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Temporal changes in the nutrient content of cattle dung in the Nebraska Sandhills ecosystem

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Temporal changes in the nutrient content of cattle dung in the Nebraska Sandhills ecosystem

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Abstract
Dung excreted by cattle composes a significant portion of the nutrient inputs in a grazed ecosystem and can have wide-ranging effects on soil properties and vegetation. However, little research has been conducted on the nutrient dynamics of excreted dung in situ that has not been disturbed prior to field sampling. In this study, we analyzed 294 dung pats (1–24 days old) collected from a Nebraska Sandhills meadow to determine water-extractable organic carbon (WEOC), water-extractable nitrogen (WEN), water-extractable phosphorus (WEP), and percent dry matter (DM) changes over time. In addition, we investigated if sample handling - frozen storage – and the formation of surface crust during dung field drying affect dung nutrient concentrations. Dung WEOC and WEN both followed exponential decay curves of nutrient loss over time and were modeled as a function of age. In contrast, WEP

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was poorly correlated with age. The percent dry matter in conjunction with sample WEOC concentration were stronger determinants of WEP than age alone. Freezing samples prior to analysis increased WEOC (37–98%) and WEN (37–123%), but lowered WEP (0.8–65%) compared to the samples from the same dung pat analyzed fresh. The dry surface crusts of dung pats had higher WEOC (98–112%) and WEN (112%) compared to moist interiors (on average, 3 cm from surface). This research provides evidence that dung nutrient concentrations decreased by 73% (WEOC) and 76% (WEN) over 24 days and shows that frozen storage and subsequent thawing for analysis, as well as crust formation during field drying, can significantly affect dung nutrient concentrations and spatial partitioning of dung nutrients.

Keywords: Crust formation, Dung, Frozen storage, Grazing, Soil

Introduction

Information regarding the nutrient contributions of cattle dung to a grazed ecosystem is essential for understanding the spatial and temporal components of nutrient cycling patterns in these ecosystems (Haynes and Williams 1993; Lovell and Jarvis 1996; Bardgett and Wardle 2003). This information is also foundational for estimating dung's contribution to soil carbon sequestration potential and monitoring changes to the physical, chemical, and biological properties of soils over time as a result of grazing and grazing management decisions. In addition, tracking spatial and temporal changes in vegetation quality and plant species community composition related to dung distribution patterns can reveal landscape-scale processes that impact ecosystem functioning (During and Weeda 1973; Aarons et al. 2004; Gillet et al. 2010; Pineiro et al. 2010).

One of the challenges with conducting research on dung nutrient dynamics and decomposition is that both are highly site-specific and dependent on multiple controlling factors, including diet of grazing animals (Sorensen et al. 2003), animal age and size, animal species, time of year (da Silva Cardoso et al. 2019), the absence or presence of dung beetles and earthworm communities (Pecenka and Lundgren 2018), and weather. Therefore, relying on averages or a general model of how nutrient cycling proceeds at both macro- and micro-scales across different ecosystems may not produce accurate models for specific sites. Compounding these issues is the fact that dung is not commonly studied in the pasture where it was deposited and absent of human manipulation. Instead, researchers have relied on the creation of artificial dung pats from bulk manure collected in cattle holding areas or have harvested dung from pastures and then re-formed the pat into a particular size, shape, or weight to facilitate controlled, long-term monitoring of changes (Weeda 1967; During and Weeda 1973; Lovell and Jarvis 1996; Aarons et al. 2004; Evans et al. 2019). While these techniques are useful for providing insight on a range of dung-related processes that might otherwise be not possible to study (Dickinson and Craig 1990; Bol et al. 2000), they may also impact the nutrient content, physical consistency and moisture content of the dung in comparison to unaltered dung from grazing animals (Eghball et al. 2002). These, in turn, can affect decomposition rates and activity of macrofauna such as dung beetles and earthworms, both of which are major contributors to dung decomposition (Evans et al. 2019; Wagner et al. 2020). To address these limitations, this study evaluated a large quantity of unaltered dung pats in a pasture, ranging in age from 1 to 24 days since deposition.

Further, analyses of dung nutrient content are often performed on samples that have been harvested in the field, frozen for transport and storage, and then thawed prior to analysis. Studies have evaluated the effect of freezing and drying of manure samples prior to analysis on water-extractable phosphorus (WEP; Vadas and Kleinman 2006), and found significant differences in analysis outcomes. Studnicka et al. (2011) found that freezing prior to analysis consistently raised WEP levels as compared to fresh sample analysis. There is ample evidence that freezing may change nutrient availability and chemical form in soils (Freppaz et al. 2007; Xu et al. 2016; Song et al. 2017); therefore, it is hypothesized that freezing dung before analysis may also create physical and chemical changes in the dung and dung nutrients (Chen et al. 2019). If so, this could lead to faulty assumptions about the nutrient composition of dung in a field setting, which would lead to inaccurate predictions of the availability and loss of nutrients from a given site. Another aspect of the nutrient dynamics of aging dung is the formation over time of a crust-like layer across the exposed top of pats. Crust forms within a short timeframe after deposition (Laubach et al. 2013) and creates two distinct layers within the pat: a very dry outer crust and the protected interior of the dung pat, which retains a much greater moisture content. The crust prevents access of water from the top of the pat and may slow the release of gaseous compounds (e.g., CO_2 and CH_4) from within the pat. This crust may also limit rainfall from entering the pat and contributing to its disintegration as it effectively sheds water once sealed (Weeda 1967; MacDiarmid and Watkin 1972; Dickinson and Craig 1990). Such stratification of moisture content and physical properties has the potential to affect the outcome of laboratory analyses depending on which layer is sampled. To the best of our knowledge, there has been no research conducted that evaluates how these layers can affect dung nutrients. We hypothesized that the crust and interior layers of dung have different moisture contents and, potentially, different nutrient contents (MacDiarmid and Watkin 1972; Holter and Hendriksen 1988).

This study evaluated a large number of unaltered dung pats (n = 294) from cattle grazing a Nebraska Sandhills pasture, across study years, sampling dates, and ages. The objectives of this research were (1) to measure changes in dung nutrients (carbon, nitrogen, and phosphorus) over time when dung is left in the pasture and (2) to evaluate how the freeze-thaw event and dung crust formation affect dung nutrient concentrations.

Materials and methods

Site description

The research site was located at the University of Nebraska's Barta Brothers Ranch, approximately 40 km southwest of Bassett, NE (42°13'13"N, 99°38'27"W) in the Nebraska Sandhills Ecoregion. The pastures where dung collection took place were part of a long-term grazing study (2010–2017) (Shropshire 2018; Wagner et al. 2020; Andrade et al. 2022), located on a subirrigated meadow site with a seasonally-high water table. These wet, interdune areas, which are characteristic of the Sandhills region, are generally high-producing areas well-suited for hay production or beef cattle grazing (Horney et al. 1996; Mousel et al. 2007). Vegetation communities at the site are dominated by cool season grasses (*Phalaris arundinacea* L., *Poa pratensis* L., *Elymus repens (*L.*) Gould), Phleum pratense* L.), rushes (*Eleocharis* and *Juncus spp*.) and sedges (*Carex spp*.), with fewer warm season grasses and forbs (Guretzky et al. 2020; Wagner et al. 2020).

Soils at this site are sandy to fine sandy loam in texture and classified as mixed, mesic Aquic Ustipsamments. The average summer temperatures range from 21 to 25 °C, and the average yearly precipitation over the past 20 years at the site is 665 mm (High Plains Regional Climate Center, 2018). Precipitation and air temperature from April to August of 2016 and 2017 in the study area are shown in **Table S1**. Total precipitation in June and July was 199% and 52% higher, respectively, in 2016 than in 2017 (Table S1). The average of the June and July maximum temperature was approximately 35.9 °C in 2016 and 38.4 °C in 2017, and the average minimum temperature was approximately 10.6 °C in 2016 and 9.2 °C in 2017 (Table S1).

