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Use of UAV Imagery and Nutrient Analyses for Estimation of the Spatial and Temporal Contributions of Cattle Dung to Nutrient Cycling in Grazed Ecosystems

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USE OF UAV IMAGERY AND NUTRIENT ANALYSES FOR ESTIMATION OF THE SPATIAL AND TEMPORAL CONTRIBUTIONS OF CATTLE DUNG TO NUTRIENT CYCLING IN GRAZED ECOSYSTEMS

by

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Nutrient inputs from cattle dung are crucial drivers of nutrient cycling processes in grazed ecosystems. These inputs are important both spatially and temporally and are affected by variables such as grazing strategy, water location, and the nutritional profile of forage being grazed. Past research has attempted to map dung deposition patterns in order to more accurately estimate nutrient input, but the large spatial extent of a typical pasture and the tedious nature of identifying and mapping individual dung pats has prohibited the development of a time- and cost-effective methodology.

The first objective of this research was to develop and validate a new method for the detection and mapping of dung using an unmanned aerial vehicle (UAV) and multispectral imagery. The second objective was to quantify change over time in water-extractable organic carbon (WEOC), water-extractable phosphorus (WEP), and water-extractable nitrogen (WEN) in naturally-deposited dung that ranged from one to twenty-four days old. In addition, pre-analysis dung storage methods (refrigeration vs. freezing) were evaluated for their impact on laboratory analyses results.

Multispectral images of pastures were classified using object-based image analysis. Post-classification accuracy assessment showed an overall accuracy of 82.6% and a Kappa coefficient of 0.71. Most classification errors were attributable to the misclassification of dung as vegetation, especially in spectrally heterogeneous areas such as trampled vegetation. Limitations to the implementation of this method for identifying and mapping cattle dung at large scales include the high degree of geospatial accuracy required for successful classification, and the need for additional method validation in diverse grassland environments.

Dung WEN concentrations ranged from 1.20 g kg^{-1} at three days of age, to a low of 0.252 g kg^{-1} at 24 days. The highest WEOC values were in day-old dung, 19.25 g kg^{-1}, and lowest in 14-day-old dung, 2.86 g kg^{-1}. WEOC and WEN both followed exponential decay patterns of loss as dung aged. WEP was lowest at 1.28 g kg^{-1} (day one) and highest at 12 days (3.24 g kg^{-1}), and dry matter and WEOC concentration were stronger determinants of WEP than age alone. Freezing consistently increased WEN and WEOC concentrations over fresh values, but WEP was inconsistent across ages in its response. This research provides new insight into dung nutrient dynamics and presents a novel method for studying them across large spatial and temporal scales.

Dedication

This thesis is dedicated to my three children:

Elliot Isaiah, Anina Dell, and Gillian Neve.

They have provided unconditional love and support during a long and demanding academic journey that required their sacrifice, understanding and patience, as well as giving up time with their mom.

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CHAPTER 1:

LITERATURE REVIEW

Introduction

Unlike the majority of other agricultural production systems, nutrient management on rangelands supporting livestock production is, for the most part, extensively managed. That is, pastures receive little to no supplemental nutrient additions in the form of inorganic fertilizers sourced from off-site (e.g. ammonium nitrate, urea, etc.). Instead, availability of nutrients to plants relies primarily on the return of nutrients to the system from dung, urine, and trampled vegetation, and on the abiotic and microbial decomposition and transformation of aboveground inputs and soil organic matter into their plant-available inorganic forms (Bardgett and Wardle, 2010; Evans et al., 2017). Therefore, the long-term sustainable management of grazed ecosystems necessitates the sustainable management of nutrient resources and the cycling processes that determine their availability, which in turn relies on the skillful (i.e. creative, adaptive and scientific) management of livestock and grazing systems (Provenza, 2003; Teague, 2018; Wilmer et al., 2018). There is an increasing urgency about this task, as more recognition is being given to the role of soil health in rangeland systems and the need for understanding its underlying ecological drivers, including nutrient cycling dynamics within different management and ecological contexts (Derner et al., 2018b). Such knowledge will be crucial in helping land managers sustain healthy rangeland systems far in to the future in the face of climate change, widespread soil loss and degradation, and escalating pressure from invasive plant species (DeLonge and Basche, 2018; Derner et al., 2018a).

The impacts of grazing on soil and nutrient cycling processes

At its most fundamental level, grazing facilitates the transformation and physical re-location of nutrients through the consumption of vegetation and its later excretion, accelerating nutrient cycling by short-cutting the decomposition process and providing nutrients in more readily-available forms (McNaughton et al., 1997; Piñeiro et al., 2010; Yoshitake et al., 2014). There are subsequent cascading effects that reverberate throughout the ecosystem, transforming communities and processes at both the micro- and macro-scale levels. For example, grazing has been shown to influence: soil nutrient levels and rates of nutrient cycling and mineralization (Augustine and McNaughton, 2006; Haynes and Williams, 1993; Odriozola et al., 2014; Schrama et al., 2013; Wang et al., 2016); soil organic carbon dynamics (Abdalla et al., 2018; Barsotti et al., 2016; Wilson et al., 2018); soil food web stability (Andrés et al., 2016); abundance and diversity of arbuscular mycorrhizal fungi (Ba et al., 2012; Eom et al., 2001; Ren et al., 2018) and other soil microbial communities (Andriuzzi and Wall, 2017; Olivera et al., 2016), to name just a handful of studied outcomes. Grazing also has the potential to alter plant community composition, structure, and dynamics (Gillet et al., 2010; Medina-Roldán et al., 2012; Volesky et al., 2004), which may lead to long-term changes in the ecosystem aboveground (Augustine, 2003; Porensky et al., 2016; Teague et al., 2011) and, subsequently, in the belowground community as it responds to changes in litter characteristics, root morphology, and nutrient inputs (Bardgett and Wardle, 2003; Hunter, 2016; Vályi et al., 2015).

Grazing-mediated outcomes are complex and often context-specific, dependent upon variables such as: grazing intensity and management, climate, vegetation type, topography, site history, and existing soil physical, chemical, and biological properties (Andriuzzi and Wall, 2017; Bardgett and Wardle, 2003; Piñeiro et al., 2010; Ren et al., 2018; Teague et al., 2013; Ward et al., 2016; Zhang et al., 2018). This makes it difficult to attribute grazing effects solely to the presence of grazing livestock in an ecosystem and can lead to conflicting outcomes in studies, which may give rise to very different pictures of how grazing and/or grazing management affect an ecosystem (Briske et al., 2011; McSherry and Ritchie, 2013; Provenza, 2003; Teague et al., 2013)

Dung Composition

The nutrient and moisture content of dung is highly variable and can be affected by an animal's diet, age, reproductive status (e.g. pregnant or lactating), an individual animal's unique physiology, or even by the time of year (Eghball, 2000; Kissinger et al., 2007; Sutton et al., 2006; van Vliet et al., 2007). Dung nutrient composition, as well as C:N ratios, can also vary by species of ruminant, so the nutrient cycling patterns associated with, for instance, the degradation of sheep or goat manure may be quite different than that of cattle manure. Additionally, micronutrients found in dung may impact soil and vegetation health (Eghball et al., 2002; Sager, 2007). These are not considered here, but are nonetheless an important component of dung's contribution to nutrient cycling on rangelands.

There are several potential challenges in synthesizing the existing research on nutrient content of cattle excreta and its effect on vegetation and soils. First, much of the published research comes from studies of cattle fed in confinement for production purposes: often either dairy cows or beef cattle in feedlots. And much of what we know about manure-soil interactions comes from studies reporting on the results of manure spread in agricultural fields after being collected from holding areas in these operations. Because diet plays a defining role in the nutrient content and physical consistency of manure, generalizing outcomes from these studies to dung from grazing animals subsisting solely on pasture may be problematic (Eghball et al., 2002). Additionally, there is a common research practice of gathering manure *en masse* from confined cattle being fed a concentrated ration, then homogenizing the samples and forming artificial pats to place on pasture for observation (Aarons et al., 2009, 2004a; Evans et al., 2019; Lovell and Jarvis, 1996). Again, due to discrepancies in diets and differences in the physical structure of the manure, using this manure to study the decomposition dynamics of as-excreted dung from cattle grazing on pasture may give rise to decomposition and nutrient cycling processes that differ from those associated with naturally-deposited dung. Finally, if formed dung pats are introduced to a system which has either never been or has not recently been grazed, it is possible that the dung-feeding and dung-dwelling earthworm, arthropod, and insect communities may not be structured as they would in a pasture that has a long history of grazing (Holter, 1979). In this review, an effort has been made to use studies utilizing dung as-deposited on pasture, but due to the limited number of studies (and the age of many of them) available on the topic, it has also drawn from more recent research that uses one of the above alternative methods or settings to study dung composition and decomposition.

A typical newly-deposited dung pat is composed of water (usually about 70-80%), ash, partially-digested vegetation, a suite of nutrients in various inorganic and organic forms, and millions of microbes, both dead and alive (Evans et al., 2019; Holter, 2016). The following discussion looks more closely at three major nutrient components of dung: phosphorus (P), nitrogen (N) and carbon (C), and how they affect and are affected by both biotic and abiotic factors within dung and within the environment they are released into.

Phosphorus

Phosphorus is found primarily in its inorganic forms in cattle manure, in about a 65% inorganic to 25% organic ratio (Aarons et al., 2004a; Sharpley and Moyer, 2000). McDowell and Stuart (2005) found slightly more skewed ratios using dung from grazing cattle: 85% inorganic to 15% organic. However, this distribution can be greatly impacted by diet and organic matter levels, and Sharpley and Moyer (2000) strongly state that both total amounts and the fractionation between organic and inorganic forms may vary substantially between studies and manure types. In addition, water-extractable phosphorus (WEP) results reported in the literature have historically been obtained using a wide range of methods that vary in the extraction ratio, type of manure (air-dried, oven-dried or fresh), length of extraction, and analysis method, which makes comparisons between studies difficult to impossible (Kleinman et al., 2002; Pagliari and Laboski, 2012; Studnicka et al., 2011; Vadas and Kleinman, 2006).

Inorganic phosphorus leaves the dung pat through leaching (primarily as dissolved reactive phosphorus (DRP) (Kleinman et al., 2005)) 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). Organic phosphorus in dung is more stable and less plant-available due to its complexation with organic matter in dung and soils; it will primarily be transported into the surrounding soil with particulate organic matter either as a result of decomposition processes that break up the pat, or due to the action of dung beetles or other soil fauna who physically move dung (Aarons et al., 2004a). There is, however, a more labile pool of organic P that is also available to plants and microorganisms (Aarons et al., 2004b). Over time, the organic P in dung will be microbially-converted to inorganic P and this will be reflected in increasing values of inorganic P in dung (Dao and Hoang, 2008).

Nitrogen

Nitrogen is perhaps the most studied nutrient in manure, given its importance to plant growth and nutrition, and overall ecosystem productivity. Nitrogen is present in dung as both organic N and inorganic ammonium (NH_4) and nitrate (NO_3), with solid manures composed primarily of organic N (Eghball et al., 2002). All N is subject to a variety of transformations and losses over time. Volatilization of NH_4 to NH_3 occurs soon after excretion while moisture and warmth are both plentiful (Hao and Benke, 2008). MacDiarmid and Watkins (1972) found that just under 5% of the total dung N from a pat was lost as NH_3 in the first five days, after which loss tapered off. And Cardoso et al. (2019) found that between 2 and 12% of total applied N was volatilized as NH_3, with significant variation between years and seasons. This is less than what is reported for NH_3 losses during composting (Hao and Benke, 2008) of manures or field spreading of manure, and may be due to the early formation of a relatively-impenetrable crust on undisturbed dung (Aarons et al., 2009; Dickinson and Craig, 1990).

Conversion of organic N to NH_4 happens within the pat as a result of microbial actions and makes inorganic N available to both microbes and plants. NH_4 can then be transformed into NO_3 via nitrification, and some losses of N_20 and N_2 may occur via denitrification of NO_3. As NH_4 is immobilized by the microbial community for growth and reproduction, NH_4 is returned to organic N and is no longer readily available (Hao and Benke, 2008).

Carbon

Carbon (C) is present in dung primarily as organic C. Soon after deposition, microbes will mineralize organic C into CO_2 via respiration, as they utilize carbon as an energy source. It has been shown that upwards of 50% of carbon is lost through mineralization (Bol et al., 2000; Yoshitake et al., 2014), and carbon is lost to the atmosphere as CO_2 in much greater amounts than is retained and incorporated into the soil. For example, Yoshitake et al. (2014) found that only between 4-9.8% of carbon returned to the soil, and Bol et al. (2000) found only 12.6% of the original dung C in the top 1-5 cm of soil. Within the pat there is certain to be use of carbon as a substrate for other microbial processes to fuel various growth and survival needs. However, the internal processes of mineralization and immobilization within the dung pat seem to be poorly accounted for in the literature.

Dung decomposition and its effect on soils and nutrient cycling

While the effects of grazing on soils and vegetation have been extensively studied, disentangling the influence of grazing (i.e. vegetation removal, trampling, compaction) from the impact of dung deposition[1] on the landscape (and on nutrient cycling specifically) remains elusive due to the complex spatial and temporal relationship

[1] The focus here is specifically on the dung input to the system. We acknowledge that urine and trampled vegetation represent additional pools of resources, also distributed across the landscape in a spatially and temporally diverse manner, but those are not explicitly considered here.

between vegetation removal and trampling, and dung and urine deposition (Andriuzzi and Wall, 2017; Cherif and Loreau, 2013; Tate et al., 2003). There are an abundance of studies available that have evaluated the impact of grazing on vegetation and soils, but many of these studies have either ignored the impact of dung deposition and distribution altogether or have generalized its potential influence across a pasture (Schrama et al., 2013). For example, in an entire chapter on rangeland soils, dung and urine inputs were not mentioned a single time as contributing factors to nitrogen or carbon cycling (Evans et al., 2017), but the influence of changing plant community composition and the impact of invasive species on soil health were covered extensively. These omissions are no doubt due, in large part, to the logistical complexity of gathering and analyzing data on vegetation and/or soils concurrently with dung distribution data, all of which are time- and labor-intensive endeavors. However, because dung can lead to changes in aboveground biomass and plant community composition (Augustine, 2003; Gillet et al., 2010; Weeda, 1977), which in turn can affect belowground insect and microbial communities and nutrient cycling processes, not considering this spatial and temporal variable while assessing the impact of grazing on plants and soils leaves out an essential piece of the holistic picture of a site's ecology.

On the other hand, there are studies focused primarily on the impact of dung deposition on soil nutrient content (and/or on the associated changes in vegetation biomass or species composition) that either remove pats to a separate study area protected from grazing (Aarons et al., 2009; Evans et al., 2019; Yoshitake et al., 2014) or ignore grazing effects (e.g. stocking density, trampling of vegetation and dung pats, cattle congregating sites, etc.) in the pasture area, which also potentially leaves out an important set of variables that may influence outcomes.

In a healthy grazed ecosystem, decomposition may begin almost immediately. Earthworms and dung beetles are attracted to the dung and will affect it primarily by removing mass (and the associated nutrients and organic matter) to other locations, mixing the dung with soil below the pat and in the surrounding area, bringing soil upward into the pat, and creating numerous channels in the dung that contribute to the structural dynamics of decomposition over time (Holter, 2016, 1979; Mohr, 1943). Studies have found increased rates of decomposition with increasing numbers of dung beetles and earthworms (Evans et al., 2019; Yamada et al., 2007; Yoshitake et al., 2014).

Moisture and temperature also play key roles in the decomposition of dung (Mohr, 1943; Yoshitake et al., 2014). As temperature increases to a maximum ideal, rates of microbial respiration and nutrient use will increase, and decomposition processes can be accelerated. Since water is needed as a substrate for chemical reactions and microbial movement both within the soil and within the dung pat, moisture content plays an essential role in decomposition rates and processes.

Although precipitation also has the potential to affect the decomposition rates of dung, both my own research and observations in the field, and observations from others (for example, see Dickinson and Craig, 1990, Aarons, 2009, and Holter, 1979) show that once the top of the dung has formed a dry crust, it is nearly impenetrable to rainfall from above. This also has the effect of locking moisture in below the crust and preserving it for a prolonged period of time and thus helping to facilitate further use and transport of the dung (MacDiarmid and Watkin, 1972). If, however, rainfall is plentiful while the dung is still moist, it will contribute to the degradation of the pat, the loss of nutrients from the pat and their leaching into the soil below (Aarons, 2009).

Multiple studies have found little to no increase in soil total N values (Dickinson and Craig, 1990; Lovell and Jarvis, 1996; Yoshitake et al., 2014) under dung pats, although increases in NH_4 and NO_3 were recorded. Lovell and Jarvis theorize that this discrepancy between organic and inorganic N source dynamics can be attributed to increased warmth under the dung pat, decrease in plant uptake from plants smothered by dung and decreased leaching, all of which would increase mineral N concentrations (either directly from the dung or as a result of increased mineralization in the soil below it). Evans et al. (2019) found a peak in NH_4 and NO_3 levels between 7 and 14 days after dung placement, with levels then dropping back to initial levels by day 56.

As mentioned previously, many studies have recorded minimal increases in organic carbon below dung pats. Bol et al. (2000) found only 15% of carbon retained in the soil from dung when tracking dung-derived carbon with tracers. However, Evans et al. (2019) found a significant increase in water-extractable organic carbon beneath dung pats compared to a control site, although they do not report the value as a percentage of initial dung carbon. When present in an ecosystem, dung beetles and earthworms are responsible for burying dung-derived carbon in the soil, and can have a substantial effect on transporting carbon below the soil surface and stabilizing it in casts. For example, Schon et al. (2015) were able to recover between 13-32% of initial dung carbon in the soil below a dung pat, depending on the earthworm assemblages in the mesocosm. Microbial transformations in dung also lead to the production of dissolved organic carbon, which can leach out of the pat into the soil below. Bol et al. (2000) found a large flux of dissolved organic carbon in the soil around 25 days after dung deposition. They also observed that the addition of dung had a priming effect on the soil microbial community, leading to an increase in DOC arising from the soil and not the dung. They theorize that the input of labile C to a system may increase microbial activity, mobilizing carbon in SOM. It is also possible that the nutrient flux of other dung nutrients (N, P, K) stimulates microbial activity, leading to greater utilization of SOM and production of DOC.

Stoichiometry may also play a role in determining how nutrient inputs from dung are utilized by plants and microbes, and may affect subsequent vegetation dynamics in response to stoichiometry mismatches between plants, soil microbes, and grazers (Cherif and Loreau, 2013; Sitters and Olde Venterink, 2018)

The impact of the dung-derived organic carbon and organic matter on soil organic matter (SOM) represents a much-studied but still unpredictable and complex interaction in grazing systems. Some studies and reviews have found increased soil organic carbon (SOC) and SOM in grazed vs. ungrazed sites (McSherry and Ritchie, 2013; Wang et al., 2016; Wilson et al., 2018), but it is challenging to attribute this solely to dung contributions. However, studies specifically looking at dung placement effect on SOC/SOM, have shown increases in both of these. For example, During et al. (1973) report that even three years after the placement of their experimental dung pats there was still a "pronounced effect" on both organic matter and total nitrogen in the soil. But correlating dung OM inputs to actual increases in soil OM is difficult due to the small increases in values weighed against total SOM (Lovell and Jarvis, 1996). As is the case with organic carbon originating from dung, dung beetles and earthworms physically move dung-derived organic matter below the surface, incorporating significant amounts into the soil and, in the case of earthworms, refining it to an even more stable, nutrient-rich addition to the soil ecosystem (Holter, 1979; Schon et al., 2015).

Although the true nature and origin of SOM has been a topic of scientific debate for some years (Lehmann and Kleber, 2015), newer analysis methods have allowed for substantive progress on the illumination of its structure and makeup, and there is increasing evidence that significant amounts of SOM are derived from microbial sources (Caruso et al., 2018; Kallenbach et al., 2016; Miltner et al., 2012). Movement of microbes between dung and soil is virtually unstudied, so we have very little insight into how the microbial community that is native to the dung pat (i.e. rumen microbes) interacts with the soil microbial community upon deposition, and how this dynamic may ultimately contribute to SOM in grassland soils. However, given the fact that approximately 50% of dung is comprised of living and dead microbes (Holter, 2016), we can assume a large contribution of nitrogen, phosphorus, and carbon from the dung microbial biomass alone.

Studies have reported mixed findings regarding soil microbial biomass (SMB), soil microbial carbon (SMC) and nitrogen (SMN) associated with the presence of dung. Lovell and Jarvis (1996) found no measureable effect on soil nutrient content or soil microbial biomass (SMB) when dung was placed on the soil surface, but significant effects on SMB, C, N, and respiration when dung was dried, then pulverized and mixed with soil. Aarons et al. (2009) and Williams and Haynes (1995) also found a significant increase in SMC associated with dung presence. It is theorized that this is a result of a microbial population explosion, with the subsequent increase in microbial biomass adding to what was initially present. If dead and decaying microbes do indeed form the basis of much of SOM, then there is a virtual gold mine of OM potential in a single dung pat that probably exceeds our current estimates (Miltner et al., 2012).

The spatial distribution of dung

Both dung distribution and grazing are non-uniform in space and time across a pasture (Auerswald et al., 2010; Augustine et al., 2013; Haynes and Williams, 1993; Tate et al., 2003) and both are influenced by a suite of factors that create unique grazing microcosms shaped by pasture size and shape (Oñatibia and Aguiar, 2018); management (Teague et al., 2011); topography (Ren et al., 2018; Zhang et al., 2018); vegetation communities and soils (Bardgett and Wardle, 2010; McNaughton et al., 1997); climate (Dubeux et al., 2014); livestock type and breed, as well as livestock behavior (Provenza, 2003).

The uneven return of herbivore dung within a pasture has been an ongoing source of frustration and research efforts for scientists and livestock managers for many years, due to the environmental and ecological issues raised by the concentration of dung in small areas. For example, Weeda (1967) referred to this unevenness as a "striking feature," and investigated the use of chain harrowing in order to promote evenness of dung distribution. This patchiness of harvest and nutrient return creates microbial and nutrient "hot spots" (Kuzyakov and Blagodatskaya, 2015) in some areas, while leaving other portions of the pasture largely devoid of nutrient inputs, which may lead to declines in soil nutrient levels and soil microbial activity (Haynes and Williams, 1999). Average number of defecations per cow per day is estimated to be between 10 and 13 (During and Weeda, 1972; Weeda, 1966), which illustrates the potential for rapid accumulation of dung and its associated nutrients in favored areas of the pasture used by cattle. In what Pineiro et al. (2010) called a spatial 'uncoupling' of nutrients, vegetation and its associated organic matter and nutrients can be harvested in one area, but returned via dung and urine to a different part of the pasture altogether. This exodus of nutrients and organic matter from certain areas, and their accumulation in other areas, can have profound effects on patterns of vegetation production and community composition, as well as on nutrient cycling processes at the pasture and/or landscape-scale (Augustine, 2003; Augustine et al., 2013; Bardgett and Wardle, 2003; Hunter, 2016; Porensky and Veblen, 2015). .

