The Role of Nutrient and Energy Limitation on Microbial Decomposition of Deep Podzolized Carbon: A Priming Experiment

Ryan E. Champiny[1] , **Kanika S. Inglett[1]**, and **Yang Lin[1]**

[1]Department of Soil, Water, and Ecosystem Sciences, University of Florida, Gainesville, FL, USA

Abstract Soil carbon decomposition is primarily driven by microbial activities and is regulated by factors which stimulate or impede microbial functions. Deep podzolized carbon (DPC), found in the United States Southeastern Coastal Plain, is situated well below the soil surface in horizons isolated from active plant input. This carbon is characterized by high C:N ratios (>30) which could reflect nutrient limitation of microbial decomposition. To uncover the energy or nutrient limitation on DPC degradation, a 90-day priming experiment was performed with soils from the surface horizon and DPC horizons (i.e., Bh1 and Bh2) received the additions of ^{13}C-labeled alanine and glucose. This resulted in prominent priming effects: addition of alanine increased basal decomposition of soil organic carbon by 918 ± 51% and 737 ± 7% in Bh2 and Bh1, respectively. Glucose relative priming was 505 ± 28% in Bh1 and 606 ± 77% of basal respiration in Bh2. These strong responses to substrate input highlight the susceptibility of DPC to loss when microbial carbon and nutrient constraints are alleviated. After 90 days, glucose addition increased the microbial biomass in DPC horizons relative to alanine addition, with the latter showing no difference from ultrapure-water control. The response of the microbial biomass indicates constraint by a lack of energy sources both by the paucity of labile substrates and reduced availability of organic matter as a result of podzolization. Our study has important implications for predicting the response of DPC in Coastal Plain soils in the context of land management and global change.

Plain Language Summary In the United States Southeastern Coastal Plain deep podzolized carbon (DPC) is found well below the soil surface, this organic matter has a high ratio of carbon to nitrogen (C:N) and is highly associated with aluminum. This suggests that microbes struggle to decompose it due to a shortage of compounds which provide nutrients such as nitrogen to create enzymes, and energy-providing compounds such as glucose. To understand how DPC breakdown can be affected by energy or nutrient availability, we conducted a 90-day incubation experiment where we added specially labeled compounds (alanine and glucose) to soils taken from the surface (A) and DPC layers (Bh1 and Bh2). We found that adding these compounds caused a significant increase in microbial activity, especially in the DPC layers. We also observed a greater increase in microbial growth when glucose was added compared to alanine, which did not show much difference from the water-addition control. This suggests that the lack of easily digestible nutrients and the protection from metal association restricts microbial growth and activity in these DPC layers. Understanding deep carbon response to changes in elemental flows is crucial for managing land and anticipating changes in response to global environmental shifts.

1. Introduction

In the Southeastern Coastal Plain of the United States (hereafter referred to as the coastal plain), there is a regionally extensive and morphologically distinct accumulation of podzolized carbon (C) that has been called deep podzolized carbon (DPC). The DPC identified in the coastal plain exhibits morphological and chemical features that distinguish it from the vadose zone podzolized C found in the region, as well as globally (Bacon et al., 2020; Champiny, Bacon et al., 2024). This C pool co-occurs with phreatic zone hydrology a meter or more below the soil surface (Bacon et al., 2020) and is estimated to contain over a billion tons (1.1×10^9 t) of C, a quantity roughly equivalent to the forest biomass covering the Carolinas (Gonzalez et al., 2018). In contrast to occurrences of DPC, podzolized C in the vadose zone in the region are shown to decrease in C content with increasing depth, and are transient across a landscape, whereas DPC is more uniform in occurrence (Banik et al., 2014; Gonzalez et al., 2018). In the coastal plain, DPC horizons are often located beneath spodic horizons in

the vadose zone (Bacon et al., 2020). The DPC is also associated with high aluminum (Al) concentrations and a nearly complete depletion of reactive iron (Fe) species (Bacon et al., 2020; Champiny, Bacon et al., 2024).

Although DPC as it is described has only been documented in the coastal plain, it may occur globally in sandy coastal plains with similar combinations of conditions, such as high Al content and phreatic conditions (Gonzalez et al., 2018). Indeed, hydromorphic, equatorial and Amazonian podzols with high resemblance to the DPC have been reported, however their morphology distinguishes them from the DPC described in the coastal plain (Farmer et al., 1983; Schwartz, 1988; Silva et al., 2012; Ishida et al., 2014; Ferro-Vázquez et al., 2020; in Montes et al., 2023). These morphological and chemical differences highlight the uniqueness of DPC compared to the podzolized C found in other parts of the world. They also reflect differences in the formation mechanisms, as the phreatic zone, where DPC is formed, experiences more persistent water saturation and anaerobic conditions than the vadose zone. These conditions, in turn, favor the accumulation of organic matter and depletion of Fe species in the DPC horizons. In fact, the low C concentration in the upper section of DPC has been linked to frequent fluctuations of water table and oxic conditions and microbial decomposition (Bacon et al., 2020; Bolivar, 2000). Despite recent progress its understanding its hydrological and chemical controls, the microbial factors influencing DPC persistence remain less well understood. Because DPC contains such a large quantity of C, understanding the factors that influence its persistence, such as those limiting microbial activity, is important for predicting the fate of this C pool under land use and climate change.

Microbes drive the belowground carbon (C) cycling in terrestrial ecosystems, therefore factors which constrain or stimulate microbial degradation are key regulators of soil C (Fontaine & Barot, 2005; Kuzyakov et al., 2000; Langley et al., 2009; Schmidt et al., 2011). Microbial energy and nutrient limitations are two such mechanisms responsible for the long-term persistence of soil C. Their importance stems from the control on the growth of microbial biomass, production of enzymes used to break down complex compounds, as well as energy to initiate chemical reactions (Fontaine et al., 2004, 2007; Schimel & Weintraub, 2003). Soil microbes rely on energy dense compounds, in particular those compounds that have a high return on invested resources, to provide energy and enable their cell metabolism (Fontaine et al., 2007; Henneron et al., 2022; Manzoni et al., 2012; Soong et al., 2020). Without access to such compounds, microbes are considered energy limited, which is prevalent in subsoils (Fontaine et al., 2007; Henneron et al., 2022).

