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**Interactions between Soil Biogeochemistry and
Native Earthworms in New Zealand**

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
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by
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Despite apparently similar burrowing and feeding behaviours to introduced Lumbricidae earthworms, native Megascolecidae, with more than 179 recognised species, have become isolated in natural vegetation remnants on the margins of agricultural land. Long-term geographic isolation has provided high endemic earthworm diversity in New Zealand, but they appear to have a poor ability to adapt to anthropogenic disturbance. Although earthworms are well known as ‘soil engineers’, there is lack of knowledge of the role of endemic earthworms in New Zealand’s soil ecosystems. The aims of the present PhD study were to identify endemic earthworm preferences for and influences on soil biogeochemistry, and to investigate interactions between the drilosphere of native earthworms and the rhizosphere of native plants.

Species of earthworm, collected from native vegetation, natural remnants and restoration sites in Canterbury and on the West Coast of South Island, were identified using DNA barcoding with 16S and COI primers. Thirteen endemic and nine exotic species were identified and, of these, eight abundant earthworms were selected for this study: 5 endemic taxa identified as *Deinodrilus* sp.1 (epigeic), *Maoridrilus transalpinus* and *Maoridrilus* sp.2 (anecic), *Megascolecidae* sp.1 and *Octochaetus multiporus* (endogeic), and 3 exotic species: *Eisenia fetida* (epigeic), *Octolasion cyaneum* and *O. lacteum* (endogeic).

Both endemic and exotic earthworms preferred agricultural soils to a native forest soil. Ryegrass litter was preferred to litter of native plants, although *Coprosma robusta* was also favoured by endemic earthworms. There was more preference for less acid soil than for high organic matter soil. Earthworm species could also be separated on the basis of their effects on soil biogeochemistry, in terms of organic matter consumption, nutrient mineralisation, soil microbial biomass and greenhouse gas emissions from the soil. Earthworm inoculation of soils increased more mobile forms of the key

nutrients N and P, and emissions of N₂O and CO₂ from an agricultural soil. Lesser differences were found between native and exotic earthworms than between functional (burrowing) groups.

Native earthworms increased growth of plants, including *L. perenne*, and had a marked interaction with root morphology of two native species of tea tree (*Leptospermum scoparium* and *Kunzea robusta*). They also stimulated microbial activity in rhizosphere soil. An anecic species, *M. transalpinus*, enhanced rates of root nodulation of a native leguminous shrub (*Sophora microphylla*), enhancing critical concentrations of nitrate, but also reducing nitrous oxide emissions. *Maoridrillus* spp. enhanced plant productivity in biosolids-amended soils, but raised some potential environmental concerns through increased N₂O emissions in <50 % biosolid treatments. They also significantly increased ammonium and nitrate in soil, microbial activity and soil concentrations of soluble copper.

The results showed that endemic earthworms could play a critical role providing soil ecosystem services in New Zealand's production landscapes. Novel habitats within agricultural management systems provide an important refuge for threatened species conservation. Enhanced restoration of native vegetation into agricultural landscapes will enhance the dispersion and sustainability of communities of native earthworms. An integrated understanding of plant growth and microbial communities with earthworm functionality is essential for effective management of soil biogeochemistry and to inform ecological restoration on former agricultural land.

Keywords: Endemic earthworms, Megascolecidae, biogeochemistry, nitrogen, greenhouse gas emissions, drilosphere, rhizosphere, ecological restoration, soil ecosystem services

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Glossary of common terms

Term	Definition
Native	A species is defined as 'native' to a given region if its presence in that region is the result of only natural processes. The region in the present thesis refers to New Zealand. The term is equivalent to 'indigenous' in more academic usage, particularly for ecology.
Endemic	A species being unique to a defined geographic location, such as an island, nation, country or other defined zone, or habitat type; organisms that are indigenous to a place (in this case, New Zealand) are not endemic to it if they are also found elsewhere.
Exotic	An exotic species is defined simply as an 'introduced species' living outside its native distributional range (in this case, in New Zealand), which has arrived by human activity. There may be negative effects of exotic species on local ecosystems.
Taxa	A group of organisms (earthworms) that are identified to be unique in terms of morphological or molecular characteristics, but not necessarily recognized as distinct species.
Earthworm	Tube-shaped, segmented animals in the order Oligochaeta, involving approximately 8,000 species from about 800 genera. Of these, nearly 3,600 species are terrestrial earthworms. They are an essential part of the soil fauna in most soils, globally, represent a significant proportion of the soil biomass and are regarded as 'soil engineers' in terms of soil health and quality. In New Zealand, there are three families (Megascolecidae, Lumbricidae, and Glossoscolecidae) and more than 200 species of endemic earthworms have been identified.
Drilosphere	An approximately 2 mm wide zone around earthworm burrows, containing more plentiful organic matter and nutrients compared to the bulk soils and with a generally higher accessibility for roots. This zone is a hot spot of soil biological activity.
Rhizosphere	A zone, from less than 1 mm from the root surface to several millimeters, of intense chemical and biological activity in soil that surrounds the root. The soil around the rhizosphere is chemically, physically and biologically different in comparison with the bulk soil since its properties have been modified by roots and their associated microorganisms and soil fauna. Many key processes associated with soil fertility occur in this zone.

Chapter 1

Introduction

1.1 General introduction

Earthworms have important functions as 'soil engineers' in soil ecosystem worldwide, mediating soil structure, organic matter dynamics and nutrient cycling, particularly nitrogen, and enhancing biological interactions with other soil biota such as microbial and plant communities (Bertrand et al., 2015). Undoubtedly, they contribute to the sustainability of agroecosystems (van Groenigen et al., 2014), but there is lack of knowledge of New Zealand endemic earthworms in this context. This is problematic in terms of conservation of biodiversity in this world biodiversity hotspot and in knowing their functional role in soil. Currently we have little understanding of the functionality of endemic earthworms in soils that we are unable to realise and leverage their potential benefits to soil ecosystem services.

Following relatively recent human colonization, ground disturbance through burning, vegetation clearance and ploughing played a major role in the demise of the native megascolecid earthworms, as happened elsewhere in the world (Edwards and Bohlen, 1996; Hendrix, 2006). Native earthworms are almost entirely absent from agricultural pastures in New Zealand, with the probable exception of only a single deeper-burrowing species, *Octochaetus multiporus* (Springett et al., 1998). In a study of over 750,000 ha of commercial farms in the region of the present study, no native earthworms were found (Fraser et al., 1996). European species of lumbricids have been introduced, often intentionally and with varying degrees of success, providing some improvement the quality of agricultural soils where more substantial populations have become established (Lee, 1961; Springett et al., 1992). Endemic earthworms have found refuge beneath small remnants and fragments of native vegetation, for example in riparian zones, along fence lines and on the borders of agricultural land (Bowie et al., 2016). In these restricted areas we have found that it is common to find coexisting assemblages of both native and introduced species (Kim et al., 2015).

The rationale of this research project was an awareness of the necessity to elucidate the importance and functional role of native earthworms in selected human-modified soils in South Island, New Zealand.

1.2 Aim and objectives

The aims of this research were to investigate (i) how native earthworms survive alongside invasive species in highly disturbed landscapes, (ii) whether and how they have adapted to the modified soil biogeochemistry of agricultural land, and (iii) whether they play a role in influencing the functionality of these soils.

The PhD research programme had the following objectives:

Objective 1. Investigation of the interactions of native and introduced earthworms with soils and plant rhizospheres in production landscapes of New Zealand [Chapter 3, Published paper]

- Remnants of native vegetation support mixed assemblages of depleted populations of native Megascolecid earthworms together with apparently increasing invasive populations of introduced Lumbricidae. This objective questions whether the survival and viability of these earthworm populations is a function of soil preference and whether there are significant differences in terms of how the two groups are influenced by and modify soil properties and plant growth. The aims of this experimental work were to elucidate the predilection of native earthworms for soils that have become nutrient enriched and otherwise modified by agriculture and forestry, and their effect on soil physico-chemistry.

Objective 2. Molecular identification and the distribution of New Zealand earthworms in human-modified Soils [Chapter 4, Draft manuscript prepared for submission]

- Unique geographic isolation has contributed a hotspot of biodiversity to New Zealand. Of the endemic earthworm fauna, it appears that a number of cryptic Megascolecidae or species that are difficult to identify using morphological keys, inhabit isolated remnants of undisturbed native vegetation. Recent studies have unearthed many new species in remote locations where no previous searches had been conducted. The aims of work reported in this chapter was to identify and describe selected taxa through DNA barcoding, elucidating both phylogenetic relationships and their distribution in human-disturbed soils.

Objective 3. Endemic earthworms in a sheep-farmed soil: implications for soil nutrients, environment and conservation [Chapter 5, Draft manuscript prepared for submission]

- The hypothesis of the research reported in this chapter was that introduced lumbricid earthworms are better-suited to agricultural land and will burrow more actively than native

megascolecids, with a larger influence on soil physicochemical conditions. This was expected to contrast with endemic megascolecid species that are likely to be naturally found in more acidic native soils with low to moderate fertility. The aims were to identify how native and exotic earthworms modify the biogeochemistry of a low fertility agricultural soil prior to farm intensification.

Objective 4. Integration of earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a mesocosm experiment [Chapter 6, Draft manuscript prepared for submission]

- Agricultural landscape matrices in New Zealand are depauperate in native flora and fauna; remnants of natural and re-planted vegetation are represented as little more than refugia in riparian zones, along fence lines and on the borders of agricultural land. These natural remnants are now significantly expanding though renewed interest in native species, and modern intensive agricultural systems that are integrating restoration of biodiversity into farm planning. The aim of this work was to investigate whether it is possible to demonstrate significant integration between a native nitrogen-fixing plant, earthworms and soil biogeochemistry.

Objective 5. Investigation of the potential role of New Zealand native earthworms (Megascolecidae) as ecosystem engineers on agricultural land [Chapter 7, Standard thesis chapter]

- Worldwide, anthropogenic disturbances have favoured colonization of exotic Lumbricid earthworms, following deforestation, cultivation and urbanization, whilst native earthworms seem to maintain their populations in undisturbed soils. However, there is no knowledge of the status and role of native species alongside pasture soils, and on the boundaries of remnant forests and stand of native vegetation. My hypothesis was that native earthworm may relocate to agricultural pastures under conditions of less intensive management and that, where this occurs, they may also have a positive influence on agroecosystems. The aim of this work was to investigate the viability and effects of one selected native species, *M. transalpinus*, on soils and plants typical of agricultural pastures in South Island.

Objective 6. Biochemical impacts of endemic *Maoridrilus* earthworms (Megascolecidae) in biosolid-amended soil [Chapter 8, Pre-submission manuscript]

- Vermicomposting using exotic species (e.g. *Eisenia fetida*) has been proposed as a cost effective and easily controllable means to increase availability of mineral N in biosolids as well as to reduce the burden of human pathogens. Despite high biodiversity in New Zealand, no studies have investigated New Zealand earthworms in the context of biosolids disposal to soil. Native earthworms may prove to be more effective than exotic species since they are adapted to local climatic and edaphic conditions; they may also confer other ecological benefits, such as food-chain continuity, and roles that their exotic counterparts may not fulfil. The aim of work presented in this chapter was to evaluate the feasibility of using native anecic earthworms to improve the quality in biosolid-amended soil, in terms of how they affect solubility of macronutrients and trace elements, and greenhouse gas emissions (N₂O and CO₂).

Objective 7. Earthworm feeding and burrowing behaviours: observational studies [Chapter 9, Standard thesis chapter]

- Coexistence of endemic and exotic species of earthworms appears to be common in New Zealand native vegetation as well as on marginal agricultural land. However, there is a lack of knowledge of the feeding and burrowing behaviour of native species. The aim of studies reported in this chapter was (i) to evaluate the relative importance of soil pH and soil organic matter to endemic *Maoridrilus* spp., (ii) to investigate native plant litter preferences, and (iii) to observe feeding activity and burrowing of earthworms behaviours in the rhizosphere of native plants.

1.3 Thesis structure

Chapter 2 provides a detailed literature review.

Subsequent chapters report experimental work carried out in laboratory, glasshouse and field studies:

- Chapter 3 and 4 were carried out in 2012 and 2013.
- Chapter 6 and 7 were implemented in 2014.
- Chapter 8 and 9 were conducted during 2015.
- Chapter 5 for molecular identification was carried out between 2012 and 2015.

Chapter 10 provides conclusions.

Each of the six experimental chapters report experimental work presented in the form of manuscripts either published, submitted or prepared for intended full or partial publication.

Chapter 2

Literature review

2.1 Importance of earthworms

Charles Darwin (1881) stated, “It may be doubted whether there are many other animals which have played so important a part in the history of the world, as have these lowly organized creatures.” Earthworms are an essential part of the soil fauna in most soils, globally representing a significant proportion of the soil biomass (Edwards, 2004) and regarded as a valuable indicator of soil health and quality (Sizmur and Hodson, 2009). Although earthworms had been regarded as pests in agricultural soil until the late 1800s, Darwin (1881) realised the importance of earthworm activity in the soils, particularly their critical role as a circulator of nutrients. Earthworms are now considered as the most important soil engineers that directly or indirectly influence the availability of resources for other organisms including plants, microorganisms, and invertebrates (Brown et al., 2000; Lavelle, 1996; Nahmani et al., 2007).

2.2 Classification as ecological categories

Earthworms can be classified into three major ecological categories, according to their burrowing and feeding activities: epigeic (litter dwellers), endogeic (topsoil dwellers), and anecic (vertical deep burrows) species (Figure 2.1). Description below are from Bouché (1977) and Lee (1985).

2.2.1 Epigeic species

These litter feeding earthworms are mostly small and dark, with a pigmented body to protect against UV light and predators. They live around crops and plants as ‘litter transformers’. They are not common in most agricultural soils and do not ingest large amounts of soil. Epigeic species show greater short-term fecundity and maturation compared to endogeic and anecic species.

Examples of representative species:

[Endemic] Lee (1959a) describes some species as being associated with mould, but examples of epigeic species in New Zealand are otherwise unknown. A potential candidate is *Maoridrilus plumbers* [also see Figure 2.3]

[Exotic] *Dendrodrilus rubidus*, *Eisenia fetida*, and *Lumbricus rubellus*

2.2.2 Endogeic species

These geophagous earthworms colonise topsoil, creating a more horizontally-oriented drilosphere with their burrows and excrement. They rarely visit the soil surface. These are medium-sized earthworms and usually have a pale white, pink or grey skin colour. They ingest large amounts of mineral soil and below-ground organic matter, particularly dead root material. One endemic endogeic species (*Octochaetus multiporus*) appears to be widespread in agricultural or pastoral soils of New Zealand.

Examples of representative species:

[Endemic] *Octochaetus multiporus*

[Exotic] *Aporrectodea caliginosa*, *Aporrectodea rosea*, *Allolobophora chlorotica*, *Octolasion cyaneum*, and *Octolasion lacteum*

2.2.3 Anecic species

These earthworms live in permanent vertical burrows connected to the surface that can be up to 2 m depth. They have moderate or large-size bodies and are mostly dark in colour, at least on the upper-side of the body. They require surface plant litter to feed on. Their burrows remain open, although they cap the top with castings and litter residues (middens) that they pull to the entrance. These species ingest substantial amounts of soil that they mix with plant residue in their guts.

Examples of representative species:

[Endemic] *Maoridrilus transalpinus*

[Exotic] *Lumbricus terrestris* and *Aporrectodea longa*

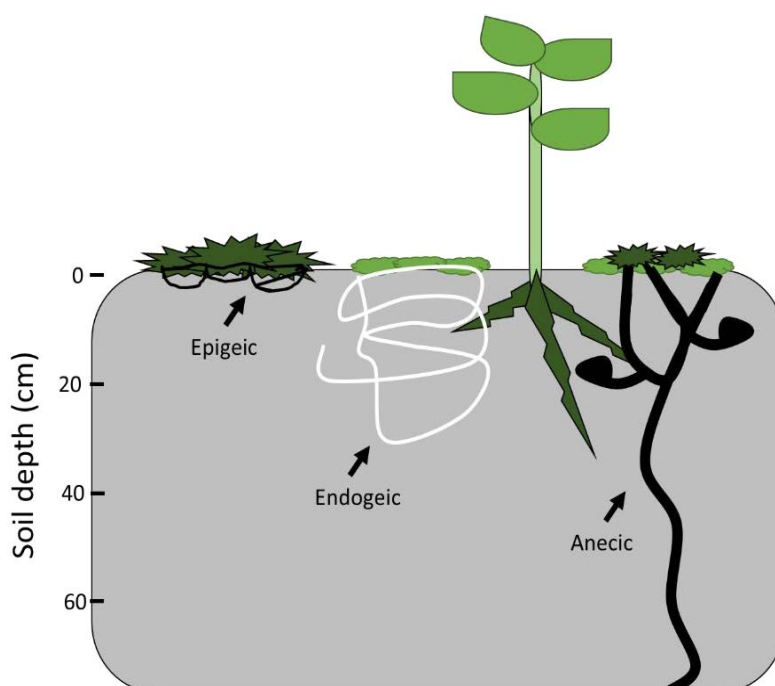


Figure 2.1 Three major ecological functional groups of earthworms: primarily based on burrowing habit and the soil horizon they inhabit.

2.3 Earthworm ecology

A variety of environmental factors influence earthworm abundance, such as temperature, moisture, soil fertility, food source, and land management practices (Edwards, 2004; Fragoso et al., 1997; Lee, 1985; van Groenigen et al., 2014).

2.3.1 Temperature and moisture

Earthworms inhabit cropping, pastoral and forest soils, mainly in tropical and temperate regions (Sharma et al., 2005). Optimum temperature for survival and colonisation ranges between 10 and 30 °C (Curry, 2004; Edwards and Bohlen, 1996; Yadav and Garg, 2011). Perreault and Whalen (2006) reported that the Lumbricidae (e.g. *Aporrectodea caliginosa* and *Lumbricus terrestris*) showed most active burrowing at 20 °C. In addition, cocoons of *A. longa* are cultured optimally at 15-20 °C (Baker and Whitby, 2003). In terms of moisture content, earthworm activity is most dynamic over 10 kPa of water retention while earthworms are unable to live below the permanent wilting point (1500 kPa) (Curry, 2004). More burrows of endogeic *A. caliginosa* and anecic *L. terrestris* are found at higher temperatures and they move more deeply, away from drier soil condition; more growth occurs and more casts are found on wetter soil (Perreault and Whalen, 2006).

Impacts of drought stress on earthworms may depend on both the species and on ecological grouping (Curry, 2004). Long-term drought can be lethal to epigeic species but less to endogeic and anecic species, which can burrow into deeper soils. Therefore, irrigation on arid agricultural soils may help to maintain viability of earthworms, particularly epigeic species (e.g. *L. rubellus*) (Lobry de Bruyn and Kingston, 1997). However, these authors also found that endogeic *A. caliginosa* could not survive following irrigation where serious soil compaction had been caused by long-term drought. In addition, excessive irrigation of high-salinity topsoil tended to be detrimental for earthworms (Owojori et al., 2009). Earthworms have adaptations to local conditions and may burrow into a deeper soil profile during extreme seasons (Curry, 2004). Wet soils with increased temperature (but below 30 °C) are better for earthworm feeding and casting (Perreault and Whalen, 2006). New Zealand pasture land often experiences seriously drought in summer that negatively affects abundance of earthworms, particularly native species of Megascolecidae. Elsewhere, James (1988) collected Megascolecidae in the surface soil of tallgrass prairie (<10 cm) when soil was wet, but found the earthworms in deeper soil (below 20 cm) under extreme drought conditions. Following deforestation and conversion of 60-70 % of native vegetation to farmland, it has been shown that less tree canopy and reduced soil organic matter on pastoral lands has accelerated evaporation of soil moisture, limiting the refuges of native earthworms to remnants of mature vegetation (Yeates and Lee, 1997).

2.3.2 Soil properties

Physicochemical soil properties such as texture, depth, pH, and organic matter are certainly responsible for earthworm distribution and abundance (Curry, 2004). Earthworms prefer moderately textured soils to sandy soil (Guild, 1948). Previous studies in Europe reported that higher clay content ranging from 5 to 25 % tended to increase populations of *Aporrectodea* spp. in both forest (Nordstrom and Rundgren, 1974) and pasture soils (Baker et al., 1992). In fact, earthworm activities are dependent on the relationship between moisture and clay content (Curry, 2004). Earthworms generally prefer less acid to neutral soil (pH 4.5 to 7.0) (Baker et al., 1992; Curry, 2004; Lowe and Butt, 2005). They seem to avoid soil of pH less than 4.5 and are totally absent in very acid soil substrates (<pH 3.5) (Edwards, 2004). Moreover, earthworm abundance is influenced by other edaphic factors such as nutrients (e.g. nitrogen, calcium, and magnesium) and by contaminants (e.g. salt and heavy metals) (Curry, 2004).

2.3.3 Food sources

Plant litter and dead roots are the main organic food source for earthworms in most ecosystems, and populations are considerably affected by the quantity and quality of these food resources. Favourable habitats such as permanent plantation and animal manure-amended soil have been shown to increase earthworm abundance (Curry, 2004). The quality of food source, particularly the C/N ratio, is critical for growth and survival. High nitrogen content enhances maturity and fecundity of earthworms (Butt, 2011; Gajalakshmi and Abbasi, 2004). In addition, soil microorganisms (e.g. fungi) can increase palatability of food materials as well as nutrient content (Bonkowski et al., 2000). However, toxic components in the food sources such as ammonia, phenolic compounds (e.g. tannins) and carbohydrate content may lessen its palatability to earthworms (Curry and Schmidt, 2007; Lowe and Butt, 2005).

2.3.4 Land management

Human activity causes massive modification of land and hence influences soil biodiversity. Of the soil biota, earthworms are influenced directly by physical disturbance (e.g. tillage and ploughing) and indirectly by changed edaphic conditions (e.g. food resources and biochemistry). Populations, growth and reproduction of earthworms can be increased with less tillage (Capowiez et al., 2009) and applications of organic amendments (Curry, 2004). Long-term intensive agriculture practices such as tillage and chemical applications (e.g. fertilizers and pesticides) alter soil chemistry and compaction which inhibit earthworm establishment in arable land (Chan, 2001; Curry, 2004; Yasmin and D'Souza, 2010). In New Zealand, conversion to agricultural has certainly disrupted earthworm habitats and has substantially reduced populations of native earthworms (Kim et al., 2015).

2.4 New Zealand earthworms

2.4.1 Origins of NZ earthworms and historical impacts

New Zealand native earthworms are in the Family Megascolecidae of the Order Megadrilacea which consists of two subfamilies: Acanthodrilinae and Megascolecinae (Figure 2.2) (Blakemore, 2006; Boyer et al., 2011a; Lee, 1959a). The Megascolecidae is of an ancient fauna that had a strong northern origin from Australia and Polynesia. In New Zealand this family has evolved in geographical isolation since the Tertiary, with accompanying changes of characteristics, like other endemic flora and fauna (Gaina et al., 1998; Lee, 1959a; Michaux, 2009).

The family Lumbricidae were absent from New Zealand but, since 19th century colonization by Europeans, at least 23 species have been introduced. Lumbricids were mainly brought from Europe through the inadvertent inflow of European soils as potting mix and as ballast in ships (Lee, 1959a). Lumbricid species including *Aporrectodea* spp., *Lumbricus* spp. and *Octolasion* spp. have become established in regions formerly inhabited by the established Megascolecidae. The clearance and burning of forest and tussock grasslands following human settlement resulted in a dramatic reduction in native earthworms (Fraser et al., 1996; Lee, 1985). Continued invasion of the exotics may be detrimental to endemic species, and Lumbricids have largely replaced endemics in agricultural soils of New Zealand (Fraser et al., 1996; Springett et al., 1992).

2.4.2 Classification of NZ earthworms

Earthworm taxonomy in New Zealand is still strongly dependent on morphological identification (Buckley et al., 2011; Lee et al., 2000; Lee, 1959a). The Acanthodrilinae is distributed throughout all of the main Islands, but the Megascolecinae is entirely restricted to northern and western regions of New Zealand (Lee, 1959a).

Of about 3,700 terrestrial earthworm species worldwide, 179 endemic species in 26 genera of the Megascolecidae have been reported in New Zealand so far (Figure 2.2), but more recent use of molecular identification methods is raising this number (Boyer, 2013; Boyer et al., 2011a; Buckley et al., 2011). Using DNA barcoding, six species of earthworms new to science were postulated by Blakemore (2011) and Boyer et al. (2011a): *Deinodrilus gorgon*, *Maoridrilus felix*, and *Octochaetus kenleei* in the subfamily Acanthodrilinae and *Aporodrilus aotea*, *Aporodrilus ponga*, and *Notoscolex repanga* in the subfamily Megascolecinae. However, the scarcity of earthworm systematic studies and geographical isolation still causes difficulties for indigenous earthworm taxonomy.

Classification of New Zealand earthworms

Phylum	Annelida
Class	Clitellata
Subclass	Oligochaeta
Order	Haplotaxida
Suborder	Lumbricina

Family	Megascolecidae						Lumbricidae		Glossoscolecidae	
	Acanthodrilinae			Megascolecinae						
	(17) ^a	129 ^b	2 ^c	(10) ^a	50 ^b	6 ^c	(8) ^a	17 ^c	(1) ^a	1 ^c
Genus	<i>Acanthodrilus</i>	1		<i>Amyntas</i>		3	<i>Allolobophora</i>	1	<i>Pontoscolex</i>	1
	<i>Decachaetus</i>	2		<i>Aporodrilus</i>	2		<i>Aporrectodea</i>	5		
	<i>Deinodrilus</i>	9		<i>Didymogaster</i>		1	<i>Dendrobaena</i>	1		
	<i>Dinodriloides</i>	1		<i>Diporochaeta</i>	11		<i>Dendrodrilus</i>	1		
	<i>Eodrilus</i>	9		<i>Megascolex</i>	2	1	<i>Eisenia</i>	2		
	<i>Eudinodiloides</i>	1		<i>Megascolides</i>	22		<i>Eiseniella</i>	1		
	<i>Hoplochaetina</i>	8		<i>Notoscolex</i>	2		<i>Lumbricus</i>	4		
	<i>Leucodrilus</i>	4		<i>Perionyx</i>	4	1	<i>Octolasion</i>	2		
	<i>Maoridrilus</i>	29		<i>Plutellus</i>	3					
	<i>Microscolex</i>	5	2	<i>Spenceriella</i>	4					
	<i>Neochaeta</i>	2								
	<i>Neodrilus</i>	5								
	<i>Octochaetus</i>	14								
	<i>Perieodrilus</i>	4								
	<i>Plagiochaeta</i>	3								
	<i>Rhododrilus</i>	31								
	<i>Sylvodrilus</i>	1								

^aTotal number of genus; ^bTotal number of native species; ^cTotal number of exotic species

Figure 2.2 Synthetic classification of New Zealand earthworms based on family and genus, according to Buckley et al. (2011, 2015), Boyer et al. (2011a), Lee et al. (2000), and Sims and Gerard (1985).

Unlike the Lumbricid species, there is a serious paucity of knowledge in relation to ecological groupings of New Zealand Megascolecidae. Lee (1959a) associated all his sampled earthworms with different soil profiles, including litter mould, topsoil or subsoil from field evaluations in his book on 'The Earthworm Fauna of New Zealand'. However, such descriptions are still providing difficulties with identifying accurate ecological groupings. Even the better known classified species have been described discordantly in some publications; for instance, *Octochaetus multiporus* has been described as either endogeic (Wüst et al., 2009) or anecic (Schon et al., 2011). Clearly, more specific ecological classifying studies are required to support improved knowledge of native earthworm taxonomy and understanding of their functions in soil.

2.4.3 Distribution of NZ earthworms

Geographical barriers in New Zealand may have interrupted the natural dispersal of endemic species, but distributions are also determined by habitat and environmental variability (Lee, 1959a). Since 19th century European colonization, native earthworms have been mainly restricted to native vegetation and to less modified environments such as marginal areas of farmland (Bowie et al., 2016; Fragoso et al., 1997; Kim et al., 2015). Several studies have described refuges both for previously identified species (Lee, 1959a, b) and for formerly undescribed species (Boyer, 2013; Buckley et al., 2015; Kim et al., 2015), but we have a highly deficient understanding of earthworm biodiversity. Of the native species, only *Octochaetus multiporus*, an endogeic deep-burrowing species that rarely comes to the soil surface, is commonly found in agricultural pasture soils (Springett et al., 1998). In contrast, 23 introduced species that are tolerant of environmental disturbance, are widespread throughout the main Islands and, with a few exceptions, they are the only earthworms found in arable and pastoral soils (Fraser et al., 1996; Springett et al., 1998). Schon et al. (2008) suggested that populations of Lumbricids are becoming more widespread with beneficial effects to agriculture. Stockdill (1982) estimated that, 23 years after introduction of Lumbricids, grass production, soil organic matter, soil water-holding capacity and drainage were improved by over 20 %. However, the numbers of Lumbricid earthworms in cropping lands appear to have been restricted by disturbance such as ploughing (Berry and Karlen, 1993; House and Parmelee, 1985; Springett et al., 1992).

2.5 Earthworm functionality in soil

Earthworm activities such as feeding, burrowing and cast production certainly affect physical, chemical, and biological soil properties, thereby enhancing soil quality (Bertrand et al., 2015; Frouz et al., 2007). Changes of soil physical properties by earthworm activity stimulates more aggregative, stable and porous soil that may help to prevent soil erosion (Francis and Fraser, 1998; Topp et al., 2001). Earthworms also modify soil biochemistry including soil pH and chemical forms of nutrients (i.e. plant-available nutrients) by modulating organic matter dynamics (Brown, 1995; Lee, 1985). Moreover, earthworm activities improve the structure and abundance of microbial communities, including fungi, actinomycetes, and bacteria within the drilosphere (Edwards, 2004; Edwards and Bohlen, 1996). These beneficial influences of earthworms can increase plant growth (Blakemore, 1997; Fragoso et al., 1997) and contribute to sustainability of agroecosystems (van Groenigen et al., 2014). Earthworm activity is also likely to play a role in vegetation dynamics and biodiversity of flora and other fauna. They may increase plant performance through the release of nutrients in the rhizosphere, the change in soil hydraulic properties, the enhancement of mutualistic microorganisms, and the production of beneficial hormones (Blouin et al., 2013; Brown et al., 2000). Vermicomposting technology is

increasingly used for disposal of organic wastes from domestic, agricultural, and industrial sources (Dominguez and Edwards, 2004).

2.5.1 Impacts on soil structure

Soil structure is an essential determinant of most soil functions, directly or indirectly influencing liquid and gas fluxes, biological activity, and plant growth (Piron et al., 2012). Earthworms contribute to the formation and sustainability of soil structure that may benefit soil biophysicochemical properties by construction of macropores, and creating stable macroaggregates and organo-mineral complexes (Amossé et al., 2015; Castellanos-Navarrete et al., 2012). The drilosphere can increase water infiltration and retention and thus is responsible for supplying water to crops, as well as preventing surface runoff and erosion (Bertrand et al., 2015; Li et al., 2012; Zaller et al., 2011a).

Pore morphology differs depending on the ecological groups (Castellanos-Navarrete et al., 2012). Endogeic and anecic species, deeper-burrowing earthworms, can create greater soil porosity than epigeic species and their casting also improves soil structural stability and resistance to erosion (Le Bayon et al., 2002). Earthworms can relieve soil compaction in agricultural land, which otherwise reduces crop yields by disturbing water infiltration and rooting capacity of plants (Yvan et al., 2012).

2.5.2 Mineralisation of nutrients

Earthworms can increase organic carbon (C) and nitrogen (N) by accelerating soil organic matter (SOM) turnover, subsequently contributing to enhancement of microbial activity and nutrients mineralisation, particularly of nitrogen (NH_4^+ and NO_3^-) (Bertrand et al., 2015). Casts and structures caused by earthworm burrowing contain more enriched OM, extractable nutrients (N, P, K, Ca, and Mg), and higher moisture content compared to bulk soil, thereby facilitating preferential local environments for microorganisms (Bertrand et al., 2015; Bhadauria and Saxena, 2009; Blouin et al., 2013; Le Bayon and Milleret, 2009). Interaction between earthworms and microbes in the casts and biostructures may enhance N_2O release from the soil surface through processes of nitrification, denitrification, and/or nitrifier-denitrification (Brown et al., 2000; Lubbers et al., 2011); nitrifying bacteria (e.g. *Nitrosomonas* and *Nitrobacter*) influence nitrification, and denitrifying bacteria (e.g. *Pseudomonas denitrificans*) increase denitrification in particularly low aerobic condition (Figure 2.3) (Barnard and Leadley, 2005; Kester et al., 1997). The decomposition of SOM in the presence of earthworms leads to increase plant-available N and crop productivity (Lubbers et al., 2011), through creations of casts, urine, and mucus which constitutes of NH_4^+ , urea, allantoin, and uric acid (Blouin et al., 2013). In addition, it would seem likely that dead tissues of earthworms may enhance plant-available N, although no substantial effects of decaying earthworm tissues on bioavailability of N were found in previous studies (van Groenigen et al., 2014; Whalen et al., 1999).

The influence of earthworm activity on N cycling (mineralisation, plant availability, and N₂O emission; see Figure 2. 3) also differs between ecological groups (Butenschoen et al., 2009; Lubbers et al., 2011). Epigeic species modify the physicochemical status of litter (e.g. reduction in C/N ratio) promoting more favourable conditions for microbial activity (Fragoso et al., 1997; Scheu, 1993). Lubbers et al. (2011) reported that the epigeic *Lumbricus rubellus* released more N₂O from the soil surface than did anecic and endogeic species. Amador et al. (2003) found that *Lumbricus terrestris* increased C and N mineralization and accumulation of nitrate in and around the drilosphere. phosphorus dynamics is also influenced by earthworm activity; biogenetic structures in the earthworm gut provide favourable conditions for phosphatase enzymatic activity of microbes (Le Bayon and Milleret, 2009). Carbohydrate compounds and raised pH (6.0-6.8) in earthworm secretions may increase soluble forms of P (mainly H₂PO₄⁻ and HPO₄²⁻) (Barois and Lavelle, 1986; López-Hernández et al., 1993). Sharpley and Syers (1976, 1977) reported that fresh casts of *A. caliginosa* had triple the amount of soluble P compared to surroundings pasture soils in New Zealand. As with N mineralization, there are different effects on P mineralization depending on earthworm species (Le Bayon and Milleret, 2009).

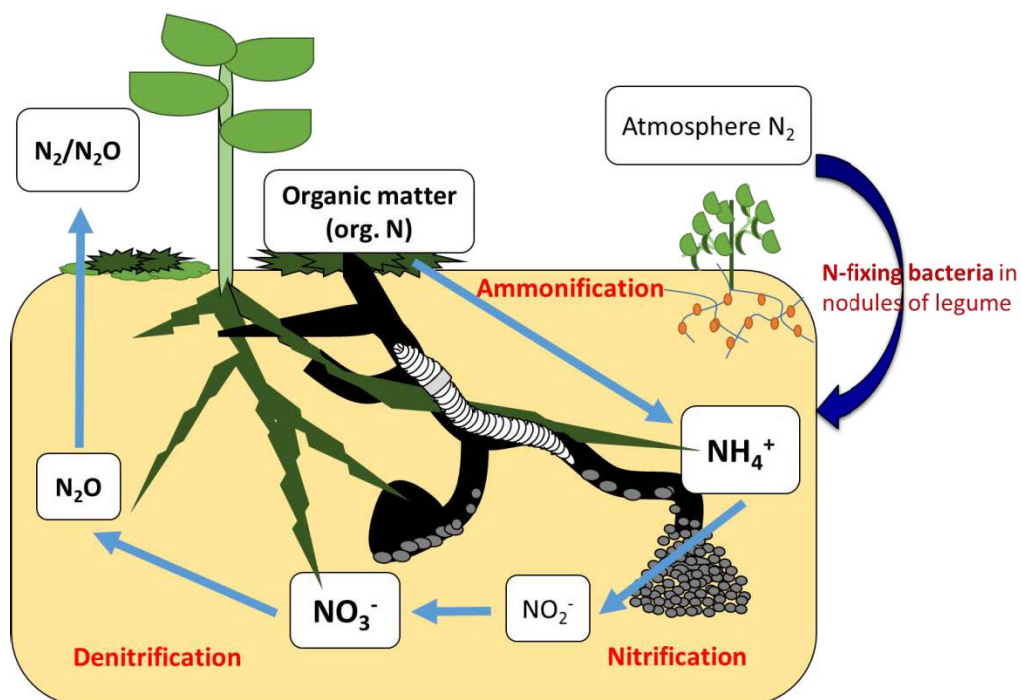


Figure 2. 3 Nitrogen cycling and the potential influence of earthworm activity in the soil profile.

2.5.3 Association with microbiota

Biotic interactions are significant factors in soil fertility and plant growth through alteration of the physicochemical soil environment and nutrient cycling (Wardle, 2002). Earthworms influence the

dispersion and activity of both nitrogen-fixing bacteria and arbuscular mycorrhizal fungi within the rhizosphere. The drilosphere is a hotspot of soil microflora and microfauna activity. Enriched organic matter and secreted mucus on burrowed walls and casts can promote diversity of microbes, particularly fungi (Wardle, 2006), with knock-on effects on plant-available nutrients and plant productivity (Brown et al., 2000; Daniel and Anderson, 1992; Perreault and Whalen, 2006).

Nitrogen-fixing *Rhizobium* spp. can be dispersed by earthworm activity which stimulates root nodulation and enhances nitrogen fixation in soil (Figure 2.3) (Doube et al., 1994; Thompson et al., 1993; Wurst, 2010). Stephens et al. (1994) reported that endogeic species (*Aporrectodea trapezoides* and *Microscolex dubius*) enhanced the colonization of alfalfa or Lucerne (*Medicago sativa*) by *Rhizobium meliloti* to 90 mm soil depth. *A. trapezoides*, increased nodulation of *R. trifolii* fivefold, although other studies reported no effects on plant growth or foliar N (Doube et al., 1994). The number of soybean nodules was found to be increased by burrowing of the anecic earthworm, *Lumbricus terrestris* (Rouelle, 1983). Conversely, other studies have found no influence of epigeic species on rhizobial dispersion below 2.7 cm from the soil surface (Madsen and Alexander, 1982).

Previous studies have found negative (Gormsen et al., 2004; Milleret et al., 2009; Welke and Parkinson, 2003) or no effects (Eisenhauer et al., 2009; Ma et al., 2006; Wurst et al., 2004; Zaller et al., 2011b) of earthworm activity on colonization of symbiotic arbuscular mycorrhiza fungi (AMF). It has been suggested this may be due to physical disturbance of AMF by burrowing (Scheu, 1987) or selective feeding on hyphae and spores (Bonkowski et al., 2000; Gange et al., 1993). However, in other studies, earthworms have been found to stimulate dispersion of AMF spores and to increase AMF populations (Cheng et al., 2006; Gormsen et al., 2004; Lee et al., 1996; Yu et al., 2005; Zarea et al., 2009a). In the presence of earthworms AMF have been shown to increase plant availability of N and P, particularly in sterile soils, and to enhance soil particle aggregation (Brown et al., 2000; McLean et al., 2006). Integration of earthworms, AMF (*Glomus intrardices*), and rhizobium (*R. melilotus*) has been found to increase yields of another species of *Medicago* (Zarea et al., 2009a).

2.5.4 Mobility of contaminants

Earthworms have the capacity to survive and reproduce in soils contaminated with heavy metals (Spurgeon et al., 1994). They can also accumulate high concentration of heavy metals within their bodies (Morgan and Morgan, 1993). The amounts of accumulated metals are variable, depending on soil properties including soil pH, cation exchange capacity (CEC), OM content, and clay size particle content (Beyer et al., 1987; Ma et al., 1983; Morgan and Morgan, 1988). Moreover, earthworms can influence the mobility and availability of metals and metalloids in soils through their activities including feeding, burrowing, and casting (Nahmani et al., 2007; Sizmur and Hodson, 2009). Heavy metals mobility is modified more in the drilosphere than in bulk soil (Sizmur and Hodson, 2009; Tomlin et al.,

1993) and earthworm activity may promote metal uptake by plants in contaminated soils (Cheng and Wong, 2002; Wang et al., 2006; Yu et al., 2005).

There are a number of mechanisms by which earthworm activity impacts metal mobility and bioavailability in contaminated soils. Increases in population of bacteria, actinomycetes, and fungi around earthworm casts results in enzymatic degradation of organic matter and discharge of organically-bound metals into soil solution, with enhanced bioavailability of metals to plants (Rada et al., 1996; Wen et al., 2004). Cutaneous mucus secretion (Ma et al., 2003; Udovic et al., 2007) may decrease soil pH that increases the mobility and bioavailability of metals (El Gharmali et al., 2002; Kızılkaya, 2004; Yu et al., 2005). Changes to dissolved organic carbon (DOC) by earthworms produce humic acids that increase the availability of metals through forming organo-metal complexes (Businelli et al., 1984; Evangelou et al., 2004; Halim et al., 2003), resulting in increased uptake by plants (Currie et al., 2005; Udovic et al., 2007; Wang et al., 2006; Wen et al., 2006).

2.6 Interaction of rhizosphere and drilosphere

Plant growth and production are dependent on the interaction between roots and biotic and abiotic components of soils (Pinton et al., 2001). The rhizosphere ranges from less than 1 mm from the root surface to several millimeters and is a highly complex environment within the soil matrix where multiple interactions occur affecting the biophysicochemical properties of the soil (Uren, 2007). The spatial territory of the rhizosphere is also influenced by soil structure, particle size, water content, and buffering capacity (Darrah, 1993; Jungk et al., 2002; Nye, 1984). The rhizosphere plays a critical role in enhancement of plant-available nutrients and water and in facilitating the existence of the soil biota (Bertin et al., 2003; Uren, 2007). The rhizosphere withdraws dissolved nutrients from the soil solution and solubilizes soil minerals such as N and P, particularly through root exudation of ions, oxygen, water, enzymes, mucilage, and carbon relating primary and secondary metabolites (Bais et al., 2006; Bertin et al., 2003; Neumann et al., 2002). Root exudates can provide a hotspot for microorganisms such as endo- and ecto-arbuscular mycorrhizal fungi (AMF), nitrogen-fixing bacteria, and plant growth-promoting bacteria (PGPB) (Bais et al., 2006; Raaijmakers et al., 2009). Clearly, it is important to improve our understanding of the dynamics and complexity of the rhizosphere.

Interactions between the rhizosphere and drilosphere occur both spatially and temporally. The drilosphere is defined as a 2 mm wide zone around earthworm burrows, and this represents a microsite often enriched in soil organic matter and nutrients and with a generally higher accessibility than bulk soil for root foraging (Brown et al., 2000; Kautz et al., 2013). AMF spores are moved throughout the rhizosphere by earthworms (Milleret et al., 2009), and fungal growth is stimulated though enhanced organic matter mineralization. Earthworms also influence plant growth by changing the spatio-

temporal availability of nutrients such as carbon, nitrogen, and phosphorus in the drilosphere (Stromberger et al., 2012).

Clearly there are complex and profound interactions between the rhizosphere and the drilosphere. The rhizosphere provides a food source for earthworms through root residues. It has been reported that living roots are consumed by earthworms, particularly by endogeic species (Lubbers et al., 2011). Undoubtedly however they primarily feed on sloughed and decaying roots as well as other products from the rhizosphere, such as mucilages, root exudates, and associated fungi, bacteria, nematodes, and protozoa (Brown et al., 2000). Reciprocally, the drilosphere provides root channels and a substrate which is preferential for root elongation and nutrient uptake. Burrow walls are smooth and cemented with mucous secretions that contain high concentrations of organic N, ammonium (NH_4^+), and nitrate (NO_3^-) (Lavelle, 1988). In addition, earthworm burrows induce the preferential flow of water and solutes resulting in abundance of NO_3^- and labile-C in the wall of earthworm burrows by allowing infiltration of water (Syers and Springett, 1983). In particular, the existence of burrow walls and casts is beneficial to root proliferation in deeper soils (Brown et al., 2000). As described previously (#2.5.2), decomposed tissues of dead earthworms would also be expected to provide a readily-available localized supply of fertilizer for plant roots.

Overall, the inextricable connection between the rhizosphere and the drilosphere is likely to have considerable significance. There are obvious benefits to an improved understanding of these interactions. Such knowledge may influence management of physical, chemical and biological soil properties, as well as plant growth and microorganism diversity to enhance plant production and maintain soil ecosystem sustainability.

