12-2016

EFFECT OF SMOOTH BROMEGRASS MANAGEMENT ON GRAZING CATTLE DUNG DECOMPOSITION, CO_2 FLUX, AND SOIL NUTRIENT MOVEMENT

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by

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A THESIS

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Master of Science

Major: Agronomy

Under the Supervision of Associate Professor John A. Guretzky

And Professor Walter H. Schacht

Lincoln, Nebraska

December, 2016

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University of Nebraska, 2016

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Rapid nutrient cycling improves forage quality and livestock production in pastures. Interseeding legumes may be a strategy to enhance N cycling, but effects of dung excreted from cattle grazing pastures with legumes on dung decomposition rates and soil N cycling have not been studied. Our objective was to evaluate how dung excreted from cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass (*Bromus inermis* Leyss.) pastures affects dung chemical composition, dry matter decomposition, CO_2 flux, and N availability in soil. Freshly deposited dung from yearling steers grazing legume-interseeded, N-fertilized, and unfertilized pastures was collected and placed as pats in a neighboring pasture where experiments were established to evaluate effects of year (2014 and 2015), season (June and August), cattle diet, and time after dung placement (3, 7, and 30 days) on dung [dry matter, C, N, water extractable C (WEC), and water extractable N (WEN) contents] and soil (WEC, WEN, NH_4-N, and NO_3-N) characteristics. Across the experiments, these characteristics were often found to depend on year, season, and diet interactions, but overall, dung from legume-interseeded pastures had greater N content, WEC, and WEN, and lower C:N ratio

than dung from N-fertilized and unfertilized pastures. Dung from legume-interseeded pastures also decomposed faster and had more CO_2 flux than dung from unfertilized pastures, but showed no differences with that from N-fertilized pastures. Soil nutrient movement was not affected by cattle diet, but may have been limited by the time of dung placement and the depth of soil analyzed. Legume interseeding distinguish itself as a positive component of pasture management with an improved potential for dung decomposition and soil nutrient movement because of nutrient rich dung.

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CHAPTER 1. LITERATURE REVIEW

Introduction

Nutrient cycling is a key process governing grassland production (Russelle, 1992). Grazing by large herbivores accelerates nutrient cycling relative to ungrazed grasslands by returning nutrients through dung and urine deposition (Russelle, 1992; McNaughton et al., 1997). More rapid N cycling through the soil-plant-animal continuum increases soil N availability, forage quality and yields, and animal gains and production (Ledgard, 2001). In the north central U.S., management strategies such as N fertilization have been deployed historically to increase N cycling and improve performance of beef cattle grazing smooth bromegrass (*Bromus inermis* Leyss.) pastures (Greenquist et al., 2009) yet in many instances these strategies remain unprofitable (Watson et al., 2012). The objective of this chapter is to review the scientific literature that discusses: *(i)* the importance and distribution of smooth bromegrass as a perennial pasture grass; *(ii)* developmental morphology of smooth bromegrass and its response to changing soil and environmental conditions; *(iii)* different management strategies for optimizing forage production and quality in smooth bromegrass pastures; and *(iv)* importance of dung return, composition, and decomposition on N cycling and how it varies with pasture management strategies.

Importance and Distribution of Smooth Bromegrass

Smooth bromegrass is a perennial cool-season grass dominant throughout pastures in the north central USA and in the south central provinces of Canada. Planting in these areas began in the late 1880s with introduction of European and Western Asian cultivars. Smooth bromegrass remains the most dominant cool-season grass in pastures of these areas due to high palatability for all livestock classes, excellent competitiveness, and high forage and livestock production (Vogel et al., 1996; Stubbendieck et al., 1997; Otfinowski et al., 2007). It has been used effectively in grazing and haying operations, as well as for soil conservation and erosion control purposes (Vogel et al., 1996; Casler, 2004; Otfinowski et al., 2007). Research in south central Alberta has indicated that it is better for haying than pasture compared to meadow bromegrass (*Bromus riparius* Rhem.) (Van Esbroeck et al., 1995). Interest in smooth bromegrass as a forage in the United States increased after it showed superior drought survival during the 1930s. Smooth bromegrass can survive extreme winter temperatures down to -28°C, allowing it to survive above 40°N latitude (Limin and Fowler, 1987; Vogel et al., 1996; Casler et al., 2000). Smooth bromegrass has been intensely studied and bred for use as forage by researchers in Canada and the US resulting in the production of varieties well-adapted to differing regions, soil types, and temperature regimes (Vogel et al., 1996).

Smooth bromegrass persists best on lightly grazed, well-drained pastures, where rhizomatous growth allows the plant to quickly fill in thin areas, and the upright, elongated tillers flower and produce seed annually. The cultivar ‘Lincoln’ was developed by the USDA and the Nebraska Agriculture Experiment Station in 1942 and remains the most common cultivar in the USA (Vogel et al., 1996; Casler et al., 2000). This shows how consistent and productive smooth bromegrass has been for producers for many years. Compared to other grasses and crops, yield improvements have been minimal since Lincoln was introduced, outside of some cultivars released for improved disease resistance and increases in forage quality (Casler et al., 2000).

Developmental Morphology and Environmental Responses

Smooth bromegrass is a tall, leafy, cool-season grass with a unique “W” constriction near the end of its leaf blades. It differs from other bromegrasses (*Bromus* sp.) in that its lemmas are awnless. Blades range from 0.5 to 1.5-cm wide and 5 to 40-cm long (Vogel et al., 1996; Stubbendieck et al., 1997; Otfinowski et al., 2007). A 7 to 20 cm panicle inflorescence is produced upon maturity with the culms reaching 0.5 to 1.0 m in height (Stubbendieck et al., 1997). Smooth bromegrass remains susceptible to repeated defoliation and negative carbohydrate balances because it produces extended vegetation culms during summer to aid in efficient light use throughout the canopy (Nelson and Moser, 1994).

Smooth bromegrass is described as both a short-day and a long-day plant because of its response to photoperiod. For floral induction, short days must be followed by long days in the developmental process. Short days are characterized by a day length that is shorter than a maximum value whereas a long day is characterized by having day length longer than a certain minimum. The length of day in hours is not truly what long and short means, but rather minimum and maximum hours required for a certain plant. Temperature virtually has no effect on floral induction in smooth bromegrass because the short day aspect is the principle factor. Vernalization or a cold period is not needed for floral induction (Heide, 1994; Vogel et al., 1996).

Although photoperiod is the principal factor in determining floral characteristics, temperature remains a key factor regulating growth. The optimum air temperature for growth of smooth bromegrass is 18-25°C while the optimum soil temperature is 18.3°C (Baker and Jung, 1968; Morrow and Power, 1979). A summer slump, or semi dormant period, occurs when temperatures exceed the optimum which commonly takes place in regions where smooth bromegrass pastures are prevalent (Anderson et al., 1941). When the temperatures fall back within the optimum during autumn, growth resumes. Even so, a range of 3 to 33°C is capable of supporting growth (Morrow and Power, 1979). Cold temperatures have little effect on survivability in established smooth bromegrass (Vogel et al., 1996). In an establishment study, when new seedlings were under ice for 60 days at -4°C, no damage was seen on the seedlings (Freyman, 1969).

The precipitation requirements for smooth bromegrass are 500 mm or more annually (Vogel et al., 1996). In Alberta, Canada, however, 280 to 450 mm has been shown to be sufficient for establishment and continued growth (Hardy BBT Limited, 1989). The limit was also seen in southeastern Nebraska on pastures fertilized with N at 0, 45, and 90 kg ha^{-1} in which precipitation limited yield when less than 280 mm (Colville et al., 1963).

Increased N fertilization has been shown to increase smooth bromegrass production and quality (Lorenz et al., 1961; Colville et al., 1963; Greenquist et al., 2009). Soil N is essential to the persistence and perpetuation of smooth bromegrass even though it is competitive in mixtures (Guretzky et al., 2004), amasses forage well (Guretzky et al., 2013), and it is very drought and cold tolerant (Vinton and Goergen, 2006). In North Dakota, fertilization of smooth bromegrass at rates of 45, 90, and 224 kg ha^{-1} across three soil moisture levels has been shown to double, triple, and quadruple (7842 kg ha^{-1} yr^{-1}) forage mass compared to unfertilized swards producing 1683 kg ha^{-1} yr^{-1} (Lorenz et al., 1961). Studies at the University of Nebraska-Lincoln have shown a 30% increase in forage mass on fertilized compared to unfertilized smooth bromegrass pasture (Greenquist et al., 2011).

Pasture Management Strategies

Pasture management strategies can affect forage production and nutrient utilization in pastures. Recently, interest has been growing for advanced understanding of pasture management strategies and their effects on nutrient and C cycling (Haynes and Williams, 1993; Soussana et al., 2004). Differing pasture management strategies are utilized to serve needs of producers and optimize smooth bromegrass persistence and production. Management strategies commonly used to affect forage production and quality in smooth bromegrass pastures include N fertilization and legume interseeding. A management concern for monoculture smooth bromegrass is that following establishment of pastures, yield can reach its peak in 2 to 5 years because the stand becomes sod-bound. One way to prevent or treat this problem is to fertilize the smooth bromegrass stand or interseed other grasses or legumes into the stand (Anderson et al., 1946; Lowe, 1950; Vogel et al., 1996; Otfinowski et al., 2007).

Nitrogen fertilization increases forage dry matter production and crude protein content of smooth bromegrass pastures (Lorenz et al., 1961; Colville et al., 1963). Smooth bromegrass fertilized at rates ranging from 0 to 224 kg ha^{-1} across three soil moisture levels increased forage production from 1683 to 7842 kg ha^{-1} yr^{-1}, respectively. Nitrogen fertilizer applied at 45, 90, and 224 kg ha^{-1} doubled, tripled, and quadrupled brome yield relative to unfertilized stands (Lorenz et al., 1961). In eastern Nebraska, it was found that between 90 to 135 kg N ha^{-1} maximized economic return (Colville et al., 1963). The optimum rate often varies with location due to differences in precipitation and growing degree days, but it is generally considered best to fertilize in early spring or split applications between spring and fall to enhance growth of smooth bromegrass when soil moisture is available (Vogel et al., 1996). Fertilization can also affect the diet quality of grasses. Fertilization on smooth bromegrass in Nebraska has also been shown to increase crude protein content from 16 to 20% at 0 to 179 kg N ha^{-1}, respectively (Snell et al., 2016). Fertilization also increased the crude protein content of smooth bromegrass from 8.96% with no fertilization to 14.83% with 179 kg N ha^{-1} (Colville et al., 1963). In Florida, N application at rates of 0 to 157 kg ha^{-1} has shown to increase in in vitro organic matter digestibility (IVOMD) and decrease neutral detergent fiber (NDF) in bermudagrass (*Cynodon dactylon* L.) (Johnson et al., 2001).

Interseeding legumes is an alternative strategy to increase forage production as well as growing cattle weight gains on pasture relative to N-fertilized and unfertilized pastures (Vogel et al., 1996). The introduction of legumes has long been a management technique to increase the amount of N available for plant uptake in pastoral systems while reducing commercial inputs of N fertilizer. Legume-interseeded pastures have been shown to have greater forage production and quality than fertilized and unfertilized pastures. Legume quality stays higher throughout the growing season while cool-season grass quality diminishes during the summer growing slump or more dormant part of the growing season when most cool-season grasses have reached maturity. At this time, the optimum temperature in which cool-season grasses grow is below the ambient temperature (Vogel et al., 1996; Sleugh et al., 2000; Otfinowski et al., 2007). Some legumes are considered cool-season plants; however, their optimum temperature extends beyond that of cool-season grasses allowing growth during warmer periods of summer.

When pastures are grazed down to a short height (grazed from 20 to 5 cm) compared to a taller height (grazed from 27 cm to 7 cm), stands of some legume species such as red clover (*Trifolium pratense* L.) and alfalfa (*Medicago sativa* L.) can decline (Carlassare and Karsten, 2002). This can occur in continuously grazed pastures when the legumes do not have a chance to mature and replenish carbohydrate reserves. A reduction in carbohydrate reserve storage due to utilization of the reserves by the plant to regrow after severe defoliation can cause a lack of regrowth the following year and increase competition from other plant species in the weakened legume stands.

A reduction in pastoral legume composition may also occur in rotationally grazed systems when there are not sufficient rest periods from year to year and if the same paddocks are grazed yearly during the same growth stage. Species such as alfalfa, birdsfoot trefoil (*Lotus corniculatus* L.), and red clover are upright-growing legumes and therefore are more susceptible to initial and subsequent defoliation by grazers as opposed to a clone-forming species such as white clover (*Trifolium repens* L.) that spreads near the ground (Beuselinck et al., 1994). Grazers, however, tend to favor legumes over grasses because of higher nutritive values. When given a choice between monoculture white clover and monoculture perennial grasses, cattle and sheep chose the legume 70% of the time (Rutter, 2006).

When grasses and legumes are at the same maturity or growth stage, legumes tend to have a greater leaf:stem ratio and more crude protein (Nelson and Moser, 1994). Legume-grass pastures also may have greater forage mass, as well as greater in vitro dry matter digestibility (IVDMD), higher crude protein, and lower NDF, than grass monocultures (Zemenchik et al., 1996; Sleugh et al., 2000). Legumes in cool-season pastures have been shown to increase digestible dry matter yield by 98% and crude protein by 111% in the pastures (Taylor and Allinson, 1983).

Interseeding legumes into grass stands also has been shown to increase N_2 fixation via the symbiotic relationship with rhizobia and provide balance to inorganic N inputs (Russelle, 1992). The fertilization of cool-season grasses with N can exceed the actual uptake of N for plants and can vary by year (Mosier, 2001). By interseeding legumes, biological fixation of N can potentially allow pastures to be more efficient with N compared to fertilized pastures. Unfertilized pastures of perennial ryegrass (*Lolium perenne* L.) mixed with white clover retained seven times more ammonia from soil and forage than ryegrass pastures fertilized with N at 420 kg ha^{-1} (Ryden et al., 1987). This becomes important when considering greenhouse gas emissions.

Lastly, pastures can also be managed without fertilization or legume interseeding. This strategy is typically utilized in pastures that are unsuitable to fertilizing or seeding such as along riparian areas or when the price of N or seed is high. Pastures that remain unfertilized have been shown to produce much less biomass and usually have lower quality. There is significantly less forage produced in unfertilized pastures than either legume-interseeded or fertilized pastures (Anderson et al., 1946; Lowe, 1950; Colville et al., 1963; Briske, 1991; Vogel et al., 1996; Brueland et al., 2003; Otfinowski et al., 2007). Insufficient N limits plant growth and metabolism. Although unfertilized pastures do not produce as much, in some cases forage quality can be high, but usually only in the early part of the growing season when vegetative growth predominates and herbage mass is low. In a five-year study in eastern NE, average daily gains of yearling steers grazing unfertilized and fertilized smooth bromegrass did not differ, although fertilized pastures had higher crude protein , total forage mass, forage mass in June, and forage mass in October than unfertilized pastures (Watson et al., 2012).