If the soil matrix does not contain enough nutrients such as nitrogen (N) to sustain microbial metabolism, the microbes are nutrient limited. In addition, a lack of N also impedes the production of microbial extracellular enzymes and subsequently limits the degradation of organic matter (Schimel & Bennett, 2004). These limitations on microbial activities are reflected in the ratio of carbon to nitrogen (C:N) in organic matter. Lower C:N ratios (higher N content) are indicative of energy limitation on microbial communities whereas higher ratios (lower N content) indicate nutrient constraint (Manzoni et al., 2012; Zechmeister-Boltenstern et al., 2015). The threshold at which the limiting factor switches between energy and nutrients varies but tends to fall between C:N mass ratios of 23–47 (Moore et al., 2006; Parton et al., 2007). Horizons containing DPC have been shown to have high C:N ratios, ranging from 29.9 in the Bh1 to 36.3 and 36.6 in Bh2 and Bh3 (Table S1 in Supporting Information S1). These high C:N values suggest that the microbial decomposition of DPC could be N limited. Previous work has shown that the water extractable fraction of DPC was depleted of species with small molecular mass ($m/z < 375$), and bulk DPC was characterized by low abundance of the labile aliphatic functional groups (Champiny, Bacon et al., 2024), suggesting energy limitation as another factor constraining microbial activity (Fontaine et al., 2004, 2007). Thus, inputs of a labile, easily degraded substrate could satisfy those energy or nutrient limitations and accelerate microbial decomposition of DPC.

Root exudates, organic material, and microbial necro-mass are potential sources of fresh and labile substrate for soil microbial communities that could facilitate the degradation of deep soil C via the process known as priming (Kuzyakov et al., 2000; Rumpel & Kögel-Knabner, 2011). Priming is a short-term acceleration of organic matter decomposition after fresh substrate is added to the soil matrix. Matter that is easily degraded or rich in N can provide an energy or N source for enzyme production and cause microbial communities to expand and attack soil organic matter that were previously protected from degradation (Bernal et al., 2016; Fontaine et al., 2004, 2007). Priming effects can be variable between ecosystems due to complexity of the interactions between biotic (roots and microbes) and abiotic (metals, minerals, chemical SOM characteristics, etc.) factors (Blagodatskaya & Kuzyakov, 2008; Chen et al., 2019; Dijkstra et al., 2013; Kuzyakov, 2010). Due to its location in deep horizons, DPC is likely to be isolated from the priming effects prevalent in surface soils. However, as land management

trends toward increased soil C inputs in attempt to sequester C, fresh C could migrate to DPC horizons and prime this already-stabilized C pool. Therefore, it is critical to evaluate the magnitude and direction of priming that is possible from DPC soils and determine how susceptible the C pool is to changes in microbial respiration.

We performed an incubation experiment in which ^{13}C-labeled alanine and glucose were added to soils containing DPC to examine the limitation of substrate quality on microbial degradation of DPC. The following hypotheses were tested:

1. The addition of glucose to soil containing DPC would induce a larger priming effect than to surface soils, with the largest priming effect occurring in the deepest DPC-containing horizons due to increasing carbon content with depth. This would indicate that the presence of fresh, easily degradable C compounds is a constraint to the degradation of DPC, as is the case with energy limitation. The larger priming effect from the Bh2 horizon would indicate that the deeper, more C-rich DPC is at a higher risk to microbial degradation if labile compounds were introduced.
2. The addition of organic N would also cause a larger priming effect to DPC than to surface horizons, indicating N availability as a key constraint in the microbial turnover of deep soil C. This C pool has been shown to have a relatively high C:N ratio, which implies low N content is a limiting factor to microbial activities.

2. Materials and Methods

2.1. Soil Collection and Preparation

Seven soil cores were collected using a JMC subsoil probe (PN425, 3 cm diameter) at the Suwannee Valley Research and Extension Center in Live Oak, FL (30°18′11.99521″ N, 82°54′32.0862″ W). The soils sampled for this study have been mapped as sandy, silicious, thermic, Aeric Alaquods within the Leon fine sands series consociation, and formed primarily from marine sediments (United States Department of Agriculture (USDA), 2017). The sampled field serves as a minimally managed overflow bahiagrass (*Paspalum notatum*) grazing pasture, receiving no mineral fertilizer. Before conversion to agriculture, the predominant vegetation community ranged from sandhill longleaf pine (*Pinus palustris*) savannah to mixed flatwoods with proximity to wetlands (United States Department of Agriculture (USDA), 2017). In the field, horizons were identified and differentiated based on color and then labeled on transparent sheaths. Upon arrival at the lab, soil sheaths were opened, and the A, Bh1, and Bh2 horizons were separated, sieved to 2-mm, and homogenized into composites by horizon. Bh1 and Bh2 were both classified as DPC horizons based on characteristic decreases in Munsell value, with Bh1 sitting above B2 horizon. Soil composites were air-dried overnight to reduce water content and enable subsequent addition of substrate solution. After air-drying, the soils were re-homogenized, and 20 g sub-samples ($n = 3$ per horizon) were oven-dried at 100°C for 24 hr to determine soil water content. Prior to homogenization, the pH of the soil horizons used for the incubation were measured using an Accumet AB200 pH meter (Fisher) via a 1:1 mass ratio of soil to ultrapure water.

