Ecological Entomology

Ecological Entomology (2018), DOI: 10.1111/een.12513

Newly released non-native dung beetle species provide enhanced ecosystem services in New Zealand pastures

SHAUN A. FORGIE,[1] QUENTIN PAYNTER,[1] ZENG ZHAO,[1] CHRISTINE FLOWERS[2] and SIMON V. FOWLER[3] [1]Landcare Research, Auckland, New Zealand, [2]University of Birmingham, Birmingham, UK and [3]Landcare Research, Lincoln, New Zealand

Abstract. 1. Whether the release of non-native insect species benefits or harms ecosystem services has been the subject of debate. In New Zealand, the release of new non-native dung beetle species was intended to enhance ecosystem services but concerns were raised over possible negative effects.

2. Field cage trials used three newly released dung beetle species to investigate two concerns: that soil disturbance from dung beetle activity increases soil losses in runoff after rainfall; and that dung burial increases survival of infective parasitic nematodes on pasture.

3. Three treatments – dung + beetles, dung-only, and controls (without dung or beetles) – were applied on each of three soil types with different permeability: sandy loam, clay loam, and compacted clay.

4. Dung beetle activity resulted in significant reductions of 49% and 81% in mean surface runoff volume, depending on simulated rainfall intensity. Amounts of sediment in the runoff did not change under an extreme rainfall simulation, but in a less extreme rainfall simulation the presence of dung beetles resulted in a 97% reduction in mean sediment amount in runoff.

5. The numbers of infective third-stage nematode larvae recovered from foliage varied considerably between soil types and through time; however, dung beetle activity reduced overall mean nematode numbers on grass around the dung pats by 71%.

6. This study adds to global evidence that dung beetles can improve agricultural ecosystem services by providing data on services that have rarely been investigated: reduced runoff/soil losses through increased soil porosity, and reductions in parasitic nematodes.

Key words. *Digitonthophagus gazella*, *Geotrupes spiniger*, *Onthophagus binodis*, parasitic nematodes, soil losses, surface runoff.

Introduction

Insects are a highly species-rich part of terrestrial ecosystems, and their positive contributions to a wide range of ecosystem services have been highlighted (Losey & Vaughan, 2006; Kremen & Chaplin-Kramer, 2007; Nichols *et al.*, 2008). About 3000 non-native insect species have been deliberately introduced into many countries to improve three ecosystem services: biological control of pests or weeds (Hajek *et al.*, 2016), pollination (Russo, 2016), or decomposition (Hanski & Cambefort, 1991). The vast majority of deliberate releases of non-native insects targeted at improving ecosystem services have been for biological control of pests or weeds (Winston *et al.*, 2014; Cock *et al.*, 2016). Nevertheless, over 100 species of non-native dung beetles have been deliberately released into at least seven countries (Hanski & Cambefort, 1991; Simmons & Ridsdill-Smith, 2011) to provide a range of beneficial ecosystem services, e.g. to reduce pests such as flies and parasitic nematodes, and to promote bioturbation, which can potentially enhance herbage yields and water quality (Hughes *et al.*, 1975; Nichols *et al.*, 2008; Beynon *et al.*, 2015). However, concerns have also been raised over possible harmful side-effects of non-native dung beetle species (e.g. Hanski & Cambefort, 1991; Simmons & Ridsdill-Smith, 2011).

Correspondence: Shaun A. Forgie, Dung Beetle Innovations, PO Box 351, Kumeu, Auckland 0841, New Zealand. E-mail: shaun@dungbeetles.co.nz

Releasing non-native dung beetles to enhance ecosystem services is functionally different from biological control of pests and weeds because successful biological control programmes result in substantial reductions in the populations of the target organism. Therefore, provided the agent is host-specific, the population of the agent should also substantially decline, minimising the risk and magnitude of any potential unwanted side-effects (Holt & Hochberg, 2001; Van Driesche *et al.*, 2008; Fowler *et al.*, 2012). By contrast, dung is a continuously replenished resource and dung beetles must therefore become permanently widespread and common to be effective at providing ecosystem services. There is a risk, therefore, that any potentially harmful side-effects of introducing dung beetles will also be permanent. Thus, it is not surprising that multiple authors have raised the issue that the deliberate release, or subsequent invasion, of non-native dung beetles could result in possible harmful side-effects, (Durie, 1975; Hanski & Cambefort, 1991; Zunino & Barbero, 1993; Lobo, 1996; Montes de Oca & Halffter, 1998; Young, 2007; Simmons & Ridsdill-Smith, 2011; Noriega *et al.*, 2017), although these hypothetical risks have rarely been quantified.

It is important that the potential benefits of introducing non-native dung beetles are quantified so that an overall evaluation of the potential risks, costs, and benefits can be made. However, despite the long history of deliberate releases of non-native dung beetle species (Hanski & Cambefort, 1991), and considerable interest in native dung beetle communities (e.g. Beynon *et al.*, 2015), there is a surprising lack of information regarding several of the ecosystem service effects of dung beetle activity (e.g. Beynon *et al.*, 2015). For example, although tunnelling by dung beetles should increase bioturbation, reduce soil compaction, and improve drainage (Beynon *et al.*, 2015), the effects of bioturbation have rarely been empirically assessed.

Another ecosystem service that dung beetles can affect involves the survival and potential re-infection of grazing animals by parasitic nematode (Nichols & Gomez, 2014). Parasitic nematodes are a major problem for livestock production, with eggs being passed in dung from infected animals, and the infective third-stage larvae migrating onto foliage which, when consumed by stock, completes the infection cycle (Sutherland & Scott, 2009). The interaction of dung beetle activity and the development of parasitic nematodes to the infective third-stage larvae (L_3) is complex (Durie, 1975) with potentially both positive and negative consequences for successful parasite transmission (Nichols & Gomez, 2014).

Hatching of parasitic nematode eggs is enhanced by increased levels of oxygen in the dung and inhibited by anaerobic conditions in the dung (Sutherland & Scott, 2009). Sands and Wall (2016) and Chirico *et al.* (2003), for example, found that numbers of nematode larvae increased shortly after pat colonisation by endocoprid dung beetles (beetles that inhabit the dung pats rather than bury dung) which may have aerated the dung causing more nematode eggs to hatch. The first two feeding stages of the larvae are desiccation-intolerant (Sutherland & Scott, 2009). Hence dung beetle burial of infected faeces could enhance nematode survival and development within the soil by creating an oxygenated environment that is buffered from both solar radiation and temperature extremes, reducing mortality from desiccation (Durie, 1975). This could theoretically create a 'time bomb' effect due to increased larval survival followed by later migration of large numbers of the desiccation-resistant L_3 nematode larvae onto pasture foliage (Durie, 1975; Waghorn *et al.*, 2002; Coldham, 2011; Nichols & Gomez, 2014). By contrast, dung beetle activity can also increase pat desiccation, reducing the survival of the early-stage larvae of parasitic nematodes. Furthermore, mechanical damage from dung beetle feeding and brood ball production can destroy nematode eggs, and deep burial of dung can prevent nematodes from migrating back to the soil surface (Nichols & Gomez, 2014).