2.7 Biosolids disposal by vermicomposting

Large quantities of organic wastes from domestic, agricultural and industrial materials raise environmental and economic issues globally. Of the organic wastes, biosolids comprise the treated solid fraction of sewage, containing high concentrations of organic matter and plant nutrients (Gartler et al., 2013) which stimulate soil microbial and enzymatic activities and enhance soil nutrients status and plant growth (Evanylo et al., 2005; Gardner et al., 2010; Madejón et al., 2006; San Miguel et al., 2012). Potentially, the biosolids can be utilized as a highly nutritious soil amendment in agricultural lands as well as for remediation and revegetation projects (Kinney et al., 2008). However, there are concerns about contaminants within biosolids, including heavy metals (Silveira et al., 2003), persistent organic pollutants (Clarke and Smith, 2011), antibiotics, pharmaceuticals and pathogens (Garrec et al., 2003; Jones-Lepp and Stevens, 2007).

Vermicomposting, which is biodegradation of sewage sludge by earthworms, has been introduced as an environmentally-friendly technique of biosolid disposal since the early 1990s.

Research and commercial projects for vermicomposting have developed in many countries including USA, Germany, New Zealand and elsewhere (Edwards and Arancon, 2004). Vermicomposting technology has been touted as being cost effective (Hand et al., 1988) with beneficial aspects of high rates of mineralization, reflecting increases in plant availability of nutrients such ammonium (NH_4^+), nitrate (NO_3^-), potassium (K), calcium (Ca), zinc (Zn), copper (Cu), and sulphur (S) (Dominguez and Edwards, 2004; Sharma et al., 2005). It has been also claimed that vermicomposting can promote microbiological activities which potentially could enhance beneficial hormones and enzymes to plants (Sharma et al., 2005). Furthermore, the vermicomposting can degrade human pathogens such as *E. coli* and *Salmonella* by releasing antibiotic materials from their coelomic fluids and from some bacteria and fungi (e.g. *Aspergillus* spp. and *Penicillium* spp.) in the intestines of earthworms (Eastman et al., 2001; Sinha et al., 2010; Yadav et al., 2010).

Selection of earthworm species is very important for vermicomposting. Most vermicomposting employ epigeic earthworm species such as *Eisenia fetida*, *E. andrei*, *Lumbricus rubellus*, *Eudrilus eugeniae* and *Perionyx excavates* that are tolerant of high temperature of composts and toxic components in the biosolids, especially heavy metals and ammonia (Artuso et al., 2010; Mitchell et al., 1980; Ndegwa and Thompson, 2001; Suthar, 2010; Yadav and Garg, 2011; Yadav et al., 2010). *E. fetida* has a particularly wide capacity of tolerance to temperature (5 to 42 °C). These epigeic species potentially have greater capacity of waste decomposition with higher fecundity than endogeic and anecic species (Gajalakshmi and Abbasi, 2004), but epigeic species only breakdown organic matter on the soil surface (Ismail, 1997). Anecic species may be more useful when biosolids and other organic wastes are applied as a soil amendment since they burrow and incorporate the wastes into soil, thereby improving nutrient recycling and physical structure through soil profile. It is likely that the presence of anecic species or combination of anecic, epigeic, or endogeic species may be most beneficial for the management of biosolids-amended soil (Sharma et al., 2005). Furthermore, there appears to be no knowledge of the existence of native epigeics in New Zealand. Only one species was found in the present study, sampled in litter of a native sandplain forest at Punakaiki (Figure 2.4).



Figure 2.4 An unidentified epigeic earthworm (approx. 20 mm, with unusual spotted markings) on *Coprosma grandifolia* leaf litter at the Nikau Reserve in Punakaiki.

Chapter 3

Interactions of native and introduced earthworms with soils and plant rhizospheres in production landscapes of New Zealand

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Interactions of native and introduced earthworms with soils and plant rhizospheres in production landscapes of New Zealand



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ABSTRACT

Native and exotic earthworms and plants co-exist on the margins of agricultural land in New Zealand. Remnants of native vegetation support mixed assemblages of depleted populations of native Megascolecidae earthworms together with apparently increasing invasive populations of introduced Lumbricidae. We question whether the survival and viability of these earthworm populations is a function of soil preference and whether there are significant differences in terms of how the two groups are influenced by and modify soil properties and plant growth. Choice chamber and mesocosm experiments, with and without plant rhizospheres, were used to study five species of native earthworms, two of which could be identified only by DNA barcoding, and four introduced exotic species. Both natives and exotics preferred agricultural soils to a plantation forest and a native forest soil. Earthworms also modified the physico-chemistry of soils and greenhouse gas emissions, with a marked interaction with root morphology of two native species of tea tree. Lesser differences were found between native and exotic earthworms than between functional groups. It is concluded that New Zealand's production landscapes provide novel habitats with clear benefits both to threatened species conservation and to soil ecosystem services.

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3.1 Abstract

Native and exotic earthworms and plants co-exist on the margins of agricultural land in New Zealand. Remnants of native vegetation support mixed assemblages of depleted populations of native Megascolecidae earthworms together with apparently increasing invasive populations of introduced Lumbricidae. We question whether the survival and viability of these earthworm populations is a function of soil preference and whether there are significant differences in terms of how the two groups are influenced by and modify soil properties and plant growth. Choice chamber and mesocosm experiments, with and without plant rhizospheres, were used to study five species of native earthworms, two of which could be identified only by DNA barcoding, and four introduced exotic

species. Both natives and exotics preferred agricultural soils to a plantation forest and a native forest soil. Earthworms also modified the physicochemistry of soils and greenhouse gas emissions, with a marked interaction with root morphology of two native species of tea trees. Lesser differences were found between native and exotic earthworms than between functional groups. It is concluded that New Zealand's production landscapes provide novel habitats with clear benefits both to threatened species conservation and to soil ecosystem services.

Keywords

Soil ecology, earthworms, nitrogen, ecological restoration, ecosystem services

3.2 Introduction

Due to a long period of geographic and evolutionary isolation, New Zealand is one of the world's biodiversity hotspots; more than 80 % of most floral and faunal groups are endemic and found nowhere else (Trewick et al., 2007). Human colonisation and introduction of mammalian pests to these islands has been relatively recent, but native biodiversity has been impacted particularly severely (Lee, 1961; MacLeod and Moller, 2006; Sparling and Schipper, 2002). Agricultural modification of landscapes, vegetation and soils has certainly been to the detriment of native earthworms (Bowie et al., 2016; Lee, 1959a; Molloy, 1988).

Megascolecid earthworms are naturally well represented in the endemic fauna of New Zealand, with 177 recognised species (Glasby et al., 2009; Lee et al., 2000; Lee, 1959a; Sims and Gerard, 1985) that are otherwise poorly described in the scientific literature, compared with the 17 species of exotic introduced lumbricids. One of only a few recent field surveys of New Zealand's native earthworms revealed extensive cryptic taxonomic diversity with about 48 additional species (Buckley et al., 2011). The province of Canterbury on South Island has 25 recorded species, many of which are dispersed through the lowland plain that has been largely converted to intensive agriculture (Winterbourne et al., 2008). Several additional species found in Canterbury by two authors of the present paper (SB and YK) are currently in the process of formal recognition subsequent to DNA barcoding.

Native earthworms apparently disappeared quickly following conversion of land to agriculture, which was then colonised intentionally or unintentionally by introduced exotic European Lumbricidae, predominantly *Aporrectodea caliginosa*, *A. longa*, *A. rosea*, *A. trapezoides*, *Lumbricus rubellus* and *Octolasion cyaneum* (Fraser et al., 1996; Lee, 1961; Springett et al., 1992, 1998). Endemic earthworms have found refuge beneath small remnants of native vegetation on the borders of agricultural land, which account for less than 1% of the vegetation cover of Canterbury (Winterbourne et al., 2008). In these restricted areas we have found that it is common to find coexisting assemblages of both native and exotic earthworms. It is recognised that ground disturbance through burning, vegetation clearance and ploughing played a major role in the demise of native megascolecids, as is the case elsewhere in the world (Edwards and Bohlen, 1996; Hendrix, 2006). However, little is known of the interdependence and interactions between soil properties and the presence, absence or combinations of natives and exotic species. This lack of knowledge has much relevance in terms of both conservation of endemic species and the potential benefits of native earthworms to soil quality and ecosystem services.

Earthworms are known to mediate structural and functional processes in soil including aggregate stability, porosity, organic matter dynamics and nutrient cycling (Al-Maliki and Scullion, 2013; Edwards, 2004; Lee, 1985). They facilitate the mineralization of nitrogen and phosphorus from organic matter, thus stimulating plant growth and development (Blakemore, 1997; Sizmur and Hodson, 2009). These beneficial effects are weighed against the potentially detrimental effect of

earthworm burrowing enhancing the preferential flow pathways for water and nitrate movement to waterways and increasing the release of greenhouse gases (Kernecker et al., 2014). Clearly, earthworms potentially have an important role both in management and mediation of the environmental footprint of production systems.

The aims of the present study were (i) to elucidate the predilection of native earthworms for soils that have become nutrient enriched and otherwise modified by agriculture and forestry, and (ii) to begin to understand the functionality and role of native earthworms alongside introduced species on marginal land, refugia and restoration plantings within production landscapes. A series of laboratory and glasshouse experiments were devised to compare the interactions of native and introduced earthworms with variously-modified soils and two native plant rhizospheres.

3.3 Materials and methods

3.3.1 Soils

Surface soils (0-15 cm) were collected from two Lincoln University farms situated close to the Lincoln University campus (Table 3.1). One is an intensively-managed, irrigated and fertilised dairy farm (referred to as DF) soil, well represented on intermediate terraces in Canterbury (Molloy, 1988). A second soil from a nearby dryland sheep farm (referred to as SF) has a lower-capacity for storing water due to a high stone content, although the collected surface horizon of soil beneath the turf was largely free of stones. Sheep-farming since the mid-19th century will have involved some degree of ploughing, top-dressing and reseeded, but this site had no recent history of fertilization or intensive management. A third Canterbury soil was collected from a relatively undisturbed plantation forest (referred to as PF) of non-native *Pinus radiata* that was established in about 1930 on land that had been used for perhaps the previous 50-80 years by European settlers for extensive sheep grazing. The original vegetation was probably degraded through burning by Maori in the centuries before this, but remnants of native plants (dominantly *Kunzea robusta*, Myrtaceae, kānuka) still exist. By way of further contrast, a fourth soil was collected from a native forest (referred to as NF) on the west coast of South Island. This soil has had little modification from its natural state, and incorporated a substantial organic component from plant litter. This location has much higher rainfall of >2000 mm, compared to mean annual regional rainfall of 630 mm at the Canterbury sites, and supports luxuriant indigenous broadleaf forest (Hahner et al., 2013; Rhodes et al., 2013).

Stones were removed from soils, using 4 mm sieves, and soils were stored for periods of up to 3 months prior to use in experimental work.

Table 3.1 Location of the four soils collected for experimental work. Distance refers to distance from Dairy Farm (DF).










	Dairy Farm (DF)	Sheep Farm (SF)	Plantation Forest (PF)	Native Forest (NF)
Location	43°38'11.27"S, 172°26'17.56"E University grounds	43°38'39.48"S, 172°23'28.07"E Gammack Estate	43°25'24.55"S, 172°18'28.14"E Eyrewell	42°8'38.39"S, 171°19'50.36"E Punakaiki
Distance (km)	0	5	25	200
Classification	Templeton (Immature Pallic)	Eyre (Immature Pallic)	Lismore (Orthic Brown)	Karoro (Sandy to Orthic Brown)
Description	Well-drained, fine sandy to silty alluvium. High WHC. Ryegrass paddock.	As for DF, but stonier, freer draining, with lower WHC. Ryegrass, Cocksfoot paddock.	Very similar to SF, but more stony. Mature <i>Pinus radiata</i>	Leached soil on sandplain of old marine and river terraces. Broadleaf, Podocarp.

3.3.2 Earthworms

Five native species of earthworms representing epigeic, anecic, and endogeic functional groups were collected from locations in South Island, New Zealand. Three of these species have been described (*Deinodrilus* sp.1, *Maoridrilus transalpinus*, and *Octochaetus multiporus*) and are known to occur in Canterbury, but the remaining two are abundant but appear to be undescribed and are likely to be new to science (Table 3.2). We also collected specimens of four exotic species of lumbricid earthworms. Three of these (*Aporrectodea caliginosa*, *Octolasion lacteum*, and *O. cyaneum*) are endogeics that are well represented on agricultural land, amongst about 19 species of exotics in New Zealand. The fourth exotic species, *Eisenia fetida* (an epigeic species), was collected from local compost heaps. The species of the present study were selected largely by virtue of ease of collection in large enough numbers by digging, abundance of adults during field sampling, most easily-recognizable morphology, and survivorship under laboratory conditions. Native species were initially identified morphologically using keys and descriptions from (Lee, 1959a, b), followed by molecular methods using a DNA barcoding approach based on the cytochrome oxidase subunit 1 (COI) and 16S rDNA regions, as described previously (Boyer et al., 2011a).

For each part of the experimental work, earthworm species were further selected on the basis of the visually most viable and healthy laboratory cultures on each set-up occasion (Table 3.3).

Table 3.2 Species of earthworms used in the experiments, their origin, ecology and some aspects of morphology. Endemic earthworms were named based on morphological identification following Lee (1959a, b). Specimens that did not match any know description were considered undescribed and were attributed a code name. Functional group was determined based on earthworm location in the soil profile as well as general morphology and behaviour.

Functional Group	Name of Species	Status	Description				Photo
			Origin	Colour	Length (mm)	Prostomium	
Epigeic	<i>Deinodrilus</i> sp.1	Native	Nikau Reserve	Dark brown with reddish head	80-120	Tanylobous	
	<i>Eisenia fetida</i>	Exotic	Compost and manure heaps	Red or brown with transverse pigmented bands	32-130	Epilobous	
Anecic #	<i>Maoridrilus transalpinus</i>	Native	Banks Peninsula, Lincoln township	Brown with dark clitellum	160-200	Tanylobous	
	<i>Maoridrilus</i> sp.2	"	Lincoln University	Pale orange with reddish brown head	120-150	Tanylobous	
Endogeic	<i>Megascolecidae</i> sp.1	Native	Nikau Reserve	Pale pink or white	60-80	Tanylobous	
	<i>Octochaetus multiporus</i>	"	Banks Peninsula, Lincoln township	Pale pink or white	180-300	Prolobous	
	<i>Aporrectodea caliginosa</i>	Exotic	"	Colour variable, dark green	30-80	Epilobous	
	<i>Octolasion cyaneum</i>	"	"	Bluish grey and bright yellow in tail	65-180	Epilobous	
	<i>O. lacteum</i>	"	Punakaiki coastal restoration area	Grey and yellow spot in tail	45-55	Epilobous	

Based on laboratory observations of the authors.

Table 3.3 Earthworm species and soils used in the experiments.

		Choice chambers		Incubation experiment	Plant-soil-earthworms mesocosms		
		I	II		Mānuka	Kānuka	Ryegrass
Earthworm species	<i>Deinodrilus</i> sp.1	√		√	√		
	<i>Eisenia fetida</i>		√				
	<i>Maoridrilus transalpinus</i>		√	√		√	√
	<i>Maoridrilus</i> sp.2		√			√	√
	<i>Megascolecidae</i> sp.1				√		
	<i>Octochaetus multiporus</i>			√			
	<i>Aporrectodea caliginosa</i>			√			
	<i>Octolasion cyaneum</i>		√				
	<i>O. lacteum</i>	√		√	√	√	√
Soils	DF	√	√				
	SF	√	√	√	√	√	√
	PF		√				
	NF	√	√				

3.3.3 Choice-chamber experiments

Simple choice chamber pieces of apparatus were constructed (Figure 3.1) to investigate whether earthworms had clear preferences for the different soils in a series of separate assays. The apparatus was made up of five 400 ml polypropylene containers; the exterior four chambers amended with 200 g of different soil treatments, with an intermediate chamber into which earthworms were introduced. A commercially-available organic compost (intelligro.co.nz) that provided a suitable medium for maintaining the cultures, was placed in a fourth chamber. A moisture content of 30 % was established and maintained in each soil by weighing. The species of earthworms in each assay was dependent of the availability, numbers and viability of cultures that were being maintained in the laboratory throughout. All comparisons between species made in this paper refer to replicate choice-chamber assays run at the same time. Using different species (Table 3.3), groups of five earthworms per species were placed in the central chamber of the apparatus which was then maintained in darkness. After a period of one week, the apparatus was emptied to observe where the earthworms were resident. Each soil was also carefully evaluated for visible evidence of burrowing activity. Representative fresh bulked sub-samples from each of the replicates, were then analysed for pH, EC and mobile fractions of N as described below.



Figure 3.1 Choice chamber apparatus for earthworm preference tests. Multiple sets of apparatus were used to run each trial at the same time, with 5 earthworms per choice chamber apparatus. Earthworms shown in *Deinodrilus* sp.1.

3.3.4 Incubation experiments

Five species of earthworm were placed separately in 250 g of wetted SF soil (30 % moisture) within 400 ml polypropylene containers (Table 3.3). A gauze covering prevented the earthworms escaping and soil moisture was maintained on a weekly basis by weighing each container and adding appropriate amounts of water. Sawdust (2 g) was added as a food source, having been previously

found to maintain earthworm viability whilst adding minimal additional nutrients into the containers. Four replicates of each treatment were maintained in the dark in an incubator at 15 °C for 3 weeks, with a randomised arrangement of the containers. The same procedure was followed in four reference pots without earthworms. Earthworm survival was monitored on a weekly basis with minimal disturbance of the soils. On completion of this experiment, earthworms were removed and the soils were sampled and analysed as described below.

3.3.5 Plant-soil-earthworm mesocosms

Uniform one-year old plants of native tea trees, *Leptospermum scoparium* (Myrtaceae, mānuka) and *Kunzea robusta* (Myrtaceae, kānuka) grown in plugs were obtained from the Department of Conservation nursery at Motukarara. Plants of each species were transplanted singly into 20 plastic plant pots filled with 1.3 L of SF soil. Five treatments consisted of three species of native earthworm, one species of exotic earthworm and plants without earthworms as a control (Table 3.3). Each treatment contained two individual earthworms, with 5 replicate pots [2 plant species x 5 earthworms treatments x 5 replicates = 50 pots]. To stop earthworms escaping, drainage holes in the bottom of the pots were sealed with a nylon mesh which was also placed over the top of the pots and sealed around the single woody stems of the plants. The pots were maintained in a glasshouse for 7 weeks, after which the pots contents were removed and compared. This experiment was carried out on two occasions with different species of earthworms as they became available in suitable numbers. In the second experiment, a further 20 pots were sown with perennial ryegrass (*L. perenne*) as an additional treatment to provide a dairy pasture comparison. Plant growth (biomass at final harvest), earthworm survival and root structure (biomass and photographic comparisons) were measured.

3.3.6 Analytical

All soils were analysed in-house by Analytical Services in the Department of Soil and Physical Sciences at the Lincoln University using standard methodologies, with ASPAC Ring Test QA procedures. Following extraction with 2M KCl using fresh soil, samples were analysed for available-N using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden), attached to a spectrophotometer (Blakemore, 1987; Clough et al., 2001). Air-dried soil samples were sieved to <2 mm using a metal sieve. Soil pH and electronic conductivity (EC) were measured using pH and EC meters (Mettler Toledo Seven Easy). Total N and C were analysed by a Vario-Max CN elemental analyser (Elementar GmbH, Germany). Oven-dried (80 °C) soil samples were analysed for loss on ignition (LOI) in a muffle furnace. Following microwave digestion of oven-dried soil, Total-P was analysed using ICP-OES (Varian 720 ES, USA). Available-P was determined as Olsen P, using 0.5M NaHCO₃ extractant and a UV160A spectrophotometer (Shimadzu, Japan) (Blakemore, 1987). Soils from the choice chamber and

incubation experiments were analysed for LOI at the beginning and end of the experiments to provide an estimate of the amount of organic matter consumed.

Gas sampling was conducted at 16 °C in the incubation experiments after 20 days in all treatments except those containing *A. caliginosa*, where high mortality rates were being recorded at the time. Lids placed on the 400ml containers left about 20 ml of headspace above the soil, from which 10 ml aliquots of gas were sampled 0, 20, and 40 min after sealing. Emission rates were calculated from regression equations. Nitrous oxide (N₂O) and carbon dioxide (CO₂) were analysed using a gas chromatograph (SRI 8610 GC, CA, USA) with a ⁶³Ni electron capture detector and flame ionisation detector, linked to an autosampler (Gilson 222 XL, USA). All methods follow those described by Clough et al. (2006).

3.3.7 Statistical analysis

Data were analysed using Minitab (Minitab Inc., State College, Pennsylvania, USA). To compare means of each earthworm species treatment, data were analysed using one-way ANOVA with Fisher's least-significant-difference post-hoc test.

3.4 Results and discussion

3.4.1 Soils

The selected soils had contrasting physicochemical characteristics as expected. The native forest soil was more acidic than the agricultural soils, with substantially higher organic matter, total N and total P (Table 3.4). Lability of both N and P presented the opposite picture, with higher concentrations of soluble nitrate and mobile P in the agricultural soils. High Olsen P in SF, despite low Total-P is an anomaly that has been described previously (Randhawa, 2003). Organic forms of N and NH₄⁺ were much more prevalent in the native forest soil, but there was much less mobile NO₃⁻. The absence of fertilisation is evident in the plantation forest soil.

Table 3.4 Properties of the four soils and compost used in experimental work. Values in blankets represent standard error of the mean (n=3). Plantation forest values are mean of two samples.

Properties	Dairy Farm (DF)	Sheep Farm (SF)	Plantation Forest (PF)	Native Forest (NF)	Compost
Texture	Silt loam	Silt loam	Silt loam	Silt loam	–
pH (1:5W)	5.6 (<0.1)	5.4 (<0.1)	5.0 (<0.1)	4.7 (0.2) [†]	5.1 (<0.1)
OM (%)	7.3 (0.2)	7.5 (0.1)	4.3 (0.1)	22.6 (0.2)	75.4 (0.6)
Total C (%)	3.3 (0.4) [‡]	3.3 (0.1)	2.4 (0.1)	10.7 (3.4) [†]	–
Total N (%)	0.2 (<0.1) [‡]	0.3 (0.0)	0.1 (<0.1)	0.7 (0.2) [†]	–
C/N Ratio	15.0 (<0.1) [‡]	12.2 (0.3)	17.2 (2.0)	16.9 (0.9) [†]	–
NH ₄ -N (mg·kg ⁻¹)	1.8 (0.5)	2.9 (0.5)	0.2 (0.2)	19.6 (1.4)	37.3 (3.5)
NO ₃ -N (mg·kg ⁻¹)	118 (12)	88.1 (0.6)	< 0.1	18.3 (0.5)	462 (28)
Total P (mg·kg ⁻¹)	596 (7) [‡]	341 (-)	335 (-)	836 (116) [†]	–
Olsen P (mg·kg ⁻¹)	23.8 (0.2)	34.4 (0.1)	4.8 (0.1)	15.5 (0.2)	–

[†] Hahner et al. (2013); [‡] Gartler et al. (2012)

3.4.2 Soil preference

The first Choice Chamber assays clearly showed that native and exotic earthworms selected agricultural soils in preference to native forest and plantation forest soils and compost (Figure 3.2). This was not a case of earthworms preferring the soil they were acclimatized to, since they had been collected from a range of different sites, none of which were dairy farm or sheep farm soil (Table 3.1). Earthworms in the early trials tended to lose weight; this varied between species, ranging from 0.2 % fresh weight losses in *Deinodrillus* sp.1 to 15.4 % weight loss in *O. lacteum*. More detailed Choice Chamber studies resolved this problem, showing earthworms did not select the most organic soils, although the amount of organic matter consumed corresponded with soil preference (Figure 3.3). In these trials, fresh weight gains over one week were 2.1 % (*M. transalpinus*), 12.1 % (*Maoridrillus* sp.2), 2.6 % (*O. cyaneum*), and 5.5 % (*E. fetida*). In addition, compost used in the first trial tended to be unpalatable to earthworms, and its hard texture had not been comminuted by earthworm feeding a week after inoculation. Plantation forest soils replaced this compost in the second Choice-chamber assay.

It is counter-intuitive that native species, or even lumbricid earthworms, would prefer the physico-chemical conditions of farmed soils to the soils of a plantation forest and a native forest. They had a predilection for less acid soils with lower C/N ratios and higher soluble P, although the actual causal factors for their preference are unknown. High levels of OM were less important, and earthworms were not sensitive to high soil NO_3^- -N. Eijsackers (2011) considered that abiotic factors such as pH, soil type and organic matter play a more important role than inherent ecological characteristics of the particular species. The findings of the present study appear to support this, with few discernible or consistent differences between native and exotic species or between functional groups. Earthworms have been found to use chemical odours to guide their foraging behaviour towards microbial food sources (Zirbes et al., 2011) and are likely to be able to detect NH_4^+ . However, it appears from the results of the present study that the likely attraction of high levels of organic matter is outweighed either by higher soil pH or avoidance of elevated NH_4^+ . The high NO_3^- -N that is quite typical of agricultural soils, but a less easy form of N for animals to detect, does not appear to be a deterrent.

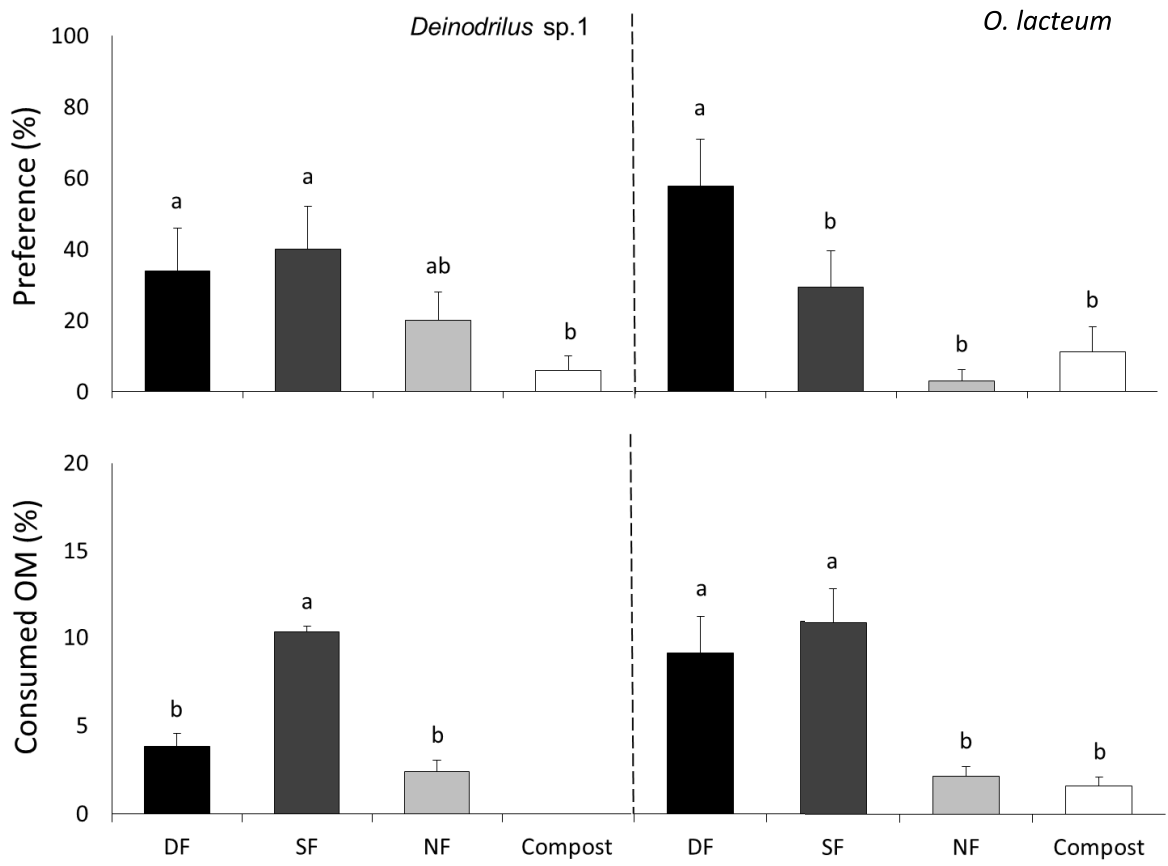


Figure 3.2 Preferred soils of two species of earthworm added to the choice chambers in Trial I, after one week. Consumption of organic matter refers to LOI changes after 7 days. Shading distinguishes DF (■), SF (▒), NF (░), and Compost (□). Values are means ± standard errors (n=5). The same letters indicate no significant difference ($p < 0.05$).

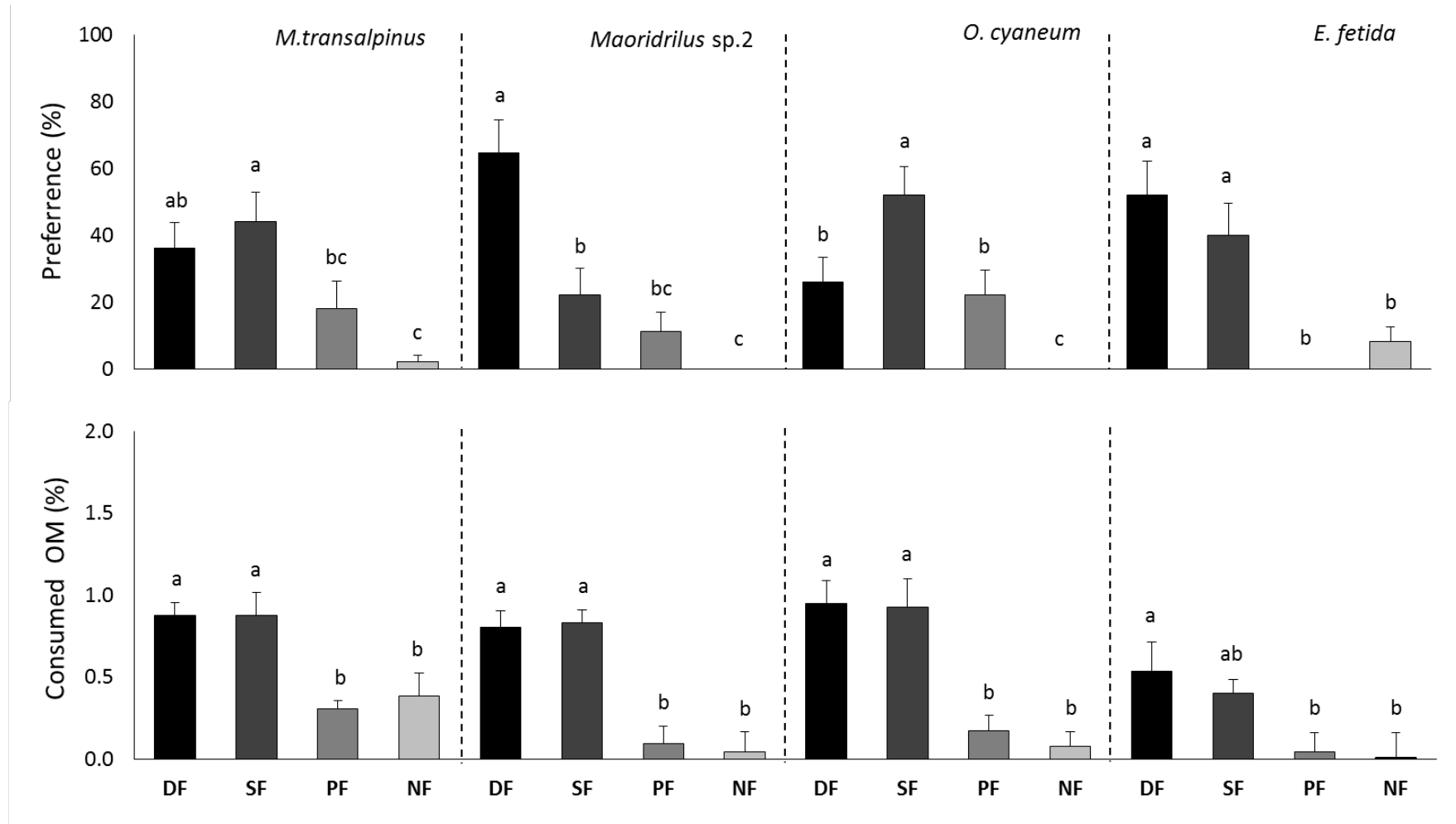


Figure 3.3 Soil preferences in choice chamber trial II. Consumption of organic matter refers to LOI changes after 7 days. Shading distinguishes DF (■), SF (■), PF (■), and NF (■). Values are means ± standard errors (n=10). The same letters indicate no significant difference ($p < 0.05$).

3.4.3 Effects on soil properties

After three weeks of the incubation in SF soil, earthworm activity had marginally increased Electrical Conductivity (EC), Microbial Biomass Carbon (MBC) and Olsen P (Table 3.5). There were no significant differences, or else only negligible differences, between the soils with and without earthworms, in terms of pH, OM and C/N ratio (data not shown). Other studies with longer incubation periods have shown a much more pronounced effect on mobile P (e.g. Scheu and Parkinson, 1994; Vos et al., 2014). In the present study, an initial soil pH of 5.4 of the sheep farm soil was reduced to 4.85-4.90 after being wetted and incubated in the reference containers (without earthworms), but was reduced no lower than pH 4.7 in earthworm treatments. In future work it would be advisable to allow for an initial period of wetting before the experiment begins. A decline in soil pH has been reported previously (Cheng and Wong, 2002), although many studies have demonstrated that earthworm activity increase soil pH towards neutrality, due to excretion of intestine and cutaneous mucus (Cole et al., 2006; Edwards and Bohlen, 1996; Schrader, 1994).

Earthworms have been shown previously to increase nitrification and denitrification activity (Parkin and Berry, 1999). They probably also have impacts on nitrate leaching in soil through both their effects on mineralisation of organic N and water movement through burrow channels, but these effects have not yet been quantified under New Zealand conditions (Fraser, 2010). In our study, increased microbial biomass led to increased OM decomposition that is reflected in increased respiration and more release of NH_4^+ from organic N in the earthworm incubated soils (Figure 3.4). This appears to have a knock-on effect causing marginal increases of soluble $\text{NO}_3\text{-N}$ associated with earthworm activity.

In this study, increased NH_4^+ concentration by *O. multiporus* tended to enhance nitrous oxide emission. This may be due to the relatively large size of this earthworm and the release of more mucus and secretion in the drilosphere wall stimulating denitrification. Release of substantially more CO_2 (by 33 %) and N_2O (by 42 %) by burrowing endogeic species has also been reported elsewhere from a detailed meta-analysis from 57 short-term studies each of up to 200 days (Lubbers et al., 2013b), although production of N_2O from denitrification requires anaerobic conditions. Of course, earthworm burrowing increases aerobic conditions, and therefore the main source of N_2O would be expected to be from nitrification (Chen et al., 2013). However, earthworms are known to also stimulate nitrification, leading to enhanced release of N_2O (Postma-Blaauw et al., 2006). Presumably the requisite anaerobic conditions could occur in microsites in the walls of the drilosphere.

The data from the present study have been extrapolated to be shown as $\text{g}\cdot\text{ha}^{-1}\cdot\text{day}^{-1}$. Of the earthworms studied, *O. multiporus* released N_2O ($9.1 \text{ g}\cdot\text{ha}^{-1}\cdot\text{day}^{-1}$), representing perhaps 1 % N_2O emissions reported elsewhere in fertilized pasture soils in New Zealand (e.g. de Klein et al., 2001). This may be of some concern since N_2O accounts for about 29 % of agricultural emissions in New Zealand

which has the highest agricultural GHG emissions for any developed country. This greenhouse gas is 310 times more potent than CO₂ and about 75 % of N₂O is emitted directly or indirectly from soils (Thorburn et al., 2012). Clearly this justifies further study.

Present-day agricultural systems seek to improve the efficiency of N usage, mainly to limit the release of reactive N to the wider environment as soluble NO₃⁻ and gaseous N₂O. It is argued that a shift is required towards NH₄⁺-dominated, low-nitrifying agricultural production systems. Enhancing the release of biological nitrification inhibitors from the roots of pasture grasses and cereals is receiving considerable attention (Subbarao et al., 2013), but management of tillage systems and interactions between the rhizosphere and earthworm communities will also play a significant role.

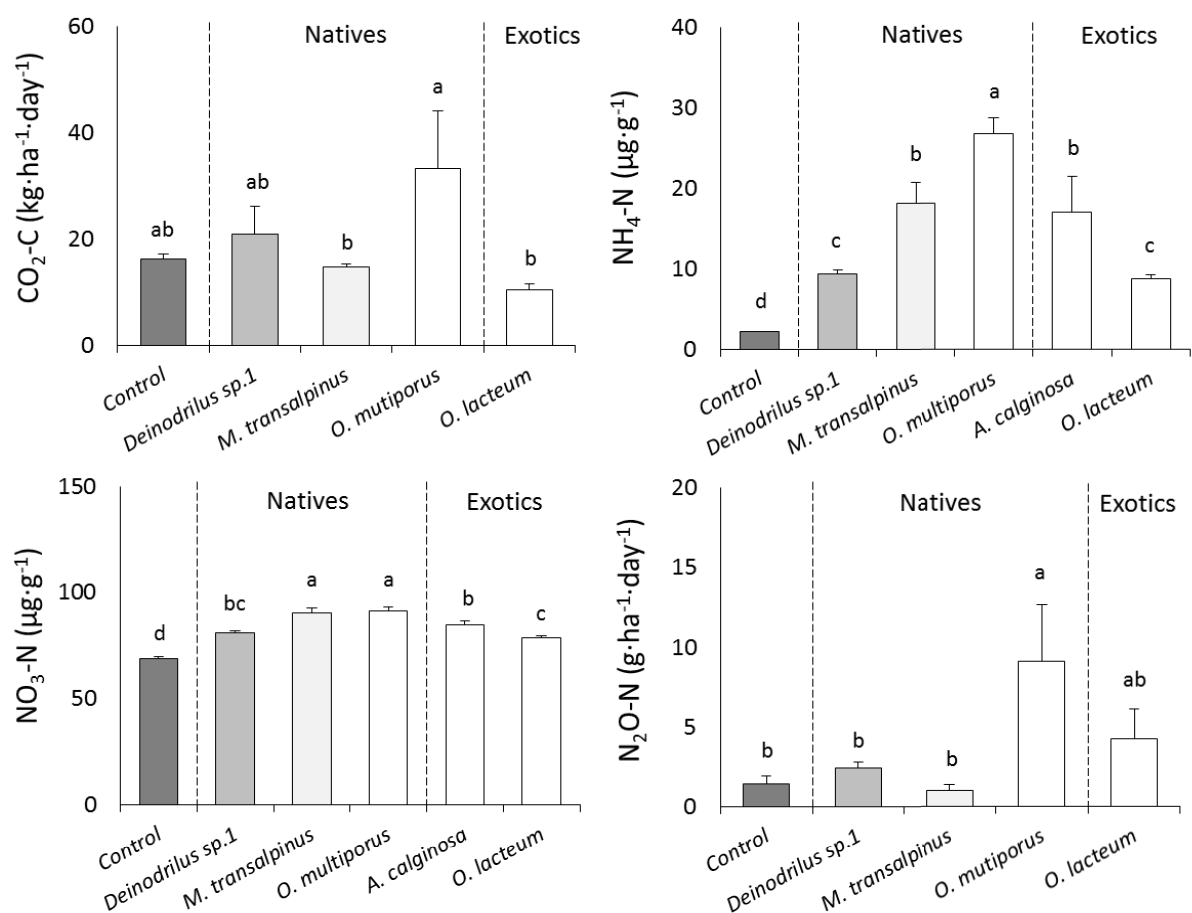


Figure 3.4 Mobile nitrogen (ammonium and nitrate) and release of N₂O (nitrous oxide) and CO₂ (carbon dioxide) in the presence of native and exotic earthworms during 3 weeks inoculation. Shading distinguishes control (■), epigeic (▒), anecic (□), and endogeic (□) species. Values are means ± standard errors (n=4 for mobile N and n=3 for gas measurement). The same letters indicate no significant difference (p<0.05).

Table 3.5 Changes of soil properties by all species of earthworm throughout incubation for 3 weeks in Dairy Farm soil. Value in brackets represent standard errors of the mean (n=4). The same letters indicate no significant difference ($p<0.05$).

Properties	Control	----- Native -----			----- Exotic -----	
		<i>Deinodrilus</i> sp.1	<i>Maoridrilus transalpinus</i>	<i>Octochaetus multiporus</i>	<i>Aporrectodea caliginosa</i>	<i>Octolasion lacteum</i>
EC (dS·m ⁻¹)	0.16 (0.00) ^c	0.19 (0.00) ^b	0.21 (0.01) ^a	0.21 (0.00) ^a	0.20 (0.01) ^{ab}	0.19 (0.00) ^b
Olsen P (μg·g ⁻¹)	34.1 (0.10) ^e	34.7 (0.13) ^{cd}	35.8 (0.24) ^a	35.2 (0.07) ^b	34.8 (0.13) ^{bc}	34.3 (0.14) ^{de}
MBC (μg·g ⁻¹)	122 (13) ^b	146 (23) ^b	161 (17) ^{ab}	141 (20) ^b	190 (46) ^{ab}	241 (54) ^a

3.4.4 Plant-soil-earthworm interactions

All tested species of earthworm increased the growth of ryegrass, and *Deinodrillus* significantly increased the above-ground growth of mānuka (Figure 3.5). When these root and shoot data were combined, they showed a general increase in growth of all plants in the presence of earthworms. More profound visual differences were evident between the root systems of different plants in the presence of earthworms (Figure 3.6). The compost around the original plant plugs of mānuka had been dispersed quite differently through the pots, and burrowing patterns evident in the soils as formed by different earthworms were also variable.

The presence of a plant rhizosphere hugely diminished the concentrations of mobile NO_3^- in soil, but raised the concentrations of NH_4^+ (Figure 3.7). These mobile forms of N tended to be higher with the additional presence of earthworms. In the presence of earthworms, there were differences between the plants: kānuka, increased NH_4^+ concentrations in soil, kānuka and mānuka raised NO_3^- , but here was no apparent effect in the presence of ryegrass. These results illustrate that we require a more detailed understanding of the interactions between soils, root systems, earthworms and soil chemistry, and their impact on the soil ecosystems of agricultural landscapes in New Zealand.

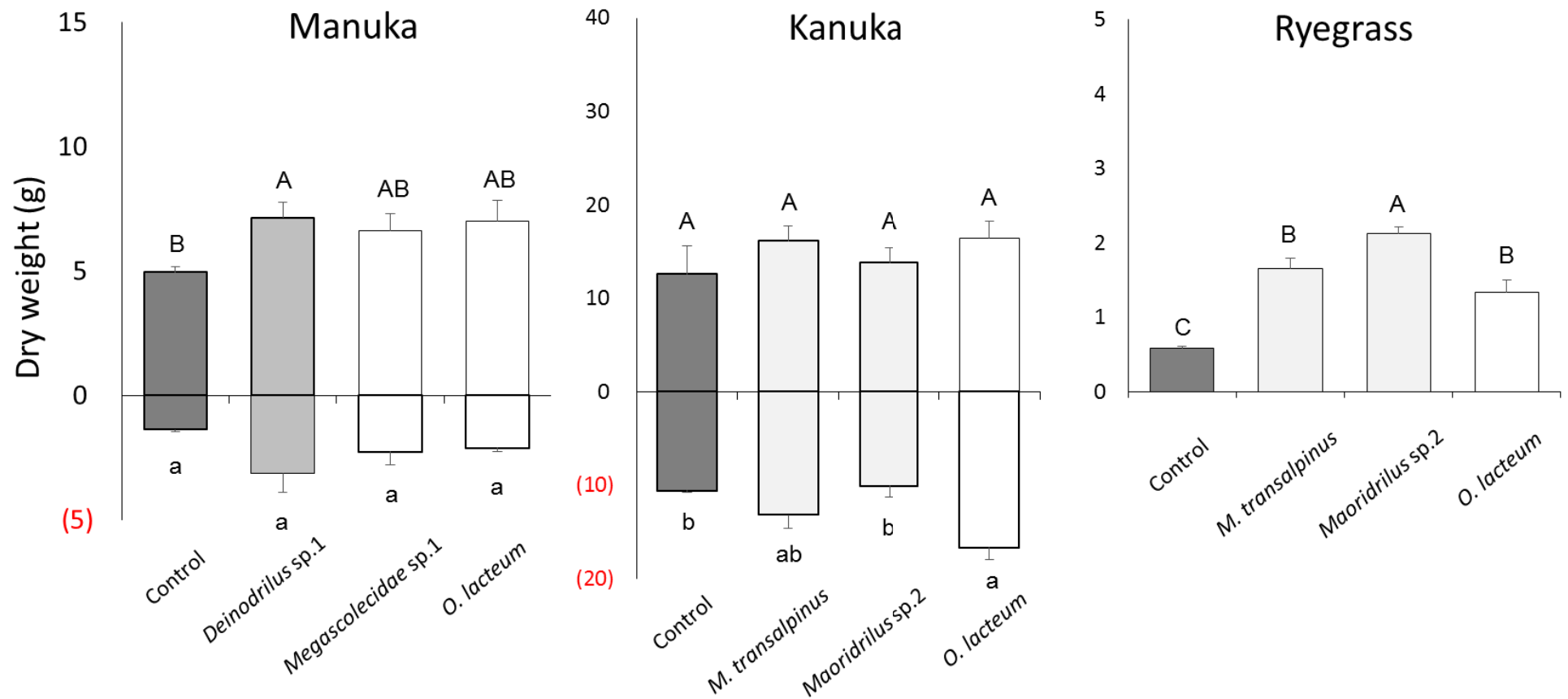


Figure 3.5 Biomass of two native plants (*L. scoparium* and *K. robusta*) and *L. perenne* in the presence of earthworms in DF soil, 7 weeks after earthworms were added. Upper bar is shoot dry weight and lower bar is root dry weight. Shading distinguishes control (■), epigeic (▒), anecic (□), and endogeic (□) species. Values are means ± standard errors (n=5). The same letters indicate no significant difference ($p < 0.05$).