Management of grazing to manage dung distribution

Over the history of rangeland science there has been no shortage of prescriptions, scholarly advice, and outright warring factions that seek to resolve the heterogeneity of grazing's impacts through various management strategies (Briske et al., 2011; Sayre, 2017; Teague et al., 2013). However, actual cattle distribution and vegetation utilization patterns are highly site-specific and hard to reproduce in other locations, even if identical grazing strategies are used (Bailey et al., 1996; Dubeux et al., 2014; Tate et al., 2003). In addition, cattle foraging behavior is complex and often difficult to both predict and control, and may operate at multiple spatial scales within a pasture, making consistent, even distribution of cattle across the landscape nearly impossible (Bailey et al., 1996). Land use history, especially in arid and semi-arid locations, may play a significant role in the responses of vegetation and soils to changes in management and can also impact spatial dynamics of grazing. This history may be difficult or impossible to obtain, leading to an information gap in how historical land use may be a driving factor in present-day plant community composition, soil microbial community composition and soil health (Sayre, 2017).

Fencing, herding, mineral and salt locations, and water source placement have all been used successfully to encourage livestock to more efficiently utilize available vegetation resources and spread dung in an orderly manner across pastures, instead of congregating in certain areas or over-grazing preferred patches of vegetation. Yet despite these efforts to simultaneously manage for homogeneity of vegetation use and homogeneity of dung distribution, there has been little evidence that the two are related (Tate et al., 2003). In fact, there is probably more evidence that segregation of grazing and ruminating/resting areas is a major driver of the spatial dynamics of dung deposition and accumulation, despite the management strategy used. Augustine et al. (2013) found highly-concentrated areas of dung in the corners of their study pasture where cattle preferred to congregate. Others have shown that cattle preferentially choose shade, watering points, certain topographical features, locations of gates, and riparian areas as lounging areas, naturally leading to an increase in dung in these areas (Bailey et al., 1996; Dubeux et al., 2014; Haynes and Williams, 1999; Tate et al., 2003). It appears that despite changing grazing management strategies and other efforts, cattle will still most often choose their preferred area(s) for lounging and ruminating based on comfort and perceived safety, and large quantities of dung will accumulate in these areas. It is hard to escape the fact that cattle are herd animals who feel safest in groups; therefore, when not grazing their time will often be spent together in communal areas. As dung density increases, this area becomes less-attractive for grazing due to avoidance of fouled vegetation, and subsequent grazing will take place away from these congregation sites, giving rise to distinctly partitioned areas for different activities.

Methods for measuring dung distribution

Historically, accounting for nutrient input on rangelands has often taken the "average" approach. That is, the expected average amount of daily manure production per animal is multiplied by the average amount of nutrients expected in that manure and the nutrients are averaged across the defined grazed area as if they are spread more or less evenly. But, as discussed above, it is well-established that nutrient concentrations are highly variable depending upon animal growth stage, reproductive status, and feed source, and that dung is not evenly spread across pastures.

Previous methods to assess distribution and density of dung in a pasture include manual mapping of dung across a pasture (Auerswald et al., 2010); transect establishment (Augustine, 2003; Tate et al., 2003); use of the line intercept method (Oliver and Young, 2012); quadrat placement either randomly or along transects (Oñatibia and Aguiar, 2018; Yoshitake et al., 2014); or simply walking and marking pats and returning at a later date to observe changes (Dubeux et al., 2014). Tate et al. (2000) used a novel technique to establish fecal loading on rangelands based on an existing method to estimate vegetation yield, but which did not explicitly map spatial locations of each dung pat. One study used true color aerial imagery (Dennis et al., 2013) from a remote control helicopter. However, this study looked primarily at urine patches using vegetation height and greenness as indicators for urine placements from a couple of weeks prior, and did not specifically address dung locations.

What is missing from the scientific toolbox is a method for evaluating dung distribution over ranch-size spatial scales and at a temporal frequency that returns usable data with an efficiency that makes the information worth collecting in the first place. The time and cost associated with manually collecting distribution data, as well as the severely-limited scope of scale when viewed against large ranch-scale landscapes, has been a hindrance to the in-depth exploration of the influence of non-uniform dung distributions on soils and vegetation. This has profound implications for rangeland monitoring and the acquisition of soil and vegetation samples which are the primary source of feedback on the health of a system, whether in relation to start or cessation of grazing, or a change in management strategies.

A UAV-based approach to mapping dung over large spatial and temporal scales

A novel approach to solving this problem could be to take advantage of the increased availability of unmanned aerial vehicle (UAV) technology and sensor capabilities for the spectral identification and mapping of dung, and the use of spatial analysis for the statistical assessment of dung distribution patterns across a landscape. A UAV offers the benefit of being able to obtain imagery at high spatial and temporal resolutions that are conducive both to the identification of dung (e.g. spatial resolutions of 6-7 cm or less per pixel) and to its mapping and monitoring at frequent intervals. In addition, there are a range of sensor options, from consumer-grade RGB cameras, to multispectral and thermal sensors, which may be of benefit for detecting dung and discriminating it from other features on the landscape (soils, vegetation).

UAV's have been used in diverse range management projects, from vegetation monitoring (Sankey et al., 2019), to characterization of site ecohydrology (Vivoni et al., 2014), to mapping invasive plants (Sandino et al., 2018). However, to my knowledge, there has not been a published study using UAV-sourced imagery for the identification and mapping of dung.

Evolving classification methods, such as geographic object-based image analysis (GEOBIA) (Blaschke et al., 2014; Hay and Castilla, 2008), combined with machine learning approaches, such as random forest and support vector machine, are making classification of images at higher spatial resolutions both more efficient and more accurate (Maxwell et al., 2018) and have already been successfully applied to a number of UAV-remote sensing projects (Pande-Chhetri et al., 2017; White et al., 2018). These methods could also be applied to the classification of dung, with perhaps a greater likelihood of success than has ever before been possible.

Conclusion

Dung from grazing ruminants comprises a substantial and influential component of total nutrient input in grazed ecosystems and has both immediate and long-lasting effects on nutrient cycling processes, soil chemical and biological properties, and vegetation dynamics. However, isolating the effects of dung and dung distribution patterns from other grazing-associated variables (e.g. trampling, harvesting of vegetation, urine excretion) is difficult due to the large spatial scales at which grazing occurs and the high temporal variability of dung deposition and decomposition.

A new method to detect dung and document its location both spatially and temporally is needed to further our knowledge of dung's important contribution to nutrient cycling in grasslands. This would facilitate improved specificity of soil sampling efforts and long-term monitoring of the interactions between dung locations, concentrated areas of dung deposition, and vegetation changes at scales that are appropriate for the investigation of pasture-scale effects. Aerial imagery sourced from a UAV combined with remote sensing classification approaches using object-based image analysis and machine learning algorithms present the potential to do just that. If successful, this type of high-resolution mapping and analysis could be combined with knowledge of dung nutrient contributions to soils and vegetation to create more accurate models of the effects of different grazing strategies and dung distribution patterns on the long-term dynamics of nutrient cycling processes across a wide range of ecosystem types.

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CHAPTER 2:

REMOTE SENSING OF CATTLE DUNG USING A UAV AND MULTI-SPECTRAL IMAGERY: DETECTION, CLASSIFICATION AND SPATIAL ANALYSIS OF DISTRIBUTION

Abstract

Knowledge and documentation of the distribution of cattle dung across grazed pastures is important for determining the spatial and temporal dynamics of nutrient cycling processes in grasslands and their effects on soils, microbial communities, and aboveground plant communities. However, in-depth investigation of these distributions at adequate spatial extents and over meaningful time periods (i.e. years) is hindered by the lack of a time- and cost-efficient method for documenting dung pat locations and monitoring them over time. In order to meet this need, we used an unmanned aerial vehicle (UAV) with a multispectral sensor to develop a novel technique of dung pat identification based on spectral reflectance and object-based image classification techniques. Imagery was collected on eleven flight dates over two different grazing treatments which utilized vastly different stocking densities. Images were then classified using supervised classification techniques with a support vector machine algorithm, and post-classification accuracy assessment was performed. Results from the classification of eleven dates of imagery showed an overall classification accuracy of 82.6 % and a Kappa coefficient of 0.71. The majority of classification errors were related to the misclassification of dung as vegetation, often in spectrally-complex areas where shadowing affected the ability of the classifier to correctly identify dung. In pastures with lower stocking densities and longer cattle residence times, dung detection was hindered initially by the presence of tall vegetation, and subsequently by the loss of

spectral signal as dung dried over time. Classification accuracy declined precipitously after dung reached 10-14 days of age. Ripley's K was successfully used to identify high-density dung areas (clusters) at varying densities and spatial extents, which facilitated the identification of dung distribution patterns under the different grazing strategies and stocking densities used in this study. The success of this method in other settings has yet to be tested, and overall classification accuracy needs to be improved before using it on a large scale. However, this new approach to high-resolution dung identification, mapping, and spatial cluster analysis presents a promising alternative to existing methods.

Introduction

Remote sensing has a long history of providing insightful data in the fields of agriculture, range management and natural resource management. It has been instrumental in the development of precision agriculture (Mulla, 2013); has aided novel methods of detecting biodiversity (Wang et al., 2016; Wang et al., 2018), and has been used for monitoring vegetation changes in rangelands (Boswell et al., 2017; Eddy et al., 2017). Most imagery has historically been obtained by either satellite or manned aircraft, but more recently unmanned aerial vehicles (UAV's), or drones, have also been used to obtain remotely-sensed data and imagery. UAV's offer the advantages of high spatial resolution imagery (e.g. centimeters instead of meters), greater flexibility in timing of obtaining imagery, cost savings (compared to owning or chartering a plane or helicopter), and a range of sensor options, from LiDAR, to multispectral, to high-resolution color imagery and 3D sensors. Previous applications of UAV technology in agronomy and natural resource management include: high-throughput phenotyping projects (Haghighattalab et al., 2016), monitoring senescence in crops (Andries B. Potgieter et al., 2017; Hassan et al., 2018), and mapping and monitoring invasive plants (Martin et al., 2018).

However, UAV's have not, to our knowledge, been used to detect and map a more neglected component of agricultural data: the distribution of cattle dung in pastures or on rangelands. In extensively managed grazinglands, where little to no inorganic fertilizer is applied to pastures, the dung and urine from cattle (and other ruminant livestock) constitute the majority of nutrient inputs back in to the system (Augustine et al., 2003; Bardgett and Wardle, 2003; Haynes and Williams, 1993; Rumpel and Rumpel, 2015). It is estimated that upwards of 80% of plant nutrients consumed during grazing are returned to the ecosystem, with only a small percentage retained by the animal (Heady, 1994). Digestion and subsequent excretion of plant-derived nutrients in both dung and urine make nutrients more available not only to plants (McNaughton et al., 1997; Piñeiro et al., 2010), but also to the soil microbial community and others that feed on dung, such as earthworms, flies, and dung beetles (Holter, 1979; Merritt and Anderson, 1977). Fresh dung is roughly 75-90% water, with the remaining contents split between inorganic ash and organic matter (Holter, 2016). The organic matter is comprised of both undigested plant matter and millions of microbes—both fungi and bacteria (Holter, 2016). Consumption of the organic matter component (aided by adequate moisture availability) by earthworms and dung beetles transports and transforms nutrient substrates further, spreading dung across a pasture and adding it to soil below the surface. This, along with belowground plant responses to grazing which can stimulate growth in microbial communities (Bardgett et al., 1998), may explain why many studies find an increase in soil organic matter content in grazed vs. ungrazed sites (Abdalla et al., 2018; Wilson et al., 2018).

The re-distribution of nutrient and mineral resources from where they were consumed (via grazing) to where they were deposited (in dung) can have landscape-scale effects on everything from the soil microbial community (Bardgett and Wardle, 2003), to water quality (Tate et al., 2003), to the phytochemistry of the plant communities in the pasture (Hunter, 2016). Thus, understanding the drivers of dung distribution patterns, as well as their long-term effects, is crucial for making grazing management decisions and managing nutrient cycling on rangelands. For instance, studies have shown that cattle congregation sites with high dung densities have lasting impacts on soils and vegetation (Augustine et al., 2003; Gillet et al., 2010; Porensky et al., 2016). As such, knowledge of the spatial distribution patterns of dung in different grazing systems can be an important component of understanding grazing behavior (Bailey et al., 1996; Dubeux et al., 2014; Tate et al., 2003), carbon sequestration in pastures (Piñeiro et al., 2010; Rumpel, 2015), pasture ecology (Yoshitake et al., 2014), the behavior of coprophagous insects (Mohr, 1943) and even the influence of commercial de-worming products on dung-feeding insects and animals (Beynon, 2012; Cooke et al., 2017). Previous research addressing these drivers has demonstrated the site-specific nature of dung distribution patterns, which may be influenced by climate, season and/or weather (Dubeux et al., 2014); topography (Ren et al., 2018; Tate et al., 2003); stocking strategy or density (Oñatibia and Aguiar, 2018), or management decisions, including placement of water and mineral, the location of shade, or the size and shape of a pasture (Augustine et al., 2013; Oñatibia and Aguiar, 2018; Sigua and Coleman, 2006).

Despite abundant knowledge about the important and complex (as well as potentially detrimental) effects of dung on soils and vegetation, and the keystone role excretion of dung plays in the nutrient cycles of rangelands, there has been relatively scant research devoted to studying the spatially-relevant and spatially-dependent cascading effects of dung within the grazed pasture ecosystem, especially over extended periods of time (years). This may be due, in part, to the inherently messy nature of dung research, as well as the amount of time and large spatial scales involved in identifying and marking the location of dung pats, and subsequently monitoring their decomposition.

Previous methods used to assess distribution and density of dung in a pasture include manual mapping of dung across a pasture (Auerswald et al., 2010); transect establishment (Augustine et al., 2003; Tate et al., 2003); use of the line intercept method (Oliver and Young, 2012); quadrat placement either randomly or along transects (Oñatibia and Aguiar, 2018; Yoshitake et al., 2014); or simply walking and marking pats and returning at a later date to observe changes (Dubeux et al., 2014). Tate et al. (2000) used a novel technique to establish fecal loading on rangelands based on an existing method to estimate vegetation yield, but which did not explicitly map spatial locations of each dung pat. One study using aerial imagery was located (Dennis et al., 2013) that utilized color imagery from a remote control helicopter. However, this study looked primarily at urine patches using vegetation height and greenness as indicators for urine patches excreted a couple of weeks prior. It is also not uncommon for 'artificial' dung pats to be created from manure from confined cattle and then placed on a pasture area separate from cattle for observation and analysis (Aarons et al., 2009; Evans et al., 2019) as a way to control the timing of dung placement and the environment, as well as to facilitate long-term monitoring in a protected space.

All of these methods are temporally and spatially limited in their scope, and give only a small glimpse into the dynamics of dung distribution and the way it influences and is influenced by vegetation communities, grazing dynamics and management strategies (Auerswald et al., 2010). On a typical ranch or grazing allotment that may encompass hundreds or thousands of hectares, it is not logistically possible to map and monitor dung at scales which are relevant or meaningful for these operations. As such, remote-sensing technology, particularly UAV-sourced imagery, holds the potential to revolutionize our ability to map and monitor dung distribution at much higher spatial and temporal resolutions than have previously been possible. With centimeter-scale resolution, high temporal frequency of repeat image data capture, and the expanded analytical possibilities that multispectral sensor data and geographic information system (GIS) integration offers, UAV imagery presents an opportunity to gain new insight and analysis options in important areas of research that have thus-far been fairly elusive and under-studied.

This research project utilized a UAV-based multi-spectral sensor to capture high-resolution images of cattle pastures in a Nebraska Sandhills meadow. These images were then classified using geographic object based image analysis (GEOBIA) with a support vector machine algorithm, and dung distribution was mapped and analyzed using spatial statistics methods. Our objectives were:

1. Design a methodology for the acquisition, processing, and analysis of multispectral aerial image data to classify and map the spatial distribution of dung pats
2. Evaluate the imagery for classification accuracy across multiple dates in order to understand how differences in image quality and ambient light conditions, as well as variation in vegetation characteristics and dung ages, may impact classification outcomes
3. Determine the efficacy of spatial statistics methods to detect variation in the distribution and clustering of dung between low and high density grazing strategies on a subirrigated meadow in the Nebraska Sandhills.

Methods and Materials

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 that were part of this research were used in a long-term grazing study (2010-2017) that investigated the effects of different grazing strategies on animal performance, vegetation characteristics, and soil properties (Shropshire, 2018) and were located on a subirrigated meadow site with a seasonally high water table. These wet, interdune areas are characteristic of the Sandhills region and are generally high-producing areas with good potential for hay production or beef cattle grazing (Horney et al., n.d.; Mousel et al., 2007). Vegetation communities 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 a lesser occurrence of warm season grasses and forbs. Soils at this site are sandy to fine sandy loam in texture and classified as mixed, mesic Aquic Ustipsamments. Average summer temperatures range from 21° C to 25° C and average yearly precipitation (past 20 years) at the site is 665 mm with approximately 40% of the yearly total falling during the summer months of June-August (High Plains Regional Climate Center, 2018).

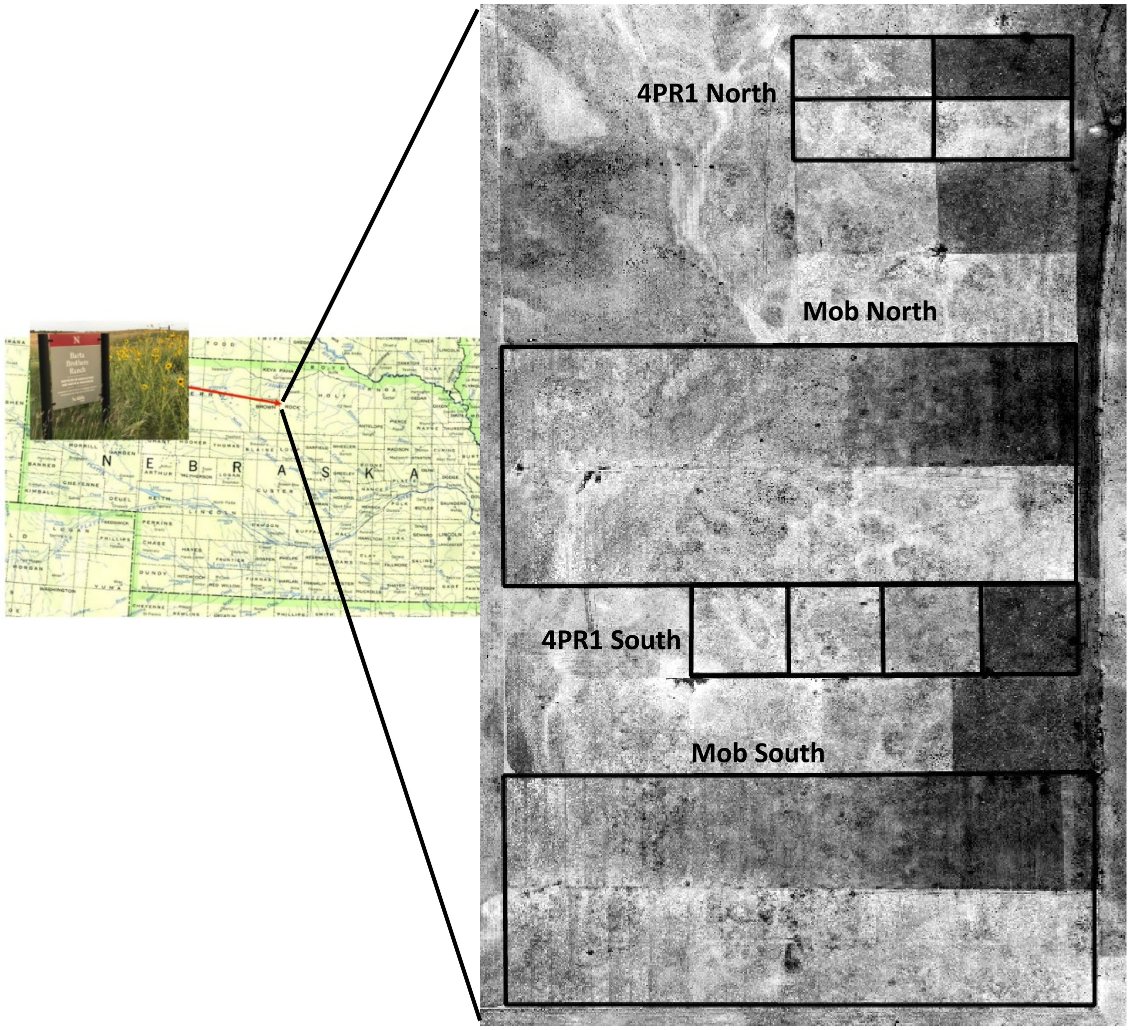


Figure 2.1. Location of study site and aerial image of the Nebraska Sandhills meadow where the grazing study took place. Only grazing treatments that were part of aerial imagery collection and analysis for this study are outlined, with pasture subdivisions for the 4PR1 replications also shown.

Grazing Treatments

The 25 ha meadow was divided into five different treatments with two replications each, arranged in a randomized complete block design (Figure 2.1). Stocking rates were held constant across the treatments (7.4 AUM ha^{-1}), but stocking densities varied between treatments due to the research objectives of the grazing study (Shropshire, 2018). Treatments included: seasonal haying (one cutting per year in July);

an ultra-high stocking density rotation (225,000 kg live weight ha^{-1}, n=32 steers) in which cattle were moved twice per day across 120 paddocks (0.06 ha each, equal to a strip 5.8 m x 98.8 m) over the 60-day grazing season; a four-pasture rotation in which cattle grazed each 0.42 ha pasture once during the season (7,000 kg live weight ha^{-1}, n=9 steers); a four-pasture rotation in which cattle grazed each 0.64 ha pasture twice during the season (5,000 kg live weight ha^{-1}; n=10 steers); and a 0.40 ha control which received no haying or grazing. Yearling steers were brought to the site each June to begin grazing and remained at the site until early to mid-August of each year, at which point they left the site. Therefore, no grazing occurred between mid-August and early June. Imagery and analysis for this study was taken from the ultra-high stocking density (hereafter referred to as "north mob" and "south mob") and the four pasture, once grazed ("4PR1 north" and "4PR1 south") treatments. The large contrast between the stocking densities and rates of rotation between these two treatments enabled the assessment of changes in dung distribution patterns between two different grazing strategies, as well as the testing of spatial statistics to successfully identify these patterns, which was one of the goals of this research.

