

Do dung beetles affect dung pat greenhouse gas emissions?

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Agroecology
March 2012

Tiedekunta/Osasto — Fakultet/Sektion — Faculty Faculty of Agriculture and Forestry		Laitos — Institution — Department Department of Agricultural Sciences	
Tekijä — Författare — Author Atte Penttilä			
Työn nimi — Arbetets titel — Title Do dung beetles affect dung pat greenhouse gas emissions?			
Oppiaine — Läroämne — Subject Agroecology			
Työn laji — Arbetets art — Level Master's thesis	Aika — Datum — Month and year March 2012	Sivumäärä — Sidoantal — Number of pages 42	
Tiivistelmä — Referat — Abstract <p>Increased concentrations of greenhouse gases in the atmosphere have accelerated global warming. Agriculture is one of the largest contributors of anthropogenic greenhouse gases (GHG), and changing consumption habits may increase this share even more. Measurements of gas fluxes from dung pats suggest that dung is a source of GHGs, but whether these GHG emissions are altered by biotic agents is so far unknown.</p> <p>In this thesis, I studied the effects of dung beetles on GHG emissions from dung pats in Viikki, Helsinki, during the summer season in 2011. A static chamber system was used to measure the fluxes of carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) from dung pats without dung beetles (n=10), with dung beetles (n=10) and a control without any dung or dung beetles (n=2).</p> <p>Overall, I discovered that the presence of dung beetles significantly affected the fluxes of GHGs from dung pats. Most importantly, fresh dung pats emitted higher amounts of CO₂ and lower amounts of CH₄ in the presence of beetles. Three weeks after the start of the experiment, emissions of N₂O showed a distinct peak in the presence of beetles – a pattern lacking in the absence of any insect fauna. For CO₂, the change in emission levels between the early and late parts of the summer actually caused pooled emission levels to almost converge between treatments.</p> <p>These patterns reveal a key impact of dung beetles on gas fluxes realized at a small spatial scale, and suggests that generally, biotic interactions may have an impact on gas fluxes from agriculture. When converted to CO₂ equivalents the differences among dung treatments were relatively small (5%), with higher emissions from the treatment without dung beetles. Therefore, the potential of dung beetles in mitigating climate change may be limited, but should not be neglected.</p>			
Avainsanat — Nyckelord — Keywords dung beetles, dung, carbon dioxide, nitrous oxide, methane, greenhouse gas fluxes			
Säilytyspaikka — Förvaringsställe — Where deposited Department of Agricultural Sciences and Viikki Campus Library			
Muita tietoja — Övriga uppgifter — Further information Tomas Roslin, Asko Simojoki, Kari Minkkinen			

Tiedekunta/Osasto — Fakultet/Sektion — Faculty Maatalous-metsätieteellinen tiedekunta		Laitos — Institution — Department Maataloustieteiden laitos	
Tekijä — Författare — Author Atte Penttilä			
Työn nimi — Arbetets titel — Title Vaikuttavatko lantakuoriaiset lantaläjien ilmastokaasupäästöihin?			
Oppiaine — Läroämne — Subject Agroekologia			
Työn laji — Arbetets art — Level Maisterintutkielma		Aika — Datum — Month and year Maaliskuu 2012	Sivumäärä — Sidoantal — Number of pages 42
Tiivistelmä — Referat — Abstract <p>Ilmakehän lisääntyneet kasvihuonekaasut ovat nopeuttaneet ilmaston lämpenemistä. Maatalous on yksi suurimmista ihmisperäisten kasvihuonekaasujen lähteistä, ja muuttuvat elintavat voivat nostaa tätä osuutta entisestään. Lantaläjistä mitatut kaasuvuot osoittavat, että lanta päästää ilmakehään kasvihuonekaasuja, mutta biottisten eliöiden vaikutus lannan kaasuvuoihin on tuntematon.</p> <p>Tässä Pro gradu -tutkimuksessa tutkin lantakuoriaisten vaikutusta lantaläjien kasvihuonekaasuvuoihin kasvukauden 2011 aikana Viikissä, Helsingissä. Hiilidioksidin (CO₂), metaanin (CH₄) ja typpioksiduulin (N₂O) mittaamiseen lantaläjistä käytettiin staattista kammimittaustekniikkaa. Mittaukset otettiin läjistä lantakuoriaisten kanssa (n=10), ilman lantakuoriaisia (n=10) ja kontrollista ilman lantaa tai lantakuoriaisia (n=2)</p> <p>Lantakuoriaisten läsnäolo vaikutti huomattavasti lantaläjien kaasuvuoihin. Tärkein tulos oli se, että tuoreet lantaläjät, joissa oli lantakuoriaisia, päästivät korkeampia määriä hiilidioksidia ja pienempiä määriä metaania kuin ne lantaläjät, joissa ei ollut lantakuoriaisia. Toinen kiinnostava tulos oli se, että kolme viikkoa kekeen alkamisesta typpiosiduuilipäästöt nousivat huomattavan korkeiksi lantakuoriaisten läsnäollessa. Ajallinen vaihtelu hiilidioksidipäästöissä aiheutti päästötasojen tasaantumista lantakäsittelyiden välillä.</p> <p>Nämä tulokset paljastavat, että lantakuoriaiset vaikuttavat kaasuvuoihin, ja että biottiset vuorovaikutussuhteet voivat vaikuttaa maatalouden ilmastokaasupäästöihin. Kun lantakäsittelyiden päästöt muunnetaan CO₂ ekvivalenteiksi, erot käsittelyiden välillä jäivät kuitenkin suhteellisen pieniksi (5%). Siksi lantakuoriaisten mahdollisuudet ilmastonmuutoksen lieventämiseen ovat rajoitetut, mutta eivät olemattomat.</p>			
Avainsanat — Nyckelord — Keywords lantakuoriainen, lanta, hiilidioksidi, dityppioksi, metaani, kasvihuonekaasu			
Säilytyspaikka — Förvaringsställe — Where deposited Maataloustieteiden laitos ja Viikin kampuskirjasto			
Muita tietoja — Övriga uppgifter — Further information Tomas Roslin, Asko Simojoki, Kari Minkkinen			

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1. INTRODUCTION

Global climate change is currently progressing at an alarming rate (IPCC 2007). Agriculture and food production are major sources of greenhouse gases (GHGs) contributing to global warming (FAO 2006). In this thesis, I examine what effect dung beetles may have on GHG emissions from dung pats. I start by examining the general processes behind climate change, and then discuss the role of agriculture in GHG emissions. Finally, I summarize prior knowledge of how biotic processes modify GHG fluxes, and end by identifying the exact objectives of my studies.

1.1 Climate change

The United Nations Framework Convention on Climate Change (UNFCCC) defines climate change as "...a change of climate that is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and that is in addition to natural climate variability observed over comparable time periods" (UN 1992). Here, the alteration in the composition of the atmosphere refers to changing GHG concentrations.

To identify the anthropogenic effect on climate, the present concentrations of GHGs in the atmosphere can be compared with pre-industrial concentrations. The concentration of the most common GHG carbon dioxide (CO₂) has increased from 280 ppm in the pre-industrial times to 379 ppm in 2005, whereas the concentration of methane (CH₄) has soared from 715 ppb to 1774 ppb (IPCC 2007). The concentration of nitrous oxide (N₂O) has increased from 270 ppb to 319 ppb, and the concentration of halocarbons was nearly zero before industrialization (IPCC 2007). This overall increase in GHG concentrations has the potential to raise global average temperatures, which may, for example, cause disruptions in the water cycles, changes in the cryosphere and sea level, and the spread of invasive species (IPCC 2007, Davidson 2009).

1.2 The effects of agriculture on climate change

The climatic effects of agriculture have been studied extensively in the past decades. FAO (2006) concluded that cattle farming produces 18% of all anthropogenic GHG emissions and 65% of the total N₂O emissions. Also in Finland, agriculture is the most important source of N₂O, with agricultural soils being the largest point sources (Pipatti et al. 1996, Monni et al. 2004). There is some uncertainty with respect to exact amounts of N₂O released from soils (Monni et al. 2004); although it has been shown that an increase in the use of N fertilizer causes an increase in N₂O emissions (Mosier et al. 1998). Also livestock manure has the potential to emit N₂O (Yamulki et al. 2000, Skiba et al. 2005, Maljanen et al. 2007, Smith et al. 2008, Wachendorf 2008 et al., Lin et al. 2009, Matthews et al. 2009).

The role of agriculture in general and of livestock in particular in mitigating climate change has been the focus of a heated debate. A global increase in meat consumption increases livestock production which directly causes an increase in GHG emissions (FAO 2006). It is then important to study all possible ways to reduce associated GHG fluxes. According to Gill et al. (2009), the management of manure may offer a particularly important strategy to mitigate climate change.