Mānuka



Plant without earthworm



Deinodrillus sp.1



Megascolecidae sp.1



O. lacteum

Kānuka



Plant without earthworm



M. transalpinus



Maoridrilus sp.1



O. lacteum



Earthworm without plant

Figure 3.6 Rhizosphere structures in SF soil after 7 weeks growth with earthworms.

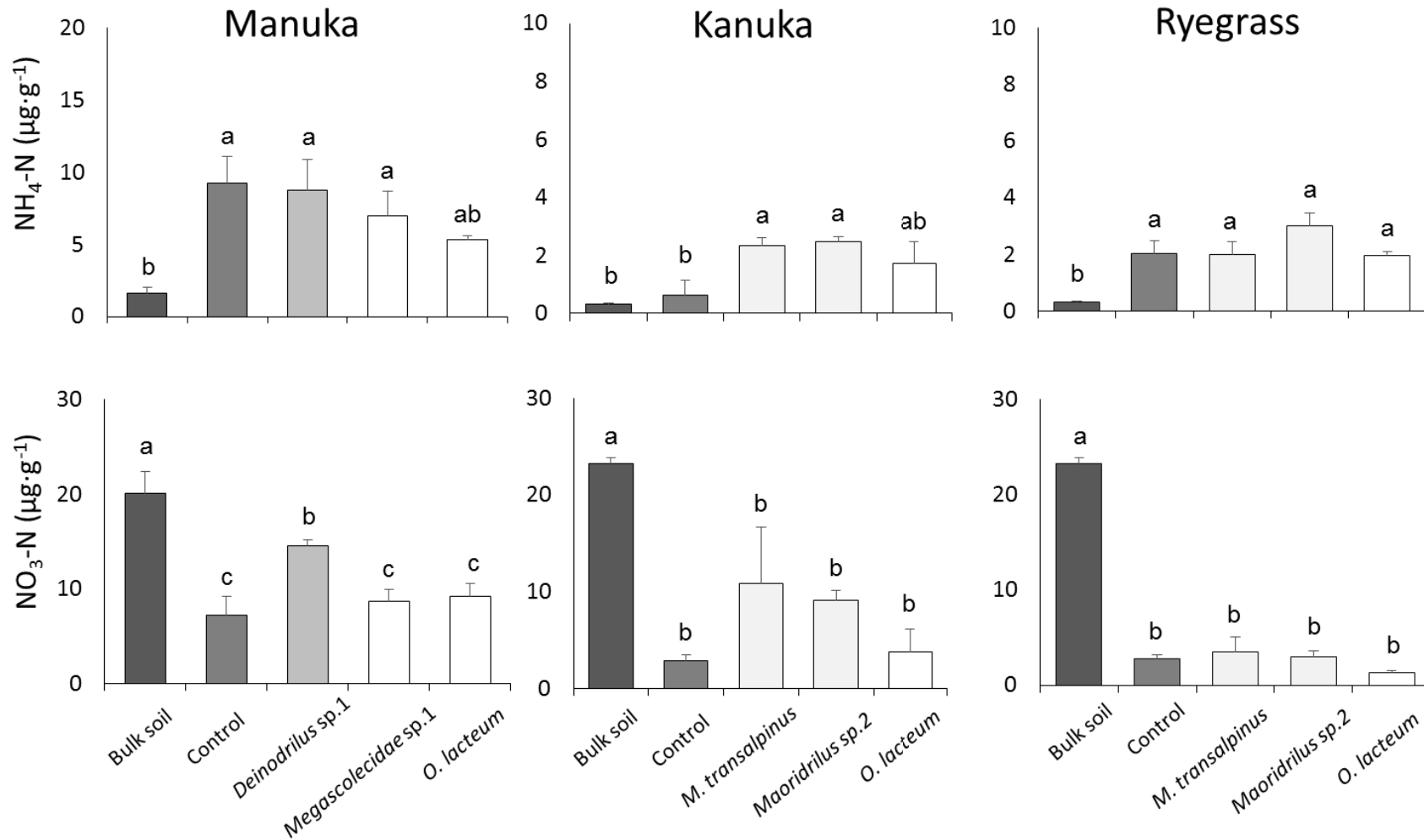


Figure 3.7 Mobile nitrogen concentration (NH₄-N and NO₃-N) in the rhizosphere SF soil of Mānuka, Kānuka and ryegrass, with native and exotic earthworms. Shading distinguishes bulk soil (darkest), control (dark), epigeic (medium), anecic (light), and endogeic (white) species. Values are means ± standard errors (n=5). The same letter indicate no significant difference ($p < 0.05$).

3.5 Conclusions

This study provides a first step towards understanding rare species of earthworm remaining in the very restricted refugia within production landscapes of New Zealand. The deeper-burrowing endogeic earthworm *O. multiporus* is the only native species that is known to survive successfully in agricultural pastures (Springett et al., 1998). However, we were unable to identify any differences, in terms of burrowing behaviours, associated with the coexistence of these disparate families of native and exotic earthworms which have apparently similar functional traits. In New Zealand there is no evidence that lumbricids have been responsible for the disappearance of native megascolecid species, nor that they competitively exclude them; both natives and exotics appear to be able coexist on the margins of agricultural land. The disappearance and current absence of native species from agricultural land appears to be related to the inability of megascolecid worms to tolerate disturbance, as suggested by Lee (1985), rather than to any agricultural modification of the physicochemistry of soil.

The inherent taxonomic and ecological characteristics of earthworms were less important than soil type to their habitation. In the present study, native species preferred the physicochemical conditions of farmed soils to the soils of a plantation forest and a native forest. Less acid soils with lower C/N ratios and higher soluble P were more important than high OM. Earthworms were not sensitive to high soil NO_3^- -N in agricultural soils. Their burrowing increased microbial biomass, mobile-P and EC in our short-term incubation studies, but increased gaseous NH_4^+ and N_2O emissions. Earthworms also influenced root morphology and sometimes increased plant growth, with raised soil NH_4^+ and NO_3^- in the presence of plant roots.

Whilst introduced Lumbricidae have colonised agricultural pastures in New Zealand, their establishment has often been less successful than agronomists have hoped (Fraser, 2010). However, reduced tillage in modern agricultural management systems may allow native earthworms on marginal land to recolonize, with concurrent benefits both to species conservation and to soil quality. Meanwhile, marginal land in agricultural landscapes provides suitable soils and a valuable habitat for earthworms.

Chapter 4

Molecular identification and distribution of New Zealand earthworms in human-modified soils

4.1 Abstract

This work aimed to identify new putative taxa of New Zealand native species, elucidate their phylogenetic relationships and describe their distribution in selected human-modified ecosystems (HMEs). A total of 15 Megascolecidae species were identified following DNA barcoding with 16S rDNA and COI (cytochrome oxidase subunit 1). In terms of phylogenetic separation, the 16S-based phylogeny clearly separated Megascolecidae from Lumbricidae. Of these DNA sequences, eight unknown or newly discovered taxa included genera of *Octochaetus*, *Maoridrilus* and *Deinodrilus*, which are available to search on the GenBank database. Adaptation to soil conditions such as pH and organic matter appeared to be a decisive factor in the dispersion of endemic earthworm.

Keywords

Native earthworms, Megascolecidae, Lumbricidae, 16S, COI, phylogenetic analysis

4.2 Introduction

In the past two centuries New Zealand native forests have been replaced by human-modified landscapes across two-thirds of the land mass, resulting in depauperation of endemic fauna including earthworms. Following conversion of native habitat to agriculture, endemic earthworm communities have largely disappeared from newly established productive soils. Introduced European Lumbricidae (mainly *Aporrectodea caliginosa*, *A. longa*, *A. rosea*, *A. trapezoides*, *Lumbricus rubellus*, and *Octolasion cyaneum*), with more tolerance to environmental disturbances, have become dominant (Fraser et al., 1996; Lee, 1985; Springett et al., 1998). Native species are often confined to protected habitats and remnants of native vegetation but they can also be found on the borders of agricultural land (Kim et al., 2015). Coexistence of native and exotic species has recently been reported where patches of native vegetation borders agricultural land (Bowie et al., 2016; Kim et al., 2015).

Of the 3,700 species of terrestrial earthworm described worldwide, 173 had been described in New Zealand prior to 2000 (Blakemore, 2006; Glasby et al., 2009; Lee et al., 2000). The earthworm species list was mainly the result of Lee's monograph published in the late 1950s (Lee, 1959a, b). Despite an extensive geographical coverage of New Zealand, Lee's work was restricted to areas that were relatively easily accessible at that time. As a result, recent studies have unearthed a number of putative undescribed native species particularly in remote locations where no previous searches had been conducted (Boyer et al., 2011a; Buckley et al., 2011).

In many cases, research on earthworm taxonomy has faced limitations due to lack of standardized morphological characters, phenotypic variability, and difficulties in diagnostic characters at juvenile or cocoon stages (Decaëns et al., 2013). Although this may be alleviated by recent developments in imagery for the description of internal morphology using Micro-Computed Tomography (Fernández et al., 2014), lack of taxonomic expertise is also limiting. In recent years, the introduction of DNA barcoding has effectively aided species discrimination, identification of new taxa, reconstruction of phylogeny, and biodiversity assessments particularly for invertebrate groups (Chang and James, 2011; Decaëns et al., 2013; King et al., 2008). DNA barcoding can be particularly useful to solve previous taxonomic confusion but also to accelerate new taxonomic acts. For example, a new species of *Hormogaster* (*H. abbatissae*) was reported by Novo et al. (2010) based on DNA barcoding. Moreover, molecular tools can be used to support phylogeography analysis for single species or a group of closely related species (e.g. Chang and Chen, 2005; Minamiya et al., 2009) as well as discriminating between native and exotic species (Cameron et al., 2008; Porco et al., 2013). DNA barcoding analyses in conjunction with phylogenetic analyses not only contribute to the discovery of new species and the identification of specimens but also enhances our understanding of earthworms' ecology taxonomy and evolutionary history (Domínguez et al., 2015).

Due to its unique geography, New Zealand is potentially home to many yet to be described Megascolecidae inhabiting isolated remnants of undisturbed native vegetation (Boyer, 2013). Buckley

et al. (2012) anticipated that about 101 cryptic taxonomic species may remain to be described and molecular tools are now instrumental to taxonomic description of native earthworms in New Zealand. Boyer et al. (2011a, 2013) and Buckley et al. (2011, 2015) used DNA barcoding and phylogenetic analysis to describe six new species of Megascolecidae (*Aporodrilus aotea*, *A. ponga*, *Deinodrilus gorgon*, *Maoridrilus felix*, *Notoscolex repanga*, *Octochaetus kenleei*) using the 16S rDNA and COI genes.

The aim of this study was to identify new taxa of New Zealand native species through DNA barcoding, elucidate their phylogenetic relationships and describe their distribution in human-disturbed soils in selected relation to soil physicochemical properties.

4.3 Materials and methods

4.3.1 Earthworm sampling

Earthworm collection was undertaken between 2012 and 2015 in remnants of native vegetation and at a number of restoration areas in South Island of New Zealand (Table 4.1). Sampling sites were located at Banks Peninsula, Bankside, Eyrewell, Lincoln, and Punakaiki. Sampling occurred mostly from the late of autumn (May) to the beginning of summer (December), to avoid the dry season when soil is hard to excavate and earthworms are more difficult to find.

Soil pits (20 x 20 x 20 cm) were dug using a spade and earthworms were hand sorted in the field. Collected earthworms were brought back to the laboratory for morphological identification following Lee (1959a, b) as well as for DNA analysis and other experimental work. Specimens were first categorised in operational taxonomic units (OTUs) based on their external morphology, size, colour and behaviour. A total of thirty two specimens representing all OTUs were then used in DNA analyses in an attempt to confirm species status.

Table 4.1 Earthworms sampling sites and GPS coordinates.

	Sampling sites	Location (latitude/longitude)
Banks Peninsula	Okuti Reserve	-43°47'07.98"S / 172°49'51.23"E
	Bossu Rd.	-43°48'59.93"S / 172°51'49.46"E
	Southern Summit Rd.	-43°44'15.41"S / 172°54'32.64"E
	Kaituna Reserve	-43°44'37.23"S / 172°41'14.82"E
	Ahuriri Reserve	-43°39'58.97"S / 172°37'26.37"E
Bankside	Bankside Scientific Reserve	-43°43'49.33"S / 172°09'34.60"E
Eyrewell	DOC Scientific Reserve	-43°22'59.07"S / 172°11'39.78"E
	Spencer Bower Reserve	-43°25'42.08"S / 172°25'48.10"E
Lincoln	Liffey Spring	-43°38'18.64"S / 172°29'06.93"E
	Lincoln University	-43°38'37.19"S / 172°27'43.77"E
Punakaiki	Nikau Reserve	-42° 8'38.39"S / 171°19'50.36"E
	Restored and unplanted land	-42° 8'26.74"S / 171°19'47.53"E

4.3.2 DNA extraction, PCR and sequencing

Molecular analyses were conducted in a molecular laboratory at Lincoln University following a modified method from Boyer et al. (2011a). Earthworms were washed in distilled water, then tissue samples (muscular body wall) were taken from behind the clitellum (mostly the tip of the tail) and preserved in 98 % ethanol. Genomic DNA was extracted using a GF-1 Tissue DNA extraction kit (Vivantis Technologies Sdn. Bhd., Malaysia) following the manufacturer's recommendation. DNA was eluted in 200 µl preheated elution buffer and stored at -20 °C until further analysis.

Universal invertebrate primers for 16S (LR-J-12887 and LR-N-13398) and COI (LC01490 and HC02198) were used to amplify ~550 and ~ 650 base pair fragments of DNA respectively (Table 4.2). PCR reactions (10 µl) consisted of 5 µl GoTaq® Green Master Mix (Promega, Madison, WI, US), 0.1 µl MgCl₂ [25 mM], 0.4 µl forward and reverse primers [10 µM], 1.5 µl DNA template and 2.6 µl super pure water. The thermocycling protocol comprised of an initial denaturation at 95 °C (4 min), 35 cycles of denaturation at 94 °C (1 min) annealing at 52 °C (1 min) and elongation at 72 °C (1.5 min), followed by a final elongation at 72 °C (10 min). Negative controls were included to detect potential contamination. PCR products were sequenced in both directions using Big Dye Terminator Cycle Sequencing Kit following the manufacturer's protocol.

All sequences were manually edited using FinchTV 1.40 (Geospiza), exported into MEGA6 (Tamura et al., 2013) for alignment using MUSCLE (Edgar, 2004) and phylogenetic analysis. Species delineation was inferred from the COI sequences using the Neighbor-Joining method described by (Saitou and Nei, 1987). A 3% delineation threshold was used based on a previous study (Boyer, 2013). Evolutionary distances were computed for COI and 16S sequences using the Maximum Composite Likelihood method (Tamura et al., 2004). DNA sequences were submitted to the GenBank database (see Appendix A for accession numbers). Both 16S rDNA and COI sequences obtained from the collected specimens were compared to sequences from Boyer (2013) and Buckley et al. (2011).

Table 4.2 Primers used for PCR and DNA sequencing in this study.

Gene target	Primer	Primer sequence	Annealing temperature	Reference
16S	LR-J-12887	5'-CGCCTGTTTAACAAAAACAT-3'	52 °C	Simon <i>et al.</i> (1994)
16S	LR-N-13398	5'-CCGGTCTGAACTCAGATCACGT-3'	52 °C	Simon <i>et al.</i> (1994)
COI	LC01490	5'-GGTCAACAAATCATAAAGATATTGG-3'	52 °C	Folmer <i>et al.</i> (1994)
COI	HC02198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	52 °C	Folmer <i>et al.</i> (1994)

4.3.3 Soil analyses

To elucidate soil properties at each collection site, 500 g of fresh soil was sampled from the pits at the time of earthworm sampling. All soils were analysed in-house by Analytical Services in the Department of Soil and Physical Sciences at Lincoln University using standard methodologies, with ASPAC Ring Test QA procedures. Available nitrogen was analysed on fresh soil following extraction with 2M KCl (Blakemore, 1987) and was determined using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden). The remaining soil was air-dried and sieved to <2 mm using a stainless steel sieve for further soil chemical analysis. Soil pH (1:5W) and electric conductivity (EC) were measured using pH and EC meters (Mettler Toledo Seven Easy). For OM content, 10 g of oven dried (100 °C) soil was processed through loss on ignition (LOI) at 550 °C in a muffle furnace (Blakemore, 1987) .

4.3.4 Statistical analysis

Soil properties such as soil pH, electronic conductivity (EC), organic matter content, mobile nitrogen (NH_4^+ and NO_3^-), were analysed using one-way ANOVA followed by a Fisher's least-significant-difference (LSD) post-hoc test. Data were analysed using Minitab 17 (Minitab Inc., State College, Pennsylvania, USA).

4.4 Results and discussion

4.4.1 Species identification

Amongst the 32 individuals, discrete taxa were identified using 16S r-DNA and COI with sequence length around 550 and 750 bp respectively. A total of 15 Megascolecidae species were identified on the neighbour-joining tree. Of these, *Amyntas cortices* and *Megascolex laingii* have been described from Australia but are considered to be exotic in New Zealand (Blakemore, 2006; Lee, 1959b). When compared to existing DNA barcodes produced by Boyer et al. (2011a) and Buckley et al. (2012) and the morphological descriptions from Lee (1959a, b), four New Zealand Megascolecidae species could be confidently identified (Table 4.3): *Octochaetus multiporus*, *Maoridrilus transalpinus*, *O. kenieei* and *Deinodrilus gorgon*. In addition, there were eight taxa for which specimens could only be identified at genus level: *Octochaetus*, *Maoridrilus*, and *Deinodrilus*. DNA sequences for these eight unknown or newly discovered taxa were submitted to the GenBank database (see accession numbers in Appendix A).

Table 4.3 Distribution of 22 earthworm taxa, 13 of endemic and 9 of exotic species collected from soils in New Zealand's South Island. Species presence is indicated by 'V'. Punakaiki is located on the West Coast while the other four sampling sites are located in the Canterbury region. Species were classified as endemic or exotic and named after DNA barcoding and morphological identification.

Classifi- cation	Species name	Sampling sites				
		Punakaiki	Bankside	Banks Peninsula	Eyrewell	Lincoln
Endemic species	<i>Deinodrilus</i> sp.1	V				
	<i>D. gorgon</i>	V	V			
	<i>Maoridrilus transalpinus</i>			V		V
	<i>Maoridrilus</i> sp.1			V		
	<i>Maoridrilus</i> sp.2					V
	<i>Octochaetus multiporus</i>			V		V
	<i>O. kenleei</i>	V	V			
	<i>Megascolecidae</i> sp.1	V				
	<i>Megascolecidae</i> sp.2	V				
	<i>Megascolecidae</i> sp.3	V				
	<i>Megascolecidae</i> sp.4				V	
	<i>Megascolecidae</i> sp.5				V	
	<i>Megascolecidae</i> sp.6					V
Exotic species	<i>Amyntas corticis</i>	V				
	<i>Aporrectodea caliginosa</i>	V	V	V	V	V
	<i>Dendrobaena octaedra</i>	V				
	<i>Fridericia magna</i>	V				
	<i>Lumbricus rubellus</i>	V				
	<i>Lumbricidae</i> sp.		V	V	V	V
	<i>Megascolex laingii</i>	V				
	<i>Octolasion cyaneum</i>	V	V	V		V
<i>Octolasion lacteum</i>	V					

4.4.2 Species distribution

A total of 13 endemic taxa were sampled from Punakaiki (6 taxa), Bankside (2 taxa), Banks Peninsula (3 taxa), Eyrewell (2 taxa), and Lincoln (4 taxa) (Figure 4.1). With regard to exotic earthworms, 9 taxa were identified, four of which were Lumbricidae: *A. corticis*, *A. caliginosa*, *D. octaedra*, *F. magna*, *L. rubellus*, *Lumbricidae* sp., *M. laingii*, *O. cyaneum*, and *O. lacteum*. These species are widespread in West Coast soils as well as in Canterbury (Table 4.3) (Hahner et al., 2013; Kim et al., 2015; Smith et al., 2016).

Punakaiki restoration area contained the greatest diversity of both native and exotic earthworms, 6 and 8 taxa respectively. This may be explained by the proximity to native forest and the smaller scale, less intensive nature of agriculture when compared to the Canterbury Plain sites. In this site, native earthworms included *Octochaetus kenieei* and *Deinodrilus gorgon*, originally discovered from tussock grassland of Waimangaroa Valley, West Coast by Boyer et al. (2011a). All other species

appear to be new indigenous Megascolecidae as they did not match the morphology of any described species and had no correspondence in the existing DNA libraries. These species are subsequently referred to as *Deinodrillus* sp.1, *Megascolecidae* sp.1, *Megascolecidae* sp.2 and *Megascolecidae* sp.3 in the present study (Table 4.3).

None of the native species found at Punakaiki were present in the Canterbury Plains. *Maoridrillus transalpinus* has been recently classified as non-threatened species due to its widespread distribution in New Zealand's South Island with a distribution spanning from the West Coast to Banks Peninsula via Arthur's Pass (Boyer, 2013; Buckley et al., 2015; Kim et al., 2015). The deep burrowing endogeic *Octochaetus multiporus* was found in Canterbury reserves (Kim et al., 2015) as well as in agricultural pastures on ridges of Banks Peninsula (Springett et al., 1998). Another dominant species was *Maoridrillus* sp.1 which is morphologically very similar with *M. transalpinus*; a significant difference is that the clitellum is visible in the former but not in the latter (Kim et al., 2015; Lee, 1959b).

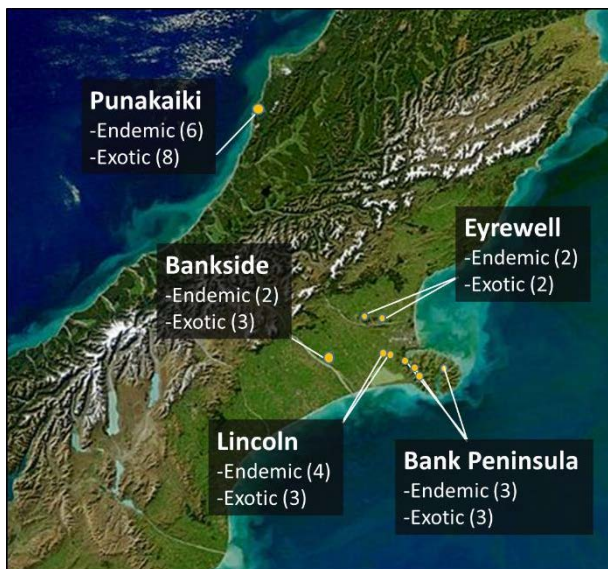


Figure 4.1 Distribution of endemic and exotic earthworms sampled in five regions of the South Island (Punakaiki, Bankside, Banks Peninsula, Eyrewell, and Lincoln). Number of species are in brackets.

4.4.3 Phylogenetic relationships

The phylogeny produced with 16S clearly separates Megascolecidae from Lumbricidae (Figure 4.2), while this distinction is not so clear in the COI-based phylogeny (Figure 4.3). This supports previous studies suggesting that 16S can outperform COI for the reconstruction of earthworm phylogeny (Pop, 2003). According to the COI-based phylogeny, 22 of the collected specimens were identified as Megascolecidae (13 taxa), 9 were Lumbricidae (9 taxa), and 1 was Enchytraeidae (1 taxa) (Figure 4.2). With 16S, 21 of the collected specimens were Megascolecidae (15 taxa) while 9 were Lumbricidae (8 taxa) and 1 was Enchytraeidae (1 taxa) (Figure 4.3). One concern of the present study is that the difference between the two trees may be partly due to the fact that the specimens used for each tree

were not exactly the same. This is clearly unsatisfactory and future works should use the same specimens.

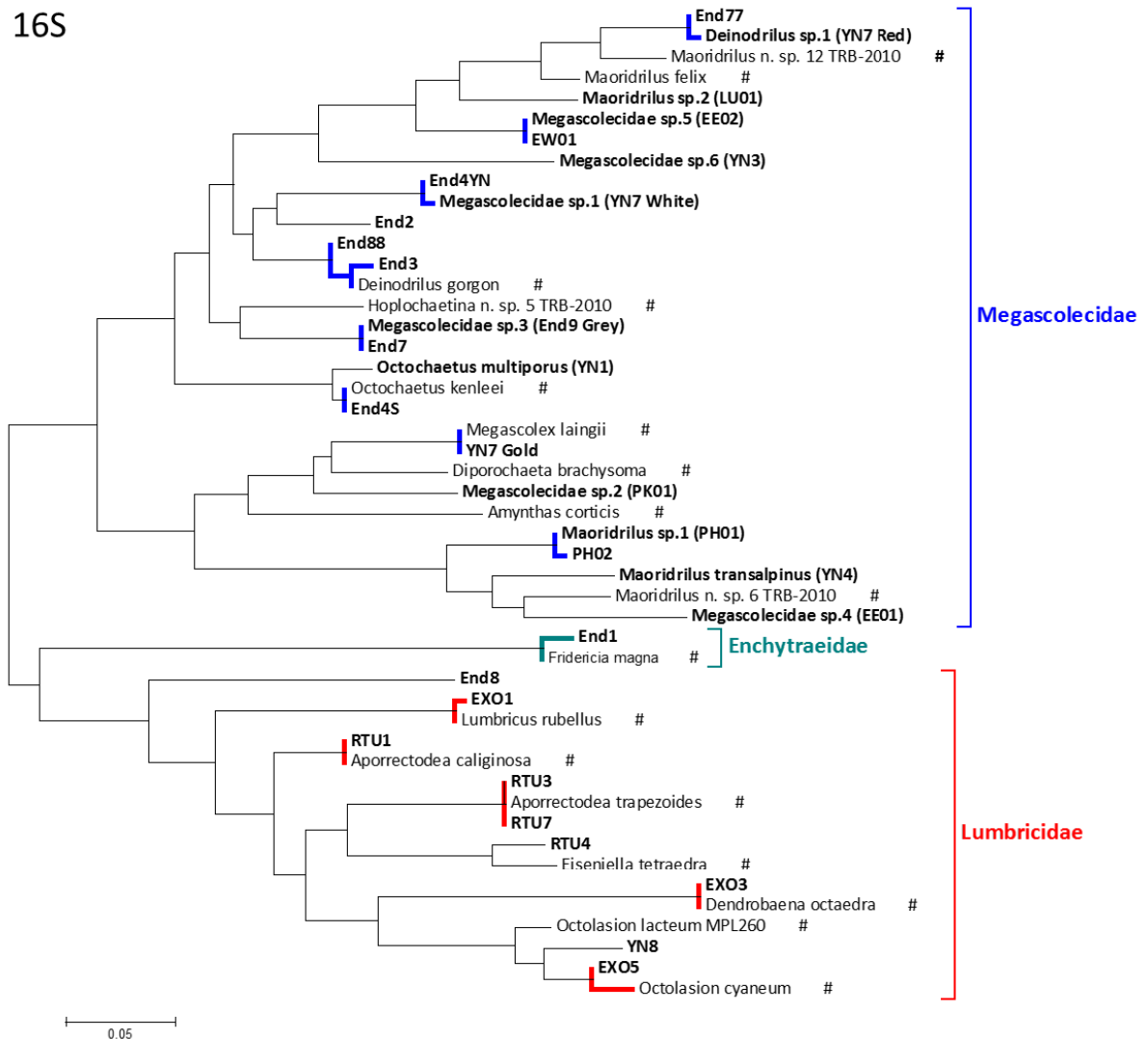


Figure 4.2 Maximum Likelihood tree based on the 16S for 31 earthworm individuals collected from Canterbury and West Coast in South Island (names in bold), along with 17 sequences from Genbank corresponding to closely related species (names followed by #). The Likelihood tree maps are drawn to scale, with horizontal branch lengths corresponding to percentage differences (see scale for 5 %). Specimens linked by blue, green and red lines correspond to individuals of the same species of Megascolecidae, Enchytraeidae and Lumbricidae, respectively (based on a conservative 3% similarity threshold).

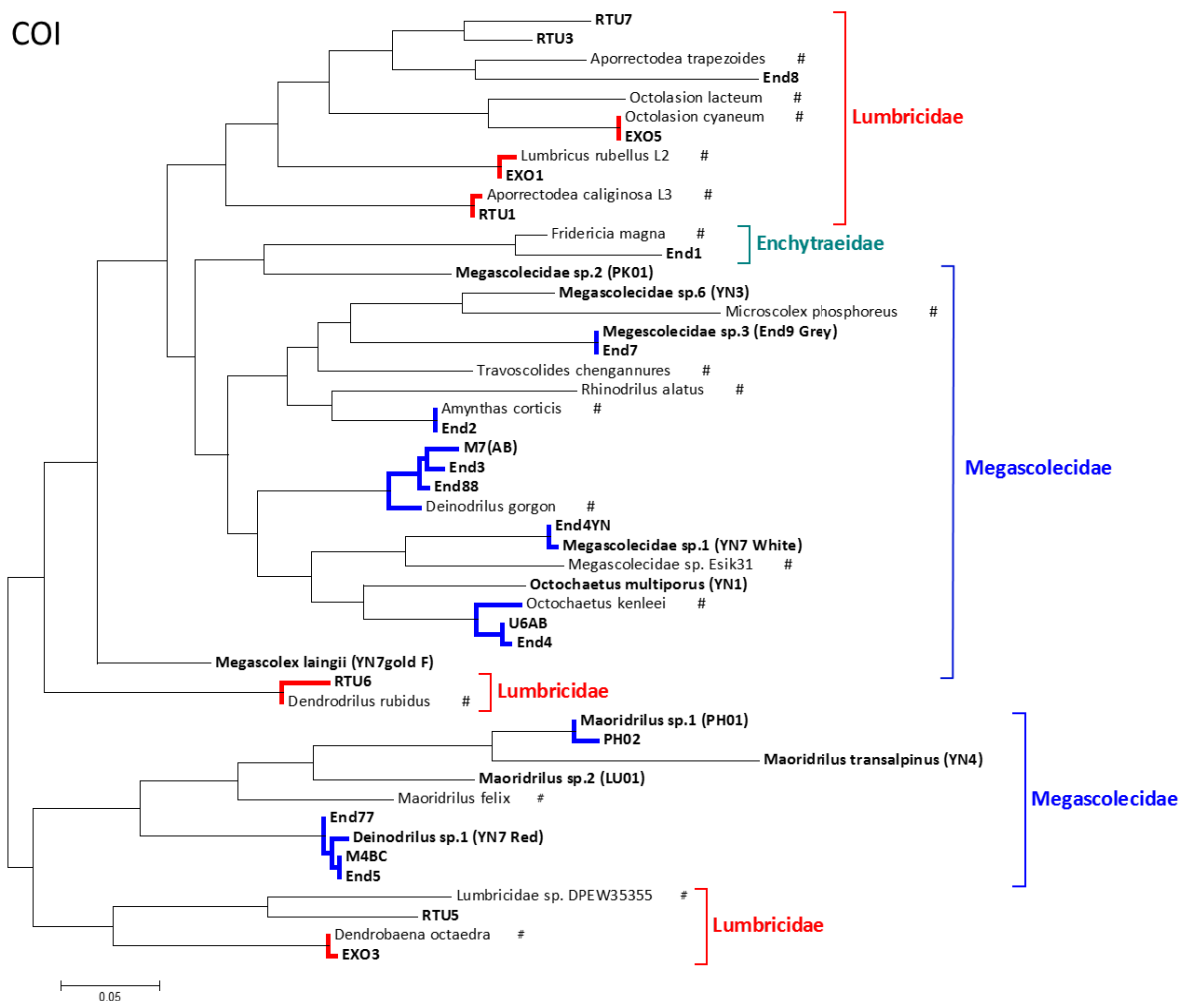


Figure 4.3 Maximum Likelihood tree based on the COI gene for 32 earthworm individuals collected from Canterbury and West Coast in South Island (names in bold), along with 17 sequences from Genbank corresponding to closely related species (names followed by #). The Likelihood tree maps are drawn to scale, with horizontal branch lengths corresponding to percentage differences (see scale for 5 %). Specimens linked by blue, green and red lines correspond to individuals of the same species of Megascolecidae, Enchytraeidae and Lumbricidae, respectively (based on a conservative 3% similarity threshold).

4.4.4 Earthworm abundance and soil chemistry

The Nikau reserve soil was more acid and contained high concentrations of mobile N (ammonium and nitrate) than soils from Canterbury reserves (Table 4.4) (Kim et al., 2015). This may be explained by high organic matter from the litter of local luxuriant broadleaf vegetation (Hahner et al., 2013; Rhodes et al., 2013), which may have promoted a greater diversity of endemic earthworms involved in a variety of ecological niches. In contrast, less acidic Canterbury soils, which largely harboured only three indigenous species (*Maoridrilus* spp. and *Octochaetus multiporus*), displayed moderate OM content of 11 - 30 %.

Soil pH and organic matter are both vital factors for earthworm feeding activity and survival (Curry, 2004). Two endemic species (*M. transalpinus* and *O. multiporus*) that are known to have a wide geographic distribution (Boyer, 2013; Buckley et al., 2015; Lee, 1959a) were collected in several of the sampling sites. Both of these species occurred in soils which contained similar OM content (12 to 30 %) but different soil pH (Figure 4.4). Temperate climate species are generally found in soil where pH is between 4.5 and 7.4 (Bouché, 1972; Satchell and Lowe, 1967). *M. transalpinus* was collected from soils of pH 5.5 to 6.3 and *O. multiporus* was found in soils of pH 5.3 to 5.9. Springett et al. (1998) also estimated that *O. multiporus* was distributed in soils of pH 4.9 in native forests to 5.7 in hill pastures. It seems that the endogeic *O. multiporus* was more likely to be able to tolerate more acidic soil than the anecic species. Exotic species such as *L. rubellus*, *L. terrestris*, *A. caliginosa*, *A. rosea*, *O. cyaneum* occurred in more acid (pH 4.7) and less organic soils (7.3 % of OM) than both endemic species. Fraser et al. (1996) recorded exotic earthworms in agricultural soils containing 4.3 to 5.5 % OM.

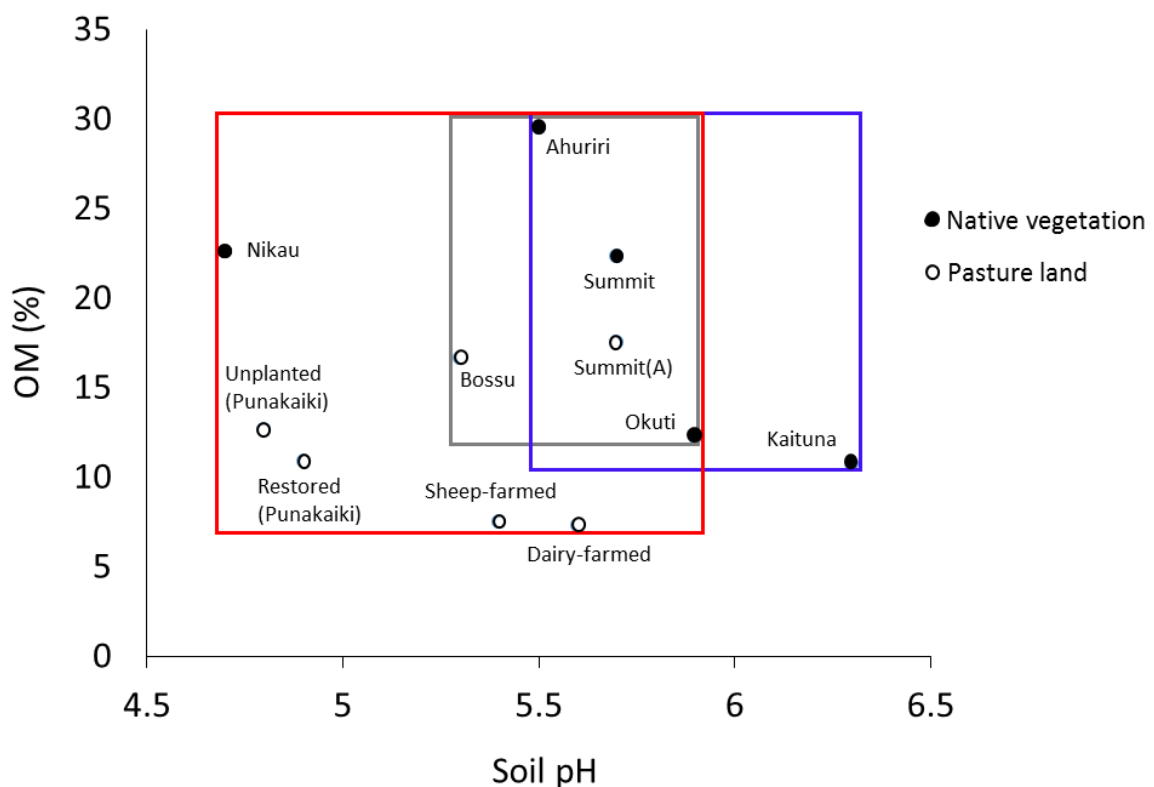


Figure 4.4 Soil pH and organic matter (OM) content in the soils at sampling sites in which native earthworms including *Maoridrilus transalpinus* (blue box) and *Octochaetus multiporus* (grey box) and exotic earthworms including *O. cyaneum*, *O. lacteum*, *L. rubellus*, *A. caliginosa*, *L. terrestris* (red box) were sampled.

Table 4.4 Physicochemical soil properties of endemic earthworm collection sites and distribution and population of those species with separation of ecological group. Each species' population was evaluated by how many earthworms in 20 x 20 x 20 cm³ were monitored during 3 years and then represented as three levels of population density; H (high ≥ 5 individuals), M (1 < medium < 5 individuals) and L (low ≤ 1 individual). The Nikau Reserve is located in Punakaiki, West Coast while other reserve forests are in Banks Peninsula. Paddock soils were collected from the volcanic ridges of Banks Peninsula (Bossu Rd.) and the margin of Ahuriri Reserve (Northern Summit Rd.). Values in brackets represent standard error of the mean (n=3). The same letters indicate no significant difference (LSD, p<0.05).

Sampling site	Soil pH (1:5W)	EC (dS·cm ⁻¹)	OM (%)	NH ₄ -N --- (mg·kg ⁻¹) ---	NO ₃ -N	Distribution of ecological species	Population density	
Mature Forest	Nikau Reserve	4.7 (0.2)	0.19 (0.03)	23 (0.2)	20 (1.4)	18 (0.5)	[Epigeic] <i>Deinodrilus</i> sp.1 / <i>D. gorgon</i>	M / M
							[Anecic] <i>Megascolecidae</i> sp.2 / sp.3	M / L
							[Endogeic] <i>O. kenleei</i> / <i>Megascolecidae</i> sp.1	M / M
	Okuti Reserve	5.9 (0.5)	0.08 (0.02)	12 (1.3)	4.0 (1.3)	9.7 (2.3)	[Anecic] <i>M. transalpinus</i>	H
							[Endogeic] <i>O. multiporus</i>	L
							Southern Summit Rd.	5.7 (0.2)
Kaituna Reserve	6.3 (0.1)	0.06 (<0.01)	11 (0.1)	0.4 (0.2)	2.6 (0.1)	[Anecic] <i>M. transalpinus</i> / <i>Maoridrilus</i> sp.1	M / M	
Ahuriri Reserve	5.5 (0.1)	0.08 (0.01)	30 (2.6)	0.9 (0.1)	3.9 (0.5)	[Anecic] <i>M. transalpinus</i>	H	
						[Endogeic] <i>O. multiporus</i>	L	
Paddock	Bossu Rd.	5.3 (<0.1)	0.04 (0.01)	17 (0.7)	1.1 (0.4)	0.4 (<0.1)	[Endogeic] <i>O. multiporus</i>	H
	Northern Summit Rd.	5.7 (0.1)	0.04 (<0.01)	18 (0.6)	0.5 (0.2)	2.1 (0.2)	[Anecic] <i>M. transalpinus</i>	M

4.5 Conclusion

A total of 179 indigenous species belonging to 26 genera of the Megascolecidae family have been described previously from New Zealand (Figure 2.2). However, many more undescribed species may be present in remote, difficult to access locations or places that have simply never been sampled before. In present study, 8 new taxa of Megascolecidae, collected beneath native vegetation in South Island, were identified through DNA barcoding. The molecular analyses provided a first insight into the distribution of native earthworms and their environmental requirements as well as phylogenetic relationships either between native and exotic species and between native species of earthworms. Widespread sampling and the sequencing of a variety of genetic markers (e.g. 16S, 28S, and COI) will also be necessary to make informed decisions for the conservation of native earthworms as 59% of the species formally described are currently classified as Data Deficient by the Department Of Conservation (Buckley et al., 2015). In addition to this, a large number of undescribed species have been reported in recent studies and a significant taxonomic effort is required to complete the list of New Zealand native earthworms. The present study has illustrated the rudimentary nature of much of our existing knowledge.

Chapter 5

Endemic earthworms in a sheep-farmed soil: implications for soil nutrients, environment and conservation

5.1 Abstract

Native and introduced earthworms co-exist on marginal agricultural land in New Zealand, despite apparently similar burrowing and feeding behaviours. Native species are not found within grazed paddocks, and appear to be entirely restricted to small fragments of native vegetation on marginal land; this is not explained by competitive exclusion. There is concern that recent and widespread intensification of farming systems and spillover of nutrients into adjacent fragments of native vegetation may be to the detriment of endemic megascolecid earthworms. We investigated how different groups of earthworms survived in a sheep-grazed soil and how they modified soil properties. Individual species could be separated on the basis of their effects on soil biogeochemistry, in terms of organic matter consumption, nitrogen and phosphorus mineralisation, soil microbial biomass and greenhouse gas emissions from soil. Earthworm inoculation often increased more mobile forms of key nutrients but differences between native and introduced species were no larger than those between behavioural groupings. The results suggested earthworms are likely to mediate changes to soil properties in situations whereby incipient spillover and the gradual accrual of nutrients from farmland occurs. Mixed communities of native and exotic earthworms are unlikely to be compromised on the periphery of sheep-farmed landscape matrices. This is a valuable habitat for survival and sustainability of endemic species of soil fauna. We consider there may be an opportunity for populations of native species to expand into more intensive farmland under conditions of reduced tillage management and enhanced fertility. Modification of rates of ammonification, nitrification, CO₂ and N₂O emissions by earthworms may or may not be beneficial to the wider environment.

Keywords

Soil ecology, earthworms, biogeochemistry, nutrient spillover, ecosystem services

5.2 Introduction

A lack of knowledge of the endemic earthworms of New Zealand is problematic in terms of conservation of biodiversity in this world biodiversity hotspot. Currently we have so little understanding of the functionality of endemic earthworms in soils that we are unable to realise and leverage their potential benefits to soil ecosystem services. Following relatively recent human colonization of both South and North islands, ground disturbance through burning, vegetation clearance and ploughing played a major role in the demise of the native megascolecid earthworms, as happened elsewhere in the world (Edwards and Bohlen, 1996; Hendrix, 2006). Native earthworms are almost entirely absent from agricultural pastures in New Zealand, with the probable exception of only a single deeper-burrowing species, *Octochaetus multiporus* (Springett et al., 1998). In a study of over 750,000 ha of commercial farms in the region of the present study, no native earthworms were found (Fraser et al., 1996). European species of lumbricids have been introduced, often intentionally and with varying degrees of success, providing some improvement to the quality of agricultural soils where more substantial populations have become established (Lee, 1961; Springett et al., 1992). Endemic earthworms have found refuge beneath small remnants and fragments of native vegetation, for example in riparian zones, along fence lines and on the borders of agricultural land (Bowie et al., 2016). In these restricted areas we have found that it is common to find coexisting assemblages of both native and introduced species (Kim et al., 2015). The province of Canterbury on South Island has 25 recorded native species, many of which are dispersed in very restricted habitats through the lowland plains (Winterbourne et al., 2008). Several additional species found in Canterbury by two authors of the present paper (SB and YNK) are currently in the process of formal recognition subsequent to DNA barcoding.

