Response of endemic and exotic earthworm communities to ecological restoration.

Running head: Endemic or exotic earthworms after restoration

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Authors contribution

SB conceived the study, coordinated the sampling and wrote the manuscript; SB, YNK conducted molecular analyses and identified the specimens based on morphology; SB, YNK, MB, NMD collected and hand sorted the specimens in the field; SB, MCL performed the statistical analyses; SB, YNK, MB, MCL, NMD edited the publication.

Implication for practice

• Restoration of native vegetation in New Zealand leads to increased recolonization of endemic earthworm that disappeared following conversion to agriculture
• Restoration of natural vegetation does not cause the disappearance of exotic earthworms, which persist for at least 30 years
• The proportion of endemic versus exotic earthworm (based on either abundance or biomass) were the best indicators of restoration age and likely good predictors of restoration success
• Native and exotic earthworms coexist under restored vegetation in New Zealand
• Continued spread of exotic earthworms through agricultural land may be detrimental to endemic species

Abstract
New Zealand has 23 exotic and more than 200 endemic earthworm species. Endemic earthworms disappeared quickly after vegetation clearance and land conversion to agriculture from the early C19th. Environmental changes associated with agronomic practices are believed to have been the main drivers for their disappearance. Exotic earthworms introduced from Europe have since largely replaced endemic earthworms into farming systems and have been intentionally propagated to increase production. Little is known about potential competition between endemic and exotic earthworms in New Zealand, and the capacity of exotic earthworms to extend their range into native habitats. Using two sites in the South Island of New Zealand, we investigated the impact of restoring native vegetation on earthworm communities. The study sites were Quail Island (Banks Peninsula), which has been undergoing native plant restoration for more than 30 years, and the Punakaiki Coastal Restoration Project (West Coast) where 130,000 native trees have been planted in retired pasture in the last seven years. At each site, soil samples (20 x 20 x 20 cm) were collected and hand sorted for earthworms. Sequential restoration plantings revealed that recolonisation by endemic earthworms increases with time after restoration at the two sample sites. With increasing age of the restoration, the biomass of endemic earthworm significantly increased, as did abundance at Punakaiki. However, exotic species did not disappear after restoration of native vegetation, even after 30 years in Quail Island. The persistence of exotic species leads to the cohabitation of the two communities and potential for interspecific competition.

Key words
Earthworm abundance; Earthworm biomass; Interspecific competition; New Zealand; Recolonisation; Soil fauna

Introduction
In recent years, there has been an increased interest in the conservation or re-introduction of keystone species that are essential for the provision of ecosystems services in restoration programs (Gibbs et al. 2008; Longland & Ostoja 2013; Cosentino et al. 2014; Hunter et al. 2015). In terrestrial ecosystems, this includes earthworms, which provide or contribute to a number of essential ecosystem services essential to restoration including topsoil creation, mineralisation of organic matter, improvement of soil structure and chemistry, soil mixing and aggregating, enhancing of soil macroporosity and water-holding capacity, sustaining of a wide range of predators etc. (for reviews, see Boyer & Wratten 2010).

New Zealand has more than 200 endemic earthworm species (Lee 1959a; Boyer et al. 2011a), and many putative new species yet to be described (Buckley et al. 2011, 2015; Boyer et al. 2013; Waterhouse et al. 2014). Endemic earthworms are all in the Megascolecidae family and mostly found under native vegetation (Lee 1959a; Springett et al. 1998). It has been reported that they
disappeared quickly from agricultural systems after land conversion first by Maori and then by Europeans in the 19th century (Lee 1961). The introduction of exotic grassland and crops with the associated soil disruption through agricultural practices are believed to have been the main drivers of endemic earthworm disappearance (Lee 1961). In addition to these native species, European earthworms (Lumbricidae) have been introduced to pastures and other agricultural land first accidentally with the introduction of European plants and the dumping of ballast soil (Lee 1961). Some of these species were later intentionally propagated to increase crop and pasture production (Stockdill 1982; Springett 1992). Exotic earthworms provide important ecosystem services in these managed agroecosystems that are otherwise devoid of native earthworms (Schon et al. 2011). Twenty three exotic species of earthworm are currently present in New Zealand; some with widespread distribution in agricultural landscapes that occupy more than 50% of New Zealand’s landmass, including Aporrectoda longa, Aporrectodea caliginosa, Aporrectodea rosea, Lumbricus terrestris, Lumbricus rubellus and Octolasion cyaneum (Lee 1961; Stockdill 1966; Schon et al. 2008).

