Complementarity of dung beetle species with different functional behaviours influence dung-soil carbon cycling

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Abstract

Decomposition of large ungulate herbivore dung and its subsequent incorporation into the soil play key roles in carbon and nutrient cycling and are important for grassland productivity. Dung beetles contribute to the initial breakdown and transport of organic matter from the dung into the soil but how they interact with the microbial community to modify decomposition processes remains poorly understood.

Using a mesocosm experiment, we investigated the individual and interactive effect of two dung beetle species with contrasting functional behaviour (dweller species: *Agrilinus ater* (De Geer 1774) vs. tunneler species: *Typhaeus typhoeus* (Linneaus 1758)) on dung C cycling (CO_2 fluxes and C transfer through the soil profile) and resultant effects on microbial activity and biomass in the soil.

Both dung beetle species contributed significantly to dung removal, reducing the C lost through microbial respiration from the whole mesocosm. However, C concentrations measured in leachates from the mesocosm were only significantly higher in the presence of the tunneler species, indicating that tunnelling activity was required to increase C transfer down the soil profile. The combined effect of the two dung beetle species resulted in the highest soil microbial respiration from the soil and in particular in the 2-10 cm depth increment, suggesting positive complementarity effects between species with different functional behaviour.

We conclude that the return of C in the form of dung in grasslands, coupled with the activity of a functionally diverse dung beetle assemblage, could result in short term fluctuations in soil microbial activity with important consequences for soil C cycling.

Key–words: CO_2 fluxes, complementarity, dung removal, functional diversity, microbial biomass, soil carbon.

1. Introduction

In grasslands the decomposition of large ungulate herbivore dung and its incorporation into soil play key roles in ecosystem carbon (C) and nutrient cycling. The way in which dung is processed is thus important for the long-term sustainability of the grassland and its productivity (Williams and Haynes, 1995; Zaman et al., 2002; Aarons et al., 2009; Yoshitake et al., 2014). On pasture stocked at rates of 700 cow days ha^{-1} y^{-1}, dung deposition adds around 22 t ha^{-1} of C (Bol et al., 2000), providing a significant input of C to soil. This C input is thought to contribute to soil C stocks in temperate grasslands with 10-16% of cow-dung C incorporated into the soil in only two months (Bol et al., 2000; Dungait et al., 2005). However, a significant proportion of dung-C is lost through microbial respiration (Lovell and Jarvis, 1996; Chen et al., 2011; Grilo et al., 2011). In addition, dung can stimulate microbial activity in the soil underneath the dung, resulting in the loss of pre-existing soil C (Bol et al., 2003). Any factor that modifies microbial decomposition of dung is therefore likely to have a strong influence on the retention of dung- and soil-C in pasture soils. One such factor could be macro-invertebrates, which are responsible for the initial breakdown and transport of organic matter from the dung into the soil (Stevenson and Dindal, 1987; Lee and Wall, 2006).

In many regions a large proportion of dung removal is mediated by dung beetles, which use the dung both for feeding and breeding (Yamada et al., 2007; Lee and Wall, 2006, Nichols et al., 2008). Adult dung beetles feed on the liquid part of fresh dung (Holter, 2000) but some also create tunnels in the soil in which they store dung for further feeding or for the creation of brood balls that host eggs and developing larvae (Cambefort and Hanski, 1991). The activity of dung beetles (Owen et al., 2006) and that of other soil macro-invertebrates such as earthworms (Hendriksen, 1997), has been reported to

increase the concentration of C in the upper soil horizon. Soil macro-invertebrates, including dung beetles, have also been reported to strongly influence greenhouse gas emissions (e.g. CO_2, N_2O and CH_4) from dung (Lubbers et al., 2013, Pentillä et al., 2013), suggesting that these organisms influence microbial activity and dung decomposition rates. The main mechanisms by which this might occur are: (1) by feeding on dung and increasing the rate at which it dries out on the soil surface, which reduces the amount of resource available for microbes, and (2) by burying dung, which increases its exposure to soil microbes and changes the environment in which it is decomposed. Dung beetle behaviour can also change the surface area of the dung, which alters its accessibility to microbes and may influence the amount of C moved further down the soil profile in leachate.

Although it is clear that dung beetles influence grassland C cycling, we lack any real understanding of how dung beetle species with different functional behaviour and interactions between them affect soil microbial biomass and activity. In temperate grasslands dung beetles are typically subdivided into two main functional types, based on their nesting behaviour: dweller species (adults and larvae feed inside the fresh dung deposits) and tunneler species (adults dig tunnels in the soil under the dung deposit and bury dung for feeding and nesting). Tunneler species have been shown to be much more effective in dung removal than dweller species (Rosenlew and Roslin, 2008; Nervo et al., 2014), but the consequences of this for soil microbial activity and biomass as well as dung C retention in soils remains unknown. Furthermore, whether the two types of species interact to have a non-additive effect on dung decomposition is unknown.

The aim of this study was to examine the individual and interacting effects of two functionally contrasting dung beetle species (a tunneler and a dweller species) on herbivore-dung decomposition, microbial biomass and respiration, and the incorporation

of C into the soil. To achieve this we used a mesocosm experimental approach that allowed investigation of interactive effects of two types of dung beetle species under controlled conditions. We test the following hypotheses: (1) dung processing and the transfer of C from the dung through the soil profile will differ in the presence of the two contrasting dung beetle species, being higher when tunneler beetles are present due to their ability to bury large amounts of dung; (2) microbial respiration from dung deposits will be modified by the action of dung beetles, being higher soon after the dung is deposited as dung beetle activity will promote aerobic conditions within the dung, and lower later on as beetle activity accelerates the drying process and depletes the amount of dung resource available for microbes; (3) the interactive effects of the two functionally contrasting dung beetle species on microbial respiration will be synergistic as a consequence of greater physical processing of dung and a potentially larger more active microbial community.

