu metal concentrations were relatively low. This essential metal may also be regulated by earthworms. In addition, in the present experiment, the internal body metal concentration was measured following 48 h of gut depuration, which may have resulted in a loss of metals from the tissues of the earthworms (Spurgeon and Hopkin, 1999).

The accumulation of Cd and Pb metals by *E. fetida* increased with the increasing levels of metals in the soil. Cd was highly accumulated by *E. fetida*. This phenomenon could be explained by its high mobility, availability in soil and its chemical analogy with Zn (Li and Thornton, 2001; Nannoni et al., 2011). However, less Pb uptake and accumulation by *E. fetida* was observed. This suggests that feeding behavior and ecological category of *E. fetida* are factors which determine the metal accumulation, although other factors may also contribute. Lukkari and Haimi (2005) showed that *E. fetida* (epigeic) appeared to be more tolerant to metals than *A. tuberculata* (endogeic) and seemed to regulate the tissue metal concentrations more strictly.

Metal toxicity involves three steps: bioavailable metal causes exposure, exposure leads to uptake, and effect results through reaction with a biological target. In *E. fetida*, the Pb and Cd accumulation increased in soil with greater soil metal concentrations. By contrast, Zn and Cu metals have a steady level of accumulation. It is likely that Cd and Pb elements do not utilise the same uptake pathways as do Zn and Cu of uptake. Li et al. (2010) provided evidence that the uptake of Cd by *E. fetida* proceeds through calcium channels, whereas Zn uptake is carrier-mediated by proteins or other sulfhydryl-containing compounds, implying that the mechanisms of Cd and Zn uptake in *E. fetida* are essentially different. Therfore, Cu, Zn, Pb and Cd may employ different mechanisms in influencing the uptake of each other. Weltje et al. (1998) compiled data on sublethal toxicity and tissue concentrations of Cu, Zn, Pb and Cd mixtures in earthworms. Mixture toxicity shifted from mainly antagonism towards nearly concentration-addition when the endpoints were based on extractable metal concentrations instead of total concentrations.

In our experiment, when plants were inoculated with earthworms, Cd and Pb metal accumulation in *E. fetida* decreased. The presence of *Z. mays* and *V. faba* can prevent and reduce the accumulation of Cd and Pb metals in earthworm tissues. Probably, plants can accumulate part of the Cd and Pb, which may create competition between the two organisms. Pb and Cd uptake by *E. fetida* was higher in the presence of *Z. mays* compared with *V. faba* for the treatment with the highest metal concentration (C3). The effect of the two plants on earthworm metal uptake was different. Actually, by producing exudates, plants can modify metal speciation and their behavior in soil, especially in the rhizosphere (Chaignon and Hinsinger, 2003; Uzu et al., 2009) and hence, as results show, plants can modify metal accumulation in earthworms.

4.4. Role of E. fetida on metal accumulation in Z. mays and V. faba

This part of the study investigated the effect of *E. fetida* on the ability of *V. faba* and *Z. mays* to extract metals and phytoremediate a metal contaminated soils. Relative metal concentrations in *V. faba* and *Z. mays* plants grown in control decreased in the order Zn > Cu > Pb > Cd. This suggests that, in natural conditions, these plants have a similar capability to assimilate capability for these elements. In the plants cultivated in the contaminated soil, however, the order of heavy metal concentrations was different. This probably occurs because some metals were present at high concentrations in the contaminated soils and, hence, they were preferentially assimilated by the plants. Plants accumulated elements to varying degrees depending on the soils and metals. *V. faba* plants accumulated higher metal concentrations of Cu and Zn than *Z. mays* which accumulated higher concentrations of Cd. These results suggests that the potential of *Z. mays* for phytoextraction is higher for Cd, and that for *V. faba* it is higher for Cu. *Z. mays* plants appeared to be a good candidate for phytoextraction. Previous reports have classified *Z. mays* as a root accumulator (Li et al., 2009; Mench and Martin, 1991). Results did suggest that maize plants tend to retain excess of Cd. The difference between the two plants could be explained by the absorption mechanisms of plants. Looking at plant accumulation of elemnts, Pignattelli et al. (2012) also found differences in the influence of the available and total concentrations between arsenic and other elements (e.g., Zn and Cd), which was attributed to the difference in the uptake mechanisms of arsenate (phosphate transporters) and metals. We can hypothesize that uptake is not only controlled by the element distribution in the soil but also by the exposure pathways. The accumulation of Cu in *V. faba* and *Z. mays* follow the same trend whatever the metal concentrations in soil. These findings may be explained because Cu is an essential micronutrient for plant nutrition and deficiency effects could be mistaken for toxic responses. Levels between 5 and 20 mg kg^{-1} plant are considered adequate for normal growth, whereas levels higher than 20 mg kg^{-1} are considered toxic (Adriano, 2001).

