BURIED SOIL CARBON VULNERABILITY TO DECOMPOSITION WITH LANDSCAPE DISTURBANCE

by

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DEDICATION

To my grandfather, Eugene ‘Bapa’ Rickard, who helped me see and reach my potential

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ABSTRACT

Buried layers of ancient soil organic carbon (SOC) can store significant amounts carbon (C). Persistence of this C is favored by burial, which disconnects the soil from atmospheric conditions and limits plant derived C inputs, thus reducing microbial activity. However, erosion exposes buried paleosols to modern surface conditions and results in influx of root-derived C through the processes of root exudation and root turnover. These C inputs stimulate microbial activity and leave paleosol C vulnerable to decomposition. Understanding turnover of ancient soil C is critical for predicting the response of this large C reservoir to environmental change and feedbacks to climate. Yet, the effects of root-derived C inputs on decomposition of buried C is not well established. With this study we aim to quantify how root derived C inputs affect decomposition of paleosol C located along varying degrees of isolation from modern surface conditions, Our field site is located in Wauneta, NE where erosion has brought a Pleistocene era soil –the Brady soil- closer to the surface. We collected Brady soil from 0.2m, 0.4m, and 1.2m below the modern surface, and conducted two controlled laboratory incubations, Soils were amended with (1) a lab synthesized ^{13}C labeled (12 atom% ^{13}C) solution to mimic root exudates (0.3 mg C g-1 soil), and (2) root litter enriched with 92% atom% ^{13}C (0.3mg C g-1 soil), in 30 day, and 240 day laboratory incubation experiments, respectively. We measured ^{13}C-CO_2 respiration from airtight microcosms throughout the incubations and used the isotopic label to partition between root derived C and Brady soil C respiration. Our data show that Brady soil C is highly vulnerable to decomposition via

soil C priming upon addition of root-derived C regardless of burial depth, indicating that exposure of paleosols to modern surface conditions may result in a positive C cycle feedback to climate.

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C	Carbon
N	Nitrogen
SOC	Soil Organic Carbon
MBC	Microbial Biomass Carbon

INTRODUCTION

Soil and climate are closely linked through the carbon (C) cycle which connects the pedosphere, biosphere, and the atmosphere (Schlesinger, 1999). Globally, more C is stored in soil reservoirs than in living terrestrial vegetation and atmospheric pools combined (Lal et al, 2004; Houghton, 2007; Batjes, 2014). Given the size of the soil C reservoir, changes to the processes that drive soil C dynamics can influence the atmospheric CO_2 concentrations and climate change (Anderson, 1991; Lal, 2019). The majority of soil C is assumed to be sequestered within the top 30cm of soil where plant roots have the greatest influence (Rasse, Rumpel and Dignac, 2005). As a result, most of the research on soil C dynamics is limited to this depth (Carillo et al, 2017; Li et al, 2019), yet some studies show that a significant amount of C can be stored below this depth as buried soil C (Deminka, Borisov, and Demkin, 2010; Rumpel and Kögel-Knabner, 2010; Chaopricha and Marín-Spiotta, 2014; Dietzel, Liebman and Archontoulis, 2017). Buried soil C is often thought to be highly stable, predominantly cycling over long-time scales (Lawrence et al, 2015). This is because buried soil C is isolated and protected from abiotic and biotic conditions that favor biological activity and C decomposition, including oxygen availability, water, nitrogen (N) and plant-derived C inputs (Chaopricha, 2013). Especially, soil organic carbon (SOC) buried beneath aggraded dust (i.e. loess) is physically protected from microbial activity via subsurface isolation and dryness, thus it may not decompose for thousands of years (Jacobs and Mason, 2005). However, landscape disturbance, like erosion which is common in soils

composed of loess (Gou et al, 2019), can expose buried SOC to modern surface conditions resulting in an influx of root derived C, which stimulates microbial activity and leaves ancient C vulnerable to decomposition (Chaopricha and Marín-Spiotta, 2014). Given the size of the buried C pool and the fact that loss of topsoil via erosion may be between 0.5 and 1.55 ha/year especially in agricultural regions (Gou et al, 2019), high levels of buried soil C may become vulnerable to decomposition. Thus, understanding turnover of buried SOC is critical for predicting the response of the large reservoir to environmental change and potential flux of CO_2 to the atmosphere though the effects of root derived C on buried SOC decomposition are not well established.

Plants growing into a buried soil can increase decomposition of buried SOC because they provide fresh C inputs (Bernal et al, 2016, Deitzel, Leibman and Achontoulis, 2017; Gross and Harrison, 2019) that are preferentially consumed by soil microbial communities, regulating microbial activity and decomposition processes (Cheng and Kuzyakov, 2005; Fischer, Ingwersen and Kuzyakov, 2010). Root derived C inputs are regulated by both the process of root exudation and root turnover, Root exudates are low molecular weight compounds consisting of (50-70%), organic acids (20-30%), and amino acids (10-20%) that are passively excreted from living roots (Kraffczyk et al, 1984, Jones, Nguyen, and Finlay, 2009). Root turnover following root senescence leads to C input in the form of root litter which consists of molecularly complex substances such as lignin that are more difficult for microbial communities to decompose (Rasse, Rumple and Dignac, 2005). Increased root derived C input typically increases soil microbial biomass and unsustainable microbial biomass growth leads to the co-metabolism of pre-existing SOC, a process referred to as priming (Kuzyakov et al, 2000).

This process can occur in buried soils with the addition of ‘fresh’ root derived C inputs, resulting in an increase in the rate of soil C decomposition and a net loss of soil C (Fontaine et al, 2007).

Predicting the vulnerability of buried soil C to decomposition as it resurfaces and is exposed to increasing quantities of root derived C influx is difficult because the magnitude of the priming effect is likely to vary across the buried soil depth profile (Chen et al, 2019; Perveen et al, 2019; Liu et al, 2020). This is because the degree of surface exposure and root proliferation varies across depths and this influences soil properties (including soil organic matter composition) that control the priming process. These properties can include the amount of soil microbial biomass, C and N availability, and the accessibility of labile soil C (Fontaine et al, 2004; Schmidt et al, 2011; Karhu et al, 2015). The effect of soil microbial biomass relative to the amount of added C substrate can regulate the magnitude of priming through the co-metabolism of substrate of preferential substrate utilization. Addition of low quantities of root derived C relative to soil microbial biomass C tends to lead to the co-metabolism of SOC and positive priming, but the addition of high quantities of C relative to microbial biomass C can result in preferential substrate utilization and low or negative priming (Blagodatskaya et al, 2007; Blagodatskaya and Kuzaykov, 2008; Liu et al, 2017). Nutrient availability is another soil property that affects soil priming where the magnitude of the priming effect is typically greater in soils with low nutrient availability (Fontaine et al, 2004; Blagodatskaya et al, 2007; Qiao et al, 2014). Finally, reduced accessibility of SOC to the soil microbial community due to physical or chemical protection of soil C may result in weak or negative priming (Chabbi et al, 2009; Salomé et al, 2010; Chen et al, 2019).

Given that erosion leads to the proliferation of plant roots across the depth profile where they impact soil processes in a variety of different soil environments, it is important to evaluate how root-derived C input affects SOC priming throughout the profile.

The Brady soil is a Pleistocene era buried soil that that is undergoing significant erosion of the modern landscape, exposing areas of the buried soil. The buried soil is located in eastern Colorado, central Nebraska and northern Kansas (Zech et al, 2003; Jacobs and Mason, 2004), and contains, at its maximum, an estimated 2.7Pg of C (Marín-Spiotta et al, 2014), comparable to the top 1m of a Savannah grassland. The Brady soil is a 1m thick paleosol overlain with up to 6m of loess that underwent pedogenesis ~13kya during a period of interglacial stability (Mason et al, 2008). Formation ceased ~9kya in the early Holocene as aeolian loess rapidly accumulated across the landscape (Jacobs and Mason, 2004). Rapid burial of former surface horizons through sediment deposition allowed for more SOC to be stabilized at depth than would typically exist from roots and dissolved organic C leaching alone (Gurwick et al, 2008; Hoffmann et al, 2013; Chaopricha and Marín-Spiotta, 2014). High frequency of fires during Brady soil formation likely led to a high level of black carbon, a persistent aromatic compound, throughout the Brady soil (Marín-Spiotta et al, 2014). The iconic dark coloration of the Brady soil is hypothesized to stem from SOC stabilized in black carbon (Marín-Spiotta et al, 2014) and in recalcitrant clay-organic matter complexes (Jacobs and Mason, 2004), typical of the Mollisols in the area. As sections of the landscape in central Nebraska continue to erode and bring the Brady soil closer to the modern surface, we hypothesize that SOC in the shallowest depth of the Brady soil will be more vulnerable to decomposition as root derived C inputs increase with proximity to the modern surface,

subsequently increasing both microbial biomass C (MBC) and C accessibility to soil microbes. To test this hypothesis, we collected Brady located along an erosional gradient in the Central Great Plains, NE, from 0.2m, 0.4m, and 1.2m below the modern surface, and conducted two controlled laboratory incubations. Soils were amended with (1) a lab synthesized ^{13}C labeled (12 atom% ^{13}C) solution to mimic root exudates (0.3 mg C g-1 soil), and (2) root litter enriched with 92% atom% ^{13}C (0.3mg C g-1 soil), in 30 day, and 240 day laboratory incubation experiments, respectively. We measured ^{13}C-CO_2 respiration from airtight microcosms throughout the incubations and used the isotopic label to partition between root derived C and Brady soil C respiration.