2. Materials and methods

2.1. Experimental design

To examine the effects of dung beetles with contrasting functional behaviour on soil microbial respiration, biomass and dung decomposition rates, we set up a mesocosm experiment at Hazelrigg Field Station in Lancaster (54°00′49.35″N/ 2°46′30.68″W). Treatments consisted of no dung (Soil only treatment), dung only (Dung treatment), dung plus the dweller species (Dweller species treatment), dung plus tunneler species (Tunneler species treatment), and dung plus both species (Dweller & Tunneler species treatment). The dweller species was *Agrilinus ater* (De Geer 1774) and the tunneler species was *Typhaeus typhoeus* (Linneaus 1758). These two species are common in grazed grasslands

111 in the area and use dung for both adult and larval feeding. We used a complete random
112 block design with five replicates of each treatment.
113 Experimental mesocosms were constructed from 11 cm diameter sections of
114 plastic pipe and were 25 cm in length to allow the tunnelling species to construct nests
115 underneath the dung. Soil (silt loam of the Brickfield 2 association (Avis and Harrop,
116 1983), %C = 3.05, %N = 0.26, pH = 6.1) was collected from the field station and sieved
117 to 4 mm. After sieving, the soil was homogenized and the same amount (1350 g) added to
118 each mesocosm. The bottom end of the pipe was covered with fine weave plastic netting
119 material to hold in the soil while allowing water to flow through the mesocosm. Plastic 45
120 mm garden mesh was formed into a cylinder and inserted into the soil at the top of the
121 mesocosms, extending to approximately 9 cm above the top of the pipe. This acted as a
122 support for a covering of fine weave plastic netting material, with a removable lid. The
123 space created at the top of the pipe allowed the dung balls to sit on the top of the soil and
124 be exposed to natural weather conditions. The fine weave plastic netting allowed the sun
125 and rain to take effect as well as preventing colonization of the dung by flies and other
126 beetles. The lid covers were removed from all mesocosms after 20 days, once it was
127 certain that the dung had ceased to attract other organisms, and to allow the beetles to
128 leave the dung before their food resource had been completely consumed, representing
129 natural behaviour (Koskela, 1972).
130 *Agrilinus ater* specimens, along with the dung used for the experiment, were
131 collected from a sheep-grazed field on Bailrigg Farm in Lancaster (54°01′08.40″N/
132 2°47′25.98″W). Tunnelling beetles (*T. typhoeus*) were collected from rough cattle grazing
133 fields at Warton Crag Nature Reserve (54°09′07.53″N/ 2°46′45.49″W). Sheep dung was
134 used for the experiment; it was collected fresh and free of beetles from the field and
135 mixed together in a bucket and then formed into balls of equal size (mean wet weight of

199.79 ± 0.02 g), and frozen for 48 hours. Dung was fully defrosted before being added to the appropriate mesocosms. Twenty representative dung balls were also oven dried at 60°C to obtain a dry weight. Beetles were added to appropriate treatments with numbers of *A. ater* determined through allometric scaling of biomass, such that $B = M^{3/4}$ (where B is the metabolic rate and M is body mass) (West et al., 1997). This allowed species assemblage numbers to be more representative of those found in the field than through biomass alone and also to standardise biomass across treatments. For treatments containing *A. ater* equal numbers of males and females were added (28 individuals per mesocosm in the single species treatment and 14 individuals in the mixed-species treatment). For treatments containing *T. typhoeus*, only females were included (2 in the single species treatment and 1 in the mixed-species treatment). Mesocosms were sealed with the mesh lids immediately after the beetles were added. The experiment was set up on the 28th May 2010 and allowed to run for 6 weeks in the field to enable both adult and larval dung beetle effects on the dung to be included (Rosenlew and Roslin, 2008).

2.2. Dung removal

At the end of the experiment, dung remaining on the soil surface was removed from the mesocosms and weighed before being dried in an oven at 60ºC and then reweighed. The amount of dung lost during the experiment was assessed in two ways: (a) comparison between the original and final wet mass of each dung ball allowed us to measure the amount of dung lost due to both dung removal by beetle activity and the drying effects of time (Rosenlew and Roslin, 2008; Slade et al., 2007) and (b) the dried weight of fresh dung balls allowed the original dry mass of each dung ball to be estimated and comparison of the original and final dry masses allowed estimation of the actual amount of dung removed by beetle and microbial activity.

2.3. In situ CO_2 fluxes

Microbial activity was measured as CO_2 fluxes at weekly intervals; from seven days after mesocosms were set up and up to 35 days. Gas fluxes were measured using a portable IRGA EGM-4 with an SRC-1 soil respiration chamber placed directly onto the top of the mesocosms. Measurements were taken over a maximum of 120 s, and sampling was started at 10 a.m. on each measurement day. In addition, immediately after the final measurement, a further measurement was taken following the removal of any dung remaining on the soil surface to allow microbial activity in the soil alone to be measured.

2.4. Soil carbon measured in leachates

The effect of dung beetles on C movement through the soil profile was measured by collecting leachates from the bottom of each mesocosm. Leachates were collected throughout the experimental period, the morning after any rain events. Due to the unusually low rainfall for this period, mesocosms were watered (simulating 20 mm rainfall events) on two occasions corresponding with week 2 and 4 of the experiment (on the 11th and 25th of June, respectively). Leachates were collected in bottles through a funnel attached to perforated saucers placed underneath mesocosms. Once collected, leachates were filtered through Whatman filter paper no. 1, refrigerated, and analyzed within one week. Total dissolved C was determined using a Shimadzu 5000A TOC analyser. The majority of C in the leachates was organic (94 %).

2.5. Basal microbial respiration and microbial biomass

At the end of the experiment, soil within the mesocosms was harvested from the 0-2 cm and the 2-10 cm depth increments, as dweller activity is restricted to the dung and the first cm of soil while tunnelers activity will also affect deeper soil horizons. These soil

samples were used to measure the effects of the beetles on soil microbial activity and biomass at different depths. Soil subsamples from each mesocosm and depth increment were adjusted to 40% moisture content and 2 g dry weight equivalent weighed into MacCartney bottles. Bottles were incubated overnight at 17 ºC, and then sealed. Gas samples (0.5 mL) were injected into an IRGA (model ADC-225-MK3; Analytical Development Co. Ltd, Hoddesdon, UK) 1 and 4 hours after sealing to give a measure of basal respiration. Microbial biomass was measured using substrate-induced respiration (SIR). The methods used were the same as for basal respiration except 0.02 g of glucose was added to each soil subsample before sealing, and gas samples were taken 1 and 3 hours after sealing (West and Sparling, 1986).

