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Impacts of Endemic *Maoridrilus* Earthworms (Megascolecidae) in Biosolids-Amended Soil

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Abstract

Biosolids can be a valuable fertilizer for agriculture and in ecological restoration, although there are concerns about contaminants. Earthworm activity, including vermicomposting of biosolids, may influence the efficacy of their use. We investigated how two New Zealand endemic anecic species of *Maoridrilus* (cf. *Eisenia fetida*) responded to biosolids amendment and affected the 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, and 50% biosolids by volume) for 21 d. All species survived in the soil–biosolids mixtures but not in 100% biosolids. The native earthworms, *Maoridrilus transalpinus* and *Maoridrilus* sp.2, increased KCl-extractable NH_4^+ and NO_3^- by up to 29%, substantially more than *E. fetida*. All species significantly increased microbial biomass carbon and $Ca(NO_3)_2$–extractable Cu but significantly decreased dehydrogenase enzymes activity in biosolids-amended soil. Concentrations of $Ca(NO_3)_2$–extractable Mg, S, Fe, Mn, Cd, Co, and Zn varied between earthworm species and with biosolids addition rates. New Zealand native earthworms exacerbated N_2O emissions from soil, whereas *E. fetida* did not. *Eisenia fetida* is clearly a preferred species for vermicomposting biosolids and is more tolerant of high concentrations of biosolids. However, New Zealand native earthworms may be more suitable for improving the fertility of soil amended with biosolids.

Core Ideas

- Locally sourced earthworms are a potential resource for the treatment of biosolids-amended soil.
- *Maoridrilus* spp. increased biosolids mineralization more than *Eisenia fetida*.
- Native earthworms enhanced microbial activity but increased N_2O emission.

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Biosolids comprise the treated solid fraction of sewage, containing high concentrations of organic matter (OM) 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 beneficial as a soil amendment on agricultural land as well as in remediation and revegetation projects (Kinney et al., 2008). Until the middle of the 1990s, approximately 30% of European biosolids were applied to agricultural land, accounting for approximately 2.4 dry Mg yr^{-1} (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 United States now regulate biosolids disposal to production lands, including cropped and grazed lands. 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 nutrient status and plant growth (Evanylo et al., 2005; Gardner et al., 2010).

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 fertilizers, 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; 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 and 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.,

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Abbreviations: APA, alkaline phosphatase activity; ASA, arylsulfatase activity; DHA, dehydrogenase enzymatic activity; EC, electrical conductivity; MBC, microbial biomass carbon; OM, organic matter; TOM, total organic matter; UA, urease activity.

2009). Most vermicomposting uses *Eisenia fetida*, *E. andrei*, and *Lumbricus rubellus*, three species that are tolerant to high compost temperature and the toxic components in biosolids, especially heavy metals and ammonia (Artuso et al., 2010; Ndegwa and Thompson, 2001; Yadav and Garg, 2011; Yadav et al., 2010). These epigeic species seemingly have a greater capacity for waste decomposition and higher reproductive rates than endogeic and anecic species, which burrow into deeper soil (Gajalakshmi and Abbasi, 2004). However, whereas soil-dwelling, surface-feeding epigeic species only break down OM on the soil surface (Ismail, 1997), anecic species not only decompose OM but also transport it from the soil surface to deeper horizons, thereby improving the recycling of OM and the structure of the soil. In this regard, anecic species may be more beneficial for the management of biosolids-amended restoration soils (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). New Zealand earthworms are genetically and morphologically distinct from their exotic counterparts. Although they are members of the Megascolecidae, they have evolved independently in the 85 million years since New Zealand separated from Gondwana (Lee, 1959a). The habitat of indigenous earthworms is restricted to native vegetation and remnants on the margin of agricultural land (Bowie et al., 2016). Restoration of native vegetation leads to increased recolonization by native earthworms that disappeared after conversion to agricultural use (Kim, 2016). No studies have investigated functionality of New Zealand earthworms in biosolids-amended restoration lands. Native earthworms may prove to be more effective than exotic species because 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, roles that their exotic counterparts may not fulfill (Waterhouse et al., 2014b).