N₂O mostly originates from a process known as denitrification, i.e. the reduction of oxidized inorganic forms of N (NO₃⁻) into N₂O and N₂ (Oenema et al. 2005). Of these, N₂ is the more frequent product in moist conditions (Drury et al. 1992). Nitrification constitutes the inverse process, comprising the oxidization of ammonia (NH₃) to nitrite (NO₂⁻), then nitrite to nitrate (NO₃⁻); (Oenema et al. 2005). N₂O is a gaseous product of both of these processes, which might be released to the atmosphere depending on the conditions, mainly pH, temperature, soil moisture content and carbon and nitrogen substrate availability (Mosier et al. 1998, Tenuta et al. 2001). The estimated release of N₂O as a byproduct from nitrification is 0.1% of the amount of NH₄⁺ present (Sorai et al. 2007). Denitrification usually occurs under anaerobic conditions (especially in the case of dung; Tenuta et al. 2001), whereas nitrification always takes place in aerobic conditions. Nonetheless, Lloyd (1993) concludes that aerobic denitrification could, in fact, be blamed for many cases of N₂O emissions.

Methane is formed under anaerobic conditions in a process called methanogenesis. This process is facilitated by methanogens (methanogenic *Archaea*) using hydrogen gas (H_2) as an energy source (Stephenson and Stickland 1933, ref. Thauer 1998). The most important agricultural source of CH_4 is livestock production (FAO 2006). This is because ruminants, such as cattle and sheep, produce CH_4 through enteric fermentation, and then release it to the atmosphere by belching. CH_4 is also released from manure deposited onto fields directly by animals or indirectly by humans. As a consequence, manure storage is a known point source of CH_4 in agriculture (Jarvis et al. 1995, Holter 1997, Smith et al. 2008). Dung pats on pasture fields release CH_4 at a high rate for the first few days, then continue emitting the gas for some weeks before the emissions decline to background levels (Jarvis et al. 1995). Jarvis et al. (1995) concludes that CH_4 emissions from dung are low (<0.2 %) when compared to the gross methane emissions from the whole livestock system but that even so, these emissions are significant.

Although CO_2 is the main anthropogenic GHG, agricultural emissions of this gas are fairly small in comparison with emissions of CH_4 and N_2O . Most of the CO_2 emissions from agriculture derive from the use of fossil fuels since growing biomass binds CO_2 rather than emits it. The agricultural CO_2 emissions therefore originate mostly from the use of machinery. (Land use change is another major cause for global CO_2 emissions, and although these emissions may not be directly attributed to agriculture, agriculture is frequently the underlying cause of land clearing; IPCC 2007). Some other farming practices such as liming can also emit CO_2 (Biasi et al. 2008). While perhaps smaller than emissions from the use of fossil fuels livestock, manure is a source of CO_2 and should not be neglected (Ma et al. 2006).

1.3 Biotic influences on greenhouse gas emissions

As all possible sources of GHGs should be investigated, biotic influences on reducing GHG fluxes provide a novel and topical field to explore. Below, I will first describe the general ecosystem services provided by my target organisms – the dung beetles. Then turn to the more specific effects of dung beetles and other macro invertebrates on GHG fluxes.

1.3.1 Dung beetles and the ecosystem services they provide

The ecosystem services provided by insects are known to be many and valuable. For the U.S alone, Losey and Vaughan (2006) recently calculated that ecosystem services provided by wild insects prevent economic losses of \$57 billion per year. Of this total, the value of removal of dung by beetles was estimated at \$380 million. Moreover, dung beetles offer further services, including nutrient cycling, secondary seed dispersal and pest control (Gillard 1967, Nichols et al. 2008). For example, dung beetles can increase the uptake of phosphorus (P) and nitrogen (N) by plants in the vicinity of dung, thereby increasing overall yields (Yamada et al. 2007). In addition, dung beetles have the ability to improve the hydrological properties of soil by facilitating drainage (Brown et al. 2010).

An example of the value of dung beetles comes from Australia. When domestic cattle were imported to Australia in large amounts in the 1880s, no local dung beetle species were able to remove the resulting dung. In the 1960s, this waste problem reached such dramatic proportions that something had to be done. As a solution, some 50 dung beetle species were introduced from Africa and Europe. Since then, these species have significantly decreased the amount of dung accumulating in the pastures and the associated fly populations. (Tyndale-Biscoe 1996). Similar issues arose in New Zealand, and it was suggested by Dymock (1993) that exotic dung beetles should be imported there, and, indeed, now they have been.

Recent changes in agricultural practices have also affected dung beetles and the services that they provide. As the number of cattle farms in Finland has dwindled due to agricultural intensification, populations of several species of dung beetles have also declined (Roslin and Koivunen 2008). It is thus fair to assume that with a decline in dung beetle abundance, associated ecosystem services may also be reduced. The decline of certain species may have a strong effect on rates of dung removal, such as in the case of *Geotrupes stercorarius* (Linnaeus, 1758) in Finland. In the presence of this species, dung is removed twice as fast as without it (Rosenlew and Roslin 2008).

1.3.2 Effects of dung beetles on greenhouse gas fluxes

Although the ecosystem services provided by dung beetles have been extensively studied, their impact on GHG emissions have not. However, stray observations indicate that they may influence gas fluxes. For example, Yokoyama et al. (1991) showed that paracoprid dung beetles reduce the volatilization of ammonia (NH_3) as they increase nitrification due to aeration and enhance denitrification. Likewise, Gillard (1967) proposed that coprophagous beetles may decrease the volatilization of NH_3 from dung pats, as the dung is buried and the nitrogen thus returned to soil before it can volatilize. Unfortunately, this study included no actual measurements of gas fluxes.

If dung beetles do decrease the volatilization of NH_3 , it may leave more inorganic N in the soil for denitrification and nitrification processes, and thus influence the whole nitrogen-cycle (Ma et al. 2006). A similar observation was offered by Yokoyama et al. (1991), who stated that dung beetles could raise the nitrate (NO_3^-) levels of dung because of increased ammonifying and nitrifying activity, thereby enhancing denitrification (Yokoyama et al. 1991).

The tentative impact of dung beetles on GHG emissions examined above may be compared to effects observed for other organisms. Giannopoulos et al. (2010) has shown that emissions of N_2O and CO_2 may increase in the presence of earthworms, with similar results reported by Rizhiya et al. (2007). Both articles infer that the increased N_2O emissions may be caused by augmented denitrification. Svensson et al. (1986) found similar results regarding denitrification in earthworm casts as potentially, the respiration of microorganisms decreases the amount of oxygen (O_2) in the soil. Whether dung beetles could have similar effects on the N_2O and CO_2 dynamics of dung is an issue unaddressed to date.

With respect to CH_4 fluxes from dung and manure, the modifying effects of living organisms are so far unknown. However, several theoretical contributions suggest that the dung associated fauna may have an effect. As CH_4 is formed in anaerobic conditions and as dung beetles (*Aphodius* sp.) have a tendency to aerate the dung (Stevenson and Dindal 1987), the activity of dung beetles might reduce emissions of

CH₄. The likely size of such effect is still open to debate, as Holter (1991) argues that the main cause of aeration of dung pats is overall desiccation, rather than the tunnels dug by beetles. In contrast, Stevenson and Dindal (1987) state that coprophagous macro invertebrates have a tendency to accelerate the rate of desiccation. Thus, the net effect of dung beetles remains to be explored.

2. OBJECTIVES OF THE STUDY

Given the wide range of potential biotic impacts on GHG emissions from cow dung, the objective of this study was to empirically quantify the impact of dung beetles on N₂O, CO₂ and CH₄ fluxes from dung pats. Based on the physiochemical considerations outlined above, my *a priori* hypothesis were that dung beetles would aerate dung pats, and thereby increase fluxes of N₂O and CO₂ but decrease fluxes of CH₄. I quantified the cumulative effect of dung beetles on net releases of GHGs from dung pats over the main growing season, thereby establishing whether these organisms may in fact mitigate or even accentuate climate change.

3. MATERIALS AND METHODS

3.1 Experimental design

To examine the effect of dung beetles on gas fluxes from dung pats, I used three treatments: 1) dung pats with dung beetles (n=10), 2) dung pats without dung beetles (n=10), and 3) controls with neither dung pats nor dung beetles (n=2). The spatial distribution of replicates within each treatment was randomized (Fig. 1) among a set of 22 chamber collars. For this purpose, I used the random number generator of Excel (version 2007, Microsoft Inc., Redmond, WA, U.S.A.).



Figure 1. Experimental design used in measuring gas fluxes. (A) Twenty-two chamber collars were placed in an agricultural field, interspersed by distances of 70 cm. (B) These chambers were randomly assigned to three different treatments: 1) dung with dung beetles (black squares; $n=10$); 2) dung without dung beetles (white squares; $n=10$), and 3) chambers containing neither dung nor beetles (grey squares; $n=2$).

