

Destabilization of buried carbon under changing moisture regimes

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Abstract

Paleosols formed by the burial of topsoil during landscape evolution can sequester substantial amounts of soil organic carbon (SOC) over millennia due to protection from surface disturbances. We investigated the moisture sensitivity of buried SOC storage in the Brady paleosol, a loess-derived soil in Nebraska, USA, where historical aeolian deposition during the Pleistocene–Holocene transition buried soils up to 6 m deep. Topsoils from erosional (up to 1.8 m depth) and depositional (up to 5.8 m depth) transects were incubated under two moisture regimes - continuous wetting (60% water-holding capacity) and repeated drying–rewetting - to assess soil organic matter (SOM) vulnerability to changing hydrologic conditions. SOC decomposition rates modeled from CO₂ fluxes were consistently higher in erosional than depositional settings, with surface re-exposure of Brady soils enhancing microbial accessibility and destabilization. A two-pool model showed that >96% of SOC was stored in a slow-cycling pool, particularly in deeply buried soils where stabilization was linked to mineral association, fine particles, and Ca-mediated flocculation. However, this pool decomposed more rapidly in

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047 shallower Brady soils (higher turnover rate relative to buried soil), reflecting
048 increased microbial responsiveness to surface-driven processes.
049 Drying–rewetting cycles caused greater C losses from Brady soils than continuous
050 wetting, despite the dominance of the slow pool and depletion of labile C. These
051 cycles also accelerated fast pool decay in modern soils and erosional transects,
052 whereas burial dampened variability in Brady soils. Although continuous wetting
053 increased overall decay in burial transects during the incubation period, wet–dry
054 cycles destabilized the slow pool, which may result in greater long-term C loss.
055 Together, these results underscore the importance of burial depth, geomorphic
056 context, and moisture regime in shaping the long-term vulnerability of ancient
057 SOC under climate change.

058 **Keywords:** paleosol, erosion, soil carbon stabilization, organic matter decomposition

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062 1 Introduction

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064 Global temperatures have risen by 1°C since the industrial era due to anthropogenic
065 CO₂ emissions, confirming human-driven climate change (IPCC 2018). Alongside
066 warming, precipitation regimes are shifting - marked by increased frequency and
067 intensity of wetting and drying events, especially in more arid ecosystems. These
068 hydrologic fluctuations can destabilize long-stored soil organic carbon (SOC) by
069 disrupting aggregates, increasing dissolution and solute mobility, and stimulating
070 microbial decomposition (Berhe et al. 2012; Min et al. 2020; Hicks Pries et al. 2023).
071 While limiting warming to below 2°C remains critical, mitigation via emissions reduc-
072 tions alone may be insufficient. Preserving or enhancing terrestrial carbon sinks,
073 especially soils, offers a complementary pathway for climate stabilization.

074 Soil organic matter (SOM) encompasses the full suite of organic compounds in soil,
075 including living biomass, particulate debris, and mineral-associated organic molecules.
076 SOC refers specifically to the carbon fraction of SOM and is the metric used through-
077 out this study to quantify carbon stocks and fluxes. While many studies examine
078 topsoil carbon dynamics, whole-soil responses to changes in climate have rarely been
079 tested (Hicks Pries et al. 2017). Subsoils hold nearly half of global SOC stocks (Job-
080 bagy and Jackson 2000) and this deep-soil carbon may be more sensitive to varying
081 environmental conditions than surface soil (Min et al. 2020). Organic inputs reach
082 subsoils via leaching of dissolved organic carbon and vertical transport of litter by
083 bioturbation. SOC in deeper horizons typically features low carbon to nitrogen (C:N)
084 ratios and long mean residence times, suggesting advanced microbial processing and
085 relative stability (Rumpel and Kögel-Knabner 2011). In contrast, topsoils buried by
086 aeolian or alluvial deposition often retain legacy carbon signatures reflecting past veg-
087 etation and climate (Marin-Spiotta et al. 2014), diverging from modern surface soils.
088 These buried soils have historically been isolated from near-surface conditions, includ-
089 ing temperature and moisture fluctuations. Previous research supports this isolation
090 effect: for instance, Chaopricha (2013) found negligible CO₂ fluxes from Brady Soil
091 collected from 4 m below the modern surface when no water was added, indicating
092 extremely limited microbial activity under such dry, oxygen-poor conditions.

The stability of buried SOC, however, depends entirely on continued isolation from surface conditions – an assumption increasingly at odds with landscape dynamics across the Great Plains. Accelerated gully erosion, agricultural tillage, and more intense precipitation events are progressively exhuming paleosols that remained protected for millennia (Mason et al. 2008; Jacobs and Mason 2007). Yet the decomposition response of this ancient carbon to re-exposure remains poorly constrained. Will millennia-old SOC decompose rapidly once oxygen and moisture access is restored, or do the same properties that enabled its long-term preservation – fine texture, mineral associations, chemical recalcitrance – confer lasting resistance? This uncertainty carries substantial implications for carbon-climate feedbacks: if re-exposed paleosol carbon proves vulnerable to decomposition, ongoing erosion across loess landscapes could convert a long-term carbon sink into an unaccounted source.

Subsurface environments typically have limited oxygen, C inputs, and water availability, all of which constrain microbial activity and promote long-term SOC persistence (Soong et al. 2021). However, this protection may be compromised under climate change scenarios involving increased rainfall, warming, and surface disturbance (Fontaine et al. 2007; Gao et al. 2020; Hicks Pries et al. 2023). While burial isolates SOM from decomposers, enhancing its stability (Berhe et al. 2007; Stacy et al. 2015; Berhe et al. 2008), soil erosion, root intrusion, and hydrologic shifts can re-expose previously protected SOM. Erosion may also replace eroded C with increased photosynthate additions due to rejuvenation of rock-derived nutrients (Berhe et al. 2018). Given the global extent of geomorphic disturbance and the potential for reactivated decomposition, buried soils may represent an extensive but under-characterized carbon pool whose long-term persistence is uncertain (Chaopricha and Marín-Spiotta 2014; Szymanski 2021; Pal et al. 2023).

SOM decomposition is mediated by geomorphic and geochemical controls. Soil texture, mineralogy, and ionic composition shape organo-mineral associations and influence microbial access. Thermal transformation of buried SOM into condensed aromatic compounds can also increase resistance to decomposition (Marin-Spiotta et al. 2011; Schmidt et al. 2011). Recent findings from Dolui et al. (in press) link persistent SOM turnover in buried soils to fine textures, higher conductivity, and enhanced organo-mineral bonding. These stabilizing mechanisms weaken with erosional exposure to surface conditions (soil moisture and oxygen) and priming of previously-stable SOM due to the introduction of fresh organic matter inputs (McMurtry et al. 2024), making SOM more vulnerable to loss.

Soil moisture dynamics are central to SOM persistence. Wetting and drying–rewetting cycles can destabilize aggregates, increase dissolved organic carbon leaching, and stimulate mineral-associated OM loss (Berhe et al. 2012; Neff and Asner 2001; Li et al. 2023). Moisture influences microbial processes by modulating water potential, oxygen diffusion, and solute transport (Chowdhury et al. 2011; Davidson et al. 2012). Texture controls water retention during drying, while aggregate structure governs accessibility under saturated conditions (Or and Tuller 1999; Ghezzehei et al. 2019).

In surface soils, especially in semi-arid and Mediterranean systems, drying–rewetting cycles produce strong mineralization pulses (Miller et al. 2005; Zhu and

139 [Cheng 2013](#)). Such cycles break down aggregates and release labile SOM, stimulating
140 priming effects ([Najera et al. 2020](#)). Soils with broader pore-size distributions may
141 retain water longer, sustaining microbial activity and potentially increasing cumulative
142 SOM loss ([Goebel et al. 2005](#)). The effects of increased rainfall also depend on
143 seasonal timing; for instance, winter precipitation can enhance subsoil C storage more
144 than spring rain due to deeper translocation of carbon ([Wahab et al. 2025](#)).

145 As soils dry, physical and chemical processes can strengthen OM–mineral interac-
146 tions. Solute precipitation, matric tension, and shifts toward stronger bonding (e.g.,
147 inner-sphere complexes) promote greater SOM–mineral affinity ([Kaiser et al. 2015](#);
148 [Kemper et al. 1987](#); [Kang and Xing 2008](#)). Reorientation of amphiphilic compounds on
149 mineral surfaces can increase hydrophobicity ([Horne and McIntosh 2000](#)), potentially
150 misleading assessments of SOM stability under drier conditions.

151 CO₂ efflux in subsoils is shaped by physical constraints - lower porosity, higher
152 bulk density, and greater water-filled pore space - which suppress microbial respiration
153 after rewetting ([Min et al. 2020](#); [Hill et al. 1985](#); [Beare et al. 2009](#); [Schrumpf et al.](#)
154 [2013](#)). Subsurface microbial communities are often dominated by drought-tolerant
155 fungi ([Bird and Torn 2006](#)), and experience fewer moisture and temperature fluctua-
156 tions than surface soils ([Rumpel and Kögel-Knabner 2011](#)). Microbial “resistance” to
157 drying manifests as reduced respiration during dry-down, while “resilience” describes
158 rapid respiration rebound after rewetting ([Leizeaga et al. 2021](#); [Griffiths and Philip-](#)
159 [pot 2013](#)). Soils with frequent drying–rewetting history tend to support more resilient
160 microbial communities ([Fierer and Schimel 2003](#); [Steenwerth et al. 2005](#)).

161 In semi-arid systems, soil inorganic carbon (SIC) also contributes to carbon dynam-
162 ics. SIC accumulates at depth through carbonate dissolution–precipitation cycles
163 ([Shariffar et al. 2023](#); [Batoool et al. 2024](#); [Cotrufo and Lavallee 2025](#)). This is seen in
164 the Brady Soil, a late Pleistocene paleosol buried by loess ca. 13,000–10,000 years ago
165 in the Great Plains of the US ([Jacobs and Mason 2007](#); [McDowell 2020](#)). SIC inter-
166 acts with SOC via aggregation and mineral associations but can be mobilized through
167 leaching under increased moisture conditions ([Naorem et al. 2022](#); [Liu et al. 2018](#);
168 [Tsy-pin and Macpherson 2012](#)).

169 Despite growing interest in buried SOM, moisture-driven decomposition patterns
170 across landforms remain unclear. This study investigates the sensitivity of modern
171 and buried SOM to moisture inputs under erosional and depositional geomorphic
172 conditions in the Brady Soil. According to the U.S. National Climate Assessment
173 (2018), the Central Great Plains region of Nebraska is projected to warm by 3.5–9.5°C,
174 with annual precipitation increasing by 2.5 cm. Erosion driven by agriculture, grazing,
175 wind, and rainfall threatens to re-expose buried SOM to surface conditions.

176 We conducted a laboratory incubation using soils collected near Wauneta,
177 Nebraska, to compare CO₂ efflux under continuous wetting versus drying–rewetting
178 regimes. The area’s semi-arid climate with seasonal moisture variability provides a
179 relevant setting to test SOM responses to hydrologic fluctuations. Our hypotheses
180 were: (i) Brady SOM is more stable and decomposes more slowly than modern SOM
181 as reduced moisture and oxygen availability limit decomposition of SOM due to iso-
182 lation from the soil surface; (ii) buried SOM in erosional settings is more vulnerable

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to loss due to exposure of previously protected SOM to surface conditions and mixing with modern carbon; and (iii) wetting will stimulate CO₂ release from previously buried soils as a result of increased substrate availability due to enhanced dissolution, solute transport, microbial decomposition, and/or aggregate disruption. By evaluating the interactions between moisture, geomorphology, and SOM dynamics, we aim to improve predictions of carbon stability under future climate and land-use change.