Native earthworms are not present in the sheep-farmed pastures of the present study although three exotic species (*Aporrectodea caliginosa*, *Lumbricus rubellus* and *Octolasion cyaneum*) were recorded whilst sampling. A recent and large-scale landscape transition towards dairy farming and more intensive production systems has occurred throughout New Zealand, which obviously has even more profound effects on the biogeochemistry of soil nutrients (Mclenaghan et al., 2014; Simmler et al., 2013). This has raised particular concern in the context of conservation of biodiversity because nutrient spillover has been shown to negatively impact and threaten the sustainability of adjacent fragments of native vegetation (Didham et al., 2015). In an earlier paper on earthworms in these fragments (Kim et al., 2015), we were surprised to find that native species actually preferred the physico-chemical conditions of soils in intensively dairy-farmed soils to both plantation forest and native forest soils. We concluded that interactions between earthworms, soils and native plant rhizospheres are likely to be particularly important in vegetation remnants and restoration plantings that provide novel native ecosystems within New Zealand's production landscapes. Since native earthworms and native vegetation generally occur together, a better understanding of structure and

functionality within the soil ecosystem is important both to maintain the very restricted remnants in this landscape and to inform ecological restoration practise.

The aim of the present study was to identify how native and exotic earthworms modify the biogeochemistry of a low fertility agricultural soil prior to farm intensification. Our hypothesis was that introduced lumbricid earthworms are better-suited to agricultural land and will burrow more actively, having a larger influence on soil physicochemical conditions. This was expected to contrast with endemic megascolecid species that are naturally found in more acidic native soils with low to moderate fertility.

5.3 Materials and methods

5.3.1 Soil and earthworm collection

Surface soils (0-15 cm) were collected from a sheep-grazed paddock situated close to the Lincoln University campus (Gammack Estate: 43°38'39.48"S, 172°23'28.07"E). This stony freely-draining Eyre soil has a low capacity for storing water, although the collected surface horizon of soil beneath the turf was largely free of stones. The mixed sward consisted predominantly of perennial ryegrass (*Lolium perenne*), cocksfoot (*Dactylis glomerata*) and clovers (*Trifolium* spp.) with several invasive weed species, quite typical of this type of extensively managed sheep pasture. Sheep-farming since the mid-19th century has involved some degree of ploughing, top-dressing and reseeded, but it is known the site had no recent history of fertilization or intensive management. This soil was selected for the experimental work because its physico-chemical properties were typical of a low fertility agricultural soil of this series in the region. Soil properties have been described in detail in Table 3.4.

The soil was thoroughly mixed and stored for less than three months prior to use in experimental work. The earthworm species used in the present study were selected largely by virtue of their easily-recognizable morphology and ease of collection in large enough numbers by digging and hand-sorting from Canterbury (Ahuriri Reserve: 43°39'58.97"S, 172°37'26.37"E) and West Coast reserve forests (Nikau Reserve: 42°8'38.39"S, 171°19'50.36"E) in South Island. For the experimental work, species were further selected on the basis of the visually most viable and healthy in maintained laboratory cultures. Native species were identified using descriptions from Lee (1959a, b). Molecular methods were used to confirm identifications using a DNA barcoding approach based on the cytochrome oxidase subunit 1 (COI) and 16S rDNA regions (Boyer et al., 2011a).

Epigeic, anecic and endogeic behavioural groups were represented by the selection of three native species (Table 5.1), previously described and recorded in Canterbury and on the West Coast of South Island (Lee, 1959a, b; Wüst et al., 2009); further details of these species were provided in an earlier paper (Kim et al., 2015). Two exotic Lumbricid earthworms, *Aporrectodea caliginosa* was collected on local farmland and *Octolasion lacteum* was sampled from natural forest of West Coast.

Both endogeics are well represented on agricultural land amongst about 19 species of introduced earthworms in New Zealand (Lee, 1959a; Springett et al., 1992).

5.3.2 Incubation experiment

Experimental work was conducted following an acclimatisation period that provided us with confidence of survivorship and an assurance that body mass was being at least maintained. Earthworms were kept in field-collected soils for at least one month. To test their effect on soil properties, the three endemic species (*Deinodrilus* sp.1, *M. transalpinus*, *O. multiporus*) and two exotic species (*A. caliginosa* and *O. lacteum*) were placed in 250 g wetted soil (30 % moisture) contained within 400 ml polypropylene containers. Each contained two earthworms of the same species and was covered with a gauze lid to prevent the earthworms escaping. Soil moisture was maintained on a weekly basis by weighing each container. A small amount of sawdust (2 g), equivalent to an increase of <0.1 % of existing soil carbon, was mixed with the soil as a food source in both treatments and controls, having been previously found to maintain earthworm viability whilst adding minimal additional nutrients into the containers. The experiment was maintained in the dark in an incubator at 15 °C for three weeks, with 4 replicates for each species and 4 reference pots with no earthworms. Earthworm survival and biomass was monitored on a weekly basis with minimal disturbance to the soils.

5.3.3 Soil analysis

Three weeks after inoculation, earthworms were removed by hand-sorting and fresh soil was uniformly mixed and stored at 4 °C for less than one week, prior to soil analyses. Samples of fresh soil were analysed for available nitrogen (N) using a FIA star 5000 triple channel analyser [Foss Tecator AB, Sweden] following published methods (Clough et al., 2001). Microbial Biomass Carbon (MBC) was analysed using TOC-500A analyser [Shimadzu Oceania Pty Ltd., Australia] following Blakemore et al. (1987). Air-dried soil samples were sieved (<2 mm) using a stainless steel sieve. Soil pH and electronic conductivity (EC) were measured using pH and EC meters (Mettler Toledo Seven Easy). At the end of the experiment dried soil samples were analysed for Loss on Ignition (LOI) in a muffle furnace at 500 °C (Blakemore et al., 1987). Based on LOI calculations, total organic matter (OM) and consumed OM by earthworms were estimated: subtracting LOI at the end of the experiments from LOI at the beginning was used to provide an estimate of the amount of organic matter consumed. Olsen P was measured at 880 nm of wavelength absorbance using UV 160A spectrophotometer (Shimadzu, Japan) (Olsen et al., 1954). Total N and C were analysed by a Vario-Max CN elemental analyser (Elementar GmbH, Germany). Cation Exchange Capacity (CEC) and exchangeable Calcium (Ca) were determined using method of the silver thio-urea (Blakemore et al., 1987). Following extraction with 0.05M Ca(NO₃)₂ soluble elements determined by ICP-OES (Varian 702-ES) (Simmler et al., 2013).

Table 5.1 Earthworm species used in this experiment, status and sampling location. Native species were named based on morphological and genetic identification following Lee (1959a, b) and Kim et al. (2015).

Species	Functional group	Native status	Sampling location	Field density (in 20 x 20 x 20 cm ³)
<i>Deinodrilus</i> sp.1	Epigeic	Endemic	-42° 8'38.39"S / 171°19'50.36"E	2 to 5
<i>Maoridrilus transalpinus</i>	Anecic	Endemic	-43°39'58.97"S / 172°37'26.37"E	≥ 5
<i>Octochaetus multiporus</i>	Endogeic	Endemic	-43°38'18.64"S / 172°29'6.93"E	2 to 5
<i>Aporrectodea caliginosa</i>	Endogeic	Exotic	- 43°38'55.05"S / 172°28'4.72"E	≥ 5
<i>Octolasion lacteum</i>	Endogeic	Exotic	-42° 8'10.99"S / 171°19'46.68"E	≥ 5

5.3.4 Gas measurement

Gas sampling was conducted using the same containers after 20 days incubation of the three native species and *O. lacteum*. Other exotic species (*A. caliginosa*) originally included in the trial were later excluded due to earthworms mortality during the experiment. After fitting a sealed lid with a syringe connector to the containers, headspace gas (10 ml) was sampled using a syringe after 0, 20, and 40 min at 16 °C in a chamber room. Nitrous oxide (NO₂) and carbon dioxide (CO₂) were analysed using gas chromatography [SRI 8610 GC, USA] with a ⁶³Ni electron capture detector (ECD) and a flame ionisation detector (FID), respectively, and linked to an autosampler [Gilson 222 XL, USA] following Clough et al. (2006).

5.3.5 Statistical analyses

Principal Components Analysis (PCA) was used to investigate patterns of variation in the dataset, with a focus on the consensus degree between soils incubated with and without different earthworm species. Relative site-to-site dissimilarities in ordination space were detected based on PCA Axis 1 and 2 scores. Values of NH₄, NO₃, Olsen P, MBC, and EC reported in Figure 3.4 and Table 3.5 were used for PCA in this study. Differences were analysed using One-Way ANOVA with Fisher's post-hoc least significant difference (LSD) test in Minitab 16. Means and standard errors of soil pH values were calculated by conversion to the equivalent hydrogen ion concentrations and subsequent back calculation to pH.

5.4 Results and discussion

5.4.1 Separation of species

Earthworm viability was successfully maintained during the 21 days incubation. Multivariate analysis of the dataset was used to investigate the overall effects of all earthworms on biogeochemistry of a low fertility agricultural soils, by separating the earthworm species on to two axes (Figure 5.1). Data from replicate containers were generally tightly grouped. Native species separated from each other along both axes. Native epigeic *Deinodrilus* sp.1 burrow least in the soil and had least effect in modifying chemical properties, as would be expected. The native anecic *M. transalpinus* was separated from other behavioural groupings on Axis 1, represented by enhancements of soil acidity (pH) and its determinant elements (S, Al, and Ca), EC and labile P (Table 5.2). The other native endogeic *O. multiporus* separated along Axis 2 from the epigeic and anecic earthworms in terms of increased nutrient solubility (NH₄, NO₃, K, and Na). Furthermore, the same endogeic ecological grouping of earthworms separated native and exotic species (OM vs AC and OL) on Axis 2. It seems that the native

O. multiporus has a larger impact on N mineralization in agricultural soils than the introduced species. More detail data is presented in following texts.

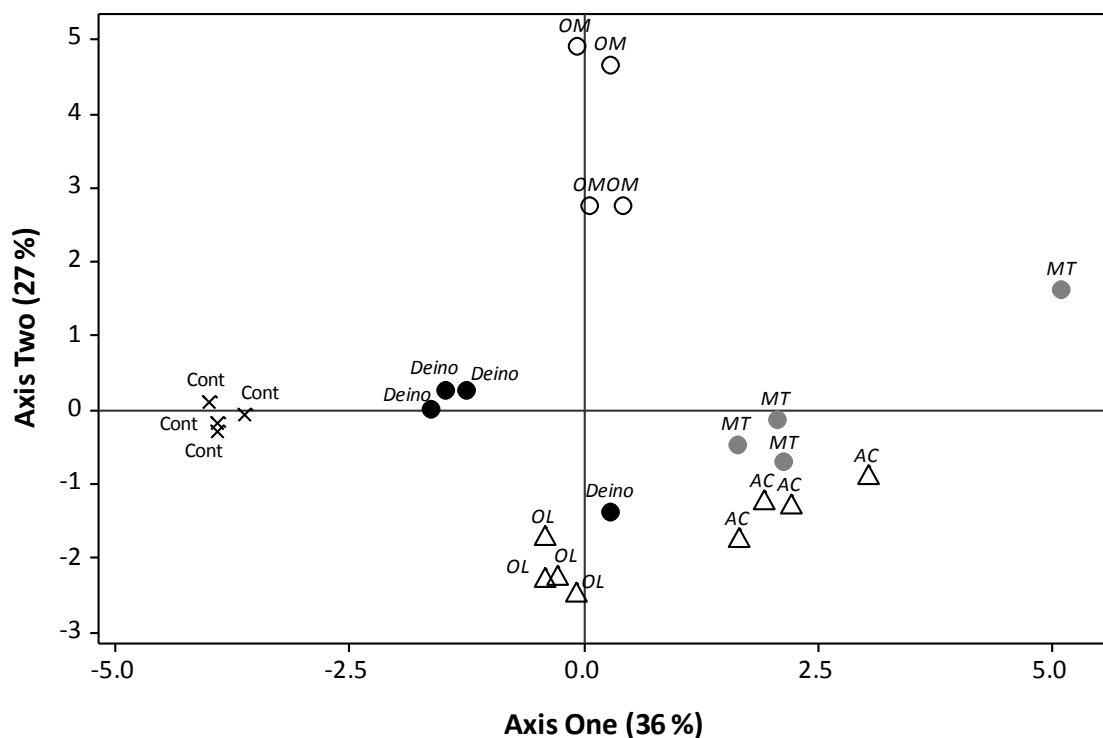


Figure 5.1 Principal Component Analysis (PCA) of soil properties after 3 weeks incubation. Symbols represent individual earthworms (n=4) of native species (●, ●, o), exotic species (△), and control soil (X). Native species are *Deinodrilus* sp.1 (*Deino*), *Maoridrilus transalpinus* (*MT*) and *Octochaetus multiporus* (*OM*). Exotic species are *Aporrectodea caliginosa* (*AC*) and *Octolasion lacteum* (*OL*).

Table 5.2 Coefficients of each variable for PC1 and PC2 through the PCA. Data from incubation experiment.

Variable	PC1 (36 %)	PC2 (27 %)
NH ₄	0.228	0.381
NO ₃	0.239	0.354
TN	0.136	-0.038
Olsen P	0.322	0.207
pH	-0.37	0.163
C/N	-0.093	0.275
MBC	0.137	-0.17
EC	0.344	0.227
CEC	0.249	0.013
Ca	0.33	-0.108
K	-0.064	0.363
S	0.329	0.199
Mg	0.195	-0.371
Na	-0.218	0.397
Al	0.342	-0.154

5.4.2 Growth and OM consumption

Native species appeared to be more active and healthy during the incubation. Biomass of all species had increased by the second week, but they subsequently tended to lose body weight (Table 5.3). This may have been due to the limited food value of sawdust, although this represented only a small proportion of the organic matter available in the pots; 2 g sawdust enhanced total soil OM by <0.5 %. There were clear differences in the amount of OM consumed by different earthworms (Figure 5.2). OM consumed was calculated from differences between LOI at the beginning and end of the experiment. Highest consumption was recorded for *M. transalpinus* which consumed more than five times more than *O. multiporus*. However, the amount consumed was related to the body mass of the particular species; *O. lacteum* was the most active feeder in relation to size. Less OM was consumed by endogeic species but, within this behavioural group, exotics consumed more OM.

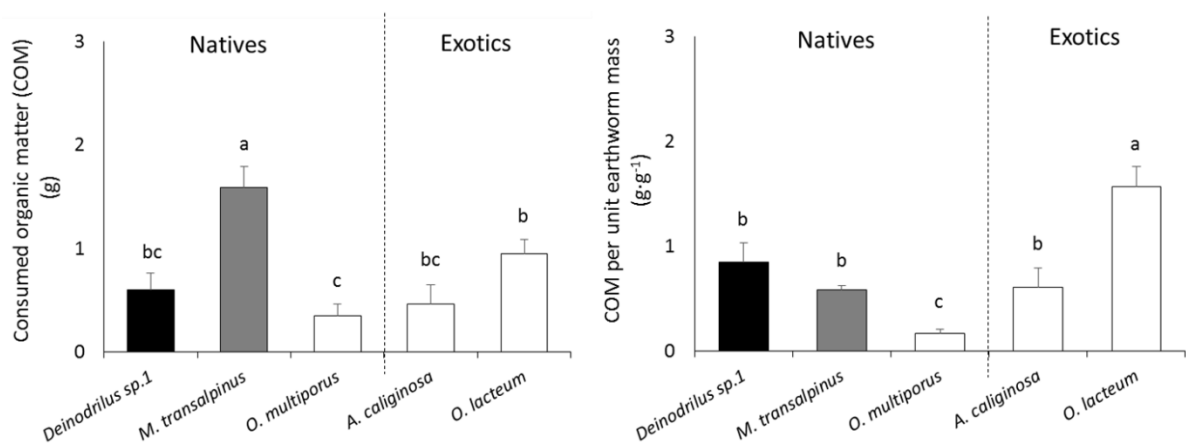


Figure 5.2 The amount of organic matter consumed (COM) by native and exotic earthworms and their feeding efficiency (COM per unit mass) after 3 weeks inoculation. Earthworms mass was measured at the end of the experiment (mean mass without voided gut). Bar values are means \pm standard error (n=4). Same letters indicate no significant difference ($p < 0.05$). Different shading indicates different feeding group species; epigeic (■), anecic (▒), and endogeic (□).

Table 5.3 Mortality and body weight variance of earthworms, and physio-chemical effects of earthworms on soil properties. Control values refer to analyses carried out following incubation. Values in blankets represent standard error of the mean (n=4). Same letters indicate no significant difference (LSD, $p < 0.05$).

	Control (no earthworms)	----- Native -----			----- Exotic -----	
		<i>Deinodrilus</i> sp.1	<i>M. transalpinus</i>	<i>O. multiporus</i>	<i>A. caliginosa</i>	<i>O. lacteum</i>
Earthworm variance						
Mortality (%)	N/A	0 (0) ^b	0 (0) ^b	17 (17) ^{ab}	50 (29) ^a	0 (0) ^b
Weight loss (%)	N/A	-9 (2) ^a	-13 (2) ^a	-7 (9) ^a	N/A	-7 (2) ^a
Soil properties						
pH (1:5W)	4.8 (0.0) ^a	4.7 (0.0) ^c	4.7 (0.0) ^e	4.8 (0.0) ^b	4.7 (0.0) ^e	4.7 (0.0) ^d
EC (dS·m ⁻¹)	0.16 (0.00) ^c	0.19 (0.00) ^b	0.21 (0.01) ^a	0.21 (0.00) ^a	0.20 (0.01) ^{ab}	0.19 (0.00) ^b
C/N	12.3 (0.1) ^{ab}	12.4 (0.1) ^{ab}	12.2 (0.1) ^{ab}	12.6 (0.2) ^a	12.1 (0.2) ^b	12.1 (0.1) ^b
TC (%)	3.41 (0.03) ^a	3.40 (0.08) ^a	3.43 (0.03) ^a	3.58 (0.06) ^a	3.51 (0.15) ^a	3.39 (0.06) ^a
TN (%)	0.28 (0.00) ^{ab}	0.27 (0.00) ^b	0.28 (0.00) ^{ab}	0.28 (0.01) ^{ab}	0.29 (0.01) ^a	0.28 (0.00) ^{ab}
CEC (me·100g ⁻¹)	7.8 (0.3) ^c	9.0 (0.2) ^{ab}	8.5 (0.2) ^{bc}	8.9 (0.2) ^{ab}	9.5 (0.4) ^a	8.6 (0.2) ^{ab}
Ca (me·100g ⁻¹)	5.4 (0.0) ^b	5.7 (0.3) ^b	6.7 (0.1) ^a	5.4 (0.1) ^b	6.6 (0.1) ^a	5.4 (0.1) ^b
K (μg·g ⁻¹)	170 (0) ^b	170 (2) ^b	169 (2) ^b	176 (2) ^a	162 (4) ^c	167 (1) ^{bc}
S (μg·g ⁻¹)	5.6 (0.1) ^d	6.9 (0.3) ^c	7.7 (0.5) ^{abc}	8.5 (0.3) ^a	8.0 (0.7) ^{ab}	7.1 (0.1) ^{bc}
Mg (μg·g ⁻¹)	225 (2) ^{cd}	228 (7) ^{bc}	246 (2) ^a	213 (3) ^d	241 (9) ^{ab}	251 (1) ^a
Na (μg·g ⁻¹)	44 (1) ^{ab}	40 (2) ^{bc}	37 (1) ^{cd}	45 (1) ^a	36 (1) ^d	36 (0) ^d
Al (μg·g ⁻¹)	18 (1) ^d	19 (0) ^{bc}	21 (1) ^a	18 (1) ^{cd}	20 (0) ^{ab}	20 (0) ^{ab}

5.4.3 Effects of earthworms on soil properties

Comparing soils with and without a legacy of intensive agriculture, Liiri et al. (2012) found earthworms had similar effects on both soils in terms of physico-chemical and biological characteristics. The sheep-farmed paddock soil of the present study was suitable to maintain viable populations of native and exotic species of earthworms of the three behavioural groups as described in an earlier paper (Kim et al., 2015). Longer-term viability clearly would be dependent on maintaining a supply of organic matter enrichment from some combination of plant litter, root exudates or animal wastes. Earthworm burrowing significantly altered the physical, chemical and biological properties of this soil that already has been modified from its original condition by farming. It would appear reasonable to assume that the effects of earthworms on soil properties may not be greatly altered by the way these soils had been previously modified by agronomic practices, although earthworm abundance is dependent on soil properties.

We found through earlier study (Chapter 3) that native species of earthworm stimulated N and P mineralisation in soil. Of the species, *O. multiporus* increased $\text{NH}_4\text{-N}$ concentration about 11 times. Although 5 % increase of available-P by *M. transalpinus* were measured, other studies with longer incubation periods have shown a much more pronounced effect on mobile P (e.g. Scheu and Parkinson, 1994; Vos et al., 2014). The present study showed broader effects of earthworms on mineralisation of key nutrients and modification of soil acidity in the previous paper; earthworms increase the mineralisation of K and Mg. In terms of acidity, pH was lowered by increased moisture and incubation in the control but earthworms also modified the lability of chemical elements that are indirect determinants of acidity, raising soil concentrations of exchangeable S and Al, and lowering exchangeable Ca. Increased EC in the presence of earthworms has been previously shown to be due to increases of exchangeable minerals such as Ca, K, Mg, Na, ammonium, or phosphate following organic matter decomposition (Balamurugan et al., 1999; Sonowal et al., 2013; Tognetti et al., 2005; Villar et al., 1993). There were marginal reductions in soluble Zn, Cd, Cu, and Co concentrations with presence of both native and exotic groups ($p < 0.05$, data not shown).

As found in previous studies, interaction with and modification of environmental factors such as pH, organic matter and nutrients seem to play a more important role than inherent ecological characteristics of earthworms (Eijsackers, 2011; Kim et al., 2015). However, in the present study, more subtle effects on soil properties were brought about by different species and behavioural groups, and between natives and exotics. Endogeic species have a deep-burrowing, permanently sub-surface dwelling behaviour, and we were able to compare native species with exotics. Of the behavioural groups, endogeic species of Lumbricidae have previously been shown to consume more soil organic matter than epigeic or anecic species (Edwards and Bohlen, 1996), similar to the present study. Our

small sample size does not justify more detailed interpretation of differences between functional groupings.

Previous studies have shown that earthworms stabilize SOM fractions within their casts, and they also increase the mineralization of organic matter in the short term by altering physical protection within aggregates and enhancing microbial activity (Bertrand et al., 2015). Exotic lumbricids in New Zealand similarly consume more organic matter and also stimulate soil microbial communities (Kim et al., 2015). Endogeics generally had a larger influence on soil properties. Exotics mobilised less N, P (Kim et al., 2015) and K, but more Mg and Na, in the soil. Within the native species, behavioural groups differed primarily in the way they modified pH and increased P mineralisation. In other studies, Natal-da-Luz et al. (2011) found that the burrowing activities of earthworms did not reduce the binding capacity of soil for trace elements, but did influence trace element concentrations of percolates.

5.4.4 Environmental concerns

Earthworms are important soil ecosystem engineers and useful indicator of soil health and quality (Edwards, 2004; Ojha and Devkota, 2014). They modulate soil properties including hydrology, organic matter dynamics and nutrient cycling, enhancing plant growth and development (Fragoso et al., 1997; Francis and Fraser, 1998; Topp et al., 2001). It is likely that they also play an important direct and indirect role in dispersion of chemicals to the wider environment. Earthworms have previously been found to stimulate activity of autotrophic nitrifiers such as NH_4^+ oxidizing bacteria, with a positive impact on nitrate and exchangeable phosphorus (Araujo et al., 2004; Blouin et al., 2013; Parkin and Berry, 1999; Suárez et al., 2004). Earthworm casts and burrows can provide favourable microhabitats, compared to bulk soil, to enhance the activities of nitrifiers (Speratti and Whalen, 2008). Clearly there is a requirement to further investigate the role of earthworms in biogeochemical cycles, and to measure their effect on the nutrient input–output balance of agroecosystems (Barot et al., 2007).

Through their effects on the microbial community, earthworms are known to also stimulate decomposition, nitrification, and denitrification in soils, leading to enhanced release of N_2O and CO_2 (Burtelow et al., 1998; Postma-Blaauw et al., 2006). Increasing the exposure of native and non-native earthworms in New Zealand to NO_3^- has been shown to increase denitrification in the alimentary canal the emissions of both N_2 and N_2O (Wüst et al., 2009), although Speratti and Whalen (2008) showed rates of nitrification could also be important. Our own earlier work showed that emission of nitrous oxide (N_2O) and carbon dioxide (CO_2) were significantly increased by endogeic earthworms. A criticism of these results reported earlier was that no account had been taken of size and mass of the different species. When recalculated to include a correction factor for biomass there were few differences between species, but all increased CO_2 and N_2O emissions. Incubation with *O. multiporus* released the largest amount of both N_2O and CO_2 (Table 5.4). N_2O release amounted to more than 9 times that of the control. *O. lacteum* also increased N_2O about 3 times than that of control. In a recent review of 57

short-term studies globally, each up to 200 days, endogeic earthworms including *A. caliginosa* were reported to enhance N₂O by 42 % and CO₂ by 33 % (Lubbers et al., 2013b).

Our earlier results (Kim et al., 2015) showed that earthworms, particularly endogeics increased the amount of NO₃-N and NH₄-N in soil. This may suggest enhanced leaching of soluble N and could be detrimental to maintain a tight N cycle in farm land. Earthworms have been found to increase leaching through their galleries to greater soil depths (Domínguez et al., 2004). Kernecker et al. (2014) evaluated the effects of introduced non-native earthworms into riparian buffers in Canada and thought they caused a decline in the reduction of NO₃ to gaseous N₂O, but increased leaching of dissolved organic carbon (DOC) and NO₃ due to preferential flow pathways. They considered that litter from native perennial grasses and forest plant species in riparian zones was less palatable than crop residues to earthworms, and would therefore release less soluble C and N through decomposition, minimizing C and N losses during the most vulnerable periods of the year with higher rainfall, lower temperatures and reduced crop growth. This would be beneficial in New Zealand, where soil beneath native vegetation is also known to be a sink for CH₄ with very low background emissions of N₂O compared to grassland (Hedley et al., 2013).

O. multitorus is the only native species currently found within agricultural paddocks, and this species appears to increase N₂O emissions. Present-day agricultural systems seek to improve the efficiency of N usage, mainly to limit the release of reactive N to the wider environment as soluble NO₃ and gaseous N₂O. N₂O accounts for about 29 % of agricultural emissions in New Zealand which has the highest agricultural GHG emissions for any developed country. This greenhouse gas is 310 times more potent than CO₂ and about 75 % of N₂O is emitted directly or indirectly from soils (Thorburn et al., 2012). It has been argued that a shift is required towards NH₄-dominated, low-nitrifying agricultural production systems for improved management of nitrogen. Subbarao et al. (2013) noted that enhancing the release of biological nitrification inhibitors from the roots of pasture grasses and cereals is receiving considerable attention in this context. Management of tillage systems and interactions between the rhizosphere and earthworm communities will play an additional and potentially highly significant role.

Table 5.4 Effects of earthworm inoculation for 21 days on N₂O and CO₂. Changes to the rate of emission was calculated using results of Kim et al. (2015), where full details are shown.

Species	Status	Rate of change	
		N ₂ O	CO ₂
<i>Deinodrilus</i> sp.1	(Native) Epigeic	2.2	1.25
<i>M. transalpinus</i>	(Native) Anecic	-1.4	-1.12
<i>O. multitorus</i>	(Native) Endogeic	9.3	2.4
<i>O. lacteum</i>	(Exotic) Endogeic	3.8	-1.34

5.4.5 Conservation of endemic species

More than 80 % of most faunal groups in New Zealand are endemic and found nowhere else (Trewick et al., 2007) and this is probably similar for earthworms, many species of which have yet to be discovered and identified. Worldwide, earthworms appear to contain a large number of morphologically similar cryptic species (Bartlett et al., 2010). In New Zealand, there are 177 recognised species of endemic megascolecid earthworms (Glasby et al., 2009; Lee et al., 2000; Lee, 1959a; Sims and Gerard, 1985) that are poorly described in the scientific literature. One of only a few recent field surveys of New Zealand's earthworms revealed extensive cryptic taxonomic diversity with about 48 additional species (Buckley et al., 2011).

The results of the present study showed that native earthworms are tolerant of chemically-modified agricultural soil, and they enhance nutrient mineralization more than exotic species. It would seem there may be an opportunity for populations of native species to expand into intensive farmland under reduced tillage management systems in the future, based on the assumption their disappearance from arable and pastoral was largely caused by ground disturbance. Pastoral land formerly used for sheep grazing often was not ploughed, but was heavily modified by native vegetation removal, burning and oversowing. The effects of land management practices on earthworm communities may be critical to their survivorship in these highly modified agricultural landscapes of lowland New Zealand. It has recently been suggested that earthworms are potential indicators of the sustainability of agricultural practices that could be actually used to optimize farming systems, helping to address criticisms concerning the negative impacts of intensive agriculture on environment and human health (Bertrand et al., 2015; Eijsackers, 2011; Lemtiri et al., 2014). A better understanding of the role of earthworms in the process of soil formation, and consequences for soil management and restoration in these environments may be requisite to their conservation. This could be beneficial to soil health like both biodiversity conservation and soil quality in agricultural systems. Only four previous research papers (Kim et al., 2015; Springett et al., 1998; Waterhouse et al., 2014a; Wüst et al., 2009) are known to have been published on the functionality of endemic earthworms in New Zealand.

5.5 Conclusions

Cultivated soils have been domesticated and highly modified from their original condition, with different biogeochemical qualities (Amundson et al., 2015). On the margins of agricultural land in New Zealand, disparate families of native and introduced earthworms co-exist with apparently similar functional traits in terms of burrowing behaviours. The incipient and gradual accrual of nutrients that spillover from intensive farming systems is further modifying soil properties and the biodiversity of adjacent fragments of native vegetation, but currently there is no evidence this is detrimental to native

earthworms. In the present study, individual species could be separated on the basis of their modification of soil biogeochemistry, with differences particularly evident in terms of organic matter consumption, nitrogen and phosphorus mineralisation, soil microbial biomass and greenhouse gas emissions.

Despite different effects of native and exotic species, both which often increased more mobile forms of nutrients in soils, these differences appeared to be no larger than those between behavioural groupings. Nevertheless there was some indication that exotic species consumed more organic matter and stimulated soil microbial communities more by *O. lacteum*, but mobilised less N, P and K in the soil. These differences require further study as sustainable management of these chemical elements is becoming increasingly important to both agriculture and the environment. We suggest that the modification of soil properties by native earthworms is likely to add to changes brought about by nutrient spillover from farmland (Didham et al., 2015). Mixed communities of native and exotic earthworms may not be compromised on the periphery of intensive farming systems. Furthermore, invasive exotic species do not appear to threaten native species (Lee, 1961); both groups of earthworms significantly modify and potentially ameliorate soil biogeochemistry, although deeper burrowing species (particularly *O. multiporus*) may enhance N₂O emissions

It has been argued that sustainable strategies for cultivated soils in the future must focus of regaining a balance of carbon inputs and losses, and release and loss of nutrients (Amundson et al., 2015). This will undoubtedly include avoidance of nutrient spillover to adjacent land and natural fragments. Conservation of native species of earthworm throughout most of lowland New Zealand is entirely dependent on the sustainability of these fragments that have become novel native ecosystems (Kim et al., 2015), but there may be no opportunity to return soil to its original condition. In this regard, it is possible that “we [may] need to love it as it is and can be, not the way it was and never will be again” (Watson, 2014). Identifying the role of native earthworms in the delivery of ecosystems goods and services is required for an integrated approach to soil management in agricultural landscape mosaics. The present work is a very early attempt to understand the functional role of New Zealand’s unique species of earthworm.

Chapter 6

Integration of earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a mesocosm experiment

6.1 Abstract

This work aimed to inform restoration trajectories in production landscapes of New Zealand in the context of protecting unique biodiversity and addressing environmental issues associated with soil nitrogen enrichment and leakage. We studied the interaction between different ecological groups of earthworms, a native rhizobium-inoculated leguminous shrub (*Sophora microphylla*) and soil biogeochemistry in a laboratory mesocosm experiment. Plants grew better in the presence of soil burrowing earthworms, and rates of root nodulation were considerably enhanced in the presence of the native megascolecid anecic earthworm *Maoridrilus transalpinus*. This species consumed more organic matter in the presence of inoculated plants whilst marginally lowering soil pH and enhancing critical concentrations of nitrate, but also reduced nitrous oxide emissions. Earthworms raised dehydrogenase enzyme activity in soil, but this was not commensurate with rates of nodulation. Our results suggest that earthworm-mediated soil aeration, modification of moisture conditions in the rhizosphere and drilosphere, and comminution of organic matter modify microbial communities and significantly impact the N cycle. We argue that an integrated understanding of plant growth and microbial communities with earthworm functionality is essential for effective management of soil N in ecological restoration on former agricultural land.

Keywords

Soil ecology, earthworms, nitrogen, nutrient spillover, greenhouse gas, ecosystem services, root nodulation

6.2 Introduction

Ecological restoration aims to construct novel native ecosystems that have some meaningful semblance of an historic post-mature vegetation and to provide habitat for faunal biodiversity (Tongway and Ludwig, 2011; Dickinson et al 2015). There is increasing realisation that management and conservation of land should include soil biodiversity as an important criterion to benefit ecosystem functioning, service provision and human health (Bardgett and Wardle, 2010). The trajectory of a restoration is obviously important, and may be reliant on the existence and development of soils suitable for establishment and sustainability of the desired type of vegetation (Smith et al., 2016). This is especially challenging in situations where land use changes have substantially modified soil structure, biogeochemistry and ecology; the soil template for restoration can be very different to the original substratum (Franklin et al., 2015).

Little is known of the requisite underlying environmental conditions to optimise the restoration trajectory within agricultural landscapes in New Zealand. This creates real challenges for restoration practitioners; re-introduction takes place in the presence of exotic weeds and animal pests, including mammals that were formerly absent from this landmass. The most troublesome invasive plants in New Zealand are legumes, including gorse (*Ulex europaeus*), brooms and lupins, although gorse often also plays an important role assisting the recovery of native vegetation on former stock-grazed pasture (Wilson, 2013). Leguminosae are poorly represented amongst the native flora, both in number of species and abundance; 4 genera and 34 species represent 1.4 % of the vascular flora (Given and Meurk, 2000), compared to 8 % worldwide (Yahara et al., 2013) and native legumes compete poorly with introduced gorse and brooms, particularly in human-modified landscapes (Wardle, 2002). One concern is the elucidation of the role played by native species of nitrogen-fixing plants in vegetation recovery. Among the New Zealand native Leguminosae, eight species of *Sophora* are shrubs or small trees (Heenan et al., 2001; Thomas and Spurway, 2002), which could have a particularly important role in global legume diversity assessment (Yahara et al., 2013). Understanding the functionality, interactions and combined influence of native legumes and soil fauna on ecosystem development presents further challenges (Bardgett and Wardle, 2010; Blouin et al., 2013). In New Zealand, the role of native earthworms in particular requires more attention (Kim et al., 2015). There are more than 200 species of native Megascolecid earthworms in New Zealand that are almost entirely unrepresented on agricultural land, even though several species of exotic Lumbricid earthworms are commonly found in farm paddocks (Fraser et al., 1996).

Agricultural landscape matrices in New Zealand are depauperate in native flora and fauna (Winterbourne et al., 2008) where remnants of natural and re-planted vegetation are only represented as little more than refugia in riparian zones, along fence lines and on the borders of agricultural land (Bowie et al., 2016). These natural remnants are now significantly expanding though renewed interest in native species, and modern intensive agricultural systems that are integrating restoration of

biodiversity into farm planning (Dickinson et al., 2015; Franklin et al., 2015). The broad aim of this research is to understand the interactions between native species of plants, soil fauna and soil physicochemistry in providing an appropriate template for restoration. In this paper we report the finding of a mesocosm experiment that investigated whether we could demonstrate significant integration of the role of a native species of nitrogen-fixing plant and earthworms.

6.3 Materials and methods

6.3.1 Establishment of pot experiment

Templeton silt loam soil (Molloy, 1988) was collected from the Lincoln University commercial dairy farm for use in this mesocosm experiment. The soil was sieved and uniformly mixed prior to planting. Four species of earthworms representing different ecological groups and burrowing behaviours were selected for this study. Two native anecic species, *M. transalpinus* and *Maoridrilus* sp.2 were collected respectively from a nature reserve (Ahuriri Reserve, Banks Peninsula) and beneath a mature stand of exotic *Quercus ilex* trees on the university campus. An exotic endogeic species (*O. cyaneum*) was also sampled from the Ahuriri Reserve; we have observed that both species commonly coexist in Banks Peninsula forests. The exotic epigeic, *Eisenia fetida*, was purchased from a local vermicomposting company. One-year-old single plants of *Sophora microphylla* (Kowhai) of uniform size were purchased from a nursery, acclimated to glasshouse growth conditions for 4 weeks, then transplanted into the dairy farm soil in 55 plastic plant pots (5 L volume), and maintained for a further 7 days before the addition of rhizobial and then earthworm inocula (Figure 6.1).

Novel *Mesorhizobium* sp. cultures (Strain ICMP 19535, Tan et al., 2015) were obtained from the International Collection of Microorganisms from Plants, Landcare Research, Auckland, NZ and incubated into Yeast Mannitol Broth (YMB) at 25 °C in the dark for a week to derive liquid cultures which were poured into 25 pots, once a week on two occasions. This strain is known to be effective on *S. microphylla*. Autoclaved YMB was added to 5 reference pots as control. One week later, four earthworms of a single species were added to each pot, with 5 replicates, and an additional 5 pots without earthworms. Weight of all species of earthworm were recorded prior to inoculation. To prevent earthworm escaping during the inoculation, the drainage holes and surface of the pots were covered with dark gauze. Pots were randomized and maintained for 7 weeks in natural light at 20 ± 5 °C. There were 5 replicates of each treatment (4 species of earthworms, with and without rhizobial inoculation, plus 5 pots with neither earthworms nor inocula). The mesocosms were lightly watered every 3 days for 8 weeks, adding the same amount of water to each pot to maintain moisture conditions subjectively judged to be optimal.



Figure 6.1 Establishment of *S. microphylla* in the glasshouse. Photographs inset show the gauze covers (top inset) and gas sampling cylinders (bottom inset).

6.3.2 Plant and soil analyses

Following the harvest of plants after 8 weeks, shoot length was measured. Roots and shoots were separated and then oven-dried (65 °C, 3 days) to measure dry weight. Earthworms were collected from rhizosphere of each pot, then weighed and assessed for viability. Number of nodules was also counted on the roots by hand-sorting. After removing surface moss (up to 2 cm depth) and mixing rhizosphere soil uniformly, 1 kg of fresh soils was collected from each pot and stored at 4 °C. Following extraction in 2M KCl, samples of the fresh soil were analysed for N (NH₄-N and NO₃-N) using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden) (Clough et al., 2001). For soil dehydrogenase enzyme activity (DHA), 2 g of fresh soil was incubated with 2 ml of 2,3,5-triphenyltetrazolium chloride (TTC) solution for 24 hours at 25 °C in darkness. After extraction with 10 ml of methanol, the supernatant was measured the absorbance at 475 nm through a UV 160A spectrophotometer (Shimadzu, Japan) (Casida et al., 1964). Air-dried soil samples were sieved (<2 mm) before determination of pH and electronic conductivity (EC). Following oven-drying (100 °C), Total Organic Matter (TOM) was analysed as Loss on Ignition (LOI) in a muffle furnace at 550 °C (Blakemore, 1987). Based on LOI calculations, OM consumed by earthworms was estimated as the difference in LOI at the beginning and end of the experiments. Following 0.5M NaHCO₃ extraction (1 g soil : 20 ml extractant), plant available-P was measured as Olsen P, spectroscopically at a wavelength of 880 nm using a UV 160A spectrophotometer (Shimadzu, Japan) (Blakemore, 1987). Total N and C were analysed using a Vario-Max CN elemental analyser (Elementar GmbH, Germany).

6.3.3 Gas sampling

Gas measurement for nitrous oxide and carbon dioxide was carried out after 50 and 55 days and the average gas data of both collections was shown in Figure 6.5. A plastic chamber (0.5 L) was installed on the soil surface. Gas (10 ml) was collected from the headspace, 0, 20, and 40 min after sealing. Collected samples were stored at 16 °C in darkness for less than one week. Nitrous oxide (NO₂) and carbon dioxide (CO₂) were analysed using a gas chromatograph (GC) (SRI 8610 GC, CA, USA) with a ⁶³Ni electron capture detector (ECD) and a flame ionisation detector (FID), respectively, and linked to an auto-sampler (Gilson 222 XL, USA). Methods follow those described by Clough et al. (2006).

6.3.4 Statistical analyses

One-way ANOVA with post-hoc test (Fisher's LSD test) was used to identify differences between the species in outcomes such as rhizosphere soil properties (soil pH, EC, OM, ratio of C/N, TC, TN, and mobile N and P), plant growth (DW), microbial activity (DHA) nodule number, and gas emission (N₂O and CO₂). Mean and standard error of soil pH values were calculated by conversion to the equivalent hydrogen ion concentrations and back calculation to the pH. All analyses were processed using Minitab 17 (Minitab Inc., State College, Pennsylvania, USA).

6.4 Results and discussion

After 8 weeks mortality rates were less than 10 % in all species, except *M. transalpinus* which had only 40 % survivorship in the rhizobia-inoculation treatment and 25 % survivorship without rhizobia-inoculation. However, several freshly-dead individuals of this species were found towards the end of the experiment, and soil in all the pots showed clear evidence of having been well worked through earthworm activity. This indicated survivorship was much higher for a large part of the experimental period, and other more recent studies we have carried out have shown high levels of survivorship in this species in similar pot experiments are seldom maintained for more than 6 weeks, unless the earthworms are provided with an abundant food source. An overall weight gain was only recorded in *Maoridrilus* sp.2. During the separation of plant roots from soil, it was observed that the two *Maoridrilus* species were invariably intertwined within the root system and there was noticeably more evidence of burrowing and mixing of the soil, compared to the other species. *Octolasion* tended to dwell beneath the roots towards the bottom of the pot, whilst *E. fetida* inhabited soil close to the soil surface. Poor survivorship of *M. transalpinus* was considered to be an acceptable limitation of this experiment; previous studies have shown that decaying earthworm bodies do not significantly contribute to the amount of N otherwise released into soil by earthworm (van Groenigen et al., 2014; Whalen et al., 1999). There were no reference pots without plants in the present study, but better

growth of Lumbricid earthworms has been previously recorded in the presence of legumes (Milcu et al, 2008).

Plants generally grew better in the presence of earthworms over the 8-week period, particularly in terms of shoot length and biomass (Table 6.1). Rhizobial inoculation appeared to lead to lower rates of shoot growth, but neither earthworms nor rhizobia modified root biomass. There was a significant interaction between the effects of these two treatments on shoot length. However the growth period was quite short in the present study, and a recent meta-analysis showed an average 23 % increase in above-ground plant biomass due to the presence of earthworms, largely through release of N from organic matter (van Groenigen et al., 2014). The amount of organic matter consumed was calculated crudely from the change in soil TOM over the duration of the experiment (Figure 6.2). Compared to the control pots, *M. transalpinus* consumed much more organic matter than other species, despite its poor survivorship and a 31 % weight loss of surviving earthworms. There was less weight loss in combination with rhizobial inoculation (data not shown), where soil organic matter consumption was even higher. Otherwise there were only small differences in soil organic matter depletion due to earthworms, and no effects of rhizobial inoculation.

