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Research

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Carbon Neutral: The Failure of Dung Beetles (Coleoptera: Scarabaeidae) to Affect Dung-Generated Greenhouse Gases in the Pasture

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Abstract

Research suggests dung beetles can churn, aerate, and desiccate dung in ways that influence the dung and soil microbes producing greenhouse gases (GHGs). We examined the impacts of the tunneling beetle, *Onthophagus taurus* (Schreber), and the dwelling beetle, *Labarrus pseudolividus* (Balthasar), on the carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) emitted from pasture-laid bovine dung as well as their sum-total ($CO_2 + CH_4 + N_2O$) effect on global warming, or their carbon dioxide equivalent (CO_2e). Despite dung beetles potential effects on CH_4 and N_2O, the existing literature shows no ultimate CO_2e reductions. We hypothesized that more dung beetles would degrade pats faster and reduce CO_2e, and so we increased the average dung beetle biomass per dung volume 6.22× above previously published records, and visually documented any dung damage. However, the time effects were 2–5× greater for any GHG and CO_2e (E = 0.27–0.77) than dung beetle effects alone (E = 0.09–0.24). This suggests that dung beetle communities cannot adequately reduce GHGs unless they can accelerate dung decomposition faster than time alone.

Key words: dung beetle, tunneling, dwelling, greenhouse gas, dung decomposition

Global CO_2e estimates for animal agriculture range from 18% (Steinfeld et al. 2006) to 51% (Goodland and Anhang 2009) depending on how direct and indirect GHG sources are calculated. Direct emissions include cattle respiration, manure emissions, and enteric fermentation from ruminants. Meanwhile, indirect emissions include the land-sparing effect (emissions from deforested, degraded, or polluted habitats; reduced environmental sequestration capacity), supply-chain emissions (storage, refrigeration, transportation, processing, food waste), and third-world impacts (food imports and their associated environmental impact outside the country-of-interest). The IPCC (Intergovernmental Panel on Climate Change) reports that animal agriculture directly produces 23% of anthropogenic CO_2e by producing 33% CH_4, 13% CO_2, and >33% N_2O globally—primarily through a combination of enteric fermentation, deforestation, and manure deposition (Jia et al. 2019). However, when also considering indirect emissions, animal agriculture produces up to 37% of global CO_2e emissions (Mbow et al. 2019). This is because animal agriculture is part of an interconnected web of industries (oil, coal, deforestation, plastic-use, etc.) and trade economies; only focusing on by-sector categories (transportation, electricity, industrial, etc.) or by-country categories (neglects importation considerations) unintentionally masks strong climate-affecting connections.

A key aspect in GHG production is habitat replacement, degradation, and/or destruction as this hinders the ecosystem's ability to provide benefits, support diverse wildlife, and sequester GHGs (Steinfeld et al. 2006, Shukla et al. 2019). For example, land-use change from 1750 to 2011 produces nearly half of the CO_2 generated by fossil fuels alone (Jia et al. 2019), hence why habitat-affecting industries are targeted for climate change reform; they hinder the global effort to reduce and reverse global warming. Animal agriculture occupies ~70% of all agricultural land (Steinfeld et al. 2006) and 30–47% of the global ice-free surface (Shukla et al. 2019), with cattle alone accounting for 65–77% of global livestock emissions (Smith et al. 2019). Cattle systems are highly interconnected with deforestation, fossil fuels, and intensive resource use as their environmental impacts includes the impact of everything they consume as well (Poore and Nemecek 2018). Thus, reducing animal agriculture's land-use footprint—often from localized pollution (i.e., waste, pesticides, antibiotics, fertilizer for feed crops), habitat replacement/destruction (deforestation, overgrazing), and resource overuse and inefficiency (land, water, oil)—is key. Converting these lands to wildlife ecosystems would both reduce and sequester existing GHGs (Poore and Nemecek 2018), and limit any consequences. Climate change affects extreme weather phenomena, such as acidic rains and oceans, glacial melting, droughts and wildfires, rising sea levels, and hurricanes/cyclones, which can easily decimate animals and habitats *en masse* (Veron 2008, Ceballos et al. 2015). This explains why five mass extinction periods, wherein ≥75% of all species went extinct, were documented throughout earth's history due to climate-induced habitat loss and ecological cascading (Veron 2008, Ceballos et al. 2015).

The two most popular solutions to reduce GHG production are: 1) reduce animal consumption and production (Mbow et al. 2019) by moving towards predominately whole foods, plant-based diets that are more sustainable (Poore and Nemecek 2018) and healthy (Willett et al. 2019); and 2) reform remaining animal operations into more resource-efficient systems. Dung beetles improve pasture health by actively dismantling and burying dung, which: 1) increases soil organic matter ('humification') and refills water tables through increased soil percolation; 2) reduces pollution, pests, and pestilence through competitive exclusion and removal; and 3) encourages cooperation among earthworms, soil arthropods, and plant species to bury seeds, enrich the soil, and/or pollinate plants (Lavelle et al. 2006, Nichols et al. 2008, Ridsdill-Smith and Edwards 2011, Doube 2018). Only recently have researchers studied the relationship between soil, dung, dung beetles, and GHGs as part of a larger resource recycling strategy (Penttilä et al. 2013, Iwasa et al. 2015, Hammer et al. 2016a, Slade et al. 2016a, Piccini et al. 2017, Evans et al. 2019). Enteric fermentation and deposited, applied, and flooded manure and dung (Mbow et al. 2019) produce 66% CH_4 of all agricultural emissions, with deposited manure and dung also producing >50% N_2O net agricultural emissions (Jia et al. 2019)—this does not include the emissions generated by applied synthetic fertilizer to animal food crops. Thus, dung management by dung beetles present an opportunity for reducing GHGs in addition to reducing disease, pollution, compaction, improving C and N storage/use, and overall sustainability.

Dung beetles generally decrease CH_4, increase N_2O, and yield no CO_2e effect—though in some cases they increased CO_2e (Fowler et al. 2020c). We propose that increasing dung beetle biomass may sufficiently alter the dung structure and reduce CO_2e. Therefore, we increased dung beetle biomass per unit dung above previously reported estimates (Table 1) and compared if major dung altering behaviors (mixing vs. burial) accelerated dung decomposition and reduced more GHGs.