2.6. Data analysis

To test the effects of dung beetle assemblages and time on CO_2 fluxes and changes in dissolved carbon in leachates over the six week period we used Linear Mixed Models (LMMs), with treatment and time as fixed factors, and mesocosm identity nested within block as a random factor to account for our experimental design and the repeated measures nature of the measurements. Fluxes of CO_2 were logarithmic transformed to improve normality. The significance of fixed effects and interactions was assessed by sequential deletion from the maximal model using maximum likelihood parameter estimation. Deviance change between models with and without individual terms was tested using chi-squared (χ^2) tests. The final model, including significant fixed effects and the random effects, was re-fitted under REML parameter estimation (Zuur et al., 2009).

At the end of the experiment, differences between treatments of dung mass loss (measured as wet and dry weight), the overall amount of dissolved C lost in leachates, the CO_2 flux from each mesocosm with and without remaining dung, and soil microbial

respiration and biomass at different soil depth increments were analysed using LMMs with block as a random and treatment as fixed factors. If normality or equal variance was not met data were log transformed for analysis. For soil microbial basal respiration and SIR in the 2-10 cm depth increment the presence of 2 outliers meant that even after transformations, data did not have a normal distribution. We therefore carried out a non-parametric test (Kruskal Wallis) for this response variable, and also conducted a LMM after removal of the outliers. To test for evidence of complementarity between dung beetle species we compared the observed effect of both species together (mixed species treatment) with that expected based on the single species treatments. Expected effects were calculated by dividing the effect of each species in the single species treatments by two, as the density of beetles in the mixed group treatment was half of that in the single species treatment, and then adding them together. This calculation was performed for each block; we then used LMM to test for significant differences between observed and expected effects. All analyses were conducted using the R statistical software (R Development Core Team, 2011) and all LMMs were fitted using the 'lme4' package (Bates et al., 2012).The 'R' package 'effects' (Fox, 2015) was used to calculate upper and lower 95% confidence intervals to determine significant differences between levels of the fixed factors.

3. Results

3.1. Dung removal

The contribution of dung beetles to dung removal was measured as wet and dry mass lost at the end of the experiment. Decrease in dung weight, measured as % mass loss of the initial dung wet weight, significantly differed between treatments ($\chi^2 = 20.350$, df = 3, $p < 0.001$) with higher loss in treatments containing the tunneler species (tunneler species

236 only and dweller & tunneler species treatment) compared to the dung only control
237 treatment (Fig. 1a). The tunneler species alone treatment also reduced dung wet mass
238 much more than the dweller species alone (Fig. 1a) but there was no difference in wet
239 dung mass loss between the dweller species alone and the dung alone treatment (Fig. 1a).
240 When dung weight loss was expressed on a dry weight basis, the presence of beetles of
241 any species significantly increased mass loss over that in the dung alone treatment (χ^2 =
242 25.934, df = 3, $p < 0.001$, Fig. 1b), but no differences were found between different beetle
243 treatments.
244
245 *3.2. In situ CO_2 fluxes*
246 We measured the individual and combined effect of dung beetle species on microbial
247 respiration from the mesocosms over time. Microbial respiration from the mesocosms
248 with dung added was higher than the soil only treatment throughout the 6-week
249 experimental period, with the magnitude of this difference declining over time (Fig. 2).
250 Microbial respiration differed significantly between treatments but the nature of that
251 difference depended on the week of measurement (Table 1). In comparison with the dung
252 only treatment, dung beetles significantly decreased microbial respiration three and four
253 weeks after the experiment began but had no effect during the first and the last two weeks
254 of the experiment (Fig. 2 & Fig.3a). At the end of the experiment, after the dung balls
255 were removed, soil microbial respiration significantly differed between treatments (χ^2 =
256 19.291, df = 4, $p < 0.001$). Microbial respiration in the soil was increased by the presence
257 of dweller and tunneler beetle species together compared with the soil only, dung only
258 and dung colonised by the dweller species only treatments (Fig. 3b). Moreover, there was
259 a significant synergistic positive effect between the two dung beetle species, with higher
260 observed CO_2 fluxes when both species were together in comparison to that expected

based on an additive effect (mean ± SE: observed= 0.67 ± 0.08, predicted = 0.46 ± 0.04; $\chi 2 = 6.450$, df = 1, $p < 0.011$). The presence of the tunneler beetle species (without the dweller beetle species) increased soil microbial respiration significantly over the soil only treatments but not over the dung only or dweller species only treatments (Fig. 3b). Finally, no significant differences in soil microbial respiration were found between the dweller species only, dung only and soil only treatments (Fig. 3b).

3.3. Soil carbon measured in leachates

We measured the amount of soluble C lost in leachates to assess the individual and combined effects of the two dung beetle species on the transfer of dung C through the soil profile. Total rainfall recorded during the period between leachate measurements in weeks 2, 4 and 6 was 37 mm, 24 mm, and 69 mm, respectively. The amount of dissolved C measured in leachates increased with time, and differed between treatments, but no significant interaction was found between week and treatment (Fig. 4a, Table 1). Treatments containing the tunneler beetle species alone lost more dissolved C in leachate than any other treatment (Fig. 4a).

The total amount of dissolved C in leachates over the entire experimental period was significantly greater in dung colonised by the tunneler beetle species alone ($\chi 2 = 30.545$, df = 4, $p < 0.001$) than in any other treatment (Fig. 4b). Treatments containing the dweller and tunneler beetle species together also resulted in higher overall losses of dissolved C than in soil only, as did dung alone (Fig. 4b). Dung with the dweller beetle species only, however, had no detectable effect over soil alone (Fig 4b).

