

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 Megascolecid 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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1. Introduction

Due to a long period of geographic and evolutionary isolation, and the former absence of mammals, 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 colonization and introduction of mammalian pests to these islands has been relatively recent, but native biodiversity has been impacted particularly severely (Lee, 1961; Sparling and Schipper, 2002; MacLeod and Moller, 2006). Agricultural modification of landscapes, vegetation and soils has certainly been to the detriment of native earthworms (Lee, 1959a; Molloy, 1988; Bowie et al., 2016).

Megascolecid earthworms are naturally well represented in the endemic fauna of New Zealand, with 177 recognized species (Lee, 1959a; Sims and Gerard, 1985; Lee et al., 2000; Glasby et al., 2009) 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 colonized intentionally or unintentionally by introduced exotic European Lumbricidae, predominantly *Aporrectodea caliginosa*, *Aporrectodea longa*, *Aporrectodea rosea*, *Aporrectodea trapezoides*, *Lumbricus rubellus* and *Octolasion cyaneum* (Lee, 1961; Springett et al., 1992; Fraser et al., 1996; Springett et al., 1998). Endemic earthworms have found refuge beneath small remnants of native vegetation on the borders of agricultural land, which account for less than 0.5% 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 recognized that ground disturbance through burning, vegetation clearance and ploughing played a major role in the demise of native

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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 (Lee, 1985; Edwards, 2004; Al-Maliki and Scullion, 2013). 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 (Kerneck 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.

2. Materials and methods

2.1. Soils

Surface soils (0–15 cm) were collected from two Lincoln University farms situated close to the Lincoln University campus (Table 1). One is an intensively-managed, irrigated and fertilized dairy farm (referred to as DF) soil, well represented on intermediate terraces in Canterbury (Molloy, 1998). 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.

2.2. 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 *Octochaetis 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 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., 2011).

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).

2.3. Choice-chamber experiments










Simple choice chamber pieces of apparatus were constructed (Fig. 1) to investigate whether earthworms had clear preferences for the different soils in a series of separate assays. 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

Table 1
Location and description of the four soils collected for experimental work. Distance is related to 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. |

Table 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 known 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 behavior.

| 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 | Bank Peninsula, Lincoln township | Brown with incompletely dark clitellum | 160–200 | Tanylobous |  |
| | <i>Maroridrilus</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> | " | Bank 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.

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), 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

Table 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>Maroridrilus</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 | ✓ | ✓ | | | | |



Fig. 1. Choice chamber apparatus for earthworm preference tests. Earthworms placed in moist conditions (< 5 ml water) in the central chamber could move freely between adjacent chambers containing four different soils. Multiple sets of apparatus were used to run each trial at the same time, with 5 earthworms per choice chamber apparatus. Earthworms shown is *Deinodrilus* sp.1.

from each of the replicates, were then analyzed for pH, EC and mobile fractions of N as described below.

2.4. 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). 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 randomized 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 analyzed as described below.

2.5. 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). Each treatment contained two individual earthworms, with 5 replicate pots [2 plant species × 5 earthworms treatments × 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.

2.6. Analytical

All soils were analyzed 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 2 M KCl using fresh soil,

samples were analyzed for available-N using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden), attached to a spectrophotometer (Blakemore et al., 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 analyzed by a Vario-Max CN elemental analyser (Elementar GmbH, Germany). Oven-dried (100 °C) soil samples were analyzed for loss on ignition (LOI) at 550 °C in a muffle furnace. Following microwave digestion of oven-dried soil, Total-P was analyzed using ICP-OES (Varian 720 ES, USA). Available-P was determined as Olsen P, using 0.5 M NaHCO₃ extractant and a UV160A spectrophotometer (Shimadzu, Japan) (Blakemore et al., 1987). Soils from the choice chamber and incubation experiments were analyzed 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 400 ml 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 analyzed using a gas chromatograph (SRI 8610 GC, CA, USA) with a ⁶³Ni electron capture detector and flame ionization detector, linked to an autosampler (Gilson 222 XL, USA). All methods follow those described by Clough et al. (2006).