The analysis of Pb metal concentrations did not reveal differences between the two plants. The extent of assimilation of heavy metals from soil depends on whether they are present in a form that can be absorbed by plants. In our study, Pb could be strongly absorbed by soil particles and, thus, it is scarcely translocated to plants, while Cd elements are relatively mobile in soil and can be more easily absorbed by *Z. mays.* The level of transfer of Pb metal to the plants was lower than the uptake of other metals. This could be due to the absorption of a higher quantity of Pb by the clay-humic complex in the soil (Smical et al., 2008). *Z. mays* can accumulate Cd and Zn elements from soil through different mechanisms, such as: absorption, ion exchange, redox reactions and precipitation-dissolution. Only the portions of elements which present availability are transferred into plants (Smical et al., 2008) or to the low affinity of plants for this element which have no role in their metabolism. Also, it may be suggested that under laboratory conditions the uptake of Pb in plants is mainly determined by crop physiological traits. However, apart from physiological functions of plants, soil physico-chemical properties also have a great contribution to the metal uptake of plants (Adriano, 1986).

The data showed a complex interaction between the elements present in the soil concentration and the plants' accumulation of them, which depended partly on the species of plant and mainly on the metal involved. Thus, the total soil concentration alone cannot explain the accumulation of any of the elements observed in the plants. When the plant tissue concentration data were compared with the available concentration, no correlation was observed for *V. faba* and *Z. mays*. The accumulation of Cd was regulated and was best described by the total concentration; this finding was obtained for both plant species. Increases in the plant tissue concentrations were observed with the highest total Cd and Zn soil concentration, particularly for *Z. mays*.

The main soil factors controlling metal solubility and bioavailability in soils are the total metal concentration, pH level, soil absorption capacity and organic matter (Adriano, 2001). Evidently, in our case, higher concentrations of observed risk elements were found in *Z. mays*. It can be said that there is a relationships between the higher concentrations of certain elements in soil (Cd and Zn), and higher accumulation of them in the plants.

The metal uptake of the two types of plant grown in contaminated soil with earthworms added varied depending on the plant species and the metal element involved. This suggests that these plants have different capacities to absorb and eliminate toxic elements, but the detailed mechanism needs to be further investigated.

Z. mays seems to have a higher assimilation capacity for Cd after the introduction of earthworms but no significant effect was observed. As suggested by Wang and Li (2006), the higher uptake of heavy metals by plants under earthworm inoculation was probably due to the increase in dry matter production stimulated by earthworms. Earthworm action increased Cd concentration in *Z. mays*, except at the Cd-0.71 (control) level, probably because the amount of Cd was low and the earthworm action was negligible in the control soil. An increase in metal concentration for plants in the presence of earthworms was also observed by Wen et al. (2004). Yu et al. (2005) found that earthworm activities increased Cd uptake and plant growth, thereby improving the phytoextraction efficiency of metal hyperaccumulators in low to medium level metal-contaminated soils. As earthworms are known to affect the distribution of microorganisms (Brown et al., 1995), we hypothesized that the effects of earthworms on metal uptake by the plants resulted in part from their impact on soil microorganisms. Microorganisms associated with plant-roots are known to be major drivers of metal speciation in soils and could promote a better efficiency in phytoremediation (Abou-Shanab et al., 2003). Dandan et al. (2007) found that the earthworm bodies and earthworm casts are rich in amino acids and proteins, and soil-available carbon. These organic materials may form chelates with heavy metals, thus contributing to enhance the transport of heavy metals from soil to plant.