Study context

Grazing at the research site began annually in early June when the steers were moved to the ranch and concluded in early August when they were removed from the site. A total of 32 to 36 yearling steers (depending on the year) grazed 120 pastures (0.06 ha and with dimensions of 5.8 m x 98.8 m each) over a 60-d grazing season at a stocking density of 225,000 kg live weight ha^{-1} in this study. Each day two pastures were grazed, with the steers moving to the first pasture in the early morning (approximately at 7 am) and then moved to the second pasture in the same day during the afternoon period (approximately at 3 pm). Each pasture was grazed for only one time per grazing season.

Table S1. Monthly total precipitation and maximum and minimum air temperature during the 2016 and 2017 growing seasons at the study site

Year	*April*	*May*	*June*	*July*	*August*
Precipitation (mm)					
2016	144.8	103.9	40.9	162.1	18.0
2017	75.9	119.9	13.7	106.7	167.4
Maximum air temperature (°C)					
2016	26.1	30.0	35.6	36.1	35.6
2017	23.3	30.6	35.0	41.7	35.0
Minimum air temperature (°C)					
2016	-3.9	-0.6	8.9	12.2	9.4
2017	-3.3	0.6	7.8	10.6	10.6

When cattle were moved from the pasture, they no longer had access to the pasture they had been in before. This grazing strategy allowed dung to be accurately classified by age for each 24-hour period without any new accumulation of dung and without any disturbance of the pats after the cattle had left the pasture.

Dung collection

Dung was collected in June and July of both 2016 and 2017 from pastures containing pats that were 1, 3, 5, 7, 10, 12 and 14 days old in 2016 and 1, 3, 5, 7, 10, and 14 days old in 2017 (**Table 1**). Dung pats were randomly chosen across each pasture, and only intact pats that had not been stepped or laid on were used. Dung pats averaged 20–30 cm in diameter, although size and shape, as well as the height of the pat, varied substantially. The total number of dung pats sampled on each harvest date is listed in Table 1. Different dung pats were randomly collected on each harvest date (Table 1). Sub-samples were collected from near the middle of the dung pat, taking care to avoid the drier, thinner edges. To investigate nutrient changes in dung over a longer period of time with a longer return sampling interval, samples were

Table 1 Summary of the total number of dung pats sampled on each harvest date at Barta Brothers Ranch, Nebraska Sandhills in 2016 and 2017

Dung age	2016				2017				
(days)	25 June	26 June	26 July	30 July	29 June	8 July	22 July	23 July	Total
1	*	4	5	5	**25**	10	*	10	59
3	*	7	5	5	*	10	*	10	37
5	*	6	5	5	*	10	*	10	36
7	4	8	5	5	*	10	*	10	42
10	6	*	5	5	*	**25**	*	4	45
12	10	*	5	*	*	*	*	*	15
14	10	*	5	*	*	10	*	10	35
24	*	*	*	*	*	*	**25**	*	25

*Indicates that no dung pat was sampled. The 25 pats that were collected in each three days in 2017 (June/29, July/8 and July/22) were used to investigate nutrient changes in dung over a longer period of time with a longer return sampling interval, Numbers in each cell are the number of dung pats sampled on a given date for a particular age group. Numbers in bold refer to the total dung collected for evaluating 24 day change in dung nutrients.

also collected at 1, 10, and 24 days old in 2017 (Table 1). In 2017, the effect of crust formation on dung nutrients was studied using pats that were 24 days old. Four pats were selected, and a portion of both the dry crust (on average, 3 cm from surface) and the still-wet interior were taken from each. After collection, fresh samples were placed in Ziploc plastic bags and stored on ice in a cooler until they arrived in the lab, one to two hours later.

Dung processing

After the samples were brought to the lab, a subsample was weighed and then dried at 60 °C for 48 h to determine dry matter (DM) content. In 2016, the remaining sample (not dried) was frozen and stored at -20 °C until nutrient analysis was conducted. In 2017, in order to evaluate the effects of the freeze-thaw event on nutrient analysis results, both fresh and frozen sub-samples of each collected dung pat were analyzed. Fresh dung samples were kept chilled in a refrigerator for 24–48 h after collection until analysis began. The remaining sample was stored at -20 °C until analysis. All frozen samples were thawed overnight prior to the start of extractions and analyses.

Laboratory analyses

Dung samples were analyzed for water-extractable organic carbon (WEOC), water-extractable nitrogen (WEN), and water-extractable phosphorus (WEP) using a 1 g dry weight equivalent sub-sample extracted in 200 mL deionized water (Kleinman et al. 2007). Flasks were shaken briefly to break up and disperse the dung sample, and then allowed to settle overnight to aid in filtering. Extracts were subsequently filtered through Fisher™ P2 (particle size retention 1–5 µm) filter paper and refrigerated at 4°C until analyses were conducted. The WEOC and WEN were determined using an OI Analytical Aurora 1030 C TOC Combustion Analyzer with an OI Analytical 1088 Rotary TOC Autosampler and a TNb module for total N (OI Analytical, College Station, TX, U.S.A.). The WEP was determined using the molybdate method of Murphy and Riley (1962) at 880 nm on a Thermo Scientific Genesys 10 S VIS Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.).

Statistical design and analysis

A split-plot experimental design was used, with date of harvest being the whole-plot factor and age of dung the split-plot factor. The repeated measures analysis was conducted to test the effects of dung age and freeze-thaw event on dung nutrient contents using PROC MIXED in SAS 9.4 (SAS, 2013). Sampling date was defined as a repeated measure variable, while age of dung for each year was considered as a fixed effect and replication as a random effect. One-way analysis of variance (ANOVA) was conducted to compare the effects of crust formation on dung nutrient contents. Statistical comparisons of the nutrient contents for all the data were obtained using an LSD Fisher test. All nutrient analysis results are reported on a DM basis. Differences were considered significant at 0.05 probability. All figures were created in R (R Core Team, 2019) with ggplot2. Models of exponential decay (using data from 296 dung pats) were created using the base R function "nls," using the self-starting model, "SSasymp," available in the "stats" package. The models of the form $Y = Y_o e^{-kage}$ were used to describe the changes in dung nutrients, where Y = amount after decay, Y_o = amount before measuring decay, e = exponential e, k = continuous decay rate, and age = age of dung (days).

Results

Change in dung nutrients over time The overall total number of dung pats of this study for all age groups and years was 294 (Table 1). The distribution of nutrient concentrations (WEOC, WEN, and WEP) and DM content for each sampling date and dung age (1–14 days old) combination can be seen in **Figs. 1 and 2**. Wide variation in nutrient concentrations was observed within the same age group in both years (Figs. 1 and 2). For example, at one day of age for both years, dung had 3.5 to 25.2 g kg^{-1} WEOC, 0.6 to 2.1 g kg^{-1} WEN, and 0.6 to 3.3 g kg^{-1} WEP, and at 14 days of age for both years, dung had 1.1 to 19.2 g kg^{-1} WEOC, 0.1 to 1.3 g kg^{-1} WEN, and 0.7 to 7.2 g kg^{-1} WEP (Fig. 1).