2.7. Statistical analysis

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

3. Results and discussion

3.1. 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 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 SP, 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 fertilization is evident in the plantation forest soil.

Table 4

Properties of the four soils 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) |
|---|-----------------|-----------------|------------------------|--------------------|
| Texture | Silt loam | Silt loam | Silt loam | Silt loam |
| pH (1:5W) | 5.6 (0.0) | 5.4 (0.0) | 5.0 (0.0) | 4.7 (0.2) † |
| OM (%) | 7.3 (0.2) | 7.5 (0.1) | 4.3 (0.1) | 22.6 (0.2) |
| Total C (%) | 3.3 (0.4) ‡ | 3.3 (0.1) | 2.4 (0.1) | 10.7 (3.4) † |
| Total N (%) | 0.2 (0.0) ‡ | 0.3 (0.0) | 0.1 (0.0) | 0.7 (0.2) † |
| C/N Ratio | 15.0 (0.0) ‡ | 12.2 (0.3) | 17.2 (2.0) | 16.9 (0.9) † |
| NO ₃ -N (mg·kg ⁻¹) | 1.8 (0.5) | 2.9 (0.5) | 0.2 (0.2) | 19.6 (1.4) |
| NO ₃ -N (mg·kg ⁻¹) | 118 (12) | 88.1 (0.6) | < 0.1 | 18.3 (0.5) |
| 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).

3.2. 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 (Fig. 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 1). Earthworms in the early trials tended to lose weight; this varied between species, ranging from 0.2% fresh weight losses in *Deinodrilus* sp. 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 (Fig. 3). In these trials, fresh weight gains over one week were 2.1% (*M. transalpinus*), 12.1% (*Maoridrilus* sp.2), 2.6% (*O. cyaneum*), and 5.5% (*E. fetida*).

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 NO₃⁻-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 NH₄⁺. 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 NH₄⁺. The high NO₃-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.

3.3. 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 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

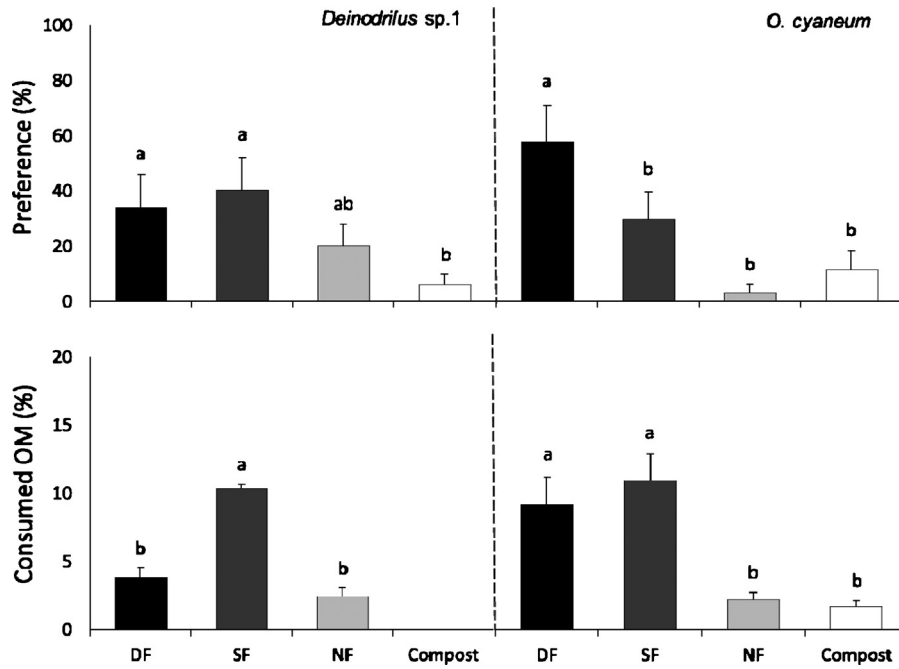


Fig. 2. Preferred soils of two species of earthworm added to the choice chambers in Trial I, after one week. Consumption of organic matter refers to LOI changes after 7 days. Shading distinguishes DF (■), SF (▒), NF (░), and compost (□). Values are means ± standard errors (n = 5). The same letters indicate no significant different (p < 0.05).