2 Methods and materials

2.1 Site and sampling

The field site is situated near Wauneta, within the loess tablelands of southeastern Nebraska, USA (40°29'52.8" N, 101°24'36" W; see Fig. 1). The region experienced reduced loess input during the terminal Pleistocene and early Holocene (13–10 ka) that permitted soil formation, leading to the development of the Brady Soil. Subsequent aridification renewed dust flux, resulting in its burial by younger loess (Johnson et al. 2007; Mason et al. 2008). Additional loess accumulation throughout the Holocene preserved weaker paleosols formed during intermittent depositional pauses (Mason et al. 2003; Miao et al. 2007). Recognized as a key paleoenvironmental and stratigraphic unit, the Brady Soil is regionally traceable across Nebraska, northeastern Colorado, and northern Kansas (Johnson and Willey 2000).

The area is characterized by broad, flat uplands and sharply incised edges, providing natural windows into stratified soil profiles. Loess cover above the Brady Soil tapers in a downwind direction across the summits where it is thickest, producing burial transects of variable thickness. The local climate is semi-arid, with an average annual temperature of 9.7°C and ca. 495 mm of precipitation, concentrated during the summer months, with occasional snow in winter. Natural vegetation includes a mix of C3 and C4 grasses, replaced by cropland on many level surfaces today, but remaining at the study site and on steeper terrain in general.

Brady Soil exposures are visible in actively eroding margins, gullies, and roadside cuts. Prior investigations, including coring and field surveys, confirm its continuity beneath the summit landform (Jacobs and Mason 2004; Marin-Spiotta et al. 2014; Mason et al. 2008). Surface soils developed in Holocene loess are weakly developed and light-colored, typically classified as Mollisols, Inceptisols, or Entisols. Although different in age, modern and buried soils share similar parent material and mineralogy due to the region's limited weathering intensity. Brady A horizons (Ab) are identified by their dark grayish brown coloration (Munsell 10YR3/2 to 10YR4/2) and silt loam texture, generally overlying Bk or Bw horizons (Szymanski 2021; Jacobs and Mason 2007; McDowell 2020).

Sampling was conducted in 2016 and 2017 across two geomorphic settings: burial transects (where the Brady Soil is deeply buried beneath Holocene loess) and erosional transects (where the Brady Soil is exposed or shallowly buried due to hillslope erosion). Within each setting, three replicate transects were established. We collected samples at three depths relative to the soil surface from each of the transects per setting (details in Fig. 1). Sampling stratigraphy relative to the present land surface was categorized using Roman numerals (Supplementary Information, Table A1). At each

231 transect position, samples were collected from (1) the modern soil surface (0–30 cm),
232 (2) the subsurface modern soil (30–60 cm, where present), and (3) the upper Brady
233 paleosol horizon (0–30 cm into the Ab horizon, at variable depth below the modern
234 surface depending on burial thickness). All samples were analyzed for physicochemical
235 properties, but only samples from the 0–30 cm depth intervals were used for incubation
236 experiments. Table A1 provides the specific sampling depths for each transect position.
237 A Giddings probe (10.2 or 8.9 cm diameter) with plastic liners was used for intact
238 sampling in burial settings, while soil pits were dug for erosional profiles. Complete
239 methods are described in (Szymanski 2021).

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241 **2.2 Soil chemical and physical analyses**

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243 General soil physical and chemical properties were determined by standard soil analyt-
244 ical methods, as described in detail in Dolui et al. (2026) and Szymanski (2021). Briefly,
245 soil pH and electrical conductivity (EC) of soil samples were determined in 1: 2 water
246 extracts using a SevenExcellence multiparameter benchtop meter (Mettler Toledo,
247 United States). Total carbon was determined by dry combustion using an ECS 4010
248 elemental combustion analyzer (Costech Analytical Technologies, Inc., USA); inor-
249 ganic carbon was removed by acidification with 1M HCl prior to TOC determination.
250 Exchangeable base cations (Ca^{2+} , Mg^{2+} , Na^{+} and K^{+}) were quantified by ICP-
251 OES (Optima 5300 DV Spectrometer, Perkin-Elmer, Germany) following ammonium
252 acetate extraction (buffered to pH 7). Sodium adsorption ratio (SAR) was determined
253 by dividing concentration of Na in soil extract by the square root of half of sum of Ca
254 and Mg concentrations (Dolui et al., in press). Particle size distribution was determined
255 using the pipette method for clay ($>2 \mu\text{m}$) and laser diffraction (Mastersizer 2000 par-
256 ticle size analyzer, Malvern Panalytical, UK) for silt and sand fractions. Radiocarbon
257 (^{14}C) analyses of bulk soil samples were conducted to determine the age and turnover
258 time of carbon in both modern and buried soils. Samples were pre-treated, combusted,
259 and then measured by accelerator mass spectrometry using an FN accelerator mass
260 spectrometer (Van de Graaff, US) at the center for accelerator mass spectrometry at
261 Lawrence Livermore National Laboratory. The $\Delta^{14}\text{C}$ results were used in a homoge-
262 neous, open-system, steady-state soil carbon decomposition model to estimate carbon
263 turnover times (Dolui et al. 2026) which were averaged for the three replicate transects.

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265 **2.3 Incubation experiments**

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267 The incubation experiment was set up to determine the effect of continuous wetting
268 and drying–rewetting on SOC fluxes using soils that were collected from the upper
269 layer of modern and Brady Soil samples at depositional and erosional transect types.
270 To isolate the effects of moisture, roots >2 mm were removed by sieving and manual
271 sorting. Samples from 0–30 cm depth from different transect numbers were homoge-
272 nized (so that they had only unique paleostatus, transect type and burial/ erosional
273 degree). Two types of water addition experiments were conducted: continuous wet and
274 drying–rewetting. Two sub-samples were taken from each composite to perform bio-
275 logical replicates of each incubation experiment, such that there was a total number
276 of 24 individual incubation vessels.

2.3.1 Experimental setup

The incubation experiments were conducted in 8 oz mason jars. Soil water holding capacity (WHC) was pre-determined by tensiometry (using pressure plates with an applied pressure of -33 kPa) and soil moisture content was monitored on a mass-basis by weighing soils weekly during the incubation. The treatments included (i) continuous wet – soils maintained at 60% WHC throughout the experiment and (ii) drying–rewetting cycles – soils were dried, then rewetted to 60% WHC. Treatment durations differed by experimental objective. The continuous wet incubation (225 days) was designed to capture the full trajectory of decomposition, enabling robust fitting of two-pool decay models that partition SOC into fast- and slow-cycling fractions. The wet-dry treatment (56 days, 8 cycles of 7-day drying followed by rewetting) was designed to assess cumulative effects of repeated moisture pulses over a timeframe comparable to a growing season. For direct comparison between treatments, we modeled CO_2 loss over equivalent 49-day windows and compared decay parameters derived from each treatment’s full duration. Each treatment had two replicates (sub-samples derived from a single composite of replicate transects per soil type), and control soils were maintained at 5% WHC.

Previously homogenized soils were sub-sampled and added to jars at an equivalent of 30 ± 0.5 g dry mass. After adding a predetermined amount of ultra-pure Milli-Q water, the jars were kept at room temperature (ca. 25°C), matching the average summer soil temperature (when most precipitation occurs) at the site (UNL Soil Temperature Data). Jars were sealed with lids fitted with rubber septa for headspace gas collection, and silicone gel was applied around the septa to prevent gas leakage.

In the continuous wet experiment, soils were maintained at 60% WHC and sealed until sampling. On average, water loss was 0.01 mL/day, ranging from 0.005 to 0.02 mL/day. Water was added after gas sampling to avoid inducing the Birch effect. After each sampling, lids were left open for one hour to allow CO_2 equilibration with ambient air.

In the drying–rewetting experiment, soils were rewetted every 7 days by slowly adding Milli-Q water to reach 60% WHC. After water addition, jars were sealed, and headspace gas was sampled 6 hours later to capture Birch effect emissions. Soils were then dried to 5% WHC by removing the lids and incubating at ca. 25°C .

2.3.2 Sampling schedule and CO_2 analysis

In the continuous wet experiment, headspace gas samples for CO_2 analysis were collected on days 1, 3, 5, 7, 11, 16, 27, 55, 82, 114, 151, and 225. After each sampling, jar lids were opened for one hour to equilibrate with ambient air.

In the drying–rewetting experiment, jars were sealed for 6 hours after water addition, and headspace gas was sampled on days 1, 7, 21, 28, 35, 42, and 49 to evaluate the Birch effect. Control samples were collected on the same days for both experiments.

Evolved CO_2 concentrations were analyzed using a Shimadzu 2014 gas chromatograph (Kyoto, Japan) with a thermal conductivity detector at UC Merced and an LI-830 infrared gas analyzer (IRGA) at Lawrence Livermore National Laboratory (LLNL).

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323 2.4 Statistical analyses

324 All statistical analyses were performed using CRAN-R 4.5.0 (R Core Team 2025). Soil-
 325 respired CO₂ measurements from incubation experiments were averaged across two
 326 biological replicates per treatment. Control samples were represented by single mea-
 327 surements due to sample constraints. Accordingly, statistical comparisons involving
 328 controls were interpreted with caution.

329 The concentration of soil-respired CO₂ was expressed as the mass of C respired
 330 per unit mass of SOC, calculated as:

$$331 \mu\text{g C} - \text{CO}_2 \text{ g soil C}^{-1} = \text{mmol air} * \frac{\mu\text{mmol CO}_2}{\text{mol air}} * \frac{10^{-3} \text{ mol air}}{\text{mmol air}} * \frac{12 \mu\text{g C}}{\mu\text{mol C}} * \frac{1}{\text{g TOC}} \quad (1)$$

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 333 For the continuously wet incubation, cumulative respiration was calculated by
 334 summing CO₂ fluxes over the 225-day experiment. Two-pool first-order decay models
 335 were fitted to cumulative respiration data according to:

$$336 C_{\text{CO}_2}(t) = C_0 \left(1 - (f_f e^{-k_f t} + f_s e^{-k_s t}) \right) \quad (2)$$

337 where $C_{\text{CO}_2}(t)$ is the cumulative mass of C (μg) respired by day t , C_0 denotes
 338 TOC, f_f and f_s represent the fast- and slow-cycling fractions of SOC (with $f_f + f_s =$
 339 1), and k_f and k_s are the corresponding decay rate constants. Models were fitted
 340 using non-linear least-squares optimization with the `minpack.lm` package (Elzhov et al.
 341 2023).

342 For the wet-dry cycling incubation, cumulative respiration was calculated by sum-
 343 ming CO₂ fluxes over wetting periods only, assuming negligible respiration during
 344 drying phases (Chaopricha 2013). The effective incubation time (t) was approximated
 345 as the cumulative duration of wetting events (2 days over a 49-day experiment).
 346 Two-pool decay models were initially fitted following the same approach as for the
 347 continuously wet incubation.

348 Preliminary model fits indicated that the fast-cycling pool contributed negligibly to
 349 respiration during wet-dry cycling (slow:fast pool ratio $\sim 0.999:0.001$), consistent with
 350 a functionally homogeneous system. Consequently, one-pool first-order decay models
 351 were also fitted for both incubation treatments:

$$352 C_{\text{CO}_2}(t) = C_0 (1 - e^{-kt}) \quad (3)$$

353 where k represents the single-pool decomposition rate constant.