Dung beetles used in the experiment were collected in pastures of the Koski Manor in Salo, Southwestern Finland ($60^{\circ} 22' 49''$ N $23^{\circ} 17' 39''$ E) on May 31st and June 1st 2011. The beetles were kept in moist paper at +4 °C until used in the experiment.

Dung for the experiment was gathered from Viikki experimental barn and manually homogenized before partitioning into pats of 1.2 l. Each pat was weighed with a kitchen scale (SKS 4512, Slnbo Kowloon, Hong Kong) before distributing into the experimental chamber collars in a grassy area on the 7th of June in Viikki (Fig. 1). The moisture content of the fresh dung was determined as the average of three samples weighed first fresh, and then dried to constant weight.

On June 7th, seven species of beetles were distributed among chambers in numbers reflecting their natural distribution in the field where they were collected from (Table 1). A temperature logger was inserted into each of two randomly chosen chambers, one with dung beetles and one without.

Table 1. Dung beetle abundances used in the experiment. Column “Total” indicates species-specific totals used in the experiment, whereas column “Per chamber” offers species-specific numbers added to each replicate chamber in treatment 1.

Beetle	Total	Per chamber
<i>Aphodius ater</i> (De Geer, 1774)	730	73
<i>Aphodius fimetarius</i> (Linnaeus, 1758)	50	5
<i>Aphodius depressus</i> (Kugelann, 1792)	40	4
<i>Aphodius erraticus</i> (Linnaeus, 1758)	410	41
<i>Aphodius haemorrhoidalis</i> (Linnaeus, 1758)	110	11
<i>Aphodius pusillus</i> (Herbst, 1789)	70	7
<i>Aphodius fossor</i> (Linnaeus, 1758)	120	12
Total	1530	153

3.2 Static chamber design

To evaluate gas fluxes from dung pats, I used the static chamber method of Crill (1988). This method was chosen because of its wide application in studies of gas fluxes, its ease of use and its accuracy (Ojanen et al. 2010, Pumpanen et al. 2004, Ma et al. 2006, Jensen et al. 1996).

The chambers were constructed following the USDA-ARS GRACEnet Chamber-based Trace Gas Flux Measurement protocol 2003 (USDA-ARS 2003), with additional instructions from Kari Minkkinen and Asko Simojoki (pers. comm.) and McGinn (2006). The 25 cm-high chamber collars were sawn out of a 0.5 mm-thick aluminum air duct pipe with a diameter of 31.5 cm. Each chamber collar was then installed 10 cm into the ground, leaving a 15 cm-high collar above ground. Between measurements the chambers were closed by a metal mesh, allowing air circulation while keeping the dung beetles from escaping. The vegetation inside the chambers was kept low by manual trimming.

To seal off the gas space inside each chamber when taking air samples for methane (CH₄) and nitrous oxide (N₂O), the chamber collar in question was covered with an air-tight lid. These lids were manufactured from modified air duct cleaning vents (TUTL D 315, Onninen Co., Hyvinkää, Finland). To circulate the air within the chamber once

closed, each lid was equipped with a 12V computer fan powered by a 0.8Ah led battery. Once the lid was installed, the volume of the chamber was 10.49 liters. Each lid held two butyl rubber caps each pierced by a Teflon tube (inner \varnothing 0.96 x outer \varnothing 1.57 mm) – one for stabilizing air pressure (150 cm) and one for taking gas samples (30cm; Figure 2). To stabilize pressure while installing the lid onto the chamber collars, one of the butyl rubber caps was first removed and then replaced.

Gas samples were drawn into 20 ml syringes (PlastikPak, Becton Dickinson and Company, Franklin Lakes, U.S.A.) through a 3-way stopcock, and then injected directly through a double septum into 3 ml vials (Exetainer, Labco Inc., Buckinghamshire, UK). For accuracy in gas measurements, each vial had been flushed twice with helium and evacuated with a vacuum pump before use.



Figure 2. A static chamber collar fitted with a lid for CH₄ and N₂O measurements.

To measure fluxes of carbon dioxide (CO_2), I used a similar lid as for CH_4 and N_2O measurements. In this case, the lid was modified to fit the gas probe of a portable device (probe 8 for PP-Systems EGM-4 Environmental Gas Monitor for CO_2 , Amesbury, MA, U.S.A.) according to instructions offered by Pumpanen et al. (2004) and Kari Minkinen (pers. comm.). The probe was installed through the center of the lid and sealed with silicone (Figure 3). To reduce the increase in partial pressure when placing the lid on to the collar before CO_2 -measurements, these lids were made higher than those used for measurements of CH_4 and N_2O (see above). Hence, during CO_2 -measurements, the volume of each static chamber was 13.37 liters. To further avoid fluctuations in pressure, a butyl rubber cap was removed while installing the lid onto the chamber, then replaced.



Figure 3. The modified probe 8 for the PPSystems EGM-4 Environmental Gas Monitor, as used in CO_2 measurements.

3.3 Measurements of CH₄ and N₂O

Gas fluxes were measured on seven occasions between June 8th and July 27th, corresponding to days 1, 6, 10, 15, 20, 30 and 50 of the experiment. On each sampling occasion, the chambers were sealed for 30 minutes with the lids described above. Gas samples were taken after 5, 10, 20, and 30 minutes of sealing, with gas contents hence reflecting the accumulation of gases. Ambient temperature was recorded during the sampling, for later scaling of gas fluxes to temperatures. Data on whether it had rained on the day preceding sampling was also recorded.

To flush the Teflon tube, the 3-way sampling valve and the syringe, a volume of 3 ml of gas was first drawn into the syringe and then ejected. Subsequently 9 ml of gas was drawn into the syringe, 1 ml of which was used to flush the 1.1x40 mm (19G) injection needle and the rest (8 ml) injected into the 3 ml vial. This procedure created an overpressure in the vial, as needed for successfully injecting the samples into the gas chromatograph. As the overpressure initially used proved excessive, causing some of the septum caps to come loose, the amount of gas injected was changed from 8 ml to 7 ml on June 22nd.

Gas contents of CH₄ and N₂O were quantified in parts per million (ppm) with an HP 5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA, U.S.A.). The quantification procedure followed Jaakkola and Simojoki (1998), with the exception that He was used as a carrier gas for both chromatographs and ArCH₄ as a makeup-gas for electron capture (EC). For methane, the readings were used as such, but for nitrous oxide, nonlinearity in the sensitivity of the electron capture detector (ECD) will cause concentration changes to be overestimated. To correct for this bias, I used a nonlinear function of the measured concentration as determined by Asko Simojoki (pers. comm.). Moreover, there is also a tendency for the N₂O readings to drift during long measurement runs. This drift was corrected for through comparison with readings from standard samples inserted at an interval of ten samples in the gas chromatograph autosampler. The following nonlinear polynomial equation was fitted to these measurements using the trendline fitting function of Excel (version 2007, Microsoft

Inc., Redmond, WA, U.S.A.), and then used as a multiplier to correct all N₂O measurements (Asko Simojoki, pers. comm.):

$$A = \frac{y_c}{y_m} = ax^3 + bx^2 + cx + d \quad (1)$$

where

A = correction factor,

y_c = correct content of standard sample,

y_m = measured content of standard sample,

x = sample position in measurement run (1-96),

and a , b , c , and d are fitted parameters.

To estimate the flux of N₂O and CH₄ in the experimental chambers, I regressed measurements of gas contents on time using the fitting algorithm of Excel (version 2007, Microsoft Inc., Redmond, WA, U.S.A.). I then used the estimated slope dc/dt to estimate the overall gas flux, as based on the following equation (Song et al. 2003):

$$J = \frac{dc}{dt} \cdot \frac{M}{V_o} \cdot \frac{T_o}{T} \cdot H, \quad (2)$$

where

J = gas flux (mg/m²*h)

$\frac{dc}{dt}$ = slope of the linear regression of gas concentration

M = molar mass of the measured gas (mg/mmol)

V_o = volume at standard condition (l)

T_o = temperature at standard condition (K)

T = temperature (K)

H = chamber height (m)

Measurements of both CH₄ and N₂O proved unsuccessful for the first sampling day (June 8th), as the double septum of the vials was too thick to be penetrated by the sampling needle of the gas chromatograph. During later measurement rounds, this

problem was eliminated by elevating the bottom of the sampling tray with the aid of acrylic sheets.

3.4 Measurements of CO₂

Once samples of N₂O and CH₄ had been drawn from each chamber (above), I measured CO₂ fluxes approximately 4 hours later, using the portable infrared gas analyzer (EGM-4, PP-Systems, Amesbury, U.S.A.). The sampling time for each chamber was set to 80s and the measuring interval was 4s. Temperatures were recorded in the shade next to each chamber. I used the linearly fitted fluxes directly from the EGM-4. To convert the flux readings from the portable gas analyzer to g/m²*d, I used the temperatures recorded and the exact volume of the chamber.

