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The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

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Abstract

This project assessed the pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry using *Bubas bison* as an example of the deep-tunnelling dung beetles introduced to Australia by CSIRO. *B. bison* is one of a group of four deep-tunnelling dung beetle species (including *Geotrupes spiniger*, *Onitis caffer*, *Copris hispanis*) that are well suited to southern Australia but are currently established over only a small portion of their potential range. The environmental and agricultural benefits of such species had not been examined previously.

There were three field experiments. The first demonstrated that complete dung burial by *B. bison* on one occasion increased pasture production (dry matter) by about 30% and that increased production has persisted for 3+ years after dung burial. Experiment 2 demonstrated that even small feral beetle populations increased pasture production. Experiment 3 provided an explanation by showing that dung burial by *B. bison* increased earthworm populations, the permeability of soil to water and the levels of nitrate, available phosphate, sulphur, carbon and organic matter in the subsoil.

We recommend that monitoring continue for 2 years, that paddock-scale validation be undertaken and that the four species of deep-tunnelling dung beetles be established throughout their potential range in southern Australia.

Executive summary

This project assessed the pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry using *Bubas bison* as one of four deep-tunnelling dung beetle species (including *Geotrupes spiniger*, *Onitis caffer*, *Copris hispanis*) introduced to Australia by CSIRO. These deep-tunnelling species are well suited to southern Australia but are currently established over only a small portion of their potential ranges. The environmental and agricultural benefits of such species had not been previously assessed and so the project was commissioned to examine *B. bison* in field trials.

The project objectives were to:

- examine the impact of dung burial by *B. bison* on pasture production and key soil characteristics at two locations on the Fleurieu Peninsula, SA
- educate producers through field days and other extension activities
- produce a draft pamphlet on the impacts of dung beetles on soil and pastures
- develop a scientific paper ready for peer review.

Further details are given in eleven appendices.

Procedures and results

Three field experiments were established on each of two contrasting soil types on collaborator properties at Ashbourne (a deep alluvial loam) and Kuitpo (a duplex soil: sandy loam over clay), SA.

Experiment 1 examined the impact of dung burial by *B. bison* on pasture production (dry matter) and the persistence of increased production after dung burial. Experiment 2 examined the impact of feral beetle populations on pasture production. Breeding populations of *B. bison* had only recently become established at the study sites: beetle numbers (relative to their potential) were low (up to 10 per trap) but increased throughout the experiment. Experiment 3 examined the impact of dung burial in soil cores on earthworm populations, subsoil chemistry and soil carbon levels. The field biology of the adult and immature beetles and their impact on soil surface properties were examined. The experiments covered a period of 33 months.

Dung burial and beetle biology

Bubas bison was released at both Ashbourne and Kuitpo in 2002 and field recoveries of F1 beetles occurred after one year at Kuitpo and after two years at Ashbourne. These were the first records of *B. bison* breeding on mainland SA. Beetle abundance was monitored using dung-baited pitfall traps and was relatively low throughout the study period. One pair of beetles can completely bury a dung pad. At both locations, beetles were most abundant from May to mid-July (achieving 60–80% dung burial), whereafter numbers decreased (achieving 15–20% dung burial).

Based on levels of abundance observed in regions where the beetle is well established, beetle numbers at Ashbourne and Kuitpo can be expected to reach at least several hundred per dung pad within the next decade. Such levels of beetle activity result in complete dung burial for 3–5+ months of the year.

A facultative third instar larval diapause (arrested development) that occurs in cool environments causes the larval beetles to spend a second year underground before emerging as adults, thereby extending the beetle's life cycle by one year. *B. bison* had a 1-year life cycle at Kuitpo and a 2-year life cycle at Ashbourne. A second form of diapause was found in adult beetles at Kuitpo but not at Ashbourne. At Kuitpo, adult beetles colonised dung pads immediately upon emergence from the soil but did not tunnel or feed for the first 4–8 weeks, whereafter normal activity was resumed.

Pasture production

The pasture growth response over 2 years (October 2005 to November 2007) to the burial of one set of dung pads was substantial: +27% (4.1 t ha⁻¹) at Ashbourne and +25% (5.0 t ha⁻¹) at Kuitpo. The pasture growth response to natural dung burial (pads placed in the field at weekly intervals)

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was +22% (2.7 t ha⁻¹). Pasture growth responses to buried dung were detected in the same year as the dung was buried and have persisted for at least 2.5 years after dung burial. No pasture growth response to unburied surface dung was detected.

The pasture growth response to the burial of cattle dung by *B. bison* over a 2 year period (2006–07) was 0.057 kg dry matter per kg of buried dung and was similar at both trial locations. This estimate is independent of stocking rate, cattle live weight, dung beetle abundance and seasonal activity and relies only upon a measure of dung burial by *B. bison*. This statistic can be used to estimate production benefits due to *B. bison* wherever the beetle will survive and bury dung.

Soil health, soil structure and subsoil chemistry

Dung burial resulted in elevated levels of nitrate, ammonia, phosphate, sulphur and soil carbon in the subsoil 20–45 cm below dung pads. Soil organic matter, soil pH and EC were also elevated. These effects were dramatic, particularly in the vicinity of the beetle tunnels, and have persisted for more than 2 years. The elevated levels of phosphate, sulphur, EC and soil pH moved from the tunnel contents+tunnel walls into the surrounding bulk soil.

Four earthworm species were recognised. Earthworm numbers and biomass increased under dung pads and increased further in soil where the dung had been buried by beetles, being found throughout the soil profile. Soil hardness decreased and the permeability to water increased where beetles buried dung.

Soil carbon

The capacity of deep-tunnelling dung beetles to increase the levels of carbon stored in the soil as soil organic matter provides an opportunity to use deep-tunnelling dung beetles to sequester atmospheric carbon (carbon dioxide) in soil as organic matter (roots and dung) and so contribute to lessening the impact of the cattle industry on global warming. In time, producers may be able to claim carbon credits for the additional carbon stored in soils as a result of dung beetle activity.

Conclusions

Adult *B. bison* emerge in May and remain active until the soil becomes dry in spring–summer. Moderate levels of beetle abundance can achieve complete dung burial. Beetle numbers in the study areas are likely to increase dramatically over the next few years, resulting in complete and rapid dung burial from May to September+. It is most likely that each of the four species of deep-tunnelling dung beetles would produce similar pasture and environmental responses.

Dung burial by *B. bison* induces substantial changes in the structure, chemistry and biology of surface and subsurface soils. The beetle tunnels, the subsoil brought to the surface, the earthworm castings in the beetle tunnels (down to 50+ cm), the decreased surface soil hardness, the increased permeability of the soil to water, and the elevated subsoil levels of N, P, S and organic matter encouraged pasture roots into the subsoil where they were scarce in the absence of dung beetle tunnels. These processes increase pasture production and levels of carbon in the subsoil. The growth response was seen soon after the dung was buried and has persisted for at least 2.5 years. Knowing how long the growth response persists is essential for quantifying the benefits of dung beetles. Evaluating the economic benefits needs to place dollar values on both the production and environmental benefits.

Key recommendations for further action

- monitor the pasture and soil health plots for a further 2 seasons
- prepare a soil nutrient budget with data for the entire soil profile
- develop a model to quantify the pasture/nutrient benefits of dung burial by *B. bison*
- validate the pasture growth and soil carbon conclusions in a paddock-scale trial
- establish deep-tunnelling dung beetles throughout southern Australia.

Who benefits and when we can expect results

Deep-tunnelling dung beetles have the potential to benefit most cattle producers in southern Australia through increasing pasture production, reducing fertiliser costs and improving water use efficiency. The general public will also benefit through reduced water pollution and elevated levels

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of carbon storage in soil organic matter (reducing greenhouse gasses). Carbon credits for C-storage in soil organic matter may generate additional income for producers in the future. Abundant beetle populations can be expected within 10 years of introducing beetles to suitable habitats. This will occur more quickly if large founder colonies are used (eg 50,000 beetles rather than 1000 beetles).

Extension and implementation: The benefits of dung beetles have been promoted through field days, *Prograzier*, ABC TV (Landline, SA Stateline), radio (Bush Telegraph, local SA radio). A beetle cropping, sales and delivery business has been established in association with SA and Tasmanian collaborators.

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Appendix 2: The effect of *Bubas bison* on pasture growth on the Fleurieu Peninsula, SA

Appendix 3: Subsoil chemical analysis, August & November 2006, May & September 2007

Appendix 4: Seasonal activity and dung burial by natural field populations of *B. bison*

Appendix 5: The effect of the dung beetle I on earthworm abundance and distribution

Appendix 6: The impact of dung burial on water infiltration

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Appendix 11: Draft pamphlet describing the impacts of dung beetles on soil and pasture characteristics

1 Background

In Australia there are over 26 million cattle (ABS 2005). Mature animals produce about 18 kilograms of wet dung each day, giving a total production of over 170 million tonnes of dung dropped onto Australian pastures each year. Dung beetles are considered to have the potential to transform this pollutant into a multi-million dollar production benefit (Bornemissza 1970, Waterhouse 1974, Fincher 1981, Doube and Dalton 2003a), to increase the permeability of soil to water (Doube, Dalton and Ford 2003), to significantly reduce the pollution of water bodies from dung-derived nutrients and organic matter (Doube 2005), and to significantly reduce diseases that are potentially fatal to humans, such as *Cryptosporidium* (Doube 2004).

However, the potential agricultural benefits of dung beetles have not been thoroughly assessed in Australia. World-wide, there are no published field data on dung beetles for Mediterranean or even-rainfall regions. One study in subtropical regions of the United States (Fincher 1981) demonstrated substantial benefits of dung beetles in a Bermuda blue-grass system using an African-derived summer-active species. Similar data for Australian agriculture will allow an evaluation of the economic and environmental benefits of dung beetles, an important component of decisions about the level of investment in dung beetle programs by research and natural resource management bodies.

Beetle-derived benefits that are demonstrated on the Fleurieu Peninsula (from this project) will be highly portable, for such benefits are likely to apply to other regions of southern Australia where this beetle species can establish and prosper.

Summer- and winter-active dung beetles have been released by CSIRO and private operators in many localities in the winter and even-rainfall regions of Australia over the past decades but, because beetles disperse relatively slowly, most regions still lack an adequate fauna; that is, one that will achieve year-round disposal of cattle dung. It is also evident that species have been released in inappropriate regions, where they perish.

The lack of dung beetles can be rectified by assessing the current and potential distributions of species to identify significant species gaps (Edwards 2007). In addition, it is necessary to test the capacity of candidate species to establish in regions where they are missing, followed by widespread introductions of successful species.

The background to the current project involved recognition that the benefits of dung beetle activity need to be established before substantial resources are devoted to redistributing species, although redistribution and increasing public awareness had already begun.

During 2003 and 2004 the Fleurieu Beef Group Inc. (FBG), in partnership with Creation Care Pty Ltd (CC) and Dung Beetle Solutions Australia (DBSA), achieved the following:

- released 60 colonies of dung beetles (*Bubas bison*, *Geotrupes spiniger* and *Onitis caffer*) on the Fleurieu Peninsula, South Australia, for field evaluation of establishment and seasonal activity. *B. bison* is now clearly established on a number of properties
- established and managed 23 dung beetle nurseries in initial trials directed towards developing mass rearing procedures for dung beetles. Developed a mass rearing protocol for *O. caffer* that requires testing.
- produced an 8-page pamphlet titled *Identifying dung beetles on the Fleurieu Peninsula* (Doube & Dalton 2003b)
- produced a 20-page booklet titled *Dung beetles: transform a pollutant into an environmental and agricultural benefit* (Doube & Dalton 2003a)
- provided a dung beetle education service to farmers in South Australia, with significant support also to interstate farmers

During 2003 to 2005 Dung Beetle Solutions Australia also established dung beetle field trials in association with DairySA (Flaxley), Central Highlands Water (Ballarat) KI NRM Board and Barwon Water (Apollo Bay). These trials examined the impact of *B. bison* and *G. spiniger* in small-scale

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plots (1m²). A limited laboratory experiment with the Australian Water Quality Centre, Bolivar, examined the effects of dung beetle activity on the survival and distribution of *Cryptosporidium* in dung. The principal findings of these trials can be summarised as follows. Dung beetle activity has:

- rapidly buried large amounts of dung
- substantially increased the permeability of soil to water
- substantially increased the depth of friable topsoil
- increased earthworm activity, especially at depth in the soil
- altered pasture composition (favouring pasture grasses over weeds)
- increased pasture production over and above that due to surface dung
- decreased levels of dissolved organic carbon (DOC) and soluble nitrogen (N) in run-off water
- substantially decreased the numbers of *Cryptosporidium* oocysts in surface dung

These production and environmental benefits needed to be confirmed and quantified in comprehensive field trials that would allow confident extrapolation to paddock-scale effects and benefits. The understanding and recommendations from such trials then needed to be transferred to farmers and water management authorities.

These trials provided a valuable testing ground for techniques that formed the basis of the current project.

The development of this project benefited substantially from the contribution of Greg Dalton (Creation Care), the Fleurieu Beef Group (FBG) chair, Geoff Davis, and members of the FBG who collaborated willingly in this and other dung beetle projects.

1.1 Purpose of the experiments

Introduced winter-active dung beetles are absent from many suitable pastures in southern Australia and yet are considered to have a substantial capacity to improve soil health and pasture productivity, but these effects needed to be quantified. This project has provided field evidence to allow quantification of the production and environmental benefits of dung beetle activity.

2 Project objectives

The project objectives were to:

- examine the impact of the dung burial by *B. bison* on pasture production and key soil characteristics at two locations on the Fleurieu Peninsula, SA
- educate producers through field days and other extension activities
- produce a draft pamphlet on the impacts of dung beetles on soil and pastures
- develop a scientific paper ready for peer review

3 Methodology

3.1 Experimental design

Two parallel trials (with an identical design) were established on Fleurieu Beef Group collaborator properties on the Fleurieu Peninsula, SA. The trials contained three complementary experiments, each with replicated plots comparing three treatments (dung + dung beetles, dung only and controls

[no dung, no beetles]). Two minor supplementary pasture growth experiments were also sampled. The trial plots were established in late winter of 2005 and assessed in 2005, 2006, 2007 and 2008. The experimental design and factors assessed are summarised in Table 1. A schematic layout of the experimental plots at each location is illustrated in Figure 1.

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Table 1 The experimental design and factors measured in each of three trials designed to evaluate the impact of the dung burial activity of the dung beetle *B. bison* on pasture growth and soil health

	Experiment 1	Experiment 2	Experiment 3
Experimental design			
Plot size	2 x 2 m	10 x 10 m	Pad size
Area sampled for plant growth	2 x 2 m	10 x 10 m	Nil
Number of replicates	4	3	4
Type of sampling	Non-destructive	Non-destructive	Destructive
Dung beetles per pad	15 pairs introduced	Natural colonisation	5 pairs introduced
Pad deposition	Once only (30 September, 1 October)	Throughout the beetle season 2006, 2007*	Once only (September)
Pad volume	3 litres	1 litre	3 litres
Factors measured			
Pasture growth	Yes	Yes	No
Persistence of green vegetation	Yes	Yes	No
Dung burial	Yes	Yes	Yes
Subsoil brought to surface	Yes	Yes	Yes
Permeability of soil to water	Yes	Yes	No
Depth of friable soil	Yes	Yes	No
Soil carbon and nitrogen levels	No	No	Yes
Earthworms: depth and number	No	No	Yes
Contents of beetle tunnels	No	No	Yes
Organic matter distribution	No	No	Yes
Dung beetle development	No	No	Yes

* also during September & October 2005

3.2 Experimental layout

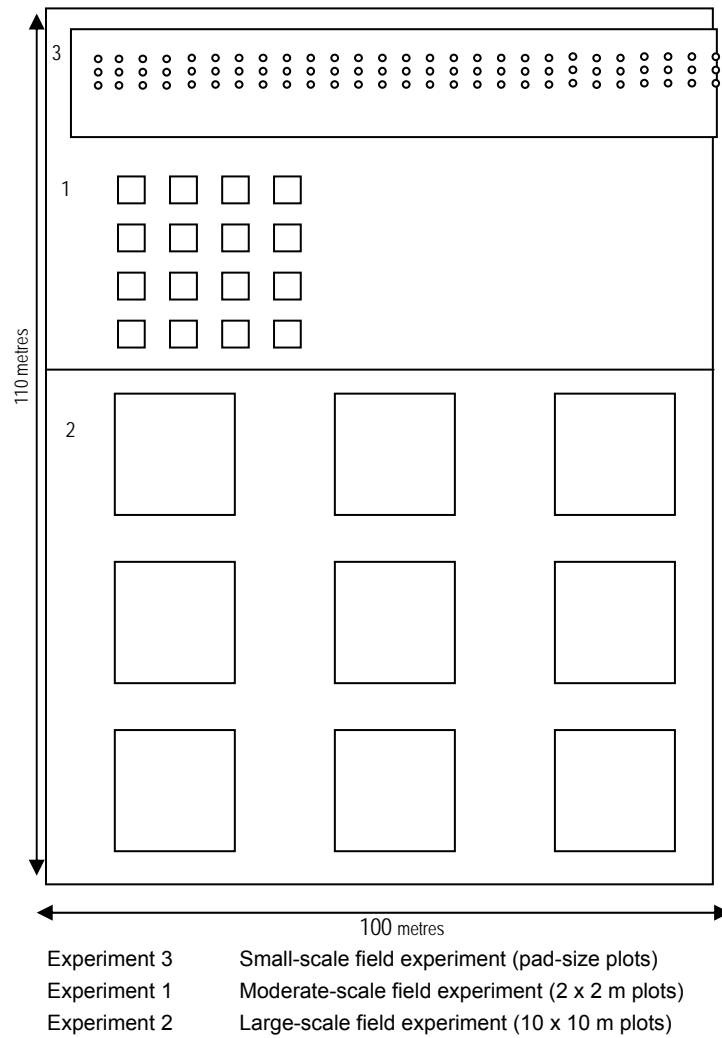
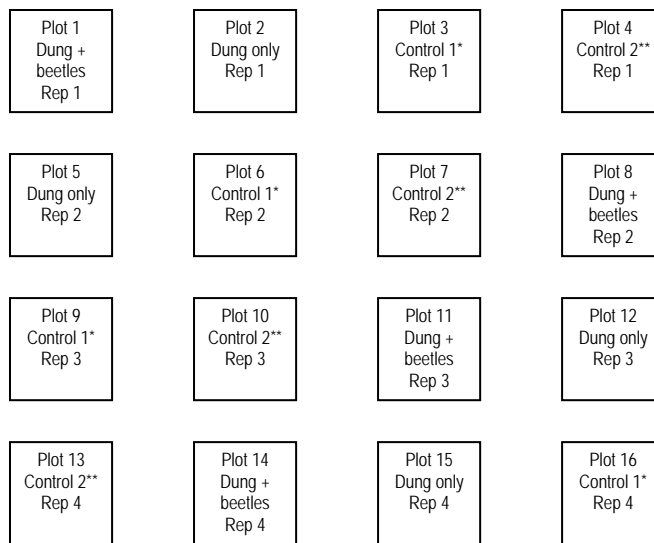


Figure 1 Schematic layout of plots in experiments 1 to 3 at each location (not to scale)

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* No dung, no beetles, with cage cover
 ** No dung, no beetles, no cover

Figure 2a Plot layout for Experiment 1 (not to scale)

1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16

Figure 2b Layout of dung pads in each +dung plot in Experiment 1 plots (not to scale)

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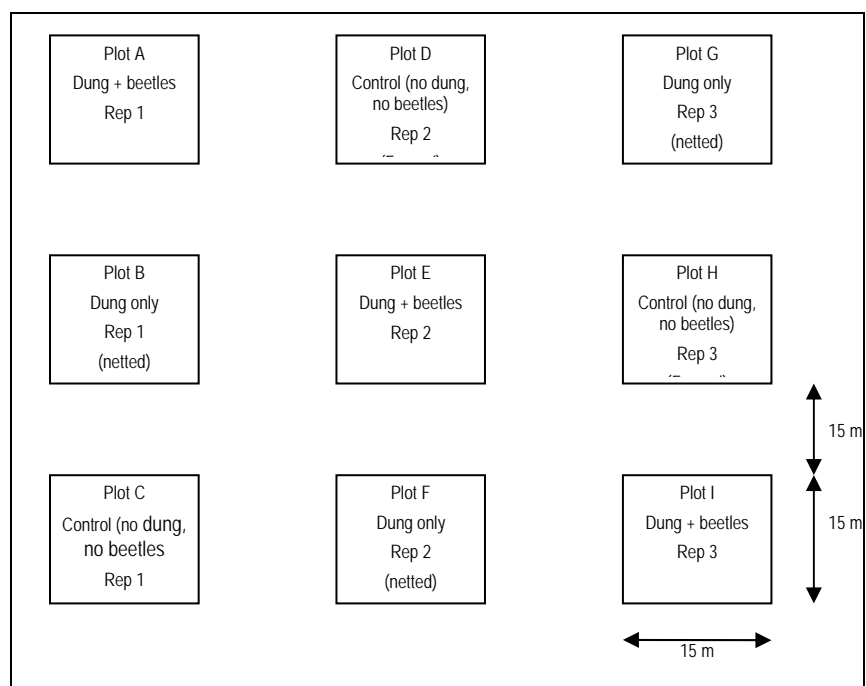


Figure 3 Plot layout for Experiment 2, the large-scale field experiment (not to scale)

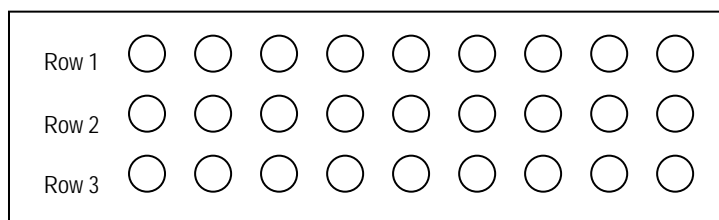


Figure 4 Plot layout for Experiment 3, the small-scale field experiment (not to scale)

The original scope of the proposed experimental protocol was more complex and included three test locations. However, the level of financial support offered by the MLA required that the scope of the original proposal be reduced to accommodate a reduced budget allocation. This was done. One test location (in the SE of SA) and some costly analyses were removed from the proposal so that the current project could be accommodated within the revised budget. The following account reports the procedures that were the outcome of the revised proposal.

The locations for the experiments were at Ashbourne, SA (a deep alluvial clay loam), and Kuitpo, SA (a duplex soil with a sandy loam over a yellow clay). Beetles for experiments 1 and 3 were sourced from Kangaroo Island in July 2005. They were maintained at moderate density in a cool environment and fed dung on a regular basis to ensure that they were ready to breed when placed in the field (late September).

3.3 Experiment 1 methods

This experiment used beetle-proof cages to confine beetles added to the dung pads and to exclude 'feral' dung beetles from the dung-only treatment. Cages, dung and beetles were installed once only, on 30 September and 1 October 2005, about half way through the dung beetle activity season.

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Immediately before setting up the experiment at location 1 (Ashbourne, a non-grazed paddock) the plots were mowed to about 5–6 cm high. At location 2 (Kuitpo) grass was 2–4 cm high due to regular grazing by cattle and was not mowed before the experiment was set up.

The experiment comprised 4 replicates of 4 treatments. The 16 plots (2 x 2 m) were established in a 4 x 4 plot design with 2 m between adjacent plots. Each row and each column contained one of each of the four treatments (Figure 2a):

- dung + dung beetles
- dung only
- control 1 (no dung, no beetles, with mesh cage)
- control 2 (no dung, no dung beetles, without mesh cage)

There were 12 plots with 2 x 2 m beetle-proof wire cages and 4 plots with no cages in order to assess the impact of the wire mesh cages on plant growth. The cages (2 x 2 m x 14 cm high) were made from 6.5 x 6.5 mm galvanised mesh (0.35 mm gauge), through which *B. bison* cannot pass. Each cage had a basal lip attached to the ground by pegs to prevent dung beetles crawling out (or in) at the edges of the cage.

In each plus-dung plot 16 pads of fresh dung (3.0 litres each) were placed in a 4 x 4 layout (Figure 2b). Each pad was numbered.

Fifteen pairs of *B. bison* in breeding condition were added to each dung pad in the dung+beetles treatment.

The number of soil casts around each pad were counted and an estimate of the amount of dung buried was made on a number of occasions after establishing the experiment. The cages remained in place on all plots for 8 weeks (to November 2005). There was little recorded activity (as indicated by baited pitfall traps) of feral *B. bison* during that period, or later that year.

Pasture growth was assessed using the procedures set out in the MLA Pasture Ruler Agricultural Note. Pasture height was monitored in all plots. When the fastest growing pasture reached about 8–10 cm high, all plots were cut back to 5–6 cm using a lawn mower. The wet and dry weights of pasture samples from each plot were determined.

The duration of the growing season at the two Fleurieu Peninsula locations was determined from pasture colour as assessed from photographic records of each plot taken at regular intervals during spring and early summer, or until the pasture hayed off.

The impact of dung and dung beetle activity on the depth of soft soil and the permeability of soil to water were assessed in spring 2005 and in autumn 2008. The depth of soft soil underneath the dung pads and in the control plots was assessed using a soil penetrometer. The permeability of soil to water was assessed using PVC tubes (15 cm diameter, 20 cm tall) hammered into the soil above the dung pads such that water could not leak from soil–tube interface. The equivalent of 50 mm of rainfall (0.5 litres) was applied to each of four 15 cm PVC tubes in each plot punched into the ground over dung pads (or where they had been) and in the control plots. The time taken for the water to soak into the soil was recorded and taken as an index of the permeability of the soil to water.

3.4 Experiment 2 methods

In this experiment, the cumulative burial by natural (feral) dung beetle populations of dung pads produced during September and October 2005 and throughout the autumn to early summer of 2006 and 2007 were monitored. In the first year (2005), there were very few feral dung beetles caught in the pitfall traps. In order to ensure that there was a degree of dung burial in the dung+beetles plots, additional beetles (1 pair per pad) were released onto each of the dung+beetles pads.

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The experiment was established in one paddock at each location. Nine large-scale field plots (10 x 10 m) were established at each location in the first year, comprising three replicates of each of three treatments (dung + natural dung beetles, dung only and controls [no dung, no dung beetles] (Figure 3). An additional dung+beetles and a dung-only plot were established at Kuitpo.

Three 1-litre dung pads were added manually at weekly intervals in progressive rows across the plots during the beetle's activity season in each season from 2005 to 2007. At the time of placement of each pad in the field, its position was marked with a numbered peg (protruding 4 cm from the soil). This procedure involved adding dung on 6 occasions in 2005 (September and October) and 24 occasions in each of 2006 and 2007 (May to October). Thus the number of pad-sites per plot was 18 in 2004 and 72 in each of 2006 and 2007, giving a total of 162 pad-sites per plot.

The number of soil casts around each pad were counted and an estimate of the amount of dung buried was made at weekly intervals for the first 12 weeks after laying down the dung pad.

All 21 plots were clearly pegged out and exposed to natural field conditions (including sunlight, rainfall and wind). In the dung-only plots, feral beetles were excluded from the added dung pads using small beetle-proof cages that covered the added dung pad and excluded the dung beetles. These beetle excluders were left in place for 8 weeks after the dung pads had been placed in the field. After that time the pads were no longer attractive to dung beetles and so remained uncolonised, despite being exposed to the feral dung beetle populations.

The dung beetle populations were monitored using dung-baited pitfall traps placed inside the 10 x 10 m enclosures and baited and emptied at weekly intervals over the dung beetle season. Three traps were placed adjacent to the dung+beetles plots.

Pasture growth was assessed using the procedures set out in the MLA Pasture Ruler Agricultural Note. When the fastest growing pasture reached about 8–10 cm high, all plots were cut back to 5–6 cm. The wet and dry weights of pasture samples from each plot were determined.

At each location, a moderate cattle density was maintained in the paddocks surrounding the experimental plots during the dung beetle season (May to October). This ensured a substantial regular supply of dung in the area surrounding the experimental plots and was considered to attract feral dung beetles to the experimental area.

3.5 Experiment 3 methods

In this experiment, small plots to monitor soil health were established at each location (in the enclosures containing Experiment 1). These plots were destructively sampled throughout the course of the experiment in order to document progressive changes in parameters (eg earthworm populations and activity down tunnels) influenced by dung beetle tunnelling and dung burial activity.

Each small plot consisted of a double coarse mesh beetle-proof bag enclosing a largely undisturbed soil profile (22 cm in diameter) buried 40–50 cm in the ground. There were three treatments (dung+beetles, dung only and controls [no dung, no beetles]). To maintain the soil profile intact, a machine to extract and replace intact cores (22 cm diameter, 50 cm deep) was developed. This machine comprised a 250-kg pile driver that hammered a steel coring cylinder into the ground, extracted it from the ground with a soil core, and allowed the soil core (bagged to make it beetle proof) to be replaced in the ground. This creates a largely undisturbed beetle-proof cage in the soil. Dung (3 litres per core) was placed on the soil surface above the core in the plus-dung plots.

At each location, four replicate plots were established. Each plot contained 27 dung pad sites (9 columns and 3 rows). Each row contained 3 replicates of the 3 treatments. In each plot the columns were numbered from 1 to 9. Adjacent dung pad sites (within plots) were separated by 1 metre (Figure 4).

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On each sampling occasion, one column (containing one of each of the three treatments) was sampled from each of the four plots (ie four replicates of each treatment were sampled). Columns 1 to 9 were sampled in turn. The order of the treatments within the columns was varied systematically so that each treatment was represented equally (3 times) in each row. Thus, at sampling, each treatment was represented. The fourth plot was organised in the same way as plot 1.

Three litres of dung were added to the soil on top of the dung+beetles and dung-only plots. Five pairs of beetles were added to each dung+beetles bag, which were then be tied to prevent beetles escaping.

The plots were sampled on six occasions: November 2005, April 2006, August 2006, November 2006, May 2007 and September 2007. The experimental arrangement provided the opportunity for 9 sampling occasions and so there are spare sets of replicates available for further sampling, if required.

On each sampling occasion at each location, four replicate bags of the three treatments were removed from the soil and laid out on a dissecting table. The bag was opened and the soil core divided into three sections (0–10 cm, 10–20 cm, >20 cm). The following parameters were assessed:

- the mass of dung remaining unburied on the soil surface
- dung beetle tunnels and their contents (dung, earthworm casts, plant roots)
- the number, species and biomass of earthworms in two depth categories (0–10 cm and >20 cm)
- the mass of the dung and dung remains (processed by beetles) buried deep in the soil
- the nutrient levels and organic matter status in the subsoil (>20 cm)
- the number and condition of eggs/larvae/pupae/adult dung beetles in the dung mass

3.6 Supplementary experiment

Two small pasture growth experiments (using 1 m² plots) were assessed twice yearly to provide supplementary data on the persistence of plant growth effects. Both experiments were established in 2002, one by the FBG (near Pt Elliott, SA) and the other by DBSA at Ballarat. The wet weight and dry weight of the pasture produced were assessed in the 1 m² plots, using the procedures set out in the MLA Pasture Ruler Agricultural Note.

4 Results and discussion

The broad results are presented in the following sections. Detailed presentation and analysis of the data are presented in the attached appendices:

Appendix 1: Background, experimental design and methods

Appendix 2: The effect of *Bubas bison* on pasture growth on the Fleurieu Peninsula, SA

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Appendix 5: The effect of the dung beetle I on earthworm abundance and distribution

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Appendix 11: Draft pamphlet describing the impacts of dung beetles on soil and pasture characteristics

4.1 Dung burial and beetle biology

Bubas bison was released at both Ashbourne and Kuitpo in 2002 and field recoveries of F1 beetles occurred after one year at Kuitpo and after two years at Ashbourne. These were the first recorded instances of *B. bison* becoming established on mainland SA. Beetle abundance was monitored using dung-baited pitfall traps at weekly intervals at the two study locations over four periods: October–November 2005, April–November 2006 and 2007 and April–May 2008. Overall, nine dung beetle species were trapped at Ashbourne and seven at Kuitpo. Overall, the abundance and biomass *Bubas bison* was greater at Ashbourne than at Kuitpo, and levels of field abundance increased at both locations over the period 2005–2008.

At both locations, beetles were most abundant from May to mid-July, whereafter numbers decreased until few to none were trapped in November. The corresponding levels of dung pad burial were about 80% and 60% of all dung pads placed in the field from May to July at Ashbourne and Kuitpo respectively, and 18% and 14% for late winter at Ashbourne and Kuitpo respectively. Few of the dung pads placed in the field during September and October were buried. In seasons/regions with a moist spring, beetle activity can continue until early summer.

During the 2007 beetle activity period the mean number of *B. bison* per trap ranged up to 10 at Ashbourne and up to 2 at Kuitpo. One pair per pad results in complete burial of the dung pad, but over an extended period of time (some weeks). Based on levels of abundance observed in regions where the beetle is well established, levels of abundance can be expected to reach at least several hundred per dung pad within the next decade. Such levels of beetle activity result in complete dung burial for 3–5 months of the year.

4.1.1 Seasonal breeding biology

A facultative third instar larval diapause (arrested development) was discovered in which diapause caused the larval beetles to spend another year underground before emerging as adult beetles. Diapause extended the duration of the dung beetle life cycle by one year, turning an annual life cycle (ie, one generation per year) into a biennial life cycle (ie, one generation every two years).

B. bison has a one-year life cycle in warmer regions and a two- (or even three-) year life cycle in cooler climates. Diapause in the third larval instar (3LL) of *B. bison* has been demonstrated in a number of other recent studies in southern Australia (B Doube, unpublished data). In Experiment 3 in this project *B. bison* has a 1-year life cycle at Kuitpo and 2-year life cycle Ashbourne; that is, at Ashbourne the majority of the larvae (98%) entered larval diapause but at Kuitpo a minority (2%) did so.

A second form of diapause can be expressed by the adult beetle soon after emergence from the soil. This is termed a facultative adult reproductive diapause. Beetles that are not in diapause colonise dung pads (by flying to them) and then dig shallow tunnels in which they feed, mate and the females mature their ovaries (eggs). Beetles leave these tunnels after some days and fly off in search of a fresh dung pad, where the same procedure is repeated. A series of such tunnels may be dug over a period of some weeks until the female beetle is ready to begin laying eggs. She then, commonly in association with a male beetle, digs a deep tunnel (to 30–50+ cm), at the bottom of which she deposits dung, in which she lays eggs. In contrast, female beetles that are in diapause colonise dung pads (by flying to them) but do not dig tunnels or feed for some (4–8) weeks. When diapause has been completed beetles then dig shallow tunnels, in which they feed, mate and the females mature their eggs, and the reproductive biology process proceeds as for beetles that are not in diapause.

4.2 Pasture production

Pasture growth responses to buried dung were detected in the same year as the dung was buried and persisted for at least three years after dung burial.

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In the 2x2 m plots (dung+beetles treatment, dung applied once, in 2005) the pasture growth response (October 2005 to November 2007) to complete dung burial was substantial: +27% (4.1 t ha⁻¹) at Ashbourne and +25% (5.0 t ha⁻¹) at Kuitpo.

In the 10x10 m plots at Ashbourne the pasture growth response to natural dung burial (pads placed in the field at weekly intervals) was +22% (2.7 t ha⁻¹) over 2 years since the project began. No pasture growth response to unburied surface dung was detected.

The pasture growth response to the burial of cattle dung by *B. bison* over a 2-year period in 2006–07 was 0.057 kg dry matter per litre of buried dung and was similar at both locations. This estimate of the impact of dung beetle activity on pasture production is independent of stocking rate, cattle live weight, dung beetle abundance and seasonal activity and relies only upon a measure of dung burial by *B. bison*. This statistic can be used to estimate production benefits due to *B. bison* wherever the beetle will survive and bury dung.

A simple model, based on a herd of 100 cattle, indicates that the increased pasture production (13 tonnes of dry matter produced over a 2-year period following initial deposition of the dung) derived from the dung produced over the activity period of *B. bison* (May to September) would be sufficient to increase gross yields by from \$2100 (hay value) to \$10,000+ if used to increase the growth rate of existing 200-kg weaner steers in forward store condition.

4.3 Soil health, soil structure and subsoil chemistry

Dung burial resulted in elevated levels of nitrate, ammonia, available phosphate, sulphur and soil carbon in the subsoil 20–45 cm below dung pads. Soil organic matter, soil pH and EC were also elevated. These effects were dramatic, particularly in the vicinity of the beetle tunnels, and have so far persisted for 2 years.

The elevated levels of nitrate and ammonia remained associated with the dung beetle tunnels but the elevated levels of phosphate and sulphur, as well as elevated pH and EC, moved from the tunnel contents+tunnel walls into the surrounding bulk soil.

Four earthworm species were recognised. Earthworm populations in unamended soil were low, and lower at Kuitpo than at Ashbourne. Earthworm populations increased under dung pads and increased substantially further in soil where the dung had been buried by beetles. At both locations the same general pattern was observed on all three sampling occasions. Overall, the biomass recovered from the dung+beetles plots was 4–5 times greater than that recovered from the control plots, and that recovered from the dung-only plots was 2–3 times greater than that recovered from the control plots.

The rate of infiltration of water in to surface soil was substantially increased by dung beetle activity. Increased permeability has persisted for at least 2.5 years.

The hardness of surface soil was substantially reduced by dung beetle activity. This effect has persisted for at least 2.5 years.

Plant roots were common in the beetle tunnels in the subsoil but were largely absent from soil that had not been influenced by dung beetle activity.

5 Success in achieving objectives

All of the project objectives have been achieved.

Detailed information on the progress of the experiments is presented in the appendices.

5.1 Field experiments

The impact of the dung burial by *B. bison* on pasture production and key soil characteristics has been documented in two locations on the Fleurieu Peninsula over three seasons. The results are presented above. Clear effects of dung beetle activity on soil health and pasture production were demonstrated (appendices 2–6).

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An economic evaluation of these impacts is required. Knowing how long the growth response persists is essential for assessing the benefits of dung beetles. Evaluating the economic benefits needs to place dollar values on both the production and environmental benefits.

5.2 Education and extension

The key findings of this study have been communicated to cattle producers and the public through:

- a series of field days with substantial numbers of growers attending
- publicising the outcomes in the press (eg, *Acre Australia*, local newspapers),
- presenting the data on two occasions at national MLA workshops on soil health
- two articles in *Prograzier*
- a substantial contribution to an ABC Landline program on dung beetles
- a dedicated ABC Stateline (SA) program on the impact of *B. bison*
- an extensive interview with Michael McKenzie on Bush Telegraph, ABC Radio National
- establishment of a beetle cropping, sales and delivery business in association with collaborators.

5.3 Publications

A draft pamphlet on the impacts of dung beetles on soil and pastures has been produced. This deals with the four species of deep-tunnelling dung beetles that are currently available in southern Australia but focuses upon *B. bison* and *G. spiniger* (Appendix 11).

A draft scientific paper is ready for peer review (Appendix 7).

6 Impact on meat and livestock industry: now and in five years time

6.1 Public awareness

A general appreciation of the benefits of dung beetles to the cattle industry has arisen in the farming community and among the general public as a result of the education and extension activities described above.

Dung beetles are now widely appreciated as beneficial animals that provide a long-term and ecologically sustainable solution to a pasture pollution problem, as well as providing numbers of production and environmental benefits. The challenge now is to maintain the momentum and to meet the demand for beetles in agriculture.

6.2 The supply of and demand for beetles

This awareness has produced a substantial demand for dung beetles throughout the Australian grazing industry. In particular the Prograzier and Landline exposure has generated numerous email and telephone enquiries to Dung Beetle Solutions Australia, for both information and supply of dung beetles.

The supply of suitable dung beetles is clearly quite limited.

DBSA has responded to this need by developing a beetle cropping, sales and delivery business in association with collaborators on Kangaroo Island (*B. bison*), SA, and in Tasmania (*G. spiniger*). Both species are now established and abundant in limited parts of their potential distribution in southern Australia. The activities of DBSA are helping to increase the beetle coverage in southern Australia.

6.3 Quantification of the economic benefits

One major current deficiency in available information is an economic evaluation of the value to Australia of the production and environmental benefits that would follow from widespread establishment of the four species of deep-tunnelling dung beetles across southern Australia.

The economic production benefits include increased pasture production and decreased requirements for chemical fertiliser (in particular nitrate and phosphate), but it is also important to evaluate the environmental benefits, such as reduced numbers of pest flies, improved water quality and reduced risk of exposure to water-borne pathogens (eg *Cryptosporidium*). These 'off-site' consequences of cattle production are now recognised as important issues for which the farming community has a responsibility.

In addition, it is possible that producers may be able to gain carbon credits for additional carbon stored in the soil organic matter as a result of dung beetle activity. This requires legislative recognition of the potential of soil organic matter to act as a mechanism for carbon sequestration and the establishment of a carbon trading mechanism that allows producers to trade such carbon credits.

6.4 Additional species

However, the supply of *O. caffer* and especially *C. hispanis* is extremely limited and requires considerable investment to develop methods for mass rearing the beetles in the field and assessing the limits to their distribution in southern Australia.

DBSA in association with the Fleurieu Beef Group and Creation Care has reviewed the constraints to mass rearing these species and produced a report on each.

6.5 In five years time

The challenges over the next five years are numerous and include:

- developing economic models that quantify the likely benefits from dung beetles
- maintaining the momentum in beetle sales and distribution
- defining the geographic limits to the distribution of *B. bison* and *G. spiniger* in Australia
- gaining support to develop and field test mass rearing procedures for *O. caffer* and *C. hispanis*
- defining the geographic limits to the distribution of *O. caffer* and *C. hispanis* in Australia

If these challenges are met, it is possible that founder colonies of *B. bison* and *G. spiniger* may be released in most of the suitable regions of southern Australia over the next decade. Substantial populations are likely to be present in areas that received starter colonies before and about the turn of the twenty-first century.

This is likely to result in a 20+% increase in the cattle carrying capacity of the regions in which *B. bison* is abundant and a decrease in fertiliser requirements. The environmental benefits are also likely to be substantial but these have not been quantified.

Procedures to mass rear *O. caffer* and *C. hispanis* may also have been validated in five years time, and mass rearing and release may be occurring on a significant scale.

7 Conclusions and recommendations

7.1 Conclusions

- Adult *B. bison* emerge in May and remain active until the soil becomes dry in spring–summer. Moderate levels of beetle abundance can achieve complete dung burial.
- Based on levels of abundance observed in regions where the beetle is well established, beetle numbers at Ashbourne and Kuitpo can be expected to reach at least several hundred per dung pad within the next decade. Such levels of beetle activity result in complete dung burial for 3–5+ months of the year.

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- *B. bison* exhibits a facultative third instar larval diapause (arrested development) that occurs in cool environments and causes the larval beetles to spend a second year underground before emerging as adults, thereby extending its life cycle by one year. This occurred at Ashbourne.
- A second form of diapause was found in adult beetles at Kuitpo but not at Ashbourne. At Kuitpo, adult beetles colonised dung pads immediately upon emergence from the soil but did not tunnel or feed for the first 4–8 weeks, whereafter normal activity was resumed.
- Establishing substantial populations of *B. bison* from a founder colony of 1000 beetles is likely to take about 10 years in regions that do not exhibit a larval diapause. Introduction of founder colonies in two successive years is required in regions where the beetle has a 2-year life cycle.
- Dung burial by *B. bison* induces substantial changes in the structure, chemistry and biology of surface and subsurface soils. The beetle tunnels, the subsoil brought to the surface, the earthworm casting in the beetle tunnels (down to 50+ cm), the decreased surface soil hardness, the increased permeability of the soil to water, and the elevated subsoil levels of N, P, S and organic matter encourage pasture roots into the subsoil where they are scarce in the absence of dung beetle tunnels.
- The capacity of deep-tunnelling dung beetles to increase the levels of carbon stored in the soil as soil organic matter provides an opportunity to use deep-tunnelling dung beetles to sequester atmospheric carbon (carbon dioxide) in soil as organic matter (roots and dung) and so contribute to lessening the impact of the cattle industry on global warming. In time, producers may be able to claim carbon credits for the additional carbon stored in soils as a result of dung beetle activity.
- The pasture growth response over 2 years to the burial of one set of dung pads was substantial: +27% (4.1 t ha⁻¹) at Ashbourne and +25% (5.0 t ha⁻¹) at Kuitpo. The pasture growth response to natural dung burial (pads placed in the field at weekly intervals over 2+ activity seasons) was +22% (2.7 t ha⁻¹).
- Pasture growth responses to buried dung were detected in the same year as the dung was buried and have persisted for at least 2.5 years after dung burial. No pasture growth response to unburied surface dung was detected.
- The pasture growth response to the burial of cattle dung by *B. bison* over a 2 year period (2006–07) was 0.057 kg dry matter per kg of buried dung and was similar at both locations. This estimate is independent of stocking rate, cattle live weight, dung beetle abundance and seasonal activity and relies only upon a measure of dung burial by *B. bison*. This statistic can be used to estimate production benefits due to *B. bison* wherever the beetle will survive and bury dung.
- It is most likely that each of the four species of deep-tunnelling dung beetle will produce similar pasture and environmental responses.

7.2 Recommendations

The recommendations are divided into two sections, one dealing with the current project and *B. bison* (which are firmly supported by the review panel that evaluated progress of MLA Project ER 211 in 2007) and the other dealing with deep-tunnelling dung beetles in southern Australia.

In relation to *B. bison* we recommend that:

1. the impact of *B. bison* on pasture growth and soil health be monitored for a further 2 seasons (finishing in December 2009) in order to document the progressive accumulation of pasture growth advantages
2. a simple model to quantify the dollar benefits of dung burial by *B. bison* be developed in association with Dr M McCaskill
3. a soil nutrient budget be prepared using data on the distribution of plant nutrients down the entire soil profile using soil samples already available, as recommended by the review panel.

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This analysis will allow us to assess the fate of dung-derived nutrients throughout the soil profile.

4. a paddock-scale validation of the pasture production benefits of dung burial by *B. bison* be established. This should include quantification of the capacity of *B. bison* to sequester carbon and reduce levels of greenhouse gasses in Australia.
5. a postdoctoral fellowship be established to examine the potential of dung beetle activity to solubilise bound phosphate

In relation to deep-tunnelling dung beetles, we recommend that:

1. a multi-agency program (including MLA, RIRDC, Land and Water Australia, Dairy Australia) be established to evaluate the capacity of deep-tunnelling dung beetles to increase levels of carbon storage in soil organic matter and thereby lessen the net impact of livestock (primarily cattle and horses) on the production of greenhouse gasses. Special attention should focus on the possibility of generating farm-based income through carbon credits for C-storage in soil organic matter.
2. mass rearing procedures for *O. caffer* and *C. hispanis* be developed and field tested and the geographic limits to the distribution of *O. caffer* and *C. hispanis* in Australia be defined experimentally

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Appendix 1: Background, experimental design and methods

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Background

In Australia there are over 26 million cattle (ABS 2005). Mature animals produce about 18 kilograms of wet dung each day, giving a total production of over 170 million tonnes of dung dropped onto Australian pastures each year. Dung beetles are considered to have the potential to transform this pollutant into a multi-million dollar production benefit (Bornemissza 1970, Waterhouse 1974, Fincher 1981), to increase the permeability of soil to water (Doube, Dalton and Ford 2003), to significantly reduce the pollution of water bodies from dung-derived nutrients and organic matter (Doube 2005), and to significantly reduce diseases that are potentially fatal to humans, such as *Cryptosporidium* (Doube 2004).

However, the potential agricultural benefits of dung beetles have not been thoroughly assessed in Australia. World-wide, there are no published field data on dung beetles for Mediterranean or even-rainfall regions. One study in subtropical regions of the United States (Fincher 1981) demonstrated substantial benefits of dung beetles in a Bermuda blue-grass system using an African-derived summer-active species. Similar data for Australian agriculture would allow an evaluation of the economic and environmental benefits of dung beetles, an important component of decisions about the level of investment in dung beetle programs by research and natural resource management bodies.

Beetle-derived benefits that are demonstrated on the Fleurieu Peninsula (from this project) will be highly portable, for any beetle-derived pasture benefits demonstrated are likely to apply to other regions of southern Australia where this beetle species can establish and prosper.

Summer and winter-active dung beetles have been released by CSIRO and private operators in many localities in the winter and even-rainfall regions of Australia over the past decades but, because beetles disperse relatively slowly, most regions still lack an adequate fauna; that is, one that will achieve year-round disposal of cattle dung. It is also evident that species have been released in inappropriate regions, where they perish.

The lack of dung beetles can be rectified by assessing the current distributions of species to identify significant species gaps and by testing the capacity of candidate species to establish in regions where they are missing, followed by widespread introductions of successful species. However, the benefits of dung beetle activity need to be established before this occurs.

During 2003 and 2004 the Fleurieu Beef Group Inc. (FBG), in partnership with Creation Care Pty Ltd (CC) and Dung Beetle Solutions Australia (DBSA), achieved the following:

- released 60 colonies of dung beetles (*Bubas bison*, *Geotrupes spiniger* and *Onitis caffer*) on the Fleurieu Peninsula, South Australia, for field evaluation of establishment and seasonal activity. *B. bison* is now clearly established on a number of properties
- established and managed 23 dung beetle nurseries in initial trials directed towards developing mass rearing procedures for dung beetles. Developed a mass rearing protocol for *O. caffer* that requires testing
- produced an 8-page pamphlet titled *Identifying dung beetles on the Fleurieu Peninsula*
- produced a 20-page book titled *Dung beetles: transform a pollutant into an environmental and agricultural benefit*
- provided a dung beetle education service to farmers in South Australia, with significant support also to interstate farmers

During 2003 to 2005 Dung Beetle Solutions Australia established dung beetle field trials in association with DairySA (at Flaxley), Central Highlands Water (Ballarat) and Barwon Water (Apollo

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Bay). These trials examined the impact of *B. bison* and *G. spiniger* in small-scale plots (1m²). A limited laboratory experiment with the Australian Water Quality Centre, Bolivar, examined the effects of dung beetle activity on the survival and distribution of *Cryptosporidium* in dung. The principal findings of these trials can be summarised as follows. Dung beetle activity has:

- rapidly buried large amounts of dung
- substantially increased the permeability of soil to water
- substantially increased the depth of friable topsoil
- increased earthworm activity, especially at depth in the soil
- altered pasture composition (favouring pasture grasses over weeds)
- increased pasture production over and above that due to surface dung
- decreased levels of dissolved organic carbon (DOC) and soluble nitrogen (N) in run-off water
- substantially decreased the numbers of *Cryptosporidium* oocysts in surface dung

These production and environmental benefits needed to be confirmed and quantified in comprehensive field trials which allow confident extrapolation to paddock-scale effects and benefits. The understanding and recommendations from such trials then needed to be transferred to farmers and water management authorities.

This project builds on the results from field trials already conducted by DBSA. These trials have also provided a valuable testing ground for techniques that form the basis of the current project.

Purpose of the experiments

Introduced winter-active dung beetles are absent from many suitable pastures in southern Australia and yet are considered to have a substantial capacity to improve soil health and pasture productivity, but these effects needed to be quantified. This project has provided field evidence to allow quantification of the production and environmental benefits of dung beetle activity.

Experimental design and measurement protocols

Two parallel trials (each with an identical design) were established in paddocks on Fleurieu Beef Group collaborator properties on the Fleurieu Peninsula SA. Each trial contained three complementary experiments, each with replicated plots comparing three treatments (dung + dung beetles, dung only and controls [no dung, no beetles]). Two minor supplementary pasture growth experiments were also sampled. The trial plots were established in late winter of 2005 and assessed in 2005, 2006, 2007 and 2008. The experimental design and factors assessed are summarised in Table 1.1. A schematic layout of the experimental plots at each location is illustrated in Figures 1.1–1.4.

Table 1.1 The experimental design and factors measured in each of three trials designed to evaluate the impact of the dung burial activity of the dung beetle *B. bison* on pasture growth and soil health

	Experiment 1	Experiment 2	Experiment 3
Experimental design			
Plot size	2 x 2 m	10 x 10 m	Pad size
Area sampled for plant growth	2 x 2 m	10 x 10 m	Nil
Number of replicates	4	3	4
Type of sampling	Non-destructive	Non-destructive	Destructive
Dung beetles per pad	15 pairs introduced	Natural colonisation	5 pairs introduced
Pad deposition	Once only (30 September, 1 October)	Throughout the beetle season 2006, 2007*	Once only (September)
Pad volume	3 litres	1 litre	3 litres
Factors measured			

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Pasture growth	Yes	Yes	No
Persistence of green vegetation	Yes	Yes	No
Dung burial	Yes	Yes	Yes
Subsoil brought to surface	Yes	Yes	Yes
Permeability of soil to water	Yes	Yes	No
Depth of friable soil	Yes	Yes	No
Soil carbon and nitrogen levels	No	No	Yes
Earthworms: depth and number	No	No	Yes
Contents of beetle tunnels	No	No	Yes
Organic matter distribution	No	No	Yes
Dung beetle development	No	No	Yes

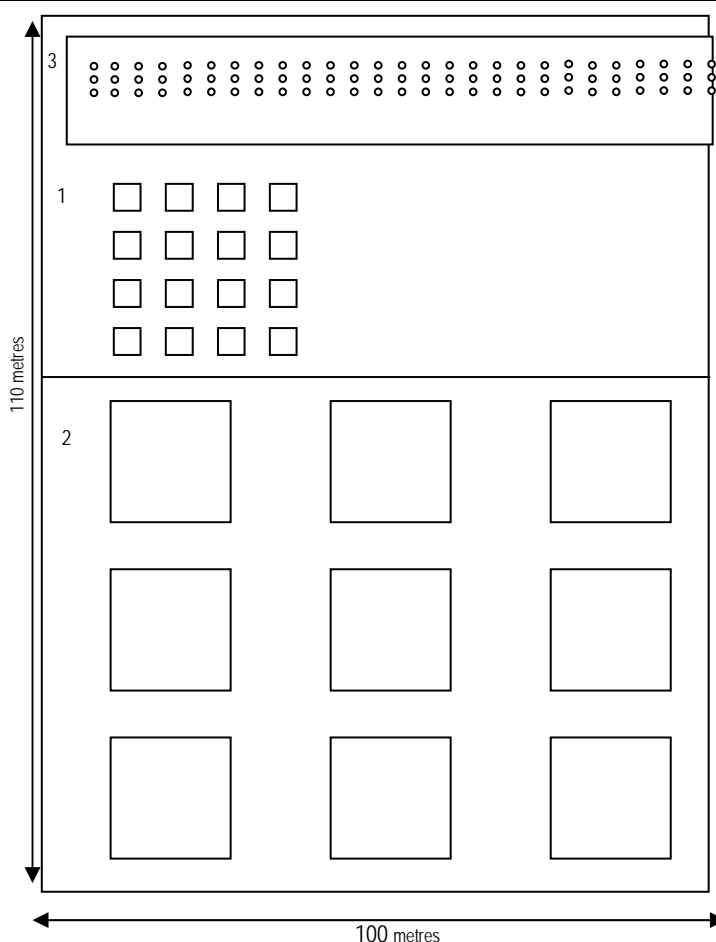
* also during September & October 2005

The original scope of the proposed experimental protocol was more complex than the current one and included three test sites. However, the level of financial support offered by the MLA required that the scope of the original proposal be reduced to accommodate a reduced budget allocation. This was done. One test site (in the SE of SA) and some costly analyses were removed from the proposal so that the current project could be accommodated within the revised budget. The following account reports the procedures that were the outcome of the revised proposal.

The locations for the experiments were Ashbourne, SA (a deep alluvial clay loam) and Kuitpo SA (a duplex soil with a sandy loam over a yellow clay).

Beetles for experiments 1 and 3 were sourced from Kangaroo Island in July 2005. They were maintained at moderate density in a cool environment and fed dung on a regular basis to ensure that they were ready to breed when placed in the field (September).

Experimental layout



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- Experiment 3 Small-scale field experiment (pad-size plots)
- Experiment 1 Moderate-scale field experiment (2 x 2 m plots)
- Experiment 2 Large-scale field experiment (10 x 10 m plots)

Figure 1 Schematic layout of plots in experiments 1 to 3 at each location (not to scale)

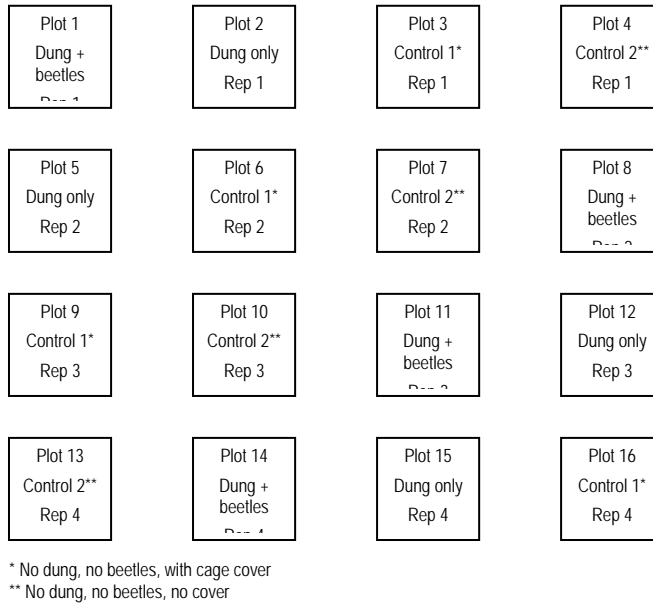


Figure 2a Plot layout for Experiment 1 (not to scale)

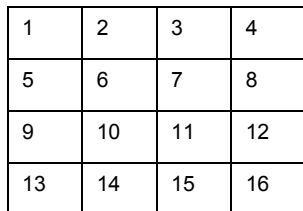
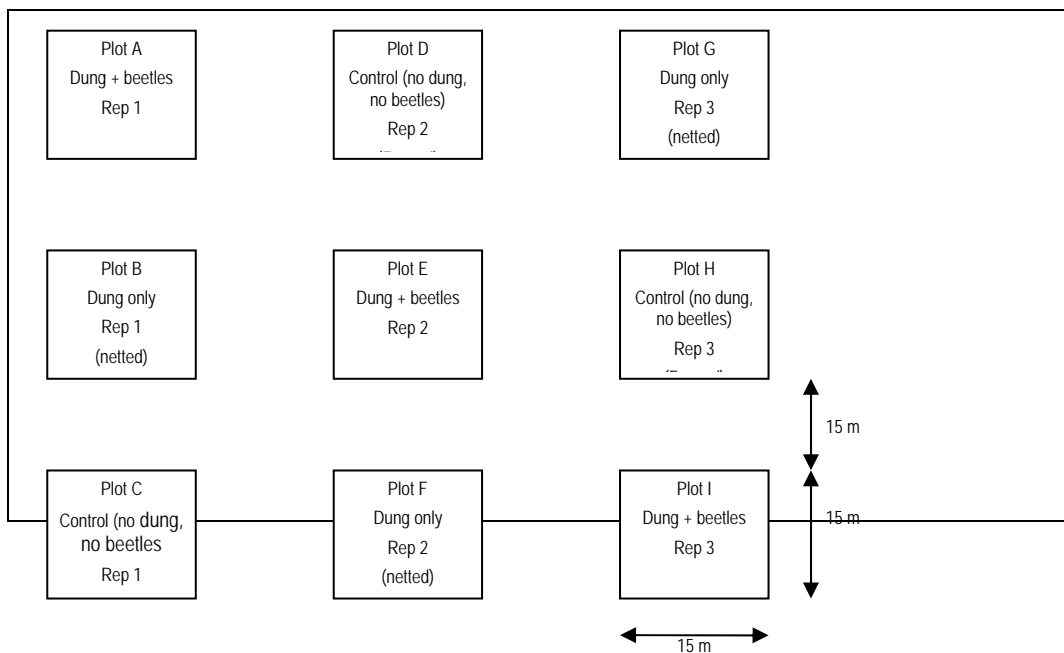


Figure 2b Layout of dung pads in each +dung plot in Experiment 1 plots (not to scale)



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Figure 3 Plot layout for Experiment 2, the large-scale field experiment (not to scale)

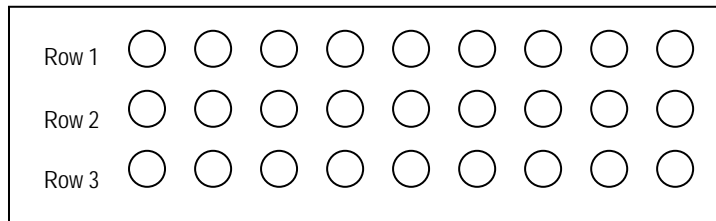


Figure 4 Plot layout for Experiment 3, the small-scale field experiment (not to scale)

Experiment 1 (2x2 m plots) methods

This experiment used beetle-proof cages to confine beetles added to the dung pads and to exclude 'feral' dung beetles from the dung-only treatment. Cages, dung and beetles were installed once only, in September 2005, about half way through the dung beetle activity season. Immediately before setting up the experiment at Ashbourne (a non-grazed paddock) the plots were mowed to about 5–6 cm high. At Kuitpo grass was 2–4 cm high due to regular grazing by cattle.

