



## Agroecology niche for New Zealand's native earthworms

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### ABSTRACT

Exotic lumbricid earthworms have had some limited success colonising productive agricultural pastures in New Zealand, in place of native megascolecid species that did not adapt to the conversion from native vegetation cover. Native earthworms in lowland intensively-farmed landscapes are now almost entirely restricted to small fragments of native vegetation on marginal land where they coexist with adventive lumbricids. In recent years, regular pasture replacement using cultivation has been largely replaced by herbicide spray-out followed by direct drilling; much less soil disturbance potentially creates a new opportunity for native earthworms to colonise pastures. Reclaiming native earthworm habitat is critical not only to protecting diversity, but also to sustaining New Zealand's unique soil ecosystem services strongly associated to role of native species; however, there have been no studies related to this. Using a mesocosm experiment, we investigated how native and exotic earthworms modify physicochemical properties of a sheep-grazed pasture soil. The pasture soil was found to be equally suitable for the growth and survival of native and exotic species. Most burrowing earthworms increased the more soluble forms of macronutrients (N, P, S, K and Mg), but differences between native and exotic species were found to be larger than between functional groups. Two species of native earthworms, *Octochaetus multiporus* and *Maoridrilus transalpinus*, modified some soil properties in similar way to exotic species, but had significantly different impacts on pH, mineralisation of nutrients, and plant availability of trace elements. Compared to control, plant available N (PAN) and S concentrations were higher by more than 100% and 40%, respectively, in soils with presence of burrowing activity of the two native earthworms. Future co-existence of communities of native and exotic earthworms in agricultural pastures appears realistic, and this would benefit conservation of native species as well as dairy production. Further research may also reveal additional and unique benefits to soil quality that could be attributable to this formerly unavailable niche for native earthworms.

### 1. Introduction

Earthworms are typical soil engineers as they have a large effect on soil functions, which can mediate soil structure, organic matter dynamic, nutrient cycling and biological interactions of belowground ecosystem (Blouin et al., 2013). Thus, there is no doubt that earthworms contribute to the sustainability of agroecosystem, but significant paucity of knowledge of New Zealand native earthworms still exists in this context. This appears to be problematic in terms of biodiversity

conservation in this global biodiversity hotspot. The history of New Zealand earthworm study was started by Lee (1961), who described the distribution and interaction of native and exotic earthworms and their impacts on soil and vegetation properties. However, since then, many researchers have focused more on exotic lumbricid earthworms, well-colonised in New Zealand agricultural land (e.g. McColl et al., 1982; Wüst et al., 2009; Schon et al., 2021). Meanwhile, only seven previous research papers have been published on the functionality of New Zealand's native earthworms, with only one or two species (Springett et al.,

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1998; Wüst et al., 2009; Waterhouse et al., 2014; Kim et al., 2015, 2017b, 2017c; Zhong et al., 2017). Currently, we have far too little understanding of the function of native earthworms in soil to identify and utilise their potential benefits as soil engineers and to soil ecosystem services. Therefore, comparative studies on the effects of more diverse species and ecological groups are needed to further broaden our insights.

Since the settlement of Europeans in 1840, ground disturbances accompanying changes in land use (deforestation and intensive farming practices) decimated native megascolecid communities (Kim et al., 2015; Boyer et al., 2016), as also happened elsewhere (Edwards and Bohlen, 1996; Hendrix, 2006). The primarily agricultural province of Canterbury in South Island alone has 25 recognised species of native earthworms (Winterbourne et al., 2008). However, native earthworms are almost entirely absent from New Zealand's agricultural pastures, with the probable exception of only a single deep-burrowing endogeic species, *Octochaetus multiporus* (Springett et al., 1998). Fraser et al. (1996) recorded a complete absence of native earthworms in a comprehensive study of agricultural pastures. European lumbricids have been introduced to improve agricultural pasture. While some effects have been limited possibly by dry New Zealand summers, areas where large populations have established have experience improved quality in agricultural soils (Lee, 1961; Springett, 1992). In contrast, in the same landscapes, native earthworms have found some refuge on less-disturbed land, in nature reserves, riparian zone and along tree/shrub fenceline boundaries of agriculture land (Fig. 1) (Kim et al., 2015, 2017a; Bowie et al., 2016). Coexistence of native and exotic species is evident in these marginal habitats (Boyer et al., 2016).

Recent large-scale landscape conversion to irrigated dairy farming and more intensive production systems has profoundly affected the status of soil biogeochemistry (McLennan et al., 2014). This has also impacted biodiversity conservation; for example, nutrient spillover from farmland has been shown to degrade adjacent native vegetation (Didham et al., 2015). In our earlier study on earthworms in these fragments we were surprised to find native species actually preferred the physicochemical condition of intensively dairy-farmed soils to native forest soils (Kim et al., 2015). We suggested that interactions between soils, earthworms, and native plant rhizospheres are likely to be particularly crucial in vegetation remnants that represent novel native ecosystems (Bowie et al., 2016; Kim, 2016). Since native earthworms and native vegetation are generally found together, a better understanding of the structure and functionality of this relationship within the soil ecosystem is clearly important to inform ecological restoration practise. Questions also remain as to whether burrowing activity of native earthworms in

paddock soil may be of benefit to the physicochemistry and agroecosystem services. Could agricultural pastures provide a significant and beneficial habitat for native species?

