

# Dung Beetle Technical Advisory Group Report

## The impact of tunnelling and dung burial by new exotic dung beetles (Coleoptera: Scarabaeinae) on surface run-off, survivorship of a cattle helminth, and pasture foliage biomass in New Zealand pastures.

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### SUMMARY

Eleven species of exotic dung-burying beetles were recently approved for release onto New Zealand agricultural pastures. Despite the formal assessment of risks and benefits by the Environmental Risk Management Authority in 2010/11, several stakeholders raised concerns over whether some of the key benefits demonstrated in overseas studies were applicable to New Zealand's pastoral environment. As a result two New Zealand-based field trials were carried out 1/ to determine the effect of dung beetle activity on surface run-off and the amount of sediments suspended in the run-off; 2/ to assess the recovery of parasitic helminths from pasture around dung from infected livestock; and 3/ measure pasture foliage biomass. The dung beetles, *Geotrupes spiniger* (trials 1 + 2), *Onthophagus binodis* (trial 2) and *Digitonthophagus gazella* (trial 2) were used in the field trials. Secure field cages were used to apply three treatments (dung+beetles, dung-only and controls, without dung or beetles) on three livestock farms with three different soil types: sandy loam, clay loam and compacted clay. Results from trial 1 showed significant reductions in surface run-off at two extreme artificial rainfall events, and in total suspended sediments at the lower level, but still extreme rainfall event. Trial 2 showed reduced levels of *Cooperia* sp. nematodes on pasture foliage in the dung+beetles treatment compared to the dung-only treatment. There was no evidence for a later peak in emergence of nematode larvae from dung buried by beetles – the so-called 'time bomb' effect. There were no significant changes in the biomass of foliar biomass between dung+beetles and dung-only treatments. The findings with run-off and nematodes are consistent with benefits that should accrue from the introduction of the 11 dung beetle species to New Zealand that were claimed in the ERMA application process in 2010/11 based on overseas data. However, the trials were probably too short in duration, and were compromised by the impending winter season, to show any increases in pasture foliar biomass from dung burial by beetles.

### INTRODUCTION

Eleven species of exotic dung-burying beetles were approved for release into New Zealand in 2011 (ERMA 2011) following an example of the ERMA-developed, world-leading, risk assessment versus benefits approach developed in New Zealand (DBSRG 2010; Hill et al 2013). However, this decision has been questioned on the basis that risks were not adequately assessed and benefits overstated.

A technical advisory group (TAG) was set up by the Dung Beetle Release Strategy Group (DBRSG) and held its first meeting in 15<sup>th</sup> May, 2012. At this meeting two field trials were approved to address two of the issues raised by stake-holders. The trials aimed to test whether, under New Zealand conditions:

1. dung beetle activity would reduce surface run-off and the amount of sediments suspended in the run-off; and,
2. dung beetle activity will reduce the numbers of the infective 3<sup>rd</sup> instar larvae of a gastrointestinal parasite on pasture foliage, and hence potential infection rates of livestock.
3. whether the processing and burial of dung by beetles resulted in an increase in above-ground pasture biomass available for stock to graze.

## METHODS

### *Sites*

The trials used three livestock farms with different soils in the greater Auckland region; Shelly Beach, South Kaipara (sandy loam); Kaipara Flats, Warkworth (compacted clays); and Kumeu, NW Auckland (clay loam).

### *Field cages*

Open ended cylinders (55cm diameter; 40cm height; 2375cm<sup>2</sup> cylinder area: Fig 1) were cut from high density plastic drums and hammered into the ground to a depth of 15 cm to ensure a tight seal preventing percolation of water between soil and cylinder. The top of each cylinder was covered with durable nylon meshing secured by a 55.0 cm plastic tapered collar which slotted tightly inside the top of the ring forming a lid. Lids were further secured to the cylinders with stainless steel screws and wing nuts. As an extra precaution, the cages were fenced off from livestock.



**Figure 1.** Cage design used for surface run-off and nematode field trials.

Cages used for rainfall simulations were each equipped with a 25 mm diameter female tank fitting with mesh filter positioned at soil surface height on the downward facing side of the cylinder. A four litre collection bucket with a PVC pipe was placed downslope from the cage to enable collection of surface run-off (Fig 2).



**Figure 2.** Rainfall simulator and cage (lid removed) setup for surface run-off experiments (in pre-trial testing before cages were set up in pasture).

Cages at each site were set up during 20-29<sup>th</sup> September 2012, and 22-28<sup>th</sup> February 2013, for surface run-off and nematode survivorship trials respectively.

### *Rainfall Simulator*

A rainfall simulation device (Fig 2) consisted of an open-topped plastic drum, the same diameter as the field cages, and a stand. One hundred 4 mm diameter holes were made in the base of the drum, and each fitted with single piece irrigation jets that had a 1mm drip diameter. Each jet was modified to accommodate a 150 mm length of nylon string. The string stopped the water flowing freely through the jets, and a small knot at the end of each strand encouraged raindrop formation to occur. Holes were dug into the ground on the uphill side of each field cage for the feet of the stand to sit in, and a digital spirit level used to ensure the drum was level. A scale was drawn on the inside of the drum to measure the water level and standardise and volume of rainfall applied to the cages.

