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QUANTIFYING THE COMBINED EFFECT OF ABIOTIC FACTORS ON THE  
DECOMPOSITION OF ORGANIC MATTER IN SEMIARID GRASSLAND SOILS

by

Elnazsadat Hosseiniaghdam

A DISSERTATION

Presented to the Faculty of

The Graduate College at the University of Nebraska

In Partial Fulfillment of Requirements

For the Degree of Doctor of Philosophy

Major: Agronomy and Horticulture

(Soil Science)

Under the Supervision of Professors Martha Mamo and Haishun Yang

Lincoln, Nebraska

August 2021

# QUANTIFYING THE COMBINED EFFECT OF ABIOTIC FACTORS ON THE DECOMPOSITION OF ORGANIC MATTER IN SEMIARID GRASSLAND SOILS

Elnazsadat Hosseiniaghdam, Ph.D.

University of Nebraska, 2021

Advisors: Martha Mamo and Haishun Yang

Grassland ecosystems play a critical role in the global carbon cycle, and their cumulative carbon dioxide (CO<sub>2</sub>) emissions can be affected by animal grazing where litter (i.e., dead plant material) is trampled into the soil. However, the subsequent effects of trampling on litter decomposition are not well understood. Meanwhile, abiotic factors, especially temperature and soil moisture, are known to strongly affect litter decomposition. Due to the very small number of published studies that have considered litter placement, either litter on top of soil or litter mixed with soil as a factor in litter decomposition under different temperature and soil moisture conditions, reported results are contradictory; thus, conclusions cannot yet be drawn regarding how grazing management can best sustain and improve the soil fertility and productivity through litter deposition in semi-arid grassland system. We hypothesize that the effect of abiotic factors on litter decomposition shifts overtime during the growing season, and litter placement and its interaction with abiotic factors alter decomposition rate and the contribution of soil organic matter (SOM) and litter to total carbon (C) output and remaining C in soil.

It was found that under cool and dry (mimic early season) and warm and dry (mimic late season) conditions, litter decomposition is restricted. Decomposition was slower for the litter on top than the litter mixed into the soil. The frequency of drying-wetting cycles combined with temperature altered the respective proportions of the SOM and litter in the total carbon decomposition. Relationships among litter input, litter

placement, and abiotic factors should be considered as upscaling factors in carbon decomposition models to improve modeling tools and land use strategies to sustain and improve soil organic matter content and thus the fertility of semiarid grasslands.

## DEDICATION

I would like to dedicate my thesis to my beloved brothers, Hassan and Amir Hosseiniaghdam, who are no longer here with us to see my graduation. I lived every moment of my lonely time with your presence in my heart; I love you and miss you so much,

This thesis is also dedicated to my beautiful Mom, who is my angel from heaven,  
Farideh Ghaffariyeh,

To my wonderful brother Dr. Jamal

No words can describe you! You are the backbone of our family during all the hard times. Thanks for being You!

To my amazing twin sister Dr. Solmaz, who has stayed home to care for my family so that I could live my dream life by studying here in the USA, I owe you my life, Soli joon,

To my kind brother Hamid, who has encouraged me whenever I was disappointed,

To my very cute and handsome little nephew Fardad joon, whose heart-melting voice was always inspiring, I have never met you in person; I hope to hug you soon,

To my sweet niece Helya, who is a piece of my heart,

And to my lovely nephews Sina and Erfan, who are my beloved ones.

They have all provided unconditional love and support throughout this academic journey. Without their endless love, sacrifice, patience, and encouragement, I would never have been able to complete my graduate studies.

I love you all, and I appreciate everything that you have done for me.

## ACKNOWLEDGMENTS

I would like to thank Dr. Martha Mamo, my major advisor at the University of Nebraska-Lincoln. Her guidance, encouragement, and support were crucial for my achievements during the time at UNL. Her high standards, in addition to professionalism, pushed me to move out of my comfort zone and made me be a better researcher.

I would like to express my deepest appreciation to Dr. Haishun Yang, my co-advisor, for being a magnificent help during the project. I could not ask for a better advisor. Thank you also for your thoughtful and timely feedback and for providing the opportunity to prosper during the last four years.

I want to extend my sincere thanks to my academic committee members, Dr. Walt Schacht, Dr. Kent Eskridge, and Dr. Michael Kaiser. They have all been more dedicated, more encouraging, and more patient than I could have ever asked.

Special thanks to Dr. Humberto Blanco, who generously offered his lab facilities and supported me in fulfilling my research project.

I very much appreciate Pam Sutton, who treated me as her daughter during the project's ups and downs. She was there for me while I was sick or whenever I needed her.

During the latter part of my journey as a Ph.D. candidate at the University of Nebraska, I was fortunate to work with our Post Doc, Gandura Abagandura, who was truly beyond a friend. She was kind, knowledgeable, and helpful during my thesis preparation. Thank you, Gandura!

Special thanks to my outstanding co-workers, Jennifer, Alex, Mohammad, Leo, Amanda, Salvador, Ashley, and Morgen. I will miss every second of the time we had

together. I wish Covid had not separated us for the last year. Thanks for being such understanding and accommodating colleagues.

Thanks should also go to the administrative team at the agronomy and Horticulture department for their devoted help. I could not accomplish my project without your support.

I would like to pay my special regards to Dr. Jeffrey Collins, who provided me with the tools that I needed to choose the right direction and successfully complete my dissertation. Your valuable guidance pushed me to sharpen my thinking and brought my work to a higher level. Jeff, without your great support and assistance, this work would hardly have been completed. I am honored to know you and our friendship. Jeff, I cannot thank you enough with simple words. I am honored to know you, Jeff.

I would like to express my deepest appreciation to Dr. Iman Kiaei, who always made me feel confident in my abilities after coming off the phone to him. Iman, many thanks for your great help and assistance in completing this work.

When I recall my time in Lincoln, some people have a special place in my heart. Tanya and her beloved family were beyond friends to me. They accepted me as a member of their family with open arms, supporting me during the tough times, and offered their love unconditionally. I love you all! You are the best American family I could wish.

When you move to a new country, you always wish to have your Mom by your side. That was a dream that came true. Annie! You are my American Mom. Thanks for always being there, supporting me, listening to my nonsense nags, and embraced me as your daughter! Love you!

I am very grateful to Aaron and Jamie, who helped me assimilate to Lincoln's new life. It was not possible without you!

I owe my deepest gratitude to my special one, Dr. Ehsan Mostaghni, who tolerated all my nonsense complaints and tears about random stuff; thanks for constantly listening to me rant and talk things out. Thanks for calming me down with your kind words. Ehsan, so many thanks for the sleepless nights we were working and studying together. Thank you for all the fun we have had during the year. Thanks for providing a happy distraction to rest my mind outside of my research. Thank you for always reminding me of the end goal.

Last but not least, I have an amazing family, unique in many ways; they deserve endless gratitude: my mother for teaching me to love people, to be always a thankful person for what I have in my life, my brother Dr. Jamal, for your tremendous support and hope you had given to me, for the strength you gave me; you have been an inspiration for making things 'perfect', my twin sister Solmaz, who is my love, who is in me every moment; your name should be on this thesis as much as mine, my brother, Hamid; thanks for the continuous encouragement and support, and attention, my Lovely niece and nephews, Helya, Sina, Erfan and Frdad, Love you all with every cell of my body. To my family, I give everything, including this.

**Thank God for blessing me surrounded by amazing people. All things are possible  
with you.**



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## CHAPTER 1: Literature review

### Grassland

Grasslands are the most common terrestrial biomes, covering about 40% of the Earth's land area (Bontti et al., 2009; Schimel, 2010). The majority of grasslands exist in semiarid regions (28%), followed by humid (23%), cold (20%), and arid zones (19%) (White et al., 2000). Generally, grassland soils account for approximately 28–37% of the global C storage (Lal, 2004; Risch et al., 2007). Owing to their large area, grasslands are a vital component of global biogeochemical cycles that can provide essential ecosystem services such as nutrient cycling and substantial amount of atmospheric C storage (Jobbágy and Jackson, 2000). As such, a slight change in the amount of soil C in grasslands may considerably influence the global C budget and its related effects on climate change (Song et al., 2012; DeLuca and Boisvenue, 2012).

Changes in climate and overgrazing have contributed to degradation of semi-arid grasslands, which has significantly decreased soil nutrient storage and pasture productivity (Liu et al., 2018; Fan et al., 2019; Yuan et al., 2019). This problem is likely to be compounded in coming years, as it is predicted that global mean temperature and precipitation regimes will change due to the rising atmospheric greenhouse gas concentrations (IPCC, 2007; Easterling et al., 2000). These temperature and precipitation changes can influence ecosystem C cycling and balance, creating a feedback cycle between the global soil C and climate change (Jobbágy and Jackson, 2000; Cox et al., 2000; Luo, 2007; Song et al., 2012). Climate change can also indirectly affect these processes via changing soil temperature and soil water availability (Shaver et al., 2000; Weltzin et al., 2003; Wan et al., 2007).

Animal grazing in grassland ecosystems can move litter into the soil through trampling. However, the subsequent effects on litter decomposition and related CO<sub>2</sub> emissions are not well understood. Due to the limited number of published studies that have considered litter placement, either litter on top of soil or litter mixed with soil, as a factor in litter decomposition and their contradictory findings, no conclusions can yet be drawn regarding how grazing management can best sustain and improve the local soil system. It is hypothesized that soil-litter mixing (due to animal trampling) will significantly enhance the litter decomposition process compared to litter on the soil surface. By measuring CO<sub>2</sub> emissions from the soil with different litter placements under various soil moisture and temperature, the effects of trampling on litter decomposition can be better understood. This would provide guidance on land management and grazing practices to optimize land productivity, soil nutrient cycling, and maximize soil C storage.

### **Soil organic matter**

Carbon in is comprised of about 60% of organic C as soil organic matter (SOM) and 40% of inorganic C pools, such as CaCO<sub>3</sub> (Reeder et al., 2004; Jin et al., 2014; Lal et al., 2015). Soil inorganic C in the form of carbonate minerals derived from parent materials or formed during pedogenic processes is dominant in arid to semiarid ecosystems (Jin et al., 2014; Lal et al., 2015). On the other hand, soil organic carbon (SOC) consists of a wide range of soil organic materials. The SOM is the fraction of the soil that includes soil organisms, plant roots, soil microbes, and plant and animal debris in various stages of decomposition in or on the soil (Baldock and Skjemstad, 1999). In other words, SOM is the partial decomposition of organic materials by microorganisms

(Paul, 2007). Thus, C inputs from plant litter (i.e., shoot and root litter), especially in grasslands, play crucial role in regulating the soil C balance (Kuzyakov and Domanski, 2000; Santos et al., 2016).

### **Plant litter input**

Plant litter from both aboveground and below ground are important sources of C input to the soil (Cotrufo et al., 2013). In different grassland ecosystems, the amount and pattern of litter fall vary depending on the various factors such as season and grazing management (Zhang et al., 2014; Scheer, 2009). Microbial litter decomposition is a critical process involving litter-derived C entering the soil (Cotrufo et al., 2013). The balance of C input amount and decomposition determines the soil C storage (Lange et al., 2015). Newly added C input by litter addition induces a rapid increase of soil microbial biomass and litter decomposition rate (Moore-Kucera and Dick, 2008; Wang et al., 2014). Xu et al. (2016) showed that a high level of microbial biomass and litter decomposition could lead to the effective incorporation of litter-derived C into the soil. Decomposition of extant SOM in response to the increased input of new litter-derived C via "priming effects" might be triggered, resulting in no change in soil C content (Kuzyakov et al., 2000).

Several physicochemical factors play a role in determining the soil's capacity to store soil C. The total amount of SOM present can limit the soil's ability to sequester C as it becomes saturated (Six et al., 2002, Castellano et al., 2015). The capacity of a soil to adsorb organic matter (OM) is also dependent on its mineralogy and surface properties, as soils with the greater surface area have a higher capacity and create a stronger attraction through formation of organo-mineral complexes (Feng et al. 2014; Wiesmeier

et al. 2015). Soil texture also affects soil C capacity, as declines in silt and clay can reduce the occlusion and adsorption of new litter-derived C within the mineral matrix (Dungait et al., 2012; Wiesmeier et al., 2015). The priming effect, induced by higher inputs of plant litter, can also offset soil C storage by promoting extant SOM decomposition (Lajtha et al., 2014; Bowden et al., 2014).

### **SOM turnover**

Although plant litter (i.e., shoot and root litter) is the major source of SOM, litter decomposition and SOM turnover are often considered as separate processes (Sollins et al., 2007). Litter decomposition is defined as the short-term mineralization process through which organic matter breaks down into smaller constituents, CO<sub>2</sub> and nutrients via physical, biological, and chemical pathways (Aerts, 1997; Parton et al., 2007). However, SOM turnover includes physical, chemical, and biological mechanisms that transform OM into stabilized forms and releases C in the form of CO<sub>2</sub> into the atmosphere through heterotrophic respiration (Chandrasekhara, 1997; Schimel, 1995; Wachendorf et al., 1997). SOM turnover is affected by organo-mineral interactions, slowing down the microbial breakdown of organic moieties due to physicochemical protection by mineral association and micro-aggregate occlusion (Parton et al., 2007; Stewart et al., 2008). During the decomposition process, turnover time increases from hours to days and hundreds of years due to the decreasing particle size and residue, which results in organic compounds in the soil becoming associated with inorganic soil particles. Therefore, the application and incorporation of OM into the soil over time can increase the proportion of stabilized SOM. Although there is much debate on the potential of grasslands to act as a sink for C, it is agreed that the balance between the



formation of stabilized SOM and SOM mineralization is a critical contributing factor of the long-term ecosystem C dynamic (Cotrufo et al., 2013; Lehmann and Kleber, 2015). However, this requires a better understanding of the feedback between SOM content, the stabilization of SOM, and microbial activity, all of which contribute to soil's capacity to sequester and store C in grassland soils (Jastrow et al., 2007).

Recent attempts to link litter decomposition and SOM turnover and stabilization by mineral association and aggregate occlusion may have reinforced their separation by implying that (1) low-quality litters (e.g., low nitrogen and high lignin) are not selectively preserved in physiochemically stabilized SOM; (2) most physio-chemically stabilized SOM is derived from microbial residues; and (3) litter quantity rather than quality is the main determinant of the amount of physio-chemically stabilized SOM (Six et al., 2002; von Lutzow et al., 2008; Stewart et al., 2008; Grandy & Neff, 2008; Gentile et al., 2011; Carrington et al., 2012; Dungait et al., 2012).

### **Litter decomposition**

The decomposition of litter, referred to dead plant material on the soil surface or mixed into the soil, is vital for grassland ecosystems' nutrient budget (Krishna and Mohan, 2017). Decomposition is the process through which organic matter breaks down into CO<sub>2</sub> and mineral nutrients via physical, biological, and chemical pathways (Aerts, 1997). Major controlling factors of litter decomposition include temperature and moisture, litter quality, and soil organisms (Aber and Melillo, 1982; Anderson, 1988). These factors interact, and their interactions need to be considered in decomposition models to better predict the organic matter decomposition dynamics (Dwivedi et al., 2019; Luo et al., 2016; Runge et al., 2019). Byproducts of litter decomposition are partly

released as CO<sub>2</sub> to the atmosphere, partially leached through the soil profile as dissolved organic C, and partly incorporated into the soil as organic matter (Bird et al., 2008; Mambelli et al., 2011). Therefore, environmental disturbances, such as changes in temperature, precipitation, management (i.e., input litter quality), and the composition of the microbial community can affect litter decomposition and overall stocks of SOM and nutrients (Berg et al., 1993; Couteaux et al., 1995; Cadish and Giller, 1997; Bohlen et al., 1997; Dechaine et al., 2005). As the decay of litter progresses through time, shifts in the constituents that can regulate the decomposition, such as litter chemical composition, and microbial community structure, can change the decomposition rate (Berg and Staaf, 1981; Kutsch et al., 2009).

### **Factors affecting litter decomposition**

Despite many studies on overall litter decomposition, relatively less information is currently available on factors that control litter decomposition and C cycling in semiarid grassland ecosystems (Jenkinson et al., 1991; Kirschbaum 1995; Bellamy et al., 2005). Generally, litter decomposition is controlled by abiotic factors such as soil physio-chemical characteristics (Coleman et al., 1999; Zhang et al. 2014), climate (Meentemeyer, 1978; Makkonen et al., 2012), UV intensity (Austin and Vivanco, 2006; Austin et al., 2016), as well as biotic features such as litter quality (Melillo et al., 1982; Trofymow et al., 2002; Makkonen et al., 2012; Zhang et al. 2014), and community and composition of soil organisms (Austin et al., 2014; Wagg et al., 2014). Although this study primarily considers the abiotic effects of litter placement in conjunction with temperature and moisture, a brief description of soil characteristics and biotic effects is given first for the sake of comparison.

## **Effect of soil characteristics on litter decomposition**

The potential storage of OC in the soil is affected by soil type. Soil type influences the soil microenvironment characteristics such as permeability, mineral surface area, the portion of the total porosity filled with water/air, and nutrient and water availability. Thus, microbial involved processes such as litter decomposition and SOM turnover are influenced by the soil microenvironment changes (Alexander, 1977; Paul and Clarke, 1989; Hassink et al., 1993; Scott et al., 1996). The decomposition of organic matter is slower in clay soils than in sandy soils. This implies that with higher clay content, more C is protected against decomposition. In support of this, Burke et al. (1989) indicated that SOC increased significantly with clay content. Similarly, Follett et al. (2012) showed that under native grassland, the rate of increases in SOC is about 1,203 kg ha<sup>-1</sup> year<sup>-1</sup> per 1% increase in clay content. Nonetheless, the corresponding rate of increase in SOC per 1% increase in clay content under cropland was 105 kg ha<sup>-1</sup> year<sup>-1</sup>, and under a conservation reserve program was 498 kg ha<sup>-1</sup> year<sup>-1</sup>. In general, the role of soil texture on decomposition is more pronounced through the physical stabilization of OM, the formation of clay-SOM complexes, and cation binding by absorbing and aggregating protect organic C from microbial decomposition (Paul, 1984; Oades, 1995; Krull et al., 2001).

Physical stabilization mechanism protects SOM from microbial decomposition through occlusion within macro- and micro-aggregates (Six et al., 2000a, b; Six et al., 2002; Feng et al., 2014). Furthermore, the organo-mineral complexes resulting from the interactions of organic matter with the mineral surface (adsorption reactions), metal cations, and other organic substances (complexation and precipitation reactions) can also

stabilize SOM by restricting enzyme and microbial access to the substrate (Vanloosdrecht et al., 1990; Scheel et al., 2008). The inaccessibility of organic substrates caused by physical protection of C from microbial attack and extracellular enzymes can be attributed to: (1) the limited microbial movement in the soil due to the sparse and heterogeneous distribution of microorganisms and substrates in soil (Young et al., 2005), (2) small pore sizes less than 0.2  $\mu\text{m}$  in diameter, which are too small and inhibit most bacterial and fungal hyphae from entering, (3) reduced diffusion of oxygen and enzyme into pore sizes of less than 0.5  $\mu\text{m}$  that limit microbial activity (Sexstone et al., 1985; Sollins et al., 1996; Zimmerman et al., 2004), and (4) hydrophobicity of numerous substrates can reduce accessibility (Bachmann et al., 2008). In this context, soil aggregate formation and sizes of aggregates, particle size, and clay minerals are considered crucial processes for increasing the mean residence time of SOM (Six et al., 2002; Yu et al., 2017).

### **Effects of biotic factors on litter decomposition**

At small scales, litter decomposability and microbial decomposer community's composition are considered the dominant biotic factors that affect litter decomposition (Olofsson and Oksanen, 2002; Vivanco and Austin, 2008; McGuire and Treseder, 2009; Strickland et al., 2009; Liu et al., 2010; Austin et al., 2014). Litter quantity and decomposability are the major regulators of OM decomposition, dependent on plant species (Swift et al., 1979; Chadwick et al., 1998; Hattenschwiler et al., 2005). During the decomposition, litter decomposability reduces because of the consumption of readily available labile compounds by decomposers at the early stage of decomposition and consequent accumulation of more recalcitrant compounds over time (Rosenbrock et al.,

1995; Horner et al., 1988; Dilly and Munch, 2001; McClaugherty and Berg., 2008; Argao et al. 2009). The main processes that explain the changes of litter over time are leaching (move of soluble material to a lower soil layer), fragmentation (creating new surface areas for decomposers through the physical breakdown of litter pieces), and chemical alteration (change of the litter composition and structure). Thus, different plant species and the changes in single plant species' chemical structure during the decomposition can alter decomposition rates in the litter decomposability aspect. The most dominant litter traits that could be effectively involved in decomposition dynamics are ash and nitrogen contents and C/N ratios (Krishna and Mohan, 2017). A high amount of ash and nitrogen and low C/N ratios and lignin contents could accelerate decomposition rates (Kucera, 1959; Krishna and Mohan, 2017). On the other hand, as decomposition is governed by microbial activity, microbial composition shifts during the decomposition process (Simpson et al., 2007). Among the soil microbes, fungi and bacteria are the leading decomposers of the OM decomposition process (Kjoller and Struwe, 1992; Dilly and Munch, 2001; Kurihara and Kikkawa, 1986; Persson, 1980). Fungi's C use efficiency is higher than bacteria, which could be the reason for the shift in microbial community structure and adaptation under stress situations (Pascoal and Cassio, 2004; Allison et al., 2010; Manzoni et al., 2012).

### **Effects of abiotic factors on litter decomposition**

It has long been documented that decomposition processes in semiarid grasslands are highly sensitive to climate change (Christensen et al., 2004; Biederman et al., 2017). Increased air temperature and altered seasonal precipitation induced by the global climate change may strongly influence both inputs and losses of organic matter in soils, plant

photosynthesis, plant production, and microbial decomposition, thus changing the soil C cycling and land-atmosphere CO<sub>2</sub> fluxes (Conant et al., 2004; Liu et al., 2016; Lu et al., 2011; Wang et al., 2019). On the global scale, the effect of climatic factors (air temperature and precipitation) on OM decomposition is more pronounced through altering other environmental parameters (e.g., soil moisture, vegetation cover, microbial activity, litter composition) (Kirschbaum, 1995; GarcíaPalacios et al., 2013; Bradford et al., 2016; Djukic et al., 2018). However, at local scales (< 1: 1 000 000), other factors such as litter decomposability, soil organisms, soil water content, topography, and land use might play a dominant role in OM decomposition (Zhang et al., 2008; Prescott, 2010; Chapin et al., 2011; Fang et al., 2012; Lee et al., 2014). This supports the fact that global climate change is multi-faceted (IPCC, 2013), which by interacting with environmental factors, may increase or offset individual factors' effects on soil C (Hobbie 1996; Wardle et al. 2009; Rillig et al., 2019). This underscores the importance of evaluating the interaction effect between the OM decomposition with other local-scale variables (Didion et al., 2016).

### **Temperature**

Studies have shown that SOC decreases with increasing temperature due to increased microbial activity (McDaniel and Munn, 1985; Lloyd and Taylor, 1994) and decomposition rates (Kirschbaum, 1995; Canadell et al., 2007). Soils in warmer climates where the decomposition rate is higher may accumulate less C than soils in cold temperatures (Lal, 2007). This suggests that decomposition could be sensitive to even small temperature changes (IRONS et al., 1994); Davidson and Janssens, 2006; Boyero et

al., 2011). However, under optimum plant growth conditions, higher favorable temperature results in higher plant biomass, leading to more organic C inputs.

Increasing the temperature in the environment with adequate moisture results in faster decomposition of SOM, less storage of C in the slow and passive pools, and greater loss of C through respiration (Canadell et al., 2007). Climate warming by affecting soil aggregation and C stabilization processes may decrease total soil C (Burke et al., 1989; Sala et al., 1996). Similarly, Guan et al. (2018) stated that soil aggregates' stability reduces in response to warming temperature in an alpine meadow. However, Poeplau et al. (2017) reported that increasing temperature could promote mineral-stabilized C in mountain grassland. Warming may also alter the soil microbial community and stimulate the decomposition of microbial compounds that contribute to soil aggregation (Lehmann et al., 2017; Rillig et al., 2002).

### **Precipitation**

Semiarid grasslands are characterized by inadequate and highly variable temporal precipitation distribution and very responsive to precipitation variability (Hawinkel et al., 2016; Knapp and Smith, 2001). It is predicted that the altered pattern of precipitation events in response to global climate change with prolonged drought periods and more intense rainfall events and temperature extremes will influence ecosystem C balance towards further soil C losses (NAST, 2001; Meehl et al., 2007; Smith, 2011; Greve et al., 2014). Temporal variation of rainfall distribution determines the temporal variability of soil moisture and the biological activity in soils. This underlines the importance of the precipitation pattern (i.e., frequency, magnitude, and timing) in C cycling and the relatively large contribution of rain-driven C fluxes to the ecosystem C balance (Knapp &

Smith, 2001; Knapp et al., 2002; Jarvis et al., 2007; Chen et al., 2012; López-Ballesteros et al., 2015). The effect of individual precipitation events on soil CO<sub>2</sub> efflux has been well studied (Cable et al., 2008; Chen et al., 2008; Aanderud et al., 2011; Rey et al., 2017). However, the impact of rainfall pulses on soil C fluxes in dry environments is still largely inconclusive (Knapp et al., 2008; Fay, 2009; Liu et al., 2019). Campos et al. (2017) showed that rainfall could increase soil C persistence by inducing aggregate formation involving C protection. In general, previous studies have shown that in semiarid grasslands, precipitation increases total soil C by regulating the following pathways:

- Increasing biodiversity (Epstein et al., 1996; Zhou et al., 2009)
- Increasing primary production (Sala et al., 1988; Lauenroth and Sala, 1992; Knapp and Smith, 2001; Austin and Sala, 2002; Huxman et al., 2004; Bai et al., 2008)
- Enhancing litter decomposition (Simmons et al., 1996; Austin, 2002; McCulley et al., 2005)
- Increasing aboveground biomass (Bai et al., 2008)
- Increasing shoot/root ratio (Schenk and Jackson, 2002; Mokany et al., 2006)
- Accelerating soil respiration (Huxman et al., 2004; Baez et al., 2012)
- Affecting soil characteristics (Zhou et al., 2009)

However, precipitation has both direct and indirect effects on ecological processes, in which the indirect effect is through complex interactions with other environmental variables (Zhou et al., 2009).



## **Soil moisture**

Soil moisture changes resulting from rainfall may affect SOM decomposition through changes in redox conditions. Under a high moisture content where a large portion of soil pores are filled with water, inadequate O<sub>2</sub> limits microbial activity, and SOM decomposition is reduced (Blodau, 2002). Furthermore, potential redox changes induced by soil moisture can alter microbial community structure and metabolic activity (Peralta et al., 2014, Picek et al., 2000). Nutrient availability to decomposers depends on water content and movement. Reduced diffusion of water and nutrients induced by low moisture content may restrict microbial activity (Kakumanu et al., 2019). Appropriate soil moisture content can affect microbial activity through the following pathways: (1) facilitating the diffusion of soluble organic C substrates to microbes' utilization (Schmidt et al., 2004; Hungate et al., 2007), and (2) improving plant production, belowground C allocation, and C substrate availability (Zak et al., 1994).

## **Drying/wetting cycles**

In semiarid grasslands, drying and wetting of soil is a typical process caused by episodic precipitation events affecting microbial decomposition and C cycling (Xiang et al., 2008; Fierer and Schimel, 2003). It has been demonstrated that immediate wetting of the dry soil can rapidly cause a short-term increase of up to five-fold in soil CO<sub>2</sub> effluxes compared with continuously moist soil (Fierer and Schimel, 2003). Hence, in these ecosystems, the boosted pulse of soil CO<sub>2</sub> stimulated by drying-wetting cycles may comprise a significant proportion of the total annual soil CO<sub>2</sub> effluxes (Fierer and Schimel, 2003; Miller et al., 2005). Studies on this topic have mainly focused on the effect of drying and wetting cycles on living microbial biomass, activity, and community

composition (Cosentino et al., 2006; Gordon et al., 2008; Bapiri et al., 2010; Schmitt et al., 2010). Soil drying restricts microbial activity and biomass growth (Halverson et al., 2000). Warming temperature along with dry soils decreases the microbial C use efficiency (CUE), limiting the microbial biomass and enhancing respiration and soil C loss. Thus, a shift in microbial community structure and adaptation such as a change in the fungal to bacterial ratio may offset the reduced CUE effect and promote microbial biomass and soil C sequestration (Jensen et al., 2003; Allison et al., 2010; Manzoni et al., 2012). However, the soil's rapid wetting increases microbial activity and biomass by promoting substrates' availability and utilizing a series of substrate resources by decomposers. These substrate resources include lysing dead microbial cells, releasing intracellular osmolytes (Schimel et al., 2007), breaking down the aggregates, and protected organic matter (Butterly et al., 2009; Fierer and Schimel, 2003; Guo et al., 2012; Zhao et al., 2010). In support of this, Jenerette and Chatterjee (2012) showed that the microbial response to a drying-wetting event is stimulated by soil wetting but is mainly controlled by resource limitation.

The magnitude of CO<sub>2</sub> pulses induced by immediate wetting changes with the number of drying-wetting cycles; a more significant pulse is produced by the first drying-wetting cycle, while subsequent drying-wetting cycles have a reduced effect or no effects on CO<sub>2</sub> pulses (Mikha et al., 2005; Yu et al., 2014). Incubation studies under multiple drying-wetting cycles have demonstrated that C amendment in the soils can alter the impact of subsequent drying-wetting cycles on soil microbial biomass and soil respiration (Shi and Marschner, 2017).