3.5 Statistical analysis of gas fluxes

To analyse how the fluxes of different compounds vary with time and treatment, we used a generalized linear mixed-effects model (GLMM). The model was fitted in SAS v. 9.2, procedure mixed (SAS Institute Inc., Cary, NC), using a repeated-measures model with chamber as the subject. To account for the dependence between consecutive measurements, we assumed a first-order ante dependence structure (TYPE=ANTE(1)). The N₂O and CH₄ fluxes of replicate 19 on the 27th of June significantly deviated from all other readings, and this data point was regarded as a measurement error and omitted from all analyses.

3.6 Additional calculations

3.6.1 Carbon dioxide equivalents

Different greenhouse gases have different Global Warming Potential (GWP) due to the warming influence of the gas in question (IPCC 2007). For this reason, the Intergovernmental Panel on Climate Change (IPCC) has estimated multipliers that translate emissions of each GHG into CO₂ equivalents, to be compared and studied in a more easily tractable way. As different gases will remain in the atmosphere for

different time spans these multipliers differ depending on the time horizon considered. The most commonly used time horizon is 100 years, giving CH₄ and N₂O multipliers of 25 and 298, respectively (IPCC 2007).

To calculate the treatment-specific gas flux for each day of the experiment, I interpolated the values in between the treatment-specific means of sampling dates 1, 6, 10, 15, 20, 30 and 50 as based on the following equation:

$$y = y_1 + \frac{y_2 - y_1}{d} * x, \quad (3)$$

where

y = daily flux (g/m² or mg/m²)

y_1 = gas flux in the beginning of the time period (g/m²*d or mg/m²*d)

y_2 = gas flux in the end of the time period (g/m²*d or mg/m²*d)

d = length of the time period (d)

x = number of the day in question

To obtain the total amount of CO₂, N₂O, CH₄, and CO₂ equivalents released through gas fluxes from each treatment, daily fluxes were then summed over the full experiment.

3.6.2 Elemental C and N loss

To compare the estimates of carbon and nitrogen lost through gas fluxes with the actual changes in C and N content of experimental dung pats I weighed the dung pats at the start and end of the experiment, and estimated changes in their relative C and N content. The total amounts of elemental C and N lost through gas fluxes were estimated by multiplying the cumulative gas fluxes derived above by the C content of CO₂ (27.3%) and CH₄ (74.9%), and the N content of N₂O (63.6%), respectively.

Seven samples of the homogenized dung used in the experiment were frozen on June 6th, dried on September 23rd, and ground on September 27th, along with the remains of the 20 dung pats used in the experiment. The C and N content of the samples were

analyzed with a Variomax CNS elemental analyzer (Elementar Analysesysteme, Stuttgart, Germany). 200-300 mg of each dung sample was placed into a crucible and dry combusted gasifying the samples, which were then separated by columns and quantified by a thermal conductivity detector (Sleutel et al. 2007).

To derive adjusted estimates of weight and elemental contents at the start and end of the experiment, we used a generalised linear mixed-effects model (GLMM). This procedure corrects for the fact that multiple measurements are taken of the same units. Least squares (LS) means and their standard errors were extracted from a model of pat weight as a function of treatment, time and treatment x time. The model was fitted in SAS v. 9.2, procedure mixed (SAS Institute Inc., Cary, NC), using a repeated-measures model with dung pat identity as the subject. Identical models were fitted to response N and C content.

To calculate how much C and N had been lost between the start and the end of the experiment, I subtracted the product of a dung pat's weight and its content of C and N (%), respectively, at the end of the experiment from the same product at the start of the experiment, i.e.

$$\Delta C = p_{t-1} * w_{t-1} - p_t * w_t, \quad (4)$$

where

ΔC = loss of element (carbon or nitrogen)

p_{t-1} = element content in the beginning of the experiment (43.6% for carbon, 2.8% for nitrogen)

w_{t-1} = average weight of a dung pat in the beginning of the experiment (LS mean from the GLMM model described above)

p_t = element content at the end of the experiment (LS mean from the GLMM model described above)

w_t = weight of dung pat at the end of the experiment (LS mean from the GLMM model described above)

To convert the total emissions of C and N as estimated from gas fluxes to a currency directly comparable to the estimated losses of C and N terms of weight, I converted gas emissions in g/m^2 to gas emissions per experimental chamber. I therefore multiplied the pooled emissions derived above by 0.078 m^2 , corresponding to the area of each experimental chamber.

4. RESULTS

4.1 Temperature and precipitation

According to Finnish Meteorological Institute (FMI) weather conditions during the summer of 2011 were close to the long-term average for 1971-2000 (FMI 2011a, FMI 2011b). Precipitation in Helsinki in June 2011 was approximately 40-50 mm (FMI 2011b), and 45-60 mm in July (FMI 2011a). Major precipitation with a potential to impact the water-filled pore space (WFPS) occurred once, on day nine before the 3rd measurements. With respect to temperature, in June it was approximately 2.5-3°C warmer (FMI 2011b) and in July 3.5-4°C warmer (FMI 2011a) than the long-term average from 1971-2000. One of the temperature loggers broke during the measurements, and therefore readings were recorded with only one logger (Fig. 10). Temperatures recorded within the dung pat were higher than the ambient temperatures at the time of sampling in Viikki (Fig. 10). However, temperatures provided no clear correlation for any of the gas fluxes.

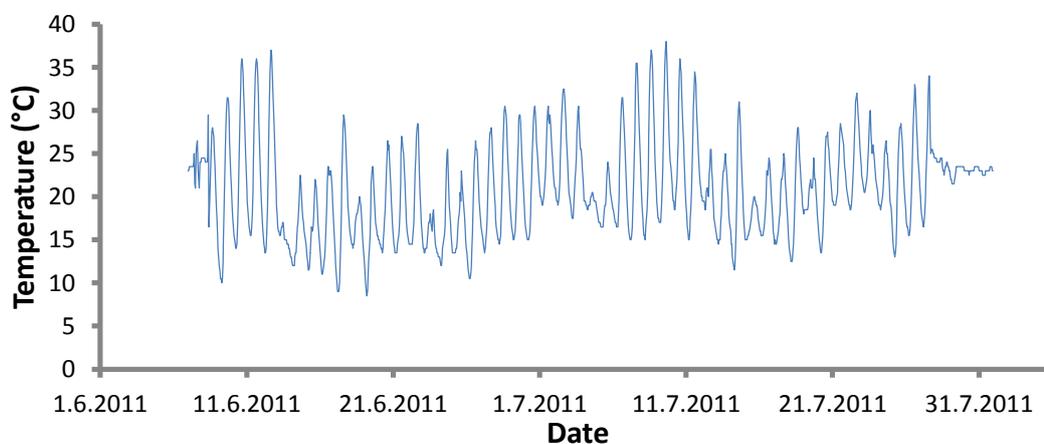


Figure 10. Temperatures recorded with a temperature logger inside a dung pat.

4.2 Temporal patterns in gas fluxes

A clear imprint of dung beetles was evident on all gas fluxes examined. This imprint was evident both in compound-specific patterns and in cumulative releases over the full course of the experiment.

4.2.1 Fluxes of CO₂

Emissions of CO₂ differed significantly among treatments (Table 2). Overall, fluxes from the control treatment were significantly lower than fluxes from treatments with dung (Table 2). In the two treatments with dung, emissions peaked markedly earlier when dung beetles were present than when they were absent (on the first versus 10th day of the experiment; Fig. 4).