The aims of the present study were to identify how native and exotic species of earthworms affect the properties of a low fertility agricultural soil prior to farm intensification, and to investigate whether native species adapt and leverage the soil properties for their survivorship. Our hypothesis is that exotic lumbricid earthworms are better-suited to agricultural land and will burrow more actively, providing a large effect on soil biogeochemical conditions. This was expected to contrast with native megascolecid earthworms that are naturally found in more acidic native soils with low to moderate fertility.

## 2. Materials and methods

### 2.1. Soil and earthworm collection

Topsoil (about 100 kg, 0–15 cm depth) was collected from a sheep-grazed paddock situated near the Lincoln University campus (Gam-mack Estate: 43°38'39.48"S, 172°23'28.07"E). This stony Eyre soil has free drainage with low water storage capacity, although the surface horizon of the soil sampled under the turf was mostly free of stones. The mixed sward consisted mainly of perennial ryegrass (*Lolium perenne*), clovers (*Trifolium* spp.), and cocksfoot (*Dactylis glomerata*) as well as several invasive weeds, quite typical of extensively-managed lowland sheep pasture. This agricultural soil was selected for the experimental work because its fertility was typically low. Soil properties were: pH 5.4, organic matter (OM) content – 75 g kg<sup>-1</sup>, moisture – 25%, total C – 33 g kg<sup>-1</sup>, total N – 3 g kg<sup>-1</sup>, and total P – 341 mg kg<sup>-1</sup> (Kim et al., 2015). The pasture has had some degree of ploughing, top-dressing and re-seeding since the mid-19th century, but no recent history of intensive land management or fertilisation.

Soil was thoroughly mixed and sieved with a 4 mm sieve prior to a microcosm study. As we expected, introduced species of earthworms including *Lumbricus rubellus*, *Aporrectodea caliginosa* and *Octolasion cyaneum*, but not native earthworms, were recorded while sampling in the sheep-farmed pastures of the present study. Earthworms were collected from sites with native and restored vegetation in the East and West Coasts of South Island (Table 1) by digging (20 × 20 × 20 cm) and hand-sorting; more than 100 individuals of each earthworm species were collected to use in this study. Native species were classified based on both morphological (Lee, 1959a, 1959b) and molecular methods, the latter using DNA barcoding of 16S rDNA regions (Kim et al., 2017a). Epigeic, anecic and endogeic behavioural groups were represented by



Fig. 1. Examples of marginal vegetation that provides a refuge for native earthworms within livestock-farmed landscape matrices in lowland New Zealand: Ahuriri scenic reserve (–43°39'58.97"S/172°37'26.37"E, left) and Lincoln University commercial dairy farm (–43°64'39.09"S/172°43'38.29"E, right).

**Table 1**

Earthworm species used in this experiment. Native species were named based on morphological and genetic identification following Lee (1959a, 1959b) and Kim et al. (2017a).

Species	Functional group	Origin	Sampling location	Field density (individuals m <sup>-2</sup> )
<i>Deinodrilus</i> sp.1	Epigeic	Native	Punakaiki Nikau Reserve (-42°8'38.39"S/ 171°19'50.36"E)	50 to 125
<i>Maoridrilus transalpinus</i>	Anecic	Native	Ahuriri Reserve (-43°39'58.97"S/ 172°37'26.37"E)	≥125
<i>Octochaetus multiporus</i>	Endogeic	Native	Southern Summit Roadside (-43°44'15.41"S/ 172°54'32.64"E)	50 to 125
<i>Aporrectodea caliginosa</i>	Endogeic	Exotic	Lincoln University Farm Field (-43°38'55.05"S/ 172°28'4.72"E)	≥125
<i>Octolasion lacteum</i>	Endogeic	Exotic	Punakaiki Restored Land (-42°8'10.99"S/ 171°19'46.68"E)	≥125

three native species (Table 1), previously described and recorded in South Island (Lee, 1959a, 1959b; Wüst et al., 2009); further details of the species are provided in earlier papers (Kim et al., 2015; Kim et al., 2017a). Two exotic lumbricid earthworms, *A. caliginosa* and *O. lacteum* were collected respectively on Lincoln University farmland (East Coast) and in a natural forest at Punakaiki on the West Coast. These two endogeics are commonly found on agricultural land, among about 19 species of exotic earthworms in New Zealand (Lee, 1959a; Springett, 1992).

## 2.2. Microcosm study

Experimental work was conducted after an acclimatisation period for the earthworms of at least one month in field-collected soils in the laboratory, to provide confidence of survivorship and maintenance of body mass. To investigate the effect of earthworms on soil properties, three native species (*Deinodrilus* sp.1, *M. transalpinus*, and *O. multiporus*) and two exotic species (*A. caliginosa* and *O. lacteum*) were placed in 400 mL polypropylene containers containing 250 g wetted soil (30% moisture). Two individuals of the same species were added into each container covered with a gauze lid to prevent the earthworm escaping. The soil moisture in each container was maintained the same as at the beginning level through water supply on a weekly basis by weighing. About 2 g of sawdust, equivalent to an increase of 1.1% of soil organic matter, was added in each container as a food source at the beginning of incubation; this limited resource was intended to avoid a large nutrient pulse to the soil. The microcosm was maintained in a dark incubator room at 15 °C for 3 weeks. The experiment comprised four replicates for each earthworm species and additional four reference containers without earthworms. Cultures were checked for mortality of earthworms on a weekly basis with minimal disturbance. Visual observation of the soil surface and a lack of smell of decay proved to be sufficient for this purpose.