354 The effects of transect type, paleostatus, degree of burial or exposure, and their
 355 interactions on SOM decomposition parameters (decay rates and pool sizes) were eval-
 356 uated using linear mixed-effects models, treating biological replicates as a random
 357 effect (controls excluded). These analyses were conducted using the `nlme` (Pinheiro
 358 et al. 2024) and `emmeans` (Lenth 2024) packages. Significant differences among fac-
 359 tor levels were assessed using pairwise Sidak-adjusted comparisons. For the wet-dry
 360 cycling experiment, similar models were applied to analyze day-1 CO₂ pulse responses.

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Total CO₂ losses during wet-dry cycling (49 days) were compared to modeled CO₂ losses over an equivalent duration under continuous wetting using linear mixed-effects models with Paleostatus, transect type, and Depth as fixed effects and replicate as a random effect. Marginal means and pairwise comparisons were used to contrast incubation treatments. For control samples, Paleostatus and transect type were treated as fixed effects and Depth as a random effect. Despite limited replication, the large magnitude of difference between control and treatment fluxes (>45-fold) indicates that moisture addition, rather than incubation artifacts, drove observed respiration patterns.

Mean cumulative CO₂ losses between control and treatment groups were further compared using a Welch test following confirmation of unequal variances via a Bartlett test, implemented in the `stats` package (RStudio Team 2019). Linear mixed-effects models were also used to evaluate the effects of transect type and paleostatus on cumulative CO₂ losses in control samples, with Depth treated as a random effect.

Multiple linear regression (MLR) was used to quantify the influence of soil physicochemical properties (soil pH, EC, TOC and TIC contents, SAR, texture, and exchangeable base cations) on modeled SOM decomposition parameters. For the continuously wet incubation, MLR models were applied to fast- and slow-pool decay rates and slow-pool fraction sizes derived from two-pool models. For the wet-dry cycling incubation, MLR analyses were conducted on decomposition rates derived from one-pool models. Model selection followed stepwise forward and backward procedures, prioritizing parsimony based on Akaike Information Criterion (AIC) values using the MASS package (Venables and Ripley 2002). Model coefficients, intercepts, R², and root mean squared error (RMSE) were calculated using the `stats` package. Pairwise Pearson correlation matrices were generated to visualize relationships among soil properties and SOM decomposition parameters using the `stats` and `ggplot2` packages (RStudio Team 2019).

3 Results

3.1 General soil properties

The physicochemical properties of soils used in the incubation experiment are shown in Table 5. The soils were relatively alkaline (pH ranging from 6.89 to 7.77), especially the Brady Soil. The clay, silt and sand content placed the soils in the texture class category of silt loam. Total organic carbon was higher in modern soils due to active biomass inputs, while inorganic carbon content was higher in the Brady Soil due to carbonate formation (cite Dolui et al i.e. paper 1). The Brady soil of the burial transect was classified as saline (mean EC of 5.41 dS m⁻¹) and the CEC of soils was moderately high, ranging from 15.17 - 23.31 and indicating the presence of higher activity clays. The turnover time of bulk soils as derived from radiocarbon-based models, was much greater in the Brady Soil (8327 - 15 654 years) compared to modern soils (576.4 - 1451 years), confirming the long-term stability of SOM in the paleosol (cite Dolui et al. i.e., paper 1). The mean CN ratio in both Brady and modern soils was relatively low (ca. 10), indicating a sufficient supply of N for plant growth and microbial activity.

415 **3.2 Effects of continuous wetting on soil CO₂ efflux**

416 Cumulative C lost from soils via respiration of CO₂ during the continuously wet incu-
417 bation are shown in Fig. 2. Raw incubation data and figures of merit of statistical
418 comparative tests are available online at DOI: doi: 10.17632/fjw646gpyf.1.

419 In the continuous wet soil treatment group, modern soils evolved significantly
420 higher CO₂ (mean of 30.6 mg CO₂-C g C⁻¹) compared to Brady Soil (mean of 15.9
421 ± 2.39 mg CO₂-C g C⁻¹) after 225 days of incubation (p < 0.01). Soils of the ero-
422 sional transect type had greater cumulative C loss (mean of 29.3 mg CO₂-C g C⁻¹)
423 compared to the depositional transect (mean of 17.3 ± 2.39 mg CO₂-C g C⁻¹, p <
424 0.01). However, the difference between cumulative C losses of erosional vs depositional
425 transect types was not significant at the greatest depth.

426 Modern soil in the erosional transect had significantly higher cumulative C losses
427 (mean of 35.9 mg CO₂-C g C⁻¹) than modern soil of the depositional transect (mean
428 of 25.3 mg CO₂-C g C⁻¹, p < 0.05). Similarly, Brady Soil of the erosional transect had
429 significantly higher cumulative C losses (mean of 22.6 mg CO₂-C g C⁻¹) compared to
430 the depositional transect (mean of 9.28 mg CO₂-C g C⁻¹, p < 0.05).

431 The Brady soil with an intermediate degree of erosion had higher cumulative CO₂
432 loss (mean of 33.2 mg CO₂-C g C⁻¹) compared to Brady Soil of other depths, while
433 the Brady soil with the greatest degree of burial had the lowest cumulative CO₂ loss
434 (mean of 4.14 mg CO₂-C g C⁻¹, differences not significant). For Brady soil of the
435 depositional transect, the magnitude of cumulative CO₂ efflux among different depths
436 was in the order of shallowest > intermediate > deepest, whereas in the erosional
437 transect, the order was intermediate > shallowest > deepest.

438 The cumulative CO₂ evolution of 60% WHC continuous wet experiments (mean
439 of 23.3 mg CO₂-C g C⁻¹) was significantly greater than control soils maintained at
440 5% WHC (mean of 0.478 mg CO₂-C g C⁻¹, p < 0.001). Among the control soils,
441 Brady Soil of the depositional transect had significantly greater C losses (mean of
442 0.561 mg CO₂-C g C⁻¹) compared to modern soils (mean of 0.253 ± 0.0683 mg CO₂-
443 C g C⁻¹, p < 0.05), but C losses in modern and Brady Soil were more similar in the
444 erosional transect (mean of 0.254 and 0.444 mg CO₂-C g C⁻¹ for modern and Brady
445 Soil respectively, n.s.).

446 Significance of differences in cumulative CO₂ losses among degree of burial/ expo-
447 sure for control samples could not be tested due to lack of replication, but we report
448 observed differences. Among the depositional transect control soils, the shallowest
449 depth Brady soil produced the greatest cumulative CO₂ loss, while modern soil col-
450 lected from the intermediate degree of burial soil produced the least cumulative CO₂
451 loss. Among the erosional transect control soils, the most CO₂ was evolved in the
452 intermediate depth Brady soils, and the lowest CO₂ was evolved in the modern soil
453 collected from the lowest degree of burial.

455 **3.3 Effect of drying and rewetting on soil CO₂ efflux**

456 Daily C lost from soils via respiration of CO₂ during the dry-rewetting incubation are
457 shown in Fig. A1 and cumulative C losses are shown in Fig. 3. Soil respiration data from
458 the dry-rewetting incubation are available online at DOI: doi: 10.17632/fjw646gpyf.1.

459
460

The largest respiration fluxes were produced on the first day, with significantly greater cumulative CO₂ loss from modern soils (mean of 0.920 mg CO₂-C g C⁻¹) compared to Brady Soil (mean of 0.729 ± 0.034 mg CO₂-C g C⁻¹, p < 0.01), but the reverse was observed at the greatest depth. Respiration pulses declined over time for all soils. Control soils had a lower day⁻¹ pulse CO₂ (mean of 0.295 mg CO₂-C g C⁻¹) compared to the dry-rewetting treatment (mean of 0.824 mg CO₂-C g C⁻¹, p < 0.001), but there was no significant difference between the cumulative CO₂ loss from control (21.9 mg CO₂-C g C⁻¹) and treatments (17.5 mg CO₂-C g C⁻¹) by the final day of incubation.

After 49 days of incubation under wet-dry cycles, the Brady Soil from both the burial and the erosional transects emitted significantly more cumulative CO₂ (0.0183 g CO₂-C g C⁻¹) than Brady Soil incubated under continuously wet conditions (0.009 ± 0.0005 g CO₂-C g C⁻¹, p < 0.001). However, there was no significant difference between cumulative CO₂ loss in modern soils incubated under wet-dry cycles versus continuous wet conditions.

The order of magnitude of total CO₂ emission after 49 days followed the order erosional modern > erosional Brady > depositional modern > depositional Brady. Soils of the erosional transect (mean of 18.3 mg CO₂-C g C⁻¹) had significantly greater cumulative CO₂ loss compared to the depositional transect (mean of 16.7 ± 0.643 mg CO₂-C g C⁻¹, p < 0.01). Modern soil had significantly greater cumulative CO₂ loss (mean of 16.7 mg CO₂-C g C⁻¹) than Brady Soil (mean of 18.3 ± 0.390 mg CO₂-C g C⁻¹, p < 0.001), but the difference was not significant at the intermediate depth. Modern soil from the shallowest depth emitted significantly more cumulative CO₂ (19.2 mg CO₂-C g C⁻¹) than the deepest layer (13.9 mg CO₂-C g C⁻¹, p < 0.05). However, the opposite was observed in Brady Soil, where the deepest layer emitted more cumulative CO₂ (15.6 mg CO₂-C g C⁻¹) compared to the shallowest layer (20.4 mg CO₂-C g C⁻¹, p < 0.05). Among the control soils, although Brady Soil emitted more cumulative CO₂ than modern soil, there was no significant difference. This was likely due to large variation of the depth-pooled samples (in absence of control replicates), as a result of the much larger CO₂ loss from Brady Soil of the erosional transect at the greatest depth compared to other samples.

3.4 Decay rates and fraction sizes of soil organic matter pools

The decomposition rate of the slow pool and size of the fast pool under continuous wetting is shown in Fig. 4 and the decomposition rate of SOM (single pool) under wet-dry cycles is shown in Fig. 5. Statistical figures of merit of linear mixed-effects models are available online at DOI : doi: 10.17632/fjw646gpyf.1.

The slow-cycling SOM pool under continuous wetting decayed ca. 2000 times more slowly than the fast-cycling pool, with a significantly lower mean decay rate in the burial transect ($8.68 \cdot 10^{-6} \text{ day}^{-1}$ i.e., 315 years turnover time, henceforth referred to as TOT) compared to the erosional transect ($1.36 \cdot 10^{-5} \pm 6.82 \cdot 10^{-7} \text{ day}^{-1}$ i.e., 201 years TOT, p < 0.01). Thus, higher decay rates correspond to faster (shorter) turnover times throughout this section. The mean decay rate of the slow-cycling SOM pool of Brady Soil ($6.86 \cdot 10^{-6} \text{ day}^{-1}$ i.e., 399 years TOT) was significantly lower than that of modern soil ($1.54 \cdot 10^{-5} \text{ day}^{-1} \pm 6.82 \cdot 10^{-7}$, i.e., 178 years TOT, p < 0.001). The

507 mean decay rate of the slow-cycling SOM pool of modern soil in the burial transect
508 was significantly greater at the greatest degree of burial ($1.53 \cdot 10^{-5} \text{ day}^{-1}$ i.e., 178
509 years TOT), compared to the lowest degree of burial ($9.97 \cdot 10^{-6} \text{ day}^{-1}$ i.e., 275 years
510 TOT, $p < 0.05$).