Materials and Methods

Experimental Design

At the beef unit (latitude 35°43′47.40″N, longitude 78°41′15.50″W) of NCSU Lake Wheeler Road Field Lab (Raleigh, NC), we set up a randomized complete block design (n = 2 blocks per experiment) of our treatments (Table 2) on fresh, untouched cattle dung (grass and hay fed) to test whether different dung beetle behaviors and abundances reduce dung-produced CO_2e. We did not standardize dung so as not to disrupt/alter its natural physical and microbial properties nor confuse dung beetle activity with human activity, thus the dung's volume (via dung width, length, and depth) was calculated instead (Supp Fig. G5 [online only]), and additional replicates were processed. We measured GHGs (CH_4, CO_2, N_2O) over a week (0, 1, 3, 5, and 7 d) using mobile GHG chambers (n = 1 treatment per block) between May and October 2018 (n = 12 experiments) totaling 24 replicates per treatment and day. On non-sampling days, cages (Fig. 1) protected treatments from dung beetle entry and exit while allowing for airflow and avoiding chamber burial (Fowler et al. 2020b). Sampling sites were enclosed with electric fences to prevent animal tampering. We photographed dung damage on 7 and 14 d for dung-based treatments and field pats, respectively (Fig. 2). We informally included naturally colonized dung pats ('field pats') in the experiment to ensure and show whether the treatment dung pats were dissembled by dung arthropods faster and more intensely than that found naturally in the surrounding area—thereby showing if natural dung beetle colonization rates are adequate for GHG reduction. See Fowler et al. (2020b) for the measurements, costs, limitations, building procedures, and verification experiments of the physical chamber designs, gas sampling strategies, and caged designs.

Table 1. A summary of reported GHG differences (+: increase, –: decrease, 0: no effect) focused on aggregate treatment effects of *t*-tests and ANOVAs ($P < 0.05$) that exclude strong and effect-masking predictors such as time or vegetation

Article	n_{dung}/d	Biomass/dung (g/L)	CH_4	CO_2	N_2O	CO_2e
Yokoyama et al. (1991a,b)	2–3	0.83[e]	–	–	+1.93×	–
Penttilä et al. (2013)	10	1.075	–1.65×	0	+27.2×	0
Iwasa et al. (2015)[a]	3	0.60[e]	–2.61×	+7.87×	+10.81×	+1.91×
Slade et al. (2016a,b)[a]	30, 20[c]	–	0	0[g]	0	0
Hammer et al. (2016a,b)	10[d]	0.31[e]	–1.59×	0	+3.02×	0[a]
Piccini et al. (2017)[b]	8	1.48	0[f]	0[h]	0[i]	0[j]
Evans et al. (2019)	32	–[e]	+3×	0	+3×	0
Fowler et al. (2020c)	24	3.09	0	0	0	0
Our data	20	3.75–7.5	–1.40×	0	+1.56×	0

These values look at how dung beetles affect the dung GHGs relative to the dung-only for each GHG. The experimental information from the current literature (2020) includes: the dung-containing sample size total per day (n_{dung}/d) and the dung beetle biomass per reported dung volumes (maximums only). An updated version from Fowler et al. (2020c).

[a] Traditional *t*-test not reported in the published paper. Based on reported and overlapping SE values or a *t*-test was performed on the available raw data (Fowler et al. 2020c - Supp Table D7 [online only]).

[b] Based only on the reported *P*-adjusted value (familywise error corrected).

[c] Numbers represent dung beetle treatments, dung-only was n = 3 for both 2016a and b.

[d] Considering only the non-antibiotic dung to avoid confounding effects.

[e] Biomass unreported, so: study-reported abundance*biomass of tunneler (Piccini, 0.20 g) or dweller (Penttilä, 0.0261 g).

[f] Five of six dung beetle treatment reported no difference, 1 treatment increased CH_4 emissions (T4).

[g] Based on fig. A2 from Slade et al. (2016b), all other GHGs come from Slade et al.'s (2016a) supplementary materials.

[h] Five of six dung beetle treatments reported no difference, 1 treatment decreased CO_2 emissions (T4).

[i] Six of six dung beetle treatments reported no difference for N_2O.

[j] Five of six dung beetle treatments reported no difference, 1 treatment decreased CO_2e emissions (T6).

Treatment Descriptions

Dung beetle nesting behaviors include (Bertone et al. 2004): 1) endocoprids or 'dwellers', which shred dung by dwelling within cavities in and around dung; 2) paracoprids or 'tunnelers', which bury brood balls (balls of dung created by dung beetles to house and feed progeny over the course of their development) beneath pats; and 3) telecoprids or 'rollers', which form, roll, and bury brood balls elsewhere. We particularly focused on the tunneler, *Onthophagus taurus*, and dweller, *Labarrus (Aphodius) pseudolividus*, since they are the most abundant dung beetles on North Carolina cattle pastures (Bertone et al. 2005) and so could be reliably gathered throughout the season. These species also represented the major dung handling behaviors (mixing vs. burial). We determined representative dung beetle biomasses by mimicking the dung damage commonly found in local pastures to simulate reasonable to intense dung beetle activities (Fig. 2). The dung-only treatments were either completely untouched ('Unmixed') or the dung (no soil or vegetation) was mixed for ~1 min on 0 d ('Mixed'). Meanwhile the pasture-only controls ('Grass') represented nearby pasture conditions. We included a mixed dung-only to further confirm/refute if mixing itself changes GHG production pathways, though recent studies thus far show that dung homogenization, translocation, and placement do not alter them (Fowler et al. 2020c).

Table 2. A summary of the treatment descriptions (Tunn = Tunneler, Dwell = Dweller) including the estimated dung beetle abundances per treatment for a single replicate (*n* = 24)

Treatment	Beetles	Dung	Grass	Biomass (g)	Tunn (#)	Dwell (#)
Unmixed		✓	✓	–	–	–
Mixed		✓	✓	–	–	–
High Tunn	✓	✓	✓	15	205	–
High Dwell	✓	✓	✓	15	–	2,489
Low Tunn	✓	✓	✓	7.5	102	–
Low Dwell	✓	✓	✓	7.5	–	1,244
Grass			✓	–	–	–

Dung Beetle Collection

Dung beetle treatments used biomass rather than abundance to avoid confounding dung beetle size and behavior. For example, *O. taurus* ('Tunn') is ~12× larger than *L. pseudolividus* ('Dwell') and so abundance varies greatly (Fowler et al. 2020a). We weighed 7.5 ('Low') and 15 g ('High') of each species (Table 2) and used the biomass-abundance conversion rate (described by Fowler et al. 2020a) to estimate beetle abundance. We wet-sieved the dung and collected dung beetles 1–2 d before experimentation (for survival stats and photographed methodology, see Fowler et al. 2020a). We replaced any dung beetles (<1%) that could not walk on 0 d with healthy dung beetles to maintain high performance activity. Dung beetles were added after GHG sampling on 0 d.

Statistics

First, we conducted power tests (packages: 'pwr', 'pwr2') in the free R statistical program (R Development Team, Geneva, Switzerland; http://www.r-project.org) to estimate the required sample sizes for 70% power given our number of contrasts (Supp Mat. P [online only]). Second, we acknowledged any heterogeneous variation, extreme outliers, and positive skewness (Erceg-Hurn and Mirosevich 2008) using Wilcox's Robust Statistics (package: WRS) (Wilcox 2013) by:

1. Winsorizing extreme outliers to allow focus on gas majority representation,
2. identifying skewed outliers using modified M-estimators,
3. bootstrapping the data (n_{boot} = 500–600) to calculate the proportion showing $P \leq 0.05$, and
4. using the Benjamini–Hochberg procedure to account for familywise error, which creates Type II errors ('false positives') by chance when evaluating multiple comparisons.