## 2.3. Analytical

After earthworm collection, fresh soil was sampled and kept at 4 °C before soil analysis. The samples were extracted with 2 M KCl solution to determine PAN, which is the sum of NH<sub>4</sub>-N and NO<sub>3</sub>-N, using a FIA star 5000 triple channel analyser (Foss Tecator AB, Sweden) (Blakemore et al., 1987). Following air-drying and sieving (<2 mm), soil pH was

measured using a pH meter (Mettler Toledo SevenEasy, USA). Soil organic matter content was determined with Loss on Ignition (LOI) method at 500 °C for 5 h (Blakemore et al., 1987). Subtracting LOI at the end of the incubation from LOI at the beginning allowed to estimate the amount of OM consumed by earthworms. Cation Exchange Capacity (CEC) and exchangeable Ca were determined with the silver thio-urea method (Blakemore et al., 1987). Following extraction with 0.05 M Ca (NO<sub>3</sub>)<sub>2</sub> the concentration of soluble elements were measured by ICP-OES (Varian 702-ES, Australia) (Simmler et al., 2013).

## 2.4. Statistical analyses

Differences among earthworm species were assessed for earthworm mortality and biomass change as well as soil chemical properties using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (LSD) post-hoc test for separation of means using agricolae R package (de Mendiburu, 2019). Omega squared ( $\omega^2$ ) from the ANOVA results was used to compare difference in effect size between behaviour groups and species origins for single soil factor. For soil pH, the detected values of pH were calculated by conversion to the equivalent H<sup>+</sup> concentrations and subsequent back calculation to pH. Principal component analysis (PCA) was performed to identify the major parameters of soil incubated with different earthworm species and the interrelations using factomineR (Husson et al., 2020) and factoextra (Kassambara and Mundt, 2020) packages. Relative site-to-site dissimilarities in ordination space were analysed on the basis of the first and second axis scores. All statistical analyses were performed using the R software (version 3.2.5).

## 3. Results

### 3.1. Growth and organic matter consumption

During 21 days of incubation, survival rate of most earthworms was successfully maintained. Visual observation based on burrowing and casting activities suggested native species were more active and healthier than exotic ones. However, individuals of all species lost body weight (Table 2). There was a large difference in the amount of OM consumed by the different species of earthworm; the greatest difference was observed by *M. transalpinus* that consumed 5 times more OM than *O. multiporus* ( $p < 0.05$ , Fig. 2). Less OM was consumed by other endogeic earthworms but, within this behavioural group, exotic species consumed relatively more OM. When looking at the amount of OM consumed per unit of biomass, the native *O. multiporus* consumed significantly less OM than other species ( $p < 0.05$ ) while the exotic *O. lacteum* consumed significantly more ( $p < 0.05$ ); thus, *O. lacteum* appeared to be the most active feeder for its size.

### 3.2. Alteration of soil properties

Burrowing and casting by earthworms had a large influence on the chemistry of the soils (Table 3). Initial soil pH of 5.4 was reduced to 4.8 after incubation, even without earthworms in the reference containers and dropped further in all earthworm treatments. Plant-available N

**Table 2**

Mortality and body weight variation of earthworms used in this study. Values in brackets represent standard error of the mean ( $n = 4$ ). Same letters within each row indicate no significant difference (LSD,  $p < 0.05$ ).

Species	Mortality (%)	Weight change (%)
<i>Deinodrilus</i> sp.1	0 <sup>b</sup>	-9 <sup>a</sup>
<i>Maoridrilus transalpinus</i>	0 <sup>b</sup>	-13 <sup>a</sup>
<i>Octochaetus multiporus</i>	17 <sup>ab</sup>	-7 <sup>a</sup>
<i>Aporrectodea caliginosa</i>	50 <sup>a</sup>	N/A <sup>†</sup>
<i>Octolasion lacteum</i>	0 <sup>b</sup>	-7 <sup>a</sup>

<sup>†</sup> Not applicable due to high mortality.



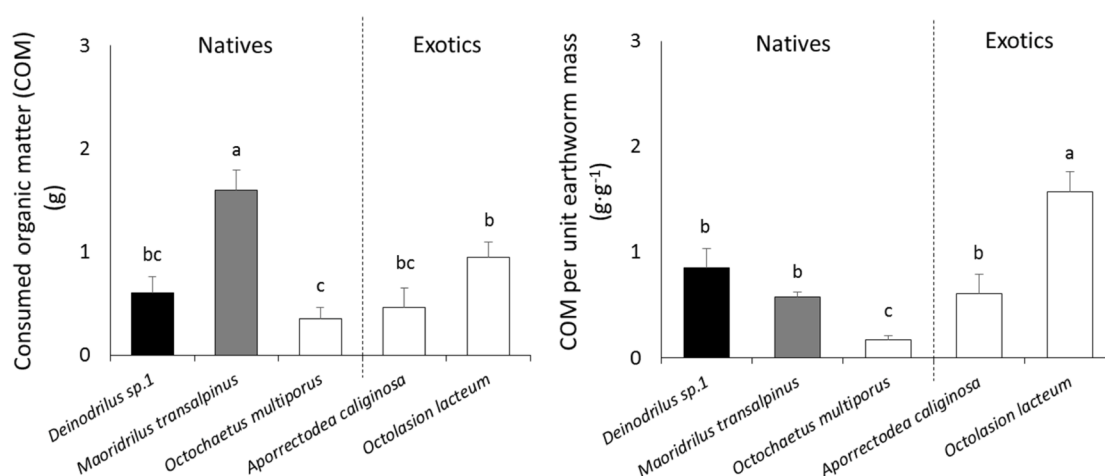


Fig. 2. The amount of organic matter consumed (COM) by native and exotic earthworms and their feeding efficiency (COM per unit mass) after 3 weeks incubation. Earthworms mass was measured at the end of the experiment (mean mass without voided gut). Bar values are means  $\pm$  standard error ( $n = 4$ ). Same letters indicate no significant difference among earthworm species (LSD,  $p < 0.05$ ). Different shading indicates different feeding group species; epigeic (black), anecic (grey), and endogeic (white).

