European Journal of Soil Biology 75 (2016) 174–179

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

Establishment of *Aporrectodea longa* and measurement of dung carbon incorporation in soils under permanent pasture

N.L. Schon [a,*], A.D. Mackay [b], R.A. Gray [b], C. van Koten [a]

[a] AgResearch, Lincoln, Private Bag 4749, Christchurch 8140, New Zealand
[b] AgResearch, Grasslands, Private Bag 11008, Palmerston North 4442, New Zealand

A R T I C L E I N F O

Article history:
Received 4 February 2016
Received in revised form
25 May 2016
Accepted 31 May 2016

Handling Editor: S. Schrader

Keywords:
^{13}C: ^{12}C isotopic ratio
Grasslands
Earthworms
Functional diversity

A B S T R A C T

Earthworm populations often have a large biomass in pasture soils and can significantly accelerate the disappearance of organic matter from the soil surface and its accumulation in soil layers. This field study investigated the influence of the anecic earthworm *Aporrectodea longa* on bulk soil C in permanent pastures two years after their introduction. We used the approach of measuring soil C and ^{13}C after the application of C-enriched dung to paddocks with and without established *A. longa* populations (~50 m^{-2}). At three sites with differing soil types, dung incorporation into the soil was limited, suggesting that densities were still too low to influence bulk soil C. In a fourth pasture where *A. longa* had been present for 23 years, and had reached a density of 190 m^{-2}, more dung-derived C was detected to 300 mm depth in the soil profile compared with the control without *A. longa*.

1. Introduction

Large quantities of carbon (C) are stored within the soil, and small changes in soil C can have a large impact on the amount of C cycled globally [1]. Thus changes in soil C stocks have significant potential to mitigate or amplify the effects of greenhouse gases. Worldwide there have been reports of decadal-scale changes in soil C, both positive and negative, across a range of ecosystem types and land use types [2,3]. Losses of soil C [2,3] are of particular concern, not only in the context of global change but also because it has implications for the ecosystem services provided by soils [4]. A reduction in soil C could negatively impact both agricultural production and the degree to which agriculture impacts other environmental conditions [5].

In agricultural pastures, large quantities of C are deposited onto the soil surface through plant senescence and in dung from grazing animals. While representing major inputs of C to soil, until incorporated into stable compounds and structures in the soil, this C is vulnerable to atmospheric C loss by decomposer respiration [6]. The other much smaller loss pathway is leaching of dissolved organic compounds [7]. Soil microbes are responsible for much of the decomposition occurring in pastures but their activity is influenced by other organisms, particularly macrofauna which perform the initial steps of dung and litter breakdown and incorporation [8]. Earthworms are particularly important not only as processers of organic material but for their role in transforming the soil physical environment. Earthworm burrowing stimulates soil aeration and consequently, microbial activity [9,10]. By consuming and excreting organic matter, earthworms also increase the surface area of organic particles available to microorganisms, distributing both within the soil and concentrating them within their casts and burrows [11,12]. The rate of organic matter decomposition and disappearance from the soil surface, both by translocation and comminution is heavily influenced by earthworm species composition and the functional groups represented in the earthworm communities [13]. There are three recognised distinct functional types, including epigeic, endogeic and anecic. Both epigeic and anecic earthworms feed on organic matter on the soil surface but anecic earthworms are functionally distinct in that they consume significantly more organic matter and burrow to much greater depths [10].

In a recent mesocosm study, anecic *Aporrectodea longa* was shown to increase bulk soil C [14]. The current study investigates the influence of *A. longa* populations introduced to grazed pasture field sites 2 and 23 years earlier, on the incorporation and

* Corresponding author.
E-mail address: Nicole.schon@agresearch.co.nz (N.L. Schon).

http://dx.doi.org/10.1016/j.ejsobi.2016.05.005
1164-5563/

accumulation of C added as dung onto the soil. The use of ^{13}C enriched dung from C4 grass-fed livestock enabled us to identify the fate of the added dung. We hypothesised that the presence of *A. longa* would increase bulk soil C with the most pronounced influence in the subsoil where *A. longa* are more active than the epigeic and endogeic earthworms already present in the soil [15].

2. Methods

2.1. Earthworm establishment

Three sites under long-term perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) pasture were selected in three different regions of New Zealand. All sites were grazed by dairy cows. The absence of anecic earthworms was verified. Site properties are given in Table 1. Location, monthly rainfall and air temperature are given in Fig. 1 (www.cliflo.niwa.co.nz, accessed June 2014). Total annual rainfall at the sites (1− 3) for July to June 2010–2011 was 2290 mm, 980 mm and 1160 mm, respectively, and for July to June 2011–2012, 2092 mm, 854 mm and 1175 mm.