### **The combined effect of abiotic factors**

The effects of temperature and precipitation on litter decomposition have been reported. Some studies demonstrated that increasing temperature and precipitation through altering the soil microenvironment and reducing thermal and moisture limitations on the microbial activity could accelerate litter decomposition (Meentemeyer, 1978; Hobbie, 1996; Luo et al., 2009; Smith et al., 2010). Moreover, the combined effect of temperature and precipitation on plant photosynthesis, growth, and belowground C input as an available substrate source for microbial activity can regulate litter decomposition. Peterjohn et al. (1994) indicated that diminished soil moisture resulted from high temperature and evaporation could limit microbial activity. Furthermore, the interaction effect of pH and soil moisture on the decomposition OM has been recognized under wet and low pH conditions, where OM decomposition is lower than in dry and acidic soils (Bradford et al., 2016; Elumeeva et al., 2018; Petraglia et al., 2018).

### **Grazing effects on litter decomposition**

SOC cycles are also affected by land management practices, such as livestock grazing (Shang et al., 2014). In grasslands, grazing is the most important land use, either by directly altering the plant species composition and soil decomposer community or indirectly by changes in soil environment conditions such as temperature and water availability, affecting litter decomposition (Solly et al., 2014). There are three simultaneous pathways of grazing effects on SOM: (1) altering the magnitude of net primary production (NPP) and its allocation to below or aboveground organs, (2) changes in nitrogen stocks, and (3) changes in organic matter decomposition (Gervasio Pineiro et al., 2010). Only the decomposition pathway was considered in this study.

Animal grazing reduces standing plant biomass, which leads to physical degradation of litter by UV solar radiation, especially in arid zones, and thus increases C loss (Austin and Vivanco, 2006). The reduction in soil cover by the plant can increase the temperature at the soil surface and subsequent moisture evaporation rate (Collins et al., 1998; Sjogersten et al., 2012; Frank et al., 2018). Altering soil microclimates can affect SOC content (Bremer et al. 1998; Burke et al. 1998) by changing the soil microbial community composition at the soil surface (Frank et al., 2003; Grayston et al., 2004; Young and Ritz, 2005). Furthermore, grazing increases soil bulk density, thus reducing water infiltration rates and increasing soil moisture variations (Abdel-Magid et al., 1987; Savadogo et al., 2007). Thus, soil management strategies can be selected to improve organic C concentrations (Brevik and Homburg, 2004; Lal, 2008; Warren, 2010).

### **Trampling**

Trampled materials induced by grazing consist of tillers from the current growing season (live) that had been broken off from the base of the plant (i.e., loose) or that remained attached but bent to a 45° angle or less from the soil surface (reference Aaron thesis). The litter, which is derived from trampling and senescence, is defined as dead plant material directly in contact with the soil surface that had become physically detached from standing vegetation (Guretzky et al., 2020). Litter mixed into the soil by trampling, which provides a significant source of nutrients for plants in semiarid grasslands with a relatively low level of plant litter input, and without inorganic nutrients added by fertilizing (Parton et al., 2007; Schlesinger and Bernhardt, 2013). Litter mixing induced by trampling into the soil can moderate soil moisture and temperature variations (Molinar et al., 2001). Due to the critical role of litter decomposition in regulating the

grassland soil nutrients cycling, there is an interest in studying its decomposition process in these ecosystems (Bornman et al., 2015). While these factors have been shown to be some of the most important controlling factors of litter decomposition in the terrestrial ecosystems (Meentemeyer, 1978; Vitousek et al., 1994; Cou<sup>^</sup>teaux et al., 1995; Austin and Vitousek, 2000; Gholz et al., 2000; Cornwell et al., 2008; Bontti et al., 2009), soil-litter mixing (SLM) represents another critical component affecting SOM decomposition (Chapman et al., 2013; Barnes et al., 2015).

Adair et al. (2008) stated that improvement in understanding dryland biogeochemical cycling depends on resolving discrepancies between decomposition rates measured in drylands and those predicted by models based on variables known to be important in these ecosystems. Barnes et al. (2015) suggested these discrepancies might be due to the influence of abiotic drivers such as soil-litter mixing (SLM) and solar radiation on plant litter decomposition. This underlines the need to evaluate the effect of litter placement on decomposition under different abiotic factors. Once the litter is in close contact with the soil surface, it will be covered with loose soil, but over time, soil films consisting of soil particles, microbes, and microbial exudates develop and adhere to the litter surface (Barnes et al., 2012; Hewins et al., 2013). These soil films, which appear to be composed of inorganic and biological constituents with fungal hyphae and microbial exudates, are dynamic and may degrade in response to temperature and moisture conditions (Hewins and Throop, 2016). Recent studies have suggested that soil-litter mixing can enhance the decomposition process by creating more favorable conditions for the development and activity of microorganisms or by changing the

microbial community composition (Throop and Archer, 2007; Barnes et al., 2012; Hewins et al., 2013; Vos et al., 2011; Chapman et al., 2013).

Soil-litter mixing can also modify microclimatic conditions (Makkonen et al., 2013). It acts as a vector for microbial colonization of litter surfaces, which increases the surface area available for microbial colonization leaching (Throop and Archer, 2009). These effects could enhance decomposition by extending windows of opportunity for microbial activity following rainfall events (Cable et al., 2011). Indeed, soil–litter mixing strongly enhanced C mineralization in laboratory experiments when the soil–litter matrix was subjected to drying–wetting cycles (Lee et al., 2014). Enhanced microbial colonization of recently detached litter may be offset by the negative effects of solar UV on microbes, but subsequent soil coverage, either as an adhering soil film or as loose soil, could partially and eventually fully shield litter from UV radiation and therefore ameliorate its adverse effects (Cockell et al., 2003; Barnes et al., 2012). Soil-litter mixing can occur through different mechanisms in grasslands. For example, high stocking density rotational grazing (so-called mob grazing), a common grassland grazing management, can increase trampled plant material into the soil by intense hoof action (Hart et al., 1993). However, there are conflicting results from studies that sought to understand the effect of mob grazing on litter decomposition. Some claimed that trampling more quickly mixes and moves the litter into the soil, increasing SOM, microbial activity, and nutrient cycling (Savory and Parsons, 1980; Hart et al., 1993). In contrast, other studies reported that mob grazing did not increase SOM, which might be caused by enhanced litter decomposition (Guretzky et al., 2020; Dunn, 2013; Beckman, 2014).

## **Effects of litter decomposition on the physical and chemical environment**

The effects of litter decomposition on the environment in semiarid grasslands can be summarized as follows: The litter layer reduces soil temperature by decreasing the soil thermal amplitude through accumulating litter interception of light and shading seedlings (Krishna and Mohan, 2017). Litter also reduces the soil evaporation by acting as a barrier to water vapor diffusion (Argao et al., 2009). The litter also retains more rainfall, thus reducing water availability to soil microbes (Vitousek and Sanford 1986). Litter fall can alter the microbial community structure because it is comprised of various plant species. In turn, each species' microbial activity might be impacted by the interaction performance of the other species (Chapman and Koch 2007; Melo et al. 2013). By providing fresh C to decomposers, litter enhances the decomposition process and increases CO<sub>2</sub> fluxes (Krishna and Mohan, 2017). Ultimately, by increasing the nutrient return to the soil, litter fall plays a crucial role in nutrient cycling of the ecosystems (Krishna and Mohan, 2017).

## **Modeling**

Given the complexity of the organic matter decomposition process, several mathematical models have been proposed to predict organic matter decomposition (Burke et al., 2003; Moorhead et al., 1996). Approaches vary greatly in spatial and temporal scales considered based on experimental data obtained (Kiehl and Ramanathan, 1983; Spangler et al., 2009). The models' temporal scale ranges from hours (Yuste et al., 2018) to years (Yang and Janssen, 2000; 2002). In turn, the choice of modeling strategy depends on the particular research objectives and the complexity of experimental data on which the model is based.

First-order reaction kinetics is often used as a basis for developing decomposition models, where the amount of decomposing residue is assumed to be related to the total amount of residue present at a given time in the process (Whitmore, 1996). However, the need to explicitly account for microbial activity and its complex effects on decomposition has led to the recent development of more complex second-order models, several of which were reviewed by Campbell and Paustian (2015). For steady-state environmental conditions like in many incubation experiments that primarily consider abiotic factors, it was found that first-order reaction models are better at predicting OM decomposition (Lawrence et al., 2009). Studies that considered complex C pools with the inclusion of microbial mass found that second-order models were needed to describe changes in decomposition rate, as microbes consume nutrients as nutrients become available during decomposition and result in non-linear changes in reactant concentration over time (Blagodatsky, 2010).

### **Reactivity and its decay over time**

During the first several weeks, the decomposition reactivity is typically high, but the reactivity becomes more predictable after this period when it demonstrates logarithmic decay over time. The initial decomposition period is typically treated separately for modeling purposes due to this very different behavior (Yang and Janssen, 2002).

Time-based models used to predict long-term SOM dynamics generally do not apply to this initial period of high reactivity. Some models attempted to predict initial decomposition behavior based on total soil respiration (Cook and Orchard, 2008). However, Yang and Janssen found better agreement by applying regressions to the



average mineralization rate  $k$  (units of  $\text{time}^{-1}$ ) rather than total soil respiration. Multiple substrates can be present under a given set of environmental conditions, each with its unique  $k$  values. Complex multi-component models consider  $k$  from each constituent element and environmental conditions, which can be accurate but difficult for average users to implement. Mono-component models attempt to find a single representative  $k$  that captures the behavior of the bulk substrate but often trade accuracy for simplicity.

To develop a model that was both accurate and simple to use, Yang and Janssen proposed a mono-component model to predict C mineralization by considering changes in  $k$  over time, thereby requiring only the initial reactivity of the substrate as an input (Yang and Janssen 2000). This initial decomposition rate was found to have a strong correlation with its slowdown over time, which allowed proper model coefficients to be determined without considering the specifics of mixtures and environmental conditions. The model was later adapted to describe a large data set with wide geographic and soil composition variations (Yang and Janssen, 2002). By classifying data into four ranges based on the magnitude of initial reactivity, it was found that regression models for each range could accurately predict mineralization over time. Temperature corrections were also applied within the model to reduce the number of unique equations needed to describe the data set with only minor penalties inaccuracy.

### **Temperature and moisture effects**

The effects of soil moisture and temperature on decomposition are understood reasonably well; however, the interactive effect of both soil moisture and temperature on different litter placement (on top/mixed into the soil) is still largely unknown (Ceccon et al., 2011; Suseela et al., 2012). Rodrigo et al. (1997) considered temperature and

moisture effects on the initial reactivity of C-N transformations using nine separate models. Linear relationships between mineralization rates and soil moisture showed poor agreement for dry climates, while temperature effects were best captured using Jenkinson's (1990) and Kirschbaum (1995) models. None of the models explicitly captured interactions between temperature and moisture.

Interactions between time, temperature, moisture, and oxygen concentration were studied to determine their effects on total C respiration over 35 days by Sierra et al. (2017). The same temperature correction used by Yang and Janssen (2000) was employed, while moisture and oxygen effects were added in terms of their respective half-saturation constants. Results showed a significant increase in decomposition rate with temperature when moisture and oxygen levels were not limiting and that moisture effects were significant when oxygen levels were not limiting. In another study on the effects of time and moisture on soil respiration from roots and microbial activity, Cook et al. (2008) found that time effects were best captured using a power function, while moisture effects could be described using linear regression.

### **Synopsis: Summary of gaps in knowledge, overall goals, and specific research objectives**

In semiarid grassland with no inorganic input, plant growth and microbial activities depend on soil nutrient availability provided by OM decomposition (Sinsabaugh et al., 1993; Parton et al., 2007). The sources for organic matter in soil are plants, animals, and microorganisms among which the plant is the main contribution to OM. Therefore, C inputs from plant litter, especially in semiarid grasslands, play a critical role in regulating the soil C balance (Krishna and Mohan, 2017). Litter layer

reduces the soil's evaporation by acting as a barrier (Argao et al., 2009). The litter layer also retains more water, thus reducing water diffusion into the soil and decreasing water availability to soil microbes (Vitousek and Sanford 1986). Litter is comprised of various plant species; thus, it can alter the microbial community structure (Chapman and Koch, 2007; Melo et al., 2013). By providing fresh C to decomposers, litter improves the decomposition process and increases CO<sub>2</sub> fluxes from the soil to the atmosphere (Krishna and Mohan, 2017).

Litter mixing induced by trampling into the soil can modify microclimatic conditions (Makkonen et al., 2013). It acts as a vector for microbial colonization of litter surfaces, which increases the surface area available for microbial colonization (Throop and Archer, 2009). This research aims to evaluate the combined effect of soil moisture, temperature, and litter placement on OM decomposition. While much research has been devoted to observing OM decomposition and its response to several variables, including temperature, moisture, and microbial activity, the role of litter placement in the soil (i.e., on top, mixed under the surface) needs clarification. In order to better understand how land management and grazing strategies can impact total CO<sub>2</sub> emission from the soil, the effects of litter placement are clarified here in conjunction with changes in temperature and moisture. This is particularly important as grazing can increase surface litter deposition. Thus, by comparing the SOM decomposition rates between soil with litter added on top (non-trampled) and mixed (trampled), land management strategies in grassland ecosystems can be modified to maintain and/or increase soil C storage based on the local climatic conditions.

In the first experiment (Chapter 2), we determined the combined effect of temperature, moisture, and soil-litter mixing on litter decomposition to clarify the ambiguity litter placement. In the second experiment (Chapter 3), we evaluated the combined effect of wet/dry cycling, soil temperature, and litter addition on the soil surface. Ultimately, in Chapter 4, we quantified and modeled the combined effect of abiotic factors and litter placement on SOM decomposition rates.

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## **CHAPTER 2: Mixing litter with soil enhances organic matter decomposition rate**

### **Abstract**

Semiarid grasslands contribute significantly to global soil carbon (C) storage. C loss from these systems via microbial decomposition is controlled by abiotic and biotic factors such as soil moisture and temperature and C input (e.g., plant litter, root exudates, dead roots, dung, or urine). Plant litter in these systems can be present above the soil surface or mixed with surface soil by trampling, especially in intensively grazed areas. Despite the importance of litter decomposition for ecosystem nutrient cycling and soil fertility, it is still not well understood how this biogeochemical process is affected by the combination of abiotic factors. A quantitative understanding of abiotic factors' interactive effect is critical for predicting soil C dynamics in response to grassland management and changes in environmental conditions. We conducted a 3-month laboratory incubation experiment to quantify the effect of soil moisture and temperature and the placement of litter on soil organic C losses under simulated Nebraska semiarid grassland conditions. The treatments included 3 litter placements (no litter, litter on top, and litter mixed with surface soil), 3 soil moisture levels (23, 37, and 50% water-filled pore space), and 3 temperatures (10, 20, and 30°C). Cumulative carbon dioxide (CO<sub>2</sub>) fluxes, as an index of organic matter (OM) decomposition, were measured every two weeks. Cumulative CO<sub>2</sub> fluxes remained nearly constant and similar among treatments at 10°C. The treatment with litter on top showed increasing (+8.5%) and decreasing (-1.8%) cumulative CO<sub>2</sub> fluxes at 20 and 30°C, respectively. Mixing litter with soil increased the cumulative CO<sub>2</sub> flux by 24% and 19.5% at 20 and 30°C, respectively. At the temperature of 20°C and 37% WFPS, soil-

litter mixing resulted in 1.2 times higher cumulative CO<sub>2</sub> than the litter on top and control treatments. Also, soil-litter mixing compared with litter on top showed a 14.3% and 21.6% increase in cumulative CO<sub>2</sub> at temperatures of 20 and 30°C. The results support the hypothesis that soil-litter mixing mitigates abiotic constraints on microbial activity and that increased accessibility of the soil decomposers to litter fragments results in elevated CO<sub>2</sub> emissions. Consequently, the litter placement effect on OM decomposition regarding alterations in abiotic factors should be considered an upscaling factor in decomposition models to improve predictions in soil C dynamics in grassland ecosystems.

## **Introduction**

In semiarid grasslands with no fertilizer application, litter and root decomposition has a significant role in the biogeochemical cycling of nutrients (Sinsabaugh et al., 1993; Parton et al., 2007), although the factors controlling litter decomposition may be different from those controlling root decomposition (Dong et al., 2020).

Litter decomposition and soil organic matter (SOM) turnover are often considered separate processes (Sollins et al., 2007). Litter decomposition includes the breakdown of OM into CO<sub>2</sub> and nutrients via physical, biological, and chemical pathways. Soil organic matter turnover relates to organo-mineral interactions slowing down the microbial breakdown of organic moieties due to physicochemical protection by mineral association and micro-aggregate occlusion, resulting in the buildup of soil organic carbon (SOC) stocks (Parton et al., 2007; Stewart et al., 2008). Changes in decomposition dynamics can result from environmental disturbances, such as changes in climate or management. Over the last decades, there has been an increasing need to understand factors that control litter

decomposition and C cycling in semiarid grassland ecosystems (Kirschbaum 1995; Bellamy et al., 2005). Several studies have documented that litter decomposition depends on abiotic factors such as temperature and moisture, as well as biotic features such as litter quality and community structure of soil organisms (Dechaine et al., 2005; Cleveland et al., 2014; Lehmann & Kleber, 2015; Campbell et al., 2016; Tamura et al., 2017). In most ecosystems, the temperature is the dominant factor influencing litter decomposition (Meentemeyer 1978; Hobbie 1996). In contrast, studies have shown that in semiarid ecosystems where water is a limiting factor, soil moisture availability is the main factor controlling decomposition (Zhang et al., 2008; Gray & Fierer, 2012). However, relative contributions of soil temperature, moisture, plant litter input, and grazing management, exerting control simultaneously in semiarid grasslands, need to be clarified (Hibbard et al., 2005; Wang and Fang, 2009).

Recent studies have suggested that soil-litter mixing can enhance OM decomposition by creating more favorable conditions for the development and activity of microorganisms or by changing the microbial community composition (Throop and Archer, 2007; Barnes et al., 2012; Hewins et al., 2013; Vos et al., 2011; Chapman et al., 2013). Soil-litter mixing can also modify microclimatic conditions (Makkonen et al., 2013) and acts as a vector for microbial colonization of litter surfaces (Throop and Archer, 2007, 2009). Soil-litter mixing can occur through different processes in grasslands. For example, high stocking density rotational grazing, a so-called mob grazing practice, can increase translocation of trampled plant material into the soil by intense hoof action (Hart et al., 1993). It has been claimed that intensified soil-litter

mixing derived from mob grazing will result in an increase in SOM, microbial activity, and nutrient cycling.

Additionally, decomposition of litter reportedly results in more fertile and active soils, which produce more vegetation (Li et al., 2013; Peterson, 2014). However, several studies reported that mob grazing did not increase SOM, which might be triggered by enhanced litter decomposition due to more favorable site-specific abiotic conditions for the microbial community (Guretzky et al., 2020; Dunn, 2013; Beckman, 2014). Thus, we aimed to clarify the combined effect of abiotic factors and soil-litter mixing on litter decomposition under simulated soil moisture and temperature conditions similar to semiarid environments. We hypothesized (1) that litter mixing with soil will increase the amount of water retained in its structure, facilitating enhanced water supply for microorganisms in semiarid ecosystems and increasing litter to soil contact, increasing litter decomposition. Despite water being considered a controlling factor of decomposition under water-limited conditions, we expected (2) that the litter layer will provide a continuous water source to decomposers due to its positive effects on water retention, causing the temperature to become the main controlling factor. We conducted a laboratory incubation experiment under growing season conditions typical of semiarid Nebraska grassland ecosystems to test and evaluate these hypotheses. This design enabled us to assess the effect of temperature and soil moisture on CO<sub>2</sub> fluxes from the soil where litter was placed on top or where mixed into the surface at different soil temperature and moisture conditions.

## Materials and Methods

### Location of Collected Soil and Litter Samples

Soil samples were collected in September 2018 from the University of Nebraska-Lincoln Barta Brothers Ranch (42°13' N, 99°38' W) located in the eastern Nebraska Sandhills. The climate of the sampling site is semiarid, with annual precipitation ranging from 430 to 580 mm and temperature averages of ~10°C. The growing season is from April through September, which receives nearly 75% of annual precipitation. The soil sampling location is a sub-irrigated meadow experimental site under short duration, high stocking density, rotational grazing management (Table A-1). The meadows are typically flat, with slopes of 0 to 3 percent. Due to the rising water table, meadows are seasonally wet in early to late spring. Soils are sandy to fine sandy loams in the Els-Valentine-Tryon association and Loup series (mixed, mesic Aquic Ustipsamments; mixed, mesic typic Ustipsamments; Mixed, mesic Typic Psammaquents; Sandy, mixed, mesic Typic Endoaquolls), containing 76, 14, and 10% sand, silt, and clay, respectively (NRCS, 2019). At the 0-10 cm depth of soil, the soil pH was measured 6.1 using the procedure given by McLean (1982). Organic C concentration was determined 2.8% by combustion (LECO Corporation, Saint Joseph, Michigan, USA) after pretreating the soil with H<sub>2</sub>SO<sub>3</sub> to remove inorganic C (Nelson and Sommers, 1996). Vegetation in the study area is dominated by grasses, redbent (*Agrostis stolonifera*), big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), prairie cordgrass (*Spartina pectinata*), smooth brome (*Bromus inermis*), kentucky bluegrass (*Poa pratensis*), and sedges. A wide variety of forbs and shrubs is ubiquitous.



### **Soil and Plant Litter Sampling**

The sampling site is a 6.8-ha field that was divided into 4 subblocks. Before soil sampling, surface litter and aboveground vegetation were removed by hand to minimize error from surface litter in soil samples. Ten soil subsamples per subblock were collected from 0-10 cm soil depth using a shovel. Subsamples per subblock were mixed to make a composite sample (four composite soil samples in total). Stones and plant materials were picked out by hand in the lab and sieved to pass through a 2-mm mesh sieve to obtain the < 2-mm fraction. Surface plant litter materials were also collected at the same time, placed in paper bags, and transferred to the lab. Samples were dried at 60°C and ground to pass through a 0.5 cm sieve to maximize size uniformity. Total nitrogen (N)  $16.1 \text{ (g kg}^{-1}) \pm 0.04$  (n=4) and C content  $400 \text{ (g kg}^{-1}) \pm 0.33$  (n=4) of litter were determined by dry combustion C analyzer (LECO Corporation, Saint Joseph, Michigan, USA).

### **Controlled Environment Incubation**

Soil column mesocosm in different treatment combinations of moisture and litter placement was set up for a period of 3 months. The experimental design was a split-block with temperature and block striped across each other and with litter placement and soil moisture as the split-plot factors. The treatment factors were: three temperatures at 10, 20, and 30°C, three litter placements: control (no litter), litter addition on the soil surface (on top), and soil-litter mixing into the uppermost 4 cm of the soil (mixed), and three levels of soil moisture with 23, 37 and 50% of water-filled pore space (WFPS). The selected temperature and soil moisture represented the ranges observed in the field during the 2014 and 2015 growing seasons at the site. To prepare soil mesocosm for the incubation, air-dried soil, 137 g was incrementally filled into acrylic cylinders to result in

the field observed surface soil (0-10 cm) bulk density of  $1.18 \text{ g cm}^{-3}$ . The cylinders were 3.81 cm in diameter and 22 cm in height. Four replicates per litter and moisture treatment combination were incubated for 91 days (growing season length) in growth chambers (Conviron A2000 PG; with  $2\text{-m}^3$  growth volume and  $1.05\text{-m}^2$  shelf area) set at 10, 20, and  $30^\circ\text{C}$  ( $n = 36$  cylinder for each chamber). The six-year end of grazing season annual (2012 to 2017) average litter, a mixture of dominant grasses at the sampling site, was  $1049 \text{ kg ha}^{-1}$ , equivalent to  $120 \text{ mg}$  per  $11.4\text{-cm}^2$  column base area. The litter was then placed above the soil surface or mixed within the top 4 cm of soil (Figure 2-1). In order to adjust the soil moisture content of each cylinder to 23, 37, and 50% WFPS (11, 18, and 26% volumetric moisture), 12, 21, and 29 ml of tap water was added to the air-dried soil (4% WFPS). Each cylinder was weighted during the incubation, and water was added every 15 days to maintain the target soil moisture level (Table 2-1).

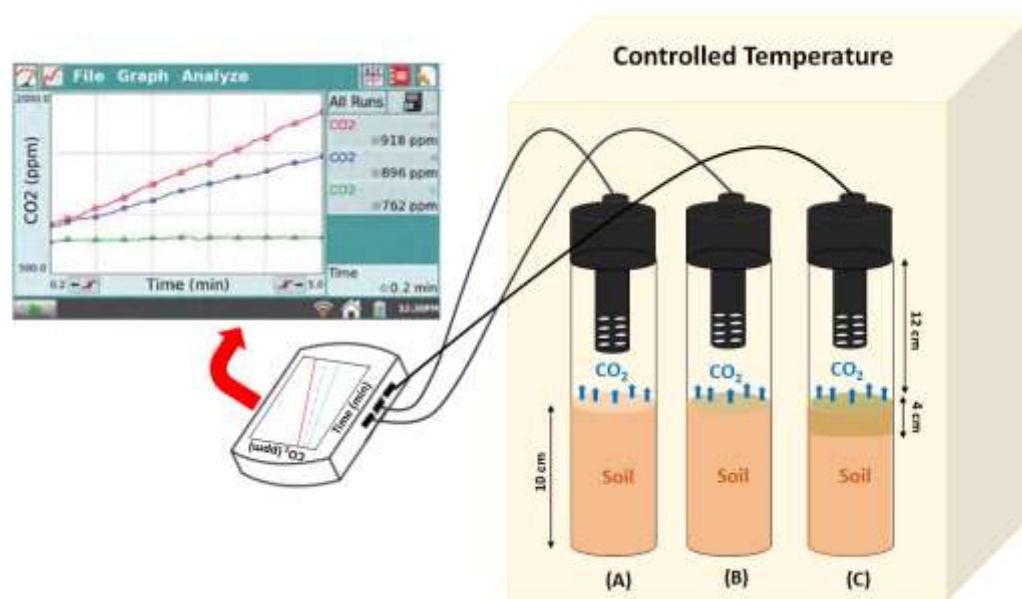


Figure 2-1. Schematic diagram of CO<sub>2</sub> measurement by using sensor (A) control; (B) litter on top; and (C) litter mixed.

Table 2-1. Average daily water evaporation (g) and standard errors from each treatment combination of moisture (water-filled pore space %) and litter (no litter, litter on top, litter-mixed) at three temperatures (10, 20, and 30°C).

	23% WFPS	37% WFPS	50% WFPS
<b>10°C</b>			
<b>No litter</b>	0.17 ± 0.11	0.19 ± 0.13	0.24 ± 0.16
<b>Litter on top</b>	0.18 ± 0.11	0.24 ± 0.13	0.27 ± 0.16
<b>Litter-mixed</b>	0.18 ± 0.11	0.21 ± 0.13	0.21 ± 0.14
<b>20°C</b>			
<b>No litter</b>	0.16 ± 0.06	0.19 ± 0.09	0.21 ± 0.10
<b>Litter on top</b>	0.15 ± 0.07	0.17 ± 0.08	0.18 ± 0.10
<b>Litter-mixed</b>	0.16 ± 0.06	0.18 ± 0.08	0.19 ± 0.09
<b>30°C</b>			
<b>No litter</b>	0.55 ± 0.07	0.64 ± 0.05	0.91 ± 0.08
<b>Litter on top</b>	0.56 ± 0.08	0.62 ± 0.06	0.86 ± 0.07
<b>Litter-mixed</b>	0.63 ± 0.08	0.74 ± 0.08	0.82 ± 0.07

### Carbon Dioxide Flux Measurements

Carbon dioxide (CO<sub>2</sub>) fluxes, as a proxy for organic C decomposition, were measured on day 1, 16, 31, 46, 61, 76, and 91 of incubation using a Vernier LabQuest 2, CO<sub>2</sub> Gas Sensor (Vernier; Beaverton, Oregon, USA). This sensor measures the amount of infrared radiation absorbed by CO<sub>2</sub> molecules. The sensor upper limit is 10,000 ppm if set to the "Low Range" and 100,000 ppm if set to the "High Range" (The low range was used in this study). The accuracy and resolution at the low range setting are ±100 ppm and ±3 ppm, respectively. To improve the accuracy of the readings, the sensor was calibrated between readings at different temperatures using outdoor atmospheric air as a reference, per manufacturer recommendation.

To measure CO<sub>2</sub> concentration, the sensor was inserted and sealed the cylinder. The CO<sub>2</sub> sampling rate and duration were set at one sampling per second for 5 minutes

for a total of 300 records for each cylinder. The CO<sub>2</sub> concentration of the incubation chamber ambient air was also measured at each sampling time. The amount of CO<sub>2</sub> in the chamber ambient air was subtracted from that of the treatment to calculate the CO<sub>2</sub> flux from the soil and litter. The CO<sub>2</sub> flux was calculated as the change in CO<sub>2</sub> concentration over time (5 minutes) and was expressed as kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup>. Because of the sensor's high sensitivity to low temperature (10°C) and consequent variations in CO<sub>2</sub> readings, we frequently calibrated the sensor to improve the readings' accuracy at that temperature. Generally, a common underestimating error of all closed chamber systems can be explained by suppressing surface flux caused by the increase in CO<sub>2</sub> concentration in the headspace and consequent reduction in the diffusion gradient of CO<sub>2</sub> (Healy et al., 1996). Cumulative CO<sub>2</sub> emission, at the end of the 91 days, was obtained by linear interpolation of the CO<sub>2</sub> flux data between successive measurements (Zibilske, 1994). Litter-derived cumulative CO<sub>2</sub> emission was calculated as the difference between total cumulative CO<sub>2</sub> (soil+litter derived CO<sub>2</sub>) from the control treatment (soil only).