The experiment comprised 4 replicates of 4 treatments. The 16 plots (200 x 200 cm) were established in a 4 x 4 plot design with 200 cm between adjacent plots. Each row and each column contained one of each of the four treatments (Figure 1.2a).

The four treatments were:

- dung + dung beetles
- dung only
- control 1 (no dung, no beetles, with mesh cage)
- control 2 (no dung, no dung beetles, without mesh cage)

There were 12 plots with 2 x 2 m beetle-proof wire cages and an additional 4 plots with no cages in order to assess the impact of the wire mesh cages on plant growth. The cages (200 x 200 x 14 cm high) were made from 6.5 x 6.5 mm galvanised mesh (0.35 mm gauge), through which *B. bison* cannot pass. Each cage had a basal lip attached to the ground by pegs to prevent dung beetles crawling out (or in) at the edges of the cage.

In each plus-dung plot 16 pads of fresh dung (3.0 litres each) were placed in a 4 x 4 layout (ca 400 litres per location) (Figure 1.2b). Each pad was numbered. Fifteen pairs of *B. bison* in breeding condition were added to each dung pad in the dung+beetles treatment (30 beetles x 16 pads = 480 beetles per field cage).

The number of soil casts around each pad and an estimate of the amount of dung buried was made on a number of occasions after establishing the experiment. The cages remained in place on all plots for 8 weeks, (to November 2005). There was no recorded activity (as indicated by baited pitfall traps) of feral *B. bison* during that period, or later that year.

Pasture growth was assessed using the procedures set out in the MLA pasture ruler *Agricultural note*. Pasture height was monitored in all plots. When the fastest growing pasture had reached about 8–10 cm high, all plots were cut back to 5–6 cm using a lawn mower. The wet and dry weights of pasture samples from each plot were determined.

The impact of dung and dung beetle activity on the depth of soft soil and the permeability of soil to water were assessed in spring in 2005 and in autumn 2008. The depth of soft soil underneath the dung pads and in the control plots was assessed using a soil penetrometer.

The permeability of soil to water was assessed using PVC tubes (15 cm diameter, 20 cm tall) hammered into the soil above the dung pads such that water could not leak from soil–tube interface. The equivalent of 50 mm of rainfall (0.5 litres) was applied to each of four 15 cm PVC tubes in each plot punched into the ground over dung pads and in the control plots. The time taken for the water to soak into the soil was recorded and taken as an index of the permeability of the soil to water.

Experiment 2 (10x10 m plots) methods

In this experiment, the cumulative burial by natural (feral) dung beetle populations of dung pads produced during October 2005 and throughout the winters of 2006 and 2007 were monitored. In the first year (2005), very few feral dung beetles were caught in the pitfall traps. In order to ensure that there was a degree of dung burial in the dung+beetles plots, additional beetles (1 pair per pad) were released onto each of the dung+beetles pads in the first year.

The experiment was established in one paddock at each location. Nine large-scale field plots (10 x 10 m) were established at each location in the first year, comprising three replicates of each of three treatments (dung + natural dung beetles, dung only and controls [no dung, no dung beetles] (Figure 1.3). An additional dung+beetles plot and a dung-only plot were established at Kuitpo.

Three 1-litre dung pads were added manually at weekly intervals in progressive rows across the plots during the beetle's activity season in each season from 2005 to 2007. At the time of placement of each pad in the field, its position was marked with a numbered peg at (protruding 4 cm from the soil). This procedure involved adding dung on 6 occasions in 2005 (September and October) and 24 occasions in each of 2006 and 2007 (May to October). Thus the number of pad-sites per plot was 18 in 2005 and 72 in each of 2006 and 2007, giving a total of 162 pad-sites per plot.

The number of soil casts around each pad were counted and an estimate of the amount of dung buried was made at weekly intervals for the first 12 weeks after laying down each dung pad.

All 21 plots were clearly pegged out and exposed to natural field conditions (including sunlight, rainfall and wind). In the dung-only plots, feral beetles were excluded from the added dung pads by use of small beetle-proof cages that covered the added dung pad and excluded dung beetles. These beetle excluders were left in place for 8 weeks after the dung pads had been placed in the field. After that time the pads were no longer attractive to dung beetles and so remained uncolonised, despite being exposed to the feral dung beetle populations.

The dung beetle populations were monitored using dung-baited pitfall traps placed adjacent to the 10x10 m enclosures and baited and emptied at weekly intervals over the dung beetle season. Three traps were placed adjacent to the dung+beetles plots.

Pasture growth was assessed using the procedures set out in the MLA pasture ruler *Agricultural note*. When the fastest growing pasture had reached about 8–10 cm high, all plots were cut back to 5–6 cm. The wet and dry weights of pasture samples from each plot were determined.

At each location, a moderate cattle density was maintained in the paddocks surrounding the experimental plots during the dung beetle season (June to October). This ensured a substantial regular supply of dung in the area surrounding the experimental plots and was considered to attract feral dung beetles to the experimental area.

Experiment 3 methods

In this experiment, small plots to monitor soil health were established at each location. These plots were destructively sampled throughout the course of the experiment in order to document progressive changes in parameters influenced by dung beetle tunnelling and dung burial activity (eg earthworm populations and activity down tunnels).

Each small plot consisted of a double coarse mesh beetle-proof bag enclosing a largely undisturbed soil profile (22 cm in diameter) buried 40–50 cm in the ground. There were three treatments (dung+beetles, dung only and controls [no dung, no beetles]). To maintain the soil profile intact, a machine to extract and replace intact cores (22 cm diameter, 50 cm deep) was developed. This machine comprises a 250-kg pile driver that hammers a steel coring cylinder into the ground, extracts it from the ground and allows the soil core (bagged to make it beetle proof) to be replaced in the ground. This created a largely undisturbed beetle-proof cage in the soil. Dung (3 litres per core) was placed on the soil surface above the core in the plus-dung plots.

At each location, four replicate plots were established. Each plot contained 27 dung pad sites (9 columns, 3 rows, Fig 1.4)Each row contained 3 replicates of 3 treatments (dung+beetles, dung only and control [no dung, no beetles]). In each plot the columns were numbered from 1 to 9. Adjacent dung pad sites (within plots) were separated by 1 metre.

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Three litres of dung were added to the soil on top of the dung+beetles and dung-only plots. Five pairs of beetles were added to each dung+beetles bag, which was then tied to prevent beetles escaping.

The plots were sampled on six occasions: November 2005, April 2006, August 2006, November 2006, May 2007 and September 2007. The experimental arrangement provided the opportunity for 9 sampling occasions and so there are spare sets of replicates available for further sampling, if required.

On each sampling occasion at each location, four replicate cores of the three treatments were removed from the soil and brought back to the laboratory. Here each core was laid out on a dissecting table. The bag was opened and the soil core divided into three sections (0–10 cm, 10–20 cm, >20 cm). The following parameters were assessed:

- the mass of dung remaining unburied on the soil surface
- dung beetle tunnels and their contents (dung, earthworm casts, plant roots)
- the number, species and biomass of earthworms in two depth categories (0–10 cm and >20 cm)
- the mass of the dung and dung remains (processed by beetles) buried deep in the soil
- the nutrient levels and organic matter status in the subsoil (>20 cm)
- the number and condition of eggs/larvae/pupae/adult dung beetles in the dung mass

Supplementary experiment

Two small pasture growth experiments (using 1 m² plots) were assessed twice yearly to provide supplementary data on the persistence of plant growth effects. Both experiments were established in 2002, one by the FBG (near Pt Elliott, SA) and the other by DBSA at Ballarat. The wet weight and dry weight of the pasture produced were assessed in the 1 m² plots. Pasture growth was assessed using the procedures set out in the MLA pasture ruler *Agricultural note*. The wet and dry weight of pasture samples from each plot were determined.

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Summary

This report summarises the growth of pastures for the first two years of the project and reports the overall pasture growth during three distinct growing seasons, namely the tail end of the 2005 growing season (October to December 2005), the 2006 growing season (February 2006 to January 2007) and most of the 2007 growing season (up to late November 2007). No subsequent samples have been taken, to mid-May 2008: pasture growth has so far been minimal due to lack of rain.

In the 2x2 m plots that used wire cages to confine and exclude dung beetles, 960 *Bubas bison* were added to each dung+beetles treatment cage. A small number of these escaped and some tunnelled under the wire flange in the 'dung-only' (intended to be beetle free) cages 4–8 weeks after the pads were placed in the field. After 8 weeks (when all the cages were removed) around 45% of the pads in the 'dung-only' cages at both Ashbourne and Kuitpo had been colonised (indicated by soil casts). These pads were subsequently buried. Thus the 2x2 m plots have four treatments: dung+beetles all dung buried, dung+beetles about 45% of the dung buried (referred to as 'dung-only' plots), controls (wire mesh cages for 8 weeks), controls (no wire mesh cages). Estimation of the effects of dung alone on pasture production relies upon data from the 10x10 m plots.

Over the first two years of the project there was no detectable effect of the presence of unburied dung upon pasture production. Over this period the pasture growth advantage due to dung beetle activity was:

- +27% (4.1 t ha⁻¹) in the 2x2 m plots at Ashbourne with 100% dung burial
- +20% (3.1 t ha⁻¹) in the 2x2 m plots at Ashbourne with 45% dung burial
- +22% (2.7 t ha⁻¹) in the 10x10 m plots at Ashbourne
- +25% (5.0 t ha⁻¹) in the 2x2 m plots at Kuitpo with 100% dung burial
- +23% (4.7 t ha⁻¹) in the 2x2 m plots at Kuitpo with 45% dung burial

It is expected that the amplitudes of the above responses to dung beetle activity will increase over the coming years.

Due to severe Cape weed infestation at Kuitpo, the 2x2 m plots were sprayed out and replanted with *Phalaris*, which has germinated and established successfully.

The value of additional pasture production

Knowing the volume of beetle-buried dung that gave rise to the increased pasture production, it was possible to estimate the increased production that could be attributed to each litre of buried dung. The average of 5 estimates was 0.057 kg DM per litre of buried dung for dry matter produced during the 2006 and 2007 growing seasons.

This estimate of the impact of dung beetle activity on pasture production is independent of stocking rate, live weight of cattle, dung beetle abundance and seasonal activity and relies only upon a measure of dung burial by *B. bison*. This statistic can be used to estimate production benefits due to *B. bison* wherever the beetle will survive and bury dung.

A simplistic model examining the economic benefit of this for a herd of 100 cattle was generated. About 0.23 million kg of dung were buried over a 123-day period (the seasonal activity period of *B. bison*) and the corresponding increased pasture production was estimated to be 13,000 kg DW of pasture. This has an estimated value of \$2100 as hay (at \$160 per tonne), or from zero up to over \$10,000 value in live weight gain, depending upon stock management practices and price per kg cattle live weight.

The 2x2 m plots at Ashbourne

Over the three seasons there was a statistically significant 27% pasture growth advantage (4.1 t DM ha⁻¹) in the dung+beetles plots over the control plots and a statistically significant 20% pasture growth advantage (3.1 t DM ha⁻¹) in the 'dung-only' (45% burial) plots over the control plots (15.4 t DM ha⁻¹).

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In the first season (2005) there was a 11% increase (0.4 t DM ha⁻¹) in pasture production in the plots with added dung over those without added dung (the control plots) (4.1 t DM ha⁻¹) but no additional effect of beetle activity.

In the second season (2006), compared with the control plots (3.8 t DM ha⁻¹), there was a significant 44% increase (1.7 t DM ha⁻¹) in pasture production in the dung+beetles plots and a significant 38% increase (1.4 t DM ha⁻¹) in production in the 'dung-only' plots (45% burial). Pasture production (0.23 t DM ha⁻¹) in the dung+beetles plots was marginally greater (P = 0.06) than in the 'dung-only' plots.

In the third season (2007), compared with the control plots (7.5 t ha⁻¹), there was a significant 30% increase (2.3 t DM ha⁻¹) in pasture production in the dung+beetles plots and marginally significant 13% increase (1.0 t DM ha⁻¹) in pasture production in the 'dung-only' (45% burial) plots. There was 15% greater pasture production (1.3 t DM ha⁻¹) in the dung+beetles plots than in the 'dung-only' plots.

During 2006 and 2007 there was minimal pasture production during autumn–winter (0.5 to 0.9 t DM ha⁻¹ in the control plots) and a significant impact of dung beetle activity on pasture production. In spring–early summer there was substantial pasture production (3.0 to 7.0 t DM ha⁻¹ in the control plots) and a significant increase in pasture production in the dung+beetles plots (51% = 1.5 t DM ha⁻¹ in 2006 and 29% = 2.0 t DM ha⁻¹ in 2007).

The 10x10 m plots at Ashbourne

Pasture production analysed over the three seasons showed a statistically significant 22% pasture growth advantage (2.7 t DM ha⁻¹) in the dung+beetles plots over that in the control plots (12.2 t ha⁻¹). There was no significant effect of surface dung on pasture production.

In the first season (2005) there was no significant effect of dung beetles or dung alone upon pasture production compared with the control plots (1.9 t ha⁻¹). In the second season (2006), compared with the control plots (4.2 t ha⁻¹), there was no significant effect of dung beetles or dung alone on dry matter production. In the 2007 season there was a significant 27% increase in pasture production (1.6 t DM ha⁻¹) in the dung+beetles plots compared with the control plots (6.0 t DM ha⁻¹). The addition of dung to the soil surface had no significant effect upon dry matter production.

During autumn–winter of 2006 and 2007 there was minimal pasture production in the control plots (0.5 t DM ha⁻¹) and little impact of dung beetles. In marked contrast, in spring–early summer there was substantial pasture production (3.7 to 6.0 t DM ha⁻¹ in the control plots) and, compared with the control plots, a marginally significant 10% (= 0.4 t DM ha⁻¹) growth advantage in 2006 and a significant 27% (P = 0.04, 1.5 t DM ha⁻¹) growth advantage in 2007 in the dung+beetles plots.

The Ashbourne data indicate that dung alone (on the soil surface) induced no long-term or short-term (3 to 6 months) pasture growth advantage. In marked contrast, the impact of dung burial by beetles on dry matter production has become increasingly evident as the trial has progressed (+39% (not significant) in season 1, +8% in season 2 and +27% in season 3).

In addition, a more finely tuned analysis was possible by considering sections of the main plots that had had different histories at Ashbourne. The pasture harvested in January 2007 (evaluating growth in the 2006 season) was assessed in two sections. In the first section (pasture not cut for 6 months) there was a 36% growth advantage (0.45 t ha⁻¹) in the dung+beetles plots over the control plots. In the second section (pasture not cut for 3 months) there was a 150% growth advantage (0.17 t ha⁻¹) in the dung+beetles plots over the control plots. In neither section was there an effect of dung alone.

The pasture assessments in August to November 2007 demonstrated a 39% increase in pasture production due to dung buried in autumn–early winter of the same year.

The 2x2 m plots at Kuitpo

Over the three seasons there was a significant 25% pasture growth advantage (5.0 t DM ha⁻¹) in the dung+beetles plots over the control plots and a significant 23% pasture growth advantage (4.7 t DM ha⁻¹) in the 'dung-only' (45% burial) plots over the control plots. Pasture production from the dung+beetles plots was not significantly greater than that in the 'dung-only' plots.

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In the first season (2005) there was no effect of added dung or dung beetle activity on dry matter production compared with that in the control plots (3.1 t ha^{-1}). In the second season (2006), compared with the pooled control plots, there was a highly significant 134% increase (3.3 t DM ha^{-1}) in pasture production in the dung+beetles plots and a highly significant 122% increase (3.0 t DM ha^{-1}) in production in the 'dung-only' (45% burial) plots over the pooled control plots (2.4 t ha^{-1}). There was no significant difference in pasture production from the dung+beetles plots and the 'dung-only' plots.

The majority of the response to dung and dung beetles occurred during autumn and winter. For the May to August period, compared to the pooled control plots (1.5 t ha^{-1}), there was a highly significant 197% increase (3.0 t ha^{-1}) in dry matter production in the dung+beetles plots and a highly significant 188% increase (2.9 t ha^{-1}) in dry matter production in the 'dung-only' (45% burial) plots. There was no significant difference in pasture production between the dung+beetles plots and the 'dung-only' plots.

In the third season (2007), compared with the control plots (1.6 t ha^{-1}), there was a highly significant 54% increase (0.9 t ha^{-1}) in dry matter production in the dung+beetles plots and a significant 38% increase (0.6 t ha^{-1}) in dry matter production in the 'dung-only' (45% burial) plots. There was no significant difference in pasture production between the dung+beetles plots and the 'dung-only' plots.

The 10x10 m plots at Kuitpo

Pasture production analysed over the three seasons showed a non-significant 11% pasture growth advantage in the dung+beetles plots over the control plots (6.8 t DM ha^{-1}), and there was no statistically significant effect of dung beetles when the data were analysed year by year. This was considered to be due in part to the substantial inter-plot variation in production due to substantial within- and between-plot variation in the density of perennial grass tussocks. Tussock density was strongly related to pasture production (June to November 2007) over the range of 2 to 10 tussocks m^{-2} .

Recommendations

1. That pasture sampling at the Ashbourne plots (2x2 m and 10x10 m) continue for an additional 2 years beyond the limit of the current project to assess the persistence of beetle-induced increased pasture production.
2. Provided that re-establishment of an even-sward perennial grass pasture at Kuitpo is successful, that pasture sampling of the 2x2 m the plots continue for an additional 2 years beyond the limit of the current project to assess the persistence of beetle-induced increased pasture productivity.
3. That beetle impact on pasture production in the 10x10 m plots at Kuitpo be undertaken on subsections of the plots with an even sward of pasture tussocks.
4. That the production benefits be validated with a paddock-scale experiment.
5. That support be provided to allow Dr Malcolm McCaskill to provide modelling expertise for an analysis of the potential economic impact of deep-tunnelling dung beetles in southern Australia.

Methods

Site selection, pasture sampling procedures and other relevant methods are detailed elsewhere.

Results

The pasture growth data for the three seasons have been analysed in more detail than presented in previous reports and the results of this analysis are presented below.

This report summarises the growth of pastures for the first two years of the experiments (October 2005 to November 2007), within which there were three distinct growing seasons, namely the tail

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end of the 2005 growing season (October to December 2005), the whole 2006 growing season and the whole 2007 growing season (up to mid-November 2007). There has been minimal pasture growth since mid-November 2007.

Ashbourne 2x2 m plots

Pasture production analysed over the three seasons (Table 2.1) showed no statistically significant difference between the two types of control (with and without cages for the first 6 weeks) ($t_3 = 1.72$, $P = 0.09$) and so these were pooled to produce one control data set (15.4 t ha^{-1}).

Importantly, there was a statistically significant 27% pasture growth advantage (4.1 t ha^{-1}) in the dung+beetles plots over the control plots ($t_{10} = 4.75$, $P = 0.0008$) and a statistically significant 20% pasture growth advantage (3.1 t ha^{-1}) in the 'dung-only' (45% burial) plots over the control plots ($t_{10} = 3.97$, $P = 0.001$) (Figure 2.1). Pasture production from the dung+beetles plots was not significantly different from that in the 'dung-only' plots (18.5 t ha^{-1}).

There were statistically significant treatment effects within years which are presented below on a year-by-year basis.

Table 2.1 The impact of dung and dung beetles on pasture production (tonnes dry matter [DM] ha^{-1}) in replicated 2x2 m plots at Ashbourne SA in the three seasons since the experiment began in early October 2005

	Pasture production (tonnes DM ha^{-1})			
	2005	2006	2007	Total
Dung+beetles	4.30	5.49	9.72	19.51
'Dung-only' (45% burial)	4.80	5.26	8.42	18.48
Control 1	4.30	4.04	7.80	16.14
Control 2	3.93	3.60	7.11	14.64

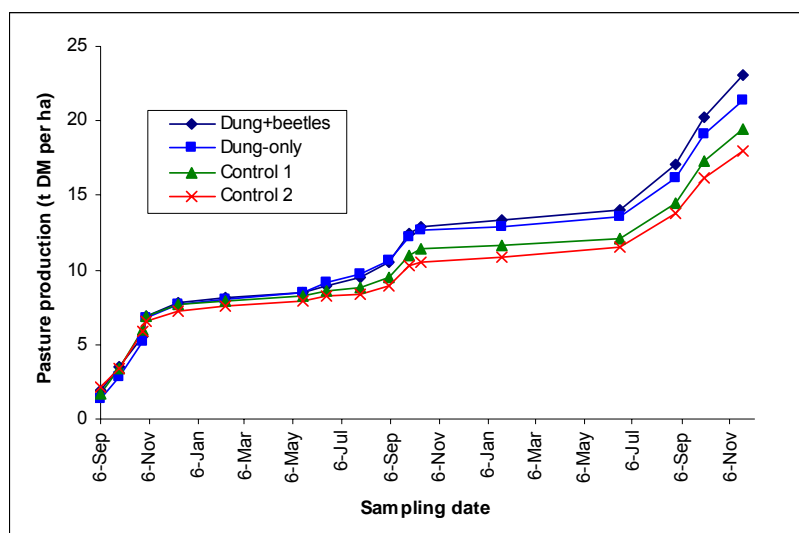


Figure 2.1 The effect of dung and the dung beetle *Bubas bison* on pasture production at Ashbourne in replicated 2x2 m plots presented as cumulative data (tonnes DM ha^{-1}) over the two years since the experiment began (October 2005) and two pre-experiment samples in September 2005

Season 1 (sampled October to December 2005)

Just before the experiment was set up, the pasture at Ashbourne was sampled, on 6 September 2005. Another sample was taken on 28 September 2005. These two samples were considered baseline samples, and were not included in the first season's pasture production. In the first season there were three sampling occasions (28 October, 18 November and 12 December). There

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was no significant difference between the pasture production from the control 1 plots and the control 2 plots ($t_3 = 0.94$, $P = 0.207$), and so the data were pooled for further comparisons.

There was a marginally significant 11% increase (0.4 t ha^{-1} , $t_{14} = 1.67$, $P = 0.059$) in dry matter production in the plots with added dung (4.6 t ha^{-1}) over the control plots (4.1 t ha^{-1}) but there was no significant additional effect of dung beetle activity on pasture production.

Season 2 (sampled February 2006 to January 2007)

In the second season there were eight sampling occasions (10 February, 17 May, 17 June, 28 July, 2 September, 29 September, 13 October 2006 and 23 January 2007, representing growth from mid-December 2005 to 23 January 2007). There was no significant difference between the pasture production from the control 1 plots and the control 2 plots ($t_3 = 1.91$, $P = 0.08$) and so the data were pooled for further comparisons.

Over the 2006 season, compared with the pooled control plots (3.82 t ha^{-1}), there was a highly significant 44% increase (1.7 t DM ha^{-1} , $t_{10} = 6.40$, $P = 0.00004$) in pasture production in the dung+beetles plots and a highly significant 38% increase (1.4 t DM ha^{-1} , $t_{10} = 5.20$, $P = 0.0002$) in pasture production in the 'dung-only' (45% burial) plots. Furthermore, there was marginally significant greater pasture production (0.23 t ha^{-1} , $t_3 = 2.07$, $P = 0.06$) in the dung+beetles plots than in the 'dung-only' plots.

Table 2.2 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Ashbourne during the 2006 growing season. The experiment began in October 2005.

	Pasture production (tonnes ha ⁻¹)								
	10 Feb	17 May	17 Jun	28 Jul	2 Sep	29 Sep	13 Oct	23 Jan	Total
Dung+beetles	0.35	0.31	0.45	0.61	1.02	1.91	0.48	0.37	5.49
'Dung-only'	0.37	0.45	0.66	0.54	0.93	1.59	0.41	0.30	5.26
Control 1	0.23	0.35	0.37	0.27	0.68	1.47	0.38	0.30	4.04
Control 2	0.29	0.35	0.28	0.22	0.57	1.25	0.32	0.32	3.60

Closer examination of the data from the eight individual sampling occasions during 2006 (Table 2.2) suggests that the majority of the plant growth and the observed responses to dung beetle activity took place during the late winter to early summer interval. Pooling the data for the period February to June 2006 (three sampling occasions) and for the period July 2006 to January 2007 (five subsequent sampling occasions) gives a clearer indication of the trends in response to dung, beetles and season (Table 2.3).

During the first half of 2006 (February–June) pasture production was minimal (0.93 t ha^{-1} in the pooled control plots). There was a pasture growth advantage in the 'dung-only' (45% burial) plots over the dung+beetles plots (0.4 t ha^{-1} , $t_3 = 3.5$, $P = 0.02$) and these data (ie the two +dung treatments, in both of which there had been beetle activity burying dung) were also pooled and compared with the pooled controls (the treatments without dung) (Table 2.3). There was a significant 39% growth advantage (0.4 t ha^{-1} , $t_{14} = 2.87$, $P = 0.006$) due to the presence of dung+beetles over the pooled control plots (Table 2.3).

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Table 2.3 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Ashbourne during autumn/early winter and spring/early summer in the 2006 growing season. The experiment began in October 2005.

Sampling occasions	Pasture production (tonnes ha ⁻¹)	
	Feb–June 2006	Jul 2006–Jan 2007
Dung+beetles	1.11	4.38
'Dung-only' (45% burial)	1.47	3.78
Pooled controls	0.93	2.89
	t-value	and significance level
Control 1 vs control 2	0.15 ns	1.27 ns
Dung+beetles vs pooled controls	1.30 ns	6.37***
'Dung-only' vs pooled controls	3.68**	3.25**
Pooled dung treatments vs pooled controls	2.87**	5.15***
Dung+beetles vs 'dung-only'	3.54*	3.75*

* P<0.05, P<0.01**, *** P<0.001

During the second half of the 2006 season (July 2006 to January 2007),¹ pasture production was substantial (2.9 t ha⁻¹ in the pooled control plots). There was a highly significant 51% pasture growth advantage (1.5 t ha⁻¹, $t_{10} = 6.4$, $P = 0.00004$) in the dung+beetles plots over the control plots, and a significant 31% pasture growth advantage (0.9 t ha⁻¹, $t_{10} = 3.3$, $P = 0.004$) in the 'dung-only' (45% burial) plots over the control plots (Table 2.3). In addition, there was a significant 16% pasture growth advantage (0.6 t ha⁻¹, $t_3 = 3.7$, $P = 0.02$) in dung+beetles plots (complete dung burial) over the 'dung-only' plots (45% dung burial) (Table 2.3).

Season 3 (sampled June to November 2007)

In the third season there were four sampling occasions (21 June, 28 August, 4 October and 23 November, representing pasture growth from February to November). There was no significant difference between the pasture production from the control 1 plots and the control 2 plots ($t_3 = 0.84$, $P = 0.23$), and so the data were pooled for further comparisons.

Over the season, compared with the pooled control plots (7.46 t ha⁻¹), there was a significant 30% increase (2.3 t DM ha⁻¹, $t_{10} = 3.55$, $P = 0.003$) in pasture production in the dung+beetles plots and marginally significant 13% increase (1.0 t DM ha⁻¹, $t_{10} = 1.89$, $P = 0.044$) in pasture production in the 'dung-only' (45% burial) plots. Furthermore, there was a non-significant 15% greater pasture production (1.3 t ha⁻¹) in the dung+beetles plots than in the 'dung-only' plots ($t_3 = 1.41$, $P = 0.13$).

Closer analysis of the data from the four individual sampling occasions during 2007 (Table 2.4) suggests that the majority of the plant growth and the observed response to dung and dung beetles took place during spring and early summer.

¹ The January 2007 pasture sample represents pasture growth during the period mid-October 2006 to January 2007, and so was included in the 2006 growing season.

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Table 2.4 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Ashbourne during the 2007 growing season. The experiment began in October 2005.

	21 Jun	28 Aug	4 Oct	23 Nov	2007 total
Dung+beetles	0.72	3.03	3.20	2.78	9.72
'Dung-only' (45% burial)	0.63	2.65	2.90	2.25	8.42
Control 1	0.37	2.48	2.77	2.19	7.80
Control 2	0.60	2.35	2.30	1.86	7.11

During the first half of 2007 (January–June) pasture production was minimal (0.49 t ha⁻¹ in the pooled control plots). There was no pasture growth advantage of in the dung+beetles treatment over the 'dung-only' (45% burial) treatment ($t_3 = 1.33$, $P = 0.138$) and so these data (ie the +dung treatments) were also pooled and compared with the pooled controls (the treatments without dung). There was a significant ($t_{14} = 1.79$, $P = 0.047$) 38% growth advantage (0.19 t ha⁻¹) due to the presence of dung (and dung beetles) over that from the pooled control plots (0.49 t ha⁻¹) (Table 2.5).

During the second half of 2006 (July to November) pasture production was substantial (6.97 t DM ha⁻¹ in the pooled control plots). There was a significant 29% pasture growth advantage (2.03 t ha⁻¹, $t_{10} = 3.03$, $P = 0.013$) in the dung+beetles plots over the control plots, and a non-significant 12% pasture growth advantage in the 'dung-only' (45% burial) plots over the control plots (0.83 t ha⁻¹, $t_{10} = 1.53$, $P = 0.079$). There was no significant pasture growth advantage in the dung+beetles plots over the 'dung-only' plots ($t_3 = 1.41$, $P = 0.127$) (Table 2.5).

Table 2.5 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Ashbourne during autumn/early winter and spring/early summer in the 2007 growing season. The experiment began in October 2005.

Sampling occasions	Pasture production (tonnes ha ⁻¹)	
	June	Aug – Nov
Dung+beetles	0.72	9.00
'Dung-only' (45% burial)	0.63	7.80
Pooled controls	0.49	6.97
t-value and significance level		
Control 1 vs control 2	1.04 ns	1.11 ns
Dung+beetles vs pooled controls	1.70 ns*	1.03**
'Dung-only' vs pooled controls	1.02 ns	1.53 ns*
Pooled dung treatments vs pooled controls	1.79*	2.67**
Dung+beetles vs 'dung-only'	1.33 ns	1.41 ns

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns*: $P = 0.06–0.07$

Ashbourne 10x10 m plots

Pasture production analysed over the three seasons (Table 2.6, Figure 2.2) showed a statistically significant 22% pasture growth advantage (2.7 t DM ha⁻¹ $t_4 = 3.50$, $P = 0.012$) in the dung+beetles plots over the control plots (12.1 t ha⁻¹), but there was no significant advantage ($t_4 = 1.52$, $P = 0.102$) in the dung+beetles plots over the dung-only plots (12.9 t ha⁻¹) (Figure 2.2). There was also no significant effect of surface dung on pasture production ($t_4 = 0.65$, $P = 0.276$) (Table 2.6).

There were statistically significant treatment effects within years which are presented below on a year-by-year basis.

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

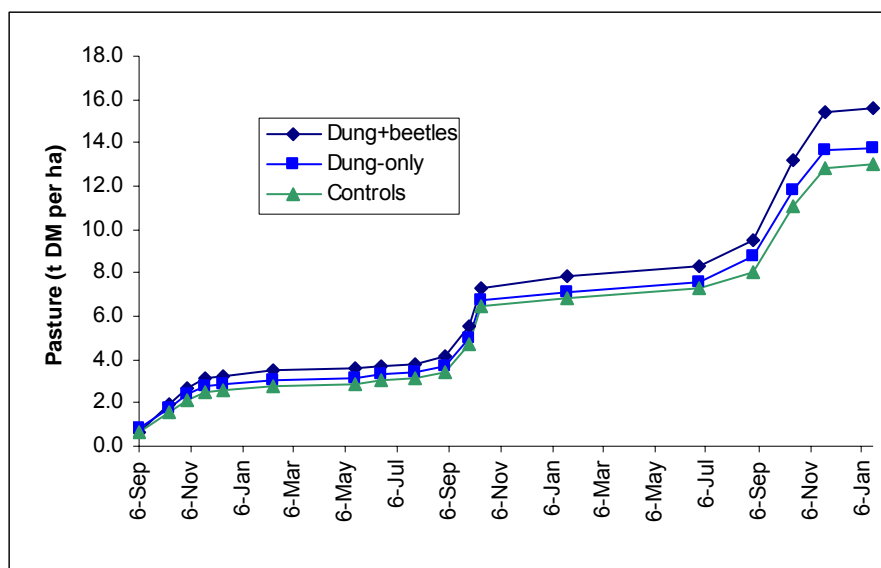


Figure 2.2 The effect of dung and dung beetles on pasture production at Ashbourne in replicated 10x10 m plots, presented as cumulative data (tonnes DM ha⁻¹) over the two years since the experiment began (October 2005)

Table 2.6 The impact of dung and dung beetles on pasture production (tonnes dry matter [DM] ha⁻¹) in replicated 10x10 m plots at Ashbourne SA in the three seasons since the experiment began in early October 2005

	Pasture production (tonnes DM ha ⁻¹)			
	2005	2006	2007	Total
Dung+beetles	2.66	4.58	7.60	14.84
Dung-only	2.10	4.26	6.51	12.87
Controls	1.91	4.23	6.00	12.15

Season 1 (sampled October to December 2005)

In the first season there were four sampling occasions (11 October, 31 October, 18 November, 12 December 2005). There was no significant effect of beetle activity ($t_4 = 1.65$, $P = 0.087$) or dung-only ($t_4 = 0.55$, $P = 0.31$) on dry matter production over the control plots (1.9 t ha⁻¹). There was a marginally significant 33% growth advantage (0.66 t DM ha⁻¹, $t_7 = 1.78$, $P = 0.059$) in the dung+beetles treatment compared to the dung-only and control plots combined (2.0 t ha⁻¹) on dry matter production.

Season 2 (sampled February 2006 to January 2007)

In the second season there were eight sampling occasions (10 February, 17 May, 17 June, 28 July, 2 September, 29 September and 13 October 2006, and 23 January 2007). Over the 2006 season, compared with the control plots (4.23 t ha⁻¹), there was no significant increase in dry matter production in the dung+beetles or the dung-only plots. There was no significant increase in dry matter production in the dung+beetles plots compared with the dung-only plots, or in the dung+beetles plots compared with the dung-only and control plots combined (4.25 t ha⁻¹, $t_7 = 1.44$, $P = 0.097$).

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

Table 2.7 The effect of dung and dung beetles on pasture growth in the 10x10 m plots at Ashbourne in autumn–winter and spring–summer of 2006

	Pasture production (tonnes ha ⁻¹)								
	10 Feb	17 May	17 Jun-	28 Jul	2 Sep	29 Sep	13 Oct	23 Jan	Total
Dung+beetles	0.20	0.10	0.14	0.07	0.35	1.41	1.80	0.51	4.58
Dung-only	0.18	0.11	0.19	0.07	0.26	1.32	1.73	0.40	4.26
Controls	0.19	0.11	0.15	0.07	0.29	1.31	1.69	0.42	4.23

Closer examination of the data from the eight individual sampling occasions during 2006 (Table 2.7) suggests that the majority of the plant growth and the observed response to dung and dung beetles took place during spring and early summer. Pooling the data for the period February to July (four sampling occasions) and the period September 2006 to January 2007 (four subsequent sampling occasions) gives a clearer indication of the trends in response to dung, beetles and season (Table 2.8).

During the first half of 2006 (January–July)² pasture production was minimal (0.52 t ha⁻¹ in the control plots). There was no pasture growth advantage in the dung+beetles plots over the dung-only plots or the control plots (Table 2.8).

Similarly, in the latter part of the season (August 2006 (sampled September) to January 2007: spring and early summer) there was a non-significant +10% pasture growth advantage (0.36 t ha⁻¹) in the dung+beetles plots compared with the control plots (3.7 t ha⁻¹). There was no effect of dung alone (Table 2.8).

Table 2.8 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Ashbourne during autumn/early winter and spring/early summer in the 2006 growing season. The experiment began in October 2005.

Sampling occasions	Pasture production (tonnes ha ⁻¹)	
	Sum Feb–Jul	Sum Sept–Jan
Dung+beetles	0.50	4.07
Dung-only	0.54	3.72
Controls	0.52	3.71
t-value and significance level		
Dung+beetles vs controls	0.24 ns	1.63 ns*
Dung only vs controls	0.22 ns	0.04 ns
Dung+beetles vs dung-only	0.32 ns	1.61 ns*]

* P<0.05, ** P<0.01, *** P<0.001

Season 3 (sampled June to November 2007)

Over the 2007 season (sampled on four occasions: 28 June, 30 August, 16 October and 22 November 2007, representing pasture growth from February to November) there was a significant 27% increase in dry matter production in the dung+beetles plots (1.59 t ha⁻¹, $t_4 = 2.73$, $P = 0.026$) compared with the control plots (6.0 t ha⁻¹), and there was a non-significant 17% greater pasture production in the dung+beetles plots than in the dung-only plots (1.09 t ha⁻¹, $t_4 = 1.24$, $P = 0.140$). The addition of dung to the soil surface had no significant effect upon dry matter production ($t_4 = 0.58$, $P = 0.297$).

Closer examination of the data from the four individual sampling occasions during 2007 (Table 2.9) suggests that the majority of the plant growth and the observed response to dung and dung beetles took place during spring and early summer. Examining the data for two periods (December to June, one sampling occasion (June 2007) and July to November 2007 (the three subsequent

² The February 2006 pasture sample represents pasture growth from mid-December 2005.

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

sampling occasions)) gave a clearer indication of the trends in response to dung, beetles and season (Table 2.10).

Table 2.9 The effect of dung and dung beetles upon pasture production in 2007 in the 10X10 m plots at Ashbourne

	28 Jun	30 Aug	16 Oct	22 Nov	Total
Dung+beetles	0.48	1.21	3.68	2.22	7.60
Dung-only	0.45	1.17	3.06	1.82	6.51
Controls	0.42	0.82	3.04	1.73	6.00

During the first half of 2007 pasture production was minimal (0.42 t ha⁻¹ in the control plots). There was no pasture growth advantage in the dung+beetles plots over the dung-only plots or the control plots (Table 2.10).

In contrast, in the latter part of the season (spring and early summer) there was a statistically significant 27% pasture growth advantage (1.5 t ha⁻¹) in the dung+beetles plots compared with the control plots ($t_4 = 2.36$, $P = 0.038$). There was no effect of dung alone on pasture production ($t_4 = 0.53$, $P = 0.314$) (Table 2.10).

Table 2.10 The impact of dung and dung beetles on pasture growth in 10x10 m plots at Ashbourne during autumn/early winter and spring/early summer in the 2007 growing season. The experiment began in October 2005.

Sampling occasions	Pasture production (tonnes ha ⁻¹)	
	June	Sum Aug – Nov
Dung+beetles	0.48	7.11
Dung-only	0.45	6.06
Controls	0.42	5.59
t-value and significance level		
Dung+beetles vs controls	0.66 ns	4.48*
Dung only vs controls	0.34 ns	1.28 ns
Dung+beetles vs dung-only	0.66 ns	2.59 ns*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns*: $P = 0.06$

Kuitpo 2x2 m plots

Pasture production analysed over the three seasons (Table 2.11) showed no statistically significant difference between the two types of control (with and without cages for the first 8 weeks) ($t_3 = 0.72$, $P = 0.26$) and so these were pooled to produce one control data set (20.4 t ha⁻¹).

There was a statistically significant 25% pasture growth advantage (5.01 t ha⁻¹) in the dung+beetles plots over the control plots ($t_{10} = 1.84$, $P = 0.047$) and a statistically significant 23% pasture growth advantage (4.7 t ha⁻¹) in the 'dung-only' plots (45% dung burial) over the control plots ($t_{10} = 2.53$, $P = 0.015$) (Figure 2.3). There was no statistically significant difference in the pasture production from the dung+beetles plots and the 'dung-only' plots.

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

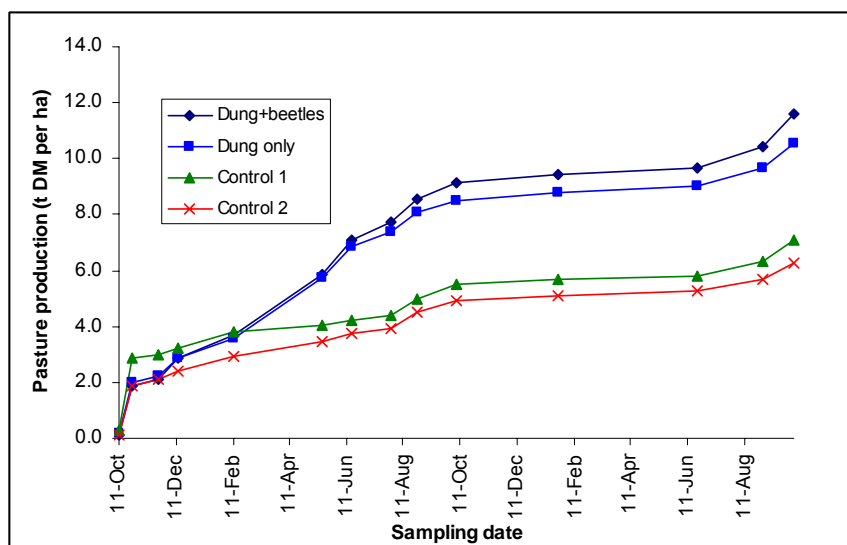


Figure 2.3 The effect of dung and the dung beetle *Bubas bison* on pasture production at Kuitpo in replicated 2x2 m plots, presented as cumulative pasture production data (tonnes DM ha⁻¹) over the two years since the experiment began (October 2005)

Table 2.11 The impact of dung and dung beetles on pasture production (tonnes dry matter [DM] ha⁻¹) in replicated 2x2 m plots (cut to 7 cm pasture height) at Kuitpo SA in the three seasons since the experiment began in early October 2005. The special cut in October 2007 harvested the organic matter between 7 cm and 1.5 cm above ground level.

	Pasture production (tonnes DM ha ⁻¹)			2007 special	Total
	2005	2006	2007		
Dung+beetles	2.95	5.71	2.44	14.28	25.38
'Dung-only' (45% burial)	2.93	5.42	2.18	14.53	25.07
Control 1	3.54	2.33	1.71	13.61	21.19
Control 2	2.67	2.55	1.45	12.88	19.55

There were statistically significant treatment effects within years which are presented below on a year-by-year basis.

Season 1 (October to December 2005)

In the first season there were four sampling occasions (11 October, 25 October, 22 November and 12 December). There was no significant difference between the pasture production from the control 1 plots and the control 2 plots ($t_3 = 1.47$, $P = 0.12$) and so the data were pooled for further comparisons. Similarly, there was no significant difference in the levels of production from the dung+beetles plots (3.0 t ha⁻¹) and the 'dung-only' (45% burial) plots (2.9 t ha⁻¹) ($t_3 = 0.12$, $P = 0.46$) and so these data were pooled (2.9 t ha⁻¹) for comparison with the control plot data (3.10 t ha⁻¹), and they did not differ from each other ($t_{14} = 0.42$, $P = 0.34$).

Season 2 (sampled February 2006 to January 2007)

In the second season there were seven sampling occasions (10 February, 16 May, 17 June, 28 July, 26 August and 6 October 2006, and 23 January 2007, representing growth from mid-December 2005 to January 2007). There was no significant difference between the pasture production from the control 1 plots and the control 2 plots ($t_3 = 0.35$, $P = 0.37$) and so the data were pooled for further comparisons.

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Over the 2006 season, compared with the pooled control plots (2.44 t ha⁻¹), there was a highly significant 134% increase (3.3 t ha⁻¹, $t_{10} = 7.14$, $P = 0.0002$) in dry matter production in the dung+beetles plots and a highly significant 122% increase (3.0 t ha⁻¹, $t_{10} = 6.87$, $P = 0.00002$) in dry matter production in the 'dung-only' (45% burial) plots over the pooled control plots (2.44 t ha⁻¹). There was no significant difference in pasture production from the dung+beetles plots and the 'dung-only' plots ($t_3 = 0.41$, $P = 0.36$) (Table 2.12).

Table 2.12 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Kuitpo during the 2006 growing season. The experiment began in October 2005.

	Pasture production (tonnes ha ⁻¹)							
	10 Feb	16 May	17 Jun-	28 Jul	26 Aug	6 Oct	23 Jan	Total
Dung+beetles	0.31	2.16	1.21	0.64	0.55	0.56	0.28	5.71
'Dung-only' (45% burial)	0.28	2.17	1.13	0.49	0.63	0.45	0.26	5.42
Control 1	0.27	0.22	0.21	0.17	0.75	0.52	0.19	2.33
Control 2	0.22	0.51	0.30	0.18	0.73	0.44	0.16	2.55

The majority of the response to dung and dung beetles occurred during autumn and winter (Table 2.12). Analysis of the data for the four sampling occasions from May to August together showed no significant difference between the pasture production from the control 1 plots and the control 2 plots ($t_3 = 0.96$, $P = 0.20$) and so the data were pooled for further comparisons.

For the May to August period, analysis revealed a highly significant 197% increase (3.0 t ha⁻¹, $t_{10} = 7.86$, $P = 0.00001$) in dry matter production in the dung+beetles plots and a highly significant 188% increase (2.89 t ha⁻¹, $t_{10} = 8.30$, $P = 0.00001$) in dry matter production in the 'dung-only' (45% burial) plots over the pooled control plots (1.53 t ha⁻¹). There was no significant difference in pasture production between the dung+beetles plots and the 'dung-only' plots ($t_3 = 0.26$, $P = 0.41$).

Season 3 (sampled June to October 2007)

In the third season (sampled June to October 2007 and representing pasture growth from February to October 2007) there were three normal pasture sampling occasions (cut to 7 cm pasture height) and a fourth, in which most of the vegetation was harvested (see below). There was no significant difference between the pasture production (normal sampling) from the control 1 plots and the control 2 plots ($t_3 = 0.80$, $P = 0.24$) and so these data were pooled for further comparisons.

Over the season (normal sampling), compared with the pooled control plots (1.58 t ha⁻¹), there was a highly significant 54% increase (0.86 t ha⁻¹, $t_{10} = 2.96$, $P = 0.007$) in dry matter production in the dung+beetles plots and a significant 38% increase (0.60 t ha⁻¹, $t_{10} = 2.22$, $P = 0.025$) in dry matter production in the 'dung-only' (45% burial) plots (Table 2.13). There was no significant difference in pasture production between the dung+beetles plots and the 'dung-only' plots ($t_3 = 0.51$, $P = 0.321$).

Table 2.13 The impact of dung and dung beetles on pasture growth in 2x2 m plots at Kuitpo during the 2007 growing season. The experiment began in October 2005.

	Pasture production (tonnes DM ha ⁻¹)				
	21-Jun-07	30-Aug-07	02-Oct-07	Total	02-Oct-07*
Dung+beetles	0.73	0.71	1.00	2.44	14.28
'Dung-only' (45% burial)	0.63	0.65	0.90	2.18	14.53
Control 1	0.38	0.53	0.81	1.71	13.61
Control 2	0.41	0.41	0.63	1.45	12.88

* an extra low cut (to 1.5 cm) following the normal height pasture cut (to 7 cm)

Kuitpo 10x10 m plots

Pasture production analysed over the three seasons (Table 2.14, Figure 2.4) showed a non-significant 11% pasture growth advantage (0.72 t ha⁻¹, $t_5 = 1.19$, $P = 0.14$) in the dung+beetles plots over the control plots (6.75 t ha⁻¹). When the data were analysed year by year, there was no

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statistically significant effect of dung beetle activity. There was also no significant difference in pasture production between the dung+beetles and the dung-only plots.

Table 2.14 The impact of dung and dung beetles on pasture production (tonnes dry matter [DM] ha⁻¹) in replicated 10x10 m plots at Kuitpo SA in the three seasons since the experiment began in early October 2005

	Pasture production (tonnes DM ha ⁻¹)			
	2005	2006	2007	Total
Dung+beetles	2.33	2.60	2.54	7.47
Dung-only	2.37	2.37	2.50	7.24
Controls	2.09	2.38	2.29	6.75

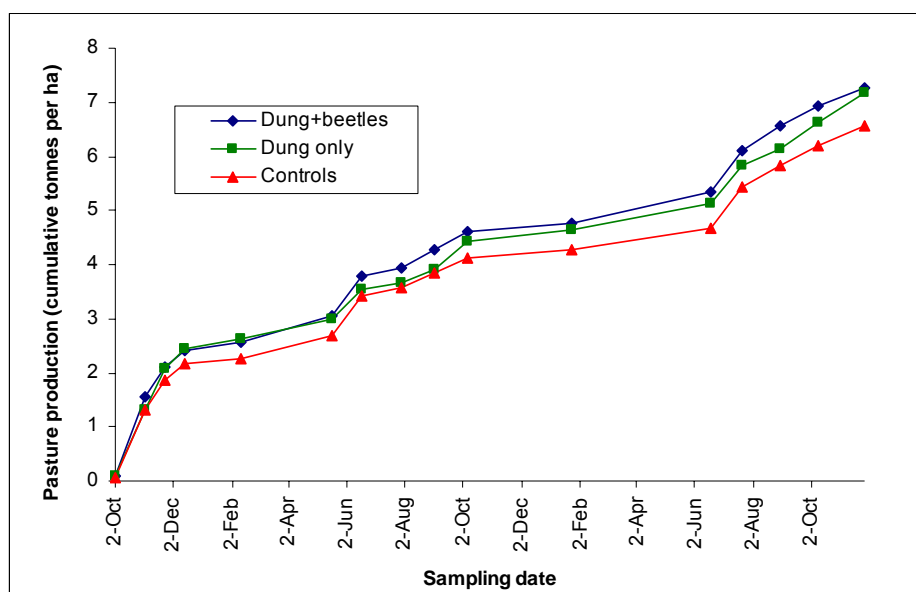


Figure 2.4 The effect of dung and the dung beetle *Bubas bison* on pasture production at Kuitpo in replicated 10x10 m plots, presented as cumulative pasture production data (tonnes DM ha⁻¹) over the two years since the experiment began (October 2005)

Season 1 (sampled November to December 2005)

In the first season (sampled November to December 2005 and representing growth from October to December) pastures were sampled on three occasions (Table 2.15)

There was no significant effect of dung or dung beetles on pasture production compared with that in the control plots (2.09 t ha⁻¹).

Table 2.15 The effect of dung and dung beetles on pasture growth in the 10x10 m plots at Kuitpo in the first season (sampled November to December 2005)

	Pasture production (tonnes ha-1)				Total
	2 Oct (pre-season)	2 Nov	22 Nov	13 Dec	
Dung+beetles	0.10	1.46	0.53	0.33	2.33
Dung-only	0.08	1.24	0.74	0.39	2.37
Controls	0.07	1.23	0.56	0.29	2.09

Season 2 (sampled February 2006 to January 2007)

Pastures were sampled on seven occasions during the 2006 season (representing pasture growth from mid-December 2005 to late January 2007 (Table 2.16). There was no significant effect of

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

dung beetles or dung alone on pasture production compared with that in the control plots (2.38 t ha⁻¹).

Table 2.16 The effect of dung and dung beetles on pasture growth in the 10x10 m plots at Kuitpo in the second season (mid-December 2005 to January 2007)

	Pasture production (tonnes ha-1)							Total
	10 Feb	16 May	17 June	28 Jul	2 Sep	6 Oct	23 Jan	
Dung+beetles	0.13	0.50	0.74	0.16	0.31	0.67	0.10	2.60
Dung-only	0.17	0.36	0.56	0.12	0.23	0.76	0.16	2.37
Controls	0.11	0.42	0.72	0.16	0.28	0.54	0.14	2.38

Season 3 (sampled June to October 2007)

Pastures were sampled on five occasions during the 2007 season (Table 2.17), representing pasture growth from February to late November 2007. Over the five sampling occasions, compared to the control plots (2.29 t ha⁻¹), there was a non-significant 11% pasture growth advantage in the dung+beetles plots (0.25 t ha⁻¹, $t_5 = 1.08$, $P = 0.165$), and a significant 9% pasture growth advantage in the dung-only plots (0.22 t ha⁻¹, $t_5 = 2.74$, $P = 0.021$). These trends are at variance with the overall results from the other plots and need to be viewed with caution.

Table 2.17 The effect of dung and dung beetles on pasture growth in the 10x10 m plots at Kuitpo in the third season (January 2007 to October 2007)

	Pasture production (tonnes ha-1)					Total
	19 Jun	21 July	30 Aug	9 Oct	26 Nov	
Dung+beetles	0.56	0.77	0.46	0.36	0.40	2.54
Dung-only	0.47	0.71	0.31	0.50	0.51	2.50
Controls	0.39	0.77	0.41	0.36	0.36	2.29

Effects of patchy perennial pastures at Kuitpo

The original planted pastures in the Kuitpo paddock from which the 10 x10 m plots were excised was a mixture of perennial pasture (predominantly ryegrass, which comprised 83% of all tussocks in February 2006) with some cocksfoot and some *Phalaris*. However, there were serious problems with Capeweed, which smothered pastures in the early part of the growing season in 2006 and 2007, despite chemical and manual weeding.

The number of perennial pasture clumps was assessed in each plot on 10 February 2006 and 2 January 2008. The number of perennial pasture plants was strongly correlated on the two occasions (Figure 2.5), although there had been an 83% increase in the number of perennial pasture clumps over that time.

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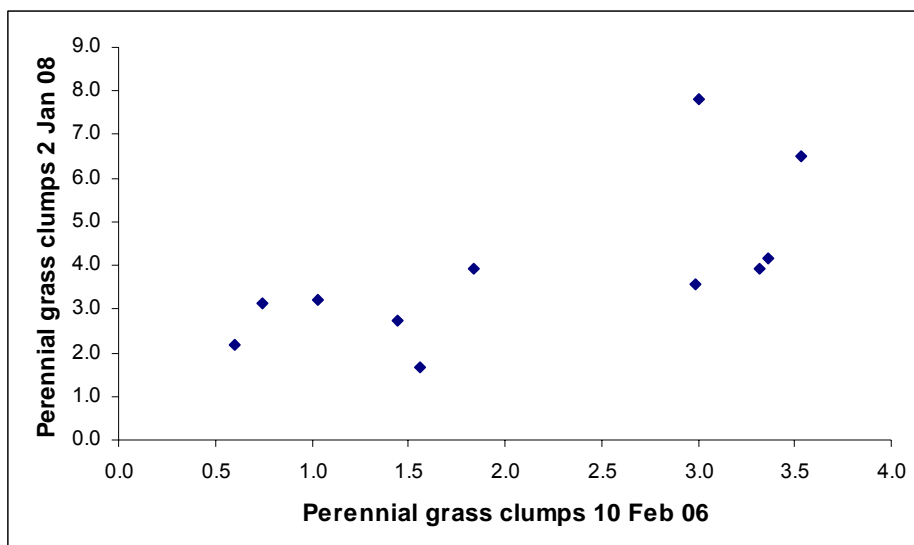


Figure 2.5 The relationship between the number of perennial pasture plant tussocks counted in February 2006 and January 2008 at Kuitpo, expressed in terms of numbers of tussocks per m² in the 11 plots (10x10 m each)

Despite this increase, the distribution of perennial pasture clumps was still very patchy within and between plots and this variation may, in part, explain why there was no detectable effect of dung beetle activity on pasture production.

In January 2008 the distribution of the tussocks was assessed within each plot by counting the number of tussocks in ten sections, each of 10 m², within each plot. This revealed a highly variable density of tussocks within plots which ranged from 2 per m² to over 10 per m² (Figure 2.6).

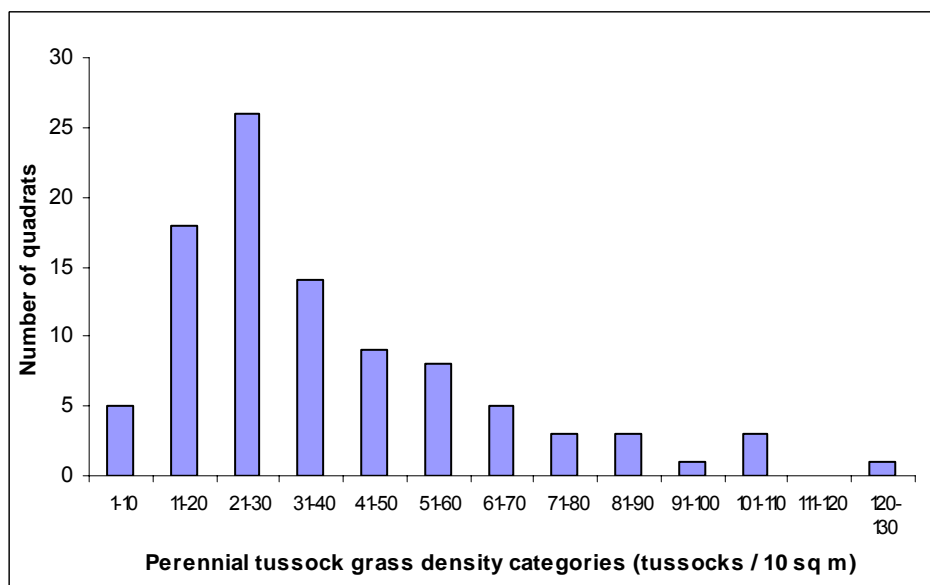


Figure 2.6 The frequency distribution of perennial pasture grass tussocks across the 10x10 m plots at Kuitpo in January 2008, expressed in terms of the number of tussocks found in each 10 m² quadrat

The pasture production in two sections of each plot (a 40 m² section and a 60 m² section) had been sampled for the period 21 July to 23 November 2007 (three sampling occasions, beginning 30 August). There was a positive relationship between pasture production over the four-month period and the density of perennial grass tussocks in the 22 sections examined (11 plots x 2 sections each) in January 2008, with a 3-fold increase in dry matter production over the range of tussock densities observed (Figure 2.7).