511 The mean decay rate of the fast-cycling SOM pool of the erosional transect (0.130
512 day^{-1} i.e., 7.68 days TOT) under continuous wetting was significantly higher (i.e.,
513 faster turnover) than that of the burial transect ($0.126 \pm 0.001 \text{ day}^{-1}$ i.e., 7.96 days
514 TOT, $p < 0.05$). The mean decay rate of the fast-cycling SOM pool of Brady Soil in
515 the erosional transect was significantly greater at the greatest degree of erosion (0.144
516 day^{-1} i.e., 6.96 days TOT) compared to the lowest degree of erosion (0.121 day^{-1} i.e.,
517 8.25 days TOT, $p < 0.01$).

518 The decay rate of SOM (one-pool model) under wet-dry cycles did not differ sig-
519 nificantly among soils of different paleostatus or transect type. In the two-pool model,
520 the decay rate of the slow-cycling SOM pool under wet-dry cycles was significantly
521 higher in the erosional transect (0.007 day^{-1} i.e., 129 days TOT) compared to the
522 burial transect ($0.009 \pm .0002 \text{ day}^{-1}$ i.e., 117 days TOT, $p < 0.05$), indicating faster
523 SOM turnover where decay rates were higher. The decay rate of the fast-cycling SOM
524 pool in the burial transect was significantly higher in modern soil ($8.11 \pm 1.94 \text{ day}^{-1}$
525 i.e., 0.123 days TOT) compared to Brady soil (0.558 day^{-1} i.e., 1.79 days TOT).

526 The slow-cycling pools of both modern and Brady Soil under continuous wetting
527 contained a much greater proportion of total SOC ($> 96\%$) than the fast-cycling
528 pool. The fraction size of the fast-cycling pool relative to the slow-cycling pool under
529 continuous wetting was significantly greater in the erosional transect (0.014) compared
530 to the burial transect (0.012 ± 0.0003 , $p < 0.01$) and significantly greater in modern
531 soils (0.0173) compared to Brady Soil (0.009 ± 0.0003 , $p < 0.001$). The fraction size
532 of the fast and slow pools tended to 0 and 1.0 respectively in all soils under wet-dry
533 cycling, nullifying the statistical comparison results among soils of different paleostatus
534 and transect types.

535

536 **3.5 Decay rates and fraction sizes of soil organic matter pools** 537 **in continuous wet versus wet-dry experiment** 538

539 A summary of SOM decomposition model parameters (one-pool and two-pool) of
540 the continuously wet and wet-dry cycling experiments is shown in Table 2. Statisti-
541 cal figures of merit of linear mixed-effects models are available online at DOI : doi:
542 [10.17632/fjw646gpyf.1](https://doi.org/10.17632/fjw646gpyf.1).

543 With the one-pool models, the continuously wet soils had a significantly higher
544 decay rate (0.00865 day^{-1} i.e., 116 days TOT) compared to the wet-dry cycles (0.00714
545 day^{-1} i.e., 140 days TOT, $p < 0.01$), indicating faster turnover under continuous
546 wetting. In the burial transect, the one-pool decay constant of the continuous wet
547 experiment was significantly higher (0.0103 day^{-1} i.e., 97.4 days TOT) than that of the
548 wet-dry cycling experiment (0.00690 day^{-1} i.e., 145 days TOT, $p < 0.001$). However,
549 there was no significant difference between the decay constant of the continuous wet
550 and wet-dry experiments in erosional transects. These trends were observed in both
551 Brady and modern soils.

552

Among the control soils with one-pool models, the continuous wet experiment had a significantly higher decay rate (0.0376 day^{-1} i.e., 26.6 days TOT) compared to the wet-dry cycling experiment (0.00146 day^{-1} i.e., 179 days TOT, $p < 0.001$), again meaning faster decomposition under continuous wetting. This was true in both burial (continuous wet $k = 0.0374 \text{ day}^{-1}$ i.e., 26.7 days TOT and wet-dry $k = 0.00372$ i.e., 268 days TOT) and erosional ($k = 0.0377 \text{ day}^{-1}$ i.e., 26.5 days TOT and wet-dry $k = 0.00746 \text{ day}^{-1}$ i.e., 134 days TOT) transects, as well as Brady (continuous wet $k = 0.0376 \text{ day}^{-1}$ i.e., 26.6 days TOT and wet-dry $k = 0.00871 \text{ day}^{-1}$ i.e., 115 days TOT) and modern (continuous wet $k = 0.0375 \text{ day}^{-1}$ i.e., 26.7 days TOT and wet-dry $k = 0.00248 \text{ day}^{-1}$ i.e., 404 days TOT) soils.

With the two-pool models, the decay rate of the slow-cycling SOM pool in the continuously wet experiment was significantly lower ($1.11 \cdot 10^{-5} \text{ day}^{-1}$ i.e., 246 years TOT) than that of the wet-dry cycling experiment (0.00812 day^{-1} i.e., 123 days TOT, $p < 0.001$), reflecting faster turnover in the wet-dry treatments. This was true in both Brady (continuously wet $k = 6.86 \cdot 10^{-6} \text{ day}^{-1}$ i.e., 399 years TOT, wet-dry $k = 0.0083 \text{ day}^{-1}$ i.e., 120 days TOT) and modern (continuously wet $k = 1.54 \cdot 10^{-5} \text{ day}^{-1}$ i.e., 178 years TOT, wet-dry $k = 0.00793 \text{ day}^{-1}$ i.e., 126 days TOT) soils, as well as both burial (continuously wet $k = 8.68 \cdot 10^{-6} \text{ day}^{-1}$ i.e., 316 years TOT, wet-dry $k = 0.00773 \text{ day}^{-1}$ i.e., 129 days TOT) and erosional (continuously wet $k = 1.36 \cdot 10^{-5} \text{ day}^{-1}$ i.e., 202 years TOT, wet-dry $k = 0.0085 \text{ day}^{-1}$ i.e., 118 days TOT) transects, and in all depth layers.

The decay rate of the fast-cycling SOM pool in modern soil was significantly higher in the wet-dry cycling experiment (8.11 day^{-1} i.e., 0.123 days TOT) compared to the continuously wet experiment (0.127 day^{-1} i.e., 7.89 days TOT, $p < 0.001$), indicating a much faster turnover response to rewetting. The decay rate of the fast-cycling pool in the erosional transect was also significantly higher in the wet-dry cycling experiment (5.148 day^{-1} i.e., 0.194 days TOT) compared to the continuously wet experiment (0.130 day^{-1} i.e., 7.68 days TOT). Separating these effects by depth revealed that the significance of the difference between the fast pool decay rate in modern soils was more evident at the intermediate and greatest depth. The decay rate of the fast-cycling SOM pool did not differ significantly between continuously wet and wet-dry cycling experiments in Brady soils or in the burial transect.

Among the control soils with two-pool models, the continuous wet experiment had significantly lower slow pool decay rate ($1 \cdot 10^{-8} \text{ day}^{-1}$ i.e., 274 000 years TOT) compared to the wet-dry cycling experiment ($k = 0.005 \text{ day}^{-1}$ i.e., 200 days TOT, $p < 0.01$), again corresponding to much faster turnover in the wet-dry treatments. This was true in both the burial and erosional transects, modern and Brady Soil and at all depths.

The decay rate of the fast-cycling SOM pool of control Brady Soil was significantly higher in the wet-dry cycling experiment (0.0556 day^{-1} i.e., 18.0 days TOT) compared to the continuously wet experiment (10.3 day^{-1} i.e., 0.0971 days TOT). This was true in both burial and erosional transects. In modern soils, however, there was no significant difference between decay rates of fast-cycling SOM in continuously wet versus wet-dry cycling experiments.

599 The fraction size of the slow-cycling SOM pool in the wet-dry cycling experiment
600 was significantly larger (0.999) than that of the continuously wet experiment (0.987,
601 $p < 0.001$) - this was true for both Brady and modern soils, across erosional and
602 burial transects and at all depths. It follows that the fraction size of the fast-cycling
603 SOM pool in wet-dry cycling experiments was significantly smaller (0.001) than that
604 of the continuously wet experiment (0.0131, $p < 0.001$) for soils of all transect types,
605 paleostatus groups and depth categories. There was no significant difference between
606 fraction sizes of the slow-cycling SOM pool among the controls of the wet-dry cycling
607 and continuously wet experiment; the fast-cycling pool was also similar among control
608 soils of the two experiments.

609

610 **3.6 Relationships between soil properties and carbon dynamics**

611

612 The true versus predicted values MLR models for prediction of decomposition rates of
613 the fast- and slow-cycling pools as well as the fraction size of the slow pool are shown
614 in the Appendix, Fig. A2. A matrix showing the correlation between these variables is
615 given in Fig. 6. The intercept, coefficients of explanatory variables, RMSE and R^2
616 of the best-performing MLR models, are summarized in Table 3 and the full equations
617 are in the Appendix (Multiple linear regression equations).

618 The MLR models for prediction of decay rates of SOM under continuous wetting
619 had better model fit ($R^2 > 0.80$) compared to those for predictions of SOM pool
620 fraction size ($R^2 = 0.44$). From the MLR equations, we deduce that soil properties with
621 the greatest coefficients had the most important effects on the SOM decay rate and
622 fraction size model parameters. While SAR increased the rate of decay and decreased
623 the size of the of the slow pool, an opposite trend is observed in the fast pool.

624 TOC and pH also increased the decay rate of the fast-cycling SOM pool under
625 continuous wetting, while the slow pool decay rate decreased with increasing TIC.
626 TOC and TIC increased the fraction size of the slow pool at the expense of fraction
627 size of the fast pool. When considering effects of these factors individually, SAR, TOC,
628 TIC and pH did not have significant correlation with SOM decomposition parameters
629 (Fig. 6). However, exchangeable Mg and K content were significantly correlated with
630 the decay rate of the fast (negative correlation) and slow (positive correlation) pools
631 (Fig. 6).

632 The true versus predicted values MLR models for prediction of the decomposition
633 rate of SOM (one-pool system) under wet-dry cycles are shown in the Appendix, Fig
634 A3. A matrix showing the correlation between these variables is given in Appendix,
635 Fig. A4.

636 The intercept, coefficients of explanatory variables, RMSE and R^2 of the best-
637 performing MLR model for prediction of the decay rate, are given summarized in Table
638 3 and the full equation is in the Appendix (Multiple linear regression equations).

639 SAR decreased the decay rate of SOM under wet-dry cycles. Clay and TOC content
640 increased the decay rate of SOM. Exchangeable Ca, Mg content decreased the decay
641 rates of both pools. Considering these parameters individually (correlation coefficients)
642 revealed a weak correlation with k. Therefore MLR, where k is modeled as a function of
643 all predictors together, demonstrates that the combination of these variables explained
644 most variance, while no single predictor explained much variance on its own.

4 Discussion

4.1 Stabilization of soil organic matter by burial

While our study characterizes decomposition dynamics using respirometry and radiocarbon-based modeling, we did not directly measure the molecular mechanisms responsible for differential SOC stability. Specifically, we did not quantify the chemical composition of mineral-associated organic matter, the nature of organo–mineral bonds, or microbial community composition. As a result, the mechanistic interpretations presented here are necessarily indirect.

The interpretations proposed in this study build on prior molecular and fractionation-based analyses conducted on the same soils and geomorphic transects (Marin-Spiotta et al. 2014; Dolui et al. 2026). That work documented enhanced mineral association and cation-mediated stabilization in buried profiles. Rather than repeating those analyses, the present study extends this framework by quantifying how these stabilization contexts translate into differences in SOC turnover rates and pool structure under contrasting moisture regimes.