Effective application of biosolids to restoration lands requires an understanding of how the native earthworms respond to biosolids. Therefore, we investigated the behavioral tolerance of native earthworms to biosolids-amended soil. We also sought to elucidate how these species affect the solubility of N and trace elements and influence greenhouse gas emission (N_2O and CO_2).

Materials and Methods

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, the by-products of sewage treatment of municipal organic wastes, were obtained from the Kaikōura Regional treatment works (42°21′47.78″ S, 173°41′20.32″ E). Stockpiled and weathered biosolids were collected and homogenized using a concrete mixer and initially passed through a 20-mm sieve. A 2-kg subsample was passed through a 2-mm nylon sieve. The gravimetric moisture content in the biosolids was 53%. Table 1 gives the properties of the soil and biosolids.

Two native earthworms, *Maoridrilus transalpinus* and *Maoridrilus* sp.2, were used in this study after classification of their morphology by Lee (1959a, b) and DNA barcoding analysis with 16S and cytochrome oxidase subunit 1 markers (Boyer et al., 2011). Approximately 100 individuals of *M. transalpinus* 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 the 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). *Maoridrilus transalpinus* was also found to have a greater capacity to break down OM compared with 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 even in human-modified soils (Kim et al., 2015). Soil samples of 20 to 30 cm depth were dug and hand-sorted for earthworms in the field.

Eisenia fetida (tiger worms) obtained from local compost heaps were added for comparison with native earthworms, particularly in terms of tolerance to biosolids toxicity. Although the native species, which weighed 5.5 to 8.0 g per individual, are up to 10 times heavier than *E. fetida* (0.8 g per individual), *E. fetida* has shown similar or greater capacity of OM decomposition than other larger Megascolecids and Lumbricids in earlier studies (Kim, 2016). Earthworms were grown in the laboratory in the soil from which they were collected. The moisture content of the substrate was maintained at 25% (w/w). Earthworms were incubated for 2 wk at 15 to 20°C in darkness. Individual worms were selected for experimentation. A visual assessment was performed to choose the healthiest (i.e., those that were glossy, elastic, sensitive to handling, and with clear clitella or prostatic pores).

Incubation Experiment

Earthworm inoculation followed an acclimatization period that demonstrated both survival and weight gain under laboratory conditions before the experimental work began. For soil mixtures, biosolids were prepared containing 0, 6.25, 12.5, 25, and 50%

Table 1. Physicochemical properties of soil and biosolids in this study. Values in parentheses represent SEM (n = 3) (n.d., not determined).

Property	Soil	Biosolids
Clay/silt/sand, %	4/20/76†	n.d.†
pH (1:5W)	5.6 (<0.1)	4.1–4.5†‡
CEC, meq/100 g	7.8 (0.3)	17‡
C, %	3.3 (0.1)	2.5–2.7†‡
N, %	0.3 (<0.1)	25–28†‡
C/N ratio	12 (0.3)	9–11†‡
NH_4^+–N, mg kg^{-1}	1.8 (0.5)	130‡
NO_3^-–N, mg kg^{-1}	118 (12)	1,352‡
Olsen P, mg kg^{-1}	34 (0.1)	123 (0.1)
P, mg kg^{-1}	732 (11)†	4,683†
K, mg kg^{-1}	2,541 (279)†	4,330 (67)†
Mg, mg kg^{-1}	3,426 (71)†	2,204 (17)†
S, mg kg^{-1}	383 (6)†	6,972 (43)†
Fe, mg kg^{-1}	17,727 (353)†	n.d.†
Cu, mg kg^{-1}	5.0 (<0.1)†	561–696†,‡
Zn, mg kg^{-1}	70 (2.0)†	878–1,048†,‡
Cd, mg kg^{-1}	0.13 (<0.1)†	2.8 (<0.1)†
Mn, mg kg^{-1}	357 (20)†	n.d.†

† Simmler et al. (2013).

