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Dung Beetles Increase Greenhouse Gas Fluxes from Dung Pats in a North Temperate Grassland

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Abstract

Soil fauna plays a critical role in various ecosystem processes, but empirical data measuring its impact on greenhouse gas (GHG) emissions from rangelands are limited. We quantified the effects of dung beetles on in situ CO_2, CH_4, and N_2O emissions from simulated cattle dung deposits. Soil in meadows of the semiarid Nebraska Sandhills was treated with three treatments (dung pats with exposure and without exposure to dung beetles, and a no dung control). A closed-chamber method was used to measure GHG fluxes at 0, 1, 2, 3, 7, 10, 14, 21, 28, and 56 d after dung placement in the early season (June–August) and late season (July–September) in 2014 and 2015. The greatest dung beetle abundance was 6 ± 2 beetles per quarter pat on Day 7; the abundance decreased to <2 ± 0.6 on Day 14 and 28 and zero on Day 56. Dung beetles increased fluxes of CO_2 by 0.2 g C d^{-1} m^{-2}, N_2O by 0.4 mg N d^{-1} m^{-2} (only in late season 2015), and CH_4 by 0.2 mg C d^{-1} m^{-2}. These increases were due to beetle-made macropores that facilitated gas transport in wet dung (initial moisture = 4.6 g g^{-1} on a dry-weight basis) within 7 d after dung placement. Seasonal environmental differences resulted in greater CO_2, N_2O, and CH_4 fluxes in the early season than in the late season. This study concluded that dung beetles increased GHG fluxes from early- and late-season dung deposits on meadows of the semiarid Nebraska Sandhills.

Core Ideas

- Dung beetle activities on dung pat enhanced CO_2–C emission.
- The effect of dung beetle activities on N_2O emission from dung pat was inconsistent.
- Soil moisture and temperature had a significant effect on GHG emission.

SOIL FAUNA (such as earthworms, flies, termites, ants, dung beetles, and other arthropods) plays a critical role in various ecosystem services. An example of ecosystem service provided by soil fauna is acceleration of organic material decomposition and nutrient cycling (Lee and Wall, 2006; Yamada et al., 2007; Freymann et al., 2008; Nichols et al., 2008; O'Hea et al., 2010). Although organic material decomposition and nutrient cycling are important for soil quality, the decomposition also produces greenhouse gases (GHGs). In a well-aerated environment, organic compounds of the decomposing organic materials are enzymatically oxidized to produce CO_2, water, energy, and decomposer biomass (Brady and Weil, 1999) as represented in Eq. [1]:

$$\begin{aligned} \mathrm{R-(C,\ 4H)} + \mathrm{2O_2} &\rightarrow \mathrm{CO_2}\uparrow + \mathrm{2H_2O} \\ &+ \text{energy}\left(478\ \mathrm{kJ\ mol^{-1}\ C}\right) \end{aligned} \qquad [1]$$

During the aerobic decomposition, proteins in the organic materials are also broken down to produce amino acids, eventually resulting in NH_4^+, NO_3^-, and SO_4^{2-}, which are available for plant nutrition and other microbial processes. The NH_4^+ and NO_3^- can be subjected to microbial process of nitrification (Eq. [2]) and denitrification (Eq. [3]) that produce N_2O (Saggar et al., 2004):

$$\begin{array}{ccccc} \mathrm{NH_4^+} & \xrightarrow[\mathrm{O_2}]{\substack{\text{ammonium}\\ \text{monooxygenase}}} & \mathrm{NH_2OH} & \xrightarrow[1/2\mathrm{O_2}]{\substack{\text{hydroxilamin}\\ \text{oxidoreductase}}} & [\mathrm{HNO}] \\ & & & & \downarrow \\ & & & \mathrm{NO_3^-} \leftarrow & \mathrm{NO_2^-} \\ & & & & \downarrow \\ & & & & \mathrm{N_2O} \end{array} \qquad [2]$$

$$\mathrm{NO_3^-} \rightarrow \mathrm{NO_2^-} \rightarrow [\mathrm{NO}] \rightarrow \mathrm{N_2O} \rightarrow \mathrm{N_2} \qquad [3]$$

Anaerobic decomposition of organic compound also produces organic acids that can be broken down further by

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Abbreviations: DM, dry matter; DSU, dung and soil underneath; GC, gas chromatography; GHG, greenhouse gas; LSMean, least squares mean; ND, no dung; UXD, unexposed dung; VWC, volumetric water content; WEN, water-extractable nitrogen; WEOC, water-extractable organic carbon; XD, exposed dung.

methanogenic bacteria to produce CO_2 and CH_4 gases (Brady and Weil, 1999), as represented in Eq. [4] and Eq. [5]:

$$4C_2H_5COOH + 2H_2O \rightarrow 4CH_3COOH + CO_2\uparrow + 3CH_4\uparrow \quad [4]$$

$$CH_3COOH \rightarrow CO_2\uparrow + 3CH_4\uparrow \quad [5]$$

Microbial decomposition of dung organic materials also produces GHGs (Saggar et al., 2004; Bellarby et al., 2013), as shown in Eq. [1–5]. Dung beetles are among the most significant invertebrate contributors to acceleration of dung decomposition in north temperate rangelands (Lee and Wall, 2006), but studies measuring beetle impact on GHG emissions are limited, and results of a few reported studies are inconsistent. Penttilä et al. (2013) and Iwasa et al. (2015) indicated that dung beetles increased CO_2 within 10 d during the early part of decomposition, yet Piccini et al. (2017) indicated that dung beetle reduced cumulative CO_2 emission during a 32-d experiment. Effects of dung beetles on N_2O emission were also found to be inconsistent; dung beetles either increased (Penttilä et al., 2013; Iwasa et al., 2015) or reduced (Slade et al., 2016; Piccini et al., 2017) N_2O emissions. Studies have shown that dung beetle activity can reduce CH_4 emissions from dung pats (Penttilä et al., 2013; Iwasa et al., 2015; Slade et al., 2016).

Factors affecting dung bioturbation and decomposition are expected to affect GHG emissions. Dung beetles feed on the liquid contents of dung and use remaining dung material for housing and food for their brood. According to nesting strategies, dung beetles are grouped in three functional groups: (i) endocoprids (dweller), the beetles live and brood inside the dung pat; (ii) paracoprids (tunneler), the beetles dig burrows and construct nesting chambers with dung materials in the soil below the dung pats; and (iii) telecoprids (ball roller), the beetles form dung balls and roll them some distance away from the dung pat before burial into soil for their brood (Halffter and Edmond, 1982; Sullivan et al., 2016). The abundance of dung beetles increased colonization of dung by arthropods communities, which are major contributors to dung degradation (Pecenka and Lundgren, 2018). Climate factors such as temperature affect dung colonization (Errouissi et al., 2004); therefore, seasonal conditions resulted in faster dung decomposition rates in late spring than in in late summer (Lee and Wall, 2006).

Consistent among a few reported studies was that dung beetles affected GHG fluxes, but the exact mechanisms of how dung properties and soil nutrients underneath the dung affect GHG emissions were not clear. Penttilä et al. (2013) and Piccini et al. (2017) speculated that dung beetles modified GHG emissions through the effects of beetle-made macropores on dung pat internal aeration and drying. Increasing aeration and O_2 enhanced CO_2 production, increased nitrification (and N_2O production), and decreased CH_4 production. These authors emphasized gas production and consumption as a governing mechanism in causing gas flux and did not discuss the importance of gas transport processes as a limiting factor. However, it is known that macropores increase gas transport in porous media such as soil (Perret et al., 1999).

Our goal was to evaluate the effect of soil fauna on the fluxes of GHG from dung pats on rangeland. Measurements of beetle colonization and properties of dung and soil over time were conducted in early and late summer to understand how new dung deposits affect GHG fluxes over the seasons in the rangeland of Nebraska's Sandhills. There are 5.1 million ha of rangeland in the Sandhills of Nebraska, and rangeland accounts for ~70% of the necessary forage used for beef and dairy production globally (Lund, 2007). Therefore, quantification of beetle effects on GHG fluxes and identification of the mechanisms of GHG fluxes from the rangeland will provide greater understanding of the overall system in the context of adaptive management needed for expected changes in climate.