### *Experimental Design*

#### 1. Surface run-off

Twenty-five square meter experimental plots were located on a gently sloping pasture on each of the three farms, each with nine cages. The slope within each cage was measured with a digital spirit level to make sure all 27 cages across the farms were on a 12 degree slope. Each plot per farm contained three treatments replicated three times: dung+beetles, dung-only and control (no dung/beetles). Each dung beetle cage received 10 newly emerged adult *Geotrupes spiniger* dung beetles (5 female, 5 males) in the beginning of October allowing the

beetles to sexually mature in situ. Treatments requiring dung were supplied equally with two litres of fresh organic cattle dung free of chemical residues sourced from Shelly Beach biodynamic farm where one of the sites was located. All collected cow pats were combined and mixed thoroughly to minimise variations in dung quality and dropped from 1 meter to create a realistic pat in each dung treatment once a week. Dung was applied towards the centre of the cage, however the preceding pat was avoided. Rainfall simulations commenced when beetles reached sexual maturity and had begun tunnelling and nest building; evident through the formation of soil casts and significant reductions in dung.

Two levels of simulated rainfall were applied. The first on 28 November 2012, used 12,500 ml applied to each cage over 10 minutes. This represented 40 mm in 10 minutes which equated to a less than 1% annual exceedence probability (aep) event (likely to occur less than once in 100 yrs) across the trial region. The second simulation commenced 18 December 2012 in which 6000 ml over 10 minutes was applied to each cage. This simulation replicated a more likely, but still extreme, rainfall event of approximately 20 mm in 10 minutes. This equates to a range between a 1.7% aep (1 in 60 yrs) in Warkworth, 1.2% aep (1 in 80yr) in Kumeu through to a 1.0% aep (1 in 100 yrs) at Shelly Beach. The run-off and suspended sediments were collected in the buckets attached to each cage. Buckets were disconnected and sealed once all the run-off had passed through the PVC tube. Water volumes were recorded and sediments were separated from the run-off water using Whatman™ filter paper and dried at 60°C for 48 hours before weighing.

At the completion of both simulation trials, adult beetles were collected and returned to the mass rearing facilities and cages + lids were kept securely in place to house any new generation adults emerging from the nests.

## 2. Nematode survivorship

Twenty-five square meter experimental plots were located beside the surface run-off plots on each farm, and set up with 9 new cages with the same combination and number of treatments (see above). Treatments requiring dung were supplied with nematode-infected dung (300 *Cooperia* sp eggs/g). The dung was obtained from nematode-infected cattle dung in an experimental herd at the AgResearch Grassland Research Centre, Palmerston North. A single 2.5 litre allocation of infected dung was dropped from 1 meter onto the pasture in the centre of the cages and each D+B treatment. Immediately following the addition of dung, the dung+beetles treatment cages received a community of dung beetles comprising 2 *Geotrupes spiniger*, 40 *Onthophagus binodis*, and 40 *Digitonthophagus gazella*. Each species had a 50:50 sex ratio.

This trial began on 14<sup>th</sup> March and ran for 84 days, allowing for sufficient time for the infective 3<sup>rd</sup> instar larvae to emerge from dung on the (Bryan 1976).

All foliage within each cage was sampled to 2-3 cm of ground level to simulate grazing height. This method collects most of the L3 larvae migrating horizontally from the faecal mass since the previous sample (Gronvold 1989, cited in Familton & McAnulty 1997).

Foliage was collected by serial sampling every 14 days for the first three sample dates then every 21 days for the remaining two sample dates to allow sufficient regrowth before cutting. To minimise cross contamination a hand held grass trimmer was washed and sterilised between treatments, and new latex gloves worn for handling each treatment sample. Clippings were individually packaged, weighed, and kept at 4°C until processing. Three litres of water was applied to each cage two days prior to the start of the trial to moisten the soil. Thereafter, sufficient rainfall fell during the trial period to prevent desiccation of the cage surface soils. To test this over the course of the trial, three 15mm diameter by 100mm deep soil cores were obtained from each plot on all sample dates. These were wet weighed, dried at 100°C for 48 hours, and then dry weighed to determine soil moisture content.

### *L3 nematode extraction and counting*

A blind sampling regime was applied to all samples to offset any bias in processing, extraction and counting. All samples (50g sub-samples were used if the weight more than 50g) were used for nematode extraction by using the Whitehead and Hemming (1965) tray method. Nematodes were collected by pouring a nematode suspension from each soaked foliage sample through two stacked Endecott™ sieves with 2 mm and 38 micron mesh respectively. Nematodes were thoroughly rinsed from the 38 micron sieve and consolidated into a 50 ml suspension. Both sieves were then washed between each treatment sample. The nematodes were transferred to a glass block for examination using a dissecting microscope. One ml subsample from each processed 50 ml nematode suspension was added to a counting slide for counting at 200 x magnification using a compound microscope. Three 1ml sub samples per 50 ml nematode suspensions were counted to provide a mean number of larvae per gram of foliage from each treatment. New plastic pipettes were used in the transfer of subsamples per 50 ml nematode suspension to eliminate cross contamination. All nematodes recovered were counted and identified using diagnostic keys. The majority were identified as *Cooperia* sp and this identification was verified by the nematologists who supplied the infected dung.

### *Data Analysis*

The data were analysed using Genstat (VSN International) to investigate the effect of treatment (dung + beetles, dung only, control) on 1) Total run-off volume (ml) and; 2) The total amount of sediment (g) in the run-off and 3) The concentration of sediment in the run-off (calculated as the total amount of sediment divided by the total volume of run-off). Separate analyses were performed for each experiment (12,500 ml and 6,000 ml simulated rainfall). A Bartlett's test for homogeneity of variances was performed prior to each analysis. Where there was homogeneity of variances among treatments, analyses of variance were performed with 'Farm' declared as a blocking term, corresponding to the three farms (Kumeu, Shelly Beach, Warkworth). Where the data failed to meet the assumptions for performing an analysis of variance, the data were analysed using Kruskal-Wallis one-way a non-parametric analysis of variance. Additionally, the Mann-Whitney test and post-hoc Mann-Whitney tests with Bonferroni correction were applied to the nematode survivorship

data. For the foliage biomass samples, the effects of site and treatment were modelled using the statistical package ‘R’ (R Development Core Team 2008).