2.2. Incubation Design

A, Bh1, and Bh2 horizons were divided into subsets of 30 g air-dried soil and were incubated under aerobic conditions in sealed 275-mL glass mason jars for a period of 90 days at 60% water holding capacity. Moisture level was maintained throughout the experiment via monitoring jar weight and periodic addition of ultrapure water. Previous studies have suggested that the lower reaches of DPC, such as Bh2, are more frequently saturated than the upper reaches, such as Bh1, resulting in the characteristic decrease in Munsell color value and increase in total C content (Bacon et al., 2020). We selected oxic conditions for this experiment because they represent a worst-case scenario for the persistence of DPC, which is typically preserved under saturated, low-redox environments in the phreatic zone. Oxic conditions also allow for comparison of the Bh horizon under different microbial substrate limitation regimes to that of surface soils which experience infrequent saturation. Each jar received a substrate treatment (glucose solution, alanine solution, or ultra-pure water as control), with five replicates per treatment, for a total of 45 jars (45 = 3 substrates × 3 soil horizons × 5 replicates). Glucose and alanine were chosen as substrate additions because they are readily utilized by microbes and are representative of the labile compounds released as root exudates (Fischer et al., 2010; Kuzyakov et al., 2000).

A fixed input of substrate can have a large impact on the magnitude of priming if the soils receiving the inputs vary in C content (Blagodatskaya et al., 2011; Blagodatskaya & Kuzyakov, 2008). To avoid this, C input was

Table 1
Soil Characteristics and Dosage of Substrate Addition

Horizon	Depth (cm)	pH	C content (%)	Mass soil (g)	Glucose added (mg)	Alanine added (mg)	C added in glucose and alanine (mg)	N added in alanine (mg)
A	0–30	5.6	1.44	30	86.6	82.5	34.64	12.5
Bh1	127–152	5.3	0.17	30	10.2	9.7	4.08	1.5
Bh2	152–172	5.3	0.38	30	22.8	21.7	9.12	3.3

Note. Soil characteristics and horizon depths are average values. The pH of the soil horizons is reported as the mean of the seven cores used for the composite horizons.

standardized by soil C content for the A, Bh1, and Bh2 horizons to ensure that each received a similar quantity of substrate relative to the other two horizons (Table 1). To increase the C content of each soil by 8%, ^{13}C-labeled glucose and ^{13}C-labeled alanine substrate were added. DPC horizons were shown in a previous experiment (Champiny, Bacon et al., 2024) to have low to undetectable levels of N at depth (<0.01%), alanine addition rates were standardized with glucose rates to add the same amount of C to each soil. Unlabeled glucose and alanine were mixed with 99 atom% ^{13}C-labeled glucose and alanine, respectively, to achieve a final ^{13}C enrichment of 2.18 atom%. The natural ^{13}C enrichment of the soils was 1.10 atom%, allowing for respired CO_2 originating from SOM to be differentiated from that of the substrates.

2.3. Respiration and ^{13}C Measurement

Microbial respiration was measured at set intervals by analyzing headspace CO_2 concentration using a LI-COR 830 CO_2 Gas Analyzer. Time zero measurements were taken immediately after substrate solution addition. Subsequent respiration measurements occurred every day for the first week of the incubation. Measurement intervals were reduced to every other day for day 8 to day 16, then every 3 days for day 16 to day 55 and were finally reduced to weekly measurement for the remainder of the incubation period. Sampling interval time was determined based on CO_2 production rates. After gas collection, sample jars were vented under a fume hood with directed airflow for a minimum of 10 min to reset the headspace CO_2 to that of the atmosphere and maintain aerobic conditions. Sample jars were then re-sealed and stored in the dark before the next sampling.

Headspace gas samples were taken after every other round of microbial respiration to measure ^{13}C enrichment level of the respired CO_2. With a week of sample collection, ^{13}C-CO_2 of the headspace sample was determined at the University of Florida Stable Isotope Mass Spectrometry Laboratory (SIMS) using a ThermoFinnigan Isotope Ratio Mass Spectrometer (MAT DeltaPlus XL). Because ^{13}C-CO_2 was not measured in all sampling days due to budgetary reasons, we adopted a locally weighted least squares regression (LOESS) to model the temporal trends of ^{13}C-CO_2 with the 'loess()' function in R (R Core Team, 2024). A loess model was constructed for each combination of substrate and soil type. The model was then used to interpolate the missing ^{13}C-CO_2 values (Figure S1 in Supporting Information S1).

2.4. Microbial Biomass

Soil microbial biomass was determined via chloroform fumigation at the end of the incubation (Vance et al., 1987b). The soils in each incubation jar were divided into two subsets of 10-g wet soil. Water content was calculated for an additional subset of the samples to correct the soil weight for the calculation of microbial biomass. One set of subsamples (fumigated block) were fumigated for 48 hr in a vacuum desiccation chamber with chloroform while the remaining subsamples (unfumigated block) were immediately mixed with 40 mL of 0.5 M potassium sulfate (K_2SO_4) solution, shaken for 1 hr, and then filtered using Whatman #42 filter paper. After fumigation, the soils were extracted with 0.5 M K_2SO_4 solution following the same procedure as the unfumigated block. The extracted solutions were analyzed for dissolved organic C (DOC) using a Shimadzu TOC-L$_{CSN}$. The difference in the DOC concentration between the fumigated and unfumigated sample blocks was used to estimate microbial biomass with a correction ratio of 0.45 (Vance et al., 1987a). Final values were reported as microbial biomass (μg C g^{-1} soil).