This effect was even more pronounced for the 7-week growing period 9 October – 26 November 2007 (a 5-fold difference, Figure 2.8).

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

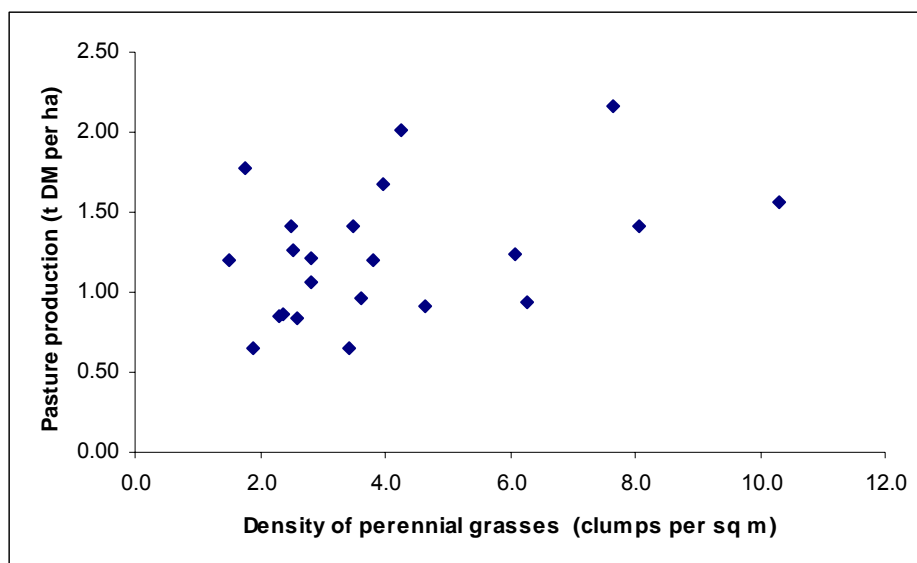


Figure 2.7 The relationship between the density of perennial pasture tussocks (expressed as the number of tussocks per m² present in January 2008) and pasture production in the 10x10 m plots at Kuitpo for the period 19 June to 26 November 2007

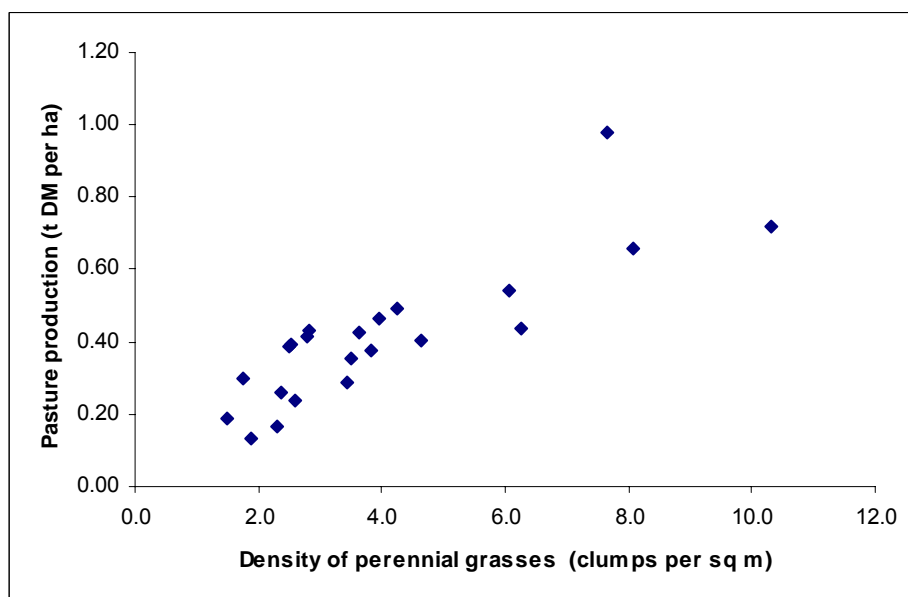


Figure 2.8 The relationship between the density of perennial pasture tussocks (expressed as the number of tussocks per m² present in January 2008) and pasture production in the 10x10 m plots at Kuitpo for the period 9 October to 26 November 2007 (one sampling occasion)

On the basis of these data on the density of perennial tussock grass clumps across the plots, a series of 12 subplots has been selected in order to continue to monitor the impact of dung beetle activity on pasture production. Subplots of 40 m² in area have been selected so that the densities of tussocks within each set of three plots (ie within treatment for each replicate) are similar to each other, and these will be used to assess the impact of the three treatments on pasture production in 2008 (Table 2.18).

Table 2.18 The density of perennial grass tussocks in the twelve 40 m² subplots selected for the 2008 evaluation of the impact of natural dung beetle populations upon pasture production. Subplots for each treatment were selected from within the plots with the same previous treatment; that is, dung-only subplots from within the 10 x10 m dung-only plots for 2005–07

Replicate	Density of perennial grass tussocks (no. m ⁻²)				Mean
	1	2	3	4	
Dung+beetles	8.2	7.5	6.3	3.3	6.3

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Dung only	8.5	6.1	3.7	3.4	5.4
Controls	8.1*	5.9	5.1	3.4	5.6

* selected from within a dung-only plot because no other high density locations were available. There has been no evidence that dung-only plots have greater pasture growth rates than control plots.

The rapidity of pasture responses to dung burial

The speed with which the pasture growth responds to dung burial is an important issue and is likely to vary with the time of year during which the dung is buried. Here we demonstrate that there was a strong within-season pasture growth response to dung buried in autumn–winter and in winter–spring. There was no pasture growth response to unburied surface dung.

The 2x2 m plots were set up in mid-spring (30 September and 1 October 2005), and dung burial occurred over the next 2–4 weeks. Pasture growth had largely finished by November. Because of this, there was little opportunity to assess the pasture growth response to dung burial by dung beetles in the year in which dung burial occurred.

In contrast, in 2006 and 2007, dung was applied to the 10x10 m plots throughout the dung beetle activity season (April to October) and so these plots provide an opportunity to assess the within-season response of pasture to dung beetle activity.

Dung burial by beetles was observed from April to November in 2006 and 2007. Since pasture growth was assessed at a variety of times throughout the year, it was possible to evaluate the pasture growth response to natural dung burial that occurred during autumn and early winter, and separately, in mid-winter to spring.

These processes are examined using Ashbourne data from the pasture cuts taken:

- in January 2007, allowing examination of the effects of dung burial by *B. bison* in autumn–winter 2006 and in winter–spring 2006
- in late winter and spring 2007 (21 July to 26 November), allowing examination of the effects of dung burial by *B. bison* in autumn–winter 2007 and in winter–spring 2007

The January 2007 sample

In 2006, each of the 10X10 m plots in the treatments with added dung (the dung-only and dung+beetles plots) contained two subsections, one to which dung had been added and one to which no dung had been added (dung was to be added to the latter section in 2007). This arrangement allowed assessment of the impact of dung and dung+beetles within plots in the same year as the dung was applied (and buried, in the case of the dung+beetles plots).

In the dung-only plots, small wire mesh cages were placed over each dung pad in order to exclude dung beetles. The presence of these cages prevented mowing for about 3 months after the cages+dung were placed in the trial plots.

This procedure created two further subsections within the +dung plots, namely one that had dung applied in autumn–winter (May–June 2006) and were mowed in October 2006 and January 2007, and one that had dung applied in the winter–spring (July–October 2006) period and were mowed in January 2007. The latter were also mowed in July 2006, immediately before beginning to apply dung at weekly intervals.

Thus each +dung plot contained three sections:

1. that having no dung applied in 2006 (5 metres wide)
2. that having dung applied in May–June 2006 and mowed in October 2006 and January 2007 (2 metres wide)
3. that having dung applied in July–October 2006 and mowed in January 2007 (2 metres wide) and previously mowed in July 2006, immediately before beginning to apply dung

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Comparable strips (2 metres wide) on the control (no dung) plots were also left un-mowed from July to October 2006.

Thus the production data from the plots in January 2007 (comprising three sections per plot), allowed a number of assessments of the impact of dung burial by beetles on pasture production. These were:

- overall pasture production (presented above)
- pasture growth response in winter–spring to dung alone and to dung burial (ie pasture production during the period July 2006 to January 2007) (presented above)
- pasture growth response in autumn–winter to dung alone and to dung burial. This can be done between plots (ie by comparing subsections of each of the 9 plots) and within plots (by comparing the +dung and no-dung sections within plots). The January 2007 sample reflects pasture growth over the period October 2006 to January 2007.

**Response to dung burial in winter–spring (July–October 2006):
6 months' growth (July 2006 to January 2007)**

A 2 m section of each plot was left un-mowed from July 2006 to January 2007 (due to the presence of beetle-excluding wire cages placed in the dung-only plots from June to October). Over that 6-month period the control plots (no dung) on average produced 1.2 t ha⁻¹ dry matter (pasture), which was not significantly different from that produced by the dung-only section of the plots (1.3 t ha⁻¹). In other words, there was no effect of dung alone on pasture production.

In contrast, the dung+beetles plots produced 1.7 t DM ha⁻¹, which was significantly greater than that from the other plots ($t_7 = 1.89$, $P = 0.049$). This represents a 0.45 t DM ha⁻¹ (36%) growth advantage in the first year; that is, the year in which the dung (+beetles) was placed in the plot.

**Response to dung burial in autumn–winter (April–June 2006):
3 months' growth (October 2006 to January 2007)**

Within-plot comparisons

Within each plot there was a 2 m subsection that had had dung added during April–June 2006, and a 5 m subsection that had had no dung added at all in 2006. Both of these sections had been mowed in October 2006 and so subsequent pasture growth allowed evaluation of the effects of dung and dung beetles *within* plots. When the subsections were sampled again in January 2007, the effects of dung and dung beetles (applied in autumn–winter 2006) on pasture production during the late spring growing season of 2006 (October 2006 to January 2007) could be evaluated.

Over this period there was no significance difference in pasture production between the two subsections of the control plots (0.21 t DM ha⁻¹, $t_2 = 0.51$, $P = 0.331$), indicating that there were no location effects within plots. Similarly, there was no significance difference in pasture production between the two subsections of the dung-only plots (producing an average of 0.17 t DM ha⁻¹, $t_2 = 0.411$, $P = 0.361$) (Table 2.19), indicating that there was no significant effect of dung on pasture production.

In marked contrast, the +dung (and beetles) section of the dung+beetles subplots produced 0.29 t ha⁻¹, which was significantly greater than that in the no-dung section of the plots (0.12 t DM ha⁻¹, $t_2 = 7.04$, $P = 0.010$) (Table 2.19). This relatively minor absolute advantage (0.17 t ha⁻¹) nevertheless represents a substantial 150% increase in pasture growth on the beetle-treated section of the plots.

Table 2.19 Effect of dung and dung+beetles on pasture production within plots at Ashbourne over the period July 2006 to January 2007

Previous history	Pasture production (t ha ⁻¹ dry matter)		
	Dung+beetles plots	Dung-only plots	Control plots
<i>Dung+beetles</i> section of plot or its equivalent in the dung-only and control plots	0.29	0.19	0.24
<i>No dung</i> section of dung+beetles plot	0.12	0.15	0.18

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Probability of a significant difference* 0.01 0.361 0.331

* paired t-tests

Overall, there were 12 no-dung-beetle sections of plots (2 for each of 6 plots) and three +dung-beetle sections. A comparison of pasture production from the +beetle subsections (n= 3) and the no-beetle sections (n = 12) was undertaken, with a similar result, in that pasture production from the +beetle sections was significantly greater (0.29 t DM ha⁻¹) than that from the no-beetle sections (0.17 t DM ha⁻¹, t₁₃ = 1.88, P=0.042).

The spring 2007 samples

In the late-winter to spring sampling in 2007 (three sampling occasions, namely 30 August, 16 October and 22 November), pasture production in two sections of each plot was assessed separately, reflecting contrasting historical effects (of application of dung). These allowed an analysis of the effects of dung burial by beetles in the current season and the effects of dung beetles one year after their dung burial activities had stopped. These subsections were:

- the area affected by dung in 2006 (a 5 m section)
- the area affected by dung in 2007 (a 2 m section). Where dung pads had decomposed the wire mesh cages were removed and where they were intact, pasture growing between the rows of wire mesh cages was harvested, leaving some part of the plot unharvested. Comparable areas in the dung+beetles plots and the control plots were also left unmowed.

In the both sections of the plots there was no significant effect of added dung upon dry matter production during spring 2007 (Table 2.20). Thus it appears that the surface dung had little influence upon pasture production.

In contrast, the spring 2007 dry matter production in the dung+beetles plots to which dung had been applied in 2006 was 24% (1.04 t DM ha⁻¹) greater than in the control plots (but with a low probability, P = 0.07), indicating that the effect of dung burial by beetles had persisted from one season to the next.

Similarly, in the section of the plots in which beetles had buried dung in the current year (autumn–early winter 2007) the spring 2007 dry matter production was 39% (3.24 t DM ha⁻¹) greater than in the control plots (but also with a low probability, P = 0.07) (Table 2.20).

Table 2.20 The effect of dung and dung beetles upon pasture production from June to September 2007

	Pasture production (t DM ha ⁻¹)	
	Dung applied 2006	Dung applied 2007
Dung+beetles	5.35	11.47
Dung-only	4.68	8.60
Controls	4.30	8.23
t-value and significance level		
Dung+beetles vs controls	1.82 ns*	1.88 ns*
Dung+beetles vs dung only	0.83 ns	1.54 ns
Dung only vs controls	0.51 ns	0.26 ns

ns* P = 0.07

Supplementary experiments

Location 1 Higgins property

The experiment was established in June 2003. At the Higgins property the plots were neither grazed nor harvested during 2003 and 2004. Initially the pasture on the plots was a mixture of Cape weed, clover and perennial grasses. Over the two years during which the pastures were not sampled, the perennial grasses became dominant, excluding most clover and all Cape weed. The

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pasture cuts in September 2006 left the soil surface in many plots relatively bare, due to massive growth of the perennial grasses excluding most other vegetation. These provided a favourable germination and establishment environment for Cape weed and so by September 2007 many of the plots contained a significant proportion of Cape weed plants, compared with the surrounding perennial grass stand (that had not been harvested in 2006) (see photo below).



Cape weed infestation in September 2007 in two adjacent 1x1 m plots that had been harvested (leaving bare ground) in 2006, surrounded by well-established perennial grasses that had not been harvested in 2006

All plots ($n = 9$) have been sampled on 5 occasions (10 August and 15 September 2005, 4 January and 3 September 2006, and 25 September 2007). Pasture on the plots was cut to about 4–5 cm and then a subsample was air-dried to estimate the moisture content of the pasture. Plant growth was calculated as tonnes of dry matter per ha by extrapolation from the 1 m² study plots.

Over the two years that the trial has been sampled (in years 3 and 4 since the trial was established in June 2003), overall there was no significant difference in yield from the dung-only plots and the control plots (Table 2.21) and so these data were pooled and compared with the yield from the dung+beetles plots.

Table 2.21 The effect of dung and dung beetles on pasture production (t DM ha⁻¹) in plots of 1 m² at Port Elliot on the Fleurieu Peninsula. Plots were established in June 2003.

	10-Aug-05	15-Sep-05	04-Jan-06	03-Sep-06	25-Sep-07	total
Dung+beetles	3.59	1.76	4.74	6.33	5.33	21.75
Dung-only	2.39	1.31	3.57	5.37	3.37	16.00
Controls	2.65	1.31	4.21	5.04	3.60	16.80
Mean	2.88	1.46	3.78	5.58	4.10	18.19

Over the two-year period there was a highly significant 38% increase (5.9 t DM ha⁻¹) in pasture production ($t_7 = 3.07$, $P = 0.009$) in the dung+beetles plots compared to the other plots.

During the 2007 growing season there was a significant 53% increase (1.8 t DM ha⁻¹) in pasture production ($t_7 = 2.71$, $P = 0.015$) in the dung+beetles plots compared to the other plots (3.5 t DM ha⁻¹).

In summary, there has been no evidence for increased pasture production in the third or fourth year of the trial in the plots in which dung had been placed on the surface in 2003 (without dung beetles). In marked contrast the pasture growth advantage in the dung+beetles plots has persisted into 2007 (the fourth year of the trial), and, overall, dung beetle activity has produced a pasture growth advantage about 6 tonnes ha⁻¹ over that 2-year period (Figure 2.9).

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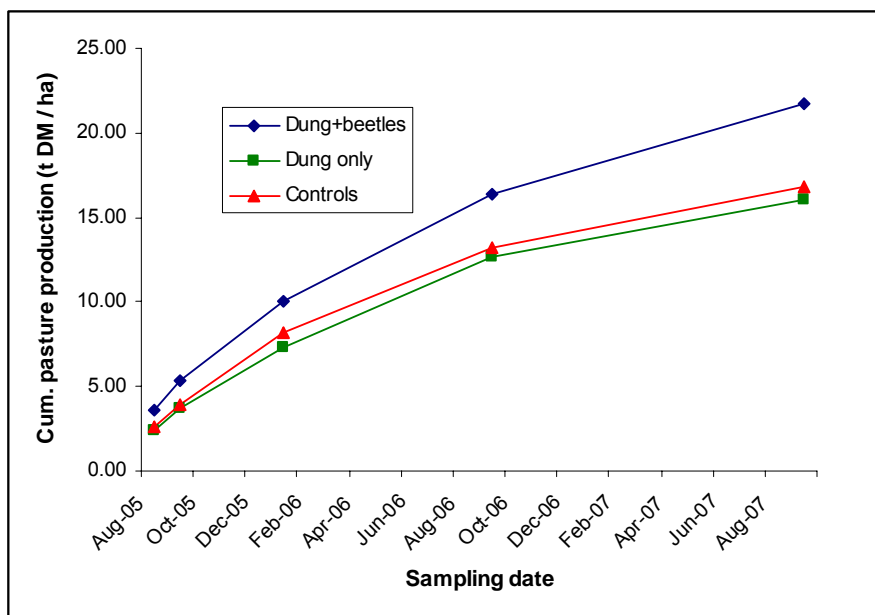


Figure 2.9 The effect of dung and the dung beetle *Bubas bison* on cumulative pasture production at supplementary location 1 (Higgins). One-metre square plots were inoculated with nine 2-litre dung pads and 10 pairs of *B. bison* per dung pad in July 2003. Pasture production from the 1 m² plots was extrapolated to tonnes of dry matter ha⁻¹.

Location 2 (Ballarat, Victoria) *Bubas bison*

The experiment was established in July 2003. All plots (n = 9) were sampled on 7 occasions (Figures 2.10 and 2.11).

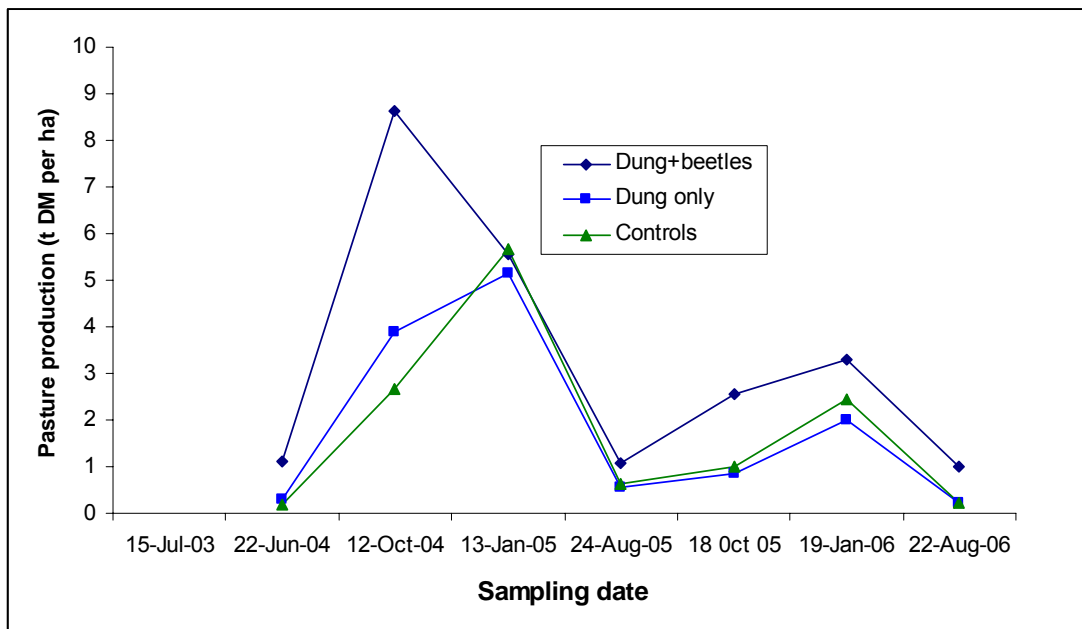


Figure 2.10 The effect of dung and the dung beetle *Bubas bison* on pasture production at Location 2 (Ballarat). One-metre square plots were inoculated with nine 2-litre dung pads and 10 pairs of *B. bison* per dung pad in July 2003. Pasture production from the 1 m² plots was extrapolated to tonnes of dry matter ha⁻¹.

On all occasions except for January 2005, there was substantially greater pasture production (dry weight) in the dung+beetles plots than in the dung-only plots or the control plots (Figure 2.10). Growth during the period October 2004 to January 2005

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was substantial for that time of year (5–6 tonnes dry matter ha⁻¹) and occurred in response to an unusually wet spring and early summer. It is not known why there was no response to dung beetle activity in this interval, when it was positive on either side (Figure 2.10), but it may be that moisture was limiting and so prevented the growth response to improved soil health. In June and October 2004 there was a positive response to the presence of dung but a statistically significant response disappeared in all subsequent sampling occasions.

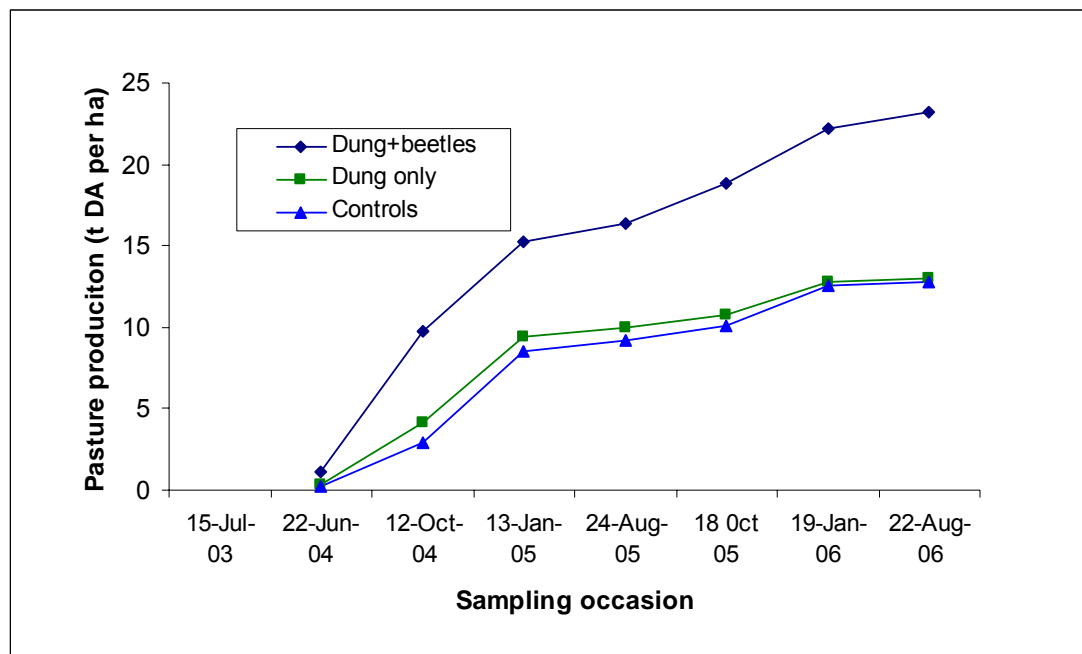


Figure 2.11 The effect of dung and the dung beetle *Bubas bison* on cumulative pasture production at Location 2 (Ballarat). One-metre square plots were inoculated with nine 2-litre dung pads and 10 pairs of *B. bison* per dung pad in July 2003. Pasture production from the 1 m² plots was extrapolated to tonnes of dry matter ha⁻¹.

Overall, the dung beetles produced a pasture growth advantage 10 tonnes ha⁻¹ over 3 years, an 80% increase over that of the dung-only (13.1 tonnes per ha) and the control plots (12.9 tonnes per ha), which did not differ significantly from each other (Figure 2.11). In other words, the effect of surface dung had disappeared by the second season but the effect of dung beetles had persisted, producing a substantial and significant growth response.

There was a poor break to the 2006 winter season and the follow-up winter rains were very mediocre and so growth over the period January to August 2006 was minimal but the dung beetle plots (1.0 tonnes ha⁻¹) maintained their growth advantage of the dung-only and control plots (0.24 tonnes ha⁻¹).

Discussion

Impact of dung beetle activity on pasture production

Baseline levels of pasture growth at Ashbourne and Kuitpo are influenced by soil type, rainfall and temperature and it is upon this platform that dung beetles have their impact on pasture production by improving soil fertility and its expression in pasture growth.

The soil at Ashbourne is a deep fertile alluvial clay loam whereas that at Kuitpo is a relatively infertile duplex soil with an acidic sandy loam over a yellow clay subsoil. The winter temperatures at Ashbourne are somewhat lower than at Kuitpo and the rainfall at Ashbourne is somewhat lower than at Kuitpo. These three factors bear in different ways upon the increased pasture production induced by the dung burial activity of dung beetles.

The 2006 results clearly indicate that the potential for increased pasture production due to dung beetle activity differed between Ashbourne and Kuitpo. For example at Ashbourne, in the 2x2 m dung+beetles plots there was a 44% (1.7 t DM ha⁻¹) increase in pasture production over that in the

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control plots (3.8 t DM ha⁻¹). In contrast, at Kuitpo there was a 134% increase (3.3 t DM ha⁻¹) in pasture production over that in the control plots (2.4 t DM ha⁻¹).

Thus, in 2006 the absolute increase at Kuitpo was greater than that at Ashbourne (3.3 and 1.7 t DM ha⁻¹ respectively), and the relative increase at Kuitpo was also greater than that at Ashbourne (134% and 44% respectively).

The data from the 2x2 m plots at Ashbourne and Kuitpo indicate that the positive pasture growth response to dung burial has persisted for at least 2 years and data from other, smaller plots (1 m²) suggest that the increased productivity is likely to persist for at least 4 years after dung burial by the beetles.

The value of additional pasture production

Because a small number of dung beetles entered the beetle-excluding cages in the 'dung-only' treatments in the 2x2 m plots (resulting in burial of 45% of the pads at both Ashbourne and Kuitpo), there were two +beetles treatments (complete burial and partial burial) in each experiment.

From the current experiments with the dung beetle *B. bison*, the pasture growth advantage due to dung beetle activity over the first two years of the experiment was:

- +27% (4.1 t ha⁻¹) in the 2x2 m plots at Ashbourne with 100% dung burial
- +20% (3.1 t ha⁻¹) in the 2x2 m plots at Ashbourne with 45% dung burial
- +22% (2.7 t ha⁻¹) in the 10x10 m plots at Ashbourne
- +25% (5.0 t ha⁻¹) in the 2x2 m plots at Kuitpo with 100% dung burial
- +23% (4.7 t ha⁻¹) in the 2x2 m plots at Kuitpo with 45% dung burial

The value of this additional pasture growth needs to be evaluated. One method that could be pursued is to model paddock performance for a variety of contrasting situations in southern Australia by the scaling-up from the plot data (see recommendations). Another is presented below.

Knowing the number of dung pads, the amount of dung buried (kg) in each experimental plot (set up using fresh local dung produced from May to September), and the increased pasture production (dry matter) from each of these plots, it is possible to estimate the increased dry matter production derived from the burial of a one-kilogram dung pad. The mean estimate based on the pasture growth in the 2006 and 2007 growing seasons was 0.057 kg DM per kg of buried dung (Table 2.22).

Table 2.22 Estimation of the pasture growth response per litre of buried dung

	# pads buried / plot	Dung buried plot (L)	Extra DM (t ha ⁻¹)	DM / m ² (kg)	DM / plot (kg)	DM / pad buried (kg)	DM / L dung buried (kg)
Ashbourne 2X2 m plots							
Full burial	16	48	3.93	0.39	1.57	0.10	0.033
45% burial	7.2	21.6	2.40	0.24	0.96	0.13	0.044
Kuitpo 2X2 m plots							
Full burial	16	48	5.16	0.52	2.06	0.13	0.043
45% burial	7.2	21.6	4.87	0.49	1.95	0.27	0.090
Ashbourne 9x9 m plots							
Full burial	216	216	1.94	0.19	15.71	0.07	0.073
Mean							0.057

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This estimate of the impact of dung beetle activity on pasture production is independent of stocking rate, cattle live weight, dung beetle abundance and seasonal activity and relies only upon a measure of dung burial by *B. bison*. This statistic can be used to estimate production benefits due to *B. bison* wherever the beetle will survive and bury dung.

This allows a simplistic modelling of the dollar benefits of increased pasture growth due to the dung burial activities of dung beetles.

For example, the dung produced by 100 mature cattle during the activity period of *B. bison* is estimated to produce a production benefit of 13 tonnes of dry matter (at 0.57 kg per kg of buried dung). The value of this can be expressed as additional hay yield. If we assume that the additional production did not substantially increase hay harvesting costs, then (assuming a 20% moisture level and a value of \$160 per tonne) the benefit of dung beetle activity would be \$2500.

Alternatively, the value of additional dry matter can be expressed as profit from carrying additional stock, or as profit from additional growth of existing stock. We illustrate this with an example using the following parameters:

- growth rates as indicated by Alan Bell (1992). Bell details the dse requirements for 200 kg steers growing at 0.0, 0.5, 1.0 and 1.5 kg live weight per day. For example, a 200 kg LW steer growing at 1.5 kg per day requires 8.25 dse per day, giving a conversion ratio of 1:5.5 (= 18% conversion), indicating abundant good quality pasture, as might occur in late-winter–spring, the time when the majority of the impact of dung beetles on pasture growth occurs.
- Net profit (\$) from the additional pasture will vary with stock growth rates and the market price of the beef. A range of growth rates (0 to 1.5 kg LW per day) and low and high sale prices of (\$1.60 and \$2.20 per kg live weight) are modelled.

A number of production scenarios are analysed using weaner steers in forward condition (beginning at 200 kg live weight) over a 4-month period (August to November). Additional profit (in response to the additional, beetle-induced pasture growth) varied in value from zero up to over \$10,000 (\$100 per animal), depending upon management (determining live weight gains) and live weight price (Table 2.23).

Consequences of beetles entering the ‘dung-only’ plots

When the project was established, there were very low numbers of feral *B. bison* in the districts where the trials were established. Large numbers (960 pairs per location) of *B. bison* were introduced to the wire cages in the dung+beetles treatment at the beginning of the experiment in the 2X2 m plots at each location (480 beetles per cage). Once the beetles had buried the dung, they were keen to escape and some did so by burrowing under the wire mesh cage flanges pinned to the soil surface. Having escaped, they went in search of new dung pads, some of which were enclosed in adjacent wire mesh cages. A small number of these beetles managed to tunnel under the wire mesh flange pinned to the soil surface and enter the ‘dung-only’ cages. This was not observed to happen, but evidence that it had happened began to appear (in the weeks following the establishment of the experiment) in the form of soil casts around the edge of dung pads in the ‘dung-only’ wire mesh cages. This was reported in the milestone 4 report, as follows:

Contamination of dung-only plots with dung beetles occurred to a small degree because the dung pads remained attractive to *B. bison* for much longer than anticipated. The moist weather and the mesh cages inhibited desiccation of the dung pads such that there was still moist dung present in the dung-only plots after 4–6 weeks in the field. Adult beetles were observed attempting to enter the beetle-proof enclosures on a number of occasions 4 to 8 weeks after the pads had been placed in the field. A small number of beetles entered the dung-only cages at this time. The number of soil casts and the degree of pad burial were assessed for all pads in the dung-only enclosures. The pads were also colonised by small numbers of the spring-active dung beetle *Onthophagus taurus*, which was too small to be excluded by the wire mesh.

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The impact of the beetles on the dung-only pads was relatively minor (an average of 16% burial) (Table 5). However, the beetle impact was not evenly distributed within the caged plots. At Location 1 the impact was more intense in the four corner pads in the cages (a mean of 35% burial) compared with the remaining pads (9% burial), whereas at Location 2, the impact on pads at the southern end of the cages was greater (33% burial) than that on the remainder of the pads (10% burial). Despite these effects, the contrast between the dung-only and dung+beetles treatments remained stark and so the comparison between the dung+beetles treatment and dung-only treatment remains valid, with the caveat that there was some level of burial in some dung-only pads.

Subsequent observations on the dung burial activity of *B. bison* revealed that one female can, over time, bury an entire dung pad. Thus even a small number of beetles entering the dung-only treatment cages can have a major impact upon the fate of the dung in the cages.

The area influenced by burial of a dung pad is also larger than originally anticipated, being 0.5 to 1.0 m² and so even a moderate number of dung pads buried in a 2x2 m plot will affect the entire plot. Evidence for effects beyond the limits of the dung pad comes from the 10x10 m plots. There, 1-litre dung pads were placed out in rows 1 m apart with 0.5 m between the dung pads within the rows. The substantial pasture growth response to the buried dung in the dung+beetles plots was clear but there was no reduced pasture growth in the 1 m between the rows, indicating that the area of influence of a buried dung pad was at least 0.5 m (half the 1 m interval between rows).

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Table 2.23 Modelling scenarios for the profit derived from an additional 13,000 dse in response to dung beetle activity for forward steers at 200 kg initial live weight. Duration 4 months (August to November inclusive)

Growth rate (kg / day)	Cost (dse / day)	Total cost (dse / beast)	No. steers supported by 13,000 dse	Final steer weight (kg)	LWG (kg steer)	Profit (\$ / beast @ \$1.60 / kg LW)	Profit (\$ / beast @ \$2.20 / kg LW)	Total return \$1.90 kg LW
0.0	3.25	400	33	200	0	0	0	0
0.5	3.3–4.6	502	26	261	62	98	135	3,100
1.0	3.3–5.3	545	24	323	123	197	271	5,600
1.5	3.3–6.0	590	22	385	185	132	181	7,700
Increased growth of existing stock								
Increased growth rate (kg / day)	Cost of extra growth (dse / day)	Total cost of extra growth (dse / beast)	No. steers supported by 13,000 dse	Final steer weight (kg)	LWG (kg steer)	Profit (\$ / beast @ \$1.60 / kg LW)	Profit (\$ / beast @ \$2.20 / kg LW)	Total return \$1.90 kg LW
0 to 0.5	0.9–1.6	151	86	262	62	98	135	10,100
0.5 to 1.0	1.2–2.6	226	58	323	123	113	158	13,600
0.5 to 1.5	1.4–3.3	292	45	385	185	132	181	15,800

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In the 2x2 m plot experiment, each of the 'dung-only' cages contained 16 x 3-litre dung pads. Only a small proportion of these would need to be buried by dung beetles to influence the pasture production over the entire 4 m² of the plot.

The data on the number of pads in each 'dung-only' cage that were colonised by at least one beetle were re-examined and it is clear that about 45% of the pads in the 'dung-only' plots at both Ashbourne and Kuitpo had been colonised by dung beetles after 8 weeks (Table 2.24). Eventually all colonised pads were buried. Therefore, at Ashbourne and Kuitpo, the average level of dung burial in the 2x2 m 'dung-only' plots was taken to be 45%. At the same time, there was complete burial of all dung pads in the dung+beetles plots.

Table 2.24 Colonisation of dung pads by *B. bison* in wire mesh cages in the 2X2 m 'dung-only' plots. There were 16 pads per cage.

Replicate	Number of pads with soil casts after 8 weeks				Total
	1	2	3	4	
Ashbourne	5	10	5	6	26
Kuitpo	6	12	8	4	30

The end result of this is that the 'dung-only' treatments cannot be viewed as treatments with no dung burial; rather they are +beetle treatments with a lower level of dung burial (20+ litres per plot) than in the original dung+beetles treatments (48 litres per plot).

In the 2x2 m experiment we therefore have four treatments, namely:

- substantial dung burial = 48 litres buried per plot in 2–4 weeks: sixteen 3-litre dung pads which were completely buried within 2–3 weeks of being placed in the field (Table 2.25)
- moderate dung burial = 20+ litres buried per plot within 2–3 months of being placed in the field
- caged control plots
- uncaged control plots

Table 2.25 The mean number (per plot) of soil casts surrounding dung pads in the dung+beetles treatments at Ashbourne and Kuitpo, 3–4 days after introducing the dung beetles

Replicate	1	2	3	4	Total
Ashbourne	3.8	4.8	3.4	3.4	3.9
Kuitpo	3.8	4.1	4.6	5.6	4.5

Pasture growth responses to different levels of dung burial

As indicated above, for the 2x2 m plots about 20 litres of dung per plot were buried in the plots with moderate levels of dung burial (previously dung-only) and 48 litres of dung were buried per plot in the substantial dung burial plots (the original dung+beetles plots).

At Ashbourne there was a significant pasture growth response to moderate levels of dung burial in 2006 (38% increase, 1.4 t ha⁻¹) and 2007 (13% increase, 1.0 t ha⁻¹). An even greater increase was observed in the original dung+beetles plots in 2006 (44% increase, 1.7 t ha⁻¹) and 2007 (30% increase, 2.3 t ha⁻¹).

At Kuitpo there was a significant pasture growth response to moderate levels of dung burial in 2006 (122% increase, 3.0 t ha⁻¹) and 2007 (38% increase, 0.6 t ha⁻¹). An even greater increase was observed in the original dung+beetles plots in 2006 (134% increase, 3.3 t ha⁻¹) and 2007 (54% increase, 0.9 t ha⁻¹).

These data indicate that the intensity of the pasture growth response increased with the increased amount of dung buried.

Seasonal changes in dung burial

In the 10x10 m plots at both locations dung pads were placed out on the plots on 24 occasions (3 x 1-litre pads per plot) at weekly intervals in 2006 and in 2007.

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At Ashbourne during the first 12 weeks 81% (in both 2006 and 2007) of all pads were buried. During the second 12 weeks 18% and 22% (in 2006 and 2007 respectively) of all pads were buried. At Kuitpo during the first 12 weeks 40% and 57% (in 2006 and 2007 respectively) of all pads were buried. During the second 12 weeks 18 % and 22% (in 2006 and 2007 respectively) of all pads were buried (Table 2.26).

From this it might be expected that there would be a seasonal change in the intensity of the pasture growth response to dung beetle activity, with a greater response in the sections of the plots in which dung was buried during the early part of the season.

Table 2.26 Seasonal changes in dung burial at Ashbourne and Kuitpo in the 10x10 m plots

	Ashbourne		Kuitpo	
	% burial	No. pads buried	% burial	No. pads buried
2006				
1–12 weeks	81	87	40	48
13–24 weeks	18	19	14	20
2007				
1–12 weeks	81	87	57	82
13–24 weeks	22	23	35	50

Rate of dung burial and smothering pastures

In the 2x2 m plot experiment dung and beetles were added to the experimental plots on 30 September 2005 at Ashbourne and 1 October 2005 at Kuitpo. Sixteen 3-litre dung pads were placed in cages of 4 m² and the pads in the dung+beetles treatment were completely buried over 2–4 weeks. The soil surface where the dung pad had been was bare with no pasture growing on it after the dung had been buried. The dung in the dung-only plots covered and smothered the pasture. The pads occupied 16–18% of the surface area inside the cages. These areas remained bare until the break of season in 2006.

Observations on Linc Willson's property at MacGillivray on Kangaroo Island, where *B. bison* has become extremely abundant, indicate that *B. bison* completely buries cattle dung within a day or so of its production over a period of several months of the year (June onwards) and there is no smothering of the pasture in such circumstances. Future experimental designs should allow for rapid dung burial that does not smother the pasture.

Speed of pasture response to buried dung in 2x2 m and 10x10 m plots

In 2005 in the 2x2 m plots, dung was buried during October and pasture growth largely stopped in November. As one might expect from the pasture smothering, there was minimal pasture growth response to buried dung at either test location.

In 2006 and 2007 in the 2x2 m plots at Ashbourne and Kuitpo there was a rapid and substantial spring-time response to dung buried in October 2005 and this occurred in plots with complete (dung+beetles treatment) and partial ('dung-only' treatment) dung burial.

The absence of a true dung-only treatment in the 2x2 m plot experiment increased the importance of the control vs 'dung-only' comparisons from the 10x10 m plots. These data failed to demonstrate a significant pasture growth response to surface dung in 2006 and in 2007, demonstrating that the effect of unburied surface dung on pasture growth is minimal at best.

In the 10x10 m trial plots, dung was applied at weekly intervals throughout 24 weeks of the year when *B. bison* was expected to be active. During the first half of the season at Ashbourne 80% of the dung pads were buried while during the second half of the season only 20% of the dung pads were buried, reflecting the reduced number of dung beetles active at that time of year. Nevertheless, at Ashbourne, there was a substantial pasture growth response in spring to dung buried by *B. bison* during autumn and early winter of the same year.

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Effects of plot size upon pasture production

The rate of pasture production from the smaller plots (4 m²) was, overall, greater than that from the larger plots at both Kuitpo and Ashbourne (Table 2.27).

Table 2.27 Total dry matter production (t DM ha⁻¹) from control plots at Ashbourne and Kuitpo 2005–07

	Ashbourne		Kuitpo	
	2x2 m plots	10x10 m plots	2x2 m plots	10x10 m plots
2005	4.11	1.91	3.10	2.09
2006	3.82	4.23	2.44	2.38
2007	7.46	6.00	1.58*	2.29
Total	15.39	12.15	7.12	6.75

* yield reduced due to Cape weed infestation

The likely explanation for the greater growth in the smaller plots is related to the method of plot management. In order to ensure that exactly 4 m² of pasture were sampled, a 2 x2 m wooden frame was placed over the plot and the surrounding vegetation was cut to ground level, providing a border around each plot of about 5 cm. This enabled an exact 4 m² pasture sample to be taken. In addition the vegetation between the plots was mowed regularly. As a consequence, it is likely that the pasture on the plots had access to more moisture and light than would have been the case if the plots had been part of a sward.

Despite this increased production, all plots were treated similarly and so the comparisons between treatments remain valid. However, the difference between the 2x2 m and 10x10 m plots may have biased to some small extent the estimates of the production response per kg of buried dung (see earlier).

Plot management at Kuitpo

The trial plot area at Kuitpo (about 1 acre) was fenced off from the main paddock in September 2005. The paddock contained a rye grass–subclover pasture which had been grazed heavily by sheep. The pasture at the time of establishing the experiment was 2–3 cm tall. In the following year (2006) the sheep were replaced by cattle. In that year the pasture in the paddock and the fenced experimental area became dominated by Cape weed. In 2007 there was an early break to the season followed by a dry spell. Both the Cape weed and clover in the plots germinated, but the dry spell killed the clover, leaving the Cape weed to flourish and become the dominant plant. To rectify this, the paddock (but not the experimental enclosure) was sprayed with Tigrex broadleaf herbicide (Bayer Corporation) in autumn 2007. This killed the Cape weed and resulted in a pasture that was a mixture of annual and perennial grasses and clover with little Cape weed evident in the pastures that grew in spring of that year.

Similar plant growth patterns were evident in the experimental plots in 2005 and 2006. By mid-2006 it was clear that Cape weed was a major but unwanted element in the pastures on the trial plots. In response to this perceived threat, the plots were sprayed with Tigrex broadleaf herbicide (Bayer Corporation) on 25 July 2006 at the recommended rate of 250 ml per 50 litres of water plus 5 ml of wetting agent, but the application rate per m² was too high on the 2x2 m plots. This killed the broadleaf plants (including the clover) but also damaged the grasses, with the result that the pasture on the plots was uneven, with some areas of some plots having little vegetative cover. The bare patches provided ideal germination sites for Cape weed in the following season (2007).

As indicated above, there was an early break to the season in 2007 followed by a dry spell. Both the Cape weed and clover germinated, but the dry spell killed the clover leaving the Cape weed to flourish. Because of the unfortunate experience with Tigrex on the 2x2 m plots in the previous season (2006), it was decided to hand weed the plots to remove the Cape weed in 2007. Despite many hours of work, this proved unsuccessful and the Cape weed became a major component of the plot vegetation, largely inhibiting successful establishment of seedling perennial grasses.

On the advice of the dung beetle project review team (Dr Tom Davison and Dr Malcolm McCaskill), the plots were sprayed with glyphosate and replanted with *Phalaris* in spring. *Phalaris* germination

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and establishment was satisfactory, and it is expected that there will be an even cover of perennial grasses over the plots in 2008.

Two pasture cuts were taken immediately before re-sowing the plots. The first was taken using the mower set at the normal height (7 cm above ground level), but the second, which immediately followed the first, was conducted with the mower set at 1.5 cm above ground level. The data from the second sampling were analysed and there was no difference between any of the treatments, yielding at an average rate of 13.8 t DM ha⁻¹.

The 2007 data for Kuitpo, while supporting the impact of dung beetles upon pasture production, was made difficult to interpret because of the uneven perennial pasture in the plots and problems with Cape weed. It is anticipated that these issues will have been resolved in 2008 and that the pastures will be even stands of grasses and clover, with the result that the variability in the data due to uneven pasture between plots will be removed, allowing the impact of dung beetles to be clearly expressed again.

Clearly these data from Ashbourne and Kuitpo show that dung burial by the dung beetle *B. bison* causes a major increase in pasture production over and above that due to surface (unburied) dung. These effects persist for at least 2 years following dung burial but may persist for many more years. If the dollar benefits of dung beetle activity are to be evaluated, it is essential that the monitoring of pasture production be continued until the effects are no longer evident. In addition, it is very important to extend the scale of these observations and undertake a paddock-scale validation of the benefits of dung beetle activity.

Appendix 3: Subsoil chemical analysis, August & November 2006, May & September 2007

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Summary

Dung burial by the dung beetle *Bubas bison* caused major increases in the levels of plant nutrients (nitrate, phosphate, sulphur) in the subsoil and also increased subsoil organic carbon, pH and electrical conductivity. In contrast, surface dung had no influence upon subsoil chemistry 11 to 23 months after the dung was applied.

The experiment was set up at Ashbourne and Kuitpo in September 2005 with four replicates of three treatments (dung+beetles, dung-only and controls (no dung, no beetles)) using cores of soil 45 cm deep with an undisturbed profile encased in a beetle-proof bag. Beetles buried the dung at 20–45 cm below the surface. Cores were extracted from the soil in August 2006, November 2006, May 2007 and September 2007. By 11 months all the buried dung had been consumed by the larval dung beetles and excreted as a black humic substance which lined the tunnels. The base section of each core (20 to 45 cm deep) was separated, weighed and sieved for the dung-only and control cores and 0.5 kg subsamples taken for chemical evaluation. In the dung+beetles cores, the dung beetle tunnels and the surrounding soil were separated from the bulk soil that was unaffected by direct beetle activity (the remainder). Both were sieved and weighed and subsamples taken for chemical analysis.

The data for 10 parameters (see below) were analysed for the four sampling occasions 11 to 23 months after the experiment was established. Changes over time are reported but are of minimal relevance to the objectives of the study. The analysis allowed an evaluation of:

- the differences between locations (Ashbourne and Kuitpo)
- the impact of surface dung on the chemistry of the subsoil
- the impact of dung buried by beetles on the average chemistry of the subsoil
- the impact of dung buried by beetles on the chemistry of the tunnels and closely surrounding soil compared with the remainder and with the control subsoil
- whether the elevated levels of compounds found in the tunnels remained there or leached out into the surrounding soil

The presence of dung on the soil surface did not affect the concentrations of any of the 10 parameters analysed at either location.

Nitrate levels in the subsoil at Ashbourne (9.8 mg kg^{-1}) were significantly higher than those at Kuitpo (5.1 mg kg^{-1}). Dung burial by *B. bison* resulted in a substantial elevation (3–4-fold) in the nitrate levels in the tunnels and adjacent soil. This effect was concentrated in the vicinity of the tunnels and not dispersed throughout the subsoil. Nitrate levels averaged over the entire dung+beetles subsoil were 2-fold higher than in the control subsoil.

Phosphate levels in the subsoil at Ashbourne (39.1 mg kg^{-1}) were significantly higher than those at Kuitpo (25.8 mg kg^{-1}). Dung burial by *B. bison* resulted in a massive elevation (15- to 20-fold) in the phosphate levels in the subsoil tunnels and adjacent soil. This effect was also dispersed throughout the dung+beetles subsoil. Phosphate levels averaged over the entire dung+beetles subsoil were 5- to 7-fold higher than in control subsoil.

Organic carbon levels in the subsoil at Ashbourne (2.2%) were significantly higher than those at Kuitpo (0.9%). Dung burial by *B. bison* resulted in a substantial elevation in the organic carbon levels in the subsoil tunnels and adjacent soil at Ashbourne (2.1% to 5.1%) and Kuitpo (0.7% to 2.8%). The high levels of organic carbon in the tunnels declined over time but there was no corresponding systematic decrease in the average levels of organic carbon in the total subsoil. Organic carbon levels averaged over the entire dung+beetles subsoil were significantly higher than those in the control soils at both Kuitpo and Ashbourne. Organic carbon did not become dispersed from the tunnels through the subsoil in the dung+beetles cores.

Sulphur levels at Kuitpo (19.5 ppm) were significantly higher than those at Ashbourne (8.1 ppm). Dung burial by *B. bison* resulted in elevation of the sulphur levels in the subsoil tunnels and adjacent soil (21.3 ppm) control subsoil (11.3 ppm). This effect was dispersed throughout the

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dung+beetles subsoil. Sulphur levels averaged over the entire dung+beetles subsoil were 60% higher than in control soils.

Ammonia levels in the subsoil did not vary between Ashbourne (3.0 ppm) and Kuitpo (2.8 ppm). Dung burial by *B. bison* resulted in a substantial elevation (7-fold) in the ammonia levels in the tunnels and adjacent soil. This effect was concentrated in the vicinity of the tunnels and not dispersed throughout the subsoil. Ammonia levels averaged over the entire dung+beetles subsoil were significantly (83%) higher than in control soils.

Potassium: Dung beetle activity did not affect the potassium concentrations in the subsoil at Ashbourne or Kuitpo on any of the four sampling occasions. The potassium levels in the subsoil at Ashbourne (273.2 ppm) were significantly higher than those at Kuitpo (51.0 ppm).

Iron levels in the subsoil at Ashbourne (1471 ppm) were significantly higher than those at Kuitpo (1023 ppm). Dung burial by *B. bison* resulted in a significant elevation in the iron levels in the subsoil tunnels and adjacent soil at both locations. The elevated iron levels were not dispersed from the tunnels through the subsoil. Iron levels averaged over the entire dung+beetles subsoil were not statistically greater than those in the control soils at Kuitpo or Ashbourne.

Conductivity of the subsoil at Ashbourne (0.073 dS/m) was significantly higher than that at Kuitpo (0.043 dS/m). Dung burial by *B. bison* resulted in a significant elevation in the conductivity of the subsoil tunnels and adjacent soil at Ashbourne and Kuitpo. At both locations the conductivity of the bulk soil surrounding the tunnels had increased (relative to the control subsoil), indicating movement of electrolytes from the tunnels into the surrounding subsoil. Conductivity averaged over the entire dung+beetles subsoil was statistically higher than that of the control soils at Ashbourne and Kuitpo.

The **pH in CaCl₂** of the subsoil at Ashbourne (6.0) was significantly higher than that at Kuitpo (5.0). Dung burial by *B. bison* resulted in a significant elevation of the pH (CaCl₂) of the subsoil tunnels and adjacent soil at Kuitpo and Ashbourne. The elevated pH (CaCl₂) was dispersed from the tunnels through the subsoil in the dung+beetles cores, indicating the movement of alkalinity into the subsoil from the tunnels. The pH (CaCl₂) averaged over the entire dung+beetles subsoil was statistically higher than that in the control subsoils at Kuitpo and Ashbourne.

The **pH in water** of the subsoil at Ashbourne (6.8) was also significantly higher than that at Kuitpo (5.9). Dung burial by *B. bison* resulted in a significant elevation in the pH (H₂O) of the subsoil tunnels and adjacent soil in some cases but the effects were far less clear than those observed for pH (CaCl₂).

In summary, dung burial by the dung beetle *B. bison* caused major increases in the levels of nitrate, ammonia and organic carbon in the vicinity of beetle tunnels, but these effects did not spread to the soil that was not directly affected by beetle activity. In contrast, the levels of phosphate and sulphur were elevated not only in the vicinity of the dung beetle tunnels but also in the soil that was not directly affected by beetle activity. In other words, the phosphate and sulphur had dispersed from the tunnels and was present throughout the subsoil in the dung+beetles cores.

Because dung beetle activity affects the surface and the subsurface (by tunnelling, lining tunnels with dung and bringing subsoil clay to the surface), a thorough analysis of the impact of dung beetles requires a nutrient budget analysis of the entire soil profile. Soil samples for the entire soil profile (0–10 cm, 10–20 cm and 20–45 cm) for May and September 2007 have been prepared and await support to be processed.

Recommendations

1. That financial support be provided to analyse the entire soil profile (surface soil, mid-soil and base soil) for the May and September 2007 samples (prepared and in storage) in order to build a nutrient budget for the impact of dung burial on soil chemistry. This will complete the account of the impact of *B. bison* on soil chemistry and is one of the primary recommendations of the review committee (Appendix 5).
2. That a further analysis of the entire core be undertaken in September 2008, 3 years after the experiment began (field cores are available)

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3. That the capacity of dung beetles to sequester organic carbon in the subsoil be examined further
4. That a PhD fellowship be established to investigate the capacity of dung beetle activity to mineralise phosphate
5. That the current data be published as a scientific paper and support be provided for Dr M McCaskill to help interpret the phosphate and nitrogen data

Background

Parallel studies in the current project have demonstrated that dung burial by the dung beetle *Bubas bison* has caused a substantial 30–40% increase in pasture growth over the 2 years since the project began. There was no corresponding increase in plots with surface-applied dung without dung burial by beetles. Apart from one field study in southern United States (Fincher 1981) and an industry report (Doube 2006), there is no previous evidence for such effects in the field, although they were assumed to be the case. There are no studies that examine the causes of such increased pasture productivity. This study is the first in the world to address dung beetle-induced changes in soil chemistry.

It appears obvious that dung burial by dung beetles should result in increased soil fertility, but the process by which this may occur needs careful consideration and will guide the types of sampling procedures used to assess its impact on soil fertility. A considerable array of soil categories could be analysed. It is incumbent upon us to make the best use of the limited resources available, and so it is necessary to be selective in choosing fractions to be analysed.

The vast majority of dung buried by *B. bison* is packed into tunnels at 20 to 45 cm below the soil surface. The tunnels that link the surface to the subsoil are lined with dung but this comprises only a small proportion of the dung buried by the beetles. Processes occurring in these tunnels (for example, the consumption by earthworms of the dung lining the tunnels and the filling of the tunnels with earthworm casts) are likely to have a significant effect on the fertility of the soil surrounding the tunnels, but, since this makes up only a minor proportion of the soil profile and the vast majority of the dung is lodged in the subsoil, the analysis of the impact of dung burial on soil fertility should initially focus on the processes occurring in the subsoil. This we have done.

A further consideration, especially in duplex soils, is the bringing of subsoil to the soil surface during dung burial. For every litre of dung buried, at least one litre of subsoil is brought to the surface. In addition, a small amount of surface soil is carried into the subsoil with the buried dung. Thus in duplex soils such as that at Kuitpo, the dung beetles provide a 'clay-spreading' service that shifts subsoil clay to the surface, and surface sand to the subsoil. Both these processes are likely to have an important impact upon soil fertility.

The experiment reported here was established in mid-September 2005 and cores were extracted and beetle development assessed in November 2005 (after 8 weeks), April 2006 (after 7 months), August 2006 (after 11 months), November 2006 (after 14 months), May 2007 (after 19 months) and September 2007 (after 23 months).

An appreciation of the seasonal biology of the adult beetles and the immatures is important to the selection of appropriate sampling occasions on which to assess the impact of dung and dung beetles on soil chemistry.

Table 2.1 Developmental stages of beetles recovered from the subsoil on six sampling occasions and the corresponding tunnel contents during the first two years of the experiment

	Dung in deep tunnels (30–45 cm)	Beetle progeny	Plant roots in beetle tunnels
November 2005			
Both locations	Damp dung packed	Eggs & young larvae	None
April 2006			
Ashbourne	Brown larval excreta	7% emerged as adults 93% in larval diapause	None
Kuitpo	Brown larval excreta	98% adults ready to emerge	None

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2% in larval diapause			
August 2006			
Ashbourne	Decomposing larval excreta	>90% in larval diapause	Extensive
Kuitpo	Decomposing larval excreta	Emerged adults 2% of total in larval diapause	Extensive
November 2006			
Ashbourne	Decomposing larval excreta	>90% in larval diapause	Extensive
Kuitpo	Decomposing larval excreta	2% in larval diapause	Extensive
May 2007			
Ashbourne	Decomposing larval excreta	None remaining	Extensive
Kuitpo	Decomposing larval excreta	None remaining	Extensive
September 2007			
Ashbourne	Decomposing larval excreta	None remaining	Extensive
Kuitpo	Decomposing larval excreta	None remaining	Extensive

Parent beetles died in spring 2005, having laid eggs in dung packed into tunnels at 30–45 cm below the soil surface. Eggs hatched into first instar larvae in spring. These developed to third instar larvae by late summer and, in autumn, most (98%) of the larvae at Kuitpo and some (10%) at Ashbourne had become adults, ready to emerge at the time of next sampling (April 2006) (Table 2.1). Most adults emerged in April–May. Most of the larvae at Ashbourne (>90%) and a few at Kuitpo remained alive in the third instar in a form of arrested development (called diapause, in which development is delayed, usually for one year), resulting in these diapausing beetles emerging as adults in autumn 2007. Thus, live beetles (larvae/pupae/adults) were present in the tunnels on the August 2006 (after 11 months), November 2006 (after 14 months) sampling occasions but had emerged as adults by the time of the May 2007 sampling occasion (after 19 months).