Materials and Methods

Site Description

Research was conducted on a shallow-groundwater-fed meadow at the Barta Brothers Ranch (42°13′28.65″ N, 99°38′19.17″ W; 773 m asl) during growing seasons of 2014 and 2015. The ranch is a 2350-ha grazing research site located in the eastern Nebraska Sandhills and operated by the University of Nebraska–Lincoln. Vegetation consists of predominantly mixed cool-season grasses [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, *Poa pratensis* L., *Bromus inermis* Leyss., *Agrostis gigantea* Roth, *Elymus repens* (L.) Gould, and *Phleum pratense* L.], less abundant warm-season grasses [*Andropogon gerardii* Vitman, *Sorghastrum nutans* (L.) Nash, *Panicum virgatum* L., and *Spartina pectinata* Bosc ex Link], mixed forbs and legumes (*Achillea millefolium* L., *Medicago sativa* L., *Potentilla recta* L., *Rudbeckia hirta* L., *Trifolium pretense* L., and *Trifolium repens* L.), and various rushes (*Juncus* L. spp.) and sedges (*Carex* L. spp.).

The climate is semiarid with long-term average (1981–2010) annual precipitation of 584 mm (NOAA, 2013), and a mean annual air temperature of 9.6°C. Eighty percent of the precipitation falls between April and September, with May and June typically being the wettest months. Soils are of the Els series, a mixed, mesic Aquic Ustipsamment with sandy to fine sandy loam texture (USDA-NRCS, 2009). Initial soil bulk density to 20-cm depth was 1.44 Mg m^{-3}; soil organic matter content ranged between 14 and 33 mg g^{-1} at the 0- to 10-cm depth and between 4 and 9 mg g^{-1} at the 10- to 20-cm depth.

Treatments

In June 2014, an experimental site (Fig. 1A) was divided into eight blocks; each block was 7.2 × 3.6 m in size. Each block was further divided into six plots (3.6 × 1.2 m in size), and one plot was randomly selected for GHG measurement (Fig. 1D). On each of the selected plots, three mesocosms were constructed and randomly designated for three levels of dung treatment (Fig. 1E); therefore, there were a total of 24 mesocosms on the site. Each mesocosm was constructed by inserting an aluminum ring (65 cm in diameter and 25 cm in height) into the soil to an average depth of 16 cm. Each mesocosm received one of the three treatments: (i) exposed dung (XD), where dung pat was placed directly on the soil surface inside the mesocosm; (ii) unexposed dung (UXD), where dung pat was placed in a wire mesh cage and then placed on the soil surface inside the mesocosm; and (iii) no dung (ND) control, where no dung was placed on the soil surface inside the mesocosm (Fig. 1E). The XD treatment enabled dung beetles or other soil fauna to colonize and/or exit the dung pat freely. The mesh (1-mm opening) cage of the

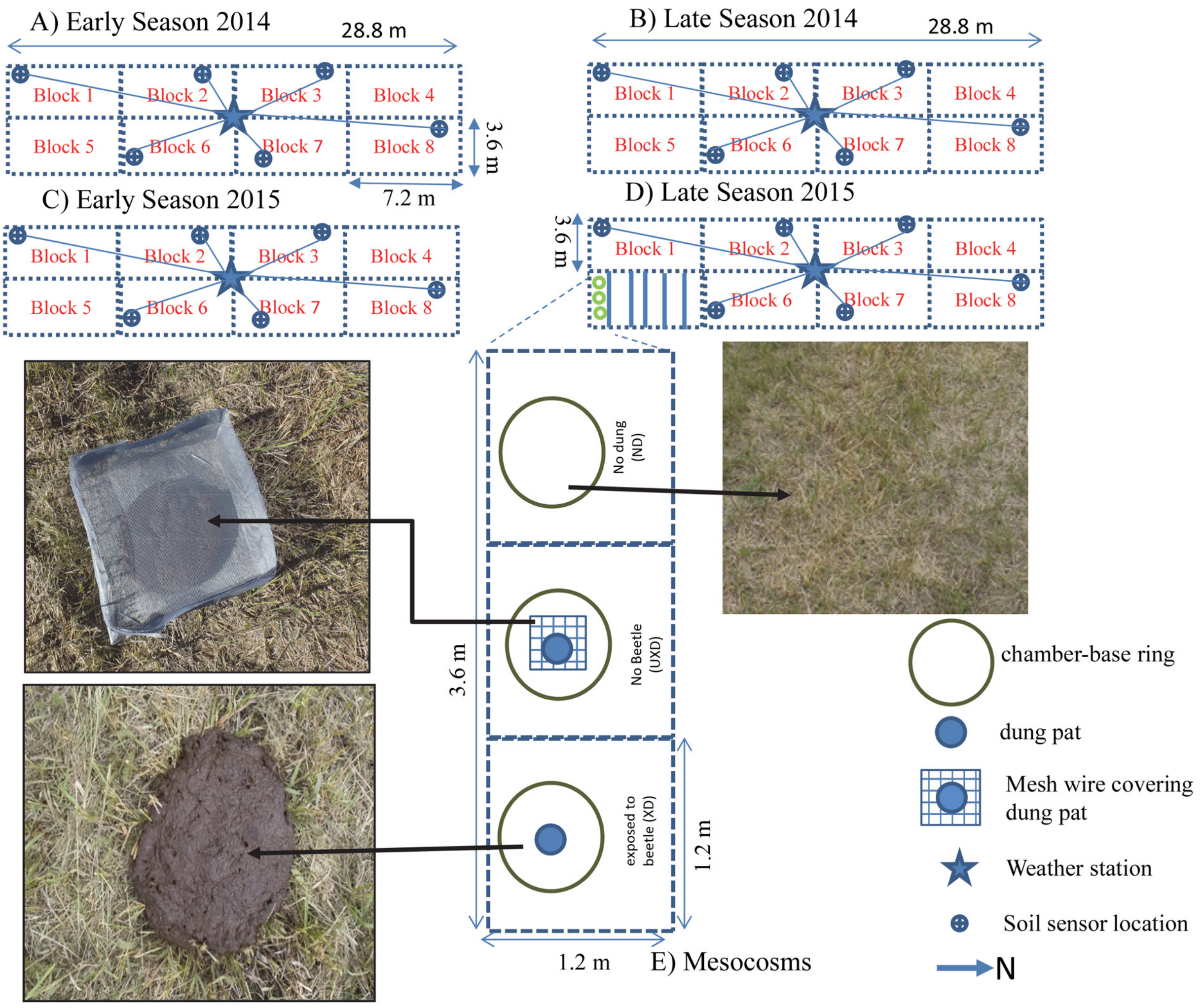

Fig. 1. Experimental layout of four year–season combination experiments for placement of exposed dung (XD), unexposed dung (UXD), and no dung control (ND) on grassed soil in the early season and in late season of 2014 and 2015 in meadows of the Nebraska Sandhills.

UXD treatment (approximate dimension of 38 × 38 × 18 cm) covered the pat from top, bottom, and the sides to prevent dung beetle or other soil fauna colonization.

To evaluate how seasonal changes in environmental condition affect decomposition and GHG emission from new dung deposits, the same experiment (Fig. 1B) was conducted in July 2014 and placed adjacent to the site constructed in June 2014. The treatment with dung application in June was identified as early season, and the treatment with dung application in July was identified as late season. Both experiments were repeated in 2015 (also in June and July) on adjacent locations (Fig. 1C and 1D). In 2014, the early-season experimental period was from 10 June to 5 August, and the late-season experimental period was from 15 July to 12 September. In 2015, the early-season experimental period was from 8 June to 3 August, and the late-season period was from 14 July to 12 September.