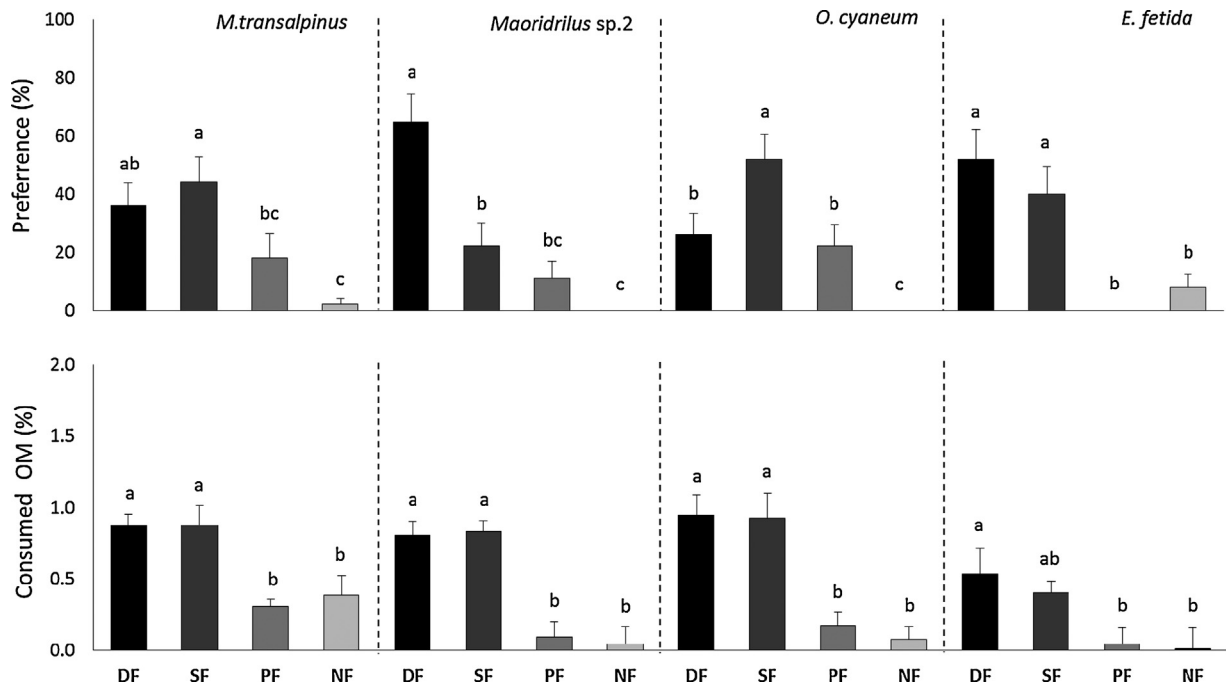


Fig. 3. Soil preferences in choice chamber Trial II. Consumption of organic matter refers to LOI changes after 7 days. Shading distinguishes DF (■), SF (▒), PF (░), and DF (□). Values are means ± standard errors (n = 10). The same letters indicate no significant different (p < 0.05).

Table 5
Changes of soil properties by all species of earthworm throughout incubation for 3 weeks in DF soil. Value in blankets represent standard errors of the mean (n = 4). The same letters indicate no significant difference (p < 0.05).

| Properties | Control | Native | | | Exotic | |
|-------------------------------|--------------------------|---------------------------|--------------------------------|-------------------------------|-------------------------------|---------------------------|
| | | <i>Deinodrilus sp.1</i> | <i>Maoridrilus translpinus</i> | <i>Octochaetus multiporus</i> | <i>Aporrectodea calginosa</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 |