Brady Soil subjected to continuous wetting exhibited lower cumulative CO₂ evolution than modern soils across both erosional and depositional transects (Fig. 2; Appendix Fig. 3). This pattern indicates reduced microbial mineralization in buried paleosols relative to surface soils under sustained moisture availability.

Multiple mechanisms may contribute to this enhanced stability, although their relative importance cannot be resolved with the current dataset. Previous work at this site reported elevated exchangeable Ca²⁺ concentrations in Brady soils and linked Ca-mediated flocculation to increased aggregate stability (Dolui et al., in press). Our results are consistent with this interpretation. Specifically, Ca concentration was a positive predictor of slow-pool size in the MLR model. However, we did not directly measure aggregation or flocculation processes. As a result, the pathway linking Ca availability to reduced decomposition remains uncertain. Reduced decay could arise from physical protection within aggregates, reduced microbial access to substrates, or changes in solute diffusivity. Within the scope of this study, Ca²⁺ therefore emerges as a statistically robust predictor of SOC pool structure, rather than a resolved mechanistic driver.

The potential contribution of pyrogenic carbon to SOC persistence in Brady soils is supported by prior molecular analyses from this site. These analyses identified condensed aromatic compounds consistent with fire-derived inputs (Marin-Spiotta et al. 2014). Pyrogenic carbon was not quantified in the present study, and its direct influence on decomposition rates cannot be evaluated here. Nonetheless, its documented persistence provides important context for interpreting the slow-cycling SOC pool observed in Brady soils. Our results are therefore compatible with a stabilization legacy established during Brady Soil formation, rather than evidence of an active pyrogenic control on contemporary decomposition.

Modern soils exhibited a slightly larger relative size of the fast-cycling SOM pool than Brady soils, which likely reflects recent organic inputs. Direct comparison of decay parameters further highlights differences in SOM persistence between soil types (Fig. 4). The slow-cycling pool in Brady soils from the most erosional transect had

691 significantly lower decay rates under continuous wetting than the corresponding pool in
692 modern soils. This provides clear evidence of greater SOM persistence in the paleosol.

693 Across all treatments, the slow pool decayed approximately 2000 times more slowly
694 than the fast pool. Brady soils exhibited turnover times (TOTs) of approximately 399
695 years, whereas modern soils exhibited TOTs of approximately 178 years. The MLR
696 models further showed a negative relationship between slow-pool decay rate (k_{slow}) and
697 both total inorganic carbon (TIC) and exchangeable Mg^{2+} (Equation A1). Although
698 CaCO_3 cementation was not pronounced, positive correlations between TOC, TIC,
699 and Ca in Brady soils from erosional transects (Dolui et al., in press) suggest that
700 carbonate-associated phases may contribute to stabilization where shallow wetting
701 promotes near-surface carbonate precipitation.

702 The association between finer texture and reduced SOC decomposition observed in
703 this study aligns with established theory linking clay-rich soils to enhanced mineral-
704 associated organic matter formation (Six et al. 2002). Clay content was a significant
705 predictor of slow-pool dynamics in the MLR models, indicating that particle size exerts
706 a first-order control on SOC accessibility. However, because mineral surface chemistry
707 and sorption energetics were not resolved, texture is interpreted here as a proxy for
708 stabilization potential rather than as a direct mechanistic control.

709 The slow-cycling SOM pool dominated total soil C, accounting for more than
710 96% of SOC (Fig. 4b). The size of this pool increased with increasing TOC and TIC
711 (Equation A1) and was accompanied by a corresponding decline in the fast-cycling
712 pool. The fast pool consistently represented a larger fraction of total SOC in modern
713 soils than in Brady soils, and in erosional transects than in burial transects. Together,
714 these patterns indicate stronger stabilization in buried paleosols and underscore the
715 central role of the slow-cycling pool in long-term SOC persistence.

716 Apparent relationships between sodium adsorption ratio (SAR), exchangeable
717 cations, decay rates, and pool sizes in the MLR models (Equations A1, A2, A3, and
718 A4; Fig. 6) should be interpreted cautiously. These correlations may reflect shared
719 depth-dependent trends rather than direct mechanistic links. Both TOC and micro-
720 bial biomass typically decline with depth (Fierer and Schimel 2003; Xiang et al.
721 2008), which contributes to reduced respiration in deeper Brady soils. At the same
722 time, Na tends to accumulate at depth through leaching (Dolui et al., in press), with
723 accumulation depth depending on precipitation and soil water status.

724

725 4.2 Destabilization of SOM by erosion and moisture variability

726

727 Greater cumulative C loss from shallower soils, combined with a smaller fast-cycling
728 SOM pool and a significantly higher slow-pool decay rate in erosional Brady soils com-
729 pared to depositional Brady soils (Fig. 4a), highlights the destabilizing effect of surface
730 exposure. This pattern indicates that erosion weakens burial-associated protection.

731 CO_2 pulses during the initial phase of wet-dry cycling were larger in erosional tran-
732 sects than in burial transects (Fig. A1). These pulses suggest enhanced mineralization
733 in Brady soils closer to the surface. One likely explanation is priming by root exudates
734 and fresh organic inputs, amplified by bioturbation (Pausch et al. 2016; McMurtry

735

736

et al. 2024). This interpretation is supported by higher fraction modern values in erosional Brady soils relative to depositional Brady soils, as well as by convergence of slow-pool fraction modern values toward those of modern soils (Dolui et al. 2026).

Enhanced decomposition in shallower Brady soils likely reflects both legacy exposure and contemporary environmental conditions. Shallower positions experience more frequent wetting and greater oxygen diffusion, which can prime microbial communities for rapid response following rewetting. Separating long-term exposure effects from ongoing environmental controls would require experimental manipulation of burial depth, which represents an important avenue for future research.

In the wet–dry experiment, erosion effects were further expressed in decay dynamics. Slow pools decayed faster in erosional transects than in burial transects, whereas fast pools decomposed more rapidly in modern soils than in Brady soils. The fraction of the slow pool was smaller under continuous wetting than under wet–dry cycling, indicating greater allocation to slow-cycling carbon under variable moisture. However, the slow pool also exhibited substantially higher decay rates under wet–dry cycling. This suggests that apparent stabilization under variable moisture is transient and may be offset over time by depletion of passive C reserves.

The accelerated decay of slow-pool SOC under drying–rewetting cycles demonstrates that burial-associated protection is not absolute, even for millennially persistent carbon. Potential mechanisms include aggregate disruption, increased solute transport, or shifts in microbial accessibility. While the present study cannot resolve these pathways, it clearly documents the outcome: enhanced decomposition of previously slow-cycling SOC under moisture variability.

Moisture variability also amplified turnover of the fast-cycling pool. Decay rates of the fast pool increased under wet–dry cycling, particularly in modern soils and erosional transects. This pattern supports the interpretation that fresh organic inputs and surface exposure accelerate labile C turnover under fluctuating moisture. The MLR model reinforces this interpretation by revealing a positive relationship between TOC and fast-pool decay rate (Equation A4). In contrast, the absence of strong moisture effects in Brady soils suggests that burial dampens moisture-driven variability in labile C decomposition.

McDowell et al. (2022) simulated soil hydrology for the Brady Soil, overlying loess, and modern soil using measured hydraulic properties and regional weather data from 2009–2019. Modeled water contents remained low and relatively constant below 1.5 m depth, including within the Brady Soil, with only rare deep wetting events following major rainfall. Paleoclimate reconstructions indicate that the depth of frequent wetting would have been even shallower during drier-than-modern periods over the past 10,000 years (Miao et al. 2007). Together, these results suggest that prolonged dry conditions played a major role in OC sequestration where the Brady Soil is deeply buried, whereas erosion and increased moisture variability could promote renewed C loss.

Geomorphic position integrates multiple stabilization controls by regulating burial depth, moisture exposure, oxygen availability, and the persistence of mineral–organic associations. In this sense, geomorphic context operates as a higher-order constraint that modulates how chemical and physical stabilization mechanisms are expressed

783 through time. These findings challenge the common assumption in soil carbon mod-
784 eling that geomorphic context can be neglected when parameterizing decomposition
785 rates. Our results show that erosional exposure accelerates slow-pool decay under con-
786 tinuous wetting, and that wet-dry cycling destabilizes slow-pool carbon regardless of
787 landscape position. For Earth system models that treat subsoil carbon as a passive
788 reservoir, these dynamics represent a substantial and underappreciated vulnerabil-
789 ity. Accurately predicting SOC responses to changing precipitation regimes therefore
790 requires explicit consideration of burial depth and exposure history, which are rarely
791 incorporated into current modeling frameworks.

792

793 **5 Conclusion**

794

795 When interpreted alongside prior molecular- and fractionation-based analyses from
796 this site (Marin-Spiotta et al., 2014; Dolui et al., in press), our results suggest that long-
797 term SOC persistence in Brady soils arises from the convergence of mineral association,
798 cation-mediated stabilization, and limited environmental exposure. The present study
799 extends this framework by demonstrating that these stabilization contexts remain vul-
800 nerable to hydrologic perturbation and geomorphic re-exposure. Although this study
801 does not resolve molecular-scale mechanisms directly, it demonstrates that decom-
802 position parameters derived from incubation and radiocarbon modeling are highly
803 sensitive to landscape history – an effect that must be accounted for even when detailed
804 chemical data are unavailable.

805 Our study demonstrates that SOC decomposition is strongly shaped by soil
806 moisture regime and landscape position, with modern and Brady soils responding
807 differently to continuous wetting versus drying–rewetting cycles. Water availability
808 exerted disproportionate effects in erosional settings where surface exposure enhanced
809 substrate diffusivity, highlighting the destabilizing role of re-exposure and moisture
810 fluctuations.

811 Drying–rewetting cycles led to greater C losses from Brady soils than continuous
812 wetting, despite modeling results indicating dominance of the slow pool and deple-
813 tion of labile C. This treatment also accelerated fast pool decay in modern soils and
814 erosional transects, while burial dampened such variability in Brady soils. Fraction
815 sizes shifted accordingly: wet–dry cycles increased the proportion of the slow pool and
816 reduced the fast pool fraction, suggesting redistribution of SOC toward more stabilized
817 forms under fluctuating moisture.

818 Depth further constrained SOM persistence, with slow pool decay constants declin-
819 ing in deeper Brady soils from depositional transects. Faster turnover in shallow layers
820 reflects greater microbial accessibility and responsiveness to surface-driven processes,
821 underscoring the importance of geomorphic context - transect type, burial depth, and
822 soil structure - over intrinsic SOM chemistry.

823 Comparisons across models showed that continuous wetting accelerated overall
824 decay, particularly in burial transects, while wet–dry cycles disproportionately destabi-
825 lized the slow pool that dominates SOC. This dual effect - rapid labile C turnover under
826 moisture fluctuations coupled with erosion of long-lived SOM persistence - points to
827 heightened vulnerability of buried carbon under future precipitation variability.

828

Overall, the fate of ancient soil carbon under climate change will depend on both its burial history and prevailing moisture regime. Integrating these contrasting controls on fast- and slow-cycling pools into Earth system models is essential for improving predictions of soil carbon vulnerability and climate feedback.

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Competing interests. The authors have no relevant financial or non-financial interests to disclose.

Data availability. Full dataset for soil incubation and statistical analyses are available at DOI: doi: [10.17632/fjw646gpyf.1](https://doi.org/10.17632/fjw646gpyf.1).