Dung was collected from grain- and pasture-fed yearling steers that did not receive insecticidal treatment. The steers' diet consisted of 70.5% bromegrass (*Bromus inermis*), 23.3% dry distiller grains plus solubles, 5.8% dry rolled corn (*Zea mays* L.), 0.28% salt, 0.05% beef trace mineral, and 0.03% vitamins A, D, and E; all rates are expressed on a dry matter (DM) basis. The steers were fed 6.9 kg DM d^{-1} while held away from any pasture for observation. Dung was stored in 19-L plastic buckets at approximately −20°C until use. Before use, the dung was thawed, homogenized, and reconstituted by adding ~4 L of tap water to each bucket. The dung was thoroughly mixed inside the bucket during application of treatments to ensure consistency across dung pats. Simulated dung pats were made by adding 1.5 L of the reconstituted dung into a 20-cm-diam. plastic ring as described by Penttilä et al. (2013). The surface area of soil covered by a dung pat was 9.5% of each 0.33-m^2 mesocosm surface area; the rest (90.5%) of the mesocosm consisted of a grassy surface area that was dung free (Fig. 1E).

Greenhouse Gas Sampling

Greenhouse gas samples were collected in accordance with GraceNet Chamber method protocols (Parkin and Venterea, 2010). Gas samples were taken at 0, 10, 20, and 30 min after placing and sealing a chamber lid onto the already installed chamber-base rings (aluminum rings of the mesocosms described above). The 30-min sampling time allowed detectable increases in GHGs in a large volume of chamber, especially N_2O and CH_4, which may have low flux (Ginting et al., 2003; Ginting and Eghball, 2005).

The chamber temperature was recorded at the end of the 30-min collection time using a thermometer placed inside the chamber. The chamber lids were made of stainless steel and had an average diameter of 66 cm, an average height of 15 cm, and a 1.3-cm-thick layer of foam board insulation covered with aluminum foil. Rubber gaskets (made of bicycle inner tubes) were taped to the outside of the lid and secured by metal screws. The rubber gasket was used to seal the seams between the chamber lid and chamber-base ring. Air circulation fans were attached to the inside of the lids with wire and powered by 9-V batteries. Septa (pierceable butyl rubber, Labco) were installed on the top of the chamber lids, through which gas samples were collected using a 30-mL syringe (Henke-Sass Wolf, Soft-Ject Luer Lock).

Gas samples were collected at approximately 9 AM, corresponding with the mean diurnal temperature to account for variations in GHG flux due to diurnal temperature changes (Parkin and Venterea, 2010). Using a syringe, 25-mL samples were taken and then transferred into pre-evacuated (to ~400 Pa) 12-mL Exetainer glass vials (Labco). Gas samples were then stored cold in insulated Styrofoam containers and transported the same day for analysis. Concentrations of CO_2, CH_4, and N_2O in each sample were determined simultaneously by gas chromatography (GC) on an automated Varian 450 GC (Agilent Technologies). Gas measurement was accomplished using He carrier gas through a Porapak QS column (50°C) equipped with a thermal conductivity detector for CO_2 detection and a flame ionization detector for CH_4 detection. Nitrous oxide was quantified with an electron capture detector (HayeSep D column and Ar/CH_4 carrier gas). The GC was calibrated at each sampling time using an external calibration method of comparing standard samples of known gas concentration and ambient air collected from the experimental site at each sampling day. Within each season, GHG sampling event was done at 0, 1, 2, 3, 7, 10, 14, 21, 28, and 56 d after dung pat placement. Henceforth, the variable for sampling events is named day and each of the 10 sampling events is named as Day 0, Day 1, Day 2, ..., and Day 56. Day 0 represents a sampling event before dung placement.

Flux Calculation

Gas flux was calculated as follows:

$$J = \frac{dC}{dt} \times \frac{M}{V^\circ} \times \frac{T^\circ}{T} \times H \times 24 \times \left(1.0 \times 10^{-6}\right) \qquad [6]$$

where J is flux (g m^{-2} d^{-1}), dC/dt is the slope of analyte gas concentration [(volume of gas/volume of air) h^{-1}; the slope is derived with simple linear regression relating gas concentration ($\mu L\ L^{-1}$) as dependent variable and length of time (h) after closure of gas collection chamber as independent variable], M is the analyte gas molar mass (44, 44, and 16 g mol^{-1} for CO_2, N_2O, and CH_4, respectively), V° is 0.0224 m^3 mol^{-1} at 273 K and 0.10 MPa, T° is 273 K, T is the chamber temperature (K), and H is the chamber height (m), derived by dividing the chamber volume (m^3) with area of mesocosm (m^2). Chamber volume is the total volume of chamber lid and chamber base.

Equivalent CO_2 values of N_2O and CH_4 were calculated based on compound-specific 100-yr atmospheric warming potentials. Multipliers of 1 for CO_2, 21 for CH_4, and 310 for N_2O were used as suggested by the Intergovernmental Panel on Climate Change (Krey et al., 2014).

Estimation of Flux from Dung and Dung–Soil Interaction

The measured flux from the dung-treated mesocosms is a sum of flux from (i) dung-free soil, (ii) dung pat, and (iii) the interaction effect of dung and soil directly underneath the dung. For convenience, the inseparable effects of dung pat and dung–soil interaction was referred to as dung and soil underneath (DSU). By assuming that the interaction of the dung-free soil and the DSU is negligible, the estimation of flux from DSU can be calculated as

$$\mathrm{FDSU}_{ystdb} = \mathrm{FM}_{ystdb} - \mathrm{FC}_{ysdb} \qquad [7]$$

where the FDSU_{ystdb} is the calculated flux from DSU for the year (y), season (s), dung treatment (t, XD or UXD), day after application (d), and block (b). The FM_{ystdb} is the measured flux of a dung-treated mesocosm, and the FC_{ysdb} is the measured flux of the ND control mesocosm. The calculated FDSU_{ystdb} were then related with physical and chemical analysis of dung and soil underneath the dung. The FDSU is in mass per day per square meter of mesocosm area; therefore, the FDSU accounts for the flux from an areal density of three dung pats per square meter of rangeland.

Dung Beetle Survey and Dung–Soil Analytes

Dynamics of dung beetle colonization after placement of dung is crucial information in understanding GHG fluxes. The beetle survey was done by placing one XD pat and one UXD pat on each of the five remaining plots in each block (Fig. 1D). The XD and UXD dung pats were harvested from each respective plot on Days 1, 3, 7, 14, and Day 28. On Day 56 (the end of the experiment), dung pats from the UXD and the XD mesocosms were harvested after gas sampling. Each harvested dung pat was homogenized and split into four quarters. One quarter was used for dweller beetle survey, using both floating and sieving survey methods (Whipple, 2011). The floatation method was performed by placing ~100 g of dung material into 1000 mL of water, followed by stirring, soaking 5 to 45 min, saturating, and stirring once again to free beetles from dung material. Beetles that floated to the water surface were then collected, counted, and identified (Whipple, 2011). The number of beetles counted in the one-quarter pat was not scaled up (not multiplied by four) to the whole pat. Our emphasis was not to find the exact enumeration of beetle in each pat at every sampling time. In this experiment, no measurements of the roller beetle, burrower beetles, and other soil fauna were made.

The other three quarters of the dung pat was used for determining of dung moisture (dry weight basis), dung DM, water-extractable organic C (WEOC), water-extractable N (WEN), and NH_4^+. We chose dung moisture on a dry-weight basis (water/DM) because dung moisture on a wet-weight basis [water/(water + DM)] is not sensitive toward water loss when dung was wet (within 7 d after dung application). Dung WEOC and WEN were obtained after 1-h extraction of field moist dung in deionized water at a water/dung ratio of 200:1.

After dung pat removal at each dung sampling, a composite of four soil cores was immediately collected below each dung pat at the 0- to 10-cm depth. Soil cores were collected using a handheld soil probe, 1.5 cm in diameter. Field-moist soil samples were sieved to pass 2-mm mesh. The field-moist soil samples were analyzed for WEOC, WEN, and KCl-extractable NH_4^+ and NO_3^-. Soil WEOC and WEN, were obtained after 1-h extraction of soil in deionized water at a water/soil ratio of 5:1.