In this study we examine two concerns that were raised prior to the introduction of new dung beetle species in New Zealand: (i) that soil disturbance from dung beetle burrowing could increase soil losses after rainfall; and (ii) that burying dung could enhance the survival of gastrointestinal nematodes of livestock, leading to greater numbers of the infective third-stage larvae on foliage in pastures (Durie, 1975). We used three soil types with different permeability, and tested the effect of dung beetle activity on: (i) surface runoff/soil loss from pasture; and (ii) the number of infective third stage larvae of a common parasitic nematode, *Cooperia* sp. (Rhabditida: Cooperiidae), on pasture foliage, and hence potential infection rates of livestock.

Methods

Sites and field cages

We selected sites on livestock farms with three soil types ranging from high to low permeabilities; sandy loam (Shelly Beach Farm; 36°34′30.58″S, 174°21′42.87″E); clay loam (Kumeu; 36°47′55.36″S, 174°30′46.54″E); and compacted clay (Kaipara Flats; 36°23′59.53″S, 174°36′33.65″E). The farms were located within 45 km of each other in the greater Auckland region.

We chose 5 × 5-m experimental sites in pasture on each of the three soil types, and fenced the sites to exclude stock. To complement the following descriptions of the trial equipment and methods we have included photographs, diagrams, and a typical site plan in the Supplementary Information (Figures S1–S4). For the field cages we cut open-ended cylinders (diameter 55 cm, height 40 cm) from high-density plastic drums. To ensure a tight seal between the soil and cage, we hammered each cylinder into the ground to a depth of 15 cm. We constructed lids for the cages by cutting a plastic collar from the same plastic drum, with a diameter slightly less than that of the top of the cage. This enabled us to bolt the collar to the top of each cylinder, and to fix a durable 2-mm nylon mesh cover that retained the beetles in the cages for the duration of the experiment. We used a digital spirit level to ensure all cages in the runoff/soil loss trial were on a 12° slope. We designed a runoff collection system that used a 25-mm-diameter female tank fitting (RX Plastics, Ashburton, New Zealand), fitted with a 2-mm mesh filter, and positioned at soil surface level at the lowest point of the cylinder. Runoff then drained down a PVC pipe leading to a 4-litre collection bucket. We used similar cages for the nematode recovery trial, but there was no hole in the cage at ground level for collecting runoff, and no runoff collecting system (tank fitting, pipe, and bucket).

We set up the surface runoff/soil loss trial for 20–29 September 2012, and the nematode recovery trial for 22–28 February 2013.

Surface runoff/soil loss trial

We set up three replicates of three treatments: dung + beetles, dung-only, and controls (no dung or beetles) on each soil type. Only *Geotrupes spiniger* Marsham (Coleoptera; Geotrupidae), a large dung beetle species, was available for this trial. In early October, each dung + beetle cage received 10 newly emerged adult beetles (50:50 sex ratio), which is at the lower end of the naturally occurring numbers recorded per dung pat where this species has been introduced in Australia (10–40 per pat; Tyndale-Biscoe, 1994).

For each set of dung pat applications we sourced organic, macrocyclic lactone-free dung from several cow pats from Shelly Beach Farm and mixed it thoroughly to minimise variations in dung quality. To simulate a natural pat, we dropped 2 litres of dung into each dung or dung + beetle cage from a height of 1 m. Each week, from early October until mid-November 2012, we added dung to the cages, aiming for the centre of the cage but avoiding any preceding pat. We removed 2-week-old dung pats, just prior to adding new dung, so there were never more than two dung pats in each cage. In the dung + beetle treatment we checked the removed dung to ensure beetles were not removed as well. We commenced rainfall simulations when we saw soil casts and substantially reduced amounts of dung in the dung + beetle cages: this indicated that beetles had reached sexual maturity, and were tunnelling and nest building.

We constructed a rainfall simulator using an open-topped plastic drum, the same diameter as the field cages. We inserted a single-piece irrigation nozzle (diameter 4 mm) into each of 100 evenly spaced 4-mm-diameter holes that we had drilled in the base of the drum. A 150-mm length of nylon string was inserted into each nozzle to stop water flowing through too freely. A small knot tied at the end of each strand encouraged simulated raindrops to form. For each rainfall simulation we used a moveable frame and digital spirit level to position the rainfall simulator horizontally over each cage. We removed the mesh top of each cage for the 10-min duration of each rainfall simulation.

In late November 2012, we applied 12 500 ml of water to each cage over 10 min. This represented 53 mm of rainfall, which equated to an extreme rainfall event with a less than 1% annual exceedance probability (aep), i.e. likely to occur less than once in 100 years across the trial region, as calculated using the NIWA High Intensity Rainfall System V3 tool (available at https://hirds.niwa.co.nz/). In mid-December 2012, we applied 6000 ml of water to each cage over 10 min. This represented a less extreme rainfall event (25 mm), which equated to 1.7% aep (1 in 60 years) at the compacted clay site (Kaipara Flats), 1.2% aep (1 in 80 years) at the clay loam site (Kumeu) and a 1.0% aep (1 in 100 years) at the sandy loam site (Shelly Beach), although the NIWA High Intensity Rainfall System V3 tool indicates that these frequencies are predicted to increase dramatically under projected climate change.

We measured the volume of runoff once flow had ceased, and assessed total sediment by passing runoff water through a previously weighed Whatman™ filter paper (Grade 1), which we then dried at 60 °C for 48 h before re-weighing.