‡ Paramashivam et al. (2016).

biosolids by volume. On a dry matter basis, these mixtures comprised 0, 4.8, 10, 20, and 43% biosolids. For each treatment, the total dry mass of soil and biosolids was 0% (532 g), 6.25% (512.5 g), 12.5% (511 g), 25% (489 g), and 50% (466 g). These substrates were placed in 1000 mL polythene containers, with two earthworms of the same species added per container, and maintained for 3 wk. 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). Containers were arranged in a randomized block design in a darkened cupboard. At the end of the experiment, survival percentage was calculated across all five replicates (10 earthworms in total). The biomass of all individual living worms as measured using a three-figure balance (Sartorius TE412).

Analysis of Soil Properties

Samples of fresh soil were analyzed for available N (NH_4^+–N and NO_3^-–N) using a flow injection analyzer star 5000 triple channel analyzer (Foss Tecator AB) after 2 M KCl extraction (Blakemore et al., 1987). For microbial biomass carbon (MBC), the soil samples were fumigated with ethanol-free $CHCl_3$ extracted with 0.5 M K_2SO_4, and MBC was determined using a TOC-500A analyzer (Shimadzu Oceania Pty Ltd.) (Blakemore et al., 1987). As an indicator of microbiological activity, soil dehydrogenase enzymatic activity (DHA) was determined based on the reduction of 2,3,5-triphenyltetrazolium chloride solution to triphenylformazan using a modified method described in Chander and Brookes (1991). A mixture of soil (2 g) and triphenyltetrazolium chloride solution (2 mL) was incubated for 24 h at 25°C in darkness. After extraction with methanol (10 mL), the mixture was shaken and centrifuged at 1880 *g*. The supernatant of hydrolysis reaction products was measured by the absorbance at 485 nm through an ultraviolet (UV) 160A spectrophotometer (Shimadzu). Air-dried soil samples were sieved to <2 mm using a stainless steel sieve. Soil pH and electrical conductivity (EC) were measured using pH and EC meters (SevenEasy, Mettler Toledo). For total organic matter (TOM), soil samples were oven-dried at 100°C, and loss on ignition was measured after combustion in a muffle furnace at 500°C. After an extraction with 0.5 M $NaHCO_3$ and color reaction with ascorbic acid, plant-available P was measured as Olsen P (Blakemore et al., 1987) at a wavelength of 880 nm using a UV 160A spectrophotometer (Shimadzu). Soluble concentrations of K, Mg, S, Fe, Mn, Cu, Cd, Co, and Zn were determined by inductively coupled plasma optical emission spectroscopy ICP–OES (Varian 702-ES) after extraction with 0.05 M $Ca(NO_3)_2$.

N_2O and CO_2 Measurement

Gas collection rings (Clough et al., 2006) were used to determine the N_2O and CO_2 that was emitted from each pot. Sampling occurred 19 and 20 d after inoculation. Nitrous oxide and CO_2 released from each chamber were collected at 20 to 22°C. Aliquots (∼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 cm^2. The gas samples were injected into glass vials and stored in a dark room for analysis (<1 wk). Nitrous oxide and CO_2 were analyzed using a gas chromatograph (SRI 8610, GC Instruments) with a ^{63}Ni electron capture detector and a flame ionization detector and linked to an autosampler (Gilson 222 XL, Sigma-Aldrich).