Dung Deposition, Decomposition, and Nutrient Cycling

Dung decomposition and soil N increase has become an important goal of pasture managers and researchers seeking to enhance nutrient availability. While dung deposited from grazing livestock is seen as a return of N to soil and a benefit to N cycling (Cowling, 1977; Russelle, 1992), the dung can smother forage and cause cattle to reject herbage around a dung pat for up to two years (Anderson et al., 1984). A factor affecting N cycling from dung is the C:N ratio. Nitrogen in dung is primarily in organic form which is ideal for microbes, but less ideal for integration of N into the soil. Mineralization is needed for N to be readily available for plant uptake and for the nutrients to be put back into the cycle for later use of cattle by consumption of vegetation (Russelle, 1992).

The deposition of dung provides complex and simple organic C chains for microbes to breakdown and utilize for energy and growth. With a C:N ratio of < 20:1, the rate of decomposition is not limited by the lack of N for microbial metabolism that is typically obtained from the soil. Because the C:N ratio of cattle dung is approximately 20:1 and continues to narrow as decomposition occurs, microbial activity and populations are optimum (Dickinson et al., 1981; Ruess and McNaughton, 1987; Aarons et al., 2009). Nitrogen is plant available at C:N ratios of approximately 17:1 in soil while soil organic matter is approximately 10:1 at which point decomposition of residues and dung slows significantly (Larney et al., 2006).

One of the most obvious results of dung on pasture is an increase in forage yield due to aforementioned N inputs from the dung. In an Australian study, after 45 days of cattle dung on soil, the 0 to 10 cm soil depth under the dung showed increased microbial biomass C compared to soil with no dung. After 112 days, in the same soil depth, pH, electrical conductivity (EC), K, and P had increased as well (Aarons et al., 2009).

The rate of decomposition is essential to the continuation of the nutrient cycle. Dung decomposition can be measured by visual loss, change in dung coverage area, DM loss, total organic C loss, total N loss, total C loss and movement into the soil, and total organic content by ash and bomb calorimetry (Dickinson et al., 1981; Omaliko, 1981; Lysyk et al., 1985; Bol et al., 2000; Aarons et al., 2009; Gillet et al., 2010). Research performed in the United Kingdom with similar annual precipitation as eastern Nebraska, showed the most rapid loss of N from dung pats during the first 20-day period was 8% of the total N. Following the rapid N loss was an extreme slowing of N loss from the dung. Ammonia volatilization and the liquid portion of the dung high in N entering the soil was postulated to be the explanation behind the initial rapid loss (Dickinson et al., 1981). Volatilization is considered to be a negative aspect of N loss to the atmosphere. The disappearance of cattle dung from pasture has been seen to range from several weeks to several years (Dickinson et al., 1981).

Dung characteristics such as moisture content can affect the rate of nutrient entry and availability in soil. Increased moisture content of dung has been shown to increase dung decomposition rate (Dickinson et al., 1981; Aarons et al., 2009). Fungi are more active decomposers at low soil moisture than bacteria and could also be applied to dung (Cook and Papendick, 1970). Other factors such as climatic conditions also are key contributing factors of decomposition rate. A study in New Zealand showed that dung deposited in autumn takes a shorter amount of time to decompose than dung deposited in spring (Weeda, 1967). Increased moisture combined with decreased temperature in fall slowed the formation of a hard crust on the pat and allowed more moisture to accelerate decomposition (Weeda, 1967). Meanwhile, decomposition rates of summer-deposited dung may differ from year-to-year due to interannual variability in climatic conditions (Dickinson and Craig, 1990).

Once dung is no longer present after decomposition, the positive effects of higher fertility and mineral content can still be seen in the soil (Aarons et al., 2009). An increase in soil fertility because of decomposition allows for higher forage yields and an expansion and robustness of nutrient cycling on a pasture (Aarons et al., 2009). Williams and Haynes (1995) showed that after three years of a deposition event from cattle, higher organic C was still detected in soil under dung compared to soil with no amended dung. However, in other research, Dickinson and Craig (1990) showed soil directly below the dung pat was not the terminus for nutrients. The increased nutrients in the soil did not equate with the loss of nutrients from dung. Some nutrients were used by surrounding vegetation while others moved laterally and downward. In an experiment by Williams and Haynes (1995), herbage uptake of nutrients from deposited dung was studied. They found that after 3 years, herbage near and under dung pats had utilized 15-65% of the nutrients from the dung (Williams and Haynes, 1995).

Dung has different characteristics depending on diet (Cook et al., 1996). The N content of dung is dependent on N content of diets (Jarvis et al., 1989). Urine contribution is more studied and more variable than dung in composition, and the N is more readily available for immediate uptake by plants than the N in dung components (Jarvis et al., 1989; Russelle, 1992). The C:N ratio of cattle dung tends to be 20:1. This ratio is lower than plant litter due to the ruminant digestive system which utilizes microbes in the gut that break down C chains in forage bound to N resulting in smaller particle sizes of forage in the dung not fully digested by the microbes. This is why cattle dung decomposes faster, increases mineralization rates, and ultimately N cycling increases (Ruess and McNaughton, 1987). Dung excreted from cattle grazing primarily cool-season grasses red fescue (*Festuca rubra* L.) and colonial bentgrass (*Agrostis capillaris* L.) had an N content of 21.95 g kg^{-1} (Bakker et al., 2004). Smooth bromegrass had an N, P, and K content of 28.4 g kg^{-1}, 3.34 g kg^{-1}, and 30.7 g kg^{-1}, respectively, in a Wisconsin study. A total of 200 kg ha^{-1}, 88.5 kg ha^{-1}, and 498 kg ha^{-1} of N, P, and K, respectively, were applied to the stands of smooth bromegrass in split applications (Casler et al., 1987). In a southeastern South Dakota study, three close pastures consisting of primarily smooth bromegrass resulted in cattle dung with N contents of 19.7 g kg^{-1}, 18.1 g kg^{-1}, and 19.4 g kg^{-1} (Lysyk et al., 1985).

CO_2 Flux

Measurement of CO_2 flux can provide an estimate of microbial respiration rate and activity as they utilize complex and simple C chains found in soil organic matter, litter, and dung. The rate and amount of CO_2 flux can give an indication of dung that will decompose and release nutrients bound with C more rapidly. Microbes affect the soil organic matter turnover rate, fertility, and nutrient cycling; therefore, the size of the population and community of microbes affects fluxes (Horwath and Paul, 1994). Soil respiration or gas flux is the transport rate at which CO_2 produced in soil by respiration diffuses across the soil-atmospheric concentration gradient. Factors that affect soil respiration and gas movement such as temperature, pressure, and moisture are taken into consideration when flux is calculated. The CO_2 flux is derived from three biological processes: autotrophic, microbial heterotrophic, and soil faunal heterotrophic activities. Autotrophic microorganisms derive their C from CO_2, but still produce some CO_2 via biological processes. Heterotrophic microbes, of which bacteria generally are considered in the soil, derive C from consuming complex organic molecules from decaying plants, organic matter, and other microbes. Lastly, soil fauna consume other microbes, plant residues, and organic matter and their metabolism adds to flux. Another part of CO_2 is derived chemically and is generated from inorganic carbonates or calcareous substrates reacting with water (Maier et al. 2011; Raich and Schlesinger 1992; Saiz et al. 2007). The three main carbon pools that are the source of soil CO_2 flux include soil organic matter, litter in and on soil, and root secretions (Kuzyakov, 2006).

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CHAPTER 2. EFFECT OF SMOOTH BROMEGRASS MANAGEMENT ON GRAZING CATTLE DUNG DECOMPOSITION, CO_2 FLUX, AND SOIL NUTRIENT MOVEMENT

Introduction

Smooth bromegrass (*Bromus inermis* Leyss.) is a cool-season grass that prevails throughout the north-central United States and south-central provinces of Canada in pastures and roadsides. The tall, leafy, and rhizomatous nature of this perennial grass creates climate resilient, highly palatable, and exceptionally productive forage for grazing and haying operations (Vogel et al., 1996; Stubbendieck et al., 1997; Casler, 2004; Otfinowski et al., 2007). Robust growth in the spring and later in the fall creates extended time periods of usable forage. Soil N is key to the persistence of smooth bromegrass despite its keen drought and cold temperature resiliency (Vinton and Goergen, 2006), competitiveness within mixtures (Guretzky et al., 2004), and high forage amassment (Guretzky et al., 2013).

Common strategies in pasture management aim to increase soil N availability for greater forage productivity and nutritive value (Harmoney et al., 2001; Zemenchik and Albrecht, 2002; Greenquist et al., 2009). Nitrogen fertilization of smooth bromegrass has been shown to increase forage mass and crude protein (Lorenz et al., 1961; Colville et al., 1963; Greenquist et al., 2009, 2011). In North Dakota, fertilization of smooth bromegrass at rates ranging from 0 to 224 kg ha^{-1} across three soil moisture levels quadrupled forage produced from 1683 kg ha^{-1} yr^{-1} to 7842 kg ha^{-1} yr^{-1}, respectively (Lorenz et al., 1961). In a 2012 eastern Nebraska grazing study on smooth bromegrass, the most economical return came from unfertilized pastures in which steers were supplemented with dry distillers grains with solubles (DDGS) which allowed the N requirements of cattle to be met (Watson et al., 2012). The other treatments in the study were unsupplemented steers grazing unfertilized pastures or fertilized pastures at 90 kg N ha^{-1} with both having negative net returns on steers (Watson et al., 2012). Differences in growing degree days and moisture from year to year can make it difficult to identify optimum fertilization requirements for smooth bromegrass (Rehm et al., 1971). Applying fertilizer to coincide with available soil moisture in the spring is preferred, but applications can also be split between spring and fall to augment growth (Vogel et al., 1996).

The practice of interseeding legumes into pasture is another management strategy which enhances forage production and forage quality of pastures. Pastoral profitability is increased by N fixation and vegetation quality with resultant higher cattle weight gain on legume-interseeded pastures than N-fertilized pastures (Vogel et al., 1996). In a symbiotic relationship with rhizobia, legumes fix atmospheric N_2 into amino acids and protein, and their incorporation into pastures improves nutritive value and seasonal distribution of forage relative to pastures managed without legumes (Russelle, 1992; Sleugh et al., 2000; Harmoney et al., 2001). The population and species of legumes in an interseeded grass pasture affects the N_2 fixed in a given paddock (Russelle, 1992). For example, alfalfa (*Medicago sativa* L.) growing in grass mixtures fixed 82 to 254 kg N ha^{-1} in a given season and averaged 182 kg N ha^{-1} annually across a four-year period (Heichel and Henjum, 1991). Others have reported a 98% increase in digestible DM and a 111% increase in crude protein yield when legumes were interseeded into cool-season grass pastures (Taylor and Allinson, 1983). Pastures interseeded with legumes also can enhance supply of forage in summer because of higher temperature tolerances of legumes

(Vogel et al., 1996; Sleugh et al., 2000; Otfinowski et al., 2007), and at similar maturities, legumes have more crude protein and a greater leaf:stem ratio compared to cool-season grasses (Nelson and Moser, 1994). Legume-interseeded pastures also have greater digestibility and lower neutral detergent fiber (NDF) compared to cool-season grass monocultures (Zemenchik et al., 1996; Sleugh et al., 2000).

In recent years, there has been growing interest in improving understanding of how pasture management influences nutrient and C cycling (Haynes and Williams, 1993; Soussana et al., 2004). Between 60% and 90% of the nutrients in forage that is consumed by livestock is returned to the pasture as either urine or dung (Haynes and Williams, 1993). Forage that is not digested by herbivores returns to pastures primarily as dung, and the size of the non-digestible C pool that returns to pastures as dung depends on forage quality and grazing pressure of a given pasture (Soussana et al., 2004). Dung deposited on a pasture is recycled into the soil, and subsequently, N and other nutrients released from dung are utilized by growing plants (Cowling, 1977; Russelle, 1992). The rate at which the dung C is decomposed is dependent upon temperature, moisture, and pasture management (Weeda, 1967; Dickinson et al., 1981; Soussana et al., 2004; Aarons et al., 2009). Increases in moisture content increases dung decomposition rate (Dickinson et al., 1981; Aarons et al., 2009), but excess soil moisture (> -300 kpa) can slow C decomposition (Clark, 1967). Climatic conditions are also significant contributing factors in the decomposition rate of dung. In a New Zealand study, dung deposited in autumn took a shorter amount of time to decompose than spring-deposited dung (Weeda, 1967). Increased moisture combined with decreased temperature in fall retarded the formation of a hard crust on the pat and allowed more moisture to accelerate decomposition (Weeda, 1967). Meanwhile, year-to-year decomposition rates of summer-deposited dung may differ because of greater interannual variability in climatic conditions often observed during this season compared to spring and fall (Dickinson and Craig, 1990).

Periods for complete dung decomposition can range between 2 weeks to 17 months (Weeda, 1967). Smothering of forage by dung causes rejection of smothered forage and surrounding forage up to two years by cattle (Anderson et al., 1984) making decomposition of dung important for determining how much forage livestock are willing or able to consume. In order for decomposition to occur, the microbial population must have sufficient nutrients for sustained metabolism. Dung provides soil microbes with simple and complex C for energy and growth. Microbes mediate soil processes such as C, N, O, S, and Fe cycles by deriving C from organic matter (Falkowski et al., 2008). This affects soil organic matter turnover rate, fertility, nutrient cycling, and in turn, the size of the population and community of microbes and their byproducts (Horwath and Paul, 1994). With a typical C:N ratio around 20:1, dung provides microbes sufficient N for metabolic activities (Dickinson et al., 1981; Ruess and McNaughton, 1987; Tang et al., 2006; Aarons et al., 2009). A soil C:N ratio of 17:1 allows N to be available for plant utilization (Larney et al., 2006). At this ratio, microbes are not as competitive with plants for N because there is sufficient N for microbial use in metabolism of simple and complex C and plants are able to access N in mineral form from the soil that microbes would otherwise use before plants could uptake the N.

Dung characteristics such as C, N, and moisture contents depend on the diet grazing animals consume (Cook et al., 1996). A higher quality diet in terms of N content and digestibility will produce higher quality dung. Urine contribution is more studied and more variable than dung in composition, and the N is more readily available for immediate uptake by plants than the N in dung (Jarvis et al., 1989; Russelle, 1992), but the rate of dung decomposition often is more closely aligned with what forage livestock consume or refuse (Anderson et al., 1984). The ruminant digestive system causes the C:N ratio to be lower in dung than in the original plant material. This creates a more rapid decomposition, increased mineralization rate, and increased N cycling from cattle dung than plant litter (Ruess and McNaughton, 1987). A grazing study in the Netherlands showed dung from cattle grazing primarily cool-season grasses red fescue (*Festuca rubra* L.) and colonial bentgrass (*Agrostis capillaris* L.) had 21.95 g N kg^{-1} (Bakker et al., 2004).