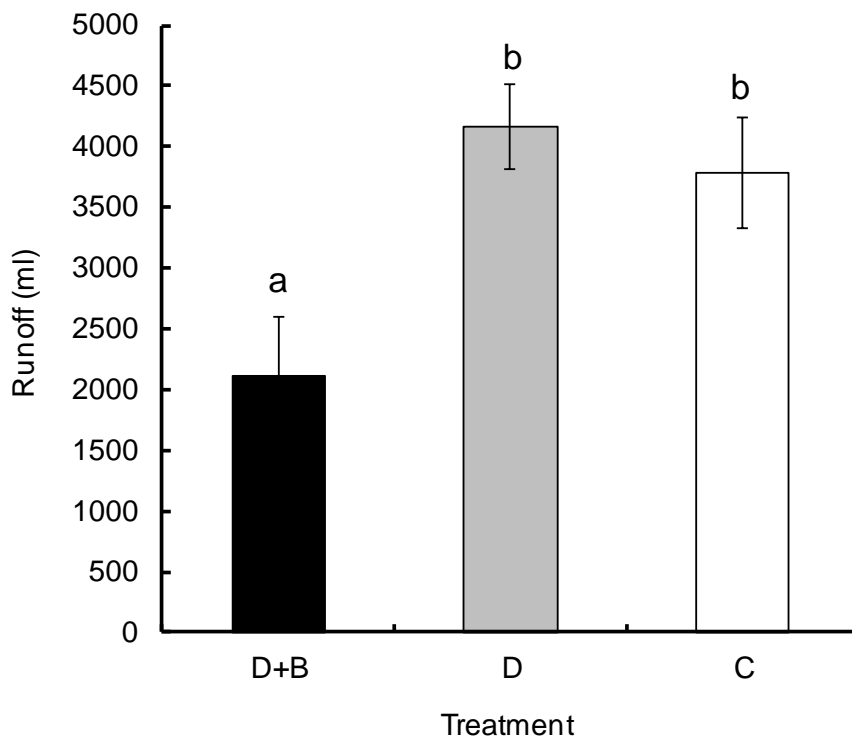
## RESULTS

### 1. Surface run-off

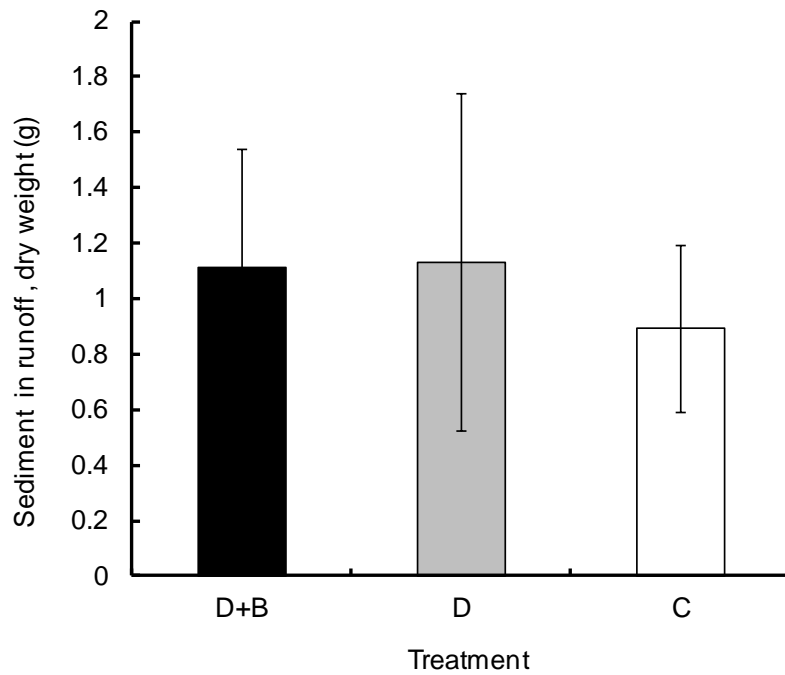
#### *Experiment 1 (12,500 ml simulated rainfall).*

Volume of run-off was similar in the dung-only and control treatments but was significantly lower in the dung+beetles treatments (Fig. 3). Across all sites, the volume of run-off (comparing dung-only with dung+beetles treatments) was reduced from a mean of 4166 ml to 2113 ml (49%). The highest reduction occurred at Shelly Beach (3050 ml versus 341 ml; 89%) and the lowest at Kumeu (4926 ml versus 3300 ml; 33%).