The impact of dung beetle activity upon soil chemistry was not assessed for the November 2005 and the April 2006 sampling occasions. In November the tunnels were packed with moist dung, the beetle young were still in the egg/first instar larval stage and there were no roots evident in the tunnels or the surrounding soil. From this we concluded that there had been little opportunity for the dung beetle activity to influence soil chemistry in a significant way. Similarly, in April 2006, following a dry summer, the tunnels were packed with dung that had been processed by the beetle larvae but there were no roots evident in the tunnels or the surrounding soil and so soil chemistry was not assessed. During that time (spring, summer and autumn) the larval dung beetles consume the dung in the tunnels, leaving behind a mass of fine-textured dark brown excreta, and form a hard protective capsule of faecal material (termed a faecal shell) in which they live until they emerge as adults.

In August 2006, part way through the 2006 winter, the tunnels were packed with dung processed by the beetle larvae and plant roots were evident in the tunnels and the surrounding soil. The chemistry of the soil was therefore assessed but only in the control and dung+beetles cores and only in the subsoil (20–45 cm). This limited sampling was done in order to establish whether there was any influence, there being no prior information on such processes. Strong effects were detected and reported in the milestone 5 report, and it was decided to examine the subsoil chemistry of all three treatments (controls, dung-only and dung+beetles) on future sampling occasions. The details of the soil samples acquired are in Table 2.2.

Table 2.2 Sampling occasions and soil fractions assessed at Ashbourne and Kuitpo in 2006–07

	in	field	Ashbourne				Kuitpo			
			Aug 2006	Nov 2006	May 2007	Sep 2007	Aug 2006	Nov 2006	May 2007	Sep 2007
Duration (months)			11	14	19	23	11	14	19	23
Controls			√	√	√	√	√	√	√	√

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Dung only	×	√	√	√	×	√	√	√
Dung+beetles: tunnels+environs	×	√	√	√	√	√	√	√
Dung+beetles: remainder	×	√	√	√	√	√	√	√
Total dung+beetles subsoil	√	√	√	√	√	√	√	√

The intensity and persistence of these effects over the following year (four sampling occasions (August 2006 to September 2007) have prompted the need to examine the impact of dung beetle activity on the soil chemistry of the whole soil profile (top 10 cm, middle 10 cm and base 20–45 cm). Duplicate samples for all three soil depths from the May and September 2007 sampling occasions have been prepared (by oven drying at 40°C) and await processing.

Methods

Parameters analysed

The following parameters were examined in the standard soil nutrient analysis conducted by CSBP Ltd, Perth. About 0.5 kg of soil was provided to represent each soil sample. Thirteen soil parameters were assessed and analysis of the latter 10 parameters is provided in this report. The texture, % gravel and colour of the soil were largely unaffected by beetle activity: The parameters assessed were:

- texture
- % gravel
- colour
- nitrate N (mg kg^{-1})
- ammonia (mg kg^{-1})
- phosphate (mg kg^{-1})
- potassium (mg kg^{-1})
- sulphur (mg kg^{-1})
- organic carbon (mg kg^{-1})
- iron (mg kg^{-1})
- conductivity (dS/m)
- pH in CaCl_2 and in water

Origin of soil samples

Four replicates of three treatments (dung+beetles, dung-only and controls (no dung, no beetles)) were established in cores in the soil at both study locations (Ashbourne, Kuitpo) at the beginning of the experiment (mid-September 2005). The new data reported here are from cores (4 replicates of each of 3 treatments) from the May and September 2007 sampling occasions.

The soil fractions are all from the base section of the cores (20–45 cm deep) and were selected to provide maximum contrast in relation to dung beetle activity. At the Kuitpo site the duplex soil had a yellow subsoil and the soil was relatively massive and not easily broken up. By early April 2006 the dung in the tunnels had been completely processed by dung beetle larvae. The tunnels were filled with dark brown beetle excreta. As a consequence, it was a relatively simple matter to identify the tunnels and the limits of the adjacent dung beetle-affected soil, although the distinction was less clear in September 2007 than in the previous autumn. At Ashbourne the tunnel contents and the soil were a similar colour, but it was still possible to recognise and separate those portions of the subsoil that had been influenced by dung beetle activity (ie the tunnel contents and the immediate surrounding soil). Thus four types of soil sample were taken from the base section of the cores. These comprised:

- from dung+beetles cores: beetle tunnel contents and the adjacent soil that appeared affected by dung beetle activity (labelled ‘tunnels+environs’ in the results below)

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- from dung+beetles cores: the remaining soil that appeared unaffected by dung beetle activity (labelled 'remainder' in the results below)
- dung-only cores: soil from cores unaffected by dung beetle activity
- control cores: soil from cores unaffected by dung beetle activity or dung alone

Soil from the cores was passed through a 2.5 mm sieve before sampling, and duplicate samples of about 500 g each were taken. The mass of the soil samples in each of the first two sample types was assessed and so, for the dung+beetles samples, it was possible to calculate the mean change in the average chemical etc (eg EC, pH) levels across the whole subsoil section (20–45 cm), as well as the levels in the beetle-affected soil and the adjacent unaffected soil separately.

Analysis

In previous reports individual samples were compared using t-tests.

For this report, there were four sets of data (from four sampling occasions), making it useful to analyse the entire data set using ANOVA. Data were analysed using Statistix version 8.2 (Analytical Software Pty Ltd). The experiment has a factorial design with replication. The differences between means were evaluated using Tukey's HSD all-pair-wise comparisons tests. Tukey's method is considered to be the most useful pair-wise comparison procedure Statistix performs.

Presentation of results

For each soil chemistry parameter, the results are presented in three components:

1. an analysis of the May 2007 (autumn) and September (winter) 2007 data results and comparison with the previously reported August and November 2006 data.
2. an analysis of the impact of dung and dung beetles on subsoil chemistry as given by ANOVAs of location (Ashbourne and Kuitpo), time (the four sampling occasions) and treatment (controls, dung only, dung+beetles)
3. a comparison (using an ANOVA (location x time x treatment)) of the soil chemistry in the dung beetle tunnels and the soil immediately surrounding them (a result of dung burial/processing by dung beetles and processing of organic matter by soil microbes) with that in the remaining subsoil from the dung+beetles cores.

Thus the four treatments are:

- tunnels and their surrounds (tunnels+environs)
- the remaining soil in the dung+beetles core subsoil (remainder)
- the dung-only core subsoil
- the control core subsoil

Results

Over the 13-month period covered by the soil sampling (August 2006 to September 2007) a number of trends were evident in the impact of dung and dung beetles on the levels of the soil chemicals in the subsoil at the two locations. For this report, there were four sets of data (from four sampling occasions).

Significant seasonal changes in the levels of nutrients in the subsoil were observed but, since there were only four sampling occasions, speculation on the causes of these differences and their seasonal predictability are of limited authority, and require a more extensive set of seasonal samples. It was not the purpose of this study to characterise seasonal changes in subsoil nutrient levels, but data on such changes arose as an inevitable consequence of the sampling strategy employed.

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Nitrate

Subsoil chemistry August 2006 to September 2007

The predominant trends in nitrate levels (Table 2.3) are:

- no effect of surface dung on the levels of subsoil nitrate
- elevated levels of nitrate in the dung+beetles subsoil throughout most of the sampling period
- elevated levels of nitrate in the tunnels+environs throughout the sampling period

Table 2.3 A comparison of the effect of dung burial activity by *B. bison* on the nitrate (ppm) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	5.3	2.0	4.3	4.5	14.5	8.0	4.0	1.3
Dung-only subsoil	na	na	4.3	4.5	10.8	6.0	3.3	1.7
Total dung+beetles subsoil	25.5	8.1	6.8	5.2	22.9	15.2	7.0	2.8
Dung+beetles: tunnels+environs	na	32.3	13.5	14.7	63.8	26.3	10.0	4.5
Dung+beetles: remainder	na	6.3	6.25	4.0	21.5	14.8	6.3	2.5

* Ash: Ashbourne; Kui: Kuitpo. na not available

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data (controls vs dung-only vs dung+beetles) for the nitrate levels in the subsoil (20–45 cm) over the four sampling occasions at both locations (Table 2.4) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment

Table 2.4 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the nitrate levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	10.35	0.0020
Time	3	8.35	0.0001
Treatment	2	8.98	0.0003
Location*Time	3	1.09	0.3576
Location*Treatment	2	1.18	0.3138
Time*Treatment	6	1.94	0.0868
Location*Time*Treatment	6	0.61	0.7193
Error	68		
Total	91		

Location Over the 13-month sampling period the nitrate levels in the subsoil at Ashbourne (9.8 ppm) were significantly higher than those at Kuitpo (5.1 ppm).

Changes over time The overall levels of nitrate in the subsoils sampled in May 2007 (12.9 ppm) were significantly higher than those in the subsoils sampled in November 2006 (5.5 ppm) and September 2007 (3.4 ppm), but not different from those in the August 2006 (8.0 ppm) sample.

Elevated subsoil nitrate levels in May 2007 may be due to nitrate mineralisation during the dry 6 months (November 2006 to May 2007), during which time there was minimal pasture growth, which

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would otherwise have mopped up the nitrate. There is no obvious reason for the elevated nitrate levels in August 2006, all the dung having been consumed by the beetle larvae months earlier. The absence of a significant time x treatment interaction indicates that all treatments moved synchronously, supporting the view that the changes in nitrate concentration are the outcome of a balance between nitrate mineralisation and nitrate consumption by plants, with consumption being minimal at times when pastures have hayed off.

Effects of dung and dung beetles The overall levels of nitrate in the dung+beetles subsoil (11.7 ppm) were significantly higher than those in the subsoil sampled from the control (5.5 ppm) treatment and the dung-only treatment (5.1 ppm), which were not different from each other.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs soil surrounding the tunnels (remainder)) for the nitrate levels in the subsoil over three sampling occasions (November 2006, May 2007 and September 2007) at both locations (Table 2.5) indicates that there was:

- a significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment
- a significant interaction between location and time
- a significant interaction between time and treatment

Table 2.5 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the nitrate levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	11.55	0.0013
Time	2	36.10	0.0000
Treatment	2	21.91	0.0000
Location*Time	2	5.93	0.0049
Location*Treatment	2	2.69	0.0776
Time*Treatment	4	6.96	0.0002
Location*Time*Treatment	4	2.63	0.0452
Error	50		
Total	67		

Concentrations in the tunnels+environs Nitrate levels in the tunnels+environs (22.1 ppm) were significantly higher than those in both the control subsoil (6.1 ppm) and the remainder subsoil (9.2 ppm).

Nutrients leaching from the tunnels Because the level of nitrate in the remainder subsoil (9.2 ppm) was not significantly different from that in the control subsoil (6.1 ppm), we conclude that, over the time period examined, the nitrate had not leached from the tunnels into the surrounding bulk soil.

Changes over time Nitrate levels in the tunnels+environs were higher in May 2007 (45.0 ppm) than in November 2006 (14.1 ppm) and September 2007 (7.3 ppm), suggesting that nitrate levels were higher at the end of a long dry period without pasture growth. This proposition needs further examination before it is accepted.

Conclusions

The presence of dung on the soil surface did not affect the nitrate concentrations in the subsoil at either location, although nitrate levels in the subsoil at Ashbourne (9.8 ppm) were significantly greater than those at Kuitpo (5.1 ppm). Dung burial by *B. bison* resulted in a substantial elevation (3–4-fold) in the nitrate levels in the tunnels and adjacent soil. This effect was concentrated in the

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vicinity of the tunnels and not dispersed throughout the subsoil. Nitrate levels averaged over the entire dung+beetles subsoil were 2-fold higher than in the control subsoil.

Phosphate

Subsoil chemistry August 2006 to September 2007

The predominant trends in the phosphate levels (Table 2.6) are:

- no effects of surface dung on the levels of subsoil nitrate
- elevated levels of nitrate in the dung+beetles subsoil throughout the sampling period
- elevated levels of nitrate in the tunnels+environs throughout the sampling period

Table 2.6 A comparison of the effect of dung burial activity by *B. bison* on the phosphate (ppm) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	12.0	4.7	22.8	8.5	19.3	8.5	15.5	9.7
Dung-only subsoil			22.0	22.8	16.3	10.5	13.0	16.0
Total dung+beetles subsoil	68.8	41.9	73.6	84.7	89.2	50.9	88.8	35.2
Dung+beetles: tunnels+environs		265.0	354.8	323.3	320.8	151.0	203.0	71.3
Dung+beetles: remainder		24.0	51.0	55.0	81.0	47.0	53.8	28.5

Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data for the phosphate levels in the subsoil over the four sampling occasions at both locations (Table 2.7) indicates that there was:

- a significant effect of location
- a highly significant effect of treatment
- a significant interaction between location and treatment

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Table 2.7 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the phosphate levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	10.91	0.0015
Time	3	3.35	0.0238
Treatment	2	74.78	0.0000
Location*Time	3	0.39	0.7601
Location*Treatment	2	2.70	0.0747
Time*Treatment	6	0.34	0.9123
Location*Time*Treatment	6	1.81	0.1105
Error	68		
Total	91		

Location Over the 13-month sampling period the phosphate levels in the subsoil at Ashbourne (39.1 ppm) were significantly higher than those at Kuitpo (25.8 ppm).

Changes over time The phosphate levels in the subsoil sampled in August 2006 (24.0 ppm) were significantly lower than in that sampled in November 2006 (41.7 ppm), after which they were similar to each other and not different from the previous levels (May 2007, 33.8 ppm; September 2007, 30.3 ppm).

Effects of dung and dung beetles The overall levels of phosphate in the subsoil sampled from the dung+beetles treatment (67.1 ppm) were significantly higher than in that sampled from the dung-only (17.7 ppm) or the control (12.6 ppm) treatment, but the latter two were not different from each other.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs soil surrounding the tunnels (remainder)) for the nitrate levels in the subsoil over the three sampling occasions at both locations (Table 2.8) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment
- a significant interaction between location and treatment
- a highly significant interaction between time and treatment

Table 2.8 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the phosphate levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	16.52	0.0002
Time	2	12.72	0.0000
Treatment	2	137.58	0.0000
Location*Time	2	2.28	0.1129
Location*Treatment	2	7.34	0.0016
Time*Treatment	4	10.21	0.0000
Location*Time*Treatment	4	1.13	0.3524
Error	50		
Total	67		

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Concentrations in the tunnels+environs Phosphate levels in the tunnels+environs (237.4 ppm) were significantly higher than the levels in both the control (14.0 ppm) subsoil and the remainder soil in the dung+beetles treatment (51.3 ppm).

Nutrients leaching from the tunnels Because the levels of phosphate in the remainder subsoil in the dung+beetles treatment (which was not directly associated with the tunnels) (51.3 ppm) were significantly higher than those in the control subsoil (14.0 ppm), we conclude that, over the time period examined, the phosphate had leached from the tunnels into the surrounding bulk soil.

Changes over time Phosphate levels in the tunnels+environs declined over the sampling period, from November 2006 (339.0 ppm) through May 2007 (235.9 ppm) to September 2007 (137.1 ppm).

Conclusions

The presence of dung on the soil surface did not affect the phosphate concentrations in the subsoil at Ashbourne or Kuitpo but the levels at Ashbourne (39.1 ppm) were significantly higher than those at Kuitpo (25.8 ppm). Dung burial by *B. bison* resulted in a massive elevation (15- to 20-fold) in the phosphate levels in the subsoil tunnels and adjacent soil. This effect was also dispersed throughout the dung+beetles subsoil. Phosphate levels averaged over the entire dung+beetles subsoil were 5- to 7-fold higher than in control soils.

Organic carbon

Subsoil chemistry August 2006 to September 2007

The predominant trends in organic carbon concentrations (Table 2.9) are:

- no effects of surface dung on the subsoil organic carbon
- elevated levels of organic carbon in the dung+beetles subsoil throughout the sampling period
- elevated levels of organic carbon in the tunnels+environs throughout the sampling period

Table 2.9 A comparison of the effect of dung burial activity by *B. bison* on the organic carbon (%) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	2.01	0.68	2.02	0.85	2.14	0.70	2.11	0.61
Dung-only subsoil			2.23	0.88	1.94	0.74	1.97	0.67
Total dung+beetles subsoil	2.56	1.10	2.66	1.54	2.54	1.21	2.55	0.97
Dung+beetles: tunnels+environs		4.12	7.20	4.25	4.80	2.70	3.35	1.38
Dung+beetles: remainder		0.79	2.27	1.20	2.49	1.15	2.32	0.89

* Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data for the organic carbon levels in the subsoil over the four sampling occasions at both locations (Table 2.10) indicates that there was:

- a highly significant effect of location
- a highly significant effect of treatment
- no significant interactions

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Table 2.10 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the organic carbon levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	300.76	0.0000
Time	3	1.46	0.2320
Treatment	2	18.94	0.0000
Location*Time	3	0.44	0.7235
Location*Treatment	2	0.17	0.8430
Time*Treatment	6	0.17	0.9843
Location*Time*Treatment	6	0.27	0.9489
Error	68		
Total	91		

Location Over the 13-month sampling period the organic carbon levels in the subsoil at Ashbourne (2.2%) were significantly higher than those at Kuitpo (0.9%).

Changes over time There were no significant differences in the organic carbon levels in the soils at the two locations from late spring 2006 (August) (1.5%) through November 2006 (1.7%) and May 2007 (1.6%) to September 2007 (1.5%).

Effects of dung and dung beetles The overall levels of organic carbon in the subsoils sampled from dung+beetles treatment (1.9%) were significantly higher than those in the dung-only (1.4%) and the control (1.4%) subsoils.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the organic carbon levels in the subsoil over the three sampling occasions (November 2006, May 2007 and September 2007) at both locations (Table 2.11) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment
- a highly significant interaction between time and treatment

Table 2.11 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the organic carbon levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	60.05	0.0000
Time	2	9.98	0.0002
Treatment	2	54.20	0.0000
Location*Time	2	0.02	0.9829
Location*Treatment	2	2.33	0.1080
Time*Treatment	4	8.39	0.0000
Location*Time*Treatment	4	0.40	0.8068
Error	50		
Total	67		

Concentrations in the tunnels+environs Organic carbon levels in the tunnels+environs (3.9%) were significantly higher than those in both the control (1.4%) subsoil and the remainder subsoil in the dung+beetles treatment (1.7%), which did not differ significantly from each other.

Nutrients leaching from the tunnels Because the levels of organic carbon in the remainder subsoil (1.7%) were not significantly different from those in the control subsoils (1.4%), we

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conclude that, over the time period examined, the organic carbon had not moved from the tunnels into the surrounding bulk soil.

Changes over time Organic carbon levels in the tunnels+environs in November 2006 (5.7%) were significantly higher than those in May (3.7%) and September (2.4%) 2007, indicating that the initial elevation in organic carbon levels in the tunnels was followed by a decline during the second year of the study. This change over time was due, in part at least, to the increasing obscurity of the limits of the tunnels as time passed. One consequence of this was that larger samples were collected (to be sure of collecting all the tunnel components) and so the organic carbon levels became diluted in relation to those observed in earlier, smaller samples.

Conclusions

The presence of dung on the soil surface did not affect the organic carbon levels in the subsoil at Ashbourne or Kuitpo but the levels at Ashbourne (2.2%) were significantly higher than those at Kuitpo (0.9%). Dung burial by *B. bison* resulted in a substantial elevation in the organic carbon levels in the subsoil tunnels and adjacent soil at Ashbourne (2.1% to 5.1%) and Kuitpo (0.7% to 2.8%). The high levels of organic carbon in the tunnels declined over time but there was no corresponding systematic decrease in the average levels in the subsoil overall. Organic carbon levels averaged over the entire dung+beetles subsoil were statistically higher than those in the control soils at both Kuitpo and Ashbourne. Nevertheless organic carbon did not become dispersed from the tunnels through the subsoil in the dung+beetles cores.

Sulphur

Subsoil chemistry August 2006 to September 2007

The predominant trends in sulphur concentrations (Table 2.12) are:

- no effects of surface dung on the levels of subsoil sulphur
- elevated levels of sulphur in the dung+beetles subsoil throughout the sampling period
- elevated levels of sulphur in the tunnels+environs throughout the sampling period

Table 2.12 A comparison of the effect of dung burial activity by *B. bison* on the sulphur (ppm) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	7.8	24.9	4.6	12.1	6.7	13.2	5.6	15.9
Dung-only subsoil			6.0	7.8	6.3	23.1	5.6	19.4
Total dung+beetles subsoil	17.9	33.8	9.2	14.4	11.0	18.4	9.1	33.9
Dung+beetles: tunnels+environs		40.3	20.3	28.1	27.0	20.5	15.5	16.3
Dung+beetles: remainder		33.5	8.4	12.8	10.4	18.4	6.9	37.6

Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data for the sulphur levels in the subsoil over the four sampling occasions at both locations (Table 2.13) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a significant effect of treatment
- a significant interaction between location and time

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Table 2.13 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the sulphur levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	49.11	0.0000
Time	3	7.51	0.0002
Treatment	2	7.62	0.0010
Location*Time	3	3.24	0.0275
Location*Treatment	2	0.26	0.7729
Time*Treatment	6	0.45	0.8420
Location*Time*Treatment	6	0.55	0.7645
Error	68		
Total	91		

Location Over the 13-month sampling period the sulphur levels in the subsoil at Kuitpo (19.5 ppm) were significantly higher than those at Ashbourne (8.1 ppm).

Changes over time The overall levels of sulphur in the subsoil from the late spring 2006 sample (August) (19.5 ppm) were significantly higher than those in the subsequent three samples (9.0 ppm, 12.0 ppm and 14.6 ppm for the November 2006, May 2007 and September 2007 samples respectively).

Effects of dung and dung beetles The overall levels of sulphur in the dung+beetles subsoil (18.3 ppm) were significantly higher than those in the dung-only (11.8 ppm) and the control (11.3 ppm) subsoils.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the sulphur levels in the subsoil over the three sampling occasions at both locations (Table 2.14) indicates that there was:

- a significant effect of location
- a highly significant effect of treatment
- a significant interaction between location and treatment

Table 2.14 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the sulphur levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	13.20	0.0007
Time	2	0.49	0.6156
Treatment	2	8.42	0.0007
Location*Time	2	1.45	0.2445
Location*Treatment	2	3.82	0.0287
Time*Treatment	4	2.12	0.0921
Location*Time*Treatment	4	1.61	0.1873
Error	50		
Total	67		

Concentrations in the tunnels+environs Sulphur levels in the tunnels+environs (21.3 ppm) were significantly higher than those in the control (11.3 ppm) subsoil but they were not significantly different from those in the remainder subsoil in the dung+beetles treatment (16.8 ppm).

Nutrients leaching from the tunnels Because the levels of sulphur in the remainder soil from the dung+beetles treatment (16.8 ppm) were significantly higher than those in the control subsoils

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(11.3 ppm), we conclude that, over the time period examined, the sulphur had leached from the tunnels into the surrounding bulk soil.

Changes over time Sulphur levels in all treatments did not change over the sampling period, from November 2006 through September 2007.

Conclusions

The presence of dung on the soil surface did not affect the sulphur concentrations in the subsoil at Ashbourne or Kuitpo but the levels at Kuitpo (19.5 ppm) were significantly higher than those at Ashbourne (8.1 ppm). Dung burial by *B. bison* resulted in elevation of the sulphur levels in the subsoil tunnels and adjacent soil (21.3 ppm) over those in the control subsoil (9.7 ppm). This effect was dispersed throughout the dung+beetles subsoil. Sulphate levels averaged over the entire dung+beetles subsoil were 60% higher than in control soils.

Ammonia

Subsoil chemistry August 2006 to September 2007

The predominant trends in ammonia levels (Table 2.15) are:

- no effects of surface dung on the levels of subsoil ammonia
- elevated levels of ammonia in the dung+beetles subsoil throughout the sampling period
- elevated levels of ammonia in the tunnels+environs throughout the sampling period

Table 2.15 A comparison of the effect of dung burial activity by *B. bison* on the ammonia (ppm) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	2.0	3.7	3.0	5.0	1.0	1.0	1.0	1.3
Dung-only subsoil	na	na	4.0	2.3	1.0	1.0	4.0	1.0
Total dung+beetles subsoil	2.8	4.5	3.8	7.9	1.1	1.3	10.6	1.4
Dung+beetles: tunnels+environs	na	16.8	17.0	30.0	4.3	7.7	28.3	3.3
Dung+beetles: remainder	na	3.8	2.8	5.0	1.0	1.0	4.3	1.0

Ash: Ashbourne; Kui: Kuitpo; na not available

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data for the ammonia levels in the subsoil over the four sampling occasions at both locations (Table 2.16) indicates that there was:

- no significant effect of location
- a significant effect of time
- a significant effect of treatment
- a highly significant interaction between location and time
- a smaller but significant interaction between location, time and treatment

Table 2.16 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the ammonia levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	0.06	0.8032

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Time	3	8.01	0.0001
Treatment	2	6.18	0.0034
Location*Time	3	6.33	0.0008
Location*Treatment	2	1.33	0.2714
Time*Treatment	6	1.60	0.1615
Location*Time*Treatment	6	2.84	0.0158
Error	68		
Total	91		

Location Over the 13-month sampling period there was no statistically significant difference in the ammonia levels in the subsoil between Ashbourne (3.0 ppm) and Kuitpo (2.8 ppm).

Changes over time There was no significant difference between the overall levels of ammonia in the subsoils sampled in August (3.1 ppm), November 2006 (4.3 ppm) and September 2007 (3.1 ppm), but levels in May 2007 were significantly lower (1.1 ppm) than those in the other samples.

Effects of dung and dung beetles There was no significant difference between the overall levels of ammonia in the subsoils sampled from the control (2.3 ppm) and the dung-only treatments (2.4 ppm), but ammonia levels in the dung+beetles subsoil (4.1 ppm) were significantly higher than those in the subsoils of the other two treatments.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the ammonia levels in the subsoil over the three sampling occasions at both locations (Table 2.17) indicates that there was:

- no significant effect of location
- a significant effect of time
- a highly significant effect of treatment
- a significant interaction between location and time
- a significant interaction between time and treatment
- a significant interaction between location, time and treatment

Table 2.17 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the ammonia levels in the subsoil at the two study locations

Source	DF	F	P
Location	1	0.25	0.6213
Time	2	7.67	0.0012
Treatment	2	27.24	0.0000
Location*Time	2	7.54	0.0014
Location*Treatment	2	0.44	0.6496
Time*Treatment	4	2.95	0.0289
Location*Time*Treatment	4	4.69	0.0027
Error	50		
Total	67		

Concentrations in the tunnels+environs Ammonia levels in the tunnels+environs (15.1 ppm) were significantly higher than those in the control (2.1 ppm) subsoil and also significantly higher than those in the remainder soil from the dung+beetles treatment (2.5 ppm).

Nutrients leaching from the tunnels Because the level of ammonia in the remainder subsoil in the dung+beetles treatment was similar to that in the control subsoil, we conclude that, over the time period examined, the ammonia had not leached from the tunnels into the surrounding bulk soil.

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Changes over time Ammonia levels in the tunnels+environs were lower in May 2007 (6.0 ppm) than in November 2006 (23.5 ppm) and September 2007 (15.8 ppm), suggesting that ammonia levels were higher in moist soil than in dry soil. This proposition needs further examination before it is accepted.

Conclusions

The presence of surface dung on the soil surface did not affect the ammonia concentrations in the subsoil at either location, nor did ammonia levels in the subsoil vary between Ashbourne (3.0 ppm) and Kuitpo (2.8 ppm). Dung burial by *B. bison* resulted in a substantial elevation (7-fold) in the ammonia levels in the tunnels and adjacent soil. This effect was concentrated in the vicinity of the tunnels and not dispersed throughout the subsoil. Ammonia levels averaged over the entire dung+beetles subsoil were significantly (83%) higher than those in the control subsoil.

Potassium

Subsoil chemistry August 2006 to September 2007

The predominant trends in potassium levels (Table 2.18) are:

- no effects of surface dung on the levels of subsoil potassium
- substantially higher levels of potassium at Ashbourne than at Kuitpo

Table 2.18 A comparison of the effect of dung burial activity by *B. bison* on the potassium (ppm) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	270.0	67.7	266.0	37.3	299.5	50.3	265.0	51.0
Dung-only subsoil			269.0	38.3	289.0	39.3	270.3	68.7
Total dung+beetles subsoil	252.3	49.2	286.1	44.6	269.0	42.8	271.3	55.8
Dung+beetles: tunnels+environs		80.5	323.0	78.0	271.5	92.3	245.8	48.8
Dung+beetles: remainder		46.8	283.8	40.3	269.0	40.5	279.8	57.0

Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data (controls vs dung only vs dung+beetles) for the potassium levels in the subsoil over the four sampling occasions at both locations (Table 2.19) indicates that there was:

- a highly significant effect of location
- no significant effect of time
- no effect of treatment
- no significant interactions

Table 2.19 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the potassium levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	976.39	0.0000
Time	3	0.26	0.8573
Treatment	2	0.21	0.8112
Location*Time	3	1.72	0.1712

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Location*Treatment	2	0.01	0.9895
Time*Treatment	6	0.56	0.7644
Location*Time*Treatment	6	0.16	0.9861
Error	68		
Total	91		

Ashbourne vs Kuitpo Over the 13-month sampling period the potassium levels in the subsoil at Ashbourne (273.2 ppm) were significantly higher than those at Kuitpo (51.0 ppm).

Changes over time The overall levels of potassium in the subsoils sampled did not change over the sampling period at either location.

Effects of dung and dung beetles There were no significant differences between the overall levels of potassium in the subsoil sampled from the dung+beetles (158.8 ppm), the dung-only (164.0 ppm) and the control (163.3 ppm) treatments.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the potassium levels in the subsoil over the three sampling occasions at both locations (Table 2.20) indicates that there was:

- a highly significant effect of location
- no significant interactions

Table 2.20 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the potassium levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	451.08	0.0000
Time	2	0.70	0.5033
Treatment	2	0.88	0.4193
Location*Time	2	0.61	0.5455
Location*Treatment	2	0.57	0.5672
Time*Treatment	4	1.55	0.2014
Location*Time*Treatment	4	0.48	0.7503
Error	50		
Total	67		

Concentrations in the tunnels+environs Potassium levels in the tunnels+environs (176.6 ppm) were not significantly different from those in the remainder subsoil in the dung+beetles treatment (161.7 ppm) or from the levels in the control (161.5 ppm) subsoil.

Nutrients leaching from the tunnels There was no elevation of potassium levels in the tunnels and so leaching of potassium into the surrounding soil had not occurred.

Changes over time Potassium levels in the tunnels+environs, in the remainder subsoil and in the control subsoil were not significantly different from each other over the whole study period.

Conclusions

Neither the presence of dung on the soil surface nor dung beetle activity affected the potassium concentrations in the subsoil at Ashbourne or Kuitpo on any of the four sampling occasions. The potassium levels in the subsoil at Ashbourne were significantly higher than those at Kuitpo.

Iron

Subsoil chemistry August 2006 to September 2007

The predominant trends in iron concentrations (Table 2.21) are:

- no effects of surface dung on the levels of subsoil iron

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- elevated levels of iron in the tunnels+environs throughout the sampling period

Table 2.21 A comparison of the effect of dung burial activity by *B. bison* on the iron (ppm) concentration in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	1183.0	811.3	1587.0	1193.8	1674.5	1207.3	1289.5	904.0
Dung-only subsoil			1589.5	1241.0	1624.3	1017.5	1288.0	817.0
Total dung+beetles subsoil	1280.8	912.0	1738.1	1081.7	1765.6	1276.2	1445.5	977.2
Dung+beetles: tunnels+environs		1460.3	2061.5	1437.0	1796.0	1553.7	1538.5	1217.3
Dung+beetles: remainder		860.0	1710.3	1036.3	1764.8	1262.0	1415.8	933.0

* Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data (controls vs dung only vs dung+beetles) for the iron levels in the subsoil over the four sampling occasions at both locations (Table 2.22) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- no significant effect of treatment
- no significant interactions

Table 2.22 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the iron levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	93.64	0.0000
Time	3	18.82	0.0000
Treatment	2	2.23	0.1158
Location*Time	3	0.47	0.7048
Location*Treatment	2	0.28	0.7553
Time*Treatment	6	0.37	0.8937
Location*Time*Treatment	6	0.35	0.9051
Error	68		
Total	91		

Location Over the 13-month sampling period the iron levels in the subsoil at Ashbourne (1471 ppm) were significantly higher than those at Kuitpo (1023 ppm).

Changes over time In late spring 2006 (August) the iron levels in the subsoil samples at the two locations were lower (1030.2 ppm) than those in November 2006 (1405.2 ppm) and May 2007 (1427.8 ppm) but not different from the subsoil sampled in September 2007 (1123.9 ppm).

Effects of dung and dung beetles The overall levels of iron in the subsoil sampled from the dung+beetles subsoil (1312.4 ppm) were not significantly different from those in the dung-only (1196.7 ppm) or the control (1231.3 ppm) subsoil.

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Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs the remainder) for the iron levels in the subsoil over the three sampling occasions at both locations (Table 2.23) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment
- no significant interactions

Table 2.23 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the iron levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	57.15	0.0000
Time	2	11.85	0.0001
Treatment	2	8.93	0.0005
Location*Time	2	0.81	0.4526
Location*Treatment	2	0.68	0.5091
Time*Treatment	4	0.38	0.8218
Location*Time*Treatment	4	0.41	0.7991
Error	50		
Total	67		

Concentrations in the tunnels+environs Overall iron levels in the tunnels+environs (1600.7 ppm) were significantly higher than those in both the control subsoil (1309.3 ppm) subsoil and the remaining subsoil from the dung+beetles treatment (1353.4 ppm).

Nutrients leaching from the tunnels Because the levels of iron in the remaining subsoil in the dung+beetles treatment (1353.4 ppm) were not significantly different from those in the control subsoil (1309.3 ppm), we conclude that, over the time period examined, the iron had not leached from the tunnels into the surrounding bulk soil.

Changes over time While there were statistically significant differences in the iron levels in the subsoil at different times of year, no clear pattern was evident.

Conclusions

The presence of dung on the soil surface did not affect the iron levels in the subsoil at Ashbourne or Kuitpo but the levels at Ashbourne (1471 ppm) were significantly higher than those at Kuitpo (1023 ppm). Dung burial by *B. bison* resulted in a significant elevation in the iron levels in the subsoil tunnels and adjacent soil at Kuitpo and at Ashbourne. The elevated iron levels were not dispersed from the tunnels through the subsoil in the dung+beetles treatment. Iron levels averaged over the entire dung+beetles subsoil were not statistically greater than those in the control soils at Kuitpo or at Ashbourne.

Conductivity

Subsoil chemistry August 2006 to September 2007

The predominant trends in soil conductivity (Table 2.24) are:

- no effects of surface dung on conductivity
- elevated conductivity in the dung+beetles subsoil throughout the sampling period
- elevated conductivity in the tunnels+environs throughout the sampling period

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Table 2.24 A comparison of the effect of dung burial activity by *B. bison* on the conductivity (dS/m) of the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	0.056	0.040	0.057	0.042	0.071	0.034	0.042	0.029
Dung-only subsoil			0.075	0.040	0.073	0.036	0.047	0.032
Total dung+beetles subsoil	0.131	0.061	0.095	0.062	0.107	0.055	0.067	0.046
Dung+beetles: tunnels+environs		0.175	0.219	0.161	0.242	0.113	0.101	0.043
Dung+beetles: remainder		0.051	0.085	0.049	0.102	0.053	0.055	0.046

* Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data (controls vs dung only vs dung+beetles) for the conductivity of the subsoil over the four sampling occasions at both locations (Table 2.25) indicates that there was:

- a highly significant effect of location
- a significant effect of time
- a highly significant effect of treatment
- a significant interaction between location and treatment

Table 2.25 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the conductivity (dS/m) in the subsoil at the two study locations

Source	DF	F	P
Location	1	61.08	0.0000
Time	3	6.02	0.0011
Treatment	2	26.60	0.0000
Location*Time	3	2.05	0.1147
Location*Treatment	2	3.37	0.0404
Time*Treatment	6	1.25	0.2904
Location*Time*Treatment	6	1.07	0.3877
Error	68		
Total	91		

Location Over the 13-month sampling period the conductivity of the subsoil at Ashbourne (0.073 dS/m) was significantly higher than that at Kuitpo (0.043 dS/m).

Changes over time There were no significant differences in the conductivity of the soils at the two locations from August 2006 (0.064 dS/m), through November 2006 (0.062 dS/m) and May 2007 (0.062 dS/m), but by September 2007 the conductivity had become significantly lower (0.044 dS/m) than on the other three sampling occasions.

Effects of dung and dung beetles The overall conductivity of the subsoil sampled from the dung+beetles treatment (0.078 dS/m) was significantly higher than in the dung-only (0.050 dS/m) and the control (0.046 dS/m) treatments.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the conductivity of the subsoil over the three sampling occasions at both locations (Table 2.26) indicates that there was:

- a highly significant effect of location

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- a highly significant effect of time
- a highly significant effect of treatment
- a highly significant interaction between location and treatment
- a highly significant interaction between time and treatment
- a significant interaction between location and time

Table 2.26 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the conductivity (dS/m) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	49.64	0.0000
Time	2	27.69	0.0000
Treatment	2	93.76	0.0000
Location*Time	2	4.56	0.0151
Location*Treatment	2	8.56	0.0006
Time*Treatment	4	11.20	0.0000
Location*Time*Treatment	4	0.82	0.5211
Error	50		
Total	67		

Concentrations in the tunnels+environs The conductivity of the tunnels+environs (0.147 dS/m) was significantly higher than that of the control (0.046 dS/m) subsoil and the remainder subsoil in the dung+beetles treatment (0.064 dS/m).

Nutrients leaching from the tunnels Because the conductivity of the remainder subsoil in the dung+beetles treatment was significantly higher than that in the control subsoil (0.046 dS/m), we conclude that, over the time period examined, ions that increase soil conductivity had leached from the tunnels into the surrounding bulk soil.

Changes over time In November 2006 and May 2007 the conductivity of the subsoil in the tunnels+environs was significantly higher (0.190 dS/m and 0.177 dS/m respectively) than in September 2007 (0.072 dS/m).

Conclusions

The presence of dung on the soil surface did not affect the conductivity of the subsoil at Ashbourne or Kuitpo although conductivity of the subsoil at Ashbourne (0.073 dS/m) was significantly higher than that at Kuitpo (0.043 dS/m). Dung burial by *B. bison* resulted in a significant elevation in the conductivity of the subsoil tunnels and adjacent soil at Kuitpo and at Ashbourne. At both locations also, the conductivity of the bulk soil surrounding the tunnels had increased (relative to the control subsoil), indicating movement of electrolytes from the tunnels into the surrounding subsoil. Conductivity averaged over the entire dung+beetles subsoil was statistically higher than that in the control soils at Kuitpo and Ashbourne.

pH (CaCl₂)

Subsoil chemistry August 2006 to September 2007

The predominant trends in pH (CaCl₂) (Table 2.27) are:

- no effects of surface dung
- no significant effect of beetle activity

Table 2.27 A comparison of the effect of dung burial activity by *B. bison* on the pH (CaCl₂) of the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

	August 2006	November 2006	May 2007	September
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Treatment	2007							
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	5.8	4.8	5.8	4.9	5.6	4.8	5.9	4.9
Dung-only subsoil			6.0	4.8	5.6	4.9	5.9	5.0
Total dung+beetles subsoil	6.5	5.1	6.3	5.5	6.2	4.9	6.3	5.1
Dung+beetles: tunnels+environs		5.6	6.8	6.0	6.5	5.3	6.7	5.1
Dung+beetles: remainder		5.1	6.2	5.5	6.2	4.9	6.2	5.2

* Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data (controls vs dung only vs dung+beetles) for the pH (CaCl₂) of the subsoil over the four sampling occasions at both locations (Table 2.28) indicates that there was:

- a highly significant effect of location
- a highly significant effect of treatment
- a significant effect of time
- no significant interactions

Table 2.28 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the pH (CaCl₂) of the subsoil at the two study locations

Source	DF	F	P
Location	1	431.11	0.0000
Time	3	4.31	0.0077
Treatment	2	30.73	0.0000
Location*Time	3	0.58	0.6308
Location*Treatment	2	1.33	0.2704
Time*Treatment	6	0.74	0.6188
Location*Time*Treatment	6	1.74	0.1246
Error	68		
Total	91		

Location Over the 13-month sampling period the pH (CaCl₂) of the subsoil at Ashbourne (6.0) was significantly higher than that at Kuitpo (5.0).

Changes over time The pH (CaCl₂) of the subsoils at the two locations was similar in August and November 2006 (5.5), and the pH (CaCl₂) in the latter was significantly higher than in May 2007 (5.3). In September 2007 the pH (CaCl₂) was again higher (5.5). Whether this reflects real changes in soil pH is not known.

Effects of dung and dung beetles The overall pH (CaCl₂) of the subsoil sampled from dung+beetles treatment (5.7) was significantly higher than that in the dung-only (5.3) and the control (5.3) treatments.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the pH (CaCl₂) of the subsoil over the three sampling occasions at both locations (Table 2.29) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment

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- a minor interaction between location and time

Table 2.29 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the pH (CaCl₂) of the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	279.59	0.0000
Time	2	9.43	0.0003
Treatment	2	50.86	0.0000
Location*Time	2	3.22	0.0485
Location*Treatment	2	0.93	0.4012
Time*Treatment	4	2.21	0.0811
Location*Time*Treatment	4	2.14	0.0890
Error	50		
Total	67		

Concentrations in the tunnels+environs The pH (CaCl₂) of the tunnels+environs (6.1) was significantly higher than that of both the remainder subsoil (5.7) and the control subsoil (5.3), which were also different from each other.

Nutrients leaching from the tunnels Because the pH (CaCl₂) of the remainder subsoil (5.7) was significantly different from that of the control subsoil (5.3), we conclude that, over the time period examined, the ions had leached from the tunnels into the surrounding bulk soil.

Changes over time The pH (CaCl₂) of the tunnels+environs was significantly higher in November 2006 (6.4) than in May (5.9) or September (5.9) 2007, but the latter two were not different from each other.

Conclusions

The presence of dung on the soil surface did not affect the pH (CaCl₂) of the subsoil at Ashbourne or Kuitpo although the pH (CaCl₂) of the subsoil at Ashbourne (6.0) was significantly higher than that at Kuitpo (5.0). Dung burial by *B. bison* resulted in a significant elevation in the pH (CaCl₂) of the tunnels and adjacent soil at both locations. The elevated pH (CaCl₂) was dispersed from the tunnels through the subsoil in the dung+beetles cores, indicating the movement of alkalinity into the subsoil from the tunnels. The pH (CaCl₂) averaged over the entire dung+beetles subsoil was statistically higher than that in the control subsoil at Kuitpo and Ashbourne.

pH (H₂O)

Subsoil chemistry August 2006 to September 2007

The predominant trends in soil pH (H₂O) (Table 2.30) are:

- no effects of surface dung
- no effects of beetle activity

Table 2.30 A comparison of the effect of dung burial activity by *B. bison* on the pH (H₂O) of the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores in mid-September 2005.

Treatment	August 2006		November 2006		May 2007		September 2007	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	6.7	5.7	6.7	5.9	6.6	5.8	6.9	5.9
Dung-only subsoil	na	na	6.8	5.8	6.5	5.7	6.9	6.0
Total dung+beetles subsoil	7.1	5.8	7.1	6.3	6.9	5.8	7.2	5.9
Dung+beetles:	na							
tunnels+environs		6.2	7.2	6.5	7.0	6.1	7.4	6.0
Dung+beetles: remainder		5.8	7.1	6.3	6.9	5.8	7.1	5.9

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* Ash: Ashbourne; Kui: Kuitpo

The impact of dung and dung beetle activity

Main treatments

Analysis of variance of the overall data (controls vs dung only vs dung+beetles) for the pH (H₂O) of the subsoil over the four sampling occasions at both locations (Table 2.31) indicates that there was:

- a highly significant effect of location
- a highly significant effect of time
- a highly significant effect of treatment
- no significant interactions

Table 2.31 Analysis of variance for four sampling occasions (August & November 2006, May and September 2007) and three treatments (controls, dung-only, dung+beetles) on the pH (H₂O) of the subsoil at the two study locations

Source	DF	F	P
Location	1	452.16	0.0000
Time	3	7.09	0.0003
Treatment	2	11.84	0.0000
Location*Time	3	1.36	0.2617
Location*Treatment	2	1.63	0.2039
Time*Treatment	6	0.74	0.6229
Location*Time*Treatment	6	0.87	0.5228
Error	68		
Total	91		

Location Over the 13-month sampling period the pH (H₂O) of the subsoil at Ashbourne (6.8) was significantly higher than that at Kuitpo (5.9).

Changes over time There were no significant differences in the pH (H₂O) of the soils at the two locations from August 2006 (6.3) through November 2006 (6.4) and in September 2007 (6.5), but in the sample from May 2007 it was significantly lower (6.2).

Effects of dung and dung beetles The overall pH (H₂O) of the subsoil sampled from dung+beetles treatment (6.5) was significantly higher than that in the dung-only (6.3) and the control (6.3) treatments.

Dung beetle tunnels and the surrounding soil

Analysis of variance of data (controls vs tunnels+environs vs remainder) for the pH (H₂O) of the subsoil over the three sampling occasions at both locations (Table 2.32) indicates that there was:

- a highly significant effect of location
- a highly significant effect of treatment
- a significant effect of time
- a minor interaction between location and time

Table 2.32 Analysis of variance for three sampling occasions (November 2006, May and September 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the pH (H₂O) of the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	230.42	0.0000
Time	2	6.75	0.0025
Treatment	2	14.27	0.0000

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Location*Time	2	3.54	0.0363
Location*Treatment	2	0.55	0.5784
Time*Treatment	4	0.63	0.6461
Location*Time*Treatment	4	0.56	0.6953
Error	50		
Total	67		

Concentrations in the tunnels+environs The pH (H₂O) of the tunnels+environs (6.9) was significantly higher than that in both the remainder subsoil (6.5) and the control (6.3) subsoil, which were also different from each other.

Nutrients leaching from the tunnels Because the pH (H₂O) of the remainder subsoil was significantly different from that in the control subsoil, we conclude that, over the time period examined, ions had leached from the tunnels into the surrounding bulk soil.

Conclusions

The presence of dung on the soil surface did not affect the pH (H₂O) of the subsoil at Ashbourne or Kuitpo, but the pH (H₂O) of the subsoil at Ashbourne (6.8) was significantly higher than that at Kuitpo (5.9). Dung burial by *B. bison* resulted in a significant elevation in the pH (H₂O) of the subsoil tunnels and adjacent soil in some cases but the effects were far less clear than those observed for pH (CaCl₂).

Discussion

Beetle biology, dung burial and subsoil chemistry

The results presented here clearly show that dung burial by the dung beetle *B. bison* and the processing of the buried dung by beetle larvae caused major increases in the levels of plant nutrients (nitrate, phosphate, sulphur) in the subsoil and also increased levels of subsoil organic carbon, pH and electrical conductivity 11 to 23 months after the dung was applied.

In marked contrast, surface dung had no influence on subsoil chemistry during that period. Similarly there was no corresponding pasture growth response to surface-applied dung (without dung beetles) in adjacent experiments at these two locations.

At Kuitpo most larvae became adult beetles and vacated their faecal shells in early winter of 2006, leaving behind a substantial mass of digested excreta and an empty faecal shell. At Ashbourne, most larvae had a two-year life cycle and so during winter of 2006 they remained in their faecal shells in tunnels, surrounded by larval excreta. These beetles emerged as adults in early winter 2007, leaving behind their faecal shells and what remained of the larval excreta.

During winter 2006, the tunnels and their contents became moist and were colonised by earthworms and substantial amounts of plant root material. At this time sampling for beetle-induced changes in soil fertility became appropriate, and so samples were taken for analysis from the cores extracted from the field in August 2006 (after being in the field for 11 months).

Since most of the changes in soil fertility associated with dung beetle activity are likely to occur in the subsoil in the dung+beetles cores, the initial comparisons were concerned to demonstrate the presence and magnitude of these responses, and so focussed on the dung+beetles cores and the control cores. The dung-only cores were not assessed in August 2006. The August data showed a massive response to dung beetle activity, and so the sampling in November 2006 was expanded (to include dung-only cores) to provide a comprehensive analysis of all three treatment subsoils.

At both Ashbourne and Kuitpo there was significant root material in the beetle tunnels and surrounding the faecal shells (containing third instar larvae when present). Considering the elevated levels of plant nutrients in the tunnels, it is most likely that roots will continue to colonise the subsoil and their contribution to subsoil carbon is likely to be increasingly important over time (years).

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As time passes a number of processes (earthworm activity in and about the tunnels, water movement (carrying dissolved plant nutrients), and plant roots growing in and about the tunnels) will disperse the effects of dung beetles from the near proximity of the tunnels to the soil between them. Further, the growth and death of plant roots in the subsoil will progressively alter its character, increasing organic carbon levels, which will, in turn, promote biological activity (eg earthworms and soil microbes) in the subsoil, which will enhance its biological fertility.

One further important process, especially in duplex soils, is the 'clay-spreading' effect of the dung burial process, in which subsoil clay is brought to the surface and mixed with surface soil, thereby improving the fertility and water-holding capacity of the surface soil. The effect of this and the dung-lined tunnels on soil fertility is not yet known.

In the dung-only treatments there was no detectable effect of dung on pasture growth or on subsoil fertility, yet the applied dung contained substantial amounts of plant nutrients (especially nitrate, phosphate and sulphur) and it seems probable that these will remain associated with the surface soil.

In order to account for the fate of nutrients and organic matter applied in the dung and to assess the impact of the dung burial process on the fertility of surface soil, it is important to construct a nutrient budget that takes account of the effects of surface and buried dung on the nutrient and organic matter distribution throughout the entire core (rather than just the base section). Duplicate cores from all three core sections (0–10 cm, 10–20 cm, 20–45 cm) from the May and September 2007 sampling occasions have been prepared and await financial support for their analysis.

How long these effects persist is not known, but it is important information that is required to understand the increased soil fertility and to assess the long-term dollar value of dung beetles in the rural economy of Australia.

Of special interest are the effects of dung burial by beetles on soil carbon and phosphate levels.

Carbon storage in soil organic matter

Global warming is now accepted as being caused by elevated atmospheric levels of CO₂ and so control of emissions and removal of CO₂ from the atmosphere are high global priorities. The commonly proposed industrial procedures to remove CO₂ from the atmosphere (or from industrial waste) and store it deep in the oceans or in the earth's mantle are yet to be validated on a scale that could influence global CO₂ levels. Furthermore, their efficiency (the cost of C-sequestration), long-term security (leakages), lag-time to establishment, and ecological impact are not yet securely established. Alternative mechanisms are urgently required.

In order for a dung beetle-based partial solution to climate change to be broadly acceptable to the community, it needs to represent a 'win-win' situation. That is, climate change benefits must be achieved using strategies that have few down-side effects and that preferably also have benefits for other areas. The use of dung beetles is an outstanding example of such a process because the potentially harmful effects are minimal. Dung beetle abundance is limited by the dung supply and so they do not have the capacity to become an environmental pest. Further, they are unlikely to have harmful effects on native Australian dung beetles because the native species occur primarily in woodland and those native grassland species that use cattle dung are now hugely more abundant than they were before the introduction of cattle.

Carbon storage in the subsoil under dung pads (as a consequence of dung burial) will be derived from the original dung pad and from plant roots colonising the subsoil via the beetle tunnels. The relative contribution of the roots will increase over time as the dung carbon is metabolised by microbes and beetle larvae and as plant roots grow into the subsoil and then senesce.

The test dung pads used in these experiments contained approximately 600 g dry organic matter (80% water content in the 3-litre pads). Assuming that organic matter is 55% carbon and 80% of the pad was buried in the subsoil, a total of 264 g of carbon would have been deposited in the subsoil under each pad. This has produced an identical elevation (0.5%) in the average levels of soil carbon in the subsoil at both Ashbourne and Kuitpo (Table 2.33).

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Table 2.33 The effects of dung burial by the beetle *B. bison* on the average levels of soil organic carbon in the subsoil at Ashbourne and Kuitpo 11 to 23 months after beetles began to bury dung

	Average levels of soil carbon (%) over 4 sampling occasions	
	Ashbourne	Kuitpo
Control cores	2.07	0.71
Dung+beetles cores	2.58	1.21
Beetle-related increase	0.51	0.50

From this, the absolute increase in carbon levels in the subsoil as a result of dung beetle activity was taken at 0.5% and so for a 12 kg subsoil core section the absolute increase in carbon in that soil can be calculated to be 60 g (0.5% of 12 kg), equivalent to about 23% of that contained in the original buried dung (264 g). This poses the possibility that dung burial by dung beetles could be responsible for sequestering substantial amounts of organic carbon.

This potential can be examined using some simple calculations.

One mature cow produces about 2 tonnes of wet dung during the annual activity season for *B. bison*. From this, about 0.18 tonnes of carbon will be placed in the subsoil (assuming 80% dung moisture, 55% C-content of organic matter, 80% dung burial over a 123-day activity period for *B. bison* (June to September)), and 0.04 tonnes of carbon will be present in the subsoil 1 to 2 years later (assuming 23% of the buried carbon remains). Thus each *B. bison* season, the dung produced by one cow, if buried by *B. bison*, will result in an additional 40 kg carbon in the subsoil. Further, it is likely that dung beetle activity will also increase levels of carbon in the surface soils, but these data are not yet available and their procurement awaits the analysis of the entire soil profile (see above).

The potential of dung beetles to sequester carbon in the subsoil becomes very substantial when one appreciates that world-wide there are 1.4 billion cattle.

A subsoil phosphate budget

Available phosphate levels in the control subsoil (no dung, no beetles) were in the range 12–23 mg kg⁻¹ at Ashbourne and 5–10 mg kg⁻¹ at Kuitpo (Table 2.6) over the four sampling occasions. In marked contrast, the levels in the tunnels+environs (ie the tunnel contents and the tunnel walls) were in the order of 200–350 mg kg⁻¹ at Ashbourne and 70–320 mg kg⁻¹ at Kuitpo, and the levels in the remainder subsoil (the soil surrounding the tunnel walls that was not affected by dung beetle activity) were in the order of 50–80 mg kg⁻¹ at Ashbourne and 25–55 mg kg⁻¹ at Kuitpo. These levels represent a massive increase in available phosphate in the subsoil (Table 2.34).

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Table 2.34 The effects of dung burial by the beetle *B. bison* on the average concentration of phosphate (ppm) in the subsoil at Ashbourne and Kuitpo 11 to 23 months after beetles began to bury dung

	Average levels of soil phosphate (ppm)	
	Ashbourne	Kuitpo
Control cores	17.4	7.9
Dung+beetles cores	80.1	53.2
Beetle related increase	62.7	45.3

The total phosphate levels in triplicate subsamples of the control subsoil from Ashbourne and Kuitpo were assessed (through CSBP), as was the total and available phosphate in the dung used to establish the experiments.

It is possible to construct a simple phosphate budget for the subsoil section of the cores from the following parameters:

- the mass of each subsoil fraction
- the total phosphate levels in the whole subsoil sample (20–45 cm)
- the levels of available phosphate in each fraction
- the amount of phosphate (total and available) in the dung that was buried
- the phosphate levels in the control and dung+beetles subsoil samples (Table 2.6)

Using these parameters it is possible to estimate that on average, over the 13 months over which the cores were sampled, available phosphate levels at Ashbourne and Kuitpo in the dung+beetles core subsoils were elevated by 878 mg and 635 mg respectively, above those in the control soils (Table 2.35). Clearly dung burial by the beetle *B. bison* caused a substantial increase in the concentrations of phosphate in the subsoil.

Table 2.35 Absolute amounts of phosphate (total and available mg) in the subsoil (20–45 cm) of cores from Ashbourne and Kuitpo (subsoil samples averaged 14 kg dry weight),

	Absolute levels of phosphate (mg per subsoil core)	
	Ashbourne	Kuitpo
Total phosphate introduced with buried dung	844	544
Available phosphate introduced with buried dung	433	315
Total phosphate in subsoil of control core	5152	4737
Available phosphate in subsoil of control core	244	110
Available phosphate in subsoil of dung+beetles core	1121	745
Elevation of phosphate in subsoil of dung+beetles cores	878	635
Available phosphate in subsoil of control cores + available phosphate introduced with buried dung	677	426
Additional phosphate in dung+beetles subsoil	444	319

This analysis provides circumstantial evidence for the view that the dung burial and dung processing activities of the dung beetle *B. bison* result in substantial mineralisation of phosphate that was previously unavailable. The source of the mineralised phosphate is not certain but it may have come from the subsoil or from the previously unavailable phosphate in the buried dung (49% and 42% of the total phosphate in the dung at Ashbourne and Kuitpo respectively was not available) or from both.

Faecal shells that encased the mature beetle larvae and faecal debris from inside the tunnels were collected, bulked and dried and duplicate samples were analysed by CSBP. These contained 4000–5000 mg kg⁻¹ of available phosphate, a huge concentration, suggesting that at least part of the phosphate that is mineralised by the dung beetle larvae comes from the phosphate bound in the dung buried in the soil.

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These findings provide a unique mechanism for increasing phosphate levels (and other nutrients) in the subsoils of pastures and should be published in collaboration with Dr M McCaskill, an expert in interpreting soil phosphate data.

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Appendix 4: Seasonal activity and dung burial by natural field populations of *B. bison*

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Summary

Abundance and seasonal activity

Bubas bison was released at both Ashbourne and Kuitpo in 2002 and field recoveries of F1 beetle occurred after one year at Kuitpo and after two years at Ashbourne. Beetle abundance was monitored using dung-baited pitfall traps at weekly intervals at two study locations (Ashbourne, Kuitpo, SA) over four periods: October–November in 2005, April–November in 2006 and 2007 and April–May 2008. Results for the four periods are compared and contrasted. Overall, nine dung beetle species were trapped at Ashbourne and seven at Kuitpo. Overall, the abundance and biomass *Bubas bison* was greater at Ashbourne than at Kuitpo, and levels of field abundance increased in both locations over the period 2005–2008.

At both locations, beetles were most abundant from May to mid-July, whereafter numbers decreased until few–none were trapped in November. Adult *B. bison* were observed in the field at both locations in 2005 but none were caught in the pitfall traps.