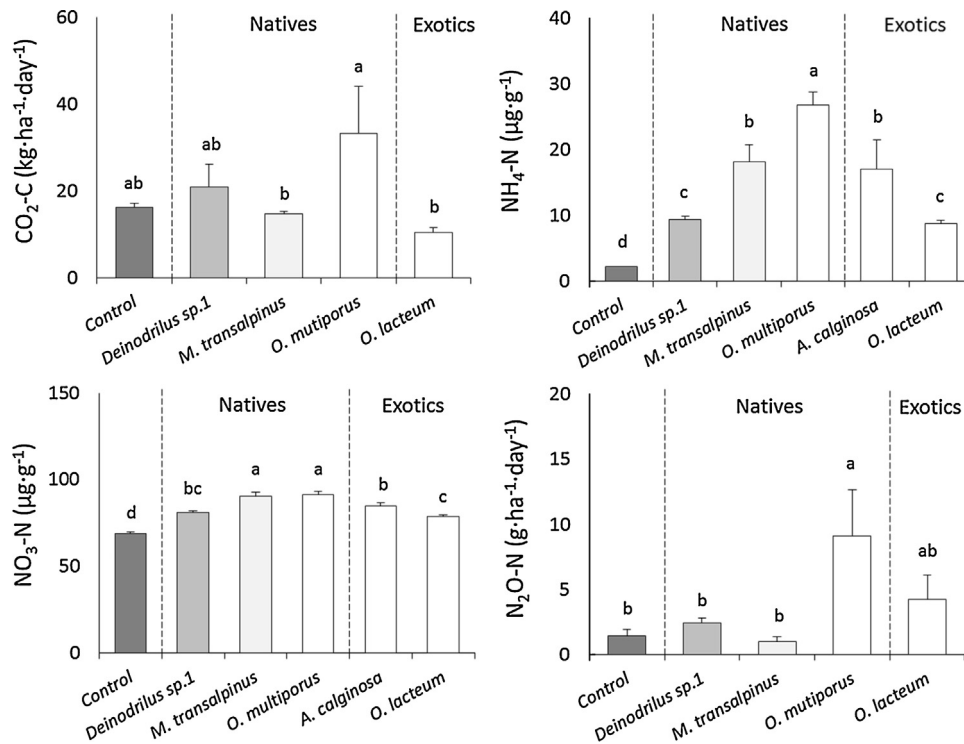


Fig. 4. Mobile nitrogen (ammonium and nitrate) and release of N₂O (nitrous oxide) and CO₂ (carbon dioxide) in the presence of native and exotic earthworms during 3 weeks inoculation. Shading distinguishes control (■), epigeic (■), anecic (■), and endogeic (□) species. Values are means ± standard errors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

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 (Schrader, 1994; Edwards and Bohlen, 1996; Cole et al., 2006).

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 mineralization 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 NH₄⁺ from organic N in the earthworm incubated soils (Fig. 4). This appears to have a knock-on effect causing marginal increases of soluble NO₃-N associated with earthworm activity.

Release of substantially more CO₂ (by 33%) and N₂O (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., 2013), although production of N₂O from denitrification requires anaerobic conditions. Of course,