Statistical Analyses

Minitab 16 (Minitab Inc.) was used for all statistical analyses. Differences in survivorship and biomass of earthworms, soil biogeochemistry, and gas emission in response to earthworm species were assessed separately for each biosolids amendment rate with one-way ANOVA using Fisher's LSD test ($n = 5$; $p < 0.05$). Two-way ANOVA analyses were performed to determine the overall effect of species, biosolids addition rate, and those interactions on soil properties, such as soil pH; EC; TOM; solubility of N, P, and trace elements; microbial activities (MBC and DHA); and N_2O and CO_2 emissions ($p < 0.05$, $p < 0.01$, and $p < 0.001$). For soil pH, descriptive statistics were calculated after conversion to the equivalent hydrogen ion concentrations and back calculation to pH. To identify effects of biosolids addition on gas release, a correlation coefficient was calculated to estimate the relationship between TOM, MBC, NH_4^+, NO_3^-, and N_2O.

Results and Discussion

Survival and Growth of Earthworms

Biosolids treatments of ≤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 control (Table 2). The high mortality of *M. transalpinus* in the control is consistent with the relatively high requirement of this species for OM as a food source (Kim et al., 2015). In an earlier trial, both *Maoridrilus* spp. died 2 d after inoculation with the 100% biosolids treatment, whereas *E. fetida* survived in 100% biosolids for over 2 mo. Mortality was probably caused by the sludge component releasing large amounts of ammonium/ammonia, inorganic salts, and toxic metals such as Cu (Table

Table 2. Survival and weight change of earthworms after 3 wk inoculation in soils with different rates of biosolids addition. Values indicate the means calculated across all the five replicates (10 earthworms in total).

Biosolids dose	Survivorship			Weight change		
	Maoridrilus transalpinus	*Maoridrilus* sp.2	*Eisenia fetida*	*Maoridrilus transalpinus*	*Maoridrilus* sp.2	*Eisenia fetida*
	%					
0%	60	90	100	−29	−6.5	−13
6.25%	100	100	100	−6.6	−3.3	28
12.5%	100	90	100	−0.8	3.1	49
25%	90	90	100	−9.9	−6.1	46
50%	90	100	100	−18	−13	61

1) (Bright and Healey, 2003; Edwards and Arancon, 2004). Therefore, we present only the results for treatments with 0 to 50% biosolids, excluding 100% biosolids.

There is a large diversity of native earthworms in the Megascolecidae of New Zealand, which comprises 179 identified species belonging to 26 genera (Kim et al., 2015). Logically, their ability to survive in biosolids may vary depending on the species. New Zealand 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-rich environments (Waterhouse et al., 2014a). However, the native *Maoridrilus* spp. used in the current study are known to survive in agricultural and high-nutrient soils, although they had been collected from soil under native vegetation in a relatively pristine environment (Kim et al., 2015).

The weight gain of *E. fetida* was proportional to the amount of biosolids added, showing that the biosolids provided an important source of OM (Adair et al., 2014; Artuso et al., 2010). *Maoridrilus* spp. lost weight in the biosolids treatments but to a lesser degree than in the control, which lacked the food source. Amendments of 12.5% appeared most suitable (Table 2); the greatest weight loss occurred with 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.

Effects on Soil Chemistry

Adding biosolids to soil increased NH_4^+–N, NO_3^-–N, Olsen P, and the $Ca(NO_3)_2$–extractable fractions of Cu (Fig. 1). The additional effects of adding earthworms were small in comparison, but all species significantly increased NH_4^+–N and soluble Cu ($p < 0.05$). It is likely that earthworms enhanced the decomposition of organic N from biosolids, releasing NH_4^+–N and some of the Cu attached to this OM. Both native *Maoridrilus* spp. significantly increased NH_4^+ and NO_3^- concentrations compared with *E. fetida* in the treatments containing 6.25 to 25% biosolids ($p < 0.05$). *Maoridrilus* sp.2 significantly increased the solubility of P in these treatments ($p < 0.05$). In the drilosphere soil, earthworm castings and mucus release ammonium, which is subsequently nitrified (Parkin and Berry, 1999) and thereby affects bacteria associated with the N cycle (Eastman et al., 2001; Edwards and Arancon, 2004). Wen et al. (2004) also found increased Cu solubility in *E. fetida* casts.