2.5. Isotope Mixing

An isotopic mixing model was used to calculate the proportion of the SOM-derived CO_2 of the total CO_2 production (*A*) (Dijkstra & Cheng, 2007):

$$A = (AT_{treatment} - AT_{sub})/(AT_{control} - AT_{sub}) \qquad (1)$$

where $AT_{treatment}$ is the ^{13}C isotope composition (in atom%) of the respired CO_2 from the substrate amended soils, AT_{sub} is the atom% of labeled substrates, and $AT_{control}$ is the atom% of the CO_2 respired from the control soil. The Priming Effect (PE) was calculated as the difference in SOM-derived CO_2 between the treatment and control incubations (Kuzyakov et al., 2000) using the following formula:

$$PE = A \times (C_{treatment} - C_{control}) \qquad (2)$$

Where $C_{treatment}$ is the respired CO_2 from the substrate amended soils, and $C_{control}$ is the respired CO_2 from the control soils. The Relative Priming Effect (RPE) is the percent change in respiration relative to the control (Chen et al., 2019), reported as a percentage and calculated using the following equation:

$$RPE = \frac{PE}{C_{control}} * 100\% \qquad (3)$$

Here we distinguish between the PE and RPE to emphasize that though these terms represent the respiration response to substrate addition, there are differences in their calculation and units of measurement. The PE is calculated in mass units and indicates the difference between the CO_2 produced in the amended and control soil (Blagodatsky et al., 2010; Kuzyakov et al., 2000). The RPE shows the intensity of the priming effect by reporting the difference as a percentage of the control respiration (Chen et al., 2019; Thiessen et al., 2013).

2.6. Statistical Analysis

For each soil, daily and cumulative CO_2 production were compared across the three substrate treatments using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) tests. To account for the differences in initial soil C and substrate addition rates across the three soils, cumulative CO_2 production was normalized by initial soil C contents. Effects of substrate addition and soil types on the cumulative CO_2 production, priming effects, and microbial biomass were compared using two-way ANOVA followed by Tukey's tests. All statistical analyses were conducted in R (R Core Team, 2024).

3. Results

3.1. CO_2 Production

Alanine and glucose additions resulted in distinct temporal patterns in CO_2 production (Figure 1). In all three soils with alanine addition, CO_2 production peaked on day 3 of the experiment and gradually decreased over time. Alanine addition resulted in higher CO_2 production than glucose addition in the first 8 days across all soils (Tukey's tests, all $P < 0.05$); however, this effect was largely reversed after day 10, as CO_2 production was lower in alanine than in glucose treatment in most days. In the A horizon, alanine addition increased CO_2 production relative to the control in most sampling days (28 out of 31-day; Tukey's tests, all $P < 0.05$). In two DPC soils, similar effects were observed in the first half of the experiment; however, CO_2 production was indistinguishable between the alanine treatment and control in most days after day 40. Unlike alanine, glucose addition did not show a sharp CO_2 peak in any soil but did show sustained CO_2 production at a level higher than the control over the incubation period.

Addition of alanine and glucose substrates greatly stimulated cumulative CO_2 production, expressed as the percent of the initial soil C, relative to the control (Figure 2). For each soil, the cumulative CO_2 production followed the order: Alanine > Glucose > Control (Tukey's tests, all $P < 0.01$). In the A horizon, alanine addition increased the cumulative CO_2 production nearly fivefold relative to the control, while glucose tripled it. In two DPC soils, both substrates induced order-of-magnitude increases in cumulative CO_2 production, as the CO_2 production from the controls were extremely low (Bh1, $0.59 \pm 0.15\%$ of initial soil C; mean $\pm$ standard error unless otherwise noted; Bh2, $0.30 \pm 0.03\%$ of initial soil C). Across soils with alanine addition, the A horizon showed the largest CO_2 production, while the Bh2 horizon produced the least (Tukey's tests, all $P < 0.001$). In soils with glucose addition, the A horizon produced more CO_2 than others (Tukey's tests, both $P < 0.001$), while
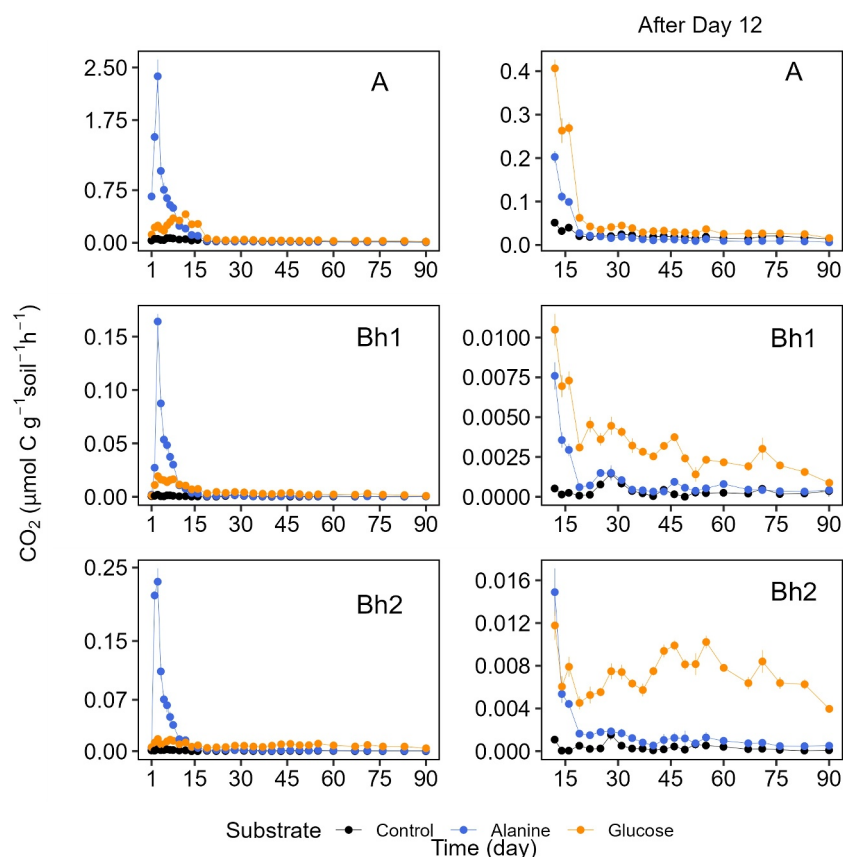
Figure 1. CO_2 production over the 90-day incubation period. Production rates as influenced by soil horizons and substrate additions over the 90-day incubation period. Control is shown in black, Alanine in blue and glucose in orange. Graphs on the right side of the figure, below the heading "After Day 12" show the CO_2 production after day 12 to better display the separation between treatments for each soil. Error bars indicate the spread of values for respiration measurements of the treatment microcosms.

there was no significant difference between the Bh1 and Bh2 horizons. Similarly, the A-horizon control released more CO_2 than other controls (Tukey's tests, both $P < 0.001$), but there was no difference between the DPC controls.