Author contributions. All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Manisha Dolui, Teneille Nel, Abbygail R. McMurtry, Stephanie Chacon, Laura M. Phillips, Teamrat Ghezzehei, Joseph A. Mason, Erika Marin-Spiotta, Marie-Anne de Graaff and Asmeret Asefaw Berhe. The first draft of the manuscript was written by Manisha Dolui and Teneille Nel. All authors commented on previous versions of the manuscript and approved the final manuscript.

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875 **Tables and figures.**

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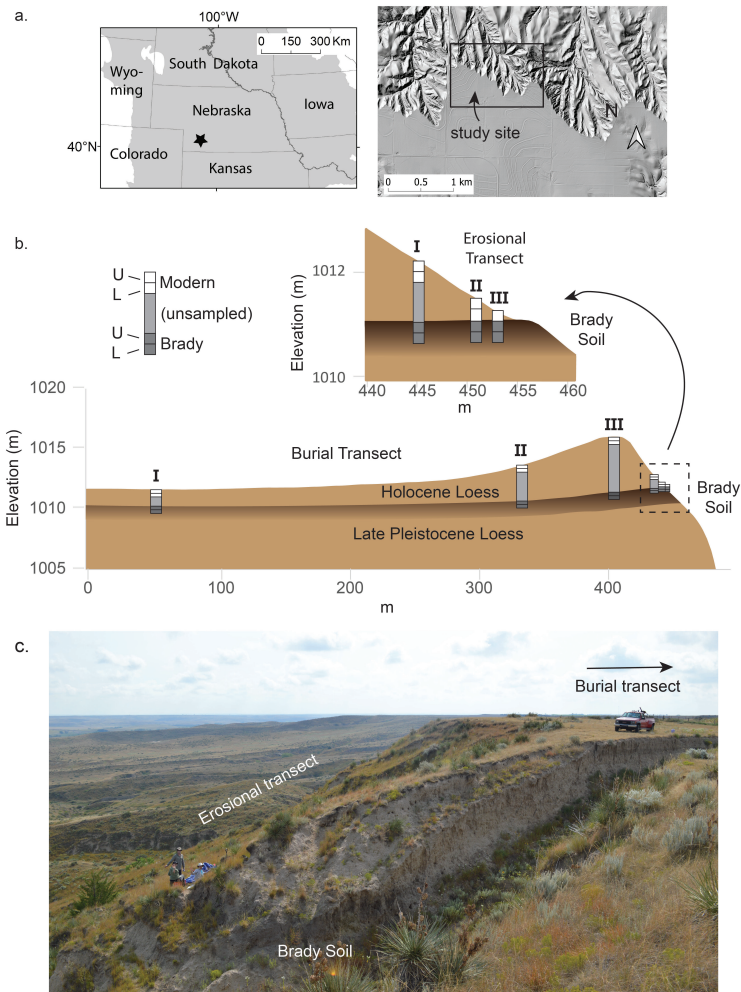


Fig. 1 Study site location, stratigraphy, and sampling scheme. a. Location of the study site within the Great Plains ecoregion (grayshade) and shaded relief map illustrating topographic setting of study site. b. Vertical cross-sections illustrating the sampling scheme relative to modern land surface topography, the Brady Soil, and loess above and below it at study site. Weakly developed horizons of modern soil and paleosols within the Holocene loess (Miao et al., 2007) not shown. Details based on one pair of burial and erosional transects but representative of all three. c. Photo illustrating topography and native grassland vegetation at one pair of transects. Truck is near deepest profile (III) on burial transect; other burial transect points are not visible. Brady Soil is exposed in an old roadcut in foreground; erosional transect is located on intact slope beyond roadcut. Figure adapted from [Dolui et al. \(2026\)](#)

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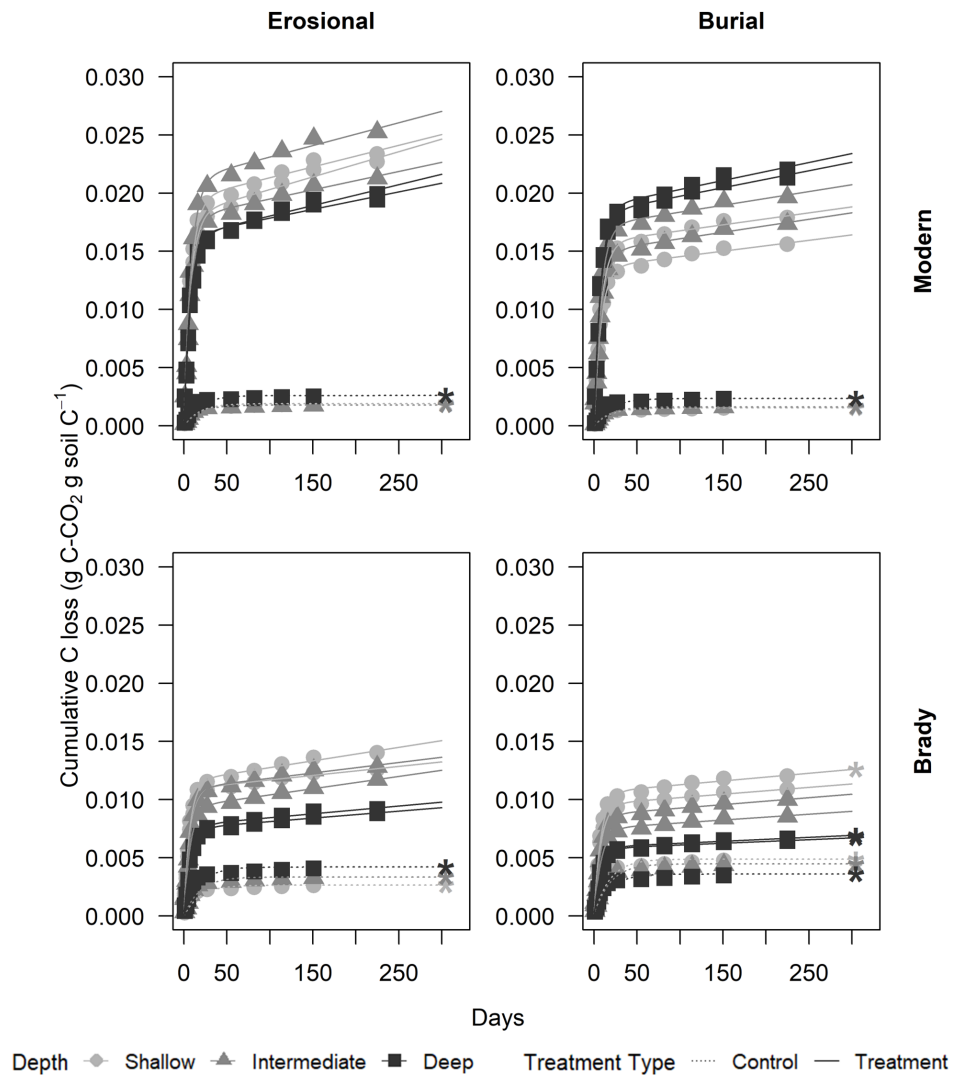


Fig. 2 Cumulative respiratory CO₂ from modern and Brady Soil sampled from three depths representing varying degrees of burial/erosional exposure (two technical replicates each). Soils were maintained at a constant moisture content of 60% water holding capacity (WHC). Lines depict a two-pool first-order decay model, fitted using a non-linear least-squares function. The dotted line represents the control (5% WHC).

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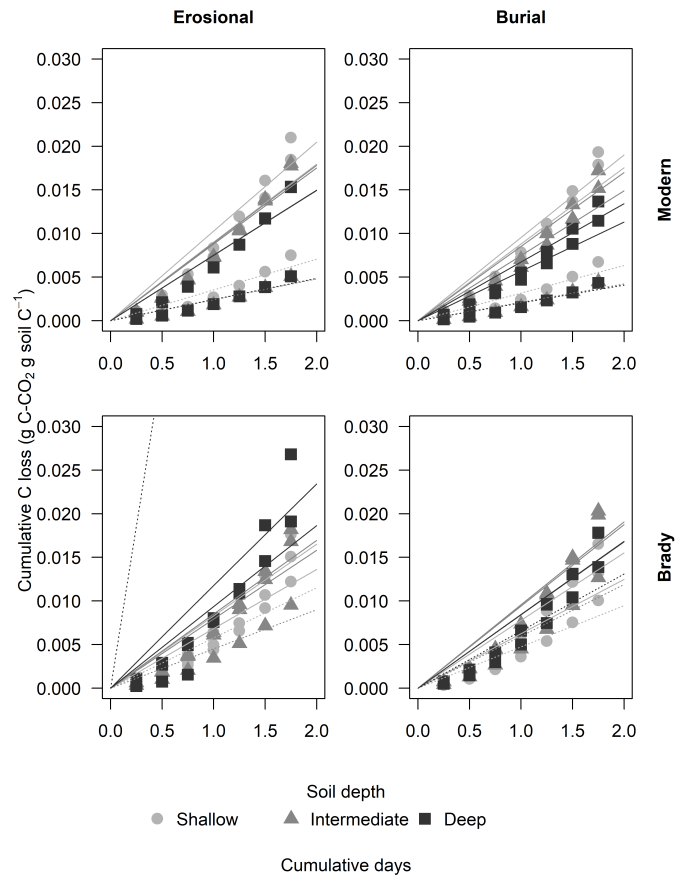


Fig. 3 Cumulative respiratory CO₂ flux from modern and Brady Soil sampled from three depths representing varying degrees of burial/erosional exposure (two technical replicates each). Soils were wetted to a moisture content of 60% water holding capacity (WHC) and allowed to dry to 5% WHC. Lines depict a one-pool first-order decay model, fitted using a non-linear least-squares function. The dotted line represents the control (constant 5% WHC); one control Brady soil sample of the erosional transect had an outlier point not shown due to scale. Asterisks (*) indicate control samples maintained at 5% WHC

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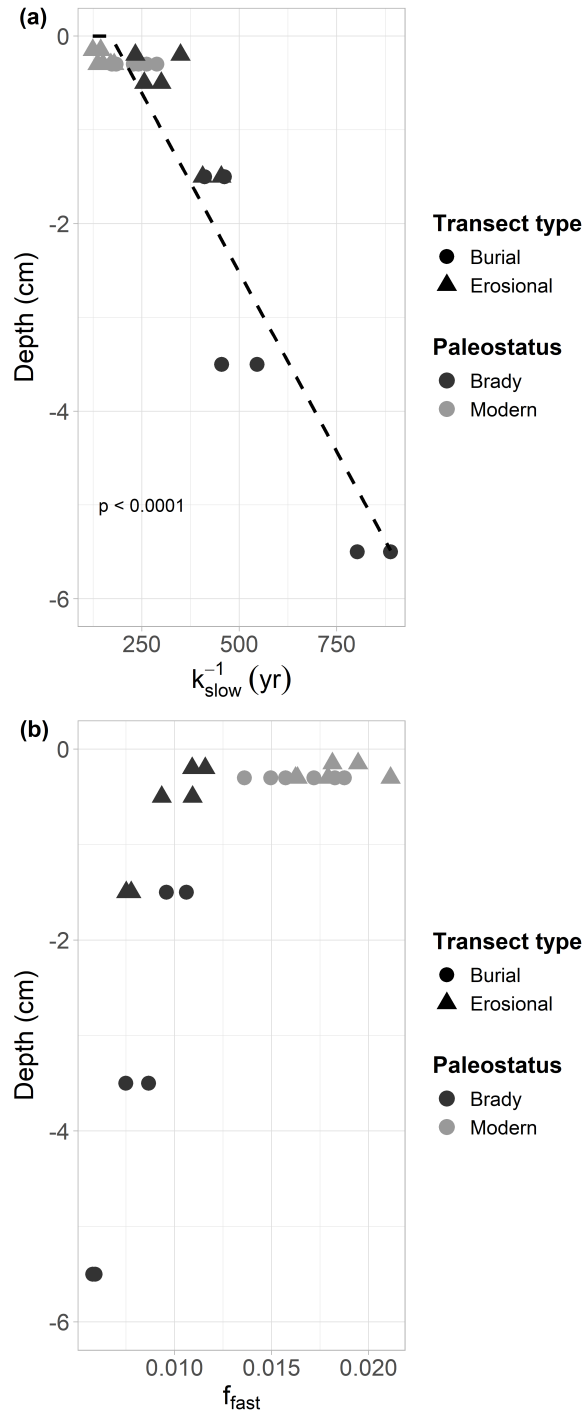


Fig. 4 Decay constant of the slow cycling pool of modern and Brady soils incubated under continuously wet conditions. Samples collected from depositional and erosional transect types. Dotted line indicates exponential decay. Figure b indicates the size of the fast-cycling pool at different depths of the modern and Brady soils in depositional and erosional transect types.