Table 6.1 Plant growth after 8 weeks, with two-way ANOVA (* $p < 0.05$; ** $p < 0.01$; * $p < 0.001$). Values in brackets represent standard error of the mean (n=5). Same letters indicate no significant difference ($p < 0.05$).**

Treatment		Shoot length (cm)	Dry weight		
			Total	Shoot	Root
			----- (g) -----		
Control	No inoculant	50.4 (2.8)	10.0 (0.9)	7.8 (0.6)	2.5 (0.3)
No Rhizobia	No earthworm	50.4 (4.3) ^c	10.0 (0.9) ^b	7.6 (0.6) ^c	2.4 (0.2) ^b
	<i>E. fetida</i>	61.8 (1.4) ^b	12.0 (1.3) ^{ab}	8.8 (0.8) ^{bc}	3.2 (0.5) ^{ab}
	<i>M. transalpinus</i>	73.4 (3.0) ^a	14.8 (0.6) ^a	11.3 (0.5) ^a	3.5 (0.2) ^a
	<i>Maoridrilus</i> sp.2	70.6 (1.5) ^a	13.9 (1.8) ^a	10.7 (1.3) ^{ab}	3.2 (0.4) ^{ab}
	<i>O. cyaneum</i>	58.8 (2.8) ^b	10.2 (0.6) ^b	7.6 (0.5) ^c	2.6 (0.2) ^{ab}
Rhizobia	No earthworm	54.4 (4.1) ^b	11.0 (1.9) ^a	8.2 (1.4) ^a	2.8 (0.5) ^a
	<i>E. fetida</i>	57.6 (1.5) ^{ab}	9.6 (0.4) ^a	6.8 (0.3) ^a	2.8 (0.3) ^a
	<i>M. transalpinus</i>	57.6 (4.0) ^{ab}	11.7 (1.4) ^a	8.3 (1.1) ^a	3.3 (0.3) ^a
	<i>Maoridrilus</i> sp.2	60.4 (3.2) ^{ab}	10.0 (0.7) ^a	7.6 (0.5) ^a	2.4 (0.2) ^a
	<i>O. cyaneum</i>	65.4 (1.5) ^a	9.9 (0.8) ^a	7.2 (0.6) ^a	2.7 (0.2) ^a
Two way ANOVA (P value)	Species	0***	0.057	0.036*	0.152
	Rhizobia	0.042*	0.021*	0.006**	0.421
	Interaction	0.002**	0.181	0.126	0.453

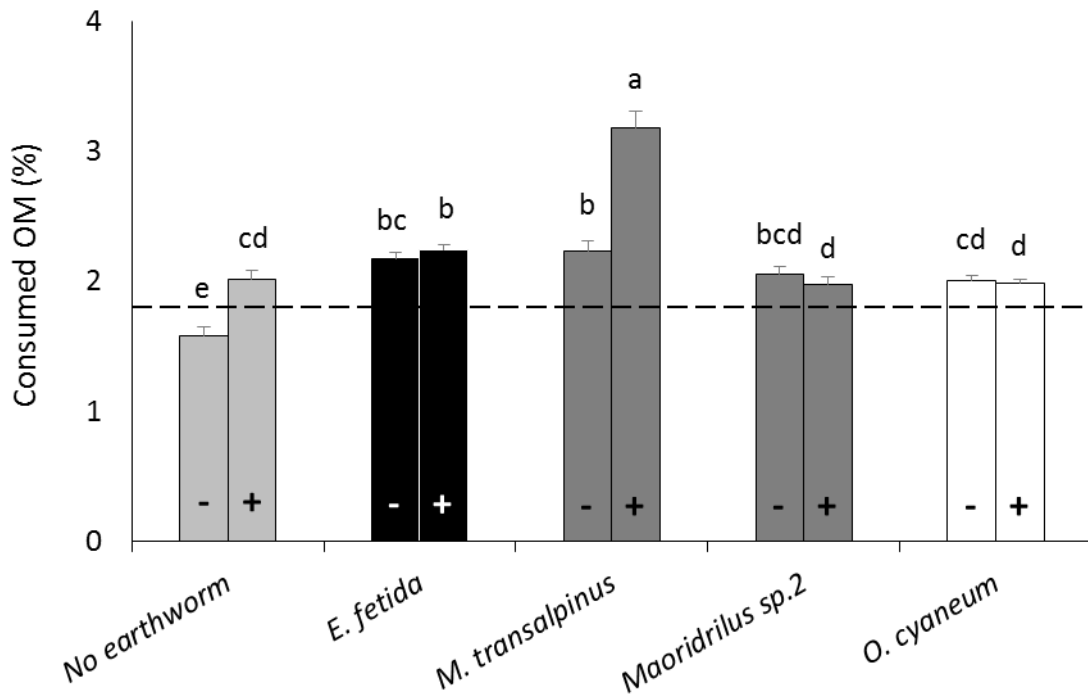


Figure 6.2 The amount of organic matter consumed (COM) by native and exotic earthworms after 8 weeks. Symbols refer to rhizobia strain present (+) or absent (-). Bars are means \pm standard error (n=5). Same letters indicate no significant difference (n=5, $p < 0.05$). Shading indicates different ecological groups of earthworms: epigeic (■), anecic (▒), endogeic (□) and control without earthworms (□). Dotted line indicates YMB addition without earthworms.

Rates of root nodulation similarly were only enhanced in the presence of *M. transalpinus* where the number of nodule per plant was twice higher in the presence of rhizobia (Figure 6.3). With other species of earthworms, the addition of the rhizobial inoculum made little difference. In pots without rhizobial inoculation there was significant root nodulation, suggesting that the soil or plants already contained appropriate and sufficient inocula. The source of the former may have been small amounts of clover in the dairy farm ryegrass sward, although Tan et al. (2015) found that closely-related *Mesorhizobium* type strains from other genera of legumes were unable to nodulate *Sophora microphylla* (which required its own specific type strains for nodulation). Genotypic data on rhizobia suggest co-evolution of rhizobial symbionts with *Sophora* in isolation from major areas of legume evolution has provided unique identities and novel characteristics (Tan et al., 2012).

Previous field studies elsewhere have shown that earthworms enhance N mineralization by reducing microbial immobilization, which may increase leaching losses of NO_3 (Blair et al., 1997; Domínguez et al., 2004). In the present study, *M. transalpinus* marginally lowered soil pH, enhanced EC, and substantially increased soil concentrations of more soluble forms of nitrogen in soil (Table 6.2). A few of the earthworms appeared to increase mobile P in soil, which was depleted in the presence of rhizobial inoculation. There was significant interaction between these two treatments. Dehydrogenase enzyme activity in soil appeared to be inhibited by altered soil pH following adding rhizobial inocula

(Figure 6.4). This was due to the broth solution contained CaCO_3 that results in an increase of soil pH (Wolińska and Stępniewska, 2012). However, DHA was enhanced by earthworm presences. Dehydrogenases provide a measure of overall soil microbial activity and play a significant role in the biological oxidation of soil organic matter, but most of this enzyme is produced by anaerobic microorganisms and would be expected to be lower under aerobic conditions (Wolińska and Stępniewska, 2012). In the presence of earthworms the bulk soil in the pots certainly was aerated through burrowing and disturbance (Edwards, 2004; Lemtiri et al., 2014), probably providing better conditions for rhizobial development and nodulation. At the same time, our observation when soil was removed from the pots indicated higher moisture in soil fractions that were obviously from earthworm burrows and drilosphere walls (Horn et al. 2006; Lemtiri et al., 2014); this may have provided locations of increase anaerobic conditions that are required for DHA production. Thus, we suggest that aeration of soil by earthworms provides improved conditions for nodulation in the rhizosphere, but also localised anaerobic conditions that favour anaerobic microorganisms and their intracellular DHA production.

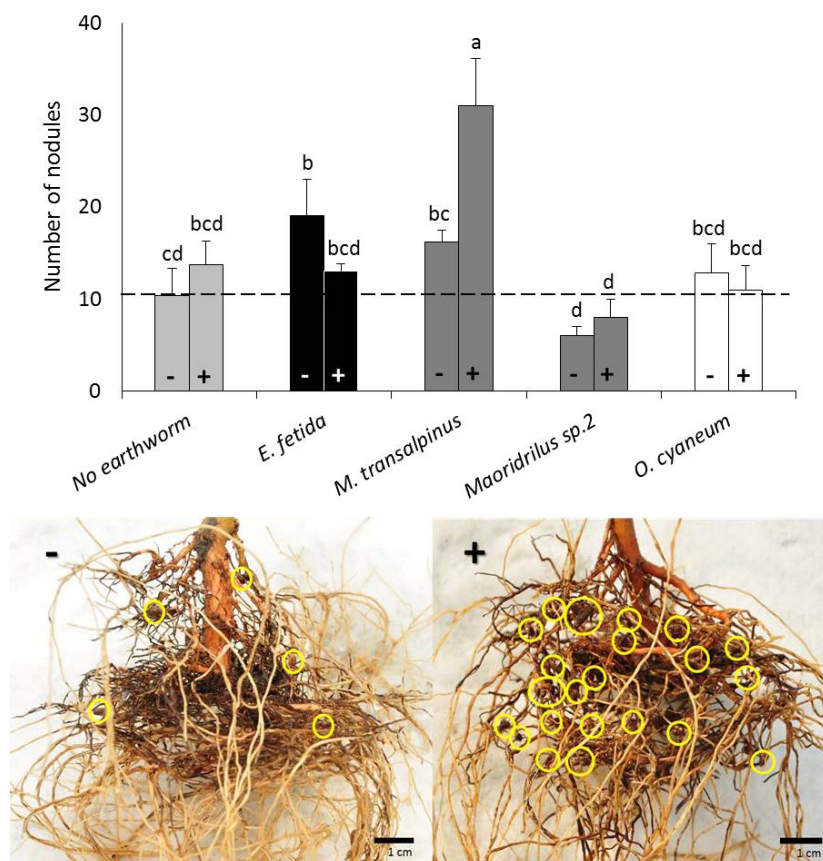


Figure 6.3 Root nodules on *S. microphylla* after 8 weeks inoculation. Symbols refer to rhizobia strain present (+) or absent (-). Bars are means \pm standard error. Same letters indicate no significant difference ($n=5$, $p<0.05$). Shading indicates different ecological groups of earthworms: epigeic (■), anecic (■), endogeic (□) and control without earthworms (□). Photographs show the effects of *M. transalpinus* activity on nodulation, marked with yellow circles. Dotted line indicates YMB addition without earthworms.

Table 6.2 Effects of earthworms and rhizobia on soil properties, with two-way ANOVA (* $p < 0.05$; ** $p < 0.01$; * $p < 0.001$). Values in brackets represent standard error of the mean (n=5). Same letters indicate no significant difference ($p < 0.05$).**

Treatment		pH (1:5W)	EC dS·m ⁻¹	Mobile N		Olsen P	Total C ----- (%) -----	Total N	Ratio of C/N
				NH ₄ -N ----- (mg·kg ⁻¹) -----	NO ₃ -N ----- (mg·kg ⁻¹) -----				
Control	No inoculant	5.4 (0.1)	0.06 (0.01)	0.32 (0.05)	19.4 (6.5)	29.3 (1.5)	2.41 (0.08)	0.22 (0.01)	10.8 (0.2)
No Rhizobia	No earthworm	5.3 (<0.1) ^a	0.08 (0.00) ^b	0.45 (0.15) ^a	29.2 (2.9) ^b	30.1 (0.3) ^b	2.50 (0.05) ^a	0.24 (0.01) ^{ab}	10.5 (0.1) ^b
	<i>E. fetida</i>	5.4 (<0.1) ^a	0.07 (0.01) ^b	0.34 (0.05) ^a	17.8 (5.2) ^b	30.3 (0.8) ^b	2.35 (0.06) ^a	0.22 (0.01) ^{bc}	10.9 (0.1) ^{ab}
	<i>M. transalpinus</i>	5.1 (<0.1) ^b	0.12 (0.01) ^a	1.13 (0.60) ^a	56.6 (2.6) ^a	32.7 (0.8) ^a	2.52 (0.06) ^a	0.24 (0.01) ^a	10.5 (0.2) ^b
	<i>Maoridrilus</i> sp.2	5.4 (<0.1) ^a	0.08 (0.01) ^b	0.51 (0.10) ^a	26.0 (3.5) ^{ab}	31.5 (1.0) ^{ab}	2.48 (0.07) ^a	0.21 (0.01) ^c	11.7 (0.5) ^a
	<i>O. cyaneum</i>	5.4 (<0.1) ^a	0.08 (0.00) ^b	0.44 (0.06) ^a	29.8 (3.4) ^{ab}	33.2 (0.5) ^a	2.41 (0.06) ^a	0.22 (0.00) ^{abc}	10.9 (0.1) ^{ab}
Rhizobia	No earthworm	5.5 (<0.1) ^a	0.06 (0.01) ^b	0.23 (0.02) ^b	14.2 (1.7) ^b	30.1 (1.3) ^a	2.42 (0.08) ^a	0.22 (0.01) ^a	10.7 (0.1) ^b
	<i>E. fetida</i>	5.4 (<0.1) ^{ab}	0.07 (0.01) ^b	0.37 (0.08) ^c	18.5 (4.2) ^b	28.5 (0.7) ^a	2.25 (0.03) ^{ab}	0.21 (0.00) ^a	10.8 (0.1) ^{ab}
	<i>M. transalpinus</i>	5.3 (<0.1) ^b	0.09 (0.01) ^a	0.83 (0.29) ^a	29.7 (6.1) ^a	29.9 (1.1) ^a	2.33 (0.08) ^{ab}	0.22 (0.01) ^a	10.8 (0.2) ^{ab}
	<i>Maoridrilus</i> sp.2	5.4 (<0.1) ^a	0.07 (0.01) ^b	0.46 (0.02) ^{bc}	17.1 (3.3) ^b	30.4 (1.0) ^a	2.39 (0.06) ^{ab}	0.20 (0.02) ^a	12.2 (1.0) ^{ab}
	<i>O. cyaneum</i>	5.4 (<0.1) ^a	0.06 (0.00) ^b	0.58 (0.13) ^b	16.0 (1.9) ^b	29.6 (0.6) ^a	2.24 (0.02) ^b	0.20 (0.00) ^a	11.0 (0.1) ^{ab}
Two way ANOVA (<i>p</i> value)	Species	0***	0***	0.133	0***	0.123	0.045*	0.017*	0.01**
	Rhizobia	0.002**	0***	0.493	0***	0.002**	0.002**	0.006**	0.379
	Interaction	0.132	0.274	0.678	0.013*	0.272	0.873	0.875	0.93

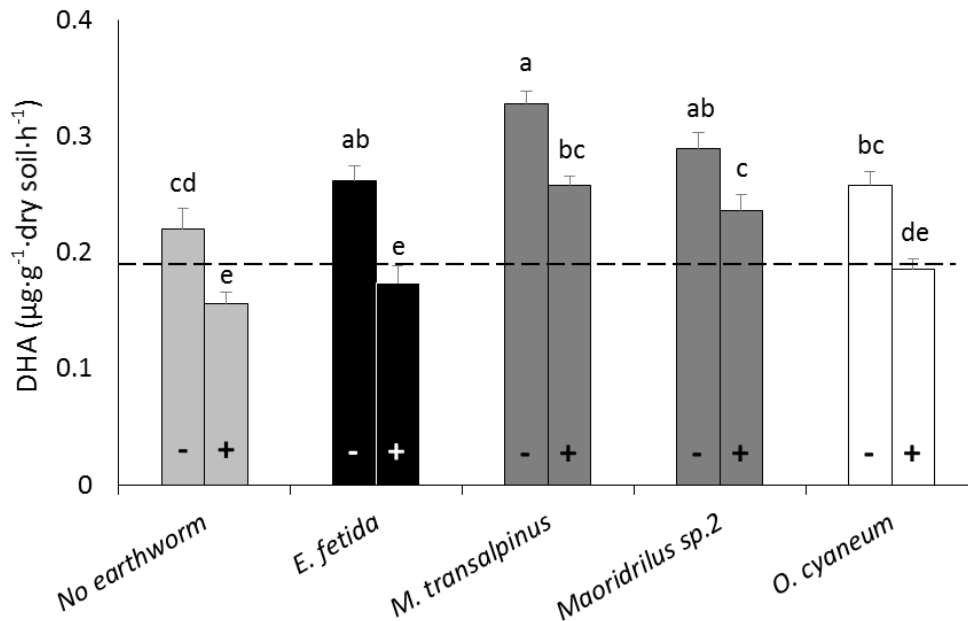


Figure 6.4 Dehydrogenase enzyme activity (DHA) in soil after 8 weeks. Symbols refer to rhizobia strain present (+) or absent (-). Bars are means \pm standard error (n=5). Same letters indicate no significant difference ($p < 0.05$). Shading indicates different ecological groups of earthworms: epigeic (■), anecic (▒), endogeic (□) and control without earthworms (□). Dotted line indicates YMB addition without earthworms.

A similar explanation may be responsible for differences in N_2O production, which was generally reduced in the presence of earthworms (Figure 6.5). Nitrification by aerobic, ammonia-oxidizing bacteria requires well-drained and aerated soils to produce nitrate from ammonium, as well as some nitrous oxide (Barnard and Leadley, 2005; Wrage et al., 2004). However, denitrification of nitrate produces more nitrous oxide, but requires anaerobic conditions. Differences in N_2O production, with and without rhizobial inoculation, reflect the same patterns as DHA, except for pots with *M. transalpinus*. This species clearly had the most substantial influence on the results of the current experiment. It appears that, when there are more rhizobia, in more aerobic conditions, there is consequently less N_2O production (Figure 6.5). Its higher level of burrowing activity appeared to provide aerobic conditions for higher nodulation that outweighed anaerobic conditions within drilosphere walls. This species consumed more organic matter, but increased aeration that provided more nitrate from its decomposition, but less conversion of NO_3 to N_2O . This differs to some previous studies that have shown increased N_2O production in the presence of earthworms in both laboratory and field conditions (Lubbers et al., 2013a, b). We have found previously that individual species could be separated on the basis of their modification of soil biogeochemistry, with differences particularly evident in terms of organic matter consumption, nitrogen and phosphorus mineralisation, soil microbial biomass and greenhouse gas emissions in Chapter 3 and 5. Obviously this becomes more

complex in the presence of plant roots; for example, Milleret et al. (2009) found that plant roots improved macroaggregate stability whereas earthworms decreased it. The present study indicates that nodulation of roots also has strong interactions and significant feedback between these variables.

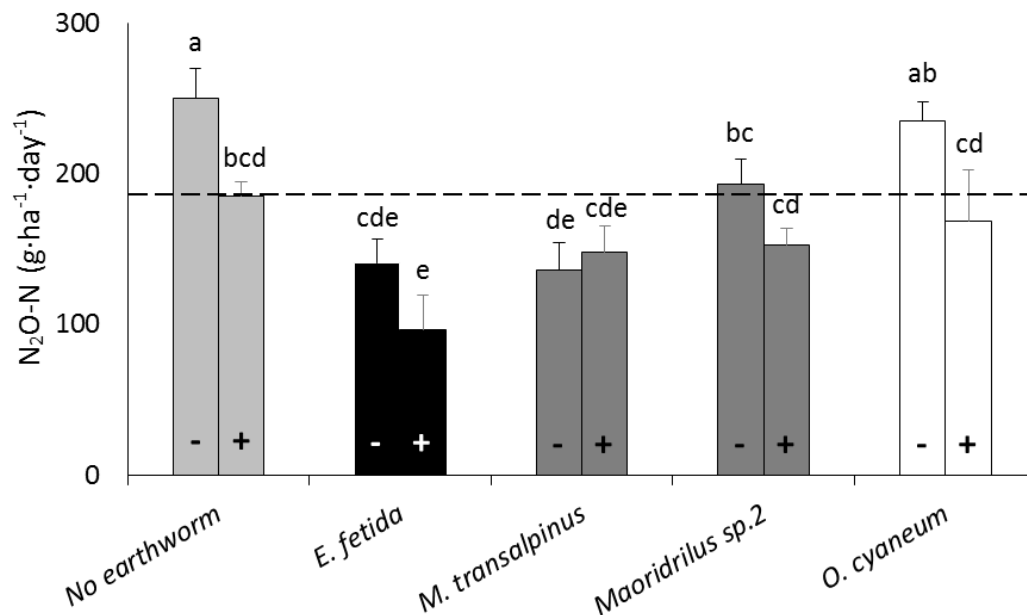


Figure 6.5 Release of nitrous oxide (N₂O) after 8 weeks. Symbols refer to rhizobia strain present (+) or absent (-). Bars are means ± standard error (n=5). Same letters indicate no significant difference ($p < 0.05$). Shading indicates different ecological groups of earthworms: epigeic (■), anecic (▒), endogeic (□) and control without earthworms (◻). Dotted line indicates YMB addition without earthworms.

In terms of restoration practice, invasive exotic legumes are known to be capable of fixing up to 200 kg N·ha⁻¹·annum⁻¹ in New Zealand (Magesan et al., 2012); an amount equivalent to current standard fertiliser applications in intensive agricultural land. Nitrate leaching does not appear to be higher under leguminous plants (Pattinson and Pattinson, 1985), but little is known of the N-fixing capacity of native legumes. Stands of exotic invasive gorse (*Ulex europaeus*) have been proven to have a role in restoration, providing nurse environments for native New Zealand plants (Burrows et al., 2015). However, this species has largely established on former agricultural land from which native earthworms have probably disappeared. The challenge remains to understand the role of both native legumes and native earthworms in the restoration trajectory, and their influence on both NO₃ and N₂O transfer to the wider environment.

The most desirable outcome would be to restore both native legumes and native earthworms into agricultural landscapes, whilst mitigating environmental concerns related to nitrogen leakage and release to the wider environment. This would have beneficial conservation outcomes and would also address environmental concerns. Results of the present study have demonstrated the interdependence between earthworms, root nodulation of *Sophora* and soil physicochemistry. Some combination of these factors mediates nitrogen cycling and the release of NO_3 and N_2O to the wider environment. This work is a first step towards a better integrated understanding of the effects of plant growth, earthworm and microbial communities on N-cycling.

Chapter 7

Potential role of New Zealand native earthworms (Megascolecidae) as ecosystem engineers on agricultural land

7.1 Abstract

Landscape conversions to agriculture has impacted soil biodiversity. In New Zealand, invasive species of earthworm improve fertility of farmland soils, but there is lack of knowledge of the functionality of native species. The aim of the present study was to identify the potential viability and impacts of native earthworm on pastoral soils, typical of NZ agricultural landscapes. In a mesocosm study, a native species *Maoridrilus transalpinus* showed high survivorship (>90 %) in native forest (NF), sheep-farm (SF), and dairy-farm (DF) soils, but all exotic *Octolasion cyaneum* died in NF soil. Concentrations of ammonium (NH_4^+) in NF soil significantly increased in presence of *M. transalpinus*. However, the effects of the rhizosphere in reducing solubility of N and P far outweighed the effects of earthworms. *M. transalpinus* decomposed more organic matter in DF soils, and substantially enhanced root morphology, soil biochemistry, and measures of plant growth (dry weight and photosynthetic pigments). Native earthworms may be more beneficial than exotic species in agricultural soils through enhancement of crop yields and providing sustainable farming practices. This would also help to safeguard the biodiversity of native fauna.

Keywords:

Earthworm, Soil biogeochemistry, Root system, Soil sustainability, Biodiversity, Agroecosystem

7.2 Introduction

Geographical isolation and conversion of native habitat to agriculture in New Zealand has restricted indigenous earthworm species into remnants of native vegetation in New Zealand (Boyer, 2013; Kim et al., 2015). Introduced European Lumbricidae are more tolerant of disturbance caused by intensive land management (i.e. tillage and application of chemical fertilizers); this exotic family of earthworms has become the dominant colonizer of agricultural soils (Fraser et al., 1996; Springett et al., 1998). Competitive exclusion of native earthworms by exotic species on arable land may have occurred, but Hendrix (2006) and Lee (1985) thought this was unlikely. This contrasts with the situation elsewhere; in California, for example, Winsome et al. (2006) considered that exotic earthworms exclude natives in highly productive pasture land with relatively high rates of growth and reproduction, but that natives can predominate and maintain their abundance in low-resource pasture land through exploiting a wider variety of organic matter. Nonetheless, coexisting assemblages of native and exotic earthworms have been documented in native forests worldwide (Hendrix et al., 2006).

In New Zealand, we have observed that native vegetation contains native Megascolecidae and introduced Lumbricidae occurring together in multiple sites, including native forests and restoration plots on Banks Peninsula and the West Coast (Kim et al., 2015). For example, the Ahuriri Scenic Reserve in Canterbury contains a diverse native vegetation that provides a habitat for the coexistence of *Maoridrillus transalpinus* and *Octolasion cyaneum*. *M. transalpinus* is dispersed from the West to East Coast of South Island (Buckley et al., 2015; Lee, 1959a, b) and is recognised as an anecic species by Kim et al. (2015). This species has beneficial effects on soil physico-chemistry comparable to exotic endogeic species (*Aporrectodea caliginosa* and *Octolasion lacteum*).

Worldwide, anthropogenic disturbances appear to have favoured colonization of exotic earthworms, following deforestation for cultivation and urbanization (González et al., 2006), whilst native earthworms seem to maintain their populations in undisturbed soils (Hendrix et al., 2006). However, in New Zealand there is no knowledge of the role of native species alongside pasture soils, and along boundaries lines of remnant forests. We hypothesize that native earthworm may relocate in modified landscapes under modern conditions of less intensive management (particularly reduced ploughing and rotation) and, where this occurs, they may also have a positive influence on agroecosystems. Exotic species have certainly been referred to as living fertilizer on agricultural land and native species may have a similar role in the future. The aim of this experimental work using mesocosms was to investigate the viability and effects of *M. transalpinus* on soils and pasture grass that are typical of South Island agricultural landscapes.

7.3 Materials and methods

7.3.1 Earthworm, soil and seed

Native *M. transalpinus* and exotic *O. cyaneum* were collected from Ahuriri Reserve (43°39'58.97"S, 172°37'26.37"E) and Okuti Reserve (43°47'7.98"S, 172°49'51.23"E) on Banks Peninsula, by digging and hand-sorting. Both species co-existed inside and outside the boundary of native vegetation, but native species have been depopulated in pastoral lands. They have easily recognizable morphology with good survivorship under laboratory conditions. Of the earthworms, mature earthworms with a distinct clitellum were selected on the basis of the visually most viable and healthy laboratory cultures on each experimental set-up occasion.

Three soils with different fertility were collected from a native forest (NF), sheep farmland (SF) and dairy farmland (DF) described previously (Table 7.1). Surface soils (0-15 cm) were dug from the Nikau reserve of West Coast for NF soil, and from two Lincoln University farms for SF and DF soils. NF soil is an acidic silt loam that contains large amounts of organic matter (Kim et al., 2015). The two other silt loam soils (SF and DF) were from stocked farmland. SF soil has lower total P and nitrate content than DF soil because it has not been fertilized under intensive management. The collected soils were sieved using a 4 mm sieve prior to use in the pot experiment.

Lolium perenne cv. Ceres ONE⁵⁰ (perennial ryegrass) was sown in this pot experiment. This has been introduced as high performance ryegrass across New Zealand, with a recommended sowing rate of 20 kg·ha⁻¹ (Agricom, 2011).

Table 7.1 Sampling locations, classification and properties of NF, SF and DF soils (Kim et al. 2015). Ahuriri Reserve data were not used in the pot experiment, but were a collection site for earthworms and were provided as a reference.

	----- Native Forest (NF) -----		Sheep Farm (SF)	Dairy Farm (DF)
Location	43°39'58.97"S, 172°37'26.37"E	42°8'38.39"S, 171°19'50.36"E	43°38'39.48"S, 172°23'28.07"E	43°38'11.27"S, 172°26'17.56"E
Classification	Mafic Brown	Sandy or Orthic Brown	Immature Pallic	Immature Pallic
Land Use	#Ahuriri Reserve	Nikau Scenic Reserve	Sheep farmland	Dairy farmland
Properties				
Texture	-	Silt loam	Silt loam	Silt loam
pH (1:5W)	5.5 (0.1)	4.7 (0.2)	5.4 (0.0)	5.6 (0.0)
Organic matter (%)	29.5 (2.6)	22.6 (0.2)	7.5 (0.1)	7.3 (0.2)
Total C (%)	-	10.7 (3.4)	3.3 (0.1)	3.3 (0.4)
C/N ratio	-	16.9 (0.9)	12.2 (0.3)	15.0 (0.0)
NH ₄ -N (mg·kg ⁻¹)	0.9 (0.1)	19.6 (1.4)	2.9 (0.5)	1.8 (0.5)
NO ₃ -N (mg·kg ⁻¹)	3.9 (0.5)	18.3 (0.5)	88.1 (0.6)	118 (12)
Total P (mg·kg ⁻¹)	-	836 (116)	341 (-)	596 (7)
Olsen P (mg·kg ⁻¹)	-	15.5 (0.2)	34.4 (0.1)	23.8 (0.2)

#Ahuriri Reserve is earthworm collection site.

7.3.2 Mesocosms

A two week acclimatization period in each bulk soil prior to the pot experiment was provided to ensure earthworm viability. The experiment was carried out using plastic plant pot (\varnothing 13 cm, 1.5 L). About 0.4 g of *Lolium perenne* cv. Ceres ONE⁵⁰ seeds, which is about triple the recommended rate (20 kg·ha⁻¹) (Agricom, 2011), was sown in pots containing either NF, SF, or DF soil. A week after germination, two earthworms of the same species were added to each pot. Each treatment had five replicates. To prevent earthworms escaping, the bottom and the top of the pots were sealed with nylon mesh. Pots were randomly arranged on a table in a greenhouse at the Lincoln University Nursery, and maintained with regular watering. The experiment was carried out at 20-25 °C in 2014. Grass was harvested 4 weeks after addition of earthworms. Pots were removed and the roots systems were photographed prior to harvest and analysis.

7.3.3 Harvest and analysis

Harvested foliage was oven-dried at 65 °C for 3 days for determination of dry weight (DW). Prior to drying, 0.1 g of fresh leaves was stored for 5 days in 10 ml of 80 % acetone to determine chlorophyll and carotenoid content. These content was determined at different wavelengths of 663, 645, and 470 nm respectively, using a UV 160A spectrophotometer (Shimadzu, Japan) and calculated as formula below (Jeoung et al., 2013):

$$\text{Chlorophyll a} = 12.7 A^{663} - 2.69 A^{645}$$

$$\text{Chlorophyll b} = 22.9 A^{645} - 4.68 A^{663}$$

$$\text{Total Chlorophyll (a + b)} = 20.29 A^{645} + 8.02 A^{663}$$

$$\text{Total Carotenoid} = (1000 A^{470} - 1.82 \text{ chl a} - 85.02 \text{ chl b}) / 198$$

Where A = wavelength

After removing surface moss and organic debris, 1 kg of fresh rhizosphere soil from each pot was collected in plastic bags after removing the bulk of plant roots. Earthworm biomass was weighed and the mortality was recorded by counting the surviving number of earthworms. Samples of fresh soils were stored at 4 °C, then extracted with 2M KCl for analysis of available N (ammonium and nitrate) using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden) (Clough et al., 2001). To analyse Microbial Biomass Carbon (MBC), soil samples were fumigated with ethanol-free CHCl₃, then extracted with 0.5M K₂SO₄ and determined using a TOC-500A analyser (Shimadzu Oceania Pty Ltd., Australia) (Blakemore, 1987). As an indicator of microbiological activity, soil Dehydrogenase

Enzymatic Activity (DHA) was determined based on the reduction 2,3,5-triphenyltetrazolium chloride (TTC) solution to triphenylformazan (TPF) using a modified method described in Casida et al. (1964) and Chander and Brookes (1991). Mixture of soil (2 g) and TTC solution (2 ml) was incubated during 24 hours at 25 °C in darkness. After adding methanol (10 ml) into the mixture, it was extracted and centrifuged at 1880 x *g* rpm for 10 minutes. The supernatant of hydrolysis reaction products was measured the absorbance at 485 nm through a UV 160A spectrophotometer (Shimadzu, Japan).

Air-dried soil samples were sieved to <2 mm using a stainless steel sieve. Soil pH and electronic conductivity (EC) were measured using pH and EC meters (Mettler Toledo Seven Easy). For total organic matter (TOM), oven-dried soil samples at 105 °C were analysed for loss on ignition (LOI) in a muffle furnace at 500 °C (Blakemore, 1987). Following 0.5M NaHCO₃ extraction, plant available-P was measured as Olsen P, spectroscopically at a wavelength of 880 nm using a UV 160A spectrophotometer (Shimadzu, Japan) (Blakemore, 1987).

7.3.4 Statistical analysis

Minitab 17 (Minitab Inc., State College, Pennsylvania, USA) was used for all statistical analysis. To compare means of each earthworm species treatment ($n=5$, $p<0.05$), a one-way ANOVA was carried out using a Fisher's Least Significant Difference test. The overall effect of species, biosolids addition rate, and those interactions with soil properties were assessed using two-way ANOVA analyses. Principal Components Analysis was used to investigate patterns of variation in the dataset, with a focus on the consensus degree between soils incubated with and without different earthworm species. Relative site-to-site dissimilarities in ordination space were detected based on PCA Axis 1 and 2 scores. For soil pH, mean and standard errors were calculated following conversion to the equivalent hydrogen ion concentrations and back calculation to pH. To identify effects of biosolids addition on gas release, a Pearson test was conducted between NH₄, NO₃, dry weight, and chlorophyll content.

7.4 Results and discussion

7.4.1 Mortality of earthworms

Native *M. transalpinus* had higher viability than exotic *O. cyaneum* in all soil treatments during the 4 week growth period (Table 7.2). NF soil was suitable for *M. transalpinus*, but lethal to *O. cyaneum*. NF soil contained similar organic matter content to the Ahuriri Reserve soil (from where earthworms were collected) (see Table 7.1), but the NF soil is considerably more acid. Earlier choice chamber trials in Chapter 3 showed *O. cyaneum* totally avoided NF soil whereas *M. transalpinus* preferentially this NF

soil and consumed substrate organic matter over a week. Both Eijsackers (2011) considered soil acidity is more responsible for mortality than other soil properties including organic matter. In the present study soil pH in NF soil significantly lower than agricultural soils of SF and DF (Table 7.4).

Table 7.2 Proportions of earthworm viability and biomass variation during four weeks mesocosms. Values in brackets represents standard errors of the mean (n=5).

Soils	Species	Viability (%)	Biomass variation (%)
NF	<i>M. transalpinus</i>	100 (± 0)	-18 (± 1)
	<i>O. cyaneum</i>	0 (± 0)	ND [#]
SF	<i>M. transalpinus</i>	100 (± 0)	-13 (± 4)
	<i>O. cyaneum</i>	60 (± 19)	-12 (± 12)
DF	<i>M. transalpinus</i>	90 (± 10)	-15 (± 7)
	<i>O. cyaneum</i>	60 (± 19)	-30 (± 15)

[#]ND (Not Determined)

7.4.2 Plant growth

Plant growth, chlorophyll and carotenoid contents all increased in the presence of earthworms (Table 7.3). Of the three soils, NF soil most significantly enhanced plant productivity, chlorophyll and carotenoid content. NF soil contains high concentration of organic matter and NH_4^+ (Table 7.1). *M. transalpinus* had a significantly large effects on chlorophyll and carotenoid content, particularly in NF and DF soils. Chlorophyll content is associated with N status in foliage, reflecting the quantity of mineral N absorbed by plants (Castelli et al., 1996; van den Berg and Perkins, 2004). Thus, it is likely that mineralization by earthworms, particularly *M. transalpinus* in this experiment, probably increased N uptake and growth. Fernández-Luqueño et al. (2010) found the same effect of earthworms on beans.

Earthworm burrowing influenced rhizosphere structures and there was a noticeably different morphology depending the species (Figure 7.1). In particular, the anecic *M. transalpinus* constructed relatively large and permanent drilospheres compared to the endogeic *O. cyaneum*. It was observed that *M. transalpinus* seemed to encourage a thicker root system around its drilosphere while *O. cyaneum* produced a complexed system of fine roots.

Table 7.3 Dry weight (DW) and chlorophyll and carotenoid content of *L. perenne* during 4 weeks of growth in mesocosms. Values in brackets represent standard errors of the means. The same letter indicates no significant difference within each soil treatment (n=5, $p<0.05$). Correlation between soil types, earthworm species, and their interaction was measured using a two-way ANOVA statistical analysis (* $p<0.05$; ** $p<0.01$; * $p<0.001$). Control pots contained ryegrass without earthworms.**

Soil	Species	DW (g)	----- (mg·g ⁻¹) -----			
			Chl. a	Chl. b	Total Chl.	Carotenoid
NF	Control	2.2 (0.1) ^b	0.8 (<0.1) ^c	0.3 (<0.1) ^c	1.0 (0.1) ^c	3.1 (0.2) ^c
	<i>M. transalpinus</i>	2.3 (0.1) ^b	1.7 (0.1) ^a	0.6 (<0.1) ^a	2.3 (0.1) ^a	7.0 (0.2) ^a
	<i>O. cyaneum</i>	2.6 (0.1) ^a	1.4 (0.1) ^b	0.5 (<0.1) ^b	1.9 (0.2) ^b	5.6 (0.3) ^b
SF	Control	0.8 (<0.1) ^b	0.3 (<0.1) ^b	0.1 (<0.1) ^b	0.3 (0.1) ^b	1.1 (0.2) ^b
	<i>M. transalpinus</i>	1.2 (<0.1) ^a	0.9 (0.1) ^a	0.3 (<0.1) ^a	1.2 (0.1) ^a	3.9 (0.3) ^a
	<i>O. cyaneum</i>	1.1 (<0.1) ^a	0.8 (0.1) ^a	0.3 (<0.1) ^a	1.0 (0.1) ^a	3.2 (0.3) ^a
DF	Control	1.5 (<0.1) ^c	0.5 (<0.1) ^c	0.2 (<0.1) ^b	0.6 (<0.1) ^c	1.9 (0.1) ^c
	<i>M. transalpinus</i>	1.7 (<0.1) ^a	1.0 (<0.1) ^a	0.4 (<0.1) ^a	1.4 (0.1) ^a	4.2 (0.2) ^a
	<i>O. cyaneum</i>	1.6 (<0.1) ^b	0.8 (0.1) ^b	0.3 (<0.1) ^b	1.1 (0.1) ^b	3.4 (0.4) ^b
Two-way ANOVA (<i>P</i> values)	Soils	***	***	***	***	***
	Earthworms	***	***	***	***	***
	Interaction	**	*	**	*	*

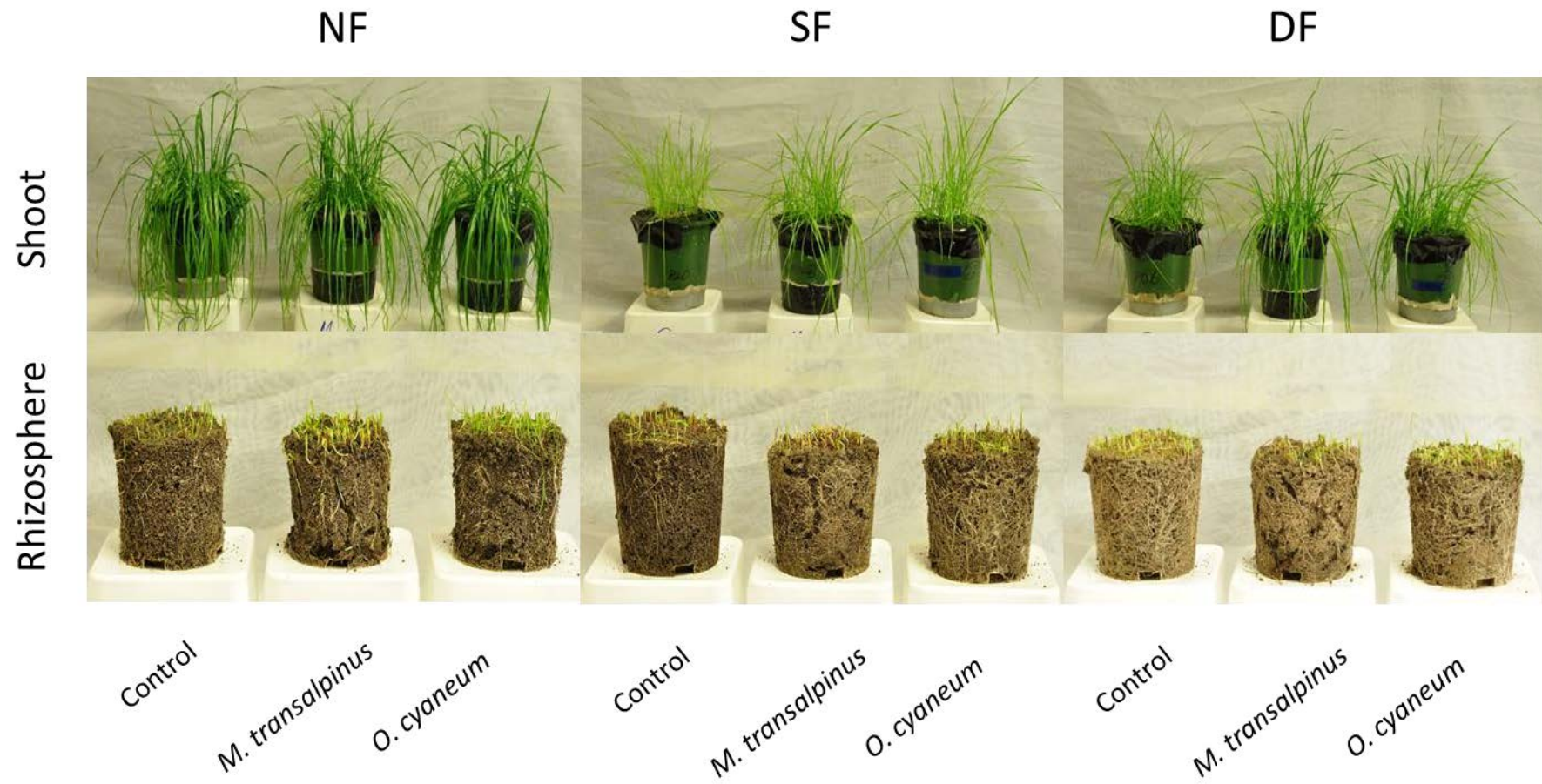


Figure 7.1 Comparison of shoot and root development in different soil pots of NF, SF, and DF in the presence of *M. transalpinus* and *O. cyaneum* after 4 weeks in mesocosms. Control pots contained ryegrass without earthworms.

7.4.3 Effects on soil properties

Total organic matter (TOM) declined in NF and SF soils over the 4 weeks (Table 7.4, cf. Table 7.1). Although *O. cyaneum* was unable to survive in NF soil that contained low pH and high concentrations of NH_4^+ , it appeared that this species consumed OM during 4 weeks inoculation (Table 7.4). A significant decrease of TOM content occurred in both forest and agricultural soils in the presence of *M. transalpinus* which had high survivorship. However, *O. cyaneum* tended to reduce TOM in DF soil. In other studies by the present author and elsewhere, earthworm activity has been shown to increase soil acidity and electronic conductivity (EC) (Cheng and Wong, 2002; Kim et al., 2015), but no similar effect was recorded in these mesocosms.

In contrast, earthworms affected microbial biomass C (MBC) and dehydrogenase enzymatic activity (DHA) during the experiment (Figure 7.2). Of those microbial activity indicators, *M. transalpinus* significantly increased both MBC and DHA in the dairy farm soil, whilst exotic *O. cyaneum* had most effect on DHA (Figure 7.2). These two measurements of soil microbiology show contrasting patterns in the 3 control soils: MBC was higher in soil with more OM (NF >SF >DF) and DHA was higher in less acidic soil (DF >SF >NF). Similar findings have been reported previously (Blońska, 2010; Moeskops et al., 2010). Overall there was significant interactions between earthworm species and soil type ($p < 0.01$). It is possible that the casts of *M. transalpinus* resulting from decomposition and digestion of organic matter in DF created a more favourable infrastructure for soil microbes. In SF pot soil, the presence of *O. cyaneum* significantly enhanced DHA compared to that of *M. transalpinus* ($p < 0.05$).