### **Statistical Analyses**

Effects of litter, moisture, and their interactions on cumulative CO<sub>2</sub> emission from the soil and litter were tested separately under three different temperatures using PROC MIXED in SAS 9.4 with mixed model procedures for a split-block design with temperature and block stripped across each other with litter placement and soil moisture as the split-plot factors followed by a pairwise differences test (adjusted by Tukey) (SAS Institute Inc., Cary, NC). We considered treatments as fixed factors and replication as a random factor. Note that temperature had no replicates then no statistical test was done on temperature effect. Significance was considered at  $p \leq 0.05$  in all analyses.

## Results

### Effects of litter placement, soil moisture, and temperature on total soil CO<sub>2</sub> fluxes and cumulative CO<sub>2</sub> emissions

The CO<sub>2</sub> fluxes over time of different treatments are shown in Figures 2-2 and 2-3. Averaged across litter treatment, CO<sub>2</sub> flux for three moisture treatments ranged from 6.7 to 14 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 10°C, from 7.6 to 28 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 20°C, and from 17.6 to 46.6 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 30°C (Figure 2-2). Averaged across moisture treatments, the fluxes from litter treatment ranged from 7.5 to 13.6 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 10°C, 6.8 to 28 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 20°C, and 18 to 49.8 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 30°C (Figure 2-3). Soil CO<sub>2</sub> flux showed similar patterns with higher fluxes for all moisture and litter treatments initially and declined over time at 20 and 30°C. The averaged soil CO<sub>2</sub> flux across the seven measurement times was 10.6, 14.4, and 27.6 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> for the 10, 20, and 30°C, respectively.

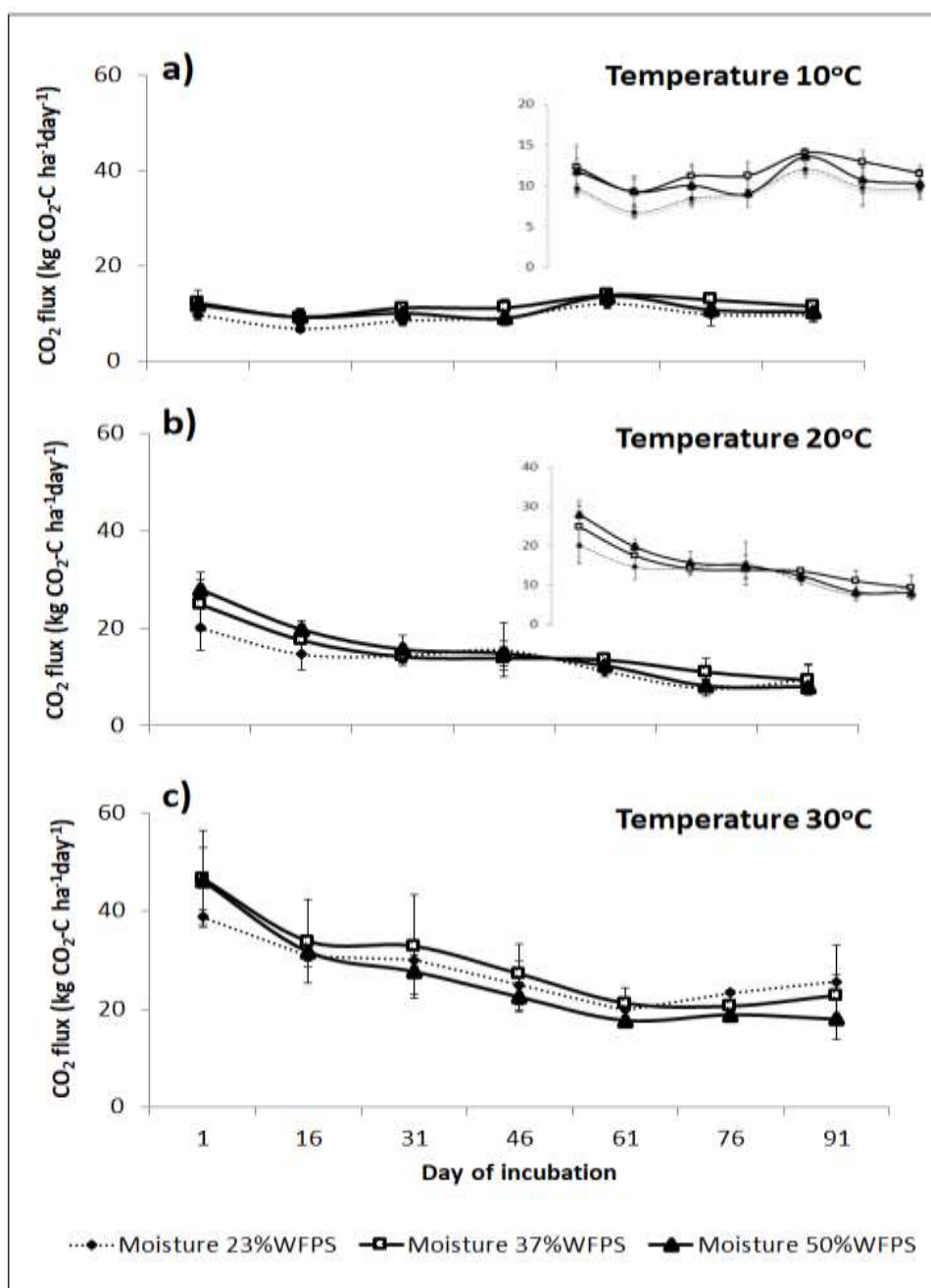


Figure 2-2. CO<sub>2</sub> fluxes over 91 days for three levels of soil moisture (23, 37, and 50% WFPS) under three temperatures with a) 10, b) 20 and c) 30°C. Error bars represent standard errors of the mean (n=12).

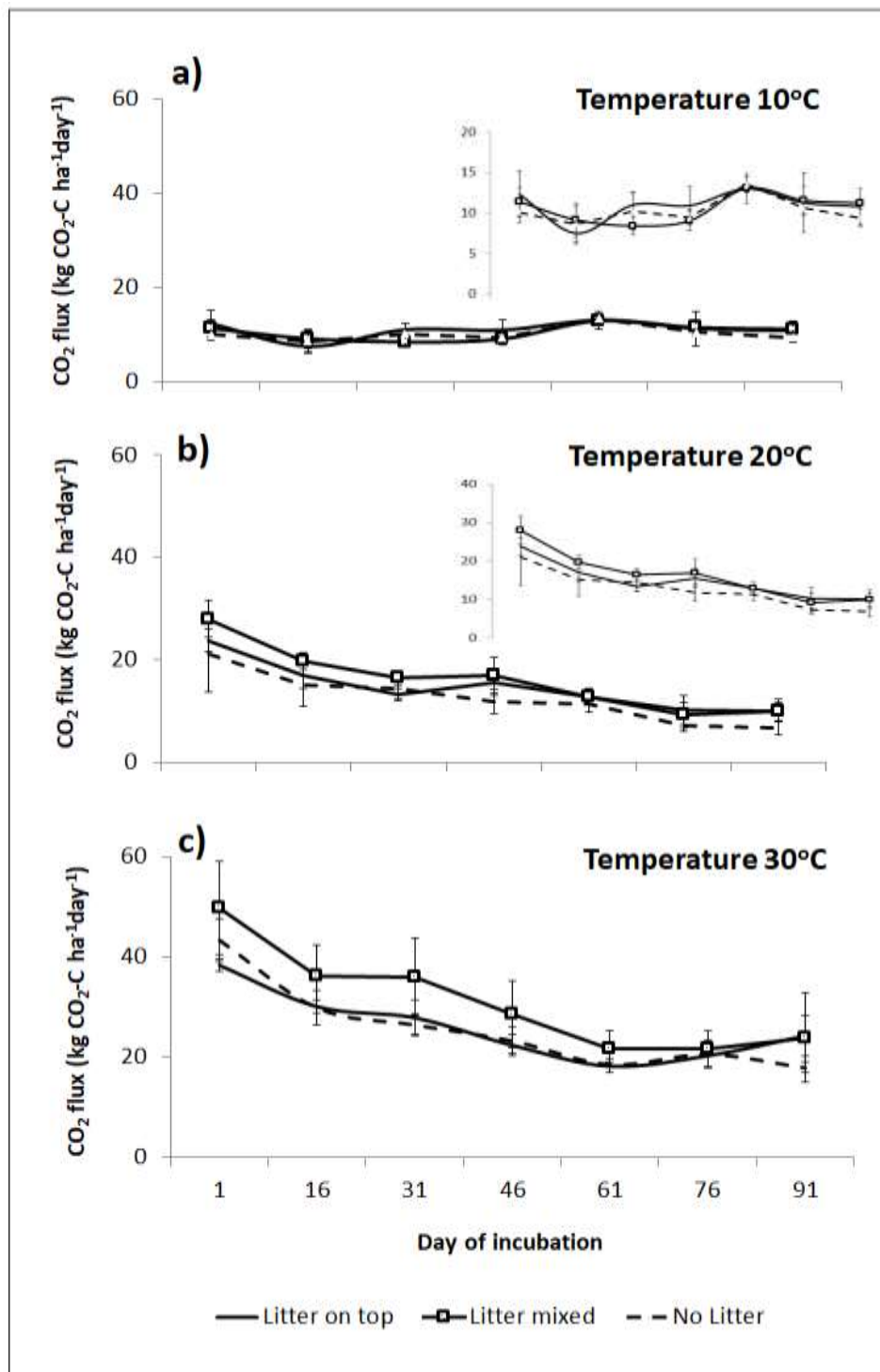


Figure 2-3. CO<sub>2</sub> fluxes over 91 days for three litter treatments (on top, mixed, and no litter) under three temperatures with a) 10, b) 20 and c) 30°C. Error bars represent standard errors of the mean (n=12).

Cumulative CO<sub>2</sub> emissions for moisture and litter treatments are given in Figs 2-4 and 2-5. The effect of moisture on cumulative CO<sub>2</sub> emissions was found to be significant at 10°C (p= 0.0004) and 30°C (p= 0.001), while the effect of litter placement treatment was significant at 20 °C (p= 0.0012) and 30°C (p= 0.0102) (Table 2-2). There was no significant interaction of litter and moisture on CO<sub>2</sub> emission (p > 0.05) (Table 2-2). The magnitude of cumulative CO<sub>2</sub> emissions at 30°C was two times greater than at 10°C. For soil moisture treatments, 23% WFPS showed the lowest cumulative CO<sub>2</sub> emission at 10°C and 30°C (763 and 1711 kg CO<sub>2</sub>-C ha<sup>-1</sup>), while 37 and 50% WFPS showed higher CO<sub>2</sub> emissions (no statistical test was done for the comparison of the treatments between different temperatures) (Figure 2-4). At the temperature of 10°C, litter placement had no significant effect on cumulative CO<sub>2</sub> emission (p= 0.8951). In contrast, larger cumulative CO<sub>2</sub> emissions were observed at 20 and 30°C for soil-litter mixing than litter placed on top treatment (no statistical test was done for the comparison of the treatments between different temperatures) (Figure 2-5).

Table 2-2. Main and interactive effects of litter and moisture on cumulative CO<sub>2</sub> emission (kg CO<sub>2</sub>-C ha<sup>-1</sup>) over 91 days of incubation under three temperatures (10, 20, and 30°C). ns: not significant differences and \*: significant differences with a p-value less than 0.05.

Factor	10°C	20°C	30°C
Litter	0.8951 ns	0.0012 *	0.0102 *
Moisture	0.0004 *	0.1385 ns	0.0010 *
Moisture * Litter	0.4557 ns	0.6077 ns	0.2093 ns



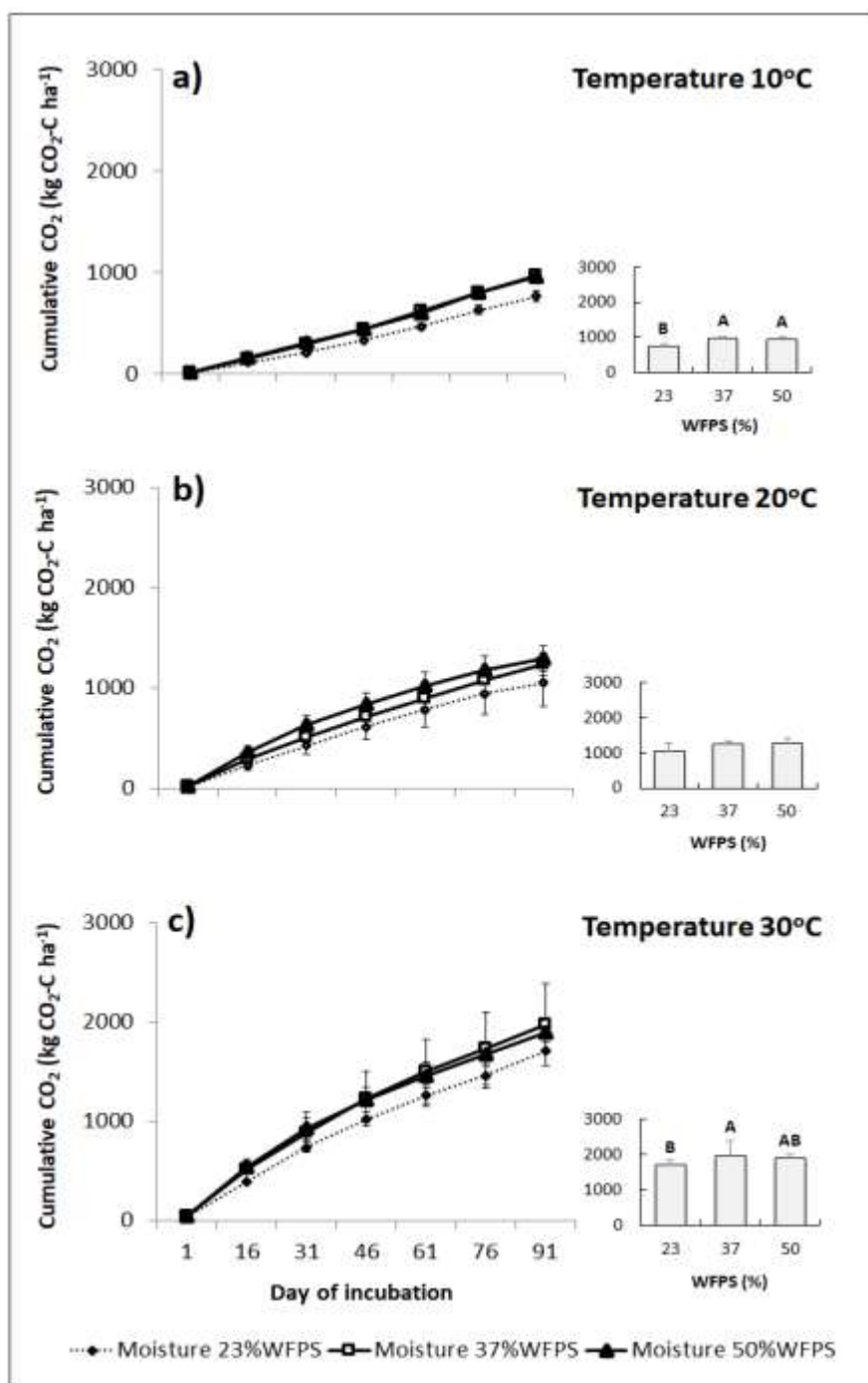


Figure 2-4. Cumulative CO<sub>2</sub> emission over 91 days of incubation under three soil moistures (23, 37, and 50% WFPS) and three temperature treatments: a) 10, b) 20 and c) 30°C. The vertical bars represent standard errors of the mean (n=12). The inserts are total CO<sub>2</sub> emissions after 91 days and the different capital letters represent mean comparison among moisture levels across litter treatment within a temperature based on Tukey's multiple comparison test ( $p < 0.05$ ).

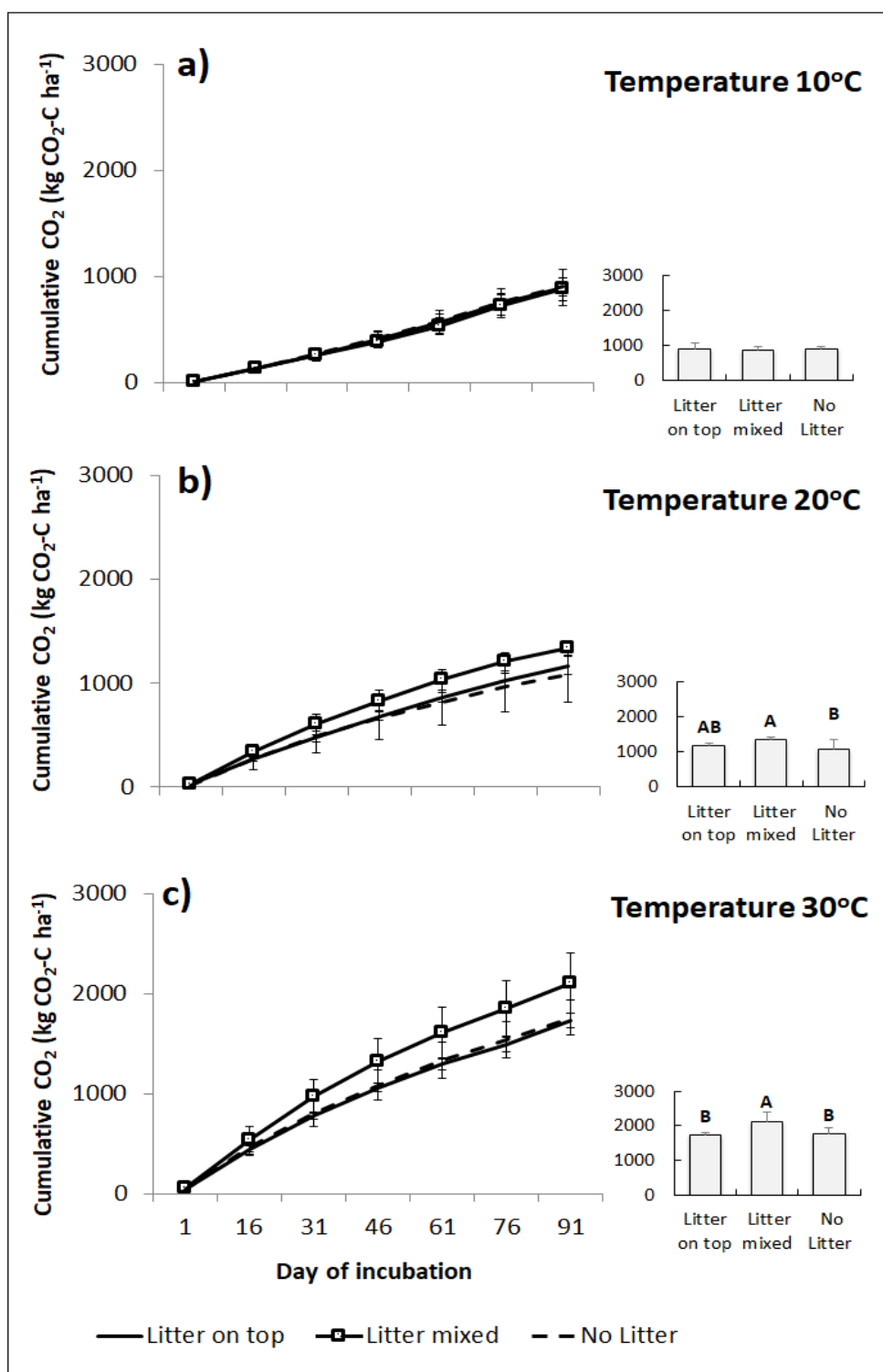


Figure 2-5. Cumulative CO<sub>2</sub> emission over 91 days of incubation from three litter treatments (on top, mixed, and no litter) under three temperature treatments: a) 10, b) 20 and c) 30°C. The vertical bars represent standard errors of the mean (n=12). The inserts are total CO<sub>2</sub> emissions after 91 days and the different capital letters represent mean comparison among litter levels across moisture treatment within a temperature based on Tukey's multiple comparison test ( $p < 0.05$ ).

### **Effects of litter placement, soil moisture, and temperature on litter decomposition**

The interaction of soil moisture and litter treatment on average cumulative CO<sub>2</sub> emission from litter decomposition was significant at all temperatures ( $p < 0.05$ ) (Table 2-3). The mean comparison across all treatments (litter placement and moisture) within each temperature is shown in Figure 2-6. Under soil moisture levels of 23 and 50% WFPS, litter decomposition was not observed for both litter placement treatments at 10°C. The negative litter-derived CO<sub>2</sub> indicates that there is no or negligible decomposition.

At 20°C, decomposition of litter either placed on top of the soil or mixed into the soil decreased with increasing soil moisture content. Comparing the effect of litter placement at a given soil moisture level showed that the litter mixed decomposed more than the litter on top treatment, however these differences were not significant ( $p > 0.05$ ). At 50% WFPS, although the negative CO<sub>2</sub> value for litter on top indicative that litter has not been decomposed, the CO<sub>2</sub> emission litter mixed treatment at the same moisture was 88.3 kg CO<sub>2</sub>-C ha<sup>-1</sup> (Figure 2-6b). At 30°C, increasing the moisture from 23 to 37% WFPS increased litter-derived CO<sub>2</sub> for the litter mixed treatment ( $p < 0.0001$ ), as the highest cumulative litter-derived CO<sub>2</sub> was observed for the litter mixed treatment at 37% WFPS (733.9 kg CO<sub>2</sub>-C ha<sup>-1</sup>) (Fig. 2-6c). While litter-derived CO<sub>2</sub> emitted from the litter on top treatment was similar for both 23 and 37% WFPS ( $p > 0.05$ ) (Figure 2-6c). At 50% WFPS, litter decomposition was not observed for litter on top treatment; likewise, CO<sub>2</sub> released from litter mixed treatment was negligible (Figure 2-6c). The greatest decomposition of litter placed on top of the soil surface was observed at 20°C and 23% WFPS (280 kg CO<sub>2</sub>-C ha<sup>-1</sup>) ( $p = 0.0467$ ) (Figure 2-6b). The proportion of averaged litter-

derived cumulative CO<sub>2</sub> across moisture levels for the litter on top treatment ranged from -15.1 to 10.1%, -13.1 to 25.8%, and -10.3 to 2.9% at 10, 20, and 30°C, respectively (Figure 2-7). However, for the soil-litter mixed treatment, the proportion of litter-derived cumulative CO<sub>2</sub> emissions ranged from -8.1 to 6%, 6.3 to 36.7%, and 1.1 to 30% at 10, 20, and 30°C, respectively.

Table 2-3. Main and interactive effects of factors litter and moisture on average cumulative CO<sub>2</sub> emission (kg CO<sub>2</sub>-C ha<sup>-1</sup>) produced by litter decomposition during the incubation periods (91 days) under three temperatures 10, 20, and 30°C. "ns and \*" denote non-significant, significant differences with a p-value less than 0.05.

<b>Factor</b>	<b>10°C</b>	<b>20°C</b>	<b>30°C</b>
<b>Litter</b>	0.1305 ns	0.0389 *	0.0094 *
<b>Moisture</b>	0.0301 *	0.0018 *	0.0256 *
<b>Moisture * Litter</b>	0.0210 *	0.0467 *	0.0177 *

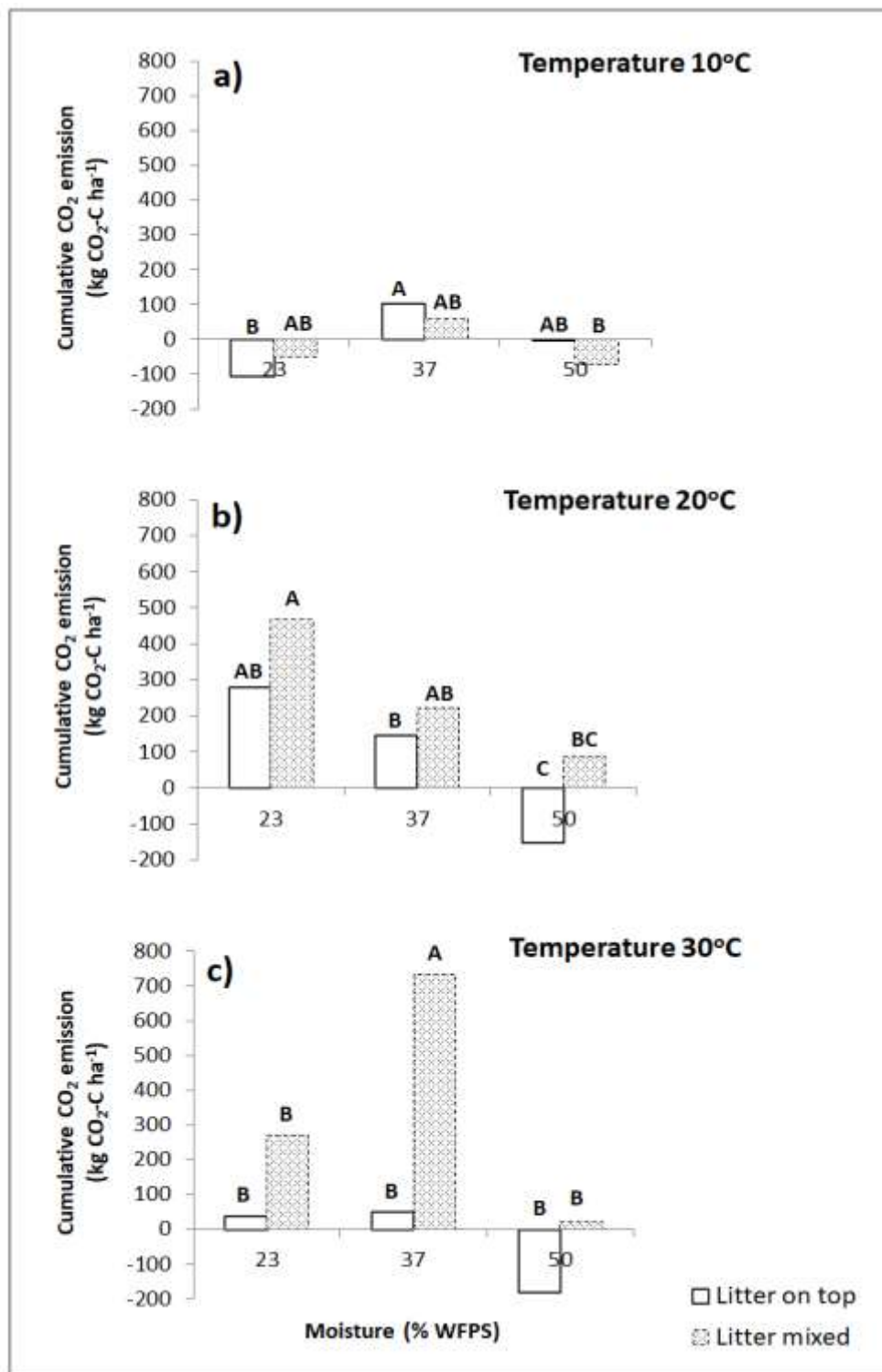


Figure 2-6. Cumulative CO<sub>2</sub> emission from litter decomposition at the end of 91 days of incubation under three temperature treatments: a) 10, b) 20 and c) 30°C. Different capital letters represent mean comparison between litter on top and litter mixed at a given moisture content and temperature based on Tukey's multiple comparison test ( $p < 0.05$ ).

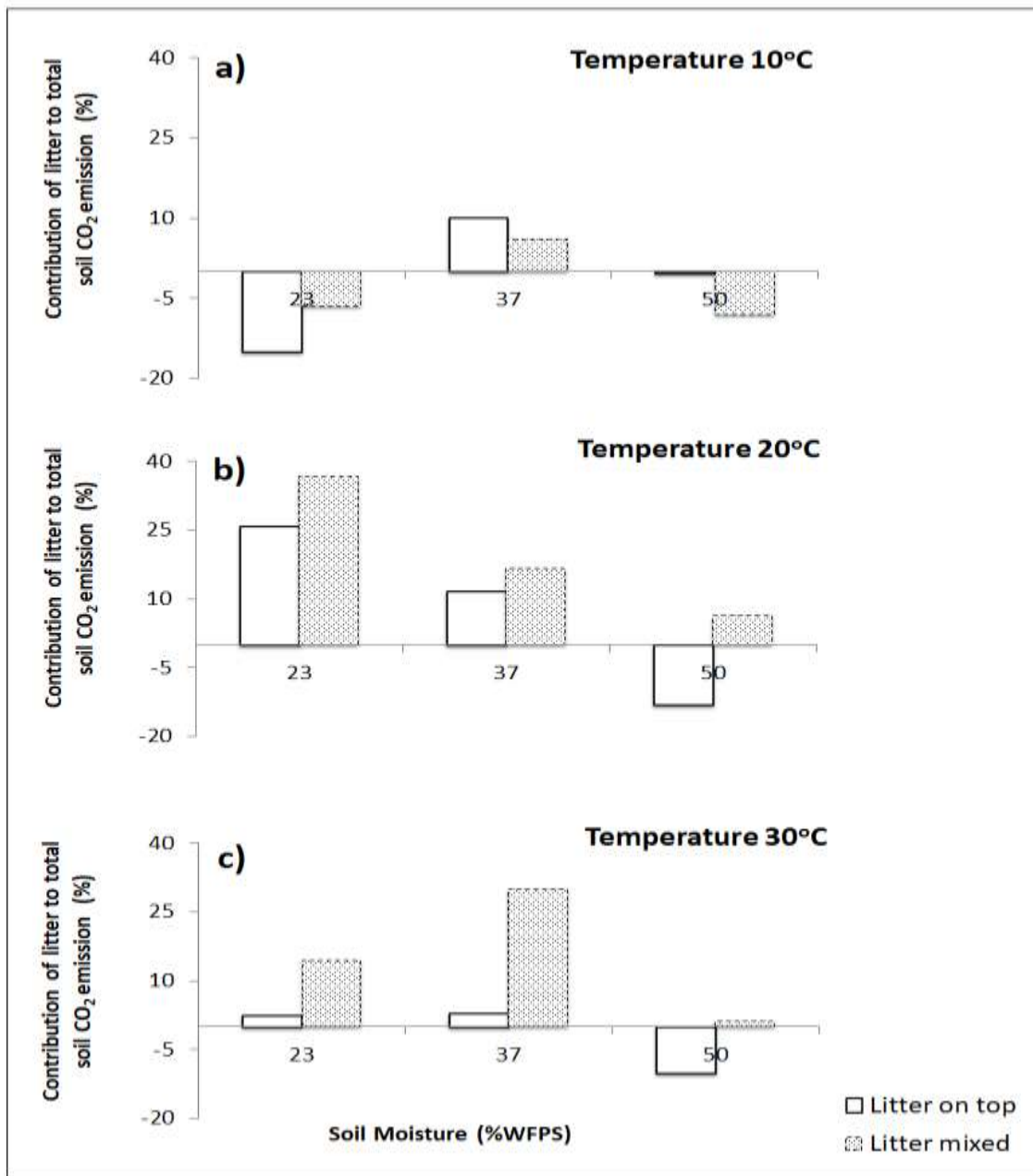


Figure 2-7. Proportion of cumulative CO<sub>2</sub> emission from litter decomposition in total cumulative CO<sub>2</sub> emission under soil moistures of 23, 37, and 50 %WFPS and litter placement (on top and mixed) at different temperature treatments: a) 10, b) 20 and c) 30°C over 91 days of incubation.