At each site, eight plots (2 m * 2 m) were established in each of three paddocks. The corners of the plots were recorded using GPS and steel-markers were placed in the ground (for relocation using a metal detector). Two treatments (*A. longa* or control) were randomly allocated to the plots. In winter (August) 2010 plots allocated to the *A. longa* treatment each received one hundred *A. longa* into four 15 cm diameter holes 15 cm deep near the centre of the plot and the soil was returned into the hole. *Aporrectoea longa* were collected by hand-sorting from a pasture in the Manawatu, and contained a mix of mature and immature earthworms. The paddocks were subjected to the normal farm management practices of each farm which included grazing by livestock and fertiliser application. One paddock at each site was an effluent paddock.

A fourth site was also sampled where *A. longa* was introduced into an Allophanic soil in 1989. This field location was in hill country in the Central Plateau, New Zealand. Although not directly comparable with the 2010 introductions of *A. longa*, the 1989 site has had a much greater time in which to influence soil C with abundant *A. longa* populations, and as such indicates what may happen with the 2010 introductions. An area of the paddock where exotic earthworms had not established, but was otherwise similar to where they had, served as a control. Total annual rainfall at this site for July to June 2010–2011 was 805 mm and for July to June 2011–2012 was 1080 mm (Fig. 1). For further site details see Schon et al. [16]. Samples to determine *A. longa* abundance were collected from three points along a 50 m '*A. longa*' and 'control' transect.

2.2. Dung application

Fresh dung was collected from cattle two weeks after the animals started a C4 plant diet (*Zea mays*). The collected dung was 20% dry matter, contained 36.3% total C and an isotopic signature ($\delta^{13}C$) of −14‰ (PDZ Europa GSL continuous flow elemental analyser and 20-20 isotope ratio mass spectrometer, Sercon Ltd., UK).

During the winter of 2011, each plot received two applications of dung one month apart. At each application 3 kg of wet dung (0.6 kg DM) were applied to mimic a large dung pat [17]. In the winter of 2012 each plot again received two applications of dung, but the quantities were increased to encourage greater dung incorporation. Each application consisted of 10 kg wet dung (2 kg DM) spread over five dung pats. A total of 26 kg wet dung was applied to each plot during the course of the study, equivalent to 5.2 kg DM.

2.3. Data collection

At the end of winter 2011 half the plots were sampled for earthworms and soil C, the other half of the plots were sampled the following winter (2012). Earthworms were collected from two duplicate 200 mm width × 200 mm long × 300 mm deep soil monoliths from each plot, hand-sorted in the laboratory and identified to species where possible.

Soil cores for C analysis were taken from the centre of each plot using an 8 cm diameter corer at site 1 and 2 and a 2.5 cm diameter corer at site 3 and separated into 0–75 mm, 75–150 mm, 150–225 mm, 225–300 mm depths. Any visible dung was removed from the soil surface before analysis. Soil was air dried, sieved to 2 mm and analysed for total C using a PDZ Europa GSL continuous flow elemental analyser, and for $\delta^{13}C$ a PDZ Europa 20-C a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., UK). Bulk density samples were collected in 2012 in 100 mm diameter, 50 mm deep steel rings. Samples were oven-dried at 105 °C for 48 h and weighed. Site 1 effluent paddock was cultivated in 2012, and another paddock had dung incorrectly applied, so only one paddock from this site was able to be used for soil C analysis.

The 1989 introduction site was sampled along the two transects for earthworms, soil C and bulk density, using methods as described above.

Table 1

Site	1	2	3	4
Site location	Waikato	Wairarapa	Southland	Central Plateau
Soil type (NSD)	Allophanic	Brown	Gley	Allophanic
Latitude	-38° 23' 46"	-39° 53' 49"	-46° 19' 35"	-39° 33'59"
Longitude	175° 5' 9"	176° 27' 33"	168° 16' 23"	175° 51' 32"
Altitude (masl)	247	185	7	480
Irrigation	No	Yes	No	No
Stocking rate (cows ha^{-1})	2.5	3.8	2.9	1.5[a]
Supplements (kg DM $ha^{-1}yr^{-1}$)	1200	10000[b]	1200	None
Aporrectodea longa introduction	August 2010	August 2010	August 2010	1989
Dung application 2011	15 June & 15 July	31 May & 7 July	21 June & 20 July	
Dung application 2012	6 June & 24 July	30 May & 26 July	15 June & 10 August	7 June & 25 July
Sampling 2011	28 September	26 September	3 October	
Sampling 2012	2 October	11 October	16 October	8 November

[a] This is not a dairy farm but a drystock farm being grazed by sheep and beef
[b] Supplements consisted of 1600 t maize silage, 700 t cereal silage, 200 t squash waste, 150 t pea & bean waste, 200 t apple waste, 700 t maize grain, 300 t barley grain, 240 t canola & soya grain and 800 t palm kernel.