Dung and soil extracts were analyzed for organic C and N on a Shimadzu 5200 liquid analyzer (Shimadzu Corporation). The NH_4^+ and NO_3^- were determined by flow injection method (Ružicka and Hansen, 1988) using a Lachat Quikchem 8000 (Lachat Instruments).

Environmental Data

Environmental data were intended to describe weather and soil conditions across each experimental site (Fig. 1). The environmental data were not associated with a particular mesocosm or dung treatment. Hourly soil and weather data were recorded on the experimental site with a data logger (Campbell Scientific CR1000). Instrument and sensors for weather data collection were installed at the center of the experimental site prior to dung application (Fig. 1). Soil sensors were installed outside the mesocosms at six (out of eight) blocks at distances of 3, 7, and 11 m from the center of the experimental site (Fig. 1A–1D). Soil sensors were buried at 10- and 20-cm depths.

Air temperature, relative humidity, and vapor pressure were measured with a Campbell Scientific WXT520 weather sensor, and precipitation was measured with a tipping bucket pluviometer (Campbell Scientific). Soil temperature and water content were measured with Campbell Scientific CR655 soil water content reflectometer sensors.

Statistical Analysis

The ANOVA of GHG fluxes and ancillary data was done using PROC MIXED procedure of SAS 9.4 (Little et al., 1996; SAS Institute, 2014). Data transformation was not necessary based on tests for normality and homogeneity of variance. The analysis fit a multisite experiment, where the ANOVA for each site (nested within year and season) was based on a split-plot experimental design with repeated measures. The whole-plot factor was treatment and the split-plot factor was day. To fit the time-series covariance structure (in which correlations decline as a function of day) on each subject (block × treatment nested within year season), the spatial power law [SP(POW)] was selected for the unequally spaced day (Days 0, 1, 2, 3, 7, 10, 14, 21, 28, and 56). The significance of fixed effects (year, season, treatment, day, and their interactions) was declared at $\alpha = 0.05$. Least squares means (LSMeans) ± SE, LSMean differences, and various group mean comparisons were also performed using the Estimate and Contrast statement of the PROC MIXED.

The data summary (means, sum, and SE) were derived using PROC MEANS procedure of SAS. Selection of environmental variables that explain the majority of variability of environmental data was done using principal component analysis on the correlation matrix. The principal component analysis was computed with the PRINCOMP procedure of SAS.

Results

Environmental Conditions

Weather and soil conditions varied with year, season, and day (Fig. 2, Table 1). A steep decrease in soil volumetric water content (VWC) occurred from the start of the early season (Fig. 2B). Rainfall (Fig. 2A) <20 mm d^{-1} resulted in a small increase in VWC, until a rainfall of 40 mm d^{-1} occurred on 10 July 2014 in the early season. Near the end of the same early season, a general trend of increasing soil temperature was observed (Fig. 2C). In 2015, the general trend of VWC decreased over time from the start of the early season to the end of the late season (Fig. 2B), and the rise in VWC was concurrent with rainfall. Soil temperatures showed an increasing trend from the start to the end of early-season period (Fig. 2C). Soil temperature drops were concurrent with rainfall events.

Dung Beetle Activities and Changes in Dung Moisture

The screen cage in the UXD treatment (Fig. 1) was effective against beetle colonization of dung pat. Out of the 192 UXD dung samples harvested in this study, only 10 pats (5.2%) contained beetles. These 10 UXD dung pats contained one or two beetles in one quarter pat. We presumed that these beetles must have been present in some of the dung pats prior to dung application and/or during dung placement. In the XD dung pats, dung beetle abundance on Day 1, 3, or 7 was greater than that on Day 14, 28, or 56 (Fig. 3). On Day 56, the XD dung pats contained no dung beetle. Across all the pats, five dung beetle species identified were *Sphaeridium scarabaeoides*, *Aphodius fimetarius*, *Onthophagus hecate*, *Onthophagus pennsylvanicus*, and *Ataenius spretulus*. The most commonly found species was *Aphodius fimetarius*.

Dung average initial moisture content was 4.6 g g^{-1} (dry weight basis). Dung moisture content decreased sharply within 7 d after dung placement. Dung moisture content in the XD and UXD treatments on Day 7 were 45 and 52% of the initial moisture content, respectively. On Day 14, dung moisture for both the XD and UXD treatment was 1.7 g g^{-1} (dry-weight basis), which was 35% of the initial moisture content; on Day 56, the remaining dung moisture was 0.7 g g^{-1} (dry-weight basis), which was 15% of initial moisture content.

Greenhouse Gas Fluxes

Carbon Dioxide

The treatment LSMeans ± SE ($n = 320$, average over 8 replications, 2 yr, 2 seasons, and 10 d) of the XD, UXD, and ND was 8.3 ± 0.2, 8.1 ± 0.2, and 7.7 ± 0.2 g C d^{-1} m^{-2}, respectively. A significant effect of treatment (Table 2) indicated that ranked CO_2 fluxes among the treatments were in the order of XD = UXD, UXD = ND, and XD > ND. The finding that XD > ND ($P > |t|$ of 0.0136) while UXD = ND ($P > |t|$ of 0.1279) reflected the effect of dung beetle activity in enhancing CO_2 flux. The effects of dung beetles in increasing CO_2 flux occurred on Days 1, 2, 7, and 21, as indicated by greater LSMeans ($n = 32$, average over 8 replications, 2 yr, and 2 seasons) of CO_2 flux in the XD than in the ND control (Fig. 4A). The CO_2 fluxes of the UXD and the ND control treatments were similar at all sampling days (Fig. 4A).

Nitrous Oxide

The significant interaction effect of year × season × treatment on N_2O fluxes (Table 2) indicated that beetles increased N_2O flux only in the late season of 2015. Treatment LSMeans ± SE ($n = 72$, average over 8 replications and 9 d) of N_2O fluxes in the late season of 2015 were 0.6 ± 0.1, 0.2 ± 0.1, and 0.2 ± 0.1 mg N d^{-1} m^{-2} for the XD, UXD, and ND treatments, respectively. Ranked N_2O fluxes among treatments were in the order XD > UXD = ND. Dung beetles effects in increasing the N_2O flux in the XD treatments occurred on Days 3 and 7 (Fig. 4B). The LSMeans ($n = 8$, average over 8 replications) of N_2O flux of the UXD and the ND control treatment were similar at all days (Fig. 4B).

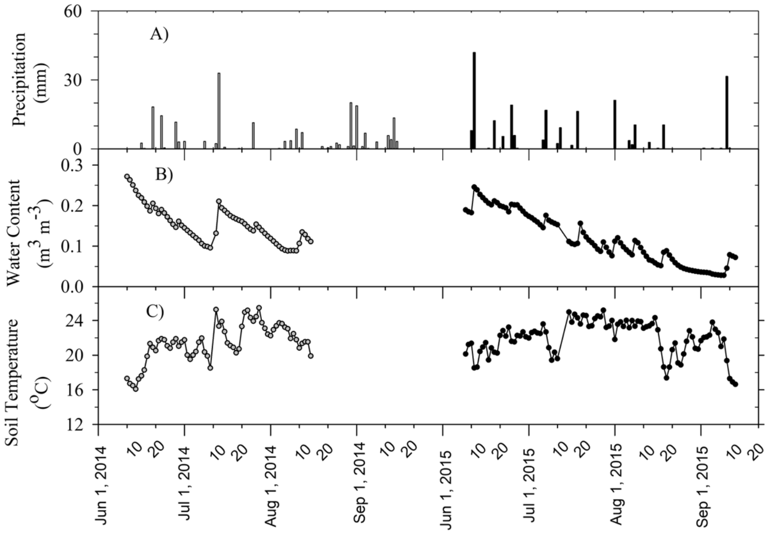

Fig. 2. (A) Daily rainfall, (B) soil volumetric water content (VWC), and (C) soil temperature at 10-cm depth during growing season from June to September of 2014 and 2015 in meadows of the Nebraska Sandhills.