3.2. Relative Priming Effects

Both alanine and glucose triggered prominent priming effects on soil C decomposition in both Bh1 and Bh2 horizons (Figure 3). Alanine treatment resulted in positive priming effects for all horizons, that is, it enhanced the decomposition of soil C. The alanine-induced priming effects followed the order: Bh2 > Bh1 > A horizons (Tukey's tests, all $P < 0.01$), where alanine addition increased soil CO_2 production by $918 \pm 51\%$ and $737 \pm 7\%$ in Bh2 and Bh1 horizons, respectively. Compared to the alanine treatment, glucose induced lower, yet notable priming effects in Bh1 ($505 \pm 28\%$) and Bh2 horizons ($606 \pm 77\%$; Tukey's tests, both $P < 0.05$). Like the alanine treatment, glucose induced a greater priming effect in the Bh2 horizon than in the Bh1 horizon (Tukey's tests, $P < 0.05$). In the A horizon, glucose addition resulted in a small positive priming effect ($34.0 \pm 8.0\%$), while alanine addition caused a slightly larger relative priming effect ($108.5 \pm 15.0\%$).

3.3. Soil Microbial Biomass and Carbon After Incubation

At the termination of the incubation experiment, substrate treatments and horizon both had significant effects on microbial biomass, but their interactions did not (Figure 4). The glucose treatment increased microbial biomass relative to the alanine treatment (Tukey's test, $P < 0.01$), while neither affected microbial biomass compared to the control. Microbial biomass was significantly higher in the A horizon than in the Bh1 and Bh2 horizons (Tukey's
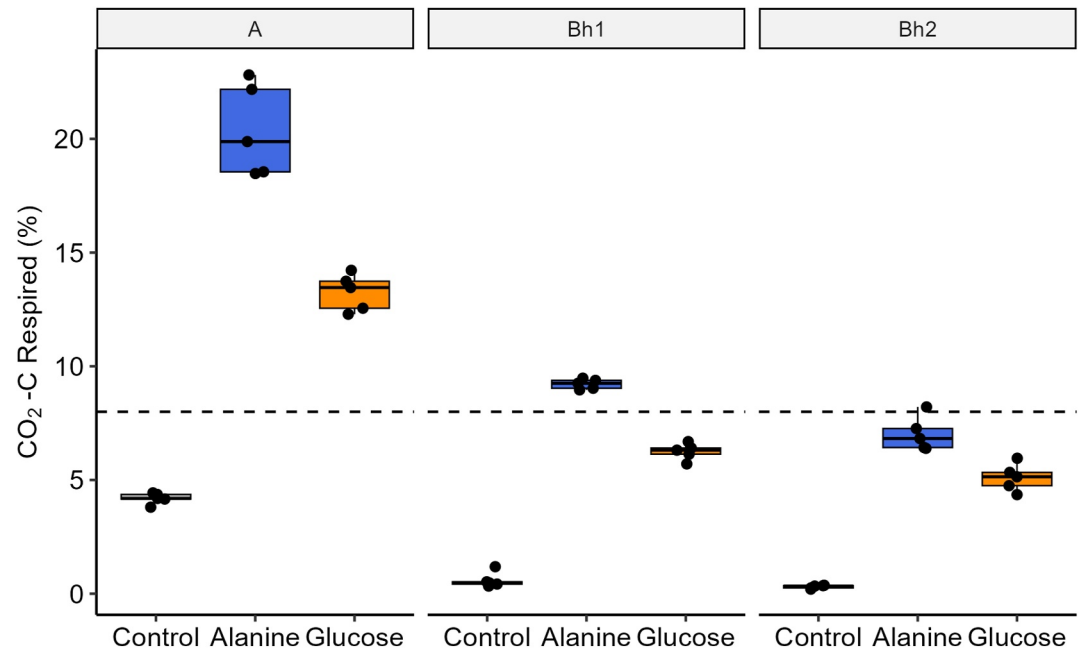
Figure 2. Cumulative CO_2-C production of the incubation jars relative to the initial soil. These percentages reflect the C loss after standardization to the initial OC contents of the soils used for the incubations. Error bars and datapoints show the range of C produced via respiration from the jars for each treatment-soil combination. Dashed line indicates 8% of initial soil C, which corresponds to the amount of alanine or glucose amendment. Control is shown in black, alanine in blue, and glucose in orange.
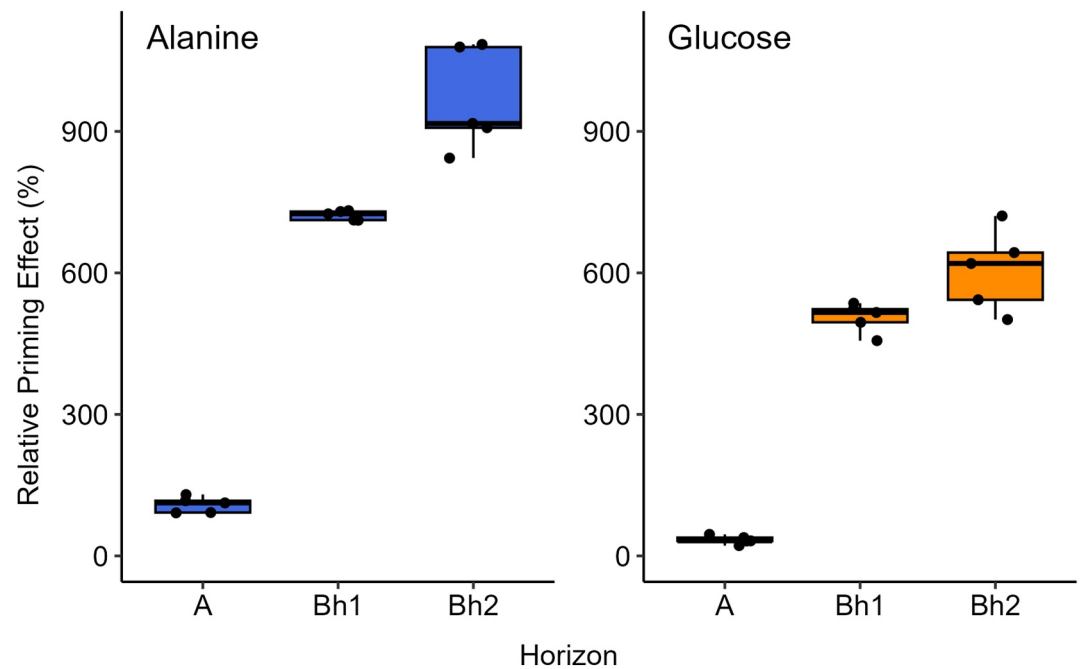