METHODS

Field Site

We collected samples of the Brady soil which is located across the loess-mantled uplands of southwestern Nebraska, USA (40° 49’ 60” N, 101° 41’ 38” W). Roadcuts revealed the dark, 1m thick layer of the Pleistocene era Brady soil beneath meters of Holocene-age Bignell loess. Horizons present in the Brady soil are Ab, ABb, and Bkb all overlain by an ACb horizon where Brady soil formation ceased and Holocene loess deposits (Bignell loess) started to accumulate (Woodburn et al, 2017). The Brady soil overlays another thick layer of loess, Peoria loess. Mean annual temperature (MAT) of the study site is 9.7º C and mean annual precipitation (MAP) is 495mm (climate data from High Plains Regional Climate Center) with strong seasonal variation and the majority of rainfall received between April and September (Jacobs and Mason, 2004). Paleo-reconstruction and isotopic ($\delta^{13}C$) signature studies of the Central Great Plains during the Pleistocene-Holocene transition mark a general warming and drying trend and a shift from C3 dominant vegetation of the Brady soil to the current C4 dominant vegetation (Jacobs and Mason, 2004; Miao et al., 2007; Woodburn et al, 2017). Current vegetation at the Old Wauneta Roadcut is shortgrass prairie grassland (a mix of C3 and C4). The modern surface soils are loess-derived Mollisols and include subgroups of Argiustolls and Haplustolls (Woodburn et al, 2017; Laura Szymanski – *personal communication*). High levels of bioturbation from plant root, invertebrates and small,

burrowing vertebrates is prevalent throughout the Brady soil and are not restricted to one time period, rather developed throughout the soil formation to the modern-day landscape (Woodburn et al, 2013).

Soil Sampling and Preparation

Sequential depths of the Brady soil (1.2m, 0.4m, 0.2m) exposed to the modern surface through erosional processes were sampled for this study. Transects were located along the southward ledge and were designed to capture differences in Bignell Loess thickness due to erosion. All transects were located adjacent to a farm in the outskirts of Wauneta, NE. Three replicate transects for three erosional depths were taken, totaling 9 samples used in this study. Soil samples were collected during the summers of 2016 and 2017. Erosional samples were taken from a soil pit dug to the depth of the bottom of the Brady soil. Along each transect, soil samples were collected from three depths: 0.2m below the modern surface, 0.4m below the modern surface and 1.2m below the modern surface. All samples were collected from the top 30cm of the Brady soil. Soils were sieved to ~2mm and visible roots were removed by hand.

pH

Soil pH was determined with a 10g subsample from each depth along a transect. Each subsample was mixed with 20mL of DI water to create a slurry and allowed to rest for 30 minutes. Before sampling with an Oakton pH 6 Acorn Series meter and pH probe (Oakton Instruments, Vernon Hills, Illinois), samples were agitated to homogenize the

slurry. Three measurements from each sample were taken and an average across all depths was calculated.

Root Exudate Incubation Design

To determine the contribution of root C to total soil C decomposition we set up an incubation study to quantify microbial activity in response to plant root exudates. We mimicked root exudates that would be found in the field by creating a lab synthesized C solution with similar relative concentrations of sugars (60%), organic acids (35%) and amino acids (5%) that are excreted from living roots (Kraffczyk et al., 1984). The stock solution (12% atom ^{13}C; delivered in a single pulse of 0.3mg C g^{-1} soil) contained: D-glucose (28 mg ml^{-1}), D-fructose (9.4 mg ml^{-1}), D-xylose (8 mg ml^{-1}), oxalate (7.8 mg ml^{1}), fumarate (14.6 mg ml^{1}), succinate (1.2 mg ml^{1}), acetate (4.0 mg ml^{-1}), L-proline (0.1 mg ml^{-1}), L-arginine (0.1 mg ml^{-1}), L-glycine (0.1 mg ml^{-1}), L-serine (0.1 mg ml^{-1}), and glutamate (0.3 mg ml^{-1}) (Kraffczyk et al., 1984; de Graaff, 2010). The root exudate mixture was titrated to 7pH.

Sieved and hand root-picked soil (48g) was divided across four scintillation vials (12g per vial) and initially brought to 30% water holding capacity (WHC) with DI water for a 72-hour pre-incubation period. All vials remained without a lid and sealed tightly in a 16oz wide mouth Mason jar modified with a rubber septum for the duration of the pre-incubation (Bernal, 2016). To maintain humidity for the duration of the incubation, 2mL of water was added to the bottom of the jar. After the pre-incubation period ended, samples were re-weighted and brought up to 30% WHC with DI water. Samples receiving additional C were rewetted up to a total of 60% WHC with exudate solution by

pipetting exudate solution over top of the wetted soil. Control samples receiving no additional C were brought up to a total of 60% WHC with de-ionized water only by pipette.

Root Litter Incubation Design

To simulate the effect of root turnover and contribution of root litter to soil C decomposition we set up an incubation to simulate microbial activity in response to plant root turnover. Sieved and hand root-picked Brady soil (80g) was separated into two specimen cups containing 40g each. Isotopically labeled *Avena barbata* (92 atom% ^{13}C; grown at University of California, Berkeley (Berkeley, California, USA) root litter material was homogenized by grinding to roughly ≤2mm. Addition of root litter was based on the 5% of the average Brady soil carbon which equaled 0.3mg C $g^{-1}C$. Once the litter was well mixed into each soil sample, soils were rewetted to 60% WHC using de-ionized water. Each specimen cup was placed in a 16oz wide mouth Mason jar and sealed by a lid fitted with a rubber septum. All specimen cups remained without a lid and sealed tightly in a 16oz wide mouth Mason jar modified with a rubber septum for the duration of the incubation. To maintain humidity between sampling events, 2mL of water was added to the bottom of the jar. Water (~1mL) was used to refresh the 2mL within the Mason jar on days 30, 60, and 120. All jars were stored at room temperature and away from light until sampling. At the end of the 240-day incubation, 4g of control soil from each depth and transect was air dried (24hours) and rewetted following the procedure for microbial biomass growth described below.

Microbial Respiration

Soil CO_2 evolution was measured in all root exudate and root litter incubations. Respiration from the root exudate incubation was measure on days: 1, 3, 15 and 30. Respiration for the root litter incubation was measured on days: 1, 3, 7, 15, 30, 60, 120, and 240. Three blank jars without soil, only water, were measured alongside each incubation to provide baseline composition to be used later in calculations. Headspace CO_2 samples were collected into 12mL exetainer vials (Labco Limited, Buckinghamshire, UK). After CO_2 extraction, jars remained open and were flushed with air for 30 minutes. Filled exetainers were quickly shipped to UC Davis Stable Isotope Facility for ^{13}C analysis. The concentration of CO_2 and the PDB^{13}C signature were determined by the Stable Isotope Facility at University of California-Davis by using a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20e20 isotope ratio mass spectrometer, Cheshire UK).

Isotope analyses allowed us to discriminate root-derived CO_2 from soil CO_2. This allowed us to further calculate primed C across depths and between treatments.

$$\delta^{13}C = ((R_{sample}/R_{standard}) - 1)*1000 \quad (1)$$

where R is the ratio of heavy (^{13}C) to light (^{12}C) isotopes in either the standard or the sample. $R_{standard}$ (PDB) is: 0.011180.

$$Q_{sb} = Q_{control} - Q_{blank} \quad (2)$$

$$\delta^{13}C_{sb} = (Q_{control}*\delta^{13}C_{control}) - ((Q_{blank}*\delta^{13}C_{blank})/Q_{sb})$$

(3)

$$Q_{sc} = ((Q_{tc}*\delta^{13}C_{tc} - \delta^{13}C_{rdc}) - (Q_{blank}*(\delta^{13}C_{blank} - \delta^{13}C_{root})))/(\delta^{13}C_{sb} - \delta^{13}C_{root})$$

(4)

$$Q_{root} = Q_{tc} - Q_{sc} - Q_{blank}$$

(5)

$$\text{Priming} = Q_{sc} - Q_{sb}$$

(6)

Where $Q_{control}$ is the total amount of measured C respired from control soils (soil with water added only) including blanks and $\delta^{13}C_{control}$ is its measured isotopic composition. Q_{blank} is the total measured amount of C respired from the blank jars (no soil, water only), and $\delta^{13}C_{blank}$ is the measured isotopic composition. Q_{tc} is the measured total C respired from soils amended with root derived C (exudates or root litter) including the blanks, and $\delta^{13}C_{tc}$ is the measured isotopic composition. Q_{sb} is the calculated amount of total C respired from the control soil minus the C derived from the blanks (equation (2)) and the $\delta^{13}C_{sb}$ is the calculated isotopic composition (equation (3)). Q_{sc} is the calculated amount of total C respired from soils amended with root-derived C minus the blanks and the root derived C (equation (4)). Q_{root} is the total amount of C respired from the root derived amendments, and $\delta^{13}C_{rdc}$ is its isotopic composition (equation (5)). The priming effect of SOC was quantified for each depth and treatment (exudates or root litter) by subtracting Q_{sb} from Q_{sc} (equation (6)).