Table 3

Chemical properties of soils after three weeks incubation with different earthworm species. Control values refer to analyses carried out following incubation under the same conditions without earthworms. Values in brackets represent standard error of the mean ( $n = 4$ ). Same letters within each row indicate no significant difference (LSD,  $p < 0.05$ ).

Soil parameter	Control (no earthworm)	Native			Exotic		
		<i>Deinodrilus sp.1</i>	<i>Maoridrilus transalpinus</i>	<i>Octochaetus multiporus</i>	<i>Aporectodea caliginosa</i>	<i>Octolasion lacteum</i>	
pH <sub>1:5w</sub>	–	4.82 (0.01) <sup>a</sup>	4.72 (0.01) <sup>c</sup>	4.68 (0.01) <sup>c</sup>	4.78 (0.01) <sup>b</sup>	4.70 (<0.01) <sup>c</sup>	4.71 (<0.01) <sup>d</sup>
CEC <sup>†</sup> (me 100 g <sup>-1</sup> )	7.84 (0.29) <sup>c</sup>	9.02 (0.24) <sup>ab</sup>	8.49 (0.24) <sup>bc</sup>	8.93 (0.17) <sup>ab</sup>	9.52 (0.37) <sup>a</sup>	8.64 (0.18) <sup>ab</sup>	8.64 (0.18) <sup>ab</sup>
PAN <sup>‡</sup> (mg kg <sup>-1</sup> )	53.4 (17.8) <sup>d</sup>	90.3 (1.4) <sup>c</sup>	108.8 (4.6) <sup>ab</sup>	118.0 (3.7) <sup>a</sup>	101.8 (4.1) <sup>b</sup>	87.6 (1.4) <sup>c</sup>	87.6 (1.4) <sup>c</sup>
Ca <sup>§</sup> (me 100 g <sup>-1</sup> )	5.37 (0.04) <sup>b</sup>	5.66 (0.31) <sup>b</sup>	6.72 (0.05) <sup>a</sup>	5.41 (0.12) <sup>b</sup>	6.60 (0.09) <sup>a</sup>	5.35 (0.08) <sup>b</sup>	5.35 (0.08) <sup>b</sup>
P <sup>¶</sup> (mg kg <sup>-1</sup> )	0.99 (0.01) <sup>d</sup>	1.03 (0.01) <sup>bcd</sup>	1.08 (0.02) <sup>a</sup>	1.01 (0.02) <sup>cd</sup>	1.05 (0.03) <sup>abc</sup>	1.08 (0.01) <sup>ab</sup>	1.08 (0.01) <sup>ab</sup>
K <sup>¶</sup> (mg kg <sup>-1</sup> )	169.59 (0.30) <sup>b</sup>	169.61 (2.04) <sup>b</sup>	169.05 (2.05) <sup>b</sup>	175.94 (1.68) <sup>a</sup>	162.10 (4.15) <sup>c</sup>	167.00 (0.55) <sup>bc</sup>	167.00 (0.55) <sup>bc</sup>
S <sup>¶</sup> (mg kg <sup>-1</sup> )	5.56 (0.11) <sup>d</sup>	6.86 (0.25) <sup>c</sup>	7.71 (0.51) <sup>ab</sup>	8.53 (0.25) <sup>a</sup>	8.00 (0.71) <sup>ab</sup>	7.05 (0.05) <sup>bc</sup>	7.05 (0.05) <sup>bc</sup>
Mg <sup>¶</sup> (mg kg <sup>-1</sup> )	225.04 (1.84) <sup>cd</sup>	228.20 (7.21) <sup>bc</sup>	246.35 (1.85) <sup>a</sup>	213.12 (2.95) <sup>d</sup>	241.08 (9.28) <sup>ab</sup>	251.17 (1.44) <sup>a</sup>	251.17 (1.44) <sup>a</sup>
Fe <sup>¶</sup> (mg kg <sup>-1</sup> )	2.06 (0.10) <sup>b</sup>	2.28 (0.09) <sup>ab</sup>	2.32 (0.18) <sup>ab</sup>	2.30 (0.08) <sup>ab</sup>	2.43 (0.09) <sup>a</sup>	2.21 (0.05) <sup>ab</sup>	2.21 (0.05) <sup>ab</sup>
Al <sup>¶</sup> (mg kg <sup>-1</sup> )	17.71 (0.62) <sup>d</sup>	19.34 (0.33) <sup>bc</sup>	21.19 (0.75) <sup>a</sup>	18.34 (0.53) <sup>cd</sup>	20.26 (0.33) <sup>ab</sup>	19.86 (0.07) <sup>ab</sup>	19.86 (0.07) <sup>ab</sup>
Mn <sup>¶</sup> (mg kg <sup>-1</sup> )	13.08 (0.29) <sup>b</sup>	13.16 (0.33) <sup>b</sup>	12.75 (0.21) <sup>b</sup>	13.43 (0.37) <sup>ab</sup>	13.03 (0.71) <sup>b</sup>	14.27 (0.03) <sup>a</sup>	14.27 (0.03) <sup>a</sup>
Cu <sup>¶</sup> (mg kg <sup>-1</sup> )	0.025 (0.004) <sup>a</sup>	0.012 (0.002) <sup>b</sup>	0.009 (0.002) <sup>b</sup>	0.015 (0.001) <sup>b</sup>	0.008 (0.001) <sup>b</sup>	0.012 (0.003) <sup>b</sup>	0.012 (0.003) <sup>b</sup>
Zn <sup>¶</sup> (mg kg <sup>-1</sup> )	5.07 (0.19) <sup>a</sup>	4.95 (0.08) <sup>ab</sup>	5.01 (0.07) <sup>a</sup>	4.68 (0.06) <sup>b</sup>	4.97 (0.22) <sup>ab</sup>	5.14 (0.03) <sup>a</sup>	5.14 (0.03) <sup>a</sup>
Na <sup>¶</sup> (mg kg <sup>-1</sup> )	43.47 (1.30) <sup>ab</sup>	40.21 (1.76) <sup>bc</sup>	37.39 (0.83) <sup>cd</sup>	44.91 (1.21) <sup>a</sup>	36.23 (0.96) <sup>d</sup>	36.32 (0.21) <sup>d</sup>	36.32 (0.21) <sup>d</sup>
Li <sup>¶</sup> (mg kg <sup>-1</sup> )	0.038 (0.003) <sup>a</sup>	0.024 (0.001) <sup>b</sup>	0.024 (0.001) <sup>b</sup>	0.027 (0.001) <sup>b</sup>	0.024 (0.001) <sup>b</sup>	0.025 (0.001) <sup>b</sup>	0.025 (0.001) <sup>b</sup>
Ni <sup>¶</sup> (mg kg <sup>-1</sup> )	0.189 (0.003) <sup>a</sup>	0.175 (0.006) <sup>ab</sup>	0.183 (0.006) <sup>a</sup>	0.181 (0.005) <sup>a</sup>	0.162 (0.005) <sup>b</sup>	0.181 (0.002) <sup>a</sup>	0.181 (0.002) <sup>a</sup>
Cd <sup>¶</sup> (mg kg <sup>-1</sup> )	0.028 (0.003) <sup>a</sup>	0.015 (<0.001) <sup>c</sup>	0.015 (<0.001) <sup>bc</sup>	0.018 (0.001) <sup>b</sup>	0.014 (0.001) <sup>c</sup>	0.015 (0.001) <sup>bc</sup>	0.015 (0.001) <sup>bc</sup>
Co <sup>¶</sup> (mg kg <sup>-1</sup> )	0.060 (0.005) <sup>a</sup>	0.047 (0.002) <sup>c</sup>	0.047 (0.001) <sup>c</sup>	0.055 (0.002) <sup>ab</sup>	0.046 (0.001) <sup>c</sup>	0.051 (0.001) <sup>b</sup>	0.051 (0.001) <sup>b</sup>