Introduction of exotic earthworms and their subsequent spread occurred after the disappearance of endemic earthworm communities; as a consequence, little is known about potential competition between endemic and exotic earthworms in New Zealand. It has been suggested that introduced European Lumbricidae do not compete with endemic Megascolecidae because they do not co-occur under agricultural landscapes (Lee 1961). However, there is very little information about the potential of exotic earthworms to colonise soil under native vegetation or the potential for endemic species to recover from land conversion. In a recent study, we reported the ability of exotic earthworms to move from pasture into adjacent native vegetation particularly into areas affected by leaching of soil nutrients from agricultural land (Bowie et al. 2016).

We hypothesise that restoration from agricultural land to native vegetation may be sufficient to restore endemic earthworm communities providing that nearby source populations are available for natural recolonisation. The aim of this study was to evaluate whether restoration of native vegetation leads to recolonisation by endemic earthworm communities, and if they are hindered by the presence of exotic species or else cause their replacement.

**Methods**

*Sampling sites*

We tested our hypothesis in two sites located on the East and the West Coast of New Zealand’s South Island. Quail Island (Otahuhua), which has been undergoing plant restoration for more than 30 years (GPS: 43.628S, 172.690E), and the Punakaiki Coastal Restoration Project (PCRP) where 130,000 trees have been replanted in the last eight years (GPS: 42.143S, 171.330E) (Fig. 1). Sequential planting programs on those sites offer the opportunity of studying native earthworm recolonisation at different ages after replanting.
Quail Island (85 ha) is a recreational reserve, administrated by the New Zealand Department of Conservation since 1875. The nearest mainland is 0.5 km NE and at low tide is connected by mudflats via the islet King Billy (Aua). Quail Island was originally covered with dry, coastal broadleaf-podocarp forest, (Genet & Burrows 1998), with tall shrub growth occupying the driest north-facing slopes (Genet 1997). The native vegetation was likely cleared by Maori and later almost entirely converted to farmland by Europeans. Over the past 30 years, considerable restoration efforts have led to the re-planting of 30 ha of native vegetation, including at least 102 species of native plants.

The PCR is a 114 ha area originally covered with coastal sandplain forest. The native vegetation was actively removed and land converted to pasture after the arrival of European settlers. A restoration program initiated in 2009 has already permitted the planting of more than 130,000 trees from over 30 species (Smith et al. 2016).

On both sites, non-restored areas were largely dominated by exotic pasture vegetation (mainly ryegrass (Lolium spp.) and clover (Trifolium spp.). Restored sites were were 5-, 10- and 30-year-old at Quail Island, whilst time since restoration was 5 years in PCR with additional remnants of native vegetation estimated to be about 300 years old since regrowth.

**Sampling and data analysis**

Sampling occurred in late November 2010 on Quail Island and in early January 2012 at Punakaiki. On both sites, three subsamples of 20 x 20 x 20 cm were excavated from 12 plots. Distance between subsamples was less than 2 m, distance between plots was least 12 m. The 36 soil cubes were hand sorted for earthworms on site and earthworms were then brought to the laboratory for further analysis. Individuals were weighed and identified as endemic or exotic earthworms using external morphological features based on Lee (Lee 1959a; b). Adult specimens were further identified to species where possible, although most endemic earthworms seemed to differ from any described species as observed in previous studies (Boyer et al. 2011b) and were therefore classified in Recognizable Taxonomic Units (RTUs) based on their morphology (Lee 1959b) as well as DNA barcoding of the COI and 16S mitochondrial genes (Boyer et al. 2011a).

Because earthworms have a very patchy distribution (Rossi 2003; Rossi & Nuutinen 2004), abundance and biomass data were calculated by pooling the data recorded from the three subsamples collected at each plot. We then used abundance and biomass ratios between endemic and exotic species to measure native earthworm recolonisation at different times after restoration. Data was analysed using the permutation version of Jonckheere-Terpstra test (JT) (Jonckheere 1954) with 10,000 permutations. This test was performed using the package clinfun of the statistical software R version 3.2.2 (R Development Core Team 2014).