Although there are studies citing variability in soil C among pasture management strategies (Franzluebbers et al., 2000; Wienhold et al., 2001), the knowledge of effects of dung from cattle and its variability as impacted by diets of cattle grazing pastures with different management strategies is relatively unknown (Russelle, 1992; Soussana et al., 2004). In recent years, there has been increased focus on measurement of soil respiration or soil CO_2 flux as a means of assessing active C cycling, but there is limited research on soil CO_2 flux responses to different pasture management strategies (Lecain et al., 2000; Frank and Dugas, 2001; Cao et al., 2004). Dung can provide soil microbes with simple and complex C sources for energy and growth and with a typical C:N ratio of around 20:1, dung will provide soil microbes sufficient N for metabolic activities (Dickinson et al., 1981; Ruess and McNaughton, 1987; Aarons et al., 2009). In pastures, CO_2 flux may vary in time with different management strategies because the composition of dung affects CO_2 released by microbial metabolism (Ajwa and Tabatabai, 1994), and microbial metabolism itself is dependent on temperature and moisture (Raich and Schlesinger, 1992; Lloyd and Taylor, 1994). During a 150 day dung decomposition period beginning in May, over 70% of dung C was mineralized and respired as CO_2 (Yoshitake et al., 2014).

To further understand the dynamics of dung decomposition from different pasture management strategies, a full cycle of nutrient cycling needs to be addressed. This study focuses on dung decomposition and dung characteristics from cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures. It was hypothesized that as decomposition increased, movement of dung N into the soil and CO_2 flux from dung of cattle grazing legume-interseeded pastures were expected to be greater than dung from cattle grazing N-fertilized and unfertilized pastures. It was hypothesized that dung from cattle grazing legume-interseeded pastures would have a greater N content than dung from cattle grazing N-fertilized and unfertilized pastures, and would result in more rapid dung DM loss. Differences in decomposition from dung of cattle grazing legume-interseeded pastures from that that in N-fertilized pastures also were expected to increase from June to August as air temperatures increased. The objectives were to determine dung decomposition, CO_2 flux, and soil nutrient movement.

Materials and Methods

Research Location

Dung decomposition experiments were conducted at the University of Nebraska-Lincoln Agricultural Research and Development Center (ARDC) near Mead, NE (41° 6’ N, 96° 30’ W, 366 m ASL). The 30-year average annual precipitation at the site is 750 mm with 40% of precipitation occurring between June and August (High Plains Regional

Climate Center, 2016). The 30-year mean (1981-2010) temperature was 22.9°C with average temperature in June, July, and August at 21.8°C, 24.1°C, and 22.8 °C, respectively (High Plains Regional Climate Center, 2016). The dung decomposition experiments were established at a grassland study site (371.6-m^2) that did not have a recent history of grazing or nutrient inputs and consisted of a mix of smooth bromegrass, big bluestem (*Andropogon gerardii* Vitman), and indiangrass [*Sorghastrum nutans* (L.) Nash]. The dominant soil was a deep, well drained Tomek silt loam (fine, montmorillonitic mesic Pachic Argiudolls) with moderately slow permeability (USDA NRCS Web Soil Survey 2016). The 0 to 10 cm portion of soil had a pH of 6.8, OM content of 5.7%, a CEC of 18.9.

Dung for the four experiments was collected from pastures grazed by yearling steers (*Bos taurus*) in a separate grazing experiment at ARDC that consisted of three smooth bromegrass pasture types: legume-interseeded, N-fertilized, and unfertilized. The legume-interseeded type was smooth bromegrass pasture interseeded in spring of 2010 with alfalfa, red clover (*Trifolium pratense* L.), and birdsfoot trefoil (*Lotus corniculatus* L.). Legumes made up 13% of the pasture over 2014 and 2015. The N-fertilized pastures were monocultures of smooth bromegrass annually fertilized in early April with urea (46-0-0) at 90 kg N ha^{-1}. The N-fertilized pastures were monocultures of smooth bromegrass annually fertilized in early April with urea at 90 kg N ha^{-1}. The unfertilized pastures were comprised largely of smooth bromegrass and had not been fertilized with N since spring 2004 (Guretzky et al., 2013). The steers annually grazed all three pasture types from late April to late September using put-and-take stocking to maintain a similar grazing pressure among pastures. Freshly deposited dung in each of the different pasture types was collected by hand in the early morning when fresh dung was abundant and temperatures were below 75°C. Dung was collected on 5 and 6 June 2014, 24 and 25 July 2014, 5 and 6 June 2015, and 23 and 24 July 2015. Approximately 38 liters of dung was collected from each pasture type during each of the four collection periods. The dung from each pasture type was placed and homogenized in separate 5-gallon buckets and stored in a walk-in cooler at 4°C.

Experimental Design

The dung decomposition experiments were established as randomized complete block designs with six blocks (Fig. 2-1a) and factorial arrangements of four pasture-type dung treatments and three dung removal or harvest times (Fig. 2-1b). The four pasture-type dung treatments were dung from the three pasture types and a no-dung control. Dung harvest times were at 3, 7, and 30 d after placement. The study site (371.6-m^2) was first divided into four separate areas to accommodate replication of experiments in June and August of 2014 and 2015 and then further subdivided into the six blocks consisting of 16, 0.9 × 0.9 m plots each with a 21.5 cm diameter center for placement of the dung treatments. The 16 plots consisted of the 12 combinations of pasture-type dung treatments (including the no dung control) (4) and dung harvest times (3), as well as four additional plots of the pasture-type dung treatments that had 10 cm diameter by 10 cm height PVC rings inserted in the center for measurement of CO_2 flux (Fig. 2-1c). The rings were inserted 24 h before placing the dung pats at a 3-cm soil depth to allow a minimum of 2 cm of clearance between the top of the dung to facilitate CO_2 gas collection. Five days before placing dung pats, the site was mowed to a 10 cm height and raked by hand to remove excess growth and litter. The center area of each plot (21.5 cm diameter) was then clipped to bare ground (< 1 cm) with hand shears to ensure ample dung-to-soil contact.

Dung placement in plots during each of the four experimental dates began within 5 d after dung collection. Each treatment of bulk dung was homogenized again for approximately two minutes by stirring with T-posts prior to measuring out dung and placing/forming pats. A 1.5 L portion of dung was measured out and an approximate 100 mL subsample was taken for moisture determination and chemical analysis. The remaining portion was formed into a pat 21.5 cm in diameter on the clipped region in the center of the plots. For the CO_2 plots, the pat was placed directly over the 10 cm diameter PVC previously inserted into the soil. Inside the PVC, dung was filled to a height of 5 cm to allow a clearance of 2 cm from the top of the dung and the top of the PVC. Initial dung had DM contents ranging from 125 to 159 g kg^{-1} and C:N ratios ranging from 16.5 to 21.8 (Table 2-1).

Measurement of CO_2 Flux

Carbon dioxide flux was measured with a LI-8100A Automated Soil CO_2 Flux System (LI-COR Biosciences, Lincoln, NE). The measurement principle of the unit is to use the rate of increase of CO_2 in a chamber to get an accurate estimate of the diffusion rate of CO_2 into the atmosphere. The program factors into calculations the barometric pressure, concentration gradients, moisture and temperature of soil, and water-corrected mass to produce the CO_2 flux. The increased concentration is also factored in because the closed chamber causes the concentration to continually increase. Water vapor dilution is also considered. A linear or empirical regression is calculated using the rate of increase and the additional factors resulting in a best-fit equation for CO_2 flux (LI-COR Biosciences, 2010).

Gas measurements began at 0900 h. Mid-morning measurements are generally suggested to account for diurnal variability of flux due to temperature (Parkin and Venterea, 2010). In 2014, CO_2 was measured at days 1, 2, 3, 4, 5, 7, 9, 11, 15, 17, 19, 21, 23, 28, and 30 after dung placement during the June experiment and days 1, 2, 3, 6, 7, 10, 14, 17, 20, and 24 after dung placement during the August experiment. In 2015, CO_2 was measured at days 1, 3, 7, 9, 13, 16, 21, 24, 28, and 30 after dung placement during the June experiment and days 1, 2, 3, 8, 13, 16, 20, 23, 27, and 32 after dung placement during the August experiment. The distance from the top of the PVC to the top of the dung and soil was measured every measurement day to ensure volumetric chamber values were updated and entered into the unit for correct calculations.

Diet Sampling, Nitrogen, and Neutral Detergent Fiber

Within two weeks before collecting dung for each experiment, diet samples of the steers were obtained using six ruminally-fistulated steers. Rumens were evacuated and the steers were then allowed to graze by treatment the legume-interseeded and N-fertilized pastures for one hour. The ingested forage was then removed from the steers, placed in plastic bags, and frozen until processing occurred. In the first step of processing the samples for lab analyses, the frozen diet samples were allowed to thaw in a 4°C refrigerator for 24 h. A hammer and chisel were then used to take random samples from the softened diet samples. Approximately 300 grams of wet diet sample from each steer were dried via lyophilization. Dried diet samples were ground through 1-mm screens using a Wiley mill and then analyzed for neutral detergent fiber (NDF) using the Van

Soest method (Van Soest et al., 1991) and for N content using combustion (LECO TruSpec N analyzer, Saint Joseph, MI). There was no difference in N content between legume-interseeded and N-fertilized diet samples (Table 2-1). The N content ranged from 2.3 to 2.9 g kg^{-1} over the two years and seasons.

Dung Harvests and Soil Sampling

On harvest days, dung was collected by gently prying pats from the soil surface. Care was taken to ensure the whole pat was collected without contamination from soil, vegetation, or litter. The bottom of the pat or pat pieces were gently scraped with a plastic serving spoon to remove any soil, vegetation, or litter adhered to the dung surface followed by hand removal of remaining undesired material. The dung pats were then placed in labeled, sealed plastic bags on ice in coolers and transported to -20°C freezers or 4°C refrigerators for storage in laboratories on UNL's East Campus. Samples were stored in freezers when lab analyses were to be delayed by more than two days.

Once dung pats were taken from the soil surface, soil cores were taken at a depth of 0 to 10 cm below where each pat had been as well as from the center of the no dung treatment plots. A trowel was used to carefully scrape the soil surface prior to coring to remove any excess dung pieces not visible to the naked eye to ensure no dung contamination of the core occurred. Four cores were taken from directly under the pat position and to make a composite sample. Soil samples also were placed in labeled, sealed plastic bags on ice in coolers and transported to -20°C freezers or 4°C refrigerators for storage until analysis.

Dry Matter Determination

Moisture was determined on the initial 100 mL subsample of the dung from each dung pat of each pasture type before placement and from the dung pats and soil samples at each harvest time. The 100 mL initial dung subsample was dried in a forced air oven at 60°C for a minimum of 72 h or until constant dry weight was obtained. Moisture and dry matter of dung pats from harvest times was determined by removing pats from plastic bags, removing extraneous soil and litter, weighing, and finally hand homogenization. By weight, half of the homogenized pat was placed in drying tins and dried in a forced air oven at 60°C for a minimum of 72 h or until constant dry weight was obtained. The remaining half was saved for chemical analysis.

Soil moisture was determined by hand homogenization of refrigerator-thawed field moist soil cores. A field-moist soil subsample of approximately 10 g was placed in drying tins and dried in a forced air oven at 105°C for a minimum of 24 h or until constant dry weight was obtained.

Dry and Wet Chemical Analyses

The dried initial dung and harvest time dung samples were prepared for chemical analysis by grinding them through 1-mm screens and then analyzed by dry combustion GC analysis on a Costech Analytical ECS 4010 (Costech Anaylytical Technologies Inc., Valencia, CA) to determine total C and total N. Water-extractable N (WEN) and water-extractable C (WEC), which are labile forms (Jandl and Sollins, 1997), from field moist samples for dung and soil were quantified using Shimadzu TOC-V CPN analyzer (Shimadzu Corporation, Kyoto, Japan). For field moist soil, KCl extractable NH_4-N and

NO_3-N were determined using SEAL high resolution analyzer (SEAL Analytical Inc., Southhampton, United Kingdom). A field moist soil equivalent to 1 g of dried soil was used for each soil sample.

Data Analysis

Effects of year, season, diet, harvest time, and their interactions on remaining dung dry matter, WEC, WEN, soil WEC, WEN, NH_4-N, and NO_3-N were analyzed using a mixed model procedure with difference of least square means using SAS 9.3 program (SAS Institute, Cary, NC). When significant interactions between year, season, and diet occurred, additional analyses were conducted by year and season to compare specific effects of dung treatments. Contrast statements were used to make specific comparisons of dung treatments (legume-interseeded vs. N-fertilized, legume-interseeded vs. unfertilized, and dung vs. no dung) when overall effects of dung treatments were significant. Significance was declared at $P < 0.05$.

Effects of year, season, diet, and their interactions on daily CO_2 flux were analyzed using a repeated measure mixed model procedure with difference of least square means also determined using SAS 9.3 program (SAS Institute, Cary, NC). Season and diet were considered fixed effects and observations (sample day) was repeated. Effects of diet on flux by day, season and year was analyzed using a mixed procedure with contrast statements to compare effects legume-interseeded vs. N-fertilized, legume-interseeded vs. unfertilized, and dung vs. no dung treatments. Significance was declared at $P < 0.05$.

Results

Weather

Precipitation in August 2014 was 84% above the 30-year mean and exceeded that which fell in June 2014 by 37%. This pattern was not consistent with the average 30-yr pattern, which showed that 17% more precipitation occurs in June than August. Precipitation also was greater than the 30-yr mean for both years and both seasons. Temperatures were cooler than the 30-yr mean for both years and in both June and August for daily high, daily low, and 24-hr measurements (Table 2-2). In June 2014, daily high, daily low, and 24-hr temperatures were 4.8%, 9.1%, and 6.5% cooler than the 30-yr mean, respectively. In August 2014, daily high and 24-hr temperatures were 3.4% and 2.2% cooler than the 30-yr mean, respectively. In June 2015, daily high, daily low, and 24-hr temperatures were 6.5%, 5.5%, and 6.1% cooler than the 30-yr mean, respectively. In August 2015, daily high, daily low, and 24-hr temperatures were 3.4%, 8.4%, and 5.2% cooler than the 30-yr mean, respectively.

Mean CO_2 Flux

The year × season × dung treatment (diet) interaction was significant for mean CO_2 flux across the 30-d experiments indicating differences in decomposition were due in part to pasture management strategy and the weather (Table 2-3). The largest factor for differences in CO_2 flux among treatments was the addition of dung itself. Addition of dung to soil increased CO_2 flux by an average of 231% compared to soil without dung in all seasons and years (Fig. 2-2). Dung from cattle grazing pastures with different N management strategies also influenced CO_2 flux, but this depended on year and season. In June 2014, there were no differences in flux from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized pastures (Table 2-3; Fig. 2-2). In August 2014, however, dung from legume-interseeded pastures had 27% and 33% greater mean CO_2 flux than N-fertilized and unfertilized pastures, respectively (Fig. 2-2). Dung from legume-interseeded pastures had 21% and 22% greater mean CO_2 flux than dung from unfertilized pastures in June and August 2015, respectively (Fig. 2-2).