Methane

The effects of treatment and treatment × day on CH_4 fluxes were significant (Table 2). Treatment LSMeans ± SE ($n = 320$, average over 8 replications, 2 yr, 2 seasons, and 10 d) of daily CH_4 fluxes of the XD, UXD, and ND were −0.0 ± 0.1, −0.2 ± 0.1, and −0.3 ± 0.1 mg C d^{-1} m^{-2}, respectively. Ranked CH_4 fluxes among treatments were in the order of XD > UXD = ND, which suggested that beetle activity increased CH_4 flux by 0.2 mg C d^{-1} m^{-2}. The LSMean ± SE ($n = 32$, average over 8 replication, 2 yr, and 2 seasons) of CH_4 flux of the XD treatment was greater than that of the UXD on Day 1; the LSMean ± SE of the XD treatment was greater than that of the ND control treatment on Days 1 and 3 (Fig. 4C).

Carbon Dioxide Equivalent of Nitrous Oxide and Methane Emission

The effects of treatment or its interaction with other factors on CO_2–equivalent of N_2O and CH_4 combined was not significant (Table 2). The LSMeans ± SE ($n = 32$, over 8 replications, 2 yr, and 2 season) among treatments at all days were similar (Fig. 4D). Treatment LSMeans ± SE ($n = 288$, average over 8 replications, 2 yr, 2 season, and 9 d) of combined CO_2–equivalent of N_2O and CH_4 among the XD (0.22 ± 0.03 g CO_2 d^{-1} m^{-2}), UXD (0.21 ± 0.03 g CO_2 d^{-1} m^{-2}) and ND (0.19 ± 0.03 g CO_2 d^{-1} m^{-2}) were not different.

Temporal Factors and Greenhouse Gas fluxes

Significant year × season × day interactions (Table 2) indicated complex effects of antecedent environmental conditions on GHG emission. The principal component analysis indicated that the first principal component explained the majority (63%) of total variability of environmental data. The second principal component explained 11% of total variability of environmental data. The first principal component indicated positive loading of antecedent soil temperature and negative loading of antecedent soil VWC. The second principal component indicated positive loading of air temperature. The principal component analysis selected soil temperature and soil VWC at 10-cm depth as a representative for the rest of the environmental factors (Fig. 5).

Discussion

Carbon Dioxide

The soil background CO_2 flux (the ND control, 7.7 ± 0.2 g C d^{-1} m^{-2}) in our study was much greater than those of fertilized and grazed native pasture on silt loam soils (2.4 and 1.9 g C d^{-1} m^{-2}, respectively) in Mandan, ND (Liebig et al., 2013). The soil background CO_2 flux in our study was also higher than the CO_2 flux of a silty clay loam agricultural soil (4–5 g C d^{-1} m^{-2}) treated with organic matter in Mead, NE (Ginting et al., 2003). Sandy soils commonly have high air permeability, which perhaps caused the higher CO_2 flux in our study compared with that of fine-textured soil.

The increase of 0.2 g C d^{-1} m^{-2} (2.6% of soil CO_2 background) due to dung beetle activity mainly occurred within days, which was coincident with dung beetle abundance within 7 d after dung application. Dung beetles fed on dung liquid nutrients; therefore, significant evaporation of dung moisture content within 7 d after dung application resulted in short-lived dung beetle abundance. Dung beetle contribution to increasing CO_2

Table 1. Means and SE of daily weather and soil (10-cm depth) variables in the early season and late season during dung experiments in 2014 and 2015 in the meadows of the Nebraska Sandhills.

Variable	2014		2015	
	Early season	Late season	Early season	Late season
Air temperature (°C)	20.8 ± 0.4	20.1 ± 0.5	21.4 ± 0.3	21.3 ± 0.4
Precipitation (mm d^{-1})	1.9 ± 0.6	2.0 ± 0.6	2.9 ± 1.0	1.7 ± 0.7
Soil volumetric water content (m^3 m^{-3})	0.17 ± 0.01	0.13 ± 0.01	0.18 ± 0.00	0.08 ± 0.00
Soil temperature (°C)	20.0 ± 0.3	22.8 ± 0.3	21.4 ± 0.0	22.3 ± 0.3

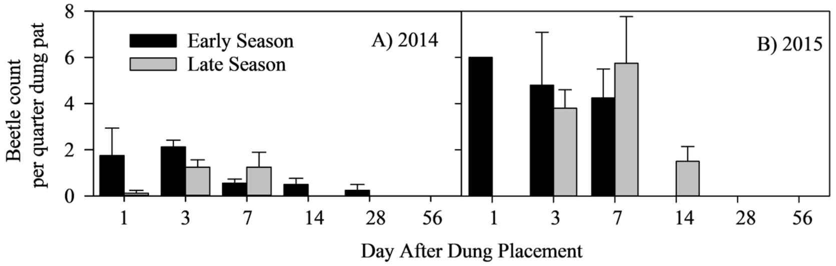

Fig. 3. Average number of dung beetles in one quarter of the exposed dung (XD) pat placed in early season and in late season of (A) 2014 and (B) 2015 on grassland soil in the Nebraska Sandhills meadows.

flux in our study was less than that observed by Penttilä et al. (2013) (2 g C d^{-1} m^{-2}) or by Iwasa et al. (2015) (1.4 g C d^{-1} m^{-2}). Short-lived colonization and low dung beetle abundance was a reason for smaller dung beetle effects than those in other studies that used a predetermined and greater amount of dung beetles in closed mesocosms (Penttilä et al., 2013; Iwasa et al., 2015; Piccini et al., 2017).

Lack of measurements of dung moisture, DM, and chemical analytes over time, such as in the studies by Penttilä et al. (2013), Iwasa et al. (2015), and Piccini et al. (2017), led to speculation of how dung beetle macropores affected dung drying, aeration, and GHG production and GHG fluxes. Our data indicated that dung moisture loss was rapid within days after dung application, and this rapid moisture loss produced a thin, dry, crust-like layer covering the dung. When exposed to dung beetle colonization, dung moisture loss increased by 7% on Day 7, and on Day 56, 85% of initial moisture was lost. Measuring only at start and end of experiment. Penttilä et al. (2013) observed that pats containing beetles lost 95% of their weight, versus 83% among pats without beetles. The presence of beetles in Penttilä et al. (2013) caused greater moisture loss because initial dung pat moisture in their study (5.0 g g^{-1}, dry weight basis) was greater than that in our study (4.6 g g^{-1}, dry weight basis). Beetle macropores function as a preferential path for vapor transport, especially when dung pats were still wet within the first week after dung pat deposition.

Trend lines relating DSU CO_2 flux and dung moisture (Fig. 6A) and trend lines relating dung DM and dung moisture (Fig. 6B) indicated which process was predominant (production or transport) in affecting CO_2 flux. The trend in Fig. 6B indicated that the rate of change in dung DM (included by production of CO_2 gas and H_2O in Eq. [1]) per unit change in dung moisture was similar between the XD and UXD treatments (Fig. 6B). However, the trend line of the DSU CO_2 flux (Fig. 6A) indicated that the rate of change of CO_2 flux per unit change in dung moisture (slope) in the XD treatment remained positive, even when moisture was >3.0 g g^{-1}, whereas the slope in the UXD treatment gradually decreased and became negative when moisture was >3.0 g g^{-1}. This suggests that when dung pat was wet, DSU CO_2 flux was more dependent on transport process than production process.

Studies have shown that gas transport was inversely related to water-filled porosity (Ball et al., 1997; Pihlatie et al., 2004; Sharma et al., 2009) and thus affected microbial activity and gas production–consumption processes (Linn and Doran 1984; Pihlatie et al., 2004). In the case of the UXD treatment, as dung moisture increased, dung water-filled porosity increased, and

Table 2. Statistical *F* and *P* > *F* values of fixed effects of year (Y), season (S), treatment (T), days after dung placement (Day), and their interactions on fluxes of CO_2, N_2O, CH_4, and CO_2–equivalents of N_2O + CH_4 from rangeland soils of Nebraska Sandhills.