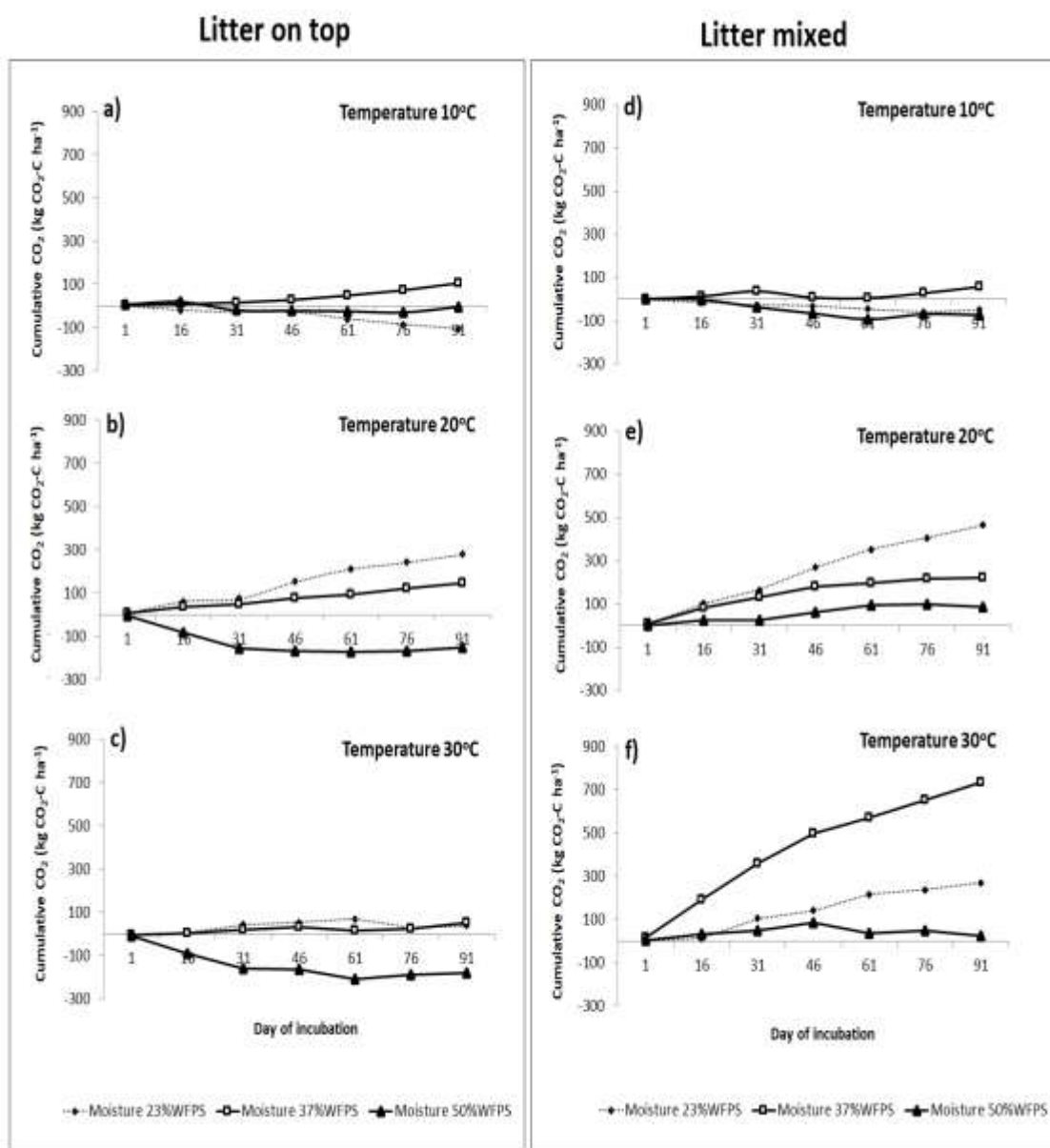


Figure 2-8. Cumulative CO<sub>2</sub> emission from litter decomposition over time with soil moisture of 23, 37, and 50% WFPS and litter placements on top and mixed at different temperatures of 10, 20 and 30°C. (Note: Y-axis is difference between total cumulative CO<sub>2</sub>-C with litter treatment and soil with no litter).

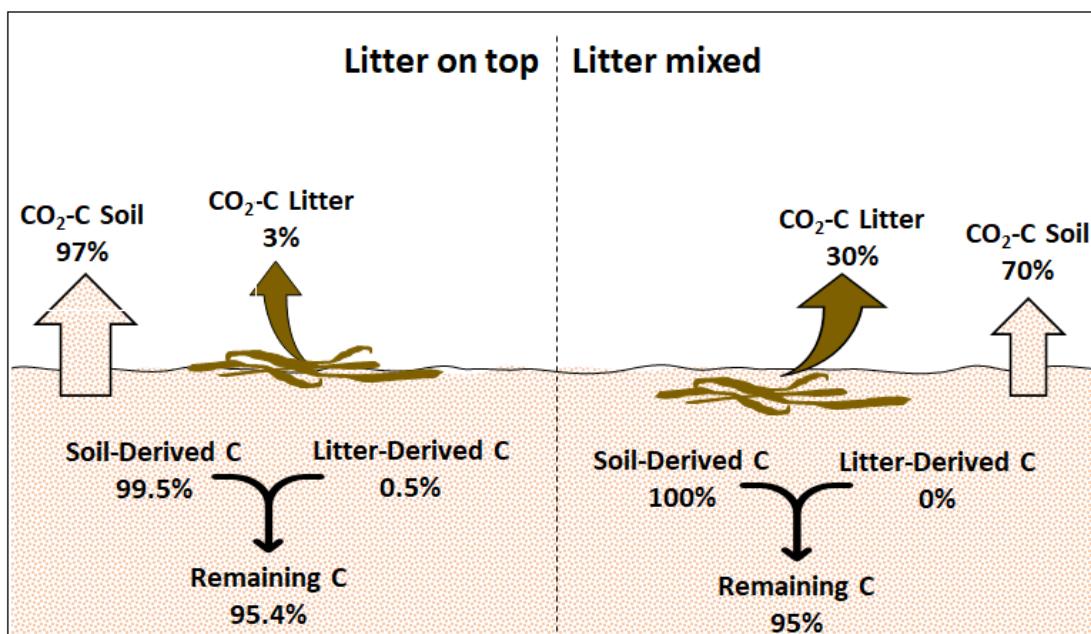


Figure 2-9. Contribution of litter (on top vs. mixed) and soil organic matter to total C output (CO<sub>2</sub>-C) and remaining C (%) in a 91-day time frame for a mid-growing season situation (30°C and 37% WFPS). Emitted CO<sub>2</sub> from only soil (%) = (emitted CO<sub>2</sub> from control treatment (g of CO<sub>2</sub>-C) \*100)/ total emitted CO<sub>2</sub> (g of CO<sub>2</sub>-C). Emitted CO<sub>2</sub> from only litter (%) = {(total emitted CO<sub>2</sub> (g of CO<sub>2</sub>-C) – emitted CO<sub>2</sub> from control treatment (g of CO<sub>2</sub>-C) \*100/ total emitted CO<sub>2</sub> (g of CO<sub>2</sub>-C)}. Total input C = initial litter C + initial SOC. Remaining C (%) = {(total input) – (total output)/ (total input)} \*100. Contribution of litter C to remaining C (%) = (initial litter C – C emitted from only litter) \*100/ remaining C. Contribution of soil C to remaining C (%) = (initial soil C – C emitted from only soil) \*100/ remaining C.



## **Discussion**

### **Effects of litter placement, soil moisture, and temperature on total soil CO<sub>2</sub> fluxes and cumulative CO<sub>2</sub> emissions**

The daily CO<sub>2</sub> fluxes were generally greater at temperatures of 20°C and 30°C (10-17 and 24-37 kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>, respectively) than at 10°C (9-12 kg CO<sub>2</sub>-C ha<sup>-1</sup> day<sup>-1</sup>). As expected, microbial activities are greater at warmer temperatures (Bray et al., 2012; Zhu et al., 2013). At temperatures of 20 and 30°C, we observed a decreasing trend of CO<sub>2</sub> fluxes over the incubation period. This can be explained by the rapid decomposition of readily available substrates (Corrigan and Oelbermann, 2010; Wu, Li, and Wan, 2013) and the simultaneous accumulation of more recalcitrant substrates decomposing at lower rates (Voriskova & Baldrian, 2013; García-Palacios et al., 2016). Shifts in microbial community structure over time might lead to an increase of decomposition (Fahrenfeld et al., 2017), as observed for the daily flux around day 90 at an incubation temperature of 30°C.

Not surprisingly, the higher fluxes observed at temperatures of 20 and 30°C resulted in higher cumulative amounts of respired CO<sub>2</sub> from all moisture and litter treatments after 91 days of incubation (804-1407 and 1608-2447 kg CO<sub>2</sub> -C ha<sup>-1</sup>, respectively) compared to those measured at 10°C (708-1020 kg CO<sub>2</sub>-C ha<sup>-1</sup>). Our results support that temperature can be considered a controlling factor in litter decomposition dynamics because microbial activities intensify with increased temperatures, leading to greater CO<sub>2</sub> emission at higher temperatures (20 and 30°C vs. 10°C) (Yin et al., 2019, Zhou et al., 2018).

In environments where water is limiting microbial activity, soil moisture will have a progressively dominant role in regulating microbial activity (Delon et al. 1, 2019). Our experiment confirmed this by larger cumulative CO<sub>2</sub> amounts at 37% and 50% WFPS compared to those at 23% WFPS. Decreasing temperature and moisture creates a less favorable environment for microorganisms, thus reducing the amount of respired CO<sub>2</sub> (Carter et al., 2015; Benbow et al., 2013). However, the independence of the cumulative CO<sub>2</sub> emission from soil moisture only at the moderate temperature of 20°C, representing the mid-season condition for our study site, suggests that decomposition is more strongly affected by water availability at higher or lower temperatures. The possible reason for this could be that at the temperature of 20°C, the water evaporation rate was approximately constant among moisture levels.

In contrast, at temperature 30°C, the water evaporation rate increased with increasing water content (Table. 2-1). In this study, it was expected that placing litter on the soil surface compared to the treatment with no litter addition (control) would enhance cumulative CO<sub>2</sub> emission by providing a fresh substrate to microorganisms (Kaspar and Singer, 2011; Lee et al., 2014; Basche et al., 2014). However, the results did not show such effect because the cumulative amount of CO<sub>2</sub> evolved was similar for litter on top and control treatment. Soil–litter mixing accelerated litter decomposition probably through enhancing biological activity by mitigating abiotic constraints on microbial activity (Barnes et al., 2015; Liu et al., 2018) and increasing litter accessibility to soil microbes (Mitchell et al., 2018, 2016; Wei et al., 2021).

### **Effects of litter placement, soil moisture, and temperature on litter decomposition**

Litter addition was postulated to stimulate microbial activity and decomposition by providing a fresh material as a substrate to decomposers (Prévost-Bouré et al., 2010). However, we observed both increasing and decreasing trends of litter-derived CO<sub>2</sub> for soil-litter mixed and litter on top treatments (Figure 2-8). This suggests that the losses of CO<sub>2</sub> from soils cannot be attributed to litter addition alone and that litter placement can shift the soil into a CO<sub>2</sub> sink or source. At 10°C, the highest CO<sub>2</sub> derived from litter decomposition was observed at 37% WFPS for both litter on top and mixed treatments with 103.2 kg CO<sub>2</sub>-C ha<sup>-1</sup> and 58.4 kg CO<sub>2</sub>-C ha<sup>-1</sup>, respectively. The different response of litter decomposition to location and soil moisture at 10°C implies that the limiting effect of low soil temperature on litter decomposition is coupled with the extreme soil moisture levels (23 and 50% WFPS). The closer contact of the litter with the soil mineral phase resulting from the mixing process seems to exert no additional stabilizing effect, as Wei et al. (2021) observed.

The current study suggests that increased decomposition and consequent higher CO<sub>2</sub> observed from soil-litter mixing treatment will not necessarily increase the soil net remaining organic C. It is shown in Figure 2-9, although mixing litter with soil increased the contribution of litter-derived CO<sub>2</sub> to the total CO<sub>2</sub>, it did not improve the net remaining C content. This suggests that litter placement could alter the relative contribution of SOM and litter to total C output and remaining C in soil. This might be explained by the soil's high sand content under this study (76%) because the formation of organic matter stabilizing organo-mineral associations and aggregates depends largely on reactive mineral particles in the silt and clay size range (Giannetta et al., 2018; Xu et al.,

2020). Our results also indicate that positive effects of soil-litter mixing on litter decomposition will become more pronounced when the environment is at the optimum for microbial activity (20°C and 37% WFPS) (Figure 2-8f). In addition, negative CO<sub>2</sub> fluxes (i.e., CO<sub>2</sub> uptake by the soil) observed at soil moisture of 23% and 50% WFPS and 10°C for both litter on top and litter-mixed treatments, and at 50% WFPS for litter on top at all temperatures suggest that soil under these environments may act as a CO<sub>2</sub> sink. This implies that litter placement and its interaction with abiotic factors controlled if this sandy soil acted as a CO<sub>2</sub> sink or source. It has been illustrated that in environments with cold temperature, high soil moisture content, low soil O<sub>2</sub> levels, and low substrate accessibility where the microbial activity is inherently low, CO<sub>2</sub> uptake by the soil can result in negative CO<sub>2</sub> fluxes (Stone, 2008; Fa et al., 2016).

Several possible inorganic processes causing negative CO<sub>2</sub> fluxes have been stated according to the ideal gas law and Henry's Law. Based on the ideal gas law, there is a negative and positive correlation of soil CO<sub>2</sub> flux with the soil air pressure and temperature, respectively (Fa et al., 2016).

Based on Henry's law, the dissolution of CO<sub>2</sub> in the soil water can be described as the following chemical equation (Schulz et al., 2006; Ma et al., 2013):



Where KH is Henry's Law constant, pCO<sub>2</sub> is the partial pressure of CO<sub>2</sub> in the soil (atm).

Inserting and sealing a small area of a cylinder, including a small headspace volume, results in a transient increase in CO<sub>2</sub> pressure of the headspace/air. Soil air shrinkage and CO<sub>2</sub> solubility rise, induced by the rise in surface air pressure and low soil temperature, can reduce surface soil CO<sub>2</sub> concentration, resulting in CO<sub>2</sub> pumping from

the air into the soil (Ball et al., 2009; Liu et al., 2014; Luo and Zhou, 2006). Moreover, increased CO<sub>2</sub> dissolution into the soil–water films induced by increasing soil air pressure and low temperature, based on Henry's Law, lead to CO<sub>2</sub> effusion from soil-air to soil–water (Suchet and Probst, 1995; Taylor, 1978). The increase of soil water content might dissolve more soil CO<sub>2</sub> (Liu et al., 2014; Fa et al., 2015). It is notable that soil CO<sub>2</sub> uptake and negative soil CO<sub>2</sub> flux be observed under the condition of high soil moisture content, high surface air pressure, and low temperature. By considering diffusion as the main mechanism of CO<sub>2</sub> transport in the soil (Oh et al., 2005), the reduced diffusion of soil CO<sub>2</sub> into the atmosphere might be another reason for the negative soil CO<sub>2</sub> flux. The wet litter layer might act as a barrier against the diffusion of CO<sub>2</sub> from the soil into the air/headspace and lead to an underestimation of the CO<sub>2</sub> concentration (Wang et al., 2013; Leitner et al., 2016). The litter layer's inhibitory effect might be more substantial in the wetter soil condition, such as at 50% WFPS. The moist litter layer induced by adding a high amount of water in a small area of the cylinder reduces water drainage speed, which leads to increased CO<sub>2</sub> dissolution in soil solution. In high moisture content (50% WFPS), slow water drainage caused by a wet litter on top could prolong the time of CO<sub>2</sub> uptake by soil. For litter mixed treatment at low temperature and wet conditions (10°C and 50% WFPS), facilitated water infiltration can provide downward pressure on soil CO<sub>2</sub> (pushes and squeezes soil CO<sub>2</sub>) (Fa et al., 2015). In addition, using the CO<sub>2</sub> gas sensor to measure CO<sub>2</sub> concentration in this study might have intensified the downward flow of CO<sub>2</sub> from the headspace into the soil (Healy et al., 1996).

Although biotic factors were not directly observed within this experimental setup, the results provide a basis to consider several hypotheses regarding litter placement

effects on fungal and microbial activities. Changes in soil abiotic factors and consequent shifts in microbial communities caused by litter placement might explain the different litter-derived CO<sub>2</sub> values from the litter on top versus litter mixed in treatments. The increased dominance of fungi is expected for litter on top treatment, resulting in a lower soil CO<sub>2</sub> emission because fungi generally have greater C assimilation efficiencies than bacteria (i.e., fungi store more C than they metabolize) (Six et al., 2006). Kaiser et al. (2010) also reported that soil moisture and temperature could change soil microbial community composition. Thus, we assume that the microbial community change induced by soil moisture and temperature alteration under the different litter placement increases the fungi to bacterial ratio under the litter on top treatment and partly explains the greater decrease in soil CO<sub>2</sub> soil-litter mixed treatment. At 30°C, the litter on top led to no significant changes in the CO<sub>2</sub> emission with increasing moisture from 23 to 37% WFPS, which suggests that the soil microbial activity was more limited by temperature. The temperature effect was lower for the litter on top treatment than for the litter-mixed treatment. The temperature response of litter-mixed treatment was significantly increased at 37% WFPS of moisture, which was likely due to the increased substrate availability at higher moisture content for microbial activity (Deng et al., 2016). This suggests that with increasing substrate availability through mixing, more enzymatic steps are getting active to break down a large pulse of fresh organic materials. Each of these steps is temperature sensitive due to microbial enzyme kinetics (Fierer et al., 2005; Curiel Yuste et al., 2007).

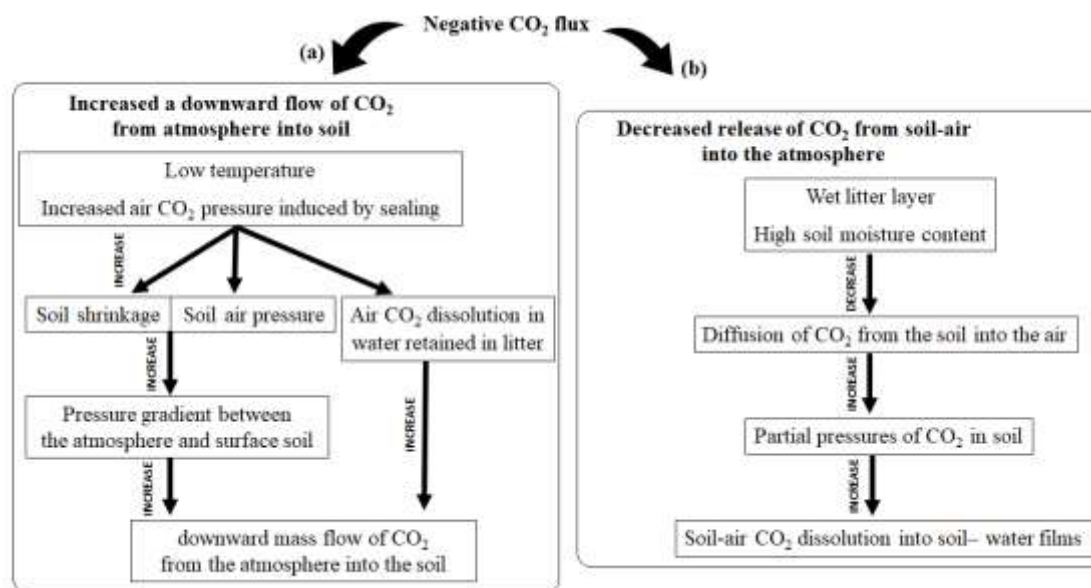


Figure 2-10. Two possible mechanisms of negative soil CO<sub>2</sub> flux in semiarid grassland soils; a) Increased downward flow of CO<sub>2</sub> from the atmosphere into soil and b) Decreased release of CO<sub>2</sub> from soil-air into the atmosphere.

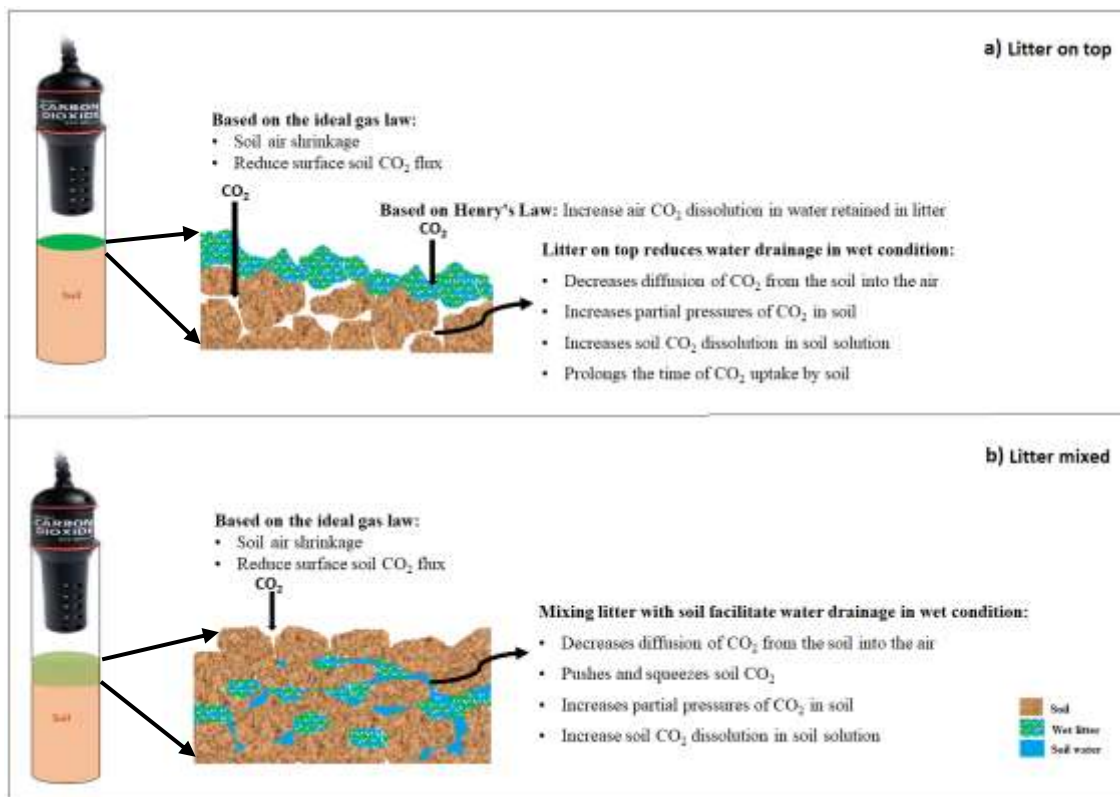


Figure 2-11. Potential processes of negative soil  $\text{CO}_2$  flux for different litter placement; a) litter on top, b) litter mixed with soil.

## Conclusion

We conclude that the observed limiting effect of deficient water on microbial decomposition of litter is coupled with soil temperature. This study showed a distinct limiting effect of warm and dry (late season) and cold and dry (early season) conditions on litter decomposition by providing unfavorable microbial decomposer conditions. The effect of abiotic factors on litter decomposition could shift over time during the growing season, and litter placement and its interaction with abiotic factors alter decomposition rate and the contribution of SOM and litter to total C output and remaining C in soil. Thus, litter placement and its interaction with abiotic factors determine if soil acted as a  $\text{CO}_2$  sink or source. Decomposition became slower for the litter on top than the litter



mixed into the soil. Therefore, we are expecting more rapid litter decomposition at optimal water and warming temperature of the growing season. Furthermore, modifying the OM decomposition model parameters with the soil temperature, moisture, and interactive effects with litter placement is needed to improve total C flux predictions under different climates and land management in semiarid grasslands. Since our research was a short-term incubation study, a long-term field study across grassland ecosystems should be considered to obtain a more holistic picture of the effect of abiotic factors on litter decomposition in semiarid grassland ecosystems.

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### **CHAPTER 3: Effect of frequency of wetting frequency and temperature on the decomposition of soil organic matter and litter**

#### **Abstract**

In semiarid grasslands, drying and wetting of surface soil is a typical process caused by irregular rainfall events that also affects microbial organic matter (OM) decomposition and carbon (C) cycling. Although the effects of drying-wetting cycles on soil processes have been studied, little is known on how surface litter and drying-wetting cycles litter affects OM decomposition in semiarid grasslands. It is expected that by retaining water in its highly porous structure, the litter layer on top of the soil surface can affect moisture fluctuations during the drying-wetting cycles. We conducted a 91-day incubation experiment at three temperatures of 10, 20, and 30°C using the soil and litter collected from a sub-irrigated meadow located at the University of Nebraska-Lincoln Barta Brothers Ranch, Nebraska Sandhills. The soils with and without litter input were subjected to two watering frequencies –high and low – with 6 and 3 drying - wetting cycles, respectively. In the high-frequency drying-wetting cycle, it is hypothesized that enhanced CO<sub>2</sub> emissions were caused partially by the litter's ability to retain moisture, which prolonged favorable conditions for microbial activity. However, the litter input effect on CO<sub>2</sub> emissions in the low-frequency drying-wetting cycle was inconsistent and varied with soil temperature.

The results showed very different effects of the drying-wetting cycles on soil organic matter (SOM) vs. litter decomposition with temperature changes. The highest average cumulative SOM-derived CO<sub>2</sub> was observed for the low-frequency of the drying-wetting

cycle at 30°C (1669 kg CO<sub>2</sub>-C ha<sup>-1</sup>), and the lowest was recorded from the high-frequency at 10°C (803 kg CO<sub>2</sub>-C ha<sup>-1</sup>).

Thus, the combined effect of frequency of drying-wetting cycles with temperature can alter the SOM and litter-derived CO<sub>2</sub> contribution to the total CO<sub>2</sub> emitted from the soil. Improving our understanding of the relationships between litter input and precipitation patterns needs to be included in the terrestrial C cycling models and land use strategies to reduce soil C release to the atmosphere in semiarid grasslands.

## **Introduction**

Arid and semiarid lands cover approximately one-third of the Earth's surface and play a key role in the global C balance (Ahlström et al., 2015; Poulter et al., 2014). Global warming is closely associated with other changes, such as increases in the frequency of intense rainfall (Goswami et al., 2006; Trenberth, 2011; Donat et al., 2017). Soil CO<sub>2</sub> emission in semiarid ecosystems is more sensitive and responsive to rainfall events than in other ecosystems (Xu et al., 2004; Borken and Matzner, 2009; Green et al., 2019). Fluctuations in soil moisture may be induced by rainfall events and affects soil microbial activity and, in turn, litter and soil organic carbon (SOC) decomposition (Xiang et al., 2008; Zhang et al., 2016; Green et al., 2019).

A rapid increase in CO<sub>2</sub> emissions immediately after wetting dry soil is called the Birch effect (Birch, 1958). This can be attributed to the increased soil microbial biomass and activity owing to the increased available substrates through processes such as lysis of living microbial cells, the release of intracellular osmoregulatory organic solutes, and disruption of aggregates, thereby releasing protected OM (Butterly et al., 2009; Zhao et

al., 2010; Guo et al., 2012). The CO<sub>2</sub> pulses associated with wetting can account for a significant proportion of the total CO<sub>2</sub> production from soil (Miller et al., 2005; Jarvis et al., 2007; Ma et al., 2012; Rey et al., 2017). However, severe drought stress can also decrease microbial activity and decomposition rate (Sinha and Cherkauer 2010; Meisner et al. 2017). In dry soils, fungi with a higher tolerance to drought stress and being more efficient in using C than bacteria are dominant microorganisms (Jensen et al., 2003). Through altering soil temperature, drying-wetting cycles can further affect microbial activity, OM decomposition, and CO<sub>2</sub> emissions (Wen et al., 2020).

In grassland, litter is constantly added to the soil, introducing fresh organic materials and altering abiotic conditions for microbial activities. The litter layer regulates soil moisture content by retaining water in its porosity structure and reducing the mineral soil's evaporation (Ogée and Brunet, 2002; O'Donnel et al., 2009; Hall et al., 2020). Nutrient diffusion to microbial community depends on soil water content; thus, in water-limited ecosystems, restriction in microbial activity caused by water and nutrient deficiency is expected (Rey et al., 2017). Despite these interactions between the litter input and abiotic factors, it remains unknown how the litter layer influences drying-wetting cycles on soil CO<sub>2</sub> emission in a semiarid grassland. Thus, identifying the interaction effects between drying-wetting and litter layer on decomposition is vital for understanding C dynamics in these ecosystems.