**Results**
A total of 513 earthworms were collected from Quail Island and 558 from Punakaiki. Six earthworm species were recorded on Quail Island, three were exotic Lumbricidae (*Apporectodea caliginosa*, *A. rosea* and *Octolasion cyaneum*) and three were endemic Megascolecidae (*Octochaetus multiporus*, *Maoridrilus transalpinus* and an undescribed Megascolecidae). At Punakaiki, five exotics Lumbricidae (*Apporectodea rosea*, *A. longa*, *Lumbricus terrestris*, *L. castaneus* and *Octolasion cyaneum*) and six endemic Megascolecidae species were found (*Deinodrilus gorgon*, *Deinodrilus* sp.1, *Octochaetus kenleei*, and three other undescribed Megascolecidae).

The number and biomass of endemic earthworms increased with time after restoration, while the number and biomass of exotic species decreased. However, results show that number of endemic or exotic earthworms and the biomass of exotic species were poor indicators of restoration age (Fig. 2a, b). The biomass of endemic species was a better indicator and the proportion of endemics (based on either abundance or biomass) were the best indicators (Fig. 3 b, c).

At both sites, the proportion of native earthworm increased with time after restoration both in terms of abundance and biomass (Fig. 3 and 4). At Punakaiki, the proportion of endemic earthworms significantly increased with restoration age, both in terms of biomass (JT=40.5, p=0.0154) and abundance (JT=43.5, p=0.0026) (Fig. 2). In Quail Island, the proportion of endemic earthworms increased significantly with age since restoration (JT=47, p=0.00342) while their contribution to the biomass did not (JT=36, p=0.2333) (Fig. 1). However, in Quail Island, exotic species did not disappear after restoration of native vegetation but remained stable at an averaged 26%, leading to the cohabitation of the two communities. In Punakaiki, old patches of native vegetation were inhabited by endemic species only, with the exception of few sampling sites located at the edge of the forest.

**Discussion**

On both sample sites, sequential replanting programs revealed that native earthworm recolonisation increased with time after restoration both in terms of abundance and biomass. Even 30 years post-restoration, exotic earthworms were still present within restoration plots on Quail Island. Earthworms substantially modify soil structure and function (Hale et al. 2005) and also influence plant growth (Brown et al. 2006), plant composition (Bohlen et al. 2004a; Wurst et al. 2005) and other soil fauna (Freligh et al. 2006; Snyder et al. 2009; Cameron et al. 2013). It has been well documented that European and Asian lumbricids invading northern temperate forests in the US have substantially altered soil nutrient storage and availability, and greatly affected populations and communities of flora and soil fauna (Bohlen et al. 2004b; Hendrix et al. 2008). Because exotic earthworms can cause such significant changes to native soils and ecosystems,
they are usually unwanted in restoration projects (Butt 2008; Snyder & Hendrix 2008; Boyer & Wratten 2010).

The above examples refer mostly to a particular situation where no native earthworm species were present prior to the introduction of exotic species, but potential direct competition between native and introduced earthworms has also been reported (Winsome et al. 2006). These authors suggest that interspecific competition has the potential to prevent American native Megascolescidae from recolonizing pastures dominated by an Exotic European Lumbricid. A similar situation could be occurring in New Zealand where at least some endemic species perform better (Kim et al. unpublished) and even show a preference for agricultural soil over native soil (Kim et al. 2015). One of the rare endemic species that can be found in agricultural soil is Octochaetus multiporus (Beddard). This deep burrowing species can be present in similar population density in pasture soils as under native forests, but Springett et al. (1998) showed that its abundance was negatively correlated with pasture production and the abundance of lumbricid earthworm. The authors suggested that this could indicate an “inability to compete with lumbricid earthworms at higher soil fertility”.

Although more than 1,000 earthworms were collected as part of this study, the limited number of replicates of soil sample pits (36 in each sampling sites) calls for caution in the interpretation of the results. Potential confounding factors may include seasonal and meteorological conditions at the time of the sampling, variation in replanting techniques over the course of the chronosequence (particularly for Quail Island), variation in the composition of earthworm communities in both sampling sites, a sampling method that may not be efficient at collecting anecic (deep-burrowing) earthworms and variation in the size of replanted areas. For example, on Quail Island, many of the 30 years old restoration plots consist of very small patches of vegetation, which may have not provided a suitable habitat to support long-term endemic earthworm communities. Recently, we reported that a small patch of native vegetation surrounded by agricultural land has been colonised by exotic earthworms, apparently linked to drainage and leaching of fertiliser (Bowie et al. 2016). Size of restored areas and distance from the edge were not recorded in the current study. Despite its potential limitations, the present study supports a growing body of literature indicating that endemic and exotic earthworms in New Zealand can thrive in similar habitats and as a consequence, may be competing for resources.

