



# Interactions between earthworm burrowing, growth of a leguminous shrub and nitrogen cycling in a former agricultural soil



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## ABSTRACT

Attempts to restore native biodiversity into agricultural landscapes in New Zealand appear to be compromised both by soil nitrogen enrichment from farming and N-leakage to the wider environment. We investigated whether interactions between native earthworms and a native rhizobium-inoculated leguminous shrub (*Sophora microphylla*) have a measurable effect on the mobility of nitrogen in an agricultural soil that has been nitrogen-enriched and colonised by exotic earthworms. Plants grew better in the presence of both native and exotic soil-burrowing earthworms. Rates of root nodulation were considerably enhanced in the presence of the native megascolecid anecic earthworm *Maoridrillus transalpinus*. This species consumed more organic matter in the presence of inoculated plants whilst marginally lowering soil pH and enhancing critical concentrations of nitrate, but also reducing nitrous oxide emissions. Earthworms raised dehydrogenase enzyme activity and microbial activity in soil, but this was not commensurate with rates of nodulation. Our results show that some combination of earthworm-mediated soil aeration, modification of moisture conditions in the rhizosphere and drilosphere, and comminution of organic matter, modify microbial communities and significantly impact the N cycle.

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## 1. Introduction

Conservation of highly endemic flora and fauna in lowland New Zealand depends on our ability to construct novel native ecosystems on soils that have been profoundly modified from their natural state by agriculture. Ecological restoration attempts to achieve some meaningful semblance of an historic naturalistic vegetation on these soils, and to provide habitat for above- and below-ground faunal biodiversity (Tongway and Ludwig, 2011; Dickinson et al., 2015). This may be reliant on an erroneous assumption of the suitability of modified soils to support the desired species (Smith et al., 2016). Even if agricultural soils provide suitable habitat, little is known of how the soils may be further modified by native biota. However, it is essential that land management and conservation incorporate soil biodiversity as an important criterion to benefit ecosystem functioning, service

provision and human health (Bardgett and Wardle, 2010). This may be especially challenging in situations where land use changes have substantially modified soil structure (Franklin et al., 2015).

Little is known of the requisite underlying environmental conditions to optimise the restoration trajectory within agricultural landscapes in New Zealand. This creates real challenges for restoration practitioners; re-introduction takes place in the presence of exotic weeds and animal pests, including mammals that were formerly absent from this landmass. The most troublesome invasive plants in New Zealand are often legumes, including gorse (*Ulex europaeus*), European brooms and American lupins, although gorse often also plays an important role assisting the recovery of native vegetation on former stock-grazed pasture (Wilson, 2013). Leguminosae are poorly represented amongst the native flora, both in number of species and abundance; 4 genera and 34 species represent 1.4% of the vascular flora (Given and Meurk, 2000), compared to 8% worldwide (Yahara et al., 2013) and native legumes compete poorly with introduced gorse and brooms, particularly in human-modified landscapes (Wardle, 2002). One concern is the elucidation of the role played by native species of

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nitrogen-fixing plants in vegetation recovery. Among the New Zealand native Leguminosae, eight species of *Sophora* are shrubs or small trees (Heenan et al., 2001; Thomas and Spurway, 2001), which could have a particularly important role in global legume diversity assessment (Yahara et al., 2013).

Understanding the functionality, interactions and combined influence of native legumes and soil fauna on ecosystem development presents further challenges (Bardgett and Wardle, 2010; Blouin et al., 2013). In New Zealand, the role of native earthworms in particular requires more attention (Kim et al., 2015). There are more than 200 species of native Megascolecid earthworms in New Zealand (Lee, 1959a,b; Boyer et al., 2011) that are almost entirely unrepresented on agricultural land, even though several species of exotic Lumbricid earthworms are commonly found in farm paddocks (Lee, 1961; Fraser et al., 1996; Springett et al., 1998).

Agricultural landscape matrices in New Zealand are depauperate in native flora and fauna (Winterbourne et al., 2008) where remnants of natural and re-planted vegetation are only represented as little more than refugia in riparian zones, along fence lines and on the borders of agricultural land (Bowie et al., 2016). These natural remnants are now significantly expanding through renewed interest in native species and through modern intensive agricultural systems that are integrating restoration of biodiversity into farm planning (Dickinson et al., 2015; Franklin et al., 2015). The broad aim of our current research is to understand the interactions between native species of plants, soil fauna and soil physicochemistry. The objectives of the mesocosm experiment reported in this paper were to investigate whether we could demonstrate significant integration of the role of a native species of nitrogen-fixing plant and earthworms in the context of nitrogen cycling and soil quality.

## 2. Materials and methods

### 2.1. Establishment of pot experiment

Surface soils (0–15 cm) were collected from the Lincoln University commercial dairy farm for use in this mesocosm experiment. The dairy farm soil (Templeton silt loam) is intensively managed, irrigated and fertilised, and is representative of intermediate terraces in the Province of Canterbury on South Island

(Molloy, 1988). The soil was sieved and uniformly mixed prior to planting. Four species of earthworms representing different ecological groups and burrowing behaviours were selected for this study. Two native anecic species, *Maoridrilus transalpinus* and *Maoridrilus* sp.2 were collected respectively from a nature reserve (Ahuriri Reserve, Banks Peninsula) and beneath a mature stand of exotic *Quercus ilex* trees on the university campus. The earthworms were identified using DNA barcoding (16S and COI), which showed that *Maoridrilus* sp.2 has not been previously recorded and may be new to science (Kim, 2016). An exotic endogeic species (*Octolasion cyaneum*) was also sampled from the Ahuriri Reserve; we have observed that both *Maoridrilus* and *Octolasion* commonly coexist in Banks Peninsula forests. The exotic epigeic, *Eisenia fetida*, was purchased from a local vermicomposting company. One-year-old single plants of *Sophora microphylla* (Kowhai) of uniform size were purchased from a nursery, acclimated to glasshouse growth conditions for 4 weeks, then transplanted into the dairy farm soil in 55 plastic plant pots (5 l volume), and maintained for a further 7 days before the addition of rhizobial and then earthworm inocula (Fig. 1).

Novel *Mesorhizobium* sp. cultures (Strain ICMP 19535; Tan et al., 2015) were obtained from the International Collection of Microorganisms from Plants at Landcare Research (Auckland, NZ) and incubated into Yeast Mannitol Broth (YMB) at 25 °C in the dark for a week to derive liquid cultures which were poured in 50 ml aliquots into 25 pots, on two occasions 7 days apart. This strain is known to be effective on *S. microphylla*. Autoclaved YMB was added to 5 reference pots in the same amounts and at the same time as a control. One week later, four adult earthworms of a single species were added to each pot, with 5 replicates, and an additional 5 pots without earthworms. Weight of all species of earthworm were recorded prior to inoculation. The drainage holes and upper surface of the pots were covered with gauze to prevent earthworms escaping during the experiment. Pots were randomized and maintained for 8 weeks in natural light at 20 ± 5 °C. There were 5 replicates of each treatment, providing a total of 45 pots (4 species of earthworms, with and without rhizobial inoculation, plus 5 pots with neither earthworms nor inocula). These mesocosms were lightly watered every 3 days for 8 weeks, adding the same volume of water to each pot to maintain likely optimal moisture conditions of 25–30% (Wever et al., 2001; Eriksen-Hamel and Whalen, 2006).



Fig. 1. Establishment of *S. microphylla* mesocosms in the glasshouse. Photographs inset show the gauze covers (top inset) and gas sampling cylinders (bottom inset).

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