During the 2007 beetle activity period the mean number of *B. bison* per trap ranged up to 10 at Ashbourne and up to 2 at Kuitpo. Based on levels of abundance observed in regions where the beetle is well established, levels of abundance can be expected to reach at least several hundred per trap within the next decade.

No *B. bison* were trapped in October–November 2005, and few were trapped in the same period in subsequent years (2006 and 2007) so these data reveal little about season-to-season increases in beetle abundance. Overall, from 2006 to 2007, the abundance of *B. bison* increased 2.9-fold at Ashbourne and 2.8-fold at Kuitpo. In mid-May 2008, the mean numbers per trap at Ashbourne and Kuitpo were 3.3 and 1.0 respectively, similar numbers to those observed in 2007.

As time progresses, *B. bison* is becoming an increasingly important component of the dung beetle fauna in the study areas. In 2007, *B. bison* comprised 48% of the biomass of beetles trapped at Ashbourne (compared with 10% in 2006) and 41% of the biomass of beetles trapped at Kuitpo (compared with 7% in 2006).

Once the mean number of beetles per trap reached about 2 (equivalent to one pair per trap, on average, with a 1:1 sex ratio), nearly all the dung pads placed in the field were buried.

The sex ratio among *B. bison* in the traps in 2007 varied with the season. In April–July the male:female sex ratios at Ashbourne and Kuitpo respectively were 2.1:1 and 1.7:1. A similar early-season preponderance of males over females was observed on Kangaroo Island in 2007 and 2008. For the remainder of the season (August–November) the male:female sex ratio was approximately 1:1 at both test locations, similar to that observed in the latter part of the 2006 season.

The implications of changing sex ratios for cropping and redistribution of dung beetles need to be considered. The standard practice has been to use 1000 field-caught dung beetles as a starter colony. A sample of 1000 beetles caught in April–July may contain as few as 300 females, whereas a comparable sample from later in the season might contain up to 500 females. Since females are the basis of each starter colony, the total number of field-caught beetles in a starter colony may need to be adjusted for the seasonal difference in the sex ratio or the beetles.

Dung burial

Dung burial by natural populations of *B. bison* was observed at the two study locations during the period May to late November in 2006 and 2007 in the fourth and fifth year following release of founder colonies. Field recoveries of the beetle occurred after one year at Kuitpo and after two years at Ashbourne. In 2007 *B. bison* was more abundant at Ashbourne than at Kuitpo, and at both locations the beetle was more abundant than it had been in 2006.

In the 10x10 m replicated field plots, pads of fresh cattle dung (1 litre each) were deposited at weekly intervals from May to October in 2006 and 2007. Dung burial and soil casts (evidence of beetle tunnelling) were observed at each pad at weekly intervals for 8–12 weeks following deposition. Pads colonised by at least one breeding female were buried completely. In the first 12

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weeks (to mid-July), about 80% (the same as in 2006) of the test pads at Ashbourne and 60% (40% in 2006) of the test pads at Kuitpo were buried. During the second half of the beetle season the proportions were 22% (18% in 2006) at Ashbourne and 35% (14% in 2006) at Kuitpo.

Thus it is clear that in 2007 both the abundance of *B. bison* and the level of pad burial increased when compared with 2006. Further increases in abundance and impact should occur over the next few seasons and it is important to observe these.

A facultative adult reproductive diapause

A facultative adult reproductive diapause is a physiological process in the adult dung beetles which is evident as a period of arrested reproductive activity that is expressed in some environmental conditions but not in others.

In the case of *B. bison* the facultative adult reproductive diapause is expressed soon after the adults emerge in autumn. Beetles that are *not in diapause* colonise dung pads (by flying to them) and then dig shallow tunnels in which they feed, mate and the females mature their ovaries (eggs). Beetles leave these tunnels after some days and fly off in search of a fresh dung pad where the same process occurs. A series of such tunnels may be dug over a period of some weeks until the female beetle is ready to begin laying eggs. She then, commonly in association with a male beetle, digs a deep tunnel (to 30–50+ cm) at the bottom of which she deposits dung in which she lays eggs. In contrast, adult beetles that are *in diapause* colonise dung pads (by flying to them) but do not dig tunnels or feed for some weeks (4–8 weeks). When diapause has been completed beetles resume normal breeding activity, with the result that the commencement of breeding is delayed in comparison with those beetles that do not exhibit an adult reproductive diapause.

The field data on the rates of appearance of soil casts around dung pads in the field over the 2006 and 2007 seasons have provided evidence for a facultative adult reproductive diapause in *B. bison*. In duplex soils such as at Kuitpo (a grey-brown sandy loam over a yellow clay), the soil casts (around the pad) derived from shallow tunnels are grey brown in colour and contain no yellow subsoil. In contrast, construction of the deep breeding tunnels brings much yellow subsoil to the surface. The timing of the appearance of casts of two different colours (at Kuitpo) allow us to identify the timing of the transition from feeding to breeding, which is not possible at Ashbourne because of the uniform colour of the soil profile.

Over the observation period (May–November of 2006 and 2007), dung burial took about 3–4 weeks following the appearance of the first soil cast. The time taken (after pads were deposited) for the soil casts to appear differed substantially between locations in the first 12 weeks of the observation period. At Ashbourne, regardless of the time of year, soil casts (indicating tunnelling activity) commonly appeared 1 to 2 weeks after the dung was placed in the field. In contrast, at Kuitpo during the first half of the beetle activity season, soil casts (indicating tunnelling activity) commonly appeared 4 to 8 weeks after the dung was placed in the field, indicating that the beetles were present but not tunnelling (ie in diapause) during the first part of the dung beetle season. The difference between the locations was not obvious in the latter part of the beetle activity season.

These data indicate the possibility of the presence of a facultative adult reproductive diapause at Kuitpo that inhibits tunnelling and feeding during the first month or so of the beetle's adult life. This was expressed at Kuitpo but not at Ashbourne and may be related in some way to soil temperature during immature development.

Recommendation

- That monitoring of the progressive increase in beetle abundance and impact be continued at both Ashbourne and Kuitpo.

Methods

Seasonal activity

Dung beetle abundance was monitored by collecting and counting the beetles trapped in pitfall traps. At each location three pitfall traps were placed equally spaced well apart at the perimeter of

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test plots. Each trap was re-baited each week during the sampling period with 1 litre of fresh dung wrapped in mesh. At the same time as re-baiting the trap, the previous week's catch of beetles was collected from the storage chamber beneath the pitfall trap. The three traps were set at each location on 8 occasions in 2005, 28 occasions in 2006, 29 occasions in 2007 and 8 occasions in 2008 (to mid-May).

As well as *Bubas bison*, a number of native (*Onthophagus mniszzechi*, *Onthophagus australis*) and introduced (*Onthophagus taurus*, *Onthophagus binodis*, *Euoniticellus fulvus*, *Geotrupes spiniger*, *Aphodius fimetarius*, *Heteronychus arator*) species were recognised and counted.

The seasonal change in the sex ratio of adult *B. bison* trapped was recorded in 2007 and 2008.

Dung burial

At both locations dung pads were placed in the field at weekly intervals from May to October in 2006 and 2007 (6 months, 24 occasions in each year) and dung burial and soil cast production observed from May until late November. At Ashbourne there were three replicate dung+beetles test plots (10x10 m) and at Kuitpo there were four replicate dung+beetles test plots (10x10 m). Within each there was a grid of nine rows (1 m apart). Each week in each plot (n=7), three one-litre dung pads were placed in a line along a row (0.5 m between the pads). Each week the line of pads across each plot was extended until a new line was required, which was placed at 1 m away from the previous line. Thus pads were progressively placed in a grid 1 m by 0.5 m apart across the plot, beginning 1 m in from one edge. Each week, each pad was inspected and its condition and the number (and colour at Kuitpo) of soil casts around the dung pad were recorded. In 2006 at both locations pads were placed in the rows beginning at the southern edge of the plots (row 1); in 2007 the pads were placed in the rows beginning at the northern edge of the plots (row 9).

Thus, in week 1 in 2007, 3 one-litre pads were placed at the beginning of row 9 in each dung+beetles plot at Ashbourne (3 plots) and at Kuitpo (4 plots). In week 2, a further 3 pads were placed in row 9 of each test plot and all 21 pads from week 1 were examined for soil casts and dung burial. In week 3, a further 3 pads were placed in row 9 of each test plot, and 42 pads were examined for soil casts and dung burial (21 from week 1 and 21 from week 2). And so on. For example, in week 12, a further 21 dung pads were placed in the field and up to (since some had been completely buried) 231 pads (week 1 to week 11 x 21 pads) were examined for soil casts and dung burial. This process continued weekly until 24 October. The plots were examined for a further 4 weeks after the last pads were put out.

The occasion on which each new soil cast appeared in each pad was recorded. At Ashbourne all casts were dark brown soil. In contrast, at Kuitpo the duplex soil (grey-brown surface soil (10 cm) and yellow clay subsoil (10–50 cm) meant that shallow feeding tunnels were indicated by grey-brown soil casts and deeper breeding tunnels were indicated by yellow soil casts. The colour of casts at Kuitpo was recorded. If a pad was colonised by one breeding female, the entire pad was buried, leaving only dry crusts of dung on the soil surface. A pad that was $\geq 90\%$ removed was considered to have been buried.

Most biological activity at dung pads had ceased within 12 weeks of pads being placed in the field, at which time they were either buried and gone, or hard dry pads sitting on the soil surface. In the hot dry spring of 2006 this occurred within 4 weeks of pad deposition, and within 6 weeks in 2007, when conditions were cooler and moister than they had been in 2006. Information for each winter dung pad (deposited May–August) was recorded each week for 12 weeks, the September dung pads were monitored for 8–12 weeks and the October pads for 4–8 weeks. Regular pad monitoring stopped in late November. This data set comprises more than 7000 separate observations during the May–November of each of the 2006 and 2007 evaluation periods.

Results

Seasonal activity

Beetle abundance was monitored at weekly intervals for the beetle's activity period and beyond at the two main study locations. None were trapped in October and November of 2005. The April to November data for 2006 and 2007 are reported and compared with each other. In 2008, none were

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trapped in April and the seasonal activity began in May with mean numbers of *B. bison* per trap being 3.3 and 1.0 for mid-May (2 trapping occasions) at Ashbourne and Kuitpo.

Over the 7-month trapping periods in 2006 and 2007, a total of nine species were trapped at Ashbourne (Table 4.1), with *Onthophagus binodis* and *Geotrupes spiniger* not being trapped in 2006. Seven species were trapped at Kuitpo, with *Onthophagus mniszechi*, *O. binodis* and *G. spiniger* not being trapped in 2006.

Overall beetle abundance and biomass per trap in 2007 were greater at Ashbourne than at Kuitpo, as in 2006 (Table 4.1). The *G. spiniger* trapped in 2007 were derived from mass releases of the beetle in autumn 2007 and so were not taken to be part of the established dung beetle fauna.

At Ashbourne in 2007 mean numbers per trap were in the range 2 to 10 beetles from April to mid-July, whereafter numbers decreased until none were trapped in November (Figure 4.1). At Kuitpo in 2007 mean numbers per trap were in the range 1 to 2 beetles from April to mid-July, whereafter numbers decreased but were marginally higher than at Ashbourne during the latter part of the season (Figure 4.1).

The abundance of *B. bison* increased from one sampling period to the next (2006 to 2007) by 2.9-fold at Ashbourne and 2.8-fold at Kuitpo.

In 2007, *B. bison* comprised 48% of the biomass of beetles trapped at Ashbourne (compared with 10% in 2006) and 41% of the biomass of beetles trapped at Kuitpo (compared with 7% in 2006) and showed a similar pattern of seasonal activity at both locations which was largely similar to that in 2006 (Figures 3.1 and 3.2), with a steady decline from autumn to spring.

Table 4.1 Total number of dung beetles trapped at Ashbourne and Kuitpo during the period 25 April to 15 November 2007. At each location three traps were emptied and re-baited with fresh dung at weekly intervals from 4 May.

Species	Total numbers trapped 2006		Total numbers trapped 2007	
	Ashbourne	Kuitpo	Ashbourne	Kuitpo
<i>Onthophagus taurus</i>	4375	2219	1148	672
<i>Onthophagus australis</i>	374	172	156	63
<i>Euoniticellus fulvus</i>	654	623	72	119
<i>Aphodius fimetarius</i>	277	39	31	10
<i>Heteronchus arator</i>	49	0	2	0
<i>Bubas bison</i>	80	28	231	78
<i>Onthophagus mniszechi</i>	6	0	1	4
<i>Onthophagus binodis</i>	0	0	2	0
<i>Geotrupes spiniger</i>	0	0	5*	152*
Total numbers	5815	3081	1648	1098
Total biomass (g live weight)	407	200	188	32

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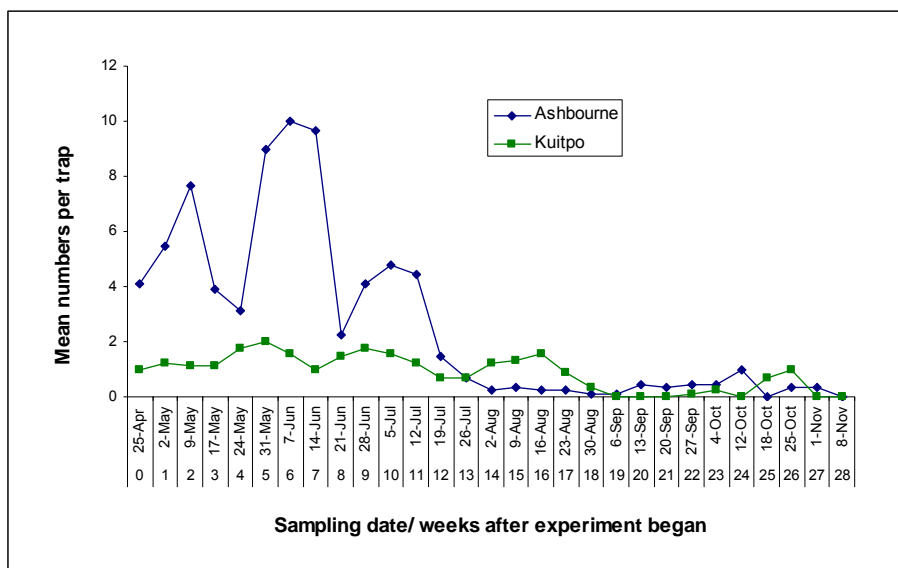


Figure 4.1 Seasonal change in abundance of *B. bison* at Ashbourne and Kuitpo in 2007. Data presented are three-point moving averages of the mean number of beetles per trap at each location. Three traps per plot were emptied and re-baited with fresh dung at weekly intervals from April to November.

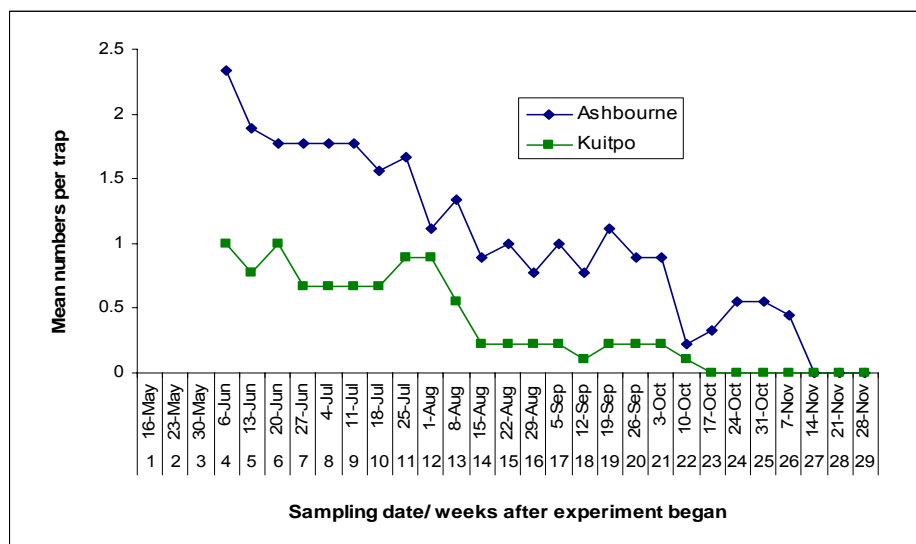


Figure 4.2 Seasonal change in abundance of *B. bison* at Ashbourne and Kuitpo in 2006. Data presented are three-point moving averages of the mean number of beetles per trap at each location. Three traps per plot were emptied and re-baited with fresh dung at weekly intervals from June to November.

The seasonal change in total biomass of all beetle species trapped (Figures 3.3 and 3.4) showed that in 2006 the biomass of beetles colonising pads during spring was substantially greater than that occurring during winter, due primarily to low numbers of *B. bison* in winter and the arrival of moderate numbers of *O. taurus*, *O. australis* and *E. fulvus* (Table 4.1) during spring. In marked contrast, in 2007 the numbers of the winter-active *B. bison* had increased and so there was a bimodal pattern of activity, with a peak in early winter (due to *B. bison*) and a peak in spring (due to the arrival of summer-active species).

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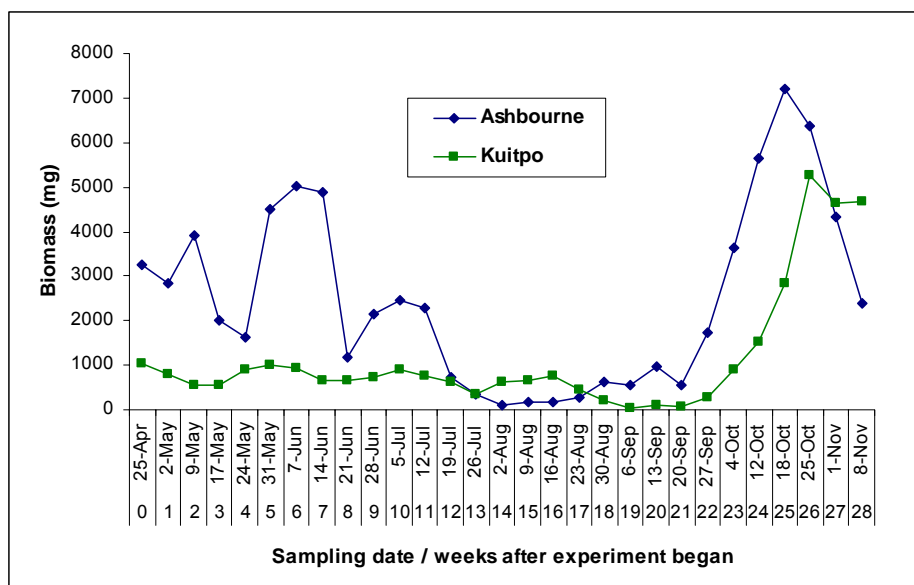


Figure 4.3 Seasonal change in mean biomass of live beetles per trap at Ashbourne and Kuitpo in 2007. Data presented are three-point moving averages of the mean biomass per trap at each location.

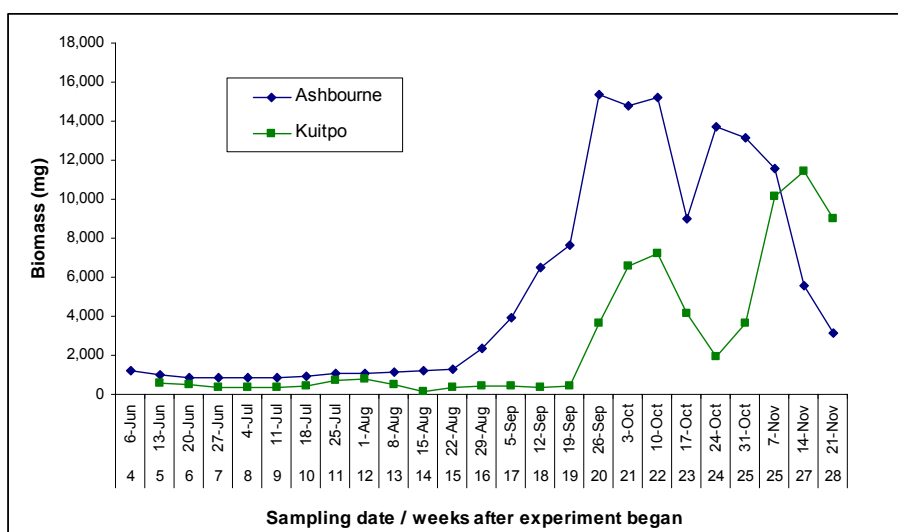


Figure 4.4 Seasonal change in mean biomass of live beetles per trap at Ashbourne and Kuitpo in 2006. Data presented are a three-point moving averages of the mean biomass per trap at each location.

The sex ratio among *B. bison* in the traps in 2007 varied with the season of the year. In April–July the male:female sex ratios at Ashbourne and Kuitpo were 2.1:1 and 1.7:1 respectively (average 1.9:1, n = 278 beetles). For the remainder of the season (August to November) the beetle numbers were low and so were pooled over the two locations and the male:female sex ratio was 1.3:1 (17 males, 14 females) (Table 4.2), similar to that observed in the latter part of the 2006 season. A similar trend in seasonal change in the sex ratio of field-caught *B. bison* was observed on Kangaroo Island in the 2007 and 2008 dung beetle season (Linc Willson, pers. comm.). In the Fleurieu study sites in 2008, a total of 20 males and 15 females were trapped in mid-May (14 and 22 May).

Table 4.2 The relative abundance of male and female *B. bison* in dung-baited pitfall traps at Ashbourne and Kuitpo during the 2007 beetle activity season

	Ashbourne			Kuitpo			
	# males	# females	M:F sex ratio	# males	# females	M:F sex ratio	# traps (each location)
April	1	0	na	1	1	1.0:1	3
May	54	22	2.5:1	18	7	2.6:1	15

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June	67	33	2.0:1	12	3	4.0:1	12
July	25	16	1.6:1	7	11	0.6:1	12
August	3	0	na	8	7	1.1:1	15
September	3	1	3.0:1	0	0	na	12
October	1	4	0.3:1	2	0	na	12
November	0	1	na	0	1	na	6
Totals	154	77	2.0:1	48	30	1.6:1	87

na not applicable

Dung burial

The beetle *B. bison* was released in 2002 at both locations and feral field populations have begun to build up after the releases, but in 2006 and 2007 at both locations beetle numbers were still far lower (a few per pad) than those observed (up to 100s per pad) in other regions where the beetle has been established for a decade or so. It is expected that beetle numbers will increase substantially over the next few years.

The number of dung pads put out each week (9 at Ashbourne and 12 at Kuitpo) that were buried after 12 weeks was expressed as a proportion of the total number of pads. One female *B. bison* will completely bury a dung pad. The proportion of pads buried changed during the beetle activity season of the beetles and the seasonal patterns of dung burial were different at the two study locations.

At Ashbourne, the level of dung pad burial was high (80–100%) in May and June but decreased substantially over the following months (Figure 4.5). At Kuitpo the level of dung burial was lower than at Ashbourne in May–June (30–70%) but persisted at these moderate levels during the period July to September, decreasing to zero in October (Figure 4.5). In the first 12 weeks (to mid-July), about 80% (the same as in 2006) of the test pads at Ashbourne and 60% (40% in 2006) of the test pads at Kuitpo were buried. During the latter half of the beetle season the proportions were 22% (18% in 2006) at Ashbourne and 35% (14% in 2006) at Kuitpo.

A comparison of the level of dung pad burial at Ashbourne and Kuitpo during the 2006 and 2007 activity seasons for *B. bison* (Table 4.3) indicates that at Ashbourne in both seasons about half of the pads placed in the field were buried by *B. bison*. At Kuitpo, in 2006 only 27% of the pads were buried, whereas in 2007 that proportion had increased to 46%, still marginally lower than at Ashbourne (Table 4.3).

Table 4.3 A comparison of the level of dung pad burial (% of dung pads buried) at Ashbourne and Kuitpo during the 2006 and 2007 activity seasons for *B. bison*

	Ashbourne		Kuitpo	
	2006	2007	2006	2007
May	89	87	72	47
June	86	97	31	71
July	67	47	27	50
August	56	29	28	43
September	6	14	15	44
October	0	17	0	0
Overall	49	52	27	46

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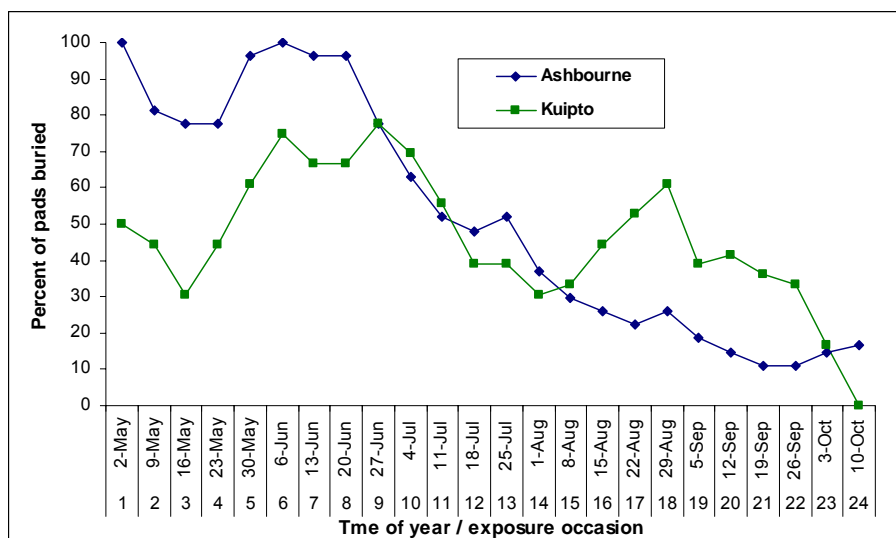


Figure 4.5 Seasonal changes in the level of dung pad burial (pads colonised by one or more females are eventually completely buried) in 2007. Data are three-point moving averages of the percent of the number of dung pads buried in each location on each exposure occasion.

The colour of the soil cast around the pad (and whether or not the pad is buried) can be used to interpret the physiological condition of the beetle(s) occupying the tunnels beneath the dung pad. Normally one beetle (of either sex), or a pair, occupies each tunnel. Kuitpo has a duplex soil, with a brown surface soil over a yellow subsoil. Beetles at the beginning of their activity period feed and mate in shallow tunnels (and bury little dung), after which they fly off to colonise new dung pads and repeat the cycle, and eventually become breeding beetles. Breeding beetles dig deep tunnels (in which they breed) and bury the entire dung pad. Because of this, at Kuitpo, shallow tunnels (occupied by feeding, non-breeding beetles, usually one pair per tunnel) are indicated by brown soil casts and minimal dung burial. In contrast, breeding beetles are indicated by casts which are initially composed of brown soil, to which yellow soil is added over time, and complete dung burial. At Ashbourne the soil is a deep brown loam with no change in colour in the first 50 cm, and so casts produced by feeding and breeding beetles cannot be distinguished on the basis of their colour, although feeding beetles produce relatively small casts and do not bury the entire pad, and so can be identified (but less reliably) on this basis.

At Kuitpo in 2007 new soil casts appeared around the edge of the pads after a period of time ranging from 1 to 12 weeks but at Ashbourne this period was usually 1 to 4 weeks. (A possible explanation for this difference, an obligate adult reproductive diapause, is discussed below.) Observing a pad over 12 weeks enabled an estimate of the number of casts produced at each pad. This is a minimum estimate because the soil casts from some tunnels are hidden inside the dung pad, and sometimes casts from two (or more) tunnels merge around the perimeter of the pad.

Dung burial was monitored at both locations from May to November in 2006 and 2007. Overall in 2007, there was an average of about 2 casts per buried pad at both locations. Dung burial took about 3–4 weeks following the appearance of the first soil cast. The time taken (after pads were deposited) for the soil casts to appear differed substantially between locations in the first 12 weeks of the observation period, being about 2 weeks at Ashbourne and about 4 weeks at Kuitpo. The difference between the locations was not obvious in the latter part of the beetle activity season. This pattern is similar to that observed in 2006.

The levels of cast production (casts per pad) at Ashbourne and Kuitpo were similar to each other in 2007 despite the beetle abundance (numbers per trap) at Ashbourne being about double that at Kuitpo (Figures 3.6 and 3.7). At both locations the levels of beetle activity and the levels of cast production decreased systematically over the winter–spring period, but with some important differences between the locations.

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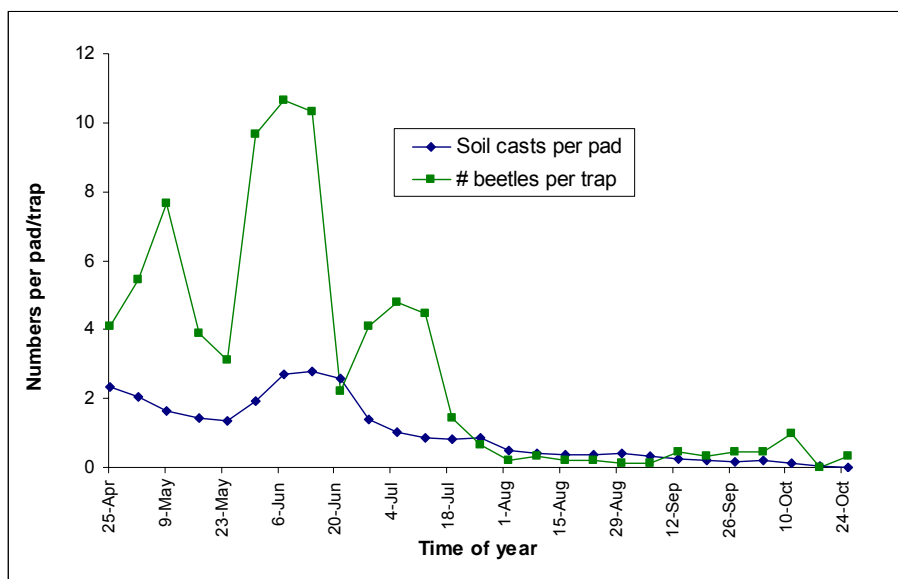


Figure 4.6 Seasonal changes in the levels of soil cast production per pad and the abundance of *B. bison* at Ashbourne in 2007, as indicated by the mean numbers per pitfall trap. Three pitfall traps were baited with fresh dung each week and the number of beetles trapped recorded one week later. The appearance of a new soil cast may occur 1 to 12 weeks after pad deposition, and the mean number of casts per pad is based upon the total number of soil casts produced around the dung pad during the observation period. Both data sets are running 3-point averages of the means on each sampling occasion.

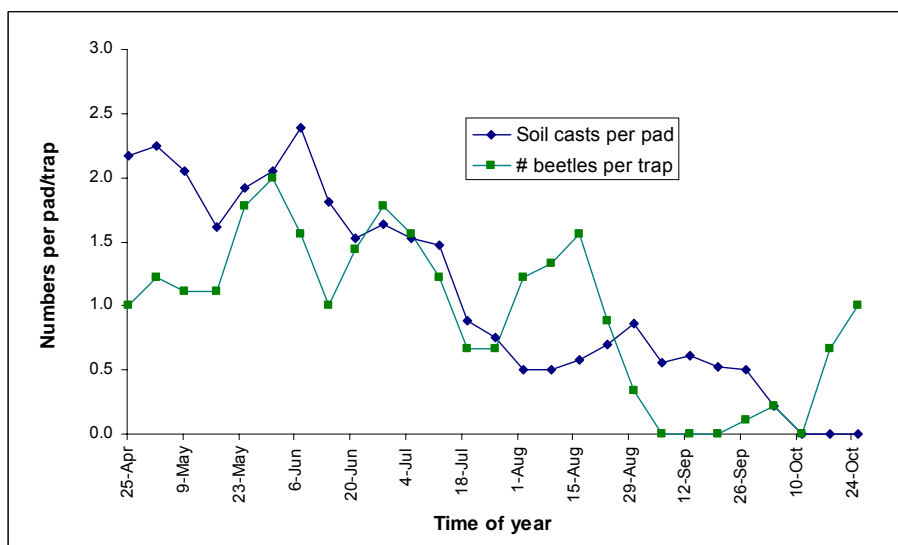


Figure 4.7 Seasonal changes in the levels of soil cast production per pad and the abundance of *B. bison* at Kuitpo in 2007, as indicated by the mean numbers per pitfall trap. Three pitfall traps were baited with fresh dung each week and the number of beetles trapped recorded one week later. The appearance of a new soil cast may occur 1 to 12 weeks after pad deposition, and the mean number of casts per pad is based upon the total number of soil casts produced around the dung pad during the observation period. Both data sets are running 3-point averages of the means on each sampling occasion.

Two forms of diapause: a larval diapause and an adult reproductive diapause

The presence of diapause (arrested development) in the third larval instar (3 LL) of *B. bison* has been demonstrated in a number of other studies (B. Doube, unpublished data). In Experiment 3 in this project, larval diapause was expressed in the field by the majority of the larvae (98%) at Ashbourne and a minority (2%) at Kuitpo. The expression of larval diapause causes the larval beetle to spend another one (or even two) year/s underground before emerging as an adult. Thus the expression of larval diapause extends the duration of the dung beetle life cycle from a one-year

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(ie an annual life cycle with one generation per year, the usual situation in warmer regions) to a two- (or even three-) year life cycle. The 2- and 3-year life cycle occurs in cooler climates. The consequences of this are dealt with elsewhere.

This physiological process is termed a *facultative diapause* because it is expressed under some circumstances (ie in cool climates) but not in others (ie in warmer climates). If diapause were expressed regardless of environmental conditions it would be termed an *obligate diapause*.

The field data on the rates of appearance of soil casts around dung pads in the field over the 2006 and 2007 seasons provide evidence for a facultative adult reproductive diapause in the beetle *B. bison*.

This form of diapause is expressed by the adult beetle soon after emergence from the soil. Beetles that are *not in diapause* colonise dung pads (by flying to them) and then dig shallow tunnels in which they feed, mate and the females mature their ovaries (eggs). Beetles leave these tunnels after some days and fly off in search of a fresh dung pad where the same process occurs. A series of such tunnels may be dug over a period of some weeks until the female beetle is ready to begin laying eggs. She then, commonly in association with a male beetle, digs a deep tunnel (to 30–50+ cm), at the bottom of which she deposits dung in which she lays eggs.

In contrast, beetles that are *in diapause* colonise dung pads (by flying to them) but do not dig tunnels or feed for some time (4–8 weeks). When diapause has been completed beetles then dig shallow tunnel in which they feed, mate and the females mature their eggs and the reproductive biology process proceeds as for beetles that are not in diapause.

This physiological process is termed a facultative adult reproductive diapause because it takes the form of arrested reproductive activity which is expressed in some environmental conditions but not in others.

In duplex soils such as at Kuitpo (a grey-brown sandy loam over a yellow clay), the soil casts (around the pad) derived from shallow tunnels are grey brown in colour and contain no yellow subsoil. In contrast, construction of the deep breeding tunnels brings much yellow subsoil to the surface. The timing of the appearance of casts of different colour (at Kuitpo) and of one colour at Ashbourne allow us to identify the presence or absence of diapause.

Field evidence for 2006

At Kuitpo in 2006 the pattern of appearance of new soil casts around dung pads in the first 10 weeks of the study (16 May to 18 July) was markedly different from that observed during the latter part of the study (25 July to 1 November) (Figures 3.8 and 3.9). In the first period, the mean (\pm SD) interval was 6.4 (\pm 3.1) weeks ($n=123$ casts), whereas that in the latter period was 2.5 (\pm 1.4) weeks ($n=58$ casts), a substantially shorter period ($P < 0.0001$). These differences are clearly illustrated by the contrasting cumulative percentage curves shown in Figure 4.10.

At Kuitpo, seasonal changes in the mean time taken for soil casts to appear around the dung pads (indicating active tunnelling) provide evidence for the presence of a facultative adult reproductive diapause which is expressed as a delay of 4–8 weeks in the initiation of tunnelling and dung burial. This was observed at Kuitpo in breeding beetles (Figure 4.8) and feeding (non-breeding) beetles (Figure 4.9) but not at Ashbourne (Figure 4.8).

These data have been examined in relation to seasonal changes in dung beetle activity. At Kuitpo, during June and July (when the mean time to appearance of the soil casts was 6 weeks), an average of 0.8 *B. bison* per pitfall trap were caught in the traps, whereas only 0.3 beetles per trap were caught in the August and September period.

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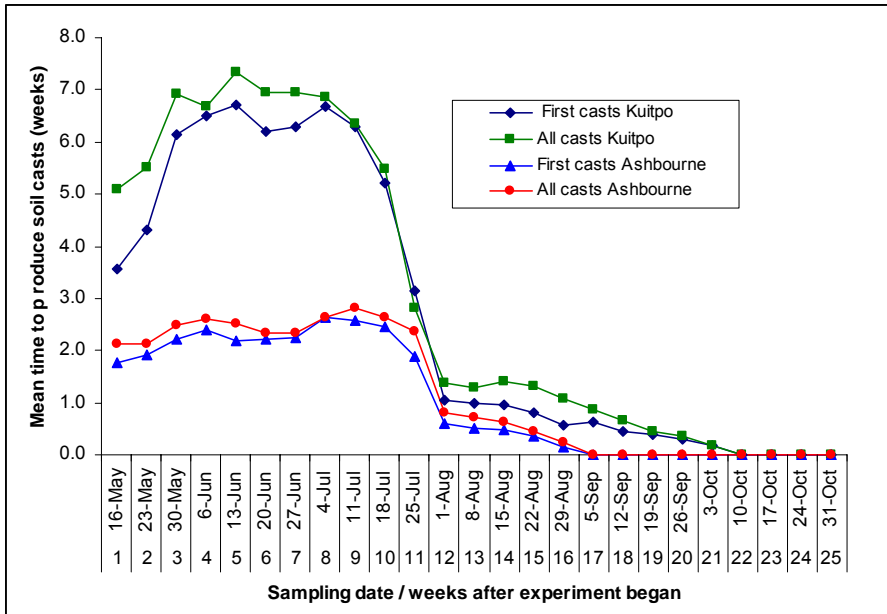


Figure 4.8 Seasonal changes in mean interval between placing dung pads in the field and the appearance of the first soil cast and of all soil casts produced by *B. bison* at Ashbourne and Kuitpo, where 9 and 12 fresh pads respectively were placed in the field each week. The appearance of new soil casts occurred 1 to 12 weeks after pad deposition, and the mean interval is based on soil casts produced around the dung pad during the observation period. All data sets are the running 3-point average of the means on each sampling occasion.

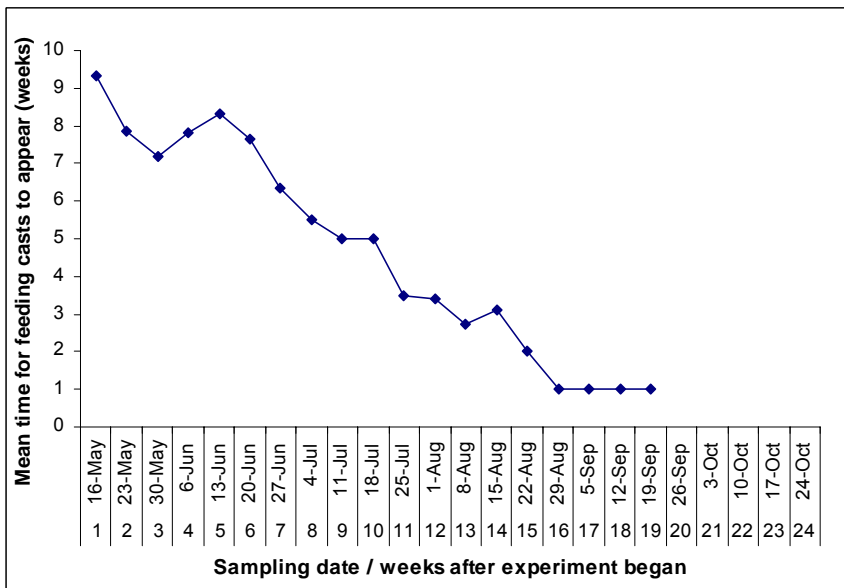


Figure 4.9 Seasonal changes in mean interval between placing dung pads in the field at Kuitpo and the appearance of soil casts produced by beetles that left the pad before breeding. These were recognised as brown (surface) soil casts without additional yellow (subsoil) casts (n=24) and the pads were not buried. This was taken as evidence of the presence of feeding (non-breeding) *B. bison*. The appearance of new soil casts occurred 1 to 12 weeks after pad deposition, and the mean interval is based on soil casts produced around the dung pad during the observation period. All data sets are the running 3-point average of the means on each sampling occasion.

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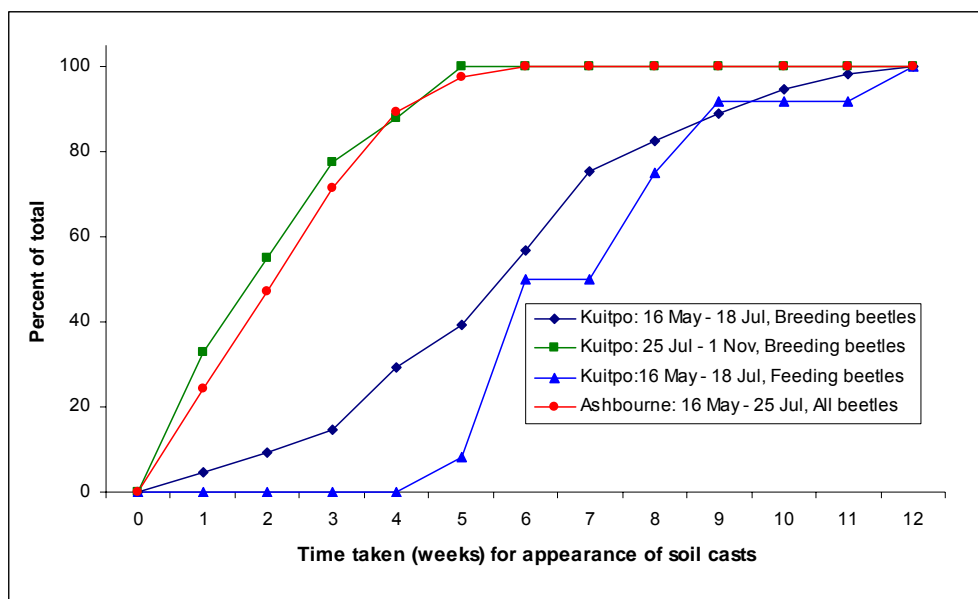


Figure 4.10 Cumulative percentages of the total number of soil casts produced in relation to the time elapsed after pads were placed in the field

The data for the delayed appearance of the soil casts could be taken to indicate that the old pads (4 to 12 weeks old) were colonised by beetles that were ready to tunnel (and so produce casts). However, in the light of the beetle abundance data, this explanation seems improbable. In the June–July period at Kuitpo, numerous beetles were colonising dung pads (as indicated by pitfall trap data) but only 17% of all casts (n=69) were produced during the first 3 weeks after pad deposition. In the latter period (August and September), 76% of all casts (n=50) were produced during the first three weeks after pad deposition (Table 4.3). These differences are highly significant both statistically ($\chi_1^2 = 38.5$; $P < 0.0001$) and biologically. The corresponding percentages for Ashbourne are 77% (of 78 casts) and 72% (of 39 casts) (Table 4.4), which are not statistically different from each other ($\chi_1^2 = 0.69$; $P > 0.05$) or from the data for the period August to September at Kuitpo ($\chi_1^2 = 0.14$; $P > 0.05$).

Table 4.4 Casts produced during the first 3 weeks after deposition from pads deposited in June–July and August–September 2006 at the 2 study locations

	# produced in 1st 3 weeks	Total casts for period	% produced in 1st 3 weeks
Kuitpo			
Casts from pads deposited in June & July	12	69	17.4
Casts from pads deposited in Aug & Sept	38	50	76.0
Ashbourne			
Casts from pads deposited in June & July	60	78	76.9
Casts from pads deposited in Aug & Sept	28	39	71.8

In marked contrast, the mean times for soil casts to appear in the early phase of the observations (6 June to 25 July) at Kuitpo (6.6 weeks (± 3.3)) and Ashbourne (2.5 weeks (± 1.2), Table 4.5) are significantly different from each other ($t=9.7$, $P < 0.0001$).

The most likely explanation is that during the early-to-mid part of the beetle activity season (June and July), the beetles at Kuitpo colonised dung pads but did not begin to tunnel (in order to feed and breed) for 4 to 8 weeks, whereas those at Ashbourne began tunnelling soon after arrival at the pad. However, at both locations in the latter half of the season (after 25 July) beetles began digging tunnels soon after arriving at the dung pad.

Table 4.5 Early and late-season comparisons of the mean time taken (weeks) for soil casts (made by breeding beetles) to appear at the two test locations in 2006

Ashbourne			Kuitpo		
mean	SD	N	mean	SD	N

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6 June–25 July	2.50	1.15	78	6.58	3.34	69
1 Aug–26 Sept	2.78	1.37	39	2.48	1.40	50

Field evidence for 2007

At Kuitpo in 2007 the pattern of appearance of new soil casts around dung pads in the first 12 weeks of the study (from 2 May) was markedly different from that observed during the latter 12 weeks of the study (Figures 3.11 and 3.12). In the first period, the mean (\pm SD) interval was 4.2 (\pm 2.5) weeks ($n=123$ casts), whereas in the second period the mean (\pm SD) interval was 2.2 (\pm 1.4) weeks ($n=58$ casts), a substantially shorter period ($P < 0.0001$). These differences are clearly illustrated by the contrasting frequency distributions shown in Figures 3.11 and 3.12.

At Kuitpo, seasonal changes in the mean time taken for soil casts to appear around the dung pads (indicating active tunnelling) provide evidence for the presence of a facultative adult reproductive diapause which is expressed as a delay of 4–8 weeks in the initiation of tunnelling and dung burial. This was observed at Kuitpo in breeding beetles (Figure 4.11) and feeding (non-breeding) beetles (Figure 4.14) but not at Ashbourne (Figure 4.11).

These data need to be examined in relation to seasonal changes in dung beetle activity. At Kuitpo, during June and July (when the mean time to appearance of the soil casts was 4.2 weeks), an average of 1.4 *B. bison* per pitfall trap were caught in the traps, whereas only 0.5 beetles per trap were caught in the August and September period.

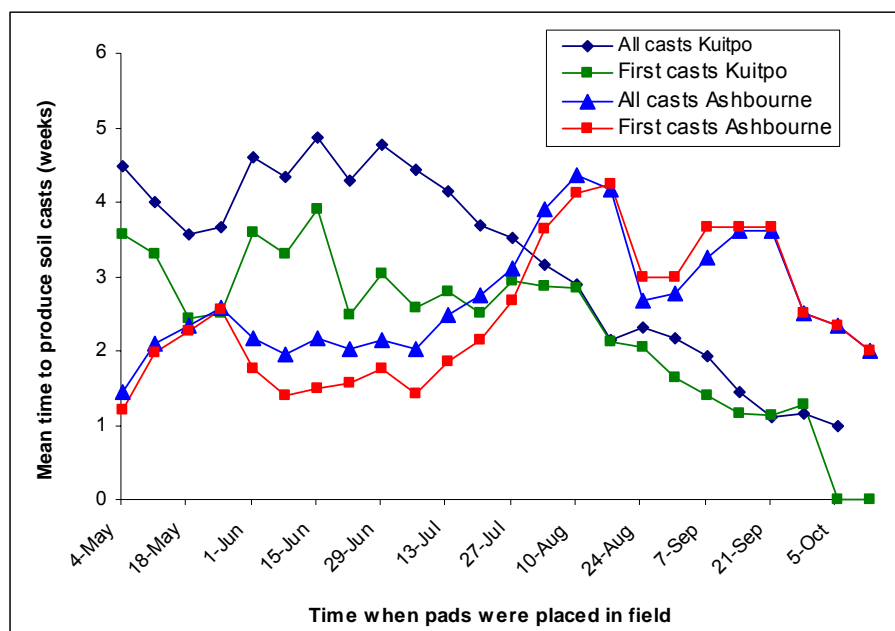


Figure 4.11 Seasonal changes in mean interval between placing dung pads in the field and the appearance of the first soil cast and of all soil casts produced by *B. bison* at Ashbourne and Kuitpo, where 9 and 12 fresh pads respectively were placed in the field each week. The appearance of new soil casts occurred 1 to 12 weeks after pad deposition, and the mean interval is based on soil casts produced around the dung pad during the observation period. All data sets are the running 3-point average of the means on each sampling occasion.

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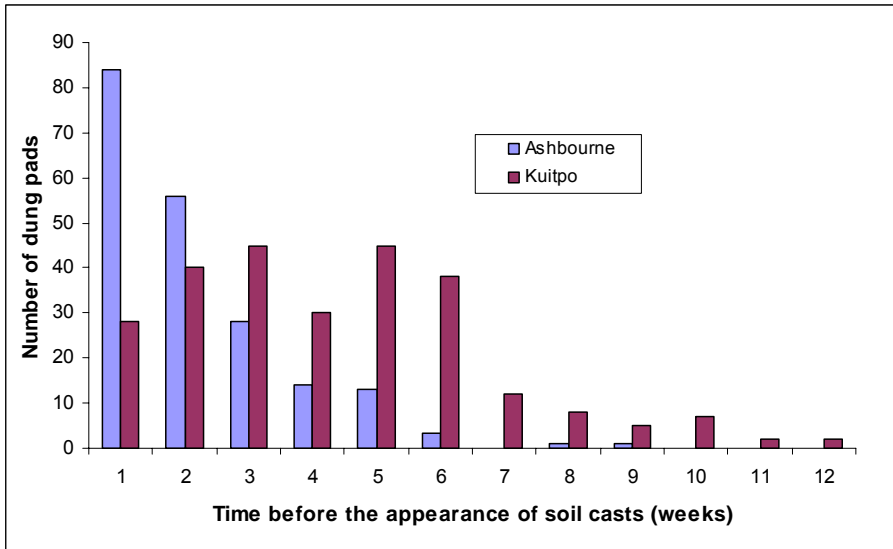


Figure 4.12 Frequency of soil casts produced in relation to the time elapsed after pads were placed in the field for the 12-week period 4 May to 19 July 2007

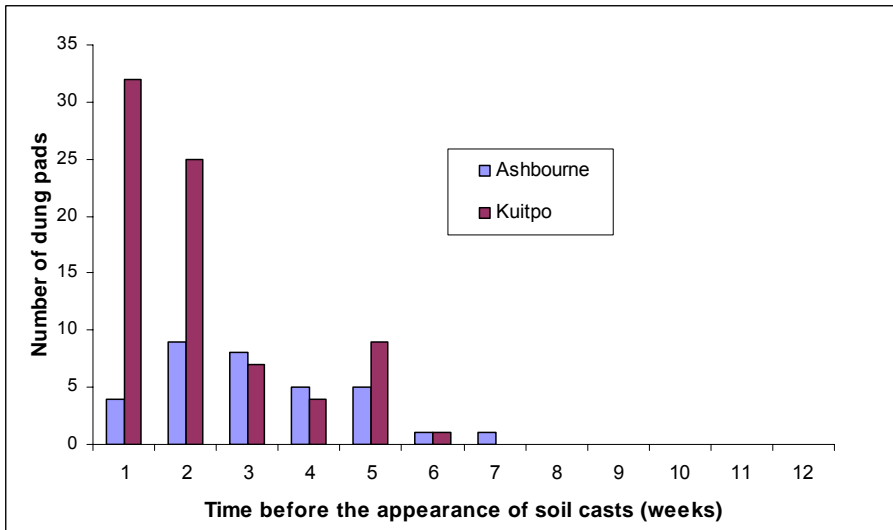


Figure 4.13 Frequency of soil casts produced in relation to the time elapsed after pads were placed in the field for the 12-week period 26 July to 24 October 2007

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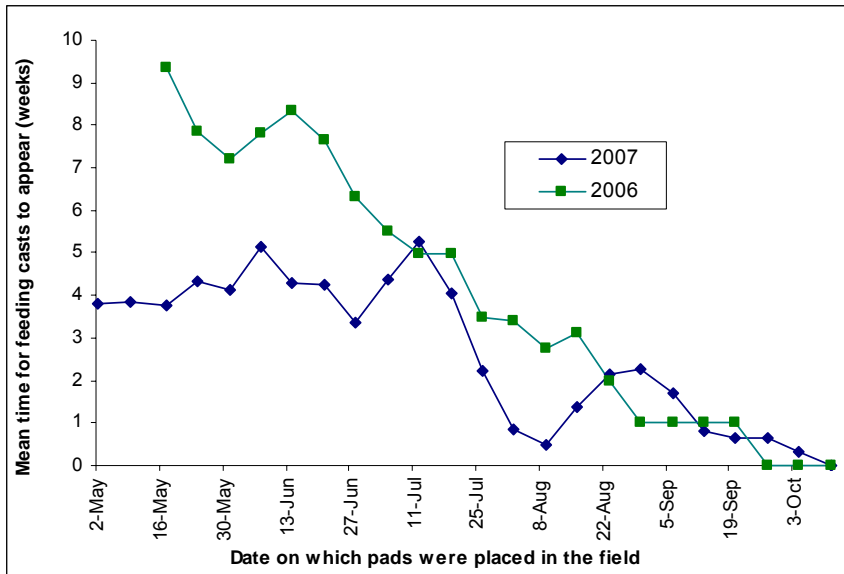


Figure 4.14 Seasonal changes in mean interval between placing dung pads in the field at Kuitpo and the appearance of soil casts produced by beetles that left the pad before breeding in 2006 and 2007. These were recognised as brown (surface) soil casts without additional yellow (subsoil) casts (n=24) and the pads were not buried. This was taken as evidence of the presence of feeding (non-breeding) *B. bison*. The appearance of new soil casts occurred 1 to 12 weeks after pad deposition, and the mean interval is based on soil casts produced around the dung pad during the observation period. All data sets are the running 3-point average of the means on each sampling occasion.

The data for the delayed appearance of the soil casts could be taken to indicate that the old pads (4 to 12 weeks old) were colonised by beetles that were ready to tunnel (and so produce casts). However, in the light of the beetle abundance data, this explanation seems improbable (see Figures 3.1 and 3.2).

The mean times for soil casts to appear in the early phase (12 weeks to mid-July) of the observations at Kuitpo and at Ashbourne are significantly different from each other ($P < 0.0001$). The most likely explanation is that during the early-to-middle part of the beetle activity season (May, June and July), the beetles at Kuitpo colonised dung pads but did not begin to tunnel (in order to feed or to breed) for 4 to 8 weeks, whereas those at Ashbourne began tunnelling soon after arrival at the pad. This conclusion is supported by the 2006 and 2007 data at Kuitpo on beetles that colonised dung pads but did not breed (ie did not produce yellow casts) (Figure 4.14).

However, at both locations in the latter half of the season (after 25 July) beetles began digging tunnels soon after arriving at the dung pad, but there was still a significant difference between Kuitpo and Ashbourne in the mean time for soil casts to appear (2 weeks vs 3 weeks, $P = 0.001$). There is no obvious explanation for this difference, which did not occur in 2006.

Beetle abundance and dung burial

The trap data for *B. bison* and the dung burial data for pads placed in the field at the same time were analysed for 2006 (see above) and the same trends were evident in 2007. These data indicate that, once the mean number of beetles per trap reached about 2–3 (equivalent to one pair per trap, on average, during the breeding phase of the annual cycle), nearly all the dung pads placed in the field were buried (Figure 4.15). This observation is supported by the evidence from the number of beetle-derived soil casts around the pads, in which pads with one substantial cast (indicating one breeding female beetle) were completely buried on most occasions.

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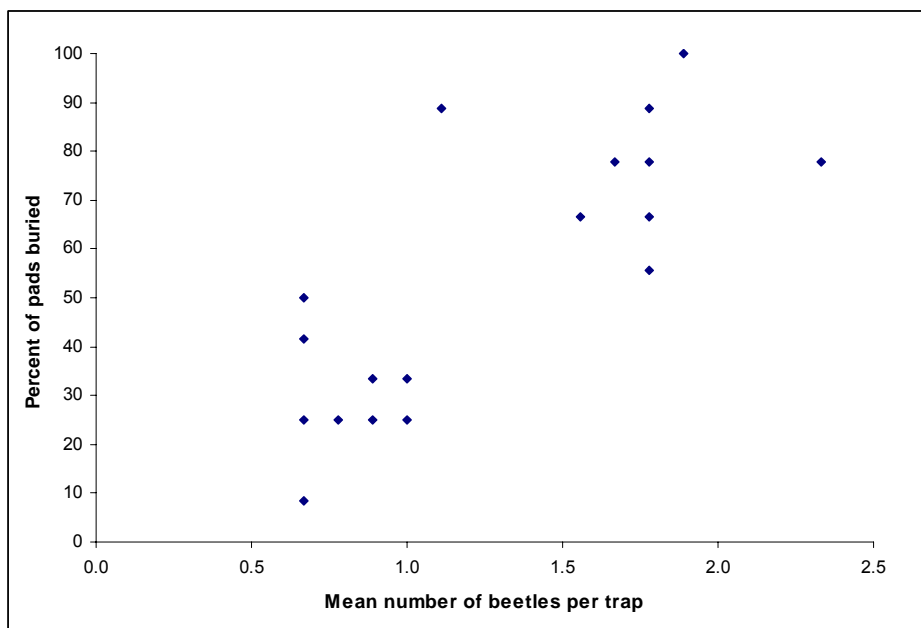


Figure 4.15 The relationship between the mean number of beetles per trap and the percentage of dung pads buried for the 9-week period 6 June – 1 August 2006. Data presented are a three-point moving average of the mean number of beetles per trap. A dung pad, once colonised, was completely buried. Data for Ashbourne and Kuitpo have been combined.

Individual pads were completely buried if colonised by one breeding female/pair. However, not all pads were colonised. Overall, the percentage of pads buried increased as the mean number of casts per pad increased, with burial of most pads occurring once a mean of two casts per pad was observed (Figure 4.16).

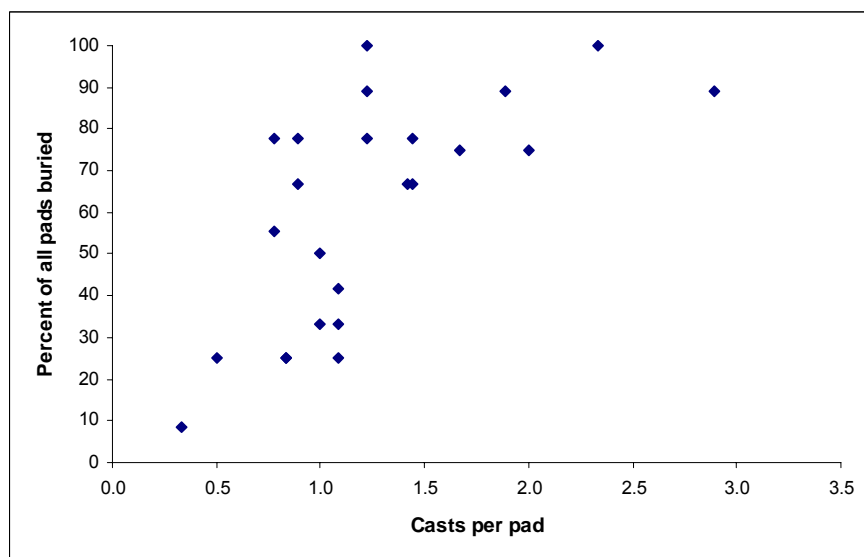


Figure 4.16 The relationship between the mean number of soil casts per pad and the percentage of dung pads buried for the 12-week period 16 May to 1 August 2006. A dung pad, once colonised, was completely buried. Data for Ashbourne and Kuitpo have been combined.