The apparent inability of endemics to survive in agricultural land has encouraged efforts to increase the dispersion of exotic earthworms in New Zealand agricultural land in recent years, despite the lack experimental evidence for this argument. At the same time, the ability of exotic earthworms to encroach under New Zealand native vegetation has only very recently been investigated (Bowie et al. 2016). The introduction of similar European species in Australia has led to concerns regarding their potential impact on native Australian soil fauna (Baker et al. 2006). Manolo & Moller (2015) recently proposed that prior to the introduction of anecic earthworms into pastures where they are absent, their effects on natural ecosystems should be
established. Although introductions of earthworms to New Zealand agricultural lands undoubtedly provides substantial benefits in terms of waste recycling, soil fertility and crop productivity (Springett 1985; Schon et al. 2011), further studies are urgently required to fully understand the potential consequences of earthworm introductions and voluntary propagation in New Zealand agricultural soils.

The proportion of endemic versus exotic earthworms (based on either abundance or biomass) were the best indicators of restoration age. Because the two study sites were considered successful restorations, these proportions are likely to be good predictors of restoration success. Proportions of endemics by far superseded raw abundance and we therefore suggest to use these and refrain from using earthworm abundance to estimate restoration success.

Acknowledgement

We are grateful to the Quail Island Restoration Trust for providing logistical help including access to the island, Rio Tinto Limited for funding the work at Punakaiki and Conservation Volunteers New Zealand for logistical support and access to the Punakaiki site. We also thank John Marris for access to the Lincoln University Entomology Museum and associated resources. Collection permit for invertebrate collection was granted by New Zealand Department Of Conservation (permit number: CA-28815-FAU).

Literature cited


Figure legends

Figure 1. Sample areas and sampling strategy (red squares indicate zoomed areas). Middle: general location. Left: Punakaiki where samples were collected along transects. Right: Quail Island where samples were collected in triangular patterns.

Figure 2. Relationship between various earthworm measurements and age of restoration (n=20). Earthworm abundance (a), biomass (b) and proportions of endemic earthworms (c) were plotted for both study sites across 30 years of restoration. Data from the mature sites in Punakaiki (300 years old) were not used because these plots do not result from active restoration (i.e. replanting) but from natural regrowth. Lines are linear regressions with R² indicated.

Figure 3. Proportion of earthworm abundance (left) and biomass (right) represented by endemic earthworms in Quail Island. Data are based on 36 soil samples (20 cm × 20 cm × 20 cm) collected from 12 plots (3 plots per treatment). The bold horizontal bars of each block show the median value, the boxes show where the middle 50% of the data lie, the whiskers show the maximum and minimum values.

Figure 4. Proportion of earthworm abundance (left) and biomass (right) represented by endemic earthworms at Punakaiki. Data are based on 36 soil samples (20 cm × 20 cm × 20 cm) collected from 12 plots (4 plots per treatment). See figure 1 for legend.
Figure 1

Punakaiki

Years since restoration
- 0 year
- 5 years
- ~300 years (natural regrowth)

Quail Island

Years since restoration
- 0 year
- 5 years
- 10 years
- 30 years
Figure 2

- **Number of exotics**
- **Number of endemic**
- **Linear (Number of exotics)**
- **Linear (Number of endemic)**

- **Biomass of exotics**
- **Biomass endemic**
- **Linear (Biomass of exotics)**
- **Linear (Biomass endemic)**

- **% of endemic biomass**
- **% of endemic abundance**
- **Linear (% of endemic biomass)**
- **Linear (% of endemic abundance)**

**R² values:**
- **0.08132**
- **0.14319**
- **0.26094**
- **0.03275**
- **0.32942**
- **0.273**
Figure 3

Abundance

Biomass
Figure 4

Abundance

Proportion of endemics

Biomass

Years since restoration

0 5 about 300