Nematode recovery trial

For each soil type we used three replicates of three treatments: dung + beetles, dung-only and controls (no dung or beetles). The AgResearch Grassland Research Centre provided cow dung containing a mean number of 300 eggs g^{-1} (wet weight) of the parasitic nematode (*Cooperia* sp.). We mixed the dung to minimise variability between the artificial pats. We dropped a single 2.5-litre pat of nematode-infected dung from a height of 1 m into the centre of each of the dung-only and dung + beetle treatment cages. We had a more diverse community of new non-native dung beetle species available for the nematode recovery trial, allowing us to use a community of dung beetle species that approximated in species composition and numbers per pat to what can be seen in climatically similar parts of Australia (Houston *et al.*, 1982; Kriticos, 2012): two *G. spiniger*, 40 *Onthophagus binodis* Thunberg (Coleoptera: Scarabaeidae), and 40 *Digitonthophagus gazella* (F) (Coleoptera: Scarabaeidae). Each species had a 50:50 sex ratio per cage. We added the dung beetle community to each dung + beetle cage immediately after the dung pat had been dropped. This trial began on 14 March and ran for 84 days, allowing sufficient time for the infective third-stage (L_3) *Cooperia* sp. larvae to emerge from dung and disperse onto pasture foliage (Bryan, 1976).

We cut foliage to within 2–3 cm of ground level using a hand-held grass trimmer every 14 days for the first three sample dates, then every 21 days for the remaining two sample dates, which allowed sufficient regrowth before cutting as conditions cooled into autumn. To minimise cross-contamination between cages and treatments, we washed the trimmer and sterilised it with 95% ethanol, and used new disposable latex gloves prior to sampling each cage. The foliage from each cage was individually packaged in sealed polythene bags, weighed, and kept at 4 °C until processing.

Dry conditions will reduce or stop the migration of L_3 nematode larvae onto herbage (Boom & Sheath, 2008). We therefore added 3 litres of water to each cage 2 days prior to the start of the trial to facilitate nematode migration using a watering can. Thereafter, sufficient rainfall occurred between sampling dates to allow nematode migration. Nevertheless, we checked whether soil moisture levels were suitable for nematode migration by taking three 15-mm-diameter by 100-mm-deep soil cores from each plot on all sample dates. These were weighed wet, and again after drying for 48 h at 100 °C.

We applied a blind sampling regime to all samples to offset any bias in processing, extraction, and counting of nematodes. If the foliage sample was less than 50 g, we processed the whole sample; for larger samples we used a 50 g subsample. We adapted a nematode extraction method based on the Whitehead and Hemming (1965) tray method for foliage use. We washed the resulting nematode suspension through two stacked Endecotts™ sieves (London, U.K.), with mesh sizes of 2 mm and 38 μm, and then rinsed the 38-μm sieve, creating a 50-ml suspension of the nematodes. Both sieves were washed between processing of each sample to avoid cross-contamination of samples.

We used a counting microscope slide at 200× magnification to score the numbers of nematode larvae in three 1-ml subsamples from each processed 50-ml nematode suspension. We used a new disposable plastic pipette to transfer each subsample from a suspension to the counting slide. We calculated the total number of nematodes per cage at each sample date from the numbers in the 1-ml subsamples, and from the foliage subsample for the larger foliage samples.

Data analysis

Surface runoff/soil loss trial. We used two-way ANOVAS to investigate the effect of treatment and soil type on total runoff volume and total sediment load in the runoff for each rainfall treatment (extreme and less extreme). We checked all variables used in the statistical analyses for homogeneity of variances (using the Fligner–Killeen test of homogeneity of variances; Conover *et al.*, 1981), and applied log transformations where appropriate. If a treatment effect was statistically significant, we used the Tukey honest significant difference (HSD) method to make pairwise comparisons to identify which means were significantly different from each other. Total sediment data in the less extreme rainfall simulation were right-skewed and zero-inflated (most values in the dung + beetle treatment were zero, but none of the values in the dung-only treatment were zero) and failed to meet the assumptions for performing an analysis of variance even after $\log(n + 1)$ transformation, so we used a Kruskal–Wallis one-way non-parametric ANOVA followed by a pairwise Wilcoxon rank sum test with Bonferroni correction to compare between treatments.

Nematode recovery trial. To investigate the influence of soil moisture we ran a repeated-measures ANOVA on the numbers of nematodes recovered on the sampling dates including soil moisture as a covariate. We calculated total nematode numbers in each cage by summing the data for all sample dates, and performed a two-way ANOVA to investigate the effect of treatment and soil type on nematode numbers. We applied a rank transformation on the nematode data prior to analysis as the raw data violated the assumptions of both normality and homoscedasticity (Olejnik & Algina, 1984).

Analyses for the surface runoff/soil loss trial were performed using the R statistical program (version 3.2.1; R Foundation for Statistical Computing, Vienna, Austria), except for the Kruskal–Wallis one-way non-parametric ANOVA, which was performed using GENSTAT (18th Edition; VSN International Ltd, Hemel Hempstead, UK), which was also used to perform the repeated-measures ANOVA in the nematode recovery trial, while the two-way ANOVA was performed using R.

Results

Surface runoff/soil loss trial.

Mean runoff volume was much greater after extreme than after less extreme simulated rainfall (Fig. 1a,c). Under both the extreme and less extreme simulated rainfalls, the mean volume of runoff varied significantly between soil types (extreme, $F_{2,18} = 24.46$, $P < 0.001$; less extreme, $F_{2,18} = 18.75$, $P < 0.001$) and treatments (extreme, $F_{2,18} = 19.00$, $P < 0.001$; less extreme, $F_{2,18} = 20.17$, $P < 0.001$) (Fig. 1a,c). Interactions between soil type and treatment were non-significant for the extreme simulated rainfall ($F_{4,18} = 1.59$, $P = 0.22$) but significant for the less extreme rainfall simulation ($F_{4,18} = 3.13$, $P = 0.04$). This weak interaction was caused by some inexplicable variation between the dung-only and control treatments: runoff volume was consistently lowest in the dung + beetle treatment and was highest in the dung-only treatment on sandy loam and compacted clay, but at the clay loam site runoff was highest in the control treatment.

Comparing dung-only with dung + beetle treatments showed that the presence of dung beetles resulted in significantly lower runoff volume under both the extreme rainfall simulation (Fig. 1a; 49% reduction) and the less extreme rainfall simulation (Fig. 1c; 81% reduction). Reductions in runoff occurred across all three soil types, but were consistently high (≥ 89%) in the sandy loam soil, rather lower (≤ 56%) in the clay loam, and quite variable between rainfall scenarios in the compacted clay soil (Table 1).

The mean amounts of sediment in runoff under the extreme rainfall simulation were not significantly different between treatments (Fig. 1b; $F_{2,18} = 0.09$, $P = 0.92$). In contrast, under the less extreme rainfall simulation there were significantly lower amounts of sediment in runoff from the dung + beetle treatments than from the dung-only and control treatments [Fig. 1d; Kruskal–Wallis one-way ANOVA; H (adjusted for ties) = 11.21, d.f. = 2, $P = 0.004$). Comparing the dung-only with the dung + beetle treatments showed that the presence of dung beetles resulted in a 97% reduction in mean sediment amount in runoff in the less extreme rainfall simulation (Fig. 1d). Breaking this overall mean down by soil type gave reductions of 73–100%, but with no obvious pattern across soils of differing permeability (Table 1).