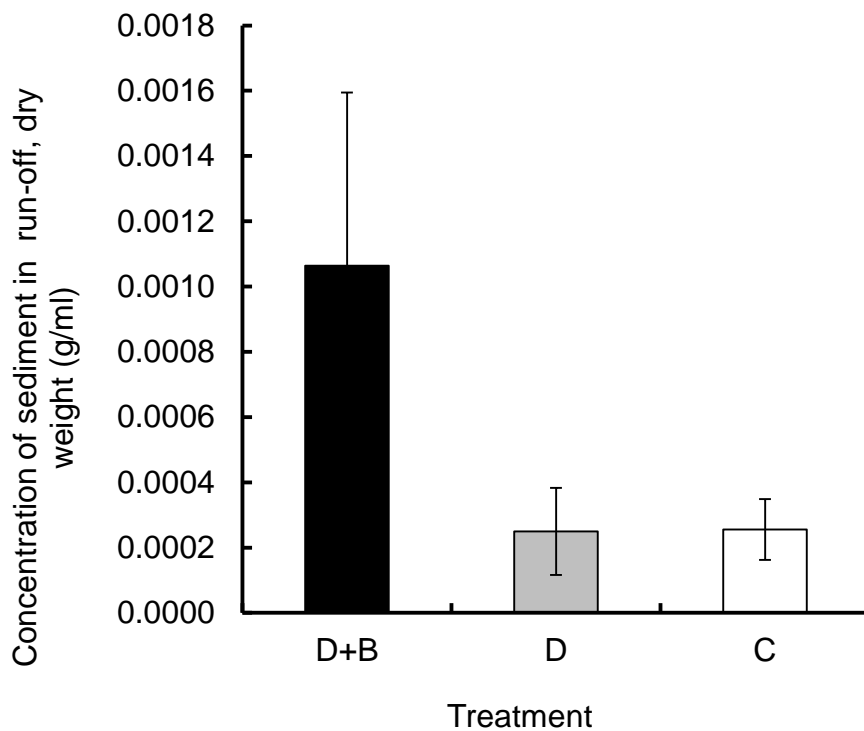
There was no significant difference in the total amount of sediment in the run-off between treatments (Fig. 4). There was a higher concentration of sediment in the reduced volume of run-off coming off the dung+beetles treatment, although the difference between treatments was not statistically significant (Kruskall-Wallis one-way ANOVA,  $H=4.413$ , d.f. = 2, Chi-square probability = 0.110; Fig. 5).



**Figure 3.** Mean amount of run-off for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the 12,500 ml simulated rainfall experiment. Columns with a different letter are significantly different (LSD). Error bars = SEM.



**Figure 4.** Mean amount of sediment in the run-off (g) for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the 12,500 ml simulated rainfall experiment. Error bars = SEM.



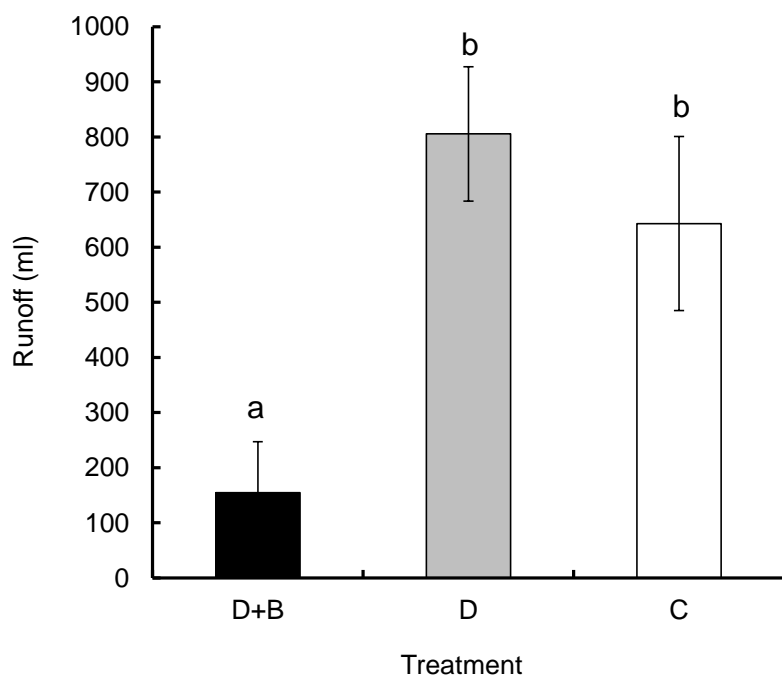
**Fig. 5.** Mean concentration of sediment ( $\pm$  SE) in the run-off (g/ml) for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the 12,500 ml simulated rainfall experiment.

*Experiment 2 (6000 ml simulated rainfall).*

As in the first rainfall simulation, volume of run-off was similar in the dung only and control treatments but was significantly lower in the dung+beetles treatments (Fig. 6.). Across all sites, run-off (comparing dung-only with dung+beetles treatments) was reduced from a mean of 806 ml to 155 ml (i.e. by approximately 80%). The highest reduction occurred at Warkworth (490 ml versus 0 ml; 100%) and lowest at Kumeu (1003 ml versus 439 ml; 56%).

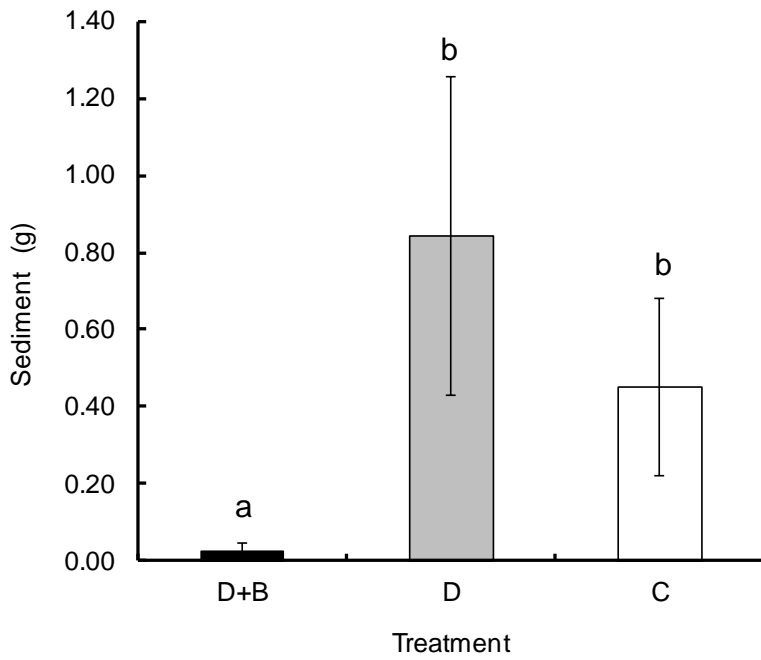
In contrast to the first rainfall simulation, there was also a highly significant difference in the total amount of sediment in the run-off between treatments (Kruskal-Wallis one-way analysis of variance;  $H$  (adjusted for ties) = 11.21, d.f. = 2, Chi-square probability = 0.004). Post-hoc Mann-Whitney tests with Bonferroni correction showed significant differences between the dung+beetles treatment and the dung-only treatment; Fig. 7). Across all sites, total sediment in run-off (comparing dung-only with dung+beetles treatments) was reduced from a mean of 0.84 g to 0.02 g (i.e. by 98%), with the highest reduction at Shelly Beach (2.2 g ml versus 0 g; 100%) and lowest at Kumeu (0.276 g versus 0.074 g; 73%).