Statistical Analyses

All analyses were completed in R Studio (R Studio Version 3.5.2). To determine differences among depths (0.2m, 0,4m and 1.2m) and between C sources (root derived C [Q_{root}], soil C [Q_{soil}], and primed C) over the course of each incubation, a two-way analysis of variance (ANOVA) was conducted followed by a TukeyHSD post-hoc analysis once significance had been determined. The level of significance for all statistical tests was $p \leq 0.05$.

RESULTS

Root Exudate Incubation

In soil amended with water only (i.e. control soils), C respiration rate for the duration of the incubation was highest in the shallowest depth compared to greater depths (fig. 1a). On day 1, each depth of the Brady soil respired peak amounts of C, with greater C respiration from the shallowest depth (0.2m below the modern surface; an average of 23.5 ± 1.6 µg C g^{-1} soil day^{-1}) compared to the deepest depth (1.2m below the modern surface; an average of 15 ± 0.09 µg C g^{-1} soil day^{-1}; $p<0.01$). Peak C respiration in the intermediate depth (0.4m below the modern surface) was not significantly different from either the shallowest or the deepest depth. By day 3, C respiration in the shallowest depth was significantly higher than in both the intermediate and deepest depths (intermediate: $p<0.05$; deepest: $p<0.02$). During the final days of the incubation, day 15 and day 30, C respiration rate remained higher in the shallowest depth compared to the deepest depth (day 15: $p\leq0.01$; day 30: $p\leq0.01$). As a result, cumulative respiration in the shallowest depth averaged 283 ± 35.2 g C g^{-1} soil day^{-1}, higher than the deepest depth which averaged 136 ± 10 µg C g^{-1} soil day^{-1} ($p\leq0.01$) (fig. 2). The percent increase in cumulative respiration from day 1 to day 30 was highest in the shallowest depth (1103%) compared to the deepest depth (685%; $p\leq0.01$) (fig. 3). The intermediate depth increased on average 679% from day 1 to day 30. When we normalized the cumulative amount of C respired by Brady soil SOC, the calculated SOC values among all depths were similar ($p=0.057$) (table 2). At the beginning of the incubation, calculated SOC values averaged

0.27%, 0.31% and 0.27% in the shallowest, intermediate, and deepest depths, respectively. By the end of the incubation, there were no significant differences among depths yet these SOC values increased to an average of 3.9%, 3.8% and 2.8%, respectively.

For Brady soil amended with ^{13}C labeled synthetic root exudates, C respiration was partitioned into four distinct components: (1) measured total C respiration (i.e. C respiration derived from both root exudates and soils), (2) calculated root exudate derived C respiration (i.e. C respiration derived from root exudates only), (3) calculated primed C. Adding root exudates to the Brady soil increased total C respiration (soil derived C respiration + root exudate derived C respiration) across all depths. Peak total C respiration rates occurred on day 1 for the shallowest depth (96.5 ± 12.2 µg C g^{-1} soil day^{-1}) which were respired to a greater extent than the deepest depth (40 ± 5.4 µg C g^{-1} soil day^{-1}; $p<0.01$) (fig. 1b). Peak respiration for the intermediate and deepest depths occurred on day 3. On day 3, the amount of C respired differed among all depths. Respiration from the shallowest depth (94 ± 1.7 µg C g^{-1} soil day^{-1}) was higher than both the intermediate depth (84 ± 1.8 µg C g^{-1} soil day^{-1}; $p\leq0.01$) and the deepest depth (73.5 ± 1 µg C g^{-1} soil day^{-1}; $p<0.01$). Respiration rate in the intermediate depth on this day was higher than the deepest depth ($p<0.01$). Total C respiration rate on day 15 remained higher in the shallowest depth compared to the deepest depth ($p<0.05$). By day 30, all depths had decreased similarly and were not significantly different. Cumulatively, the shallowest depth (667 ± 32.5 µg C g^{-1} soil; $p<0.01$) and intermediate depth (585 ± 19.9 µg C g^{-1} soil, $p<0.05$) respired higher amounts of total C compared to the deepest depth (452 ± 36.3 µg C g^{-1} soil day^{-1}) (fig. 2). Despite low values, total cumulative C respiration in the deepest

depth increased on average 1031% between day 1 and day 30, significantly higher than the shallowest depth (a 591% increase; $p<0.01$) and the intermediate depth (a 796% increase; $p<0.05$). Higher amounts of cumulative total C were respired by soils amended with root exudates than in the control soils (water only). Total cumulative C respiration differed 121% between the amended shallowest depth and the control shallowest depth with amended soils respiring more total C compared to the control soil ($p<0.01$). In the intermediate depth, total C respiration differed 107% between amended soils and control soils with amended soil respiring higher total C relative to the control soil ($p<0.01$). Total C respiration differed 189% between amended and control soils at the deepest depth with the amended soil respiring more total C than the control soil ($p<0.01$). The percent difference of the deepest depth was higher than the percent difference of the shallow soil ($p\leq0.01$). When we normalized the cumulative amount of C respired in amended soils by Brady soil SOC there were no significant differences among depths. At the beginning of the incubation, calculated SOC values averaged 1.1%, 1.0% and 0.7% in the shallowest, intermediate, and deepest depths, respectively. Values increased to 9.0%, 10.9%, and 9.3%, respectively by the end of the incubation with the intermediate soil significantly higher than the shallowest depth ($p\leq0.05$).

Exudate derived C respiration on day 1 was highest in the shallowest depth compared to both the intermediate depth ($p\leq0.05$) and the deepest depth ($p<0.01$) (fig. 1c). The shallowest depth respired on average 53 ± 7.6 µg C g^{-1} soil day^{-1} compared to the intermediate depth which respired an average of 32 ± 2.6 µg C g^{-1} soil day^{-1} and the deepest depth which respired an average of 17 ± 3.2 µg C g^{-1} soil day^{-1} on day 1. Peak exudate derived C respiration occurred on day 3 for all depths with higher values in the

shallowest depth compared to the deepest depth ($p \leq 0.05$). On day 15, exudate derived C respiration rate decreased to a similar amount across all depths. By day 30, despite low exudate derived C respiration values for all depths, the deepest depth and intermediate depth respired more than the shallowest depth (deepest: $p<0.05$; intermediate: $p<0.05$). Cumulatively, the shallowest depth respired more exudate derived C than the deepest depth ($p \leq 0.05$) (fig. 2). Exudate derived C respiration in the shallowest depth totaled an average of 320 ± 5 μg C g^{-1} soil day^{-1} compared to the deepest depth which respired an average of 313 ± 3.1 μg C g^{-1} soil day^{-1}. Cumulative exudate derived C respiration in the intermediate depth totaled 263 ± 22.3 μg C g^{-1} soil day^{-1}. Exudate derived respiration at the deepest depth increased 1289% between day 1 and day 30, significantly higher than both the shallowest (422%; $p<0.01$) and intermediate depths (745%; $p<0.05$). As a percent of total C respiration, root exudate derived C respiration made up an average of 48% in the shallowest depth, significantly less than respiration in the deepest depth which made up an average of 58% ($p \leq 0.01$) (table 2). Exudate derived respiration in the intermediate depth made up for an average of 54% of the total C respired. Cumulatively, the percent increase in root exudate derived C from day 1 to day 30 was higher in the deepest depth (1289% increase) than in the intermediate depth (745% increase; $p<0.05$) and the shallowest depth (422% increase; $p<0.01$) (fig. 3).