Daily CO_2 Flux Patterns

Plots treated with dung differed from plots without dung on all but three measurement days: day 7 during the June 2014 experiment; day 3 during the August 2014 experiment; and day 3 during the June 2015 experiment. In the August 2014 experiment, the most occurrences of significant differences were observed. Some of the measurement days in which differences were not significant or had very low fluxes coincided with weather events such as precipitation and low temperatures (Fig. 2-3, Fig. 2-4). Peak CO_2 flux readings occurred on similar days after dung placement in the June experiments. In the August experiments, however, peaks occurred later during the experimental period in 2014 compared to 2015. Differences in daily CO_2 flux from dung of legume-interseeded and N-fertilized pastures occurred on 12 measurement days across the four experiments (Fig. 2.4). Dung from legume-interseeded pastures had greater flux in 10 of the 12 instances. Significant differences in CO_2 flux from dung of legume-interseeded and unfertilized pastures occurred on 22 measurement days across the four experiments (Fig. 2-4). Dung from legume-interseeded pastures had greater flux in 19 of the 22 instances. In all experimental periods, except the June 2014 experiment, differences occurred within the first 3 days.

Diet Sampling, Nitrogen, and NDF

The NDF values from the diet samples only resulted in a significant diet effect. Legume-interseeded pastures had 12.1% lower NDF content than N-fertilized pastures. The major determining difference of the diet effect was between the 2014 legume-interseeded and the 2015 N-fertilized diet. The N content values did not result in any significant diet differences or interactions.

Dung Composition and Decomposition

Dung pat decomposition as measured by DM remaining showed several significant differences among diets within season (Table 2-4). In the June 2014 (Fig. 2-5a) and June 2015 (Fig. 2-5c) experiments, diet had a significant effect on DM remaining, but neither of the August (Fig. 2-5b, Fig. 2-5d) experiments showed differences with diet. Day had a significant effect on DM remaining in all seasons and years. The diet × day interaction was significant in the June 2015 experiment. Dry matter remaining from dung of legume-interseeded pastures was less and differed from dung of N-fertilized pastures only on day 3 by 18%. On day 30 of the August 2015 experiment, DM remaining in dung differed between legume-interseeded and unfertilized treatments by 9% less remaining for legume-interseeded.

The N content in the dung was affected by diet and sampling day in the June 2014 experiment and diet × day interactions in the August 2014, June 2015, and August 2015 experiments (Table 2-4). In the June 2014 experiment, N content in dung from legume-interseeded pastures was 5.4% and 11.9% greater than N content in dung from N-fertilized and unfertilized pastures, respectively (Fig. 2-6a). Across the August 2014 experiment, dung from legume-interseeded pastures had 5.5% and 14.5% more N content than dung from N-fertilized and unfertilized pastures, respectively (Fig. 2-6b). On all days, N content in dung from legume-interseeded pastures differed from that of unfertilized pastures, but only on days 0, 3, and 30, did it differ from that of N-fertilized pastures. Across the June 2015 experiment, N content in dung from legume-interseeded pastures was 8% greater than that in N-fertilized pastures due to differences on days 0 and 3, but dung from legume-interseeded and unfertilized pasture differed only on day 0 (Fig. 2-6c). Across the August 2015 experiment, N content in dung from legume-interseeded pastures was 20.5% greater than that from unfertilized pastures with differences occurring on all days (Fig. 2-6d), but it was greater than that of N-fertilized pastures only on days 0 (5.4%) and 3 (5.6%).

The dung C:N ratio was significantly different among diets in all seasons and years as well as among days (Table 2-4). The diet × day interaction was significant in the June and August 2014 experiments. In the June 2014 experiment, dung from legume-interseeeded pastures had a 14.7% and 6.5% lower C:N ratio than dung from unfertilized and N-fertilized pastures, respectively (Fig. 2-7a). The C:N ratio of dung from legume-interseeded pastures was lower from dung of N-fertilized pastures on all days and from unfertilized pastures on days 3 through 30. Across the August 2014 experiment, the C:N ratio of dung from legume-interseeeded pastures was 7.7% and 12.9% less than dung from N-fertilized and unfertilized pastures, respectively (Fig. 2-7b). The C:N ratio of dung from legume-interseeded pastures differed from dung of unfertilized pastures on all days and from N-fertilized pastures on days 0, 3, and 30. Across the June 2015 experiment, the C:N ratio of dung from the legume-interseeeded pastures was 11.0% less than dung from N-fertilized pastures (Fig. 2-7c), but never differed from dung of unfertilized pastures. Across the August 2015 experiment, the C:N ratio of dung from legume-interseeeded pastures differed from dung of unfertilized pastures by 6.5% (Fig. 2-7d), but differed from dung of N-fertilized and unfertilized pastures only on days 0 and 3.

A year × season × diet interaction affected WEC. In the June 2014 experiment, dung from legume-interseeded pastures was 64.8% and 54.3% greater in WEC than dung from N-fertilized and unfertilized pastures, respectively (Fig. 2-8a). Across the season of August 2014 and across the season of June 2015 experiments there were no significant differences among diets (Fig. 2-8b, Fig. 2-8c). In the August 2015 experiment, dung from legume-interseeded pastures had 25.8% greater WEC than dung from unfertilized pastures (Fig. 2-8d). Overall, WEC in dung from legume-interseeded and N-fertilized pastures became more similar as the experiments continued as evident by greater differences on day 3 than day 7 and by no differences on day 30. In contrast, WEC in dung from legume-interseeded and unfertilized pastures differed more during the middle of the experiments.

A season × diet × day and a year × season × day interaction also occurred for WEN. In June and August 2014 experiments, WEN in dung from legume-interseeded pastures was 75.0% and 48.7% greater than dung from N-fertilized pastures, respectively (Fig. 2-9a, Fig. 2-9b). In the June 2015 experiment, WEN in dung from legume-interseeded pastures exceeded that in N-fertilized pastures by 40.2% (Fig. 2-9c). In the August 2015 experiment, WEN in dung from legume-interseeded pastures was greater than from dung of N-fertilized pastures by 22.1% (Fig. 2-9d). In all experimental periods, day 3 and day 7 had greater WEN than day 30. In the June 2014 experiment, day 3 and day 7 were 63.7% and 57.8% greater than day 30, respectively. In the August 2014 experiment, day 3 and day 7 were 47.2% and 51.7% greater than day 30, respectively. In the June 2015 experiment, day 3 and day 7 were 70.4% and 60.5% greater than day 30, respectively. In the August 2015 experiment, day 3 and day 7 were 63.0% and 56.4% greater than day 30, respectively. In the June 2015 and August 2015 experiments, day 3 had greater WEN than day 7 by 25.1% and 15.2%, respectively. The only time differences did not occur between WEN in dung from legume-interseeded pastures and N-fertilized pastures within year and season and day, occurred in the June 2015 experiment on day 30. The only time differences between WEN in dung from legume-interseeded pastures and unfertilized pastures, did not occur was in the June 2015 experiment on day 30 and in the August 2015 experiment on day 3.

Soil Changes Under Dung

A year × season × day interaction in WEC occurred over the experiments (Table 2-5). In the June 2014 experiment, WEC was greater on day 3 than day 30 by 26.9% (Fig. 2-10a). In the August 2014 experiment, WEC on day 30 was 28.7% and 19.5% greater than day 3 and 7, respectively (Fig. 2-10b). In the June 2015 experiment, day 3 and day 7 were greater than day 30 by 68.7% and 66.0%, respectively (Fig. 2-10c). In the August 2015 experiment, day 7 was 22.2% greater than day 3 (Fig. 2-10d). By year and season, the only difference in soil WEC under dung was between legume-interseeded and N-fertilized pastures in June 2014. There were no differences between dung and no dung treatments by year and season. June experiments consistently had greater WEC than August experiments for dung and no dung treatments.

A year × season × day interaction in WEN occurred over the experiments (Table 2-5). In the June 2014 (Fig. 2-11a) and June 2015 (Fig. 2-11c) experiments, soil WEN on day 3 and 7 was greater than day 30. In the June 2014 experiment, day 3 and day 7 were 54.6% and 52.5% greater than day 30, respectively, and in the June 2015 experiment, were 41.6% and 37.9% greater than day 30, respectively. In the August 2014 experiment, however, day 30 was 24.2% and 15.2% greater than day 3 and day 7, respectively (Fig. 2-11b). In the August 2015 (Fig. 2-11d) experiment, day 7 was greater than both day 3 and day 30 by 35.2% and 40.9%, respectively. The only difference between soil WEN in year and season by diet was in the August 2015 experiment when soil WEN under dung was 21.9% greater than soil WEN under no dung control.

All effects and interactions were significant for NO_3-N including a year × season × diet × day interaction (Table 2-5). There were differences between June and August experiments for every diet (Fig. 2-12a). The June 2014 experiment highlighted the NO_3-N significant occurrences for all diets. Over the two August experiments, there were no differences across diets including controls.

For NH_4-N, all effects and interactions except the year × season × diet and the year × season × diet × day interaction were significant (Table 2-5). A year × season × day interaction occurred over the experiments. In the June 2014 (Fig. 2-13a) and August 2014 (Fig. 2-13b) experiments all days differed. In the June 2015 (Fig. 2-13c) experiment, day 30 differed from both day 3 and 7. In the August 2015 (Fig. 2-13d) experiment, day 7 differed from both day 3 and 30. When contrasting diets in year and season, there was greater soil NH_4-N under dung from legume-interseeded pastures than from unfertilized in both the June 2014 and the August 2015 experiments by 38.5% and 24.1%, respectively. August experiments consistently had greater NH_4-N than June experiments for dung and no dung treatments.

Discussion

Our objective was to evaluate how different smooth bromegrass pasture management strategies for grazing cattle affected dung decomposition and nutrient movement. Measurements of CO_2 flux showed that dung produced from cattle grazing legume-interseeded pastures had a faster loss of dry matter than dung from cattle grazing unfertilized pastures. This finding did not support for our hypothesis that as decomposition rates increased, CO_2 flux from dung of cattle grazing legume-interseeded would be greater than dung from cattle grazing both N-fertilized and unfertilized pastures. Faster decomposition allows for more ready availability of N and other key nutrients for plant uptake and growth (Aarons et al., 2004, 2009). The rate of C movement as seen in CO_2 flux, WEC in dung, and WEC into the soil from the dung during the decomposition process varied by diet. This is consistent with a study by Bol et al. (2000) in which C was measured in the soil and leachates. The CO_2 flux showed more differences in August than June between the legume-interseeded and N-fertilized treatments. However, this was not consistent and therefore did not support the hypothesis of increased differences in August compared to June. This was not expected due to the legumes having higher temperature tolerances than cool-season grass and at similar maturities have more crude protein and greater leaf:stem ratios (Nelson and Moser, 1994; Vogel et al., 1996; Sleugh et al., 2000; Otfinowski et al., 2007). A possible explanation for the inconsistency is the weather discontinuity in both precipitation and temperature from the 30-yr mean (Table 2-1). This may have changed growth characteristics and rates of maturity for the legumes and the smooth bromegrass. Instances of low CO_2 flux measurements coincided with precipitation and low temperatures as well (Fig. 2-3).

The DM remaining after 30 days showed that dung from legume-interseeded and N-fertilized pastures decomposed at similar rates, but dung from legume-interseeded pastures did have faster decomposition than that from unfertilized pastures. This did not agree with the hypothesis or the results of the CO_2 flux that dung from legume-interseeded pastures would have more rapid DM loss than dung from N-fertilized pasture. The DM remaining did, however support the hypothesis in that dung from legume-interseeded pastures would have more rapid DM loss than dung from unfertilized pastures which agreed with the CO_2 flux as well. The ability to capture all the CO_2 respiration from microbes may have caused the disagreements between DM and CO_2 flux. The activity on the edges of the dung pat due to the respiration of roots, dying roots, and the rich liquid portion of the dung on the soil surface and the portion of dung in contact with the soil. Additionally, the N content of the diet samples from the ruminally fistulated steers showed that there were no differences between the consumed forage of legume-interseeded and N-fertilized treatments. This likely contributed to the inconsistency of dung decomposition between those diets. The quality of dung was highest in the legume-interseeded as shown by the consistently lower C:N ratio, greater N content, and greater WEN which supported the hypothesis that dung from cattle grazing legume-interseeded pastures would have a greater N content than dung from cattle grazing N-fertilized and unfertilized pastures. This did not translate into the more rapid DM loss, as seen above. This higher quality dung agrees with a study by Jarvis et al. (1989) in which higher N content in the available diet resulted in higher N content in the dung. A lower C:N ratio which was observed 30 days after placement also indicates potential for dung from legume-interseeded pastures to have faster decomposition across its entire life compared to that from N-fertilized and unfertilized pastures. The legume-interseeded C:N ratio was at or below 20:1 across all years and seasons. When the above differences occurred, legume-interseeded always had the lower C:N ratio value. The C:N ratio became lower as a result of decomposition due to microbial population activity and possible growth reducing the overall C content which agrees with other studies (Dickinson et al. 1981; Howard and Paul, 1994; Bansal and Kapoor, 2000).

Additionally, dung from legume-interseeded pastures had significantly greater WEC than dung from other treatments only during the June 2014 and August 2015 experiment. However, dung from legume-interseeded pastures had significantly greater WEC early in the decomposition periods than dung from N-fertilized pastures when examining differences in days and then became similar at the end of the decomposition period. The greater WEN occurred in dung from legume-interseeded pastures than from N-fertilized pastures ranged from 22.1% to 75.0% by year and season. These properties indicate more potential for rapid decomposition to occur and for more nutrients to be available for use by surrounding plants in a faster time period in the same growing season. The higher WEC early in each experiment coupled with DM loss of the pats allows for smothered forage to reemerge more quickly to reduce forage loss and rejection by cattle (Cowling, 1977; Anderson et al., 1984; Russelle, 1992). Additionally, the much greater WEN shows more microbial presence and allows for a higher concentration of N to be available to microbes and has the potential to move into the soil for microbes residing in the soil.

The soil WEC indicated that movement of the more labile fraction of C was not seen despite the greater concentration in dung from legume-interseeded pastures. Thus, these data did not support the hypothesis that nutrient movement from dung of cattle grazing legume-interseeded pastures would be greater than that from N-fertilized and unfertilized pastures. It is difficult to track all C from dung and into the soil (Bol et al., 2000). Variations in the concentration of WEC and WEN of the soil across time were consistent with the control or no dung indicating trends were due to climatic factors. Differences in soil WEN below dung from pastures was not seen despite higher WEN in the dung from legume-interseeded pastures than dung from other treatments which did not provide support for our hypothesis and was not consistent result with the finding of significantly higher WEN in the dung itself. This finding did not show that N from dung moved into the soil directly below the dung pat although in August of 2015, soil WEN under dung was 21.9% greater than soil WEN under no dung control. The amount of WEN in the dung is low compared to the N content of the dung, but is much higher in the substrate rich liquid portion of the dung that is direct contact with the soil. With the soil type and the fact that cores were taken from 0 to 10 cm, could have caused a lack of differences occurring because of the concentration being too low making small differences unable to be detected. A more shallow depth may have revealed the differences of DM, WEC, WEN, and N content that were seen in the dung during the decomposition periods. The soil NH_4-N data showed that dung placed in August consistently had greater conversion of dung N into the more plant available form; a result which was expected due to higher temperatures during this time period. The transformation between NH_4-N and NO_3-N was only clearly seen on day 3 of the June

2014 in the soil. Dung from legume-interseeded and N-fertilized pastures allowed for more plant available N during the decomposition period than dung from the unfertilized pastures due to higher dung WEN and lower dung C:N ratios. The continual use of nutrients by plants may have also contributed to the lack of differences in the soil. The movement and form of N, however, is hard to determine due to many various forms in which in occurs (Russelle, 1992), as was also seen in the lack of confirmed WEN movement from the dung and into the soil. Only 20 to 30% of the initial dung nutrients was removed by the end of the study. With a short study, therefore, nutrient movement into the soil was not seen.