Imagery Acquisition and Post-Flight Data Processing

Figure 2.2 summarizes the workflow for the entire process from imagery acquisition to final spatial analysis. A senseFly eBee SQ (senseFly SA, Lausanne, Switzerland (www.sensefly.com)) and Parrot Sequoia multispectral sensor (Parrot SA, Paris, France (www.parrot.com)) were used to fly the research site and collect both RGB (i.e. true color) images and multispectral images. The Sequoia contains a 16 Megapixel RGB camera, as well as four individual bands that record reflectance in the green, red,

red edge, and near infrared wavelengths. Band centers are located at 550 nm, 660 nm, 735 nm, and 790 nm, with ranges of 530-570 nm; 640-680 nm; 730-740 nm; and 770-810 nm, respectively. The sensor is integrated with an irradiance sensor which, when combined with calibration target readings taken before a flight, uses at-sensor radiance to calculate absolute surface reflectance across dates and flying conditions. This sensor also houses the GPS unit, the inertial measurement unit (IMU) and magnetometer. Radiometric calibration was performed prior to each flight using an Airinov calibration panel supplied as part of the Sequoia system. senseFly eMotion flight control software was used for flight planning, execution, and in-flight operations, as well as for initial processing of the flight and geotagging of images.

Flights took place within two to three hours of local solar noon, except when wind or weather conditions necessitated data collection either slightly before or after this ideal time period. Flight altitudes were consistently around 70 m above ground level (AGL) and stayed constant regardless of topographical variation, which resulted in a ground sampling distances (GSD) of between 6-7 cm for all flights and images. Flight line overlap was maintained at 75% for each flight. Normal flight times for a single flight that covered one entire mob pasture (6.8 ha)

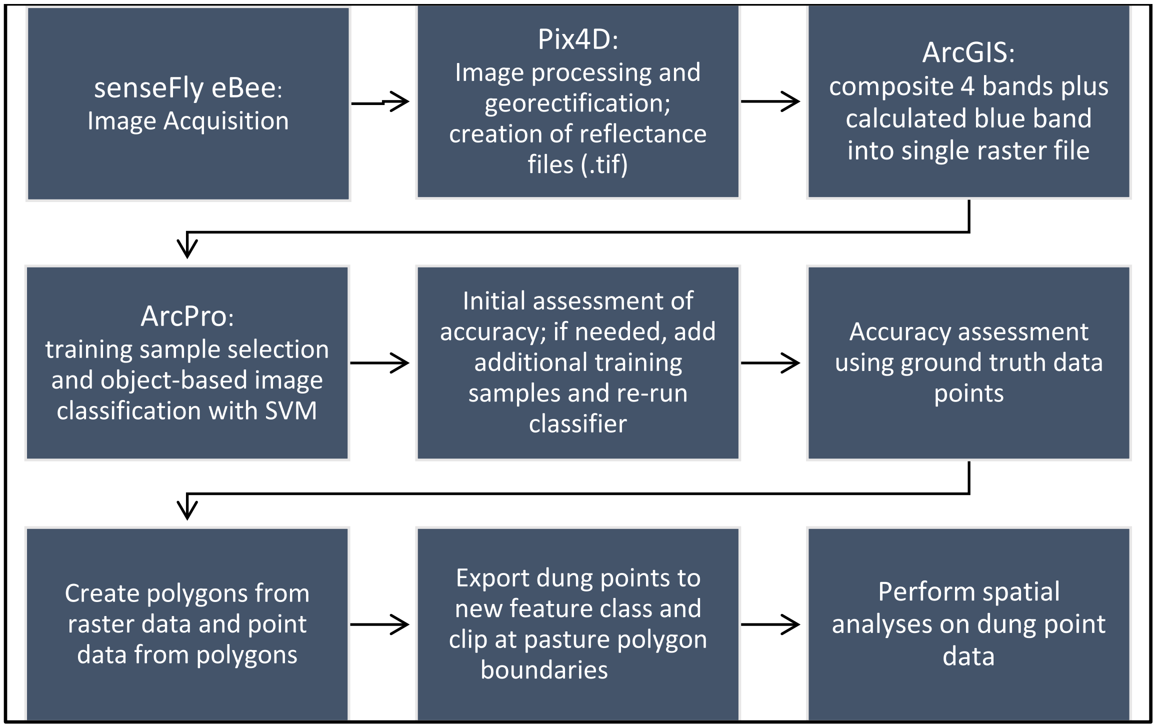


Figure 2.2. Image acquisition, classification, and accuracy assessment workflow

and one replication of the 4PR1 treatment (1.7 ha) ranged from 35 to 50 minutes, depending upon wind conditions and the exact flight pattern on a given day. Ground control points were selected by identifying fixed, readily-identifiable features (e.g. wood corner posts, corners of exclosure cages, water tanks) on the landscape and their GPS locations recorded using a Trimble Geo 7X unit (Trimble, Inc., Sunnyvale, CA, USA). These points were then used in Pix4D to increase horizontal accuracies during image processing (to achieve final accuracies of 15-40 cm).

Table 2.1. Summary of UAV flight dates, dung ages, and ground truth data points used in the image classification accuracy assessments

Date	**Pasture**	**Age of Dung Being Classified (days)**	**No. of Ground Truth Points**
June 30, 2017	Mob 22	1-15 days	49
July 7, 2017	Mob 11	7 days	25
July 7, 2017	Mob 11	1-4 days	13
July 10, 2017	4PR1S	3-14 days	39
July 10, 2017	Mob 22	1-2 days	21
July 10, 2017	4PR1N	1-14 days	40
July 21, 2017	Mob 22	1-7 days	24
July 21, 2017	Mob11	1-12 days	25
August 6, 2017	Mob 22	1-7 days	57
August 6, 2017	Mob11	1-10 days	37
August 8, 2017	4PR1N and S	1-15 days	52

Post-flight processing (georectification and addition of ground control points, image stitching/mosaicking, and reflectance calculations) occurred in Pix4D (Pix4D SA, Lausanne, Switzerland). The resulting individual reflectance files were then exported to ArcMap (ESRI, Redlands, California) and stacked together into a multi-layered GeoTIFF that contained all 4 bands, plus an 'artificial' blue reflectance band calculated using reflectance values from the visible green band and the NIR band ((visible green*3 + NIR)/4; calculated using Raster Calculator in ArcMap). This file was then used as the base for all further image analysis. The blue band was added as an extra dimension due to the findings from previous research (Shine, unpublished data) with a hyperspectral sensor that showed that the greatest spectral variation between dung and soil occurred in the visible blue spectrum, which may make it particularly important in distinguishing between dung and soil. However, a brief examination of the outcomes of classification results for four-band composite images (without the visible blue band) vs. five-band composite images did not reveal any striking loss or gain of accuracy or clarity in dung discrimination. However, subsequent analyses continued to use the 5-band composites to provide as much spectral resolution as possible during the classifier training process.

Image Classification

Supervised classification of the 5-band image was performed in ArcPro 2.4.0 (ESRI, Redlands, CA, USA) using the image classifier tool with a support vector machine (SVM) machine learning algorithm. Random tree (RT) and maximum likelihood (ML) analyses were also performed for initial comparison between the accuracies of the three techniques. The ML classifier did not approach the accuracies of the SVM and RT classifiers, so it was immediately eliminated as an option. This is consistent with results from other imagery classification studies that have found poorer performance from ML compared to more advanced classification techniques, like SVM (Maxwell et al., 2018). After classifying imagery from three different flight dates with both RT and SVM, it was determined that RT usually under-classified dung (false negative classification error) and SVM dung classification often spilled over into other classes (false positive classification error), but overall the support vector machine classifier outperformed the random tree. In refining training sample selection, it was found that optimizing selection for one classifier did not necessarily improve the accuracy of the other classifier. In addition, sometimes RT worked better in one part of the image, and SVM worked better in another. For these reasons, additional comparisons between classifier method accuracy were halted in order to focus on training the SVM model over the long term to better understand what was needed to make training sample selection more accurate and efficient.

Table 2.2. Description of classes used for classifier training

Class	Description
Dung	Readily-identifiable dung pats from a range of ages (1-10 days old)
Soil	Bare soil patches within pastures and along farm roads; gopher mounds
Wet Soil	Saturated soil found near watering points
Water	Watering troughs
Lush Vegetation	Ungrazed vegetation with a relatively homogeneous spectral signature
Trampled Vegetation	Vegetation that was trampled or showed trampling lines, not grazed
Grazed Vegetation	Heavily-grazed areas with little vegetation left
Fencelines	Vegetation beneath fencelines which was not heavily grazed or trampled
Cows	Cows visible in the final image
Cages	Exclosure cages present in the pasture used for vegetation sampling

Object-based classification was chosen over pixel-based, in order to include information relating to size, shape, texture, and location of features (especially dung) in the training data (Hay and Castilla, 2008; Pande-Chhetri et al., 2017). Continuing advances in the science of geographic object-based imagery analysis (GEOBIA) have helped to make this method a preferred option for image classification work (Maxwell et al., 2018), and it was particularly applicable to this analysis which required classification at vastly different spatial scales and the separation of small objects from a background of varying spectral characteristics which sometimes were nearly-identical to the object itself (Blaschke et al., 2014). This allowed for the selection of the same area for different classes, which appeared to have led to excellent results in the final classification. For instance, a large area could be delineated as “grazed vegetation,” but within that polygon addition training samples could be selected as “dung” or “soil”. This technique was used most frequently in the challenging areas of grazed and trampled vegetation where the classifier either under- or over-classified dung based on the dominant spectral signature of the vegetation it was found on.

In each image set, training samples were selected across the image to represent the eight thematic classes shown in Table 2.2. Between 200 and 300 dung training samples were selected to use in the model training process for each image file. In terms of absolute number, polygons of classes other than dung made up a fraction of the dung samples (approximately 5-25 polygons of each class). In terms of total number of pixels, however, they greatly outnumbered the pixels contained collectively in all dung training samples (dung pixels usually represented less than 1% of the total number of pixels selected for training). Figure 2.3 shows a representative image with multiple training data class locations highlighted. Although not every class is visible in this image snapshot, it still provides an accurate overview of what training sample selection looked like in a typical image.

Accuracy assessment

Accuracy assessment of the classified images (6 dates; see Table 2.1) was performed manually due to the slight discrepancies in geolocation between ground truth data and image data, even after post-flight corrections using ground control points. With higher locational accuracies (1-2 cm) this analysis could have been automated in ArcPro by assessing the matches between GPS point labels and the corresponding pixels or polygons. However, the small size of the target object (i.e. dung), the geolocational accuracy limits of both the Trimble GPS unit and the eBee/Sequoia data, and the lack of real-time kinematic (RTK) or post-processing kinematic (PPK) processing options

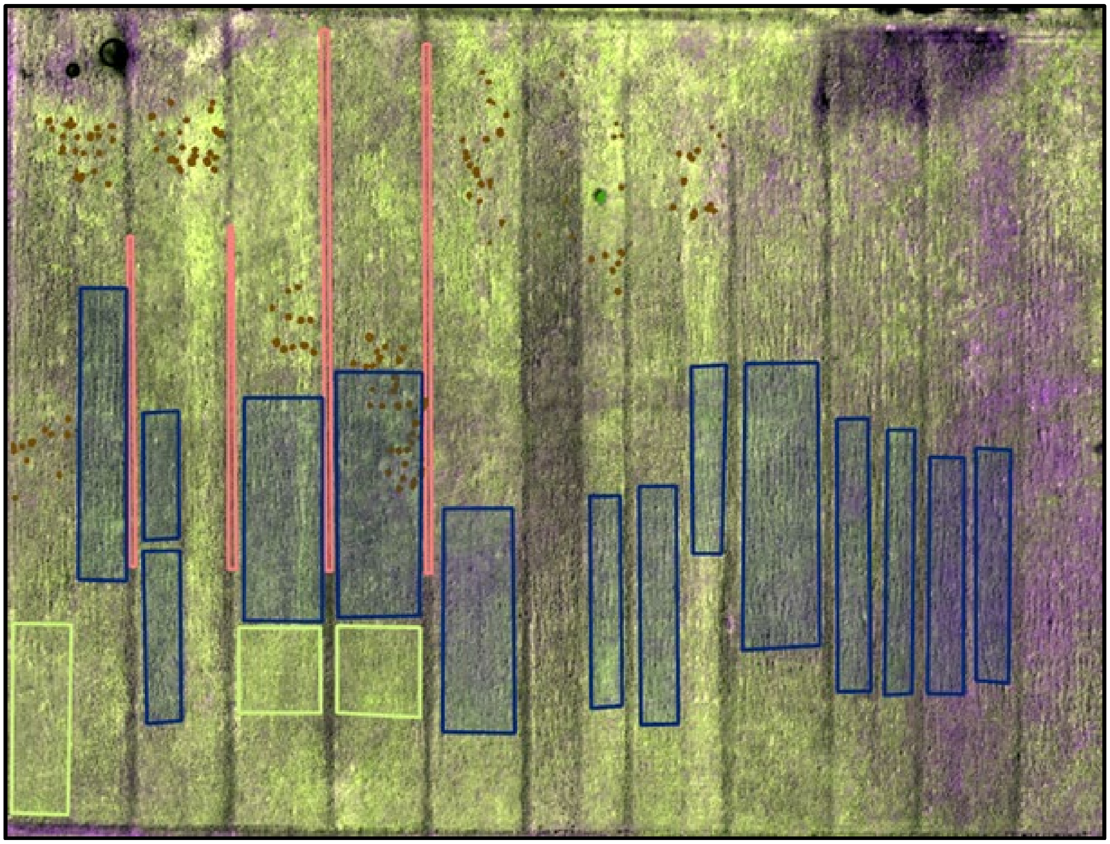


Figure 2.3: Example of training data selection in an image of the south mob pasture (July 21, 2017). Colors represent different training classes. Pink = "fencelines"; blue = "trampled vegetation"; light green = "grazed vegetation"; brown = "dung"; black (visible in top left corner) = "soil".

presented enough variation between mapped locations within the GIS that this was not possible for this project. These discrepancies fell within the normal range of accuracies expected for the UAV imagery and the Trimble GPS points, and were consistently within 0-40 cm of each other. When verifying the accuracy of a classification, pixels (and groups of pixels) were deemed correctly-labeled when they fell within 7 pixel lengths (42-49 cm, depending on GSD) of the GPS ground truth point which referred to them. This allowed for some leeway in accommodating the limits of the precision of the equipment that was used to collect the data, without over-extending the accommodation to pixels that were too far from the GPS point. On one image date, the accuracies were reduced below this expected threshold, but distance discrepancies were consistent across the image in direction and magnitude. Therefore, for this image set, an additional 2-3 pixel lengths were allowed in order to capture the true performance of the classifier, regardless of the poor geolocational accuracy. In the mob treatments, only ground truth points corresponding to dung that was 10 days old or less were evaluated for accuracy. After dung passed this age, classification errors rose precipitously and the imagery was no longer useful for accurately detecting dung (see discussion). Ground truth data for vegetation was not collected in the field with the GPS unit, so an alternate visual interpretation method of checking for classification accuracy had to be used. Accuracy assessment points (2000 per image set) were randomly generated in ArcPro across the classified raster. Of these points, all four of the vegetation categories were selected as potential ground truth data and approximately 25 points in each pasture, per image date, were used as substitute ground truth data. Accuracy was not assessed within vegetation classes (e.g. "trampled vegetation," "lush vegetation," etc.), but on correct classification as vegetation as an aggregated, general class.

Spatial Analysis

Classified raster data has limited use in spatial analyses. Because each pixel is still classified as just one distinct class, the data is not in a form amenable to object-based analysis of distribution or clustering patterns. Therefore, after classification and accuracy analysis was performed for each of the image dates, the raster data was converted to polygons using the raster-to-polygon tool in ArcPro, which transformed each group of similarly-classified pixels (i.e. segments) into a polygon. This transformation was necessary in order to then create point data from the polygons, which subsequently allowed the dung distribution to be modeled and analyzed as discrete points, a task not possible using raster data alone.

After all of the polygons were transformed into points, the subset of points which were classified as dung were extracted from the shapefile and a new feature class layer was formed that contained only dung data. Then, each pasture was clipped from the larger image so that the subsequent spatial analysis was confined to one replication of a grazing treatment at a time. The density-based clustering tool with the defined distance (DBSCAN) method was used to identify clusters. With this tool the spatial scale of clusters and the density of dung pats within them could be set manually after iteratively exploring different combinations of distance and density. This clustering tool is not affected by the size of the area being analyzed, as it is only identifying clusters that meet the space parameters set by the analyst. This allowed clusters to be identified within the two different grazing treatments, even though the total areas of each treatment, and the area of each rotation within each replication, varied significantly (the effect of pasture sizes and shapes on dung distribution patterns that led to the results of the clustering analysis is explored further in the discussion section of this paper). There are other tools available for clustering analysis within ArcPro, but these take into account point attribute data (i.e. a value associated with the point in addition to its location) when assigning "hot spots" or "cold spots" of clustering. Since there was no attribute data of significance to the determination of clustering of dung, these tools were not evaluated for their performance.

The multi-distance spatial cluster analysis tool (Ripley's K) was assessed for its correlation to the magnitude of clustering across imagery dates and pastures. Ripley's K also measures degree of clustering, but it does so at a selected number of user-specified distances (e.g. every 3 meters) in order to assess how clustering changes over different spatial scales, and at what distance(s) it is most significant (Mitchell, 2009). It looks at all the neighboring points present at a specified distance, not just at the nearest point as in the nearest neighbor index. This analysis has the advantage of producing visual output in the form of a graph that plots the expected K values (or their transformed counterpart value, L(d)) as well as the observed K values from the study area and does so across all distances used in the analysis so that it is evident at which distance clustering peaks. Distributions can be graphed alongside each other in order to compare clustering patterns over time or between multiple data sets.

Results

Classification

The primary way to measure the success of an imagery classification project is through the creation of a confusion matrix, or an accuracy assessment in table form (Jensen, 2005). This matrix not only shows the overall accuracy of a classification, but also breaks down the results into individual categories so that classification errors can be assessed between and within classes, giving more insight into which class categories are the most problematic. In addition, scores are calculated for the producer's accuracy and the user's accuracy, which measure errors of omission and commission, respectively.

Our analysis showed promising overall accuracy, 82.6%, with even better results for individual classes, especially for dung (Table 2.3). The corresponding Kappa coefficient was .72, indicating "substantial" agreement between ground truth data and the classification after accounting for statistical chance in agreement of the results

(Congalton, 1991). The results for individual classified vegetation categories were not assessed for accuracy in this analysis because the focus was on delineating dung from vegetation and soils, not classifying specific vegetation patterns or types. As a result, the accuracy assessment results for a generalized vegetation class were calculated.

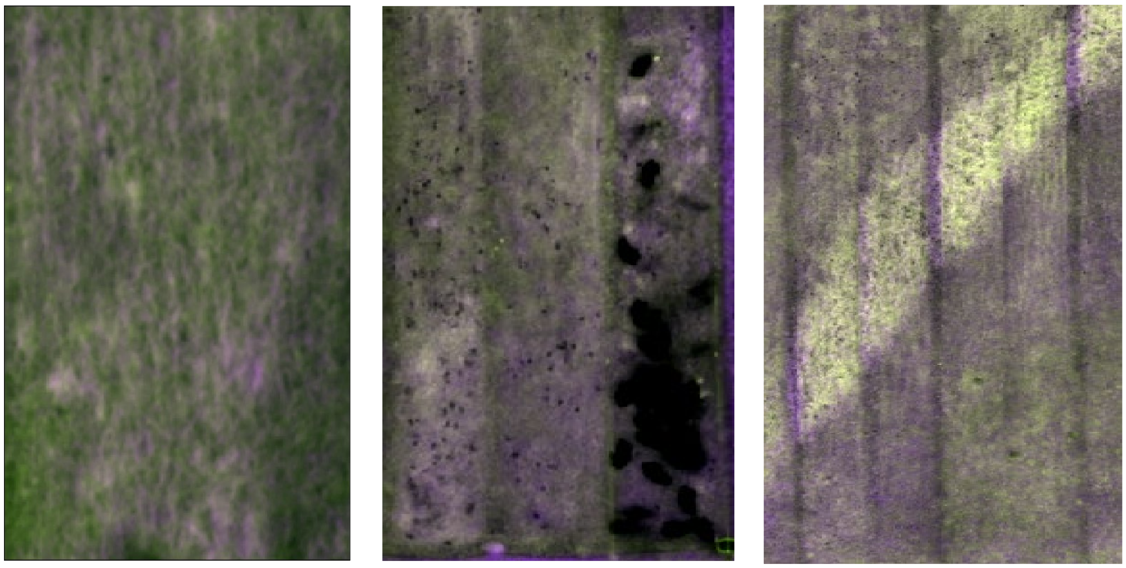
There were several unexpected findings related to classification that had not been anticipated during the ground truth data collection phase of the project. First, because the spectral signatures of dung and vegetation are typically quite distinct, it was not expected that there would be many classification errors between dung and vegetation. However, this was not the case and, in fact, the majority of dung mis-classifications were due to it being assigned to a vegetation class, not to a soil class, as we had expected (due to the very similar spectral signatures between the two). In particular, the dark shadowing found under fencelines where cattle were not able to graze as heavily (the lines that separated each 24 hour grazing period, not the 12 hour periods) mimics a reflectance pattern characteristic of dung, which leads the classifier to easily misinterpret these areas (Figure 2.4c). This is likely due to the similarity in NIR absorption characteristics in shadowy areas, and possibly also to the lower reflectance values in the red edge band. Figure 2.5 graphically demonstrates how closely aligned the spectral signatures are for “old dung” and “fencelines.” Adding “fencelines” as a class during the training phase helped reduce this source of error, had the effect of minimizing the number of pixels classified as soil in these areas, and increased the classifier’s accuracy in correctly assigning bare soil and gopher mounds to the “soils” class.