Soil priming was positive across all depths for the first three days of the incubation (fig. 1d). On day 1, respiration rates in the shallowest depth responded the most to C addition with an average of 20 ± 3.1 μg C g^{-1} soil day^{-1} compared to the deepest depth (8 ± 1.4 μg C g^{-1} soil day^{-1}) ($p \leq 0.01$). From the intermediate depth, soil priming averaged 14 ± 0.9 μg C g^{-1} soil day^{-1}. There were no significant differences

among depths by day 3 of the incubation though soil C priming remained positive. Positive soil priming rate decreased to a similar amount among all depths by day 15. By day 30, soil priming remained negative with no significant differences between depths. Cumulatively, soil priming from all depths were similar (shallowest: 63 ± 1.2 µg C g^{-1} soil day^{-1} on average; intermediate: 67 ± 5.3 µg C g^{-1} soil day^{-1} on average; deepest: 53 ± 5 µg C g^{-1} soil day^{-1} on average) (fig. 2). As a percent of total C respired, primed soil C made up for 11% on average across all depths of the Brady soil (fig.3).

Root Litter Incubation

In the control soils, C respiration rate reached its peak on day 1 for all depths of the Brady soil. On day 1, there was greater C respiration in the shallowest depth (55 ± 6.2 µg C g^{-1} soil day^{-1}) compared to the deepest depth which respired 26 ± 2 µg C g^{-1} soil day^{-1} ($p<0.01$) (fig. 4a). Control C respiration rate remained highest in the shallowest depth at 27 ± 2.1 µg C g^{-1} soil day^{-1} by day 3 compared to both the intermediate depths (20 ± 0.3 µg C g^{-1} soil day^{-1}; $p\leq0.01$) and deepest depths (14 ± 0.2 µg C g^{-1} soil day^{-1}; $p<0.01$). The intermediate depth also respired more C than the deepest depth on this day ($p<0.05$). Between day 7 and day 60, C respiration rate in the shallowest depth remained higher than C respiration in the deepest depth (day 7: $p\leq0.01$; day 15: $p<0.05$; day 30: $p<0.05$; day 60: $p<0.05$). Rate of respiration declined similarly among all depths for the final days of the incubation, day 120 and 240. Cumulatively, control soil C respiration in the shallowest depth was higher than C respiration in the deepest depth ($p<0.01$) (fig. 5). Cumulatively, C respiration in the shallowest totaled 605 ± 82.9 µg C g^{-1} soil day^{-1} on average, significantly more C than the deepest depth (308 ± 33.8 µg C g^{-1} soil day^{-1} on average; $p<0.05$). Control soil C respiration in the intermediate depth totaled an average

of 543 ± 50.3 µg C g^{-1} soil day^{-1}, not significantly different from either the shallowest or the deepest depth. The percent increase in cumulative control soil C respiration from day 1 to day 240 was similar across all depth, averaging a 1112% increase. When we normalized the cumulative amount of C respired by the amount of Brady soil SOC there were no differences among depths (table 3). The first day of the incubation, calculated SOC values averaged 0.63%, 0.64% and 0.47% in the shallowest, intermediate, and deepest depths respectively. On the final day, these values increased to 6.9%, 8.6% and 5.7%, respectively, with no significant differences.

For Brady soil amended with ^{13}C labeled *Avena barbata* root litter, C respiration was partitioned into four distinct components: (1) measured total C respiration (i.e. C respiration derived from both root exudates and soils), (2) calculated root exudate derived C respiration (i.e. C respiration derived from root exudates only), (3) calculated primed C. Adding root litter to the Brady soil increased total C respiration among all depths. Peak total C respiration rate occurred on day 1 for all depths (fig. 4b). Greater total C respiration occurred in the shallowest depth (68 ± 9.2 µg C g^{-1} soil day^{-1}) compared to the deepest depth (37 ± 1.6 µg C g^{-1} soil day^{-1}; $p<0.05$) on day 1 while the intermediate depth respired 54 ± 6.1 µg C g^{-1} soil day^{-1} and was not significantly different from the other depths. On day 3, greater amounts of total C respiration continued in the shallowest depth relative to the deepest depth ($p\leq0.01$). All depths decreased to a similar level by day 7 (19 ± 2 µg C g^{-1} soil day^{-1} on average). The shallowest depth continued to respire higher amounts of total C compared to the deepest depth until day 30 (day 15: $p<0.05$; day 30: $p<0.05$). There were no significant differences in total C respiration rate among depths between days 60 and 240. Cumulatively, all depths of the Brady soil respired similar

amounts of total C by day 240 (fig. 5). The cumulative total C respiration for the shallowest, intermediate, and deepest depths totaled 1046 ± 171.1 µg C g^{-1} soil day^{-1} on average, 828 ± 124.9 µg C g^{-1} soil day^{-1} on average, and 619 ± 11.4 µg C g^{-1} soil day^{-1} on average, respectively with no significant differences. The percent increase in cumulative total C respiration among depths was similar, averaging 1486% between day 1 and day 240. Comparatively, there were no differences in cumulative total C respiration between the shallowest depth of the litter amended soil and the control soil. The same result was found in the intermediate depth. However, the cumulative total C respiration in the deepest depth was higher in the root litter amended soils than in the control soils ($p<0.01$). Total cumulative C respiration on day 240 in the shallowest depth differed on average 53% between the litter amended and control soils. In the intermediate depth, total C respiration different on average 40%. Between the amended soils and control soils in the deepest depth there was a 68% difference on average. When we normalized the cumulative amount of total C respired in amended soils at the end of the incubation, there were no significant differences among depths (table 3). On the first day of the incubation, calculated SOC values averaged 0.78%, 0.85%, and 0.67% in the shallowest, intermediate, and deepest depths, respectively. By the final day, values increased to 11.96%, 13.05%, and 11.25%, respectively likely due to the amount of root litter added.

Litter derived C respiration rate was similar across all depths for the entirety of the 240-day incubation (fig. 4c). Peak respiration rate values occurred on day 1 for the shallowest (14 ± 2.7 µg C g^{-1} soil day^{-1}) and intermediate (11 ± 2.2 µg C g^{-1} soil day^{-1}) depths while peak respiration for the deepest depth occurred on day 3. On day 1, litter derived C respiration in the deepest depth averaged 6 ± 0.4 µg C g^{-1} soil day^{-1}. There

were no significant differences among depths on day 3 of the incubation though values in the shallowest and intermediate depths decreased (to 11 ± 0.2 µg C g^{-1} soil day^{-1} and 10 ± 0.3 µg C g^{-1} soil day^{-1}, respectively) while values increased in the deepest depth to 10 ± 0.7 µg C g^{-1} soil day^{-1}. Respiration rate continued to decrease similarly among all depths until the final day of the incubation. On day 240, despite low values, litter derived respiration rate in the deepest depth (0.3 ± 0.02 µg C g^{-1} soil day^{-1}) was higher than in the shallowest depth (0.2 ± 0.02 µg C g^{-1} soil day^{-1}; $p \leq 0.01$). Cumulatively, there were no significant differences among depths (fig. 5). For the shallowest, intermediate, and deepest depths, litter derived C respiration totaled 329 ± 29 µg C g^{-1} soil day^{-1} on average, 298 ± 36.1 µg C g^{-1} soil day^{-1} on average, and 282 ± 10.4 µg C g^{-1} soil day^{-1}, respectively. As a percent of total C respired, root litter derived C respiration made up for 46% of the total respiration in the deepest depth, higher than the percent in the shallowest (34%; $p<0.01$) and intermediate (38%; $p \leq 0.05$) depths (table 3). Cumulatively, the percent increase in root litter derived C respiration from day 1 to day 240 was higher in the deepest depth (4354% increase) than it was in the intermediate (2506% increase; $p<0.01$) and the shallowest depth (2201% increase; $p<0.01$) (fig. 3).

The rate of soil C priming on day 1 of the incubation was negative in the shallowest depth (-0.9 ± 1.3 µg C g^{-1} soil day^{-1}) and positive for the intermediate (2.2 ± 2.2 µg C g^{-1} soil day^{-1}) and deepest depths (4.2 ± 1.1 µg C g^{-1} soil day^{-1}), though there were no significant differences (fig. 4d). On day 3, soil priming reached its highest magnitude, which was similar across all depths. Priming on this day increased to 4 ± 0.2 µg C g^{-1} soil day^{-1}, 6 ± 0.7 µg C g^{-1} soil day^{-1} and 6.5 ± 0.9 µg C g^{-1} soil day^{-1} in the shallowest, intermediate, and deepest depths, respectively. Soil C priming rate decreased

similarly across all depths until day 30. On day 30, the rate of soil priming in the intermediate depth was 1.5 ± 0.2 µg C g^{-1} soil day^{-1}, higher than both the shallowest depth ($p<0.05$) and the deepest depth ($p\leq0.01$). For the duration of the incubation, soil priming decreased similarly across all depths. On day 240, soil C primed in the shallowest and deepest depths 0.1 ± 0.2 µg C g^{-1} soil day^{-1} on average, while the intermediate depth respired -0.8 ± 0.5 µg C g^{-1} soil day^{-1} on average. Cumulatively, high variability in values led to no significant differences among depths (fig.5). Soil priming in the shallowest depth totaled 112 ± 59.4 µg C g^{-1} soil day^{-1} on average. The intermediate depth cumulatively primed -13 ± 85.9 µg C g^{-1} soil day^{-1} on average. Soil priming in the deepest depth totaled 28 ± 26.3 µg C g^{-1} soil day^{-1} on average. As a percent of total soil C respiration, soil priming made up -3-10% of the total C respired across all depths (table 3). By day 240, variability in these data were high, however on day 120 soil priming values were still similar among depths yet averaged between 7.5-10% of the total C respired.