3.4. Soil microbial respiration and biomass

We measured microbial basal respiration and SIR in the soil at two depth increments to assess the individual and combined effect of the two dung beetle species on soil microbial activity and biomass respectively. In the 0-2 cm soil depth increment the level of microbial respiration significantly differed between treatments (χ^2= 22.648, df = 4, p < 0.001). The presence of dung beetles significantly increased microbial respiration compared to the soil alone treatment (Fig. 5a). Despite numerical increases, the presence of beetles did not increase microbial respiration enough to cause a significant difference between beetle treatments and dung alone (Fig. 5a). A similar trend was found for microbial biomass, with SIR values being higher in the upper 2 cm of soil in mesocosms with dung added compared to the soil only treatment, but no difference found between any other treatment (χ^2 = 27.255, df = 4, p < 0.001).

In the 2-10 cm soil depth increment the combined activity of the dweller and tunneler beetle species together resulted in a significantly higher microbial respiration compared with the soil only, dung only and dung colonised by the dweller beetle species only treatments (Fig. 5b; LMM without 2 outliers: χ^2 = 29.699, df = 4, p < 0.001; Kruskal Wallis non-parametric test including 2 outliers: χ^2 = 15.399, df = 4, p < 0.005). In addition, there was a significant synergistic effect between dung beetle species, with higher observed CO_2 fluxes when both species were together in comparison to that expected based on an additive effect (mean ± SE: observed= 1.20 ± 0.19, predicted = 0.81 ± 0.13; χ^2 = 3.881, df = 1, p < 0.049). In contrast, there was no significant difference in SIR between treatments in the 2-10 cm depth increment (LMM without 2 outliers: χ^2 = 5.663, df = 4, p = 0.226; Kruskal Wallis non parametric test including 2 outliers: χ^2 = 7.492, df = 4, p = 0.112).

4. Discussion

Our results showed that both the presence of dung beetles, and in some cases, the interaction between the two dung beetle species, affected the fate of dung C by influencing microbial activity and C transfer into the soil. Both types of beetles reduced the C lost through microbial respiration from the dung deposits and increased microbial respiration from the soil. However, the differential use of dung by the dweller and tunneler species meant that the presence of tunneler beetles was required to significantly increase C transfers through the soil profile and enhance microbial respiration deeper in the soil. Interestingly, the combined effect of the two dung beetle species resulted in a synergistic, positive effect on soil microbial respiration. This suggests that complementarity between species with contrasting functional behaviour could be important for facilitating dung transfer into the soil and stimulating microbial activity.

4.1. Dung removal

In general, the tunneler beetle species was the most effective in removing dung; their presence resulted in an 80 % loss of wet mass and in 21 % loss of dry mass compared to that caused by the dweller beetle species (77.8% wet mass and 16% dry mass loss). However, this superiority was only significant when measured as wet mass loss, suggesting that this result was largely driven by differences in the effect of each type of dung beetle on moisture loss from the dung rather than actual organic matter removal. Owen et al. (2006) found that after 40 days dung pads colonised by *Aphodius fossor* beetles (a dweller species) showed no differences in external surface area and moisture content compared with uncolonised dung and beetles were observed to re-entered dung pads from the underside of the pad, near the dung-soil interface, a behaviour that would conserve moisture. Sustained dung moisture could result in high levels of food availability and optimal moisture conditions for larval survival in the dung. In contrast,

tunnelling species physically remove dung and transport it under the soil for adult feeding and larval provision, which accelerates water evaporation from the dung deposit (Brown et al., 2010). Our results therefore suggest that in cold temperate grasslands dwellers can be as functionally important as tunnelers for dung mass removal, rather than less important as previously suggested (Rosenlew and Roslin, 2008, Nervo et al., 2014).

4.2. Microbial activity and biomass

Dung beetles significantly affected how dung-C was processed by microbes. Mesocosm respiration rates during the third and fourth week of the experiment were significantly lower in the presence of dung beetles compared to the dung alone treatment. This was probably because the physical breakdown of dung by beetle activity reduced resource availability for the microbes in the dung deposit and accelerated dung desiccation, reducing microbial activity (Penttilä et al., 2013). In addition, it appeared that the tunneler beetle species increased soil microbial activity underneath the dung (Fig 3b), and increased the amount of dissolved C leached from the dung (Fig. 4). Together, this suggests that tunnelling activity facilitates the transfer and use of dung-C in the soil to a greater extent than when only the dweller beetle species was present.

Interestingly, the presence of the dweller and tunneler beetle species together had a synergistic, positive effect on soil microbial respiration. Complementarity and/or facilitation in dung removal have been previously reported between functionally different dung beetle species (Slade et al. 2007) and between dung beetles and other coprophagous invertebrates (Holter, 1079; O'Hea et al., 2010). However, to our knowledge, this is the first experimental evidence that such functional species complementarity promotes microbial respiration and modifies C cycling. The amount of organic matter removed from the dung deposit as well as the amount of C measured in leachates was similar in the

360 treatment with the dweller and tunneler beetle species together and with the tunneler
361 species only, so it appears that the mechanism behind this complementarity effect was not
362 due to resource availability. A possible explanation is that each species stimulated the
363 activity of different components of the microbial community. *Aphodius* beetles (dwellers)
364 have been reported to increase bacterial density through substrate mixing (Lussenhop et
365 al., 1980), while tunnelers have been shown to enhance fungal growth (Yokohama et al.,
366 1991). The complementarity effect was most evident in the 2-10 cm soil horizon, with the
367 dweller and tunneler species together being the only treatment to significantly increase
368 soil microbial activity above dung alone, as well as being 25% greater than the tunneler
369 species alone (Fig. 4b). That such functional complementarity occurs at this depth is
370 important for grassland productivity, as around 44% of grass root biomass is concentrated
371 in the top 10 cm of soil (Jackson et al., 1996). Further research is required to fully
372 understand this complementarity and its potential effects on pasture health.
373 Finally, despite the obvious effect of beetles on microbial activity, below the 0-2
374 cm depth increment there were no significant effects of dung or beetle treatments on soil
375 microbial biomass measured as SIR. This discrepancy between activity and biomass
376 results is consistent with the idea that, whereas microbial activity is influenced rapidly by
377 the input of labile C, soil microbial biomass is determined by the long term input of stable
378 organic C (Bardgett et al., 1998). Additionally, dry conditions during the experiment may
379 have affected the ability of microbes to process the extra C into biomass, resulting in no
380 significant differences among microbial biomass in treatments containing dung.
381
382 *4.3. Dissolved carbon transferred into the soil*
383 In general, the presence of dung increased the dissolved C content of leachates, consistent
384 with previous findings (Haynes and Naidu, 1998; Zaman et al., 2002; Arons et al., 2009;