Effects†	NDF‡	DDF§	CO_2	N_2O	CH_4	N_2O + CH_4 CO_2–equivalent
Y	1	28	50.3***	1.25	3.66	0.55
S	1	28	8.80**	23.7***	7.79**	16.4***
Y × S	1	28	1.55	11.4**	3.79	8.39**
T	2	56	3.28*	0.68	3.07*	0.10
Y × T	2	56	0.58	0.35	0.25	0.75
S × T	2	56	1.03	2.47	0.59	2.96
Y × S × T	2	56	2.81	3.56*	1.09	1.38
Day	9	756	74.1***	84.9***	16.2***	27.8***
Y × Day	9	756	56.6***	89.6***	8.31***	27.2***
S × Day	9	756	53.2***	92.8***	10.3***	29.2***
Y × S × Day	9	756	33.2***	97.3***	7.64***	35.4***
T × Day	18	756	0.91	1.20	2.03**	0.45
Y × T × Day	18	756	1.30	1.34	1.06	1.21
S × T × Day	18	756	1.07	0.95	0.90	1.24
Y × S × T × Day	18	756	0.90	1.21	1.11	0.66

*, **, *** Significant at the 0.05, 0.01, and 0.001 probability level, respectively.

† Three treatment levels are unexposed dung), exposed dung, and no dung. Two levels of season were early season (June–August) and late season (July–September). Gas measurements were made 0, 1, 2, 3, 7, 10, 14, 21, 28, and 56 d after dung placement in the early and late seasons.

‡ NDF, numerator degrees of freedom.

§ DDF, denominator degrees of freedom.

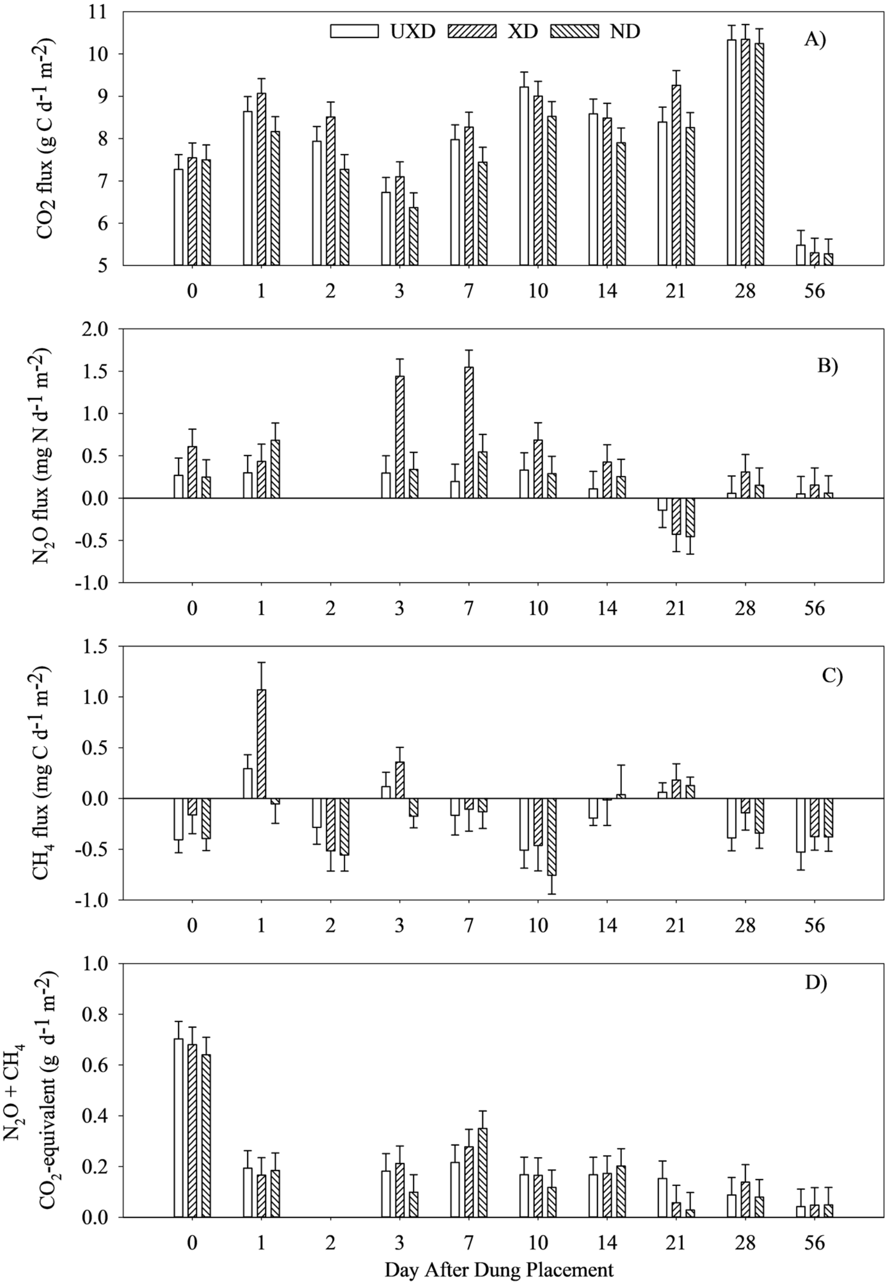

Fig. 4. The least squares means (LSMeans) ± SE of (A) CO_2, (B) N_2O during late-season 2015, (C) CH_4, and (D) CO_2–equivalence of N_2O and CH_4 fluxes from soil mesocosms with exposed dung (XD), unexposed dung (UXD), and no dung (ND) control treatments on meadows of the Nebraska Sandhills.

thus the gas transport decreased. In the XD treatment, however, the existence of beetle-made macropores provided a preferential path for gas transport. The trend line of CO_2 flux (Fig. 6A) suggested that the role of beetle-made macropores in a gas transport mechanism became more important when dung moisture was >2.0 g g^{-1} (i.e., on early days after dung deposition).

The absence of dung beetle effects on the contents of dung WEOC and the lack of differences in trend line slope of WEOC across dung moisture content between the XD and UXD treatments (Fig. 6C) indicated that dung beetles had no effect on oxidation of dung WEOC (CO_2 production). This was also the case with soil WEOC. Linear regression (Fig. 6D) indicated that soil WEOC explained little variability in CO_2 fluxes. One reason is that the time of fluctuation in the DSU CO_2–flux and the fluctuation in soil analyte contents were not synchronized. For example, beetles contributed to peak differences in the DSU CO_2 flux (compared with the control) on Days 1, 2, 7, and 21(Fig. 4A), whereas the peak differences in soil WEOC occurred on Day 14. It appears that beetle effects on increasing soil WEOC content occurred through a separate process from beetle effects on the DSU CO_2 fluxes.

Nitrous Oxide

The soil background N_2O flux (the ND control, 0.37 ± 0.05 mg N d^{-1} m^{-2}) in our study was smaller than that (0.48–2.4 mg N d^{-1} m^{-2}) of the rotational paddock grazing in eastern Nebraska (Jackson et al., 2015). The soil background N_2O flux was similar to those of less intensive agricultural soils (0.1–0.5 mg N d^{-1} m^{-2}) but lower than those of more intensive agricultural soils (2–3 mg N d^{-1} m^{-2}) in Nebraska (Ginting and Eghball,

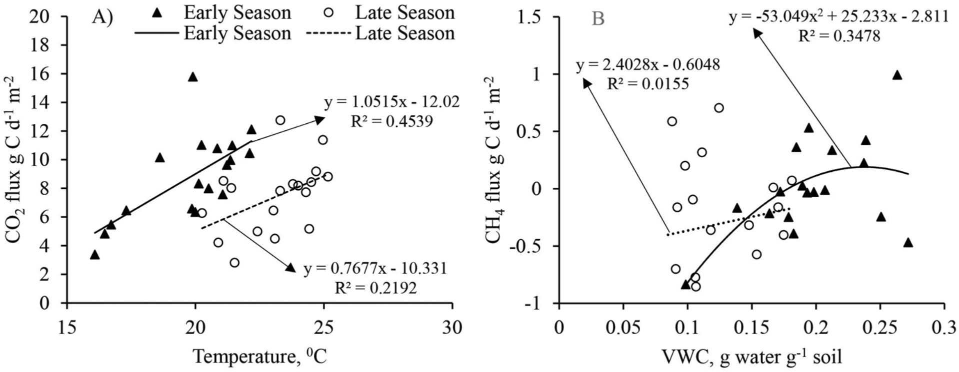

Fig. 5. Relationship of (A) CO_2 flux with soil temperature at 10-cm depth, and (B) CH_4 flux with soil volumetric water content (VWC) at 10-cm depth.