Table 2.3. Error matrix of the accuracy assessment results from all image analysis dates (6) using supervised classification and a support vector machine algorithm

Class	Dung	Soil	Vegetation	Total	**User's**
Dung	221	11	6	238	0.93
Soil	13	52	1	66	0.79
Vegetation	70	15	278	363	0.77
Total	304	78	285	667	
Producer's	0.73	0.67	0.98		
Overall Accuracy:				82.6%	
Kappa:				0.71	

Interestingly, both the RT and SVM classifiers rarely misclassified dung on areas which hadn’t been grazed (which were part of the original flight mission—peripheral areas that were photographed without good overlap were subject to much spectral distortion). In other words, in areas of lush vegetation with deep shadowing, dung was rarely assigned to pixels in these areas. This may have been a result of the strong vegetation signal that was consistent across the canopy, even when shadowed areas existed below, which made the vegetation type more distinct from dung. It was also evident from early classification attempts that trampled vegetation was another prominent source of error, mis-classifying vegetation pixels as dung, and vice-versa, and that another vegetation category would need to be added for trampled vegetation. Image exploration using known areas of trampling (e.g. tire tracks in a meadow area with adequate vegetation cover) revealed that these areas showed a complex mix of reflectance patterns, with spectral characteristics of abundant vegetation, soils, and dung (corresponding to, respectively, high NIR

a. b. c.



a. Trampling lines in mob pastures

b. Cows and the shadowing effect from their movements during UAV flight

c. Fenceline effect and change in reflectance with underlying wet soil

Figure 2.4. Three common causes of classification error: a. trampled vegetation and its complex spectral pattern; trampled areas are dark green and standing vegetation is light purple. Note especially the large amount of variation in reflectance qualities (represented by different hues) across short distances. b. presence of cows and shadows from their movements during UAV image capture over multiple flight lines. c. fenceline effect on vegetation height and structure under temporary fencelines and its effect on spectral reflectance, and the change in reflectance due to the wetter soils (bright yellow-green stripe) underlying an ephemeral stream through the pasture.

reflectance (both soils and healthy veg) and high NIR absorption (shadowed areas deep in the vegetation). Figure 2.4 shows an up-close image of cattle trampling tracks in one of the mob pastures. It is easy to see how diverse this area is spectrally, and at what a fine spatial scale it changes over, producing dung-sized shadowed areas that easily could be mis-classified. Again, once this category was added there was a significant reduction in the number of vegetation pixels mis-classified as dung.

The most successful classification of dung occurred when it was found on areas that had a consistent spectral response pattern across pixels (e.g. an expanse of homogeneous vegetation). In this instance, the dung 'object' is more readily identifiable by the computer-based classification method as something that is its own entity, separate from the background class. When the spectral picture is more complex, it becomes more challenging to assign a class to an object (dung) that is already spectrally-heterogeneous (wet interior, dry edges). Figure 2.5 shows the spectral signatures of the most common classes and how closely some of them overlap spectrally.

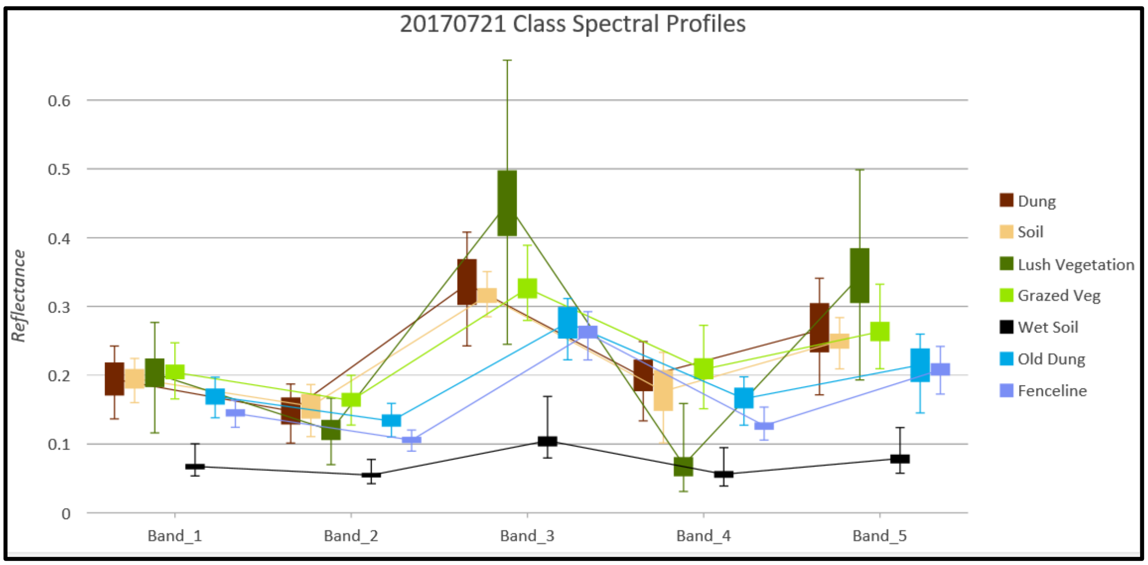


Figure 2.5. Spectral signatures of selected classes. Bands are as follows: 1: visible blue, 2: visible green, 3: near infrared (NIR), 4: visible red, 5: red edge

Another classification pitfall that was encountered was that of obtaining imagery while cattle are still in the photo (Figure 2.4b). The stitched final image contained shadowy cows in different locations across the pasture due to their movement between drone flight passes. Depending on how active the cattle were during the drone flight, this can create substantial noise in the image and cover-up underlying details of the vegetation and dung patterns due to the shadowing. Cattle (Black Angus, in this instance) are apparently very dung-like in their reflectance characteristics and were classified as dung until a separate category was created for them. Training sample selection of cows was most successful when it included a generous boundary around the animal that also captured their dark shadow on the ground around them. Otherwise, those areas would have been classified as dung.

Our results show that there is a limited window of time for UAV-acquired imagery to be useful in assessing distribution in productive pasture areas. Dung must be fresh enough to send a strong spectral signal that distinguishes it from soil or other features (via moisture characteristics), but at the same time vegetation must be at a height and density that allows detection of dung. However, in other pasture or rangeland settings that are characterized by shorter, sparser vegetation, dung may be much easier to detect both immediately after grazing begins and for a longer time period after initial deposition, if re-growth of grazed vegetation is slow. In this sub-irrigated meadow, there was a window of 7-10 days during which dung was most easily identified. This was fine for the mob grazing trial, where cattle were moved off of the grazing strip after 24 hours, but in the four-pasture rotation where stocking density was lower and residence time in the paddock was longer, it was challenging to see dung in the deep vegetation during the first few days. By the end of the rotation 15 days later, dung distribution was apparent, but a significant proportion of total dung would have dried or been decomposed enough that it was no longer visible to the sensor (or to the human eye). For example, in the June 30^{th} imagery of the south mob pasture, 47% of dung was classified correctly prior to day 14, but after day 14 (i.e. dung was two weeks old) only 17% of dung was accurately classified. In order to fully capture and map dung distribution over time in these pastures, UAV imagery would need to be acquired at the beginning, middle and end of the 15 day period.

While dung and soil were not as frequently mis-classified between classes as they were with vegetation, there are definitely still concerns about this source of error. Wet and dry soil (which was present primarily as gopher mounds at this location) had to be assigned their own classes due to their very different spectral signatures (Figure 2.5). If enough training samples were included in both classes then the confusion between dung and soils was minimized. In other settings soil type and moisture will also likely have significant impacts on the effectiveness of detection and classification of dung. If soil moisture is high, and there is a significant amount of bare soil visible, NIR absorption is increased and makes discerning patches of dung from bare soil more difficult. Along those same lines, soils with high organic matter content and/or high clay content will reflect both visible and NIR light differently than sandy, low-OM soils (Askari et al., 2015; Wight et al., 2016), also changing the algorithm needed to discern dung from soil.

An additional area of classification confusion was in the most heavily-trafficked areas (corners, watering points), where there was a tendency for the classifier to produce nearly solid blocks of dung pixels when they should have been easily-delineated as pats or classified as soil (Figures 2.6 and 2.7). This was particularly evident in the mob pastures with ultra-high stocking density. There are two possible causes of this error. First, the soil signal is probably much stronger in these areas due to heavy trampling and grazing which allows the soil to be ‘seen’ by the sensor. The similarity between soil and dung then leads the classifier to blanket-classify large areas as dung. However, there is another possibility for this error. Because there is so much trampling, walking, resting and ruminating in this area, it is possible that there is actually a large amount of dung that has been spread more thinly across the surface of the pasture after being excreted. This dung is not visible to the naked eye, but the spectral signature is still intact enough that the algorithm correctly classifies it as dung. Returning to these areas within a short timeframe after classifying the image would reveal if this was in fact the case.

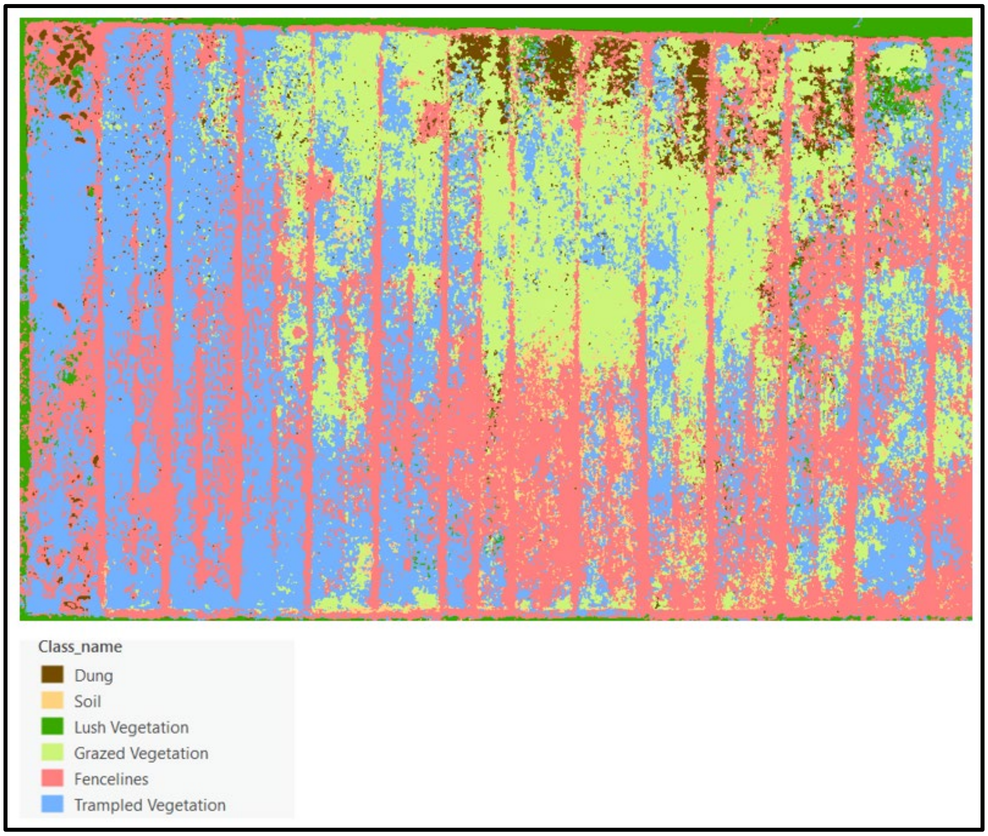


Figure 2.6. Example of a classified image from the mob grazing treatment

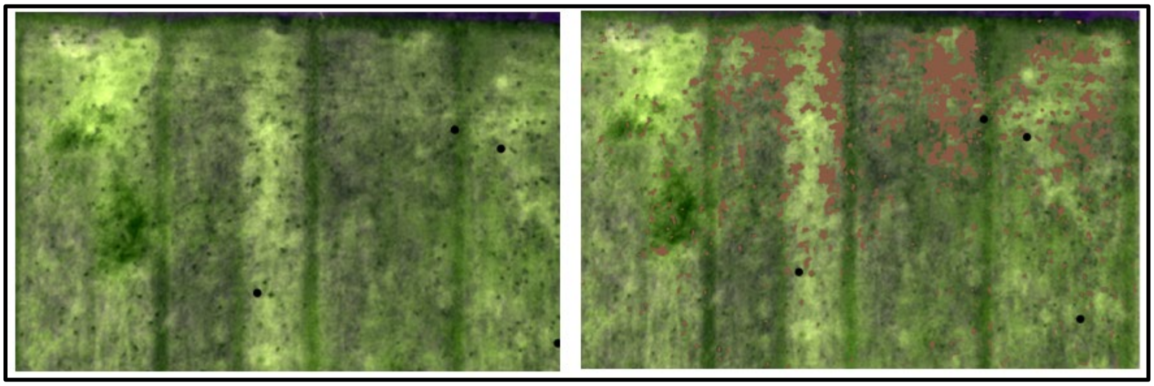


Figure 2.7. Over-classification of dung in highly-grazed and trampled areas of the pasture. On the left is a false-color image (3 of 5 bands loaded to different color guns); on the right is the classified image with only dung-classified pixels colored (brown pixels). It is easy to see that many more pixels were assigned a "dung" classification than were actually dung, based on the image on the left.

II. Spatial Analysis

The process of converting raster to point data gave a highly-accurate rendering of actual dung locations. In areas where classification produced groupings of dung-classified pixels that were not exclusively dung (i.e. pixels that were not dung were classified as dung; see Figure 2.7), the transformation from raster to point data served as a way to visually minimize error, as each of those larger polygons of contiguous dung pixels became a single point. This allowed for an easier overall visual inspection of the accuracies of points vs. actual dung visible in the image. However, this is still an inaccurate representation of a dung location, and had significant effects on the clustering analyses performed later on (as discussed below).

Another advantage of transforming raster data for dung into point data is that it makes it possible to increase the accuracy of the final shapefile by manually performing an accuracy analysis of the point data against the orthomosaic and eliminating points that are not clearly associated with dung on the ground, though this may present its own set of issues in cases where dung is detected correctly by the sensor and classifier, but not by the human eye.

Results from the density-based clustering analysis clearly showed differences in distribution across mob-grazed strips and the 4PR1 pastures. Figure 2.8 shows three iterations exploring the process of choosing optimal distances and dung points to accurately represent clustering at a meaningful spatial scale. Because there is no definition of what qualifications a grouping of dung pats has to meet in order to be considered a 'cluster,' the results of the analysis had to be weighed against knowledge of what is likely to be significant clustering for not only the system under study, but for other grazing strategies as well. A distance of 6.1 m was chosen as a large enough area that multiple animals could potentially be present for an extended time in, and a density of approximately 12 dung pats per meter gave rise to statistically-significant clusters (2.8C) that were neither too large and poorly-defined (Figure 2.8A), nor so small that the

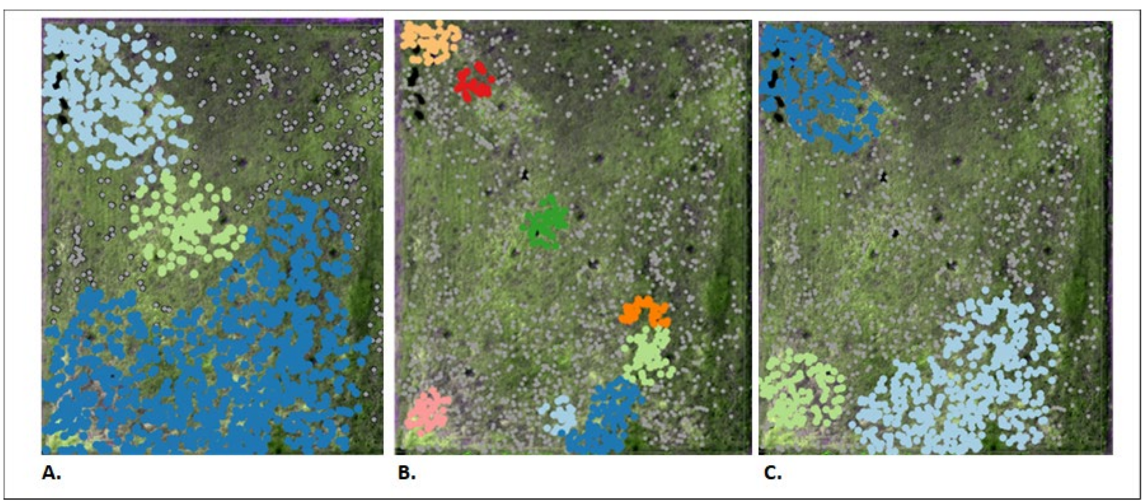


Figure 2.8. Results of density-based clustering using parameters of A. 50 points in 6.1 m., B. 25 points in 3 m. and C. 75 points in 6.1 m. Colors only represent different clusters and have no analytical or classification significance.

clusters could have been the result of a short duration of dung accumulation by a few animals, instead of from heavy and/or repeated utilization (Figure 2.8B). Additionally, a distance of 6 m is approximately the width of each grazing strip in the ultra-high stocking density treatment. This allowed an assessment of clustering to be conducted at a scale that corresponded to the smallest grazing unit (both spatially and temporally) being used in this study, which was important for our research objectives.

While the goal of this project was primarily to assess the potential for a new methodology in detecting and mapping dung, we also hoped to gain insight into thec changing patterns of dung distribution between different grazing strategies, a high-stocking density treatment with fast rotations and a low-stocking density treatment with longer residence times in each pasture. This proved to be more challenging than anticipated due to the early inability to detect dung in the 4PR1 pastures (due to thick vegetation) and the loss of detection capabilities after dung reached 7-10 days of age. In addition, as mentioned previously, relatively small (in terms of spatial area) errors in classification of dung can have large impacts on the assignment of clustering for a given date and over a range of dates in the same pasture, which makes assessment of changes in clustering over time nearly impossible in the absence of highly-accurate classifications, or post-classification intervention and point editing as suggested earlier.

In Figure 2.9 the results of a particularly over-zealous classification scheme of dung are shown. This leads to two analysis challenges: first, it gives the impression of one giant cluster spanning nearly 3 weeks of grazing rotations, which is not accurate. Second, it obscures the underlying true clusters that do exist, prohibiting the utilization of the correct data for spatial analysis. These classification errors also impact the statistical measure of distribution that we carried out: multi-distance spatial clustering (Ripley's K). Again, the differences in classification errors between dates changes not only the location of clusters, drawing into question the validity of results, but also the statistical significance of the clustering.

Figure 2.9: Over-classification of dung in a mob pasture. Colors represent independent clusters and have no other analytical or classification significance.

Despite these shortcomings, it was obvious from both the non-classified raster image, and the density-based clustering analysis after classification, that the ultra-high stocking density treatment did not lead to consistently dispersed (i.e. more evenly distributed) dung distributions. Figure 2.12 is a good representation of typical dung patterns in the mob. Most often dung was concentrated on the side of the strips nearest the water source; although there were dates where the heaviest clustering occurred in the middle or opposite end of the grazing strip. The 4PR1 treatments also consistently showed clustering, usually in the corners of the paddock. The last date of imagery in 2017 (8/6) surprisingly showed no clustering in the last 4PR1 rotation, however.

We found that Ripley's K was closely-aligned with the density-based clustering results and gave a good indication of how much clustering took place, with the added benefit of assessing how it changed over increasing distances. Figure 2.10 shows the image from August 8, 2017, of the 4PR1 north pasture. The density based cluster analysis found no clustering at the end of this rotation. Ripley's K (Figure 2.11) mirrored this result, showing that clustering was close to what was expected for a random distribution until a search distance of 12 m. was reached, at which point the observed values fell below the expected, indicating a more dispersed pattern. In the north mob treatment from that same date, we find similar good agreement between the cluster map and Ripley's K, with clustering relatively stable across the examined distances (Figures 2.12 and 2.13).

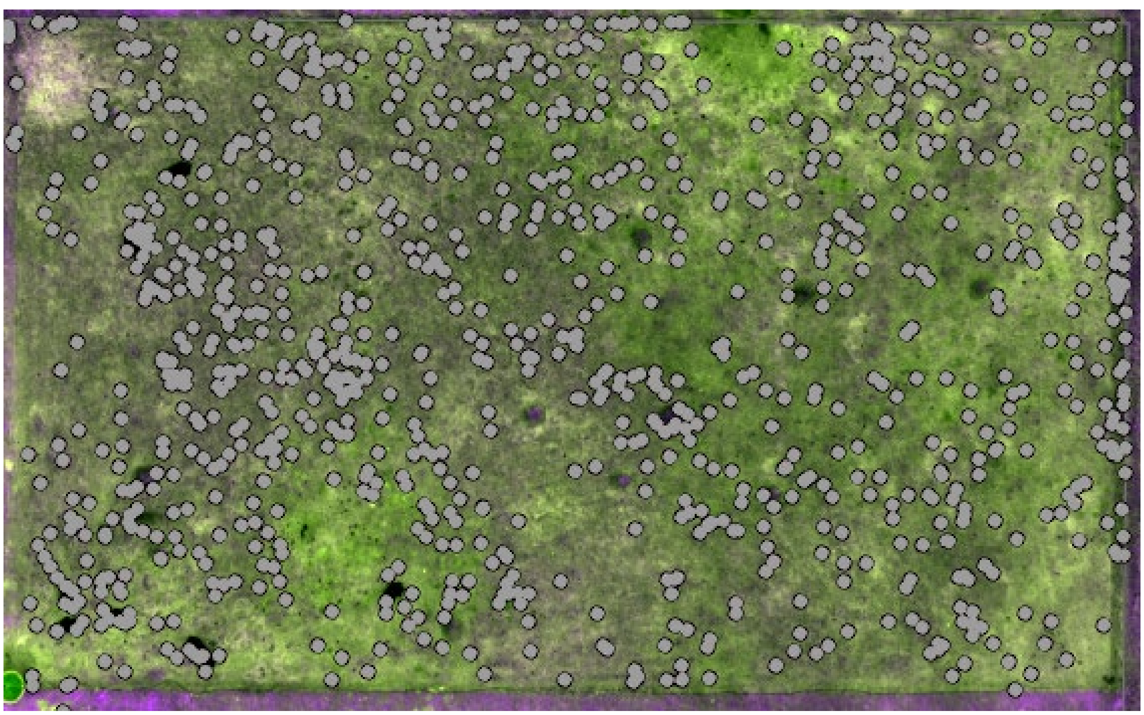


Figure 2.10: Density-based cluster analysis of 4PR1 North from 8/6/2017, showing no statistically-significant clusters (grey dots are dung locations).