Means and analysis of variance for dung nutrient concentrations and DM for 1–14 days age in both 2016 and 2017 are shown in **Fig.**

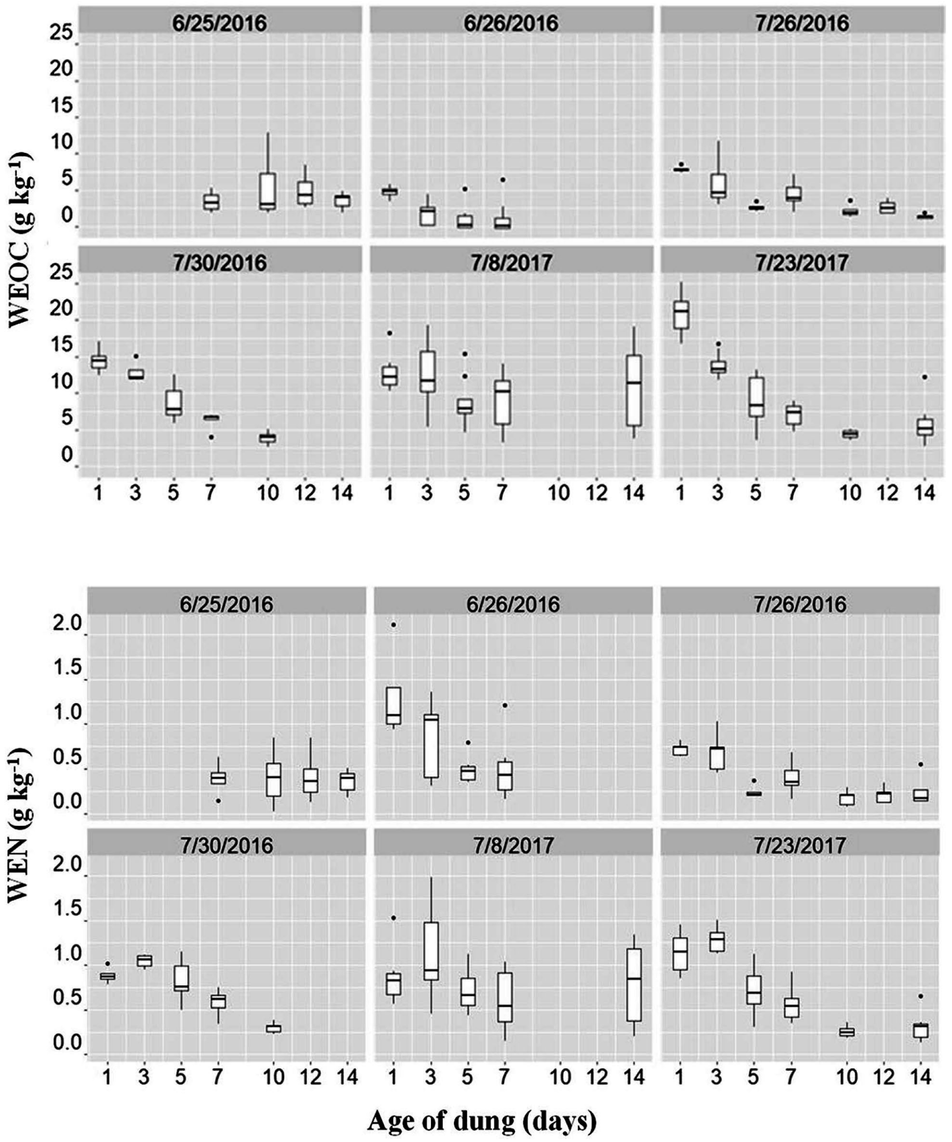

Fig. 1 Water-extractable organic carbon (WEOC) and water-extractable nitrogen (WEN) in dung across sampling dates and dung ages (1–14 days) in 2016 and 2017. Vertical lines indicate standard error of the mean

3 and **Table 2**, respectively. The concentrations of WEOC and WEN fell over time and were significantly lower at the end of the experiment in both years by 69% (WEOC) and 65% (WEN) in 2016 and by 51% (WEOC) and 45% (WEN) in 2017 than they were at day one (Fig. 3 A-D and Table 2). On the other hand, the change in WEP was not consistent (it both increased and decreased) over time, with its dung

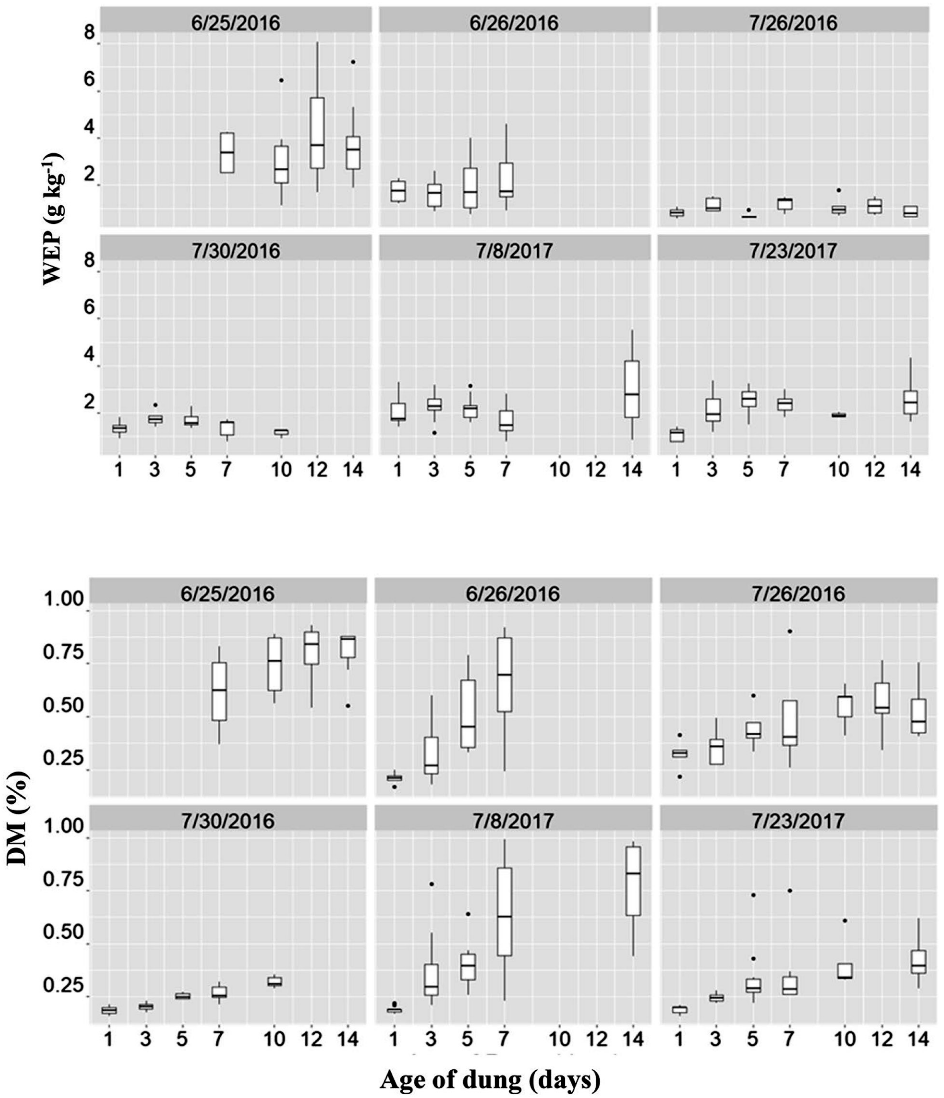

Fig. 2 Water-extractable phosphorus (WEP) and dry matter (DM) in dung across sampling dates and dung ages (1–14 days) in 2016 and 2017. Vertical lines indicate standard error of the mean.

concentration being significantly higher at the end of the experiment by 118% in 2016 and by 78% in 2017 compared to the one day age (Fig. 3 E and F; Table 2). Variances tended to decrease over time (i.e., with increasing age) for WEOC and WEN; however, WEP variance increased over time (Fig. 3 A–F). Compared to the one day of age, dung DM increased significantly by 196% (2016) and by 218% (2017) at the end of the experiment (Fig. 3 G and H; Table 2).