DISCUSSION

Adding water, root exudates and root litter to the Brady soil triggered microbial activity and respiration across all depths. Our experiment yielded three main results: (1) Adding water only (i.e. our control soils) triggered microbial respiration to a greater extent in the shallowest depth than the deepest depth, (2) that adding root derived carbon inputs increased C respiration across all depths with higher respiration in the shallowest depth relative to the deepest depth yet there was a greater percent increase in the deepest depth relative to the shallowest depth and (3) that soil priming was greater in soil amended with root exudates than in soil amended with root litter. Rewetting subsurface soils (Xiang et al, 2008) that have similar drought-like conditions to the Brady soil stimulated CO_2 respiration, confirming that the addition of water to dry, deeply buried soils can reactivate previously dormant microorganisms (de Nobili et al, 2006; Demkina et al, 2010; Sanuallah et al, 2016; Hicks Pries et al, 2018). While studies show that rewetting deep, dry soils activates the soil microbial community (Xiang et al, 2008; Min et al, 2019), few studies have evaluated how rewetting paleosols will influence microbial activity. Demkina et al (2010) and Chaopricha (2013) demonstrated that rewetting ancient, buried paleosols activates the soil microbial community, generating quantitatively low levels of CO_2 respiration up to a depth of 6.5m below the surface. In our study, adding water alone was enough to stimulate microbial respiration, though greater total CO_2 respiration (i.e. CO_2 derived from soil C + root derived C) occurred after adding root exudates and root litter, indicating that the soil microbial community

was able to quickly metabolize the added fresh C. These data support previous studies on ancient, buried paleosols that show the effect of fresh, labile C inputs on reactivating previously dormant soil microorganisms (de Nobili et al, 2001; Demkina, Borisov, and Demkin, 2010; Kryazhevskikh et al, 2012). Our data also reinforce the idea that reconnecting ancient, buried paleosols to conditions that promote microbial activity, quickly restores microbial activity and C cycling processes across the depth profile with the response rate and magnitude mediated by the availability and accessibility of these substrates to the microbial community.

While microbial activity was stimulated by the addition of water and root exudates across all depths, the magnitude of response differed among depths. In both the water only and the root exudate incubations, cumulative soil C respiration (i.e. respiration derived from control soils), and cumulative root exudate derived C respiration in the shallowest depth (0.2m below modern surface) was greater than in the deepest depth (1.2m below modern surface). In the control soils, more soil C respiration in the shallowest depth relative to the deepest depth may be explained by pre-existing differences in Brady SOC content, because soil C respiration rates were similar among depths when we normalized C respiration by Brady SOC concentration in each depth (table 1). While there were no differences in Brady SOC, values trend toward greater SOC in the shallowest depth. The higher rate of CO_2 respiration in the shallowest depth is likely the result of a greater influx of plant derived C to the shallowest depth. This is supported by the amount of root biomass discovered during sample preparation. The shallowest depth had five times more root biomass than the deepest depth (table 1). Estimates indicate that erosion has left the shallowest depth exposed to surface conditions

for approximately 1,000 years (Joe Mason – *personal communication*). During this time, plant roots have grown into the Brady soil providing a steady source of fresh C, oxygen, nitrogen (N) and a pathway for water to infiltrate. These factors are known to promote microbial activity (Lal and Shukla, 2004; Haichar et al, 2014; Hopkins et al, 2014; Ramierez et al, 2012; Xu and Wan, 2008; Zhu et al, 2016; Soong et al, 2019).

In contrast to the root exudate incubation, there were no differences in root litter derived C respiration among depths, indicating that there was either lower substrate use in the shallowest depth, greater substrate use at the deepest depth, or a combination of both leading to a similar amount of root litter respiration across depths. Differences in the impact of depth on the decomposition of root exudates and root litter may be caused by differences in the chemical composition of the root exudate mixture and the *Avena barbata* root litter, which affects how microbial communities at each depth metabolize the substrates. Root exudates are low molecular weight compounds that are more easily decomposed by microbial communities compared to root litter, which is a more complex source of energy for microbial communities and is more difficult for microbial communities to break down (Rasse, Rumple, and Dignac, 2005; Kramer and Gleixnor, 2008; Müller et al, 2016; Turner et al, 2017). As a result, root litter decomposition is likely mediated by fungi which typically metabolize substrates with more complex chemical structures, whereas root exudate decomposition tends to be mediated by bacteria (Turner et al, 2017; Schlatter et al, 2018). Given that abiotic conditions in the shallowest depth, including more N and greater C availability relative to the deepest depth, likely favors bacterial decomposition leading to exudate breaking down faster in the shallowest depth compared to the deepest (Demkina et al, 2010; Polyanskaya et al,

2016; Sanaullah et al, 2016; Turner et al, 2017). Conversely, the microbial community in the shallowest depth may not be able to easily access the root litter, suppressing decomposition of litter at this depth, while more fungal dominated decomposition at the deepest depth may stimulate greater decomposition of root litter compared to the shallowest depth (Sanaullah et al, 2016). This leads to a convergence of root litter decomposition rates across depths and explains why root litter decomposition is similar across depths.

To evaluate differences in the proportional use of root exudates and root litter among depths, we calculated the cumulative percent increase in root derived C respiration for the duration of each incubation. For the root exudate incubation, the cumulative percent increase is the percent change between days 1 and 30 in each depth and for the root litter incubation, the cumulative percent increase is the percent change between days 1 and 240 in each depth. The percent increase in root exudate derived C respiration in the deepest depth was nearly three times higher than in the shallowest depth (fig. 3). In the root litter incubation, the percent increase in root litter derived C respiration was nearly twice as high in the deepest depth than it was in the shallowest depth (fig. 3). These results indicate that root derived C is a critical substrate that regulates microbial growth at depth. The relative greater use of root exudates and root litter in the deepest depth relative to the shallowest depth of the Brady soil indicates that microbial communities at this depth preferentially metabolize the added root derived C, supporting results in other studies (Bernal et al, 2016; Sanaullah et al, 2016; Hicks Pries et al, 2018). Greater reliance on fresh root C inputs in the deepest depth relative to the shallowest depth of the Brady soil is likely driven by more recalcitrant Brady soil C at

depth. Soil in the deepest depth is more recalcitrant based on age of soil C, mineral associated SOC (Jacobs and Mason, 2004) and the amount of black carbon stabilized in the Brady soil (Marín-Spiotta et al, 2014). Together, these data show that the microbial communities in the deepest depth of the Brady soil are quick to utilize fresh C inputs and begin respiring CO_2. Substrate induced growth of the soil microbial community is the first step toward mobilizing Brady soil C, thus highlighting the vulnerability of these buried soils to decomposition.

Addition of root exudates to soil induced positive priming for the first 15 days of the incubation (fig. 1d). While soil C priming was positive and similar in magnitude for all depths for most of the incubation duration, the rate of C priming on the first day of the root exudate incubation was greater in the shallowest depth compared to the deepest depth. This initial difference between depths on the first day may be explained by the amount of SOC in the shallowest depth as a result of greater root C input to the shallower soils compared to deeper depths, which influences the availability and accessibility of SOC to the soil microbial community (Fontaine et al, 2007; Bernal et al, 2016; Kumar, Kuzyakov, and Pausch, 2016). While there were no significant differences in the amount of SOC among depths, values trend toward more labile SOC in the shallowest depth as the result of more modern plant root inputs (table 1). Furthermore, our data indicate that greater water infiltration, via root growth and high levels of bioturbation (Woodburn, 2014), and soil C input have led to a progressive decomposition and mobilization of Brady soil C (Xiang et al, 2008), making it more accessible to the soil microbial community to mobilize SOC (Blagodatskaya et al, 2008). Given that we did not conduct ^{14}C respiration analysis to confirm the respired CO_2 was Brady soil C derived, we cannot

be sure that the higher rate of priming in the shallowest depth was the result of Brady soil C decomposition. Rather, this primed C may be derived from a more modern, labile SOC source. Regardless, the greatest depth was still capable of positively priming despite reduced exposure to modern surface conditions indicating that Brady soil C is vulnerable to decomposition via the priming effect. Cumulative amounts of primed SOC totaled 9-12% of the total C respired, similar to values seen in modern soils (de Graaff et al, 2014). These data indicate that paleosol C along an erosional gradient is vulnerable to decomposition among all depths and is capable of being mobilized with root exudate inputs.