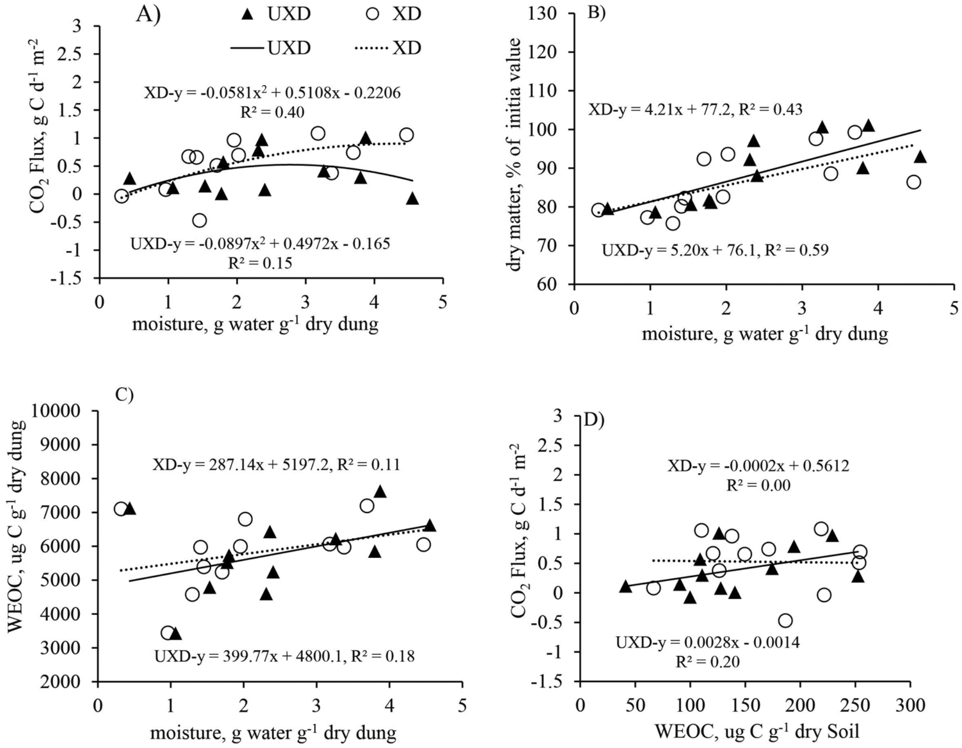

Fig. 6. Relationship of (A) CO_2 flux from dung with soil underneath the dung (DSU), (B) dung dry matter with dung moisture content, (C) dung water-extractable organic C (WEOC) with dung moisture content, and (D) CO_2 flux of the DSU with soil WEOC content at 0- to 10-cm depth beneath the dung on meadows of the Nebraska Sandhills. Symbols in the figure indicate soil mesocosms with exposed dung (XD) and unexposed dung (UXD).

2005). The N_2O background flux in our study was also lower than those of unfertilized (0.4–1.1 mg N d^{-1} m^{-2}) or fertilized (0.6–3.2 mg N d^{-1} m^{-2}) corn–soybean [*Glycine max* (L.) Merr.] rotation in Iowa (Iqbal et al., 2015).

Our data in late-season 2015 and those of Penttilä et al. (2013) and Iwasa et al. (2015) indicated that dung beetle activity increased N_2O flux. Penttilä et al. (2013) observed episodic high flux on Days 15, 20, and 30; however Penttilä et al. (2013) and Iwasa et al. (2015) did not elaborate on the mechanism of these episodic high-flux events due to a lack of physical and chemical measurements of dung and soil at the time of gas measurements. In our study, dung beetle activity (XD vs. UXD) increased the daily means of the DSU N_2O flux by 0.4 mg N d^{-1} m^{-2}. The increase was mainly occurred on Days 3 and 7, coincident with dung beetle abundance during the late season of 2015, the driest season among the four year–season combinations (Table 1).

Our results indicated that there was a strong relation between dung WEN (also dung NH_4) and dung moisture during the late season of 2015. In both relations, the slopes of WEN and NH_4 with dung moisture (Fig. 7A and 7B) were the same between the XD and UXD treatments. This similarity indicated that dung beetles did not affect dung N mineralization, a process that produced necessary substrate (NH_4 and NO_3) in nitrification and denitrification, as shown in Eq. [2] and Eq. [3] (Saggar et al., 2004). The lack of differences in dung N mineralization indicated that dung beetles affected N_2O flux during the late season of 2015 by modification of the moisture-dependent gas transport processes, as previously described.

Increased soil N analytes, WEN, NO_3, NH_4, showed a poor relation with beetle effects on the DSU N_2O fluxes as demonstrated by the relation of DSU N_2O flux and soil WEN content and soil NO_3 (Fig. 7C and D). Similar to those with CO_2 fluxes, the time of fluctuation in the DSU N_2O flux and the fluctuation in soil analyte contents were not synchronized. For example the beetle contributed to peak differences in DSU N_2O flux (compared to the no-dung control) on Day 3 and Day 7 (Fig. 4B), yet, the peak differences in soil analytes occurred on Day 14. This further indicated that beetle effects on increasing soil N analytes were a separate process of beetle effects on dung analytes.

Methane

Negative values of daily means of soil background CH_4 flux (the ND control, -0.3 ± 0.1 mg C d^{-1} m^{-2}) in our study showed that grassland soils are a CH_4 sink (Mosier et al., 1991; Hartmann et al., 2011). The increase in CH_4 flux due to dung beetle activity was contrary to those in other studies (Penttilä et al., 2013; Iwasa et al., 2015; Piccini et al., 2017); however, these reported works did not explain how beetles reduced CH_4 fluxes. Our results did not align with these other reported work on beetle-made macropore effects in reducing CH_4 fluxes. Our study showed that the DSU CH_4 flux increased as dung moisture increased in both the XD and UXD treatments (Fig. 8A), and the difference between the XD and UXD treatments was larger for greater dung moisture content. The increase of CH_4 flux from beetle activity was mainly from high-flux events on Days 1 and 3, when dung was still wet (dung moisture was >3.0 g g^{-1} on Days 1 and 3). Presumably, tunneling through wet manure, dung beetles released CH_4 from anaerobic pockets of CH_4 production (Eq. [4] and Eq. [5]), resulting in dung beetle effects peaking on CH_4 flux on Days 1 and 3.

Dung beetle effects on soil WEOC, had little relation with dung beetle effects on DSU CH_4 fluxes (Fig. 8B). Similar to those with CO_2 and N_2O fluxes, the event of fluctuation in DSU CH_4 flux and fluctuation in soil analyte contents were not synchronized.