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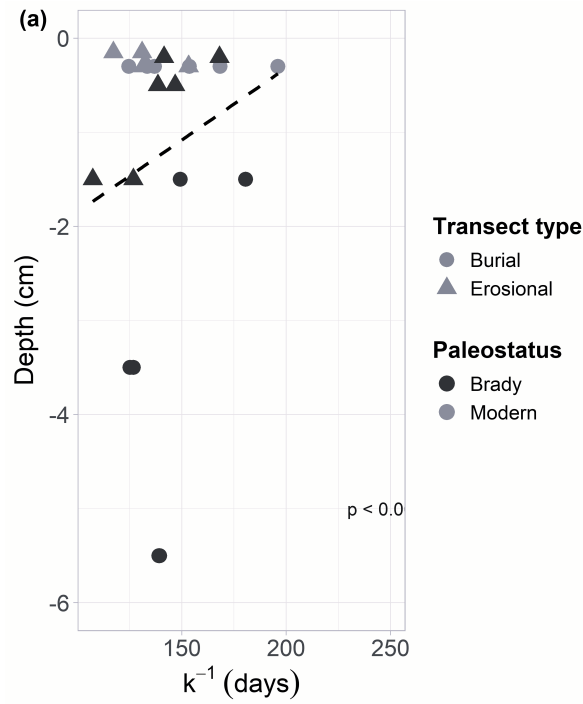


Fig. 5 Decay constant of modern and Brady soil organic matter incubated under wet-dry cycling conditions. Samples collected from depositional and erosional transect types. Dotted line indicates exponential decay.

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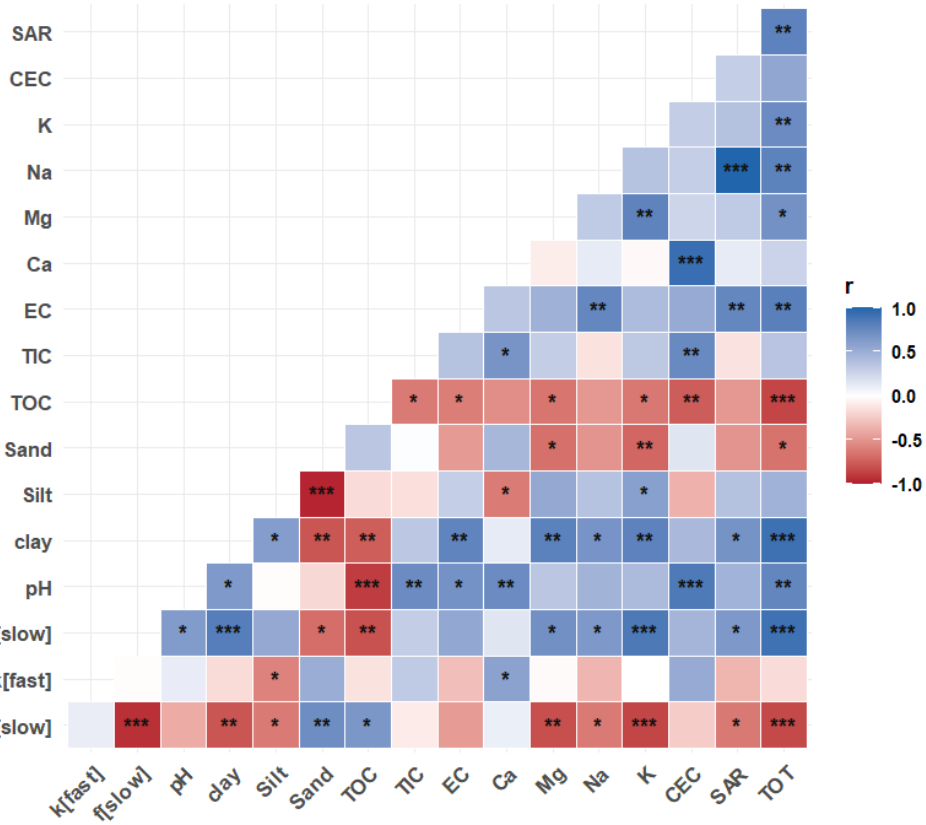


Fig. 6 Correlation between soil physicochemical properties and modeled soil organic matter decomposition parameters (decay constant, k, and fraction of C in slow pool, f) for fast- and slow-cycling pools in modern and Brady soils incubated under continuously wet conditions. Decomposition parameters were obtained by multiple linear regression across burial and erosional transects. The statistical significance of each correlation is denoted by asterisks (*** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$).

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Table 1 General physicochemical (mean \pm standard deviation) of soils used in the incubation experiment, grouped by transect type and paleostatus

Transect type Paleostatus	Burial		Erosional	
	Brady	Modern	Brady	Modern
pH	7.77 \pm 0.04	6.89 \pm 0.32	7.68 \pm 0.12	7.41 \pm 0.10
Clay (%)	9.48 \pm 0.75	6.13 \pm 0.64	7.62 \pm 0.44	6.29 \pm 0.48
Silt (%)	59.31 \pm 0.44	56.24 \pm 5.76	55.73 \pm 4.88	53.67 \pm 1.51
Sand (%)	31.24 \pm 0.94	37.62 \pm 6.39	36.64 \pm 5.22	40.08 \pm 1.92
TOC content	0.45 \pm 0.06	1.03 \pm 0.20	0.56 \pm 0.10	0.93 \pm 0.17
TIC content	0.24 \pm 0.11	0.09 \pm 0.03	0.28 \pm 0.04	0.25 \pm 0.03
EC (dS m ⁻¹)	5.41 \pm 0.32	2.26 \pm 0.43	3.05 \pm 0.47	3.57 \pm 0.35
CEC (cmolc kg ⁻¹)	22.86 \pm 0.54	15.17 \pm 3.54	23.31 \pm 3.52	20.59 \pm 1.83
Turnover time (y)	15654 \pm 2440	576.4 \pm 126	8327 \pm 2732	1451 \pm 673
CN ratio	10.00 \pm 0.45	9.24 \pm 0.21	10.96 \pm 0.20	9.76 \pm 0.25

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1260 **Table 2** Soil organic matter (SOM) decomposition parameters (decay rate k of one-pool model, decay
1261 rates of fast- (k_{fast}) and slow-cycling (k_{slow}) SOM pools, and fraction sizes of fast (f_{fast}) and slow
1262 (f_{slow}) pools, grouped by experiment, transect type, and paleostatus.

1263	1264	CW				WD			
1265	1265	Burial		Erosional		Burial		Erosional	
1266	1266	Brady	Modern	Brady	Modern	Brady	Modern	Brady	Modern
1267	k (one pool)	0.0107	0.0099	0.0069	0.0072	0.0071	0.0067	0.0074	0.0074
1268	k_{slow}	<0.0001	<0.0001	<0.0001	<0.0001	0.0080	0.0074	0.0086	0.0084
1269	k_{fast}	0.1250	0.1264	0.1333	0.1270	0.6931	6.3419	0.4222	9.8728
1270	f_{slow}	0.9920	0.9836	0.9903	0.9818	0.9990	0.9990	0.9990	0.9990
1271	f_{fast}	0.0080	0.0164	0.0097	0.0182	0.0010	0.0010	0.0010	0.0010

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Table 3 Summary of multiple linear regression models showing key positive (+) and negative (-) predictors of model parameters (proportions of fast and slow cycling fractions of TOC, i.e., f_f and f_s and the corresponding decay rate constants k_f and k_s), model performance (R^2), and root mean square error (RMSE).

Model parameter	Key (+) predictors	Key (-) predictors	R^2	RMSE
k_{slow}	clay, Ca, EC	SAR, Mg, K, pH, TOC, TIC	0.98	7.01×10^{-7}
k_{fast}	SAR, clay, Ca, Mg, TIC	K, EC, pH	0.95	1.41×10^{-3}
f_{slow}	SAR, Mg, K, pH, TIC	clay, Ca, EC, TOC	0.97	8.38×10^{-4}
f_{fast}	clay, Ca, EC, TOC	SAR, Mg, K, pH, TIC	0.97	8.38×10^{-4}
$k_{one-pool}$	clay, K, pH, TOC	SAR, Ca, Mg	0.94	2.15×10^{-4}

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1335 **Appendix A**

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Table A1 Sampling depth intervals (standard deviation in brackets for $n = 3$) of modern (surface) and Brady (burial) soils along two types of transects, where degrees of burial or exposure increase from degrees I to III. Table adapted from Dolui et al., in press.

Depth (cm)	Transect Type					
	Erosional		Degree		Burial	
	III	II	I	I	II	III
Modern	0-19 (2.5)	0-19 (3.2)	0-18 (3.5)	0-30 (0.6)	0-29 (0.6)	0-29 (0.0)
Paleostatus	NA	19-38 (3.6)	18-36 (3.5)	30-60 (0.6)	29-59 (2.3)	29-59 (2.3)
	20-50 (1.2)	50-80 (0.0)	150-180 (0.0)	100-130 (4.0)	300-330 (9.3)	550-580 (6.7)
	50-80 (0.4)	80-120 (0.0)	180-210 (2.9)	130-160 (4.5)	330-360 (2.6)	580-610 (5.6)

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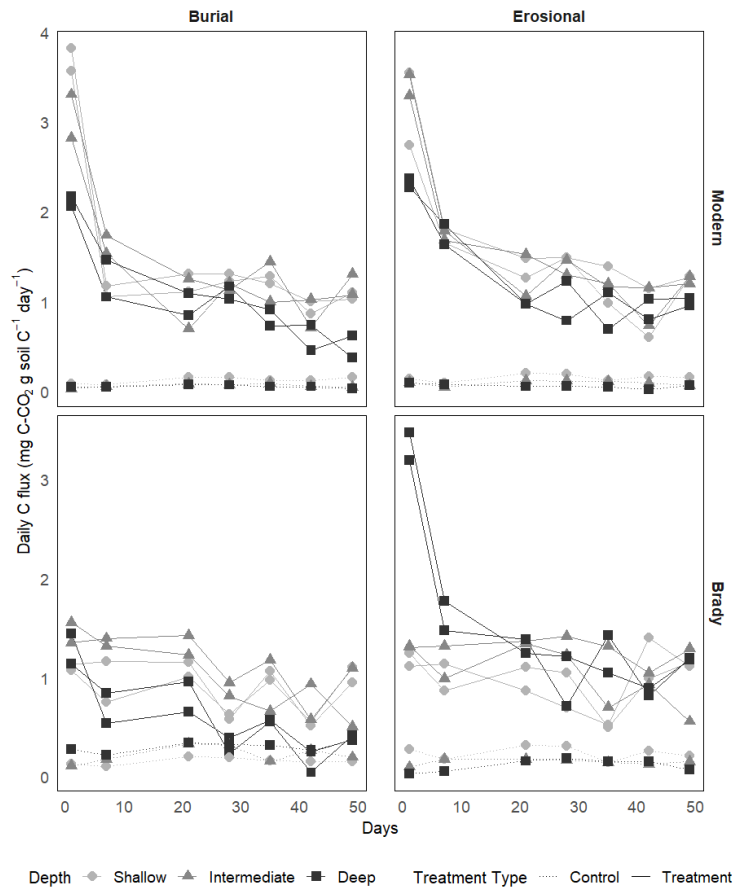


Fig. A1 Daily respiratory CO₂ flux from modern and Brady Soil sampled from three depths representing varying degrees of burial/erosional exposure (two technical replicates each). Soils were wetted to a moisture content of 60% water holding capacity (WHC) and allowed to dry to 5% WHC. The dotted line represents the control (constant 5% WHC).