Nematode recovery trial.

There were peaks in recovered L_3 nematodes from the cut foliage on each soil type on the two successive samples at days 28 and 42 (Fig. 2). A repeated-measures ANOVA on nematode numbers revealed that there were significant effects of sampling date ($F_{4,23} = 9.93$, $P = 0.009$) and treatment ($F_{2,4} = 13.35$, $P = 0.017$) but no significant effect of soil moisture ($F_{1,23} = 0.18$, $P = 0.43$), and no significant sampling date–treatment interaction ($F_{8,23} = 0.72$, $P = 0.56$). The average moisture contents (range) at the sandy loam site, the clay loam site and the compacted clay site, through the course of the trial, were 37.1% (34.3–39.1), 28.7% (26.3–33.5), and 31.4% (27.5–34.2), respectively. The variability in the numbers of recovered nematodes across the sampling dates made the comparison between treatments difficult, so the analysis was continued using summed nematode numbers across all sample

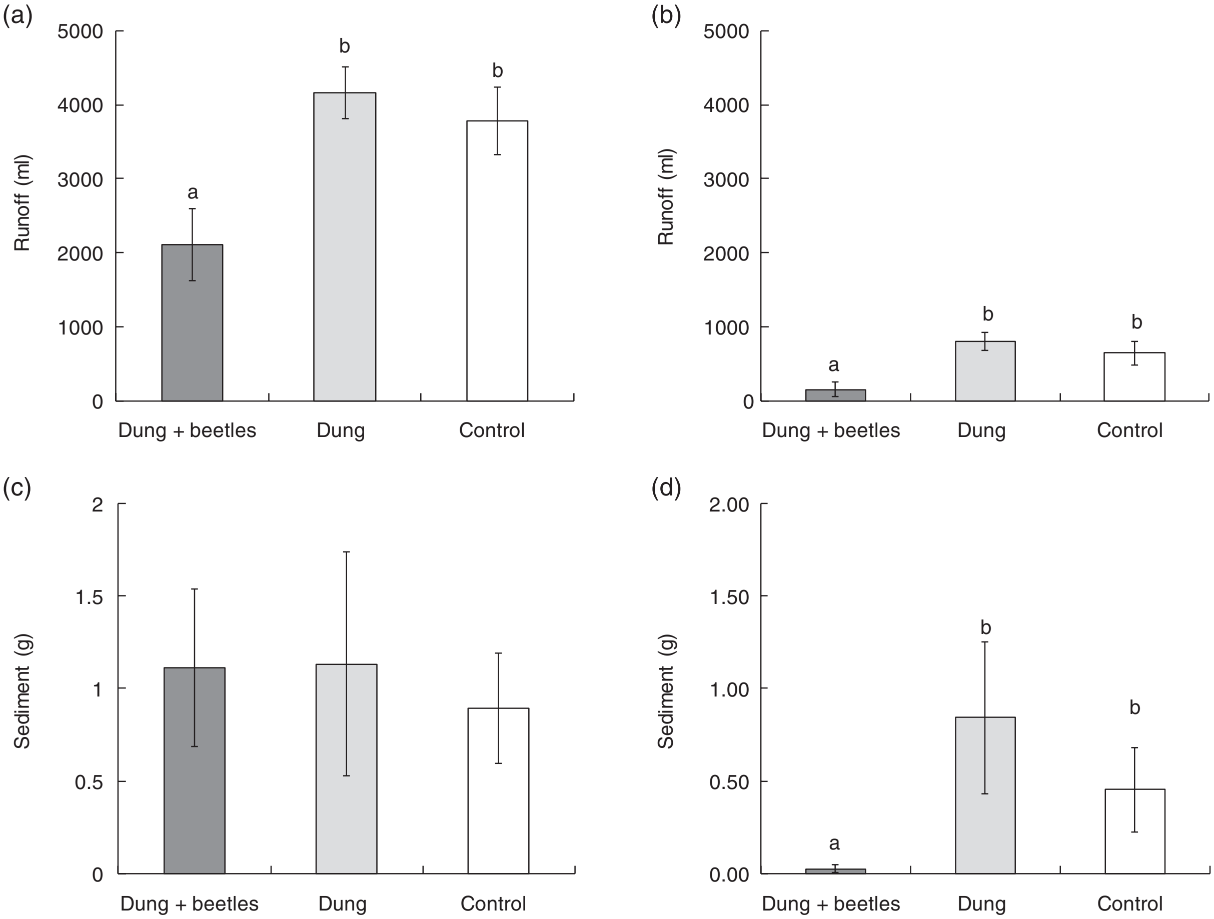

Fig. 1. Mean volume of runoff (a, b) and dry weight of sediment (c, d) in the runoff for each treatment (dung + beetles, dung-only, control) in the extreme rainfall simulation (a, c) and the less extreme rainfall simulation (b, d). Columns with a different letter are significantly different (Tukey's honest significant difference). Error bars = SEM.

dates. This showed that the total numbers of L_3 nematodes recovered from cut foliage did not vary significantly between soil types ($F_{2,18} = 0.56$, $P = 0.58$), but there was a significant treatment effect (Fig. 3; $F_{2,18} = 37.08$, $P < 0.001$). *Post hoc* tests (Tukey HSD) showed significant differences in summed L_3 nematode numbers between all three treatments (Fig. 3). Despite little difference between total nematode numbers in the dung only and dung + beetle treatments at the sandy loam site (Fig. 3a) compared with the substantial differences between these treatments at the clay loam and compacted clay sites (Fig. 3b,c), the interaction between soil type and treatment was not significant ($F_{4,18} = 1.68$, $P = 0.20$). Across all sites, the mean number of nematodes in the dung + beetle treatment was 71% lower than in the dung-only treatment.

Discussion

Limited availability of beetles restricted the level of replication in this study. Nevertheless, we detected significant impacts of dung beetle activity on both surface runoff and the infective stages of parasitic nematodes.

Surface runoff/soil loss trial

We found that dung beetle activity resulted in reductions in runoff in both extreme and less extreme rainfall simulations, although there was some variability in the scale of the reduction across the different soil types. Nevertheless, there were no cases where dung beetle activity increased runoff, and in some cases in the less extreme rainfall simulation the reduction in runoff was 100% as a result of dung beetle activity. Two previous studies show similar effects: Brown *et al.* (2010), found that dung beetle activity increased infiltration and soil porosity; and Doube (2008) reported that water infiltration was significantly faster in plots where *Bubas bison* L. dung beetles had buried dung.