Lead (Pb) levels were lower in all soils despite the presence of earthworms. Lead is not required for the metabolisms of soil organisms and is considered as a non-essential metal. For Cu, Zn and Pb, the results showed that addition of earthworms did not affect plant accumulation. In this study, it was shown that the earthworm *E. fetida* was only one factor among others affecting Cd uptake by *Z. mays* in naturally contaminated soils. Our findings can be explained by the metal impact which depends not only on the environmental factors such as pH, temperature, quantity and quality of organic matter and nutrient availability, but also on the microorganisms sensibility. Our results show that earthworms and especially *E. fetida* could potentially be used in phytoremediation as they considerably enhance Cd uptake by *Z. mays*.

5. Conclusion and perspectives

In summary, the results of the present study showed that survival and growth of *E. fetida* is insensitive to Cu, Zn, Pb and Cd metal mixtures, and this remains so even given the addition of *V. faba* or *Z. mays*. However, negative effects of contaminated soils on cocoon production were observed. The introduction of plants did not affect significantly cocoon production or weight per cocoon. These results suggested that the adult earthworms used had a tolerance for the concentrations of metals tested.

This study showed that inoculating metal-contaminated soils with *E. fetida* decreased Cd and Zn availability in those soils. The only contrary result was observed for levels of Cd and Zn in the presence of *V. faba*. Total Zn and Cu concentrations were accumulated in *E. fetida* tissues and Cd and Pb concentrations increasing with increasing of metals in soil. These findings suggest that earthworms are likely to employ able to employ different pathways for the uptake of different contaminants. Furthermore, the addition of *Z. mays* or *V. faba* reduced the accumulation of Cd and Pb metals in earthworm tissues. This is probably because plants can accumulate part of the available Cd and Pb concentrations, effectively creating an uptake competition between the types of organism. In plants, the accumulation of contaminants varied between the two species: *V. faba* plants accumulated higher Cu and Zn concentrations, whereas *Z. mays* accumulated higher Cu concentrations. After the addition of *E. fetida*, higher uptake of Cd metals by *Z. mays* was observed, indicating that *E. fetida* earthworm activity enhances Cd uptake for this plant species.

Our study showed that metal accumulation is a complex process that cannot be predicted by measuring the available fraction of contaminants alone. The final tissue concentrations after exposure to the contaminants depended on the physiological characteristics of the organisms (earthworms or plants), not only on its regulation pathways but also on its exposure routes. Nevertheless, while further research tp establish the optimum species and combinations of them to use is needed, this study suggests that improving phytoextraction treatment of industrial sites polluted with a mixture of metals by use of earthworms and plants is possible.

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CHAPTER 5

DISCUSSION AND PERSPECTIVES

Agricultural practices and earthworm communities

This is the first detailed, field-based study of earthworm communities under different agricultural practices in Wallonia, Belgium. Experiments were designed to assess earthworm community-soil property interactions under different practices and environmental conditions in order to determine the potential of earthworms for use in soil restoration.

The effects of soil physico-chemical properties and environmental factors on earthworm communities are discussed and comparisons are drawn with relevant published literature.

This research has demonstrated that, under given field conditions (specified in Chapter 2), the abundance, biomass and diversity of earthworms were affected by the exportation of crop residues from the soil. Soil tillage coupled with the removal of crop residues from the soil surface may be responsible for the decline in earthworms, especially deep-burrowing species. This was reiterated by Curry and Byrne (1997) and Söchtig and Larink (1992), who studied the role of earthworms in straw decomposition in a winter cereal field. Under RT, the abundance and biomass of earthworms were higher in comparison with CT. In CT, there is a rapid depletion of soil OM and accelerated crop residue decomposition (Eriksen et al., 2009); this can lead to a decrease in food supply for many earthworms, particularly endogeic species. Earthworm communities benefit from tillage practices which return a high proportion of crop residues to the soil, particularly when residues remain on the soil surface. Belgian arable soils, to which OM is added in the form of crop residues can support large earthworm populations despite tillage application and repeated cultivations (November 2012) before sampling (April 2012). Some species appear to be dependent on the amount of OM in the field (e.g. *Allolobophora rosea rosea*). Other species were less affected by cultivation and crop residues exportation (e.g. *Aporrectodea caliginosa meridionalis*, *Aporrectodea caliginosa*). Earthworms consume certain types of plant litter with quality rather than quantity being an important factor (Gallagher and Wollenhaupt, 1997; Curry, 2004). The quality of litter is positively related to the quantity of hemicellulose, nitrogen and other nutrients available (Lavelle and Spain, 2001). Our research suggests that the combination of reduced tillage to a depth of 10 cm and the incorporation of a crop residues to a depth of 25 cm increased earthworm populations because of the quantity, quality and availability of the food supply.