Lastly, because the analyses are identical and yielded the same conclusions (Supp Table D1 [online only] vs. Supp Table D2 [online only]), we have provided a simplified treatment layout combining all dung beetle treatments (High Tunn/Dwell and Low Tunn/Dwell, where '/' means 'or') into a single treatment ('Beetle').

Fig. 1. An example of the cages (3.7 L) within a fenced-in cattle pasture. Both the window screens and dirt-covered skirt prevented dung beetle entry or exit but allowed natural airflow. Cage designs, assembly, costs, and limitations are further discussed in Fowler et al. (2020b).

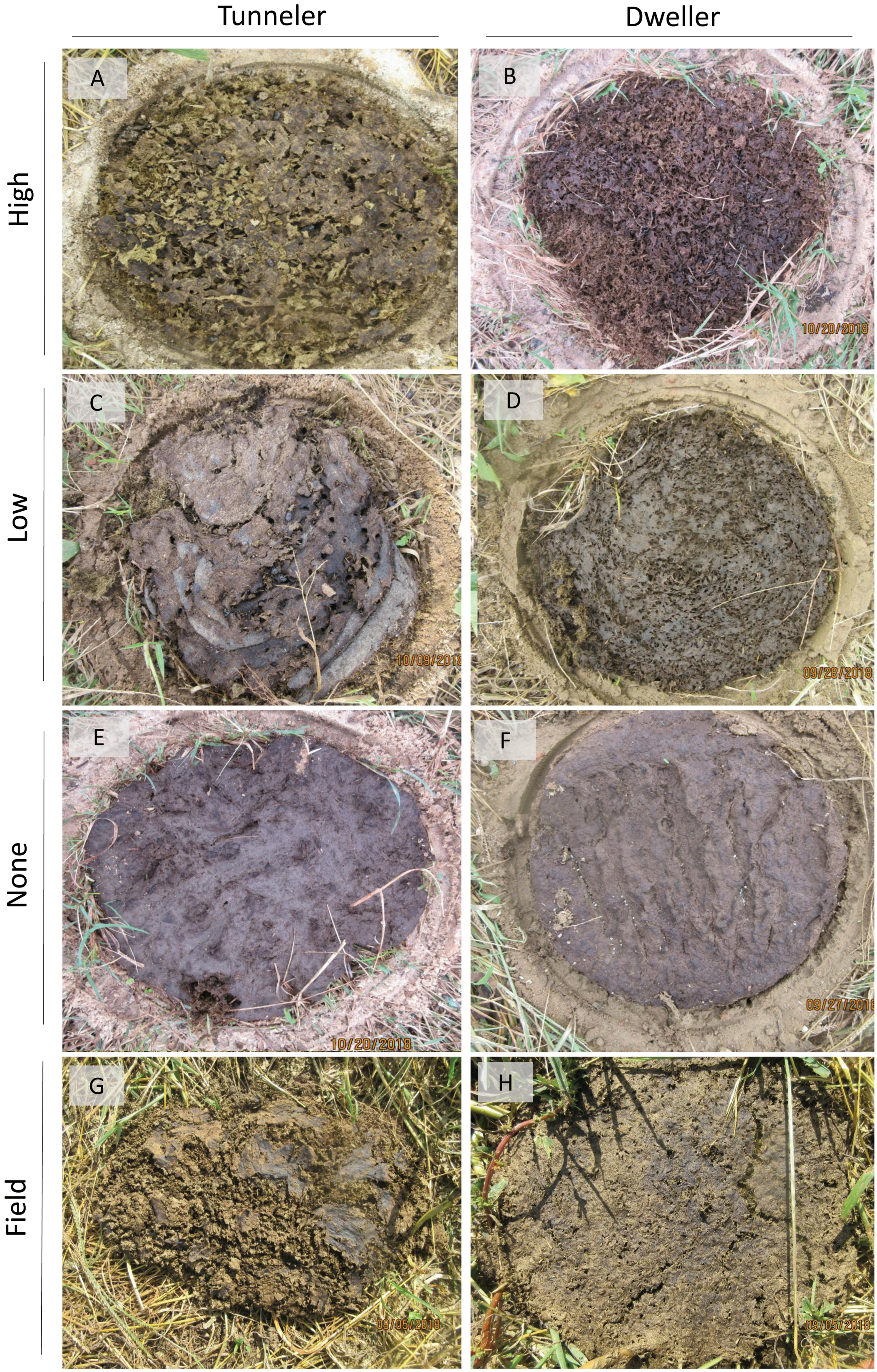

Fig. 2. Typical examples of the various treatment intensities ('Low' or 'High') of Tunneler and Dweller damage, as described in Table 2, on Unmixed dung at 7 d ('None'), with naturally colonized (no-cage) dung pats recorded at 14 d ('Field'). The lettered labels (A–H) describe both the treatment (y-axis) and dung beetle activity (x-axis) assigned to each dung pat.

Conversion, Tables, and Graphs

We converted our original GHG measurements (ppm) into fluxes (mg gas/m^2/d) using sampling time, headspace volume (Chamber Volume – Dung Volume), and temperature expansion ratios, and by applying the modified Hutchinson and Mosier model (package: HMR) to calculate linear/nonlinear HMR fluxes (Venterea and Parkin 2012). Total CO_2e were calculated as the sum of the global warming potential impact factor over 100 yr (IPCC 2007), as follows: CH_4 (=25 CO_2e), CO_2 (=1 CO_2e), and N_2O (=298 CO_2e). We included treatment, time, and treatment:time interactions (marginal means), graphs, and tables for both the original treatment designation (Supp Mat. G [online only] and Supp Table D1 [online only]) and the simplified beetle designation (Figs. 3–6; Supp Table D2 [online only]). The relevant statistics—mean-based analyses of variance (ANOVA) and median-based effect sizes—are presented on all figures, but we have also provided supplementary tables (Supp Mat. D [online only], including pseudoreplication, effect size, and comparison tables provided in Supp. Table D3–D5), and graphs (Supp Mat. G [online only]).

Results

Both the beetle design (Figs. 3–6) and original design (Supp Figs. G1–G3 [online only]) supplement one another by testing if dung beetles affected GHGs overall, and, if so, which groups. We examined two types of statistics here to present a more nuanced view of our data: the *P*-value and effect sizes. The *P*-value is the likelihood that the between-group mean difference (≠0) seen is potentially a false positive a certain percentage (~5% when $\alpha = 0.05$) of the time given accurate ANOVA assumptions and sufficient power (Colquhoun 2014). Meanwhile the effect *size* reflects the *magnitude* of reported differences to help aid interpretative conclusions (a small/weak, medium, and large/strong explanatory measure of effect is considered at least *E* = 0.15, 0.30, and 0.50, respectively; Wilcox et al. 2013).