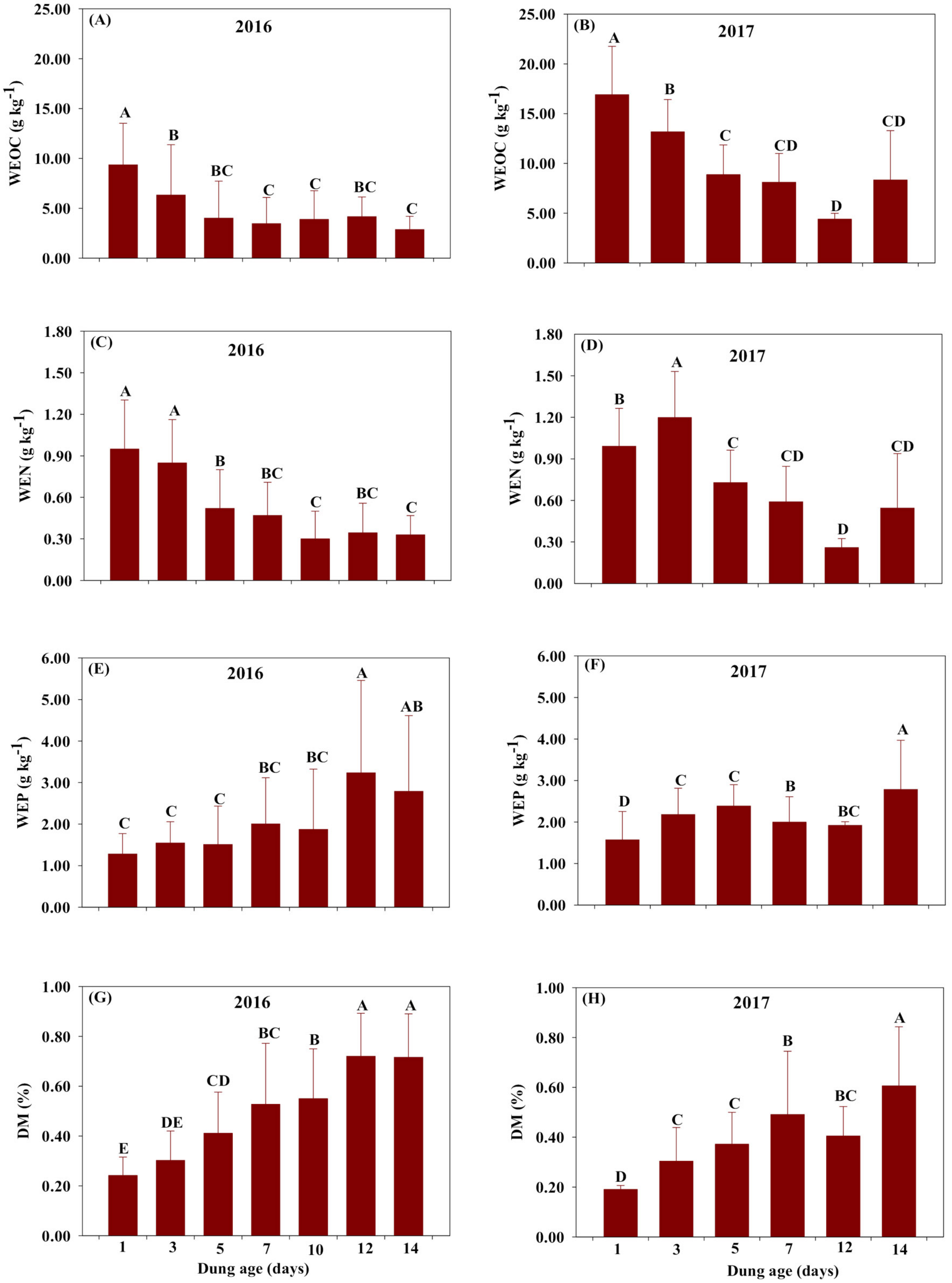

Fig. 3 Water-extractable organic carbon (WEOC) (**A** and **B**), water-extractable nitrogen (WEN) (**C** and **D**), water-extractable phosphorus (WEP) (**E** and **F**), and dry matter (DM) (**G** and **H**) in dung across sampling dates and ages (1–14 days) in 2016 and 2017. Upper case letters indicate a significant difference among age of dung. Vertical lines indicate standard error of the mean.

Table 2 Significance of F values for changes in dung water-extractable organic carbon (WEOC), water-extractable nitrogen (WEN), water-extractable phosphorus (WEP), and dry matter (DM) for 1–14 days age in 2016 and 2017 collected at Barta Brothers Ranch, Nebraska Sandhills.

	DF	*Sum of squares*	*F-value*	*p-value*
2016				
WEOC	6	454.68	6.47	< 0.0001
WEN	6	6.23	14.86	< 0.0001
WEP	6	47.16	4.11	0.0009
DM	6	3.21	16.04	< 0.0001
2017				
WEOC	5	1367.36	17.60	< 0.0001
WEN	5	7.37	15.66	< 0.0001
WEP	5	16.61	5.51	0.0001
DM	5	2.09	12.99	< 0.0001

To show the nutrient change in dung over a longer period, the means and standard deviations of dung nutrient concentrations and DM for 1, 10, and 24 days age in 2017 are presented in **Fig. 4.** Similarly, WEOC and WEN concentrations decreased over the three days of sampling and were significantly (p-values = < 0.0001 for WEOC and WEN) lower at 24 days of age by 73% (WEOC) and 76% (WEN) compared to the day-one concentrations (Fig. 4). WEP also significantly decreased (p-value = < 0.0001) by 50% at 24-days age compared to one-day age when sampling was conducted over a longer period of time (Fig. 4). Variances tended to decrease over time for WEOC and WEN; however, WEP variance increased over time. Compared to one-day age, dung DM increased significantly (p-value = < 0.0001) by 71% at 24 day age (Fig. 4).

Models of nutrient change over time

Both WEOC and WEN were modeled using exponential decay functions with all statistically significant parameters at p-value = < 0.05 (**Fig. 5**). The WEOC and WEN loss rates were expressed as $13.07e^{-0.140\text{age}}$ and $1.24e^{-0.189\text{age}}$, respectively, with values for R^2 being 0.66 for WEOC and 0.89 for WEN (Fig. 5). The WEP showed little to

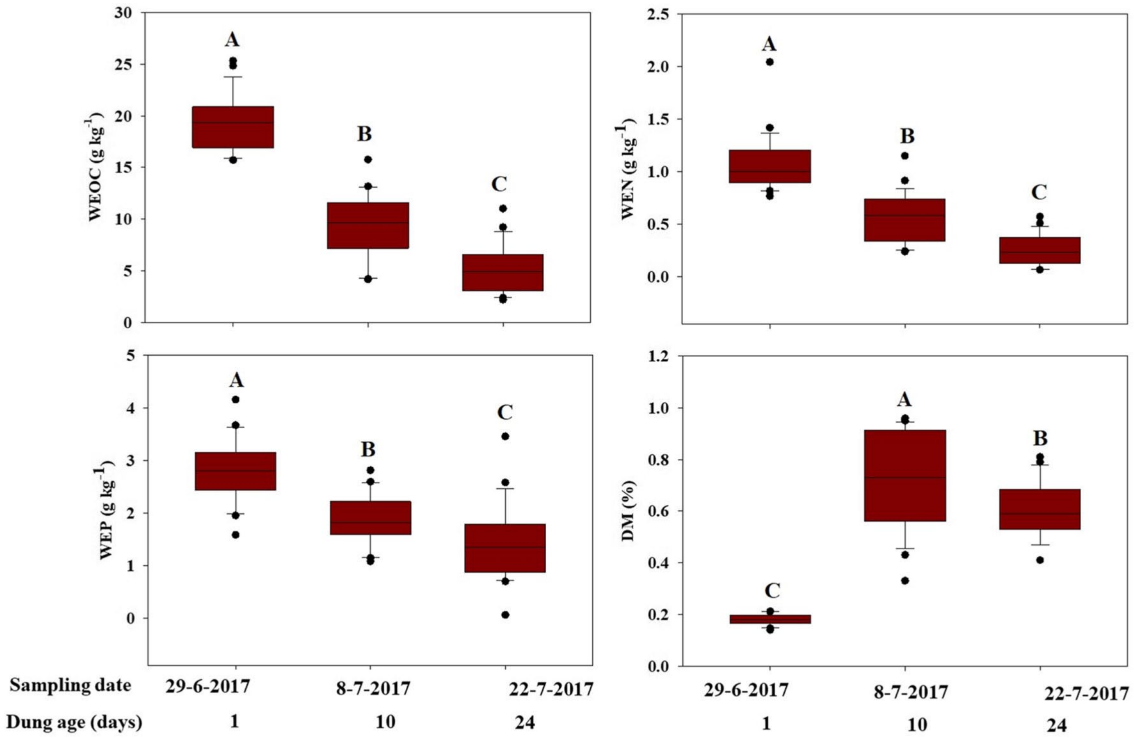

Fig. 4 Water-extractable organic carbon (WEOC), water-extractable nitrogen (WEN), water-extractable phosphorus (WEP), and dry matter (DM) in dung across age (1–24 days) in 2017. Upper case letters indicate a significant difference among age of dung. Vertical lines indicate standard error of the means.