The analysis on sediment concentration revealed that there was also a highly significant difference in the concentration of sediment in the run-off between treatments (Kruskal-Wallis one-way analysis of variance;  $H$  (adjusted for ties) = 11.11, d.f. = 2, Chi-square probability = 0.004). Post-hoc Mann-Whitney tests with Bonferroni correction showed significant differences between the dung+beetles treatment and the dung-only treatment; Fig. 8).

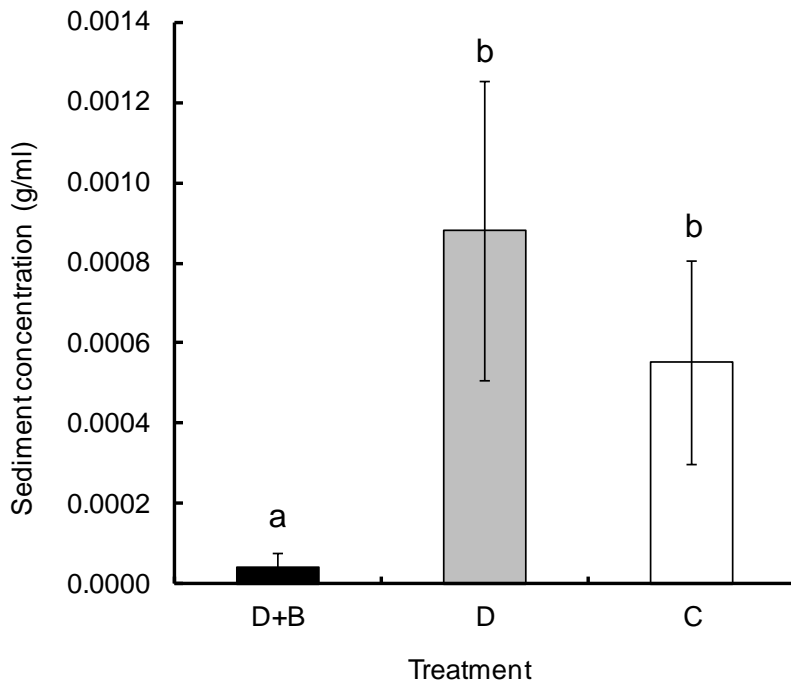


**Figure 6.** Mean amount of run-off for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the 6,000 ml simulated rainfall experiment. Columns with a different letter are significantly different (LSD). Error bars = SEM.





**Figure 7.** Mean total sediment in the run-off for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the 6,000 ml simulated rainfall experiment.. Error bars = SEM.



**Figure 8.** Mean concentration of sediment ( $\pm$  SE) in the run-off (g/ml) for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the 6,000 ml simulated rainfall experiment.

## 2. Nematode survivorship

Mean numbers of *Cooperia* sp at each sampling date for the three sites are shown in Figure 9. At Kumeu and Warkworth there were early peaks in nematode numbers in the dung-only treatments compared to the dung+beetles. At the Shelly Beach site the result was different with a slightly later, smaller peak, in nematode numbers that was very similar in both the dung-only and dung+beetles treatments. Cumulative totals of recovered nematodes are shown in Figure 10. A Kruskal Wallis test for the cumulative totals from the 5 sample dates with all sites combined revealed a significant effect of treatment on *Cooperia* numbers (Kruskal-Wallis chi-squared = 20.4225,  $p < 0.001$ ). Post-hoc Mann-Whitney tests with Bonferroni correction showed significant differences between dung+beetles and dung-only ( $p = 0.0107$ ); and between dung+beetles and control (C) ( $p < 0.01$ ) and dung-only and control ( $p = 0.0012$ ).