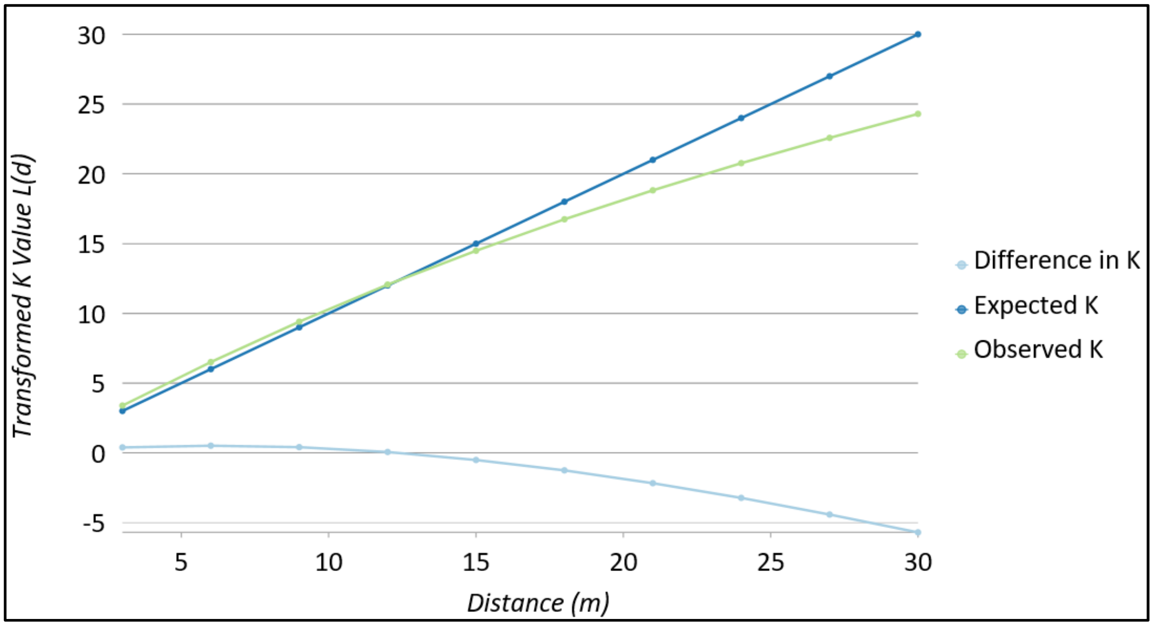


Figure 2.11: K Clustering Analysis for 4PR1 South, 8/6/2017

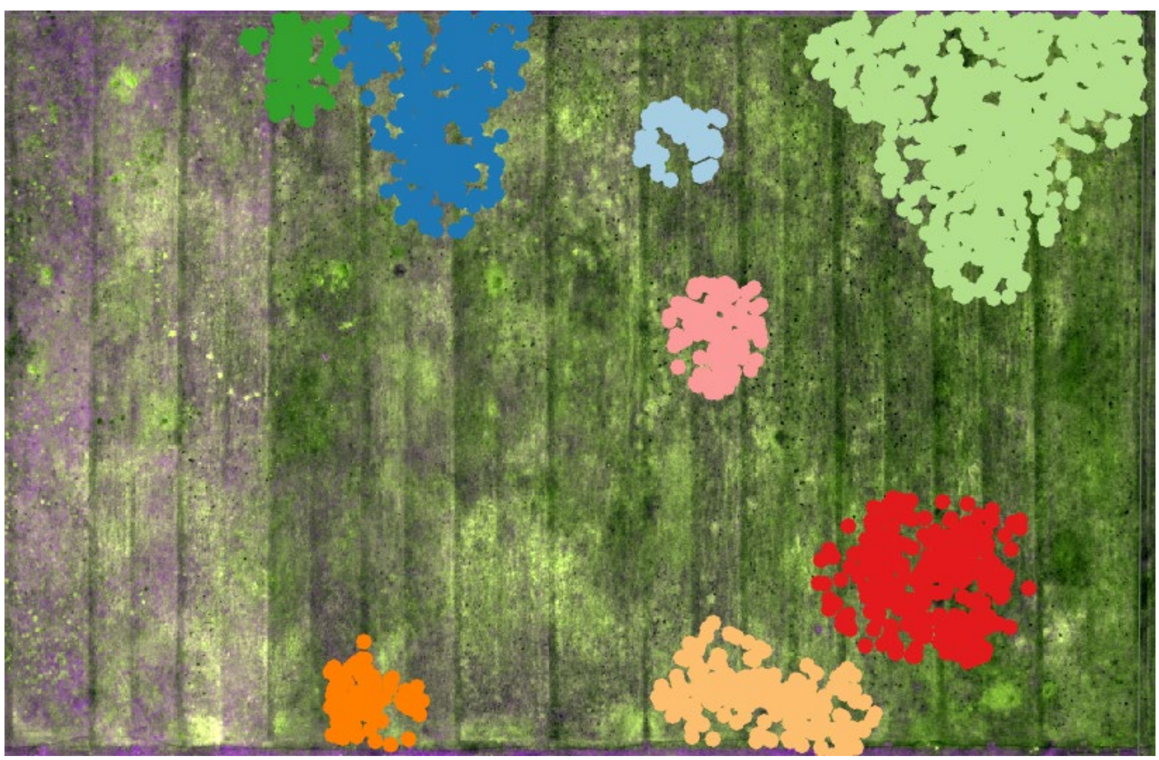


Figure 2.12: Density-based clustering analysis for north mob treatment, 8/6/2017

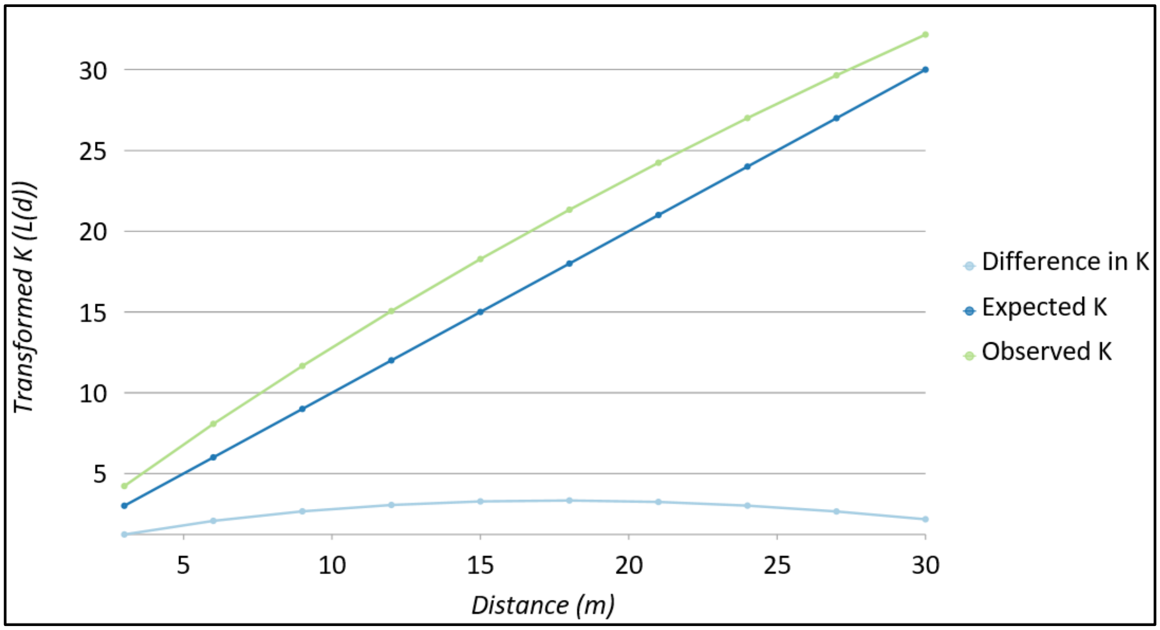


Figure 2.13: K Clustering Analysis for the north mob, 8/6/2017

Discussion

Scale has always been an essential component of both the philosophical and the scientific discussions surrounding the management, ecology, and sustainability of grazinglands (Sayre, 2017; Kothmann et al., 2009). This research is no exception; scale once again must be addressed to answer the most basic questions regarding dung nutrient and organic matter inputs, and what they mean for rangeland health and grazing management. At what scale does clustering become meaningful? At what scale is it no longer meaningful? If our analysis stops at the pasture fence, are we missing landscape-scale patterns that continue on the other side? Oñatibia et al. (2018) highlights the difficulty of disentangling scale (in this case, paddock size) from other factors such as increasing vegetation heterogeneity at larger paddock sizes, stocking density, and watering locations. They also address the non-linearity inherent in grazing effects at different scales. In this research project, there are likely confounding effects of pasture size and shape on dung distribution that make a true, unbiased analysis of dung distribution based on stocking density alone a challenge. Future research should continue to explore the density-dung distribution relationship within the framework of identical pasture sizes and shapes to remove confounding variables (Augustine et al., 2013). Spatial modeling of dung with highly-accurate classification maps and across ranch-scale pasture sizes may be one way to continue to develop more accurate models of dunging and grazing behavior and their effect on nutrient re-distribution patterns. In addition, aerial imagery may help to reveal previously-unnoticed patterns in vegetation and dung distribution at different spatial scales, facilitating the scaling of ecological investigations on rangelands by changing both the grain and extent of our viewpoint (Wiens, 1989).

There is a popular assumption that higher stocking densities lead to more even dung distribution. It is reasoned that more homogeneous vegetation use and higher numbers of cattle in a small space must lead to more even distribution of dung and urine. But as Tate et al. (2003) point out, spatial patterns of vegetation use do not mimic dung distribution patterns, and this assumption ignores basic cattle biology and behavioral science, as well as abundant research on the preferences of cattle for specific lounging and ruminating areas (e.g. near watering points or mineral feeders) that naturally give way to higher densities of dung accumulation over time in certain areas (Augustine et al., 2013; Bailey et al., 1996; Oñatibia and Aguiar, 2018). Our early assessments of dung clustering in two different grazing management strategies show that increasing stocking density does not automatically lead to more even dung distribution, and support previous research findings that the drivers of when and where dung accumulates are both predictable (corners, watering points, shade) and complex (heterogeneity of vegetation and associated quality factors across a pasture, paddock size and shape, etc.). Image analysis and classification of dung presents the opportunity to more objectively study and statistically evaluate these claims and beliefs at scales that are relevant to rangeland management and livestock production (Sayre, 2012).

Perhaps one of the most promising aspects of being able to create spatially-accurate dung maps is the potential for geographical analysis across dates, both within a grazing season and across years. For example, prior to grazing an area, imagery could be collected and classified into vegetation categories, with ground truth validation for species composition and abundance. After cattle are removed from the area, the two imagery layers could be combined to perform spatial regression analysis on the relationship between vegetation type, severity of grazing, and patterns of dung distribution. If this was to be performed over a series of years, deep insight could be gained into how nutrient re-distribution affects grazing preferences and vegetation communities across large areal expanses and at spatial resolutions that heretofore would have been impossible to capture with data collection solely on the ground. Additional map layers with soils information, topography, or other relevant data could be added in to the analysis, as well, to increase the dimensionality of data. Accurate dung maps could guide soil sampling efforts in the field, predict the potential for non-point source pollution of water by manure in susceptible areas when combined with hydrology data (Oliver and Young, 2012; Tate et al., 2000; Vadas et al., 2011), and serve as a locational guide and spatial record-keeping resource for entomologists studying dung-dwelling and dung-feeding insect ecology (Holter, 2016).

There is a large body of work surrounding the impact of grazing on soil properties and the soil microbiological community, but given the complexity of ecosystem variables and processes at play in any given pasture, authors often report that it is challenging or impossible to separate out the most meaningful influences of aboveground drivers, such as dung and urine, trampling, and grazing, on belowground processes (Bardgett and Wardle, 2003; Schrama et al., 2013). Tying specific dung locations and their effects on associated soil microbiological communities and soil physical and chemical properties at a very fine scale may help refine our understanding of these impacts (Ford et al., 2013; Odriozola et al., 2014). This type of layer analysis has been called for in previous research (Auerswald et al., 2010; Tate et al., 2003, 2000) and would be beneficial across a wide range of disciplines and study topics. We also feel that there is the potential to use this imagery to assess trampling patterns and possibly estimate the amount of vegetation (and nutrients) being returned to the soil via trampling pathways. Trampling was evident in our images, but whether or not it would also be evident in other vegetation communities, or at other times of the year, remains questionable. A reliable method for translating the amount of trampling into a measure of biomass would also have to be derived, which may be difficult.

In terms of imagery acquisition, classification, and analysis, subsequent research could address several points that were not able to be rigorously evaluated in this project. More comprehensively assessing the impact of the inclusion of the visible blue band would help determine just how important it is to either use a calculated band or a sensor with a blue band embedded in it. Pixel resolution is another topic that could be explored further, as well. Achieving the highest resolution possible was the aim in this study, but flights over larger pasture areas than those in this study would realistically need to be at higher altitudes to efficiently collect imagery given the constraints of data collection and storage limitations, flight times, and battery life, which would lead to lower pixel resolutions. It is possible that a coarser image (i.e. an image with pixel resolution of 8-9 cm instead of 6-7 cm) may prove to be more accurate by removing finer spatial details that can make classification more challenging for a machine classifier. There are a wide variety of machine learning classification methods that could be applied to this problem, and it is possible that an alternate method such as artificial neural network (ANN) would produce better classification results. Image enhancement techniques, such as band ratioing or smoothing (Jensen, 2010), could prove to be beneficial for the visual identification of dung in an original reflectance raster file, and for analysis purposes, which makes this another area worth exploring.

Ideally, a single algorithm could be developed to apply to any imagery set which would reliably classify dung, regardless of the physical site characteristics. However, after classifying the images used in this research it seems unlikely that this is truly feasible given the huge amount of variation in reflectance values for any given class between image dates, due to both image quality issues that arise during acquisition and processing, and the inherently diverse spectral properties of different soils and vegetation. At present, an individualized, site-specific approach to classifying dung using supervised classification methods via a GIS platform is recommended as the ideal way to assess dung distribution patterns across a variety of ecological sites. If geolocational accuracies of 2-3 cm could be obtained using RTK or PPK technologies, then training and accuracy checking could be much more streamlined and analysis completed more quickly.

However, the additional investment of time and financial resources needed to obtain high-precision imagery would have to be weighed against the goals of each project to determine whether or not it would result in accuracy improvements such that these expenditures would be justified.

Each run of the classifier that involved adding more training samples to a previously-classified image became more accurate, and classification also became more successful and more accurate as analyst experience accumulated, suggesting that expert knowledge and familiarity with both with the study site and the classification process will be an important component of the evolving use of this methodology, as it is in other imagery classification workflows (Arvor et al., 2013; Hoffman, 2018).

To summarize, at present, the major limitations to the application of this technology on a widespread scale are:

1. Limits of spatial and classification accuracies
2. High frequency of data capture required for accurately identifying and classifying dung over time
3. Size of land areas being studied
4. Technology acquisition, accessibility, and learning curve
5. Data storage
6. Ground truth data collection and knowledge of study area
7. Diversity of ecological site characteristics across grazinglands/rangelands, including weather patterns, soil type and moisture, vegetation communities, seasonality of vegetation growth and topography

Conclusion

The results of this research show that using multispectral imagery from a UAV for the identification, mapping, and spatial analysis of dung distribution holds potential to change the scales at which land managers and scientists are able to monitor and analyze a variety of nutrient cycling processes, animal grazing behavior, and landscape-scale ecological interactions. However, classification consistency and accuracy across flight dates and between different pastures in the same image set is, for now, a significant barrier to obtaining a useful data layer to be used in additional spatial analyses. There are also unexplored questions regarding the proposed methods' widespread applicability on different rangeland and pasture types in different climates, as well as which spectral bands are optimal to use for identifying dung and discriminating it from soil and vegetation.

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CHAPTER 3:

TEMPORAL CHANGES IN THE NUTRIENT CONTENT OF CATTLE DUNG IN A GRAZED ECOSYSTEM

Abstract

Dung excreted by cattle on extensively-managed grasslands comprises a substantial proportion of the nutrient inputs available to these grazed ecosystems, and the influx of nutrients and organic matter associated with dung can have profound effects on the aboveground and belowground plant, insect, and microbial communities. Although manure has been analyzed extensively for its nutrient content, relatively little work has been performed on dung *in situ* after excretion and without human manipulation prior to analysis. In this study, we analyzed over 200 dung pats (1-24 days old) that were collected from a Nebraska Sandhills meadow over two grazing seasons and determined percent dry matter (DM), water-extractable nitrogen (WEN), water-extractable organic carbon (WEOC) and water-extractable phosphorus (WEP). In addition, we investigated the effects that freezing samples at -20°C prior to analysis had on the subsequent analyses of nutrient content. WEN and WEOC both followed exponential decay curves of nutrient loss over time and were modeled as a function of age. WEN concentrations ranged from a mean high of 1.20 g kg^{-1} at three days of age, to a low of 0.252 g kg^{-1} at 24 days. The highest WEOC values were also found at one day of age, 19.25 g kg^{-1}, with a low of 2.86 g kg^{-1} in 14-day-old dung. WEP either remained relatively constant or increased slightly by 24 days of age, and percent dry matter along with sample WEOC concentration were stronger determinants of WEP than age alone. Highest mean WEP was 3.24 g kg^{-1} at 12 days and lowest was 1.28 g kg^{-1} in one-day-old dung. Split plot analysis of the effects of

sample age and date of harvest showed age was significant for all analytes and DM, but date of harvest and interaction of date and age were not consistently significant across analytes. Freezing samples prior to analysis increased WEN and WEOC 37% - 124% compared to the same samples analyzed fresh, but WEP responded inconsistently across sample age groups. WEN and WEOC had a linear response to freezing based on fresh values (R^2 = 0.67 and 0.71, respectively). Frozen WEP was not linearly related to fresh WEP, but the addition of DM and age to the linear model resulted in R^2 of 0.59.

Introduction

Knowledge 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 systems (Bardgett and Wardle, 2003; Haynes and Williams, 1993; Lovell and Jarvis, 1996). This knowledge is also foundational for estimating 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 community composition that stem from heterogeneity of vegetation use and dung deposition can reveal landscape-scale processes that impact ecosystem functioning (Aarons et al., 2004b; During et al., 1973; Gillet et al., 2010; Piñeiro et al., 2010).

One of the challenges of conducting research on dung nutrient dynamics and dung decomposition is that both are highly site-specific and dependent upon several controlling factors (Eghball et al., 2002; Holter, 2016, 1979; Mohr, 1943). Dung nutrient content and decomposition can be affected by livestock diet (Sørensen et al., 2003), animal age and size, animal species, time of year (Cardoso et al., 2019), absence or presence of dung beetle 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 other sites.

Compounding these issues is that dung is not commonly studied in the pasture where it was deposited, absent of human manipulation. Instead, researchers have relied on the creation of artificial dung pats from bulk manure collected in holding areas, or have harvested dung off of pastures, and then reformed the pat into a particular size, shape, or weight to facilitate controlled, long-term monitoring of changes (Aarons et al., 2004b, 2004a; During et al., 1973; Evans et al., 2019; Holter and Hendriksen, 1988; Lovell and Jarvis, 1996; Schick et al., 2019; Weeda, 1977). While these techniques are useful for providing insight on a range of dung-related processes that might otherwise be impossible to study (Bol et al., 2000; Dickinson and Craig, 1990), it does raise the question of how accurately nutrient content and decomposition of dung *in situ* is reflected by these studies, given what we know about the influence of dung moisture and nutrients, animal diet, and wider ecological context (e.g. dung beetle and earthworm assemblages) on both. For example, Weeda (1967) found that the disappearance of dung was determined by two major factors: hard crust formation on the top of the dung and the consistency of dung. Both of these factors could be altered by cattle diet, the manipulation of dung in the field or by the bulk homogenization of dung from holding areas (Eghball et al., 2002). Subsequently, changes in the consistency and moisture content of dung, in comparison to unaltered dung, may lead to either increased or restricted decomposition activity by the microbial population, and by macrofauna such as dung beetles and earthworms, both of which are major contributors to the disappearance of dung and its incorporation in soils (Nichols et al., 2008).

If rates of decomposition change, this may affect the microbial dynamics that determine mineralization or immobilization rates of nutrients due to a relative increase or decrease in the flow of available nutrients (Dao and Schwartz, 2010; Sitters and Olde Venterink, 2018). This subsequently affects the long-term retention of nutrients in soils, and their availability to plants (Aarons et al., 2009; Yamada et al., 2007; Yoshitake et al.,

2014) and may change the potential for groundwater contamination by nutrients such as nitrate and dissolved reactive phosphorus (DRP) (Kleinman et al., 2017, 2005) .

Laboratory analyses of manure 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. Multiple studies have evaluated the effect of freezing and drying of samples prior to analysis on water-extractable phosphorus (WEP); (Studnicka et al., 2011; Vadas and Kleinman, 2006), and found significant differences in concentrations. Studnicka et al. (2011) found that freezing manure prior to analysis consistently raised WEP levels compared to fresh sample analysis. However, to our knowledge, there have been no studies directed at evaluating whether or not this freeze-thaw event after dung pat harvest may affect the results of other nutrient concentrations, such as carbon and nitrogen. There is ample evidence that freezing may change soil nutrient availability and chemical form (Freppaz et al., 2007; Song et al., 2017; Xu et al., 2016), therefore it is hypothesized that freezing manure before analysis may also bring about physical and chemical changes in the manure (for example, see Chen et al., 2019). If so, this could lead to assumptions about the amounts and forms of nutrients that are present in dung in a field setting, and thus lead to inaccurate predictions of the availability and loss of nutrients from within a given site.

The first objective of this research was to evaluate changes in dung nutrients (nitrogen, phosphorus and carbon) over time when dung is left on pasture, unaltered, after being excreted by grazing cattle and prior to collection for analysis. To our knowledge, there are no existing studies that have evaluated such a large number of dung pats in the field (n=244), across study years (2), sampling dates (8), and ages (1 day to 24 days).

Therefore, this work presents an important body of knowledge that can contribute to our understanding of how dung nutrient levels change over time in a grazed ecosystem.

The second study objective was to compare water-extractable nutrient concentrations in fresh vs. frozen dung. We hypothesized that concentrations of dung nutrients would be altered by differences in sample storage prior to analysis, specifically, whether samples were analyzed fresh from the field or after being frozen and then thawed. To test this hypothesis we analyzed fresh dung pats that had never been frozen, then re-analyzed the same samples after a period of freezing, and compared nutrient values from both types of samples.