Conclusions

Interseeding legumes is a pasture management strategy that can have multiple benefits. These benefits include reducing the need for N-fertilizer application while improving forage quality, forage production, and animal performance relative to pastures managed without legumes. This research found dung from legume-interseeded pastures had a lower C:N ratio, greater N content, WEC, and WEN than dung from N-fertilized and unfertilized pastures, and thus, legume interseeding distinguish itself as a positive component of pasture management with an improved potential for dung decomposition and soil nutrient movement because of nutrient rich dung. More labile C and N in dung from legume-interseeded pastures provided richer substrate for microbial use and decomposition at the interface of dung and soil. These values indicate that dung from legume-interseeded pastures will tend to decompose more quickly with greater potential for soil nutrient movement, and thus benefits to nutrient cycling more than from other pasture management strategies. The combination of improved potential for dung decomposition and soil nutrient movement in nutrient rich dung from legume-interseeded pastures grazed by cattle displays another advantage of interseeding legumes into cool-season grass pastures.

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Figures and Tables

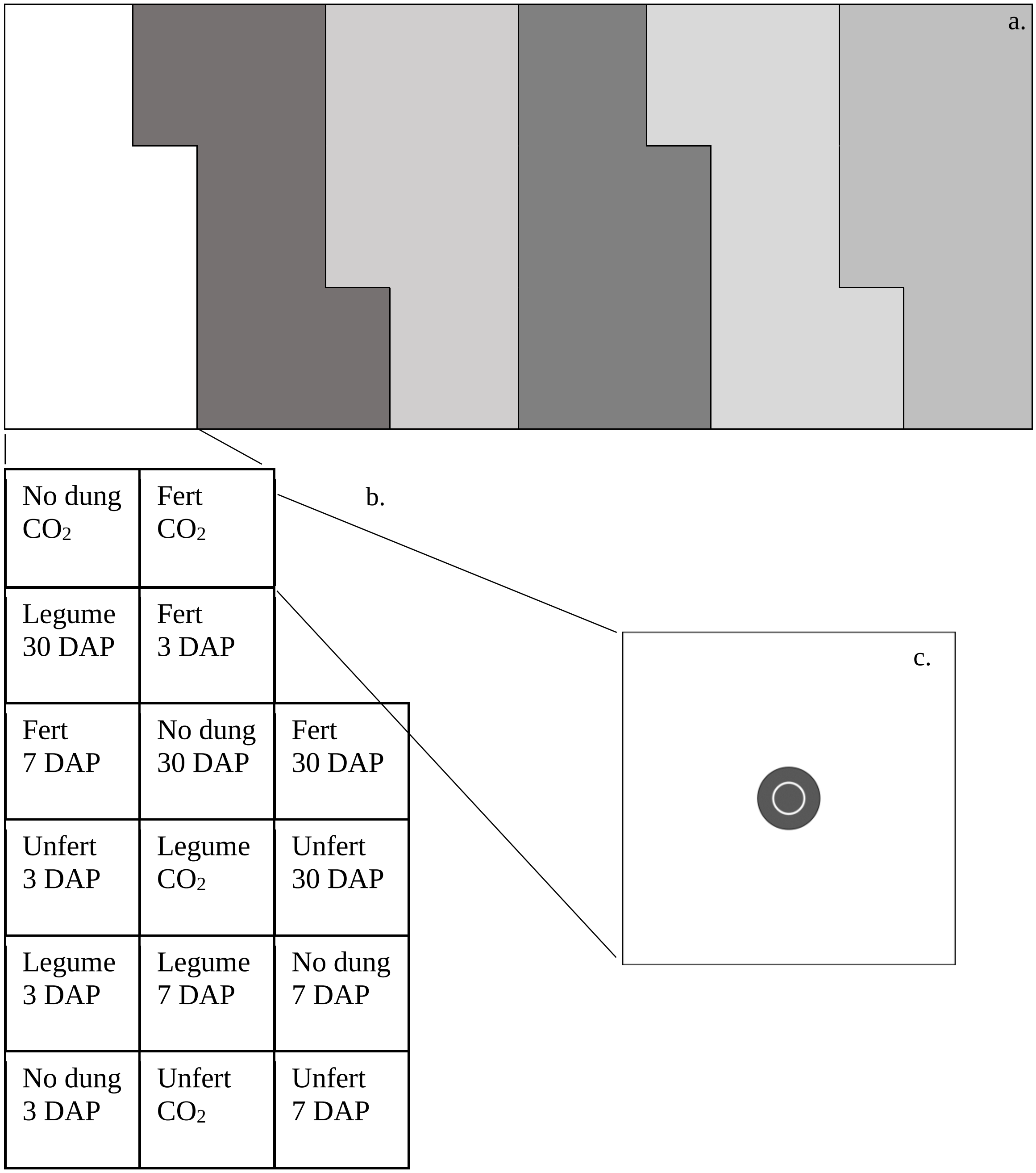

Figure 2-1. Plot diagram for one experimental period organized as part of a randomized complete block design. Dung from cattle grazing legume-interseeded (Legume), N-fertilized (Fert), and unfertilized (Unfert) smooth bromegrass pastures and one control treatment (No dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska was placed in the plots. Plots with CO_2 had PVC rings (c.) for CO_2 flux measurements. Day after placement (DAP) represents when the dung was removed from the plots.

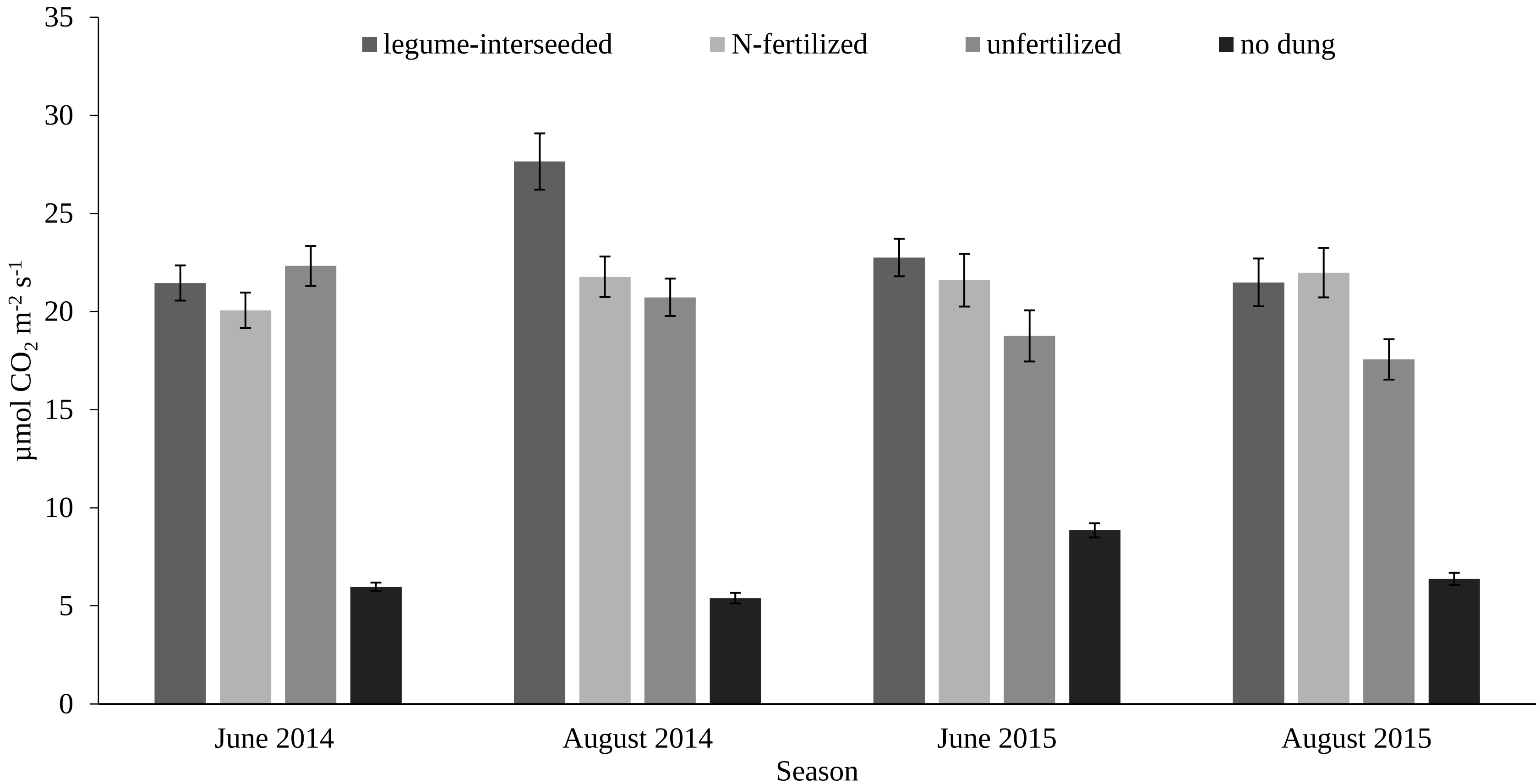

Figure 2-2. Average CO_2 flux (µmol CO_2 m-^{2} s-^{1}) from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures and one control treatment (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

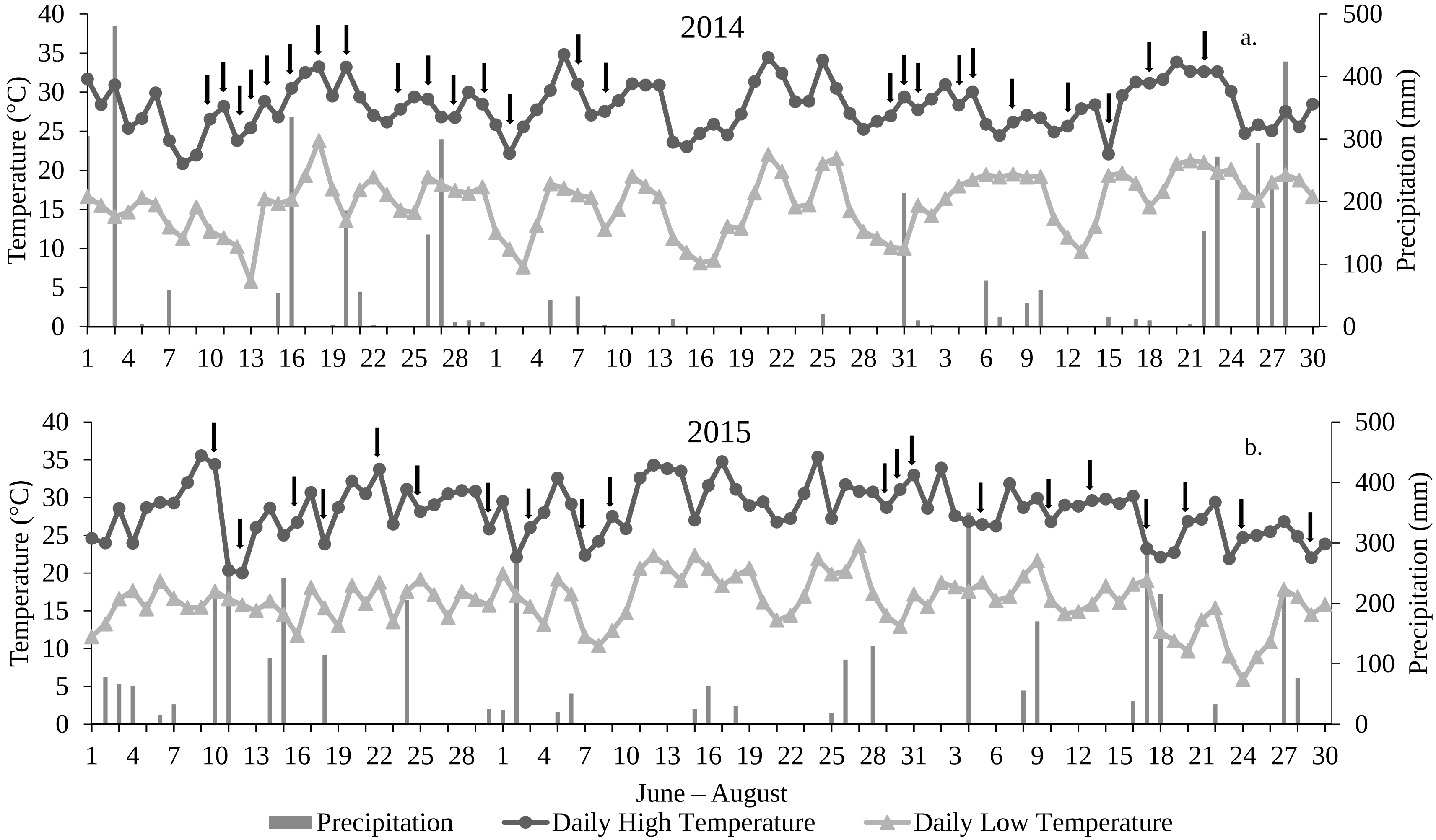

Figure 2-3. Daily high and daily low temperatures and precipitation events during June through August of 2014 and 2015. Arrows indicate days in which CO_2 flux was measured.