no mean response across time, and it did not conform to the same exponential decay model as WEOC and WEN (Fig. 5). In contrast to WEOC and WEN, age was not a significant predictor of WEP concentration (p-value = 0.99). Percent DM and WEOC were strong predictors of WEP (**Fig. 6**), with the trend line present for visual pattern detection only, not as a representation of the actual model. In Fig. 6, WEOC concentration is mapped to the data points as a continuous color scale to better visualize how it interacts with WEP and DM. Although both WEOC and DM were statistically significant (p-value = < 0.001), R^2 was 0.21.

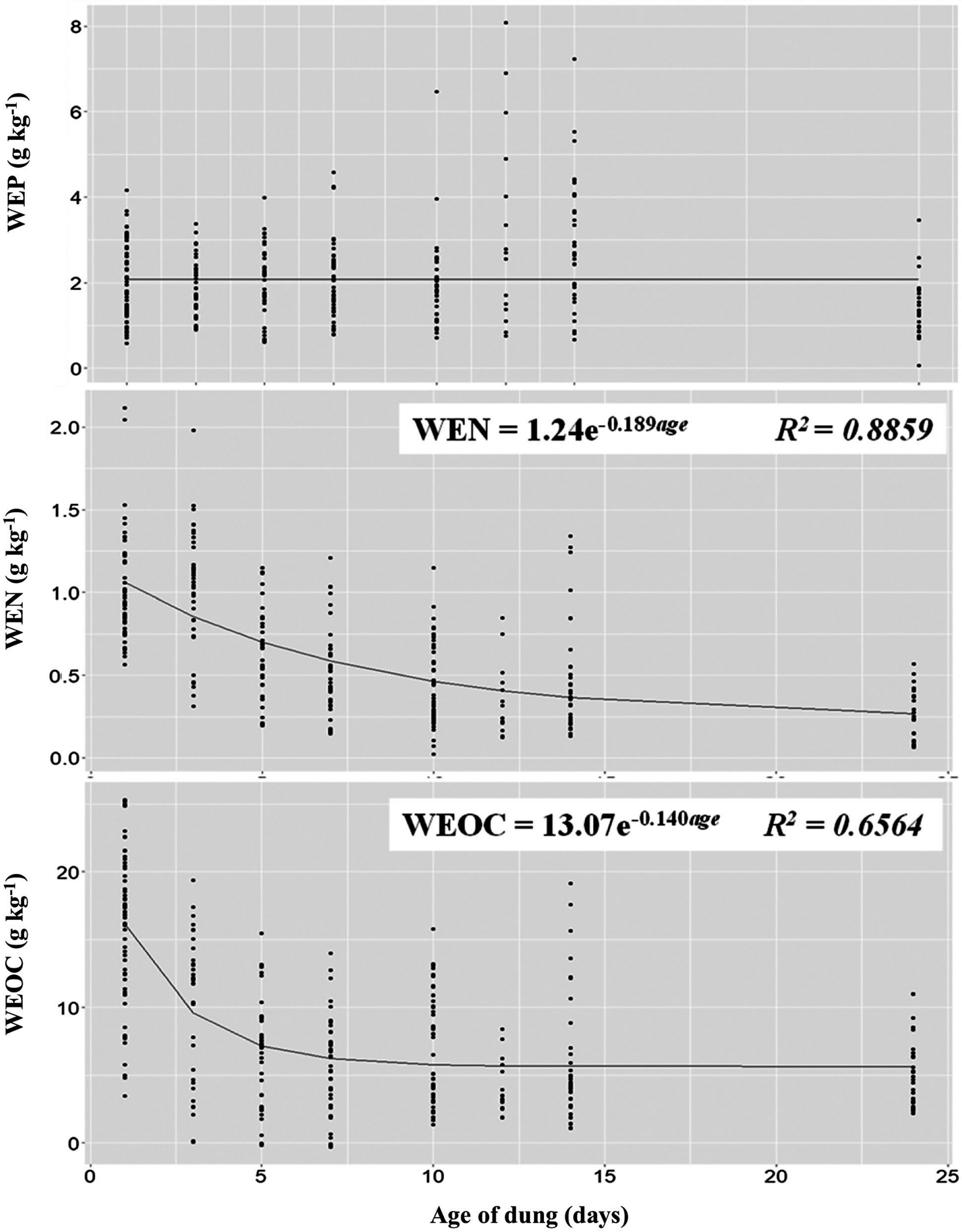

Fig. 5 Change in water-extractable organic carbon (WEOC), water-extractable nitrogen (WEN), water-extractable phosphorus (WEP) in relation to dung age in 2016 and 2017. Exponential decay function was used to model the nutrient changes.

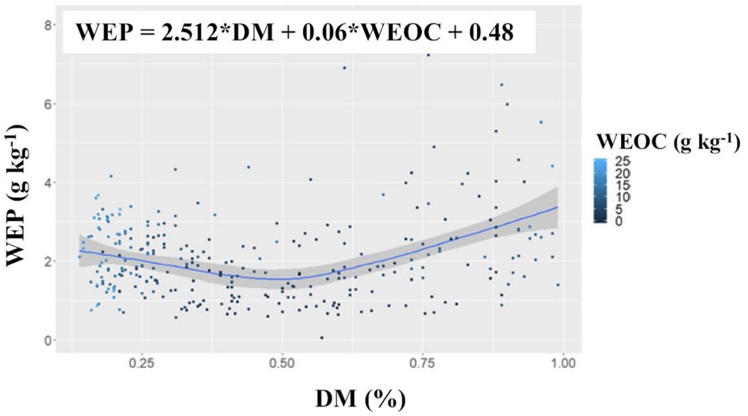

Fig. 6 Relationship of water-extractable phosphorus (WEP) to percent dry matter. Water-extractable organic carbon concentrations (WEOC) for each sample are indicated by the color scale given on the right. The trend line is shown for visualization purposes only and does not represent the model equation.

Nutrients in samples analyzed fresh and after freezing

Means and analysis of variance for dung nutrient concentrations for samples analyzed fresh and the same pat analyzed after freezing and thawing at 1, 3, 5, 7, 14, and 24 days of age in 2017 are presented in **Fig. 7** and **Table 3**, respectively. Water-extractable organic carbon values were significantly higher in the samples analyzed after freezing (37-98%) compared to the samples from the same pat analyzed fresh for five of the six dates (Fig. 7 A and Table 3). Similarly, WEN values significantly increased (37-123%) in the samples analyzed after freezing compared to the samples from the same pat analyzed

Table 3 Analysis of variance for changes in water-extractable organic carbon (WEOC), water-extractable nitrogen (WEN), and water-extractable phosphorus (WEP) in samples analyzed fresh and after freezing and thawing at 1, 3, 5, 7, 14, and 24 days age in 2017 collected at Barta Brothers Ranch, Nebraska Sandhills.

	p-value					
Dung age (Days)	1	3	5	7	14	24
WEOC	< 0.0001	0.0016	0.0027	0.0004	0.0960	0.0015
WEN	0.4926	0.1251	< 0.0001	< 0.0001	< 0.0001	0.0861
WEP	0.0591	0.0143	0.0003	0.4727	0.9556	0.0553