We found that the total amounts of sediment in the runoff were either the same when dung beetles were present as they were in dung-only or control treatments (extreme rainfall simulation), or were significantly reduced (less extreme rainfall simulation) for all soil types By contrast, Brown *et al.* (2010) measured higher inorganic soil losses in plots with dung + beetles versus controls with no dung when simulated rainfall was applied 2–3 days after dung deposition. This difference may in part reflect the contrasting experimental approaches; Brown *et al.* (2010) measured the

Table 1. Summary data comparing dung-only and dung + beetle treatments at each study site.

Trial/treatment	Mean values (SEM) Sandy loam (Shelly Beach)	Clay loam (Kumeu)	Compacted clay (Kaipara Flats)
Runoff volume (ml): extreme rainfall*			
Dung only	3050 (76)	4297 (435)	5150 (436)
Dung + beetles	341 (256)	3330 (511)	2667 (309)
Change with beetles	−89%	−22%	−48%
Runoff volume (ml): less extreme rainfall*			
Dung-only	923 (261)	1003 (142)	490 (119)
Dung + beetles	26 (16.7)	439 (202)	0 (0)
Percentage change with beetles	−97%	−56%	−100%
Sediment amount (mg): extreme rainfall			
Dung-only	0.33 (0.11)	0.12 (0.05)	2.95 (1.39)
Dung + beetles	0.43 (0.21)	0.42 (0.20)	2.49 (0.82)
Percentage change with beetles	–	–	–
Sediment amount (mg): less extreme rainfall*			
Dung-only	2.12 (0.78)	0.28 (0.12)	0.04 (0.02)
Dung + beetles	0 (0)	0.07 (0.06)	0 (0)
Percentage change with beetles	−100%	−73%	−100%

∗Treatment effect significant.

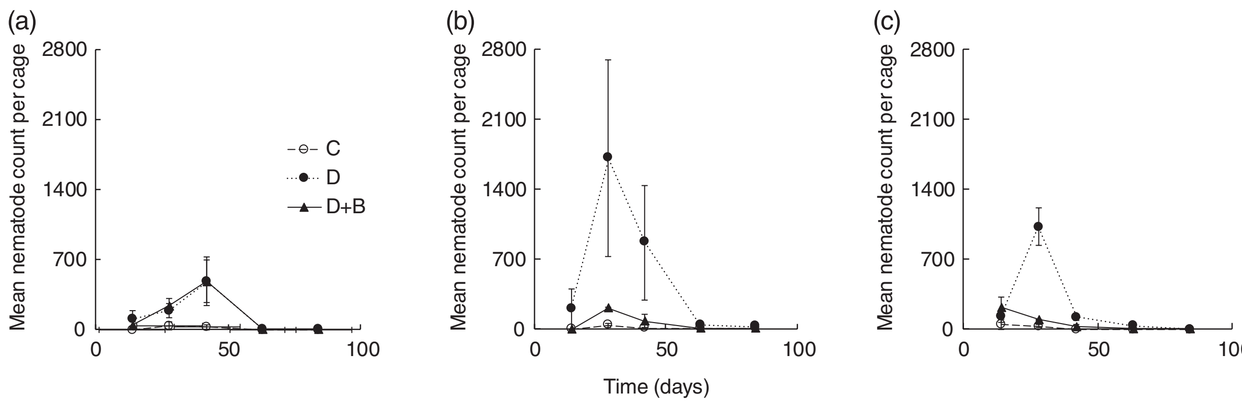

Fig. 2. Mean numbers of nematode larvae (*Cooperia* sp.) per cage in the three treatments: (C, control; D, dung-only; D + B, dung + beetles) on each sampling date at the three sites: (a) sandy loam (Shelly Beach); (b) clay loam (Kumeu); (c) compacted clay (Kaipara Flats). Error bars = SEM.

pre-runoff amount (i.e. the volume of rainfall required to saturate the soil and induce runoff), guaranteeing that runoff occurred. The current study applied a set volume of simulated rainfall and in the less extreme rainfall simulation, the runoff (and associated suspended sediment) was reduced to very low levels or zero in the dung + beetle treatment. Initially, the increased concentration of sediment in runoff is not surprising because the burying activity of dung beetles leaves small piles of loose soil on the pasture surface (J. Brown, pers. comm.). A similar effect is reported with earthworms (Sharpley *et al.*, 1979). With dung beetles, this increase in sediment in runoff due to soil casts is temporary: Brown *et al.* (2010) found no effect of dung beetles when sediment loss was measured 6 months after the dung treatment was applied. Similarly, Doube (2005) showed that dissolved organic carbon and dissolved nitrate were significantly lower in runoff from dung + beetle treatments after 3 months compared with dung-only treatments.

Two other major differences between our study and that of Brown *et al.* (2010) are that the latter only measured inorganic sediment in runoff and did not have a dung-only treatment that could be compared with the 'dung + beetles' treatment. To resolve some of the conflicting results on soil losses or sediment in runoff after dung beetle activity, a more detailed set of experiments are needed. These could measure total sediment loss (inorganic and organic) and nutrient content in surface runoff from rainfall events at short (days) to longer (weeks/months) time intervals after dung pats have been deposited in the presence or absence of dung beetles, under a range of environmental conditions. Nevertheless, all studies to date concur that the overall impact of dung beetles is to cause net reductions in volume of runoff, and hence have the potential to reduce overland flows of potential pollutants. This could potentially have major benefits; for example, Dymond *et al.* (2016) modelled the potential impact of dung beetles on the fate and transport of *Escherichia coli* (Migula) and concluded that introduction of dung beetles would minimise sources connected to waterways by overland flow, based on dung removal alone. The current study indicates that by reducing