385 Yoshitake et al., 2014). The additional effect of beetles on transfer of dung C deeper into
386 the soil was dependent on beetle functional behaviour, with the largest quantities of
387 dissolved C in leachates in the treatment with the tunneler beetle species only and the
388 lowest in the treatment with the dweller beetle species only. The higher C content of
389 leachates in the tunneler species alone treatment is likely due to facilitation of water flow
390 down the tunnels and water movement past the buried dung. Similarly, the burial of dung
391 beneath the soil surface by earthworms has been found to raise the levels of C in soil
392 (Hendriksen, 1997). The lower dissolved-C content of leachate from the dweller species
393 alone treatments may be due to these beetles promoting microbial respiration within
394 dung deposits, which could result in more C being lost through respiration and hence
395 lower C availability for leaching (Steven and Dindal, 1987). This is supported by the fact
396 that at the end of the experiment respiration was similar from all mesocosms with beetles,
397 but a greater proportion of this was from the dung in the dweller species alone treatment
398 (Fig. 3). However, the difference between dweller and tunneller beetle species in the
399 amount of C recorded in leachates suggests that although the tunneler species was more
400 efficient at transferring C into the soil, it may also have increased its vulnerability to loss
401 from the soil via leaching.
402 In conclusion, our results show that dung beetles and their functional behaviour
403 differently contribute to C transfer from the dung into the soil, affecting dung
404 decomposition, carbon cycling and soil microbial respiration. Importantly, the presence
405 of both types of species was needed to achieve the highest levels of soil microbial
406 respiration, suggesting complementarity between species with contrasting functional
407 behaviour in stimulating the soil microbial community. Together, this suggests that
408 changes in dung beetle assemblages could have a significant effect on the way in which
409 dung C is cycled in grasslands. It remains to be seen whether our results can be

transferred to other species, and whether differences in the relative abundance of functional types can significantly affect dung C processing and retention in grassland systems.

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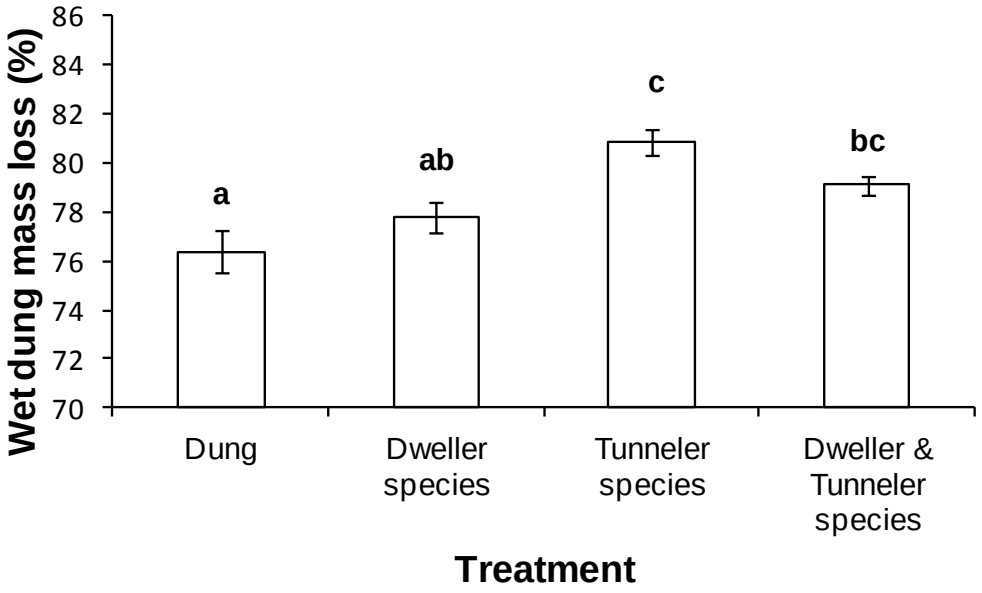
562 Table 1. Results of linear mixed models (χ^2 provided, see methods) for the effect of week
563 of measurement and treatment on the microbial respiration (CO_2 flux) and the dissolved
564 carbon measured in leachates from the mesocosms. Mesocosms were nested within
565 blocks as random factors in the analysis to account for repeated measures. Total sample
566 size was 150.
567

	CO_2 flux			**Dissolved carbon**		
Term	**χ^2**	**df**	**p**	**χ^2**	**df**	**p**
Treatment	172.490	24	< 0.001	40.629	12	< 0.001
Week	208.830	25	< 0.001	97.033	10	< 0.001
Treatment*Week	112.080	20	< 0.001	6.210	4	0.624