Figure 3. Relative priming effect. Presented as a percentage of basal respiration, for alanine and glucose treatments on soil from A, Bh1, and Bh2 horizons. The A horizon is shown in gray, Bh1 in blue, and Bh2 in orange.
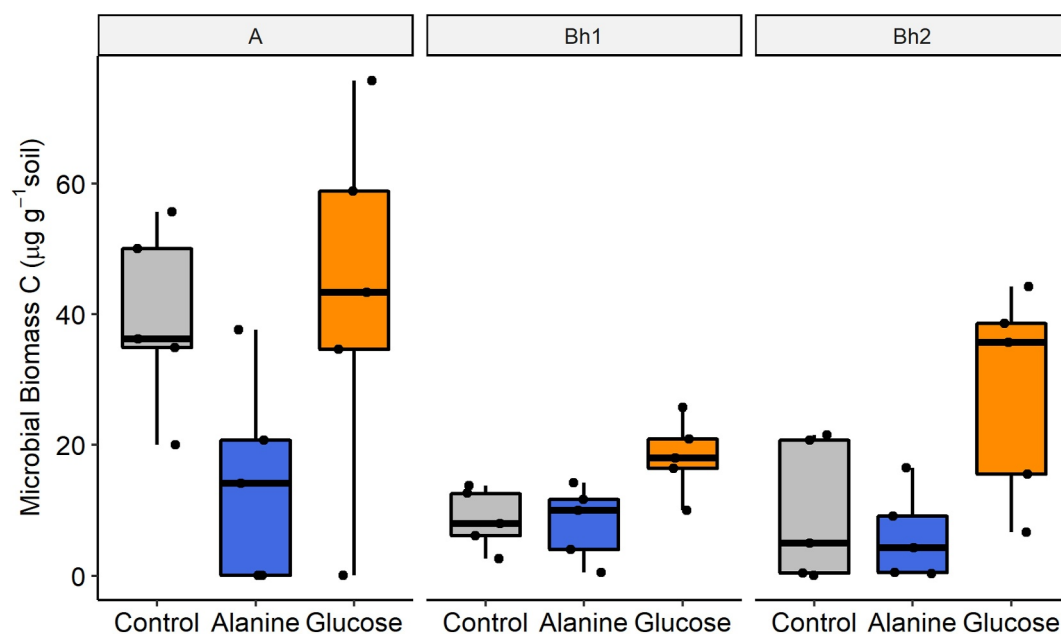
Figure 4. Microbial Biomass C. Microbial biomass separated by soil horizon and treatment. Control treatments are shown in gray, alanine in blue, and glucose in orange.

tests, both $P < 0.01$), but there was no difference between the two DPC horizons. The δ^{13}C enrichment of the soils after the incubation period was higher in the Bh1 and Bh2 soils than in the A horizon (Tukey's tests: Bh1, $P < 0.05$; Bh2, $P < 0.01$) for both alanine and glucose treatments (Figure S2 in Supporting Information S1).

4. Discussion

4.1. Microbial Limitation and DPC Persistence

Our primary finding is that microbial nutrient and energy limitations both contribute to the persistence of DPC. Both DPC horizons had priming effects that were order-of-magnitude larger than the surface horizon in the presence of fresh C and N substrate (Figure 3), indicating that the availability of labile compounds is a major constraint to the degradation of DPC under aerated conditions. Priming effects were more prevalent in deeper soils, which were depleted in labile OM, compared to surface soil. This finding is consistent with past studies that showed stronger priming effects in subsoil with more refractory OM than in surface soil (Bernal et al., 2016; Chen et al., 2022; Karhu et al., 2016; Zhang et al., 2023). However, the intensity of the priming responses in both DPC horizons were much higher than those reported in the past studies. For instance, a recent meta-analysis found that glucose induced an average priming effect of 197% in subsoils (Bastida et al., 2019). Glucose additions to temperate surface soils have been shown to trigger high intensity priming effects like those observed here (Jilling et al., 2021; Keiluweit et al., 2015). The addition of glucose indirectly supports the release of C from metal-organic associations by supporting enzyme production in the microbial biomass (Chari & Taylor, 2022; Jilling et al., 2021).

The high relative priming observed in the DPC horizons compared to the A horizon can be attributed to the difference in their basal respirations observed under control conditions. Both DPC horizons showed very low respiration in the control with less the equivalent of 0.6% initial soil C released as CO_2 over the duration of the 90 days incubation (Figure 2). In contrast, the equivalent of over 4% initial soil C was released as CO_2 in the A horizon jars under control conditions. Microbial biomass concentrations were also low in both Bh controls (∼5–20 μg C g^{-1} soil), suggesting that the turnover of microbial biomass, as seen in studies reporting apparent priming, might not be the primary mechanism driving the observed effects (Blagodatskaya & Kuzyakov, 2008). Future studies using three part tagging as suggested in Blagodatskaya and Kuzyakov., 2008 can offer more concrete data on the separation between real and apparent priming for DPC. In sum, our results demonstrate that DPC is vulnerable to degradation after the removal of labile C or N limitations on microbial decomposition.