Adding biosolids to soil caused only a marginal acidification of reference soils (<0.1 pH unit) but significantly increased EC, TOM, and $Ca(NO_3)_2$–extractable concentrations of Cd, Zn, and S (Table 3). There was an additional effect of earthworms on these variables in the 12.5 to 25% treatments. All earthworms increased the concentration of $Ca(NO_3)_2$–extractable Fe, Mn, and Co in soil. In 12.5 and 25% biosolids treatments, the two native species significantly increased EC and $Ca(NO_3)_2$–extractable Cd and S ($p < 0.05$), and *Maoridrilus* sp.2 substantially increased $Ca(NO_3)_2$–extractable Mg ($p < 0.05$). 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 (Hait and Tare, 2012; Kale, 2004). Hait and Tare (2012) found that

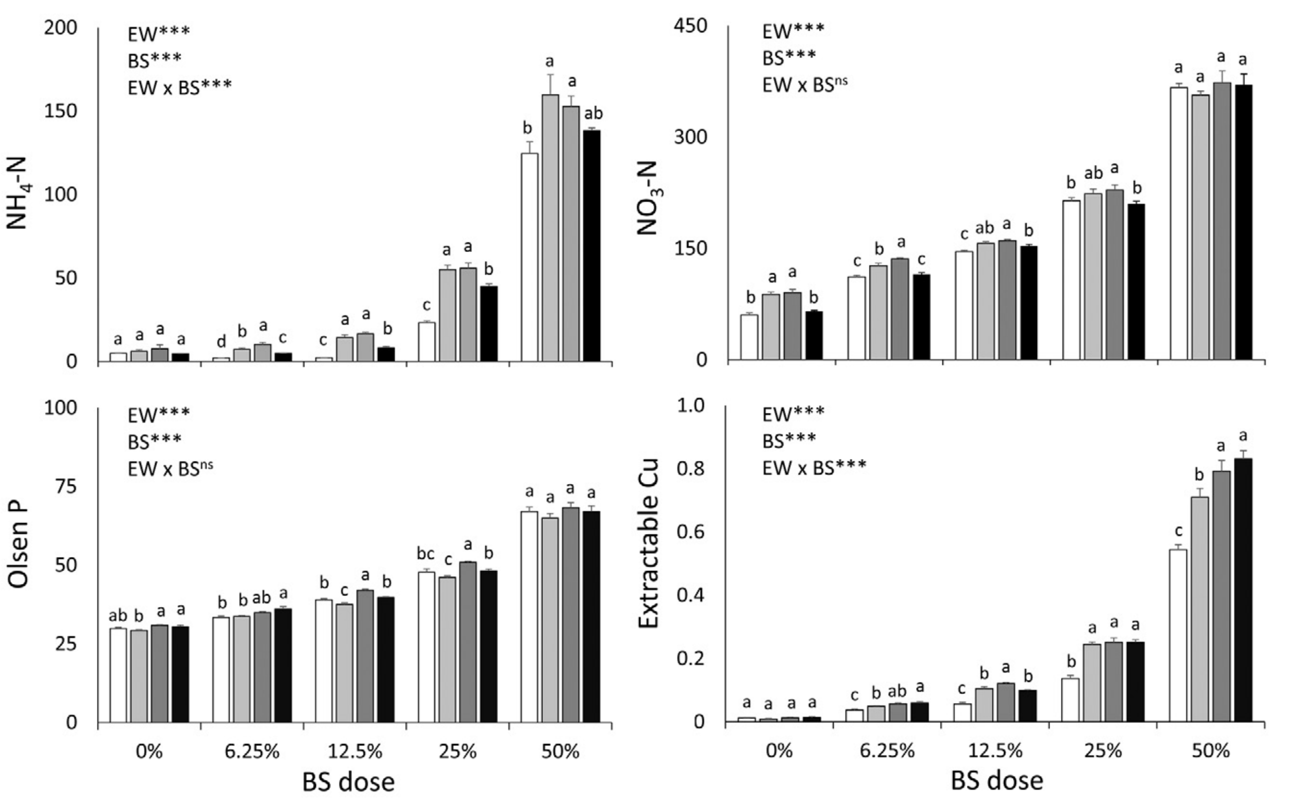

Fig. 1. Extractable concentrations (μg g⁻¹) of N, P, and Cu released from varying proportion of biosolids without earthworms (white bars) and inoculated with *Maoridrilus transalpinus* (light shaded bars), *Maoridrilus* sp.2 (dark shaded bars), and *Eisenia fetida* (black bars). Same letter indicates no significant difference within each biosolids addition rate (*n* = 5; *p* < 0.05). Overall correlations between earthworms (EW), biosolids rates (BS), and their interactions (EW × BS) are represented after two-way ANOVA (p* < 0.05; ***p* < 0.01; ****p* < 0.001; ns, not significant at *p* > 0.05).**

E. fetida increased soluble Fe and Mn but decreased soluble Co. In contrast to the other biosolids components, *Maoridrilus* sp.2 and *E. fetida* markedly decreased concentration of $Ca(NO_3)_2$–extractable Zn in the treatments. These species may have accumulated some of this Zn (not measured).

Table 3 shows the TOM reduction in the presence of earthworms. Although the native earthworms were up to 10× larger than *Eisenia*, they consumed the same or less OM from each biosolids-amended soil. The higher weight gain of *E. fetida*, as well as the presence of cocoons, indicated that this species was more likely to use the uptake of organic nutrients, particularly N, from biosolids for their metabolic activity and reproduction.

Microbiological Activity

Adding biosolids increased MBC (Fig. 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, which did not increase in proportion to the amount of