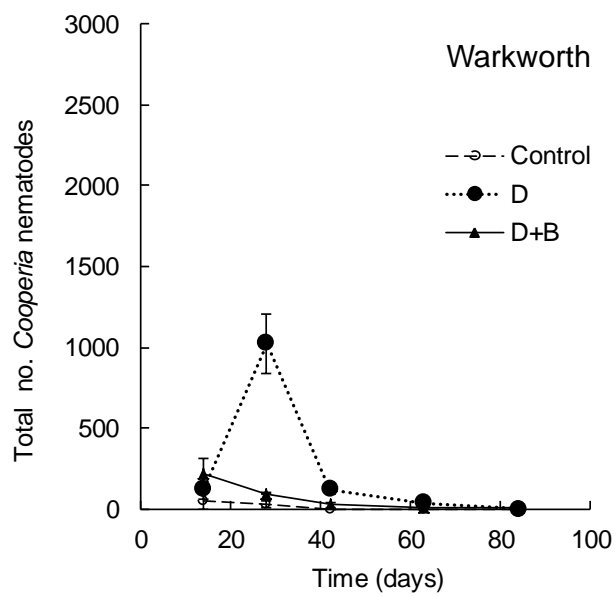
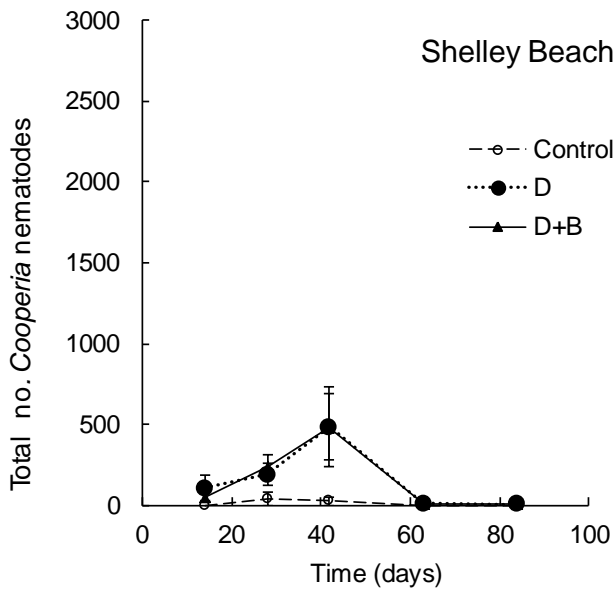
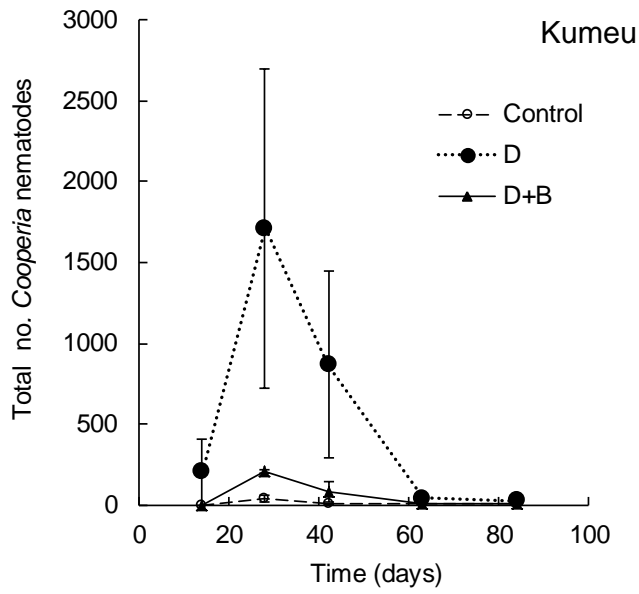


Figure 9. Mean numbers of nematode larvae (*Cooperia* sp) in the three treatments on each sampling date at the three sites.

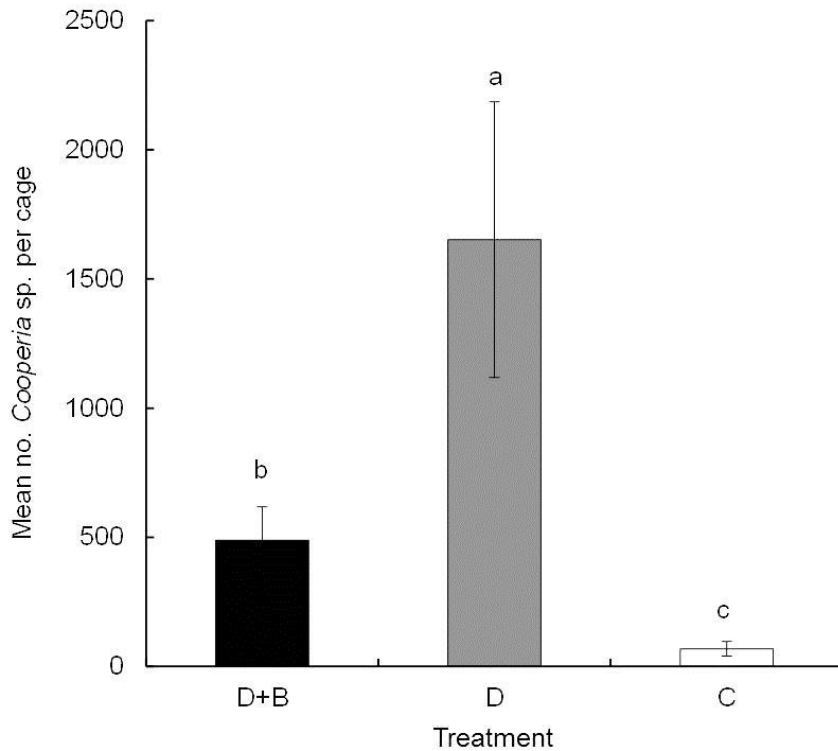


Figure 10. Mean cumulative totals of *Cooperia* sp. per cage from the 5 sample dates with all sites combined for each treatment (dung+beetles = D+B, dung-only = D, control = C) in the nematode survivorship experiment. Columns with a different letter are significantly different (LSD). Error bars = SEM.

### 3. Biomass of harvested grass and soil moisture

Site had the largest influence on grass yield ( $F_{2,18} = 19.131$ ,  $P < 0.001$ ) although treatment was also statistically significant ( $F_{2,18} = 4.48$ ,  $P < 0.05$ ). However, the treatment effect was relatively minor: grass yield was highest in the dung-only treatment and was significantly lower in the control treatment. The dung+beetles treatment, however, was not significantly different from either the dung-only or the control treatments (Fig. 11).

Soil moisture (in the top 10 cm of the soil horizon in the control plots) is shown in Figure 12. Soil moisture was reasonably consistent across sites and dates, with the exception of the 4th sample at Kumeu where soil moisture was low. The average moisture content at Kumeu (clay loam), Shelly beach (sandy loam) and Warkworth (compacted clays) through the course of the trial were 37.1%, 28.7%, and 31.4% respectively.