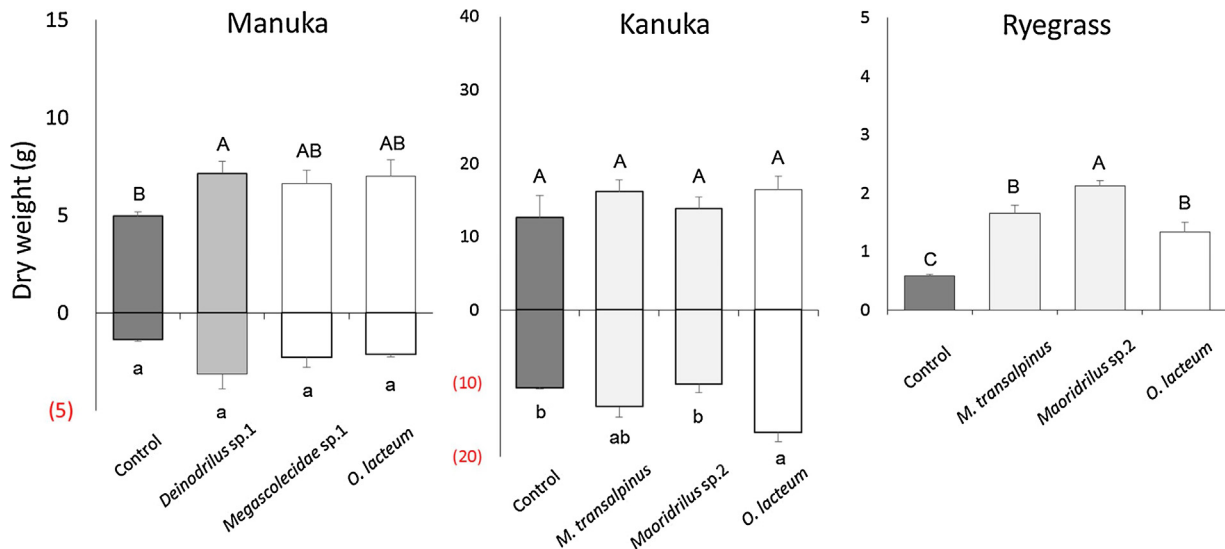


Fig. 5. Biomass of two native plants (*L. scoparium* and *K. robusta*) and *L. perenne* in the presence of earthworms in DF soil, 7 weeks after earthworms were added. Upper bar is shoot dry weight and lower bar is root dry weight. Shading distinguishes control (■), epigeic (■), anecic (■), and endogeic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

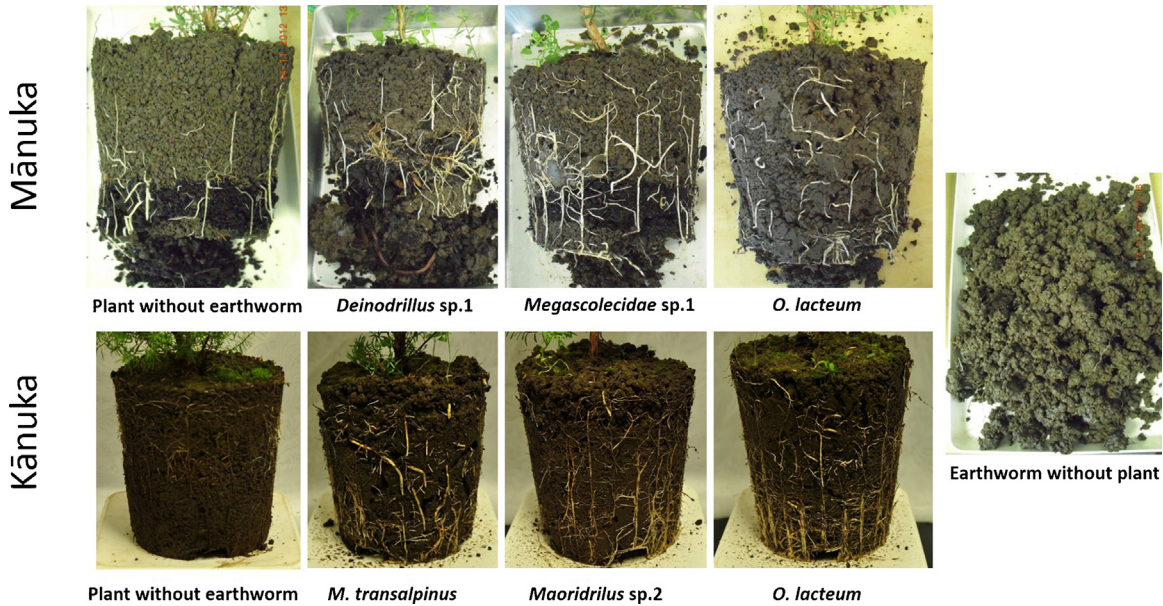


Fig. 6. Rhizosphere structures in SF soil after 7 weeks growth with earthworms. Soil structure is modified by a combination of roots and earthworms. Rooting patterns differ with earthworm species, and compost from the original rooting plugs is variously dispersed through the plant pots.