Methods and Materials

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) that investigated the effects of different grazing strategies on animal performance, vegetation characteristics, and soil properties (Shropshire, 2018). The pastures were located on a subirrigated meadow site with a seasonally high water table. These wet, interdune areas are characteristic of the Sandhills region and 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 a lesser occurrence of warm season grasses and forbs. Soils at this site are sandy to fine sandy loam in texture and classified as mixed, mesic Aquic Ustipsamments. Average summer temperatures range from 21°C to 25°C and average yearly precipitation (over the past 20 years) at the site is 665 mm with approximately 40% of the yearly total falling during the summer months of June-August (High Plains Regional Climate Center, 2018).

Study context

Grazing treatments at the research site began annually in early June, when steers were moved to the ranch, and concluded in early August, when they were removed from the site. In this treatment, 32-36 yearling steers (depending on the year) were grazed on 0.06 ha strips (dimensions of 5.8 m x 98.8 m), giving a stocking density of 225,000 kg live weight ha^{-1}. Moves to new strips occurred twice per day, at approximately 7 am and 3 pm. When cattle were moved in the morning, they no longer had access to the pasture they had been in the day before, but afternoon moves allowed the use of both the morning strip and the new afternoon strip, until the next morning. 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 pats after the cattle had left that pasture.

Dung collection and processing

Dung was collected in June and July of both 2016 and 2017 (Table 1). Dung was analyzed for nutrient content across a 24-day age range. In addition, a separate set of twenty-five pats was followed from one to twenty-four days of age, with each pat being sampled three times over this period.

During the first two collection periods in 2016 (June 25 and June 26), dung was collected from every age group between one and fourteen days of age (i.e. fourteen different age groups, one for each 24-hour period of time of deposition). However, on each subsequent sampling date in both 2016 and 2017, dung was collected only from grazing strips containing pats that were 1, 3, 5, 7, 10 and 14 days old. This change allowed a greater number of dung pats to be sampled from evenly-spaced age groups across a fourteen-day window, as opposed to fewer pats from every single day. For purposes of statistical analysis, pats from these first two sampling dates were grouped with dung samples in the age category closest to its own age from the consistently sampled age groups (i.e. 1, 3, 5, 7, 10, 12 and 14 days). Sample dung pats were randomly chosen across each pasture, and only intact pats that had not been stepped on or laid on were used. Sub-samples were collected from near the middle of the dung pat, taking care to avoid the drier, thinner edges. 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.

Table 3.1: Summary of the total number of dung pats sampled on each harvest date. Numbers in each cell are the number of dung pats sampled on a given date in a particular age group.

	2016				2017				
Dung Age (days)	**25-Jun**	**26-Jun**	**26-Jul**	**30-Jul**	**29-Jun**	**8-Jul**	**22-Jul**	**23-Jul**	**Total**
1		4	5	5	25 (LTNM)	10		10	59 (34)*
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 (LTNM)		4	45 (20)*
12	10		5						15
14	10		5			10		10	35
24							25 (LTNM)		25 (0)*
								TOTAL:	294 (219)

*Totals in parentheses exclude LTNM data, which was analyzed separately
LTNM=long-term nutrient management study (same 25 pats sampled 3 times in 24 days)

In 2017, in addition to the random sampling described above, another set of dung pats was added for a second study (hereafter referred to as the "LTNM" study). These pats were identified and marked at 24 hours of age, sampled, and then re-sampled at 10 days of age and 24 days of age. Physical samples from these dung pats were also taken from as near to the center of the pat as was possible, given repeated sampling of the same pat.

After samples were brought to the lab, a sub-sample was weighed and then dried at 60°C for 48 hours to determine moisture and dry matter content. In 2016, the remaining sample (not dried) was immediately frozen and stored at -20°C until nutrient analysis took place in spring, 2017. These samples were thawed overnight prior to the start of extractions. In 2017, fresh dung samples were kept chilled in a refrigerator for 24-48 hours until analysis began; therefore, this dung was not frozen prior to analysis. In order to evaluate the effect(s) of the freeze/thaw cycle on nutrient analysis results, these samples were then frozen at -20°C. They were then thawed and re-analyzed in the spring of 2018 using the same methods as the two previous sets of analyses.

Laboratory analyses

Dung samples were analyzed for water-extractable phosphorus (WEP), water-extractable nitrogen (WEN) and water-extractable organic carbon (WEOC) 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 then filtered through Fisherbrand P2 (particle size retention 1-5 μm) filter paper and refrigerated until analyses took place. The WEP was determined using the molybdate method of Murphy and Riley (1962), at 880 nm on a Thermo Scientific Genesys 10S VIS Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). The WEN and WEOC were analyzed on an OI Analytical Aurora 1030C TOC Combustion Analyzer with an OI Analytical 1088 Rotary TOC Autosampler and TNb module for total nitrogen (OI Analytical, College Station, TX, U.S.A.).

Statistical Design and Analysis

Data from randomly-sampled pats of different ages during 2016 and 2017 were analyzed as a split-plot experimental design. Date of harvest was the whole-plot factor and age of dung was the split-plot factor. Year (2016, 2017) was considered a random factor in the analysis. Data from the LTNM project were analyzed separately with a repeated measures experimental design, where each identified pat was considered its own block and each nutrient being analyzed was the repeated measures factor in each type of analysis. All nutrient analysis results are reported on a dry matter basis.

All analyses were performed in the statistical software package R (R Core Team, 2019) and figures were created with ggplot2 (Wickham, 2016). Models of exponential decay were created using the base R function “nls”, with a self-starting model, “SSasymp”, available in the “stats” package.

Results

Change in dung nutrients over time

The means and standard deviations of dung nutrient concentrations for each age group in both 2016 and 2017, as well as the LTNM study, are shown in Table 3.2. Figures 3.1 to 3.4 give more detailed information regarding the distribution of nutrient concentrations and dry matter content for each sampling date and dung age combination. Data from the LTNM study are not included in these graphs and are shown separately in Figure 3.5. Our findings were consistent with many other studies of dung nutrient composition that have shown wide variation in nutrient concentrations, even when samples are taken from cattle of the same ages and weights, with the same diet. For example, at one day of age, dung was found to contain anywhere from 1.3 to 2.8 g kg^{-1} of WEP and 9.4 to 19.2 g kg^{-1} WEOC. Levels of all of the nutrients fell over time, except for phosphorus. In both 2016 and 2017, WEP sharply rose between days 10 and 14. However, in the LTNM study, WEP values fell consistently from day 1 to day 24. Means of the variances tended to decrease over time (i.e. with increasing age) for WEOC and WEN; however, WEP variance increased over time. Despite the means of variance generally decreasing as dung aged, the distributions of the variances resisted clustering and were often dispersed across a wide range of values.

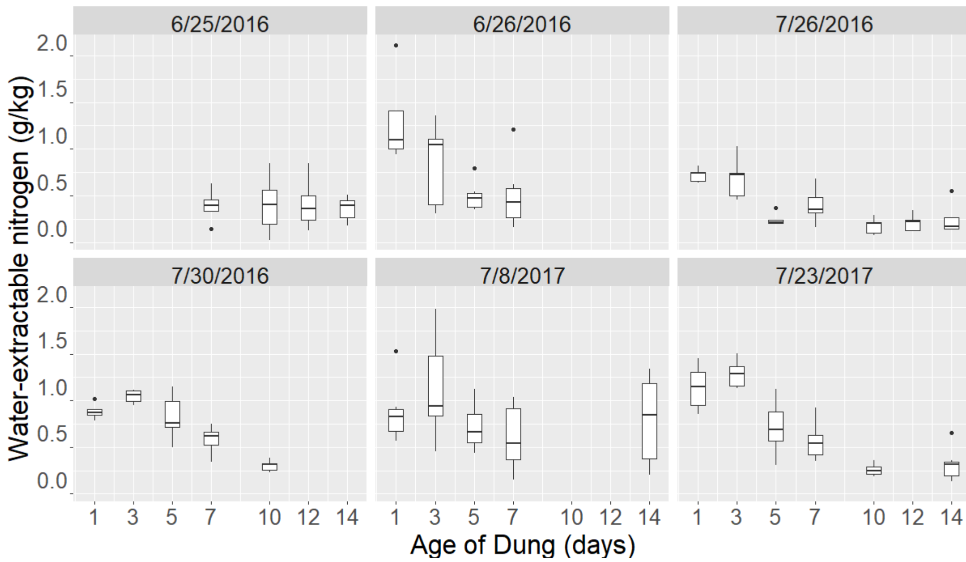


Figure 3.1. Water-extractable nitrogen (g kg^{-1}) in dung across sampling dates and dung ages

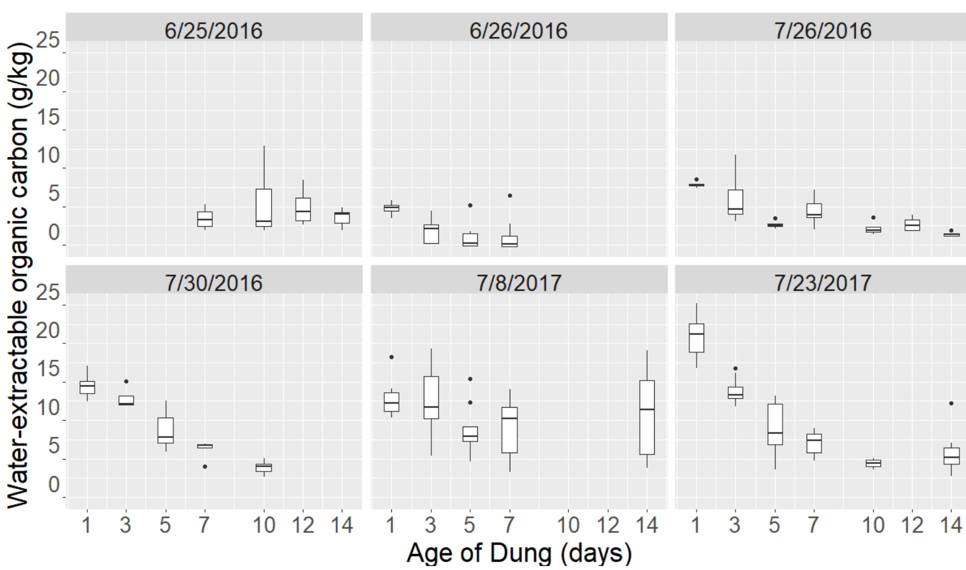


Figure 3.2. Water-extractable organic carbon (g kg^{-1}) in dung across sampling dates and dung ages

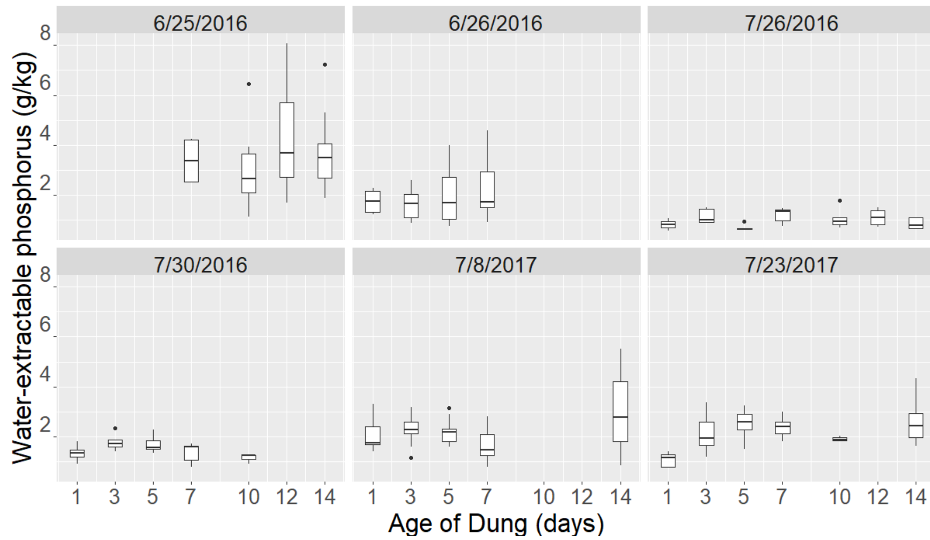


Figure 3.3. Water-extractable phosphorus (g kg^{-1}) in dung across sampling dates and dung ages

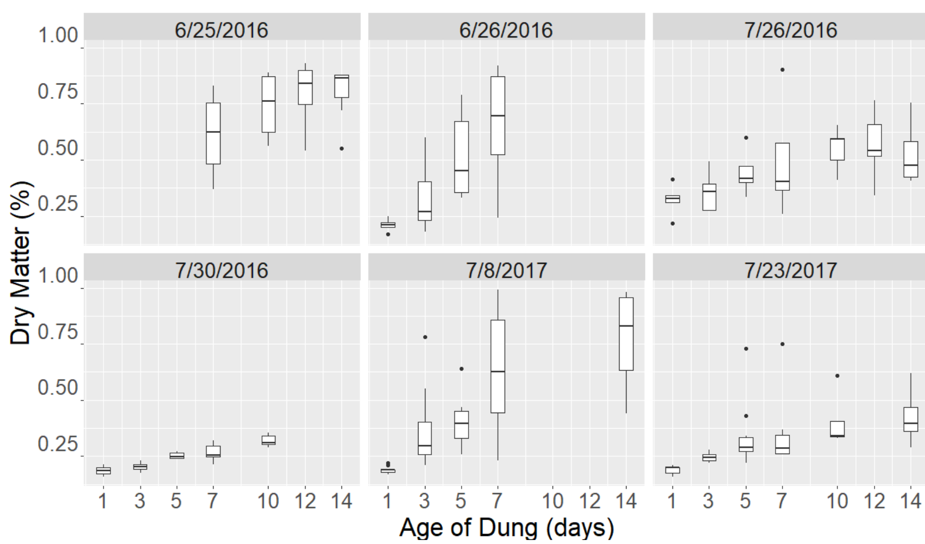
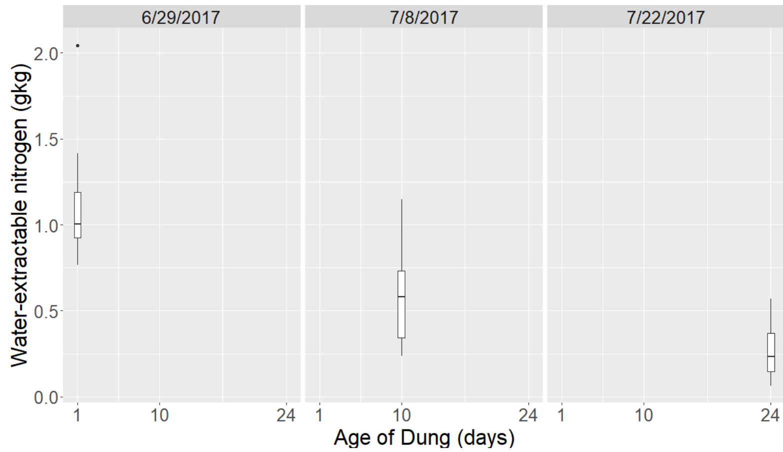
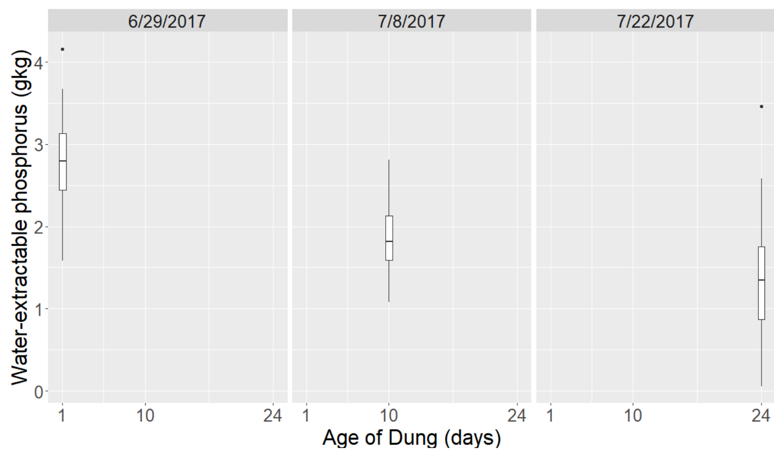


Figure 3.4. Percent dry matter content of dung across sampling dates and dung ages

a.



b.



c.

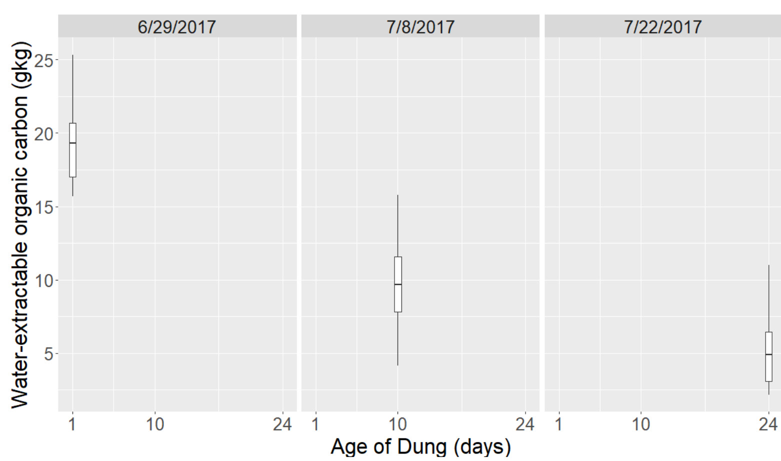


Figure 3.5. Concentrations of WEN (a), WEP (b), and WEOC (c) (g kg^{-1}) at the three sampling dates of the LTNM study.

Table 3.2. Means and standard deviations of nutrient and dry matter analyses across years and for the LTNM study. WEP: water-extractable phosphorus; WEOC: water-extractable organic carbon; WEN: water-extractable nitrogen; DM: dry matter

	2016						
Analyte	1 d.	3 d.	5 d.	7 d.	10 d.	12 d.	14 d.
WEP (g kg^{-1})	1.281 ± 0.51	1.543 ± 0.53	1.510 ± 0.95	2.004 ± 1.14	1.873 ± 1.50	3.236 ± 2.30	2.792 ± 1.89
WEOC(g kg^{-1})	9.352 ± 4.33	6.324 ± 5.21	4.013 ± 3.83	3.463 ± 2.68	3.882 ± 2.97	4.162 ± 2.04	2.857 ± 1.37
WEN (g kg^{-1})	0.949 ± 0.37	0.848 ± 0.32	0.521 ± 0.29	0.469 ± 0.25	0.300 ± 0.21	0.344 ± 0.22	0.329 ± 0.14
DM (%)	0.242 ± 0.08	0.302 ± 0.12	0.411 ± 0.17	0.528 ± 0.25	0.550 ± 0.21	0.720 ± 0.178	0.717 ± 0.180

	2017					
Analyte	1 d.	3 d.	5 d.	7 d.	10 d.	14 d.
WEP (g kg^{-1})	1.569 ± 0.7	2.181 ± 0.65	2.386 ± 0.53	1.997 ± 0.63	1.920 ± 0.10	2.786 ± 1.21
WEOC (g kg^{-1})	16.918 ± 4.98	13.183 ± 3.32	8.869 ± 3.07	8.099 ± 2.98	4.399 ± 0.677	8.341 ± 5.09
WEN (g kg^{-1})	0.992 ± 0.28	1.199 ± 0.34	0.728 ± 0.24	0.590 ± 0.26	0.260 ± 0.075	0.545 ± 0.40
DM (%)	0.191 ± 0.016	0.304 ± 0.138	0.372 ± 0.131	0.492 ± 0.26	0.405 ± 0.137	0.607 ± 0.24

	LTNM		
Analyte	1 d.	10 d.	24 d.
WEP (g kg^{-1})	2.797 ± .59	1.907 ± 0.47	1.392 ± 0.71
WEOC (g kg^{-1})	19.246 ± 2.73	9.262 ± 3.18	5.165 ± 2.38
WEN (g kg^{-1})	1.067 ± 0.27	0.569 ± 0.24	0.252 ± 0.15
DM (%)	0.180 ± 0.02	0.722 ± 0.19	0.616 ± 0.11

Models of nutrient change over time

Model creation was attempted using only non-LTNM data and again using all data, including the LTNM (Figures 3.6-3.8). However, because there were so few sampling dates (n=3) for the LTNM data, a model could not be created from this data alone, so all resulting models are based on the combined data. R^2 values are not given for model evaluation purposes due to the problematic nature of using R^2 in conjunction with nonlinear models (Spiess and Neumeyer, 2010).

Both WEN and WEOC were modeled using exponential decay functions with all statistically significant parameters, either at $P < 0.001$ or $P < 0.05$ (Figures 3.6 and 3.7). However, WEP showed little to no mean response across time, and it did not conform to the same exponential decay model as WEN and WEOC. Unlike with WEN and WEOC, age was not a significant predictor of WEP concentration ($P = 0.99$). Figures 3.8 and 3.9 show the change in mean WEP over dung ages in the complete data set (3.8) and without the LTNM data (3.9) for comparison with the WEN and WEOC data.