<sup>†</sup> Cation exchange capacity.

<sup>‡</sup> 2M KCl extractable nitrogen.

<sup>§</sup> 0.01 M AgTU extractable calcium.

<sup>¶</sup> 0.05 M Ca(NO<sub>3</sub>)<sub>2</sub> soluble elements.

increased in the presence of both native and exotic earthworms; native deep burrowing species (endogeic and anecic) provided the most pronounced effect. The presence of native *M. transalpinus* and *O. multiporus* increased PAN by 104% and 121%, respectively, compared to control soil. In addition, the solubility of S was increased by 39% and 53% by both native species, respectively. There was also increased CEC and exchangeable Ca, P, K, Mg and S in the presence of both native and exotic earthworms, with marginal reduced concentrations of soluble Zn, Cd, Cu and Co ( $p < 0.05$ , Table 3). The solubility of K, Mg, Fe, Cu, Ni and Cd showed the larger effect size by species origin rather than by behavioural group (Table 4). On the other hand, higher omega-squared ( $\omega^2$ ) of other nutrients including PAN and exchangeable Ca, P and S were found by behavioural group than by the origin.

### 3.3. Separation of species

Multivariate analysis of soil properties separated each species into distinct groups on the two PCA axes (Fig. 3; Table 5). Native species were separated from each other along both axes. Native epigeic *Deinodrilus sp.1* burrowing least in the soil had minimal effect on modifying chemical properties, as would be expected. The native anecic *M. transalpinus* was split from other behavioural groupings on Axis 1, represented by increasing soil acidity (pH) and its determinant soluble elements (S, Al, and Ca) and labile P. The other native endogeic *O. multiporus* clearly was split along Axis 2 from the epigeic and anecic earthworms in respect of increased nutrient solubility (PAN and K). Furthermore, the same endogeic grouping of earthworms separated native and exotic species (*O. multiporus* vs *A. caliginosa* and *O. lacteum*) on Axis 2. It seems that the native *O. multiporus* had a larger influence than the exotic species on N mineralisation.