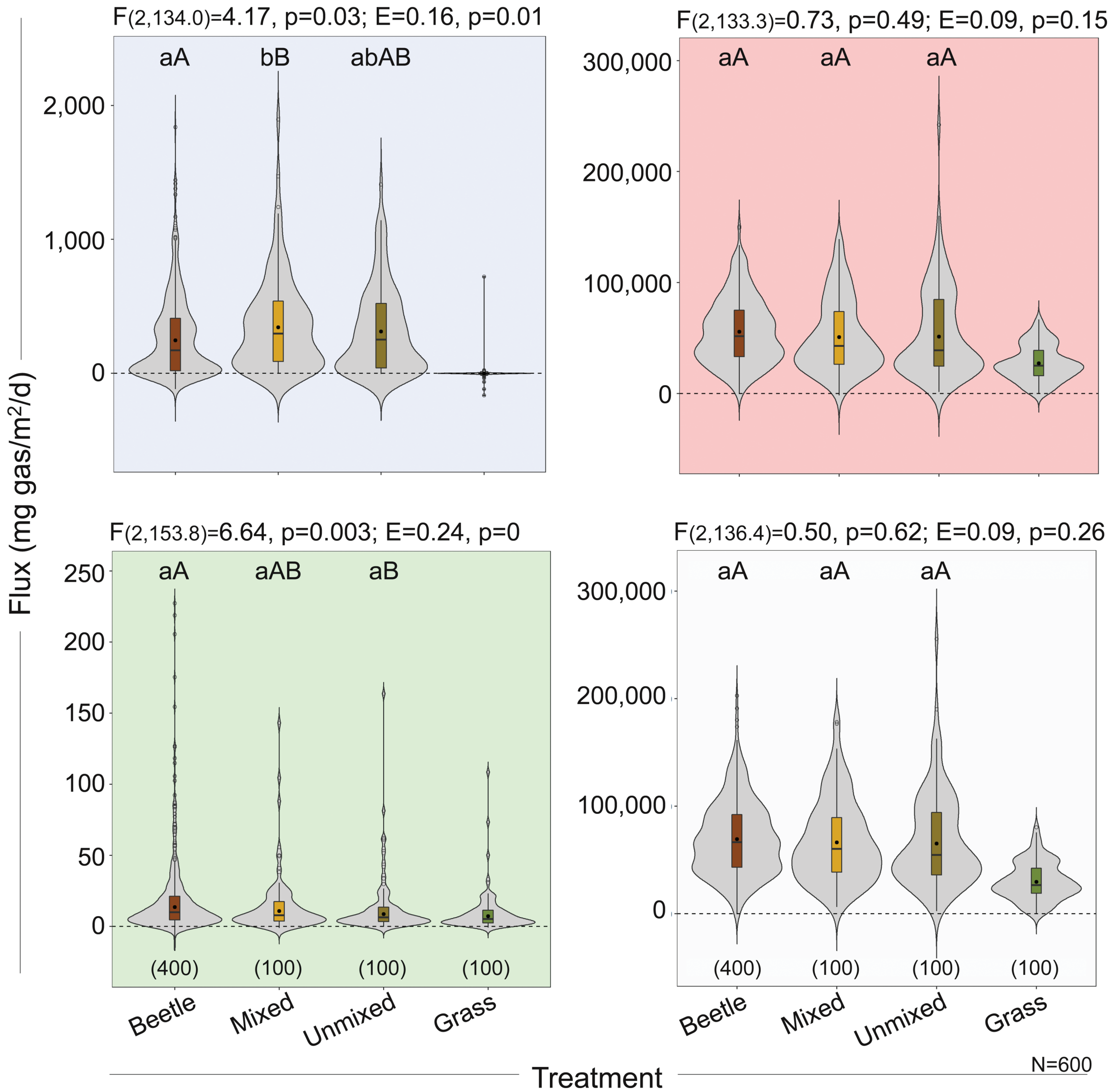

Fig. 3. Violin box plots of HMR Flux by Treatment. Each quadrant represents a GHG including CH_4 (top left), CO_2 (top right), N_2O (bottom left), and CO_2e (bottom right) with their respective omnibus mean-based ANOVAs ($F_{\text{df num, df den}}$) and median-based explanatory effect (*E*) sizes shown above. Pairwise comparisons of ANOVAs (lowercase letters) and effect sizes (uppercase letters) are shown within the graph. Sample sizes for each treatment (*n*) are shown underneath each box plot, with the total sample size (*N*) shown along the x-axis. Differing letters between groups show differences ($P \leq 0.05$). Exact means, medians, and measures of variations are given in Supp Table D2 (online only).