Percent dry matter and WEOC were strong predictors of WEP. Figure 3.10 gives the model and shows the relationship between percent dry matter and WEP, with the trend line present for visual pattern detection only, not as a representation of the actual model. In this figure WEOC concentration is mapped to the data points as a continuous color scale in order to better visualize how WEOC interacts with WEP and DM. Although both WEOC and DM were highly statistically significant ($P < 0.001$)

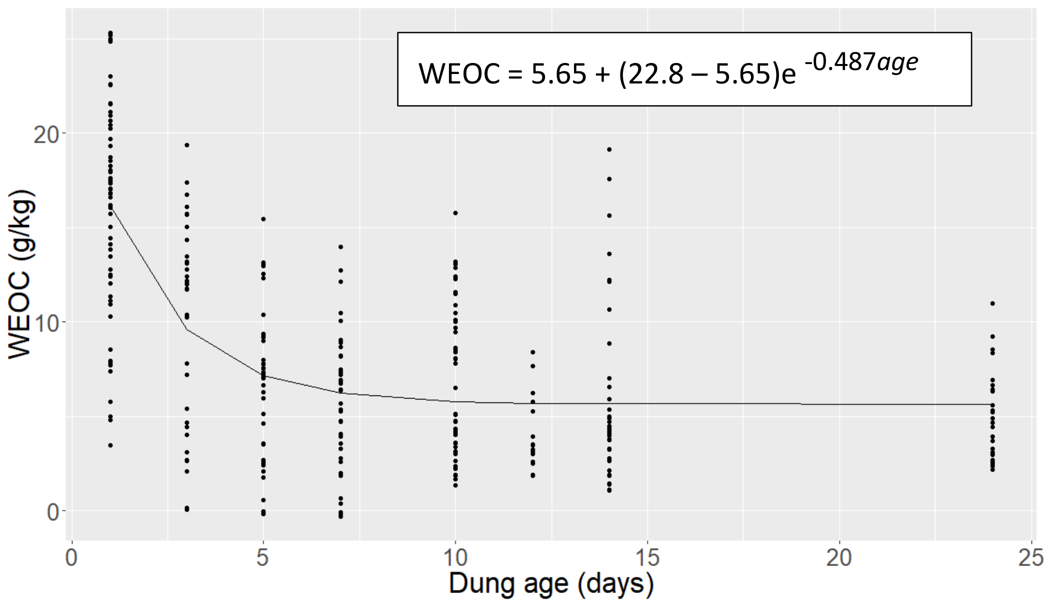


Figure 3.6. Exponential decay model for WEOC over 24 days as a function of dung age

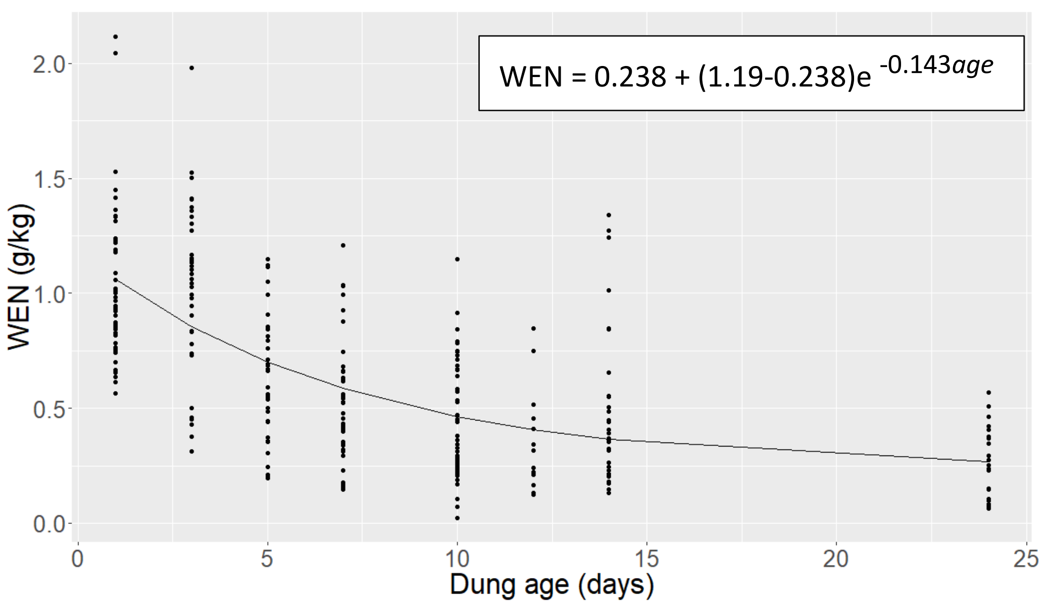


Figure 3.7. Exponential decay model for WEN over 24 days as a function of dung age

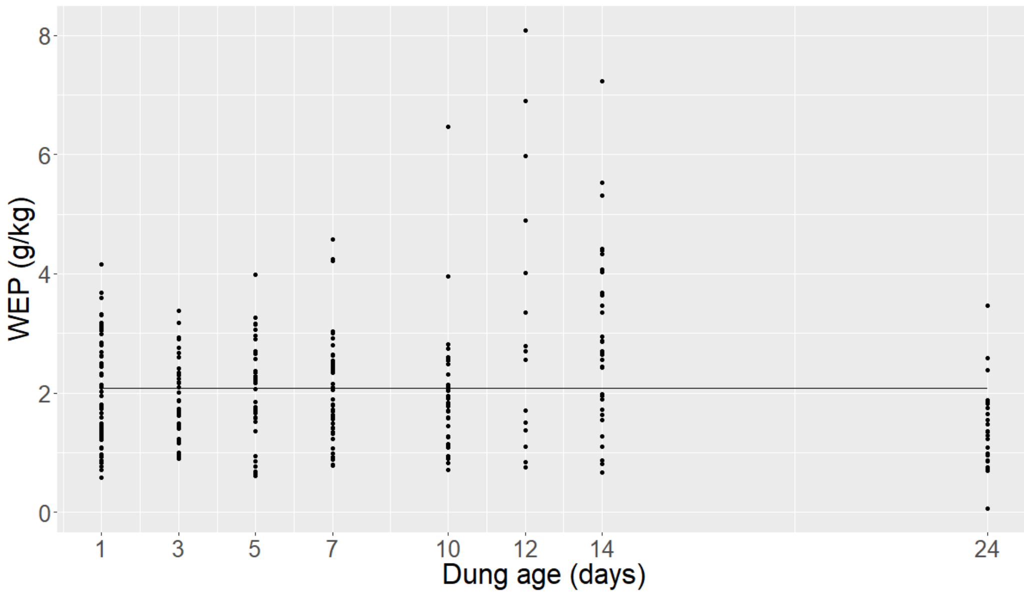


Figure 3.8. Change in water-extractable phosphorus (WEP) in relation to dung age in combined 2016 and 2017 data, including LTNM samples

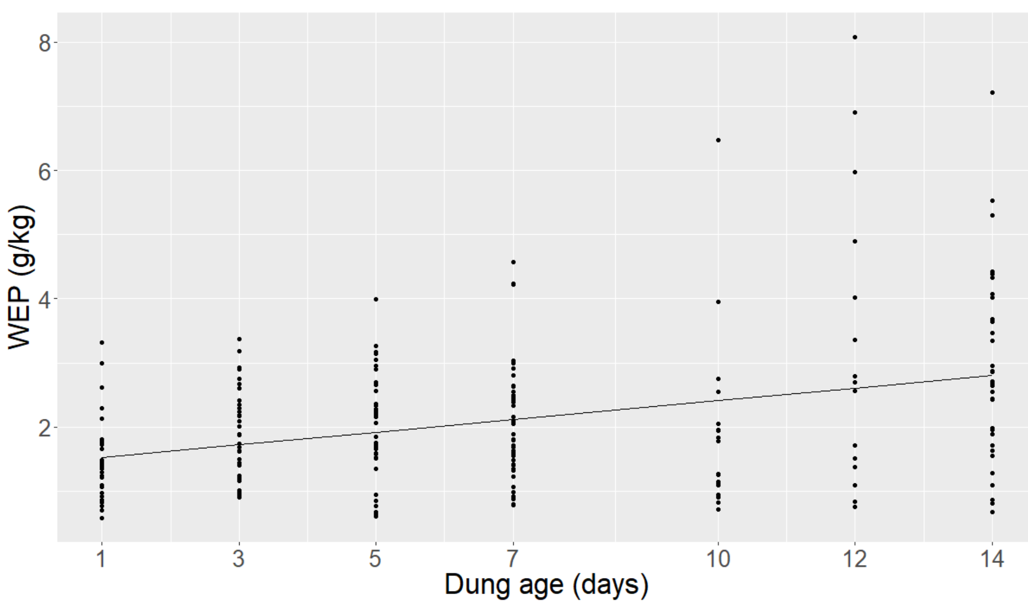


Figure 3.9. Change in water-extractable phosphorus (WEP) in relation to dung age in 2016 and 2017 data, without LTNM samples

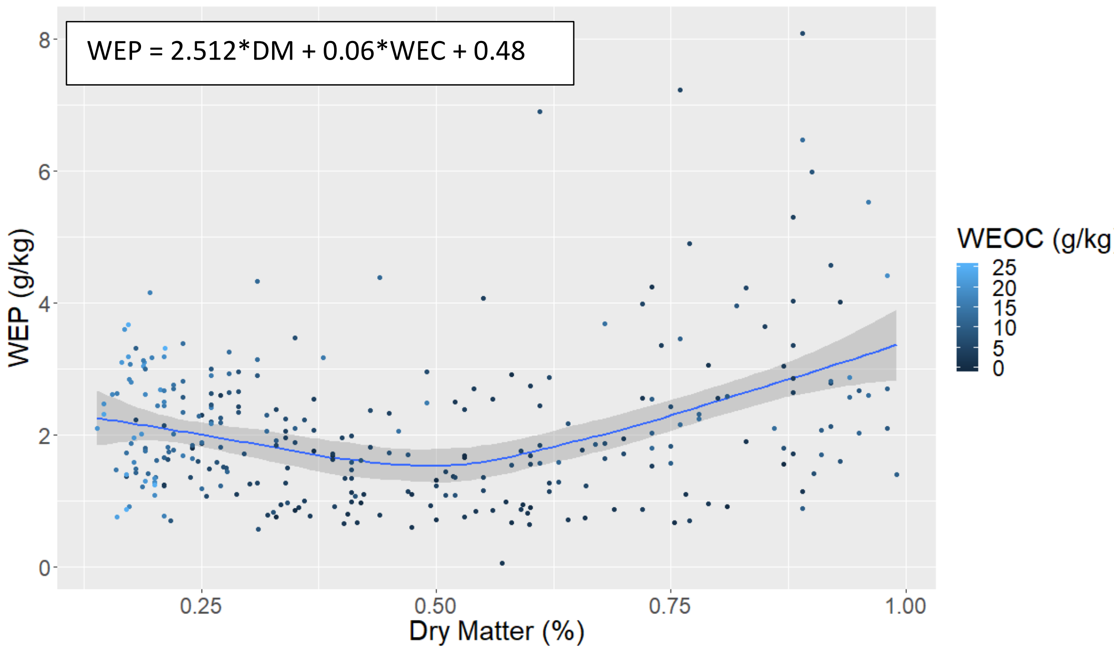


Figure 3.10. Relationship of water-extractable phosphorus (WEP) to percent dry matter. Water-extractable organic carbon concentrations for each sample are indicated by the color scale given on the right. The trend line is shown for visualization purposes only and does not represent the model equation.

in both the complete data set and that without the LTNM samples, R^2 was still very low: 0.16 with the LTNM data and 0.21 without it.

The WEN model that contained LTNM data was nearly identical to the one without, with an only slightly lower standard error of regression (0.296 vs. 0.316) and very similar Akaike Information Criterion (AIC) scores of 123 and 121, respectively. ANOVA showed no significant difference between the models ($P = 0.99$). Although the WEOC models with and without LTNM data had larger differences in AIC (1719 vs. 1291, respectively), ANOVA also showed no statistically significant difference between the two models ($P = 0.80$).

Split-plot ANOVA

Analysis of the effects of both dung age and the date of harvest, with year included in the model as a random effect, are shown in Table 3.3.

Table 3.3. Statistical F values and their corresponding P > F values of the fixed effects of age, sampling date, and age-sampling date interaction on dung water-extractable P (WEP), water-extractable organic carbon (WEOC), water-extractable N (WEN) and percent dry matter (DM).

Analyte	Age	Date	Age*Date
WEP (g/kg)	3.25**	22.88*	N/S
WEOC (g/kg)	33.70***	22.61***	5.60***
WEN (g/kg)	25.19***	N/S	N/S
DM (%)	19.33***	20.33***	2.27**

* $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$; N/S = not statistically significant

The variable "date" not only serves as a whole plot factor for the actual date of collection, but could also be used as a proxy for location, since different ages of dung were gathered from different areas of the grazing study, which spanned two, 6.48 ha treatment replications. However, specific harvest locations within the two replications were not recorded, so analyzing the effects of dung location separate from date of harvest was not possible in this study.

IV. Differences between fresh and frozen sample nutrient analyses in 2017

There were statistically significant differences between all but two of the age-nutrient pairings. WEOC values increased after freezing by 3.4 to 6.8 g kg^{-1} (37% - 98%), and WEN increased by 0.26 to 0.8 g kg^{-1} (37% - 123%). However, WEP only increased on two out of 7 dates and decreased by 0.8% to 65% on all other dates.

Table 3.4. Change in mean dung nutrient concentrations between dung samples analyzed fresh and dung samples analyzed after freezing.

Dung Age (days)	Fresh WEP (g/kg)	Frozen WEP (g/kg)	Change (g/kg)	Percent change	Fresh WEN (g/kg)	Frozen WEN (g/kg)	Change (g/kg)	Percent change	Fresh WEC (g/kg)	Frozen WEC (g/kg)	Change (g/kg)	Percent change
1	2.251	0.785	1.466	-65.12%	1.034	1.845	0.812	78.51%	18.211	25.012	6.800	37.34%
3	2.181	1.539	0.642	-29.42%	1.199	1.643	0.444	37.03%	13.183	18.263	5.080	38.53%
5	2.386	1.580	0.806	-33.77%	0.728	1.132	0.403	55.36%	8.869	12.526	3.657	41.23%
7	1.997	1.851	0.145	* -7.28%	0.590	0.949	0.358	60.75%	8.099	12.037	3.938	48.63%
10	1.908	2.394	0.485	25.43%	0.554	0.906	0.352	63.55%	9.042	14.241	5.199	57.50%
14	2.786	2.764	0.022	* -0.78%	0.545	0.808	0.263	48.24%	8.341	11.776	3.435	41.17%
24	1.392	3.004	1.612	115.79%	0.252	0.563	0.312	123.71%	5.165	10.267	5.102	98.77%

*All differences in means significant at $P < 0.01$ except for * cells

The difference (percent change) between fresh and frozen samples did not appear to follow a clear or consistent trend between ages of dung, although all three nutrients increased dramatically in the 24-day frozen samples than in the other age groups.

The change in values between fresh and frozen analyses for both WEOC and WEN followed a linear trend (Figures 3.9 and 3.10). Conversely, WEP frozen values were not linearly related to fresh values alone, and there was no significant correlation between the two (Figure 3.11). However, the addition of percent dry matter 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.

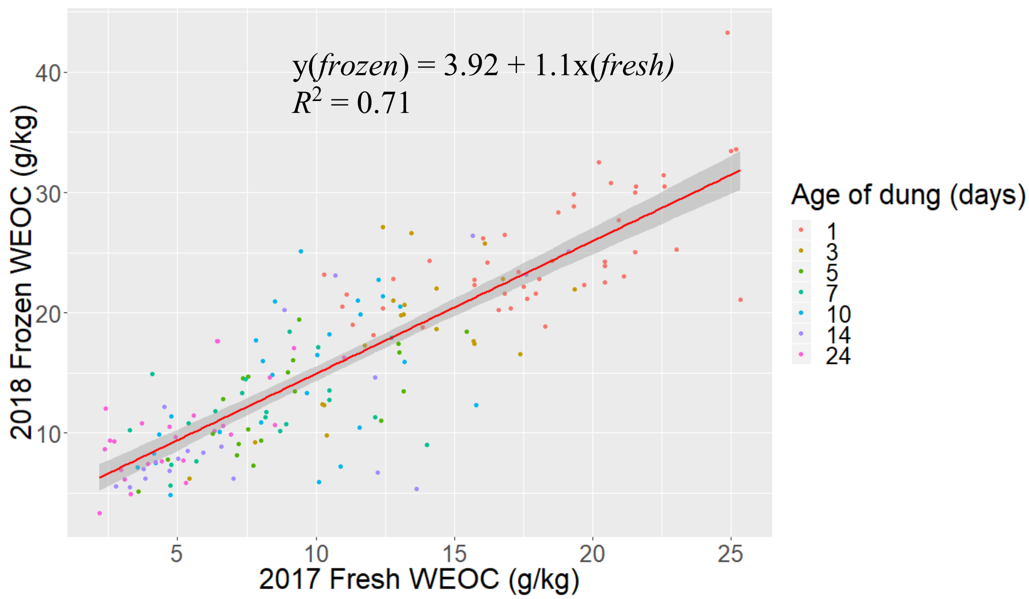


Figure 3.11. Change in water-extractable organic carbon (WEOC) between samples analyzed fresh and the same samples analyzed after freezing

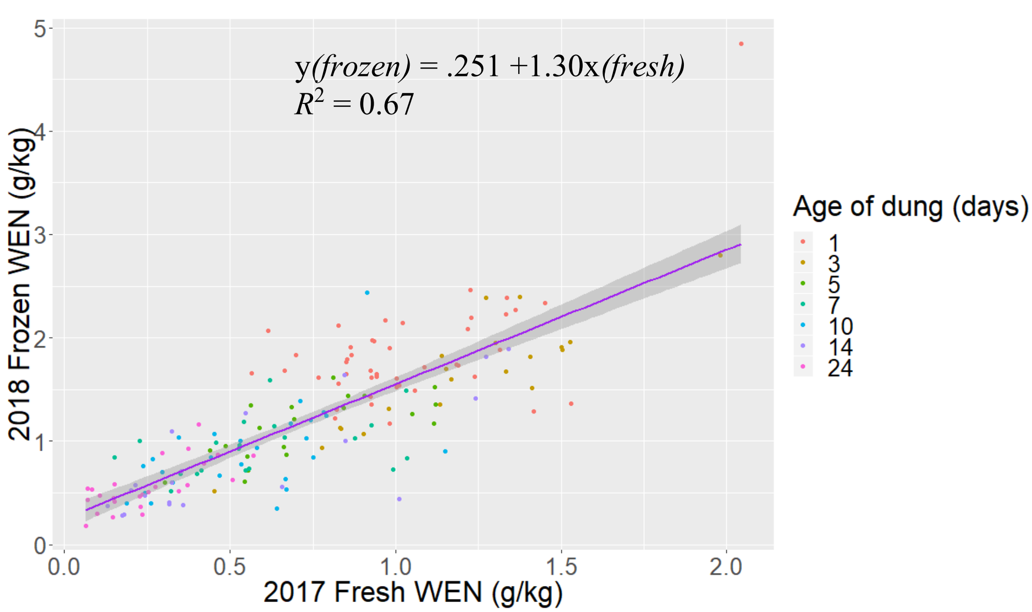


Figure 3.12. Change in water-extractable nitrogen (WEN) between samples analyzed fresh and the same samples analyzed after freezing

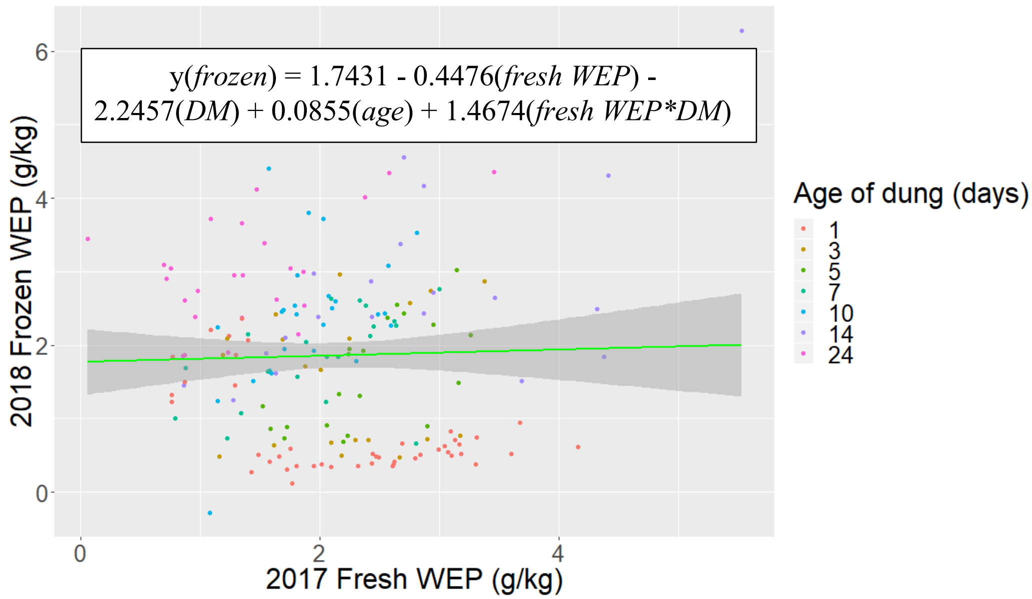


Figure 3.13. Change in water-extractable phosphorus (WEP) between samples analyzed fresh and the same samples analyzed after freezing. The equation given is the statistical model using fresh dung WEP value (fresh WEP), percent dry matter (DM), and dung age in days (age) that yielded an R^2 of 0.59.

Discussion

In this study, WEN and WEOC were lost from dung pats at a rate consistent with an exponential decay function. This pattern mirrors other research results that have shown similar outcomes when monitoring dung pats over time (Aarons et al., 2004a; Dickinson and Craig, 1990; Evans et al., 2019). 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 loss of dung WEP, WEN, and WEOC over time. In 2016, the loss of WEOC and WEN were at 70% and 65%, respectively, by 14 days of age. In the Evans et al. (2019) study, WEOC and WEN had decreased by only 25% and 42%, respectively, which was closer to our findings from

2017, when losses were only at 50% and 45% for WEOC and WEN. However, there was a pronounced spike in both WEOC and WEN values in the day 14 data (see Appendix A, Figures A.2 and A.3), and data from this sampling day has a wide range of values with a number of large outliers that would have skewed the mean upwards (Figures 3.1 and 3.2). This may have been due to chance via random selection of dung pats, or it is possible that the vegetation in the grazing strip where the day 14 data was gathered differed significantly in quality from other areas of the pasture and caused mean values to rise in this dung. If rate of loss is instead based on day 10 data, the 2016 and 2017 data show remarkably similar loss rates of 70 and 73% for WEOC and 65 and 73% for WEN. The LTNM nutrient loss on day 10 (no day 14 values collected) was lower, at 52% for WEOC and 46% for WEN.

The changes in WEP were inconsistent across dung ages, with poor model prediction based on age alone. In both the 2016 and 2017 data, WEP rose consistently across time, more than doubling in mean concentration in 2016 and almost doubling in 2017. However, in the LTNM data from 2017, WEP maintained a consistent downward trend across the 24 days the dung was monitored. There are several possible explanations for this discrepancy in findings. First, because the same 25 pats were monitored over time in the LTNM study, concentrations of WEP would have been similar and more correlated than those of randomly selected pats in each age class. This may have better modeled WEP dynamics across time. Second, there were no data for the time period between day 10 and day 24 for the LTNM experiment. In both 2016 and 2017, a pronounced spike in values occurred in WEP concentrations between days 10 and 14.