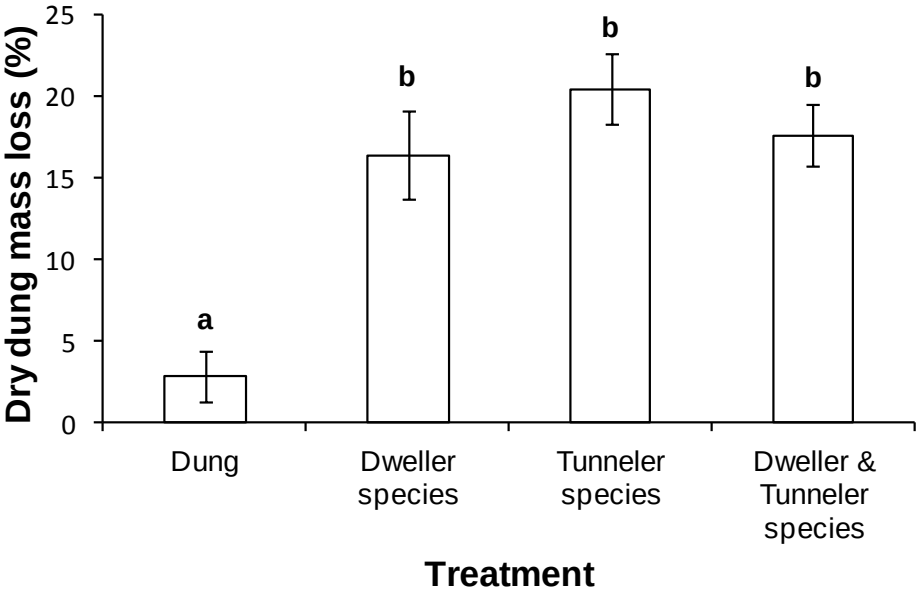
568
569

570 **a)**



571

572 **b)**

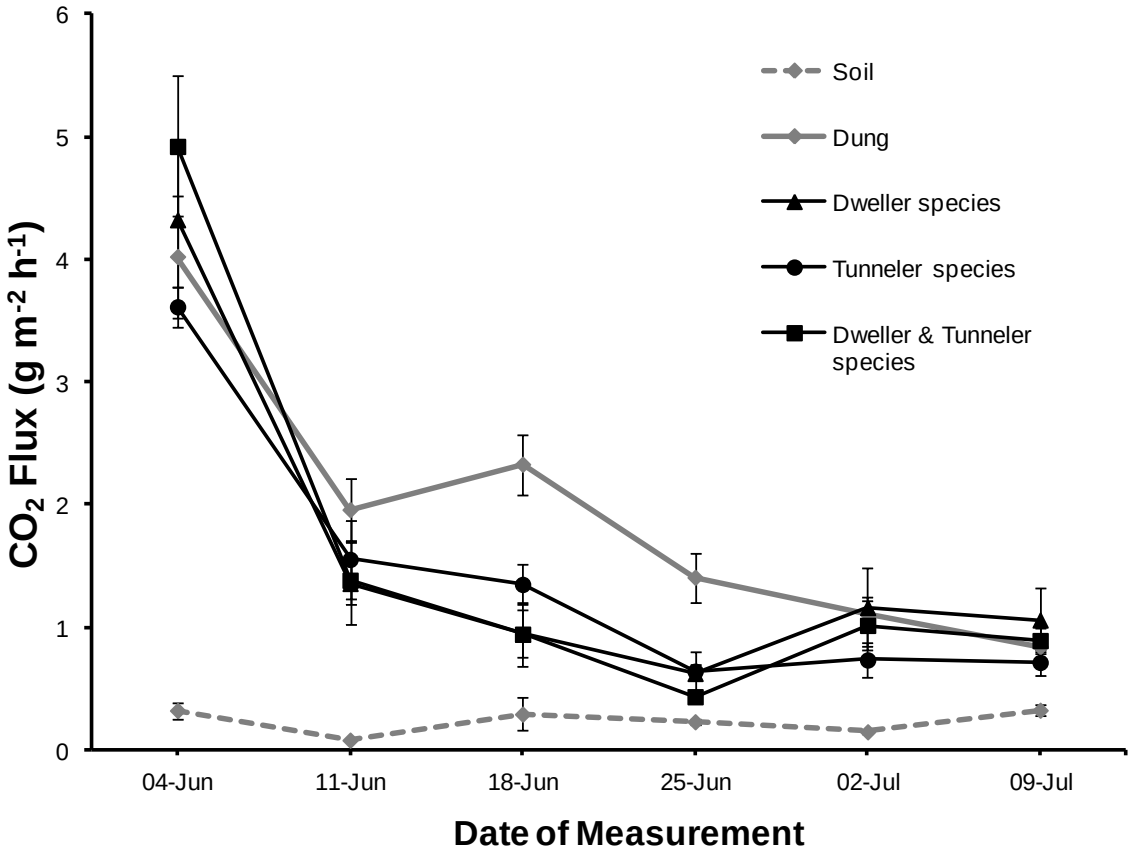


573

574 **Figure 1.** Effects of treatments on the amount of (a) wet mass and (b) dry mass of dung
575 lost at the end of the experiment. Bars represent mean ± SE. Different letters indicate
576 significant differences between treatments based on 95% CI of parameters estimates.

577

578

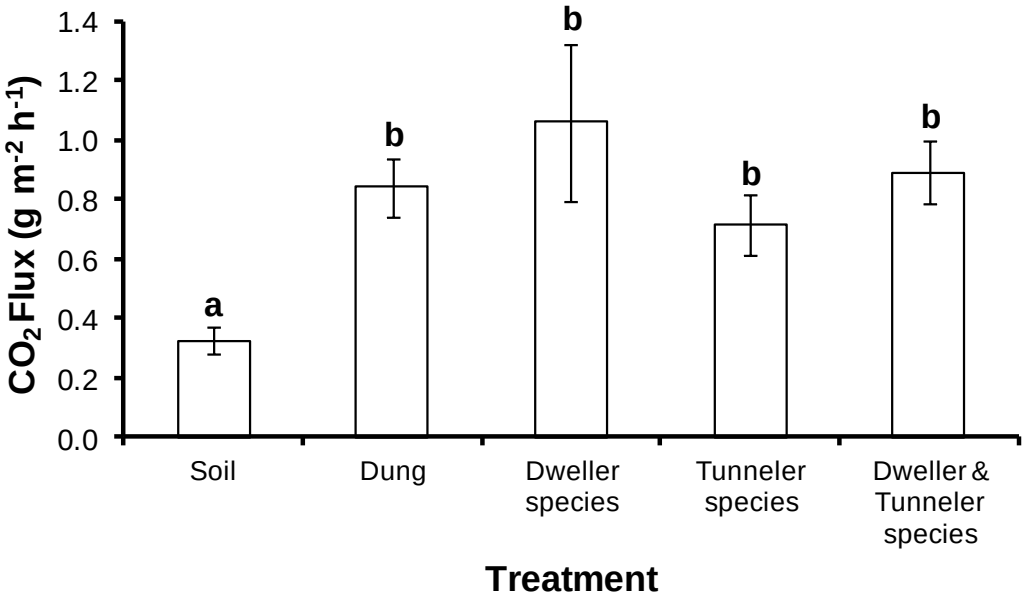


579

580 **Figure 2**. Effects of treatments on changes in microbial respiration (CO_2 flux) over the
581 six weeks of the study. Bars represent mean ± SE.