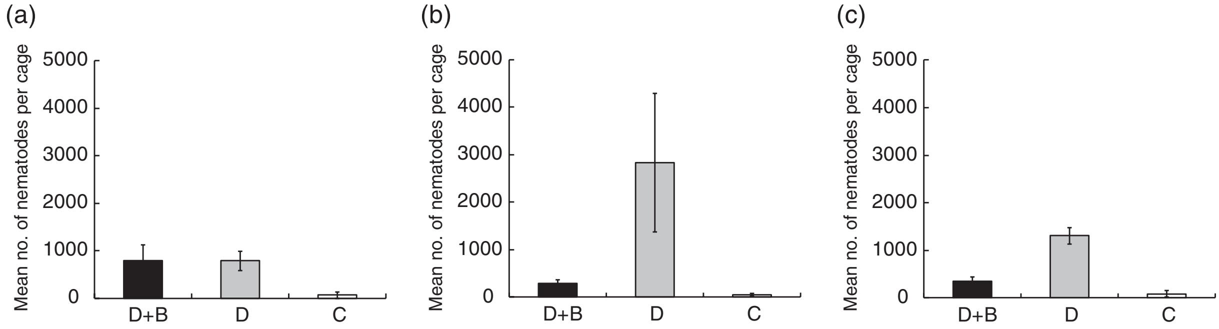

Fig. 3. Mean totals of L_3 *Cooperia* sp. nematodes per cage (summed across the five sample dates) for each treatment (C = control; D = Dung-only; D + B = dung + beetles) in the nematode recovery trial for: (a) sandy loam (Shelly Beach); (b) clay loam (Kumeu); (c) compacted clay (Kaipara Flats). Error bars = SEM.

runoff, the impact of dung beetles on overland flow of faecal material may be greater than Dymond *et al.* (2016) estimated. However, there is also the issue of the fate of the additional volumes of water infiltrating the soil after dung beetle activity. In particular, the tunnels of deep-burrowing dung beetles could potentially create routes for preferential flows (Tompkins *et al.*, 2012), taking infiltrated water directly into groundwater in an analogous way to the concerns over deep-burrowing, anecic earthworm species (Lachnicht *et al.*, 1997; Bardgett *et al.*, 2001; Dominguez *et al.*, 2001; Bardgett & Wardle, 2010). Trials using large, deep soil cores (lysimeters) are planned for New Zealand and Australia to address this issue for deep-burrowing dung beetles, but preliminary results in New Zealand have been very encouraging with no elevated levels of C, N or *E. coli* in leachate collected from the base of 600-mm-deep soil cores in an allophanic soil when dung + beetle treatments were compared with dung-only treatments (Aislabie *et al.*, 2016).

Nematode recovery trial

Our results indicate that dung beetle activity generally reduced numbers of the infective L_3 larvae of the parasitic nematode *Cooperia* sp. on pasture foliage compared with situations where dung was left undisturbed on the pasture surface. Moreover, we found no evidence of the so-called ‘time bomb’ effect when enhanced nematode survival in buried dung compared with dung on the surface could, in theory, result in higher numbers of L_3 on grass foliage (Coldham, 2011). Although the interaction between farm/soil type and treatment was not statistically significant, peak L_3 numbers were similar between the dung + beetle and dung-only treatments on the sandy loam soil. Further studies to investigate whether impacts of dung beetles on parasitic nematodes vary according to soil type are therefore desirable. For example, sandy soils allow more migration of L_3 nematodes from buried dung than do heavier clay soils (Lucker, 1936; Lucker, 1938). Nevertheless, the results of the current study are consistent with the majority of international studies which show decreases in L_3 numbers as a result of dung beetle activity (Reinecke, 1960; Bryan, 1973; Waterhouse, 1974; English, 1979; Bryan & Kerr, 1989; Hutchinson *et al.*, 1989; Sands & Wall, 2016).

Conclusions and caveats

Although limited in factors such as scale/replication, time frame, geographic range and dung beetle species, our results add to the literature showing that there are important ecosystem service benefits from dung beetles (e.g. Slade *et al.*, 2007; Beynon *et al.*, 2015; Manning *et al.*, 2016; Manning *et al.*, 2017; Piccini *et al.*, 2017). These findings are relevant both to agricultural systems where native dung beetle faunas are at risk from intensification of agriculture (Hutton & Giller, 2003) or systems where there are naturally impoverished dung beetle faunas, with active release programmes aiming to increase species richness (Edwards, 2009; Forgie *et al.*, 2014). If an entire country has a naturally impoverished pastoral dung beetle fauna, then many species of dung beetle may require to be introduced to achieve improvements in ecosystem services over seasons and in pastures that will vary in factors such as farming types, soils, or climates (Tyndale-Biscoe, 1994; Edwards, 2009). Furthermore, evidence suggests that more diverse dung beetle communities can provide better benefits to ecosystem services than comparatively species-poor communities, but that species composition effects can be complex and warrant further study (Slade *et al.*, 2007; Beynon *et al.*, 2015; Manning *et al.*, 2016; Manning *et al.*, 2017; Piccini *et al.*, 2017; Slade *et al.*, 2017).

Acknowledgements

Jacqueline Beggs (Auckland University) and members of the Dung Beetle Technical Advisory Group commented on the experimental design and/or earlier versions of the manuscript. Comments from two anonymous referees greatly improved the manuscript. Ian Sutherland and Sheralee Cleland (both AgResearch) supplied dung containing known amounts of nematode eggs. Members of the Dung Beetle Release Strategy Group provided the field sites, fencing, land access, and other general assistance. Funding was provided by the New Zealand Government through a Sustainable Farming Fund grant from the Ministry of Primary Industries (no. 09-079) and by Landcare Research core funding from the Ministry of Business, Innovation and Employment.

The project was designed by SAF, QP and SVF; data were collected by SAF and CF; nematode extractions and scoring

were done by Z Zhao; data analysis was done by Q. Paynter; and writing of the manuscript was done (in order of contribution) by QP, SVF, and SAF.

In the interest of full disclosure, SAF was employed by Dung Beetle Innovations, a company that sells dung beetles to New Zealand landowners, at the time of writing the manuscript but not during experimental design or analysis.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/een.12513

Figure S1. Cross-section and further explanation of a cage used in the runoff/soil loss trial. See also the photograph in Figure S3.

Figure S2. Cross-section and further explanation of the apparatus designed to provide artificial rainfall in the experimental cages. See also the photograph in Figure S3.

Figure S3. Field cage, with mesh lid in place (left image) and rainfall simulator in place above a field cage set up in the laboratory. Note the PVC pipe leading to a bucket on the downslope to collect runoff (right image). The mesh lids were removed while the rainfall simulation was carried out, and then replaced.

Figure S4. Example of the experimental setup at a field site. D, dung-only; D + B, dung + beetles; C, control. Note that treatments were allocated randomly and the positions of the different treatments varied between sites.

References

Aislabie, J., McLeod, M., McGill, A., & Rhodes, P. (2016). *Quantifying Dung Beetle Benefits in NZ Livestock Pastures: Final Leaching Report*. Unpublished report by Landcare Research for Agrilink New Zealand Ltd. LC2976. 32 p. Landcare Research, Hamilton, New Zealand.

Bardgett, R.D. & Wardle, D.A. (2010) *Aboveground-Belowground Linkages: Biotic Interactions, Ecosystem Processes, and Global Change*. Oxford University Press, Oxford, U.K.