**Table 4**

Analysis of effect size between different behaviour groups (epigeic, endogeic, and anecic) and origins (native and exotic) based on the omega-squared ( $\omega^2$ ) metric for single factor ANOVA model.

Soil parameter	Behaviour group			Origin		
	F	p	$\omega^2$	F	p	$\omega^2$
pH	17.17	<0.001	0.67	16.84	<0.001	0.57
CEC <sup>†</sup>	6.31	0.003	0.40	7.83	0.003	0.36
PAN <sup>‡</sup>	2.95 <sup>ns</sup>	0.058	0.20	3.84	0.038	0.19
Ca <sup>§</sup>	5.04	0.009	0.34	1.39 <sup>ns</sup>	0.272	0.03
P <sup>¶</sup>	5.11	0.009	0.34	5.57	0.011	0.28
K <sup>¶</sup>	0.08 <sup>ns</sup>	0.970	-0.13	6.10	0.008	0.30
S <sup>¶</sup>	9.40	<0.001	0.51	10.00	0.001	0.43
Mg <sup>¶</sup>	1.62 <sup>ns</sup>	0.216	0.07	4.96	0.017	0.25
Fe <sup>¶</sup>	1.76 <sup>ns</sup>	0.186	0.09	2.76 <sup>ns</sup>	0.086	0.13
Al <sup>¶</sup>	7.13	0.002	0.43	5.16	0.015	0.26
Mn <sup>¶</sup>	1.58 <sup>ns</sup>	0.226	0.07	1.54 <sup>ns</sup>	0.238	0.04
Cu <sup>¶</sup>	11.82	<0.001	0.57	18.34	<0.001	0.59
Zn <sup>¶</sup>	0.39 <sup>ns</sup>	0.763	-0.08	2.04 <sup>ns</sup>	0.155	0.08
Na <sup>¶</sup>	1.98 <sup>ns</sup>	0.149	0.11	8.96	0.002	0.40
Li <sup>¶</sup>	27.29	<0.001	0.77	41.44	<0.001	0.77
Ni <sup>¶</sup>	1.88 <sup>ns</sup>	0.165	0.10	3.71	0.042	0.18
Cd <sup>¶</sup>	33.36	<0.001	0.80	58.79	<0.001	0.83
Co <sup>¶</sup>	6.93	0.002	0.43	7.69	0.003	0.36

<sup>†</sup> Cation exchange capacity.

<sup>‡</sup> 2M KCl extractable nitrogen.

<sup>§</sup> 0.01 M AgTU extractable calcium.

<sup>¶</sup> 0.05 M Ca(NO<sub>3</sub>)<sub>2</sub> soluble elements.

## 4. Discussion

### 4.1. Influences of native earthworms on soil chemistry

The sheep-farmed pasture soil used in the present study was suitable for maintaining the survival of native and exotic earthworms of the three behavioural groups, despite limited space and lack of food source, at least in the short term. In this respect, higher populations of the earthworms in natural soils would be dependent on maintaining a supply of OM enrichment from some combination of plant litter, root exudates or animal wastes. In the soil of the present study, the organic matter source is likely to have provided a pulse of decomposition from residual root fractions and litters that had been collected during the dry period of summer, following the soil being wetted and incubated. Also, it is likely that sawdust did not have a significant role. Consistently, all species of earthworms did lose their biomass during the incubation period, which

may have been due to the limited nutritious value of sawdust, equivalent to an increase of less than 0.5% of existing soil carbon.

Earthworm burrowing significantly altered the physicochemical properties of this soil. Liiri et al. (2012) compared soils with and without a legacy of intensive agriculture, finding that earthworms had similar influences in terms of modification of soil biogeochemistry. We found that native *O. multiporus* and *M. transalpinus* stimulated N and P mineralisation (Table 3) but other studies with longer incubation periods have showed a much more pronounced effect on N and P availability (e.g. Scheu and Parkinson, 1994; Vos et al., 2014). The present study also showed broader effects of these two native earthworms on the mobility of essential mineral elements, but native epigeic *Deinodrilus* sp.1 did not show similar effects. Deficiencies of various nutrients (e.g. Fe, Mg, S, B, Cu and Co etc.) for plants and animals are widespread in New Zealand soil (Dickinson et al., 2015). Given this fact, the marked enhancements of bioavailability of some minerals such as Ca, K, S or Mg by burrowing or casting of two native species would suggest that the presence of native

**Table 5**

Loadings for variables assessed through Principle Component Analysis (PCA) in this study.