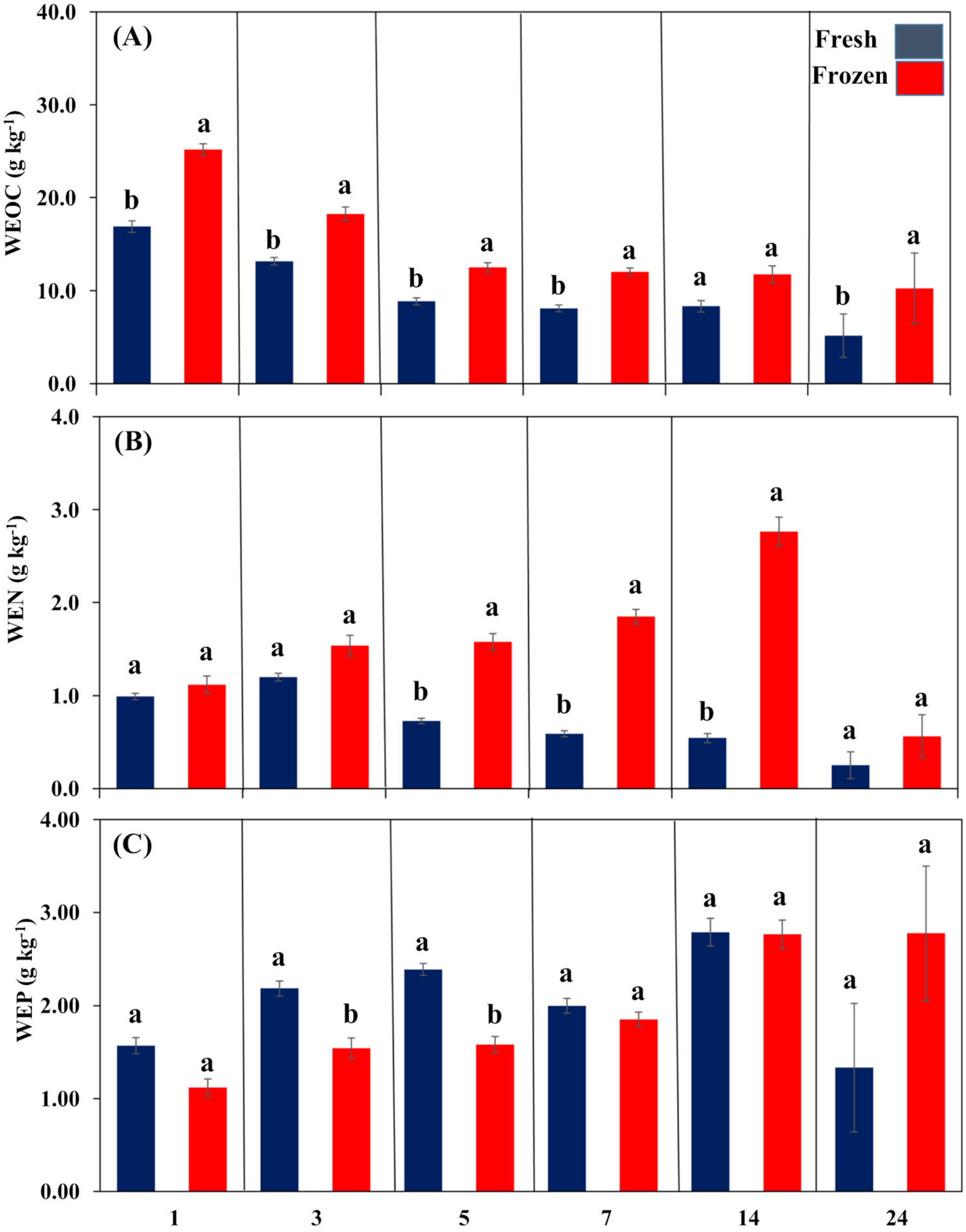

Fig. 7 Change in water-extractable organic carbon (WEOC, A), water-extractable nitrogen (WEN, B), and water-extractable phosphorus (WEP, C) between samples analyzed fresh and the same pat analyzed after freezing and thawing at 1, 3, 5, 7, 14, and 24 days age in 2017. Lower case letters indicate a significant difference among age of dung. Vertical lines indicate standard error of the mean.

fresh on three of the six dates (Fig. 7 B and Table 3). However, the trend for WEP was the opposite, with the samples analyzed after freezing having significantly lower WEP values (0.8–65%) than the samples from the same pat analyzed fresh for two of the six dates (Fig. 7 C and Table 3). The change in values between fresh and frozen sample analyses for both WEOC and WEN followed a linear trend, with R^2 values of 0.71 and 0.67, respectively (**Fig. 8** A and B). Neither age nor percent DM was significant when added to these models. Conversely, for WEP, sample concentrations determined after freezing were not linearly related to fresh WEP concentrations, and there was no significant correlation between the two. However, the addition of DM and age as independent variables yielded a statistically significant model for the prediction of frozen WEP values with an R^2 value of 0.59 (Fig. 8 C).

Differences between crust and interior nutrient concentrations

The analyzed pats had crusts with an average dry matter percent of 0.71. The moist interiors of the pats had, on average, a dry matter percent of 0.27. Means of nutrient concentrations for dung crust and the interior analyzed fresh and after freezing in 2017 are presented in **Fig. 9**. Comparing crust and interior in fresh and frozen samples showed that WEOC was 112% higher (p-value = 0.0015) in fresh crust compared to fresh interior and 98% higher (p-value = 0.0009) in frozen crust compared to frozen interior (Fig. 9 A). No differences were observed for WEN between crust and interior fresh samples (p-value = 0.0860); however, WEN was 112% higher (p-value = 0.0066) in frozen crust compared to frozen interior (Fig. 9 B). For WEP, there were no differences observed between crust and interior samples either in the samples analyzed after freezing (p-value = 0.0553) or those analyzed fresh (p-value = 0.9494) (Fig. 9 C).