To elucidate how the litter layer influences the effects of drying-wetting cycles on the decomposition of SOM and litter, we conducted a controlled laboratory incubation study for 91 days under different temperatures with semi-arid grassland soil.

By assuming that under high-frequency rainfall treatment, the partial drying before wetting creates a less unfavorable condition for microbial activity than long-term dryness of low-frequency rainfall; we hypothesized: (1) a high frequency of rainfall with shorter duration drought stress would result in a lower sudden increase in CO<sub>2</sub> emission pulse compared to the low frequency with more prolonged drought stress; (2) after receiving rainfall, wet litter layer on top of the soil surface may provide a steady small flow of water and soluble OC to decomposers; also the dry litter layer acting as a barrier to water vapor diffusion might lessen the drought stress and soil moisture fluctuation. Hence, the augmented CO<sub>2</sub> release upon wetting (CO<sub>2</sub> pulse) in low-frequency rainfall events would be reduced; and (3) the decrease in the magnitude of the CO<sub>2</sub> pulse upon wetting induced by litter layer, is expected to be greater for low-frequency rainfall than high-frequency treatment. Here, substrate availability and shorter drought stress would be more pronounced under high-frequency pulses.

## **Materials and Methods**

### **Location of Collected Soil and Litter Samples**

Soil samples were collected in September 2018 from a sub-irrigated meadow located at the University of Nebraska-Lincoln Barta Brothers Ranch (42°13' N, 99°38' W) of Eastern Nebraska Sandhills. The sampling site climate is semiarid (Potvin and Harrison, 1984). Average annual rainfall ranges from 580 mm in the east to 430 mm in the west, and temperature averages ~10°C. About 75% of the average rainfall falls during the growing season between April and September, with May and June being the wettest months (HPRCC, 2011). The sub-irrigated meadow, typical of the eastern



Nebraska Sandhills in topography, soils, and vegetation, makes up approximately 10% of the Sandhills area. The meadows are typically flat, with slopes of 0 to 3 percent with a shallow water table that stays within 25 to 150 cm of the soil surface during the growing season (Schacht et al. 2000). Soils are sandy to fine sandy loams in the Els-Valentine-Tryon association and Loup series (mixed, mesic Aquic Ustipsamments; mixed, mesic typic Ustipsamments; Mixed, mesic Typic Psammaquents; Sandy, mixed, mesic Typic Endoaquolls), containing 76, 14, and 10% sand, silt, and clay, respectively (NRCS, 2019). Vegetation in the meadows is dominated by grasses, redtop bent (*Agrostis stolonifera*), big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), prairie cordgrass (*Spartina pectinata*), smooth brome grass (*Bromus inermis*), kentucky bluegrass (*Poa pratensis*), and sedges. A wide variety of forbs and shrubs is ubiquitous.

### **Soil and Plant Litter Sampling**

The soil sampling location was an experimental site under short duration, high stocking density, rotational grazing management (Table A-1). The sampling location (6.8 ha) was divided into 4 subblocks. Before soil sampling, surface litter and aboveground vegetation were removed and ten soil subsamples per subblock were collected from 0-10 cm soil depth using a shovel. Subsamples from each subblock were mixed to make a composite sample (in total, four composite soil samples). Soil samples were transferred to the lab, air-dried at room temperature (22°C). Stones and plant materials were picked out by hand in the lab and sieved to pass through a 2-mm mesh sieve to obtain the < 2-mm fraction.

At the 0-10 cm depth of soil, the soil pH was measured 6.1 using the procedure given by McLean (1982). Organic C concentration was determined 2.8% by dry combustion C analyzer (LECO Corporation, Saint Joseph, Michigan, USA) after pretreating the soil with  $\text{H}_2\text{SO}_3$  to remove inorganic C (Nelson and Sommers, 1996).

Surface plant litter materials were also collected at the same time, placed in paper bags, and transferred to the lab. Samples were dried at  $60^\circ\text{C}$  and ground to pass through a 0.5 cm sieve to maximize size uniformity. Total nitrogen (N)  $16.1 (\text{g kg}^{-1}) \pm 0.04$  ( $n=4$ ) and C content  $400 (\text{g kg}^{-1}) \pm 0.33$  ( $n=4$ ) of litter were determined by dry combustion C analyzer (LECO Corporation, Saint Joseph, Michigan, USA).

### **Controlled Environment Incubation**

Rainfall events in semiarid ecosystems are naturally highly variable, and direct manipulations of water pulses are logistically challenging in the field. As a result, soil column mesocosm in different treatment combinations of watering frequency with litter input was set up for 3 months under controlled conditions. The treatments included three temperature regimes to evaluate the effects of varying rainfall pulses and litter input. The experimental design was a split-block with temperature and block stripped across each other and with litter input and watering frequency as the split-plot factors. The treatment factors were: three levels of temperatures (10, 20, and  $30^\circ\text{C}$  representing the range of coolest, moderate, and highest soil temperature during the growing season), two levels of litter (no litter addition control and surface addition on top), and two levels of watering frequency (high and low with 6 and 3 drying - wetting cycles, respectively with the same

total water input). Each treatment combination was replicated four times, resulting in 48 column mesocosms.

### **Soil Incubation and Manipulating Soil Wetting Pulses**

The growing season in our study area typically begins in late April and ends in early August. Based on the local precipitation data of the growing season from 2010 to 2017, the frequency of the rainfall depth  $\leq 5$  mm during the growing season is higher than other rainfall depths (61% of the time). The total amount of rainfall received at  $< 5$  mm was 33 mm during these years. We used this total rainfall (33 mm) to create two wetting treatments that differed in pulse size and frequency.

The incubation experiment was conducted using acrylic cylinders of 3.81 cm in diameter and 22 cm in height. To prepare soil columns for incubation, air-dried soil samples containing 2% of volumetric moisture (4% WFPS) were adjusted to 13% of volumetric moisture, which is the lower threshold moisture level observed during the growing season (23% WFPS), before packing soil in cylinders. Air-dried soil 137 g containing initial moisture content of 23% WFPS was packed into the cylinders to a height of 10 cm to have a bulk density of  $1.18 \text{ g cm}^{-3}$  as in the field. The six-year end of grazing season annual (2012 to 2017) average litter, a mixture of dominant grasses at the sampling site, was  $1049 \text{ kg ha}^{-1}$ , equivalent to 120 mg per  $11.4\text{-cm}^2$  column base area. The litter was then placed above the soil surface. The high-frequent pulse received 5.5 mm of water every two weeks (6 times during the 91-day experimental period) with the first watering at day 15, then days 30, 45, 60, 75, and 90. For the low-frequent treatment received 11 mm of water every four weeks (3 times during the 91-day experimental

period) with the first watering at day 30, then days 60, and 90. Water was applied using standard spray bottle gradually onto the surface.

### **Carbon Dioxide Flux Measurements**

To quantify the effect of wetting frequency and litter layer on top of the soil surface on CO<sub>2</sub> flux, as a proxy of decomposition, during the incubation period, was measured at day 1, 16, 31, 46, 61, 76, and 91 for both high and low frequency treatments using a Vernier LabQuest 2, CO<sub>2</sub> Gas Sensor (Vernier; Beaverton, Oregon, USA) (Figure 3-1). The sensor was set up on a low range in which the accuracy and resolution were +/- 100 ppm and 3 ppm, respectively. Following the manufacturer's recommendations, calibration was done using atmospheric air CO<sub>2</sub> concentration (400 ppm) as a reference. The range of the readings was within the reference tolerance of 400 +/- 100 ppm. To measure CO<sub>2</sub> flux, the sensor was inserted to seal the cylinder, and the sampling rate and duration were set at one sample per second for 5 minutes, yielding a total of 300 reading for each measurement. The CO<sub>2</sub> concentration of the chamber ambient air concentration was also measured at each sampling time to compensate for the headspace CO<sub>2</sub> concentration (~400 ppm) by subtracting from the column readings to calculate the net soil and litter's CO<sub>2</sub> flux (Hubb, 2012). The CO<sub>2</sub> flux was calculated as the change in CO<sub>2</sub> concentration (mgL<sup>-1</sup>) divided by the time of sensor measurement sampling periods (5 minutes) using either linear or quadratic regression for the best regression fit (Kutzbach et al., 2007; Pérez-Priego et al., 2010; Wagner et al., 1997). The CO<sub>2</sub> flux data between successive measurement periods were linearly interpolated to calculate the cumulative CO<sub>2</sub> emission at the end of the 91 days (Zibilske, 1994). Litter-derived

cumulative CO<sub>2</sub> emission was calculated as the difference between the CO<sub>2</sub> emission from treatments with litter and the control treatment (soil only).

### **Statistical Analyses**

The effects of litter input and frequency of drying-wetting cycles on total cumulative CO<sub>2</sub> emission from the soil were tested under three different temperatures using PROC MIXED in SAS 9.4 with mixed model procedures for a split-block design with temperature and block stripped across each other with litter placement and soil moisture as the split-plot factors followed by a pairwise differences test (adjusted by Tukey) (SAS Institute Inc., Cary, NC). We considered treatments as fixed factors and replication as a random factor. Note that temperature had no replicates then no statistical test was done on temperature effect. Significance was considered at  $p \leq 0.05$  in all analyses.



Figure 3-1. Vernier CO<sub>2</sub> Gas Sensor was placed inside each cylinder's headspace to measure CO<sub>2</sub> emissions in (mgL<sup>-1</sup>) of each treatment over time.

## Results

### Effects of wetting frequency cycles on total soil CO<sub>2</sub> fluxes

The average total CO<sub>2</sub> flux, which is derived from SOM plus litter decomposition across CO<sub>2</sub> recording days during the 91 days of incubation for the high-frequency wetting, was 11.1, 18.3, and 22.8 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 10, 20, and 30°C, respectively. For the low-frequency wetting, it was 10.9, 19.8, and 28.6 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 10, 20, and 30°C, respectively (Figure 3-2). For the treatment with litter on top, the average total CO<sub>2</sub> flux was 11.2, 19.7, and 27.4 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 10, 20, and 30°C, respectively.

For the control treatment with no litter (only soil), the average total CO<sub>2</sub> flux was 10.8, 18.3, and 24.0 kg CO<sub>2</sub>-C ha<sup>-1</sup>day<sup>-1</sup> at 10, 20, and 30°C, respectively (Figure 3-3).

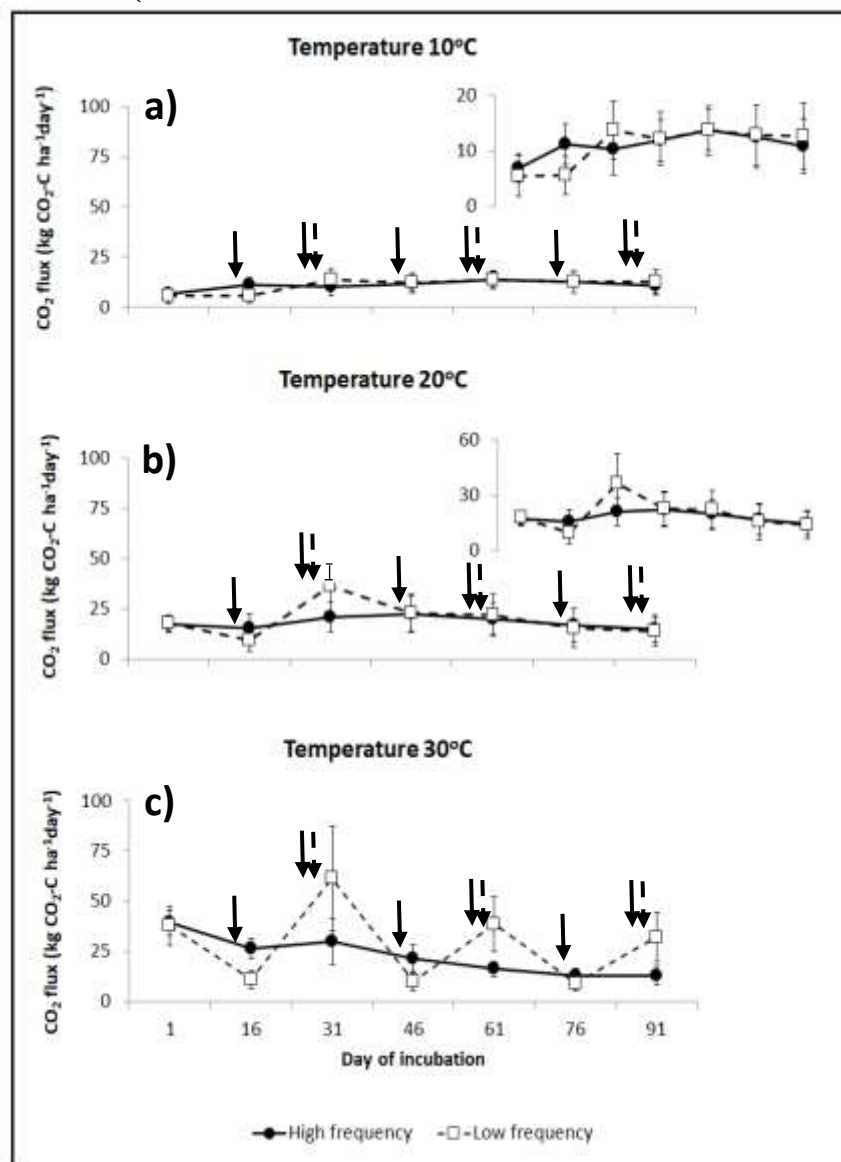


Figure 3-2. CO<sub>2</sub> fluxes over 91 days for different rainfall pulses (high and low frequencies) under three temperatures with a) 10, b) 20, and c) 30°C. Solid-line arrow represents the watering day of high frequency (every 15 days; on day 15, 30, 45, 60, 75, and 90 of incubation) and dash-line arrows are for low frequency (every 30 days; on day 30, 60, and 90 of incubation). The two inserts at 10 and 20°C represent the CO<sub>2</sub> flux with a small Y-axis scale to magnify the overlapped plots. Error bars represent standard errors of the mean (n=8).

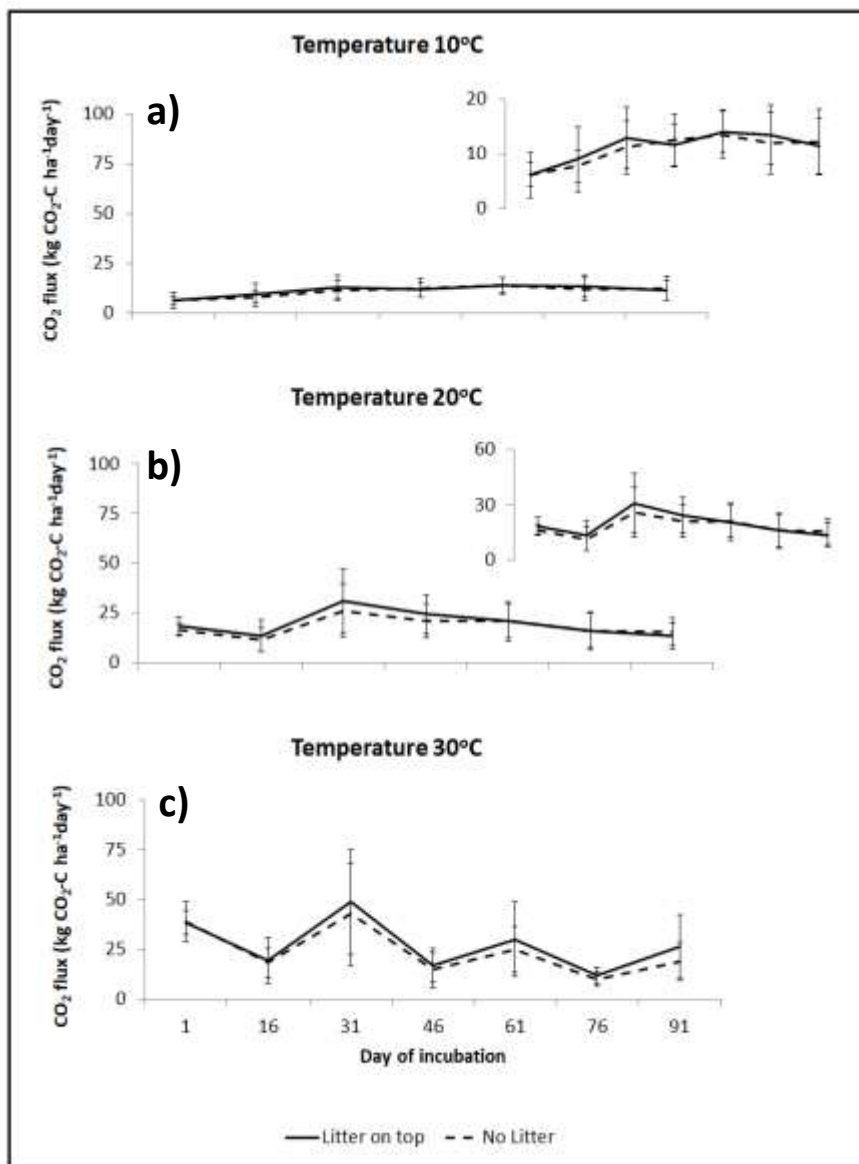


Figure 3-3. CO<sub>2</sub> fluxes over 91 days for different litter treatments (on top and control) for three temperatures with a) 10, b) 20, and c) 30°C. The two inserts at 10 and 20°C representing the CO<sub>2</sub> flux with a small Y-axis scale to magnify the overlapped plots. Error bars represent standard errors of the mean (n=8). High frequency watering: Day 15, 30, 45, 60, 75, and 90 of incubation; Low frequency watering: Day 30, 60, and 90 of incubation).



### **Effects of water or wetting frequency cycles on total cumulative CO<sub>2</sub> emission from litter plus SOM decomposition**

The cumulative CO<sub>2</sub> emission from different wetting frequencies and litter treatment at each sampling date is shown in Figure 3-4. The interaction of wetting frequency and litter input on CO<sub>2</sub> emissions was significant for all temperatures ( $p < 0.05$ ) (Table 3-1). At 10°C, CO<sub>2</sub> emitted from the litter treatment was 28% higher than control for the high-frequency water addition ( $p < 0.05$ ) but was 19% lower than the control for the low-frequency water addition treatment ( $p > 0.05$ ) (Figure 3-5). At 20 and 30°C, litter input had a positive effect on CO<sub>2</sub> emission however this increase was insignificant ( $p < 0.05$ ) within each wetting frequency. At 20°C, the litter input increased CO<sub>2</sub> emission by +10% and +9% over the no litter control? in high and low frequencies, respectively. However, at 30°C, this effect was most substantial in the high-frequency (+16%) than in the low-frequency treatment (4%). At 30°C, the highest cumulative CO<sub>2</sub> emission occurred in the low-frequency with litter ( $1,738 \pm 616$  kg CO<sub>2</sub> -C ha<sup>-1</sup>), and the lowest value was observed from the control treatment in the high-frequency ( $1358 \pm 364$  kg CO<sub>2</sub> -C ha<sup>-1</sup>).

The cumulative CO<sub>2</sub> emission increased with temperature, except for the control treatment with high-frequency pulses where CO<sub>2</sub> emissions decreased (-3%) with temperatures from 20°C to 30°C (no statistical test has done to compare the temperature effect). This suggests that the interactive effect between high-frequency

wetting and high temperature becomes the limiting factor as water evaporates more at higher than at lower temperatures.

Table 3-1. Main and interactive effects of litter and wetting frequency on cumulative CO<sub>2</sub> emission over 91 days of incubation under three temperatures (10, 20, and 30°C). ns: not significant differences and \*: significant differences with a p-value less than 0.05.

Factor	10°C	20°C	30°C
Litter	0.6039 ns	0.2424 ns	0.0244 *
Wetting frequency	0.0814 ns	0.0727 ns	0.0447 *
Wetting x Litter	0.0097 *	0.0061 *	< 0.001 *

### Effects of drying-wetting cycles on cumulative CO<sub>2</sub> emission from SOM decomposition

The average cumulative CO<sub>2</sub> emitted from the treatment without litter input, which representing only SOM decomposition at the end of the incubation for the high-frequency wetting, was 803, 1396, and 1358 kg CO<sub>2</sub>-C ha<sup>-1</sup> at 10, 20, and 30°C, respectively (Figures 3-4a, b, and c). However, in the low-frequency wetting, cumulative CO<sub>2</sub>-C emitted was 882, 1518, and 1669 kg CO<sub>2</sub>-C ha<sup>-1</sup> at 10, 20, and 30°C, respectively (Figure 3-4 d, e, and f). The highest cumulative SOM-derived CO<sub>2</sub> was observed for the low-frequency at 30°C (1669 kg CO<sub>2</sub>-C ha<sup>-1</sup>) (Figure 3-5c), and the lowest was in the high-frequency at 10°C (803 kg CO<sub>2</sub>-C ha<sup>-1</sup>) (Figure 3-5a) (no statistical test has done to compare the temperature effect).

### **Contribution of the litter-derived CO<sub>2</sub> to total cumulative CO<sub>2</sub>**

The individual contribution of the SOM and litter-derived CO<sub>2</sub> to total cumulative CO<sub>2</sub> emission depended on the interactive effect between wetting cycles and temperature. The contribution of litter-derived CO<sub>2</sub> to total cumulative CO<sub>2</sub> in the high-frequency watering accounted for 23, 10, 15% of total cumulative CO<sub>2</sub>-C flux at 10, 20, and 30°C, respectively. Under low-frequency treatment, this contribution was -34, 7, and -3% at 10, 20, and 30°C, respectively (Figure 3-6). The litter decomposition response to different wetting cycles within each temperature showed that all temperatures except for 20°C, the litter-derived CO<sub>2</sub> is significantly higher in high-frequency watering than the low-frequency watering treatment ( $p < 0.05$ ) (Figure 3-6).

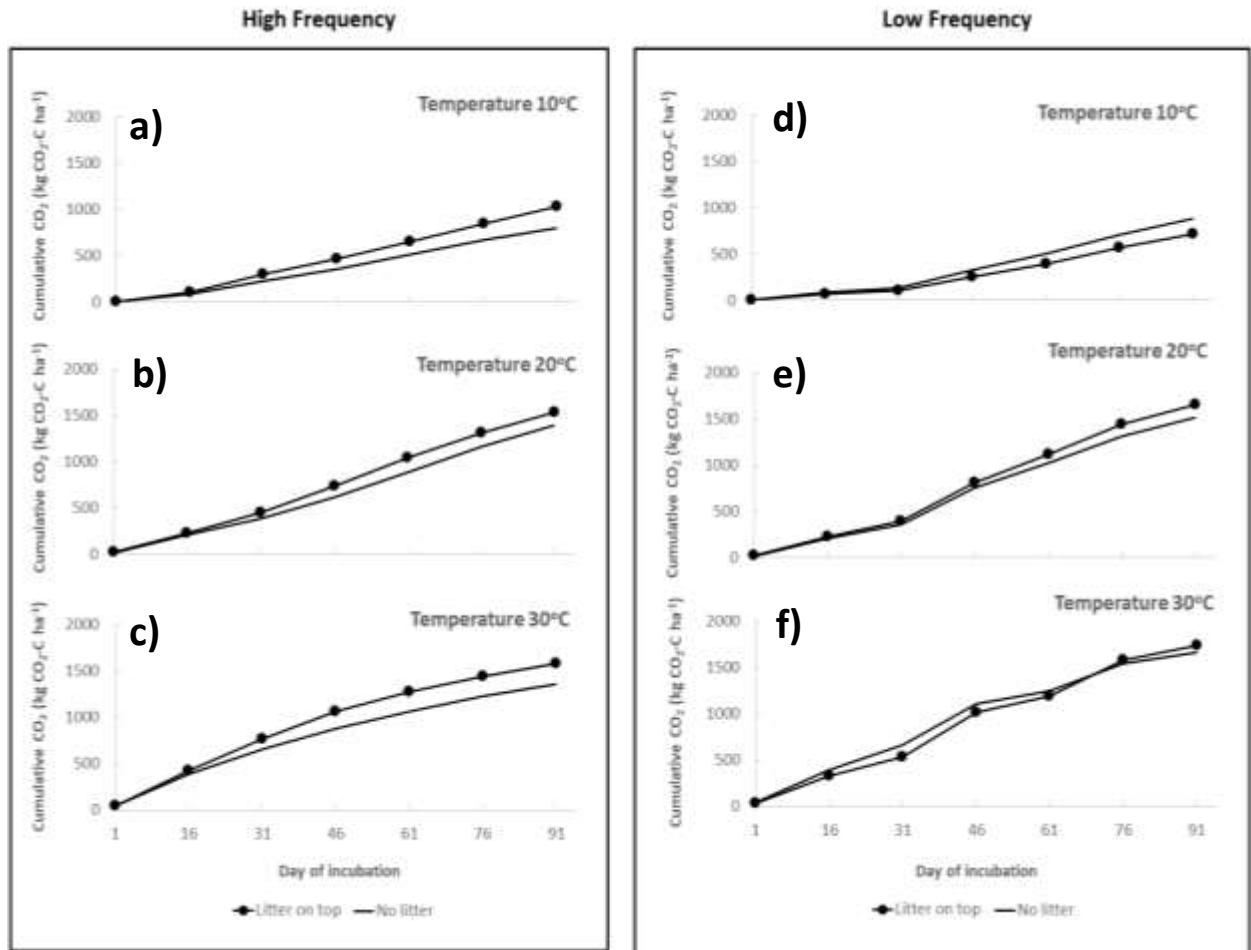


Figure 3-4. Cumulative CO<sub>2</sub> emission from the soil over time with the high and low frequency of wetting cycles and litter on top and control at temperatures of 10, 20, and 30°C. High frequency watering: Day 15, 30, 45, 60, 75, and 90 of incubation; Low frequency watering: Day 30, 60, and 90 of incubation).

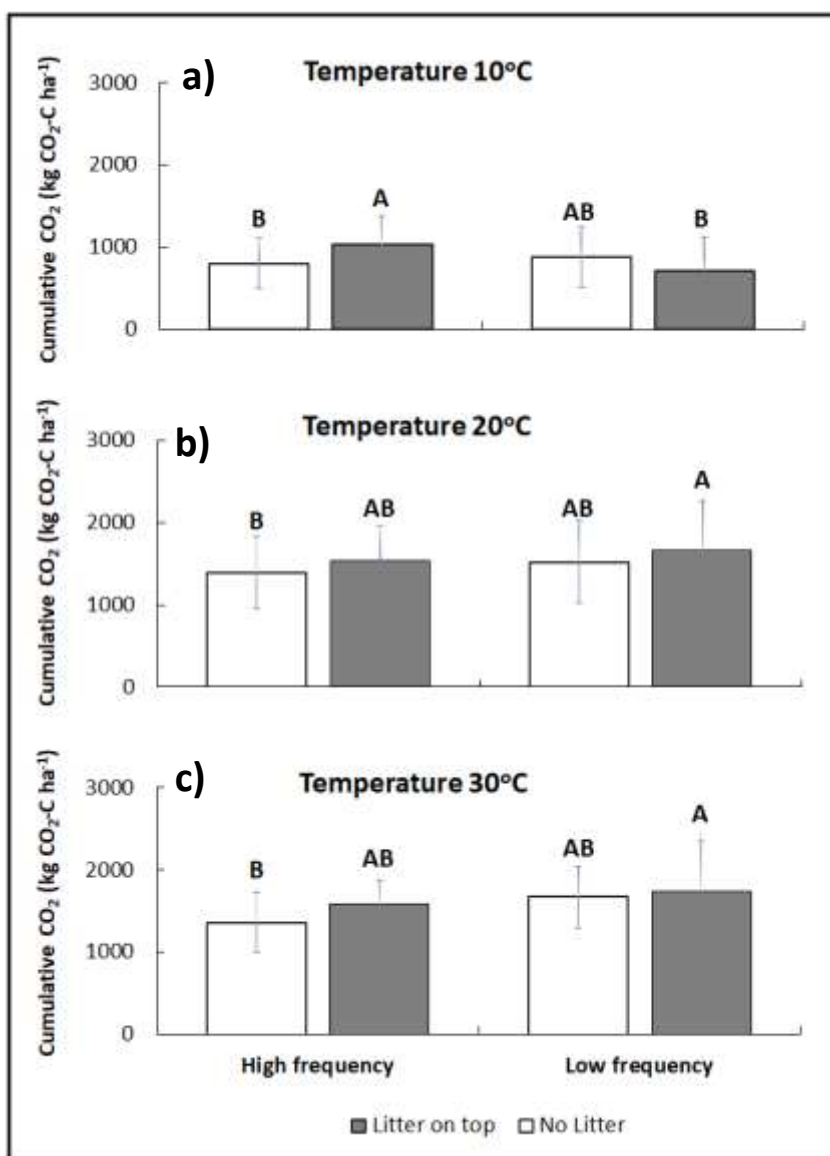


Figure 3-5. Analysis of two-way interaction between wetting cycles (high and low frequencies) and litter treatments (on top and control) for three temperatures with a) 10, b) 20, and c) 30°C. Error bars represent standard errors of the mean (n=8). Different capital letters represent statistical differences across treatment and within each temperature based on Tukey's multiple comparison test ( $p < 0.05$ ).