Experiments were conducted over four years. This effect was decreased and then partially neutralised by the effect of crop residues incorporation after a few years. After four years, the exportation of crop residues significantly affects the earthworm communities and the effect of the tillage system eventually diminishes. Two possible pathways can explain this result: (i) despite tillage systems, and when crop residues were present, earthworms can resist and continue their temporary development. Crop residues in the soil surface provide a moist and cool microclimate conducive to earthworm survival and reproduction. In addition, earthworms have adapted and acclimated to field conditions; (ii) the effect of crop residues exportation became more important after few years.

The various ecological groups of earthworms have different burrowing and feeding habits and respond differently to agricultural practices. All ecological groups were affected by the exportation of crop residues. Endogeic species (e.g. *A. c. caliginosa*, *Allolobophora chlorotica chlorotica typica*), which feed on organic material on the soil surface, were less affected by the CT system but more affected by the exportation of crop residues. *A. c. caliginosa* and *A. rosea* are both endogeic earthworms and they share the same habitat. They therefore compete for the same trophic resources. The dominancy of *A. c. caliginosa* over *A. rosea* can be explained through competition for food. Anecic species draw OM from the soil surface (at which they feed) into their burrows. This activity may increase the amount of OM available to the endogeic *A. c. caliginosa* and *Allolobophora chlorotica*, which are known to feed exclusively within the soil profile (top 10 cm) . However, it is likely that OM, drawn into the anecic burrows, should not be easily accessible to endogeic species due to the almost continuous anecic presence within the burrow. It may be more likely that endogeic species are benefitting from feeding upon the casts of anecic species deposited within the soil profile. The casts of anecic species may contain a more concentrated and easily ingested food source (in terms of particle size) than crop residues present in the soil profile; and can be used by epigeic species. The presence of some anecic species, which enhance endogeic or epigeic earthworms, can be considered as a form of commensal association between ecological groups. The presence of epigeic species in our field is still negligible compared with other ecological groups. Despite their ability to colonise unstable environments, epigeic species play only a small role in soil formation and fertility, and appear more affected by CT and exportation of crop residues. Its is to a great extent the soil dwelling species, and deep burrowing speceis in particular, that are influential in soil amelioration. This is the reason for their use, with varying degrees of success, in many land restoration projects. Addition of crop residues will provide a food supply for earthworms and also helps to stabilise soils. If adverse environmental conditions (tillage, exportation of crop residues) exist within degraded soils, inhibiting earthworm establishment, it is then possible to alter the environment to facilitate earthworm survival and development. Findings obtained in our experiments revealed some significant differences in the dynamics of earthworms. Abundance and biomass of earthworms were significantly affected by the application of tillage (after two years), and by crop residues exportation (after four years). This implies that the duration of the experiment is a critical factor in achieving relevant results regarding the negative effect of tillage associated with crop residue exportation. During the experimentation period (4 years), temperature and rainfall changes were significant; this may explain the observed changes in the abundance, biomass and diversity of earthworm communities. The effect of crop residues exportation on earthworm communities is the most marked and important effect, and it is accentuated by the application of tillage. All ecological groups have been negatively affected by these agricultural practices.