The Evans et al. (2019) study also showed a spike in WEP values at day 14 before they began decreasing again. It is possible that if data had been collected at day 14 for the LTNM study there would have also been a spike in values, prior to a decline as age increased. Interestingly, all three datasets had nearly the exact same mean WEP value at day 10, despite having drastically different initial values ranging from 1.3 to 2.8 g kg^{-1} at one day of age.

An increase in WEP across time would not be unusual in dung, as organic P is converted to inorganic P after deposition and may accumulate in the pat prior to microbial utilization or physical removal from the pat through the actions of dung beetles or earthworms (Aarons et al., 2009, 2004b; Holter and Hendriksen, 1988). Aarons (2004b) found only moderate decreases in phosphorus over 40 days, and Dao and Schwartz (2010) found an increase in inorganic P over time in suspensions of manure sourced from dairy cattle. Although that study showed a correlation of organic P transformation to inorganic P with increasing C:P levels, no such relationship or explanatory effect was found in this study.

The comparison between fresh and frozen sample nutrient concentrations showed that there is a linear relationship between fresh and frozen values for WEN, WEP, and WEOC. For WEOC (R^2 = 0.71) and WEN (R^2 = 0.67) this relationship was simple, with frozen values being predicted solely from fresh values. Neither age nor percent dry matter were significant when added to these models. Phosphorus results were not as consistent, and there was not a simple linear relationship between fresh and frozen values. However, when both age and percent dry matter were added to the model, R^2

rose to 0.59. It appears that older dung (with higher DM values) followed a more predictable response of increased WEP amounts (Figure 3.11) with age. It is also evident that WEP in fresher dung (ages 1 day to 7 days) is less-responsive to freezing than dung aged 10-24 days of age, as demonstrated by the samples from 1-day-old dung. This complex relationship between dry matter, age, and WEP needs further investigation in order to better understand how these variables are driving the response in WEP values.

The results from the study of fresh and frozen samples confirmed our hypothesis that changes would occur due to the freezing and thawing process. This relationship should continue to be investigated, 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 comprises a large proportion of the nutrient inputs for crop production.

One unanticipated result of the freezing study was the response of carbon and nitrogen, both individually and coupled, as shown by the water-extractable C:N ratio. As expected, this ratio rose as dung aged and dry matter increased, while moisture decreased. This is exemplified in the LTNM data, where C:N ratios were at 18.4, 16.9 and 21.8 at 1 day, 10 days and 24 days of age, respectively. However, after freezing, the mean C:N ratios decreased at each time period, to 14.2, 15.8 and 17.8. The decrease in C:N ratio was the result of larger increases in the amounts of WEN between fresh and frozen analyses, although WEOC also consistently increased, as well. In the 24-day-old dung pats, WEOC increased by 119% and WEN increased by 179%. At 10 days, WEOC increased by 30% and WEN 70%, and in the initial sampling at 1 day of age, WEOC increased by 28.8% and WEN by only 32.3%, leading to the rather narrow change in C:N ratio compared to the other two dates.

Studies of the effects of freeze-thaw cycles on soil nutrient content have consistently shown increases in dissolved organic nitrogen (DON), dissolved organic carbon (DOC), and dissolved organic matter (Freppaz et al., 2007; Xu et al., 2016). In a meta-analysis of the effects of freeze-thaw cycles on soil carbon and nitrogen, Song et al. (2017) showed that across nearly 50 studies, DON and DOC increased, on average, 27.5% and 37.3% after being subjected to freezing temperatures. This finding is consistent with our own, 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 labile nitrogen and carbon compounds. 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 strength of this analysis lies in the fact that for the randomly sampled dung pats (i.e. not the LTNM data), a large number of samples were able to be analyzed in order to help minimize the effects of high rates of variance between samples at any given age. In addition, data were gathered from dung pats which had not been physically altered in any way until the sample was taken. This means that decomposition processes were able to proceed without interference, theoretically leading to a more accurate reflection of nutrient dynamics at a certain dung age within a given pat (however, we are not aware of any research that either supports or refutes this claim). The weakness of this analysis is that for each date a new set of samples was gathered, which means that the initial concentrations of any given nutrient in a particular dung pat spanned a wide range of values which may have differed greatly from the starting points of the dung pats used in the analyses of other days.

Conversely, data from the LTNM study showed changes across time within each dung pat, which can more accurately assess change in nutrient content with age based on any given starting point. However, this comes at the cost of disturbing the pat via removing a sample for each sampling date, potentially altering the decomposition process by removing organic matter and nutrients, as well as potentially increasing the surface area of the dung if a hole is left behind after removing a sample. If samples are taken from the middle of the pat, a greater amount of surface area is exposed which may change evaporation of moisture and/or volatilization rates, or may affect the use of dung by important arthropod and earthworm decomposers. If samples are taken from the edges of the pat, it is likely that the dung will be thinner and drier in these areas, and thus less-representative of what is happening in the rest of the pat.

There is often a substantial crust that forms over the top of the dung within a short timeframe after deposition (24-48 hours, depending on weather conditions). Over time, if the pat remains undisturbed, the difference in moisture content between the exterior and interior of the pat widens. In addition, it is also likely that two very different sets of processes are taking place due to temperature and moisture differences between the drier exterior and the moist interior. As observed by Holter and Hendriksen (1988), and MacDiarmid and Watkin (1972), it appears that decomposition proceeds by physical removal and consumption of dung organic matter from below the pat, where moisture and temperature levels remain ideal for the microbial, insect, and arthropod communities to access nutrients. At the same time, the crust prevents access from the top of the pat and may slow release of gaseous compounds (e.g. NH_3, N_2O, CO_2) from within the pat. The crust also prevents rainfall from entering the pat and contributing to its disintegration, as it effectively sheds water once sealed over (Dickinson and Craig, 1990; MacDiarmid and Watkin, 1972; Weeda, 1967).

This segregation between layers of dung may complicate the outcome of laboratory analyses due to different moisture contents and, potentially, different nutrient contents between the two. However, to our knowledge, there has been no research conducted that characterizes these differences. In order to investigate this further, a small set of sub-samples was taken from one of the pastures used in this study. Four dung pats, 22 days old, were selected and from each pat a portion of the dry crust was taken and a sample of the still-wet interior was also gathered. Results of the nutrient and moisture analyses are shown in Table 3.5. Although the sample size is too small for a robust statistical analysis, it is informative to see the stark contrast in nutrient concentrations between the crust and the interior. The disparity in values was maintained even after freezing for WEN and WEOC. WEP increased dramatically, but the differences that existed in the fresh data were evened out post-freezing between wet and dry samples.

Table 3.5. Difference in nutrients and dry matter between dung crust and the interior at 22 days of age. All nutrient values are given in g $kg^{-1}$and were calculated on a dry matter basis.

Sample	%DM	Fresh WEP	Frozen WEP	Fresh WEN	Frozen WEN	Fresh WEOC	Frozen WEOC
1_wet	0.24	1.591	5.451	0.272	0.841	4.398	10.941
1_dry	0.74	2.034	5.408	0.598	1.042	8.777	14.61
2_wet	0.24	1.219	3.755	0.221	0.551	4.112	6.704
2_dry	0.61	1.338	3.785	0.38	1.045	6.978	15.741
3_wet	0.31	1.078	4.529	0.131	0.4	2.895	7.105
3_dry	0.74	1.744	4.731	0.335	1.056	6.156	17.192
4_wet	0.27	0.797	4.31	0.145	0.462	2.843	6.374
4_dry	0.73	2.688	4.252	0.518	0.864	8.383	14.168

Though these preliminary data provide only a small glimpse of what is taking place in the crust and interior of cattle dung as it ages, it is an important component of the whole nutrient-cycling picture to know that nutrients are concentrated in the crust of dung. It is possible that this crust then becomes a long-term reservoir of organic matter-associated nutrients that are less easily-accessible than the moist interior is immediately, but which serves as a pathway to long-term organic matter accumulation in grazed pastures and a stable sequestration pathway for nitrogen, phosphorus, and carbon (During et al., 1973; During and Weeda, 1973). Others have reported on the increased release of labile C and N in dry soils as compared to wet soils (Haney et al., 2012), and it is possible that this same mechanism is at work here.

Because WEP, WEN and WEOC account for the most labile, easily-accessible nutrients for microbes, future research should incorporate additional analyses such as total N and total C to understand more fully how these different fractions change over time. It has been shown that WEOC makes up approximately 1-2% of total carbon in manure (Katoh et al., 2015) and WEP makes up approximately 25-40% of total manure P (Dao and Schwartz, 2010). The proportion of total nitrogen which is extractable as WEN or water-extractable organic nitrogen (WEON) is somewhat more difficult to determine from the existing scientific literature, but Reeves and Van Kessel (2002) show that about 40-50% of total manure N is in an organic form, although this may be greater in solid dung as opposed to slurry or semi-solid manures where NH_4 is often found in higher proportions (He, 2013). WEN may include both inorganic N (NO_3, NH_4) and organic nitrogen forms, and there appears to be scant research devoted to determining WEN in total as a proportion of total N. Determining these ratios in dung deposited directly on pasture may provide additional insight into the different pathways and residence times that dung pat layers and their associated nutrients have in an ecological system. In addition, monitoring the change in microbial C and N, as well as biomass, would further illuminate intra-pat ecological dynamics of microbial substrate use and perhaps provide better insight into how the microbial community shifts over time. It is plausible that dung microbial communities undergo similar dynamics as soil microbial communities, and there is a shift to K-strategist dominance after r-strategists have utilized all easily-available nutrients in a fresh pat (Kuzyakov and Blagodatskaya, 2015; Rinkes et al., 2014). Whether these functional groups arrive *in situ* with the pat, or migrate from the soil upwards, and how the two communities interact, is still relatively unknown and deserves additional attention, as well.

One of the objectives of this study was to be able to qualify whether or not the nutrient contents and the progression of their change over time was impacted by artificial dung pat creation, but to our knowledge there is only one other study which measured this same suite of water-extractable nutrients and used pre-formed dung pats. We have drawn some comparisons and contrasts with the Evans et al. (2019) study throughout the paper, but given the wide variation in dung nutrient values, making any definitive statements regarding how artificial vs. natural dung pats behave over time would be shortsighted. The question remains an important one, however, and it would be a benefit to have additional studies that compared the two methods at the same pasture location, using the same analysis techniques, so that the only variable was whether the dung pat had been artificially-created from bulk feedlot manure, or had been deposited on pasture directly from a grazing animal.

Conclusion

Our findings from the analyses of over 240 individual dung pats across two different years and seven age groups mirror findings from other studies that have evaluated the change in nutrients over different ages in dung in several important ways. First, we found that phosphorus did not behave like nitrogen and carbon and either shows an inconsistent pattern of depletion over time, or actually increases in concentration as dung ages. We showed that carbon and nitrogen follow an exponential decay model of loss over time, although there exists a tremendous amount of variation in their concentrations between individual samples at each sampling date.

We have also contributed new information to the study of nutrient cycling in grazed ecosystems by documenting significant changes in the concentration of WEN and WEOC, and sometimes WEP, in dung samples that have been frozen prior to analysis, when compared to their levels in the same fresh samples. In a preliminary exploration of the differences in nutrient contents between dry crusts of dung pats and the still-moist interiors, we found that nutrients are concentrated in the dry crust and hypothesized that this 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 and 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. Future research should also seek to expand upon this work by isolating the impacts of vegetation quality and pasture location from larger whole plot or blocking factors such as date of collection and weather variables. Cattle dung is and will continue to be an essential part of soil fertility in both pasture ecosystems and in cultivated agroecosystems, 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.

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APPENDIX A

Additional Graphs Related to Dung Nutrient Dynamics

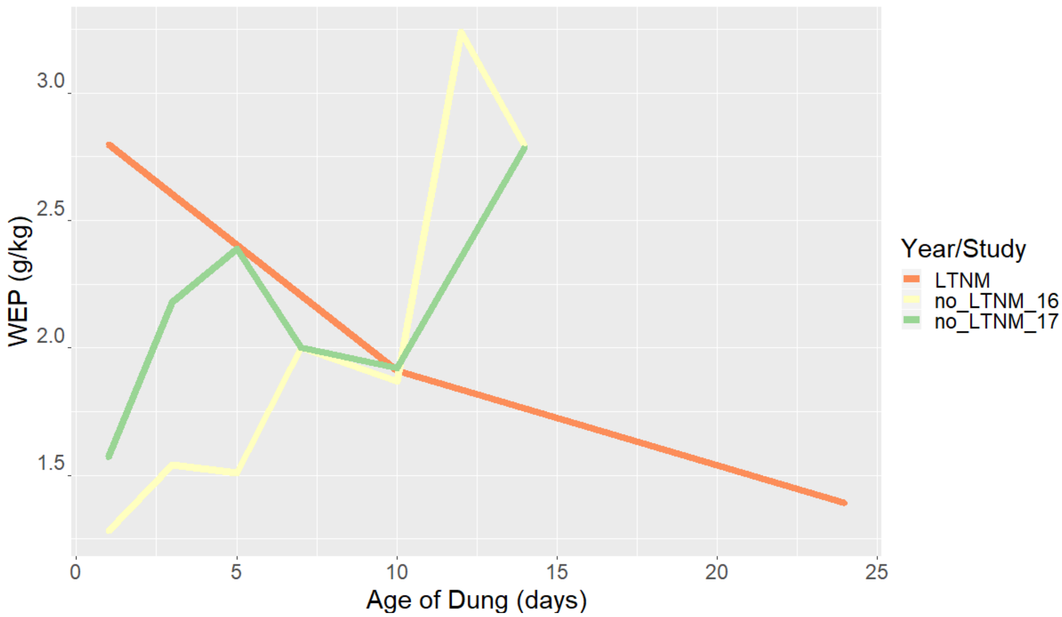

Figure A.1: Comparison of the mean values of WEP concentration between the Long Term Nutrient Management study (LTNM), 2016 samples (no_LTNM_16), and 2017 samples (no_LTNM_17)

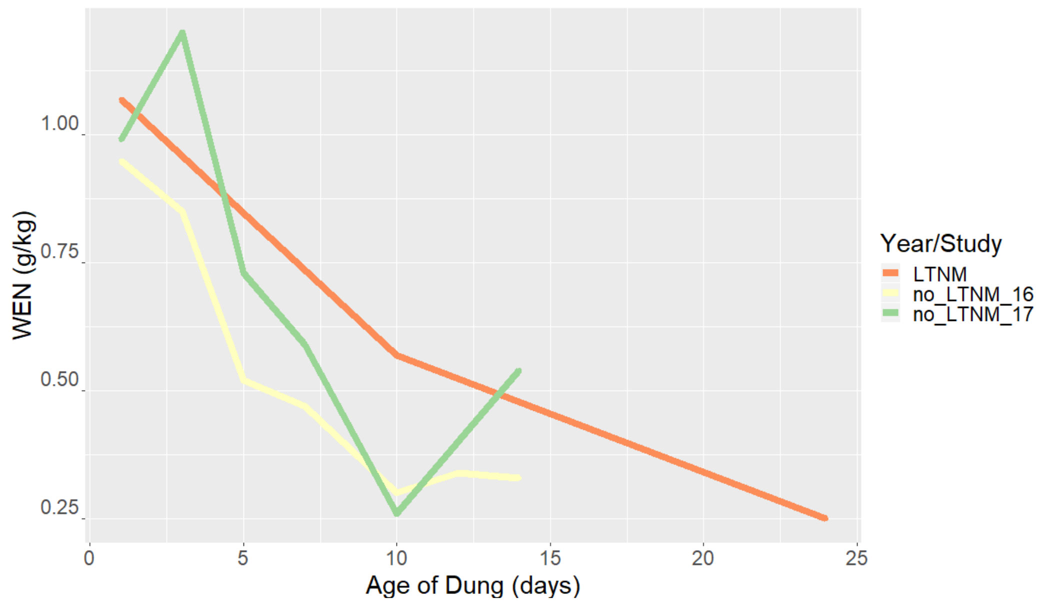

Figure A.2. Comparison of the mean values of WEN concentration between the Long Term Nutrient Management study (LTNM), 2016 samples (no_LTNM_16), and 2017 samples (no_LTNM_17)

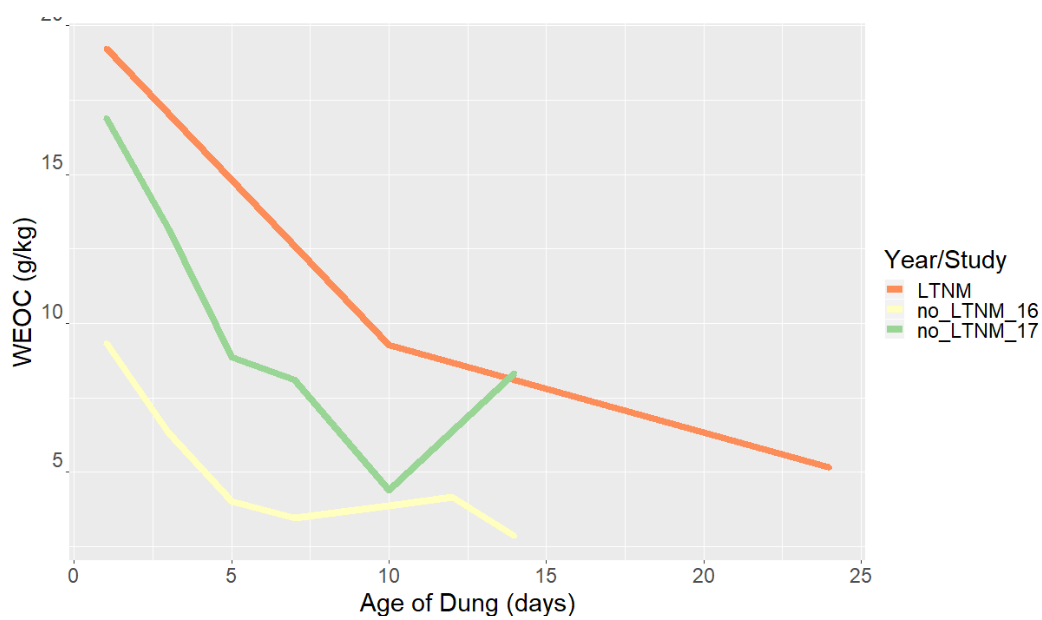

Figure A.3. Comparison of the mean values of WEOC concentration between the Long Term Nutrient Management study (LTNM), 2016 samples (no_LTNM_16), and 2017 samples (no_LTNM_17)

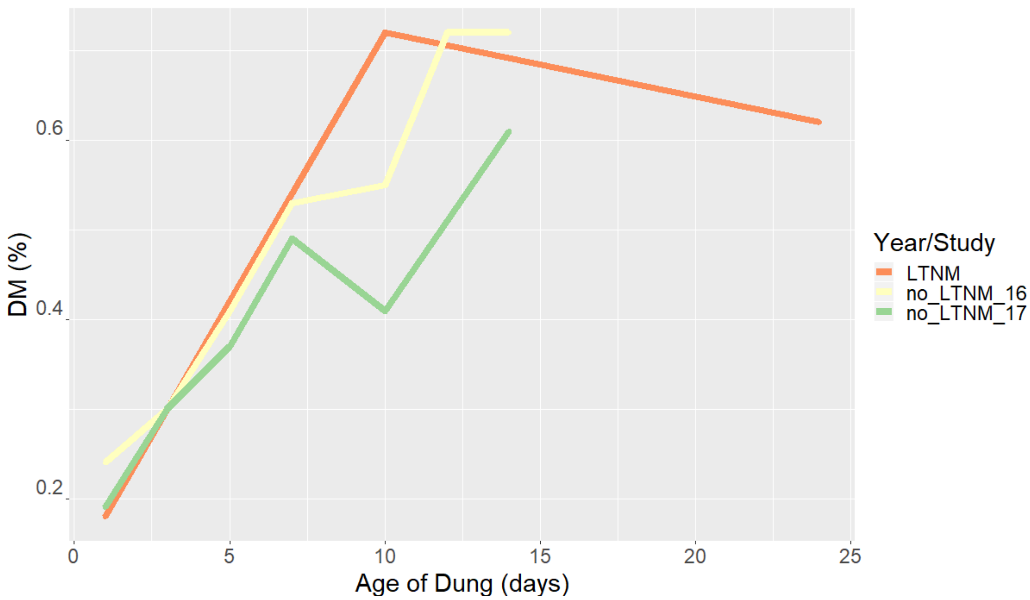

Figure A.4. Comparison of dry matter content (%) between the Long Term Nutrient Management study (LTNM), 2016 samples (no_LTNM_16), and 2017 samples (no_LTNM_17)

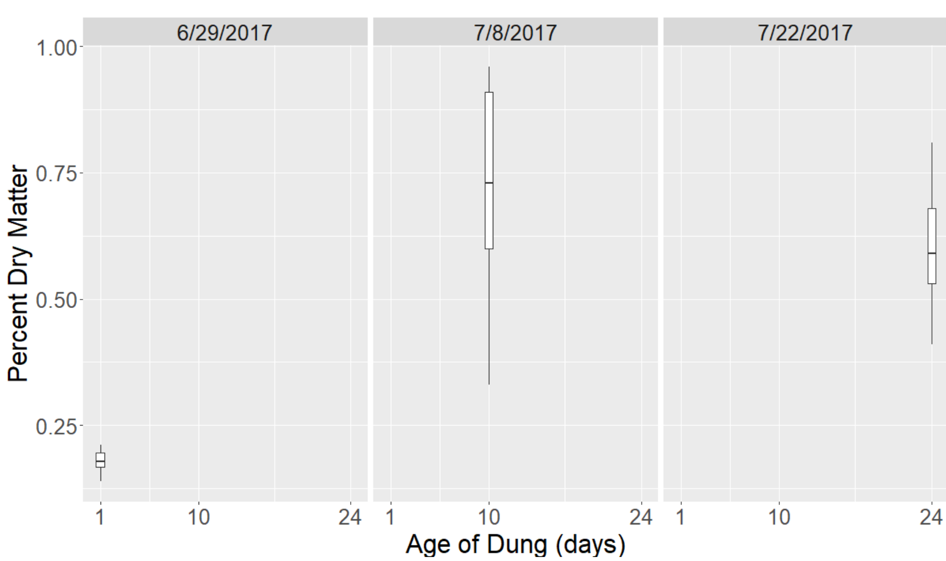

Figure A.5. Dry matter content in the LTNM study over sampling dates and dung ages