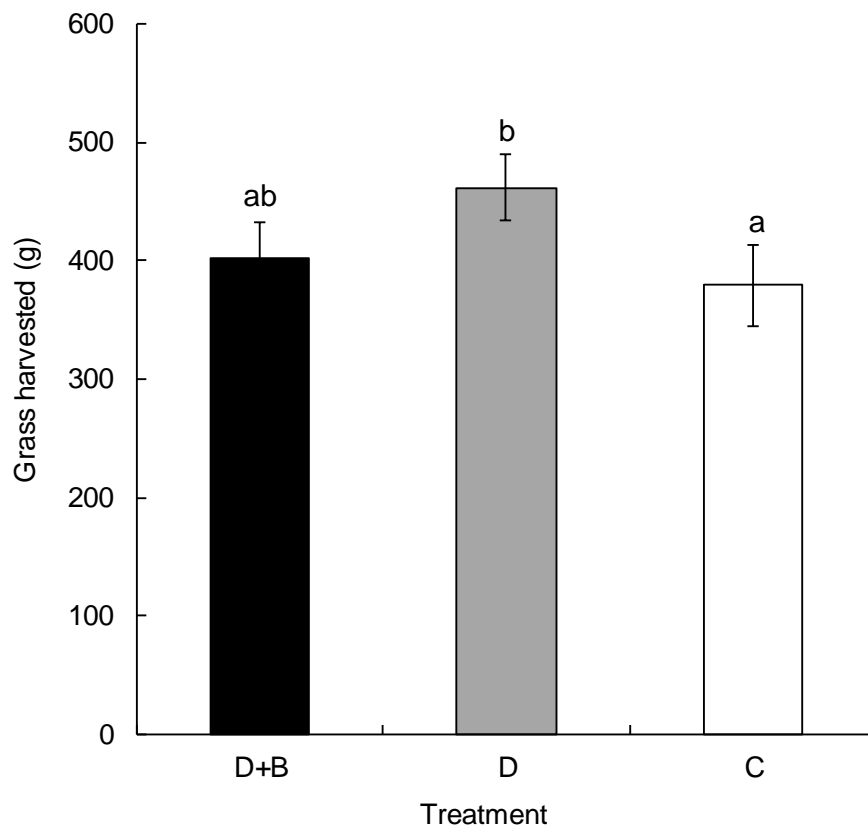


Fig. 11. The cumulative total yield of grass harvested at each of the three field sites (weight of samples 1-5 combined). A different letter above a column indicates a significant difference between treatments (Tukey HSD).

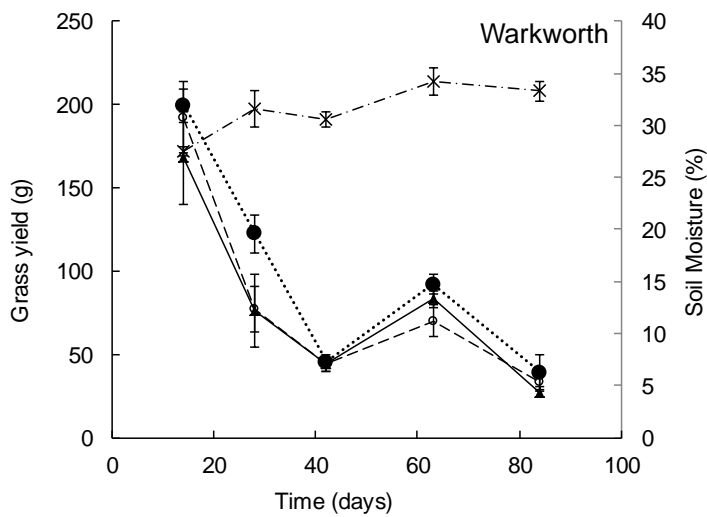
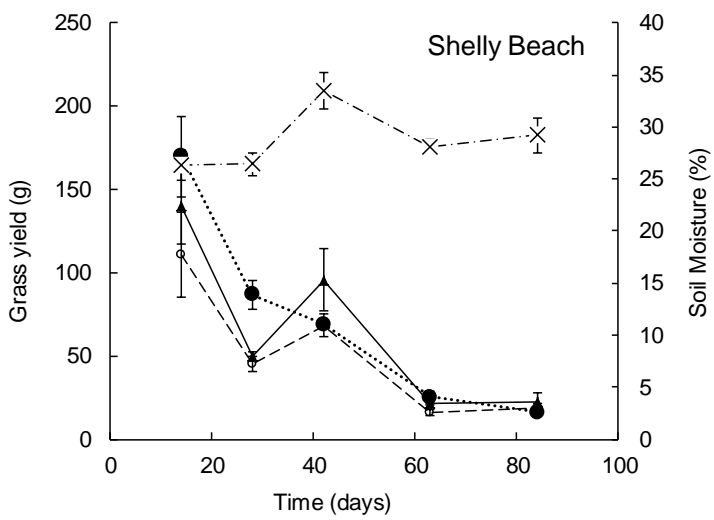
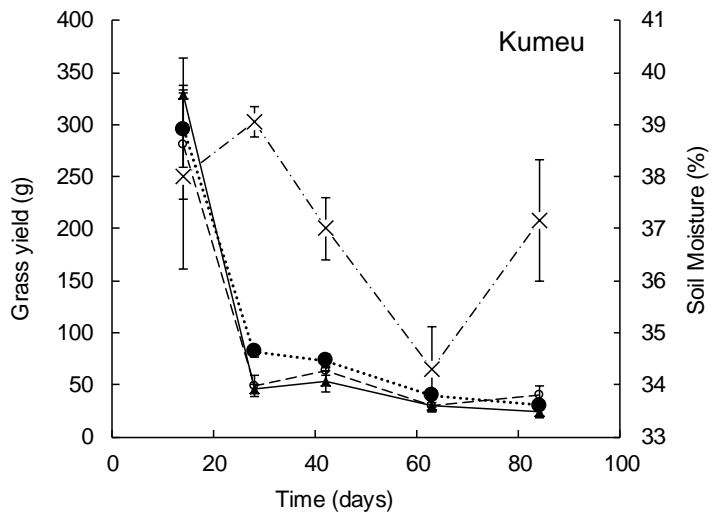