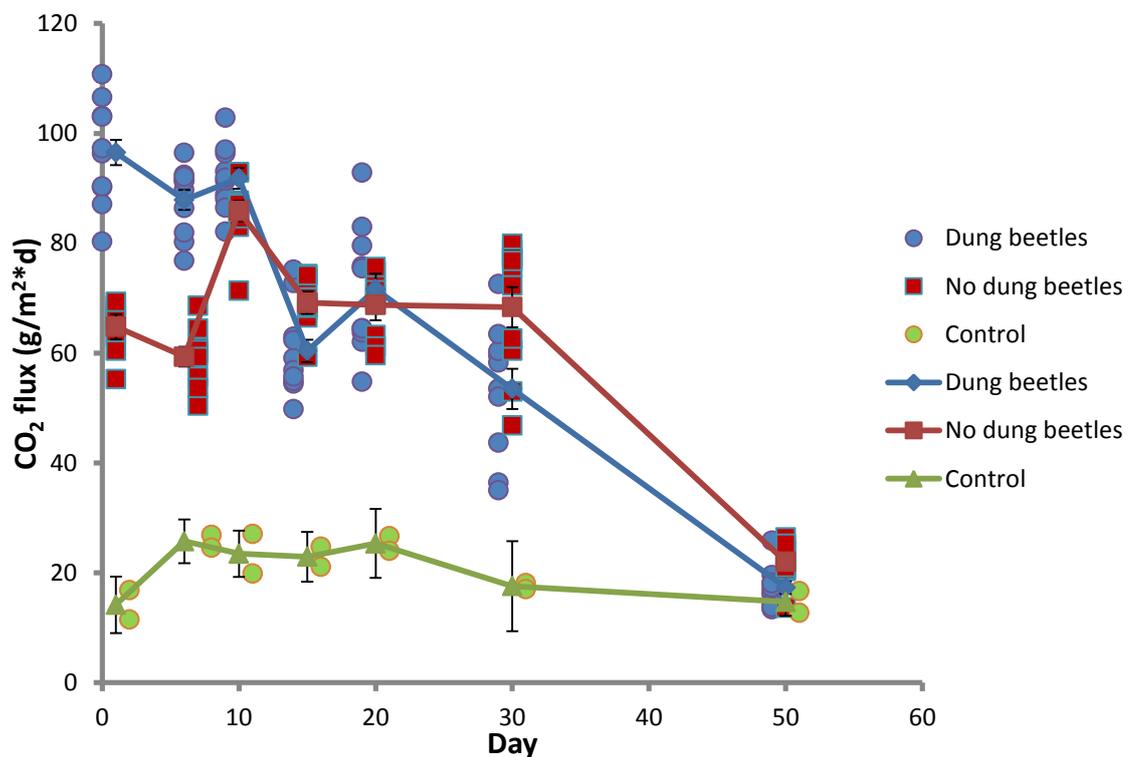


Figure 4. CO₂ fluxes observed during the 50-day experiment. Individual symbols refer to empirical observations, with treatments identified by color. Symbols with error bars (+SE) refer to least squares means estimated by a GLMM model (for details, see text). To reveal overlapping data points, empirical values were slightly offset in the horizontal dimension.

Table 2. Generalized linear mixed-effect model of changes in CO₂ fluxes over time. Type 3 *F*-tests of fixed effects are given.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	23.5	95.03	<.0001
Measurement	6	31.4	138.44	<.0001
Measurement*Treatment	12	42.2	40.72	<.0001

4.2.2 Fluxes of CH₄

Fluxes of CH₄ differed significantly among treatments (Table 3). In particular, the treatment without dung beetles showed a five-fold increase in the amounts of emitted CH₄ in the beginning of the experiment than did the other treatments (Figure 5). The control treatment served as a net sink of methane throughout most of the experiment, with a positive flux observed only on day 10. Towards the end of the experiment, the methane emissions leveled out across all treatments. At this stage, all fluxes were close to zero or slightly negative (Fig. 5).

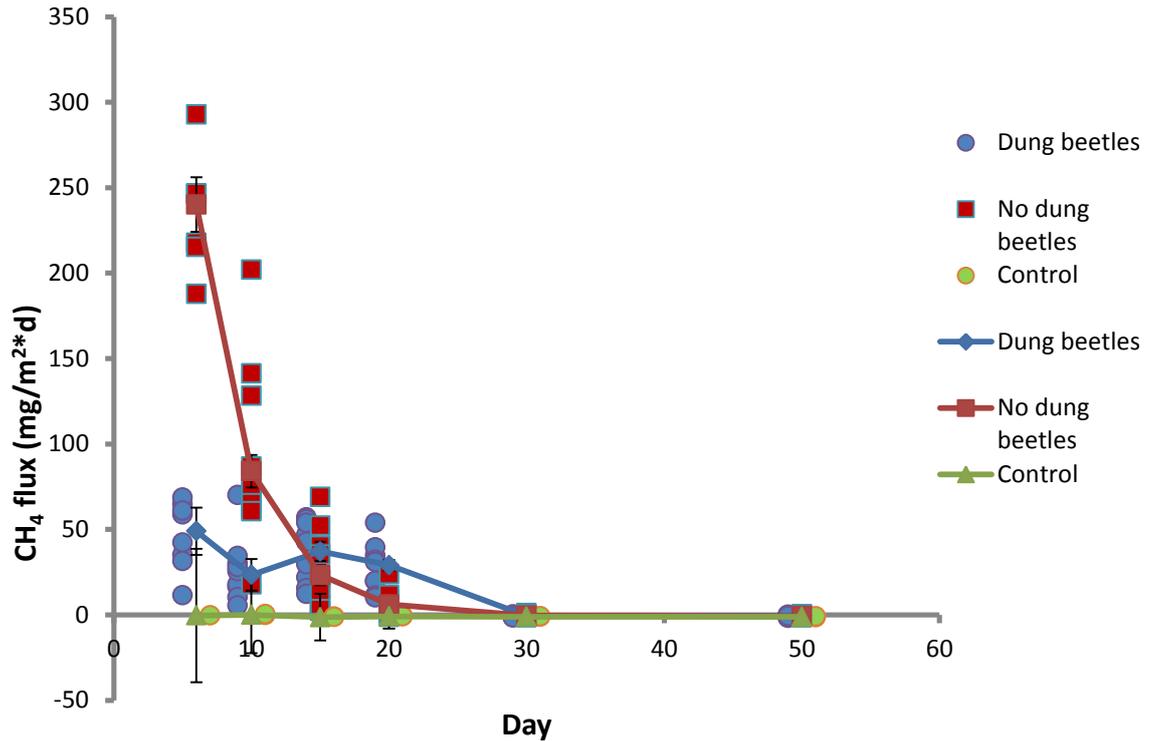


Figure 5. CH₄ fluxes observed during the 50-day experiment. Individual symbols refer to empirical observations, with treatments identified by color. Symbols with error bars (+SE) refer to least squares means estimated by a GLMM model (for details, see text). To reveal overlapping data points, empirical values were slightly offset in the horizontal dimension.

Table 3. Generalized linear mixed-effect model of changes in CH₄ fluxes over time. Type 3 *F*-tests of fixed effects are given.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	21.3	28.60	<.0001
Measurement	5	33.7	11.29	<.0001
Measurement*Treatment	10	41.1	12.91	<.0001

4.2.3 Fluxes of N₂O

Fluxes of nitrous oxide were relatively low over time (Fig. 6), but differed among treatments (Table 4). The most pronounced difference among treatments occurred as a distinct spike in N₂O emissions from the dung pats with dung beetles at day 20 (Fig.

6). Again, the control acted as a sink of N_2O , with a positive flux observed only on the last day of the measurements (day 50; Fig. 6).

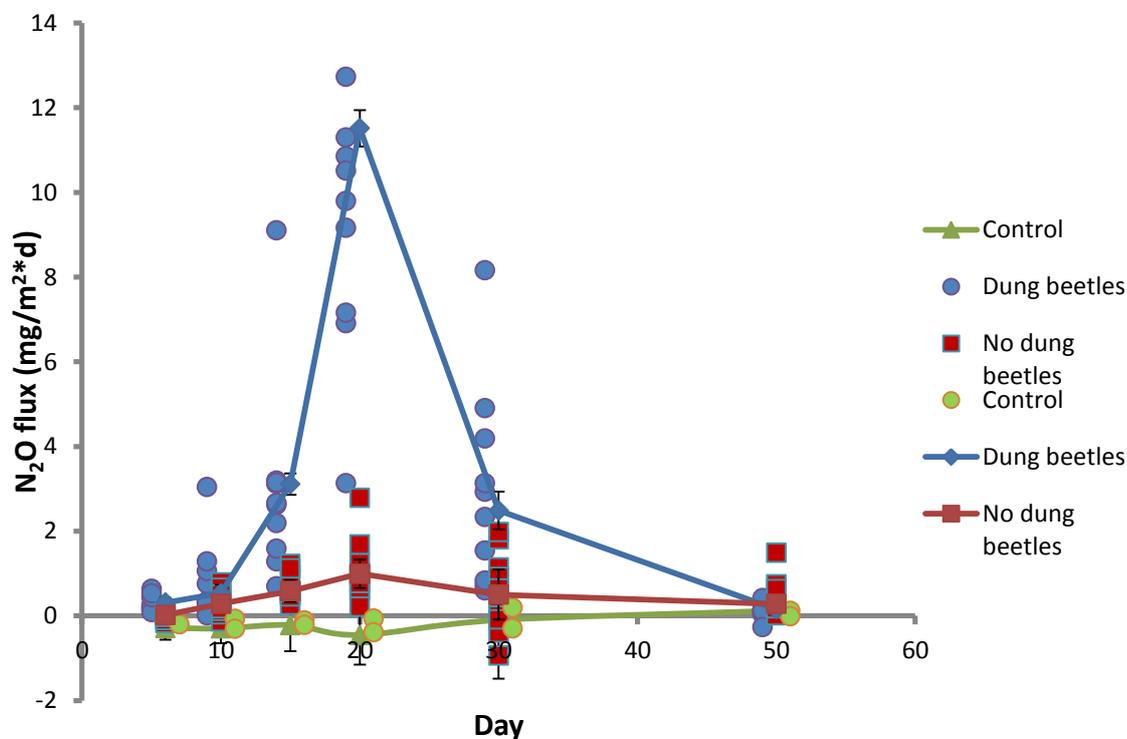


Figure 6. N_2O fluxes observed during the 50-day experiment. Individual symbols refer to empirical observations, with treatments identified by color. Symbols with error bars (+SE) refer to least squares means estimated by a GLMM model (for details, see text). To reveal overlapping data points, empirical values were slightly offset in the horizontal dimension.