Table 3. Physicochemical variations in soil properties with earthworms and biosolids after 3 wk inoculation and reference soils without earthworms. Same letters (within each column) indicate no significant differences in values of each biosolid 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 × BS) using two-way ANOVA.

Biosolids dose	Species	Soil properties†										
		pH	EC	TOM	K	Mg	S	Fe	Mn	Cd	Co	Zn
		1:5W	dS m^{-1}	%	mg kg^{-1}							
0%	Reference	5.18 (0.01)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
	Maoridrilus transalpinus	5.19 (0.02)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
	Maoridrilus sp.2	5.15 (0.01)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
	Eisenia fetida	5.26 (0.02)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
6.25%	Reference	5.08 (<0.01)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
	M. transalpinus	4.96 (0.01)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
	Maoridrilus sp.2	4.93 (0.02)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
	E. fetida	4.97 (0.01)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	4.88 (<0.01)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
	M. transalpinus	4.85 (0.01)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
	Maoridrilus sp.2	4.77 (0.01)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
	E. fetida	4.82 (0.01)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	4.70 (0.01)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
	M. transalpinus	4.75 (0.02)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
	Maoridrilus sp.2	4.63 (<0.01)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
	E. fetida	4.67 (0.01)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	4.47 (<0.01)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
	M. transalpinus	4.50 (0.01)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
	Maoridrilus sp.2	4.43 (<0.01)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
	E. fetida	4.48 (0.01)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 × BS	***	***	***	**	***	*	ns	**	*	***	***

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† EC, electrical conductivity; TOM, total organic matter.

‡ Not significant.