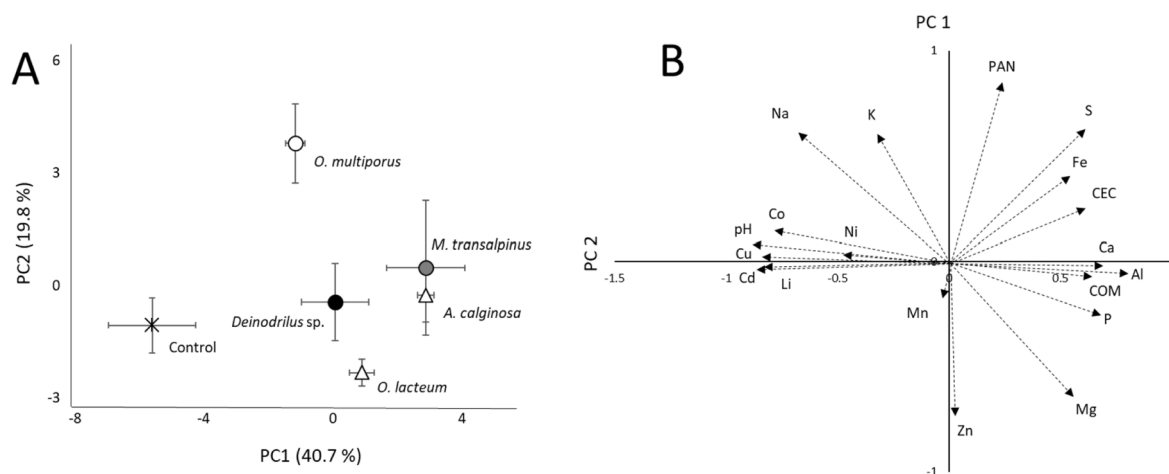
Soil parameter	PC1	PC2	PC3
pH	-0.920	0.085	-0.058
COM	0.661	-0.064	0.387
CEC <sup>†</sup>	0.624	0.256	0.21
PAN <sup>‡</sup>	0.251	0.826	-0.334
Ca <sup>§</sup>	0.702	-0.016	-0.113
P <sup>¶</sup>	0.700	-0.257	0.486
K <sup>¶</sup>	-0.338	0.614	0.507
S <sup>¶</sup>	0.621	0.635	0.031
Mg <sup>¶</sup>	0.572	-0.647	0.380
Fe <sup>¶</sup>	0.544	0.415	0.194
Al <sup>¶</sup>	0.820	-0.055	0.284
Mn <sup>¶</sup>	-0.043	-0.170	0.706
Cu <sup>¶</sup>	-0.883	0.022	0.249
Zn <sup>¶</sup>	0.021	-0.754	0.361
Na <sup>¶</sup>	-0.706	0.623	0.080
Li <sup>¶</sup>	-0.869	-0.017	0.186
Ni <sup>¶</sup>	-0.496	0.036	0.666
Cd <sup>¶</sup>	-0.899	-0.030	0.132
Co <sup>¶</sup>	-0.822	0.154	0.387

<sup>†</sup> Cation exchange capacity.

<sup>‡</sup> 2M KCl extractable nitrogen.

<sup>§</sup> 0.01 M AgTU extractable calcium.

<sup>¶</sup> 0.05 M Ca(NO<sub>3</sub>)<sub>2</sub> soluble elements.



**Fig. 3.** Principal Component Analysis (PCA) of soil properties after 3 weeks incubation. Symbols represent centroids of soils (n = 4) incubated with native species (circle), exotic species (triangle), and no earthworms (X). Native species are *Deinodrilus* sp.1, *Maoridrilus transalpinus* and *Octochaetus multiporus*. Exotic species are *Aporrectodea caliginosa* and *Octolasion lacteum*. A) Mean component scores (±standard deviation) for first two principal components of earthworm species. B) Loading plot of soil parameters.

earthworms have the potential to further improve the fertility of NZ pastoral soil, away from native vegetation areas.

Earthworm casts from soil passed through their gut are towards a neutral pH, probably due to the pH buffering role of organic molecules generated in the gut (Hitinayake et al., 2018). Earthworms also prefer less acidic to neutral soil (pH 4.5–7.0) and can adjust the soil pH between 5 and 8 with some species tolerating more acidic soils (Curry, 2004; Lowe and Butt, 2005). In the present study, soil pH value was generally reduced by increased moisture and with incubation, but varied significantly between earthworm species. Such differences in pH level may be attributed that the decomposition ability of OM sources and the composition of secreted mucus, which differs between species. The earthworms also changed the lability of chemical elements that are indirect determinants of acidity, increasing soil levels of soluble S and Al. Such increase in the minerals is attributed to organic matter decomposition by the earthworm activities (Villar et al., 1993; Balamurugan et al., 1999; Tognetti et al., 2005; Sonowal et al., 2013).

In lumbricid earthworms, endogeics (permanent sub-surface species) have a larger effect on soil properties by consuming more OM than epigeic or anecic species (Edwards and Bohlen, 1996). However, this differed in the present study, and subtle influences on soil properties were brought about by different behavioural groups. On the other hand, better benefits in improvement of nutrient availability were found by native species of earthworms, such as *O. multiporus* (e.g. N, K and S) and *M. transalpinus* (e.g. P and Ca). Within the endogeic functional group, we were able to compare native species with exotics. Exotic endogenic species such as *A. caliginosa* and *O. lacteum* with relatively greater OM consumption (see Fig. 2) did mobilise less N, K, and S than native endogeic *O. multiporus* (Table 3). In addition, the higher omega-squared values of key nutrients (N, Mg and Fe) by the origin rather than by behavioural group (Table 4) suggest that the chemical parameters of agricultural soil might be affected more by native species rather than exotic species, regardless of the known functional role according to the behaviour group. In this study, due to various limitations of microcosm use (e.g. stocking density, biomass loss and short experimental duration etc.), it was difficult to fully assess practical functions of earthworms in agricultural fields. Additionally, another weakness of this microcosm study is that the three behavioural groups access the soil to an equal degree, unlike the situation in real soil profiles in a field situation. Future co-existence of communities of native and exotic earthworms in pasture lands appears realistic, and this would benefit conservation of native species. Therefore, further studies are needed to determine whether native earthworms and their interactions with exotic earthworms and/or other soil organisms have unique synergy in improving the quality of New Zealand agricultural soils.