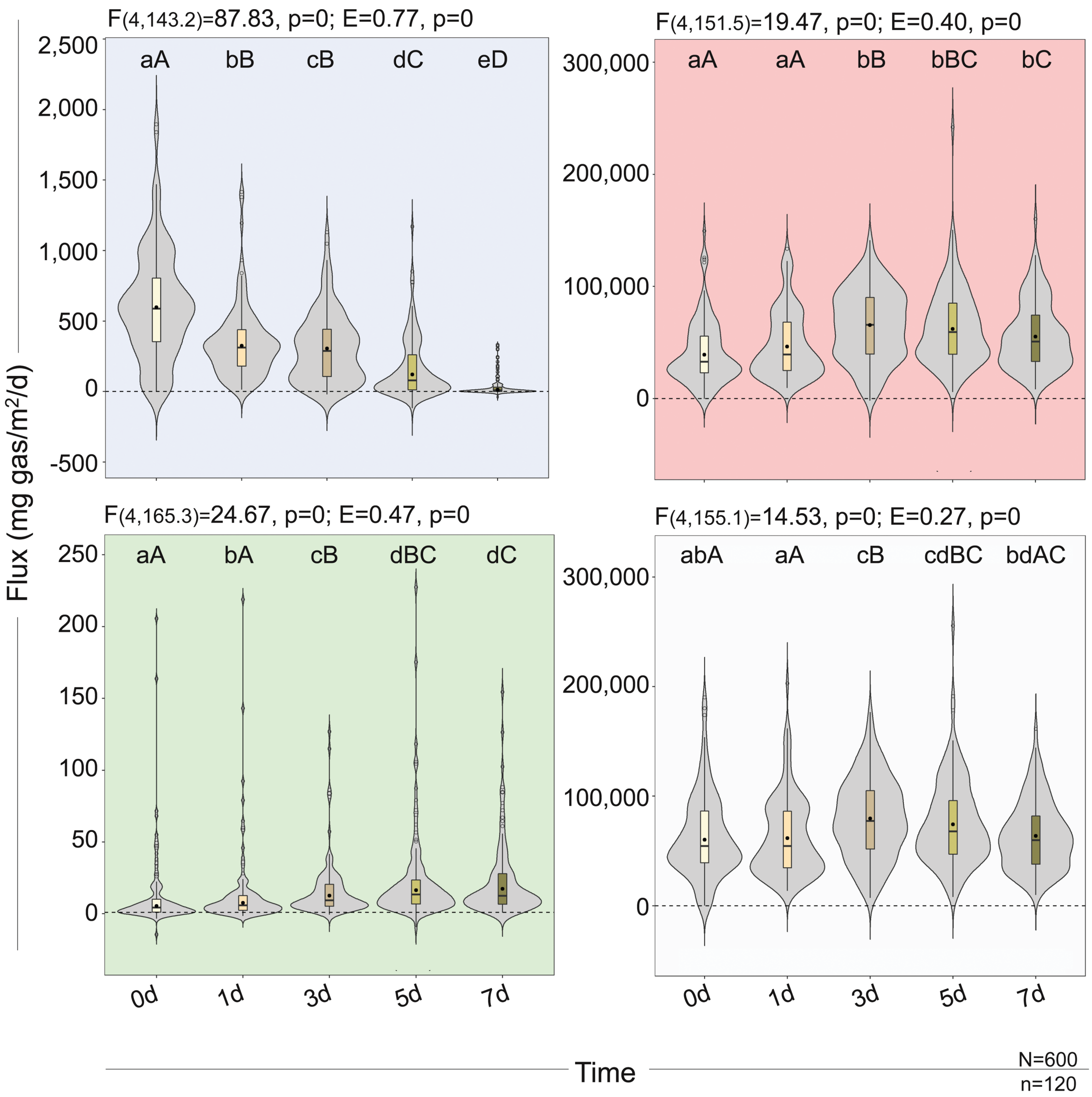

Fig. 4. Violin box plots of HMR Flux by Time. Each quadrant represents a GHG including CH_4 (top left), CO_2 (top right), N_2O (bottom left), and CO_2e (bottom right) with their respective omnibus mean-based ANOVAs ($F_{\text{df num, df den}}$) and median-based explanatory effect (E) sizes shown above. Pairwise comparisons of ANOVAs (lowercase letters) and effect sizes (uppercase letters) are shown within the graph. Sample size (n) and total samples (N) are shown along the x-axis. Differing letters between groups show differences ($P \leq 0.05$). Exact means, medians, and measures of variations are found in Supp Table D2 (online only).

Methane

By 7 d, all dung-based groups reduced CH_4 by >94% compared with its 0 d flux; however, other dung-based treatments did not differ from the Unmixed dung-only (Fig. 3), thus suggesting this reduction was strongly time-related (Fig. 4). For example, time reduced CH_4 by 1.85–1.96× for 1 and 3 d, by 4.98× on 5 d, and by 49.83× on 7 d; meanwhile, dung beetles only reduced CH_4 by 1.40× across a week—not even surpassing the effect of a single day. The largest beetle reductions were seen on 3 d and 5 d when the Unmixed and Mixed dung showed 1.90–2× and 1.28–3.97× greater mean CH_4 fluxes than dung beetle treatments, respectively (Fig. 5). Thus, dung beetle groups inhibited CH_4 spikes (trajectories without peaks, Supp Fig. G3 [online only]) while dung-only encouraged it (trajectories with peaks—Fig. 6). Considering that vegetation was a weak sink (−1 to −3 mg $CH_4/m^2/d$) and dung increased CH_4 flux by ~300×—we can surmise that dung was the predominant CH_4 source until 7 d, when it nears vegetative fluxes (Fig. 6). Ultimately, time was the main effect—it explained 60% of the variation, yielded nearly 50-fold CH_4 reductions ($E = 0.77$), predicted negative CH_4 trajectory ($R^2 = 0.68$; $y = -90.30x + 426.60$), and closely approximated interaction effects ($E = 0.75$), while treatment did not ($E = 0.16$).

Carbon Dioxide

Dung presence enhanced CO_2 generation, producing 1.94× CO_2 overall or 1.53–2.51× on any given day than grass-only. Yet, theory holds that dung beetles significantly aerate the dung, but beetle treatments did not significantly increase CO_2 overall (Fig. 3), though time did (Fig. 4). However, dung beetles did produce 1.55× more CO_2 than the Unmixed dung-only on 1 d (Fig. 5) before declining, but all treatments showed this same negative-skewed bell curve (Fig. 6). While dung beetles had larger CO_2 fluxes more frequently ($P = 0.01$) compared with Unmixed dung-only (Supp Fig. G3 [online only]), it did not compare with the time effect. For example, 1.67×, 1.59×, and 1.41× more CO_2 was produced on 3, 5, and 7 d compared with 0 d. Thus, time ($E = 0.40$), rather than treatment