Fig. 12. Graphs showing the yield of grass harvested at each sample date for each of the three field sites (dung+beetles ●; dung-only ▲, controls ○). The soil moisture content in the control cages on each date is also shown (×)

## DISCUSSION

The reduced surface run-off after artificial rain in the presence of dung beetle was probably a result of increased soil porosity due to the dung beetle tunnels. In the most extreme of the two rainfall simulations, the total amount of sediment in the run-off was similar in the treatment with beetles present versus dung-only. As there was less run-off volume in the presence of beetles, then the sediment concentration clearly had to be higher, but this increase was not statistically significant. In Brown et al (2010) the sediment concentration in run-off from cages with beetles was significantly higher with beetles in comparison to control with no dung, although as in the highest rainfall trial in this study, the total sediment in run-off from the treatment with beetles was not higher. An important difference between the two studies is that Brown et al (2010) did not include a dung-only treatment. It is not surprising that dung beetle activity can cause an increased concentration of sediment in run-off because even if most of the actual dung is buried, the burying activity leaves small piles of loose soil on the pasture surface (J. Brown pers. comm.). The first rainfall simulation in this trial represented an extremely high quantity of rain (52 mm) in a 10 minute period (likely to happen less than once in every 100 years). The second rainfall simulation applied half the volume of rain used in the first simulation, but was still was an event likely to happen only once every 60-100 years depending on the site. In this simulation the reductions in run-off volume and sediment (either as a total or as concentration) were very large in the dung beetle treatment versus dung alone. Both simulations used the equivalent of extreme rainfall events and hence were probably underestimating the benefits in reduced run-off and sediment content in more typical rainfall events. The results here are consistent with other studies showing increased infiltration rates of rainfall with dung beetle activity (Richardson & Richardson 2000; Waterhouse 1974). Extrapolating from these small scale trials, the extra water infiltrating into the soil should reduce the impact of flooding and droughts, with every additional 25 mm of rainwater absorbed adding over 254,000 l/ha to the soil (Richardson & Richardson 2000). There are also likely to be benefits from less pollutants entering waterways e.g. from dung, urine or fertiliser (Doubé 2008; Fincher 1981).

Cumulative results from the nematode survivorship trial across all sites clearly indicate dung beetle activity reduced numbers of the infective 3<sup>rd</sup> instar larvae of the gastrointestinal nematode (*Cooperia* sp) on pasture foliage compared to the treatment where dung was left undisturbed on the pasture surface. Reduced larval nematode numbers on foliage should translate to reduced infection rates of stock because the consumption of pasture foliage is provides the infection pathway. This result is consistent with the majority of overseas studies comparing dung+beetles and dung-only treatments (Bryan 1973; Bryan & Kerr 1989; English 1979; Hutchinson et al 1989; Reinecke 1960; Waterhouse 1974). Of particular significance was the lack of any 'time bomb' effect where buried dung, being protected from the sun, might lead to greater survival of nematode larvae, and then a later peak in larval numbers on foliage when the larvae migrate back to the surface (Coldham 2011). This is also consistent with international studies: where dung was buried by dung beetles (Bryan 1973, 1976; Coldham 2011; Fincher 1973; Grønvold et al 1992) or earthworms (Grønvold 1987) all studies failed to produce any significant 'time bomb' effect. The results across two of the

farms used in this study were comparable, but there were no differences in larval nematode numbers on pasture foliage in the dung+beetles and dung-only treatments at Shelly Beach Farm. This site had a sandy soil compared to the other two farms, and nematode migration from buried dung in such soils has been shown to be greater than in heavier soils, provided there is sufficient moisture (Lucker 1936, 1938). Conversely, if conditions are dry then larval nematode survival in sandy soils is likely to be poorer because the 1<sup>st</sup> and 2<sup>nd</sup> instar larvae are easily killed by a lack of moisture in such well-drained soils. Variable weather conditions over the period of the trial could therefore explain both the reduced level of larval nematodes on pasture foliage at Shelly Beach compared to the other farms (because of, at-times, poorer survival) and the lack of the main treatment effect (because of easier migration from buried dung when moisture levels were sufficient). This is consistent with the timing of the Shelly Beach peak in larval nematode numbers, which occurred in the 3<sup>rd</sup> sample of the trial when soil moisture levels also peaked (Figs 9 and 12). Importantly, there was no evidence that dung beetle activity resulted in higher numbers of nematode larvae on pasture foliage at any site or sampling date.

The lack of difference in pasture foliage biomass between the dung+beetles and dung-only treatments was not unexpected because previous studies have shown that the positive impacts of dung beetle activity on both above and below ground plant biomass required several months to manifest (Miranda et al 2003; Bang et al 2005; Nichols et al 2008). For example, Bang et al (2005) found that higher herbage yields were obtained in a beetles+dung treatment, compared to a dung-only treatment, after five months. Moreover, delays setting up the current study meant that grass yield declined dramatically in all treatments, due to the onset of winter (Fig. 12). For this reason, the data should be considered to be preliminary as further sampling in spring (i.e. after c. 160 days) is more likely to detect an impact of dung beetle activity.

These trials provide the first quantitative assessments of several of the possible benefits to be gained from the widespread establishment of additional species of dung beetles in New Zealand.

## **ACKNOWLEDGEMENTS**

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