Table 7.4 Total organic matter (TOM), pH and electronic conductivity (EC) of soil after 4 weeks. Values in brackets represent standard errors of the means. The same letter indicates no significant difference within each soil treatment (n=5, $p < 0.05$). Correlation between soil types, earthworm species, and the interaction between both soils and earthworms were measured using a two-way ANOVA statistical analysis (* $p < 0.05$; ** $p < 0.01$; * $p < 0.001$, ^{NS}not significant). Control pots contained ryegrass without earthworms.**

Soil	Species	TOM	pH	EC
		(%)	(1:5W)	($\text{dS}\cdot\text{m}^{-1}$)
NF	Control	18.1 (0.2) ^a	4.4 (<0.1) ^b	0.13 (0.01) ^a
	<i>M. transalpinus</i>	17.3 (0.1) ^b	4.4 (<0.1) ^a	0.13 (0.01) ^a
	<i>O. cyaneum</i>	17.0 (0.3) ^b	4.4 (<0.1) ^b	0.12 (<0.01) ^a
SF	Control	6.7 (<0.1) ^a	5.4 (<0.1) ^a	0.04 (<0.01) ^a
	<i>M. transalpinus</i>	6.3 (<0.1) ^b	5.4 (<0.1) ^a	0.03 (<0.01) ^b
	<i>O. cyaneum</i>	6.0 (0.2) ^b	5.4 (<0.1) ^a	0.04 (<0.01) ^{ab}
DF	Control	7.5 (0.1) ^a	5.5 (<0.1) ^a	0.04 (<0.01) ^a
	<i>M. transalpinus</i>	7.1 (<0.1) ^b	5.5 (<0.1) ^a	0.05 (<0.01) ^a
	<i>O. cyaneum</i>	7.5 (0.1) ^a	5.5 (<0.1) ^a	0.05 (0.01) ^a
Two-way ANOVA (p values)	Soils	***	***	***
	Earthworms	***	NS	NS
	Interaction	**	**	NS

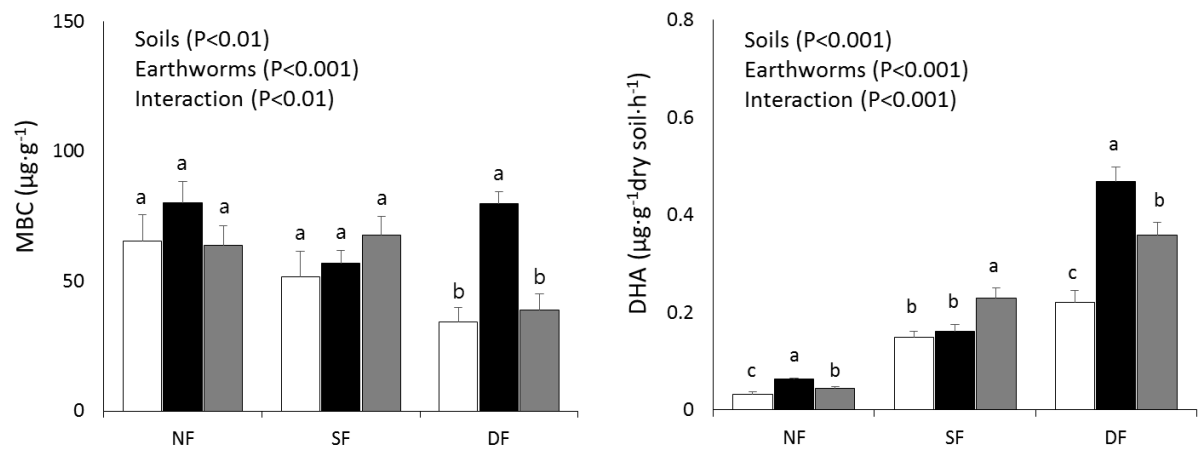


Figure 7.2 Microbial Biomass C (MBC) and Dehydrogenase Enzyme Activity (DHA) of rhizosphere soils after 4 weeks. Different shading indicates different treatments of species; control (□), *Maoridrilus transalpinus* (■) and *Octolasion cyaneum* (▒). Bar value is means \pm standard errors. The same letter indicates no significant difference ($n=5$, $p<0.05$). Two-way ANOVA results are inserted.

7.4.4 Mineralization of N and P

Ryegrass substantially depleted $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and Olsen P in the two agricultural soils (Figure 7.3 cf. Table 7.1). In contrast $\text{NH}_4\text{-N}$ was greatly enhanced in NF, likely due to decomposition of organic matter. The effects of earthworms were negligible compared to the effects of the root system, with the exception of reduced $\text{NO}_3\text{-N}$ in NF. In Chapter 3, similar declines of nitrate were found in rhizosphere soils due to the presences of native plants and ryegrass, but there was no influences of earthworm activity.

There were high correlations between dry weight of ryegrass and extractable NH_4 ($r=0.827$, $p=0$) and NO_3 ($r=0.755$, $p=0$) and between chlorophyll content and extractable NH_4 ($r=0.692$, $p=0$) and NO_3 ($r=0.435$, $p=0.003$). Thus, mineralized N by earthworms may be absorbed by plant roots directly, thereby increasing plant yields and physiological activity. Overall, the effects of soil fertility outweighed solubility of nitrogen and phosphorus differences due to earthworms.

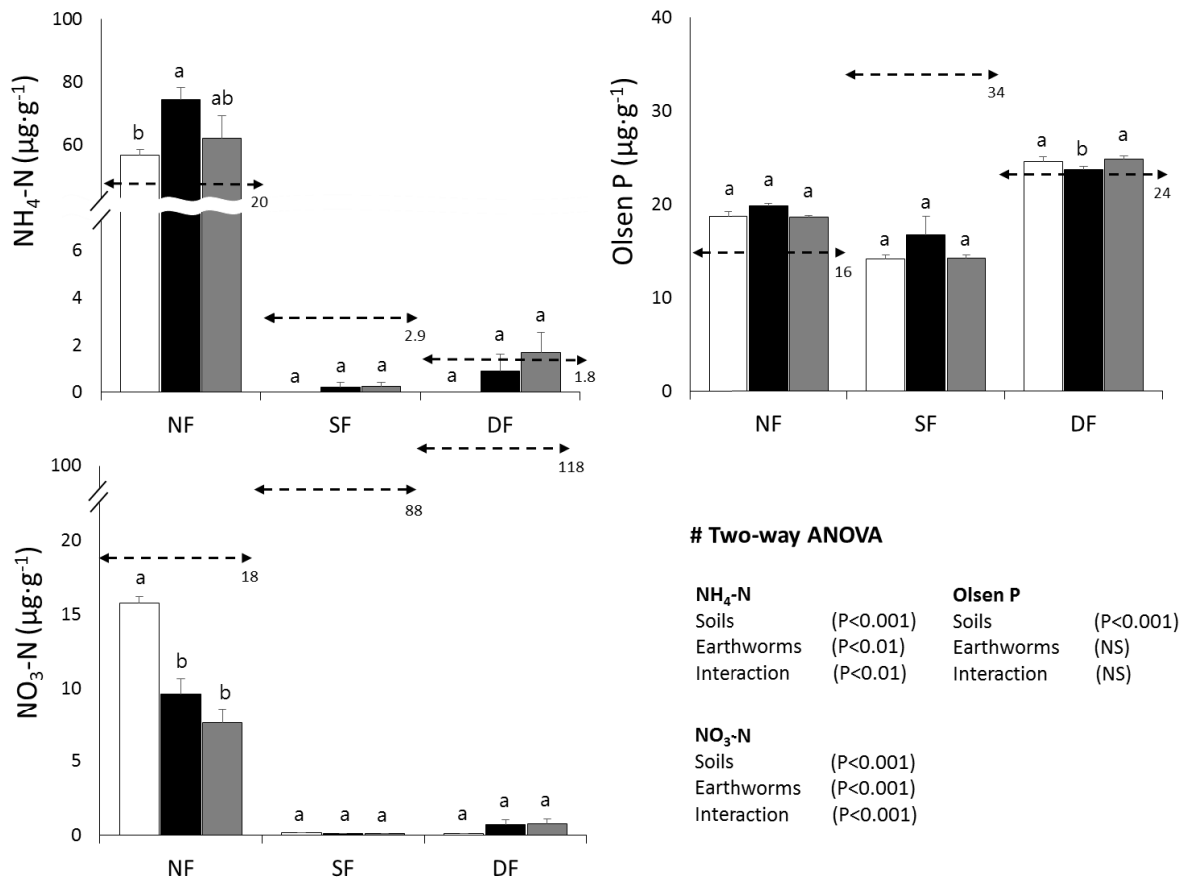
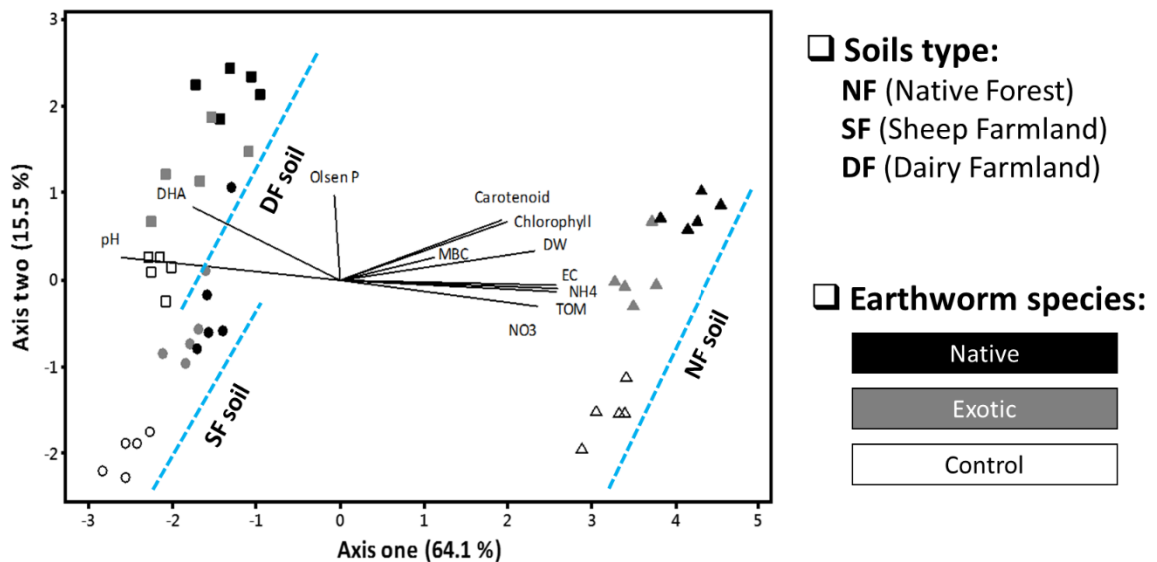


Figure 7.3 Extractable concentrations of nitrogen (NH₄-N and NO₃-N) with 2M KCl, phosphorus (Olsen P) with 0.5M NaHCO₃ in the rhizosphere soils after 4 weeks. Different shading indicates different treatments of species; control (□), *Maoridrilus transalpinus* (■) and *Octolasion cyaneum* (▣). Bar value is means ± standard errors (n=5). The same letter indicates no significant difference ($p<0.05$). Data for bulk soil are represented using dotted arrows and Two-way ANOVA results are inserted.

7.4.5 Overall effects

Multivariable analyses of the combined dataset (Figure 7.4) strongly separated the soils from the three sites in relation to pH and organic matter (Axis 1). Compared to agricultural soils (SF and DF), a richness of OM in the native forest soil had more influence on N mobility of rhizosphere, plant pigments and productivity. Furthermore, the individual species of earthworm certainly separated along both axes. On Axis 1, *M. transalpinus* is distributed more to right-hand side (cf. control and *O. cyaneum*) indicating enhanced N mineralization (NH₄⁺ and NO₃⁻) and electronic conductivity (EC) associated with the native species. The second axis also separated *M. transalpinus* on more upward trajectory than other treatments, related to increased P solubility (Olsen P), microbial activity (DHA), and plant growth. It is possible that the native *M. transalpinus* has more influence on soil biogeochemistry and plant productivity on agricultural system, than the introduced species.



	DW	Chlorophyll	Carotenoid	NH ₄	NO ₃	Olsen P	MBC	DHA	pH	EC	TOM
PC1	0.33	0.28	0.27	0.37	0.33	-0.01	0.16	-0.25	-0.37	0.37	0.37
PC2	0.19	0.39	0.41	-0.06	-0.18	0.56	0.15	0.49	0.15	-0.04	-0.09

Figure 7.4 Multivariate analysis of the dataset separated the earthworm-incubated pot soils and the species on two axes. Principal Component Analysis (PCA) of soil properties after 4 weeks incubation. Coefficients of each variable for PC1 and PC2 through the PCA. Symbols represent individual pot soils (n=5) of NF (Δ), SF (\circ), and DF (\square). Different colours indicate earthworm species: *M. transalpinus* (black), *O. cyaneum* (grey) and control (white).

7.5 Conclusion

Earthworms can increase plant growth through their capacity to enhance decomposition of organic matter to mineralize nutrients. This was evident in increased growth of ryegrass in the present study. The drilosphere may provide micro-environments which further stimulate nutrient cycling and the release of plant-available nutrients. The results showed that the morphology of the ryegrass rhizosphere is modified by earthworms, with differences apparent between different species of earthworms. Clearly, this affects soil microbiology and physicochemistry. In the current study, native *M. transalpinus* provided benefits to the rhizosphere of ryegrass in the dairy farm soil (DF), by enhancing microbial activity; however, the effects of the rhizosphere in depleting soil N and P far outweighed the effects of earthworms in increasing soil N and P. The disappearance of organic matter in SF and NF soils in the presence of earthworms is likely to have played a significant role in providing nutrients for ryegrass growth.

Although the growth of *L. perenne* depended on soil properties, the presence of native *M. transalpinus* was significantly associated with yields of the pasture and indicators of plant photosynthesis (chlorophyll and carotenoid content). Moreover, burrows of *M. transalpinus* in dairy-farmland (DF) soil considerably stimulated more microbial activity (i.e. DHA) compared to exotic *O.*

cyaneum, which is known to be a dominant component of the soil fauna in temperate agricultural soils globally. It can be inferred from the higher viability of *M. transalpinus* in arable soil (DF) that there may be increased abundance and a functional roles for native species of earthworms on agroecosystems, particularly with less intensive land management that includes reduced cultivation.

Chapter 8

Biochemical impacts of endemic *Maoridrilus* earthworms (Megascolecidae) in biosolid-amended soil

8.1 Abstract

Biosolids can be a valuable fertiliser for agriculture and in ecological restoration, although there are concerns about biological and chemical contaminants. Earthworm activity, including vermicomposting of biosolids, may influence the efficacy of their use. We investigated how *E. fetida* and two New Zealand endemic anecic species of *Maoridrilus* affect mobility of nutrients and trace elements, as well as greenhouse gas emissions in biosolids-amended soil. Earthworms were incubated with mixtures of biosolids-amended soil (0, 6.25, 12.5, 25, 50, and 100 % biosolids by volume) for 21 days. All species survived in the soil-biosolids mixtures but not in 100 %. The native earthworms, *Maoridrilus transalpinus* and *Maoridrilus* sp.2 (an undocumented species) increased KCl-extractable NH_4 and NO_3 by up to 29 %, substantially more than *E. fetida*. All species significantly increased microbial biomass carbon (MBC), dehydrogenase enzymes activity (DHA) and $\text{Ca}(\text{NO}_3)_2$ -extractable Cu in biosolids-amended soil. Concentrations of $\text{Ca}(\text{NO}_3)_2$ -extractable Mg, S, Fe, Mn, Cd, Co and Zn varied between earthworm species and with biosolids addition rates. Both native species significantly increased N_2O emissions from soil, more so than *E. fetida*. *Maoridrilus* earthworms have the potential to enhance plant productivity in biosolids-amended soils, but may raise additional environmental concerns.

Key words

native earthworm; sewage sludge; mineralisation; microbial activity; nitrous oxide; restoration land

8.2 Introduction

Biosolids comprise the treated solid fraction of sewage, containing high concentrations of organic matter and plant nutrients (Gartler et al., 2013). Biosolids also commonly contain contaminants, including heavy metals (Silveira et al., 2003), persistent organic pollutants (Clarke and Smith, 2011), antibiotics, pharmaceuticals and pathogens (Garrec et al., 2003; Jones-Lepp and Stevens, 2007). Nonetheless, biosolids can be beneficially used as a soil amendment on agricultural land as well as in remediation and revegetation projects (Kinney et al., 2008). Until the middle of 1990s, some 30% of European biosolids were applied to agricultural land, accounting for some 2.4 million dry tonnes per year (Chang et al., 2002; Silveira et al., 2003) and 0.1 % of the US agricultural land was treated with biosolids (NRC, 2002). With increased concerns about risks from chemical contaminants and pathogens, Europe and the US now regulate biosolids disposal to production lands including cropped and grazed lands. It has been shown that the addition of biosolids to soil can result in the accumulation of trace elements, especially Cd, Cu and Zn (Silveira et al., 2003) and can result in reduced soil fertility or breaches of food safety standards (Wang et al., 2003). Such concerns are reduced when biosolids are used for rebuilding degraded soils (Robinson et al., 2011; Waterhouse et al., 2014a), where biosolids can stimulate soil microbial and enzymatic activities, enhancing soil nutrients status and plant growth (Evanylo et al., 2005; Gardner et al., 2010; Madejón et al., 2006; San Miguel et al., 2012). Clearly, there is considerable uncertainty about the efficiency of biosolid amendments to soil.

Most N in biosolids is present as organic N, which is unavailable to plants; organic N slowly mineralizes to plant-available ammonium (NH_4^+) and nitrate (NO_3^-) (Claassen and Carey, 2007). Thus, unlike mineral fertilisers, the addition of biosolids may not result in the release of sufficient N for optimal plant growth in the short term. However, earthworms enhance mineralization thereby increasing the fraction of plant-available N in biosolids (Edwards and Arancon, 2004; Edwards and Batey, 1992; McDaniel et al., 2013; Yadav and Garg, 2011). Vermicomposting has been proposed as a cost effective and easily controllable means to increase mineral N in biosolids as well as to reduce the burden of human pathogens (Eastman et al., 2001; Yadav et al., 2010). Vermicomposting may also reduce N_2O greenhouse gas emission from soils amended with biosolids (Fernández-Luqueño et al., 2009). Most vermicomposting employs *Eisenia fetida*, *E. andrei*, and *Lumbricus rubellus*, three species that are tolerant to high compost temperature and the toxic components in the biosolids, especially heavy metals and ammonia (Artuso et al., 2010; Mitchell et al., 1980; Ndegwa and Thompson, 2001; Suthar, 2010; Yadav and Garg, 2011; Yadav et al., 2010). These epigeic species seemingly have greater capacity for waste decomposition and higher reproductive rates than endogeic and anecic species, which burrow into deeper soil (Gajalakshmi and Abbasi, 2004). However, whilst soil-dwelling surface-feeding epigeic species only breakdown organic matter on the soil surface (Ismail, 1997), anecic species not only decompose organic matter, but also transport it from the soil surface to deeper horizons

thereby improving the recycling of organic matter and the structure of the soil. In this regard, anecic species may be more beneficial for the management of biosolids-amended soil (Sharma et al., 2005).

There is little knowledge of native earthworms in New Zealand despite their high diversity in this country (Buckley et al., 2011). Restricted refugia of native vegetation and remnants on the margin of agricultural land provide the remaining of habitats for indigenous earthworms (Bowie et al., 2016). Restoration of native vegetation leads to enhanced recolonization of native earthworm that disappeared following conversion to agriculture (Boyer et al., 2016, submitted manuscript), but no studies have investigated the functionality of New Zealand earthworms in biosolids-amended restoration land. Native earthworms may prove to be more effective than exotic species since they are adapted to local climatic and edaphic conditions (Sharma et al. 2005). Native earthworms also confer other ecological benefits, such as food-chain continuity and strong relationships with other native biota, and roles that their exotic counterparts may not fulfil (Waterhouse et al. 2014b).

For effective application of biosolids in restoration lands, an understanding of how the native earthworms respond to biosolids is required. Therefore, we aimed to investigate behavioural tolerance of native earthworms to biosolid-amended soil. We also sought to elucidate how these species affect the solubility of N and trace elements, and influence greenhouse gas emission (N₂O and CO₂).

8.3 Materials and methods

8.3.1 Soil, biosolids, and earthworm collection

Soil (Templeton silt loam) was collected from the margin of the Lincoln University Commercial Dairy Farm (43°38'11.27"S, 172°26'17.56"E). The top 15 cm was sampled. Stones and plant residue were removed using a 4 mm sieve. Biosolids were obtained from the Kaikōura Regional treatment works (42°21'47.78"S 173°41'20.32"E). About 160 kg of stockpiled and weathered biosolids were collected and homogenized using a concrete mixer and initially passed through a 20 mm sieve. A 2 kg sub-sample was passed through a 2 mm nylon sieve and then used in this study. A gravimetric moisture content (θ_g) in biosolids equaled 53 %. Table 8.1 gives the properties of the soil and biosolids.

Two native earthworms, *Maoridrilus transalpinus* and *Maoridrilus* sp.2 were used in this study following classification by their morphology and DNA barcoding analysis. Some 100 individuals of the species were collected from the Ahuriri Reserve in Banks Peninsula (43°39'58.97"S, 172°37'26.37"E). *Maoridrilus* sp.2 is probably new to science and was sampled below *Quercus ilex* trees bordering to Lincoln University rugby ground (43°38'37.19"S, 172°27'43.77"E). Earlier studies indicated that both *Maoridrilus* spp. are anecic species (Kim et al., 2015). *M. transalpinus* was also found to have a greater capacity to breakdown organic matter compared to other native and exotic endogeic and epigeic species (Kim et al. 2015). These species are easily found and could be readily collected in large numbers

(Kim et al. 2015). Soil samples of 20-30 cm depth were dug and hand-sorted for earthworms in the field.

Eisenia fetida (tiger worms) obtained from local compost heaps was added to compare with the native earthworms, particularly in terms of tolerance to biosolids toxicity. Although the native species (5.5 - 8.0 g per individual) have up to ten times higher mass than *E. fetida* (0.8 g per individual), *E. fetida* showed similar or greater capacity of OM decomposition than other larger Megascolecids and Lumbricids in my earlier studies. Following two weeks in laboratory culture at 15-20 °C in darkness, individual worms were selected for experimentation. A visual assessment was carried out to choose the most healthy, i.e. those that were glossy, elastic, sensitive to handling and with clear clitella or prostatic pores.

Table 8.1 Physicochemical properties of soil and biosolids in this study. Values in brackets represent standard errors of the means (n=3). (n.d. = not determined).

Properties	Soil	Biosolids
Clay/silt/sand (%)	4/20/76 ^a	n.d. ^a
pH (1:5W)	5.6 (<0.1)	4.1 – 4.5 ^{a,b}
CEC (meq/100g)	7.8 (0.3)	17 ^b
C (%)	3.3 (0.1)	2.5 – 2.7 ^{a,b}
N (%)	0.3 (<0.1)	25 – 28 ^{a,b}
C/N ratio	12 (0.3)	9 – 11 ^{a,b}
NH ₄ -N (mg·kg ⁻¹)	1.8 (0.5)	130 ^b
NO ₃ -N (mg·kg ⁻¹)	118 (12)	1352 ^b
Olsen P (mg·kg ⁻¹)	34 (0.1)	123 (0.1)
P (mg·kg ⁻¹)	732 (11) ^a	4683 ^a
K (mg·kg ⁻¹)	2541 (279) ^a	4330 (67) ^a
Mg (mg·kg ⁻¹)	3426 (71) ^a	2204 (17) ^a
S (mg·kg ⁻¹)	383 (6) ^a	6972 (43) ^a
Fe (mg·kg ⁻¹)	17727 (353) ^a	n.d. ^a
Cu (mg·kg ⁻¹)	5.0 (<0.1) ^a	561 – 696 ^{a,b}
Zn (mg·kg ⁻¹)	70 (2.0) ^a	878 – 1048 ^{a,b}
Cd (mg·kg ⁻¹)	0.13 (<0.1) ^a	2.8 (<0.1) ^a
Mn (mg·kg ⁻¹)	357 (20) ^a	n.d. ^a

^aSimmler et al. (2013); ^bParamashivam et al. (2015)

8.3.2 Incubation experiment

Earthworm inoculation followed an acclimatisation period that demonstrated both survival and weight gain under laboratory conditions before the experimental work began. Mixtures of soil: biosolids were prepared containing 0, 6.25, 12.5, 25, 50, and 100 % biosolids by volume. On a dry matter basis, these mixtures comprised 0, 4.8, 10, 20, 43, and 100 % biosolids. These substrates were placed in 1000 mL polystyrene containers, with two earthworms of the same species added per container, and maintained for three weeks. Each container was lined with gauze to prevent the earthworms from escaping and placed in the dark at room temperature (18 °C). Soil moisture was maintained at 30 %

after weighing each container weekly. For each soil treatment, there were five replicates and five additional pots without earthworms were used as reference (control).

8.3.3 Soil properties analyses

After 3 weeks inoculation in the culture room, the survival and biomass of all species in each treatment were measured. Samples of fresh soil were analysed for available N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden) following 2M KCl extraction (Clough et al., 2001). For microbial biomass carbon (MBC), the soil samples fumigated with ethanol-free CHCl_3 were extracted with 0.5M K_2SO_4 and MBC were determined using a TOC-500A analyser (Shimadzu Oceania Pty Ltd., Australia) (Blakemore, 1987). As an indicator of microbiological activity, soil dehydrogenase enzymatic activity (DHA) was determined based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) solution to triphenylformazan (TPF) using a modified method described in Casida et al. (1964) and Chander and Brookes (1991). Mixture of soil (2 g) and TTC solution (2 ml) was incubated for 24 hours at 25 °C in darkness. After extraction of the mixture with methanol (10 ml), it was shaken and centrifuged at 1880 x *g*. The supernatant of hydrolysis reaction products was measured the absorbance at 485 nm through a UV 160A spectrophotometer (Shimadzu, Japan). Air-dried soil samples were sieved to <2 mm using a stainless steel sieve. Soil pH and electronic conductivity (EC) were measured using pH and EC meters (Mettler Toledo Seven Easy). For total organic matter (TOM), soil samples were oven-dried at 100 °C and loss on ignition (LOI) was measured after combustion in a muffle furnace at 500 °C (Blakemore, 1987). Following an extraction with 0.5M NaHCO_3 , plant available-P was measured as Olsen P, at a wavelength of 880 nm using a UV 160A spectrophotometer (Shimadzu, Japan) (Blakemore, 1987). Soluble concentrations of K, Mg, S, Fe, Mn, Cu, Cd, Co, and Zn were determined by ICP-OES (Varian 702-ES) following extraction with 0.05M $\text{Ca}(\text{NO}_3)_2$ (Simmler et al., 2013).

8.3.4 N₂O and CO₂ measurement

Gas emissions were sampled twice on the 19th and 20th days after inoculation; average gas data from both sampling events is shown in Figure 8.3. Nitrous oxide (NO_2) and carbon dioxide (CO_2) released from each chamber were collected at 20-22 °C. Aliquots (ca 10 ml) of headspace gas were collected at 0, 25, and 50 min after sealing. The headspace volume was 0.2 L and soil surface area was 64 cm². The gas samples were injected into glass vials and stored in dark room for analyses (<1 week). NO_2 and CO_2 were analysed using a gas chromatograph (SRI 8610 GC, CA, USA) with a ⁶³Ni electron capture detector and a flame ionisation detector and linked to an auto-sampler (Gilson 222 XL, USA).

8.3.5 Statistical analyses

Minitab 16 (Minitab Inc., State College, Pennsylvania, USA) was used for all statistical analysis. To compare means of all data in each earthworm species treatment ($n=5$, $p<0.05$), a one-way ANOVA was

carried out using a Fisher's Least Significant Difference test. The overall effect of species, biosolids addition rate, and those interactions with soil properties were assessed using two-way ANOVA analyses ($p < 0.05$, $p < 0.01$, and $p < 0.001$). For soil pH, descriptive statistics were calculated following conversion to the equivalent hydrogen ion concentrations and back calculation to pH. To identify effects of biosolids addition on gas release, a correlations coefficient (R) was conducted between pH, TOM, MBC, NH_4 , NO_3 , N_2O , and CO_2 .

8.4 Results and discussion

8.4.1 Survival and growth of earthworms

Biosolids treatments of $\leq 50\%$ did not significantly reduce earthworm survival. All species had $>90\%$ viability during the experiment except for *M. transalpinus*, which had 40% mortality in the treatment without biosolids additions (Table 8.2). This high mortality of *M. transalpinus* in the reference is consistent with the relatively high requirement of this species for organic matter as a food source (Kim et al., 2015). In the 100% biosolids treatment, both *Maoridrilus* spp. died two days after the inoculation. Mortality was probably caused by the sludge component releasing large amounts of ammonium/ammonia, inorganic salts, and toxic metals such as Cu (see Table 1) (Bright and Healey, 2003; Edwards and Arancon, 2004). All results are therefore presented for treatments with 0-50% biosolids, excluding 100% biosolids. *E. fetida* survived in 100% biosolids for over 2 months.

There is a large diversity, 179 species belonging to 26 genera of Megascolecidae, of native earthworms in New Zealand (Buckley et al., 2011) and their ability to survive in biosolids may vary depending on the species. Native soils tend to be acidic and low in nutrients (de Freitas and Perry, 2012; Sparling and Schipper, 2002), and endemic earthworm species may be largely intolerant of nutrient enrichment (e.g. Waterhouse et al. 2014a). However, the species used in the current study are known to survive in agricultural and high nutrient soils (Kim et al., 2015). They had been collected from soil under native vegetation in a relatively pristine environment.

Introduction of the earthworms into experimental containers appeared to inhibit the growth of all species. The weight gain of *E. fetida* was proportional to the amount of biosolids added, showing that biosolids can provide an important source of organic matter (Adair et al., 2014; Artuso et al., 2010). *Maoridrilus* spp. lost weight in the biosolids treatments, but to a lesser degree than in reference containers, which lacked the food source. Amendments of 12.5% appeared most suitable (Table 8.2); the greatest weight loss was in the two highest rates of biosolids (25 and 50%) probably due to biosolids-borne contaminants; the 12.5% biosolids treatment provided the best balance between nutrition and toxicity.

Table 8.2 Survival and weight change of earthworms after 3 weeks inoculation in soils with different rates of biosolids amendment. Values in brackets indicate standard error of the means (n=5). Values with the same letters (within each column) are not significantly different at the 5% level.

Biosolids dose	----- Survivorship (%) -----			----- Weight change (%) -----		
	<i>M. transalpinus</i>	<i>Maoridrilus</i> sp.2	<i>E. fetida</i>	<i>M. transalpinus</i>	<i>Maoridrilus</i> sp.2	<i>E. fetida</i>
0 %	60 (24) ^b	90 (10) ^a	100 (0) ^a	-29 (17) ^b	-6.5 (1.5) ^{bc}	-13 (1.7) ^c
6.25 %	100 (0) ^a	100 (0) ^a	100 (0) ^a	-6.6 (3.0) ^a	-3.3 (2.6) ^{ab}	28 (4.5) ^b
12.5 %	100 (0) ^a	90 (10) ^a	100 (0) ^a	-0.8 (3.1) ^a	3.1 (1.3) ^a	49 (4.3) ^a
25 %	90 (10) ^{ab}	90 (10) ^a	100 (0) ^a	-9.9 (6.2) ^{ab}	-6.1 (3.8) ^{bc}	46 (10) ^a
50 %	90 (10) ^{ab}	100 (0) ^a	100 (0) ^a	-18 (4.6) ^{ab}	-13 (1.0) ^b	61 (4.9) ^a

8.4.2 Effects on soil chemistry

Adding biosolids to soil increased NH₄-N, NO₃-N, and the more soluble fractions of P and Cu (Figure 8.1). The additional effects of adding earthworms were small in comparison, but all species significantly increased NH₄-N and soluble Cu ($p < 0.05$). It is likely that earthworms enhanced the decomposition of organic nitrogen from biosolids, releasing NH₄-N and some of the very high concentrations of Cu attached to this organic matter. In drilosphere soil, earthworm castings and mucus release ammonium (Needham, 1957), which is subsequently nitrified (Edwards and Lofty, 1980; Parkin and Berry, 1999), and affects bacteria associated with the N cycle (Eastman et al., 2001; Edwards and Arancon, 2004). Wen et al. (2004) also found increase of Cu solubility in *E. fetida* casts.

Adding biosolids to soil caused only a marginal acidification of reference soils (<1 pH unit) but significantly increased EC, TOM, and soluble concentrations of S, Zn and Cd (Table 8.3). There was an additional effect of earthworms on these variables, but only when biosolids amendment was below 50 %. There appears to be evidence of inhibition or toxicity at higher amendment rate. However, earthworms also significantly modified the chemistry of the amended soils. High concentrations of Zn in the added biosolids (see Table 8.1) appear to have been absorbed within *Maoridrilus* sp.2. Zn is an essential nutrient that may have been retained in the tissues, unlike Cd and Cu, although certain species of earthworms do appear to be able to regulate both metals (Morgan and Morgan 1999; Dai et al. 2004; Suthar and Singh 2009; Adair et al. 2014). Earthworms can solubilized total metal by digestion and excretion but *Maoridrilus* sp.2 released less soluble fraction of Zn to soil.

Otherwise, all earthworms increased the amount of soluble Fe, Mn and Co in soil. In 12.5 and 25 % biosolids treatments, the two native species significantly enhanced EC and soluble K, S, and Cd ($p < 0.05$) and *Maoridrilus* sp.2 substantially increased Mg mobility ($p < 0.05$). In earlier studies, *E. fetida* reduced total organic C and decreased soil pH, but increased EC (Yadav and Garg 2011). Increased plant-available K, Mg, and S by *Eudrilus eugeniae*, *E. fetida*, and *Perionyx excavatus* has been observed in soils amended with organic wastes (Kale 2004; Hait and Tare 2012). Hait and Tare (2012) found that *E. fetida* increased soluble Fe and Mn but decreased soluble Co.

Different effects of the different earthworm species on soil properties in the present study may be related to not the larger size of the native earthworms but the efficiency of nutrient mineralisation. With regards of TOM reduction in the presence of earthworms (see Table 8.3), while the native earthworms were up to 10 x larger than *Eisenia*, they consumed the same or less amount of OM from each biosolid-amended soils. Otherwise, *E. fetida* was more likely to use the uptake of organic nutrients, particularly N, from biosolids for their metabolic or fecundity activities. This exotic species gained more weight with increase of biosolids rates (see Table 8.2) and also produced cocoons, but not in all pots, during the inoculation.

8.4.3 Microbiological activity

Adding biosolids increased MBC through adding an organic substrate (Figure 8.2), but there was a large additional effect of earthworm activity on microbial biomass. There appears to be a contradictory effect of biosolids on DHA, that did not increase in proportion to the amount of biosolids added. Earthworm activity caused a significant decline in DHA, but it is unlikely that less of the substrate was being oxidized in the presence of earthworms. Instead, this may be a differential response amongst other microbial groupings related to urease (UA), alkaline phosphatase (APA) and arylsulfatase (ASA) activities (Kızılkaya and Hepser, 2004; Mulongoy and Bedoret, 1989). DHA measures respiratory enzymes that activate oxidation-reduction reactions. As total oxidative activity of soil microflora can be reflected through a DHA measurement, the soil DHA may be regarded as good indicator of microbiological activity like MBC (Skujinš, 1973). Similar results were reported by Kızılkaya and Hepser (2004), who found significant increases of UA, APA and ASA, while reduced DHA in biosolids treatments with *L. terrestris*. These authors also suggested positive associations between soil enzyme activities (UA, APA, ASA) and nutrients mineralization (N, P, and other elements) in the casts. We could infer that there were enhancement of UA, APA and ASA in the present study. Another factor reducing DHA may be more aerobic condition by burrowing and Cu solubility associated with biosolids (Fernández-Calviño et al., 2010; Levyk et al., 2007).

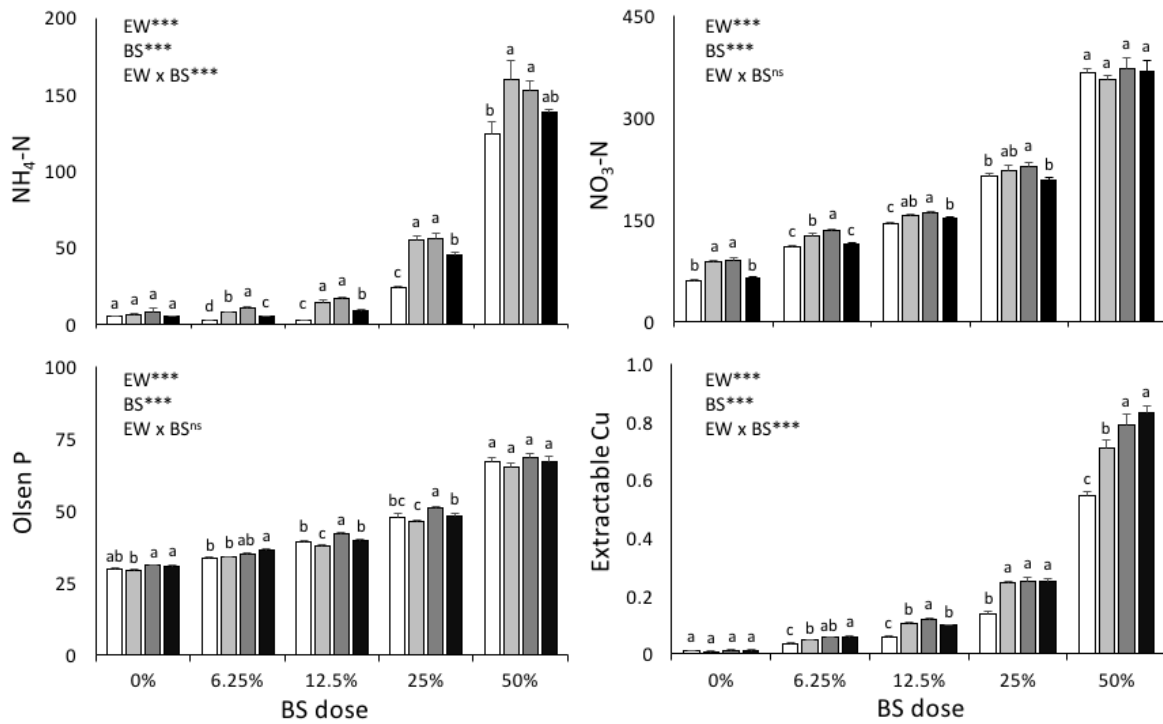


Figure 8.1 Extractable concentrations ($\mu\text{g}\cdot\text{g}^{-1}$) of nitrogen, phosphorus, and copper released from varying proportion of biosolids without earthworms (\square), and inoculated with *M. transalpinus* (\square), *Maoridrilus sp.2* (\square), and *E. fetida* (\blacksquare). Same letter indicates no significant difference in each biosolids addition rate ($n=5$, $p<0.05$). Overall correlations between earthworms (EW), biosolids rates (BS), and their interactions (EW x BS) are represented following Two-way ANOVA analysis ($*p<0.05$; $**p<0.01$; $***p<0.001$).

Table 8.3 Physicochemical variations in soil properties with earthworms and biosolids following 3 weeks inoculation, and reference soils without earthworms. Same letters (within each column) indicate no significant differences in values of each biosolids application rate (n=5, p<0.05). All data are determined the effects of earthworm species (EW), biosolids application rate (BS), and their interactions (EW x BS) using two-way ANOVA.

Biosolids dose	Species	pH (1:5W)	EC (dS·m ⁻¹)	TOM (%)	----- (mg·kg ⁻¹) -----							
					K	Mg	S	Fe	Mn	Cd	Co	Zn
0 %	Reference	5.1 (<0.1) ^b	0.17 (<0.01) ^{bc}	7.0 (<0.1) ^a	206 (2) ^{bc}	170 (1) ^a	10 (0.2) ^b	0.9 (<0.1) ^b	21 (0.5) ^b	0.02 (<0.01) ^a	0.07 (<0.01) ^b	44 (7.5) ^b
	<i>M. transalpinus</i>	5.2 (<0.1) ^b	0.19 (0.01) ^{ab}	6.5 (0.1) ^b	211 (2) ^b	155 (2) ^b	11 (0.8) ^{ab}	1.1 (0.1) ^a	19 (0.5) ^b	0.02 (<0.01) ^b	0.06 (0.01) ^b	31 (2.4) ^b
	<i>Maoridrilus</i> sp.2	5.1 (<0.1) ^b	0.21 (<0.01) ^a	6.1 (0.2) ^b	221 (5) ^a	173 (2) ^a	13 (1.6) ^a	1.0 (<0.1) ^{ab}	26 (1.0) ^a	0.02 (<0.01) ^a	0.09 (<0.01) ^a	36 (5.1) ^b
	<i>E. fetida</i>	5.3 (<0.1) ^a	0.16 (<0.01) ^c	6.2 (0.3) ^b	199 (1) ^c	158 (4) ^b	8.8 (0.1) ^b	1.1 (0.1) ^{ab}	21 (1.1) ^b	0.02 (<0.01) ^a	0.08 (<0.01) ^b	66 (9.2) ^a
6.8 %	Reference	5.1 (<0.1) ^a	0.23 (0.01) ^c	7.7 (0.1) ^a	211 (3) ^b	172 (1) ^a	33 (0.9) ^a	0.9 (<0.1) ^c	20 (0.1) ^b	0.04 (<0.01) ^b	0.06 (<0.01) ^c	73 (1.5) ^a
	<i>M. transalpinus</i>	5.0 (<0.1) ^b	0.26 (<0.01) ^b	7.5 (0.1) ^a	206 (1) ^b	151 (1) ^b	33 (0.7) ^a	1.2 (0.1) ^{ab}	25 (0.6) ^{ab}	0.04 (<0.01) ^b	0.09 (<0.01) ^b	74 (4.0) ^a
	<i>Maoridrilus</i> sp.2	4.9 (<0.1) ^b	0.28 (<0.01) ^a	7.3 (0.2) ^a	251 (2) ^a	173 (2) ^a	36 (1.0) ^a	1.1 (0.1) ^{bc}	29 (0.6) ^a	0.04 (<0.01) ^{ab}	0.12 (<0.01) ^a	56 (4.0) ^b
	<i>E. fetida</i>	5.0 (<0.1) ^b	0.23 (<0.01) ^c	7.4 (<0.1) ^a	215 (1) ^b	177 (8) ^a	37 (3.8) ^a	1.4 (0.1) ^a	29 (2.9) ^a	0.05 (<0.01) ^a	0.11 (0.01) ^a	55 (10.6) ^b
12.5 %	Reference	4.9 (<0.1) ^a	0.27 (0.02) ^c	8.5 (0.4) ^a	205 (6) ^{bc}	161 (4) ^c	49 (2.3) ^c	0.9 (0.1) ^b	24 (0.6) ^c	0.06 (<0.01) ^b	0.07 (<0.01) ^c	81 (2.0) ^a
	<i>M. transalpinus</i>	4.8 (<0.1) ^b	0.32 (0.01) ^b	8.1 (0.1) ^{ab}	213 (2) ^{ab}	155 (1) ^c	63 (1.8) ^{ab}	1.3 (0.1) ^a	28 (1.2) ^b	0.07 (<0.01) ^a	0.11 (<0.01) ^b	78 (4.0) ^a
	<i>Maoridrilus</i> sp.2	4.8 (<0.1) ^d	0.38 (<0.01) ^a	8.3 (0.1) ^{ab}	221 (3) ^a	178 (3) ^a	69 (3.5) ^a	1.2 (0.1) ^{ab}	33 (0.7) ^a	0.07 (<0.01) ^a	0.12 (<0.01) ^a	53 (2.4) ^b
	<i>E. fetida</i>	4.8 (<0.1) ^c	0.29 (0.01) ^{bc}	7.9 (0.1) ^b	203 (2) ^c	171 (2) ^b	60 (1.5) ^b	1.5 (0.2) ^a	33 (0.7) ^a	0.07 (<0.01) ^a	0.13 (<0.01) ^a	60 (3.1) ^b
25 %	Reference	4.7 (<0.1) ^b	0.43 (0.01) ^c	11 (0.5) ^a	197 (7) ^b	158 (5) ^b	97 (6) ^b	0.9 (<0.1) ^b	31 (1.2) ^c	0.11 (<0.01) ^c	0.10 (0.01) ^c	109 (5.2) ^a
	<i>M. transalpinus</i>	4.8 (<0.1) ^a	0.45 (<0.01) ^b	9.4 (0.3) ^b	222 (3) ^a	160 (1) ^b	136 (4) ^a	1.4 (0.1) ^a	37 (0.5) ^b	0.14 (<0.01) ^{ab}	0.14 (<0.01) ^b	111 (3.4) ^a
	<i>Maoridrilus</i> sp.2	4.6 (<0.1) ^c	0.50 (0.01) ^a	9.3 (0.1) ^b	211 (6) ^{ab}	174 (6) ^a	125 (9) ^a	1.0 (0.1) ^b	41 (2.3) ^{ab}	0.13 (<0.01) ^b	0.15 (0.01) ^b	93 (7.1) ^a
	<i>E. fetida</i>	4.7 (<0.1) ^b	0.40 (0.01) ^d	9.2 (0.1) ^b	212 (7) ^{ab}	170 (1) ^{ab}	120 (3) ^a	1.4 (0.1) ^a	44 (1.2) ^a	0.14 (<0.01) ^a	0.18 (<0.01) ^a	103 (7.6) ^a
50 %	Reference	4.5 (<0.1) ^a	0.72 (0.01) ^b	16 (0.7) ^a	225 (2) ^a	193 (7) ^a	283 (2) ^a	1.1 (0.1) ^b	54 (1.7) ^b	0.31 (0.01) ^a	0.17 (0.01) ^c	187 (8.9) ^a
	<i>M. transalpinus</i>	4.5 (<0.1) ^a	0.68 (0.01) ^b	12 (0.3) ^b	217 (2) ^a	176 (2) ^b	286 (7) ^a	1.5 (0.1) ^a	53 (1.5) ^b	0.32 (0.01) ^a	0.21 (0.01) ^b	175 (6.5) ^{ab}
	<i>Maoridrilus</i> sp.2	4.5 (<0.1) ^b	0.77 (0.02) ^a	13 (0.2) ^b	216 (4) ^a	196 (3) ^a	290 (6) ^a	1.4 (0.1) ^a	61 (2.1) ^a	0.33 (0.01) ^a	0.25 (0.01) ^a	164 (4.4) ^b
	<i>E. fetida</i>	4.5 (<0.1) ^a	0.62 (<0.01) ^c	13 (0.3) ^b	216 (7) ^a	193 (2) ^a	286 (7) ^a	1.5 (0.0) ^a	59 (1.5) ^a	0.33 (0.01) ^a	0.25 (0.01) ^a	159 (1.8) ^b
Two-way ANOVA (p values)	EW	***	***	***	ns	***	**	***	***	***	***	***
	BS	***	***	***	ns	***	***	***	***	***	***	***
	EW x BS	***	***	***	**	***	*	ns	**	*	***	***

*p<0.05, **p<0.01, ***p<0.001, ns (not significant)

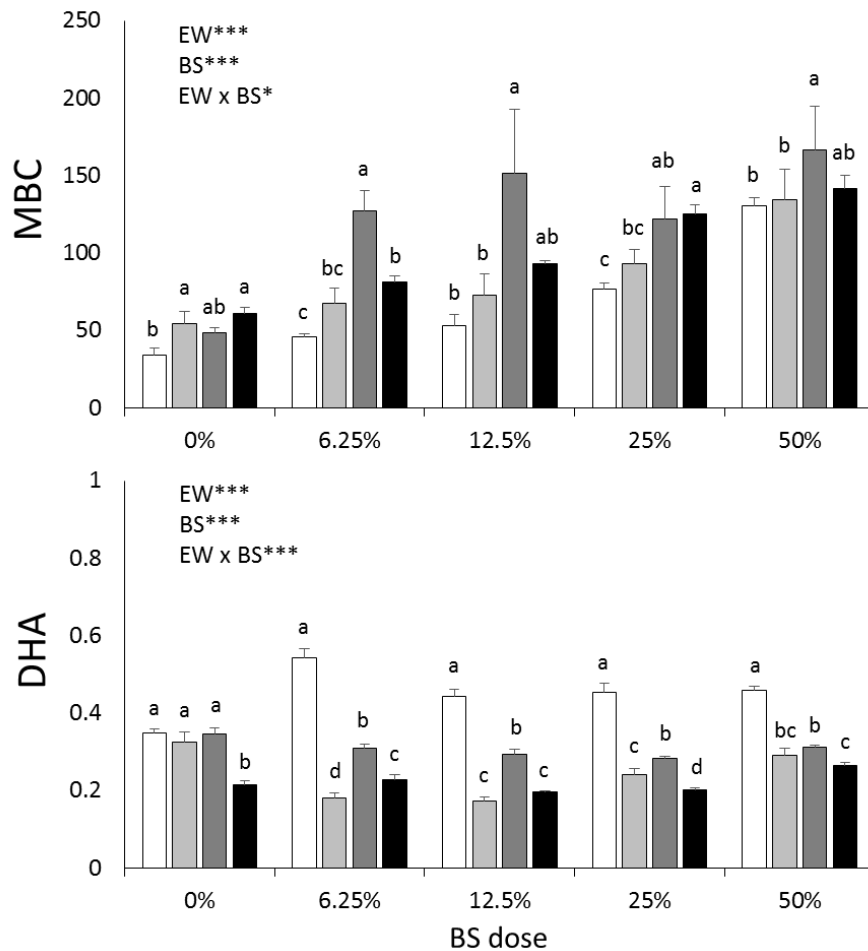


Figure 8.2 Effects of earthworms on Microbial Biomass Carbon (MBC, $\mu\text{g}\cdot\text{g}^{-1}$) and dehydrogenase enzyme activity (DHA, $\mu\text{g}\cdot\text{g}^{-1}\cdot 24\text{h}^{-1}$) at varying proportion of biosolids without earthworms (□), and inoculated with *M. transalpinus* (▤), *Maoridrilus sp.2* (▥), and *E. fetida* (▧). Same letters indicate no significant difference in each biosolids application rate ($n=5$, $p<0.05$). Correlations between earthworms (EW), biosolids rates (BS), and their interactions (EW x BS) are represented by Two-way ANOVA analysis (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

8.4.4 GHG emissions

Adding biosolids influenced the release of greenhouse gas (GHGs), especially N_2O and CO_2 (Figure 8.3), due to changes in the soil's chemical and physical properties (Fernández-Luqueño et al., 2009). Emission of nitrous oxide from soil is produced via the partial reduction of NO_3^- under low-oxygen conditions (Barnard and Leadley, 2005; Wrage et al., 2004). The addition of biosolids also increased MBC in proportion to the amount of added organic matter (Table 8.4) which was correlated with increased $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, and also to increased greenhouse gas emissions. More N_2O appears to be released in the presence of native earthworms (Figure 8.3); both *Maoridrilus* spp. emitted >2.5 times more N_2O than the reference and *E. fetida* ($p<0.05$) in 6.25 and 12.5 % biosolids treatments. However in 50 % biosolids amendment, the earthworm-free treatment released much more nitrous oxide than earthworm-presence treatments. This may be due to the biosolids originally containing

large amount of NH_4 and NO_3 , leading to increased denitrification. Although *E. fetida* stimulated N mineralisation, this species inhibited more N_2O emission compared to the native species. It may be due to the epigeic earthworm increased more aeration in biosolids-amended soil. Contreras-Ramos et al. (2009) reported that, compared to bulk soil, presence of *Eisenia fetida* released 14 times less N_2O from 5 % biosolids-amended soil. In the present study, a considerable amount of N_2O gas was released from the 50 % biosolids treatment that contained high concentration of NO_3^- .