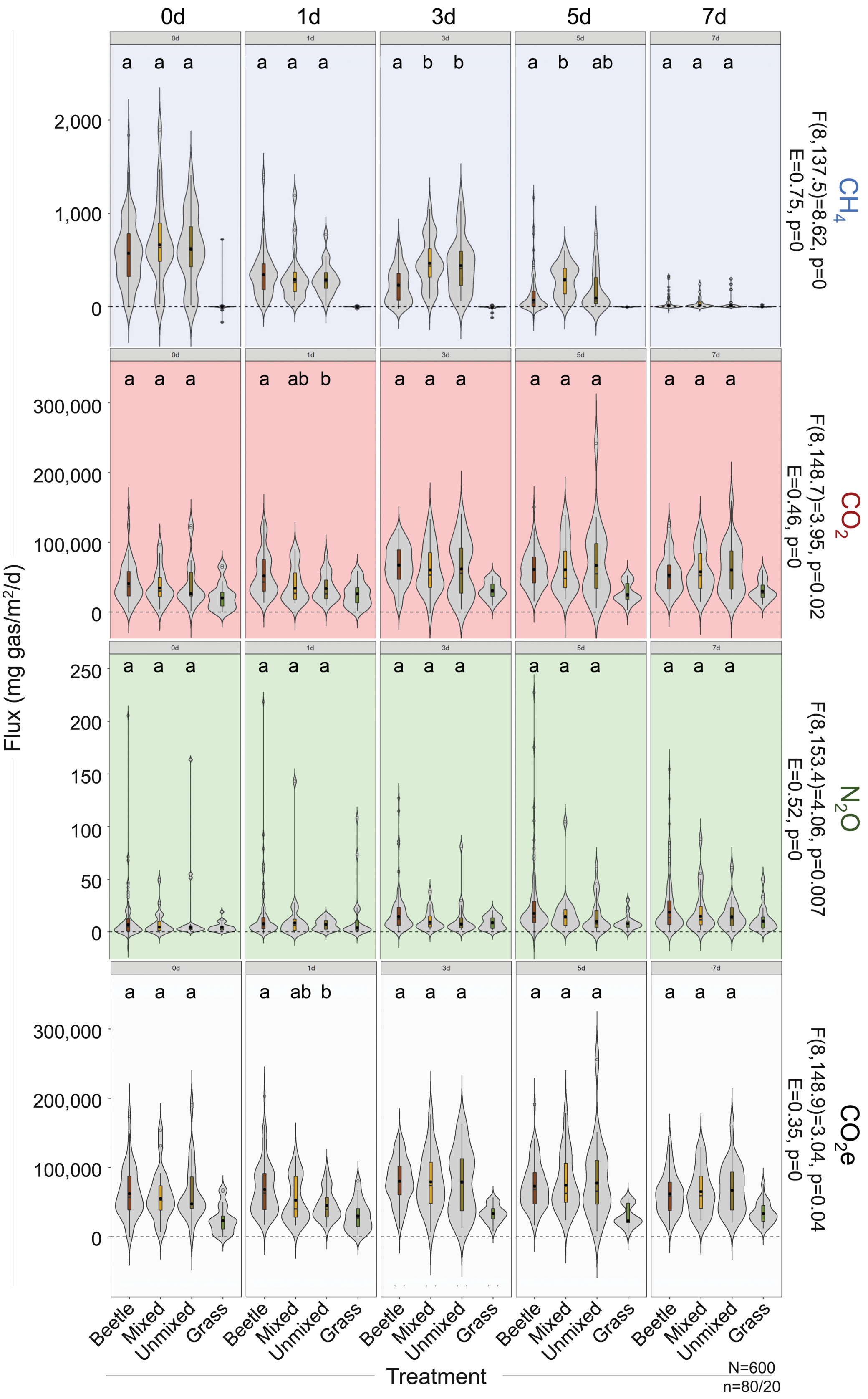

Fig. 5. Violin box plots of HMR Flux by Treatment within Time. Each quadrant represents a GHG with their respective omnibus mean-based ANOVAs ($F_{\text{df num, df den}}$) and median-based explanatory effect sizes (*E*) shown on the graph's right. Pairwise comparisons of ANOVAs (lowercase letters) are shown within the graph. Sample size (*n* = beetle/other groups) and total samples (*N*) are shown along the x-axis. Differing letters between groups show differences ($P \leq 0.05$). Exact means, medians, and measures of variations are found in Supp Table D2 (online only).

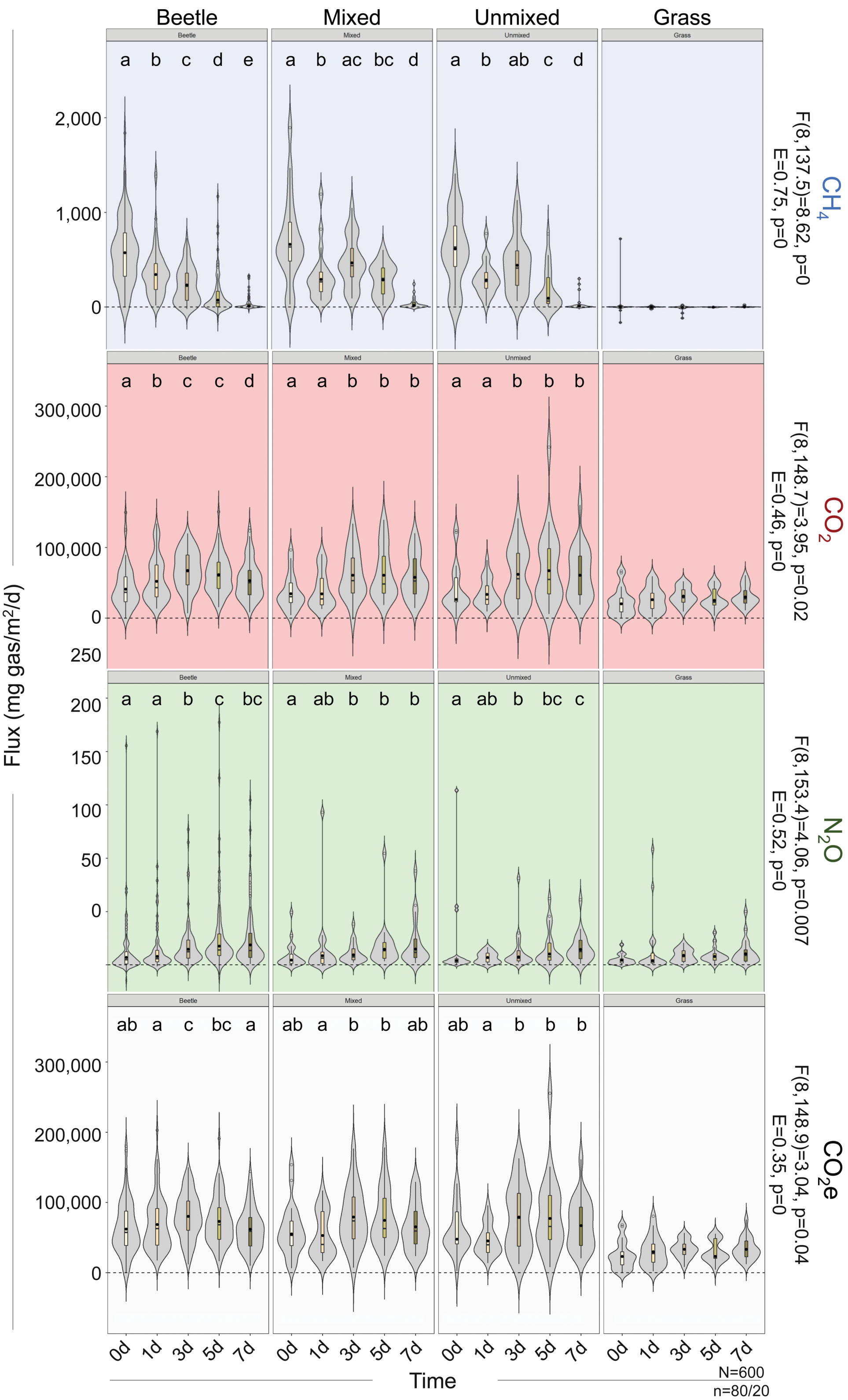

Fig. 6. Violin box plots of HMR Flux by Time within Treatment. Each quadrant represents a GHG with their respective omnibus mean-based ANOVAs ($F_{\text{df num, df den}}$) and median-based explanatory effect sizes (*E*) shown on the graph's right. Pairwise comparisons of ANOVAs (lowercase letters) are shown within the graph. Sample size (*n* = beetle/other groups) and total samples (*N*) are shown along the x-axis. Differing letters between groups show differences ($P \leq 0.05$). Exact means, medians, and measures of variations are found in Supp Table D2 (online only).

($E = 0.09$), predicted a positive CO_2 trajectory ($R^2 = 0.11$; $y = 4{,}354x + 35{,}070$) and more closely approximated the interactive effect ($E = 0.46$).