In the root litter incubation, there were no significant differences among depths in both the rate and cumulative amounts of soil C priming. In general, the magnitude of soil C priming was lower in this incubation compared to the root exudate incubation. This result agrees with Bernal et al, (2016) who showed that priming was lower in magnitude in deep soils amended with aboveground litter than in soils amended with alanine (a component of our root exudate cocktail). In addition, they showed that the magnitude of priming was similar across depths ranging from 0m – 3m when aboveground litter was added to soils. Differences in the magnitude of our two incubations is potentially caused by differences in the chemical composition of the root exudate mixture and the root litter amendments which influences the microbial community active in the amended root derived C decomposition process. Easily available root derived substrates, like our root exudate mixture, tend to favor fast growing bacteria while root litter or more complex substrates tend to favor slow growing fungi (Blagodatskaya et al, 2007; Shazad et al, 2019). Fast growing bacteria is more likely to prime SOC in order to sustain biomass

growth while slow growing fungi incorporate C into biomass slower, reducing the need to co-metabolize SOC to maintain growth (Six et al, 2006). The rapid uptake of root exudate C by fast growing bacteria may explain the differences in soil priming on day 1 of the root exudate incubation and not in the root litter incubation. Our root exudate mixture contained a mix of substrates and this diversity may have promoted microbial biomass growth and SOC priming (de Nobili et al, 2001; de Graaff et al, 2011). In our root litter incubation, priming values were highly variable compared to the root exudate incubation suggesting that the heterogeneity of the root litter added may have occluded differences in the magnitude of soil primed among depths. Together, our data suggest that soil C is mobilized throughout the Brady soil with root derived C input though the magnitude of priming is more driven by root derived C substrate (i.e. root exudate or litter) rather than depth.

CONCLUSION

Our results show that all depths of the Brady soil are vulnerable to decomposition with the addition of root derived carbon. Drought is likely to increase in the Great Plains, NE as in many parts of the world (IPCC, 2018) which may not only increase erosion and exposure of the Brady soil to surface conditions but it may also increase root death, reduce quantity of root exudation and change the composition of root exudation (Williams and de Vries, 2019). A change in the composition of root exudates can make up for the reduced quantity and can stimulate microbial activity as respiration (de Vries, 2019). Thus, it is crucial to understand C dynamics as increasing surface conditions and environmental changes leave soil C in the Brady layer vulnerable to decomposition and positively priming SOC. Soil priming is an important mechanism for buried soils that reduce C sequestration with the addition of plant derived C inputs. This is reported across ecosystems however our study in one of the few studies that reveals how positive soil C priming can deplete ancient, buried soil C reservoirs as they become increasingly exposed to surface conditions.

While modern root derived C can lead to turnover and priming of buried C, it can also promote soil C stabilization (Spohn et al, 2018). Once root derived C enters the soil, its fate is dependent on the distribution of that C to either microbial residue or to CO_2 respiration. Microbial residue formation is important for root derived C stabilization because the majority of C stabilized in soil is derived from microbial residues that were initially formed from the assimilation of root exudates (Kallenbach et al, 2016). The

mechanisms behind these responses especially within soils at depth are not well studied though their implications can have a significant impact on C storage or efflux within the biosphere. Soil priming is an important mechanism to buried soils that reduced C sequestration upon the addition of plant derived C inputs. This has been reported across ecosystems however this is one of the few studies that reveals how positive soil C priming can deplete ancient, buried soil C reservoirs as they become increasingly exposed to surface conditions. Future research should study microbial carbon use efficiency based on the root derived C addition, the microbial growth rate across depths and should research where C is stabilized in soil fractions (i.e. course particulate organic matter, fine particulate organic matter, silt and clay).

TABLES

Table 1. Brady soil C within each depth: 0.2m, 0.4m and 1.2m below the modern surface. Lowercase letters indicate significant differences ($p<0.05$) between each depth. Values represent means ± SE (n=9).

Depth (m)	δ13 C	SOC (mg C/g soil)	Root Weight (mg/g soil)
0.2	17.64 ± 0.43	7.03 ± 0.52	0.67 ± 0.164 a
0.4	18.21 ± 0.38	5.57 ± 0.54	0.31 ± 0.046 ab
1.2	18.30 ± 0.31	4.60 ± 0.69	0.14 ± 0.041 b

Table 2. Fraction of C respired from soils amended with water only relative to total Brady SOC, and the fraction of C respired from root exudates and primed C relative to total CO_2 respiration. Lowercase letters indicate significant differences between depths. Values are means ± SE (n=9).

Depth (m)	normalized cumulative C respiration		
	Control C	Exudate C	Primed C
0.2m	0.04 ± 0.002	0.48 ± 0.03 a	0.10 ± 0.006
0.4m	0.04 ± 0.004	0.54 ± 0.02 ab	0.11 ± 0.008
1.2m	0.03 ± 0.003	0.58 ± 0.00 b	0.12 ± 0.006

Table 3. Fraction of C respired from soils amended with water only relative to total Brady SOC, and the fraction of C respired from Avena barbata root litter and primed C relative to total CO_2 respiration. Lowercase letters indicate significant differences between depths. Values are means ± SE (n=9).

Depth (m)	cumulative C respired normalized		
	Control C	Litter C	Primed C
0.2m	0.85 ± 0.05	0.32 ± 0.02 a	0.09 ± 0.039
0.4m	1.01 ± 0.20	0.36 ± 0.02 a	0.04 ± 0.094
1.2m	0.71 ± 0.15	0.46 ± 0.02 b	0.05 ± 0.044

FIGURES

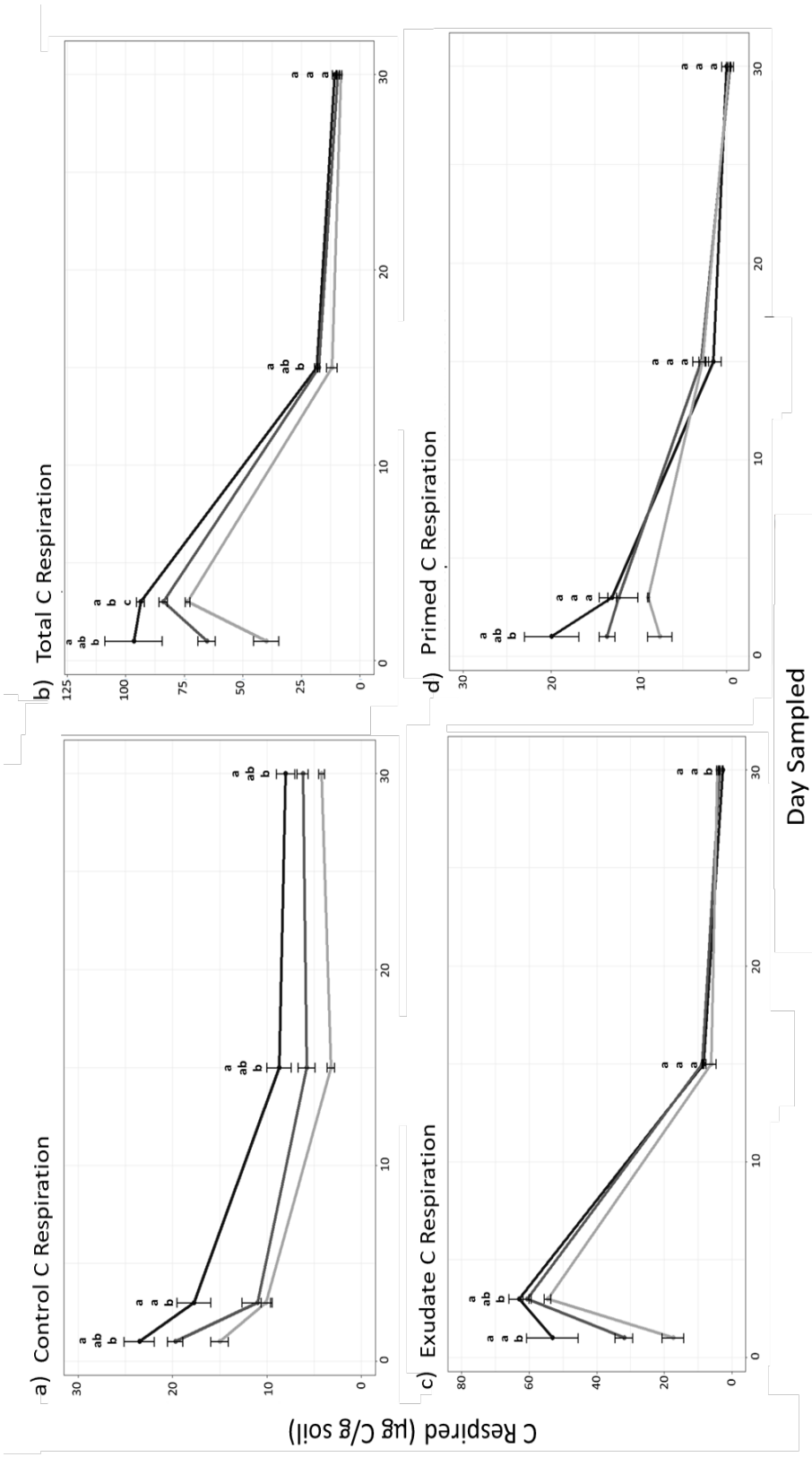

Figure 1a, b, c, d. Respiration rate in the root exudate incubation of the Brady soil from day 1 to 30 from the a) control (i.e. water only) soils, b) total C (exudate C + soil C), c) root exudate derived C and d) primed SOC. Samples were collected along an erosional gradient: 0.2m below the modern surface, 0.4m below the modern surface, and 1.2m below the modern surface. Lower case letters indicate significant difference among depths on each day CO_2 was sampled. Values are means ± SE (n=9)