Respiratory CO_2 emissions from biosolids-amended soils were markedly reduced in the presence of earthworms, particularly *Maoridrilus* sp.2 ($p < 0.05$). Reasons for this may include differences in the formation of stable aggregates in the presence of earthworms. According to a meta-analysis by Lubbers et al. (2013b), earthworms increase CO_2 by 33% in a number of field and laboratory studies by stimulating OM decomposition. However, the long-term effects of earthworms can improve stability and storage of soil C by protecting carbon in microaggregates formed in large macroaggregates (Bossuyt et al., 2005; Pulleman et al., 2005; Six et al., 2004), which may decrease net CO_2 emissions (Lubbers et al., 2013b). Furthermore, since this coincides with less DHA, we consider that a better explanation may be the release of toxic concentrations of Cu into soil solutions to which there are different sensitivities amongst microbial groupings.

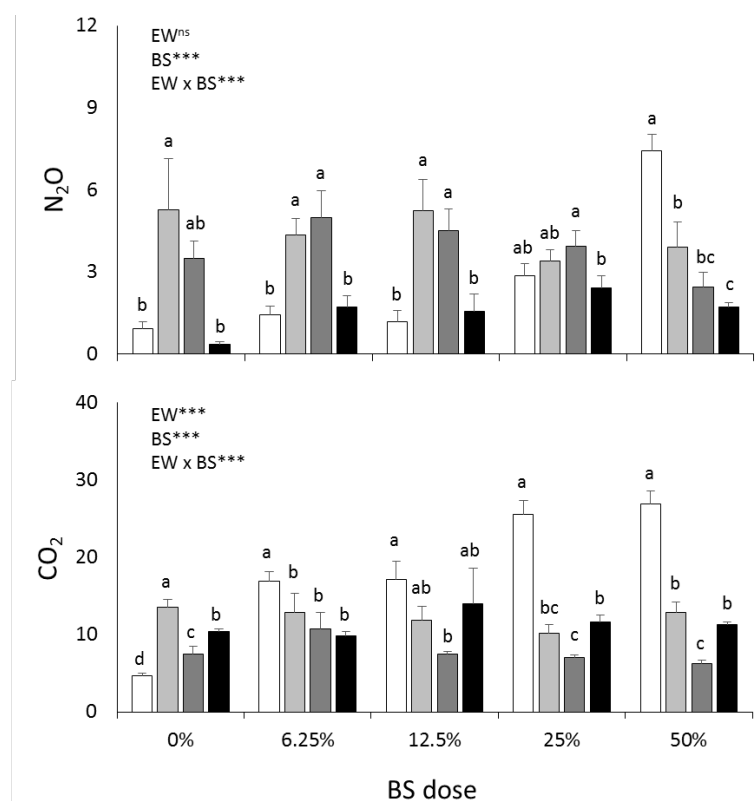


Figure 8.3 Effects of earthworms on nitrous oxide (N_2O , $\text{g}\cdot\text{ha}^{-1}\cdot\text{day}^{-1}$) and carbon dioxide (CO_2 , $\text{kg}\cdot\text{ha}^{-1}\cdot\text{day}^{-1}$) at varying proportion of biosolids without earthworms (□), and inoculated with *M. transalpinus* (◻), *Maoridrilus* sp.2 (◻), and *E. fetida* (◼). Same letters indicate no significant difference in each biosolids application rate ($n=5$, $p < 0.05$). Correlations between earthworms (EW), biosolids rates (BS), and their interactions (EW x BS) are represented by Two-way ANOVA analysis ($*p < 0.05$; $**p < 0.01$; $***p < 0.001$).

Table 8.4 Correlations coefficient (R) of soil biogeochemical properties and N₂O on gas emissions. MBC: microbial biomass carbon, TOM: total organic matter.

	TOM	MBC	NH ₄	NO ₃
MBC	0.59****			
NH ₄	0.87****	0.65****		
NO ₃	0.93****	0.69****	0.94****	
N ₂ O	0.27**	0.24*	0.17**	0.23*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

8.5 Conclusion

Eisenia fetida is clearly a preferred species for vermicomposting at biosolids and is much more tolerant of high concentrations of biosolids that were responsible for high mortality of native anecic earthworms. However, native earthworms may be much more suitable to improve the physicochemical conditions of biosolid-supplemented soil. They burrow more deeply into soil and, at optimal amendment rates of 12.5%, native earthworms showed the greatest behavioural tolerances in terms of survivorship and weight gain to other treatments. This could substantially enhance the availability of key nutrient including mobile N, P, K, S, and Mg. These are much larger individuals but consume the same or less organic matter than the much smaller *Eisenia*. As is common, concentrations of Zn and Cu were both highly elevated in biosolids in the present study. Earthworms substantially enhanced the mobility of Cu in soil, although *Maoridrilus* sp. 2 appeared to limit the availability of Zn through enhanced uptake.

Adding biosolids to soil provided an organic matter substrate that improved nutrition and increased MBC, but there appeared to be a detrimental effect on DHA that may be connected to Cu toxicity and more aeration. Furthermore native species of earthworms increased N₂O emissions from soil. The results provide confidence to investigate the potential benefits of native species of earthworms on restoration lands amended with biosolids.

Chapter 9

Earthworm feeding and burrowing behaviours: observational studies

9.1 Introduction

Earthworm populations and distribution principally depend on biogeography and local climate, together with influences of quantity and quality of organic resources and soil properties. The quality of plant litter is important to maintain earthworm abundance (Curry, 2004); for example, high N in the litter enhances maturity and fecundity of earthworms (Butt, 2011; Gajalakshmi and Abbasi, 2004; Rajapaksha et al., 2013b). In addition, rhizosphere products such as dead roots and microbiota (e.g. fungi, bacteria, algae, amoebae, protozoa, and nematodes) can be the preferred nutritional resources for earthworms (Curry and Schmidt, 2007; Lee, 1985; Neilson and Boag, 2003). Furthermore, deep-burrowing earthworms (endogeic and anecic species) may incidentally consume living roots (i.e. fine and hair roots) when feeding mineral rhizosphere soil (Baylis et al., 1986; Curry and Schmidt, 2007; Gunn and Cherrett, 1993).

Studies of earthworm feeding ecology have been implemented previously through a variety of experiments such as choice chambers (Frúnd et al., 2010; Neilson and Boag, 2003; Rajapaksha et al., 2013a), palatability assays (Doube et al., 1997; Schönholzer et al., 1998), and litter bag studies (Hendriksen, 1990). Stable isotope assays (^{15}N and ^{13}C) have informed N and C assimilation rates by analysis of gut component, which has refined conventional classification of ecological groups (Briones et al., 2004; Curry and Schmidt, 2007; Uchida et al., 2004). Enhancement of microbial communities by earthworm digestion has been estimated using terminal-restriction fragment length polymorphism (T-RFLP), RNA profiles, and fluorescent in situ hybridisation (FISH) (Egert et al., 2004; Horn et al., 2006; Singleton et al., 2003).

Although these studies have improved our understanding of feeding preferences and interactions between earthworms and food web (Curry and Schmidt, 2007), there are no known studies of New Zealand endemic earthworms. The same exotic species as those in New Zealand are also widespread across temperate regions, but endemic species (Megascolecidae) in New Zealand have been isolated in specific regions of the country since the Tertiary. Anthropogenic disturbance of landscapes has restricted colonisation of Megascolecidae, which are often restricted to remnants of native vegetation and/or tussock grasslands (Boyer et al., 2011b; Brockie and Moeed, 1986; Kim et al., 2015). However, Kim et al. (2015) found that some endemic earthworms, actually prefer agricultural

soils to their native mature forest soils. Lesser acid soil were found to be preferred more so than soils with high amount of organic matter (Chapter 3).

More recently coexistence of endemic and exotic species of earthworms appears to be common in New Zealand native vegetation as well as on marginal agricultural land (Kim et al., 2015). However, there is a lack of knowledge of the roles of coexisting mixed assemblages of native and exotic, and of the potential benefits of endemics. The aims of this study were (i) to evaluate the relative importance of soil pH and soil organic matter to endemic *Maoridrilus* spp., (ii) to investigate native plants litter preferences, and (iii) to observe feeding activity and burrowing behaviours of earthworms in the rhizosphere of native plants.

9.2 Materials and methods

9.2.1 Soil, earthworm and plant litter

Surface soils (0–15 cm) were collected from Lincoln University sheep farmland (referred as SF; 43°38'39.48"S, 172°23'28.07"E) and from a remnant of coastal sand plain forest in West Coast (referred as NF; 42°8'38.39"S, 171°19'50.36"E). Stones and plant remains were removed from soils using 4 mm sieves. Two endemic species of earthworms were collected by digging soil pits (20 x 20 x 20 cm) from Ahuriri reserve in Canterbury in South Island of New Zealand: the anecics *Maoridrilus transalpinus* (-43°39'58.97"S, 172°37'26.37"E) and *Maoridrilus* sp.2 (-43°38'37.19"S, 172°27'43.77"E). Exotic species *Octolasion cyaneum* (endogeic) were sampled from beneath same native vegetation and *Eisenia fetida* (epigeic) was collected from local compost heaps. All earthworm species were further selected on the basis of the visually most viable and healthy laboratory cultures on each set-up occasion. Leaf litter of 6 native plant species were selected from Lincoln University Dairy Farm native vegetation (43°38'38"S 172°26'02"E): 3 monocotyledons (*Carex secta*, *Cortaderia richardii*, and *Phormium tenax*) and 3 dicotyledons (*Coprosma robusta*, *Kunzea robusta*, and *Olearia paniculata*). Withered-yellowing and -necrotic leaves were removed from living plants and ryegrass (*Lolium perenne*) was also sampled at the same location. After oven-dried at 60 °C for 3 days, the litters were crushed and ground to 1 mm for the preference trial, in order to have a comparable and consistent texture of all plant litters. Litter properties including C, N, C/N ratio, ADF (acid detergent fibre), macro- and trace elements were also analysed (Table 9.1).

Table 9.1 Litter properties of native plants including 3 monocotyledon and 3 dicotyledon species and an exotic ryegrass (*L. perenne*). Values in brackets indicate standard errors of the means (n=3). The same letters within each row indicate no significant difference ($p<0.05$).

Properties	----- Monocots -----				----- Dicots -----		
	<i>Carex secta</i>	<i>Cortaderia richardii</i>	<i>Phormium tenax</i>	<i>Lolium perenne</i>	<i>Coprosma robusta</i>	<i>Kunzea robusta</i>	<i>Olearia paniculata</i>
C (%)	44 (0) ^c	45 (0) ^{bc}	48 (1) ^a	40 (0) ^d	45 (1) ^{bc}	49 (2) ^a	47 (0) ^{ab}
N (%)	0.7 (0.1) ^{bc}	0.8 (0.1) ^b	0.5 (0.1) ^c	2.2 (0.0) ^a	0.7 (0.1) ^{bc}	1.9 (0.1) ^a	0.7 (<0.1) ^{bc}
C/N	65 (9) ^b	58 (6) ^b	102 (19) ^a	19 (0) ^c	61 (5) ^b	26 (1) ^c	68 (4) ^b
ADF (%)	47 (2) ^b	48 (1) ^b	57 (1) ^a	26 (1) ^d	29 (1) ^{cd}	27 (3) ^d	33 (0) ^c
Ca (mg·kg ⁻¹)	5675 (612) ^{cd}	3479 (347) ^d	12508 (3291) ^{cd}	4925 (315) ^d	33689 (1433) ^a	15076 (7543) ^{bc}	23817 (274) ^b
K (mg·kg ⁻¹)	2772 (785) ^b	1672 (376) ^b	3400 (522) ^b	37295 (3740) ^a	4330 (224) ^b	4897 (1319) ^b	5362 (900) ^b
Mg (mg·kg ⁻¹)	1094 (62) ^{cd}	1157 (79) ^{cd}	1003 (76) ^d	1838 (77) ^b	2346 (60) ^a	1370 (157) ^c	2395 (191) ^a
P (mg·kg ⁻¹)	634 (73) ^c	338 (10) ^c	4556 (115) ^c	3884 (463) ^a	691 (46) ^c	1524 (279) ^b	913 (103) ^{bc}
S (mg·kg ⁻¹)	825 (68) ^d	1419 (46) ^c	640 (74) ^d	2829 (103) ^a	2195 (156) ^b	1681 (148) ^c	682 (28) ^d
Cu (mg·kg ⁻¹)	2.0 (0.3) ^{cd}	1.8 (0.1) ^{de}	1.3 (0.2) ^e	5.5 (0.4) ^a	2.6 (0.3) ^{bc}	2.9 (0.3) ^b	2.3 (0.2) ^{bcd}
Zn (mg·kg ⁻¹)	15 (2) ^{de}	21 (2) ^{cd}	11 (2) ^e	29 (2) ^{ab}	23 (2) ^{bc}	34 (3) ^a	28 (4) ^{abc}
Mn (mg·kg ⁻¹)	558 (8) ^a	151 (35) ^{cd}	122 (15) ^d	204 (11) ^c	155 (6) ^{cd}	282 (36) ^b	202 (27) ^c

9.2.2 Choice-chamber and glass-sided panel experiments

Simple choice chamber pieces of apparatus were constructed with 5 plastic jars (designed as shown in Figure 3.1) to investigate whether earthworms had clear preferences for the different soil conditions in a series of separate assays, as previously described (Chapter 3, Kim et al., 2015). A moisture content of 30 % was established in each soil. The species of earthworms in each assay was dependent on the availability, numbers and viability of cultures that were being maintained in the laboratory throughout. All comparisons between species made in this paper refer to replicate choice-chamber assays run at the same time. Using different species (Table 9.2), groups of five earthworms per species were placed in the central chamber of the apparatus which was then maintained in darkness. After a period of one week, the apparatus was emptied to observe where the earthworms were resident. Each soil was also carefully evaluated for visible evidence of burrowing activity. There were two trials for earthworm preference to (1) different soil pH levels and to (2) different litters of native plants.

The behaviour of endemic *M. transalpinus* and exotic *O. cyaneum* in the rhizosphere were also observed through simple mesocosm experiments. Vermarium apparatus were manufactured (100 (width) x 50 (height) x 5 (breadth) cm) with glass and timbers (Figure 9.1). SF soil (silt loam) was, uniformly tamped down in the chamber. One-year old seedlings of *Cortaderia richardii* and *Phormium tenax* were planted in a separate chamber.

Prior to planting and adding earthworms, some chambers were vertically divided with timber, to compare effects of earthworm presence on root growth. Plants were positioned with roots evenly divided each side of the chambers. Biosolids or plant litters as food sources were placed in three different places in each half of the soil profile, at least 30 cm from the planting point (Figure 9.1). The buried OM were enclosed with a cellulose (paper tissue) wrapper. Plant were acclimatised time in the soil matrix for a month and then five earthworms were added to one side of the chamber and visually monitored for 2 months.

Table 9.2 Earthworm species and soils used in the experiments.

	Species name	Group	--- Choice Chamber ---		Glass-sided chamber
			pH preference	Litter preference	
Earthworms	<i>Eisenia fetida</i>	Epigeic	√	√	
	<i>Maoridrillus transalpinus</i>	Anecic	√	√	√
	<i>Maoridrillus</i> sp.2	Anecic	√		
	<i>Octolasion cyaneum</i>	Endogeic	√	√	√
Soils	NF	Karoro	√		
	SF	Eyre		√	√

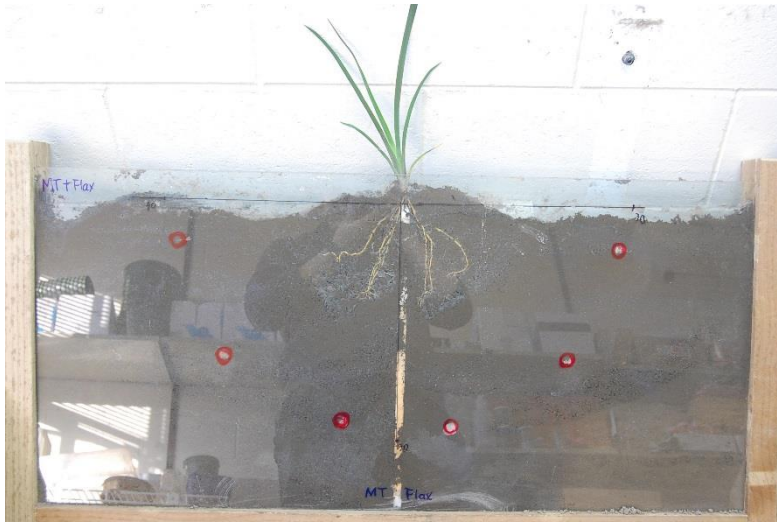


Figure 9.1 Photo of glass chamber vermarium in which *Phormium tenax* was planted on SF soil, prior to earthworm addition. Biosolids were encapsulated within tissue paper and placed at 6 points, indicated by red circle.

9.2.3 Soil pH preference

Four species of earthworms were included in this trial (Table 9.2). Soil pH was adjusted to four different levels by adding calcium carbonate (lime) to NF soil: pH 4.7, 5.2, 5.7, and 6.2. Based on a formula of Edmeades et al. (1985), the required adjustment was newly calculated through several preliminary tests to assure an accuracy: Adjusted Soil pH wanted = $0.6545 \times (\text{additional lime amount } \text{g} \cdot \text{L}^{-1}) + 4.1067$. The adjusted soil substrates were stored in plastic containers (50 L) for more than 2 months to become homogenised soil pH after the lime addition. Soil pH was analysed prior to this choice-chamber trial. There were ten replicates in the soil pH preference trial.

9.2.4 Litter preference

This litter preference trial were carried out to identify the most palatable litters in three separate trials:

- Trial A preference between monocot species (*Carex secta*, *Cortaderia richardii*, and *Phormium tenax*)
- Trial B preference between dicot species (*Coprosma robusta*, *Kunzea robusta*, and *Olearia paniculata*)
- Trial C preference between *Lolium perenne* and two native species, selecting the most preferred plant litter by *M. transalpinus* from each trial A and B.

SF soil (200 g) mixed with 1 g litter powder was placed in each chamber, to which was added *E. fetida*, *M. transalpinus*, and *O. cyaneum*. Bulk soil also was established as reference (control) in the 4th chamber. There were five replicates in each trial.

9.2.5 Statistical analysis

Minitab 17 (Minitab Inc., State College, Pennsylvania, USA) was used for all statistical analysis. To compare means of each earthworm species treatment ($n=5$, $p<0.05$), a one-way ANOVA using a Fisher's Least Significant Difference test and two-way ANOVA were carried out. For soil pH, mean and standard errors were calculated following conversion to the equivalent hydrogen ion concentrations and back calculation to pH. To elucidate effects of litter properties on earthworm preference and feeding activity, a Pearson test was conducted between C, N, C/N, ADF, Ca, K, Mg, P, S, Cu, Zn, and Mn.

9.3 Results and discussion

9.3.1 Choice chamber

Soil pH preference

Increased pH of soil certainly influenced earthworm preference and their feeding activity (Figure 9.2; Table 9.3). The amount of organic matter consumed (COM) differed depending on earthworm species. Addition of more lime (calcium carbonate) was more attractive to all species of earthworms, but less OM was consumed at the highest pH level of 6.2. Interestingly, two endemic *Maoridrilus* spp. preferred all raised soil pH treatments whilst the exotics (*O. cyaneum* and *E. fetida*) totally avoided in soil at pH 4.7. *Maoridrilus* spp. and *O. cyaneum* preferred similar pH of their habitat soils, which ranged pH 5.2 to 5.7. In contrast, *E. fetida* preferred a more neutral pH of soil as found previously (Edwards and Bohlen, 1996; Gajalakshmi and Abbasi, 2004), although it consumed more OM of pH 5.2 and 5.7. All earthworms showed significant weight changes after a week: *M. transalpinus* (5 %), *Maoridrilus* sp.2 (2 %), *O. cyaneum* (-13 %), and *E. fetida* (-2 %).

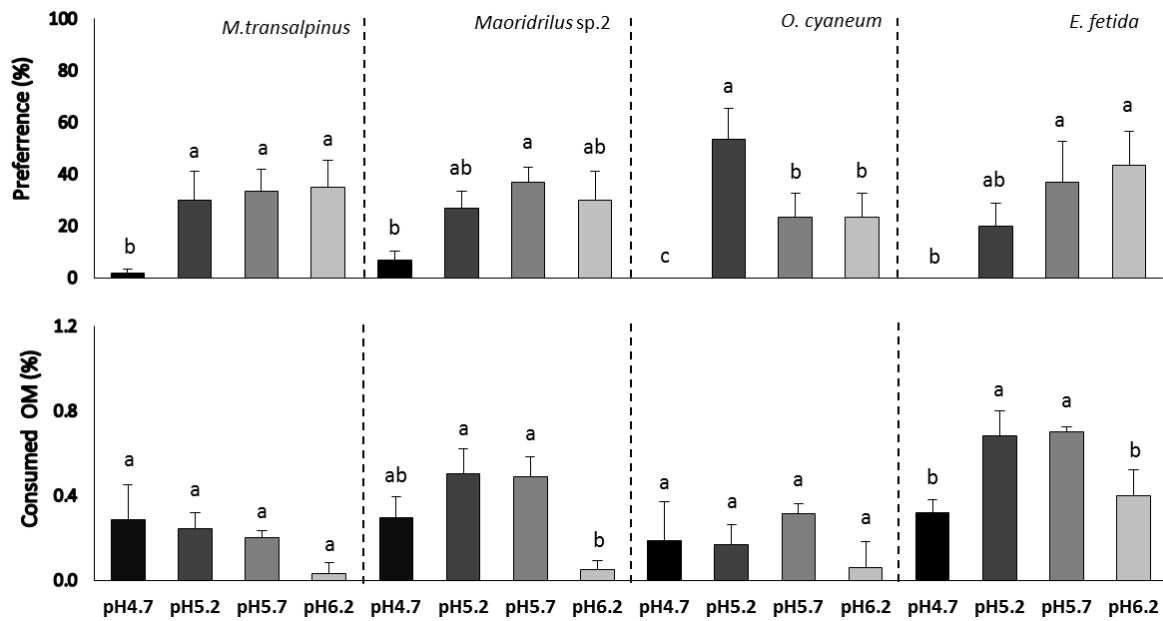


Figure 9.2 Soil pH preferences in choice chamber. Consumption of organic matter refers to LOI changes after one week inoculation with 5 earthworms in each set of choice chamber. Shading distinguishes pH 4.7 (■), pH 5.2 (■), pH 5.7 (■), and pH 6.2 (■). Values are means \pm standard errors (n=5, sets number of choice chamber). The same letters within each earthworm species indicate no significant difference ($p < 0.05$).

9.3.2 Litter preference assays

A. Monocot species choice (Trial A)

There was no striking preference of any species of earthworms to litter of the three monocot plant species, although only *Eisenia* preferentially selected chambers with litter. The other two species appeared to avoid these chambers (Figure 9.3). The amount of organic matter consumed (COM) was significantly influenced both by different earthworm species and different plant species (Table 9.3). *M. transalpinus* and *E. fetida* consumed significantly more organic matter from choice chambers containing litters of *C. secta* and *P. tenax*, compared to *C. richardii* and bulk soil. There were no significant effects of litter properties on earthworm preference for monocots (Table 9.4). Litter properties influenced the amount of organic matter consumed by all species of earthworms (Table 9.4); high concentrations of Ca and K in the litters were likely to be more palatable, but litters with more of N, Mg, S and Zn were less palatable.

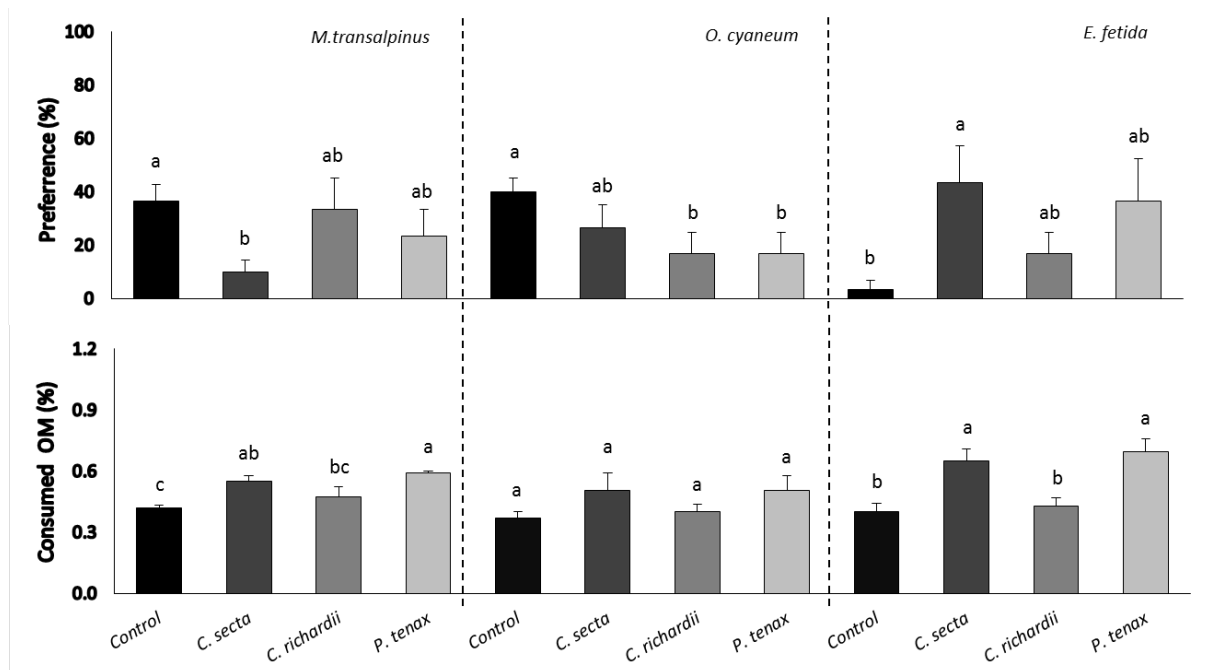


Figure 9.3 Plant litter preferences amongst native monocot species in choice-chamber (Trial A). Consumption of organic matter refers to LOI changes after one week inoculation with 5 earthworms in each set of choice chamber. Shading distinguishes control (■), *Carex secta* (■), *Cortaderia richardii* (■), and *Phormium tenax* (■). Values are means \pm standard errors (n=6, sets number of choice chambers). The same letters within each earthworm species indicate no significant difference ($p < 0.05$).

B. Dicot species choice (Trial B)

Each dicot species had significantly different preferences for earthworms (Table 9.3). Like monocot species (Trial A), *M. transalpinus* and *O. cyaneum* avoided litter treatments while *E. fetida* showed clear preference for litter amendments (Figure 9.4). Within litter treatments, *C. robusta* was least disagreeable to all species of earthworms ($p < 0.05$). This may be due to this litter containing more Ca and S and less K (Table 9.3). *M. transalpinus* and *O. cyaneum* completely avoided choice chambers amended with *K. robusta* litter. This clear avoidance maybe strongly associated to high concentration of tannins or volatile oils in foliage of *K. robusta* (Dickinson et al., 2015). These authors suggested that the tannins in plant foliage may be beneficial as a browse crop to livestock, but degrade palatability to earthworms, as also found by Curry (2004).

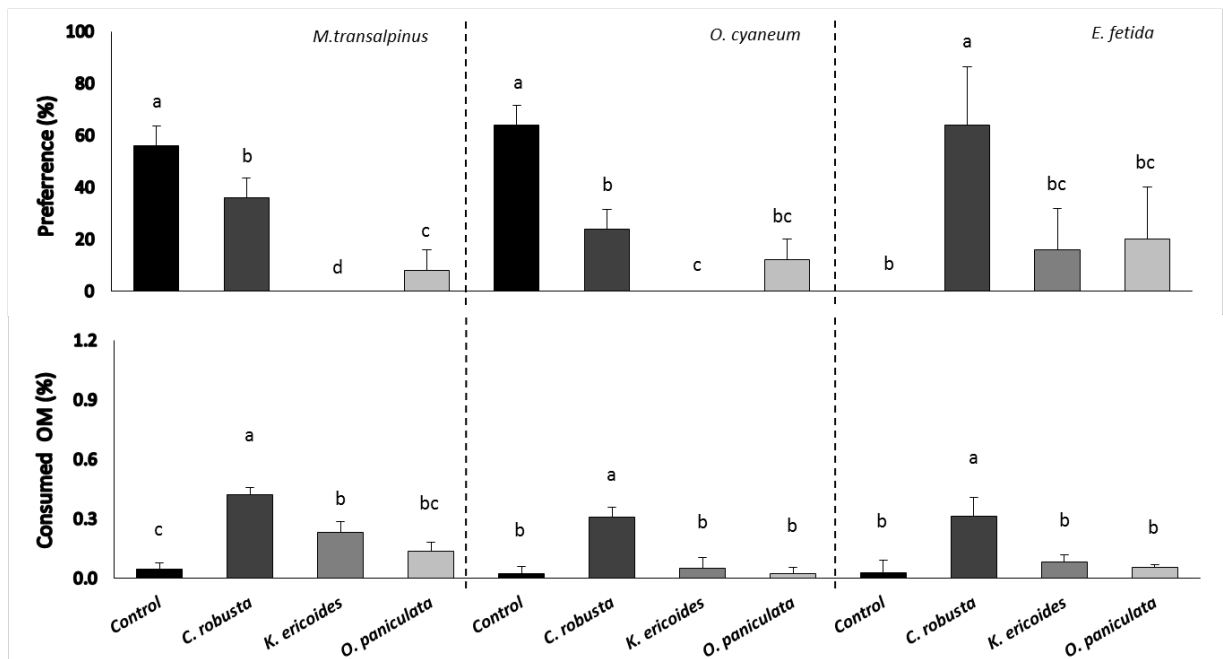


Figure 9.4 Plant litter preferences amongst three native dicot species in choice-chamber trial B. Consumption of organic matter refers to LOI changes after one week inoculation with 5 earthworms in each set of choice chamber. Shading distinguishes control (■), *Coprosma robusta* (■), *Kunzea robusta* (■), and *Olearia paniculata* (■). Values are means \pm standard errors (n=6, sets number of choice chambers). The same letters within each earthworm species indicate no significant difference ($p < 0.05$).

C. Native species vs *L. perenne* choices

Plant species differed significantly in terms of earthworm preferences between *C. richardii* (native monocot), *C. robusta* (dicot), and *L. perenne* (exotic monocot) (Table 9.3). *M. transalpinus* and *E. fetida* significantly preferred litter of *L. perenne*, containing triple the amount of N than native plants (Table 9.1, Figure 9.5). In addition, both species consumed the largest amount of *C. robusta* litter, followed by *L. perenne* and *C. richardii*. In contrast, *O. cyaneum* did not show specific preference. Neilson and Boag (2003) reported that *O. cyaneum* preferred only bulk soil rather than other food sources. However, *O. cyaneum* digested double the amount of OM from litter treatments of *C. robusta* and *C. richardii* compared to bulk soil in the current study.

High Ca content in litters, particularly of *C. robusta*, can increase soil pH (Zhong et al., pers. comm.) and thus may improve habitat conditions for *M. transalpinus*. This may explain why *M. transalpinus* were found most often beneath *C. robusta* in native vegetation when we sampled. In addition, endemic earthworm showed a preference for high concentrations of K, P, S, Cu, Zn, and Mn and negative correlation with C and C/N ratio of plant litter (Table 9.3). High N in litter also tended to be unpalatable to *M. transalpinus* and *E. fetida* in this study (Table 9.4), although Gajalakshmi and Abbasi (2004) reported that higher N ratio enhanced maturity and fecundity of earthworm. In the present study, there were no evident effects of lignin content associated with acid-detergent fibre

(ADF) on earthworm preference. Others have concluded that most species of earthworm are not preferentially attracted to rich lignin or high C/N ratio, carbohydrates and polyphenols in plant litter (Curry and Schmidt, 2007; Ganesh et al., 2009; Tian et al., 2000).

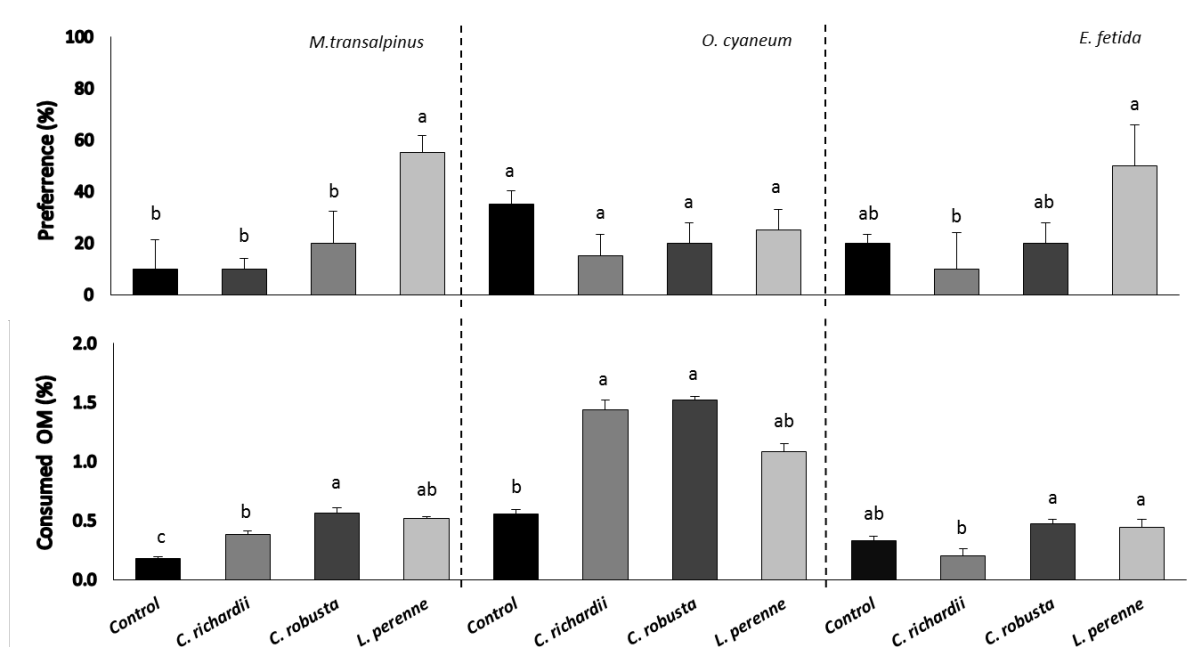


Figure 9.5 Plant litter preference amongst two native species and an exotic ryegrass in choice-chamber trial C. Consumption of organic matter refers to LOI changes after a week inoculation. Shading distinguishes control (■), *C. richardii* (■), *C. robusta* (■), and *L. Perenne* (■). Values are means \pm standard errors (n=6). The same letters within each earthworm species indicate no significant difference ($p < 0.05$).

Table 9.3 Two-way ANOVA (p) and Pearson test (r) in earthworm preferences to pH and plant litter and amount of consumed organic matters (COM) in choice chamber assays during a week inoculation (NS Not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Preference	Treatment	Two-way ANOVA (P)		Pearson correlation (r)
		Preference	COM	Preference x COM
Soil pH (n=5)	Earthworm	NS	***	0.095
	pH	***	***	
	Interaction	NS	NS	
a. Monocot (n=6)	Earthworm	NS	***	0.118
	Plant	NS	***	
	Interaction	NS	NS	
Litter Experiment	Earthworm	NS	*	0.112
	Plant	***	NS	
	Interaction	**	NS	
c. Natives vs Ryegrass (n=6)	Earthworm	NS	***	0.008
	Plant	*	**	
	Interaction	NS	*	

Table 9.4 Correlation between litter properties and earthworm's preference and consumed organic matter (COM). Values were Pearson correlation score (r) with P-value (^{NS} $p>0.05$; * $p<0.05$; ** $p<0.01$; * $p<0.001$). Abbreviation in brackets is of each species of earthworm: MT (*Maoridrilus transalpinus*), OC (*Octolasion cyaneum*), and EF (*Eisenia fetida*).**

Litter properties	----- Monocot species -----		----- Dicot species -----		----- Natives vs Ryegrass -----	
	Preference	COM	Preference	COM	Preference	COM
C	NS	NS	-0.73**(MT), -0.61*(OC)	-0.68*(OC), -0.60*(EF)	-0.65*(MT)	NS
N	NS	-0.6*(MT), -0.66*(EF)	NS	NS	NS	-0.60*(MT), -0.77**(EF)
C/N	NS	NS	NS	NS	-0.65*(MT)	NS
ADF	NS	NS	NS	NS	NS	NS
Ca	NS	0.58*(MT), 0.61(EF)	0.74***(MT), 0.61*(EF)	0.57*(MT), 0.71**(OC), 0.62*(EF)	NS	NS
K	NS	0.64*(MT), 0.75**(EF)	-0.60*(MT)	-0.82***(MT), -0.78**(OC), -0.70*(EF)	0.66*(MT)	NS
Mg	NS	-0.62*(MT), -0.69**(EF)	0.52*(OC)	NS	NS	0.59*(MT), 0.73**(EF)
P	NS	NS	-0.65**(MT), -0.59*(OC)	NS	0.66*(MT)	NS
S	NS	-0.63*(MT), -0.76**(EF)	NS	0.77*(MT), 0.69*(OC), 0.61*(EF)	0.62*(MT)	0.66*(EF)
Cu	NS	NS	NS	NS	0.67*(MT)	NS
Zn	NS	-0.64*(MT), -0.73**(EF)	-0.72**(MT), -0.61*(OC)	-0.66*(OC), -0.58*(EF)	0.67*(MT)	NS
Mn	NS	NS	-0.69**(MT), -0.61*(OC)	NS	0.66*(MT)	NS

9.3.3 Earthworm behaviour in rhizosphere (Glass-sided chambers)

Both *M. transalpinus* and *O. cyaneum* burrowed and navigated accurately towards the OM amendments of biosolids and plant litters (Figure 9.6, *O. cyaneum* not shown). In the absence of OM inclusions on one half of the chamber, the earthworms readily crossed the vertical timber barriers towards the inclusions, moving across above the soil surface and probably following the rhizospheres. It seems that the earthworms have a distinct olfactory sense to detect food material such as plant and microbiota (Zirbes et al., 2011). The best evidence of this was the direct connection of drilospheres to all the locations of the embedded litter and biosolids; the inclusions had been replaced by empty spaces following two months of inoculation (cf. Figure 9.1). Plant root systems tended to follow the burrowing pathways of the earthworms which create favourable environments for root development. (Figure 9.6). Earthworm burrowing can facilitate root proliferation by enhancing nitrogen and microbial status of the soil (Blouin et al., 2013; Brown et al., 2000) and by increasing water infiltration (Bertrand et al., 2015). Unlike the endogeic *O. cyaneum*, anecic *M. transalpinus* tended to preferentially burrow adjacent to roots forming a more complex drilosphere around the rhizosphere than in surrounding bulk soil. This may be due to the rhizosphere containing more food sources of carbon and a higher microbial community richness (Bertin et al., 2003; Uren, 2007).

9.4 Observational

This study showed that earthworm preference are variable, depending on soil properties, (especially soil pH), and the quality of food resource. I reported previously (Chapter 3, Kim et al., 2015) that native species of earthworms preferred the physicochemical conditions of farmed soils to forest soils; less acid soils with lower C/N ratios and higher soluble P were more important than soil with high OM. In this study, all earthworms preferred less acid soils that had been amended with lime. Between the litters of native plants, there was selectivity between species; *C. robusta* litter containing high concentrations of Ca and S was most palatable of the species tested. The nutrient content of litter (Ca, K, Mg, S, and Zn) considerably influenced litter palatability and earthworm preference, particularly of endemic species *M. transalpinus*. However, there was no clear correlation between litter preference and the amount of litter consumed (Table 9.3).