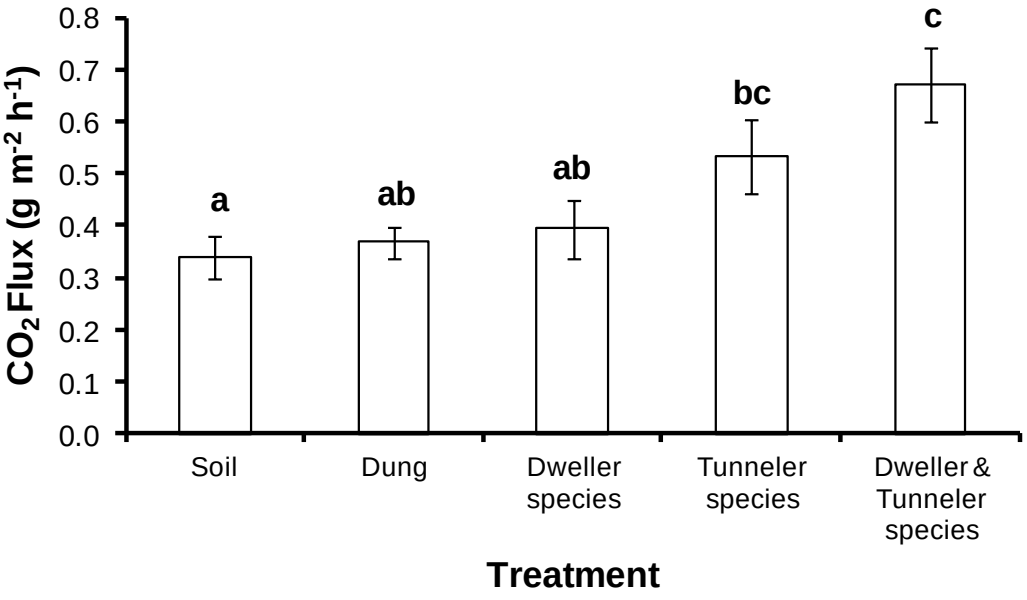
582

583 **a)**



584

585 **b)**

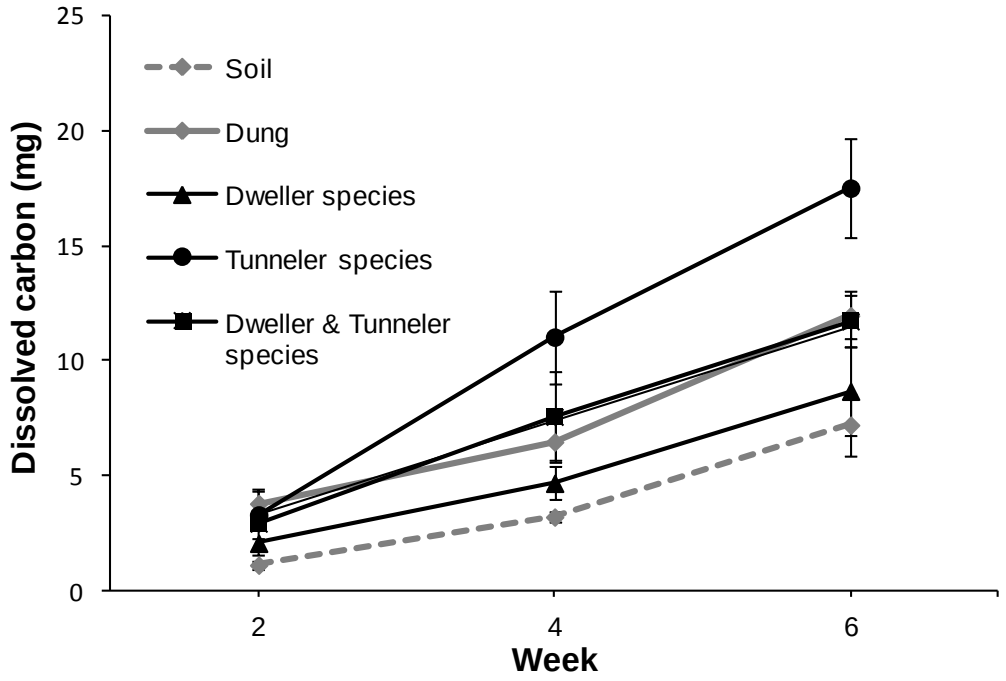


586

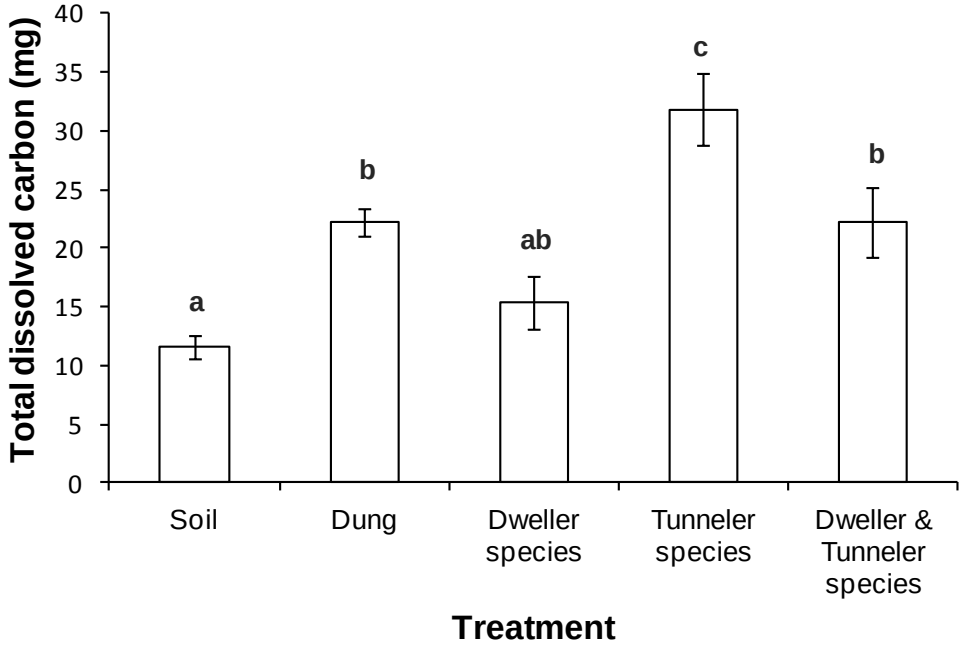
587 **Figure 3.** Effects of treatments on microbial respiration (CO_2 flux) (a) from the whole
588 mesocosm and (b) from the soil after the remaining dung on the soil surface has been
589 removed at the end of the experiment. Bars represent mean ± SE. Different letters indicate
590 significant differences between treatments based on 95% CI of parameters estimates.

591

592 **a)**

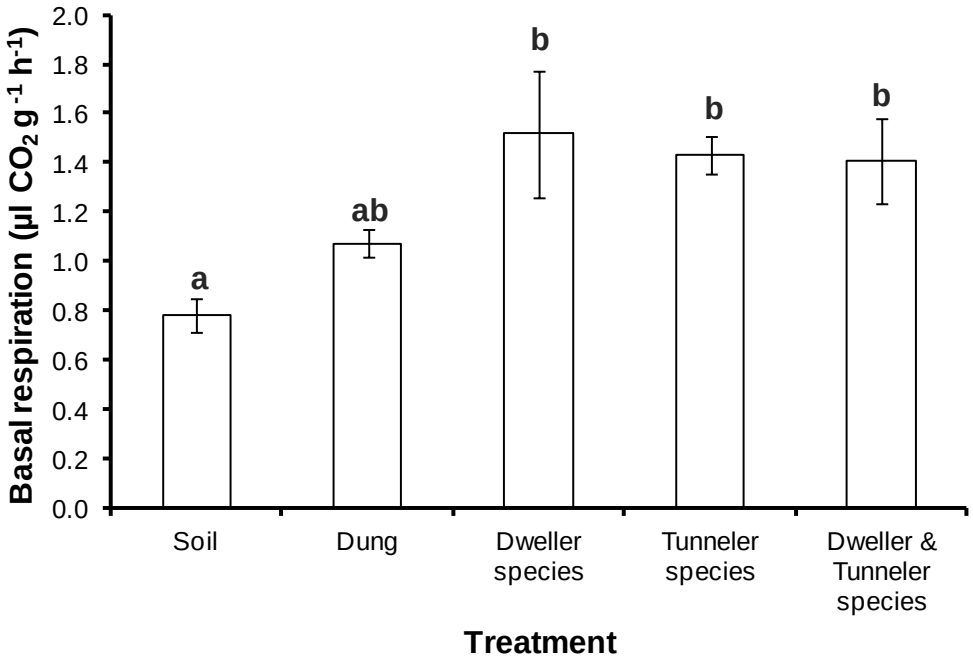


593
594
595 **b)**
596



597
598 **Figure 4**. Effects of treatments on (a) changes in dissolved carbon collected from