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 $g\ ha^{-1}\ day^{-1}$ and represent perhaps 1% N_2O emissions reported elsewhere in non-fertilized soils (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 CO_2 and about 75% of N_2O is emitted directly or indirectly from soils (Thorburn et al., 2013). 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 NO_3^- and gaseous N_2O . It is argued that a shift is required towards NH_4^+ -dominated, low-nitrifying agricultural production systems. Enhancing the release of biological

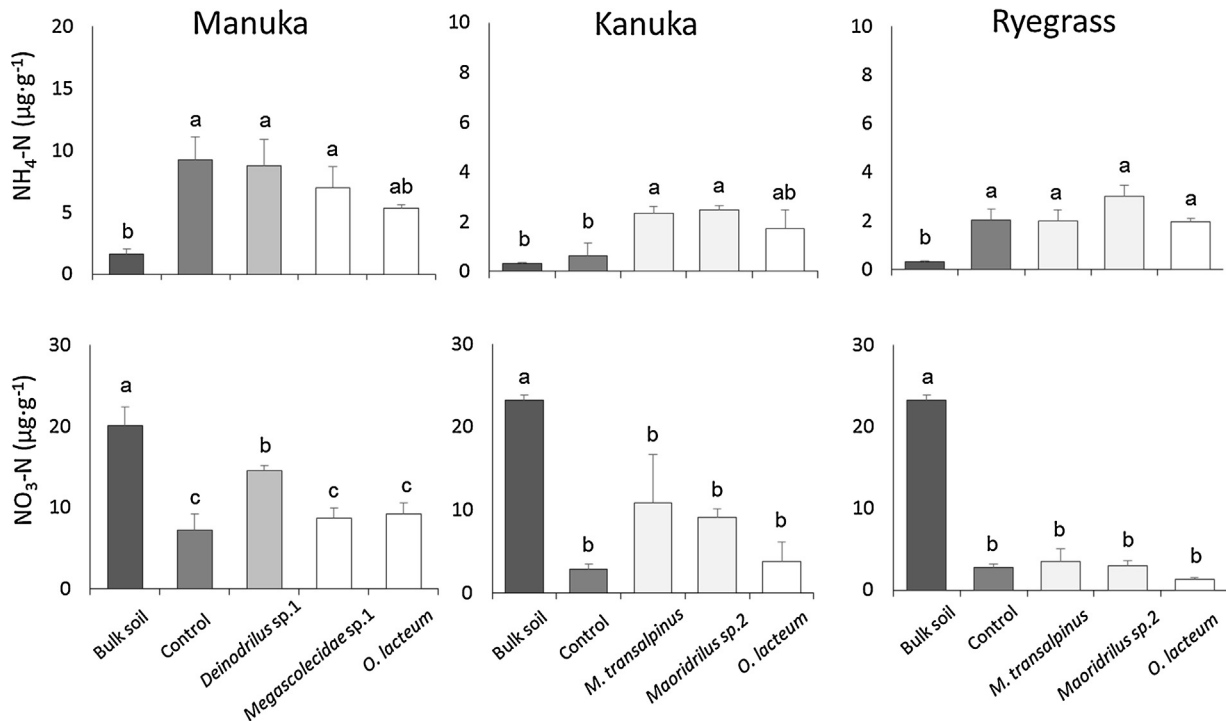


Fig. 7. Mobile nitrogen concentration (NH_4-N and NO_3-N) in the rhizosphere SF soil of Mānuka, Kānuka and ryegrass, with native and exotic earthworms. Shading distinguishes bulk soil (■), control (□), epigeic (▒), anecic (▓), and endogeic (□) species. Values are means \pm standard errors ($n = 5$). The same letter indicate no significant difference ($p < 0.05$).

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.

3.4. Plant–soil–earthworm interactions

All tested species of earthworm increased the growth of ryegrass, and *Deinodrilus* significantly increased the above-ground growth of mānuka (Fig. 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 (Fig. 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^+ (Fig. 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 the interactions between soils, root systems, earthworms and soil chemistry, and their impact on the soil ecosystems of agricultural landscapes in New Zealand.

4. Conclusions

This study provides a first step towards managing 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 and feeding 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 physico-chemistry 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 physico-chemical 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. Their borrowing 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 colonized 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.

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