There was a significant positive relationship between the mean number of beetles caught in pitfall traps over one week and the mean number of soil casts appearing about the dung pads placed in the field at the same time (Figure 4.17). Overall, there appeared to be about 1.2 beetles in the pitfall traps for each soil cast. The sex ratio was 1:1. Other data, from pad excavations, indicate that each tunnel was commonly occupied by a pair of beetles. Thus it appears that the casts observed were made by solitary beetles, or that the trap catches underestimated the number of

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beetles colonising pads, or that there were soil casts associated with dung pads that were not observed (eg those within the pad).

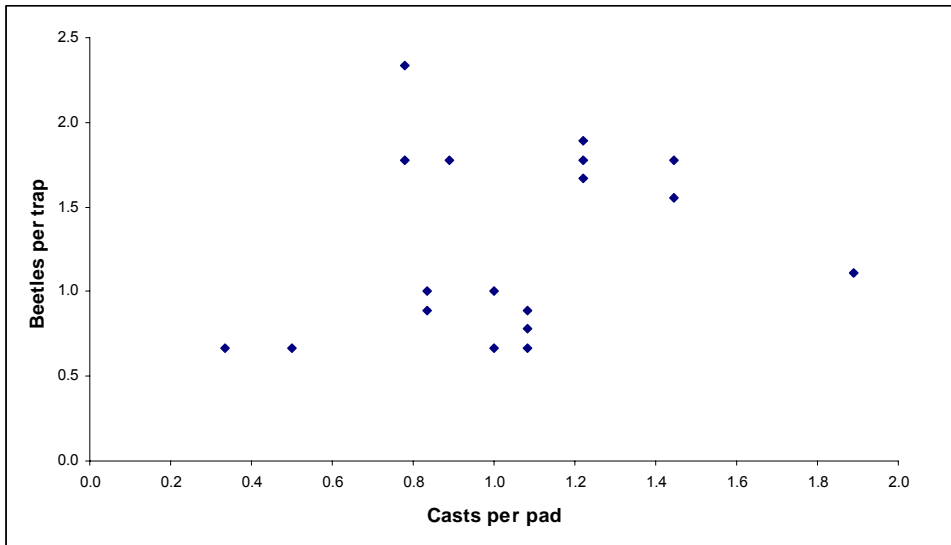


Figure 4.17 The relationship between the mean number of beetles per trap and the mean number of soil casts per pad for the 9-week period 6 June to 1 August 2006 (the traps were baited at the same time as pads were placed in the field). Data presented are a three-point moving average of the mean number of beetles per trap. Data for Ashbourne and Kuitpo have been combined.

Discussion

Increasing abundance of *B. bison*

Beetle abundance was monitored at weekly intervals from May to November 2006 and 2007 and the seasonal pattern of abundance was similar in both years, although the absolute abundance of *B. bison* increased from one sampling period to the next over the two sampling seasons (2006 to 2007) by 2.9-fold at Ashbourne and 2.8-fold at Kuitpo. The scarcity of beetles in October–November 2005 and the appearance of moderate numbers in May 2008 also support the general trend of increasing levels of abundance over time.

In May this year (2008) high number of *B. bison* have been reported in the field in for the first time on the Fleurieu Peninsular (pers. comm.. Mr Mark Higgins, Pt Elliott, SA, a producer-collaborator). Beetles were released there in 2002. This grower-feed-back is the first report of large numbers of *B. bison* on the mainland in SA, although the beetle has been known to be established on the Peninsula since 2003.

We do not know the potential maximum abundance of *B. bison* but unpublished data (daily catches from 3 traps over 4 months) from Mr Linc Willson on Kangaroo Island in an environment similar to that at Kuitpo and using the same type of dung beetle traps caught several hundred *B. bison* per trap per day during the peak activity season in 2007, and similar levels of activity have been observed in previous years. Under these circumstances, the dung was completely removed within some days of deposition. There was obvious dung beetle activity over the period May to November. High levels of beetle activity were also observed in Western Australia in June 2007 by B. Doube.

Considering these data, it seems very likely that the abundance of *B. bison* in the two study areas will increase considerably over the next few years, with a corresponding increase in the rapidity and extent of dung burial during winter, and in the impact upon pasture productivity. The beetle is also likely to become abundant throughout the peninsula over the next decade.

How long to peak levels of beetle abundance?[OK by BMD]

The maximum potential abundance of *B. bison* in any one locality is largely determined by four factors, These are

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- the number of cattle present (determining the daily rate of dung production)
- the duration of the beetle breeding season (beetles stop breeding when the soil dries out)
- the suitability of the soil for breeding and survival of the immature stages of development
- the intensity of competition between beetles for dung

A mature beast produces 20+ kg of dung per day and, under optimal conditions, this can produce 10+ beetle progeny per kg of dung. The daily production of dung from one mature beast therefore has the potential to give rise to 200 beetles under optimal conditions (ie in a deep loamy soil). This number will be reduced if the soil type reduces beetle survival. For example, the mean number of survivors in a clay or a duplex soil is about half that in deep loams and the number in sandy soils is about 1% of that in deep loam (BMD unpublished data). Thus the daily production of beetles from the dung from one mature beast fall to about 100 beetles in clay soils and to 2 in deep sand.

A short beetle breeding season may be 100+ days long and an extended breeding season may be up to 200 days long. Thus each season in ideal conditions the dung from one mature beast has the potential to give rise to 20,000 to 40,000 beetles. A herd of 500 cattle therefore would have the potential to produce between 10m and 20m beetles annually, provided that competition between beetles for dung was not limiting beetle reproduction.

However, the limit of the dung supply stops the exponential growth in beetle numbers that one might expect to follow from this reproductive potential. Once beetles have become abundant, competition between beetles for dung becomes intense and substantially reduces the reproductive potential of the dung beetle population. The nature of this competitive interaction has been defined for *Onthophagus binodis* but not for *B. bison*. This needs to be understood in order to understand the population dynamics of the beetle in the field.

An example from Kangaroo Island, SA, helps understand how populations of *B. bison* build up. Two starter colonies each of 1000 *B. bison* were introduced to adjacent properties on KI in 1993. By 2001 (after 8 generations) the beetles had reached substantial numbers and were burying most of the winter dung and during the years 2006, 2007 and 2008 the beetles were extremely abundant, achieving populations of 100+ per dung pad during May and June each year. The effect of this on dung was profound. Horse dung in the home paddock was completely buried within 12 hours and an assessment of dung burial in the cattle paddocks on 26th June 2008 indicated that all of the cattle dung produced had been buried within 5 – 8 days of its production.

Thus on Kangaroo Island where the beetle has one generation per year, a starter inoculation of 2000 beetles produced substantial populations within 7-8 years and what are presumed to be maximal populations within 10 or 11 years.

In the study sites at Ashbourne and Kuitpo, beetles were released in 2002 and moderate number have been observed in May and June of 2008 (BMD unpublished data), five years after 1000 beetles were released at each location, corroborating what was observed on Kangaroo Island. Field populations at the Ashbourne and Kuitpo field sites increased about 2.7-fold from the 2006 to the 2007 (in their 4th and 5th years) and levels of activity in 2008 appear to be greater again (in the 6th year).

From these observations we predict that, in regions where the beetle has one generation per year, a starter colony of 1000 dung beetles will produce moderate numbers in 7 to 8 years and large numbers in 10 to 11 years. In regions where the beetle has a two-year life cycle, moderate number will be achieved in 14 – 16 years, and then only in every second year. To resolve this, beetles need to be released in two successive years so that beetles will be present in all years.

If however, the starting inoculum were 50 starter colonies (not one), the time to achieve large numbers of beetles would be shortened by 4-5 years where there is a 1-year life cycle and by 8-10 years where there is a 2-year life cycle. Considering that pasture production is likely to be increased by 20+% and that fertiliser demand is likely to be significantly reduced by beetle activity, the economics of starting to 50 rather than one starter colony appear to be overwhelming.

Adult reproductive diapause

Both the 2006 and the 2007 data indicate that there may be an adult reproductive diapause in *B. bison* which is expressed at Kuitpo but not at Ashbourne as delayed dung burial and breeding activity.

Evidence consistent with the presence of a facultative adult reproductive diapause in *B. bison* has been observed in beetles imported from Spain to quarantine laboratories in Geelong, Australia (K. Wardhaugh pers. comm.). Adult beetles were trapped in early spring in Spain and brought directly to Australia, where they began to breed immediately once given a supply of fresh dung. In marked contrast, adult beetles trapped in the field in Spain in the following (Spanish) autumn and brought directly to Australia, failed to feed, bury dung or breed for 6–8 weeks even though provided with a regular supply of fresh dung. Subsequently they bred in a manner similar to the beetles in the earlier shipment.

It is likely that we are observing the same phenomenon in the dung beetles at Kuitpo, in that they emerge in autumn and colonise dung pads, but do not produce soil casts (evidence of tunnelling and feeding/breeding) for about 6–8 weeks. Why this occurs among the beetles at Kuitpo and not those at Ashbourne is not known, but may be related to some environmental cues (eg average temperature). There is no reason to believe that the two populations are genetically different.

Whether this possible adult reproductive diapause is induced in the larval or the adult stage is not known, but in most instances in the entomological literature, the expression of adult diapause is induced well before the emergence of the adults.

Beetle sex ratios

The male:female sex ratio among adult beetles taken from their faecal shells (ie before emergence) is clearly 1:1. This is based upon a sample of many hundreds of beetles and clearly demonstrates that the sex ratio among the beetles in the field is 1:1. However, behavioural differences between the sexes may lead to a different sex ratio in beetles caught at dung-baited pitfall traps.

All adult *B. bison* live for only one season, dying before summer arrives. At Ashbourne and Kuitpo and on Kangaroo Island, the sex ratio among *B. bison* caught in pitfall traps in 2007 changed with the season, with a male:female sex ratio of about 2.4:1 in the early part of the beetle activity season (May to mid-July) and approximately 1:1 in the latter part of the season (after July), although numbers were so low at that time of year that it is difficult to be confident of the real sex ratio in the field.

Despite this, it seems probable that the sex ratio in beetle trap catches shifts from a predominance of males early in the season to an even sex ratio in the mid-season and possibly a predominance of females later in the season.

There are a number of possible explanations for the shifting sex ratio.

One explanation may be that males emerge earlier in the season than do females and that their early emergence allows them to feed and mature sexually before the females emerge, thereby ensuring that they are ready to mate with the females immediately they emerge. Alternatively the males may leave the feeding–mating tunnel once the female has been mated, while the female remains behind in the tunnel in order to continue to feed and mature her ovaries. This would mean that the females spent longer underground than did the males, thereby generating a predominance of males in the trap catches.

Once females have been mated, they can reproduce without the presence of a male, although there is a degree of collaborative provisioning of the nest if males are present. The apparent low male:female sex ratio later in the season indicates that the males die sooner than do the females.

These findings have implications for cropping and redistribution of dung beetles. The standard practice has been to use 1000 field-caught dung beetles as a starter colony. A sample of 1000 beetles caught in April–May may contain as few as 300 females, whereas a comparable sample from later in the season may contain 500 or more females. Since females are the basis of each starter colony, the total number of field-caught beetles needs to be adjusted for the seasonal difference in the sex ratio of the beetles.

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In conclusion it is clear that we are observing a progressive year-by-year increase in beetle abundance and their impact upon dung at both Ashbourne and Kuitpo. We recommend that this progression be monitored over the next few years so that we can know what to expect in other, similar regions into which *B. bison* is introduced.

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Site selection

The two study sites (Location 1, Ashbourne; Location 2, Kuitpo) were selected and established as the locations for the two main pasture production experiments (see previous reports). An experiment to monitor the impact of dung and dung beetles on soil health, and especially earthworm populations (Experiment 3), was established at each location in late winter 2005.

Methods

Beetle-proof soil cores were established in the field at both locations. There were four replicates of each of three treatments (dung+beetles, dung only and controls [no dung, no beetles]). Specific details are presented in previous reports.

On each sampling occasion, one core from each replicate of each treatment (12 cores per location) was removed from the soil and taken to the laboratory where each core was divided into three sections (0–10cm, 10–20 cm and 20–45 cm: termed surface, mid and base sections). The soil in each section (a total of 72 sections per sampling occasion) was examined carefully and the earthworms present were removed and preserved in 70% alcohol. Each earthworm was subsequently weighed, identified to species and classified as an adult, a sub-adult or a juvenile. Four species of earthworm and one platyhelminth species were recognised.

The sampling occasions (reported separately) for which earthworm data are presented are as follows:

- sampling occasion 1, November 2005 (after 6 weeks)
- sampling occasion 2, April 2006 (after 7 months)
- sampling occasion 3, August 2006 (after 11 months)

The analysis was complex, involving assessing the effects of 3 treatments (with 4 replicates each) at each of two locations on five earthworm species. In addition three developmental stages were recognised for each earthworm species and both biomass and numerical abundance were assessed. All of these factors were assessed at three soil depth categories. In summary there were 2 locations x 3 treatments x 4 replicates x 5 species, each with 3 size categories for both numerical abundance and biomass at each of 3 soil depth categories, making a total of 1718 categories for analysis on each sampling occasion.

Results for November 2005

Species abundance and biomass

Sites were sampled on 14 November 2005 at Location 2 and 17 November 2005 at Location 1.

Location 1 (Ashbourne)

At Ashbourne (Location 1) a total of 303 earthworms and 32 platyhelminths were recovered from 12 cores. The dominant species was *A. rosea*, which made up 72% of the numbers and 53% of the biomass. *A. trapezoides* and *A. caliginosa* made up 5–11% of the numbers and 10–18% of the biomass. *M. dubius* was scarce (Table 5.1).

Earthworms within species were relatively patchily distributed across the test plots, with *A. rosea* being relatively scarce in plot 4 (only 6% of the total of *A. rosea*) and *A. trapezoides* scarce in plot 2 (6% of the total). The platyhelminths had a tendency to be concentrated in plots 1 and 4 (both next to the fence), with 78% of all individuals in those two plots (Table 5.1).

Table 5.1 Total numbers and total biomass of earthworms and platyhelminths recovered from soil cores (n=12) taken from the soil at Ashbourne in November 2005. There were four replicate plots adjacent to each other at the study site

Replicate plot number	Numbers of earthworms & platyhelminths					% total	% total e/worms
	1	2	3	4	Total		

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<i>A. trapezoides</i>	6	1	5	5	17	5.1	5.6
<i>A. caliginosa</i>	5	9	12	11	37	11.0	12.2
<i>A. rosea</i>	77	77	72	14	240	71.6	79.2
<i>M. dubius</i>	6	0	0	3	9	2.7	3.0
Platyhelminths	10	2	5	15	32	9.6	
Total	104	89	94	48	335	100.00	
Total earthworms	94	87	89	33	303		100.00
Biomass of earthworms & platyhelminths (g live weight)							
Replicate plot number	1	2	3	4	Total	% total	% total e/worms
<i>A. trapezoides</i>	2.9	1.1	2.0	3.0	9.5	10.0	12.0
<i>A. caliginosa</i>	2.7	5.9	4.8	3.1	16.5	17.4	20.8
<i>A. rosea</i>	12.7	17.7	16.1	3.2	49.7	52.3	62.6
<i>M. dubius</i>	2.4	–	–	1.2	3.6	3.8	4.5
Platyhelminths	6.0	1.0	1.5	7.1	15.6	16.4	
Total	26.7	25.7	24.4	17.6	94.9	100.0	
Total earthworm biomass	20.7	24.7	22.9	10.5	79.3		100.0

Location 2

At Kuitpo (Location 2) a total of 446 earthworms and 38 platyhelminths were recovered from 10 sample cores (two were not processed). The dominant species was *A. trapezoides*, which made up 44% of the numbers and 58% of the biomass. *A. rosea* made up 43% of the numbers and 28% of the biomass. *A. caliginosa* was relatively scarce and *M. dubius* was not found (Table 5.2).

The earthworm *A. trapezoides* was relatively evenly distributed across the test plots (Table 5.2). In contrast *A. caliginosa* and *A. rosea* were virtually absent from plots 3 and 4 (plots on the uphill side of the slope) whereas platyhelminths had a tendency to be concentrated in plots 3 and 4, with 97% of all individuals in those two plots.

The four plots were placed in a row about 20 m from the fence line. Each plot was about 20 m long and separated from its adjacent neighbour by 5 m, and so the plots occurred over a 100 m stretch of paddock which sloped uphill but appeared homogeneous.

Soil moisture was assessed at each of the three soil depths for each of the cores sampled. There were no obvious trends across the 100 m transect in the moisture levels of the surface or the mid-level soils (Table 5.3) but the base soils were drier in plots 3 and 4 than in plots 1 and 2 (Table 5.3). No obvious explanation is available for the strongly disjunct distribution patterns observed for *A. caliginosa* and *A. rosea* but it may be that the drier soils favour the platyhelminths and are relatively hostile to the earthworms. Platyhelminths have also been recorded as predators of earthworms.

Table 5.2 Total numbers and total biomass of earthworms and platyhelminths recovered from soil cores (n=10) taken from the soil at Kuitpo in November 2005. There were four replicate plots adjacent to each other at the study site

Replicate plot number	Numbers of earthworms & platyhelminths					Total	% total	% e/worms
	1	2	3	4				
<i>A. trapezoides</i>	65	40	32	77	214	44.2	49.6	
<i>A. caliginosa</i>	10	13	0	0	23	4.8	5.5	
<i>A. rosea</i>	93	112	3	1	209	43.2	44.9	
<i>M. dubius</i>	0	0	0	0	0	0.0	0.0	
Platyhelminths	0	1	29	8	38	7.9		
Total	168	166	64	86	484	100.0		
Total earthworms	168	165	35	78	446		100.0	

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Replicate plot number	Biomass of earthworms & platyhelminths (g live wt)					% total	% e/ worms
	1	2	3	4	Total		
<i>A. trapezoides</i>	36.0	17.9	12.8	37.8	104.5	58.3	65.2
<i>A. caliginosa</i>	2.3	4.3	0.0	0.0	6.6	3.7	4.1
<i>A. rosea</i>	21.8	26.5	0.5	0.5	49.3	27.5	30.7
<i>M. dubius</i>	–	–	–	–	–	–	–
Platyhelminths	0.0	0.6	14.9	3.3	18.8	10.5	
Total	60.1	49.3	28.2	41.6	0.0	100.0	
Total e/worm biomass	60.1	48.7	13.3	38.3	160.4		100.0

Soil moisture: effects of plot location, soil depth and treatment

At the time of sampling (November 2005) soil moisture was in the range 13–25% at Location 1 and 12–30% at Location 2 (Table 5.3). The moisture profile down through the soil varied between locations.

At Ashbourne, the surface soil was moister than the two subsoil strata, which did not differ from each other. This is presumed to reflect recent rainfall and the even soil texture down the soil profile to 50 cm.

At Kuitpo, the surface and the base strata had similar water contents, presumably reflecting the recent rainfall (on the sandy loam surface soil) and the high water-holding capacity of the clay subsoil. The mid stratum had a lower moisture content, which was taken to reflect the presence of substantial quantities of fine gravel in the mid stratum. There was a clear trend in base-level moisture levels across the plots, in which plots 1 and 2 had a substantially higher moisture level (27.8%) than plots 3 and 4 (15.3%). This may help explain the low numbers of *A. caliginosa* and *A. rosea* in plots 3 and 4 (Table 5.2).

Table 5.3 Effect of site location, plot location and soil depth on soil moisture

	Soil moisture (% water relative to the dry weight of soil)				
	Plot 1	Plot 2	Plot 3	Plot 4	Mean
Ashbourne: L1					
Surface	21.8	18.8	25.4	25.0	22.8
Mid	16.5	14.7	16.3	15.4	15.7
Base	14.9	13.3	16.1	18.4	15.6
Mean	17.8	15.6	19.2	19.6	18.0
Kuitpo: L2					
Surface	21.3	26.5	18.8	23.9	22.6
Mid	12.4	17.1	13.0	15.3	14.5
Base	29.7	25.9	17.1	13.5	21.6
Mean	21.2	23.2	16.3	17.6	19.5

The effect of dung and dung beetles on earthworm abundance and biomass

These results indicate a strong response of the earthworm populations to dung during the first 7 weeks of the experiment. The pattern of results was similar at both locations in that *A. trapezoides*, *A. caliginosa* and *A. rosea* were more abundant in the presence of dung than in the controls. In addition, the abundance and biomass of *A. rosea* appeared to be further increased by the activities of the dung beetles, as was that of *A. trapezoides* at Location 2.

The following trends in numerical abundance emerge from the data (Tables 2.4 and 2.5):

- *A. trapezoides* seems to be more abundant in the presence of dung, and its abundance is further increased by dung beetle activity at Location 2.
- *A. caliginosa* appears to be more abundant in the presence of dung at Location 1 but not at Location 2

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- *A. rosea* appears to be substantially more abundant in the presence of dung and its abundance is further increased by dung beetle activity.
- Platyhelminth abundance may be reduced in the presence of dung beetle activity at Location 2.

Table 5.4 The effect of dung and dung beetle activity on the total abundance and biomass of earthworms and platyhelminths at the Ashbourne study location, November 2005

Numbers	Dung+beetles	Dung only	Controls	Total	% total	% total e/worms
<i>A. trapezoides</i>	4	7.5	3	14.5	4.8	5.3
<i>A caliginosa</i>	12	17.3	2	31.3	10.4	11.5
<i>A rosea</i>	149	63	7	219.0	72.9	80.6
<i>M. dubius</i>	1	6	0	7.0	2.3	2.6
Platyhelminths	7	10.5	11	28.5	9.5	
Total	173	104.3	23	300.3	100.0	
Total earthworms	166	93.75	12	271.8		100.0

Biomass	Dung+beetles	Dung only	Controls	Total	% biomass	% total e/worm biomass
<i>A. trapezoides</i>	2.5	4.1	1.5	8.1	9.6	11.5
<i>A caliginosa</i>	5.5	7.5	1.0	14.0	16.6	19.8
<i>A rosea</i>	33.4	11.4	1.0	45.8	54.3	64.8
<i>M. dubius</i>	0.3	2.5	0.0	2.8	3.3	3.9
Platyhelminths	2.3	5.6	5.8	13.7	16.2	
Total	44.0	31.2	9.3	84.5	100.0	
Total e/worm biomass	41.7	25.5	3.5	70.7		100.0

Table 5.5 The effect of dung and dung beetle activity on the total numbers and biomass of earthworms and platyhelminths at the Kuitpo study location, November 2005

Numbers	Dung+beetles	Dung only	Controls	Total	% total	% total e/worms
<i>A. trapezoides</i>	98	78	38	214	44.2	48.0
<i>A caliginosa</i>	1	16	6	23	4.8	5.2
<i>A rosea</i>	124	50	35	209	43.2	46.9
Platyhelminths	2	18	18	38	7.9	
Total	225	162	97	484	100.0	
Total numbers	223	144	79	446		100.0

Biomass	Beetles	Dung	Controls	Total	% biomass	% total e/worm biomass
<i>A. trapezoides</i>	52.9	34.3	17.3	104.5	58.3	65.2
<i>A caliginosa</i>	0.4	3.6	2.6	6.6	3.7	4.1
<i>A rosea</i>	26.6	16.5	6.2	49.3	27.5	30.7
Platyhelminths	1.1	8.6	9.1	18.8	10.5	
Total	81.0	63.0	35.2	179.2	100.0	
Total e/worm biomass	79.9	54.4	26.1	160.4		100.0

Average size of adults

The average size of adults is important for understanding the population dynamics of earthworms because the potential fecundity of individuals increases with size. Earthworms feed on organic matter in soil and so it is likely that an increased food supply (dung) would result in earthworms growing to a larger size. The live weight of all adult and sub-adult earthworms was recorded and so

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it was possible to investigate the effect of dung and dung beetles on the size of earthworms (as indicated by their live weight).

Statistical analysis of the effects of soil depth and treatment on the size of earthworms requires a moderate sample size (> about 50) in order for there to be sufficient individuals in each category to allow a useful comparison. This criterion was met by only three sets of data (*A. trapezoides* at Kuitpo and *A. rosea* at both locations). The results for these are presented below.

A. trapezoides, Location 2 (Kuitpo)

Adult earthworms from the dung+beetles plots were larger than those from the dung-only plots ($P < 0.001$) and the control plots ($P < 0.01$) and there was no difference between the latter two ($P > 0.05$) (Figure 1). These data indicate that the presence of dung mixed through the soil by dung beetle activity created an environment that was highly favourable for earthworm growth and development, with the result that the adults were able to grow to a larger size than in the dung-only and control cores.

There was no significant effect of dung or beetles on the mean weight of the sub-adult beetles (Figure 5.1), indicating that the transition from sub-adult to adult occurred at the same earthworm size in all treatments and was not affected by the food supply.

I interpret these data as indicating that the transition from sub-adult to adult occurred at the same earthworm size in all treatments but that the presence of dung mixed through the soil by dung beetle activity created an environment that was highly favourable for earthworm growth and development and so the adults were able to grow to a larger size than in the dung-only and control cores.

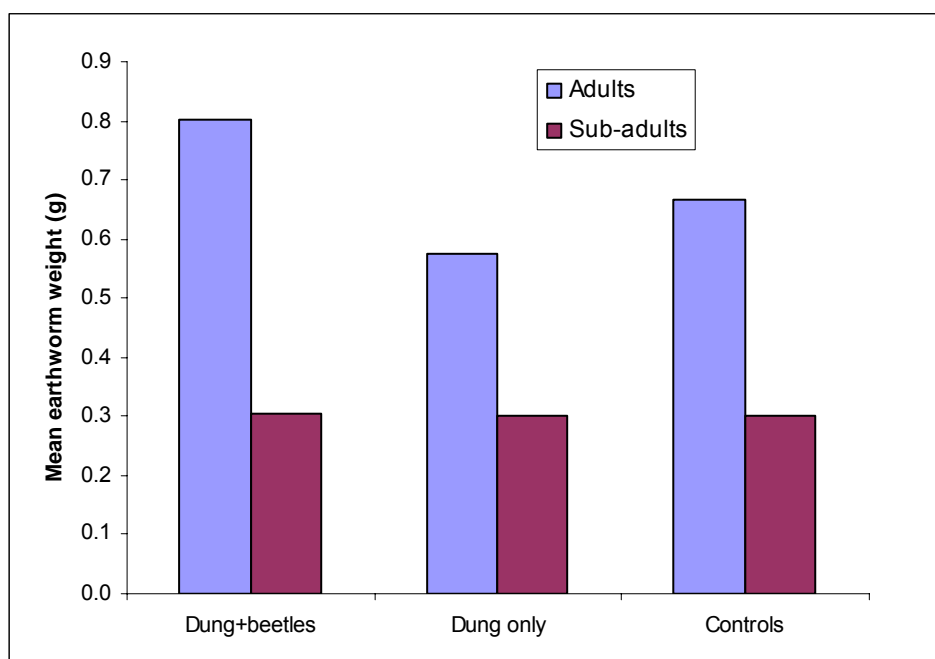


Figure 5.1 Mean weight of adult and sub-adult *A. trapezoides* at Location 2 (Kuitpo)]

A. rosea, Location 2 (Kuitpo)

The earthworm *A. rosea* was dispersed throughout the soil profile. An analysis of the average weight of adult earthworms indicated that there was no consistent change in size with soil depth for adults or sub-adults in any of the three treatments (Table 5.6). However, there was a strongly significant effect ($P < 0.001$) of treatment on the size of the adult earthworms but not on the size of the sub-adults ($P > 0.05$). The adult earthworms in the dung-only treatment plots were substantially heavier (+23%) than the adults in the dung+beetles treatment plots ($P < 0.001$), which in turn were heavier (+12%) than those in the control plots ($P < 0.001$).

The presence of earthworm food (cattle dung) appears to have created an environment that was highly favourable for earthworm growth and development with the result that adults became

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substantially larger than in the control treatment but this effect was reduced in the presence of dung beetle activity. The mechanism by which this reduction occurs is not known.

There was no significant effect of dung or beetles on the mean weight of the sub-adult beetles (Figure 5.2), indicating that the transition from sub-adult to adult occurred at the same earthworm size in all treatments and was not affected by the food supply.

Table 5.6 The effect of soil depth, dung and dung beetle activity of the mean weight (\pm SD) of adult and sub-adult *A. rosea* at Location 2 (Kuitpo)

	Dung+beetles		Dung-only		Controls [no dung, no beetles]	
	Adults	Sub-adults	Adults	Sub-adults	Adults	Sub-adults
Surface	0.33 \pm 0.11	0.16 \pm 0.06	0.20 \pm 0.00	0.15 \pm 0.06	0.26 \pm 0.09	0.13 \pm 0.05
Mid stratum	0.29 \pm 0.10	0.11 \pm 0.04	0.39 \pm 0.15	0.10	0.24 \pm 0.08	0.10 \pm (na)
Base stratum	0.31 \pm 0.11	0.13 \pm 0.05	0.40 \pm (na)	–	0.40 \pm (na)	–
Overall mean	0.31\pm0.10	0.14\pm0.06	0.43\pm0.13	0.15\pm0.05	0.25\pm0.08	0.12\pm0.04

na not applicable

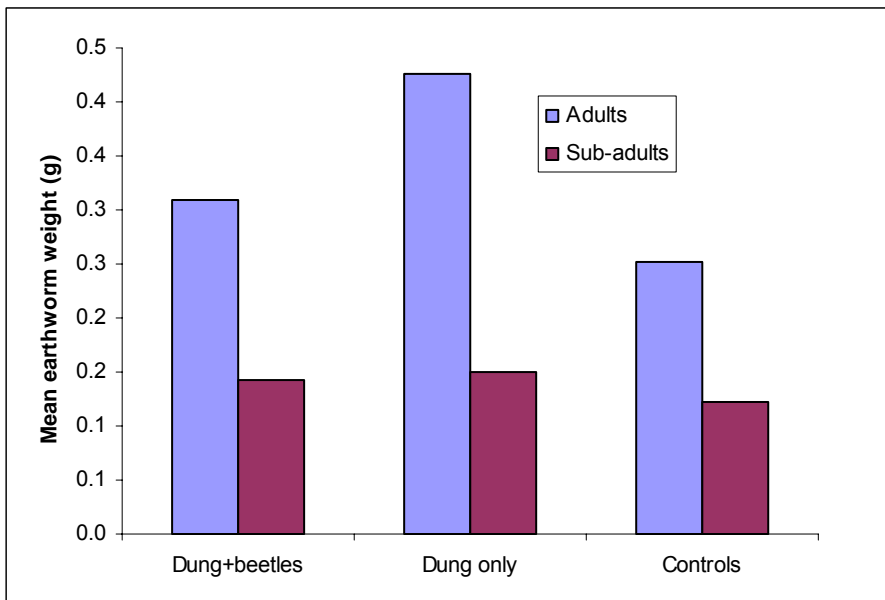


Figure 5.2 Mean weight of adult and sub-adult *A. rosea* at Location 2 (Kuitpo)]

A. rosea, Location 1 (Ashbourne)

The earthworm *A. rosea* was dispersed throughout the soil profile. An analysis of the average weight of adult earthworms indicated that there was no consistent change in size with soil depth for adults or sub-adults in any of the three treatments (Table 5.7). However, there was a strongly significant effect ($P < 0.001$) of treatment on the size of the adult earthworms but not on the size of the sub-adults ($P > 0.05$). The adult earthworms in the dung+beetles treatment plots were substantially heavier (+21%) than the adults in the dung-only treatment plots ($P < 0.01$).

Table 5.7 The effect of soil depth, dung and dung beetle activity of the mean weight (\pm SD) of adult and sub-adult *A. rosea* at Location 1 (Ashbourne)

	Dung+beetles		Dung-only		Controls [no dung, no beetles]	
	Adults	Sub-adults	Adults	Sub-adults	Adults	Sub-adults
Surface	0.33 \pm 0.00	0.13 \pm 0.00	0.19 \pm 0.08	0.12 \pm 0.06	–	0.10 \pm 0.00
Mid stratum	0.32 \pm 0.13	0.16 \pm 0.07	0.29 \pm 0.14	0.20 \pm 0.00	–	0.13 \pm 0.06
Base	0.36 \pm 0.11	0.23 \pm 0.13	0.30 \pm 0.12	0.10 \pm 0.00	0.30 \pm (na)	–
Overall mean	0.34\pm0.12	0.16\pm0.09	0.28\pm0.15	0.13\pm0.06	0.30\pm (na)	0.12\pm0.04

na not applicable

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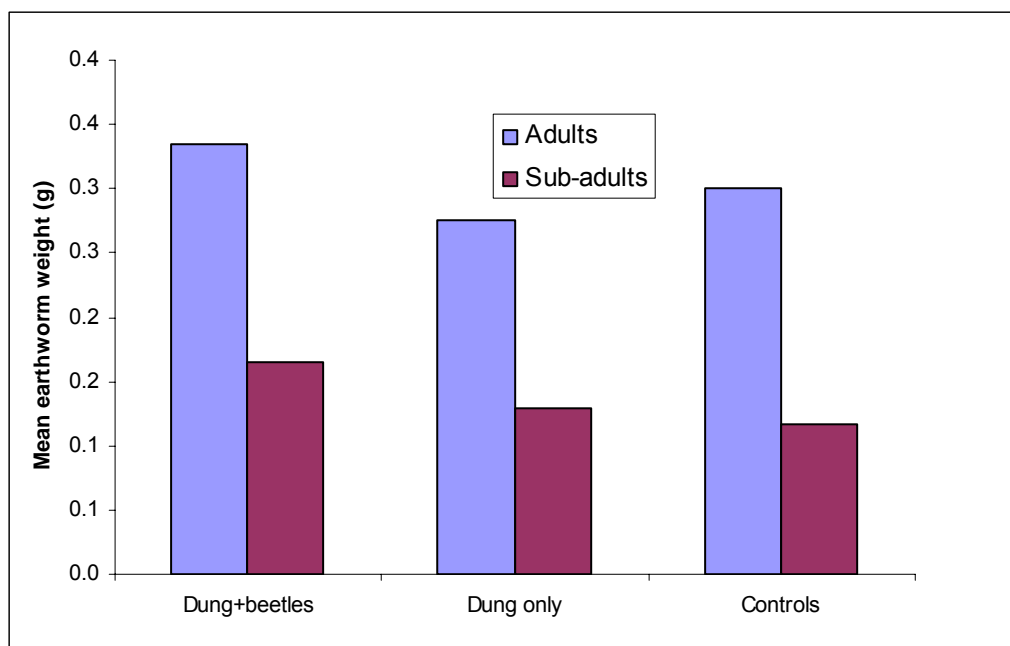


Figure 5.3 Mean weight of adult and non-adult *A. rosea* at Location 1 (Ashbourne)]

Species abundance in relation to soil depth and treatment

The distribution of earthworms through the soil profile during winter indicates the distribution of suitable food (organic matter). However, in late spring/early summer, as the soil dries out, earthworms undergo an annual migration in which they move deep into the soil, where they spend the summer curled up inside a small chamber at 30–50 cm below the soil surface. The data have been collated and sorted but have not yet been analysed statistically.

Location 1 (Ashbourne)

A number of apparent trends emerge from the data (Table 5.8). These are:

- *A. trapezoides* was confined largely to the surface stratum at Location 1 (Ashbourne).
- *A. caliginosa* was found throughout the soil profile.
- *A. rosea* was found throughout the soil profile, with 41% in the surface soil, 40% in the mid stratum and 18% in the base stratum. *A. rosea* found in the surface soil in the dung+beetles, dung-only and control cores contained 25%, 58% and 25% of the total earthworms in each treatment respectively. This distribution suggests either that *A. rosea* was naturally more widely distributed than were the other species, or that *A. rosea* had begun its annual migration to deeper soil, or a mixture of both. Juvenile earthworms were found primarily in the surface and sub-surface soil strata (0–20 cm).
- Platyhelminths were found largely in the lower two strata.

Table 5.8 Earthworm distribution in relation to developmental stage and soil depth at Location 1 (Ashbourne) for the November 2005 sampling occasion

	Numerical abundance				Biomass (g)			
	Adult	Sub-adult	Juvenile	Total	Adult	Sub-adult	Juvenile	Total
<i>A. trapezoides</i>								
Surface	3	8	1	12	3.0	3.6	0.3	6.9
Mid	1	0	2	3	0.5	–	1.1	1.6
Base	2	0	0	2	1.0	–	–	1.0
Total	6	8	3	17	4.5	3.6	1.4	9.5
<i>A. caliginosa</i>								

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Surface	9	6	1	16	4.8	1.7	0.1	6.6
Mid	11	4	1	16	6.9	0.4	0.6	7.9
Base	3	2	0	5	1.7	0.3	–	2.0
Total	23	12	2	37	13.4	2.4	0.7	16.5
<i>A. rosea</i>								
Surface	30	38	31	99	7.0	4.8	2.3	14.1
Mid	51	28	18	97	16.2	2.8	1.4	20.4
Base	33	10	1	44	11.7	3.3	0.3	15.3
Total	114	76	50	240	34.9	10.8	4.0	49.7
<i>M. dubius</i>								
Surface	3	0	0	3	1.2	–	–	1.2
Mid	4	0	0	4	1.7	–	–	1.7
Base	2	0	0	2	0.7	–	–	0.7
Total	9	0	0	9	3.6	–	–	3.6
Platyhelminths								
Surface	6	0	0	6	3.0	–	–	3.0
Mid	9	0	0	9	2.9	–	–	2.9
Base	17	0	0	17	9.7	–	–	9.7
Total	32	0	0	32	15.6	–	–	15.6

Location 2 (Kuitpo)

A number of apparent trends emerge from the data (Table 5.9). These are:

- *A. trapezoides* was found primarily in the surface and sub-surface soil strata (0–20 cm) with 87% in the surface soil, 11% in the mid stratum and 1% in the base stratum. This distribution was not due to the earthworms moving down into the subsoil as part of the annual migration to the base stratum (which occurs each summer) because in the control (no dung) cores all the earthworms were found in the surface soil, indicating that the annual migration had not yet begun. There was no effect of dung beetle activity on the vertical distribution of earthworms, with 6% and 27% of the earthworms being in the lower two strata in dung+beetles and dung-only treatments respectively. Juvenile earthworms were found primarily in the surface soils.
- *A. caliginosa* was found exclusively in the surface soils. Only one juvenile *A. caliginosa* was recognised. Juvenile *A. caliginosa* and *A. trapezoides* have a very similar appearance. It is likely that *A. caliginosa* juveniles were present but were classified as *A. trapezoides*.

Table 5.9 Earthworm distribution in relation to developmental stage and soil depth at Location 2 (Kuitpo) for the November 2005 sampling occasion

	Numerical abundance				Biomass (g)			
	Adult	Sub-adult	Juvenile	Total	Adult	Sub-adult	Juvenile	Total
<i>A. trapezoides</i>								
Surface	97	57	33	187	68.0	18.0	3.7	89.7
Mid	21	3	0	24	11.9	0.8	0.0	12.7
Base	2	0	1	3	2.0	–	0.1	2.1
Total	120	60	34	214	81.9	18.8	3.8	104.5
<i>A. caliginosa</i>								
Surface	15	7	1	23	5.4	1.0	0.2	6.6
Mid	0	0	0	0	–	–	–	–
Base	0	0	0	0	–	–	–	–
Total	15	7	1	23	5.4	1.0	0.2	6.6
<i>A. rosea</i>								
Surface	33	28	30	91	9.6	4.3	2.8	16.7
Mid	69	10	8	87	22.8	1.1	0.5	24.4

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Base	23	4	4	31	7.4	0.5	0.3	8.2
Total	125	42	42	209	39.8	5.9	3.6	49.3
Platyhelminths								
Surface	3	0	0	3	0.8	–	–	0.8
Mid	9	0	0	9	4.8	–	–	4.8
Base	26	0	0	26	13.2	–	–	13.2
Total	38	0	0	38	18.8	–	–	18.8

- *A. rosea* was found throughout the soil profile, with 44% in the surface soil, 42% in the mid stratum and 15% in the base stratum. This distribution may have been due to the earthworms moving down into the subsoil as part of the annual migration to the base stratum (which occurs each summer) because in the control (no dung) cores 51% the earthworms were found in the lower two strata, or it may be that *A. rosea* was naturally more widely distributed than were the other species. Juvenile earthworms were found in each stratum but primarily in the surface soil.
- The distribution of *A. rosea* through the soil profile was strongly influenced by dung beetle activity (in the dung+beetles treatment 23% were located in the base stratum compared with 2–3% of the other treatments).
- *M. dubius* was not recovered.
- Platyhelminths were found largely in the lower two strata.

Results for April 2006

Species abundance and biomass

Location 1 (Ashbourne)

At Ashbourne (Location 1) a total of 106 earthworms and 4 platyhelminths were recovered from 12 sample cores. The dominant species was *A. rosea*, which made up 69% of the numbers and 41% of the biomass. *A. trapezoides* and *A. caliginosa* each made up 11 and 16% of the numbers and 23 and 34% of the biomass respectively. *M. dubius* was not found (Table 5.10).

Earthworms within species were relatively patchily distributed across the test plots, but numbers were so low that statistically significant trends were not evident: these may appear when the data for a number of sampling occasions are pooled and analysed.

Table 5.10 Total numbers and total biomass of earthworms and platyhelminths recovered from soil cores (n=12) taken from the soil at Ashbourne in April 2006. There were four replicate plots adjacent to each other at the study site.

Replicate plot number	Numbers of earthworms & platyhelminths				Total	% total	% total e/worms
	1	2	3	4			
<i>A. trapezoides</i>	0	1	7	4	12	10.9	11.3
<i>A. caliginosa</i>	7	4	4	3	18	16.4	17.0
<i>A. rosea</i>	28	16	17	15	76	69.1	71.7
<i>M. dubius</i>	0	0	0	0	0	0.0	0.0
Platyhelminths	4	0	0	0	4	3.6	
Total	39	21	28	22	110	100	
Total earthworms	35	21	28	22	106		100.0
Replicate plot number	Biomass of earthworms & platyhelminths (g live weight)				Total	% total	% total
	1	2	3	4			

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							e/worms
<i>A. trapezoides</i>	0.0	0.4	2.2	1.5	4.1	22.6	23.2
<i>A caliginosa</i>	2.6	1.3	1.2	1.1	6.2	34.2	35.1
<i>A rosea</i>	2.8	1.5	1.7	1.5	7.4	40.7	41.7
<i>M. dubius</i>	–	–	–	–	–	–	–
Platyhelminths	0.5	0.0	0.0	0.0	0.5	2.5	2.5
Total	5.8	3.2	5.1	4.1	18.1	100.0	
Total e/worm biomass	5.4	3.2	5.1	4.1	17.7		100.0

Location 2 (Kuitpo)

At Kuitpo (Location 2) a total of 75 earthworms and 2 platyhelminths were recovered from 12 sample cores. The dominant earthworm species was *A. rosea*, which made up 74% of the numbers and 44% of the biomass. *A. trapezoides* made up 22% of the numbers and 54% of the biomass. *A. caliginosa* was scarce and *M. dubius* was not found (Table 5.11).

Earthworms within species were patchily distributed across the test plots, with the majority of all species found in plots 1 and 2. This supports the trend found in the November 2005 sampling.

Soil moisture was assessed at each of the three soil depths for each of the cores sampled. There were no obvious trends across the 100 m transect in the moisture levels of the surface, mid-level or subsoils at this time of this year (Table 5.12) but soil moistures at other times of the year differ across the plots and so may influence earthworm reproduction.

Table 5.11 Total numbers and total biomass of earthworms and platyhelminths recovered from soil cores (n=12) taken from the soil at Kuitpo in April 2006. There were four replicate plots adjacent to each other at the study site

Replicate plot number	Numbers of earthworms & platyhelminths					% total	% e/ worms
	1	2	3	4	Total		
<i>A. trapezoides</i>	5	6	2	4	17	22.1	22.7
<i>A caliginosa</i>	1	0	0	0	1	1.3	1.3
<i>A rosea</i>	20	33	4	0	57	74.0	76.0
<i>M. dubius</i>	0	0	0	0	0	0.0	0.0
Platyhelminths	0	0	2	0	2	2.6	
Total	26	39	8	4	77	100.0	
Total earthworms	26	39	6	4	75		100.0

Replicate plot number	Biomass of earthworms & platyhelminths (g live wt)					% total	% e/ worms
	1	2	3	4	Total		
<i>A. trapezoides</i>	2.2	6.4	0.7	1.0	10.3	53.5	54.1
<i>A caliginosa</i>	0.2	0.0	0.0	0.0	0.2	1.0	1.1
<i>A rosea</i>	3.5	4.2	0.8	0.0	8.5	44.4	44.9
<i>M. dubius</i>	–	–	–	–	–	–	–
Platyhelminths	0.0	0.0	0.2	0.0	0.2	1.0	
Total	5.9	10.6	1.7	1.0	19.2	100.0	
Total e/worm biomass	5.9	10.6	1.5	1.0	19.0		100.0

Soil moisture: effects of plot location, soil depth and treatment

At the time of sampling (April 2006) soil moisture was in the range 8–20% at Location 1 and 7–24% at Location 2 (Table 3). The moisture profile down through the soil varied between locations.

At Ashbourne, the surface soil was moister than the two subsoil strata, which did not differ from each other. At Kuitpo, the base stratum had a higher water content than the other two strata, The

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mid stratum had a lower moisture content than the upper and lower strata, which was taken to reflect the presence of substantial quantities of fine gravel in the mid stratum.

Table 5.12 Effect of site location, plot location and soil depth on soil moisture

Soil moisture (% water relative to the dry weight of soil)					
Ashbourne: L1	Plot 1	Plot 2	Plot 3	Plot 4	Mean
Surface	16.6	18.7	18.7	20.0	18.5
Mid	8.8	7.5	9.3	10.7	9.0
Base	8.8	7.6	8.1	12.2	9.2
Mean	11.4	11.2	12.0	14.3	12.2
Kuitpo: L2	Plot 1	Plot 2	Plot 3	Plot 4	Mean
Surface	13.3	17.4	14.7	12.1	14.4
Mid	10.3	7.8	13.3	6.8	9.6
Base	16.5	17.4	24.3	16.5	18.7
Mean	13.3	14.2	17.4	11.8	14.2

The effect of dung and dung beetles on earthworm abundance and biomass

The pattern of results was similar at both locations in that *A. trapezoides*, *A. caliginosa* and *A. rosea* were more abundant in the presence of dung than in the controls. In addition, the abundance and biomass of *A. rosea* appeared to be further increased by the activities of the dung beetles.

These results indicate that the strong response of the earthworm populations to dung and dung beetle activity observed after 7 weeks of the experiment (see above) persisted for at least 9 months. It is likely that the impact of dung and dung beetles will be more pronounced after winter (2006), when the increased reproductive potential of the earthworms becomes reflected in the next generation of earthworms.

Despite the relatively low levels of abundance observed, the following trends in numerical abundance emerge from the data (Tables 2.13 and 2.14):

- *A. trapezoides* seems to be more abundant in the presence of dung, and at Location 2 there was an additional effect of dung beetles.
- *A. caliginosa* appears to be more abundant in the presence of dung at Location 1; at Location 2 it was barely present.
- *A. rosea* appears to be substantially more abundant in the presence of dung and its abundance was further increased by dung beetle activity.
- Platyhelminth numbers were too low to assess the impact of dung or dung beetles.

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Table 5.13 The effect of dung and dung beetle activity on the total abundance and biomass of earthworms and platyhelminths at the Ashbourne study location, April 2006

Numbers	Dung+beetles	Dung only	Controls	Total	% total	% total e/worms
<i>A. trapezoides</i>	4	7	1	12	10.9	11.3
<i>A caliginosa</i>	6	12	0	18	16.4	17.0
<i>A rosea</i>	52	15	9	76	69.1	71.7
Platyhelminths	3	0	1	4	3.6	
Total	65	34	11	110	100.0	100.0
Total earthworms	62	34	10	106		

Biomass	Dung+beetles	Dung only	Controls	Total	% total biomass	% total e/worm biomass
<i>A. trapezoides</i>	1.6	1.6	0.9	4.1	22.6	23.2
<i>A caliginosa</i>	1.8	4.4	0.0	6.2	34.2	35.1
<i>A rosea</i>	4.7	2.1	0.6	7.4	40.7	41.7
Platyhelminths	0.4	0.0	0.1	0.5	2.5	
Total	8.5	8.1	1.6	18.1	100.0	
Total e/worm biomass	8.1	8.1	1.5	17.7		100.0

Table 5.14 The effect of dung and dung beetle activity on the total numbers and biomass of earthworms and platyhelminths at the Kuitpo study location, April 2006

Numbers	Dung+beetles	Dung only	Controls	Total	% total	% total e/worms
<i>A. trapezoides</i>	9	5	3	17	22.1	22.7
<i>A caliginosa</i>	1	0	0	1	1.3	1.3
<i>A rosea</i>	30	19	8	57	74.0	76.0
Platyhelminths	0	0	2	2	2.6	
Total	40	24	13	77	100.0	
Total earthworms	40	24	11	75		100.0

Biomass	Beetles	Dung	Controls	Total	% total biomass	% total e/worm biomass
<i>A. trapezoides</i>	7.4	1.9	1.0	10.3	53.5	54.1
<i>A caliginosa</i>	0.2	0.0	0.0	0.2	1.0	1.1
<i>A rosea</i>	4.1	3.1	1.3	8.5	44.4	44.9
Platyhelminths	0.0	0.0	0.2	0.2	1.0	
Total	11.7	5.0	2.5	19.2	100.0	
Total e/worm biomass	11.7	5.0	2.3	19.0		100.0

Average size of adults

The numbers of adults at Ashbourne and Kuitpo were too low to assess the impact of treatment on the mean weight of earthworms.

Species abundance in relation to soil depth and treatment

The distribution of earthworms through the soil profile during winter indicates the distribution of suitable food (organic matter). In late spring/early summer, as the soil dries out, earthworms undergo an annual migration in which they move deep into the soil, where they spend the summer curled up inside a small chamber at 30–50 cm below the soil surface. In autumn, when this sample was taken, earthworms are in the process of leaving their protective cocoon deep in the soil and migrating to the surface to feed and breed during winter. This vertical migration is triggered by high soil moisture. In this sample, numbers of earthworms, especially *A. rosea* at Ashbourne, were still in their cocoons in the base section of the soil profile.

The data have been collated and sorted but have not been analysed statistically.

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Location 1 (Ashbourne)

A number of apparent trends emerge from the data (Tables 2.15 and 2.16). These are:

- All species were more abundant in the base stratum than in the upper strata.
- *A. rosea* was found throughout the soil profile, with 20% in the surface soil, 18% in the mid stratum and 62% in the base stratum. The base soil in the dung+beetles, dung-only and control cores contained 58%, 60% and 89% of the total *A. rosea* in each treatment respectively.
- There were too few *A. trapezoides*, *A. caliginosa* and platyhelminths present to allow meaningful analysis.

Table 5.15 Earthworm distribution in relation to developmental stage and soil depth at Location 1 (Ashbourne) for the April 2006 sampling occasion

	Numerical abundance				Biomass (g)			
	Adult	Sub-adult	Juvenile	Total	Adult	Sub-adult	Juvenile	Total
<i>A. trapezoides</i>								
Surface	0	2	0	2	0.0	0.8	0.0	0.8
Mid	1	3	0	4	0.9	0.8	0.0	1.7
Base	0	6	0	6	0.0	1.6	0.0	1.6
Total	1	11	0	12	0.9	3.2	0.0	4.1
<i>A. caliginosa</i>								
Surface	1	0	0	1	0.8	–	–	0.8
Mid	2	3	0	5	1.0	0.8	0.0	1.8
Base	7	2	3	12	2.6	0.7	0.3	3.6
Total	10	5	3	18	4.4	1.5	0.0	6.2
<i>A. rosea</i>								
Surface	0	6	9	15	0.0	0.9	0.2	1.1
Mid	0	13	1	14	0.0	1.3	0.1	1.4
Base	6	30	11	47	1.5	3.0	0.4	4.9
Total	6	49	21	76	1.5	5.2	0.7	7.4
Platyhelminths								
Surface	1	0	0	1	0.1	–	–	0.1
Mid	1	0	0	1	0.1	–	–	0.1
Base	2	0	0	2	0.3	–	–	0.3
Total	4	0	0	4	0.5	–	–	0.5

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Table 5.16 The effect of dung beetle activity on the numbers and biomass and their distribution in relation to soil depth at Ashbourne (sampled April 2006)

	Numerical abundance				Biomass (g)			
	Dung +beetles	Dung only	Control	Total	Dung +beetles	Dung only	Control	Total
<i>A. trapezoides</i>								
Surface	1	1	0	2	0.7	0.1	–	0.8
Mid	1	2	1	4	0.2	0.6	0.9	1.7
Base	2	4	0	6	0.7	0.9	–	1.6
Total	4	7	1	12	1.6	1.6	0.9	4.1
<i>A. caliginosa</i>								
Surface	0	1	0	1	–	0.8	–	0.8
Mid	2	3	0	5	0.5	1.3	–	1.8
Base	4	8	0	12	1.3	2.3	–	3.6
Total	6	12	0	18	1.8	4.4	–	6.2
<i>A. rosea</i>								
Surface	11	3	1	15	0.8	0.2	0.1	1.1
Mid	11	3	0	14	1.1	0.3	–	1.4
Base	30	9	8	47	2.9	1.6	0.5	4.9
Total	52	15	9	76	4.7	2.1	0.6	7.4
Platyhelminths								
Surface	0	0	1	1	–	–	0.1	0.1
Mid	1	0	0	1	0.1	–	–	0.1
Base	2	0	0	2	0.3	–	–	0.3
Total	3	0	1	4	0.4	–	0.1	0.5

Location 2

A number of apparent trends emerge from the data (Table 5.17). These are:

- *A. trapezoides* was found exclusively in the surface and sub-surface soil strata (0–20 cm).
- Only one *A. caliginosa* was recovered (in the base stratum of the dung+beetles treatment), and *M. dubius* was not recovered.
- *A. rosea* was found primarily in the surface stratum (75%), with some recovered from the mid (16%) and base (9%) strata. Juvenile earthworms were found only in the surface stratum.
- For *A. rosea*, dung increased its abundance and beetle activity further increased its abundance: this was primarily due to differences in the number of adults (Table 5.18).

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Table 5.17 Earthworm distribution in relation to developmental stage and soil depth at Location 2 (Kuitpo) for the November 2005 sampling occasion [taken from

	Numerical abundance				Biomass (g)			
	Adult	Sub-adult	Juvenile	Total	Adult	Sub-adult	Juvenile	Total
<i>A. trapezoides</i>								
Surface	4	9	1	14	2.5	3.1	0.1	5.7
Mid	0	3	0	3	–	4.6	–	4.6
Base	0	0	0	0	–	–	–	–
Total	4	12	1	17	2.5	7.7	0.1	10.3
<i>A. caliginosa</i>								
Surface	0	0	0	0	–	–	–	–
Mid	0	0	0	0	–	–	–	–
Base	1	0	0	1	0.2	–	–	0.2
Total	1	0	0	1	0.2	–	–	0.2
<i>A. rosea</i>								
Surface	3	27	13	43	1.1	4.3	0.4	5.8
Mid	0	9	0	9	–	1.7	–	1.7
Base	1	4	0	5	0.4	0.6	–	1.0
Total	4	40	13	57	1.5	6.6	0.4	8.5
Platyhelminths								
Surface	2	0	0	2	0.2	–	–	0.2
Mid	0	0	0	0	–	–	–	–
Base	0	0	0	0	–	–	–	–
Total	2	0	0	2	0.2	–	–	0.2

Table 5.18 Numbers and biomass by treatment and soil depth, Kuitpo, April 2006

	Numerical abundance				Biomass (g)			
	Dung +beetles	Dung only	Control	Total	Dung +beetles	Dung only	Control	Total
<i>A. trapezoides</i>								
Surface	6	5	3	14	2.8	1.9	1.0	5.7
Mid	3	0	0	3	4.6	–	–	4.6
Base	0	0	0	0	0.0	–	–	–
Total	9	5	3	17	7.4	1.9	1.0	10.3
<i>A. caliginosa</i>								
Surface	0	0	0	0	–	–	–	–
Mid	0	0	0	0	–	–	–	–
Base	1	0	0	1	0.2	–	–	0.2
Total	1	0	0	1	0.2	–	–	0.2
<i>A. rosea</i>								
Surface	23	15	5	43	2.6	2.3	0.9	5.8
Mid	4	4	1	9	0.7	0.8	0.2	1.7
Base	3	0	2	5	0.8	–	0.2	1.0
Total	30	19	8	57	4.1	3.1	1.3	8.5
Platyhelminths								
Surface	0	0	2	2	–	–	0.2	0.2
Mid	0	0	0	0	–	–	–	–
Base	0	0	0	0	–	–	–	–
Total	0	0	2	2	–	–	0.2	0.2

Results for August 2006

Species abundance and biomass

Location 1 (Ashbourne)

At Ashbourne (Location 1) a total of 414 earthworms and one platyhelminth were recovered from the 12 sample cores sampled on 28 August 2006. The dominant species was *A. rosea*, which made up 83% of the numbers and 59% of the biomass. *A. trapezoides* and *A. caliginosa* made up 7% each of the numbers and 12 and 26% of the biomass respectively. *M. dubius* made up 2% of the numbers (Table 5.19).

Earthworms within species were relatively patchily distributed across the test plots, with *A. rosea* being relatively scarce in plot 4 (10% of the total of *A. rosea*) and *A. trapezoides* being relatively scarce in plots 3 and 4 (each 10% of the total).

Table 5.19 Total numbers and total biomass of earthworms and platyhelminths recovered from soil cores (n=12) taken from the soil at Ashbourne in August 2006. There were four replicate plots adjacent to each other at the study site.