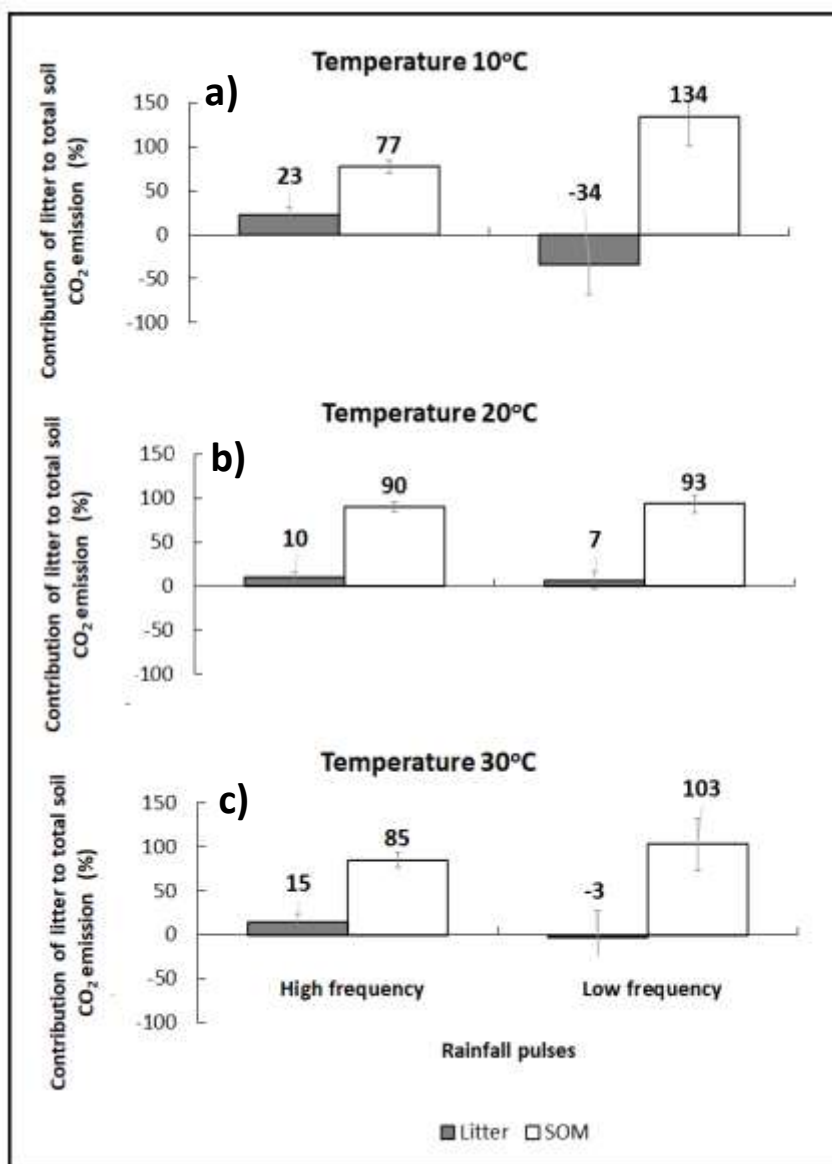


Figure 3-6. The proportion of cumulative CO<sub>2</sub> emission from litter decomposition and soil organic matter in total CO<sub>2</sub> emission under the high and low wetting frequency at temperatures of a) 10, b) 20 and c) 30°C over 91 days of incubation.

## **Discussion**

### **Effects of drying-wetting cycles on total soil CO<sub>2</sub> fluxes**

At temperatures of 20 and 30°C, the maximum CO<sub>2</sub> flux was recorded on day 31, one day after the first wetting at low frequency. Thereafter, the CO<sub>2</sub> flux decreased and showed no difference from the high-frequency wetting treatment. This implies that at 20 and 30°C in this incubation setup, drought stress may have limited microbial activity, and immediately after receiving water, decomposers became active, and consequently, CO<sub>2</sub> increased. Mikha et al. (2005) and Yu et al. (2014) demonstrated that the first drying-wetting cycle produces a large pulse of soil CO<sub>2</sub>; however, the subsequent drying-wetting cycles have little or no effect on soil respiration. Fierer and Scheme (2003) attributed the increase of microbial biomass following drying-wetting cycles to the rapid microbial growth contributed to the rise in OM availability and the subsequent persistence of a semi-dormant state. Zhang et al. (2007) inferred that after wetting the dry soil, the soil microbial community's adjustment cause increases in CO<sub>2</sub> flux. Diatta et al. (2019) showed that in Days 30 and 45 following drying- wetting cycles; microbial biomass dropped significantly to the same level before drying-wetting cycles. Thus, based on Diatta et al. (2019) results, resiliency of the microbial communities in soils could be considered as a potential reason of the observed trend of CO<sub>2</sub> flux in response to drying- wetting cycles in this study.

### **Effects of watering frequency cycles on total cumulative CO<sub>2</sub> emission from litter plus SOM decomposition**

Previous studies have reported that CO<sub>2</sub> emission from treatment with litter input was higher than that without litter input (Zhang et al., 2017; Liang et al., 2021). In this study, compared to the control treatment, the litter layer increased CO<sub>2</sub> emission mostly in the high frequency watering. At 10°C, a decrease in cumulative CO<sub>2</sub> emission was observed under the low-frequency watering. This highlights that the combined effect of litter and wetting cycles depends on soil temperature. The increase in cumulative CO<sub>2</sub> with litter input suggests that leached litter C with frequent water addition might have shifted the microbial community composition and increased microbial activity (Cleveland et al. 2006). Litter can improve microbial activity favored CO<sub>2</sub> emission by providing available labile C for decomposers and mitigating soil water loss by evaporation (He et al., 2014; Benesch et al., 2015). The water-saturated litter layer can explain the decreasing effect on CO<sub>2</sub> emission at 10°C under the low-frequency watering treatment with a large amount of water input limiting microbial activity compared to the high-frequency treatment with smaller amounts of water input. Additionally, this saturated litter layer could inhibit the CO<sub>2</sub> released from the soil to the atmosphere, which could cause measured CO<sub>2</sub> in the headspace to be less than the actual value.



### **Effects of water frequency on cumulative CO<sub>2</sub> emission from SOM decomposition**

Drying-wetting cycles have been considered an important factor influencing soil CO<sub>2</sub> emission by releasing labile organic C from previously protected soil organic matter and microbial biomass pool and accelerating C mineralization (Mondini et al., 2002; Wu and Brookes, 2005; Jia et al., 2014; López Ballesteros et al., 2016; Li et al., 2018; He et al., 2019). At 20°C and 30°C, low-frequency water addition increased CO<sub>2</sub> emission more than high-frequency treatment with a smaller amount of water, which was consistent with previous results (Schwinning and Sala, 2004; Moyano et al., 2013; Morillas et al., 2017). However, the contribution of the SOM-derived CO<sub>2</sub> compared to litter-derived CO<sub>2</sub> is less affected by the water addition frequency. This is in line with previous studies that drying-wetting cycles slowed the decomposition of litter more than that of SOM (Magid et al., 1999; Lopez-Sangil et al., 2018; Prein et al., 2016). Chen et al. (2008) found that only rainfall events larger than 10 mm could significantly increase the soil CO<sub>2</sub> emissions in semiarid grasslands. Our study also found that low-frequency wetting of 11 mm water also increased the CO<sub>2</sub> released from the SOM, although not statistically significant than the high frequency-wetting. Low frequency of drying-wetting cycles can increase soil moisture and concentration of available substrates for bacterial and fungal activity, increasing soil CO<sub>2</sub> emissions (Liang et al., 2016; Shi and Marschner, 2017; Liang et al., 2021). Xiang et al. (2008) stated that the pulse of CO<sub>2</sub> upon wetting is mainly dominated by the substrate provided by physically protected SOM.

Results showed that cumulative CO<sub>2</sub> emission increased with increasing temperature within each watering frequency. This can be attributed to the high microbial

activity at warmer temperatures. Bai et al. (2019) showed that soil bacterial and fungal groups had high activities when soil temperature was in a range of 15–45 °C. This suggests that the effects of wetting cycles on CO<sub>2</sub> emission are coupled with soil temperature and soil moisture content (Liu et al., 2017; Morillas et al., 2017; Sorensen et al., 2018).

### **Contribution of the litter-derived CO<sub>2</sub> to total cumulative CO<sub>2</sub>**

Most studies have tested the effect of drying-wetting cycles on total CO<sub>2</sub> emission from the soil in the presence of litter. But to our knowledge, the effect of wetting frequency on litter decomposition has not been well examined. Our results showed the different response of the fresh litter layer decomposition to wetting frequency or cycles. The different response of the litter layer decomposition to wetting frequencies can be attributed to the litter layer's porous structure, which provides a favorable environment for microbes to increase litter decomposition by retaining water and providing a more continuous supply for microbial activity.

In the high-frequency treatment, cumulative CO<sub>2</sub> derived by litter decomposition was higher than the low-frequency wetting cycle. With shorter dry periods, fluctuations in soil moisture were less severe, which increased microbial activity. In support of this possible reason, Zhang et al. (2017) and He et al. (2014) have stated that the litter layer moisture content should have remained higher under the high frequency wetting cycle, causing a consistent increase in CO<sub>2</sub> emission during the incubation (Zhang et al., 2017; He et al., 2014).

In contrast, in the low frequency at extreme temperatures, the observed negative values imply that litter decomposition was inhibited, and a downward flow of CO<sub>2</sub> into the soil had occurred. The negative litter-derived CO<sub>2</sub> emission at temperature 10°C might be attributed to the lower microbial activity at cold temperature. At 30°C, the negative values can be caused by the water limitation, which severs the drought stress for microbes. When a large amount of water was added at low frequency to a small area of the cylinder, complete water intake into the soil took longer than high-frequency pulses with smaller amounts of water. This suggests that the relatively faster water flow speed into the soil, translocate some of the substrates from the litter layer down to the microbial sites deeper in the soil, resulting in higher microbial activity and litter-derived CO<sub>2</sub> emission due to the increased availability of substrate (Yang et al., 2020). Another possible reason for negative litter-derived CO<sub>2</sub> emission is that oxygen availability to the microbes might become limited when a thin layer of the soil surface was saturated temporarily after the addition of larger water pulse. Under less favorable temperatures like 10°C and 30°C, rainfall pulse and frequency seem to play larger roles in the litter decomposition process.

### **Conclusion**

The response of SOM decomposition to wetting frequency or cycle is different than the response of litter decomposition. This means that drying-wetting cycles affect the contribution of SOM and litter-derived CO<sub>2</sub> to total CO<sub>2</sub> emission from the soil. Although the litter layer enhanced the decomposition and CO<sub>2</sub> emission, the magnitude of this increase depended on the wetting frequency and soil temperature. Under lower

temperatures (10°C) and prolonged drought stress caused by the low wetting frequency, litter decomposition was negligible. The high-frequency wetting cycle with a smaller amount of water likely provided continuous moisture supply and soluble C flow to decomposers, enhancing litter decomposition. Litter saturation may limit the oxygen availability of decomposers and consequently inhibit litter decomposition when large pulses of water are applied at low frequency. Our research results have important implications for predicting the soil C cycling of semiarid grassland ecosystems under variable rainfall input and length of period between rainfall occurrences. In this study, although the possible reasons for the effect of the wetting cycles on SOM and litter decomposition were described, further studies are needed to determine the mechanisms controlling these contributions.

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## **CHAPTER 4: Modifying a mono-component decomposition model for the combined effect of soil temperature and moisture**

### **Abstract**

This study aims to improve modeling on the effects of abiotic factors on litter decomposition with different placements of litter. The effect of temperature on organic matter decomposition is expressed by  $Q_{10}$  values and with a two-factor model accounting for temperature and moisture. To describe the changes in decomposition rates over time, log-linear regression was applied to the average reactivity rate coefficient ( $K$ ) for each case. Temperature corrections were also applied to simplify the model, although accuracy was reduced by up to 30% in the worst cases. To improve the accuracy of correction factors in the model, multiple linear regression was applied to  $K$ , the initial reactivity  $R$ , and  $K$ 's decay rate  $S$ , with temperature and moisture as independent variables. Results obtained by this hybrid method were compared with existing models and demonstrate a better agreement with the specific effects of litter placement on carbon (C) decomposition under different environmental conditions over time. Finally, a correlation between  $R$  and  $S$  was applied to further generalize the model, which predicted soil C residue within 2% of the experimental data while using only temperature and moisture as inputs for each litter placement.

### **Nomenclature**

$K$  = Average rate coefficient ( $\text{time}^{-1}$ ) over a specific period

$k$  = Instantaneous rate coefficient ( $\text{time}^{-1}$ )

$R$  = Average rate constant from time 0 to 1 ( $\text{time}^{-1}$ )

$S$  = Decay rate of  $K$  or  $k$  (no dimension)

$F_{\text{CO}_2}$  = CO<sub>2</sub> flux (kg C-CO<sub>2</sub>/ha/day)

T = Temperature (Celsius)

t = Time (day)

w = Water-filled pore space (WFPS, %)

Y = Mass of carbon (grams)

## **Introduction**

Grassland ecosystems are important for the global C cycle, as they occupy 40 % of the global land surface (Bontti et al., 2009) and store 295 GT of C (Bolin et al. 2000). Grasslands are more vulnerable to the impact of management on soil C storage because of the frequent human interventions (Soussana et al. 2004). Grazing management is responsible for major processes of C cycling through defoliation, dung and urine return, and trampling (Zhao et al., 2017; Chen and Frank, 2020). Among the grazing management practices, mob grazing is one of the grazing practices used in the USA, though its effects on soil C balance and CO<sub>2</sub> emission are still little known. Previous studies have shown that mob grazing promotes the incorporation of litter (i.e., dead plant material) into the soil through trampling (Mancilla-Leyton et al., 2013), which provides a better environment for microorganisms and promotes litter decomposition (Hewins et al., 2017; Lee et al., 2014). However, in grasslands under alterations in environmental conditions or management, it is important to understand the effect of trampling on litter decomposition, a critical process in regulating CO<sub>2</sub> emission, and nutrient cycling and soil fertility (Wardle, 2002; Vitousek, 2004). CO<sub>2</sub> release from the soil through autotrophic (live roots and associated rhizosphere communities) and heterotrophic (soil organic matter and litter decomposition) pathways are controlled by different factors. In



the current study, only the heterotrophic pathway derived from soil organic matter and grass litter decomposition was considered. Decomposition, in this case, is controlled by abiotic factors, mainly soil temperature and moisture (Curtin et al., 2012; Taggart et al., 2012; Benbi et al., 2014).

### **Overview of modeling approaches**

Given the complexity of the organic matter decomposition process, several mathematical models have been proposed to predict organic matter decomposition (Burke et al., 2003; Moorhead et al., 1996). Approaches vary greatly in mathematical formulation, as well as spatial and temporal scales, depending on the data used for model development and the intended uses of the models. Litter decomposition has been modeled for time intervals ranging from hours (Fernández-Alonso et al., 2018) to years (Yang and Janssen, 2000; 2002). In turn, the choice of modeling strategy will depend on the particular research objectives and the complexity of experimental data on which the model is based.

First-order reaction kinetics is often used as a basis for developing decomposition models, where the rate of decomposition is assumed to be related to the total amount of residue present at a given time (Whitmore, 1996). However, the need to explicitly account for microbial activity and its complex effects on decomposition has led to recent development of more complex second order models, as reviewed by Campbell and Paustain (2015). For steady-state environmental conditions like in many incubation experiments that primarily consider abiotic factors, it was found that first-order reaction models are better at predicting organic matter (OM) decomposition (Lawrence et al., 2009). Studies that considered complex C pools with the inclusion of microbial mass

found that second-order models were needed to describe changes in decomposition rate, as nutrients are consumed by microbes as they become available during decomposition and result in non-linear changes in reactant concentration over time (Blagodatsky, 2010).

### **Reactivity and its decomposition rate over time**

During the first several weeks, the decomposition rate is typically very high. Thereafter, the rate becomes more predictable (Yang and Janssen, 2002). For modeling purposes, the initial period of decomposition is typically treated separately due to this very different behavior. To account for temperature effects, the  $Q_{10}$  is commonly used, which describes the change in decomposition rate for a 10°C change in temperature (Kirschbaum, 1995). Wang et al. (2016) studied the combined effects of moisture, temperature, and elevation on the initial decomposition rates of soil organic matter. A two-factor non-linear model was applied to data from each sampling location, which demonstrated that temperature and moisture effects accounted for 80-90% of the differences in observed decomposition samples (Wang et al., 2016).

Models to predict long-term soil C balance are generally applied beyond this initial period of high reactivity. Some models attempt to predict this behavior based on total soil respiration (Cook and Orchard, 2008), while Yang and Janssen found a better agreement by applying regressions to the average decomposition rate ( $K$ , unit of  $\text{time}^{-1}$ ) rather than total soil respiration. When multiple substrates are present, each substrate has its own unique  $K$  value. Complex multi-component models consider  $K$  for each constituent element along with environmental conditions, which can be very accurate but also difficult for average users to implement. Mono-component models attempt to find a

single representative  $K$  that captures the behavior of the bulk substrate but often trade accuracy for simplicity.

To develop a model that was both accurate and simple to use, Yang and Janssen (2000) proposed a mono-component model by considering changes in  $K$  over time, requiring only the initial decomposition rate of the substrate and its decay over time as the independent variables. This initial decomposition rate ( $R$ ) was found to have a strong correlation with how it slows over time, which allowed proper model coefficients to be determined without considering the specifics of mixtures and environmental conditions.

The model was later applied to describe a large data set with wide variations in geography and soil composition (Yang and Janssen, 2002). By classifying decomposing materials into four groups based on their magnitude of initial reactivity, it was found that regression models could accurately predict their decomposition over time. Temperature corrections were also applied within the model to reduce the number of unique equations needed to describe the data set with only minor penalties on accuracy.

### **Temperature and moisture effects**

The effects of soil moisture and temperature on litter decomposition are understood reasonably well. However, the interactive effect of soil moisture and temperature on litter decomposition is less understood (Ceccon et al., 2011; Suseela et al., 2012). Rodrigo et al. (1997) considered temperature and moisture effects on the initial reactivity of C-N transformations using nine separate models. Linear relationships between decomposition rates and soil moisture showed poor agreement for dry climates, while temperature effects were best captured using models proposed by Jenkinson (1990)

and Kirschbaum (1995). None of the models considered has explicitly captured interactions between temperature and moisture.

Interactions between time, temperature, moisture, and oxygen concentration were studied to determine their effects on total C respiration over a 35-day period by Sierra et al. (2017). The same temperature correction as used by Yang and Janssen was employed, while moisture and oxygen effects were added in terms of their respective half-saturation constants. Results showed a strong interaction between decomposition rate and temperature when moisture and oxygen levels were not limiting, and that moisture effects were significant when oxygen levels were not limiting. Other studies on the effects of time and moisture on soil respiration from roots and microbial activity were performed by Cook and Orchard, (2008). It was found that time effects were best captured using a power function, while moisture effects could be described using linear regression.

### **Objectives of this study**

The objective of this study is to improve current decomposition models to accurately capture the interactions between litter placement, soil moisture, and temperature, considering both initial reactivity and their subsequent behavior through a typical growing season.

### **Materials and methods**

#### **Controlled Environment Incubation**

The data for this study were from the experiment that was aimed at quantifying decomposition rates of grass litter and existing soil organic matter under different litter placement, soil temperature, and moisture. Since decomposition in open fields would be affected by a large number of interacting factors, the experiment was carried out as

incubation under controlled conditions. The soil and litter used in the incubation were collected from the University of Nebraska-Lincoln Barta Brothers Ranch (42°13' N, 99°38' W) in Eastern Nebraska Sandhills. The climate of the sampling area is semiarid, with annual precipitation ranging from 430 to 580 mm and an annual average temperature of ~10°C. The litter was a mixture of species including redtop bent (*Agrostis stolonifera*), big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), prairie cordgrass (*Spartina pectinata*), smooth brome grass (*Bromus inermis*), kentucky bluegrass (*Poa pratensis*), and sedges. The incubation experiment was carried out in microcosms placed in a growth chamber under controlled temperature and soil moisture content. The experimental design was a split-block with temperature and block stripped across each other and with litter placement and soil moisture as the split-plot factors. The treatment factors were three temperatures 10, 20 and 30°C; three litter mixing modes; control (no litter), litter addition on top of the soil (on top), and litter mixing into the uppermost 4 cm of the soil (mixed); and three levels of soil moisture: 23, 37 and 50% of water-filled pore space (WFPS). The soil moisture and temperature levels represent typical soil field conditions of that area during the growing season. Air-dried soil, 137 g was placed in acrylic cylinders and packed to 10 cm depth to conform to the field's soil bulk density. The cylinders measured 3.81 cm in diameter and 22.0 cm in height. Four replicates per litter and moisture treatment combination were incubated for 91 days (typical growing season length) in growth chambers. Carbon dioxide (CO<sub>2</sub>) fluxes were measured using Vernier LabQuest 2, CO<sub>2</sub> Gas Sensor (Vernier, Beaverton, OR) every two weeks on days 1, 16, 31, 46, 61, 76, and 91 of the incubation. Note in this chapter experimental data from chapter 2 were used.

### Mathematical models of decomposition rate

Based on first-order reaction kinetics, the rate of decomposition at a time  $t$  is given generically by the relation:

$$\frac{dY}{dt} = -KY \quad (1a)$$

where  $Y$  is the amount of decomposing material, and  $K$  is the decomposition rate coefficient (time<sup>-1</sup>) at time  $t$ . Integrating Equation (1a) from time 0 to  $t$  (day), the resulting expression is:

$$Y_t = Y_0 e^{-Kt} \quad (1b)$$

Where  $Y_t$  is the remaining material amount at time  $t$  and  $Y_0$  is the amount of material at time 0. As Yang Janssen (2000) reported, the decomposition rate  $k$  is not a constant. Instead, it decreases over time linearly in the double logarithmic scales:

$$\log(K) = \log(R) - S * \log(t) \quad (2)$$

where  $K$  is the average decomposition rate from time=0 to  $t$ ,  $R$  is the average  $K$  from time=0 to time=1, or 'initial reactivity', and  $S$  (no dimension) represents the speed of decrease of  $K$  over time (Yang and Janssen, 2000).

The temperature sensitivity ( $Q_{10}$ ) of OM decomposition can be described as the ratio of the rate constants at two temperatures differing by 10°C (Ellert and Bettany, 1992; Kirschbaum, 1995; Stanford et al., 1973);

$$Q_{10} = (K_2/K_1)^{10/(T_2-T_1)} \quad (3)$$

$T_1$  and  $T_2$  are the lower and higher temperatures (°C), and  $K_1$  and  $K_2$  are the average rate constant at  $T_1$  and  $T_2$ .

Based on Equation 2 (Yang and Janssen, 2000), the temperature sensitivity of the decomposition rate ( $Q_{10}$ ) over time ( $t$ ) is calculated as:

$$Q_{10} = (R_2/R_1)^{\frac{10^\circ\text{C}}{T_2-T_1}} \times t^{\frac{10^\circ\text{C}(S_1-S_2)}{T_2-T_1}} \quad (4)$$

where subscript 1 refers to the lower temperature ( $T_1$ ) and subscript 2 the higher temperature ( $T_2$ ), and R and S coefficients are found at  $T_1$  and  $T_2$ .

The combined effects of temperature and moisture on total measured  $\text{CO}_2$  flux from the soil ( $F_{\text{CO}_2}$ ) at the initial time step (day 1 to day 17 in the experiment) were modeled based on the method of Wang et al. (2016), who considered only the first 14 days of incubation in their model. This approach obtained models for  $F_{\text{CO}_2}$  by directly considering the soil respiration rate rather than calculated Y values, which was also applied here to enable better comparison between results obtained by Wang et al. (2016) and those obtained here. Note that this was the only equation where soil respiration was used in place of Y. In this case, non-linear regression was applied to the following relation:

$$F_{\text{CO}_2} = ae^{bT}(cw^2 + dw + f) \quad (5)$$

Where a, b, c, d, and f are fitted parameters.

Time effect on decomposition was first considered independently from environmental factors using R and S based on the method of (Yang and Janssen, 2000).

After determining proper values for S by linear regression (Equation 2), the decomposition average rate coefficient K (1/time) between time 0 and t (day), and actual instantaneous rate coefficient k (1/time) at time t, are calculated by:

$$K = Rt^{-S} \quad (6)$$

$$k = (1 - S)K \quad (7)$$

Remaining residue  $Y_t$  at any time t is related to the initial residue  $Y_0$  at time  $t = 0$  by

$$Y_t = Y_0 \exp(-Rt^{1-S}) \quad (8)$$

The percentage of the remaining residue ( $Y_t$ ) calculated using CO<sub>2</sub> flux (raw data) to construct this model is shown in Figure 4-1.

To consider temperature effects within the model of Yang and Janssen (2000), a temperature coefficient ( $f$ ) was calculated as follows. For a known value of  $R$  at 10°C,  $f$  is calculated by:

$$f = \begin{cases} 2^{\frac{T-10}{10}} & \text{if } 10^\circ\text{C} < T < 27^\circ\text{C} \\ 4 & \text{if } 27^\circ\text{C} < T < 35^\circ\text{C} \end{cases} \quad (9)$$

Using the subscript "10" to indicate values obtained at 10°C, the temperature corrected values are found by:

$$K = R_{10} \cdot f \cdot (f \cdot t)^{-S} \quad (10)$$

$$k = (1 - S) \cdot R_{10} \cdot f \cdot (f \cdot t)^{-S} \quad (11)$$

$$Y_t = Y_0 \cdot \exp(-R_{10} \cdot (f \cdot t))^{1-S} \quad (12)$$

In addition, two other methods were proposed below to account for both temperature and moisture effects. The first method assumes linear functions of  $T$  and  $w$  with a single interaction term, similar to earlier proposed equations (Rodrigo et al, 1997), allowing  $R$  to be calculated for each litter placement by the relation:

$$R = a_R T + b_R w + c_R T w + d_R \quad (13)$$

where  $a_R$ ,  $b_R$ ,  $c_R$ , and  $d_R$  are coefficients to be obtained by regression of  $R$  with  $T$  (temperature) and  $w$  (moisture). For clarity, coefficient subscripts are included to reference the dependent variable to which they apply. The remaining amount ( $Y_t$ ) at time  $t$  is then calculated using Equations 8-12, while  $S$  remains unchanged from the values obtained by direct regression on Equation 6.



To further reduce the number of equations required to predict decomposition, a similar approach was used to predict S based on temperature and moisture by the relation:

$$S = a_s T + b_s w + c_s T w + d_s \quad (14)$$

where  $a_s$ ,  $b_s$ ,  $c_s$ , and  $d_s$  are coefficients obtained by regression of S with T (temperature) and w (moisture). The amount of residue is then calculated using Equations 8-12, while R remains unchanged for each case.

To further generalize the model, the second approach was proposed following Yang and Janssen (2002), where a relation between R and S was considered to have the form:

$$R = a e^{bS} \quad (15)$$

Where S is obtained by Equation 14, with a and b as fitted parameters.

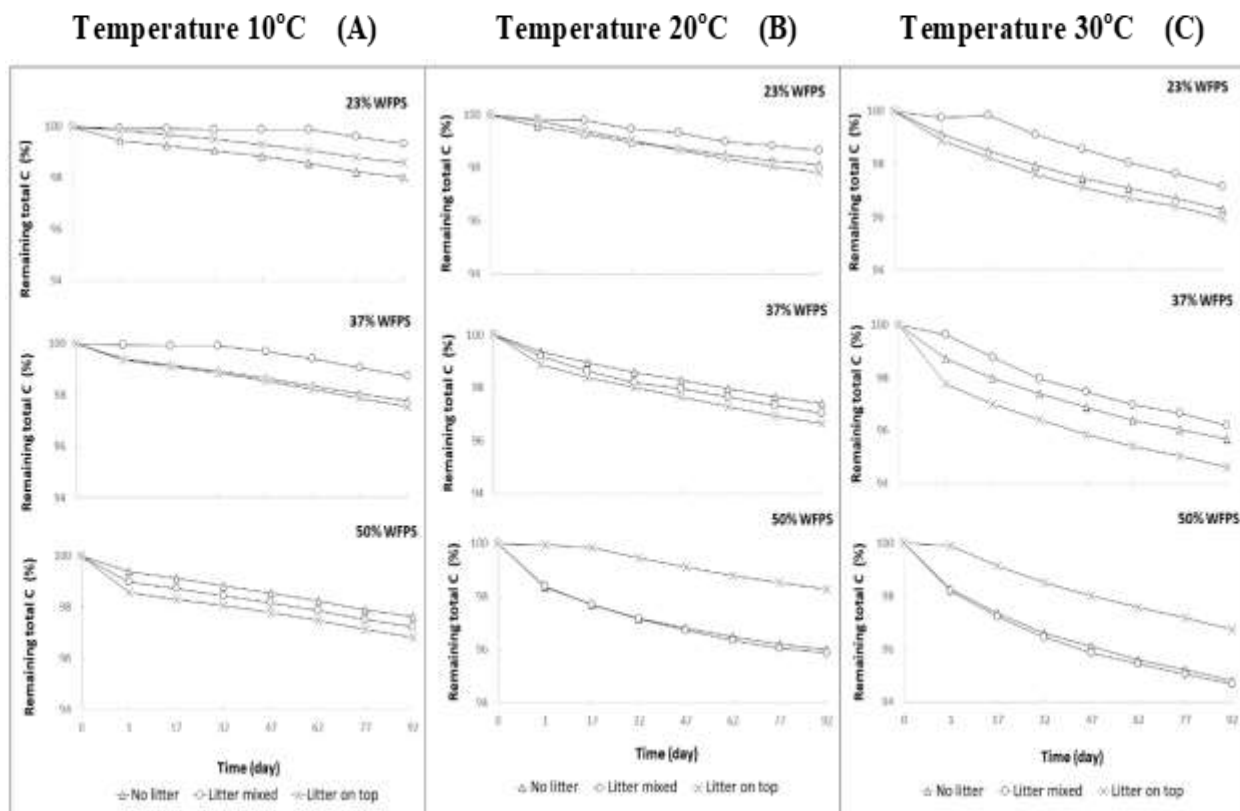


Figure 4-1. Remaining carbon (%) over time in soil incubated under constant moisture content (23, 37, and 50% WFPS) at three temperatures of 10, 20, and 30°C: 1A, 1B, and 1C, respectively.