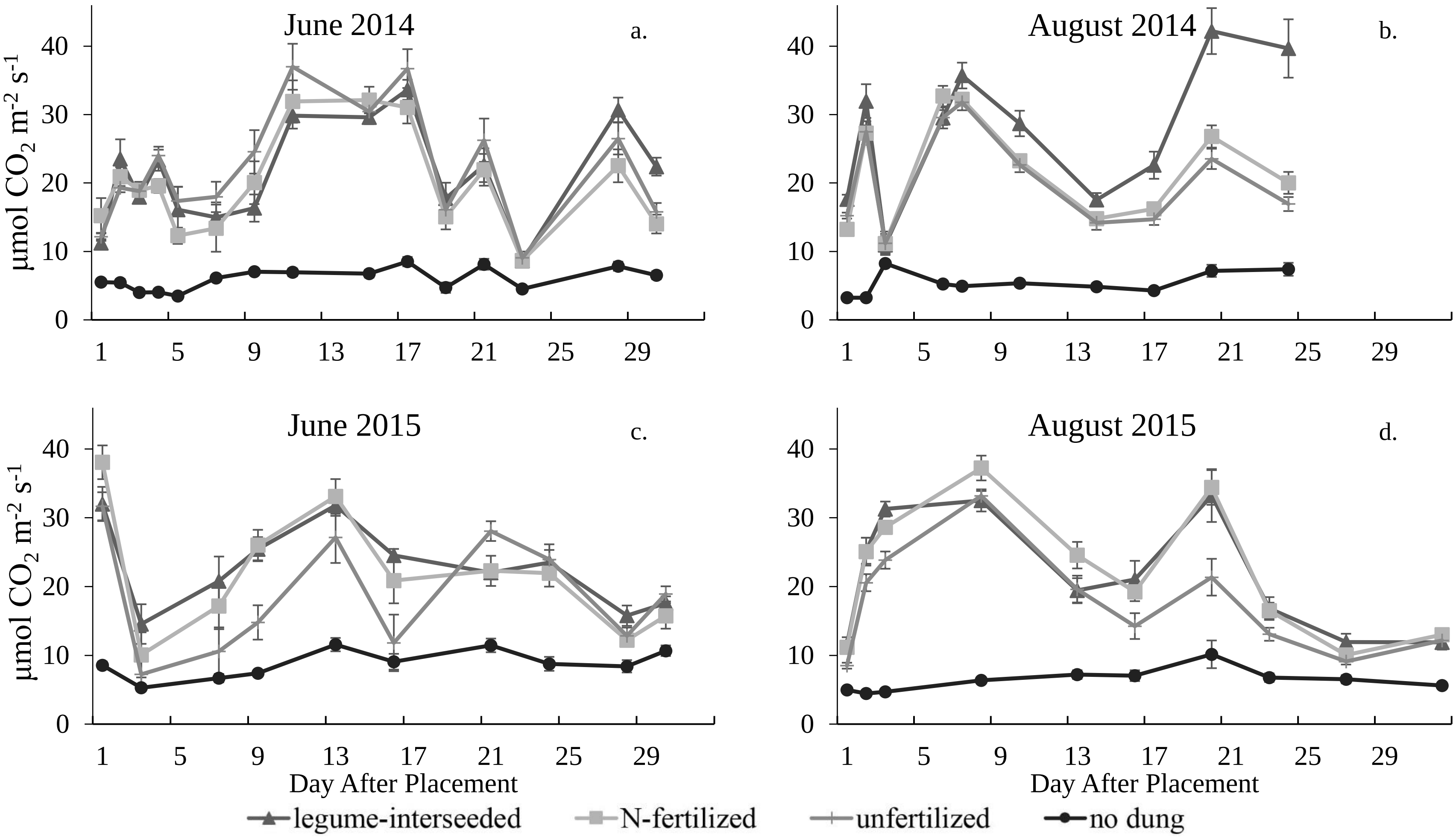

Figure 2-4. Daily CO_2 flux after placement of dung from cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures and one control treatment (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

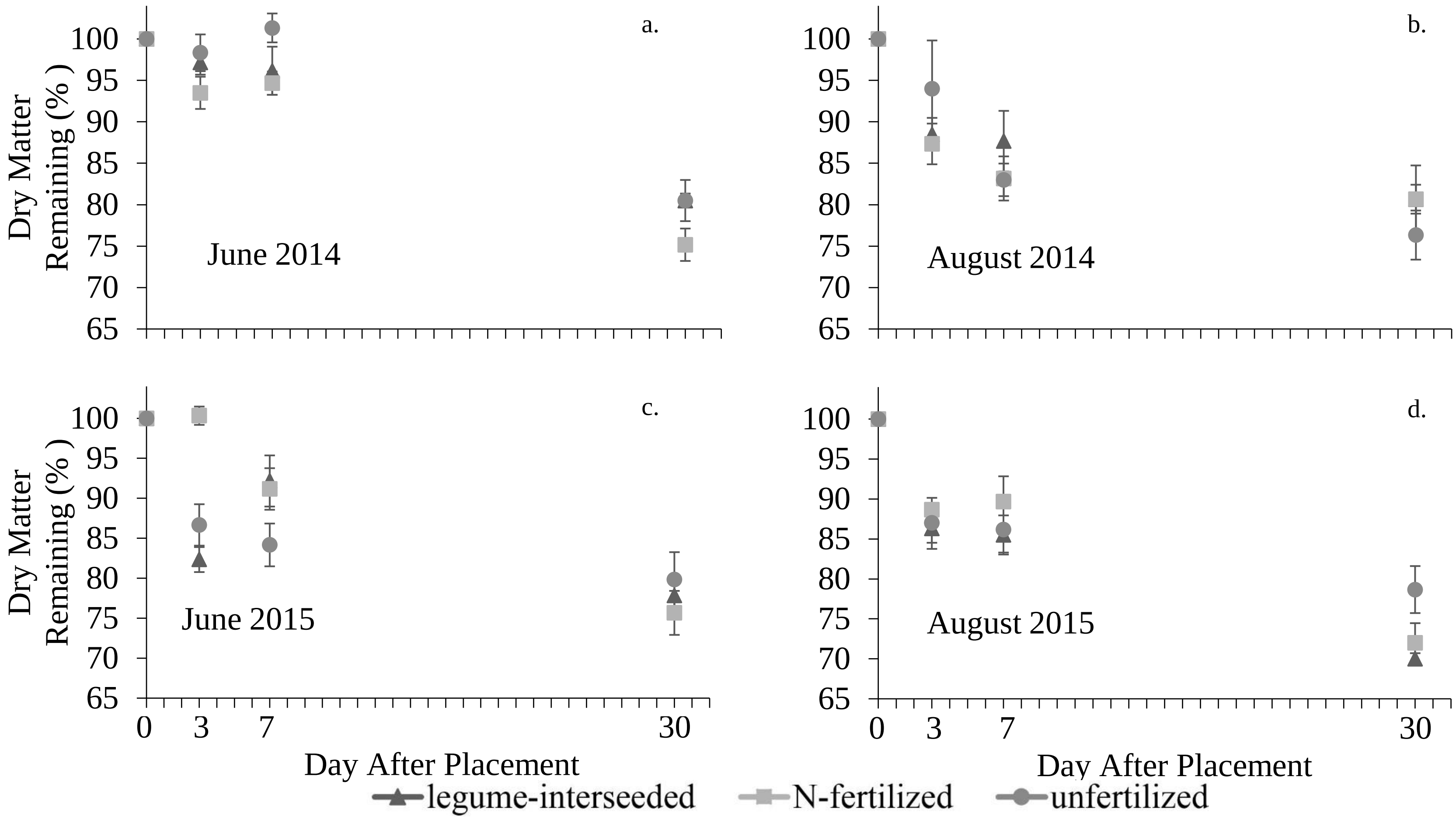

Figure 2-5. Mean percentage of dry matter remaining from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

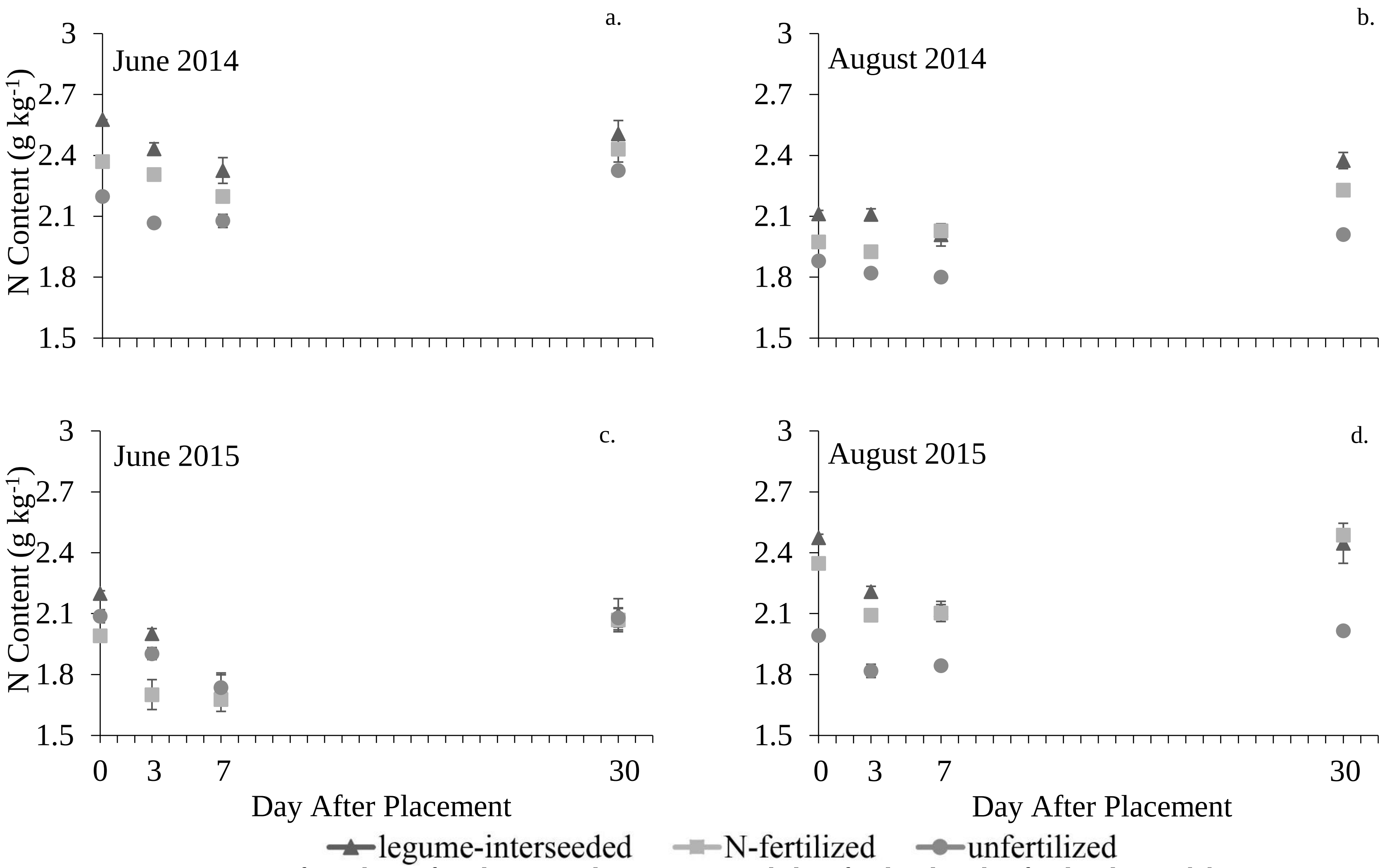

Figure 2-6. Mean N content from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

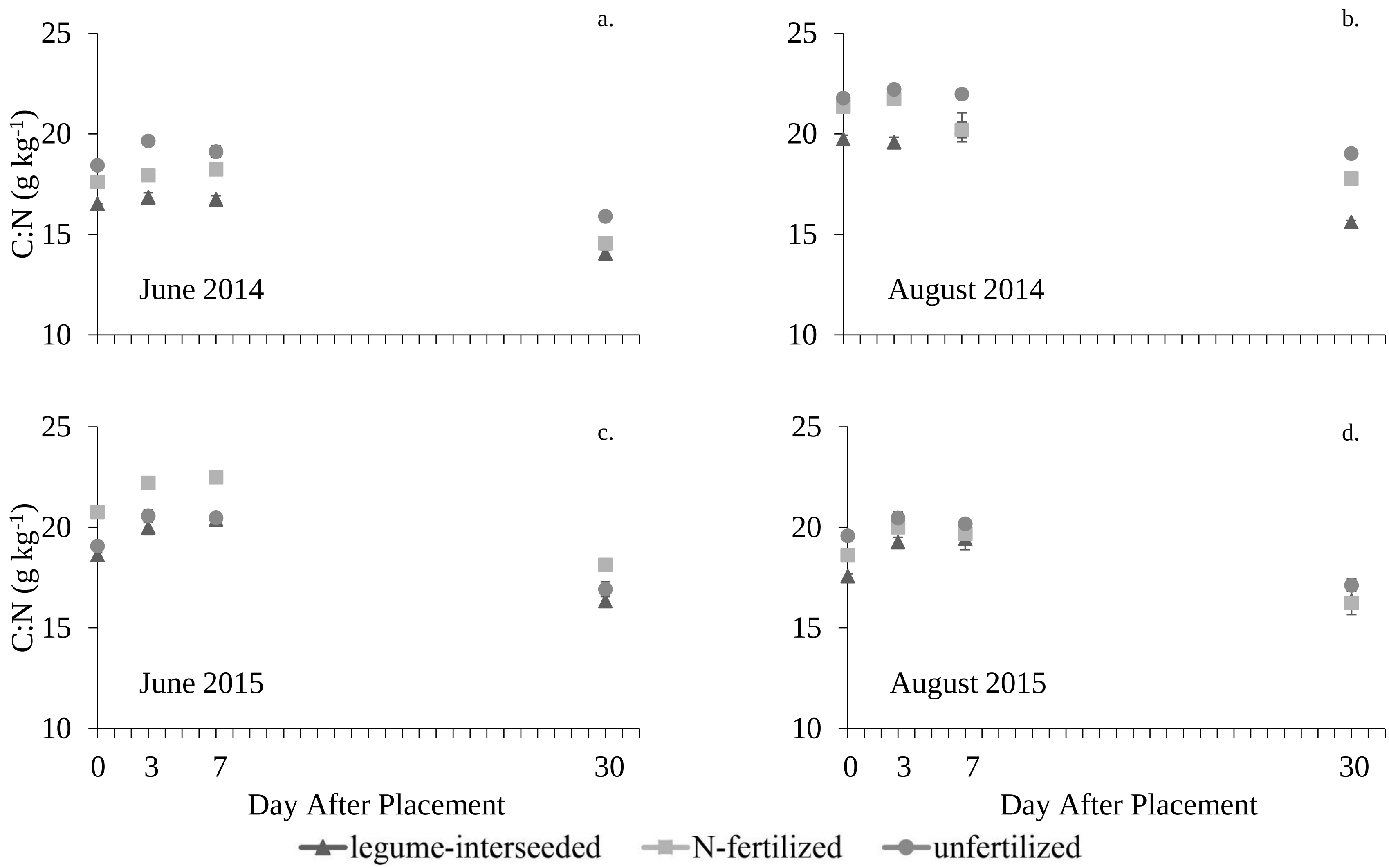

Figure 2-7. Mean C:N ratio from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