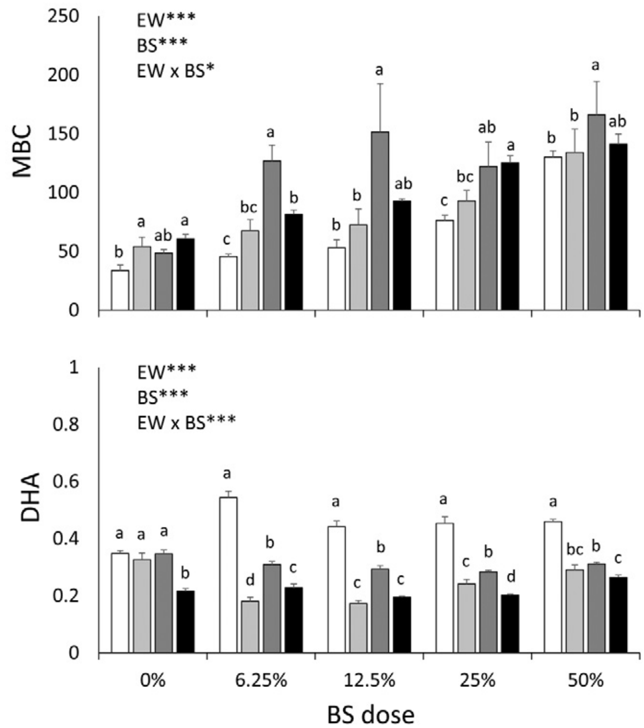

Fig. 2. Effects of earthworms on microbial biomass carbon (MBC; μg g^{-1}) and dehydrogenase enzyme activity (DHA; μg g^{-1} 24 h^{-1}) at varying proportion of biosolids without earthworms (white bars) and inoculated with *Maoridrilus transalpinus* (light shaded bars), *Maoridrilus* sp.2 (dark shaded bars), and *Eisenia fetida* (black bars). Same letters indicate no significant difference within each biosolids application rate ($n = 5$; $p < 0.05$). Correlations between earthworms (EW), biosolid rates (BS), and their interactions (EW × BS) are represented after two-way ANOVA (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

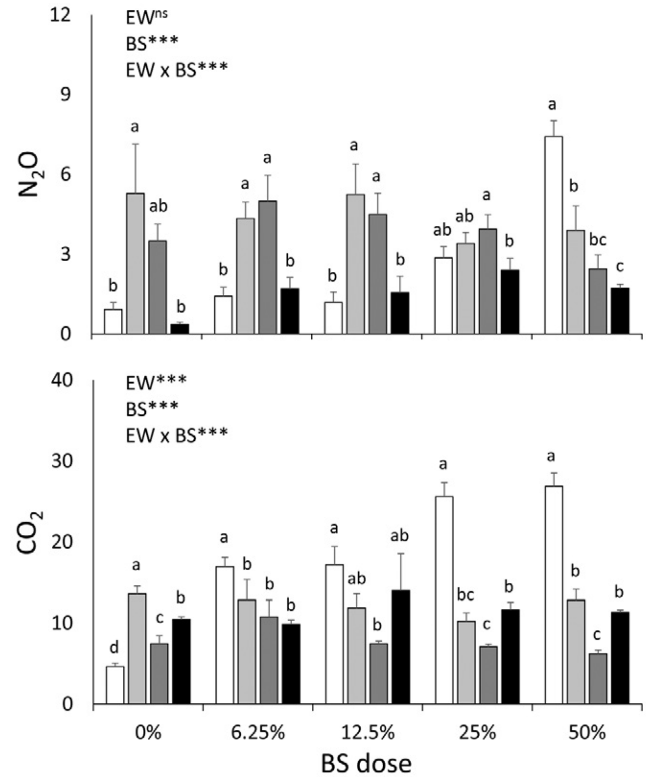

Fig. 3. Effects of earthworms on N_2O (g ha^{-1} d^{-1}) and CO_2 (kg ha^{-1} d^{-1}) at varying proportions of biosolids without earthworms (white bars) and inoculated with *Maoridrilus transalpinus* (light shaded bars), *Maoridrilus* sp.2 (dark shaded bars), and *Eisenia fetida* (black bars). Same letters indicate no significant difference within each biosolids application rate ($n = 5$; $p < 0.05$). Correlations between earthworms (EW), biosolids rates (BS), and their interactions (EW × BS) are represented after two-way ANOVA (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ns, not significant at $p > 0.05$).