## Results and Discussion

Results for  $Q_{10}$  as a function of R and S given by Equation 4 are shown in Figure 4-2. For litter mixed treatment, the averaged  $Q_{10}$  across three levels of soil moisture content for the temperature range of 10 to 20°C and 20 to 30°C were  $2.0 (\pm 0.48)$  and  $1.4 (\pm 0.15)$ . This suggests that the decomposition of soil organic matter plus litter in the litter mixed treatment is more sensitive to the rising temperature from 10 to 20°C than from 20 to 30°C. In contrast, there were no differences between the averaged  $Q_{10}$  across three soil moisture content levels between the control and litter on top treatments under both temperature levels. The range of  $Q_{10}$  we found in this experiment was similar to those reported for grasslands (2.1 to 2.7) (Luo et al. 2001). Compared with the no litter

treatment (control), adding litter on the soil surface did not affect the  $Q_{10}$  value, while mixing litter with soil increased the  $Q_{10}$  value (+33.3%) for the temperature range of 10 to 20°C but decreased it (-6.7%) for the temperature range of 20 to 30°C.

Additionally, the effect of soil moisture on  $Q_{10}$  depended on the temperature and the placements of litter. For the temperature range of 10 to 20°C, the average  $Q_{10}$  over the incubation period for litter on top treatment was the highest at 37% WFPS ( $1.7 \pm 0.14$ ) and lowest at 50% WFPS ( $1.2 \pm 0.00$ ). Rising temperature from 20 to 30°C decreased the  $Q_{10}$  value of the 37% WFPS to  $1.5 \pm 0.02$  but increased the  $Q_{10}$  value of the 50% WFPS ( $1.6 \pm 0.03$ ), whereas the average  $Q_{10}$  value for the low moisture (23% WFPS) of litter on top was 1.6 for both temperature levels.

For the litter mixed treatment, the average  $Q_{10}$  over the incubation period was different for the two ranges of temperatures. By rising temperature from 10 to 20°C, the highest  $Q_{10}$  was observed at 23% WFPS ( $2.3 \pm 0.54$ ), and the lowest was at 37% WFPS ( $1.5 \pm 0.12$ ). However, at the temperature range of 20 to 30°C, this response was reversed as the highest and lowest  $Q_{10}$  were found from 37 and 23% WFPS,  $1.5 (\pm 0.05)$  and  $1.3 (\pm 0.24)$ . Rising temperatures from 20 to 30°C decreased the  $Q_{10}$  value of litter mixed treatment at the low and high moisture content (23 and 50% WFPS). It decreased the  $Q_{10}$  for 23% WFPS from 2.3 to 1.3 and 50% WFPS from 2.1 to 1.4; however, no changes were observed for 37% WFPS.

For the no litter treatment (control) at the lower range of temperature (10 to 20°C), the highest average of  $Q_{10}$  during the incubation period was observed for 50% WFPS ( $2.0 \pm 0.35$ ), and the lowest was for 37% WFPS ( $1.1 \pm 0.07$ ). However, for rising temperature from 20 to 30°C, this response was reversed as the highest and lowest  $Q_{10}$

were found from 37 and 50% WFPS,  $1.7 (\pm 0.14)$  and  $1.2 (\pm 0.09)$ , respectively. Rising temperature from 20 to 30°C decreased the  $Q_{10}$  value of no litter treatment for moisture content 50% WFPS from 2.0 to 1.2. In contrast, the  $Q_{10}$  values of 37% WFPS increased from 1.1 to 1.7 and in 23% WFPS from 1.2 to 1.6. Along with the previous studies (Luo et al., 2001; Zhou et al., 2009). Our results showed that with increasing soil temperature,  $Q_{10}$  tends to become lower. Generally, soil moisture effects on  $Q_{10}$  are most likely due to the confounding influences of litter placement on moisture distribution between litter and soil.

At lower soil moisture (23% WFPS), litter addition either into the soil or on top of the soil increased  $Q_{10}$ , probably because of more favorable conditions for microbial respiration induced by the litter addition (Figure 4-2a). At the higher soil moisture content (50% WFPS, Figure 4-2c), litter mixing increased  $Q_{10}$ , which could be attributed to the possibility that diffusivity of soluble substrates increases with increasing water content and substrate accessibility to decomposers (Davidson and Janssens, 2006). However,  $Q_{10}$  decreased when adding the litter on top of the soil surface at 50% WFPS (Figure 4-2c). The  $O_2$  limitation for microbial activity may explain this because of the saturated condition caused by wet litter on top of the soil surface. In addition, litter on top is less accessible to decomposers in soil, while reduced diffusion of the soluble substrate could be another possible reason for the low  $Q_{10}$  value (Linn and Doran, 1984).

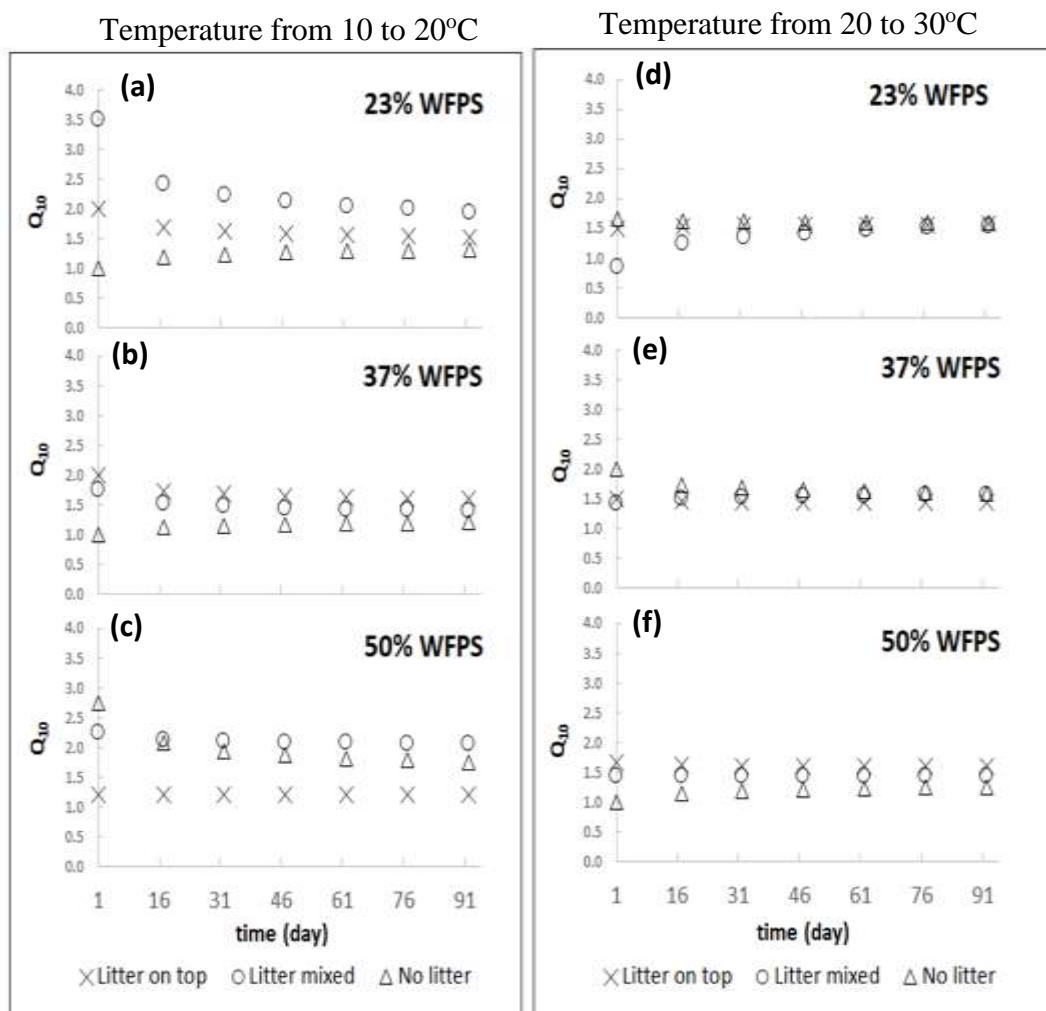


Figure 4-2.  $Q_{10}$  values over time for three litter placements, three soil moisture contents, and two temperature intervals from 10 to 20°C and 20 to 30°C.

The temperature sensitivity is used in most models to predict changes in C balance as a function of changes in the soil temperature (Kirschbaum 1995; Friedlingstein et al., 2006). However, some researchers argue that the  $Q_{10}$  function lacks a theoretical justification and that it may be biased in estimating decomposition (Johnson and Thornley, 1985; Lloyd and Taylor, 1994). Many models (e.g., Raich and Potter 1995) use a constant  $Q_{10}$  even though  $Q_{10}$  values often vary depending on temperature range

(Holland et al. 2000; Luo et al., 2001; Xu and Qi 2001; Davidson and Janssens, 2006), quantity and quality of soil organic matter (Taylor et al., 1989; Wan and Luo, 2003), substrate availability (Davidson and Janssens, 2006), and soil moisture (Davidson et al., 1998; Hui and Luo, 2004). A major limitation of these models is the uncertainty in estimates of  $Q_{10}$  values for soil temperature  $>20^{\circ}\text{C}$  (Del Grosso et al., 2005).

The interactive effects of soil temperature and moisture on decomposition are demonstrated through alterations in microbial composition, microbial activity, substrate supply, and substrate availability (Davidson and Janssens, 2006; Wagai et al., 2013). Combined effects of temperature and moisture on the initial reactivity rates for each litter placement given by Equation 5 are shown in Figure 4-3, along with residual data showing the fit quality for each case Figure 4-4. Fitted parameters obtained by non-linear regression are listed in Table 4-1, along with values for  $r^2$ , F-statistics, and p-values for each litter placement.

Table 4-1. Regression coefficients of Equation 5 for different litter placement.

	<b>Litter on top</b>	<b>Litter mixed</b>	<b>No litter</b>
<b>A</b>	0.01	0.01	0.03
<b>B</b>	0.05	0.07	0.07
<b>C</b>	-0.15	-0.57	0.04
<b>D</b>	13.26	46.04	-1.38
<b>F</b>	273.78	-345.84	148.89
<b>r<sup>2</sup></b>	0.98	0.98	0.96
<b>F</b>	403	361	159
<b>P</b>	< 0.0001	<0.0001	<0.0001

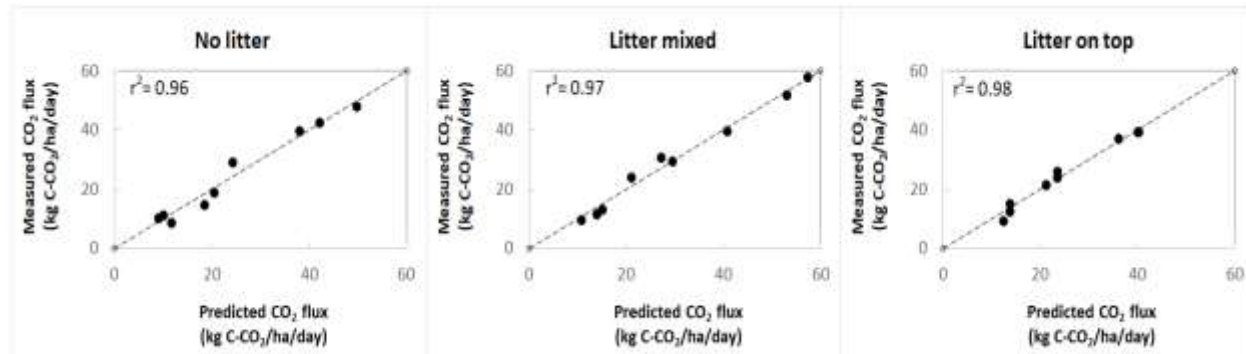


Figure 4-3. Measured and predicted values for CO<sub>2</sub> flux obtained by Equation 5. Note that the dashed line represents the 1:1 relationship between predicted and observed data. All the corresponding probabilities (p-value) were less than 0.0001.

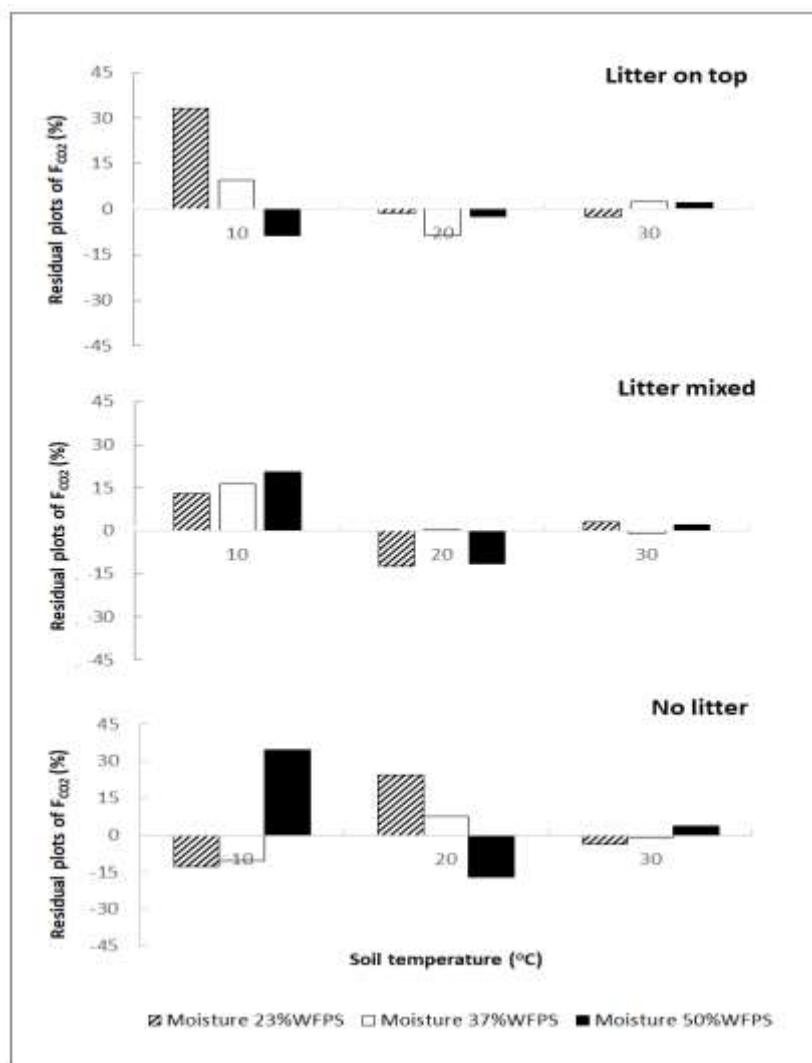


Figure 4-4. Percentage of the deviation between measured and predicted  $CO_2$  flux ( $F_{CO_2}$ ) by Equation 5 for three litter placements.

For total  $CO_2$  flux at the initial time step, the two-factor model, including soil temperature and moisture, showed a good agreement with observed values. The lowest  $r^2$  value, in this case, was 0.96, which occurred for the no litter control treatment.

Temperature and moisture interactions were most significant for the litter on top treatment ( $F = 403$ ) and least significant for the no litter treatment ( $F = 159$ ). All litter treatments showed  $p$ -values  $< 0.00001$ . For the litter on top treatment, the model fitted the changes of decomposition rate well for all moisture and temperature levels except for the



cool (at 10°C) and dry condition (23% WFPS) with 33% overestimation of total soil respiration. For litter mixed and control treatments, the performance of this model regarding the different levels of moisture and temperature was more complex than the litter on top treatment. Although we did not observe a trend in estimating the relationships between these factors and decomposition rate for litter mixed and control treatments, the worst fit was related to the high moisture content (50% WFPS) and low temperature (at 10°C) in control treatments with 34% overestimation. The average errors for all soil treatments at 10°C for 23 and 50% WFPS were 11% and 15%, respectively. Our findings confirmed that the interaction between temperature and moisture had a significant effect on decomposition rate at different litter treatment. However, there is still a need to capture the effect of these factors more accurately.

To capture the behavior of decomposition rates over time, a full regression was applied to Equation 2, which yielded unique model coefficients for each combination of litter placement, temperature, and moisture, resulting in 27 equations. Coefficients from each individual regression are listed in Table 4-2. The criteria for evaluating the quality of regressions performed on Equation 2 are the distribution of  $r^2$  values obtained for each case and the model's ability to predict C residue  $Y$  over time. For all cases,  $r^2$  values ranged from 0.74 to 0.99 except for litter on top at temperature 10°C and moisture 23% WFPS which is the worst case with  $r^2 = 0.33$ . The values of R and S differ among soil moisture and temperature within each litter placement.

At 10°C, R increased with moisture levels from 23% to 50% WFPS in the litter on top treatment but showed no change for control and mixed litter treatments with moisture levels at or above 37% WFPS. At temperature 20°C, R increased with moisture

from 37% to 50% WFPS for litter on top and litter mixed from 23% to 37% WFPS. However, for all litter treatments at temperature 30°C, R increased with increasing moisture content.

Although we observed that S generally increases with soil moisture, it decreased for litter mixed at 20°C from 23% to 37% WFPS and the control treatment at temperature 10°C with moisture between 37% and 50% WFPS. Overall, the averaged R-value across the three-soil moisture and three litter placement levels increased with increasing temperature and moisture (Table 4-3). Adding litter on top did not change the R-value compared to the control treatment but mixing litter with soil increased R by +20%. The S values increased with increasing moisture; however, the temperature effect did not follow an increasing trend, as the highest averaged S value was calculated for temperature 20°C (0.14). Furthermore, compared to litter on top and control treatments, litter mixed treatment had the highest S value of 0.14. Against our expectation of a positive relationship between R and S values, the results demonstrated that R and S values change differently with temperature and moisture. Hence, consequential effects of litter placement through altering soil temperature and moisture had different effects on R and S values.

Table 4-2. Coefficients obtained by linear regression of Equation 6, with  $r^2$  values for each soil mixture, moisture, and temperature.

Temperature (°C)	Moisture (%WFPS)	Litter on top			Litter mixed			No litter		
		R (day <sup>-1</sup> )	S	Adjusted $r^2$	R (day <sup>-1</sup> )	S	Adjusted $r^2$	R (day <sup>-1</sup> )	S	Adjusted $r^2$
10	23	0.0002	0.03	0.33	0.0002	0.07	0.78	0.0003	0.13	0.96
10	37	0.0003	0.08	0.93	0.0004	0.12	0.97	0.0004	0.12	0.98
10	50	0.0005	0.15	0.97	0.0004	0.15	0.96	0.0004	0.10	0.99
20	23	0.0004	0.09	0.89	0.0007	0.20	0.88	0.0003	0.07	0.75
20	37	0.0006	0.13	0.90	0.0007	0.17	0.85	0.0004	0.08	0.83
20	50	0.0006	0.15	0.86	0.0009	0.17	0.83	0.0011	0.20	0.85
30	23	0.0006	0.08	0.74	0.0006	0.07	0.77	0.0005	0.08	0.74
30	37	0.0009	0.14	0.86	0.0010	0.15	0.80	0.0008	0.13	0.84
30	50	0.0010	0.16	0.86	0.0013	0.17	0.82	0.0011	0.15	0.81

Table 4-3. The average value of R and S of the three temperatures, moistures, and three litter placements.

Moisture (%WFPS)	R (day <sup>-1</sup> )	S	Temperature (°C)	R (day <sup>-1</sup> )	S	Litter placement	R (day <sup>-1</sup> )	S
23	0.0004	0.09	10	0.0003	0.11	On top	0.0006	0.11
37	0.0006	0.12	20	0.0006	0.14	Mixed	0.0007	0.14
50	0.0008	0.16	30	0.0009	0.12	Control	0.0006	0.12

The relative frequency of  $r^2$  values obtained from the full set of regressions performed on Equation 2 is shown in Figure 4-5. The control treatment had more instances of  $r^2 > 0.95$ , whereas the litter on top treatment had the fewest. For the mixed litter and control treatments, most regressions yielded  $r^2$  values less than 0.85, while the top litter treatment showed better fitting overall.

Fitted values of K and S obtained by regression on Equation 6 were used to calculate the remaining residue ( $Y_t$ ) over time using Equation 8. In this case, fitted values of Y were compared with those calculated directly from experimental  $CO_2$  flux measurements. The agreement between observed and model-predicted values of total C residue and the percent differences between them are shown in Figures 4-6 and 4-7, respectively. The total residue was predicted with errors well below 2%. Samples showed the highest errors at earlier stages of the experiment.

Comparing the predicted and observed values for  $Y_t$  in Figure 4-6, it can be seen that the predicted values for  $Y_t$  fell consistently under the 1:1 line representing the ideal case of perfect model agreement, which indicates a slight but consistent overprediction by the model. To investigate possible sources of this bias, predicted values of K were also compared to those calculated directly from observed flux values. However, no bias for K values was observed. It is possible that data used in the regression had not fully reached steady decomposition rates and that starting the regression for a later initial time step may yield slightly different results. However, as shown in Figure 4-7, the total error of the predicted  $Y_t$  was still within 2%.

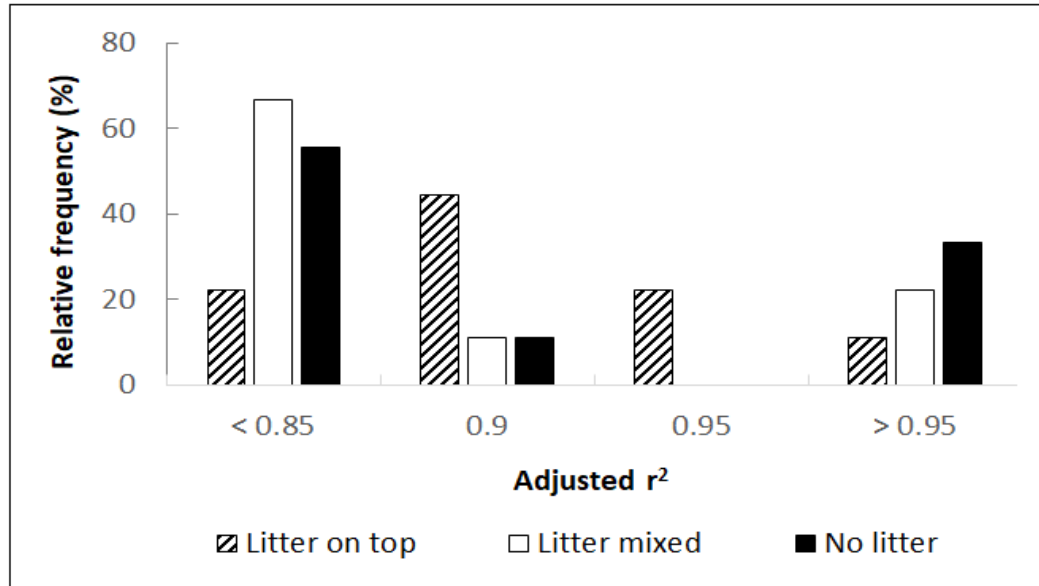


Figure 4-5. Distribution of  $r^2$  values for linear regression of  $\log(K)$  to  $\log(t)$  at constant temperature and moisture.

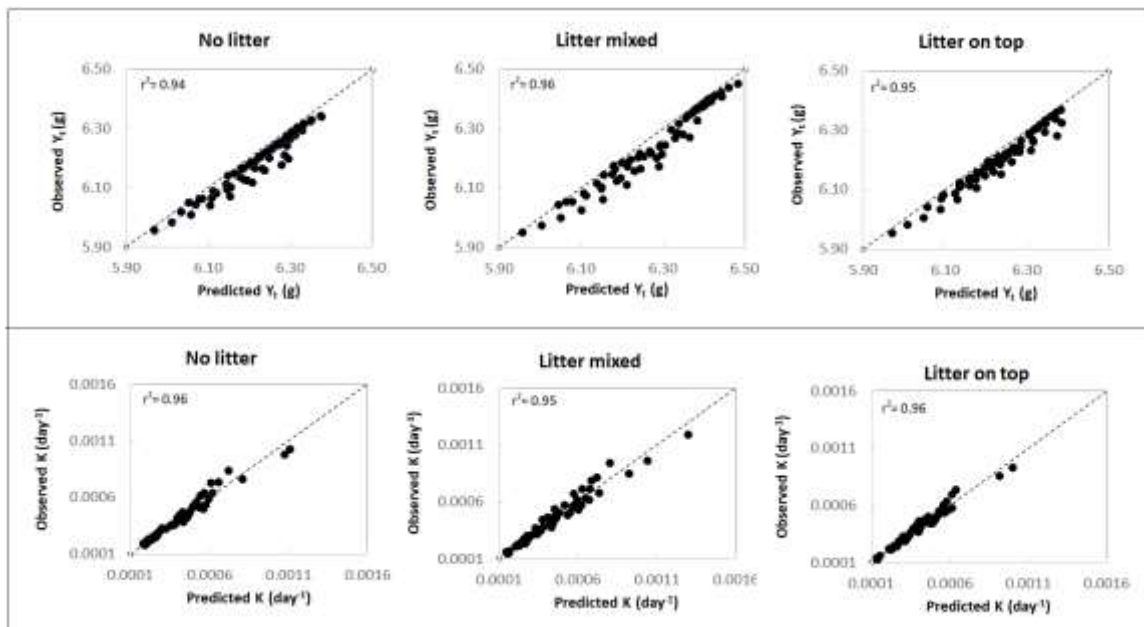


Figure 4-6. Observed and model predicted values for total remaining carbon (g) and K ( $\text{day}^{-1}$ ) for three litter placements using Equation 8. Note that the dashed line represents the 1:1 relationship between predicted and observed data. All the corresponding probabilities (p-value) were less than 0.0001.

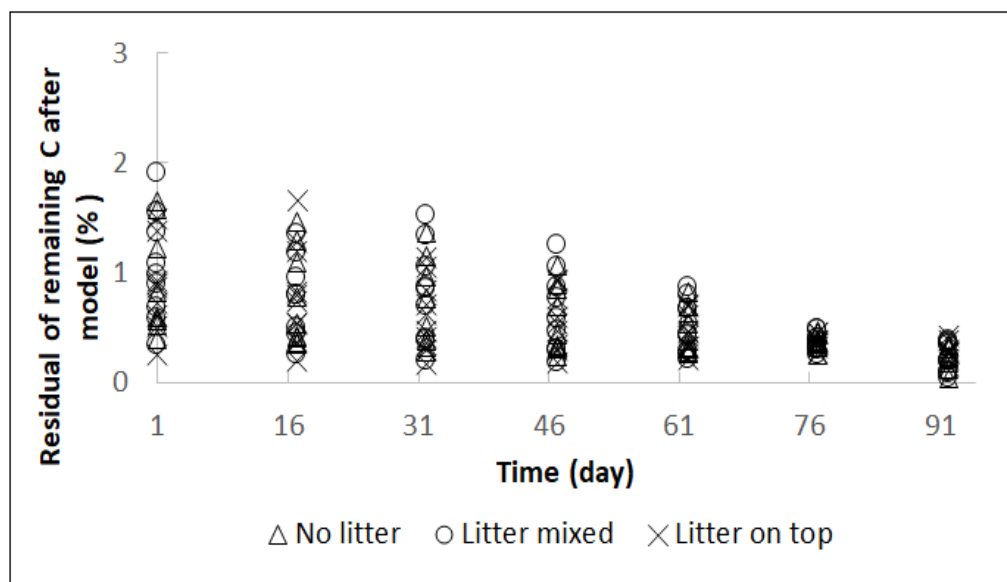


Figure 4-7. The percent difference between observed and model-predicted remaining C using Equation 8 with unique regression coefficients for each temperature and moisture level in Table 4-3.

Since the model was shown to be a good predictor using individual regression coefficients for each condition, we tested whether it might be generalized using the temperature corrections described in Equations 9-12. Figure 4-8 shows the model agreement with and without temperature corrections. Although this temperature correction simplifies the number of required model inputs by eliminating the need for unique regression coefficients to be obtained for each temperature, this approach introduces significant modeling errors for the data considered here, in some cases under-predicting C residue by more than 30%.

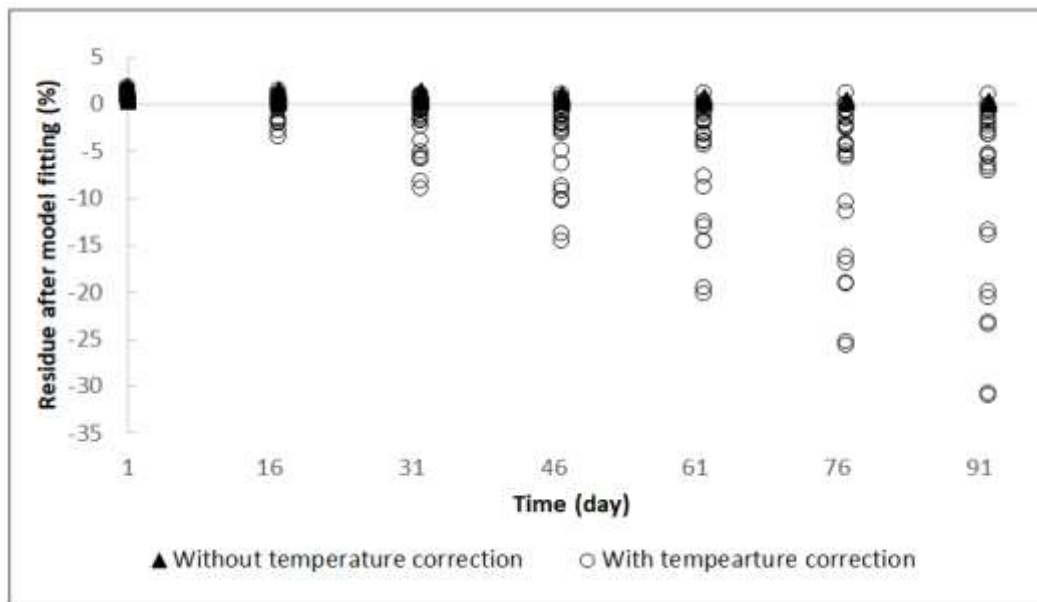


Figure 4-8. The residual after model fitting with and without temperature corrections for remaining carbon ( $Y_t$ ) calculated by Equations 8 and 12, respectively.