The high priming intensity observed in the substrate treatments may be a result of the OM-stabilization provided by metal association. Metals inhibit microbial attack of complexes OM molecules and preserve them from degradation, resulting in a slow cycling C pool (Kleber et al., 2015; Matus et al., 2014; Torn et al., 1997). Studies have shown variable priming intensities in soils with short-range order (SRO) minerals and in spodic material, both rich in Fe and Al (Bernal et al., 2016; Finley et al., 2018; Jilling et al., 2021; Keiluweit et al., 2015; Rasmussen et al., 2007). Priming intensity varied among the studies, with more complex substrate addition, such as litter (Rasmussen et al., 2007), resulting in lower intensity priming compared to labile substrates such as glucose and alanine (Bernal et al., 2016; Jilling et al., 2021; Keiluweit et al., 2015). Metal composition of podzolized OM has been shown to vary regionally, with Al ions becoming the dominant complexing metal in podzols that develop in sandy, coastal plains occurring while Fe dominates in northern podzols (Mokma & Buurman, 1982; Schwartz, 1988). The metal-OM complex protects OM from degradation by inhibiting enzyme and microbial access and is known to play a central role in the persistence of OM (Conant et al., 2011; Mikutta et al., 2006; Rasmussen et al., 2007). While both substrate treatments resulted in large relative priming effects, only glucose stimulated a change in the microbial biomass of the DPC horizons while alanine resulted in no change. It is possible that the addition of labile substrates relieved the inaccessibility of the metal-associated OM by promoting microbial biomass growth (Finley et al., 2018). This would provide an explanation for the increased microbial biomass of the glucose treatments in both DPC horizons. Our results show a stronger intensity response with labile substrate addition, indicating the stability of this C pool is influenced by substrate limitation on the microbial biomass. While the DPC is likely stabilized from microbial attack by the lack of exposure to labile substrates, the role of metal protection of complexed OM should not be overlooked. Further research constraining priming response of this soil pool, and similar global occurrences of deep carbon, with varying substrate additions will aid in furthering understanding of the limits of DPC stability.

Although both substrates triggered prominent priming effects in DPC horizons, they had differential effects on soil microbial dynamics. Glucose addition resulted in significantly higher microbial biomass than alanine treatment by the end of the experiment (Figure 4). Thus, glucose helped to sustain an increase in microbial biomass over the long term, which subsequently enabled microbial decomposition of DPC. Alanine addition, on the other hand, had no significant impacts on microbial biomass by the end of the experiment, which is consistent with the result that the CO_2 production rate was indistinguishable between the alanine treatment and control in the second half of the experiment (Figure 1). Alanine addition did induce stronger priming effects on soil C decomposition than glucose. Interestingly, most of the alanine-induced effects on the microbial decomposition of DPC occurred in the first few days of the experiment, again highlighting the transient nature of the alanine effects.

These differential effects of alanine and glucose on microbial dynamics may reflect several biogeochemical mechanisms. First, unlike glucose, alanine relieved both the energy and N limitation of soil microbes. Even with glucose addition, low N availability, as indicated by the high C:N ratios, still likely constrained microbial decomposition of DPC. Thus, microbes could be more responsive to alanine than glucose. Second, as an amino acid, alanine can be readily utilized to produce extracellular enzymes that catalyze the decomposition of organic matter (Hamer & Marschner, 2005). Third, alanine undergoes deamination to produce ammonium and pyruvate, which is a direct precursor for the citric acid cycle, while glucose must undergo glycolysis before becoming pyruvate (Hamer & Marschner, 2005; Mason-Jones et al., 2018). Therefore, alanine is likely more effective in jumpstarting microbial metabolism compared to glucose and therefore enables a more rapid increase in microbial respiration.

Our results also have implications for understanding the dynamics of DPC under different redox conditions, even though we did not manipulate redox conditions. Interestingly, the cumulative CO_2 productions seen in the two DPC controls were extremely small (<0.6% of initial soil C). Given the importance of phreatic zone conditions, namely persistent saturation, to DPC stabilization (Bacon et al., 2020; Champiny, Bacon et al., 2024), this finding suggests that short-term aerobic conditions, as implemented in this experiment, might not be sufficient to stimulate robust microbial activity. While the phreatic zone is characterized by infrequent fluctuations in water level, there is still a degree of fluctuation that may expose the upper reaches of DPC to aeration. During sample collection for this and previous studies, we discovered that the top 2 m of soil were not water saturated. The DPC horizons have potentially experienced similar aeration events in the past such that short-term aeration events alone are insufficient to stimulate significant microbial respiration. It is possible, however, that the aerobic conditions of the incubation contributed to the high priming intensity observed in DPC horizons for both alanine and glucose treatments. Further

redox manipulation can provide more concrete data on how DPC will react under varying gradients of oxygen availability with respect to release of greenhouse gases.

The extent of N and labile C limitation on the microbial community appeared to be stronger in the deeper DPC horizon, as indicated by the greater priming responses in Bh2 horizons compared to the A and Bh1 for both treatments (Figure 3). Past research suggested that the magnitude of priming effects was proportional to the dose of substrate relative to soil C or microbial biomass (E. Blagodatskaya & Kuzyakov, 2008). While the addition of glucose was standardized against soil C, the microbial biomass was comparable between Bh1 and Bh2, suggesting that the relative amount of substrate C to microbial biomass C may have been higher in Bh2. As a result of using soil C content to standardize the relative proportion of added substrate, the Bh2 also received a higher quantity of N relative to the Bh1 in the alanine treatment. Nevertheless, it is still likely that the extent of substrate limitation on microbial respiration increases with distance from the soil surface and roots. As the distance increases, or as DPC becomes deeper, the inputs of labile substrate will decrease, and any microbes present will encounter greater energy and nutrient limitation. This suggests that the combination of depth and substrate limitation contributes to the stability of DPC.