biosolids added. Earthworm activity caused a significant decline in DHA. This may be a differential response among other microbial groupings related to urease activity (UA), alkaline phosphatase activity (APA), and arylsulfatase activity (ASA) (Kızılkaya and Hepsen, 2004). Similar results were reported by Kızılkaya and Hepsen (2004), who found significant increases of UA, APA, and ASA but reduced DHA in biosolids treatments with *L. terrestris*. The authors suggested positive associations between soil enzyme activities (UA, APA, and ASA) and nutrient mineralization (N, P, and other elements) in the casts. We could infer that there may be an increase of UA, APA, and ASA in the presence of earthworms. Another factor reducing DHA may be the increase in aeration caused by burrowing and the increased Cu solubility associated with the mineralization of biosolids (Fernández-Calviño et al., 2010).

N_2O and CO_2 Emissions

Adding biosolids influenced the release of N_2O and CO_2 (Fig. 3) due to changes in the soil's chemical and physical properties (Fernández-Luqueño et al., 2009). More N_2O was released in the presence of native earthworms (Fig. 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 N_2O than earthworm-present treatments.

Emission of N_2O from soil occurs via the partial reduction of NO_3^- under low-oxygen conditions (Wrage et al., 2004). The addition of biosolids also increased MBC in proportion to the amount of added OM (Table 4), which was correlated with increased NH_4^+–N and NO_3^-–N, and also to increased N_2O emissions. Although *E. fetida* stimulated N mineralization, this species inhibited N_2O emission compared with the native species. This may be due to the epigeic earthworm increasing aeration in biosolids-amended soil. Contreras-Ramos et al. (2009) reported that, compared with bulk soil, the presence of *E. fetida* released 14 times less N_2O from 5% biosolids-amended soil.

Respiratory CO_2 emissions were significantly reduced in the presence of earthworms, particularly *Maoridrilus* sp.2 ($p < 0.05$). According to a meta-analysis by Lubbers et al. (2013), earthworms increase CO_2 by 33% in a number of field and laboratory studies by stimulating OM decomposition. However,

Table 4. Correlations coefficient of soil biogeochemical properties and N_2O on gas emissions.

	TOM†	MBC‡	NH_4^+	NO_3^-
MBC	0.59***			
NH_4^+	0.87***	0.65***		
NO_3^-	0.93***	0.69***	0.94***	
N_2O	0.27**	0.24*	0.17**	0.23*

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Total organic matter.

‡ Microbial biomass C.

earthworms can improve stability and storage of soil C by protecting C in micro-aggregates formed in large macroaggregates (Bossuyt et al., 2005), which may decrease net CO_2 emissions (Lubbers et al., 2013).

Conclusions

Eisenia fetida is clearly a preferred species for vermicomposting biosolids and is much more tolerant of high concentrations of biosolids that were responsible for the high mortality of native anecic earthworms. However, native earthworms may be more suitable for improving 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 behavioral tolerances in terms of survivorship and weight gain to other treatments that could substantially enhance the availability of key nutrients, including mobile N, P, K, S, and Mg. They are much larger individuals but consume the same or less OM as the much smaller *Eisenia*. Earthworms substantially increased the $Ca(NO_3)_2$–extractable Cu in soil, although *Maoridrilus* sp.2 reduced $Ca(NO_3)_2$–extractable Zn. Adding biosolids to soil provided an OM substrate that improved nutrition and increased MBC but reduced DHA. New Zealand native earthworms increased N_2O emissions from soil. Future work should investigate the performance of native earthworms to biosolids-amended soils under different vegetation types.

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