Table 4. Generalized linear mixed-effect model of changes in N_2O fluxes over time. Type 3 *F*-tests of fixed effects are given.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	33.3	75.05	<.0001
Measurement	5	29.9	38.49	<.0001
Measurement*Treatment	10	33.2	42.09	<.0001

4.2.4 Fluxes of carbon dioxide equivalents

Fluxes of CO₂ equivalents differed significantly among treatments (Table 5). As absolute fluxes of CO₂ were much higher than fluxes of CH₄ and N₂O (see Figs 4-6, above), qualitative patterns were dominated by the former.

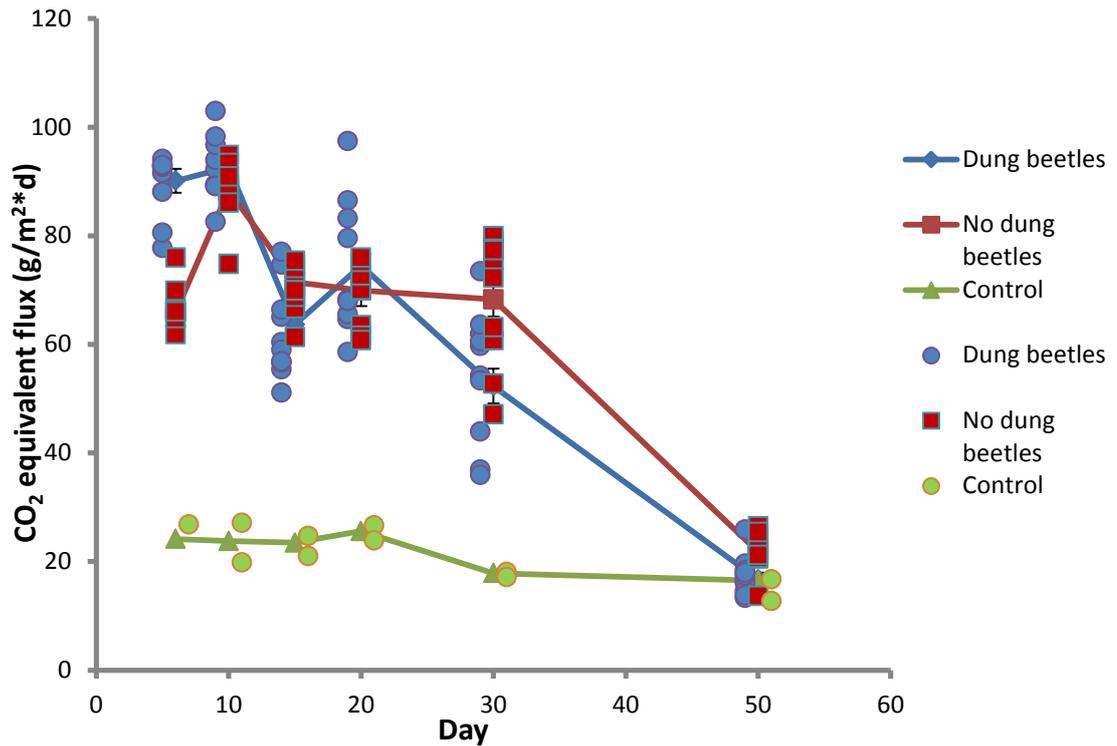


Figure 7. Fluxes of carbon dioxide equivalents observed during the 50-day experiment. Individual symbols refer to empirical observations, with treatments identified by color. Symbols with error bars (+SE) refer to least squares means estimated by a GLMM model (for details, see text). As measurements of CH₄ and N₂O were not available for the first measuring date, only results for days 6-50 are shown. To reveal overlapping data points, empirical values were slightly offset in the horizontal dimension.

Table 5. Generalized linear mixed-effect model of changes in fluxes of CO₂ equivalents over time. Type 3 *F*-tests of fixed effects are given.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	2	27.2	74.50	<.0001
Measurement	5	37.8	183.44	<.0001
Measurement*Treatment	10	44.5	32.43	<.0001

4.3 Cumulative Fluxes over the full experiment

Over the 50-day experiment, cumulative emissions of CO₂ were similar in the presence and absence of dung beetles, but approximately three times higher in the presence of dung than in the dung-free control (Table 6). In contrast, dung beetles had a strong but opposite effect on the cumulative emissions of methane and nitrous oxide: in the presence of beetles, the summed fluxes of methane were less than half in the absence of beetles, but for nitrous oxide, they were six times higher. Based on these opposite effects, differences in overall emissions of CO₂ equivalents were relatively subtle, being *ca.* 5 % higher with than without dung beetles (Table 6). The ground surface of the control treatment acted as a weak sink of both methane and nitrous oxide (Table 6).

Table 6. The cumulative fluxes of gases as pooled over the experiment. For CH₄, N₂O and CO₂ equivalents I only had access to measurements from day 6 onwards, for CO₂ measurements were available for the full course of the experiment. Therefore, the first column refers to fluxes over a 50-day time period, the last three columns to fluxes over a shorter 44-day time frame.

Treatment	CO ₂	CH ₄	N ₂ O	CO ₂ equivalents
Dung beetles	2922 g/m ²	737 mg/m ²	145 mg/m ²	2704 g/m ²
No Dung beetles	2966 g/m ²	1727 mg/m ²	22 mg/m ²	2862 g/m ²
Control	992 g/m ²	-43 mg/m ²	-7 mg/m ²	978 g/m ²

4.4 Total loss of mass, C and N

During the course of the experiment, dung pats in the treatment with dung beetles lost an average of 67.4% of their dry mass, whereas in the absence of beetles, the mass of dung pats actually increased by an average of 6.2% (Fig. 8).

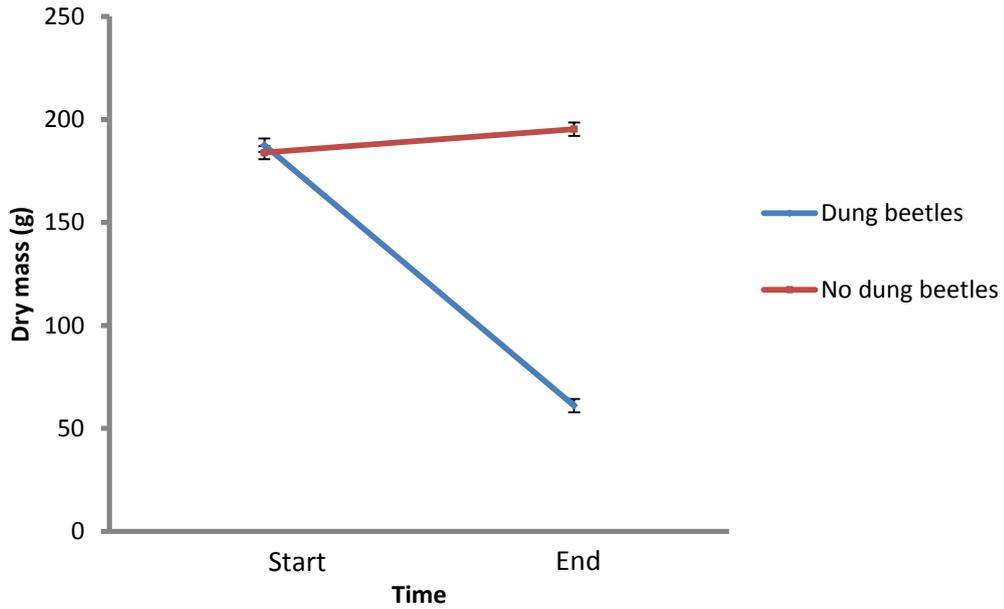


Figure 8. Changes in the mass of experimental dung pats over the 50-day experiment, with treatments identified by color. Shown are least squares means (\pm SE) estimated by a GLMM model (for details, see text).

Table 7. Generalized linear mixed-effect model of changes in mass over time. Type 3 *F*-tests of fixed effects are given.

Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	18	439.71	<.0001
Time	1	18	295.53	<.0001
Time*Treatment	1	18	424.03	<.0001

The initial content of carbon and nitrogen in the experimental pats was 43.6% (\pm SE 0.08) and 2.8% (\pm SE 0.03), respectively. By the end of the experiment, the average content was slightly lower, being 35.9% (\pm SE 1.2) for carbon and 2.4% (\pm SE 0.08) for nitrogen in the presence of dung beetles, and 40.9% (\pm SE 0.1) and 3.1% (\pm SE 0.03), respectively, in their absence. These figures translate into an average loss of 59.8 g (73%) of C and 3.7 g (72%) of N per dung pat in the presence of dung beetles, and a loss of 0.38 g (0.5%) of C and an addition of 0.89 g (17%) of N to pats without dung beetles (Fig. 9, Tables 8 and 9).

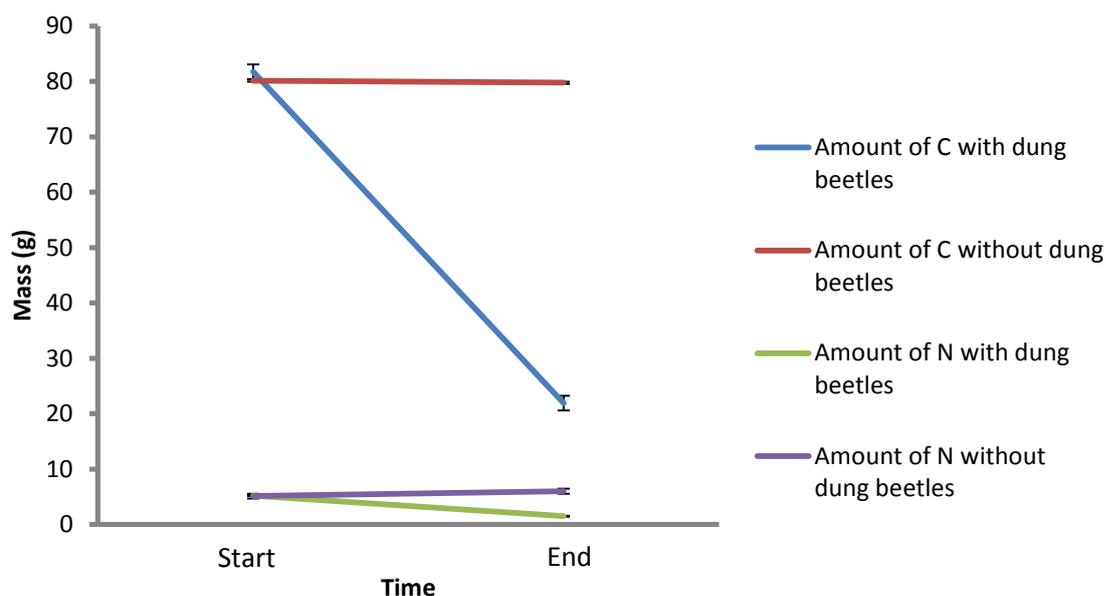


Figure 9. Changes in the amount of carbon and nitrogen per experimental dung pat over the 50-day experiment, with treatments identified by color. Shown are least squares means (\pm SE) as estimated by a GLMM model (for details, see text).

Table 8. C and N losses (g) estimated from gas fluxes as compared to the losses estimated from changes in mass and elemental content of experimental dung pats.

Treatment	Losses from gas flux estimates		Losses from mass estimates	
	C	N	C	N
Dung beetles	62.2 g	0.007 g	59.8 g	3.7 g
No dung beetles	63.2 g	0.001 g	0.38 g	-0.89 g
Control	21.0 g	-0.0004 g		

Table 9. Generalized linear mixed-effect models of changes in a) C content and b) N content over time. Type 3 *F*-tests of fixed effects are given.

(a)				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	18	454.69	<.0001
Time	1	18	510.71	<.0001
Time*Treatment	1	18	497.76	<.0001

(b)				
Effect	Num DF	Den DF	F Value	Pr > F
Treatment	1	18	470.39	<.0001
Time	1	18	240.25	<.0001
Time*Treatment	1	18	635.25	<.0001

5. DISCUSSION

In this study, I discovered that the presence of dung beetles significantly affects the fluxes of GHG compounds from dung pats. Most importantly, fresh dung pats emitted higher amounts of CO₂ and lower amounts of CH₄ in the presence than absence of beetles. Three weeks after the start of the experiment, emissions of N₂O showed a distinct peak in the presence of beetles – a pattern lacking in the absence of any insect fauna. Neither variation in temperature nor precipitation seemed to account for any of the patterns in gas fluxes, as neither wet nor warm spells were reflected as gas fluxes. These findings reveal a key impact of dung beetles on gas fluxes realized at a small spatial scale, and suggests that, biotic interactions may have an impact on gas fluxes from agriculture.

5.1 Dung pats release high amounts of greenhouse gases

My measurements identified dung as a major source of GHGs. When comparing gas fluxes between chambers with and without dung, I found considerable releases of CO₂, CH₄ and N₂O. These findings agree with those of Lin et al. (2009) and Ma et al. (2006), who also reported that treatments with dung emitted significantly more CO₂ than controls. Likewise, several studies have concluded that CH₄ emissions have a tendency to be high after dung application and then rapidly decrease (Jarvis et al. 1995, Holter

1997, Lin et al. 2009, Ma et al. 2006). When Jarvis et al. (1995) measured CH₄ emissions from livestock they concluded that the dung of grazing dairy cows can emit approximately 1700 mg CH₄ m². These fluxes are notably similar to the accumulated CH₄ fluxes that I measured from dung pats of approximately the same size. With respect to nitrous oxide, my results agree with previous studies which have observed a tendency of livestock excreta to emit N₂O (Yamulki et al. 2000, Skiba et al. 2005, Maljanen et al. 2007, Smith et al. 2008, Wachendorf et al. 2008 and Lin et al. 2009).

5.2 Dung beetles increase CO₂ fluxes from fresh dung

Dung beetles significantly affected the fluxes of greenhouse gases from treatments with dung. In terms of CO₂, these effects were centered on the first week of the experiment. As was the case for all other gases measured, the difference in fluxes of CO₂ from dung pats with and without dung beetles changed with time: dung pats with beetles released particularly high amounts of CO₂ during the early part of the experiment (days 1 and 6), but these differences disappeared over time.

Most likely, high fluxes during the first few days relates to respiration by the beetles, with decreasing emissions reflecting progressing mortality. Nonetheless, the differences among treatments were relatively subtle compared to overall fluxes (Fig. 4), suggesting multiple sources of CO₂. For example, it could be that the dung stimulated the microbes in the soil, thus releasing more CO₂ (Bol et al. 2003), or that an increase in biomass, both above and below ground, caused some of the CO₂ emissions (Ma et al. 2006). Microbial activity is high in dung, and much of the observed fluxes may therefore have originated from respiration by microorganisms (Chadwick and Pain 1997). Notably, CO₂ fluxes from dung pats without beetles rapidly increased after day 5, and might be related to the start of aerobic decomposition with the desiccation of the dung. Such a pattern would agree with the observation by Stevenson and Dindal (1987) that CO₂ is volatilized from the substrate in aerobic decomposition. CO₂ fluxes are also dependent on water-filled pore space (WFPS) (Stevenson and Dindal 1987), and while both treatments would be affected by increasing WFPS, the different sized pores created by beetles could facilitate faster drying of dung pats in that treatment. Therefore, the treatment without dung beetles

could retain moisture, thus facilitating decomposition for a longer time. The rain on the 9th day of the experiment may have resulted in the increase of CO₂ emissions observed in both treatments on day 10. CO₂ is also temperature-dependent, but the temperature rise in July (Fig. 10) did not translate into any detectable result in the increase of CO₂ emissions, possibly because the conditions were already too dry.

After the 20th day of the experiment, CO₂ emissions from dung pats lacking dung beetles surpassed those from pats with dung beetles. This might be due to the fact that whilst the aerobic decomposition continued in the beetle-free pats (see above), many of the dung beetles had emigrated or died from the treatment containing beetles, and so were no longer releasing CO₂ through respiration. Finally, at this point in time, the dung beetles may also have caused the pats to dry out to a level effectively hindering decomposition. Overall, the results show that dung beetles have an effect on the temporal dynamics CO₂ emissions from dung.