Numbers of earthworms & platyhelminths							
Replicate plot number	1	2	3	4	Total	% total	% total e/worms
<i>A. trapezoides</i>	7	16	3	3	29	7.0	7.0
<i>A. caliginosa</i>	12	6	2	10	30	7.2	7.2
<i>A. rosea</i>	93	50	164	39	346	83.4	83.6
<i>M. dubius</i>	1	6	2	0	9	2.2	2.2
Platyhelminths	0	0	1	0	1	0.2	
Total	113	78	172	52	415	100	
Total earthworms	113	78	171	52	414		100.0

Biomass of earthworms & platyhelminths (g live weight)							
Replicate plot number	1	2	3	4	Total	% total	% total e/worms
<i>A. trapezoides</i>	2.5	4.6	0.5	1.3	8.9	12.3	12.3
<i>A. caliginosa</i>	8.9	3.9	1.0	4.7	18.5	25.5	25.6
<i>A. rosea</i>	14.2	9.7	14.8	4.0	42.6	58.7	59.0
<i>M. dubius</i>	0.3	1.3	0.6	0.0	2.2	3.0	3.0
Platyhelminths	0.0	0.0	0.4	0.0	0.4	0.6	
Total	25.9	19.5	17.3	10.0	72.6	100.0	
Total e/worm biomass	25.9	19.5	16.9	10.0	72.2		100.0

Location 2 (Kuitpo)

At Kuitpo a total of 271 earthworms and one platyhelminth were recovered from the 12 sample cores. The dominant earthworm species was *A. rosea*, which made up 81% of the numbers and 61% of the biomass. *A. trapezoides* made up 13% of the numbers and 23% of the biomass. *A. caliginosa* and *M. dubius* were relatively scarce (Table 5.20).

The earthworm *A. trapezoides* was relatively evenly distributed across the test plots (Table 5.20). In contrast, *A. rosea* were virtually absent from plots 3 and 4 (plots on the uphill side of the slope). The four plots were placed in a row about 20 m from the fence line. Each plot was about 20 m long and separated from its adjacent neighbour by 5 m, and so the plots occurred over a 100 m stretch of paddock which sloped uphill but appeared homogeneous.

Soil moisture was assessed at each of the three soil depths for each of the cores sampled. There were no obvious trends across the 100 m transect in the moisture levels of the surface, mid-level or base soils (Table 5.21).

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Table 5.20 Total numbers and total biomass of earthworms and platyhelminths recovered from soil cores (n=12) taken from the soil at Kuitpo in August 2006. There were four replicate plots adjacent to each other at the study site. New R1 D entered

Numbers of earthworms & platyhelminths							
Replicate plot number	1	2	3	4	Total	% total	% e/ worms
<i>A. trapezoides</i>	15	4	5	10	34	12.5	12.5
<i>A caliginosa</i>	4	0	7	0	11	4.0	4.1
<i>A rosea</i>	32	167	8	14	221	81.3	81.5
<i>M. dubius</i>	2	1	2	0	5	1.8	1.8
Platyhelminths	0	0	1	0	1	0.4	
Total	53	172	23	24	272	100.0	
Total earthworms	53	172	22	24	271		100.0
Biomass of earthworms & platyhelminths (g live wt)							
Replicate plot number	1	2	3	4	Total	% total	% e/ worms
<i>A. trapezoides</i>	4.6	0.4	1.6	5.3	11.9	22.8	23.0
<i>A caliginosa</i>	2.5	0.0	3.8	0.0	6.3	12.1	12.2
<i>A rosea</i>	3.9	23.0	1.6	3.3	31.8	61.4	61.7
<i>M. dubius</i>	0.4	0.1	1.1	0.0	1.6	3.1	3.1
Platyhelminths	0.0	0.0	0.3	0.0	0.3	0.6	
Total	11.4	23.5	8.4	8.6	51.9	100.0	
Total e/worm biomass	11.4	23.5	8.1	8.6	51.6		100.0

Soil moisture: effects of plot location, soil depth and treatment

At the time of sampling (late August 2006) soil moisture was in the range 13–24% at Location 1 and 11–25% at Location 2 (Table 5.21). The moisture profile down through the soil varied between locations.

At Ashbourne, the surface soil was moister than the two subsoil strata, which did not differ from each other. This is presumed to reflect recent rainfall and the even soil texture down the soil profile to 50 cm.

At Kuitpo, the surface and the base strata had similar water contents, presumably reflecting the recent rainfall (on the sandy loam surface soil) and the high water-holding capacity of the clay subsoil. The mid stratum had a lower moisture content, which was taken to reflect the presence of substantial quantities of fine gravel in the mid stratum. There was no clear trend in base-level moisture levels across the plots.

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Table 5.21 Effect of site location, plot location and soil depth on soil moisture in late August 2006

Soil moisture (% water relative to the dry weight of soil)					
Ashbourne: L1	Plot 1	Plot 2	Plot 3	Plot 4	Mean
Surface	20.7	23.8	24.1	16.1	21.2
Mid	15.7	17.8	14.3	15.3	15.8
Base	13.0	13.6	14.4	17.9	14.7
Mean	16.5	18.4	17.6	16.4	17.2
Kuitpo: L2	Plot 1	Plot 2	Plot 3	Plot 4	Mean
Surface	22.5	19.8	25.3	20.0	21.9
Mid	13.5	12.9	13.0	11.3	12.7
Base	17.2	22.2	19.9	13.4	18.2
Mean	17.7	18.3	19.4	14.9	17.6

Effect of dung and dung beetles on earthworm abundance and biomass

The pattern of results for *A. rosea* was similar at both locations in that it was more abundant in the presence of dung than in the controls and its abundance was increased further by the activity of dung beetles (Tables 2.22 and 2.23).

For *A. caliginosa* it appears that at both locations there was no detectable effect of dung alone (although numbers were low) but its abundance was increased by the activity of dung beetles.

For *A. trapezoides* there appeared to be no effect of dung or beetles at Kuitpo, but at Ashbourne its abundance was increased by the activity of dung beetles (although numbers were low).

Table 5.22 The effect of dung and dung beetle activity on the total abundance and biomass of earthworms and platyhelminths at the Ashbourne study location, August 2006

Numbers	Dung+beetles	Dung only	Controls	Total	% total	% total e/worms
<i>A. trapezoides</i>	15	9	5	29	7.0	7.0
<i>A. caliginosa</i>	16	8	6	30	7.2	7.2
<i>A. rosea</i>	181	111	54	346	83.4	83.6
<i>M. dubius</i>	3	6	0	9	2.2	2.2
Platyhelminths	0	0	1	1	0.2	
Total	215	134	66	415	100.0	
Total numbers	215	134	65	414		100.0
Biomass	Dung+beetles	Dung only	Controls	Total	% total biomass	% total biomass e/worm
<i>A. trapezoides</i>	3.8	3.6	1.5	8.9	12.3	12.3
<i>A. caliginosa</i>	10.9	5.2	2.4	18.5	25.5	25.6
<i>A. rosea</i>	22.5	13.3	6.9	42.6	58.7	59.0
<i>M. dubius</i>	0.9	1.3	0.0	2.2	3.0	3.0
Platyhelminths	0.0	0.0	0.4	0.4	0.6	
Total	38.1	23.4	11.2	72.6	100.0	
Total biomass	38.1	23.4	10.8	72.2		100.0

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Table 5.23 The effect of dung and dung beetle activity on the total numbers and biomass of earthworms and platyhelminths at the Kuitpo study location, August 2006

Numbers	Dung+beetles	Dung only	Controls	Total	% total	% total e/worms
<i>A. trapezoides</i>	11	10	13	34	12.5	12.5
<i>A. caliginosa</i>	8	3	0	11	4.0	4.1
<i>A. rosea</i>	141	60	20	221	81.3	81.5
<i>M. dubius</i>	4	1	0	5	1.8	1.8
Platyhelminths	1	0	0	1	0.4	
Total	165	74	33	272	100.0	
Total earthworms	164	74	33	271		100.0

Biomass	Beetles	Dung	Controls	Total	% total biomass	% total e/worm biomass
<i>A. trapezoides</i>	4.0	4.5	3.4	11.9	22.8	23.0
<i>A. caliginosa</i>	4.7	1.6	0.0	6.3	12.1	12.2
<i>A. rosea</i>	19.8	9.6	2.5	31.8	61.4	61.7
<i>M. dubius</i>	1.5	0.1	0.0	1.6	3.1	3.1
Platyhelminths	0.3	0.0	0.0	0.3	0.6	
Total	30.3	15.8	5.8	51.9	100.0	
Total e/worm biomass	30.0	15.8	5.8	51.6		100.0

Average size of adults

The average size of adults is important for understanding the population dynamics of earthworms because the potential fecundity of individuals increases with size. Earthworms feed on organic matter in soil and so it is likely that an increased food supply (dung) will result in earthworms growing to a larger size. The live weight of all adult and sub-adult earthworms was recorded and so it was possible to investigate the effect of dung and dung beetles on the size of earthworms (as indicated by their live weight).

Statistical analysis of the effects of soil depth and treatment on the size of earthworms requires a moderate sample size (> about 50) in order for there to be sufficient individuals in each category to allow a useful comparison. This criterion was met by only two sets of data (*A. rosea* at both locations). The results for these are presented below.

A. rosea, Location 1 (Ashbourne)

The presence of dung resulted in adult earthworms being about 20% larger than those in control soil. There was no additional effect of dung beetle activity on earthworm size (Table 5.24, Figure 5.4).

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Table 5.24 The effect of soil depth, dung and dung beetle activity of the mean weight (\pm SD) of adult and sub-adult *A. rosea* at Location 1 (Ashbourne) in August 2006

	Dung+beetles		Dung-only		Controls [no dung, no beetles]	
	Adults	Sub-adults	Adults	Sub-adults	Adults	Sub-adults
Surface	0.31 \pm 0.00	0.14 \pm 0.00	0.27 \pm 0.12	0.12 \pm 0.04	0.26 \pm 0.07	0.12 \pm 0.04
Mid stratum	0.32 \pm 0.06	0.16 \pm 0.05	0.32 \pm 0.04	0.11 \pm 0.04	–	0.13 \pm 0.07
Base	0.32 \pm 0.10	0.14 \pm 0.05	0.30 \pm 0.00	0.00 \pm 0.05	0.30 \pm (na)	–
Overall mean	0.32\pm0.08	0.15\pm0.05	0.31\pm0.06	0.12\pm0.05	0.26\pm0.07	0.12\pm0.05

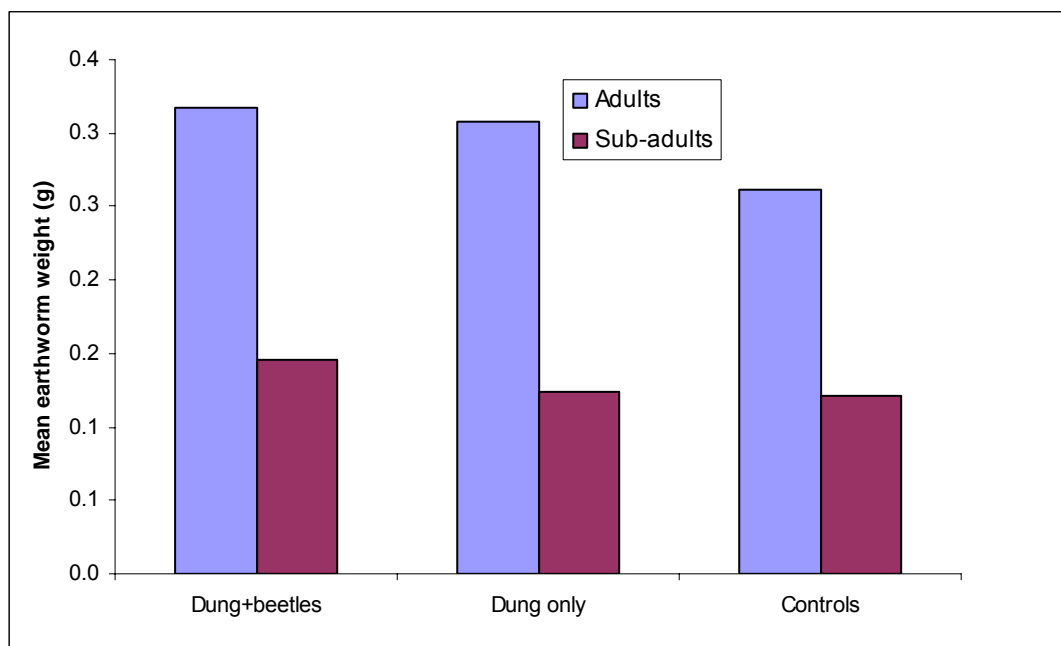


Figure 5.4 Mean weight of adult and sub-adult *A. rosea* at Location 1 (Ashbourne)] in August 2006

A. rosea, Location 2 (Kuitpo)

The earthworm *A. rosea* was dispersed throughout the soil profile. An analysis of the average weight of adult earthworms indicated that there was no consistent change in size with soil depth for adults or sub-adults in any of the three treatments (Table 5.25, Figure 5.5).

Table 5.25 The effect of soil depth, dung and dung beetle activity of the mean weight (\pm SD) of adult and sub-adult *A. rosea* at Location 2 (Kuitpo) in August 2006

	Dung+beetles		Dung-only		Controls [no dung, no beetles]	
	Adults	Sub-adults	Adults	Sub-adults	Adults	Sub-adults
Surface	0.26 \pm 0.07	0.13 \pm 0.05	0.21 \pm 0.11	0.13 \pm 0.05	0.27 \pm 0.08	0.08 \pm 0.03
Mid stratum	0.26 \pm 0.07	0.12 \pm 0.04	0.08 \pm 0.15	0.05 \pm 0.07	–	0.15 \pm 0.07
Base stratum	0.28 \pm 0.09	0.13 \pm 0.05	–	–	0.20 \pm (na)	–
Overall mean	0.27\pm0.08	0.12\pm0.04	0.19\pm0.12	0.12\pm0.06	0.26\pm0.08	0.11\pm0.05

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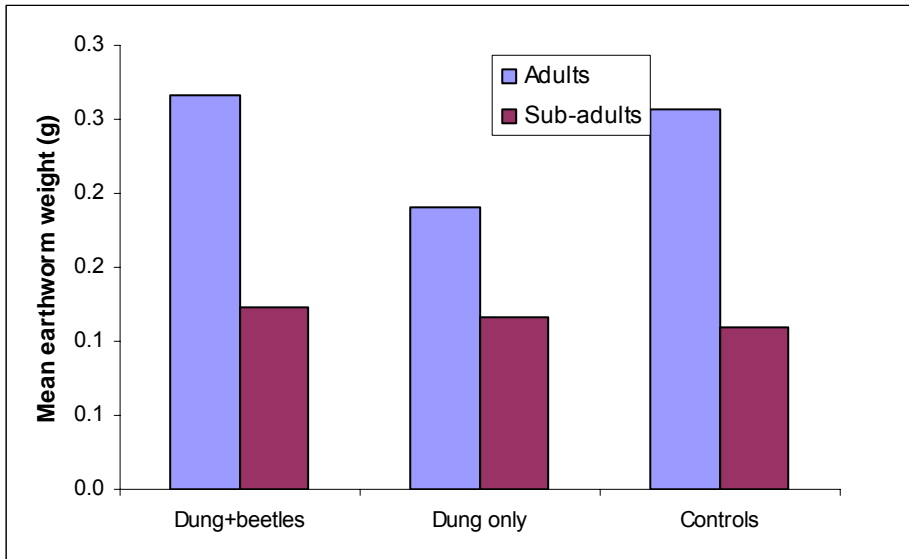


Figure 5.5 Mean weight of adult and sub-adult *A. rosea* at Location 2 (Kuitpo)] in late August 2006

Species abundance in relation to soil depth and treatment

The annual migration in which earthworms move deep into the soil where they spend the summer had not begun at the time of sampling (August 2006), since earthworms in the base soil were not coiled up in spherical chambers and did not have empty intestines (the characteristics of the over-summering condition).

The data have been collated and sorted but have not been analysed statistically.

Location 1 (Ashbourne)

A number of apparent trends emerge from the data (Tables 5.26 and 5.27). These are:

- *A. trapezoides* was dispersed through the soil profile at Ashbourne, where its biomass was higher in the +dung treatments than in the controls but there was no additional effect of dung beetle activity.
- *A. caliginosa* was found throughout the soil profile: its biomass was higher in the +dung treatments than in the controls and there was an additional effect of dung beetle activity.
- *A. rosea* was also found throughout the soil profile, with 38% in the surface soil, 28% in the mid stratum and 34% in the base stratum: its biomass was higher in the +dung treatments than in the controls and there was an additional effect of dung beetle activity.
- For all three species, the relative abundance in the mid stratum was lower than in the surface or the base stratum. This is presumed to reflect the distribution of organic matter, with the plant root zone being concentrated in the top 10 cm and the majority of the buried dung having been placed in the base section.

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Table 5.26 Earthworm distribution in relation to developmental stage and soil depth at Location 1 (Ashbourne) for the August 2006 sampling occasion

	Numerical abundance				Biomass (g)			
	Adult	Sub-adult	Juvenile	Total	Adult	Sub-adult	Juvenile	Total
<i>A. trapezoides</i>								
Surface	4	13	0	17	2.5	4.3	0.0	6.8
Mid	0	2	0	2	0.0	0.2	0.0	0.2
Base	2	4	4	10	1.1	0.7	0.1	1.9
Total	6	19	4	29	3.6	5.2	0.1	8.9
<i>A. caliginosa</i>								
Surface	10	4	0	14	7.6	0.9	0.0	8.5
Mid	1	0	0	1	0.5	0.0	0.0	0.5
Base	14	1	0	15	9.2	0.3	0.0	9.5
Total	25	5	0	30	17.3	1.2	0.0	18.5
<i>A. rosea</i>								
Surface	15	39	76	130	7.6	6.2	1.5	15.3
Mid	20	35	43	98	6.4	5.6	1.2	13.2
Base	19	45	54	118	6.4	6.2	1.5	14.1
Total	54	119	173	346	20.4	18.0	4.2	42.6
<i>M. dubius</i>								
Surface	7	0	0	7	1.6	0.0	0.0	1.6
Mid	1	0	0	1	0.3	0.0	0.0	0.3
Base	1	0	0	1	0.3	0.0	0.0	0.3
Total	9	0	0	9	2.2	0.0	0.0	2.2
Platyhelminths								
Surface	1	0	0	1	0.4	0.0	0.0	0.4
Mid	0	0	0	0	0.0	0.0	0.0	0.0
Base	0	0	0	0	0.0	0.0	0.0	0.0
Total	1	0	0	1	0.4	0.0	0.0	0.4

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Table 5.27 The effect of dung beetle activity on the numbers and biomass and their distribution in relation to soil depth at Ashbourne (sampled August 2006)

	Numerical abundance				Biomass (g)			
	Dung +beetles	Dung only	Control	Total	Dung +beetles	Dung only	Control	Total
<i>A. trapezoides</i>								
Surface	7	6	4	17	2.7	2.7	1.4	6.8
Mid	0	1	1	2	0	0.1	0.1	0.2
Base	8	2	0	10	1.1	0.8	0	1.9
Total	15	9	5	29	3.8	3.6	1.5	8.9
<i>A. caliginosa</i>								
Surface	6	4	4	14	4.5	2.5	1.5	8.5
Mid	1	0	0	1	0.5	0	0	0.5
Base	9	4	2	15	5.9	2.7	0.9	9.5
Total	16	8	6	30	10.9	5.2	2.4	18.5
<i>A. rosea</i>								
Surface	65	26	39	130	6.6	3.3	5.4	15.3
Mid	33	51	14	98	6.5	5.5	1.2	13.2
Base	83	34	1	118	9.4	4.4	0.3	14.1
Total	181	111	54	346	22.5	13.3	6.9	42.6
<i>M. dubius</i>								
Surface	1	6	0	7	0.3	1.3	0	1.6
Mid	1	0	0	1	0.3	0	0	0.3
Base	1	0	0	1	0.3	0	0	0.3
Total	3	6	0	9	0.9	1.3	0	2.2
Platyhelminths								
Surface	0	0	1	1	0.0	0.0	0.4	0.4
Mid	0	0	0	0	0.0	0.0	0.0	0.0
Base	0	0	0	0	0.0	0.0	0.0	0.0
Total	0	0	1	1	0.0	0.0	0.4	0.4

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Location 2 (Kuitpo)

A number of apparent trends emerge from the data (Tables 2.28 and 2.29). These are:

- *A. trapezoides* was found throughout the soil profile at Kuitpo, with 71% in the surface soil, 12% in the mid stratum and 18% in the base stratum. Its biomass was higher in the +dung treatments than in the controls but there was no additional effect of dung beetle activity. The relative abundances in the mid and base strata were lower than in the top stratum.
- *A. caliginosa* was found throughout the soil profile and its biomass was higher in the +dung treatments than in the controls (no earthworms were recovered) and there was an additional effect of dung beetle activity.
- *A. rosea* was found throughout the soil profile, with 58% in the surface soil, 28% in the mid stratum and 14% in the base stratum: its biomass was higher in the +dung treatments than in the controls and there was an additional effect of dung beetle activity.

Table 5.28 Earthworm distribution in relation to developmental stage and soil depth at Location 2 (Kuitpo) for the August 2006 sampling occasion

	Numerical abundance				Biomass (g)			
	Adult	Sub-adult	Juvenile	Total	Adult	Sub-adult	Juvenile	Total
<i>A. trapezoides</i>								
Surface	6	14	4	24	4.3	3.0	0.2	7.5
Mid	3	1	0	4	1.8	0.2	–	2.0
Base	3	3	0	6	1.8	0.6	–	2.4
Total	12	18	4	34	7.9	3.8	0.2	11.9
<i>A. caliginosa</i>								
Surface	2	1	0	3	1.3	0.3	–	1.6
Mid	3	2	0	5	1.8	1.0	–	2.8
Base	3	0	0	3	1.9	0.0	–	1.9
Total	8	3	0	11	5.0	1.3	–	6.3
<i>A. rosea</i>								
Surface	38	32	59	129	9.7	4.0	1.9	15.6
Mid	28	16	18	62	7.3	1.9	0.6	9.8
Base	19	10	1	30	5.2	1.3	0.0	6.5
Total	85	58	78	221	22.2	7.2	2.5	31.8
<i>M. dubius</i>								
Surface	5	0	0	5	1.6	–	–	1.6
Mid	0	0	0	0	–	–	–	–
Base	0	0	0	0	–	–	–	–
Total	5	0	0	5	1.6	0.0	0.0	1.6
Platyhelminths								
Surface	0	0	0	0	–	–	–	–
Mid	0	0	0	0	–	–	–	–
Base	1	0	0	1	0.3	–	–	0.3
Total	1	0	0	1	0.3	0.0	0.0	0.3

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Table 5.29 Numbers and biomass by treatment and soil depth, Kuitpo, August 2006

	Numerical abundance				Biomass (g)			
	Dung +beetles	Dung only	Control	Total	Dung +beetles	Dung only	Control	Total
<i>A. trapezoides</i>								
Surface	7	7	10	24	1.6	3.4	2.5	7.5
Mid	1	2	1	4	0.8	0.7	0.5	2.0
Base	3	1	2	6	1.6	0.4	0.4	2.4
Total	11	10	13	34	4.0	4.5	3.4	11.9
<i>A. caliginosa</i>								
Surface	0	3	0	3	–	1.6	–	1.6
Mid	5	0	0	5	2.8	–	–	2.8
Base	3	0	0	3	1.8	–	–	1.8
Total	8	3	0	11	4.6	1.6	–	6.2
<i>A. rosea</i>								
Surface	55	58	16	129	4.4	9.2	2.0	15.6
Mid	57	2	3	62	9.0	0.4	0.3	9.8
Base	29	0	1	30	6.3	–	0.2	6.5
Total	141	60	20	221	19.8	9.6	2.5	31.8
<i>M. dubius</i>								
Surface	4	1	0	5	1.5	0.1	–	1.6
Mid	0	0	0	0	–	–	–	–
Base	0	0	0	0	–	–	–	–
Total	4	1	0	5	1.5	0.1	–	1.6
Platyhelminths								
Surface	0	0	0	0	–	–	–	–
Mid	0	0	0	0	–	–	–	–
Base	1	0	0	1	0.3	–	–	0.3
Total	1	0	0	1	0.3	–	–	0.3

Summary

The same general patterns of relative abundance of earthworms between replicates were observed on all three occasions (Table 5.30). At Ashbourne plot numbers 1 and 3 had the greatest populations of earthworms and plot 4 had the least. At Kuitpo, plots 1 and 2 had the greatest numbers of earthworms and plots 3 and 4 had the least. These patterns are taken to reflect the relative suitability of the plots for earthworm breeding and survival.

Table 5.30 Summary of the total number of earthworms recovered from the cores on the three sampling occasions

Replicate plot number	1	2	3	4	Total
Ashbourne					
November 2005	94	87	89	33	303
April 2006	35	21	28	22	106
August 2006	113	78	171	52	414
Kuitpo					
November 2005	168	165	35	78	446
April 2006	26	39	6	4	75
August 2006	53	172	22	24	271

The same general patterns of relative abundance between species were observed on all three occasions at Ashbourne (Table 5.31), where *A. rosea* was the dominant species on all three sampling occasions and *A. caliginosa* was marginally more abundant than *A. trapezoides*. At Kuitpo, *A. rosea* was the dominant species on the second and third sampling occasions, and the proportion of *A. trapezoides* diminished over time.

Table 5.31 Summary of the relative numbers (percent of total on each occasion) of earthworm species recovered from the cores on the three sampling occasions

Sampling date	November 2005	April 2006	August 2006
Ashbourne			
<i>A. trapezoides</i>	5.6	11.3	7.0
<i>A. caliginosa</i>	12.2	17.0	7.2
<i>A. rosea</i>	79.2	71.7	83.6
<i>M. dubius</i>	3.0	0.0	2.2
Kuitpo			
<i>A. trapezoides</i>	49.6	22.7	12.5
<i>A. caliginosa</i>	5.5	1.3	4.1
<i>A. rosea</i>	44.9	76.0	81.5
<i>M. dubius</i>	0.0	0.0	1.8

Data from next year's sampling will reveal whether there is any systematic shift in the relative abundance of species in response to the dung and dung beetle activity.

Appendix 6: The impact of dung burial on water infiltration

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Summary

The effect of dung beetle activity on the permeability of the surface soil to water was assessed 2.5 and 32 months after the dung and beetles were added to the plots. At Ashbourne, on an alluvial loamy soil, dung burial increased the permeability of the surface soil to water and this effect lasted at least 32 months. At Kuitpo, on a duplex soil (sandy loam over a yellow clay), dung burial had increased the permeability of the surface soils to water 2.5 months after the dung and beetles were added to the plots and the effect may have persisted for 32 months.

The effect of dung and dung beetle activity on the softness of the soil surface was assessed for 32 months after the dung and beetles were added to the plots, and dung burial activity (tunnelling and bringing subsoil to the surface) substantially increased the depth of soft surface soil. This persisted for at least 32 months after the dung and beetles were applied to the soil.

Background and methods

The effect of dung and dung beetle activity on the permeability of the surface soil to water was assessed for Experiment 1 in December 2005 (approximately 10 weeks after the dung and beetles had been added to the plots) and in May 2008, 32 months after the dung was inoculated. The effect of dung and dung beetle activity on the softness of the soil surface was assessed for Experiment 1 in May 2008, 32 months after the dung was inoculated.

The permeability to water of the soil beneath the dung pads and in the control plots was examined using PVC cores (15 cm in diameter) punched into the soil and filled with 0.5 litres of water. The time taken for the water to soak into the soil was recorded and taken as an index of the permeability of the soil to water. Cores were inserted into the soil such that they did not leak when water was placed in them. Three PVC cores were inserted into each of the 16 plots at both locations. A total of 48 readings were taken at each location on each occasion.

Soil hardness was assessed by using a simple hand-held penetrometer that was inserted into the soil with moderate consistent pressure. The depth to which the penetrometer entered the soil was assessed. Five readings were taken per plot, giving a total of 20 readings for each treatment at each location.

Because some dung beetles had entered the 'dung-only' field cages and buried some of the dung pads (see Appendix 2, Pasture growth), the comparisons were between the control plots ($n = 8$), plots with 45% burial (4 plots) and plots with 100% burial (4 plots). In the 'dung-only' plots (45% burial by *B. bison*), about half of the 10-week old dung pads had obviously been buried by *B. bison* at the time of testing in 2005, but it was also clear from examination of the soil surface in the plots at the time of testing (and from previous observation) that the spring–summer active dung beetle *O. taurus* had been tunnelling in the pads and the soil beneath them. *O. taurus* was not excluded by the wire mesh used to exclude *B. bison*.

Results

December 2005

Water infiltration

In December 2005 there was no significant difference between the rates of infiltration of water into the soil in the plots with 45% and 100% dung burial ($P > 0.05$). This was so at both locations (Tables 1 and 2, Figure 1). Similarly, there was no significant difference between the rates of infiltration of water into the soil in the two control treatments (with and without wire mesh cages) ($P > 0.05$) (Tables 1 and 2).

However, at both locations, there was a highly significant ($P < 0.001$) reduction in the time taken for the water to soak into the soil in the plots where beetles had buried dung. At both locations the plus-dung treatments took about 1.5 minutes for the 0.5 litres to soak into the soil whereas in the controls it took about 4–5 minutes at Ashbourne and 11–13 minutes at Kuitpo.

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Table 1 The effect of dung and dung beetles on the permeability of soil to water at Ashbourne in December 2005 as indicated by the mean time taken (minutes) for 0.5 litres of water to soak into each plot. Each data point is the average of three readings in each plot.

Replicate	1	2	3	4	Mean
100% burial	1.5	2.0	1.3	0.8	1.4
45% burial	2.6	1.3	1.9	1.1	1.7
Control 1 (cage)	2.7	3.8	2.6	6.3	3.9
Control 2 (no cage)	2.5	11.8	4.4	2.2	5.2
Mean	2.3	4.7	2.6	2.6	3.0

At Kuitpo, one of the dung+beetles plots had a rate of water infiltration that was not different from that in the control plots ($P>0.05$) but was significantly greater (11.8 minutes) than that of the other three dung+beetles plots (1.2–2.6 minutes) ($P<0.001$). Close examination of the bare surface in the plot in replicate 1 showed that the tunnelling activity of the dung beetles had brought a substantial quantity of clay soil to the surface and that rain had spread the clay over the soil surface. In the other plots (plots 2 to 4) the soil surface was fissured with clear cracks over the bare soil surface despite the presence of subsoil clay brought to the surface by dung beetle activity.

We interpret this to indicate that, in plot 1, the clay surface acted as a barrier to the infiltration of water into the soil where dung beetles had been active. For this reason, and because this plot was an outlier among the dung+beetles plots, the data from this plot were omitted from the analysis presented above.

Table 2 The effect of dung and dung beetles on the permeability of soil to water at Kuitpo in December 2005 as indicated by the mean time taken (minutes) for 0.5 litres of water to soak into each of plot. Each data point is the average of three readings in each plot.

Replicate	1	2	3	4	Mean
100% burial	11.8	2.6	1.2	1.4	4.3
45% burial	2.0	1.7	5.5	2.4	2.9
Control 1 (cage)	9.2	5.8	8.8	19.8	10.9
Control 2 (no cage)	21.5	7.5	9.3	12.3	12.6
Mean	11.1	5.6	6.2	9.0	8.0

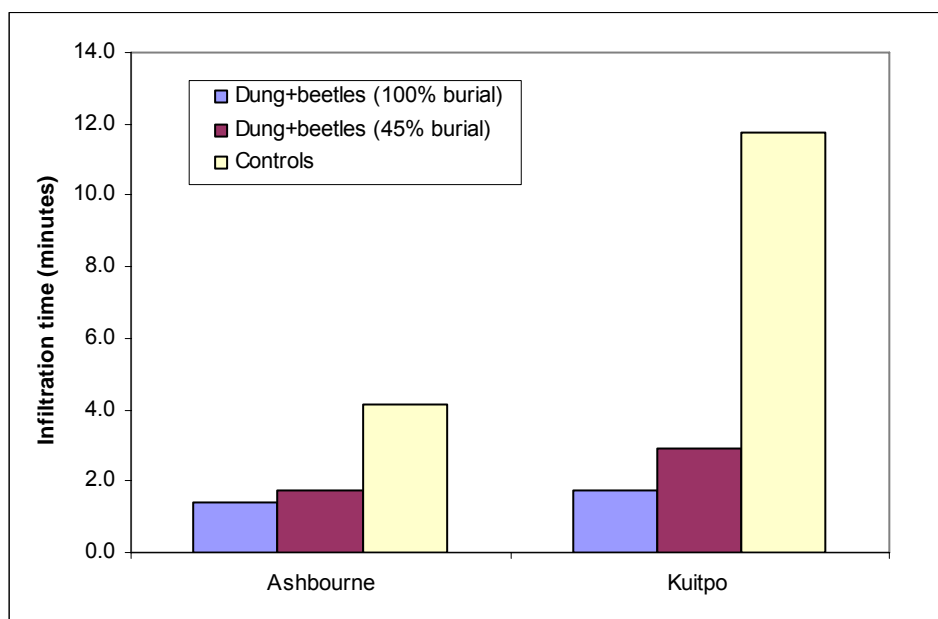


Figure 1 The effect of dung and dung beetles on the permeability of soil to water at the two study locations (Ashbourne and Kuitpo) in December 2005. The dung beetles were introduced to the dung pads 11 weeks before the readings were taken.

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May 2008

Water infiltration

In May 2008 there was no significant difference between the rates of infiltration of water into the soil in the plots with 45% and 100% dung burial ($P > 0.05$). This was so at both locations (Tables 3 and 4). Similarly, there was no significant difference between the rates of infiltration of water into the soil in the two control treatments (with and without wire mesh cages) ($P > 0.05$) (Tables 3 and 4).

Table 3 The effect of dung and dung beetles on the permeability of soil to water at Ashbourne in May 2008 as indicated by the mean time taken (minutes) for 0.5 litres of water to soak into each plot. Each data point is the average of three readings in each plot.

Replicate	1	2	3	4	Mean
100% burial	1.4	4.5	4.8	3.0	3.4
45% burial	3.6	3.4	3.8	3.6	3.6
Control 1 (cage)	13.9	6.4	3.4	4.3	7.0
Control 2 (no cage)	1.9	7.7	4.8	2.3	4.1
Mean	5.2	5.5	4.2	3.3	4.5

Table 4 The effect of dung and dung beetles on the permeability of soil to water at Kuitpo in May 2008 as indicated by the mean time taken (minutes) for 0.5 litres of water to soak into each of plot. Each data point is the average of three readings in each plot.

Replicate	1	2	3	4	Mean
100% burial	50.4	49.5	3.8	2.3	26.5
45% burial	15.3	5.6	41.5	113.6	44.0
Control 1 (cage)	50.0	13.9	8.6	110.4	45.7
Control 2 (no cage)	11.2	51.7	23.3	53.0	34.8
Mean	31.7	30.2	19.3	69.8	37.8

However, at Ashbourne, there was a significant ($P < 0.05$) reduction in the time taken for the water to soak the soil in the plots where beetles had buried dung. The plus-dung treatments took about 3.5 minutes for the 0.5 litres to soak into the soil, whereas in the controls it took about 5.6 minutes (Figure 2).

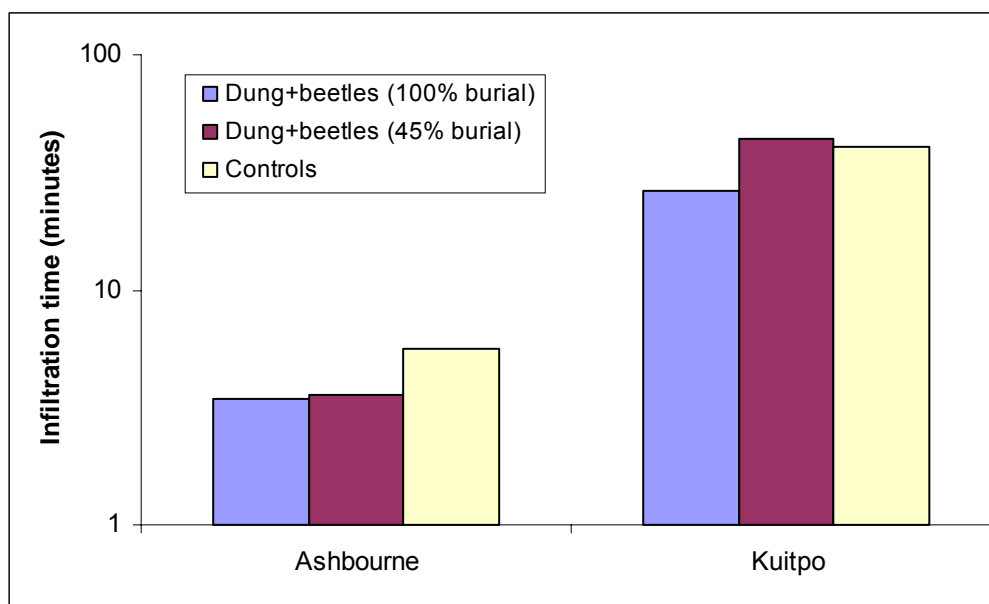


Figure 2 The effect of dung and dung beetles on the permeability of soil to water at the two study locations (Ashbourne and Kuitpo) in May 2008. The readings were taken 32 months after the dung beetles were introduced to the dung pads, by which time there was no clear evidence of where the dung pads had been placed.

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At Kuitpo, there was a statistically non-significant suggestion that water infiltration into the soil was faster in plots affected by dung burial but there was great variation between readings within and between plots (Table 4). A frequency analysis of the distribution of different time intervals (Figure 3) also suggests that there was a higher frequency of faster infiltration readings in the plots with dung burial but this was not statistically significant. It was not possible to identify with certainty the specific locations in which the dung burial had occurred within the plots (being nearly 3 years since the dung was buried) and so some cores may have been placed on soil that was not directly affected by dung burial by the beetles.

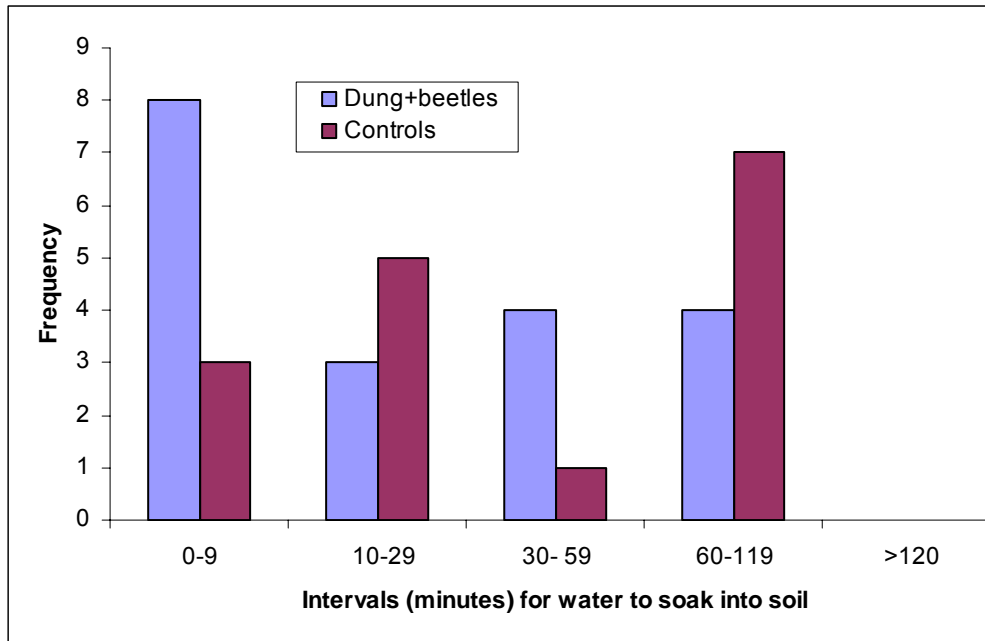


Figure 3 The frequency distributions of infiltration intervals (ie the time taken for 0.5 L of water to enter the soil at Kuitpo in May 2008

Soil hardness

At Ashbourne dung burial by the beetle *B. bison* significantly increased the depth of soft friable surface soil in the plots (13.3 cm) compared with the control plots (9.7 cm) ($t_{67} = 3.29$, $P = 0.0008$) and there was no significant difference between the two dung beetle treatments ($t_{36} = 0.27$, $P = 0.39$) (Figure 4). The effect persisted for at least 32 months.

At Kuitpo dung burial by *B. bison* significantly increased the depth of soft friable surface soil in the 100% burial plots (13.0 cm) over that in the 45% burial plots (9.4 cm) ($t_{38} = 2.95$, $P = 0.003$) and both of these were significantly deeper than that in the control plots (6.5 cm) ($t_{38} = 6.02$, $P = 0.000003$ for 100% burial; $t_{38} = 4.10$, $P = 0.0001$ for 45% burial) (Figure 5). The effect persisted for at least 32 months.

At the time of assessment, there had been little autumn rain and so the soils at both locations were hard and dry.

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry

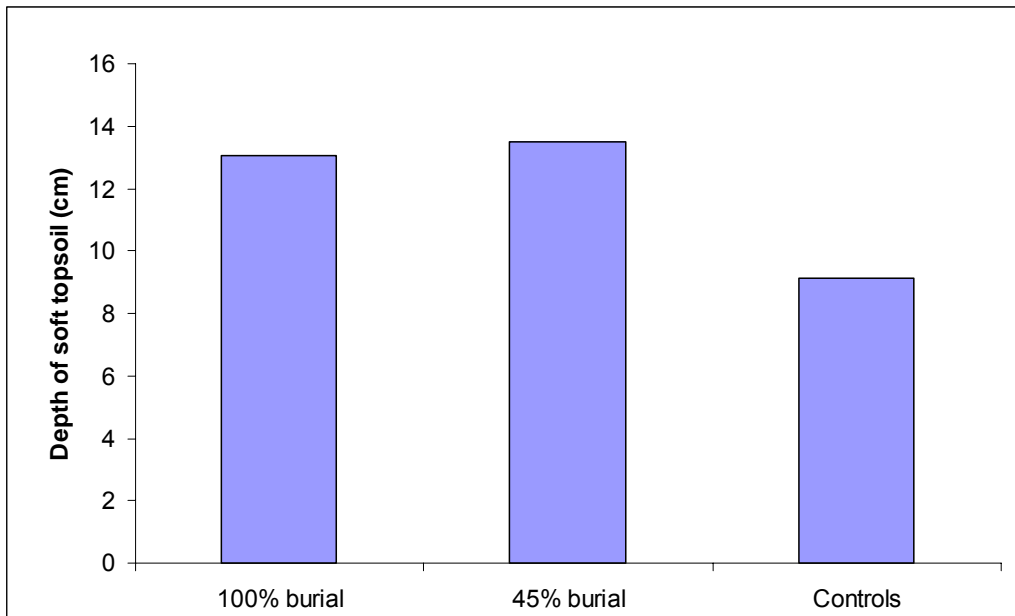


Figure 4 The effect of dung burial by *B. bison* on the depth of soft surface soil at Ashbourne as indicated by a simple hand-held soil penetrometer in May 2008. The dung beetles were introduced to the dung pads 32 months before the readings were taken, at which time there was no clear evidence of where the dung pads had been placed.

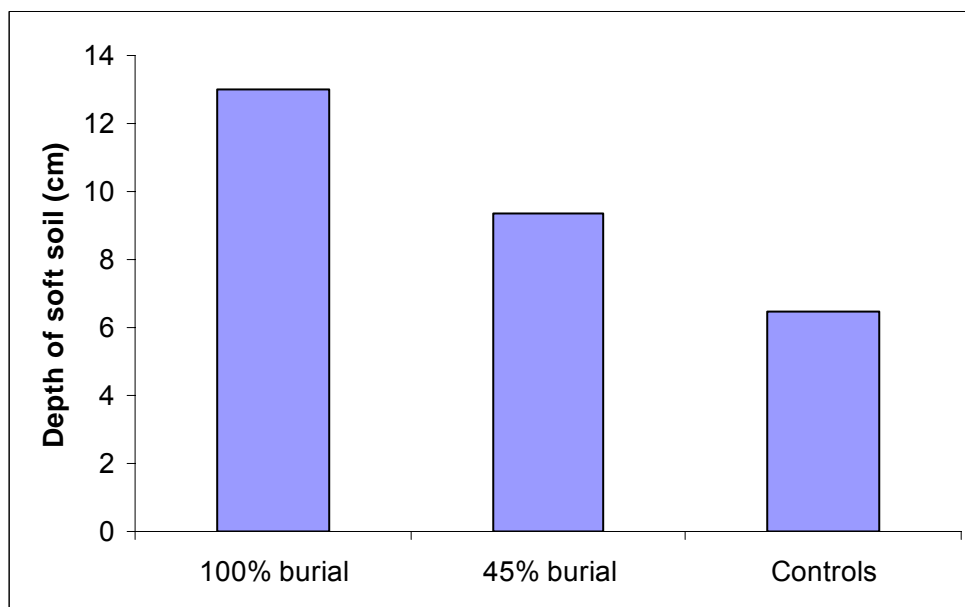


Figure 5 The effect of dung burial by *B. bison* on the depth of soft surface soil at Kuitpo as indicated by a simple hand-held soil penetrometer in May 2008. The dung beetles were introduced to the dung pads 32 months before the readings were taken, at which time there was no clear evidence of where the dung pads had been placed.

Discussion

The dung burial activities of the beetles brought much subsoil to the surface as well as creating a series of vertical tunnels. This clearly had a substantial influence on the permeability of the soils to water and on the depth of soft surface soils.

The effects of dung burial by the winter-active beetle *B. bison* on the relative rates of infiltration of water into soil was assessed 2.5 and 32 months after dung burial was initiated in two contrasting types of soil. Two levels of dung burial were examined (45% and 100%) and there were two types

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of controls (with and without wire mesh cages at the beginning of the experiment). The presence of the mesh cages had no effect upon the permeability of soil to water.

The soil at Ashbourne, a deep alluvial clay loam soil, was considerably more permeable (2- to 10-fold greater) than the duplex soil at Kuitpo, a sandy loam over a yellow clay (Table 5). Further, the rate of infiltration in 2005 was substantially greater than that observed in 2008, and this difference is thought to be due to the soil being extremely dry at the time of testing in May 2008 whereas in December 2005 the soil was still relatively moist (though not measured) as indicated by the fact that there had been had extended spring rain and the pasture had continued to grow into December.

Table 5 The effect of dung and dung beetles on the permeability of soil to water at Ashbourne and Kuitpo as indicated by the mean time taken (minutes) for 0.5 litres of water to soak into each plot. Each data point is the average of twelve readings in each treatment.

	Ashbourne		Kuitpo	
	December 2005	May 2008	December 2005	May 2008
Dung burial by beetles	1.5	3.5	3.6	35.3
Controls: no dung burial	4.6	5.6	11.8	40.3

Parallel data from a similar experiment in the Ballarat region of Victoria (BM Doube unpublished data) examined the impact of two beetle species (*B. bison* and *Geotrupes spiniger*) in a number of soils over several years and found that dung burial substantially increased the permeability of soils to water. For example, on a clay loam soil in the Ballarat region (Figure 6) the dung burial activity of *B. bison* decreased the infiltration time for 0.5 litres of water from nearly 6 minutes in the controls to 3 minutes in the dung+beetles treatment (Figure 63). There was no statistically significant effect of the presence of dung alone. This effect was substantially reduced (but still statistically significant) in soils that were very wet (close to waterlogged).

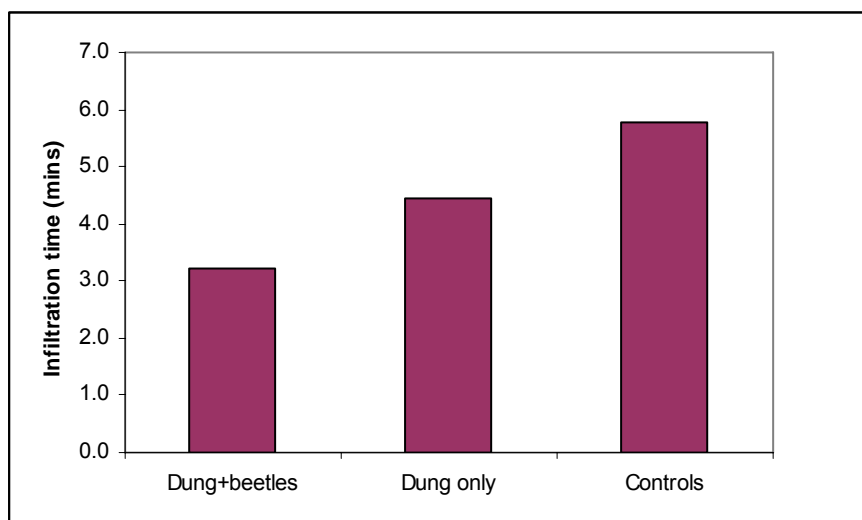


Figure 6 The effect of dung and dung beetles on the permeability of soil to water in Ballarat, 18 months after the experiment was established. The dung and beetles were applied in July 2003 and this test was conducted in January 2005.

These data suggest that in clay loam soils, dung burial by *B. bison* induced a substantial and significant increase in the permeability of the soil and this effect lasted for at least 2-3 years after the beetles had finished burying the dung.

Results in other soils may be different. For example, at the Kuitpo study site, on a duplex soil (sandy loam over yellow clay), the results were more variable. Initially, there was increased permeability in the soil directly beneath where the dung pad had been buried but this effect appears to have been lost over time in this soil. This may be related to the clay subsoil that the beetles brought to the surface and which may seal the soil surface and so impede permeability to water.

Appendix 7: Draft scientific paper (phosphate analysis)

Note: Dr Malcolm McCaskill has agreed to co-author this paper and has provided feedback on the attached manuscript but this has not yet been incorporated.

Title

The impact of dung burial by the dung beetle *Bubas bison* (L.) on phosphate levels in two contrasting subsoils on the Fleurieu Peninsula, South Australia

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Abstract

The European dung beetle *Bubas bison* has become established in a small portion of its potential range in southern Australia, where it is active from April to September and, where abundant, completely buries most cattle dung pads produced from June to October. Dung is buried in tunnels at 20 to 50 cm. Beetle larvae eat their way along the dung tunnels, filling them with friable dark brown humic excreta.

An experiment to examine the effect of this process on available phosphate levels in the subsoil (20–45 cm) was established in early October 2005 at two sites on the Fleurieu Peninsula, South Australia (Ashbourne and Kuitpo). There were three treatments (dung+beetles, dung-only and controls (no dung, no beetles)) established in undisturbed cores of soil (15 cm in diameter and 45 cm deep) encased in a beetle-proof mesh bag. The experiment was set up using 3-litre dung pads and 4 pairs of *B. bison* per pad in the dung+beetles treatments. Cores were sampled on 4 occasions (August 2006, November 2006, May 2007 and September 2007). All the dung in the dung+beetles cores had been consumed by the beetle larvae by August 2006. The base section of each core (20 to 45 cm deep) was separated, sieved and weighed for the dung-only and control cores and subsamples taken for chemical evaluation. For the dung+beetles cores, the dung beetle tunnels and the surrounding soil were separated from the bulk soil that was unaffected by direct beetle activity (the remainder). Both were sieved and weighed and subsamples taken for chemical analysis.

The presence of dung on the soil surface did not affect the phosphate concentrations in the subsoil at Ashbourne (17 mg kg⁻¹) or Kuitpo (8 mg kg⁻¹). Soil phosphate levels were elevated (5- to 7-fold higher than in the controls) throughout the base section of the dung+beetles cores (14 kg dry weight, 20–45 cm) and this effect was particularly marked in the subsoil tunnel contents and their walls, where the phosphate levels were 17- to 26-fold higher than in the control soils.

A subsoil phosphate budget was constructed using total phosphate in the Ashbourne and Kuitpo subsoil sections (5150 and 4740 mg / soil core respectively), and the total and available phosphate in the Ashbourne dung (840 and 430 mg / soil core respectively) and the Kuitpo dung (535 and 315 mg / soil core respectively). The available phosphate in the subsoil sections of the cores from Ashbourne and Kuitpo respectively was 440 mg and 320 mg greater than that in the base sections of the control cores. This provided circumstantial evidence that the dung burial and dung processing activities of the dung beetle *B. bison* may result in significant mineralisation of previously unavailable phosphate. The source of the mineralised phosphate could be the subsoil or the phosphate bound in the buried dung. High levels of available phosphate in the dung beetle excreta taken from subsoil tunnels in cores from Ashbourne and Kuitpo (5070 and 4160 mg kg⁻¹ respectively), suggest that at least part of the mineralised phosphate was derived from the buried dung. The importance of this to the Australian pastoral industry is discussed.

Introduction

Numerous species of dung beetle have been introduced to Australia by CSIRO in the years following the first introduction in 1976 (Edwards 2007). The benefits proposed included pest fly control, parasite control, improved pasture production and water infiltration (Waterhouse 1974,

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Bornemissza 1970, Doube and Dalton 2003). The biology of southern African and Australian dung beetle communities has been reviewed by Doube (1991) and Doube et al (1991). The agronomic benefits were assumed to be obvious but there was little scientific data for this view except that derived from a simplistic laboratory pot trial (Bornemissza 1970, Waterhouse 1971, AMRC 1975) and one field study on Bermuda grass in the United States (Fincher 1981). In addition, Hughes et al (1975) speculate that dung beetles might increase soil phosphate levels, but provide no evidence. Here we provide field-based evidence demonstrating that the activities of deep tunnelling dung beetles may substantially increase the levels of available phosphate in the subsoil.

Despite soils having moderate levels of total phosphate (commonly 300–500 mg kg⁻¹) (references), pasture growth in Australian soils is often limited by low levels of available phosphate (refs) and so phosphate fertiliser, usually supplied as superphosphate, has been a key ingredient of successful pasture production over the past century (Friend et al + others refs). Any biological mechanism that increases the levels of available phosphate in Australian pastoral soils is likely to be an important factor increasing both pasture growth and farm production.

Maybe discuss Response to phosphorus fertiliser compared under grazing and mowing: JWD Cayley and MC Hannah

Why b bison

Cattle dung is an important potential source of phosphate for pastures but studies on the movement of phosphate from dung to the soil (Ellenbank studies) have found that, while there is some dispersion of faecal phosphate into the soils surrounding the dung pad, this has had little impact upon pasture productivity (Refs). Most studies on faecal phosphorus have found that, unless the dung was mixed with the soil, levels of available faecal phosphorus were low (Walston 1955, Watkin 1957, Broomfield 1961, Gunary 1968 quoted in Daring and Weeda 1972).

Here we report two field experiments in contrasting soil types that demonstrate substantial increases in levels of available phosphate in the subsoil (20–45 cm) in response to the dung burial activities of the European dung beetle *Bubas bison*. The objective of the experiments was to assess the effect of dung burial and processing of buried dung on the phosphate levels in pasture subsoils.

The biology of *B. bison* in the field

An appreciation of the field biology of *B. bison* is useful in interpreting its potential to increase the fertility of soils through dung burial.

In Europe *B. bison* is reported to have a univoltine life cycle (Kirk date) but in southern Australia it exhibits a one- two- or three-year life cycle (or a mixture of all three), depending upon ambient temperature (BM Doube unpublished data). The beetle breeds successfully in duplex soils, loams and clays but not in deep sand (BM Doube unpublished data) and shows a strong preference for pasture over woodland (Doube and Dalton 2003).

Adults emerge in autumn and initially feed and mate in shallow tunnels beneath the dung pads. When ready to reproduce, they commonly work in pairs and construct a dung-lined tunnel to 20–60 cm below the surface. The female packs the tunnels with dung and lays a single egg in each of a series of small chambers along the dung-filled tunnel. Further tunnels are excavated and packed with dung (and more eggs are laid) until the entire pad is buried or the remaining dung is too dry to bury. Once a pad is colonised by a breeding female, it will be buried completely, although this process can take a number of weeks, depending upon the size of the dung pad and ambient conditions. When beetles are abundant there can be dozens of breeding females per pad, and such pads are buried within a day or so of production. The end result is complete dung burial and a network of dung-filled tunnels (about 2 cm in diameter) at 20–60 cm in the subsoil beneath the dung pad site. About 5–10 larvae (ranging up to 20) are commonly produced per litre of buried dung. Females are considered to have a potential fecundity 60–80 eggs (AMRC date), and can colonise numbers of dung pads during their adult life. Breeding continues until ambient conditions become unfavourable (eg hard, dry soils that inhibit tunnelling) in spring or early summer, whereafter the spent beetles die.

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Eggs laid during winter hatch in spring and the larvae eat all the dung in the tunnels over the following months, filling the tunnels with loosely packed faecal material, then producing a protective capsule from faeces (a faecal shell), which protects the third instar larvae from desiccation in mid-summer. Pupae are formed in early autumn and adults emerge in late autumn in the same year, or the following year for individuals exhibiting a 2-year life cycle. In colder environments than examined in these experiments, a small proportion of the larvae exhibit a 3-year life cycle.

Methods

Identical experiments were set up in early October 2005 at two sites, Ashbourne (a deep alluvial dark brown clay loam soil) and Kuitpo (a duplex soil with a sandy loam over a yellow clay subsoil). In each there were four replicates of three treatments (dung+beetles, dung alone and controls (no dung, no beetles)). These were sampled on four occasions (11, 14, 20 and 23 months after establishing the experiment), making a total of 48 cores sampled from each site (4 occasions x 3 treatments x 4 replicates).

The experiment was set up as follows. At each site 48 (4 blocks of 12 cores = 4 replicates) undisturbed soil cores (15 cm in diameter, 45 cm deep) were extracted from the soil profile, encased in a beetle-proof mesh bag and reinserted into the hole in the soil from which they had been extracted. The top of each beetle-proof bag protruded from the soil such that it could be tied off (sealing it against beetle exit) after dung (alone) or dung and dung beetles had been placed on the top of the soil column. Three litres of fresh dung were placed on the top of each core in the dung-only and dung+beetles treatments, and four pairs of *B. bison* were placed on the top of each core in the dung+beetles treatment. All bags in all treatments were then tied off.

Beetles buried the dung at 20–45 cm below the surface over the following 2–4 weeks. Cores were extracted from the soil in August 2006, November 2006, May 2007 and September 2007. All the dung had been consumed by the larval dung beetles by August 2006. For the dung-only and control cores, the base section of each extracted core (20–45 cm deep) was separated, sieved and weighed, and 0.5 kg subsamples taken for chemical evaluation. For the dung+beetles cores, the dung beetle tunnels and the surrounding soil in the base section were separated from the bulk soil that was unaffected by direct beetle activity (the remainder). Both fractions were sieved and weighed and 0.5 kg subsamples taken for chemical analysis. Subsamples (in duplicate) from each fraction were assessed for soil moisture by weighing, drying for 48 h in a drying oven, and then reweighing. The dry weight of each fraction was then calculated. Thus, four types of soil sample were taken from the base section of the cores. These comprised:

- from dung+beetles cores: dung beetle tunnel contents and the adjacent soil that appeared affected by dung beetle activity (labelled 'tunnels+environs' in the results below)
- from dung+beetles cores: the remaining subsoil that appeared unaffected by dung beetle activity (labelled 'remainder' in the results below)
- dung-only cores: subsoil from cores unaffected by dung beetle activity
- control cores: subsoil from cores unaffected by dung beetle activity or dung alone

The standard soil nutrient analysis was conducted by CSBP Ltd, Perth. About 0.5 kg of soil was provided to represent each soil sample. Only the data for phosphate are presented here.