Effects of Environmental Variables on Greenhouse Gas Flux

The increase in soil temperature resulted in increased CO_2 flux (Fig. 5A) in the early season and late season. The ANOVA (Table 2) and trend lines indicated that CO_2 flux was greater in the early season than in the late season. Higher temperature during the late season did not result in greater CO_2 flux than those in the early season because of lower VWC in the late

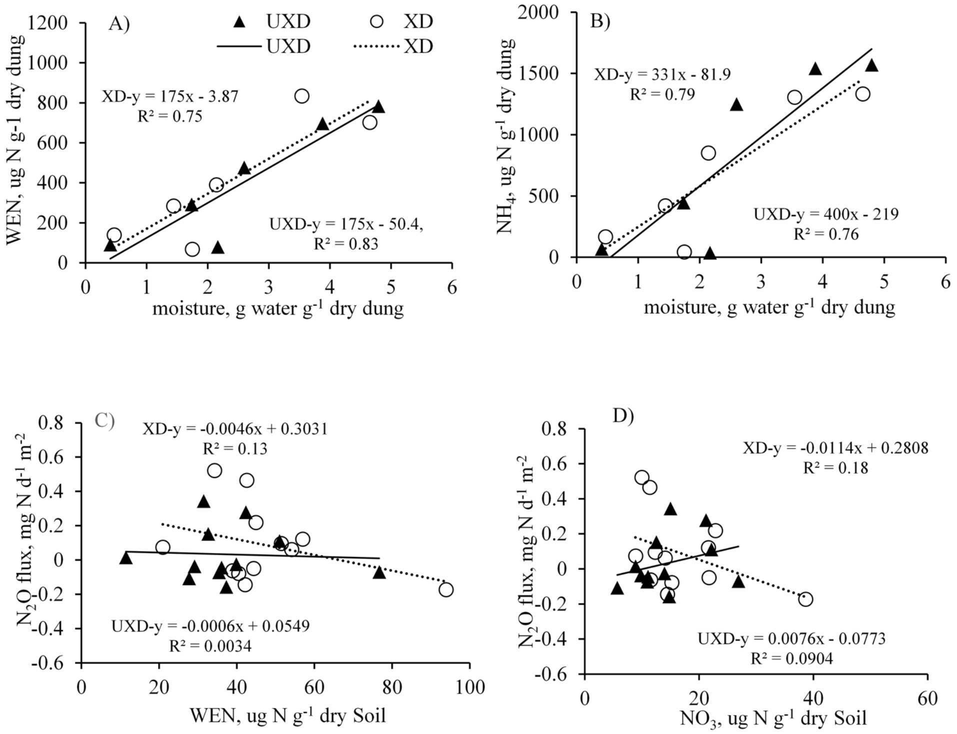

Fig. 7. Relationship of (A) dung water-extractable N (WEN) content and (B) dung NH_4 content with dung moisture content; relationship of N_2O flux from dung and soil underneath the dung with (C) WEN and (D) NO_3 of soil at 0- to 10-cm depth underneath the dung pat on meadows of the Nebraska Sandhills. Symbols in the figure indicate soil mesocosms with exposed dung (XD) and unexposed dung (UXD).

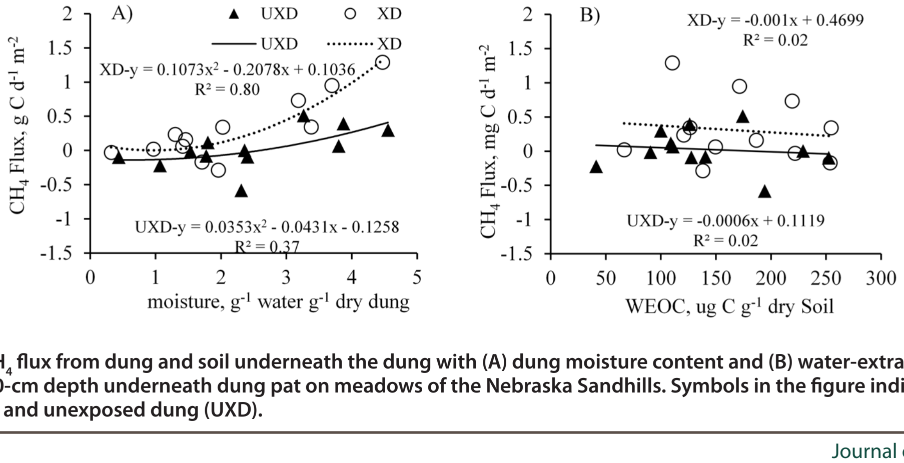

Fig. 8. Relationship of CH_4 flux from dung and soil underneath the dung with (A) dung moisture content and (B) water-extractable organic C (WEOC) of soil at 0- to 10-cm depth underneath dung pat on meadows of the Nebraska Sandhills. Symbols in the figure indicate soil mesocosms with exposed dung (XD) and unexposed dung (UXD).

season. When VWC was limiting in the late season, increasing temperature was less conducive for plant root and microbial activities. Other studies also reported a strong soil VWC and soil temperature effect on the fluxes of CO_2 (Lloyd and Taylor, 1994; Smith et al., 2003; Balogh et al., 2011).

The ANOVA (Table 2) indicated that N_2O flux was greater in the early season (0.6 ± 0.1 mg N d^{-1} m^{-2}) than that in the late season (0.2 ± 0.1 mg N d^{-1} m^{-2}); however, the effects of soil temperature and/or VWC on N_2O flux were not clear. Previous studies have shown that N_2O emission was dependent on a wide range of physical variables, including soil moisture and temperature (Pihlatie et al., 2004; Saggar et al., 2004; Uchida et al., 2011).

The ANOVA (Table 2) indicated that CH_4 flux was higher in the early season than in the late season. The soil VWC explained the variability in CH_4 flux in the early season better than that in the late season because of the narrow range of soil VWC in the late season (Fig. 5B). The trend lines suggested that CH_4 flux increased as soil VWC content increased. These results supported the association between soil moisture and methanogenesis in other studies (Schnell and King 1996; Chadwick et al., 2000; Jones et al., 2005). The trend line indicated that the rate of increase in CH_4 flux diminished as the soil VWC increased in the early season.

Rangeland Management for Greenhouse Gas

Our study presents two management challenges for the meadows in the semiarid Nebraska Sandhill: (i) background soil CO_2 flux (7.7 g C d^{-1} m^{-2}) was high compared with other agricultural soils; (ii) dung beetle activity increased CO_2, N_2O, and CH_4 emission within 7 d after dung deposition, when dung was wet. Several studies reported plant production during the 5-mo growing season in the meadows of semiarid and sandy soils of the Nebraska Sandhills. Potvin and Harrison (1984) reported 724 g m^{-2} of aboveground biomass production; Mousel et al. (2007) reported aboveground yields of 290 and 340 g m^{-2} for C_3 and C_4 grass, respectively. Aboveground plant production of grazed meadows adjacent to our study site in the same years (2014 and 2015) ranged from 400 to 600 g m^{-2}, or 2.7 to 4.0 g d^{-1} m^{-2}. Assuming a root/shoot ratio of 1:1 (Mousel et al., 2007), and 58% C in dried plant tissues, we estimated that plant productivity (total above- and belowground productivity ranging from 3.1–4.6 g C d^{-1} m^{-2}) was lower than the background soil CO_2 flux. The estimate suggested that CO_2 emission management must increase meadow net primary productivity to compensate for the excess of soil CO_2 flux.

Reducing background soil CO_2 flux is a rather difficult task (because of its dependence on soil and environmental factors). Managing plant–animal (grazing) and soil–plant systems are among possible options to increase plant net primary productivity. Grazing duration, frequency, and/or intensity are reported to affect grassland aboveground primary productivity (Burboa-Cabrera et al., 2003; Volesky et al., 2004) and belowground net primary productivity (Gao et al., 2008). Any option for GHG management on grazed meadows must consider and evaluate C costs in increasing plant productivity, and the increase of CH_4 and N_2O from dung beetle activities. Examples of processes in soil–plant management that generate C costs are fertilizer manufacturing, fertilizer transport and land application, increased CO_2 flux from root respiration, and increased soil N_2O fluxes from N fertilizer application.

Conclusions

Dung beetle activity increased fluxes of CO_2 on Days 1, 2, 7, and 21, N_2O on Day 3 and Day 7 of late-season 2015, and CH_4 on Days 1 and 3. The increase in GHG flux within 7 d after dung application was due to beetle-made macropores that facilitated gas transport in initially wet dung. Seasonal differences resulted in greater CO_2, N_2O, and CH_4 fluxes in the early season than in the late season. This study concluded that dung beetles increased GHG fluxes from early- and late-season dung deposits on meadows of the semiarid Nebraska Sandhills.

Conflict of Interest

The authors declare no conflict of interest.

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