Bardgett, R.D., Anderson, J., Behan-Pelletier, V., Brussaard, L., Coleman, D., Ettema, C. *et al.* (2001) The influence of soil biodiversity on hydrological pathways and the transfer of materials between terrestrial and aquatic ecosystems. *Ecosystems*, **4**, 421–429.

Beynon, S.A., Wainwright, W.A. & Christie, M. (2015) The application of an ecosystem services framework to estimate the economic value of dung beetles to the U.K. cattle industry. *Ecological Entomology*, **40**, 124–135.

Boom, C. & Sheath, G. (2008) Migration of gastrointestinal nematode larvae from cattle faecal pats onto grazable herbage. *Veterinary Parasitology*, **157**, 260–266.

Brown, J., Scholtz, C.H., Janeau, J.-L., Grellier, S. & Podwojewski, P. (2010) Dung beetles (Coleoptera: Scarabaeidae) can improve soil hydrological properties. *Applied Soil Ecology*, **46**, 9–16.

Bryan, R. (1973) The effects of dung beetle activity on the numbers of parasitic gastrointestinal helminth larvae recovered from pasture samples. *Crop and Pasture Science*, **24**, 161–168.

Bryan, R. (1976) The effect of the dung beetle, *Onthophagus gazella*, on the ecology of the infective larvae of gastrointestinal nematodes of cattle. *Crop and Pasture Science*, **27**, 567–574.

Bryan, R. & Kerr, J. (1989) Factors affecting the survival and migration of the free-living stages of gastrointestinal nematode parasites of cattle in central Queensland. *Veterinary Parasitology*, **30**, 315–326.

Chirico, J., Wiktelius, S. & Waller, P.J. (2003) Dung beetle activity and the development of trichostrongylid eggs into infective larvae in cattle faeces. *Veterinary Parasitology*, **118**, 157–163.

Cock, M.J., Murphy, S.T., Kairo, M.T., Thompson, E., Murphy, R.J. & Francis, A.W. (2016) Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. *BioControl*, **61**, 349–363.

Coldham, J. (2011) *Dung Beetles and Internal Parasites of Sheep*. Final report on project B.PRS.0502/ S2005/NO3. Meat & Livestock Australia Ltd, Sydney, Australia.

Conover, W.J., Johnson, M.E. & Johnson, M.M. (1981) A comparative study of tests for homogeneity of variances, with applications to the outer continental shelf bidding data. *Technometrics*, **23**, 351–361.

Dominguez, J., Edwards, C.A. & Dominguez, J. (2001) The biology and population dynamics of *Eudrilus eugeniae* (Kinberg)(Oligochaeta) in cattle waste solids. *Pedobiologia*, **45**, 341–353.

Doube, B. (2005) *The Impact of the Activity of the Late Summer/Autumn-active Dung Beetle* Geotrupes spiniger *on Soil and Water Properties in the Field*. Final report, Report for DairySA, 23 p. Dung Beetle Solutions Australia, Adelaide, Australia.

Doube, B. (2008) *The Pasture Growth and Environmental Benefits of Dung Beetles to the Southern Australian Cattle Industry*. Meat & Livestock Industry, North Sydney, Australia. ISBN: 2101379822.

Durie, P. (1975) Some possible effects of dung beetle activity on the infestation of pastures by intestinal worm larvae of cattle. *Journal of Applied Ecology*, **12**, 827–831.

Dymond, J.R., Serezat, D., Ausseil, A.-G.E. & Muirhead, R.W. (2016) Mapping of *Escherichia coli* sources connected to waterways in the Ruamahanga catchment, New Zealand. *Environmental Science & Technology*, **50**, 1897–1905.

Edwards, P. (2009) *Introduced Dung Beetles in Australia 1967–2007: Current Status and Future Directions*. Dung Beetles for Landcare Farming Committee, Sinnamon Park, Australia. ISBN: 9780980627107.

English, A. (1979) The effects of dung beetles (Coleoptera-Scarabaeinae) on the free-living stages of strongylid nematodes of the horse. *Australian Veterinary Journal*, **55**, 315–321.

Forgie, S.A., Dymock, J.J. & Tompkins, D.M. (2014) No evidence that brushtail possums (*Trichosurus vulpecula*) forage on dung beetles (Coleoptera: Scarabaeidae) in New Zealand. *New Zealand Journal of Zoology*, **41**, 95–102.

Fowler, S.V., Paynter, Q., Dodd, S. & Groenteman, R. (2012) How can ecologists help practitioners minimize non-target effects in weed biocontrol? *Journal of Applied Ecology*, **49**, 307–310.

Hajek, A.E., Hurley, B.P., Kenis, M., Garnas, J.R., Bush, S.J., Wingfield, M.J. *et al.* (2016) Exotic biological control agents: a solution or contribution to arthropod invasions? *Biological Invasions*, **18**, 953–969.

Hanski, I. & Cambefort, Y. (1991) *Dung Beetle Ecology*. Princeton University Press, Princeton, New Jersey.

Holt, R. & Hochberg, M. (2001) Indirect interactions, community modules and biological control: a theoretical perspective. *Evaluating Indirect Ecological Effects of Biological Control* (ed. by E. Wajnberg, J. K. Scott and P. C. Quimby), pp. 13–37. CABI Publishing, Wallingford, U.K.

Houston, W.W.K., Feehan, J.E. & Runko, S. (1982) Methods for harvesting large numbers of dung beetles (Coleoptera: Scarabaeidae). *Australian Journal of Entomology*, **21**, 217–219.

Hughes, R.D., Ferrar, P., Macqueen, A., Durie, P., McKinney, G.T. & Morley, F.H.W. (1975) Introduced dung beetles and Australian pasture ecosystems: papers presented at a symposium during the meeting of the Australia and New Zealand Association for the Advancement of Science at Canberra in January 1975. *Journal of Applied Ecology*, **12**, 819–837.

Hutchinson, G., Abba, S. & Mfitilodze, M. (1989) Seasonal translation of equine strongyle infective larvae to herbage in tropical Australia. *Veterinary Parasitology*, **33**, 251–263.

Hutton, S.A. & Giller, P.S. (2003) The effects of the intensification of agriculture on northern temperate dung beetle communities. *Journal of Applied Ecology*, **40**, 994–1007.

Kremen, C. & Chaplin-Kramer, R. (2007) Insects as providers of ecosystem services: crop pollination and pest control. *Insect Conservation Biology: Proceedings of the Royal Entomological Society's 23rd Symposium* (ed. by A. J. A. Stewart, T. R. New and O. T. Lewis), pp. 349–382. CABI Publishing, Wallingford, U.K.