Earthworm burrows were strongly associated with feeding activity. Although there is a lack of knowledge about earthworm sensory responses, such as olfactory and visual reactions to food sources, it seemed that earthworms can forage for the organic resources beneath soils with a very high capacity for accurate navigation. Visual observations also indicated that a strong relationship between rhizosphere and drilosphere was formed through intensive burrowing around roots (see Figure 9.6.B). Thus, a mutually-beneficial relationship develops: earthworms modify the rhizosphere structure, and roots preferentially forage around drilospheres.

Overall, the endemic species *M. transalpinus* preferred agricultural soils and litter of *L. perenne* rather than native soils and vegetation. Of course, the preference and behaviour of *M. transalpinus* in this study may not be representative of other endemic earthworms, but the results indicated that native earthworms could burrow and colonise in agricultural land, similar to introduced earthworms, perhaps unless the land is under intensive land management practices such as regular ploughing or tillage.

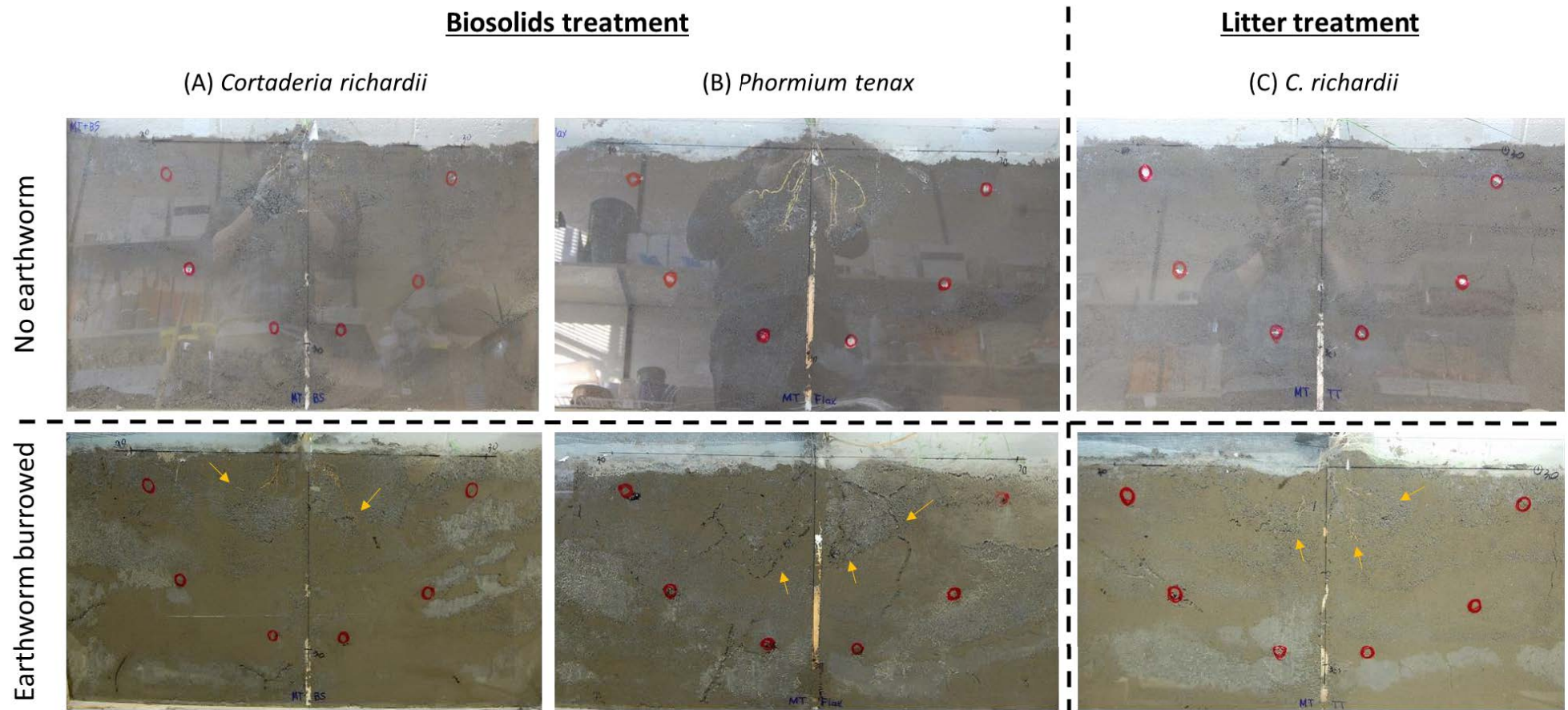


Figure 9.6 Influence of *M. transalpinus* on the rhizosphere of native plants (*Cortaderia richardii* and *Phorimium tenax*) 2 months after inoculation. First row shows rhizosphere prior to earthworm addition and second row show the rhizosphere following earthworm activity. Organic materials as food sources were buried at six points, indicated by red circle: biosolids treatment in column (A) and (B) and litter treatment of *C. richardii* in column (C). Yellow arrows points out lengthened roots along the drilosphere.

Chapter 10

Synopsis and Conclusions

10.1 Synopsis

10.1.1 Responses of native earthworms to agriculturally-modified soils

The preference of native earthworm for modified agriculture soil over native unmodified soils is an unexpected findings of this research. New Zealand lowlands have been mostly converted to agricultural landscapes since European settlement. In the province of Canterbury on South Island less than 0.5 % of native vegetation cover remains (Winterbourn, 2008). It is known that introduced species of Lumbricid earthworms are the dominant fauna in agricultural soils, having replaced native Megascolecidae (Fraser et al., 1996; Springett et al., 1998). Restricted refugia of native vegetation and remnants on the margins of agricultural land provide the remaining habitats for indigenous earthworms (Bowie et al., 2016). This PhD study (Chapter 3 and 9) has shown that native earthworms, and particularly *Maoridrilus transalpinus*, prefer fertile agricultural soils to highly organic native forest soils. The most important factors is found to be soil pH; earthworms also preferred native forest soils with increased pH from lime addition.

Among the litters of different plant species, native earthworms preferred ryegrass litter to native plants (*Carex secta*, *Cortaderia richardii*, *Phormium tenax*, *Kunzea robusta*, *Olearia paniculata*, and *Coprosma robusta*) (Chapter 9). This may be due to high concentrations of N, K, P, S, and Cu in ryegrass foliage improving palatability to native earthworms (Gajalakshimi and Abbasi, 2004). High C/N ratio, tannins and volatile oils in native plant foliage of Kanuka were certainly unpalatable to all earthworms as found in other studies (Curry and Schmidt, 2007; Dickinson et al., 2015). By way of exception, native *C. robusta* containing high Ca concentration, like ryegrass, seems to be mostly consumable by native *M. transalpinus*. It can be a supportive reason why *M. transalpinus* colonized well around rhizosphere of *Coprosma* in native vegetation.

Native earthworms also tended to influence soil biogeochemistry more than exotic species, particularly in terms of N mineralization by burrowing and casting (Chapter 3 and 5). I found, using mesocosms, that they can sustain their survivorship on pastoral system with accompanying improvement of plant-availability of nutrients, microorganisms communities, and ryegrass productivity (Chapter 7). In the pastoral soils (Figure 10.1), survivorships of native *M. transalpinus* certainly surpassed that of exotic *O. cyaneum*. The native species also enhanced biogeochemical conditions like microbial activity (dehydrogenase enzyme activity), aeration, aggregation, and mobility of N (NH_4^+ and NO_3^-) and P (Olsen P) in the rhizosphere soil, more than the exotic species. In the species

tested in the present work, native earthworms appeared to be superior to introduced species in terms of their effects on root morphology, photosynthetic pigments (chlorophyll content), and ryegrass yields.

Although exotic Lumbricidae have colonized agricultural pasture in New Zealand, agronomists have often had difficulty with establishment of introduced species (Fraser, 2010). In this regard, physical disturbance of soils by ploughing and tillage probably far outweighs the chemical modification of soils from fertilizer application. As with exotic species, soil pH and quality and quantity of food materials are critical to sustain native earthworms (Curry, 2004; Edwards, 2004).

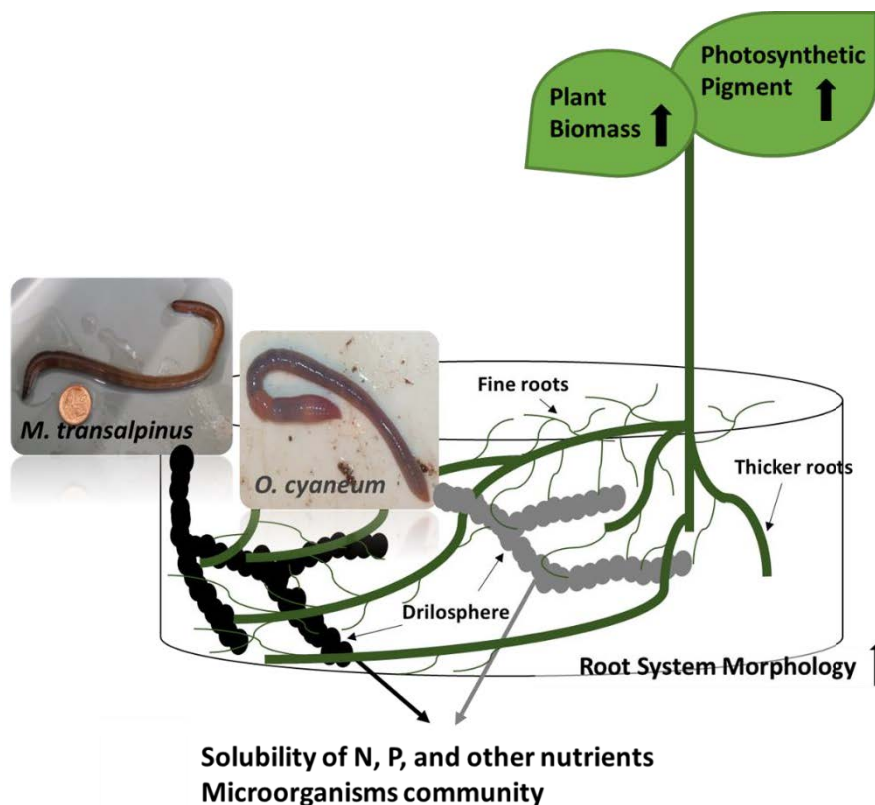


Figure 10. 1 Overall functionality of both native and exotic earthworms on soil biogeochemistry and plant growth under pasture system.

10.1.2 Identification and distribution of native earthworms

Megascolecidae earthworms have evolved in isolation since the Tertiary period in New Zealand, but anthropogenic activity in the past two centuries has depauperated abundance of native species from the lowlands (Lee, 1959a). About 173 native species of earthworms had been reported prior to 2000, often using morphology identification. Following introduction of DNA barcoding techniques in the present, six taxa of Megascolecidae species were newly identified. Molecular tools can be used to investigate phylogenetic relationships between a group of closely related species and between native and exotic species (Minamiya et al., 2009; Porco et al., 2013). More than 100 cryptic taxonomic

species may remain to be described their phylogeny in New Zealand (Buckley et al., 2012). Matching molecular techniques with morphological identification requires further work. In this study, 13 native species were collected from five locations in South Island (Figure 4.1) including Punakaiki, Eryewell, Bank Peninsula, Bank side, and Lincoln. The collected native species could be clearly separated from Lumbricidae groups through a tree map of 16S primer DNA barcoding (Figure 4.2). I confirmed that *Maoridrilus* spp. and *O. multiporus* were dominant species particularly in Canterbury vegetation, and the rest species may be new taxa of species and also needed to be investigate their phylogeography and taxonomy.

10.1.3 Role of native earthworms in soil biogeochemistry and native plant growth

As soil engineers, earthworms improve soil quality by altering soil structure (aggregation and water infiltration), stimulating organic matter dynamics and nutrient cycling (e.g. N, P, K etc.) and enhancing biological interactions with microorganisms and plants (Bertrand et al., 2015; Frouz et al., 2007). The present study has shown that native earthworms increase N mineralization (ammonification and nitrification) from decomposition of soil organic matter.

Native *O. multiporus* and *M. transalpinus* are shown to influence N cycling in an agriculturally-disturbed soil, to a greater extent than exotic Lumbricids (Chapter 3). Both native species increased NH_4^+ concentration by 3 and 2 times, respectively, to the exotic *O. lacteum*. They also enhanced more nitrate concentration, and particularly *O. multiporus* released significant more N_2O gas emission from the soil. In this regards, the deep burrow *O. multiporus* stimulates N dynamic to the Lumbricids in agricultural soils. Moreover, the native species influence more mineralization of key nutrients such as K, Mg, Ca, and S.

However, the differences between ecological grouping species outweigh the differences between native and exotic species, in terms of effects on soil biogeochemistry (see Figure 5.1). Regardless of species origins, all anecic and enecic species of earthworms influenced more on solubility of P, Ca, S and Al, electronic conductivity (EC), and soil pH than epigeic species. Only the impacts of native *O. multiporus* tended to differ to that of the exotic species, particularly on mineralization of N and K.

With biosolids amendments, native *Maoridrilus* spp. had more effective capacity to solubilize nutrients, particularly N mineralization, than exotic *Eisenia fetida* (Figure 10.2). *Maoridrilus* spp. significantly increased NH_4 and NO_3 as well as microbial biomass C that tended to encourage more nitrous oxide (N_2O) emission from the biosolids-amended soil. Casts of the native earthworms contained more solubility of nutrients such as P, K, S and Cu, whilst Zn solubility reduced probably by

absorption in their tissue. The increase of Cu solubility and pH reduction in the presence of native earthworms suppressed soil enzyme activity (DHA). Like DHA, respiratory CO₂ emission of microorganisms appeared to be decreased by casting and Cu toxicity.

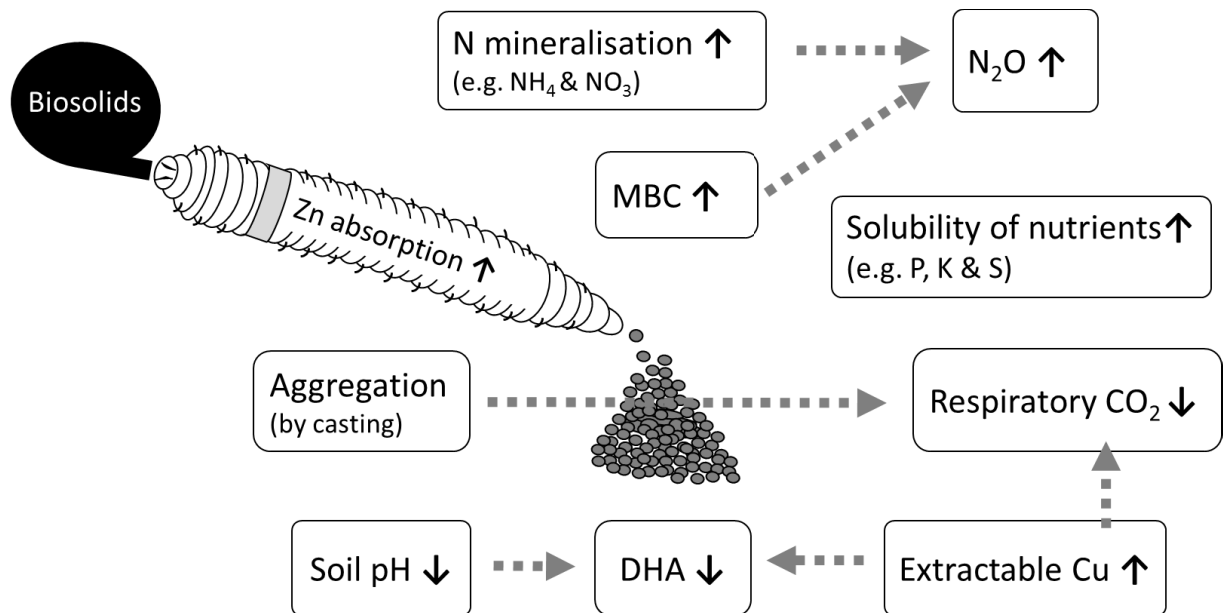


Figure 10.2 Tentative interpretation of results of inoculation experiment. Effects of *Maoridrilus* spp. in biosolid-amended soil are indicated on arrows.

There were strong interactions between native earthworms (*Deinodrilus* sp.1, Megascolecidae sp.2, and *Maoridrilus* spp.) and the native plants Manuka, Kanuka, and Kowhai. Although there was no significant variation in plants growth during the mesocosms, the burrowing activity of native earthworms modified root morphology more than exotic species (Figure 3.6). *Maoridrilus transalpinus* produced considerable amount of drilosphere in potted soils of Kowhai that resulted in enhancement of microbial activity, N solubility, and nodulation in the rhizosphere soils (Figure 10.3). Although there were no variations in plant growth, the native species significantly increased NH₄ and NO₃ concentrations in rhizosphere soils with added rhizobia, compared to exotic earthworms. It seems that *M. transalpinus* effectively dispersed rhizobia and facilitated more nodulation around the roots of Kowhai. In addition, N₂O emission appeared to be suppressed by increase of nodulation and increased aeration within the drilosphere of *M. transalpinus*.

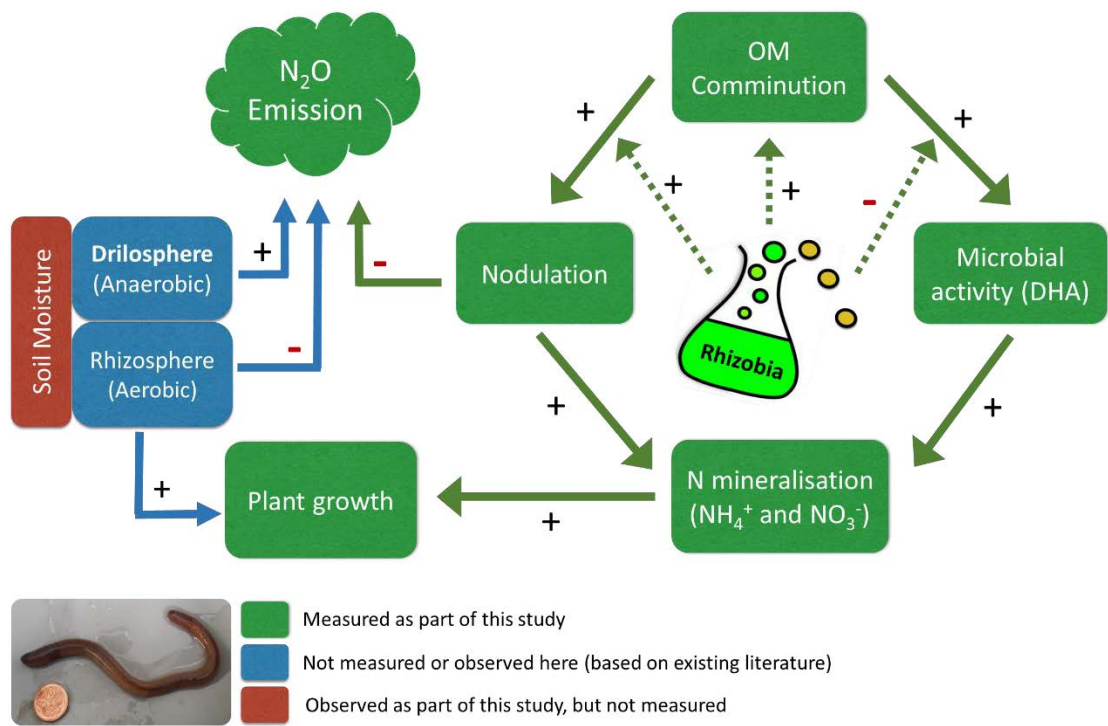


Figure 10.3 Tentative interpretation of results of mesocosm experiment, based on data of *Maoridrilus transalpinus*.

10.1.4 Benefits of native earthworms on New Zealand ecosystem

New Zealand is one of worldwide biodiversity hotspots; long-term geological isolation has led to the evolution of a highly endemic flora and fauna (Lee, 1959a). Undoubtedly native earthworms interact with lots of species of native plants, animals, and microorganisms. Their linkages and interactions may not be provided by exotic species. Disappearance of native earthworms and replacement by exotics may cause wider problems in terms of conservation of biodiversity in New Zealand. More than 200 species of native earthworms may provide multiple but as yet unknown benefits to NZ ecosystems, although they are often highly restricted in distribution, such as in vegetation remnants on the margins of agriculture. There has been insufficient study of native species to understand their role in soil ecosystems.

This study improved our understanding of the functionality of native earthworms and their relationships with native plants (e.g. Manuka, Kanuka, and Kowhai) and microorganisms (e.g. *Mesorhizobium* sp. ICMP 19535) through mesocosm studies. Of the native species, *M. transalpinus* and *O. multiporus* were shown to be more beneficial than invasive species of Lumbricidae, in terms of nutrients mineralization and plant productivity. Native species could play a crucial role in conservation of soil biodiversity and sustainability of ecosystem in New Zealand.

10.2 Overall conclusions

Native Megascolecidae earthworms have persisted in remnants of native vegetation on the margins and borders of intensively-managed agricultural pasture land, despite their disappearance from farm paddocks. Earlier work has shown that exotic Lumbricidae have extensively colonized agricultural land with varying degrees of success, but little attention has been given to any aspect of the ecology of endemic species, for which only a very restricted habitat is likely to remain. Nevertheless, digging for earthworms at multiple locations as part of the present study has shown that native species are widespread in this marginal habitat and also that they frequently co-exist with exotic lumbricids. Although it was not one of the main objectives of this study, I found no evidence of competitive exclusion of either family of earthworms. I did find evidence that both native and exotic earthworms can persist and colonize restoration sites where native vegetation has been re-established. This is an important point, in view of a clear recent trend over little more than the last few decades towards native plant restoration within these landscapes that remain highly depauperate of floral and faunal biodiversity.

Disappearance of native species in various parts of the world is recognized to be associated with a lack of tolerance to disturbance (burning, ploughing and removal of native vegetation) and the resultant changed environmental conditions (e.g. extreme temperatures and less soil moisture). I consider that the same is likely to apply to the agricultural landscapes of New Zealand, but my original hypothesis was that the two families also interact differently with soil biogeochemistry. I expected to be able to demonstrate different range of tolerances or preferences to different soil conditions, and different effects of native and exotics on modification of soil physicochemistry. Native species were often more tolerant of acid soils which was a stronger limiting factor than soil organic matter content. However, like exotics, the native species studied also had a preference for agriculturally-modified soils and pasture grass litter. Burrowing and feeding behaviours appeared similar between both families, but food supply was critical. Observational studies indicated a high degree of precision in foraging abilities, and this would be a worthwhile area for further study.

Experimental studies in this thesis focused on functionality and interactions with the rhizosphere of native plants. *Maoridrillus* spp. had a marked effect on organic matter decomposition, nutrient cycling (especially nitrogen speciation and mobility), microbial activity, rhizosphere morphology and plant growth. Differences between ecological groups of earthworms were often more substantial than differences between the families, and between the native and exotic species. Nonetheless, there were significant differences between individual species that affected important environmental variables including nitrate leaching from soil and nitrous oxide emissions from soils. Contribution to knowledge from the present study falls short of being able to predict reliable management options that might involve the manipulation of earthworm populations.

Conclusions of the seven experimental chapters are drawn together in the context of the Aims and Objectives described in Chapter 1 (pp. 2-4).

The aims of this research were to investigate (i) how earthworms have survived alongside invasive species in highly disturbed landscapes, (ii) whether and how they have adapted to the modified soil biogeochemistry of agricultural land, and (iii) whether they play a role in influence the functionality of these soils.

- This research project has provided fresh insights into the status and ecology of native earthworms in human-modified soils in New Zealand. It was found that their exclusion from agricultural pastures is not due to an inability to adapt to modified soil physicochemistry. It is considered most likely that they were not resistant to vegetation clearance, land disturbance or the ensuing environmental conducts (e.g. changed temperature and moisture).

Objective 1. Investigation of the interactions of native and introduced earthworms with soils and plant rhizospheres in production landscapes of New Zealand [Chapter 3]

- Native and exotic earthworms are found to co-exist in agricultural landscapes in New Zealand. They are shown to modify plant growth, nitrogen mobility and greenhouse gas emission. The main differences between earthworm functionality were found to be between different ecological groups, rather than between taxonomic groups. I argue that a move towards sustainable agricultural systems and current restoration practices will probably enhance the dispersion of native earthworms.

Objective 2. Molecular identification and the distribution of New Zealand earthworms in human-modified Soils [Chapter 4]

- A total of 15 undescribed Megascolecidae taxa from native vegetation, restoration plots and agricultural pasture was identified using DNA barcoding. Eight taxa were identified in genera of *Octochaetus*, *Maoridrilus* and *Deinodrilus*. In terms of phylogenetic separation, the 16S-based phylogeny clearly separated Megascolecidae from Lumbricidae. Compared to native taxa, exotic earthworms were disturbed across wider environmental conditions, with more resistance to acidic soil and low organic resources. This work illustrated the rudimentary nature of our knowledge of earthworm taxonomy and soil ecology in New Zealand.

Objective 3. Endemic earthworms in a sheep-farmed soil: implications for soil nutrients, environment and conservation [Chapter 5]

- Native species were found to coexist with exotics in remnants of native vegetation within intensive sheep-farmed landscapes, and they could survive in modified pasture soils. This work indicated that native earthworms are unlikely to be compromised by a gradual accrual of nutrients, they increased soil concentrations of exchangeable minerals including N, P, Ca, K, Mg, and Na. Individual species of both natives and exotics could be separated on the basis of their modification of soil biogeochemistry. This work supports the idea that less intensive farm management systems (e.g. with reduced tillage) may allow the expansion and increased diversity of native species.

Objective 4. Integration of earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a mesocosm experiment [Chapter 6]

- The native legume *Sophora microphylla* grew better in the presence of soil burrowing earthworms. The native earthworm *Maoridrilus transalpinus* modified soil biogeochemistry (e.g. enhancing nitrate and dehydrogenase enzyme activity) and rates of root nodulation, but also reduced nitrous oxide emissions. The finding of this experiment indicated that earthworm-mediated soil aeration, modification of moisture conditions in the rhizosphere and drilosphere, and comminution of organic matter modify microbial communities and influence the N cycle. I argue that the functionality of native earthworms could be valuable for effective management of soil N in ecological restoration on former agricultural land.

Objective 5. Investigation of the potential role of New Zealand native earthworms (Megascolecidae) as ecosystem engineers on agricultural land [Chapter 7]

- The native *Maoridrilus transalpinus* had high survivorship on agriculture soils within pasture management systems. This species appeared to play an equivalent or more substantial role to the exotic *Octolasion cyaneum* in the rhizosphere of ryegrass. Modification of morphology of the drilosphere and root system, stimulation of nutrient dynamics (e.g. nitrogen) and microbial communities, and enhancement of plant-availability of nutrients were evident. These effects increased plant biomass and photosynthetic pigments. This further supports the argument that native earthworms may have a functional role in modern and future agroecosystem management.

Objective 6. Biochemical impacts of endemic Maoridrilus earthworms (Megascolecidae) in biosolids-amended soil [Chapter 8]

- Two native *Maoridrilus* spp. proved to be efficacious in the context of biosolids disposal to land. They increased mobile N, microbial communities, and soluble Cu in soil. Both species also increased N₂O emissions from soil, and more so than did the compost earthworm *E. fetida*. *Maoridrilus* earthworms have the potential to enhance plant productivity in biosolids-amended agriculture and ecological restoration soils, but this work showed they may raise additional environmental concerns in terms of greenhouse gas emissions.

Objective 7. Earthworm feeding and burrowing behaviours: observation studies [Chapter 9]

- Earthworm preference was shown to be variable depending on soil pH and availability of native plant litters. All earthworms preferred lesser acid soils amended with lime. Of the native plant litters, *C. robusta* litter was the most palatable of the species tested. Nutrient content of litter (N, Ca, K, P, S, and Zn) influenced litter palatability and earthworm preference, particularly of endemic *M. transalpinus*. These studies indicated that earthworm sensory abilities, such as olfactory and visual reactions to food sources, allowed foraging for food resource in soils with surprisingly accurate capacity of navigation. Earthworms were also shown to modify rhizosphere structure, and there was a knock-on effect of plant root preferentially growing around drilospheres.

10.3 Suggested further study

Further work on native earthworms is justified both in terms of the importance of their conservation and their role as soil engineers in mediating soil fertility, soil sustainability and environmental biogeochemistry. Large gaps in knowledge exist at every level, including the need to identify cryptic taxa of earthworms. In addition to this, the outcomes from this study is based on a focus on only a few native species. Clearly this is highly limited and is unlikely to be representative all native earthworms in New Zealand. Further studies should concentrate more on:

- Identification of native earthworm diversity by classifying new cryptic species and surveying their distribution.
- Co-existence and competition of natives with exotic species.
- Opportunties for Invasion of agricultural lands by invasive species with reduced tillage.
- Tolerances of native species to disturbed soils, physically and chemically.
- Interactions of native species with other native biota (flora, soil invertebrates, macrofauna, and microflora).
- Influence of native earthworms on commercial crop productivity (e.g. winery, fruits, and vegetables).

10.4 Publications

Five chapters presented in this thesis are draft manuscripts prepared for journal submission. One is already published.

Chapter	Objective	Title	Proposed Journal
3	1	Interactions of native and introduced earthworms with soils and plant rhizospheres in production landscapes of New Zealand	Applied Soil Ecology (published)
4	2	Molecular identification and the distribution of New Zealand earthworms in human-modified soils	New Zealand Journal of Ecology
5	3	Endemic earthworms in a sheep-farmed soil: implications for soil nutrients, environment and conservation	Applied Soil Ecology
6	4	Integration of earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a mesocosm experiment	Pedobiologia
8	6	Biochemical impacts of endemic <i>Maoridrilus</i> earthworms (Megascolecidae) in biosolids-amended soil	Biology and Fertility of Soils

During this PhD study, two posters were presented

- 20th World Congress of Soil Science (20WCSS) in 2014:
Biogeochemical role of native and exotic earthworms in New Zealand soil
- New Zealand Ecological Society Conference in 2015:
Endemic earthworms and biogeochemical impacts on New Zealand soils.

See Appendix B for attachments of these posters.

Appendix A

DNA sequences data

Octochaetus multiporus (YN1)

16S, GenBank accession number KP780262 (submitted 12 Feb 2015)

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COI, GenBank accession number KP780261 (submitted 12 Feb 2015)

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Maoridrilus transalpinus (YN4)

16S, GenBank accession number KP828823 (submitted 20 Feb 2015)

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COI, GenBank accession number KP771668 (submitted 12 Feb 2015)

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***Maoridrilus* sp.1 (PH01, 02)**

16S, GenBank accession number KP771674 (submitted 12 Feb 2015)

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COI, GenBank accession number KP771669 (submitted 12 Feb 2015)

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***Maoridrilus* sp.2 (LU01)**

16S, GenBank accession number KP771675 (submitted 12 Feb 2015)

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COI, GenBank accession number KP771670 (submitted 12 Feb 2015)

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***Deinodrilus* sp.1 (YN7_Red)**

16S, GenBank accession number KP771676 (submitted 12 Feb 2015)

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COI, GenBank accession number KP771671 (submitted 12 Feb 2015)

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***Megascolecidae* sp.1 (YN7_White)**

16S, GenBank accession number KP771677 (submitted 12 Feb 2015)

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***Megascolecidae* sp.3 (End9_Grey)**

16S, GenBank accession number KP771678 (submitted 12 Feb 2015)

GCTAGAATGAACGGATAAACGAAATAAAAACCTGTCTCAATCAACAAACCTAAAAATTAATATCTACATGAAGA
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COI, GenBank accession number KP771673 (submitted 12 Feb 2015)

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GAGCTATTACTATACTTCTAACAGATCGAAACTTAATACCTCCTTTTTCGACCCAGCCGGGGGGGGGAGA

***Megascolex laingii* (YN7_Gold)**

16S was already submitted by Buckley et al. (2011)

COI, GenBank accession number KP828824 (submitted 20 Feb 2015)

GGTGCTGGCATAAGACTTCTTATTTCGAATTGAATTAAGACAACCTGGAGCATTCTAGGAAGAGATCAACTATA
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Appendix B

Poster presentations

- Biogeochemical role of native and exotic earthworms in New Zealand soil (2014). The 20th World Congress of Soil Science (20WCSS). Jeju, Korea.



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Introduction

Earthworms are important ecosystem engineers and useful indicators of soil health and quality, stimulating mineralization of nitrogen and phosphorus in soil and plant growth and development.

Megascolecid earthworms are well represented in the endemic fauna of New Zealand with more than 196 species, but they have disappeared from farmed paddocks which are being colonised by exotic European Lumbricid earthworms.

Aims: To identify how native and introduced earthworms influence soil properties including nutritional status and relationship with native plant rhizospheres.

Materials and Methods

A. Earthworms

- Collected by digging from native forest and agricultural land.
- Species ID using DNA barcoding with COI & 16S rDNA methods.
- **Natives:** *Octochaetus multiporus*, *Deinodrilus* sp., *Maoridrilus transalpinus*, + two unnamed species.
- **Exotics:** *Octolasion cyaneum*, *O. lacteum*, & *Aporrectodea caliginosa*.

B. Incubation experiment

- 250 g soil (30% moisture) + 2g sawdust as food source.
- Incubated at 15°C, 3 weeks, 4 replicates.

C. Plant-soil-earthworm mesocosms

- Native plants: mānuka (*Leptospermum scoparium*), kānuka (*Kunzea ericoides*) + ryegrass (*Lolium perenne*)
- 7 weeks, 1.5 L pots of dairy farm soil in glasshouse trial.

D. Analyses

- Soil pH, EC, NH₄⁺, NO₃⁻, Olsen P, CEC, exchangeable cations.
- N₂O and CO₂ gas emissions
- Statistics: 1-way ANOVA with Fisher's LSD (p<0.05).

Results

Table 1. Changed soil properties by earthworm species in incubation experiments. Values are means (± standard error) (n=4). Same superscripts indicate that there is no significantly different (p<0.05).

	Control	<i>O. multiporus</i>	<i>Deinodrilus</i> sp.	<i>M. transalpinus</i>	<i>A. caliginosa</i>	<i>O. lacteum</i>	<i>O. cyaneum</i>
pH (1.5M)	4.9 (0.00) ^a	4.8 (0.01) ^b	4.7 (0.01) ^c	4.6 (0.01) ^d	4.7 (0.00) ^a	4.7 (0.00) ^f	4.7 (-) ^f
EC (dS-m ⁻²)	0.16 (0.003) ^a	0.21 (0.004) ^a	0.19 (0.003) ^b	0.21 (0.008) ^a	0.20 (0.006) ^{ab}	0.19 (0.002) ^b	0.21 (-) ^{ab}
NH ₄ ⁺ (µg/g)	2.2 (0.04) ^a	26.8 (2.00) ^a	9.4 (0.57) ^c	18.2 (2.60) ^b	17.1 (4.34) ^b	8.7 (0.48) ^f	26.7 (-) ^{ab}
NO ₃ ⁻ (µg/g)	69.1 (0.87) ^a	91.2 (1.86) ^a	81.0 (0.87) ^{ab}	90.6 (2.18) ^a	84.8 (1.86) ^b	78.9 (0.95) ^f	80.9 (-) ^{ab}
Olsen P (µg/g)	34.1 (0.10) ^a	35.2 (0.07) ^b	34.7 (0.13) ^{ab}	34.7 (0.24) ^a	35.8 (0.13) ^{ab}	34.8 (0.14) ^{ab}	34.5 (-) ^{ab}

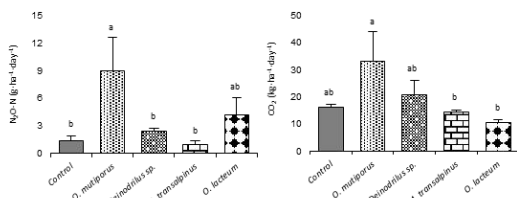


Fig. 1. N₂O and CO₂ emissions from incubation experiment with earthworms. Means ± standard errors (n=3). Same letters indicate no significant difference (p<0.05).

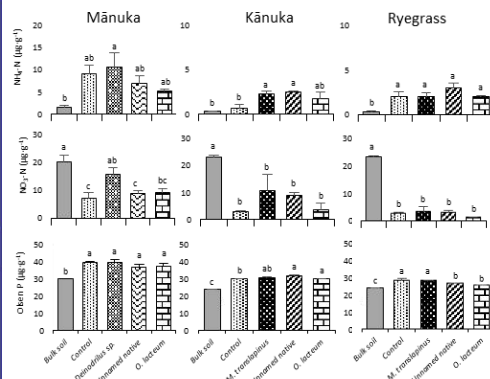


Fig. 2. NH₄-N and NO₃-N in rhizosphere soils of two native (mānuka and kānuka) and ryegrass, with presence of native and exotic earthworms. Bar value are means ± standard errors (n=4). Same letters indicate no significant difference (p<0.05).

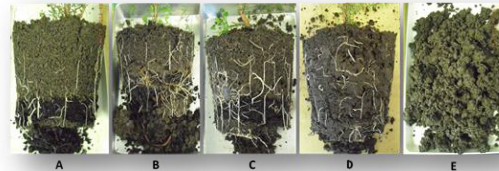


Fig. 3. Rhizospheres of mānuka with different earthworm species. A (control), B (*Deinodrilus* sp.), C (unnamed native), D (*O. lacteum*). E shows surface earthworm casts.

- Pronounced impact on N (but not P) mineralization
- Significant enhancement of CEC, exchangeable-Ca and exchangeable-S compared to bulk soil (p<0.05).
- Exchangeable fractions of Cu and Ni significantly reduced by earthworm activity (p<0.05).
- Significant different root system morphologies were developed by plants growing with different species of earthworms.
- Both native and exotic earthworms improved the growth of native plants and ryegrass.

Conclusions

- ✓ Earlier disappearance of native earthworms was caused by intolerance to disturbance.
- ✓ Modern agricultural systems using reduced tillage may allow better survival of native earthworms.
- ✓ Incorporating native earthworm communities into agricultural landscapes and other human-modified soils may have functional and biodiversity benefits.



- Endemic earthworms and biogeochemical impacts on New Zealand soils (2015). New Zealand Ecological Society Conference in Canterbury, New Zealand.

Endemic earthworms and their biogeochemical impacts in New Zealand soil ecosystem

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Introduction

- About 207 earthworms of Megascolecidae species in New Zealand.
- Native species coexist with exotic species in remnants of native vegetation on agricultural farms.
- Due to intensive farming system, incipient spillover of nutrients into adjacent fragments of native vegetation disturbs sustainability of endemic earthworms.
- Earthworms mediate changes to soil properties, including N and P mineralization.
- They also increase plant production and stimulate microbial activity.

The aim of the present study was to identify how native and exotic earthworms modify the biogeochemistry of agricultural soils and rhizosphere ecosystem.

Materials and Methods

Table 1. Earthworm species used in this experiment, status and sampling location. Native species were named based on morphological and genetic identification following Lee (1959) and Kim et al. (2015).

Species	Functional group	Native status	Sampling location	Field density (in 20 x 20 x 20 cm ³)
<i>Deinodrilus</i> sp.1	Epigeic	Endemic	42° 8'38.39"S / 171°19'50.36"E	2 to 5
<i>Maoridrilus transalpinus</i>	Anecic	Endemic	43°39'58.97"S / 172°37'26.37"E	≥ 5
<i>Maoridrilus</i> sp.2	Anecic	Endemic	43°38'37.19"S / 172°27'43.77"E	≥ 5
<i>Octochaetusa multiporus</i>	Endogeic	Endemic	43°38'18.64"S / 172°29'6.93"E	2 to 5
<i>Aporrectodea caliginosa</i>	Endogeic	Exotic	43°38'55.05"S / 172°28'4.72"E	≥ 5
<i>Octolasion lacteum</i>	Endogeic	Exotic	42° 8'10.99"S / 171°19'46.68"E	≥ 5

- Earthworm sampling:
 - Digging and hand-sorting (see Table 1). *Eisenia fetida* was purchased.
- Incubation experiment:
 - 250 g immature Pallic soil (30 % moisture) + 2 g sawdust as food source
 - Incubated at 15 °C, 21 days, 4 replicates.
- Plant-rhizobia-earthworm mesocosms:
 - Native plants; Kowhai (*Sophora microphylla*)
 - Rhizobia strain additive: ICMP 19535
 - 5 L pots of dairy farm soil, 8 weeks at Lincoln University glasshouse.
- Molecular identification:
 - DNA barcoding with 16S rDNA and COI regions (Boyer et al., 2011).
- Soil analysis: soil pH, EC, NH₄-N & NO₃-N, Olsen P, trace elements, microbial biomass C.
- N₂O and CO₂ gas emissions.
- Plant analysis: Dry weight (root, shoot) and nodule counting.

Results

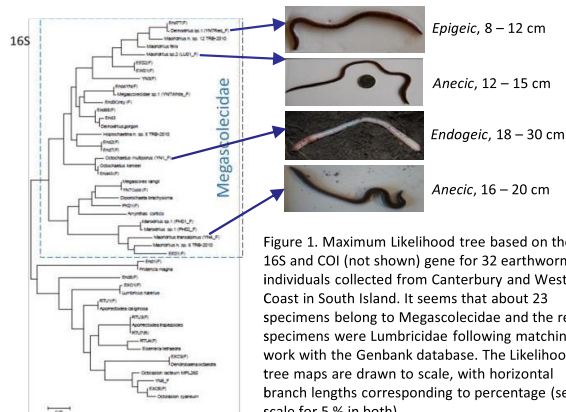


Figure 1. Maximum Likelihood tree based on the 16S and COI (not shown) gene for 32 earthworm individuals collected from Canterbury and West Coast in South Island. It seems that about 23 specimens belong to Megascolecidae and the rest specimens were Lumbricidae following matching work with the Genbank database. The Likelihood tree maps are drawn to scale, with horizontal branch lengths corresponding to percentage (see scale for 5 % in both).

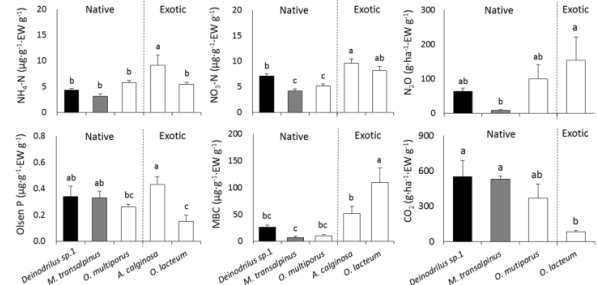


Figure 2. Enhancement of ammonium, nitrate, Olsen P, Microbial Biomass C, and GHGs in soil in presence of native and exotic species. Results are calculated per unit earthworm mass, as change over 21 days. Earthworms mass was measured at the end of the experiment (mean mass without voided gut). Different shading indicates different feeding group species; epigeic (■), anecic (■), endogeic (□).

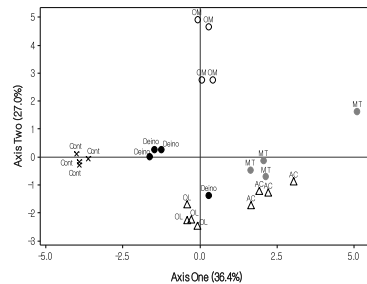


Figure 3. Principal Component Analysis of soil properties after 3 weeks incubation. Symbols represent individual earthworms (n=4) of native species (□, □, ○), exotic species (△, △, ○), and control soil (×). Native species are *Deinodrilus* sp.1 (Deino), *M. transalpinus* (MT) and *O. multiporus* (OM). Exotic species are *A. caliginosa* (AC) and *O. lacteum* (OL).

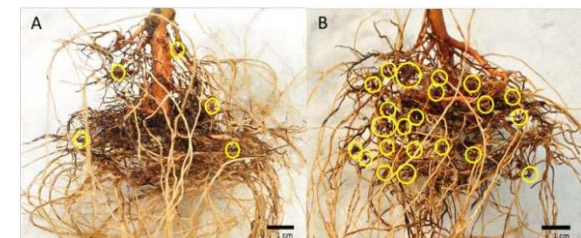


Figure 4. Effects of *M. transalpinus* activity on nodulation following the rhizobia strain addition after 8 weeks. (A) is control in absence of *M. transalpinus* and (B) is in presence of the species. Nodules were marked with yellow circles.

- More than 10 Megascolecidae species were newly identified.
- Pronounced impacts on mineralization of N and P and microbial activity.
- *M. transalpinus*, anecic species, were separated by measures of soil pH and its determinant elements (S, Al, and Ca), EC and labile P.
- Significantly positive influences on nodulation, particularly by *M. transalpinus*.

Conclusion

- Both native and exotic earthworms significantly modify and potentially ameliorate soil biogeochemistry, although deeper burrowing species (particularly, *O. multiporus*) may enhance N₂O emissions.
- Individual species could be separated on the basis of their modification of soil biogeochemistry.
- Identifying the role of native earthworms in the delivery of ecosystems goods and services is required for an integrated approach to soil management in agricultural landscape mosaics.

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