Tillage and crop residue management significantly affect the SOC of agricultural soils. In our experiments, the adoption of RT for four years was found to potentially increase SOC concentrations, particularly in the first centimetres of soil. The concentration of SOC diminished with an increase in depth regardless of the tillage system, and this trend is in accordance with other studies (Dong et al., 2009; Du et al., 2010; Mishra et al., 2010). Higher SOC concentrations in the surface layer under RT than those under a CT system can be attributed to a combination of less disturbance and reduced litter decomposition due to less soil-crop residue interaction. Tillage depth under different systems can affect residue location, and thus influence the depth distribution of SOC. Agricultural practices change the quality and quantity of C inputs to soil and soil physico-chemical properties that affect C decomposition. Numerous studies have shown that long-term cultivations of soil decrease soil C and adoption of conservational tillage (e.g., no-tillage, reduced tillage and direct drilling) only reduces the rate of soil C decline, and did not lead to soil C increase (Thompson, 1992; Dalal et al., 1995; Valzano et al., 2001; Dalal et al., 2007). Vegetation types determine the vertical distribution of soil C (Dixon et al., 1994). Tillage may incorporate aboveground fresh organic matter (crop residues) into soil, which provides nutrients and energy for microbial growth and therefore stimulates the decomposition of soil C, including inert organic C (Fontaine and Barot, 2005; Fontaine et al., 2007). The application of RT practice and a crop residue incorporation system may prevent soil degradation and C loss through minimising soil disturbance, increasing the input of biomass C to the soil, and reducing the decomposition and removal of biomass C from crop land. Replacing conventional tillage with conservation tillage (e.g., no-till, reduced tillage, and direct drilling) has been reported to improve soil conditions and significantly increase soil C content (Dalal et al., 1991; Cavanagh et al., 1991; Chan et al., 2002; Pankhurst et al., 2002; Valzano et al., 2005; Rahman et al., 2007).

In our experiments we demonstrated that after four years of a RT system associated with incorporation of crop residues, some of the chemical parameters of soils were significantly modified when compared with a CT system and exportation of crop residues. The P and K contents were higher for winter wheat under RT associated with crop residue incorporation than under CT. The crop residues incorporated into the soil are broken up and the soluble fraction of their compounds can explain the increase of nutrients in the topsoil. Ginting et al. (1998) found higher concentrations of P in the top layer under RT management. This result is in contrast to the findings of Vogeler et al. (2009) who showed that tillage practice did not affect the P content of the soil. The higher soil nutrients in RT compared with CT can be attributed to the presence of crop residues (accumulation of OM) and associated activities of beneficial soil microorganisms. The lower values of soil organic C, P, K, Ca and Mg measured for CT could be due to inversion of top soil (0 – 20 cm) during ploughing which brought less fertile subsoil to the surface in addition to possible leaching. Earthworms are known to play a major role in increasing available nutrients for plants and other soil organisms, through the decomposition of OM both within the soil and at its surface. Our results showed that soil physico-chemical properties could differ among agricultural practices and in the presence or absence of earthworms. The incorporation of crop residues into the soil led to an increase in nutrient availability. Moreover, nutrient release due to earthworm activity is temporally and spatially synchronised with plant activity. The short-term increase in nutrient availability in the presence of earthworms is well documented, but the long-term effect of earthworms on SOM content is less clear (Lavelle et al., 1992; Don et al., 2008). Incorporation of crop residues into the soil profile by earthworms might lead to a partial protection of surface litter within the soil OM. The availability of some of the water soluble nutrients (e.g., K, Ca, Mg) is enhanced as SOM and litter pass through the earthworm gut, because these nutrients are solubilised and dissolved from soil minerals during the grinding/rearrangement of organo-minerals during gut transit. All ecological groups, each with distinct burrowing and casting behaviour, had a positive effect on plant growth, which also argues against soil structure improvement as a major pathway, and in favour of enhanced nutrient mineralisation. In our research, no significant relationships were found between physico-chemical properties and earthworm parameters. Some trends were observed but the relationships between earthworm populations and soil nutrient availability are complex. Earthworms influence soil nutrient availability directly by ingesting and processing soil and indirectly through their impact on the microbial community (Frelich et al., 2006). The lack of correlation between physico-chemical variables and earthworm communities indicates that other environmental factors (soil type, climate, etc) must be given consideration. Significant positive earthworm activity effects on nutrients occur across a range of climate regions, soil textures, soil OM contents and soil C/N ratios. When crop residues were exported from soil, the activity of earthworms decreased. Low residue quality and residue supply are most likely to be the constraining factor for reaching the full potential of earthworm activity (Lavelle and Spain, 2001).