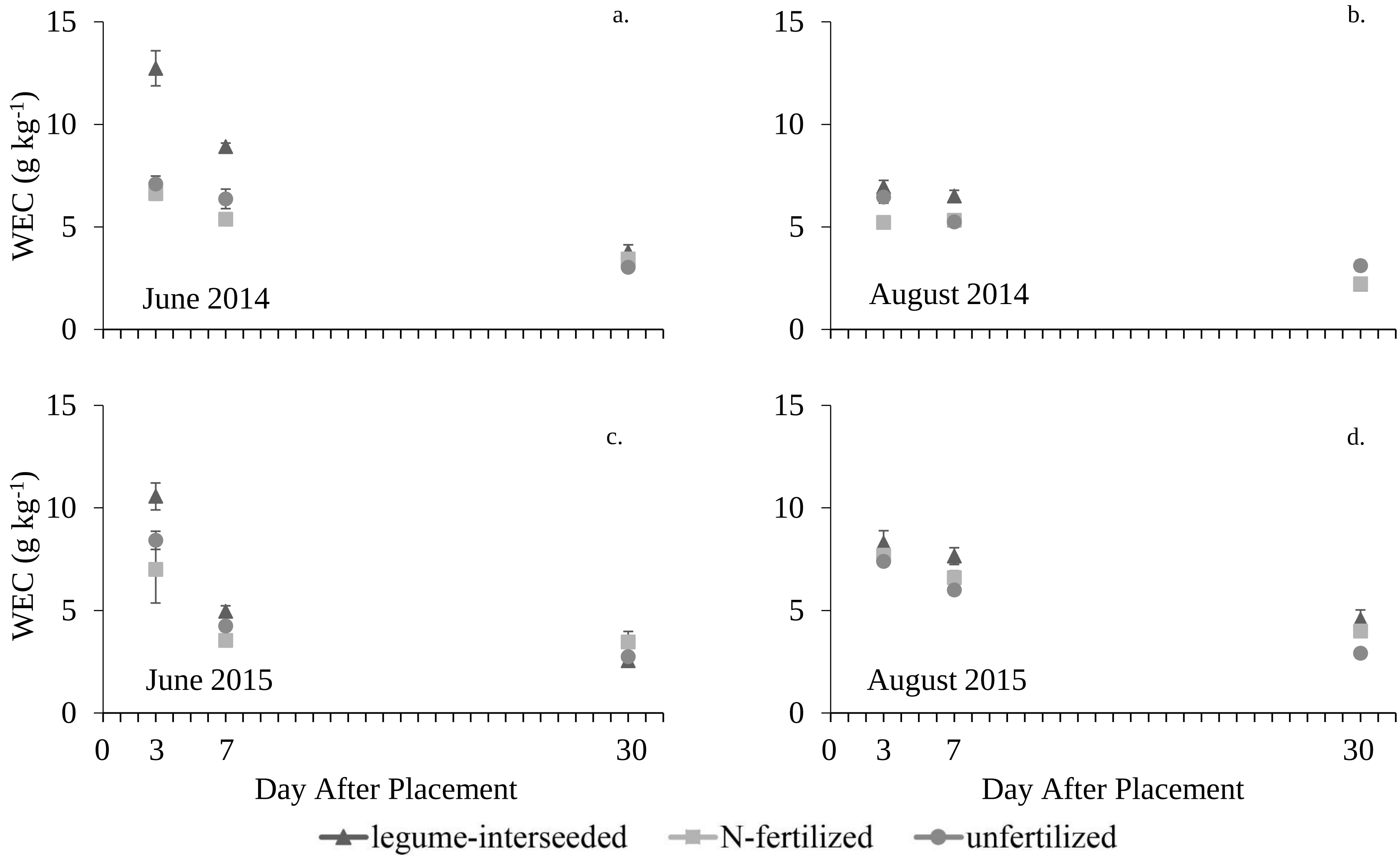

Figure 2-8. Mean water extractable C (WEC) from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

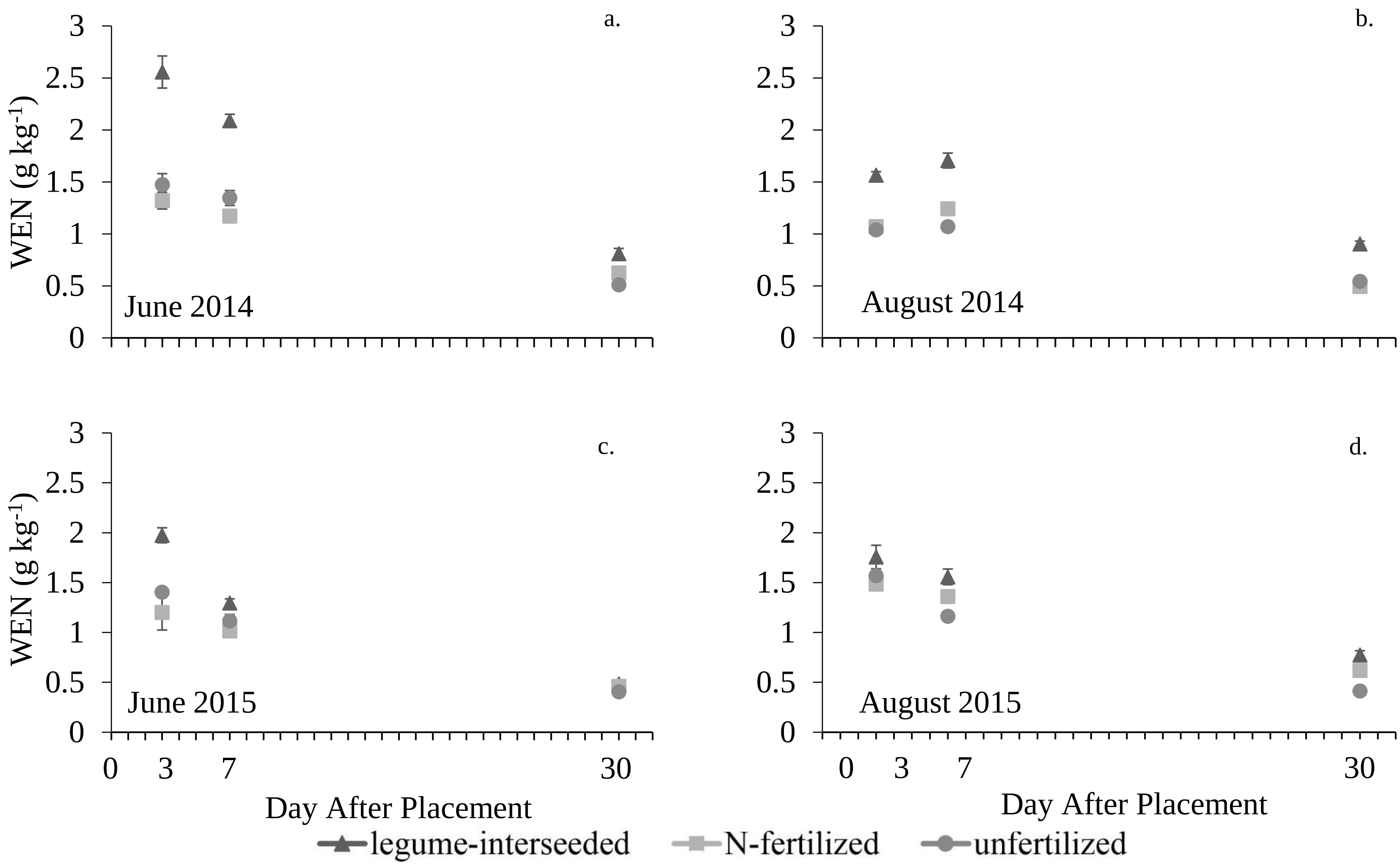

Figure 2-9. Mean water extractable N (WEN) from dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

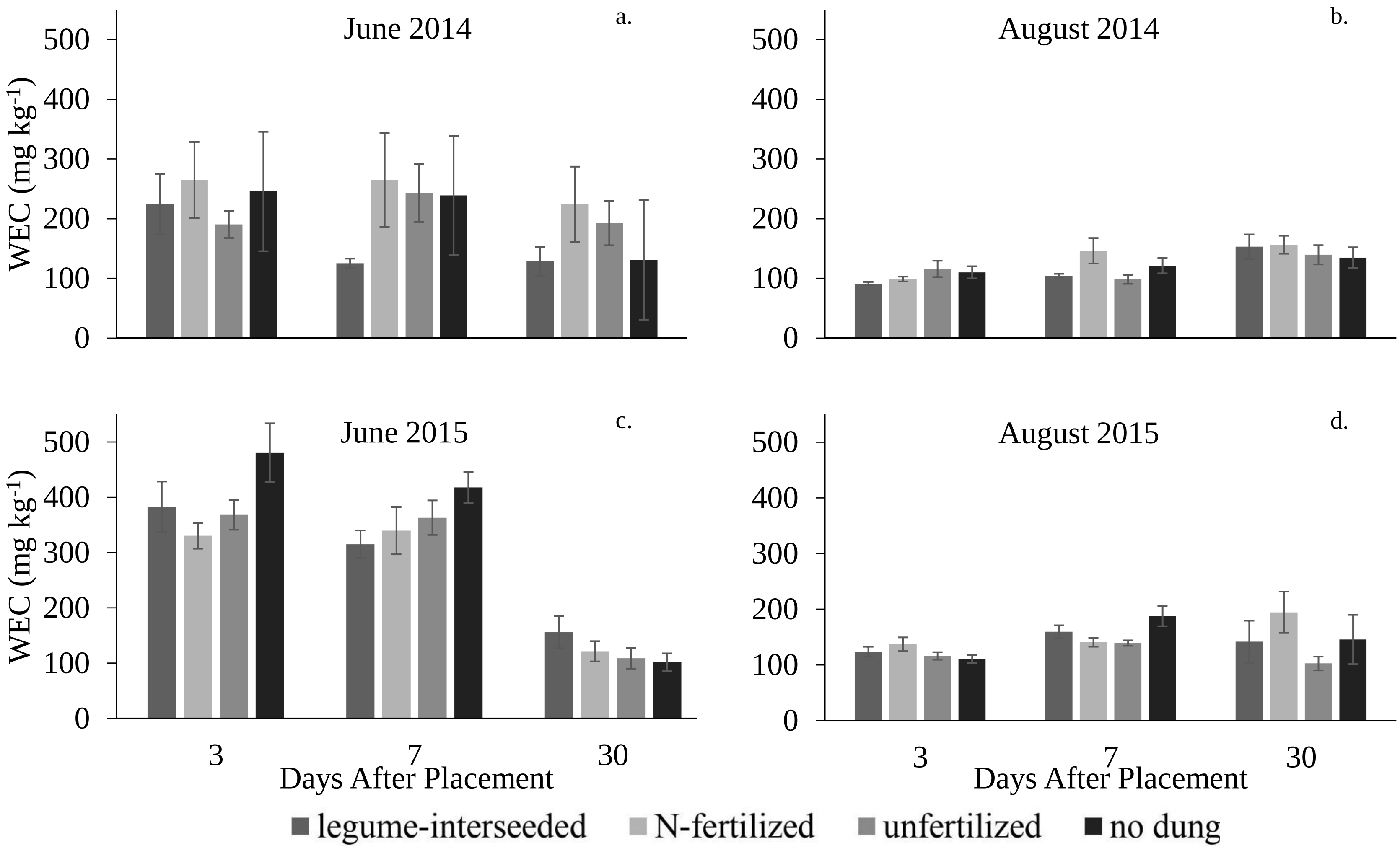

Figure 2-10. Mean water extractable C (WEC) from soil under dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures and one control (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

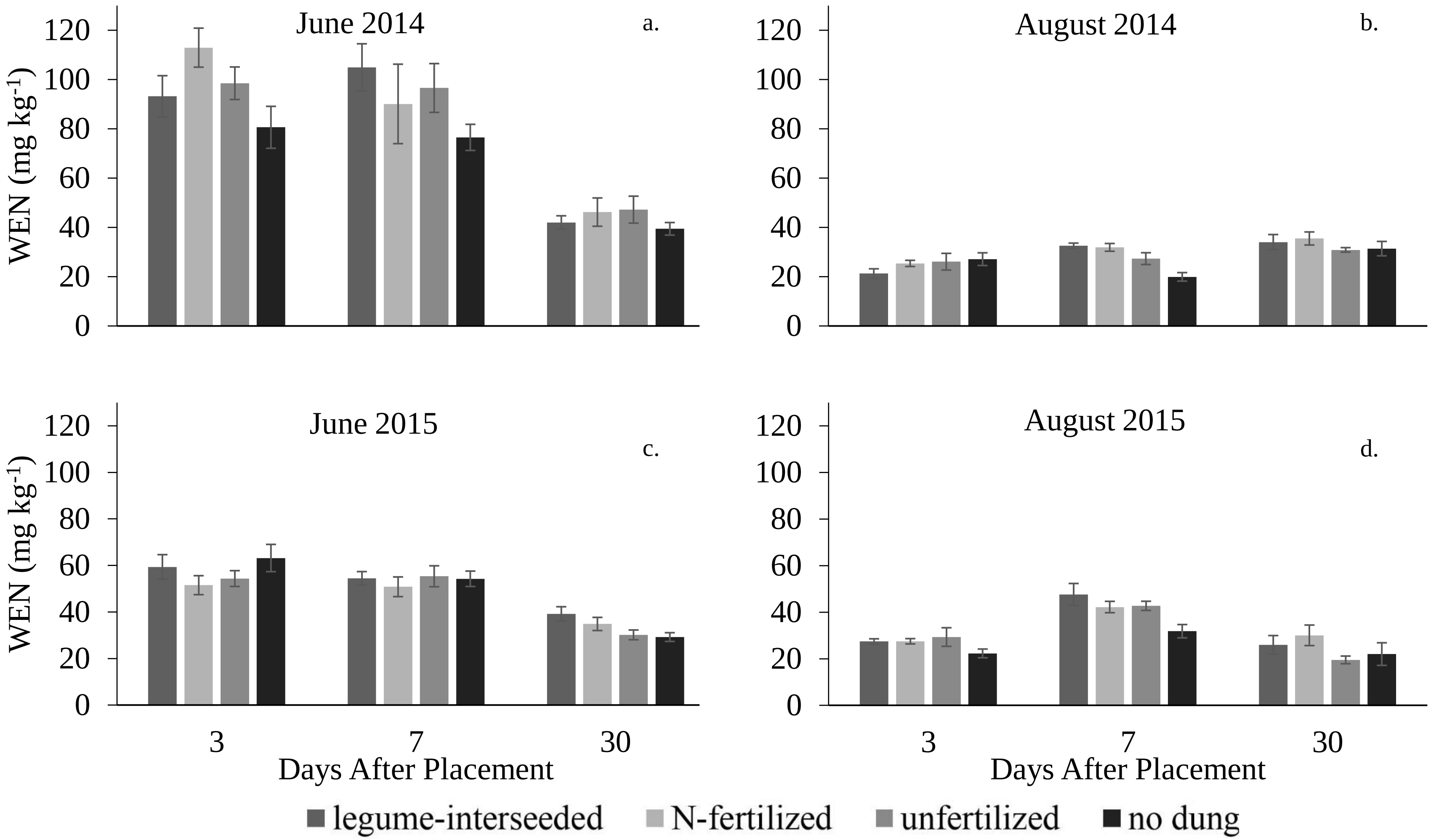

Figure 2-11. Mean water extractable N (WEN) from soil under dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures and one control (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