Nitrous Oxide

Cumulatively, dung beetles produced 1.56× and 1.67× greater N_2O means and medians than the Unmixed dung-only, but only the medians were statistically larger (Fig. 3). This is because dung beetles produced larger N_2O maximums, smaller N_2O minimums, and larger N_2O fluxes more frequently ($P < 0.005$; Supp Fig. G6 [online only]) than the Unmixed dung-only (Fig. 6), despite means showing different results (Fig. 5). Tunneler treatments were responsible for the largest fluxes, especially on 1 d (Supp Table D1 [online only] and Supp Fig. G1 [online only]). Mean–median inconsistencies indicate that reliable mean differences are infrequent and/or weak when comparing beetle and dung-only treatments ($E = 0.24$). This mean difference arises as dung beetle groups increased N_2O emissions from 3–5 d (Supp Table D1 [online only]) and dung-only treatments only did so from 5–7 d (Fig. 6; Supp Table D2 [online only]). These earlier arising N_2O emissions, when compared with Grass-only, suggests a beetle–time interaction—albeit, a minor influence. Comparatively, time surpassed treatment effects by increasing both mean and median N_2O fluxes (Fig. 4) across a week (Fig. 6). From 0 d onward, both means and medians increased by ~1.5×, ~2.4×, and ~3.3× on 1, 3, and 5—7 d, respectively ($E = 0.47$). However, four of the seven treatments are dung beetle-based and all dung beetle groups raised N_2O fluxes preemptively (Fig. 6), thus indicating that time's effect may be part of a dung beetle–time interaction. As with the other GHGs, dung presence increased total N_2O generation by 1.62× compared to Grass-only, with dung-based treatments generating 1.6–2× more N_2O on any given day except on 0 and 3 d (<1.25×). Interestingly, all treatments, including the Grass-only, had both N_2O and CO_2 follow the same increasing pattern ($R^2 = 0.14$; $y = 1.76x + 2.23$) over time (Fig. 6), particularly for the dung beetle groups.

Total Greenhouse Gas Effect

CO_2e is the sum of each GHG's relative effect rather than their total volume. For example, CH_4 and N_2O accounted for less than 0.5% of the total gases collected, but contributed 9.26 and 11.18% of the total CO_2e, respectively (Supp Fig. G4 [online only]). Comparatively, CO_2 accounted for nearly 80% of the total CO_2e, and so CO_2e and CO_2 are identical (Figs. 3 and 5) or nearly so (Figs. 4 and 6). Once again, treatment had no reliable effect on CO_2e (Fig. 3), except on 3 d in which dung beetles produced 1.06× more CO_2 than the Unmixed dung-only (Fig. 5). Time, meanwhile, showed a negative-skewed bell curve (Fig. 4) in which CO_2e weakly increased over time for all treatments ($R^2 = 0.008$; $y = 1{,}794x + 56{,}960$), except for 1 d, wherein dung-only treatments were at their lowest (Fig. 6). So while the dung-only treatments tended toward a higher frequency of larger CO_2e fluxes (Supp Fig. G3 [online only]), this effect was inconsistent (Fig. 3) and weak ($E = 0.09$), especially when compared with time's consistent (Fig. 4), strong effect ($E = 0.27$), and its close relationship with the interaction effect ($E = 0.35$).

In summary, both the beetle design and original design analyses revealed the same conclusions: dung beetles weakly reduced CH_4, inconsistently increased N_2O, and had no effect on CO_2 and CO_2e when compared with either the Mixed or Unmixed treatments. Tunneler treatments (Supp Fig. G1 [online only]) were likely responsible for the greatest CH_4 reductions and N_2O productions compared with the Mixed or Unmixed treatments. However, Beetle-Unmixed and Mixed-Unmixed contrasts prominently explain the dung beetle effect and homogenization effect, respectively. By these standards, dung beetles did not reduce CH_4 or CO_2e nor did mixing affect any GHG.

Discussion

Dung beetle activity disassembles, dries, and/or buries dung (Fowler et al. 2020c) over time (Fig. 2), which changes the microbial community through the mixing of dung and soil (Yokoyama et al. 1991a, Slade et al. 2016b). Furthermore, this releases and increases dung nutrients (Yokoyama et al. 1991a, Nichols et al. 2008, Evans et al. 2019) and moisture (Penttilä et al. 2013, Evans et al. 2019, Fowler et al. 2020c) in surrounding soils; this sparks soil microbial consumption (Fowler et al. 2020c), particularly for N_2O-producing microbes within the soil (Yokoyama et al. 1991a), because of the increased dung surface area. Therefore, we hypothesized that more constant activity (manual mixing on 1 d vs. dung beetle activity for 7 d), greater abundance (7 g vs. 15 g dung beetles), and soil-disrupting activities (dwelling vs. tunneling) would result in treatment-based GHG reductions—this was not the case.

GHG Trends Across the Literature

The production of CH_4 and N_2O occur under similar anaerobic conditions (Sylvia et al. 2005), so it is surprising that the dung-only treatments saw brief CH_4 spikes during the week whereas dung beetle-based treatments solely decreased CH_4 over time—with the situation reversed for N_2O (Figs. 5 and 6). Periodic CH_4 (Penttilä et al. 2013, Slade et al. 2016a, Evans et al. 2019) and N_2O spikes (Penttilä et al. 2013, Iwasa et al. 2015, Slade et al. 2016a, Fowler et al. 2020c) are common and likely related to dung/soil moisture (Penttilä et al. 2013, Evans et al. 2019) and/or soil disturbance (Fowler et al. 2020c)—abiotic factors heavily influenced by dung's natural decay process. Understandably, since dung beetles affect both the dung and soil matrix simultaneously, studies often focus on these two GHGs, despite beetle related CH_4 reductions and N_2O productions occurring only 50–63% of the time, respectively (Table 1). While our Tunneler treatments hint at this similar trend when compared to the Unmixed dung-only (Supp Fig. G1 [online only]), the effect is too weak. Meanwhile, CO_2 is produced under aerobic conditions and saw different trends.

Generally, dung-only showed highly variable CO_2 trajectories, with some studies showing increases (Slade et al. 2016b, Evans et al. 2019, Fowler et al. our data) or decreases (Penttilä et al. 2013, Iwasa et al. 2015, Piccini et al. 2017, Fowler et al. 2020c) despite using identical methodologies in some cases. Thus, CO_2 does not follow a familiar process as it does for CH_4 and N_2O. More importantly, if dung beetles aerated the dung, we would expect more dung-based CO_2 from increased microbial respiration; however, only 12.5% of studies showed that dung beetles increased CO_2 production (Table 2) and our results showed only a weak effect on 1 of 5 d (Fig. 5). Explanations for this increase included: 1) dung beetle respiration (Iwasa et al. 2015), 2) enhanced gas transport (Evans et al. 2019), or 3) increased microbial populations and subsequent respiration from fresh resources (Fowler et al. 2020c). While gas transport is unknown, our recent trace gas analysis indicates CO_2 changes were not based on dung beetle respiration (Fowler et al. 2020c, Piccini et al. 2017). In fact, CH_4, N_2O, and CO_2 fluxes dwarfed vegetative controls by >300×, 1.94×, and 1.62×, respectively; this suggests that microbial consumption and respiration of dung-specific resources is likely the primary source of increased GHGs generally. So while dung beetle microtunnels partially explain CH_4, N_2O, and CO_2 increases in wet dung (Evans et al. 2019)—it does not explain why dried dung (Fig. 2) showed both linear increases (Fowler et al. our data) and decreases (Fowler et al. 2020c) in CO_2 over time using identical methodologies. Like many biological processes, CO_2 production is unclear and is guided by a myriad of processes for which there may be multiple reasons for an experiments' reported trajectory.