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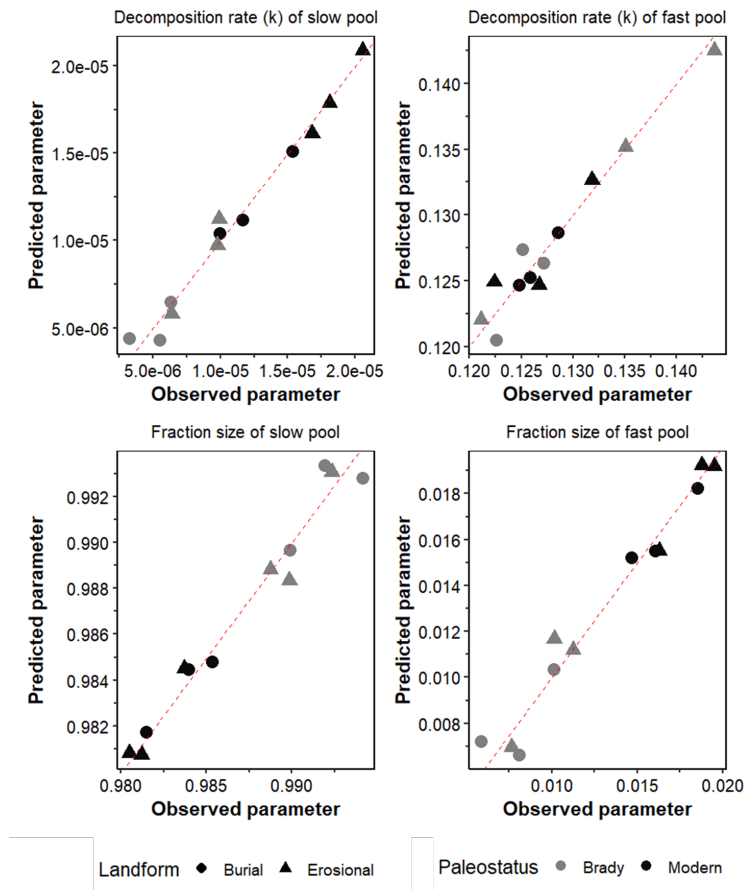


Fig. A2 True versus predicted soil organic matter decomposition parameters (decay constant, k , and fraction of C in slow pool, f) for fast- and slow-cycling pools in modern and Brady soils incubated under continuously wet conditions. These parameters were obtained by multiple linear regression across burial and erosional transects. Red dotted line represents $y = x$.

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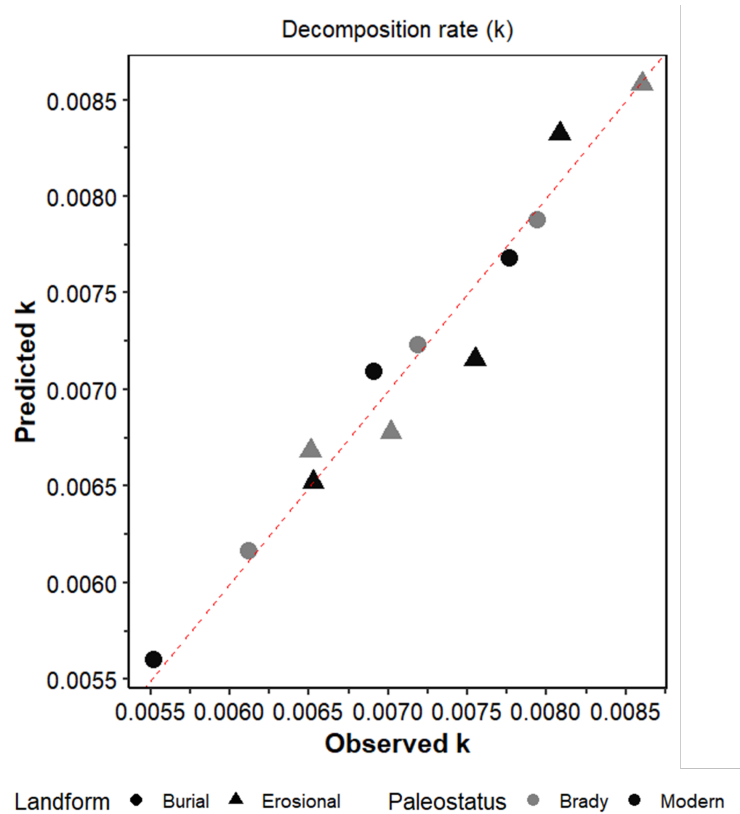


Fig. A3 True versus predicted soil organic matter decay constant, k) in modern and Brady soils incubated under wet-dry cycling conditions. These parameters were obtained by multiple linear regression across burial and erosional transects. Red dotted line represents $y = x$.

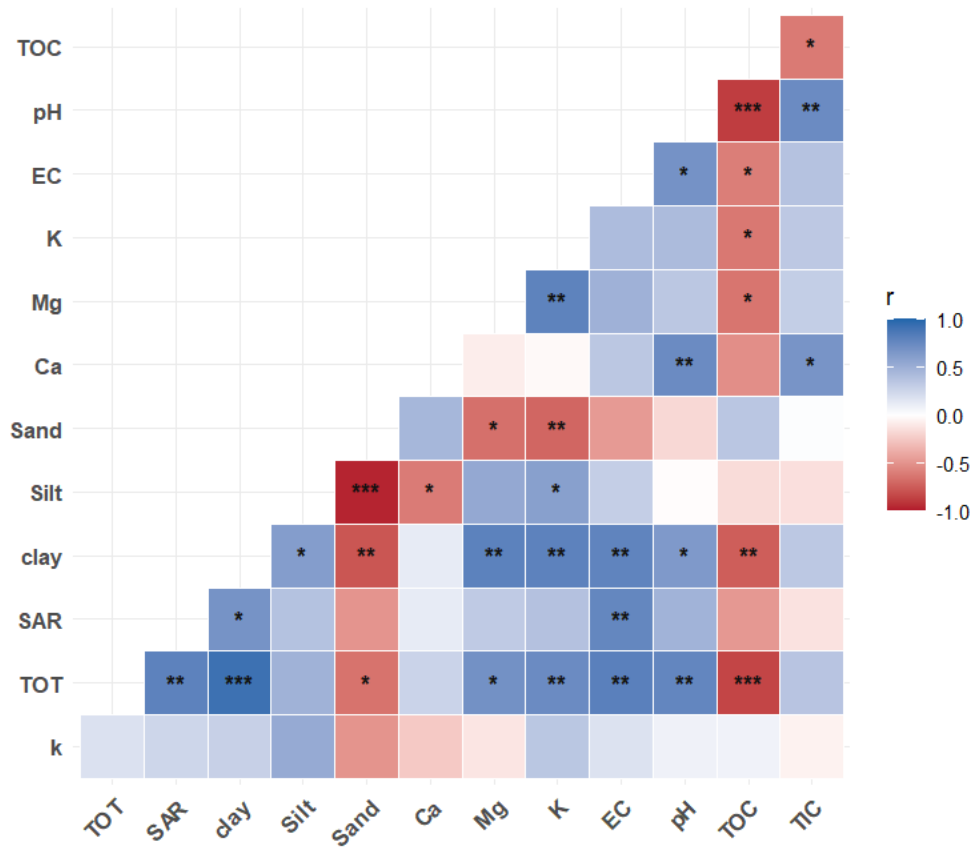


Fig. A4 Correlation between soil physicochemical properties and modeled soil organic matter decomposition parameters (decay constant, k , and fraction of C in slow pool, f) for fast- and slow-cycling pools in modern and Brady soils incubated under wet-dry cycles. Decomposition parameters were obtained by multiple linear regression across burial and erosional transects. The statistical significance of each correlation is denoted by asterisks (*** for $p < 0.001$, ** for $p < 0.01$, * for $p < 0.05$).

1611 **A.1 Multiple linear regression equations**

1612 $k_{slow} = 0.0000532 - 0.0000979 \cdot SAR + 0.00000225 \cdot clay + 0.000000395 \cdot Ca$
1613 $- 0.00000622 \cdot Mg - 0.00000271 \cdot K + 0.00000347 \cdot EC - 0.00000618 \cdot pH$ (A1)
1614 $- 0.00000687 \cdot TOC - 0.0000275 \cdot TIC$

1616 $RMSE : 0.000000701, R^2 : 0.98$

1618 $k_{fast} = 0.215 + 0.0189 \cdot SAR + 0.00344 \cdot clay + 0.0026 \cdot Ca + 0.00204 \cdot Mg$
1619 $- 0.000302 \cdot K - 0.00513 \cdot EC - 0.0196 \cdot pH + 0.0146 \cdot TIC$ (A2)

1621 $RMSE : 0.00141, R^2 : 0.95$

1623 $f_{slow} = 0.976 + 0.0732 \cdot SAR - 0.000362 \cdot clay - 0.000241 \cdot Ca + 0.00138 \cdot Mg$
1624 $+ 0.00283 \cdot K - 0.00288 \cdot EC + 0.00174 \cdot pH - 0.000707 \cdot TOC$ (A3)
1625 $+ 0.0298 \cdot TIC$

1628 $RMSE : 0.000838, R^2 : 0.97$

1630 $f_{fast} = 0.0243 - 0.0732 \cdot SAR + 0.000362 \cdot clay + 0.000241 \cdot Ca - 0.00138 \cdot Mg$
1631 $- 0.00283 \cdot K + 0.00288 \cdot EC - 0.00174 \cdot pH + 0.000707 \cdot TOC$
1632 $- 0.0298 \cdot TIC$
1633 $RMSE : 0.000838, R^2 : 0.97$ (A4)

1638 $k(one - pool) = -0.00641 - 0.00308 \cdot SAR + 0.000599 \cdot clay - 0.000141 \cdot Ca$
1639 $- 0.0015 \cdot Mg + 0.00118 \cdot K + 0.00159 \cdot pH$ (A5)
1640 $+ 0.002 \cdot TOC$

1643 $RMSE : 0.000215, R^2 : 0.94$

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