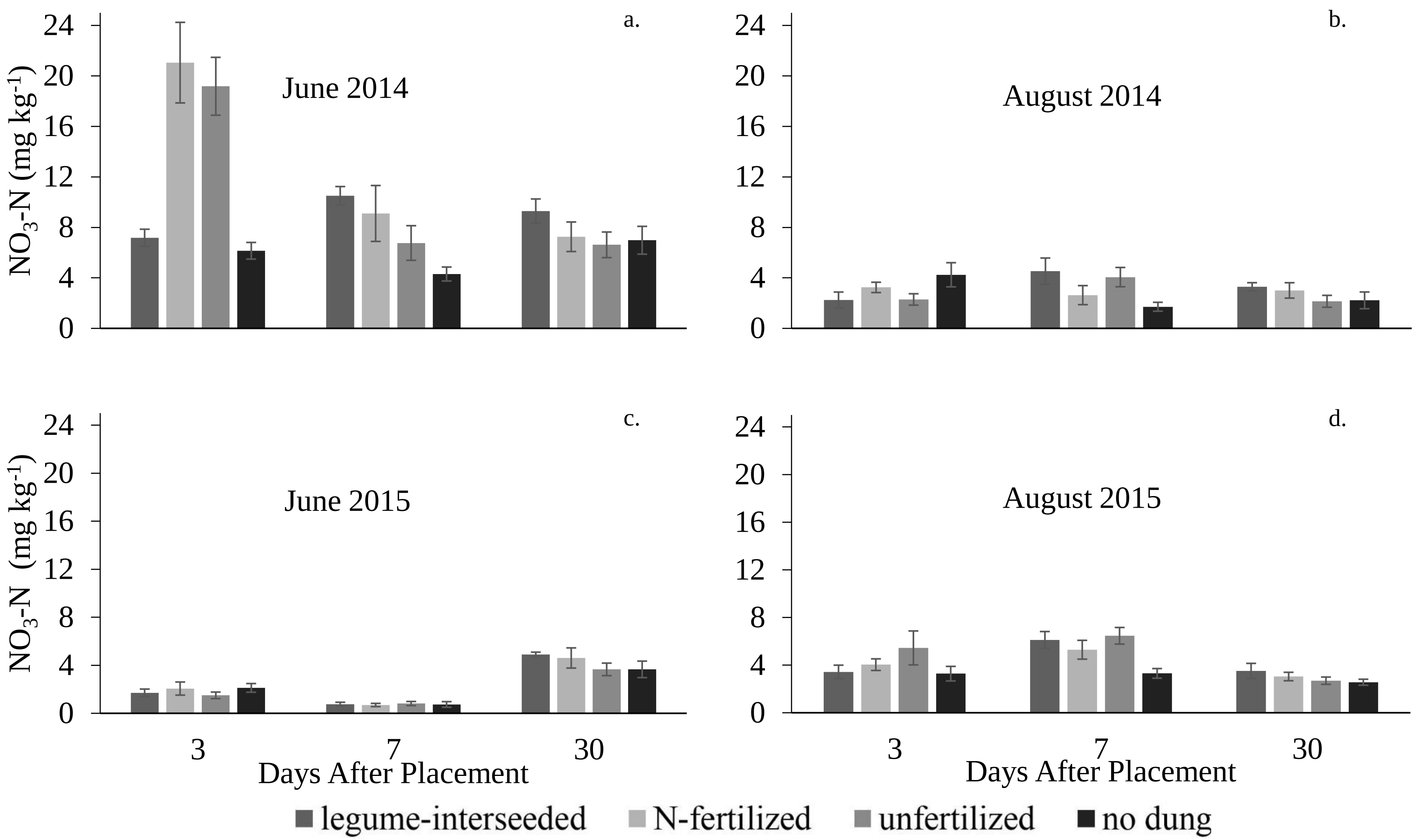

Figure 2-12. Mean NO_3-N from soil under dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures and one control (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

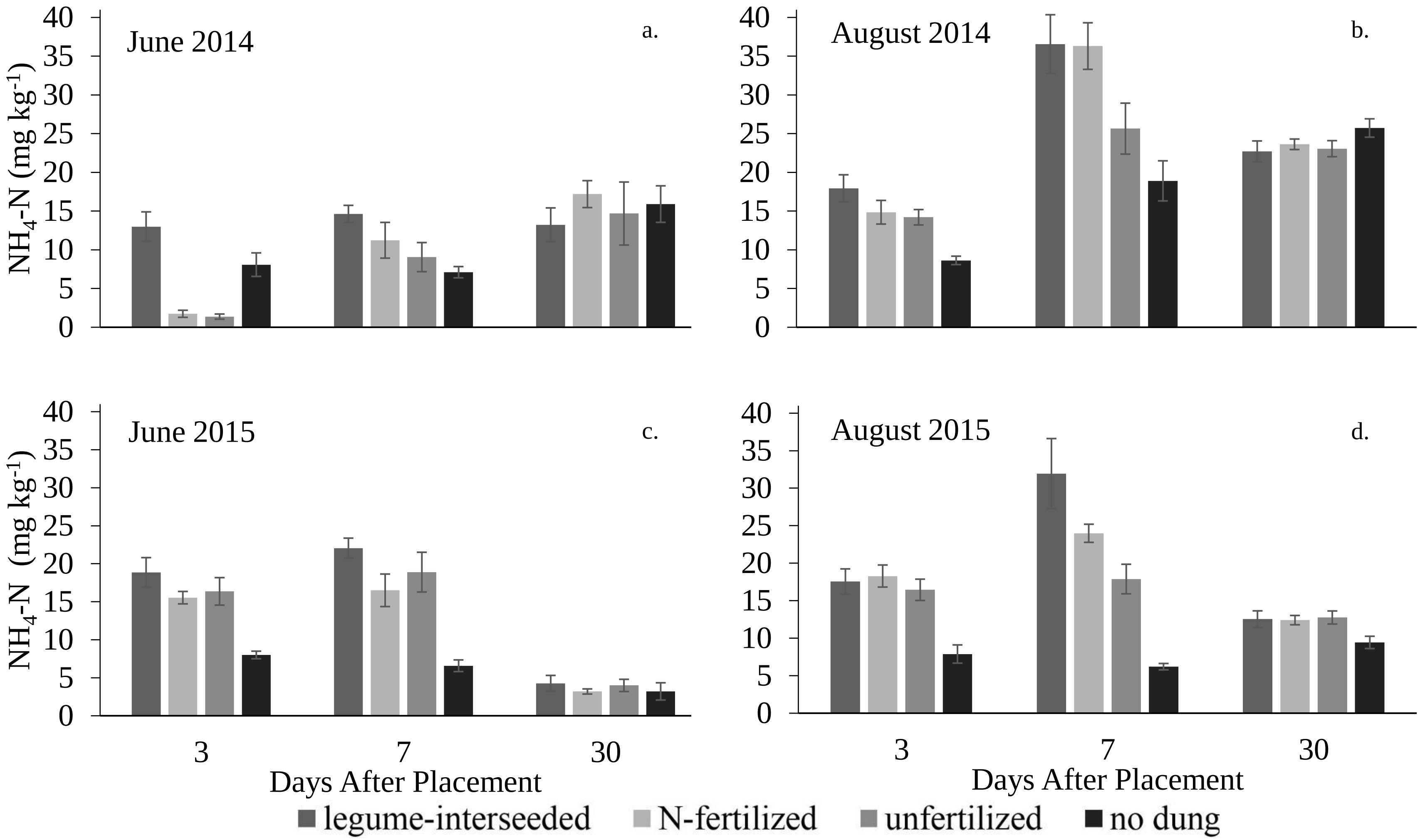

Figure 2-13. Mean NH_4-N from soil under dung of cattle grazing legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pastures and one control (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

Table 2-1. Initial dung traits [dry matter (DM), nitrogen content (N), and C:N ratio] and consumed diet sample traits [nitrogen content (N)] of cattle as affected by time of collection (year and season) and diet of cattle grazing legume-interseeded (LI), N-fertilized (NF), and unfertilized (UN) smooth bromegrass pastures.

			Pasture diet		
Year	Season	Trait	LI	NF	UN
			Dung		
			g kg^{-1}		
2014	June	DM	146	150	159
		N	25.8	23.7	22.0
		C:N	16.5	17.6	18.4
	August	DM	152	147	148
		N	21.1	19.7	18.8
		C:N	19.7	21.4	21.8
2015	June	DM	146	151	138
		N	22.0	19.9	20.9
		C:N	18.6	20.7	19.0
	August	DM	132	141	125
		N	24.7	23.5	19.9
		C:N	17.6	18.6	19.6
			Consumed Diet		
			g kg^{-1}		
2014	June	N	2.3‡	2.3	
	August	N	2.4	2.4	
2015	June	N	2.9	2.6	
	August	N	2.7	2.8	

‡Diet samples were collected from ruminally fistulated steers in another study using only LI and NF pastures.

Table 2-2. Temperature and precipitation at the University of Nebraska Agriculture Research Development Center (ARDC) near Mead, Nebraska. Mean daily high and low temperature, mean 24-hr period temperature, mean daily precipitation, and total precipitation for the experimental periods and months are given. Mean daily high and low temperature, mean 24-hr period temperature, mean daily precipitation, and total precipitation for 30-yr means are given for months of the experiment.

			Temperature			**Precipitation**	
Year	Season	**Experimental period**	**Daily High**	**Daily Low**	**24-hr period**	**Daily Mean**	**Total**
			°C			mm	
2014	June	9 Jun - 9 Jul	28.0	15.0	21.5	41.3	1280
	August	29 Jul - 28 Aug	28.4	16.6	22.5	56.7	1758
2015	June	9 Jun - 9 Jul	27.5	15.6	21.6	56.6	1755
	August	28 Jul - 27 Aug	28.4	15.2	21.8	52.4	1626
1981-2010	June	9 Jun - 9 Jul	29.4	16.5	23.0	36.2	1123
	August	28 Jul - 28 Aug	29.4	16.6	23.0	29.9	958

Table 2-3. Analysis of variance of mean CO_2 flux (μmol CO_2 m^{-2} s^{-1}) from dung of cattle on three grazing diet treatments [legume-interseeded (Legume), N-fertilized (Fert), and unfertilized (Unfert) smooth bromegrass pasture] and one control treatment (no dung) during two years (2014 and 2015) and two seasons [late spring (June) and mid-summer (August)] at Mead, Nebraska.

Effect	df†	F value‡
Year (Y)	1,20	2.79
Season (S)	1,20	0.08
Y × S	1,20	7.97*
Diet (D)	3,60	280.00***
Y × D	3,60	8.00***
S × D	3,60	4.70**
Y × S × D	3,60	3.51*
Orthogonal Contrast¶		
Dung and No Dung		
June 2014	1,15	187.00***
August 2014	1,15	557.00***
June 2015	1,15	85.20***
August 2015	1,15	344.00***
Legume and Fert		
June 2014	1,15	0.98
August 2014	1,15	39.60***
June 2015	1,15	0.53
August 2015	1,15	0.29
Legume and Unfert		
June 2014	1,15	0.43
August 2014	1,15	55.00***
June 2015	1,15	5.94*
August 2015	1,15	18.10***

† Numerator followed by denominator df .

‡ *, **, and *** indicate significance at $P \leq 0.05$, 0.01, and 0.001 probability levels.

¶ Single df orthogonal contrasts that compared effects of dung and no dung, dung from legume-interseeded and N-fertilized pastures, and dung from legume-interseeded and unfertilized pastures on mean CO_2 flux during each year and season.

Table 2-4. Analysis of variance of mean percent dung dry matter remaining (DMrem), total nitrogen (TN), and C:N ratio from dung of cattle on three grazing diet treatments (legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pasture) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

		Effect		F Value		
			df			
				DMrem	TN	C:N
2014	June	Diet	2,40	6.19**†	28.55***	102.26***
		Day	2,40	84.74***	20.62***	262.64***
		Diet*Day	4,40	0.51	1.32	2.76*
	August	Diet	2,55	0.25	106.55***	66.71***
		Day	3,55	29.27***	63.73***	104.30***
		Diet*Day	6,55	0.99	4.25**	4.10**
2015	June	Diet	2,55	4.28*	12.09***	61390***
		Day	3,55	51.32***	52.68***	132.04***
		Diet*Day	6,55	6.29***	2.33*	0.41
	August	Diet	2,55‡	1.53‡	141.48***	14.47***
		Day	3,55‡	73.60***‡	54.56***	72.80***
		Diet*Day	6,55‡	1.41‡	3.06*	1.15

†*, **, and *** indicate significance at ≤ 0.05, 0.01, and 0.001 probability levels.
‡Degrees of freedom for DMrem are 54 instead of 55 due to a lost sample.

Table 2-5. Analysis of variance of mean water extractable C WEC (mg kg^{-1}), water extractable N WEN (mg kg^{-1}), NO_3-N (mg kg^{-1}), and NH_4-N (mg kg^{-1}) from soil under dung of cattle on three grazing diet treatments (legume-interseeded, N-fertilized, and unfertilized smooth bromegrass pasture) and one control treatment (no dung) during two years (2014 and 2015) and two seasons (June and August) at Mead, Nebraska.

Effect	df	F Value			
		WEC	WEN	NO_3-N	NH_4-N
		-mg kg^{-1}-	-mg kg^{-1}-	-mg kg^{-1}-	-mg kg^{-1}-
Year (Y)	1,20	10.98**†	63.53***	103.73***	17.71***
Season (S)	1,20	55.39***	374.77***	61.79**	130.49***
Y × S	1,20	4.38*	84.51**	194.20***	29.51***
Diet (D)	3,60	2.91*	5.46**	12.35***	43.60***
Y × D	3,60	3.32*	1.98	5.14**	7.95***
S × D	3,60	1.24	0.25	5.46**	5.79**
Y × S × D	3,60	2.73	3.66*	10.93***	0.91
Day	2,40	24.71***	82.14***	8.14**	50.47***
Y × Day	2,40	17.71***	3.84*	13.87***	72.60***
S × Day	2,40	44.98***	75.58***	17.17***	14.03***
Y × S × Day	2,40	10.31***	29.21***	18.66***	13.57***
D × Day	6,119	2.29*	1.59	9.76***	17.97***
Y × D × Day	6,119	0.53	1.36	6.34***	2.33*
S × D × Day	6,119	1.89	0.59	6.58***	4.22**
Y × S × D × Day	6,119	0.88	1.68	8.96***	1.43
Orthogonal Contrast					
Dung and No Dung					
June 2014	1,63	0.00	3.38	10.24**	0.03
August 2014	1,63	0.00	3.10	0.46	6.36*
June 2015	1,63	2.03	0.08	0.07	15.00***
August 2015	1,62	0.32	5.91*	7.51**	35.35***
Legume and Fert					
June 2014	1,63	7.85**	0.08	3.35	2.82
August 2014	1,63	1.98	0.51	0.46	0.07
June 2015	1,63	0.18	1.23	0.00	2.02
August 2015	1,62	0.74	0.05	0.18	1.79
Legume and Unfert					
June 2014	1,63	2.27	0.00	0.96	6.11*
August 2014	1,63	0.02	0.28	0.81	2.56
June 2015	1,63	0.01	0.85	0.60	0.72
August 2015	1,62	1.49	0.95	0.54	6.20*

†*, **, and *** indicate significance at ≤ 0.05, 0.01, and 0.001 probability levels.