#### 4.2. Potential role of native earthworms in pasture agroecosystems

Earthworms regulate hydrology, OM dynamics, nutrient cycling, and improving plant growth and development (Fragoso et al., 1997; Francis and Fraser, 1998; Topp et al., 2001; Edwards, 2004; Ojha and Devkota, 2014). Native earthworms, particularly endogeics considerably increased N mineralisation in soil (Table 3). This may suggest enhanced leaching of soluble N and could be detrimental to maintain a tight N cycle in agricultural land, as is known to be the case elsewhere (Domínguez et al., 2004). Kernecker et al. (2014) evaluated that earthworms caused a decrease in the reduction of NO<sub>3</sub> to gaseous N<sub>2</sub>O, but increased leaching of dissolved organic carbon and NO<sub>3</sub> to the environment due to preferential flow pathways. Therefore, minimising the C and N losses, especially during the most vulnerable periods of the year with higher precipitation, lower temperature, and reduced crop growth, would be beneficial in New Zealand lowlands where soil beneath native vegetation is also known as a sink for methane with very low background emissions of N<sub>2</sub>O compared to grassland (Hedley et al., 2013).

Like elsewhere, the New Zealand agricultural system strives to improve the efficiency of N usage in order to reduce NO<sub>3</sub> leaching loss

and N<sub>2</sub>O emission from dairy farm. Nitrous oxide accounts for about 17% of the total GHG emissions in New Zealand which originates mainly from pastoral agriculture (de Klein et al., 2006). *O. multiporus* is the only native species commonly found in New Zealand's agricultural paddocks, and this species appears to increase N<sub>2</sub>O emissions from the agriculture soil (Kim et al., 2015). Nevertheless, considering that capacity of biological nitrification inhibition in roots of pasture grasses would result in improved management of nitrogen (Subbarao et al., 2013), the root rhizosphere interactions with earthworm communities under proper soil management will play a highly significant role in maintaining the tight nutrient cycle. As reported by Kim et al. (2017b), for example, two native anecic *Maoridrillus* spp. incubated in pot soil of legume plant (*Sophora microphylla*) resulted in less N<sub>2</sub>O emission, and this seemed to be due to the provision of more aerobic condition and improvement of nodulation by high level of their burrowing activity.

Moreover, New Zealand pasture lands have widely been issued about cadmium contamination of agricultural soils through application of Cd-rich phosphate fertilisers or biosolids (Simmler et al., 2013); the average level of pastoral soil is 0.43 mg Cd kg<sup>-1</sup>, which is 2.7 times greater than the background level (Taylor et al., 2007). Consistently, this is a major concern for human health because grazing livestock animals can absorb Cd via consumption of herbaceous plants. Earthworms can affect the bioavailability of metal(oids) in soil through feeding, burrowing, and casting (Sizmur and Hodson, 2009). In particular, the earthworm activities increase dissolved organic carbon and reduce soil pH that would enhance the mobility and bioavailability and plant uptake of metals (Wen et al., 2004; Yu et al., 2005). However, native earthworms in the present study did reduce the solubility of Cd in the incubated soil, which would contribute to reducing the concern about toxic Cd from the dairy industry. Stürzenbaum et al. (1998) elucidated a metal binding mechanism in earthworms that it may be attributed to their capacity to accumulate in their tissues by retaining insoluble calcium phosphate granules or chloragosomes, and this do not inhibit essential biochemical mechanisms in the cytoplasm. Also, the formation of stable metal-humic complexes in earthworm casts can immobilize the metals in the soil (Kang et al., 2011).

#### 4.3. Conservation of native species

Our findings suggest that native species of earthworms are tolerant to modified pasture soils, and they increase nutrient mineralisation more than introduced exotic species. It is likely that the population of native species will expand into intensive farmland under reduced tillage management systems, based on the assumption that the cause of their disappearance from arable and pastoral lands was mainly due to ground disturbance (Chan, 2001). In addition, an appropriate policy is needed to gradually expand the distribution of native earthworms and improve the potential of their advance into wider farmland by increasing the density of the native vegetations around or on pasture farmland. A better understanding of the functional role of native earthworms in the process of soil formation, as well as consequences for soil management and restoration in such environments may be necessary for their conservation. This could be beneficial both to biodiversity conservation and to soil quality in New Zealand agricultural systems.

## 5. Conclusions

The present study is an early attempt to understand the functional role of native species of earthworm in New Zealand agricultural soils. On the periphery of agricultural land, divergent families of native and exotic earthworms coexist with apparently similar burrowing behaviours. Although spillover of fertilisers and enhanced fertility of soil are known to have impacted the biodiversity of adjacent fragments of native vegetation, there is currently no evidence that this is harmful to native earthworms. Our findings suggest that the modification of soil properties by native earthworms is likely to be more significant than exotic

earthworms; *O. multiporus* and *M. transalpinus* enhanced the bioavailability of nutrients including N, P, S, K, and Ca. We also found that differences between native and exotic species in their effect size on the solubility of K, Mg and Fe were greater than differences between burrowing groupings. As such, the results suggest that soil fertility of agricultural lands may be influenced more by native species rather than exotic species. This justifies further research, in view of the increasing importance of sustainable and efficient management of these elements in agriculture and the wider environment. Identifying the impact of native earthworms alone and in combination with exotic earthworms in the provision of ecosystem services is needed for an integrated soil management plan in New Zealand agricultural landscape mosaics.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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