These data and the analysis allowed examination of

- The differences between sites (Ashbourne and Kuitpo)
- The impact of surface dung on the chemistry of the subsoil
- The impact of beetle buried dung on the average chemistry of the subsoil
- The impact of beetle buried dung on the chemistry of the tunnels and closely surrounding soil compared with the remainder and with the control subsoils
- Whether the elevated levels of compounds found in the tunnels remained there or leached out into the surrounding soil. Subsample analysis

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The soil fractions were all from the base section of the cores (20–45 cm deep) and were selected to provide maximum contrast in relation to dung beetle activity. At the Kuitpo site the duplex soil had a yellow subsoil and the soil was relatively massive and not easily broken up. Unpublished sampling of additional cores showed that by early April 2006 all the dung in the tunnels had been completely processed by dung beetle larvae (which became adults and departed in autumn 2006 or 2007). The tunnels were filled with dark brown beetle excreta. As a consequence, it was a relatively simple matter to identify the tunnels and the limits of the adjacent dung beetle-affected soil, although the limits of the tunnels were less clear in September 2007 than in the previous autumn. At Ashbourne the tunnel contents and the soil were a similar colour, but it was still possible to recognise and separate portions of the subsoil that had been influenced by dung beetle activity (ie the tunnel contents and the immediate surrounding soil).

All the subsoil from the cores was passed through a 2.5 mm sieve before subsampling, and subsamples of about 500 g each were taken. The mass of the soil samples in each of the first two sample types was assessed and so, for the dung+beetles treatment samples, it was possible to calculate the mean change in the average phosphate levels for three fractions:

- in the beetle-affected soil (tunnel contents + surrounding soil)
- in the adjacent unaffected soil
- across the whole subsoil section (20–45 cm)

Data analysis

There were four sampling occasions, two locations, three treatments and four replicates. The data were analysed by ANOVA using Statistix version 8.2 (Analytical Software). The experiment has a factorial design with replication. The differences between means were evaluated using Tukey's HSD all-pair-wise comparisons tests. Tukey's method is considered to be the most useful pair-wise comparison procedure Statistix performs.

On the first sampling occasion (August 2006) in the Ashbourne sample, the tunnels and environs were not treated separately from the remainder. A complete set of data for the comparison of the tunnels and environs compared with the remainder of the dung+beetles core subsoils was available only for the latter three sampling occasions. For these a separate ANOVA was conducted on the data comparing phosphate levels in the control soils, the dung+beetles tunnels+environs soil and the dung+beetles remainder soil.

Results

Effects of location, time, dung and dung beetle activity

The effect of dung and dung beetles on the phosphate concentration averaged over the entire subsoil component of the soil profile for each location and sampling date was analysed by ANOVA (location x time x treatment x replicate).

There was no systematic trend in the mean phosphate levels in the control soil over time (August 2006 to September 2007, Table 1), although the levels at Ashbourne (mean 17.4 mg kg⁻¹, range of means 12.0 to 22.8 mg kg⁻¹) were significantly higher (Table 2) than those in the control soil at Kuitpo (mean 7.9 mg kg⁻¹, range of means 4.7 to 9.7 mg kg⁻¹) (Table 1). There was no systematic change over time in the phosphate levels in the soil affected by dung beetle activity at either site (Table 1).

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Table 1 A comparison of the effect of dung burial activity by *B. bison* on the concentration of available phosphate (mg kg^{-1}) in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007. Beetles and dung were added to the cores at the beginning of October 2005.

Treatment	August 2006		November 2006		May 2007		September 2007		Mean	
	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*	Ash*	Kui*
Controls subsoil	12.0	4.7	22.8	8.5	19.3	8.5	15.5	9.7	17.4	7.9
Dung-only subsoil			22.0	22.8	16.3	10.5	13.0	16.0	17.1	16.4
Total dung+beetles subsoil	68.8	41.9	73.6	84.7	89.2	50.9	88.8	35.2	80.1	53.2
Dung+beetles: tunnels+environs		265.0	354.8	323.3	320.8	151.0	203.0	71.3	292.9	202.7
Dung+beetles: remainder		24.0	51.0	55.0	81.0	47.0	53.8	28.5	61.9	38.6

Ash: Ashbourne; Kui: Kuitpo

There was no significant effect of surface dung on subsoil phosphate levels at either test site (Table 1). In marked contrast, there was a highly significant effect of dung beetle activity on the levels of phosphate in the subsoil at both sites (Table 2). At Ashbourne the mean levels in the dung+beetles core subsoils was 80.1 mg kg^{-1} (range 68.8 to 89.2 mg kg^{-1}), which was 4.6 times greater than in the control subsoils. Similarly, at Kuitpo the mean level in the dung+beetles core subsoils was 53.2 ppm (range 35.2 to 84.7 mg kg^{-1}), which was 6.8 times greater than in the control subsoils (Table 1).

Table 2 Analysis of variance for four sampling occasions (2006 and 2007) and three treatments (controls, dung-only, dung+beetles) on the phosphate levels (ppm) in the subsoil at the two study locations

Source	DF	F	P
Location	1	10.91	0.0015
Time	3	3.35	0.0238
Treatment	2	74.78	0.0000
Location*Time	3	0.39	0.7601
Location*Treatment	2	2.70	0.0747
Time*Treatment	6	0.34	0.9123
Location*Time*Treatment	6	1.81	0.1105
Error	68		
Total	91		

Dung beetle tunnels and the surrounding soil

Analysis of the contrast in available phosphate levels in the control soils compared with the tunnels+environs and with the soil surrounding the tunnels (remainder) in the subsoil (20–45 cm) over the three sampling occasions at both locations (Table 3) indicates that there was a highly significant effect of location, time and treatment, a significant interaction between location and treatment and a highly significant interaction between time and treatment (Table 3).

Of considerable importance are the substantially elevated phosphate levels in the tunnels +environs (walls) compared with the remainder of the dung+beetles core subsoil. At Ashbourne the mean level in the tunnels+environs was 292.9 mg kg^{-1} (range 203.0 to 354.8 mg kg^{-1}) which was 4.7 times greater than in the 'remainder' of the dung+beetles core subsoil. Similarly, at Kuitpo the mean phosphate level in the dung+beetles core subsoils was 202.7 mg kg^{-1} (range 71.3 to 323.3 mg kg^{-1}), which was 5.3 times greater than in the 'remainder' of the dung+beetles core subsoil (Table 1).

Table 3 Analysis of variance for four sampling occasions (2006 and 2007), three treatments (controls, beetle tunnels and environs, remainder of subsoil) on the phosphate levels (ppm) in the subsoil at two locations on the Fleurieu Peninsula

Source	DF	F	P
Location	1	16.52	0.0002
Time	2	12.72	0.0000
Treatment	2	137.58	0.0000
Location*Time	2	2.28	0.1129

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Location*Treatment	2	7.34	0.0016
Time*Treatment	4	10.21	0.0000
Location*Time*Treatment	4	1.13	0.3524
Error	50		
Total	67		

Because the levels of phosphate in the remainder subsoil in the dung+beetles treatment (which was not directly associated with the tunnels) (48.6 mg kg^{-1}) were significantly higher than those in the control subsoil (12.6 mg kg^{-1}), we conclude that, over the time period examined, the phosphate had leached from the tunnels into the surrounding bulk soil.

Discussion

This study of phosphate in subsoils is part of a wider study of the impact of dung burial by the dung beetle *B. bison* on the chemistry of the subsoil. This ongoing study has shown that dung beetle activity induced a substantial increase in subsoil nitrate, ammonia and sulphur as well as increasing levels of subsoil organic carbon, pH and electrical conductivity over the time period examined (BM Doube unpublished data). This paper focuses upon the effects on subsoil phosphate.

Parallel studies at the two study sites in SA on the impact of dung burial on pasture production have shown major increases in pasture production over at least 2 years in response to dung burial by *B. bison*. Part of the explanation for this may lie in the elevated levels of subsoil plant nutrients (phosphate, nitrate, sulphur) that have also persisted for at least 2 years after dung burial.

Another aspect of the same study (BM Doube unpublished data) has shown that there was no detectable pasture growth response to the presence of unburied surface dung. It is noteworthy that in the current study there was no detectable effect of unburied surface dung on subsoil phosphate levels.

Further, and in contrast to other nutrients (eg nitrate), the phosphate appears to have moved from the restricted location of the tunnel contents and tunnel walls into the bulk soil surrounding the tunnels. It seems likely that this will encourage root growth into the bulk soil.

Phosphate movement through the soil

In this study available phosphate released in the dung beetle tunnels had partially dispersed from the tunnels into the surrounding bulk soil in the core. Tunnels were networked through the core, especially at the base, indicating that phosphate had moved some distance (possibly a few cm) into the soil surrounding the tunnels over a 2-year period.

McCaskill and Cayley (date) and McCaskill et al (date), in a study of surface-applied phosphate in long-term field trials, found that 80% of the applied phosphate remained in the top 43 cm of the soil profile after 17 years, and that superphosphate application over 17 years significantly increased total phosphate concentration in the 0–5 cm and 5–10 cm layers, but had no significant effect at lower depths in the profile, indicating a slow rate of dispersal into the subsoil. Improving subsoil fertility is a key aspect of improving pasture productivity and so it appears that dung beetle activity may provide an important ecosystem function that will promote increased subsoil fertility.

The lack of movement of phosphate into the subsoil was also demonstrated by Haynes & Williams (1992), who, in an irrigated grazing trial in New Zealand, found that with increasing superphosphate rates inorganic phosphorus accumulated primarily in a form adsorbed to Aluminium hydrous oxides and as calcium phosphate compounds while organic phosphorus accumulated in both labile forms and forms associated with humic compounds. In the fertilised sites both inorganic and organic phosphorus accumulated in the soil profile to a depth of 20 cm.

Malcolm maybe also discuss Phosphorus leaching from cattle dung and fertiliser in a krasnozem D Nash, C Murdoch - Australian and New Zealand National Soil Conference. Soil ..., 1996

A subsoil phosphate budget

[should at least some of this be in the results??]

Available phosphate levels in the control subsoils (no dung, no beetles) were in the range 12–23 mg kg⁻¹ at Ashbourne and 5–10 mg kg⁻¹ at Kuitpo (Table 1). In marked contrast, the levels in the tunnels+environs (ie the tunnel contents and the tunnel walls) were in the order of 200–350 mg kg⁻¹ at Ashbourne and 70–320 mg kg⁻¹ at Kuitpo, and the levels in the remainder (ie the soil surrounding the tunnel walls) were in the order of 50–80 mg kg⁻¹ at Ashbourne and 25–55 mg kg⁻¹ at Kuitpo. These levels represent a massive increase in available phosphate in the subsoil (Table 4).

The total phosphate levels in triplicate sub samples of the control subsoil from Ashbourne and Kuitpo was assessed (through CSBP) as was the total and available phosphate in the dung used to establish the experiments.

Knowing the mass of each subsoil fraction, the total phosphate levels in the bulk soil, the levels of available phosphate in each fraction, and the amount of phosphate (total and available) in the buried dung, it is possible to construct a simple phosphate budget for the subsoil cores.

Table 4 A comparison of the effect of dung burial activity by *B. bison* on the concentration of available phosphate (mg kg⁻¹) in the subsoil (20–45 cm) in cores sampled between August 2006 and September 2007 (averaged over 4 sampling occasions). Beetles and dung were added to the cores at the beginning of October 2005.

	Average levels of soil phosphate (mg kg ⁻¹)	
	Ashbourne	Kuitpo
Control cores	17.4	7.9
Dung+beetles cores	80.1	53.2
Beetle-related increase	62.7	45.3
Faecal shells/tunnel contents recovered from subsoil	5071.5	4159.5

Analysis of the available phosphate levels in the control and dung+beetles treatment subsoils (Table 5) indicates that, on average, during the 12 months over which the cores were sampled, available phosphate levels at Ashbourne and Kuitpo in the dung+beetles core subsoils were elevated by 878 mg and 635 mg respectively, above those in the control soils (Table 5). Clearly the dung burial by the beetle *B. bison* caused a substantial increase in the concentrations of phosphate in the subsoil (Table 5).

Table 5 Absolute amounts of phosphate (total and available mg) in the subsoil cores from Ashbourne and Kuitpo (subsoil cores averaged 14 kg dry weight), assuming 80% burial in the subsoil

	Absolute levels of phosphate (mg per subsoil core)	
	Ashbourne	Kuitpo
Total phosphate introduced with buried dung	844	544
Available phosphate introduced with buried dung	433	315
Total phosphate in subsoil control core	5152	4737
Available phosphate in control core	244	110
Available phosphate in dung+beetles core	1121	745
<i>Elevation of phosphate in dung+beetles cores</i>	<i>878</i>	<i>635</i>
Available phosphate in control cores + available phosphate introduced with buried dung	677	426
<i>Additional phosphate in dung+beetles subsoil</i>	<i>444</i>	<i>319</i>

This analysis provides circumstantial evidence for the view that the dung burial and dung processing activities of the dung beetle *B. bison* results in substantial mineralisation of phosphate that was previously unavailable. The source of the mineralised phosphate is not certain but it may have come from the subsoil or from the previously unavailable phosphate in the buried dung (49%

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and 42% of the total phosphate in the dung at Ashbourne and Kuitpo respectively was not available) or from both.

Faecal shells that encased the mature beetle larvae and faecal debris from inside the tunnels were collected, bulked and dried and duplicate samples were analysed by CSBP. These contained 4000–5000 mg kg⁻¹ of available phosphate, a huge concentration, suggesting that at least part of the phosphate that is mineralised by the dung beetle larvae comes from the phosphate bound in the dung buried in the soil.

Beetle biology and subsoil modification

The results clearly show that dung burial by the dung beetle *Bubas bison* and the processing of the buried dung by beetle larvae caused major increases in the concentrations of available phosphate in the subsoil. In marked contrast, surface dung had no influence upon available phosphate in the subsoil during the period of the experiments. Similarly, there was no corresponding pasture growth response to surface-applied dung (without dung beetles) in adjacent experiments at the two sites although there was a major increase in pasture production on plots where dung was buried by the beetles.

At Kuitpo most larvae became adult beetles and vacated their faecal shells in early winter 2006, leaving behind a substantial mass of digested excreta and an empty faecal shell. At Ashbourne, most larvae had a two-year life cycle and so during winter 2006 they remained in their faecal shells in tunnels surrounded by larval excreta. These beetles emerged as adults in early winter 2007, leaving behind their faecal shells and what remained of the larval excreta.

During winter 2006, the tunnels and their contents became moist and were colonised by earthworms and substantial amounts of plant root material. There was significant root material in the beetle tunnels and surrounding the faecal shells (containing third instar larvae when present) at both Ashbourne and Kuitpo. Considering the elevated levels of available phosphate in the tunnels, it is most likely that roots will continue to colonise the subsoil and their contribution to subsoil phosphate may be increasingly important over time (years).

As time passes a number of processes (earthworm activity in and about the tunnels, water movement (carrying dissolved plant nutrients), and plant roots growing in and about the tunnels) will disperse the effects of dung beetles from the near proximity of the tunnels to the soil between the tunnels. Further, the growth and death of plant roots in the subsoil will progressively alter its character, increasing organic carbon levels, which will, in turn, promote biological activity (eg earthworms and soil microbes) in the subsoil, which will enhance its biological fertility.

Acknowledgements

We wish to thank MLA for financial support for this project and Mr G Dalton () for supporting the establishment of the project. Ms Cherry Macklin and ??? are thanked for the use the land for the Ashbourne and Kuitpo study sites.

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Appendix 8: Review of MLA Project ER 211

Project Number. ER 211

Project Leader. Bernard Doube

Review Team: Malcolm McCaskill, Soil and Water Scientist DPI (Victoria), Hamilton and Tom Davison, Environment Program Coordinator Southern Beef, MLA.

Review conducted on site in South Australia on 21 May 2007.

Purpose of review

1. Review what has been achieved to date in the research program including the methods used in the research program
2. Review the implications of the research findings for the southern beef industry and suggest what options if any, for future work.

Summary of review

1. The project team exhibit great enthusiasm for their work and have put in a lot of work above and beyond that expected from the project proposal.
2. This study is the first in Australia to investigate the relationship between the role of dung beetles and their effect on pasture growth and soil fertility changes over time.
3. In response to demand for dung beetles from beef producers and catchment management authorities, the project team have developed a small dung beetle collection and distribution business.
4. The project has provided strong evidence that dung beetles support increased pasture growth, improved soil structure and improved soil fertility.
5. However, due to the small-scale nature of the study with large quantities of dung spread onto small areas, the evidence that dung beetles cause paddock-level benefits is still weak, and relies on modelling.
6. This case needs to be proven at a paddock scale to take into account the spatial variability of dung beetles and their potential impact on pasture growth.
7. There is a case for some work to be supported to determine if dung beetles have a role in carbon sequestration in soil.
8. A hypothesis was presented that deep burial of dung released unavailable P from deeper in the soil.
9. A modelling approach to determine likely benefits from dung beetles is recommended prior to a paddock scale study being implemented.
10. Limited plot work continue to determine data for future modelling work if funding permits.

Recommendations

Next 2 months

1. That additional funding be provided to analyse topsoil samples collected at the release sites, so soil fertility can be described in a way that beef producers can relate to.
2. An article be prepared for the MLA Prograzier magazine on dung beetles, with contact details for how producers can obtain dung beetles. The review team suggests that Bruce Munday be approached for this task, because he is located in Adelaide.

Next 6 months

1. A cost-benefit analysis should be conducted as a consultancy in November or December 2007 after the final pasture growth results have been collated. Skills required include modelling, economics, biometrics, soil nutrient cycling, pasture measurement and GIS (for the area of applicability). Some individuals may have several of these skill areas. A modelling template that could be used as part of the cost-benefit analysis is described in Appendix 5. The cost/benefit analysis should also review detailed plans for a field

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experiment, to ensure that the experimental design can capture statistically significant differences in pasture growth due to dung beetles.

2. That MLA facilitate discussions with the Australian Greenhouse Office to fund a study of the carbon sequestration benefits of dung beetles.

Next 1-2 years

1. A grazing experiment be funded to examine the paddock-level benefits of dung beetles. A potential site on Kangaroo Island was suggested to the review team, where there is already a good population of dung beetles including *Bubas bison*. The “control” (no dung beetle) treatment would need to be implemented by using substances toxic to dung beetles. A field study of this scale may require the involvement of additional funding and research delivery partners, and this would take time to negotiate.
2. To minimise any potential loss of staff and expertise, it would be worthwhile minimising the time gap between when the current MLA-funded project B-ERM.0212 finishes in June 2008, and the next project.
3. A booklet on dung beetles developed for South Australia, should be adapted to a wider southern Australian audience in a follow-up project. This publication should be made available more widely, such as through the MLA website.
4. Within MLA there is a need to co-ordinate investments in dung beetles with respect to other proposals.
5. MLA should look to include dung beetles as part of a postgraduate study of phosphorus in soils. The case for introducing dung beetles does not rely on proving the hypothesis that deep-burying dung beetles cause release of otherwise unavailable P from deeper in the soil, so for dung beetle research it is a low priority. However, if MLA is involved postgraduate study in phosphorus forms in soil, or in suggesting topics to university supervisors for ARC or CRC-supported scholarships, this topic should be included.

Appendix 9: Project ER211 extension activities

Milestone 3

A draft article describing the project was written and submitted for publication in the mlaProGrazier summer issue.

Milestone 4

A Field Day was held on 21 December 2005 at the site of the field trial at Location 2. Representatives of the Fleurieu Beef Group, Creation Care, local producers, collaborators, dairy discussion groups (DairySA) and the Department of Agriculture were invited and most attended. A colour handout was provided and was submitted to MLA with milestone 4.

Milestone 5

A second field day was held on 23 September 2006 in conjunction with the Goolwa_LAP Agricultural Bureau and Creation Care. Presentations at the Kuitpo Hall followed by inspection of soil cores and the field trials at Location 2. The field day was well publicised in the rural and local press and more than 80 people attended. A colour handout was provided and was submitted to MLA with milestone 5. A report on the field day was also provided in the milestone 5 report (appendix 5).

This meeting was reported in a 2-page section of the Stock Journal.

Milestone 6

Presentations detailing the progress of the project were given to the New Springs Landcare group, the Parawa Agricultural Group and the Fleurieu Beef Group. Between 20 and 50+ people attended each meeting. A draft media release was submitted to MLA.

Address to the Parawa Agricultural Bureau, October 2006

About 25 farmers attended. The guest speaker was Dr B. Doube, who presented an account of current progress in dung beetle research in southern Australia, emphasising the contribution of the MLA-funded project, and canvassing attitudes towards research into developing 'beetle-friendly' irrigation strategies.

Address to the New Springs Landcare Group, November 2006

About 20 farmers attended. The guest speaker, Dr B. Doube, presented an account of current progress in dung beetle research in southern Australia, emphasising the contribution of the MLA-funded project, and canvassing attitudes towards research into developing 'beetle-friendly' irrigation strategies.

Address to the Fleurieu Beef Group, February 2007

Over 50 farmers attended. The guest speaker, Dr B. Doube, presented an account of current progress in dung beetle research in southern Australia, emphasising the contribution of the MLA-funded project, and outlining recent results in relation to

- Pasture growth benefits of *B. bison*
- Effects of *B. bison* on soil biology in the subsoil (earthworms and plant roots)
- Effects of *B. bison* on chemistry of the subsoil

A copy of the handout for the FBG meeting was submitted to MLA with the milestone 6 report.

Milestone 7

The program has gained considerable exposure on national television and radio as well as through local radio and a 2-page feature in mlaPrograzier.

The Australian dung beetle project was featured on ABC Landline in 2007 and was replayed over the Xmas – New Year break as one of the ‘best of Landline for 2007’. The program featured the Ashbourne field experiment.

Dr Doube was interviewed on ABC Bush Telegraph by Michael McKenzie and spoke at length about the results of the MLA project.

In May 2007 Dr Doube presented an invited paper in Canberra at a Healthy Soils Australia workshop. In July 2007 Mr Adam Tassicker, a DBSA employee, attended a Healthy Soils Australia conference on the Gold Coast to demonstrate the benefits of dung beetles.

In March 2008 Dr Doube presented a talk at a Dairy Australia workshop on climate change, held in at the Flaxley Dairy Research Centre, SA.

In July 2008 Dr Doube was featured on ABC Stateline SA where he presented details of the potential of dung beetles to affect climate change by reducing levels of greenhouse gasses through sequestration of carbon in soil organic matter.

Appendix 10: Project ER211: Selected draft media releases

Prograzier article 1: October 2005

The environmental and production benefits of the dung beetle *Bubas bison* in southern Australia

Dr Bernard Doube, Dung Beetle Solutions Australia

Why do we need to introduce dung beetles?

In Australia there are about 26 million cattle, which produce over 170 million tonnes of dung each year. This dung is a huge source of environmental pollution – it fouls pastures and contaminates waterways with unwanted nutrients, organic matter and human pathogens such as *Cryptosporidium*.

While there are native dung beetles in Australia, most of them are not well adapted to consume cattle dung. Between 1965 and 1985, CSIRO introduced 57 species of foreign dung beetles, and 26 of these have become established permanently. These introduced species can bury large volumes of dung through all seasons of the year, with many apparent benefits for soil, water and pasture, as well as biological control of the bush fly.

However, there are many regions in Australia, especially in southern Australia, where introduced dung beetles could provide these benefits but where few species have become established and abundant. Winter-active beetles are especially important in southern Australia because at that time of year (the wet season), the dung produced is high in nutrients (for example, nitrogen and phosphorous) that pollute run-off water at present but could become a fertiliser if incorporated into the soil.

Bubas bison is a promising, introduced (from southern Europe), winter-active dung beetle that is established in a series of pockets in WA, SA, Vic and NSW. In these localities in winter the beetle buries the dung of sheep (which tend to produce dung sausages, not pellets, in winter) and cattle but experimental analysis has focussed primarily on cattle dung.

During 2002–2005 Dung Beetle Solutions Australia (DBSA), worked in association with a number of organisations (The Fleurieu Beef Group, the EPA, Dairy SA and a number of Water Authorities), to examine the biology of *B. bison* and its impact on pasture production, soil health and water quality. These preliminary studies have shown that dung beetle activity can:

- rapidly buried large amounts of dung
- substantially increased the permeability of soil to water
- substantially increased the depth of friable topsoil
- increased earthworm activity, especially at depth in the soil
- altered pasture composition (favouring pasture grasses over weeds)
- increased pasture production over and above that due to surface dung
- decreased levels of soluble organics and nitrogen (N) in run-off water
- substantially decreased the numbers of pathogens in surface dung

Why do we need quantify the benefits of dung beetles?

While it is clear that beetles are beneficial, there are no published estimates of the \$\$ value of dung beetles to the Australian environment or grazing industry. Such information is necessary in order to quantify the benefits that would flow from widespread and abundant dung beetle populations in southern Australia.

In response to this need, the MLA has provided funds for DBSA to quantify the environmental and pasture production benefits of dung beetles for Mediterranean and even-rainfall regions of

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southern Australia. The trial will run for 3 years (2005-2008) and will quantify the plant growth responses (yield and pasture composition) and the soil health benefits (permeability to water, soil aeration, deep placement of dung nutrients and organic matter).

What does *B. bison* do?

Adult beetles emerge from the soil in autumn, fly to dung pads, dig shallow tunnels (10 cm) and feed on dung juices and mature their ovaries. They then fly to new pads, dig deep tunnels (up to 50 cm) at the bottom of which they bury large amounts of dung in which they lay eggs. Having buried one dung pad, beetles then fly to another pad and repeat the burial/egg laying process. Many pads may be colonised by an individual beetle before it die in late spring. In the buried dung, the eggs hatch into grubs (larvae) which feed on the buried dung, grow and turn into adults which emerge in the following autumn. Abundant populations commonly bury a dung pad within a few days of its production.

Field trials on the Fleurieu Peninsula

In winter/spring of 2005, two parallel field trials have been set up in contrasting soil types on the properties of Fleurieu Beef Group collaborator at Ashbourne (a deep alluvial loam) and Kuitpo (a duplex soil [sand over clay]) SA where *B. bison* has recently become established. Beetle numbers are increasing each year, but the beetle is not yet abundant. At each location, field plots have been set up to quantify the impact of dung beetle activity on pasture growth and composition, the hydraulic properties of soil, earthworm activity and abundance, and the nutrient status of soil, especially sub soil.

In these trials DBSA is measuring the changes that dung beetles cause in the soil under individual dung pads (tunnels, earthworms, plant roots, soil nutrients, soil organic matter,). They are also using small plots (2m x 2m) and larger plots (10m x 10m) to put a \$\$ value on the increased pasture production with known numbers of beetles per pad (introduced into beetle-proof cages on the small plots) and with natural colonisation (larger plots).

How can cattle producers set up dung beetle populations?

Methods for acquiring, introducing and establishing dung beetle populations are explained in the booklet *Dung beetles: transform a pollutant into an environmental and agricultural benefit* by Dr Bernard Doube and Greg Dalton (2003). Knowing which beetles are present on your property can be assessed by using the booklet *Identifying dung beetles on the Fleurieu Peninsula* by the same authors. The booklets were produce by DBSA in partnership with Fleurieu Beef Group Inc. (FBG) and Creation Care Pty Ltd (CC). Copies are available on request to Dr Bernard Doube, Dung Beetle Solutions Australia, 37 Cave Ave Bridgewater, SA.

Media release 2: milestone 6

Dung beetles increase levels of soil nutrients

1 February 2007

A new South Australian research project is measuring the pasture production and soil health benefits of the winter-active dung beetle *Bubas bison*.

Bubas bison, large black dung beetle that flies at dusk and dawn, was introduced to Australia from southern Europe by CSIRO about 25 years ago. It is now well established in pockets in WA, SA, Vic and NSW. In these localities it is active for 3 to 5 months each year, and buries huge amounts of dung.

The project, managed by Dr Bernard Doube (ex CSIRO scientist) and his company *Dung Beetle Solutions Australia*, is run in collaboration with the Fleurieu Beef Group, a producer organisation that promotes rural R&D on the Fleurieu Peninsula, SA.

“Despite the huge sums of money spent bringing dung beetles to Australia, there are no sound data on the effects of dung burial on the levels of soil nutrients,” Dr Doube said. “If we could

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demonstrate that dung beetles improve soil fertility, we could have a good case for spreading the beetle throughout southern Australia.”

The field trials were set up in winter of 2005 and already results are flowing in.

“One of the most interesting discoveries has been the effect of dung beetle activity on soil nutrient levels. *Bubas bison* digs a vertical tunnel to about 45 cm deep and lines it with dung. The rest of the dung pad is stacked in underground chambers at the bottom of the tunnel.” Dr Doube said.

“Earthworms, dung beetle larvae and soil microbes feed on the buried dung and so, in places where dung beetles have been active, the chemical and biological composition of the subsoil can be transformed.”

“One experiment at Kuitpo has been running for 16 months. There the phosphate levels in the subsoil (20 to 45 cm deep) beneath where beetles have been active have increased from 9 ppm to 320 ppm in the beetle tunnels and the soil surrounding the tunnels. Furthermore the phosphate appears to be quite mobile and has moved into the subsoil that has not been directly affected by dung beetle activity.”

“We all believe that dung beetles improve soil health, but now we are learning how dung burial by dung beetles increases subsoil nutrient levels. This may provide a long-term improvement in subsoil fertility.”

“We are already seeing improved pasture growth in response to this, but we expect that the big benefits will become clear over the next few years.”

The trial will continue for at least 3 years.

Prograzier article 2: September 2007

Dung beetles boost pasture production

Bubas bison (see photo) is a large winter-active dung beetle introduced to Australia by CSIRO over 20 years ago. The beetle flies to fresh dung pads at dusk and dawn. Huge populations now occur in some areas in southern Australia and all are derived from about 1500 beetles released in WA in 1983–86 (at Dardanup and Kojonup). However, many regions in southern Australia are suitable for the beetle but remain uncolonised.

Despite extensive work by CSIRO and other research agencies, there were no sound field data demonstrating the pastoral and environmental benefits of dung beetles in Australian soils. This is a key issue. If the benefits are substantial, there is a good case for widespread re-distribution of *B. bison* and other similar species. Dung Beetle Solutions Australia (DBSA), an Adelaide-based R&D business, had initiated small-scale field trials in SA and Vic in 2003, but more detailed information was required.

In winter 2005 two MLA-funded 3-year field trials were established on the Fleurieu Peninsula SA to assess the pasture production benefits of *B. bison*, on a brown alluvial loam soil and a duplex soil. The trials were established at two locations where the beetle had recently established small self-perpetuating populations.

The trials measure the pasture growth responses (extrapolated to tonnes of dry matter per ha) to the addition of dung+beetles and dung-only in comparison with control plots (no dung, no beetles). The project is looking to explain pasture growth benefits in terms of altered soil structure, improved subsoil fertility (dung is buried 30 to 50 cm deep) and improved soil biology (eg earthworms).

One trial has been established using small plots (4 m²) to which large dung pads and beetles were added (once only) in October 2005. All the dung was buried after a week or so.

The other trial uses larger plots (100 m²) to which three 1-litre dung pads are added to each plot each week over a 5-month period (May to October) each year: these pads are colonised by natural field populations of the dung beetle. Because beetle populations have not yet become abundant, only a portion (40–80%) of the pads have been buried. The growth responses observed are therefore underestimates of what is likely to occur in a few years time when beetle numbers have built up. In both trials pasture growth has so far been assessed on 13 occasions since the trial was set up.

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There have been significant pasture responses on both soil types, with the greater response occurring on the duplex soil. However, here we present results from the deep alluvial brown loam site, where Cherry Macklin is the owner and collaborator.

What we found

Over the current 2-year trial period (September 2005 to August 2007):

- In the small plot trial the pasture production in the control plots (no dung, no beetles) averaged 20.3 t per ha. Addition of dung alone increased pasture production by 3.3 t per ha over that in the control plots. Production in dung +beetles plots was 5.3 t per ha higher than in the controls and 2.0 t per ha higher than in the dung-only plots.
- In the 100 m² plot trial, 30 pads were added to each +dung plot October-November 2005, 72 pads were added in 2006 and 48 pads have been added in 2007 (to August). Over half were buried by *B. bison*. In 2005 or 2006 no significant effect of dung alone or dung+beetles was detected. For the period January to August 2007, the pasture production was not elevated in the dung-only plots. But in the dung+beetles plots, dung beetle activity has increased pasture production by 31% per ha over that in the dung-only plots and by 44% (0.5 t per ha) over that in the control plots.
- Dung burial by the beetles has had a major impact on the chemical fertility of the subsoil (20–50 cm). There were strongly elevated levels of nitrate, phosphate, organic carbon and sulphur in beetle-affected subsoil.
- Dung burial by the beetles had a major impact on the earthworm populations. In beetle-affected soils earthworms were found in substantially greater numbers and biomass, and occurred throughout the soil profile (to 50 cm deep), while in the control soil worms were less abundant and were found in surface soil only.

In conclusion

Dung beetle activity has increased pasture production in both trials and the elevated pasture production levels have persisted for at least 2 years, whereas the effects of dung alone were either absent or were detected only in the first year. Other trials in SA and Victoria indicate that the effects of dung beetle activity persist for at least 3 years. Elevated levels of soil nutrients and earthworm populations have persisted for at least 2 years in the soils affected by dung beetle activity, and may help explain the presence and persistence of improved soil fertility in response to dung beetle activity.

Prograzier revision: November 2007

Dung beetles, the humble recyclers of manure, have emerged as vital players in improving the sustainability of agriculture.

That's according to South Australian beef producer, Cherry Macklin who has labelled the small invertebrates "nature's little helpers and recyclers".

The hive of action under the dung pads begins when the beetles dig tunnels to depths of up to 50 cm. The beetles fill the subsoil tunnels with brood dung in which the female beetles lay eggs which hatch into larvae that feed on the buried dung, then change into adult beetles and emerge from the soil.

Today the beetles are becoming more recognised as a low cost method for recycling nutrients and improving soil aeration by burying and feeding on the 'scrumptious' juices and bacteria found in dung pads.

Cherry has a 200 hectare property at Ashbourne, south-east of Adelaide, where she breeds about 100 Murray Grey x Angus cows and on sells the calves at six to nine months for the vealer market. Much of the property also consists of native scrub.

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In a good year, the property, which has a mixture of deep loam and duplex soils, has an average rainfall of about 26 inches.

Cherry purchased the property in 2003 with the aim to grow beef as naturally as possible using no irrigation and minimal chemicals.

“When I first purchased the place the soils were very acidic and had no microbe activity,” Cherry said.

“I decided to find a “natural” way to re-invigorate the soils so contacted Dr Bernard Doube from Dung Beetle Solutions Australia in Adelaide.

“He came and assessed the property’s trees and soil type and then recommended a particular species of dung beetles that would suit the region’s geographical and climatic limits.”

In June 2003, Cherry released 1000 *Bubas bison* beetles and after just one year there were obvious signs of their work.

“Usually it takes two to three years before any colony is well established and for farmers to see visual results,” Cherry said.

She said after the first year there were lots of soil casts around dung pads found near the beetles’ initial release site and there were obvious holes where the beetles had been working.

The beetles were released in the middle of the property (in an area of about 0.5 hectares) and once established, will continue for ever to benefit the soil, as long as cattle dung is being produced.

Cherry said their ability to fly often means neighbouring farms also reap the beetles’ benefits.

Bubas bison is a large winter-active dung beetle introduced from Europe into Australia more than 20 years ago.

Bernard said huge populations now occurred in some areas of southern Australia and all were derived from about 1500 beetles that were released in Western Australia in 1983-86.

After releasing the beetles on Cherry’s property, Bernard said the aim was to measure the pasture growth responses (tonnes of dry matter per hectare).

“We wanted to explain pasture growth benefits in terms of altered soil structure, improved subsoil fertility (dung is buried 30 to 50 cm deep) and improved soil biology (such as earthworms),” Bernard said.

He said on the 100 m² experimental plots at Cherry’s property, dung beetle activity had increased pasture production by 22% compared with dung-only plots over the June to October 2007 period, ie, from 3.5 to 4.2 tonnes dry matter per ha. Of great significance was the observation that the beetle-induced increased pasture production was still clear 2 years after the beetles had buried the dung pad, while the response to dung had long vanished. This was due to long-term changes in soil structure and fertility caused by beetle activity.

For every 100 head of cattle in one year the amount of unburied dung is equivalent to the cost of about 0.8t of urea and 1.2 tonnes of single super so after paying just \$350 for 1000 beetles, Cherry said it was money well spent.

“Dung burial by the beetles has had a major impact on the chemical fertility of the subsoil (20-50 cm). There were strongly elevated levels of nitrate, phosphate, organic carbon and sulphur in beetle-affected soil,” Bernard said.

Cherry said the tunnelling behaviour of dung beetles increased the soil’s capacity to absorb and hold water, and their dung-processing activities enhance soil nutrient cycling.

“Pasture production has also improved, I haven’t sown any seed since I bought the property but as the weeds have now come under control the existing pastures, clover and ryegrass, have been rejuvenated,” she said.

Cherry now only has to fertilise biannually and at a much smaller rate per hectare. This year she spent \$10,000 on 30 tonnes of fertiliser at an application rate of 350 kilograms/ha.

One important point Cherry stressed was that dung beetles were very susceptible to chemicals, such as drenches. She said, to date, Cydectin drench was the only chemical that didn’t kill the larvae and young adult beetles.

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Cherry rotationally grazes her cattle and has found it doesn't hinder the dung beetle populations as the beetle simply flew to the new food source in the new paddock.

"In fact, I have found we now have a more even pasture production," she said.

Last year, Bernard released another 6000 *Geotrupes spiniger* beetles, that are summer and autumn active, in an attempt to have beetles working year round on Cherry's property.

Cherry said a summer active introduced beetle – *Onthophagus taurus* – already existed in the area and its numbers also had significantly increased. During summer, introduced dung beetles can destroy a pad in two to three days, and so also remove the breeding ground of the dreaded bush fly. The bush fly larvae require five to six days to mature within a dung pad.

Appendix 11: Draft pamphlet describing the impacts of dung beetles on soil and pasture characteristics



Introducing and managing deep-tunnelling dung beetles in southern Australia

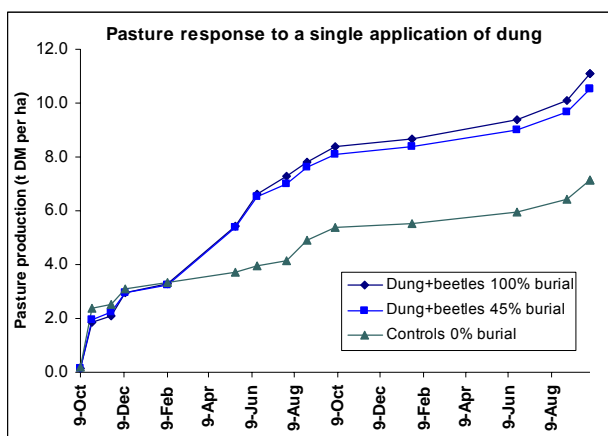
Bernard Doube, Dung Beetle Solutions Australia

This pamphlet explains the agricultural and environmental benefits of the deep-tunnelling dung beetles *Bubas bison* (active from May to October/November) and *Geotrupes spiniger* (active from December to May) and how to purchase, release and manage starter colonies in southern Australia.

It also introduces two additional deep tunnelling species, *Onitis caffer* and *Copris hispanus* (active in autumn and spring), which need to be mass reared and dispersed across southern Australia. More detailed information is available on the DBSA website (www.dungbeetlesolutions.com.au)

Dung burial by *B. bison* and *G. spiniger* increases pasture production

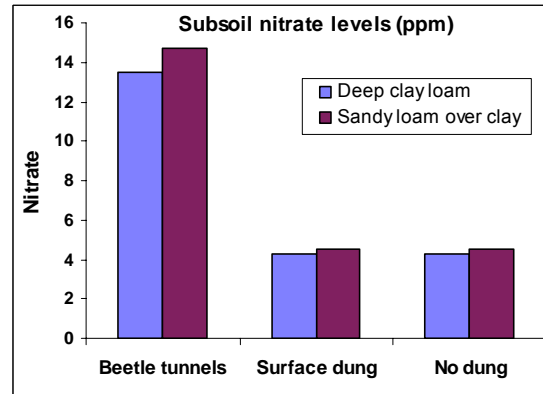
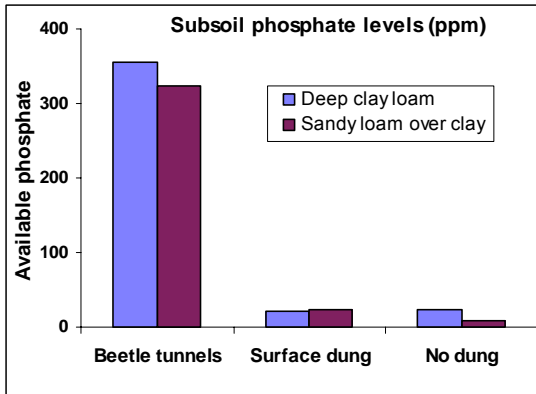
Where these species are well established and abundant, each completely buries cattle dung within days of its production over several months each year. Breeding occurs in dung buried in tunnels in the subsoil (20–50+ cm).



Dung burial by *Bubas bison* increased pasture production (the figure above shows cumulative production in tonnes of dry matter per hectare) in 2x2 m and 10x10 m field plots on the Fleurieu Peninsula, SA, over 2 years. The photo shows greater pasture growth in the dung beetle test plots than in the control plots. The increased rate of pasture growth has persisted for three years so far in one trial.

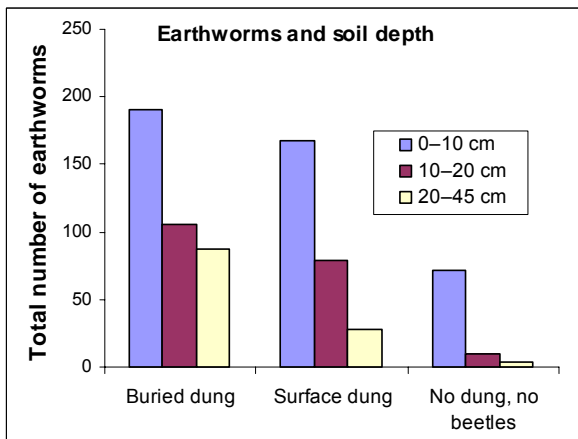
Deep-tunnelling dung beetles increase plant nutrients in the subsoil

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry



Deep-tunneling dung beetles such as *B. bison* and *G. spiniger* increase levels of plant nutrients in the subsoil. These figures show the effects on subsoil 16 months after dung was buried by *B. bison*. Similar results were obtained for subsoil levels of ammonia, sulphur and organic carbon. These effects have persisted for at least 2.5 years. Dung burial also increases levels of soil carbon and contributes to the removal of greenhouse gasses from the atmosphere.

Dung burial increases earthworm numbers



Dung burial by *Bubas bison* increases earthworm abundance and the depth at which they work

Plant roots and an earthworm associated with dung buried by dung beetles in the subsoil

Free clay spreading by deep-tunnelling dung beetles



Beetles bring subsoil to the surface



Beetle tunnels in the surface soil

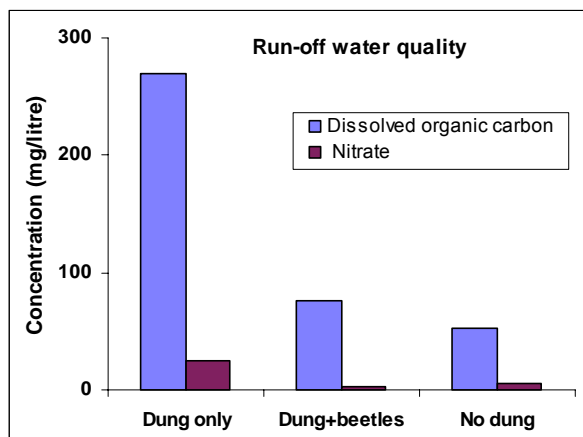


Subsoil on the surface a year later

For every litre of dung buried by *B. bison*, just over 1 litre of subsoil is brought to the surface. We estimate that each year normal beetle populations on Kangaroo Island bring about 300 tonnes of subsoil (from 30 to 50+ cm) to the soil surface for every group of 100 cattle.

Dung burial improves the quality of run-off water

The pasture growth and environmental benefits of dung beetles to the southern Australian cattle industry



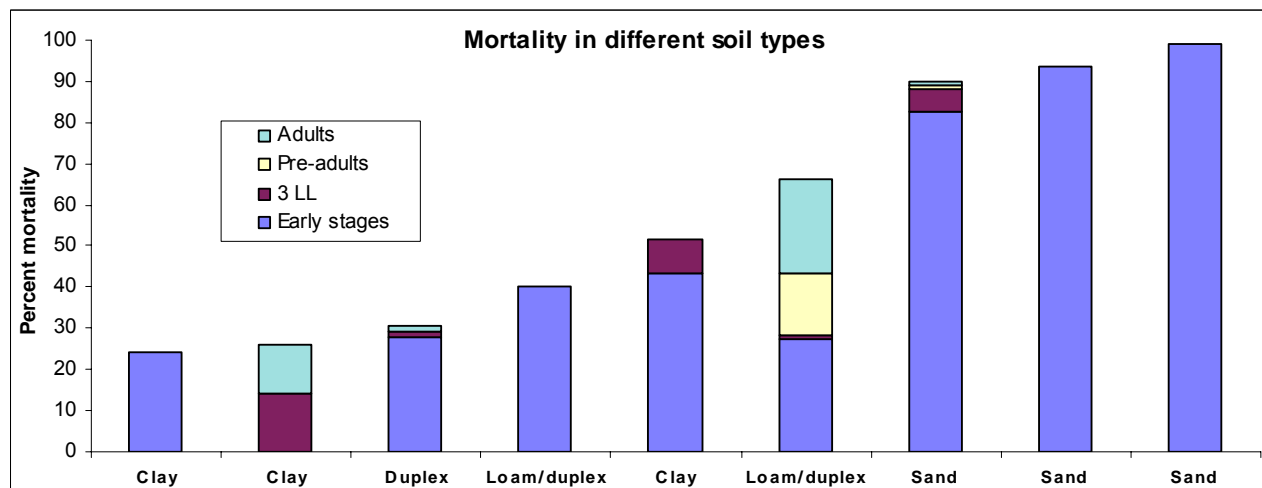
Three months after dung had been buried by beetles, a rainfall simulator (above) was used to test the levels of organic pollutants in run-off water. The levels of pollutants were high in the run-off water from plots that had dung on the surface but no beetles, but pollutant levels were similar in run-off water from plots where beetles had buried dung and from plots that had no dung at all, indicating complete removal of the pollution where beetles had buried the dung.

Dung burial increases rates of water infiltration into soil

The disruption of the surface soil by dung beetle tunnels increases the permeability of the soil to water. Increased rates of water infiltration into dung beetle-affected soil have persisted for nearly 3 years after dung burial.

Soil type preferences of *B. bison* and *G. spiniger*

Soil type has a major influence on the survival of *B. bison*; for example, it cannot breed well in deep sand. This is illustrated by the results of an experiment testing beetle survival in different soil types on the Fleurieu Peninsula.



B. bison will not establish in extensive regions of deep sand but can persist in a mosaic landscape with patches of deep sand interspersed with other, favourable soil types (loam and clay). *G. spiniger* breeds best in well-drained alluvial soils.

Seasonal activity

In South Australia, and presumably elsewhere in southern Australia, adult *B. bison* emerges from the soil in May each year, and then spend some weeks feeding, mating and maturing eggs in shallow tunnels (up to 10+ cm deep). They then dig deep tunnels (to 50+ cm) in which they breed. Breeding continues in successive dung pads until the adults die in spring or early summer. The adult emergence in autumn is not influenced by rainfall. In warmer regions the beetle has a 1-year

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life cycle but in cooler climates a portion of the population (up to 100%) has a 2-or 3-year life cycle in which the larvae enter a form of arrested development ('diapause'), delaying the emergence of the adult beetles by one or even two years. In these areas it is best to release beetles in successive years to ensure that they are present in all years.

G. spiniger has a one-year life cycle in which the adults emerge from the soil between December (in warm climates) and February (in cooler climates).

When they are abundant, both species bury all available dung within a day or so of its production. Both species will breed in cattle and horse dung.

Potential distribution of *B. bison* and *G. spiniger* in Australia

The potential distribution of *B. bison* and *G. spiniger* in southern Australia has been estimated using the known distribution of these species in Europe and Asia. Maps of the potential distribution of the beetles are given in *Introduced dung beetles in Australia 1967–2007, current status and future directions* by Dr Penny Edwards (https://wic004tv.server-secure.com/vs154616_secure/resources).

Both species are, as yet, poorly distributed throughout their potential range.

Beetle-friendly irrigation strategies for *B. bison*

The larvae of *B. bison* (found at 30 to 50+cm in the subsoil) drown in heavy soils that become excessively wet (by rainfall or irrigation) in spring and summer. However, recent field experiments on irrigated dairy properties in SA have shown that regular light irrigation throughout the summer can maintain pasture production without killing the dung beetle larvae. These beetle-friendly irrigation schedules conserve water, do not compromise pasture production and allow the dung beetles to prosper.

Other deep-tunnelling species for southern Australia

Two additional dung beetle species, *Onitis caffer* (from South Africa) and *Copris hispanus* (from Europe), are established in very limited parts of their potential distribution in southern Australia. These species are active in autumn and spring. They need to be mass reared and released in large numbers to capture the benefits flowing from deep-tunneling dung beetles in southern Australia.

There are other promising species which have not yet established in Australia. One of these is the spring-active European beetle *Bubas bubalis*, which should colonise cooler regions where *B. bison* does not prosper.

Establishing and managing dung beetles

Once you have decided that your property will benefit from dung beetles, you can purchase and establish them using some simple steps. You need the right species, at the right time of year, and in the right place, and then you need to manage them over the next few years to maximise their chances of establishing well and generating large populations.

Starter colonies

Purchasing a starter colony (usually 1000+ beetles) from a professional beetle supplier is usually the most convenient way to obtain beetles. These are harvested from field populations in regions where beetles have become abundant. In warmer regions *B. bison* has a one-year life cycle, but in colder regions a two-year life cycle is predominant. In regions where *B. bison* has a two-year life cycle, starter colonies will need to be released in two successive years to ensure that beetles are active in successive winters. *G. spiniger* has a one-year life cycle.

Selecting release sites

It is essential to select a good site for your beetles, for they will not breed successfully if they are released into a hostile environment. Choose a well-drained patch of pasture, preferably on a loamy soil or a light clay. Avoid deep sand and regions that become waterlogged. Ensure that cattle have been present for some days before you release the beetles.

Stock management

Make sure cattle are around when and where you release the beetles. Also make sure the cattle have not been drenched or sprayed with toxic chemicals for some time (depending on the chemical) before you release the beetles. Some veterinary chemicals used for parasite control make the dung toxic to dung beetles. Most of the parasite-control chemicals of the macrocyclic lactone type (for example, the avermectins) have been shown to be toxic to dung beetles, but one of these (moxydectin) appears to cause minimal damage to dung beetles.³

To avoid killing your dung beetles with toxic chemicals:

- use drenches that are not toxic to dung beetles
- if a toxic drench is unavoidable, use it only at a time when adult dung beetles are scarce
- ensure that drench and insecticide withholding periods are met before releasing dung beetles

When to release *B. bison* and *G. spiniger*

Beetles should be released near the beginning of their breeding season so that they have maximum breeding capacity. For *B. bison* this will usually be May and June and for *G. spiniger* it will usually be January to March.

How to release dung beetles

Select your release site on pasture on a loam or light clay soil where cattle have been present in recent weeks. When you receive your beetles, keep them cool. Divide the starter colony (of about 1000) into handfuls of about 20 beetles and release these onto the top of large, fresh (1–2 days old) dung pads spread over about one acre.

Management after release

It is essential that cattle remain in the vicinity of the release site for 8–10 weeks after you release the beetles to provide them with a regular supply of fresh dung. Do not treat cattle with beetle-toxic chemicals during this time.

³ The pamphlet *Consider your dung beetles when using parasiticides*, published by the National Heritage Trust and AgForce, provides an analysis of the dung beetle toxicity of chemicals used for parasite control.

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It is also important that stock are present in the vicinity of the release site in May–June in the two years after the release of *B. bison*, and in January–February for *G. spiniger*. This will ensure that there is a ready supply of food (dung) for the beetles that emerge from the starter colony (1 and 2 years after release).

About the author

Dr Bernard Doube was a Principal Research Scientist with CSIRO for 29 years and has had extensive research experience with dung beetles in South Africa and Australia. He is now Principal of Dung Beetle Solutions Australia, which collaborates with water authorities, federal agencies (eg MLA), universities and other organisations to research the influence of dung beetles on water quality, grazing systems and carbon sequestration in southern Australia.

Dung beetle FAQs

Q: Why do I need dung beetles?

A: Dung beetles can provide a self-renewing environmentally friendly solution to dung accumulation while at the same time improving pasture production, water quality and carbon storage in the soil.

Q: How many dung beetles do I need for a starter colony?

A: A usual starter colony is about 1000 beetles. Successful establishment can occur with fewer beetles but the chances of success are diminished and the time taken to breed up large numbers will be extended.

Q: How quickly will my dung beetles become established? How long after releasing a starter colony can I expect to see dung beetle activity on my property?

A: On some rare occasions, beetles (eg *B. bison*) have been recovered in the year following their release, but normally it takes at least several years before beetles become common and obvious in dung pads.

Q: How long will it take for *B. bison* to breed up to numbers that bury most of the dung produced during the winter?

A: It depends... In one instance on cattle country on Kangaroo Island, beetles were obvious and burying much dung five years after the release of 2000 *B. bison*. After 7–8 years, nearly all the dung in the paddocks surrounding the release area was being buried during the beetle's active season. Other introductions have established more slowly. If you begin with several starter colonies large numbers will build up more quickly.

Q: How does the 2-year life cycle of *B. bison* affect establishment?

A: The 2-year life cycle of *B. bison* in many cool locations, eg Central Victoria, means that starter colonies need to be released in two successive years to ensure that beetles are active each winter. Beetles released in winters of 2007 and 2008, for example, will emerge in winters of 2008 and 2009 respectively.

Q: How do dung beetles spread?

A: Dung beetles spread by flying between dung pads. Adult *B. bison* and *G. spiniger* fly for about 20 minutes just on dark and again just on light in the morning. During a season an individual beetle may live in many (possible 10 or more) dung pads.

Q: How fast do dung beetles spread?

A: It depends... At one test site on the Fleurieu Peninsula SA, *B. bison* appeared to be concentrated over about 4 ha in the year following release and then to be dispersed over 200 ha in the following season. Eight years after release of *B. bison* on KI, small numbers were recovered 6 km from the release site. However, if dung is not available or is rare in an area, it is likely that beetles could travel some kilometres in a season.

Q: Can dung beetles become a problem?

A: Dung beetles have highly specific food preferences and feed only on dung: they do not feed on other materials such as compost. Their abundance is limited by the dung supply and so over time a natural balance develops between dung beetle populations and the local dung supply.

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Q: Are there dangers associated with the introduction of foreign dung beetles; for example, disease dispersal?

A: There is no known history of transfer of dung-borne stock diseases (such as Johnes' disease) associated with dung being transported from one property to another (eg by stock transport vehicles) and so it seems unlikely that dung beetles could act as disease vectors.

Q Is toxic dung a serious problem for dung beetles?

A: Again it depends....Dung containing beetle-toxic chemicals kills adult dung beetles, reduces their egg-laying capacity and kills their young, and can decimate your dung beetle populations. Beetle-friendly chemicals are available and should be used when dung beetles are active.

Q Do dung beetles have predators?

A: Yes. Ibis, crows, foxes and other vertebrates find dung beetles to be both delicious and nutritious.

Securing and releasing your starter colony of dung beetles

A starter colony will contain 1000+ dung beetles. These will be packed in a light substrate such as damp vermiculite in a beetle-proof mesh bag. A disposable ice pack will be included with each bag of beetles to keep them cool and so minimise stress during shipment.

The beetles will be dispatched by the quickest feasible method available to your area, such as the Australia Post overnight Express Post service. Australia Post guarantees overnight delivery within their 'Next business day networks' but outside these networks delivery may take longer. We will contact you when your beetles are ready for dispatch and discuss with you the quickest means of delivery.

2008 Price list for *Bubas bison*

1–9 starter colonies: \$450 each + GST

10 or more starter colonies to one address: \$400 each + GST

A tax invoice will be included with your order.

Payment can be made by electronic money transfer (preferred) or cheque as indicated on the tax invoice.

Ordering your starter colony

Starter colonies can be ordered by telephone, post or email by contacting:

- **Bernard** **Doube**
37 Cave Ave, Bridgewater SA 5155; phone 08 8339 4158; email: bernardo@internode.on.net
- **Linc** **Willson**
Sec 3, MacGillivray, Kangaroo Island SA 5223; phone 08 8553 8203; email: linc.willson@bigpond.com
- **Kevin** **Johnson**
Private Mail Bag 77, Keith SA 5267; phone 08 8757 8291; email: kevlyn@activ8.net.au

Delivery

B. bison is usually available between May and July. *G. spiniger* is usually available between in February and March.

We will advise you by telephone when the beetles are ready for dispatch, and the dispatch date.

Releasing your beetles

The beetles should be released as soon as possible after they have arrived. Beetles can be released under all weather conditions and at any time of day or night. They will be ready to fly at dusk and at dawn.

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Release the beetles onto fresh dung pads (up to 2 days old) over an area of about one acre, placing a handful (about 20) on top of each dung pad. Ensure that cattle remain in the vicinity of the release site for at least some weeks after releasing the beetles. Do not be concerned if the beetles remain inactive for some time after release. A few beetles may die before release. This is normal.

Who supported the dung beetle research reported here?

Meat and Livestock Australia, MLA project ER211

Central Highlands Water

The Western Australian Water Corporation

The Fleurieu Beef Group and other producer organisations

DairySA

The Adelaide Mt Lofty, the Murray Darling and the Kangaroo Island Natural Resources Management Boards

Please note: The information in this pamphlet is based upon scientific evidence and is provided in good faith. However, we do not guarantee the successful establishment of populations of dung beetles.