Kriticos, D. (2012) Regional climate-matching to estimate current and future sources of biosecurity threats. *Biological Invasions*, **14**, 1533–1544.

Lachnicht, S., Parmelee, R., McCartney, D. & Allen, M. (1997) Characteristics of macroporosity in a reduced tillage agroecosystem with manipulated earthworm populations: implications for infiltration and nutrient transport. *Soil Biology and Biochemistry*, **29**, 493–498.

Lobo, J. (1996) Diversity, biogeographical considerations and spatial structure of a recently invaded dung bettle (Coleoptera: Scarabaeoidea) community in the Chihuahuan Desert. *Global Ecology and Biogeography Letters*, **5**, 342–352.

Losey, J.E. & Vaughan, M. (2006) The economic value of ecological services provided by insects. *Bioscience*, **56**, 311–323.

Lucker, J. (1936) Extent of vertical migration of horse strongyle larvae in soils of different types. *Journal of Agricultural Research*, **52**, 353–361.

Lucker, J.T. (1938) Vertical migration, distribution, and survival of infective horse strongyle larvae developing in feces buried in different soils. *Journal of Agricultural Research*, **57**, 335–348.

Manning, P., Slade, E.M., Beynon, S.A. & Lewis, O.T. (2016) Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. *Agriculture, Ecosystems & Environment*, **218**, 87–94.

Manning, P., Slade, E.M., Beynon, S.A. & Lewis, O.T. (2017) Effect of dung beetle species richness and chemical perturbation on multiple ecosystem functions. *Ecological Entomology*, **42**, 577–586.

Montes de Oca, E. & Halffter, G. (1998) Invasion of Mexico by two dung beetles previously introduced into the United States. *Studies on Neotropical Fauna and Environment*, **33**, 37–45.

Nichols, E. & Gomez, A. (2014) Dung beetles and fecal helminth transmission: patterns, mechanisms and questions. *Parasitology*, **141**, 614–623.

Nichols, E., Spector, S., Louzada, J., Larsen, T., Amezquita, S. & Favila, M.E. (2008) Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. *Biological Conservation*, **141**, 1461–1474.

Noriega, J.A., Delgado, O., Blanco, J.I., Gámez, J. & Clavijo, J. (2017) Introduction, establishment, and invasion of *Digitonthophagus gazella* (Fabricius, 1787)(Coleoptera: Scarabaeinae) in the savannas of Venezuela and Colombia. *Natural Resources*, **8**, 370–381.

Olejnik, S.F. & Algina, J. (1984) Parametric ANCOVA and the rank transform ANCOVA when the data are conditionally non-normal and heteroscedastic. *Journal of Educational Statistics*, **9**, 129–149.

Piccini, I., Arnieri, F., Caprio, E., Nervo, B., Pelissetti, S., Palestrini, C. *et al.* (2017) Greenhouse gas emissions from dung pats vary with dung beetle species and with assemblage composition. *PLoS One*, **12**, e0178077.

Reinecke, R. (1960) A field study of some nematode parasites of bovines in a semi-arid area, with special reference to their biology and possible methods of prophylaxis. *Onderstepoort Journal of Veterinary Research*, **28**, 365–464.

Russo, L. (2016) Positive and negative impacts of non-native bee species around the world. *Insects*, **7**, 69.

Sands, B. & Wall, R. (2016) Dung beetles reduce livestock gastrointestinal parasite availability on pasture. *Journal of Applied Ecology*, **54**, 1180–1189. https://doi.org/10.1111/1365-2664.12821.

Sharpley, A., Syers, J. & Springett, J. (1979) Effect of surface-casting earthworms on the transport of phosphorus and nitrogen in surface runoff from pasture. *Soil Biology and Biochemistry*, **11**, 459–462.

Simmons, L.W. & Ridsdill-Smith, T.J. (2011) *Ecology and Evolution of Dung Beetles*. Wiley-Blackwell, Oxford, U.K.

Slade, E.M., Mann, D.J., Villanueva, J.F. & Lewis, O.T. (2007) Experimental evidence for the effects of dung beetle functional group richness and composition on ecosystem function in a tropical forest. *Journal of Animal Ecology*, **76**, 1094–1104.

Slade, E.M., Kirwan, L., Bell, T., Philipson, C., Lewis, O.T. & Roslin, T. (2017) The importance of species identity and interactions on multifunctionality depends on how ecosystem functions are valued. *Ecology*, **98**, 2626–2639.

Sutherland, I. & Scott, I. (2009) *Gastrointestinal Nematodes of Sheep and Cattle: Biology and Control*. John Wiley & Sons, Chichester, U.K.

Tompkins, D., Forgie, S., Aislabie, J., Nugent, G., Gourlay, H., McGill, A., *et al.* (2012) *Informing the Infectious Disease Risks of Dung Beetle Releases into New Zealand*. Landcare Research Internal Report LC908, prepared as part of the Capability Fund CF1112 95 04, 17 p. Landcare Research, Lincoln, New Zealand.

Tyndale-Biscoe, M. (1994) *Cropping and Distribution of Exotic Dung Beetles in South Australia*. Final Report, Dairy Research and Development Corporation, Australia.

Van Driesche, R., Hoddle, M. & Center, T.D. (2008) *Control of Pests and Weeds by Natural Enemies: An Introduction to Biological Control*. Blackwell Publishing, Malden, Massachusetts.

Waghorn, T.S., Leathwick, D., Chen, L.-Y., Gray, R. & Skipp, R. (2002) Influence of nematophagous fungi, earthworms and dung burial on development of the free-living stages of *Ostertagia* (*Teladorsagia*) *circumcincta* in New Zealand. *Veterinary Parasitology*, **104**, 119–129.

Waterhouse, D. (1974) The biological control of dung. *Scientific American*, **230**, 100–109.

Whitehead, A. & Hemming, J. (1965) A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology*, **55**, 25–38.

Winston, R.L., Schwarzländer, M., Hinz, H.L., Day, M.D., Cock, M.J.W. & Julien, M.H. (2014) *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*, 5th edn. USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia. FHTET-2014-04.

Young, O.P. (2007) Relationships between an introduced and two native dung beetle species (Coleoptera: Scarabaeidae) in Georgia. *Southeastern Naturalist*, **6**, 491–504.

Zunino, M. & Barbero, E. (1993) Escarabajos, ganado, pastizales: Algunas consideraciones deontológicas. *Folia Entomológica Mexicana*, **87**, 95–101.

Accepted 26 January 2018
Associate Editor: Simon Leather