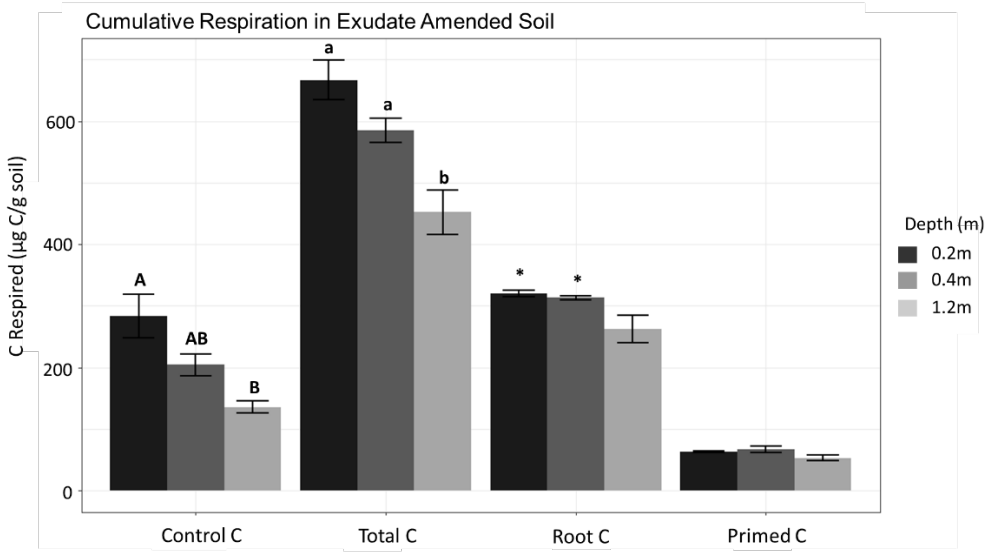

Figure 2. Total quantity of C respired during the root exudate incubation (30 days). Cumulative values for the control soils (water only), total amount of C (i.e. exudate derived + soil derived), exudate derived C only, and the amount of C primed are represented. Letters and asterisks indicate significant differences ($p<0.05$) among depths in each group. Values are means ± SE (n=9).

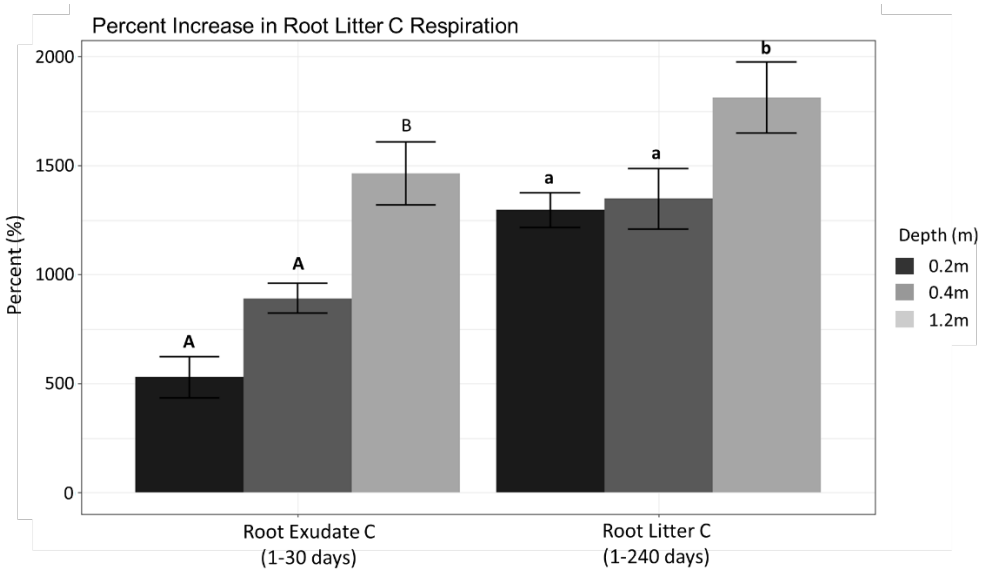

Figure 3. The relative change in root derived respiration from day 1 to the final day of the incubation. Uppercase letters indicate significant differences within the root exudate incubation. Lowercase letters indicate significant differences in the root litter incubation. Values are means ± SE (n=9).

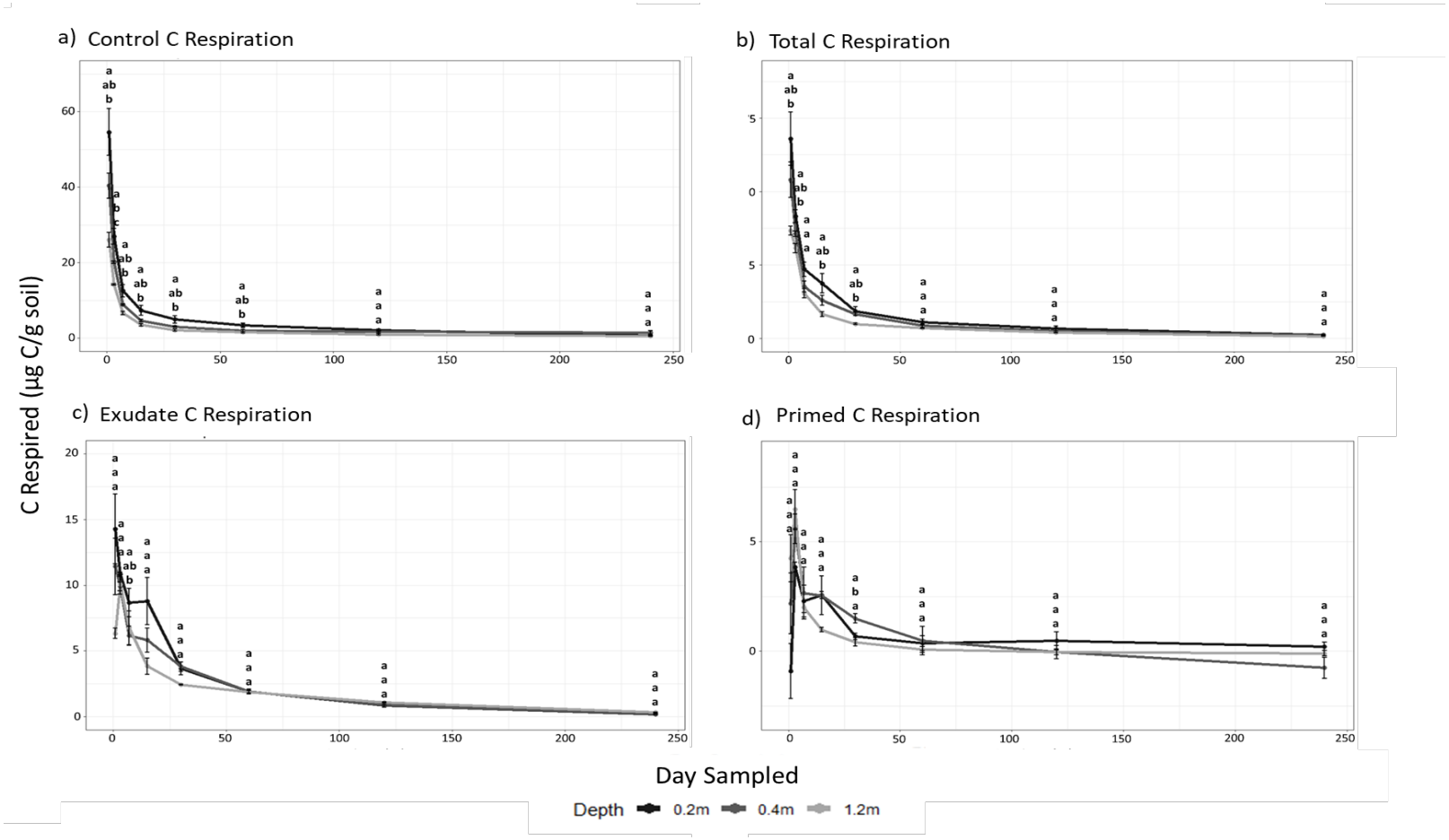

Figure 4 a, b, c, d. Respiration rate in the *Avena barbata* root litter incubation Brady soil from day 1 to 240 from the a) control (i.e. water only) soils, b) total C (litter C + soil C), c) root litter derived C and d) primed C. All sampled depths of the Brady soil are represented: 0.2m below the modern surface, 0.4m below the modern surface, and 1.2m below the modern surface. Lower case letters indicate significant difference among depths on each day CO_2 was sampled. Values are means ± SE (n=9)