599 leachates over the six week duration of the study (measures taken every 2 weeks) and (b)
600 total amount of dissolved carbon collected from leachates throughout the experiment.
601 Bars represent mean ± SE. Different letters indicate significant differences between
602 treatments based on 95% CI of parameters estimates.
603

a)



b)

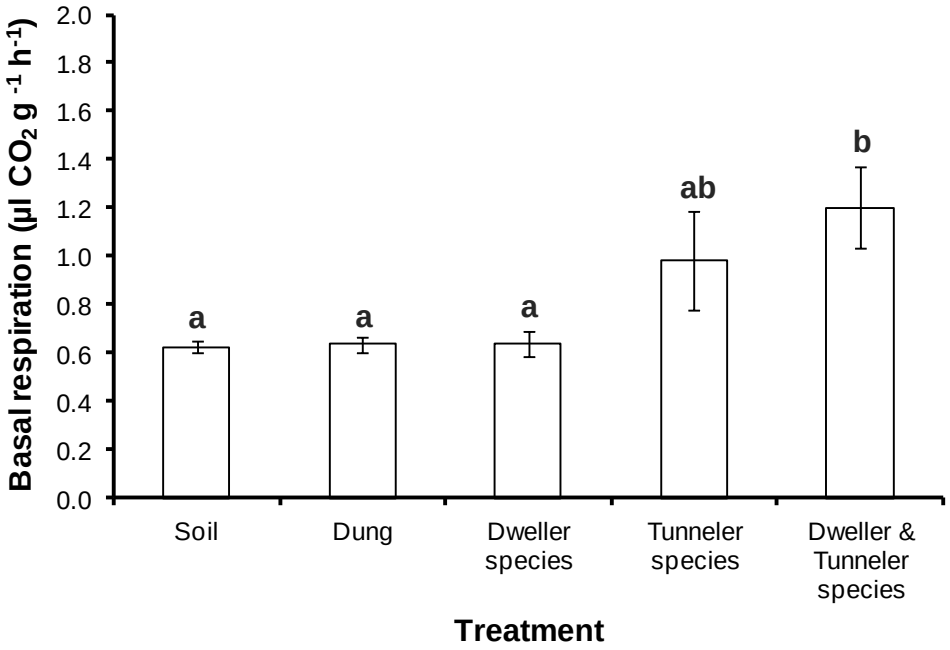


Figure 5. Effects of treatments on microbial basal respiration in (a) the 0-2 cm soil depth increment and (b) the 2-10 cm soil depth increment. Bars represent mean ± SE. Different letters indicate significant differences between treatments based on 95% CI of parameters estimates.