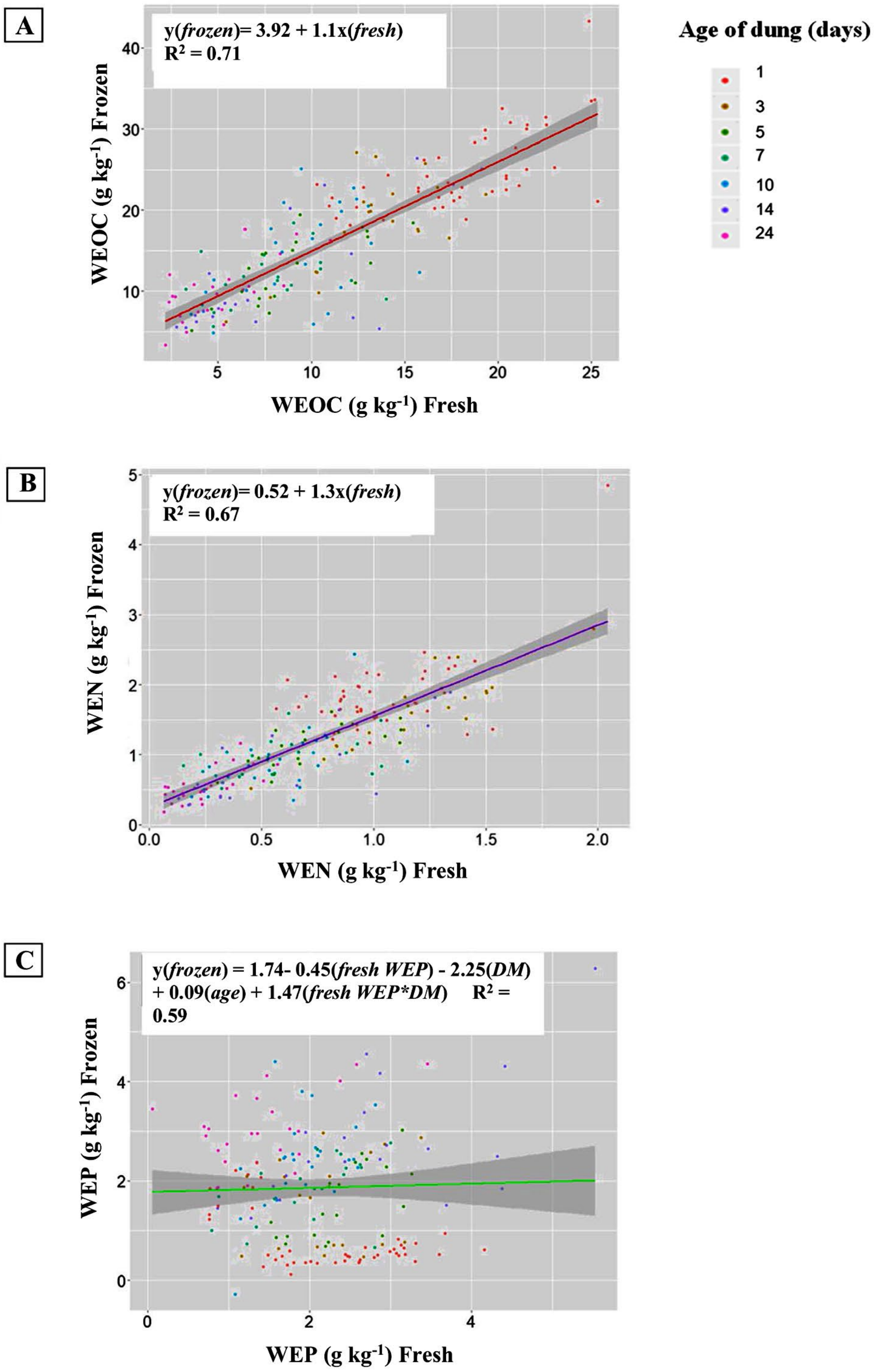

Fig. 8 Change in water-extractable organic carbon (WEOC, A), water-extractable nitrogen (WEN, B), and water-extractable phosphorus (WEP, C) between samples analyzed fresh and the same pat analyzed after freezing and thawing. Note, the equation given in C is the statistical model using fresh dung WEP value (fresh WEP), percent dry matter (DM), and dung age in days (age).

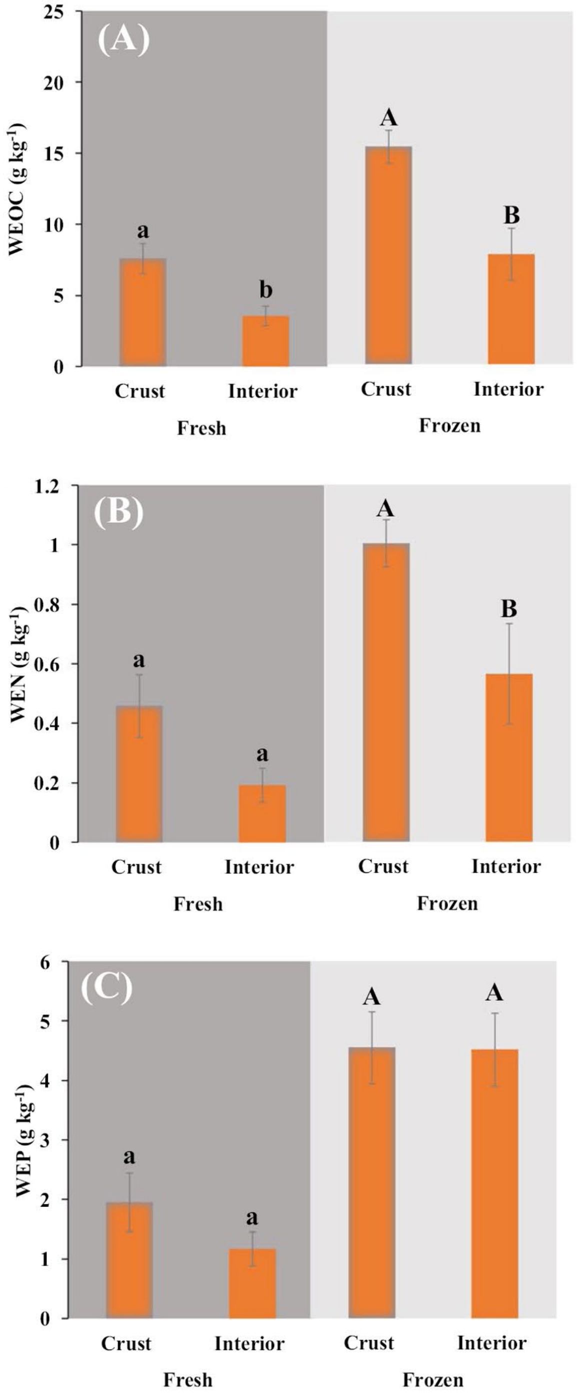

Fig. 9 Differences in water-extractable organic carbon (WEOC, A), water-extractable nitrogen (WEN, B), and water-extractable phosphorus (WEP, C) between dung crust and the interior analyzed fresh and after freezing and thawing in 2017. Lower case letters indicate a significant difference among fresh and upper case letters indicate a significant difference among frozen. Vertical lines indicate standard error of the mean.

Discussion

Evaluation of dung age effects on carbon, nitrogen, and phosphorus levels

Understanding the temporal changes in the nutrient content of dung is important due to its effects on soils, forage productivity and ecosystem services on rangelands and in pastures (Zhu et al. 2018; Evans et al. 2019; Carpinelli et al. 2020). Wide variation in nutrient concentrations were observed within the same dung age classes over June and July in both years. Although the initial nutrient profiles of dung pats can be affected by vegetation changes, the study site vegetation was relatively homogenous across the sampling area, and all dung samples were collected within approximately one month in each study year, both of which would have helped to minimize differences in initial dung nutrient content due to vegetation type and quality. Forage production was evaluated annually in our site from 2010 to 2017 (averaging 5107 kg ha^{-1}) and changes over years in production were not significant (Guretzky et al. 2020; Andrade et al. 2022). Although other researchers (Stephenson et al. 2019) have found that variable inter-annual precipitation affected plant production on uplands in the Sandhills, soil water availability and plant production did not vary much among years on our study site because it was subirrigated meadow. The change in dung DM over time was possibly due to water loss from dung through either evaporation or movement of water into the adjacent soil (Yoshitake et al. 2014).

The gradual decrease in dung C and N in this study may be attributed to the fact that these two nutrients are subject to a variety of transformations and losses over time. For examples, volatilization, nitrification, and denitrification may occur soon after excretion causing N to be lost from the dung (MacDiarmid and Watkins 1972; da Silva Cardoso et al. 2019). Also, ammonium (NH_4) can be immobilized by the microbial community for growth and reproduction, returning to its organic form and thus is no longer readily available (Hao and Benke 2008). Both N and C can also be lost from dung over time due to mineralization processes which accelerate in response to increases in precipitation and air temperature. Microbes can mineralize organic C and N, as they utilize these two substrates as an energy source, converting them to gases lost to the atmosphere. It has been shown that more than 50% of C is lost through mineralization (Bol et al. 2000; Yoshitake et al. 2014) and is lost to the atmosphere as CO_2 in much greater amounts than is retained and incorporated into the soil. Lovell and Jarvis (1996) and Du et al. (2021) reported that higher precipitation stimulated soil fauna, increasing C and N mineralization and thus causing an increase in the loss of dung nutrients. These findings suggest that most of this loss occurred soon (24 days) after the dung was deposited in the field. Other research supports this suggestion, finding that the N concentration in the soil beneath a dung pat (MacDiarmid and Watkin 1972) and CO_2 emission from a dung pat (Iwasa et al. 2015; Evans et al. 2019) increased markedly over the first 10 and 6 days, respectively, after the dung was deposited. Changes in weather conditions can also influence soil fauna abundance and activity, which may affect dung nutrient cycling dynamics (Dominguez et al. 2015; Schick et al. 2019; Wagner et al. 2020).