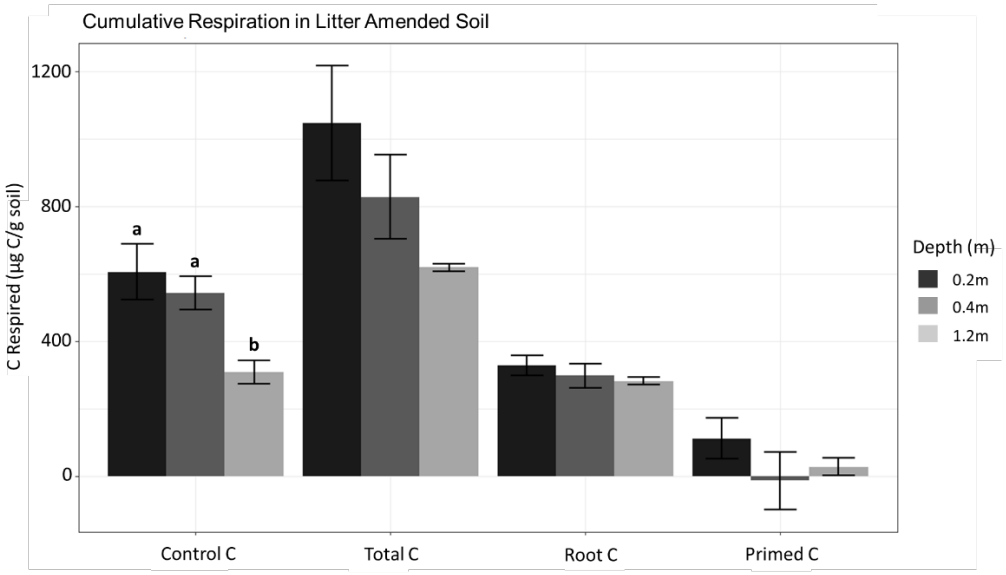

Figure 5. Total quantity of C respired for during the root litter incubation (240 days). Cumulative values for the control soils (water only), total amount of C (i.e. litter derived + soil derived), litter derived C only, and the amount of C primed are represented. Lowercase letters indicate significant differences ($p<0.05$) among depths in each group. Values are means ± SE (n=9).

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APPENDIX A

Methods Not Addressed in the Results

The following methods were completed yet due to Covid-19 shutdowns and delays were not analyzed by the time this thesis was written. These methods are included to serve as accurate documentation for future research and analysis.

Soil Fractionation

Incubated soil from the root litter incubation was used to determine soil C concentration and soil C content. A 12g subsample of soil from each depth was fractionated by density into course particulate organic matter (CPOM), fine particulate organic matter (FPOM), silt and clay. This was done by taking wetted soil directly from root litter incubation (wetted equivalent to 10g dry weight; ~12g soil). The subsample was mixed with 50 mL of 5g L^{-1} sodium hexametaphosphate (NaHMP) in a specimen cup and placed on an Eberbach E6000 reciprocal shaker (Belleville, Michigan) for 24 hours CPOM and FPOM fractions were isolated by pouring the slurry over a stacked 250µm sieve and 53µm sieve in a Nalgene basin, rinsing any remaining matter with a squirt bottle filled with de-ionized water. Large aggregates were gently crushed and any remaining material on the 250µm sieve was flushed into a separate, pre-tared tin. This became the CPOM fraction. Material left on the 53µm sieve was similarly rinsed until liquid running into the basin was clear and flushed into a pre-tared tin, becoming the FPOM fraction. The remaining slurry in the Nalgene basin containing silt and clay was separated by centrifuge. Slurry was poured into four 250mL centrifuge bottles, balancing each bottle with de-ionized water to within 1g of each other. Bottles were centrifuged for 1:20 min. The remaining supernatant after centrifuge was poured into a 1L glass bottle. The silt fraction at the bottom of the centrifuge bottles was flushed with a squirt bottle of de-ionized water into a pre-tared tin. The residual clay-containing supernatant now in the 1L glass bottle was treated with 1ml 0.25M $CaCl_2$ + $MgCl_2$ solution per 100ml of remaining liquid. This remaining liquid was balanced between four centrifuge bottles as described above and centrifuged for 10 min to isolate the final clay

fraction. Supernatant was poured off and remaining fraction in the bottle was flushed into a pre-tared tin. All fractions were oven dried at 65ºC overnight. After drying, each fraction was brushed out of the tin into a glass vial. All samples were homogenized using a ball mill. CPOM (10mg) and FPOM (50mg), silt and clay (50mg) were weighed and placed into silver capsules. To reduce inorganic C present in each fraction, 80µl de-ionized water was added to each fraction sample in the silver capsule and left in a vacuum desiccator with 30mL hydrochloric acid for 22 hours. Capsules were placed in an oven at 60C for 3 hours following fumigation. Once dry and sealed, capsules were shipped to UC Davis Stable Isotope Facility for ^{13}C analysis.

Microbial Biomass C

We used the fumigation-extraction method to determine microbial biomass C. A subsample of incubated soil was collected from 24hrs, 72hrs, day 30 and day 240 of the root litter incubation as well as 24hrs, 72hrs, day 15 and day 30 of the root litter incubation. Control soils were collected for each of the noted above dates. Unincubated soil was wetted to 60%WHC and immediately processed to determine baseline MBC. Soil (24g) was split into two equal parts, one of which (12g) was immediately extracted using 0.5M potassium sulfate (K_2SO_4). To this end, 50mL of K_2SO_4 was added to the sample and left to shake on the Eberbach E6000 reciprocal shaker (Belleville, Michigan) for 1 hour. After 1 hour, the soil slurry was poured over a Whatman no.1 filter paper with a specimen cup underneath to catch the liquid extract. Filtering lasted for ~3hours or until no liquid remained in the funnel. The other sample (12g) was placed in a 25mL beaker in a vacuum desiccator along with 20mL chloroform and 4-5 boiling chips. The vacuum desiccator was evacuated in a fume hood until chloroform boiled and vented. This was repeated two more times, not venting after the final boil. After the final chloroform boil, the desiccator was sealed off, covered with a garbage bag and left to sit in the fume hood for 72 hours. After 72h, the vacuum was released, and fumigated soils were transferred to specimen cups. Fumigated soils

were extracted using K_2SO_4 with the same protocol as described above. Liquid extracts were stored in a -20ºC freezer until further analysis.

To analyze microbial biomass, 1.5mL of the extract was pipetted into a 10mL plastic centrifuge tube. Tubes were placed into glass receptacles for freeze drying on the Labconco Freezone 2.5L Freeze Dry System (Hampton, NH). All samples were freeze dried at -40ºC for 48hours. Dried extract was scraped from the centrifuge tube and packaged into tin capsules for shipment and analysis at UC Davis Stable Isotope Facility.

Microbial Biomass Growth

Short term incubation involving both root litter and root exudate derived C inputs were used to determine differences in microbial carbon use efficiency (CUE) during peak respiration times. To determine CUE, 38g of soil from each depth were given either ^{13}C labeled *Avena barbata* root litter (as from the incubation above) or the ^{13}C exudate mixture (as above). Unlike previous incubation design, no pre-incubation was included in the root exudate incubation. The root litter incubation design remained the same. This was to directly compare the effects of the readily available exudates versus the more complex root litter. After the 15-day incubation with root exudates, all control soil samples were dried overnight. Dry, unamended soil (4g) was divided between four vented 12-ml exetainers for each depth and transect and re-wetted with 99% atom ^{18}O (20% ^{18}O mixed with 80% DI water) up to 60% WHC. Soil samples incubated for 24h, 48h, 72h. At each time point, soil in the exetainer was collected and immediately frozen at -80ºC for DNA analysis. Method based on Spohn et al, 2014.