Results for the combined temperature and moisture correction given in Equation 13 are shown in Figure 4-9. This method was much more accurate than the temperature correction shown in Figure 4-8 and predicted residue within 2% of error. Hence, predicting  $R$  as a function of temperature and moisture showed better model accuracy than previous models based on temperature alone.

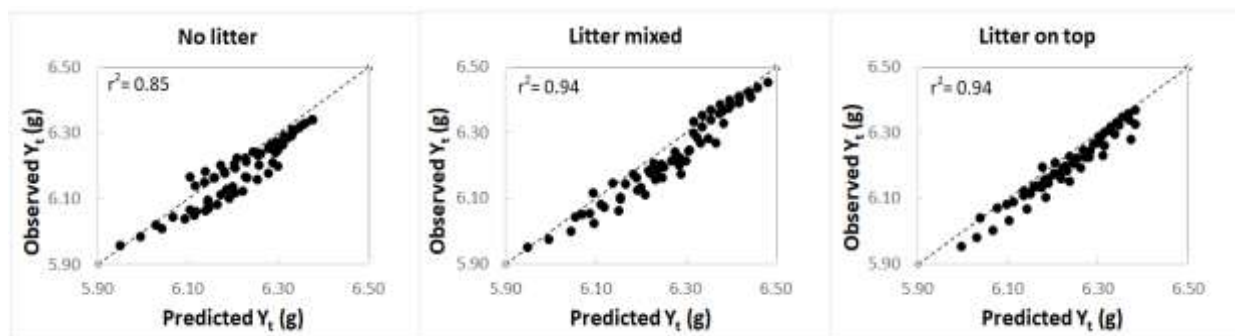


Figure 4-9. Comparison between observed and predicted remaining carbon ( $Y_t$ ) calculated by Equation 13 with temperature and moisture corrections on R parameter. Note that the dashed line represents the 1:1 relationship between predicted and observed data. All the corresponding probabilities ( $p$ -value) were less than 0.0001.

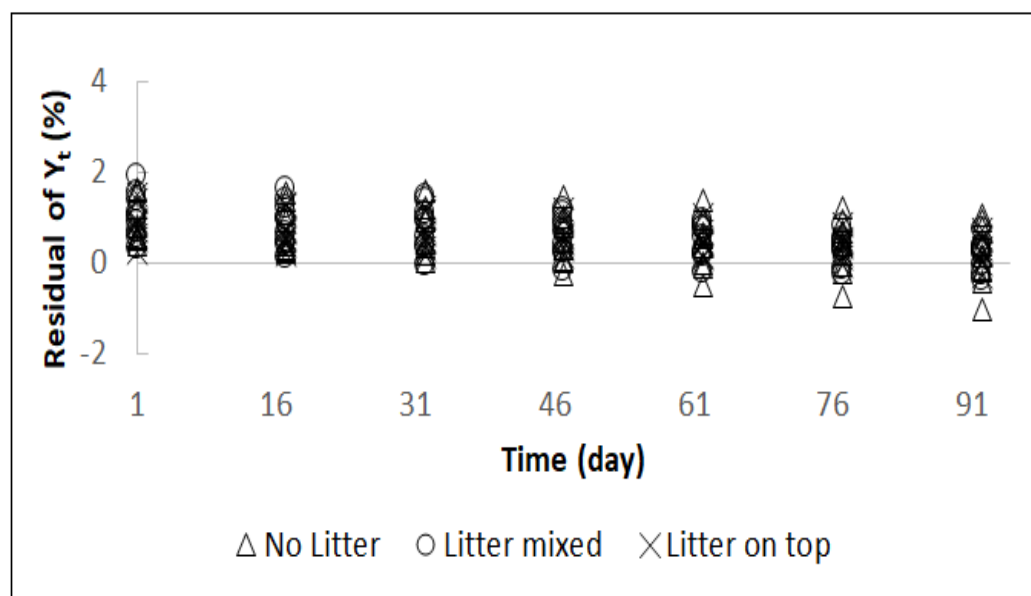


Figure 4-10. Residual after model fitting with temperature and moisture corrections on R parameter for remaining C ( $Y_t$ ) calculated by Equation 13.

Since R was predicted well as a function of temperature and moisture, we hypothesized that applying the same method to correct S could further reduce the total



number of model inputs. The combined temperature and moisture correction given in Equation 14 are shown in Figures 4-11 and Figure 4-12. In this case, correcting both R and S for temperature and moisture had virtually no different effect on model accuracy.

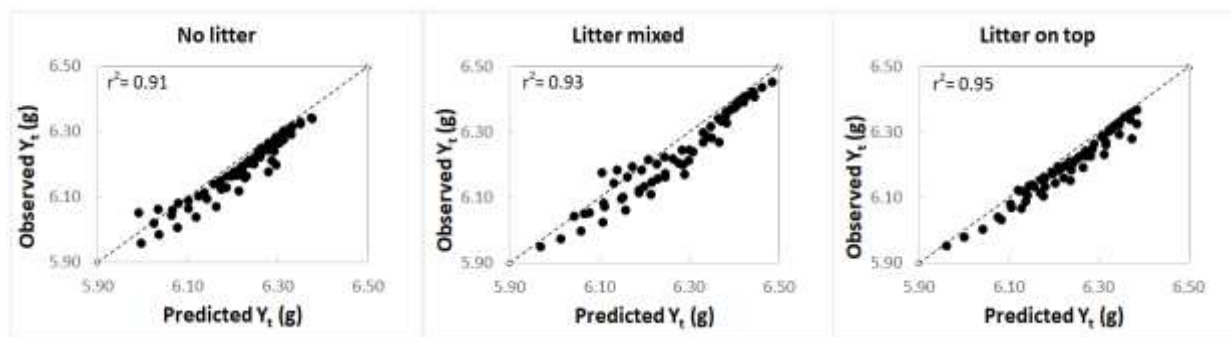


Figure 4-11. The model fitting with both temperature and moisture corrections on the S parameter for the remaining C ( $Y_t$ ) was calculated by Equation 14 for three litter placements. Note that the dashed line represents the 1:1 relationship between predicted and observed data. All the corresponding probabilities (p-value) were less than 0.0001.

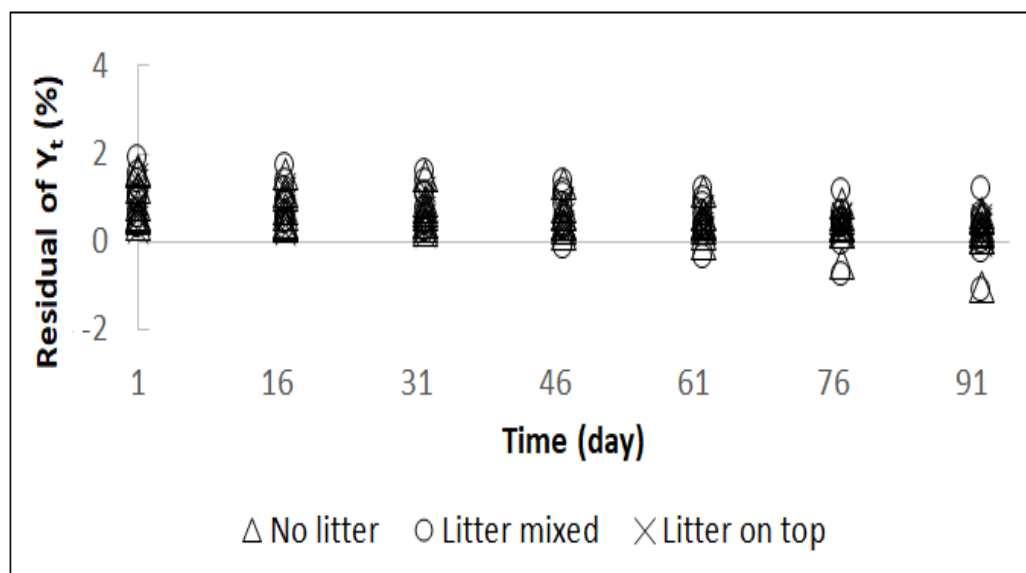


Figure 4-12. Residual after model fitting with temperature and moisture corrections on S parameter for remaining C ( $Y_t$ ) calculated by Equation 14.

Previous studies concluded that the R and S of decomposing substrates are positively related and affected by substrate characteristics as well as by environmental conditions (Yang and Jansen, 2002). Following the insights in Yang and Janssen (2002), the relationship between R and S was used to reduce further the number of model inputs needed to describe the data set shown in Figure 4-13. The fitted values of S with temperature and moisture effects obtained by Equation 14 were replaced in Equation 15 to calculate the global R values. The global R and fitted S values were used to calculate  $Y_t$  by Equation 8 for each litter placement. The accuracy of the proposed method shown in Figure 4-8 was similar to that obtained by applying the full set of regressions to obtain unique model parameters for each case. Hence, this proposed method allowed better predictions of total C residue over time for each litter placement using only temperature and moisture as model inputs and applying the correct regression coefficients for each litter placement.

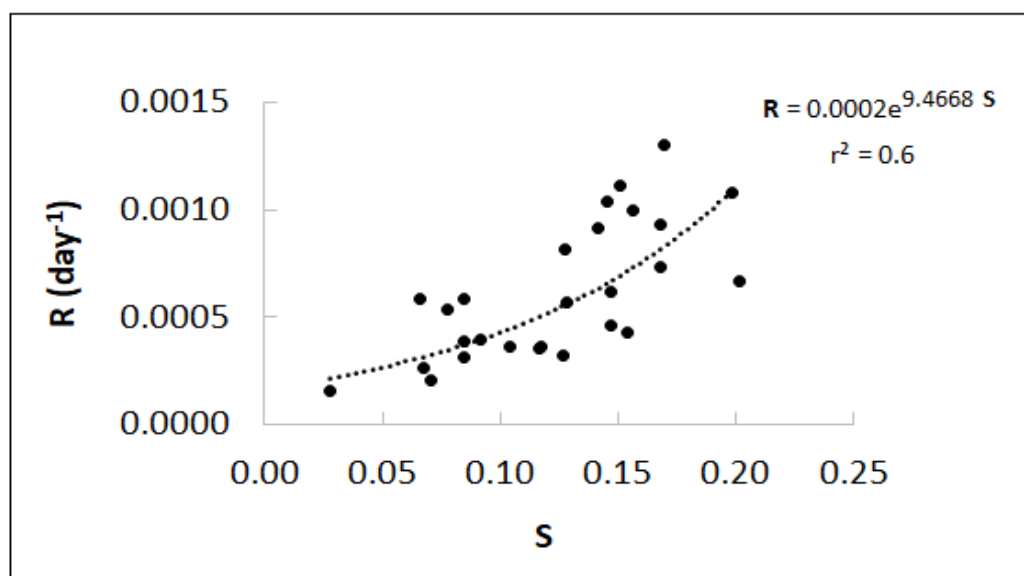


Figure 4-13. Relationship of R and S (unitless) for all 27 cases.

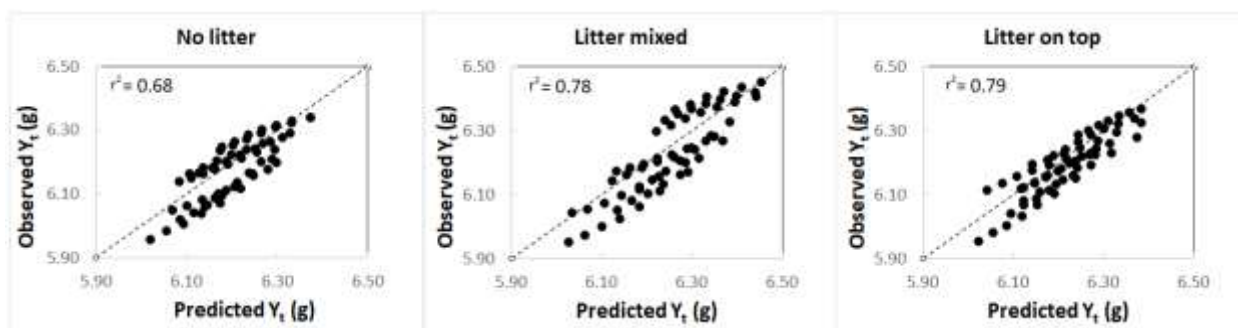


Figure 4-14. The model fitting using the proposed moisture and temperature corrections obtained using Equations 14 and 15 for three litter placements. All the corresponding probabilities (p-value) were less than 0.0001.

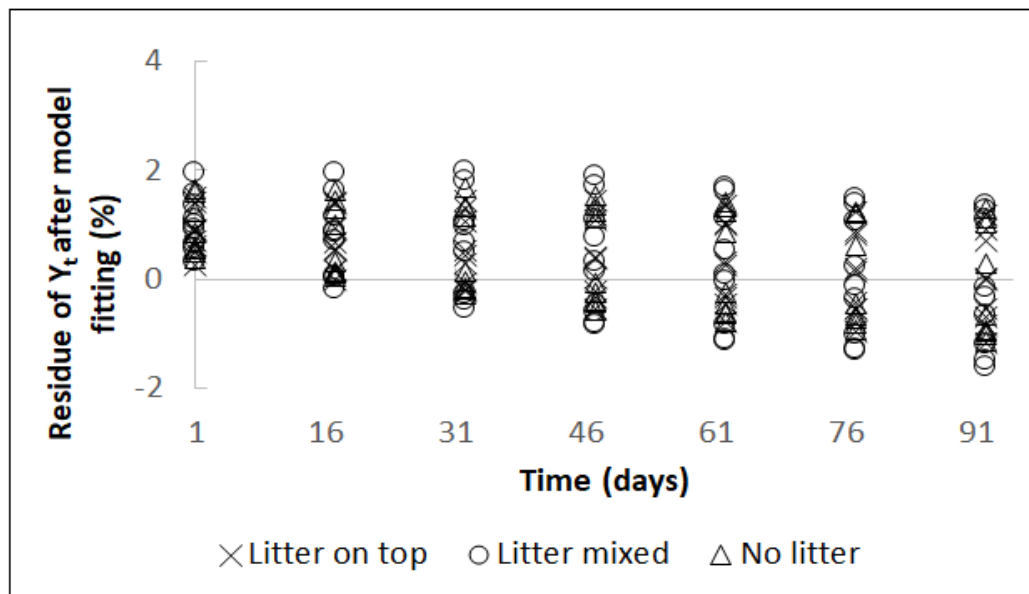


Figure 4-15. The residual after model fitting using the proposed moisture and temperature corrections obtained using Equations 14 and 15.

## Conclusion

The combined effects of temperature and moisture on OM decomposition for different placements of litter were investigated using a variety of first-order models. Using  $Q_{10}$  to determine the temperature sensitivity, it was found that for low temperature and moisture levels, such as 10 °C and 23% WFPS, the mixed litter treatment showed

high-temperature sensitivity. The top litter treatment was substantially less sensitive to temperature changes for the same temperature range when soil moisture was increased to 50% WFPS. At high temperatures with low moisture, the top litter treatment was most sensitive to temperature changes. In all cases, the temperature sensitivity was more pronounced during the initial phase of the incubation but became relatively constant over time.

A non-linear two-factor model was used to capture temperature and moisture effects for each litter placement for the initial time step. Substantial agreement was found between predicted and observed values. In all cases, the model suggested a strong interaction between temperature and moisture on total CO<sub>2</sub> flux during the initial incubation period for both litter treatments ( $361 < F < 403$ ), while the control treatment with no litter showed less interaction ( $F = 159$ ).

Changes in OM decomposition rates over time were modeled by performing regressions to obtain 27 unique equations for each litter placement, temperature, and moisture level considered. To reduce the number of equations required to describe the data set, common temperature corrections were applied. However, they introduced significant errors into the model (up to 30%). A new approach was tested to first determine R and S as linear functions of temperature and moisture, which predicted the remaining C within 3% of the experimental data. The model was further reduced by considering R as an exponential function of S, which allowed accurate predictions of remaining soil C over time while requiring only one Equation for each litter placement using temperature and moisture as model inputs. Results of this model were in the same order of accuracy as was observed by the full set of regressions using unique regression

coefficients for each tested condition. Overall, the method proposed here accurately captured the interactive effects between temperature, moisture, and litter placement using a simple model. Further research is warranted to determine the general validity of this modeling approach, as its final form could be easily implemented by average users in the world of agriculture and land management.

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### **Future suggestions**

A long-term field study across grassland ecosystems with different soil textures might be considered to obtain the overall picture of the effect of abiotic factors on litter decomposition in semiarid grassland ecosystems.

Due to the gradual mixing of added litter from the soil surface into the soil, a model that considers the mixing fraction changes over time and accurately predicts the decomposition similar to the field condition is recommended.

Further research is warranted to determine the general validity of this modeling approach, as its final form could be easily implemented by average users in the world of agriculture and land management.

## Appendix A

Table A-1. Sampling site history at Barta Brothers Ranch (42°13' N, 99°38' W) of Eastern Nebraska Sandhills

Landscape Location	Grazing treatment	Animals	Grazing season
Meadow	<p>Grazing treatments were applied in a randomized complete block design with two replications in 2010.</p> <p>The ultra-high stocking density of ~ 500 AU ha<sup>-1</sup> (200,000 lb/acre) was achieved by small paddock size (0.06 ha) and short grazing periods (0.5- day). The stocking rate was (3 AUM/acre).</p>	<p>A 120-paddock rotational grazing system grazed annually by 36 yearling cattle from 2010 through 2017.</p>	<p>Cattle grazed for a 60-day grazing season from early June to early August.</p>

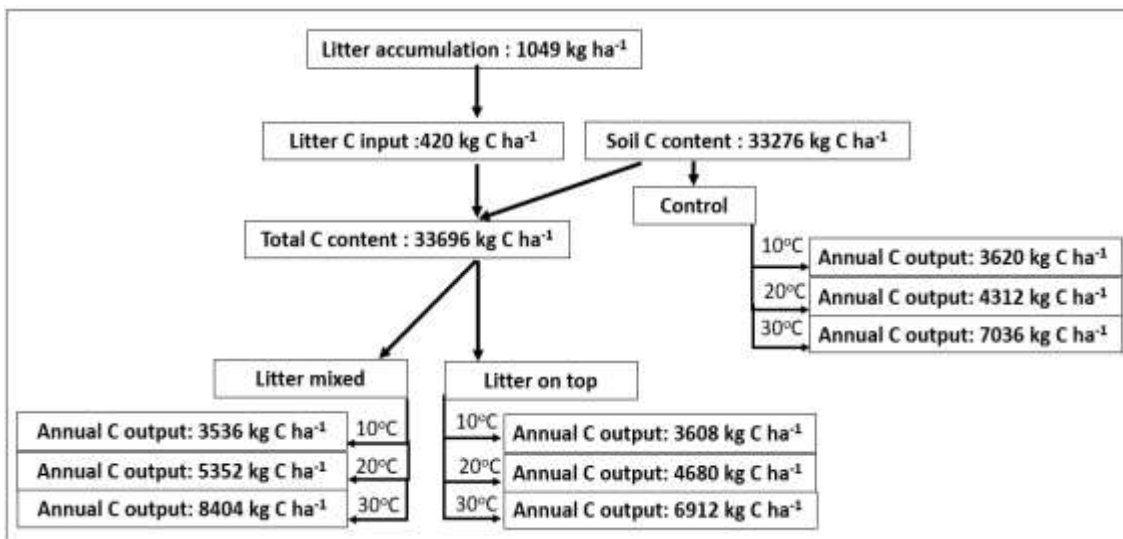


Figure A-1. Total C input and output (kg ha<sup>-1</sup> year<sup>-1</sup>) from the treatments with litter on top, mixed in, and control.

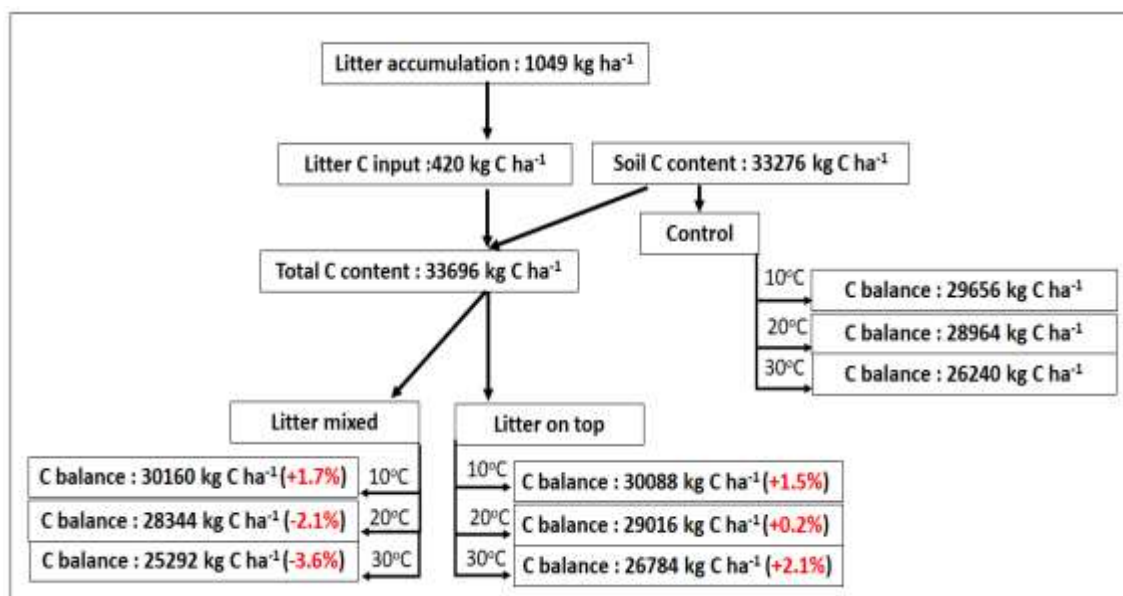


Figure A-2. Litter placement effect (mixed and on top) on the calculated C balance (kg ha<sup>-1</sup> year<sup>-1</sup>) and its percentage change compared to the control treatment at the given temperature (red color numbers) of the study area.

## Appendix B

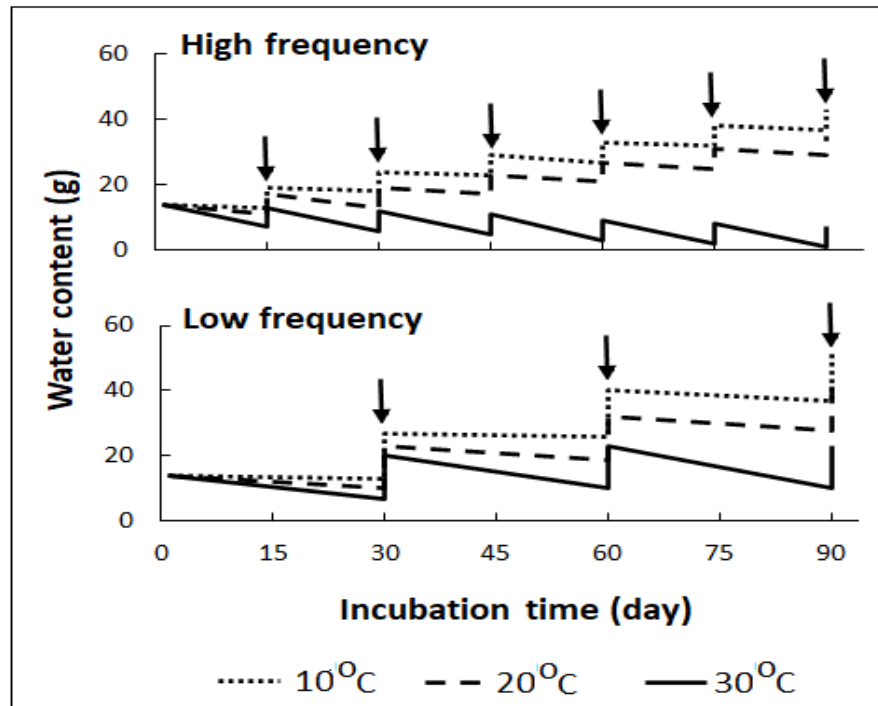


Figure B-1. Soil moisture profile during the incubation period for high and low-frequency pulses under three temperatures of 10, 20, and 30°C. Arrows indicate watering days. High and low-frequency pulses watering were done 6 times (5.5 mm of water per watering time) and 3 times (11 mm of water per watering time) during the incubation period. At the end of the incubation under both frequencies, in total, 33 mm water was added.



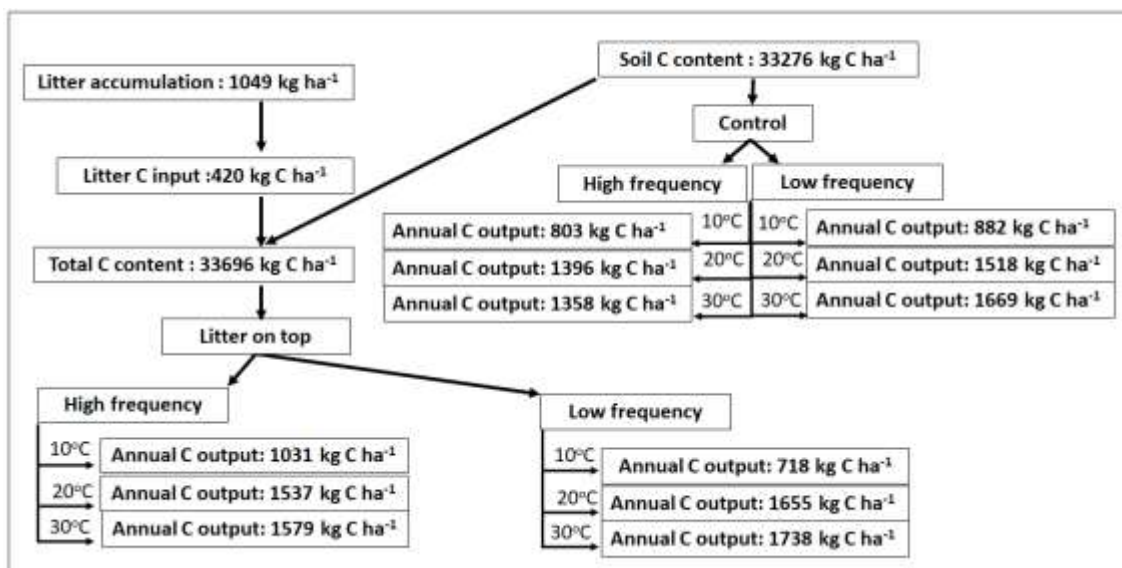


Figure B-2. Total C input and output (kg ha<sup>-1</sup> year<sup>-1</sup>) from the treatments with litter on top and control.

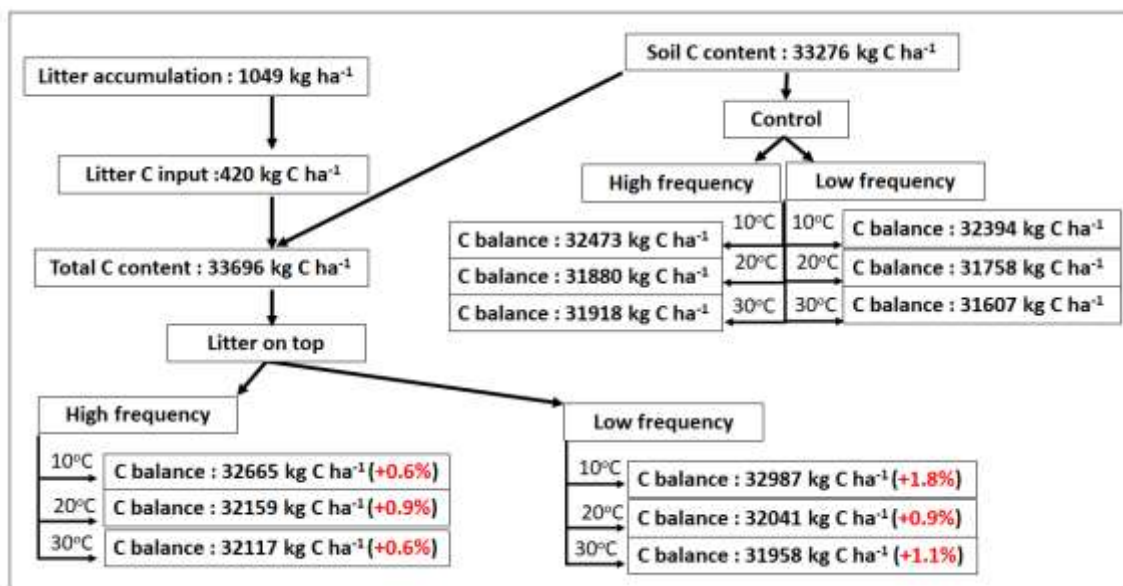


Figure B-3. Litter on top effects on the calculated C balance (kg ha<sup>-1</sup> year<sup>-1</sup>) and its percentage change compared to the control treatment at the given temperature and watering frequency (red color numbers) of the study area.

### Appendix C

Table C-1. Average  $Q_{10}$  values across the incubation period and standard errors ( $n = 7$ ) from each treatment combination of moisture (water-filled pore space %) and litter (on top, mixed, and no litter) at two ranges of temperatures (10 to 20°C and 20 to 30°C).

	Litter on top		Litter mixed		No litter	
Temperature (°C) \ Moisture (%WFPS)	10 to 20	20 to 30	10 to 20	20 to 30	10 to 20	20 to 30
23	1.6 ± 0.17	1.6 ± 0.02	2.3 ± 0.54	1.3 ± 0.24	1.2 ± 0.11	1.6 ± 0.03
37	1.7 ± 0.14	1.5 ± 0.02	1.5 ± 0.12	1.5 ± 0.05	1.1 ± 0.07	1.7 ± 0.14
50	1.2 ± 0.0	1.6 ± 0.03	2.1 ± 0.07	1.4 ± 0.0	2.0 ± 0.35	1.2 ± 0.09

Figure C-1. A summary chart for the modeling step by step.