Dung WEP levels fluctuated (decreased and increased) over time in this study. Phosphorus leaves the dung pat through leaching (primarily as dissolved reactive phosphorus (Kleinman et al. 2007)) and can be taken up by plants, used by microorganisms, or lost from the system entirely through entry into the soil water and groundwater (Vadas et al. 2011). On the other hand, the organic P in dung can be microbially-converted to inorganic form through a mineralization process, which will be reflected in increasing values of inorganic P in dung (Dao and Hoang 2008).

Estimation of carbon, nitrogen, and phosphorus losses from dung over time using models

Dung WEN and WEOC decreased at a rate consistent with an exponential decay function which appears to be a consistent rate and pattern of transformation and/or loss from the pat irrespective of nutrient levels at the time of deposition. This observation is consistent with other research results that have shown similar outcomes when monitoring dung pats over time (Dickinson and Craig 1990; Aarons et al. 2004). Although the type of decay function was similar, rates of loss of WEOC and WEN in this study were greater when compared to rates of analyte loss in Evans et al. (2019), a study that also measured change in reconstituted dung WEP, WEN, and WEOC over time on subirrigated meadow in the Nebraska Sandhills. Based on the current literature, rates of loss of dung nutrients over time are likely to vary based on site characteristics, climatic conditions, and dung type (Cai et al. 2013). In this study, the changes in WEP were inconsistent across dung ages, with poor model prediction based on age alone. There are several possible explanations for this discrepancy in findings. In both 2016 and 2017, a pronounced spike in values occurred in WEP concentrations between days 10 and 14, which may cause poor WEP model prediction across time. The reasons for the high spike in WEP levels between days 10 and 14 are not known. However, the random selection of dung pats for each sampling date may have caused mean WEP values to rise in the dung pats collected in the day 14 data. Evans et al. (2019) also showed a spike in WEP values at day 14 before they began decreasing again.

Effects of the freeze-thaw event on dung nutrient concentrations

The determination of manure nutrients can optimize the benefits of manure application. However, because these dung nutrient analyses are time-consuming and research facilities are far from the field with the dung samples, much of this testing occurs on frozen and subsequently thawed dung (Pratt et al. 2014). The comparison between our samples analyzed for WEOC and WEN after freezing compared to the samples from the same pat analyzed fresh showed that both parameters increased after being subjected to freezing and the relationship was simple, with frozen values being predicted solely from fresh values (Fig. 8). Neither age nor percent DM were significant when added to these models. Studies of the effects of freeze-thaw cycles on soil nutrient content have consistently shown increases in dissolved organic nitrogen (DON) and carbon (DOC) (Freppaz et al. 2007; Xu et al. 2016). In a meta-analysis of the effects of freeze-thaw cycles on soil C and N, Song et al. (2017) showed that across nearly 50 studies, DON and DOC increased, on average, by 27.5% and 37.3%, respectively, after being subjected to freezing temperatures. This finding is consistent with our study, and likely points to freezing as a disruptive mechanism in cattle manure that lyses microbes, breaks down plant matter, and decreases particle size, all of which would lead to increases in water-extractable N and C. Chen et al. (2019) examined the effects of freezing on pig manure and also found similar mechanisms at work: increase in fine particle size, increase in available P, and a 30% increase in DOC.

The response of dung WEP to freezing was not consistent, and there was not a simple linear relationship between fresh and frozen values. However, the model improved with age and when percent DM was added ($R^2 = 0.59$). These findings do not agree with the results reported by Studnicka et al. (2011), who found that samples analyzed after freezing had similar WEP concentrations compared to the samples from the same pat analyzed fresh. It appears that older dung (with higher DM values) followed a more predictable response of increased WEP amounts with age. Our results also suggest that WEP in fresher dung (ages 1 day to 7 days) is less-responsive to freezing than dung aged 10–24 days, as demonstrated by the samples from 1-day-old dung. This complex relationship between DM, age, and WEP needs further investigation to better understand how these variables are driving the response in WEP values. The results from the study of the freeze-thaw event confirmed the hypothesis investigated here that changes would occur due to the freezing and thawing process. This relationship should be the focus of continued investigation, given its potential implication for estimations of nutrient cycling on grasslands, as well as the potential estimation of manure nutrients for land application in other agricultural systems where manure composes a large proportion of the nutrient inputs for crop production.

Effects of crust formation on dung nutrient concentrations

In the field, there is often a substantial dry crust that forms over the top of the dung within a short time-frame after deposition (24–48 h, depending on weather conditions). The crust varies in thickness depending upon the size, shape and thickness of the dung pat, and may not be consistent across the pat due to its structural heterogeneity. Over time, if the pat remains undisturbed, the difference in moisture content between the exterior and interior of the pat widens. These temperature and moisture differences between the drier exterior and the moist interior may lead to asynchronous nutrient cycling dynamics over time.

In this study, the comparison of WEOC and WEN between the crust and the interior showed that both parameters were higher in the former than the latter, potentially due to moisture differences between the drier exterior and the moist interior. As observed by MacDiarmid and Watkin (1972) and Holter and Hendriksen (1988), decomposition proceeds by physical removal and consumption of dung organic matter from below the pat, where moisture and temperature levels remain better for the microbial, insect, and arthropod communities to access nutrients. At the same time, the crust slows release of gaseous compounds from within the pat (Radcliffe and Rassmussen 2000). The crust also slows rainfall from entering the pat and contributing to its disintegration (Weeda 1967; MacDiarmid and Watkin 1972; Dickinson and Craig 1990). This crust then likely becomes a long-term reservoir of organic matter-associated nutrients, and may act as a pathway to long-term organic matter accumulation in pastures and a stable sequestration pathway for N, P, and C (During and Weeda 1973). A similar dynamic has been reported in soils, where dry soils had an increased release of labile C and N compared to wet soils (Haney et al. 2012). Although not significant, a similar trend was also observed with WEP contents, with the crust having higher WEP than the interior, suggesting that more samples may need to be included to capture changes in WEP. This study is one of the first of its kind providing preliminary data on the effects of dung crust formation on nutrient release. Future research should include a larger subset of dung samples with formed crust to better record their effects on nutrient dynamics.

Management implications

Rangeland managers, such as those in the Sandhills, have commonly thought that manure builds soil organic matter content and enhance forage production. Understanding how dung nutrients change over time will improve ranchers' ability to manage their lands both to enhance and maintain soil health and to increase forage production. Knowledge regarding the nutrient changes of cattle dung in a grazed ecosystem is essential due to its effects on soils, forage productivity, and other ecosystem services on rangelands. This study has demonstrated that C and N levels in dung decreased over 24 days. This information can potentially help in understanding how dung can contribute to storing C within the soil, better monitor changes in soil properties over time, and guide managers in making accurate decisions regarding grazing management. In addition, the findings that frozen storage and subsequent thawing as well as crust formation during field drying affected dung nutrient concentrations suggest that the outcomes of analyses of dung nutrient contents may change depending on sample handling and crust formation. This information is important especially for managers who want to know the impact of environmental and management factors on manure nutrient levels and soil organic matter.

Conclusion

Cattle dung is an essential part of soil fertility in pasture ecosystems, thus it is imperative that we continue to deepen our understanding of the complex factors that drive dung nutrient availability, utilization, and loss across a wide range of environments. We have contributed new information to the study of nutrient cycling in pasture ecosystems by analyzing individual dung pats across different years and age groups. Over time, P concentrations fluctuated (decreased and increased), whereas the concentrations of C and N decreased. We also documented that freezing samples prior to analysis changed the concentrations of N and C, and sometimes P, in dung samples when compared to their levels in the same fresh samples. In a preliminary exploration, nutrient concentrations were higher in dry crusts than in the still-moist interiors of dung pats, suggesting that dry crusts may serve as a pathway for the long-term retention of nutrients and organic matter in grazed ecosystems. Additional research is needed with substantially larger sample sizes across different pasture types to confirm that this is a pattern present beyond our study site, and, if so, to understand this contribution to soil chemical and physical properties over the long term.

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