5.3 Dung beetles decrease CH₄ fluxes from fresh dung

Perhaps the most drastic impacts of beetles on gas fluxes from dung were found for methane. Initial emissions from six-day old dung pats showed a five-fold increase compared to pats with beetles (Fig. 5). Since CH₄ is formed under anaerobic conditions, the difference between the two treatments can likely be traced to the aerating effect of dung beetle tunnels (Stevensson and Tindal 1987). By digging holes, beetles may quickly increase the availability of oxygen in the deeper parts of the pats. The observed effect is then contrary to the prediction of Svensson et al. (1986) and Yokoyama et al. (1991), who both assumed that the respiration of organisms would actually make the dung anaerobic by consuming available O₂ reserves. In this context, respiration by microorganisms may well deplete oxygen resources, but for dung beetles, the net effect seems to be the opposite.

That dung beetle activity so clearly decreased methane emissions refutes the earlier result of Holter (1991), who inferred that progressive desiccation from the surface would be the main cause of oxygenation even in dung pats inhabited by beetles. In the

pats without dung beetles, a pattern more consistent with desiccation from the surface was observed: when CH_4 levels decreased in these pats, the CO_2 levels concomitantly increased (compare Figures 4 and 5). These concerted changes likely reflect a shift from anaerobic decomposition to aerobic decomposition. Together, the differential patterns observed between pats with and without beetles suggest that dung beetles promote the oxygenation of the dung pat interior. Dung beetles may therefore have a quite different effect than earthworms, which are suggested to promote anaerobic decomposition (Rizhiya et al. 2007).

5.4 Dung beetles cause a spike in N_2O emissions

Of all the compound-specific patterns observed, the temporal pattern in N_2O emissions is the most intriguing. The presence of dung beetles was associated with a spike in gas emissions around day 20, whereas emissions from pats without beetles were much less variable. Sporadic peaks in N_2O fluxes have been witnessed before (Kanerva et al. 2005). However, these patterns are hard to explain, as the formation of N_2O by microbes is based on such complex processes (Oenema et al. 2005). Some tentative relationships may still be proposed.

Denitrification is likely a major contributor of N_2O emissions and Yokoyama et al. (1991) have suggested that dung beetles may enhance this process. If dung beetles aerate the dung (as the CH_4 results would lead us to assume), there will be more O_2 available in the presence than absence of beetles, thereby raising the question of how denitrification could be enhanced by beetles if they simultaneously increase O_2 levels. By contrast, Stevansson and Dindal (1986) propose that microorganisms decrease O_2 levels. Müller et al. (2004) found that even in soil where oxygen is present, the major pathway for N_2O production is the reduction of NO_3^- . It is also possible that dung beetles enhance NO_3^- levels at the same time as they aerate their substrate (Yokoyama et al. 1991), in which case conditions in the dung could be optimal for aerobic denitrification. Lloyd (1993) concludes that in the case of aerobic denitrification, most of the NO_3^- would be incompletely reduced to N_2O (more so than under anaerobic denitrification), which seems supported by the fact that the conditions in the dung are no longer anaerobic. Also Khalil et al. (2004) regards aerobic denitrification as a

possible pathway for N₂O production. Another option is that anoxic microsites or micro niches within the dung might offer sites for denitrification (Mørkved et al. 2007), even though dung beetle activity creates oxygenated conditions in other parts of the pat. Also, Drury et al. (1992) suggest that N₂O is a more frequent product of nitrification and denitrification processes in drier conditions and we assumed that the decreasing CH₄ emissions with dung beetles indicated a drier dung pat.

The source of the observed N₂O emissions could also be in nitrification (Kebreab et al. 2006). Nonetheless, the amounts of N₂O released from nitrification are usually small, and it is thus unlikely that nitrification could account for any major spike in N₂O emissions, although this possibility cannot be neglected. On the one hand, the beetles might first aerate the dung and then eventually decrease the amount of O₂, thus first promoting nitrification and then anaerobic denitrification. On the other hand, the release of N₂O from nitrification can also increase when O₂ levels are decreased (Khalil et al. 2004). Rizhiya et al. (2007) claim that earthworms increase anaerobic decomposition and therefore denitrification. While the process appears somewhat opposite in the case of dung beetles, both taxa seem to increase N₂O production. Drake and Horn (2006) conclude in a review that earthworms create optimal conditions for denitrifying bacteria to function in their gut, mostly because of the anoxicity. Whether dung beetles could have the same effect is unknown, but offers another avenue for future research.

5.5 Where have all the elements gone? On sources of error and scope for improvement

To verify my findings on gas fluxes, I calculated how much carbon and nitrogen had actually disappeared from the dung pats. If all the losses of these elements had occurred in measured gaseous forms, then the cumulative estimates of elements lost in a volatile form should have matched the observed losses from remaining dung. Indeed, the figures matched fairly well for the dung pats with beetles, particularly for carbon, but for the dung pats lacking beetles, they differed substantially. The latter result was due to the fact that the beetle-free pats were found to lose virtually no

mass, but actually to gain some. This unexpected finding must have been caused by some anomaly arising in the experiment. Upon closer inspection, some plants and plant roots were found to be attached to the bottom of the dung samples, which might partly explain the peculiar weight gain.

Another factor contributing to the mass increase might be earthworms, as Holter (1977) has found these organisms to add soil and casts into the dung. However, no earthworm activity was directly observed in the experiment. Fungi have the potential to infiltrate cellulose and decomposing wood and increase their mass. Indeed, such processes caused some inconsistencies in the measurements of Tuominen (1981) in an experiment on cellulose decomposition. Hence, in my experiment, fungal growth in the dung without dung beetles could have led to the observed increase in mass. If so, it might be that the fungi took carbon from outside of the dung pat, in which case the CO₂ emitted from this source would not have resulted in any decrease in the carbon content of the dung pat. Fungal growth in the treatment with dung beetles might then have been disrupted by the activity of dung beetles, for example by the mixing of dung and soil at the soil surface, or by the beetles actively consuming the fungi.

In terms of nitrogen losses, another unexpected pattern was found. While Yamada et al. (2007) found a temporal decline in the nitrogen contents of dung both with and without dung beetles (although this loss was found to be higher in the presence of dung beetles); in my experiment the N content of pats without dung beetles did not decrease but actually increased. In part, this increase might be associated with the increase in dung mass referred to above, which would have distorted the calculations of N content as a product of weight and concentration. Nonetheless, even the absolute concentration of N increased in the dung pats without dung beetles, thus refuting this interpretation. If the assumptions above about the fungal growth are accurate, then it might be that the fungi, of which some are rich in N (Christias et al. 1975) are the actual cause for the rise in N content. In fact, an increase of N is interesting as it has also been reported in some other experiments; for example, Palviainen et al. (2004) states that N accumulated in logging residue in a three-year experiment.

Since the carbon figures from estimates from mass and gas flux measurements matched fairly well (see above) with the treatment with dung beetles, it leads to the assumption that nitrogen is apparently lost from the dung pats in some other form than nitrous oxide; most likely volatilized as N_2 and/or NH_3 or used by surrounding plants as nutrients, leaving scope for further explorations. It should be noted that also the control was a source of carbon. Therefore, the elemental carbon lost from the dung should not match the cumulative C lost in gas fluxes, but as the cumulative C lost in gas fluxes with the cumulative C lost from the control subtracted. However, the dung covered a large part of the soil surface within the chambers, inhibiting soil respiration, and thus, the accurate amount of C lost from soil respiration in the treatments with dung is difficult to estimate.

6. CONCLUSIONS

When pooled over the full season, total emissions of greenhouse gases differed between dung pats with and without beetles. For CH_4 they were larger in the absence of dung beetles, for N_2O they were smaller. For CO_2 , the change in emission levels between the early and late parts of the summer actually caused pooled emission levels to almost converge between treatments. When converted to CO_2 equivalents, the differences were relatively small (only 5%), with higher emissions from the treatment without dung beetles. Together, these results imply that dung beetles do have the potential to reduce overall GHG fluxes, although, the potential for dung beetles in mitigating climate change seems to be limited. However, it should be noted that my experiment specifically targeted the effects of *Aphodius* beetles, and that the effects of other dung beetles (such as *Geotrupes*; cf. Rosenlew and Roslin 2008) remain to be established. FAO estimates that within the near future, global meat consumption will radically increase (FAO 2006), which means a substantial increase in dung and in GHG emissions. Therefore, it is important to conduct further research on how different dung fauna species compositions would affect the GHG emissions from dung.

7. ACKNOWLEDGEMENTS

First, and foremost, I would like to thank Tomas Roslin for giving me the opportunity to work with dung beetles, and for never ending, never tiring guidance, assistance and the right amount of pressure throughout my thesis work. My deep appreciation extends to Asko Simojoki and Kari Minkkinen for assistance and guidance with gas measurements. Also, thank you to Bess Hardwick for help in hunting dung beetles and to Eleanor Slade for linguistic assistance and to Miira Jääskeläinen for help with the gas chromatograph.

I thank Alekski Kiema and Olavi Väärämäki for help in metalwork in the construction phase of the experimental chambers.

I would like to thank Vanamo, Finland's Association of Biology for the thesis grant I received.

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