Overall, dung beetles weakly decreased CH_4 and increased N_2O, while time strongly decreased CH_4, increased N_2O, and increased CO_2 over time (E = 0.27–0.77). Time effects were ~2–5× greater than any dung beetle effect alone (E = 0.09–0.24), nor were interaction effects greatly enhanced by dung beetle presence (E = 0.35–0.75), except for N_2O. Dung beetles interacting with dung increased N_2O compared with dung-only, which suggests an unexplored potential link between the soil and dung decay processes (Fowler et al. 2020c). Theoretically, dung beetles can drastically affect dung/soil-generated GHGs, since both time and dung beetles similarly affect dung (Penttilä et al. 2013) and soil moisture content (Evans et al. 2019, Fowler et al. 2020c), but it is likely that this effect will only show when dung beetles accelerate dung decay as strongly as time does—which ours and previous experiments do not reflect (Table 2).

Potential Reasons for Negligible Dung Beetle Effects

Perhaps there is no dung beetle effect on CO_2e because of: 1) low dung beetle biomass per liter dung; 2) low productivity (dung damage) and cage efficiency; or 3) unrepresentative dung beetle populations.

1. *Dung Beetle Biomass*. While abundance is a useful metric, it weakly correlates with dung beetle ecosystem services and functionality when compared with biomass (Nichols et al. 2008) as individual numbers cannot predict large weight differences between and within species. For example, Piccini et al. (2017) measured the mass of the small tunneler, *Onthophagus coenobita*, and the larger tunneler, *Copris lunaris*, which showed a 10× difference in individual biomass despite being from the same functional group. Therefore, we created the largest dung beetle abundances our treatments could naturally sustain throughout the late spring, summer, and fall. Our limiting dung beetle species was *L. pseudolividus* and collecting >50 g dung beetle per experiment throughout the season was unreasonable. Thus, we chose biomasses similar to and 2× as large as the heaviest treatments to date (Fowler et al. 2020c) to reveal any differences, but no practical GHG differences were found despite our 15 g treatment being 6.22× larger (min: 2.42×, max: 23.94×) than the mean reported beetle biomass per dung (Table 1).
2. *Productivity*. Since no other visual dung pat damage data exist for other studies (Penttilä et al. 2013; Iwasa et al. 2015; Hammer et al. 2016a,b; Slade et al. 2016a, b; Piccini et al. 2017; Evans et al. 2019), we hypothesized greater dung beetle biomass per unit dung would also increase dung damage—it did (Fig. 2). Both the Unmixed and Mixed dung-only pats were completely solid throughout (Fig. 2E and F) and, when broken apart, gooey on the inside, which is the ideal control. Meanwhile, Tunneler treatments showed shredded damage (Fig. 2A) and/or dung tunnels or soil mound combos that increased when tunneler biomass increased. Dweller treatments were shredded at higher abundances as well (Fig. 2B), though lacked the rigor of tunneling damage despite identical biomasses. Low Dwell treatments showed pockmarked dung pats (Fig. 2D). Because of the protective measures taken with the cages (Fig. 1), the difference in visual damage between all treatments (Fig. 2), and the progressively worsening dung damage over a week—it is unlikely that dung beetle escape was an issue nor a reason for seeing minimal GHG changes.
3. *Population Representation*. Most studies, including this one, were assumed to adequately represent rigorous dung beetle populations when this may not be the case. In our experiment, our treatment pats were ideally over-colonized (Fig. 2A and B) compared with the surrounding field pats (Fig. 2G and H), thus giving our treatments the best chance at finding any GHG differences from dung beetle activity, or so we thought. On one rare occasion, we happened upon an unusual dung pat (Supp Fig. A [online only]) completely dissembled within 2 d—an activity not reported by any other authors (Penttilä et al. 2013; Iwasa et al. 2015;Hammer et al. 2016a,b; Slade et al. 2016a, b; Piccini et al. 2017; Evans et al. 2019; Fowler et al. 2020c), but frequently reported in wild, tropical habitats (chapter 17: Hanski and Cambefort 2014; Jones 2017) and in studies showing decimation of local fly larvae populations (Nichols et al. 2008). We found this pat (Supp Fig. A [online only]) after setting up the electric fences surrounding our experimental site on 0 d where the curious cattle lingered and defecated just outside of our sampling site. We marked the dung pats to informally compare our treatment pats to field pats (Fig. 2A and B vs. Fig. 2G and H). Even the damage sustained by 15 g dung beetles per liter dung did not compare to this intense, wild dung beetle activity. Caging dung beetles may potentially force them to withstand unfavorable conditions over time that they may not have chosen for themselves (=reduced activity)—especially when considering that dung beetles show soil, dung age, dung quality, diel, and seasonal preferences (chapter 18: Hanski and Cambefort 2014). Additionally, field pats may show rapid dung decomposition (Supp Fig. A [online only]) because of a constant daily influx of attracted dung beetles, thereby any single dung pat may experience greater beetle biomasses over time compared with restricted, caged dung pats. As evidence, when determining our experimental biomasses for this study (7.5 and 15 g), we tested various tunneler biomasses (5, 10, 15, 20, and 25 g) and noticed that >15 g dung beetle biomass showed similar dung damages to our highest biomass treatment (Fig. 2, High Tunn). Initially, we thought this meant a certain dung beetle biomass threshold guaranteed adequate dung damage, but alternatively it could mean that limited space (extreme competition) or forced cohabitation with non-preferred, aging dung result in somewhat dissembled dung pats (Fig. 2 vs. Supp Fig. A [online only]). We therefore wonder if studying the dung beetle effect from extremely active populations (disassembling dung pats within days) at their leisure may reveal their true GHG reductive potential.

Conclusion

Our experiment, despite reporting the highest reported dung beetle biomass per dung pat (Table 1) with observed dung damage (Fig. 2), saw no practical dung beetle effect on any GHG nor on CO_2e. It is possible that dung beetles do not play a significant role in reducing dung-generated GHGs, but after finding that dung beetles can dissemble dung pats within 2 d (Supp Fig. A [online only]), it is also possible that current reported dung beetle abundances and biomasses may be insufficient in impacting GHG emissions. Thus, studying extremely active dung beetles in and around wildlife habitats may finally explain why current research reports negligible dung beetle effects on dung-generated GHGs (Fowler et al. 2020c).

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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