Increasing the number of earthworms in agricultural fields is a key component of programmes that promote soil quality and sustainability, such as RT associated with crop residue incorporation. This agricultural system was used in our experimental parcels over four years with an increase of earthworm abundance, biomass and diversity. A positive effect on nutrient availability was also highlighted under this system. The effect of earthworm activity on nutrient availability was difficult to identify, while certain trends were observed in the presence of earthworms under the RT system for SOC, K, P and Ca concentrations. This practice is increasingly being adopted in Europe and Africa. Adoption of agricultural practices that retain crop residues, minimise soil disturbances (conventional tillage and exportation of crop residues) and rely more extensively on foodweb interactions for plant nutrition is expected to ameliorate the impact of agriculture on soil organisms.

The present study adds to the knowledge that agricultural practices, particularly tillage and exportation of crop residues, negatively affect the earthworm communities under agricultural soils. With regard to the ecological importance of earthworms through soil engineering, it is likely that, when attempting to assess the ecosystem services rendered by earthworm communities to the soil, earthworm abundance, biomass and diversity can have important effects for soil functioning (nutrient stimulation, increased microbial activity, soil carbon balance, etc). Little evidence is available in the literature on how earthworms participate to increase nutrient elements and participate in organic matter decomposition in the first centimetres of soil in the presence of crop residues, so this pattern would need to be verified at the field scale over several years.

The role of earthworms on phytoremediation of metal contaminated soils

In this study, a model system using the earthworm *E. fetida* and the plants *V. faba* and *Z. mays*, was used to determine the potential effects of earthworms on metal uptake by plants. This section discusses the results of the establishment and monitoring of microcosms experiment, designed to provide information on the ability of earthworms and plants or their combination in remediating contaminated soils contaminated by heavy metals. Experiments described in Chapter 4 demonstrated that earthworms and the tested plants affect the availability of metals in soil. Furthermore, results from experiments indicated that earthworms and plants accumulate metals differently. Under certain conditions, mutualistic (beneficial) interactions occurred between the earthworm and plant species. Such beneficial interactions, if verified in the field, would support the greater use of a combination of earthworms and plants in land restoration schemes.

During the experimental period, the survival rate of the *E. fetida* species was was 90% in the uncontaminated soils. These observations indicate that the environmental conditions used in the experimental conditions, used in the experiment were acceptable for culturing the *E. fetida* species. Results demonstrated that the high survival rate of earthworms in contaminated soils could be explained by the low concentrations of metals or the decrease of the availability of metals (Lock and Janssen, 2003). The addition of plants (*V. faba* and *Z. mays*) did not affect the survival rate or weight of earthworms. We can suppose that metallic particles adhered quickly on the root-surface and penetrated into plant tissues, which would explain the inhibited development seen after 28 days of exposure for both plants and this would make metals less available. In our laboratory microcosms, the influence of toxic elements on earthworm survival and weight were not observed. It was suggested that the length of the experiment (42 days) had not been sufficient for the uptake of toxic elements by the earthworms to reach lethal levels. Despite the non-significant difference observed in earthworm traits (mortality and weight) after the addition of plants, the earthworm behaviour differed depending on plants used. The weight of earthworms increased with *V. faba* plant and decreased with *Z. mays*. *V. faba* plants could participate in a nutrient stimulation process which results in making nutrients more available to earthworms.

Recorded cocoon production and cocoon weight rates for *E. fetida* showed that these two parameters were differently affected by soil contamination and by the presence of the two plants. It is possible that *E. fetida* in polluted soils and stressed environment is "forced to choose" on which process to spend its energy: cocoon production or cocoon weight. The presence of plants *V. faba* and/or *Z. mays* played an important role in *E. fetida* reproduction. The cocoon production and cocoon weight were two parameters, which can depend on the intensity (concentrations) of metals and their availability in soils, and on the interactions between earthworm and plants in soils. The resource allocation could create competition between plants and earthworms. Some earthworm populations can tolerate MTE concentrations well above the critical concentrations known to induce lethal effects in more typical populations (Sturzenbaum et al., 1988). The increased tolerance of these populations was shown to be linked to the up-regulation of certain genes. The presence of MTE is known to induce the production of the stress-related Metallothionein proteins. These proteins sequester MTE and are crucial in reducing its toxic effects (Landis and Yu, 1995). Therefore, it was proposed that *E. fetida* earthworms may have been better adapted to the levels of MTE in soils.

In this study, the effects of the earthworms *E. fetida* on MTE uptake and accumulation in the two plants *V. faba* and *Z. mays* were investigated through tissue analyses after 42 days of exposure. The analyses of MTE concentrations in *E. fetida* tissues indicated an enhancement of the Cu and Zn uptake due to the plant (*V. faba* and *Z. mays*) presence. This accumulation seems to principally result from a greater availability of the two metals. The concentrations of Pb and Cd metals increased in the earthworm tissues with increasing levels of metals in the soil, particularly at high concentrations (C3). This can be explained by the high mobility of Pb and Cd and their chemical speciation. The addition of plants decreased the metal uptake by earthworms. This suggests that plants (*V. faba* and *Z. mays*) can accumulate some Cd and Pb, which may create competition between the two organisms. Additionally, by producing exudates, plants can modify metal speciation and their behaviour in soil, especially in the rhizosphere (Chaignon and Hinsinger; 2003; Uzu et al., 2009). Hence, as these results show, plants can modify metal availability and accumulation in *E. fetida* earthworms.

When we investigated the effects of *E. fetida* on the accumulation of MTEs in plant tissues (*V. faba* and *Z. mays*), results showed that the two plants accumulated MTEs to varying degrees depending on the soils and metals. *V. faba* plants accumulated higher Cu and Zn metals in their tissues, whereas *Z. mays* accumulated higher concentrations of Cd. This suggests that the potential of plants for phytoextraction is different depending on the plant species and the metals in the soil. These differences can be attributed to the differences in the uptake mechanisms of metals by plants. Our results showed complex interactions between MTE in the soil and the plants' accumulation of them, which depended partly on the plant species and mainly on the metal involved. The main soil factors controlling metal solubility and bioavailability in soils are the total metal concentration, pH levels, soil absorption and OM (Adriano, 2001). The addition of *E. fetida* earthworms showed that the metal uptake by plants depends on the plant species and the metal element present in the soil. These plant species have different capacities to absorb, accumulate and eliminate toxic elements, but the detailed mechanism is still unclear and must be further investigated. *E. fetida* activity appeared to increase Cd concentrations in *Z. mays*. It has been previously reported that following earthworm activity, the bioavailability of several MTEs such as Zn, Pb, Cd, Cr, and Co is changed significantly (Devliegher and Verstraete, 1996; Ma et al., 2002; Abdul Rida, 1996). Similar results were obtained with a range of MTE (Wen et al., 2004) with a greater accumulation of these elements in the root system when earthworms *E. fetida* were present. The authors explained these observations by enhancement of the water-soluble forms of MTE. Moreover, the presence of *E. fetida* earthworms seems to boost the uptake machinery of MTE by plants.

To conclude, our results showed that earthworms and especially, *E. fetida,* can potentially be used in phytoremediation as they considerably enhanced MTE uptake by the plants.

In this experiment, the phytoremediation process associated with earthworm activity can be considered as a green technology to clean up soils polluted by heavy metals. The effectiveness and efficiency of the technique depends on the amount of metal extracted from the soil by plants and earthworms. In turn, the quantity extracted depends on both the metal concentration in the different parts of the plant (aerial part and root) and in earthworms. Earthworms may be an interesting alternative to enhance phytoremediation. In fact, specific earthworm activities can participate in improving phytoremediation and preserving soil quality. Nevertheless, for the practical use of phytoextraction with earthworms for polluted soil remediation, some issues must be deeply studied by conducting field trials; i. e. biomass and metal accumulation amounts in natural conditions and the confinement of earthworms in the polluted soil area. Likewise, additional research is ongoing in order to better ascertain changes in the chemical partitioning of metals in soil that has passed through the earthworm gut, and to assess the effects of different earthworm species.

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