4.2. Implications for Land Use and Management

Our results have further implications for predicting the fate of DPC under current and future management practices and global change. Given the strong N limitation on the decomposition of DPC, land management practices that alter the flow of nutrients into the deep soil, such as nutrient-rich leachate, could relieve the nutrient limitation of microbial activities and stimulate decomposition of DPC. The influx of nutrients from root exudates from tree roots, or nutrient rich leachate from agriculture, could also meet the nutrient requirements and stimulate the release of CO_2 from this deep C pool. Additionally, the downward transport of C from root exudates or decaying organic material could relieve the energy limitations to microbial activity and stimulate the loss of DPC. Short-term changes in oxygen availability appeared to have a relatively small impact on DPC stability compared to the effect of nutrient and C availability, more research is required to ascertain the impact of oxygen availability on the degradability of this deep soil C.

Extrapolation of our findings may improve the understanding of other instances of phreatic zone podzolized C globally and elucidate deep C cycling responses to altered biogeochemical flows. Although DPC has been reported only in the United States, podzolized C is likely to be found in similar environments globally. For example, some equatorial and Amazon podzols intersect with the phreatic zones and could induce similar microbial energy or nutrient limitation on the C persistence. By exploring the occurrence of phreatic zone podzols in other parts of world, future studies can better document the importance of DPC in the global C cycle and examine factors driving DPC persistence.

In conclusion, our study shows that microbial nutrient and energy limitations are important mechanisms responsible for the persistence of DPC. The microbial biomass is constrained by a lack of energy sources both by the paucity of labile substrates and by the reduced availability of organic matter as a result of podzolization. The input of fresh labile substrates via root exudates or fertilizer outflow could relieve those limitations and allow microbial biomass to accumulate. Our study has important implications for protecting DPC in the context of land management and global change.

Data Availability Statement

All data generated for use in this study and the R file used to create the LOESS models may be accessed in the Zenodo data repository (Champiny, Inglett et al., 2024) https://doi.org/10.5281/zenodo.13722652. Figures were created in R studio version 4.3.3 (R Core Team, 2024) available at www.R-project.org, using the ggplot2 package (Wickham, 2016), available at https://ggplot2.tidyverse.org. Statistics were run using the Tidyverse package (Wickham et al., 2019) available at https://doi.org/10.21105/joss.01686.

References

Bacon, A. R., Gonzalez, Y. N., & Anderson, K. R. (2020). Morphologic and hydrologic distinctions between shallow and deep podzolized carbon in the southeastern United States Coastal Plain. *Geoderma*, *361*(11407), 114007. https://doi.org/10.1016/j.geoderma.2019.114007

Banik, C., Harris, W. G., Ellis, R., Hurt, W., & Balboa, S. (2014). Relations of iron, aluminum, and carbon along transitions from Udults to Aquods. *Geoderma*, *226–227*(1), 332–339. https://doi.org/10.1016/J.GEODERMA.2014.03.016

Bastida, F., García, C., Fierer, N., Eldridge, D. J., Bowker, M. A., Abades, S., et al. (2019). Global ecological predictors of the soil priming effect. *Nature Communications*, *10*(1), 3481. https://doi.org/10.1038/s41467-019-11472-7

Bernal, B., McKinley, D. C., Hungate, B. A., White, P. M., Mozdzer, T. J., & Megonigal, J. P. (2016). Limits to soil carbon stability; Deep, ancient soil carbon decomposition stimulated by new labile organic inputs. *Soil Biology and Biochemistry*, *98*, 85–94. https://doi.org/10.1016/j.soilbio.2016.04.007

Blagodatskaya, E., Yuyukina, T., Blagodatsky, S., & Kuzyakov, Y. (2011). Three-source-partitioning of microbial biomass and of CO2 efflux from soil to evaluate mechanisms of priming effects. *Soil Biology and Biochemistry*, *43*(4), 778–786. https://doi.org/10.1016/J.SOILBIO.2010.12.011

Blagodatskaya, E., & Kuzyakov, Y. (2008). Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: Critical review. *Biology and Fertility of Soils*, *45*(2), 115–131. https://doi.org/10.1007/s00374-008-0334-y

Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., & Kuzyakov, Y. (2010). Model of apparent and real priming effects: Linking microbial activity with soil organic matter decomposition. *Soil Biology and Biochemistry*, *42*(8), 1275–1283. https://doi.org/10.1016/j.soilbio.2010.04.005

Bolivar, J. G. P. (2000). *The genesis of carbon sequestration in subtropical spodosols*. University of Florida. Retrieved from https://ufdcimages.uflib.ufl.edu/UF/00/10/08/16/00001/thesis2.PDF

Champiny, R. E., Bacon, A. R., Brush, I. D., McKenna, A. M., Colopietro, D. J., & Lin, Y. (2024). Unraveling the persistence of deep podzolized carbon: Insights from organic matter characterization. *Science of the Total Environment*, *906*, 167382. https://doi.org/10.1016/j.scitotenv.2023.167382

Champiny, R. E., Inglett, K. S., & Lin, Y. (2024). The role of nutrient and energy limitation on microbial decomposition of deep podzolized carbon: A priming experiment [Dataset]. *Zenodo*. https://doi.org/10.5281/zenodo.13722652

Chari, N. R., & Taylor, B. N. (2022). Soil organic matter formation and loss are mediated by root exudates in a temperate forest. *Nature Geoscience*, *15*(12), 1011–1016. https://doi.org/10.1038/s41561-022-01079-x

Chen, L., Liu, L., Qin, S., Yang, G., Fang, K., Zhu, B., et al. (2019). Regulation of priming effect by soil organic matter stability over a broad geographic scale. *Nature Communications*, *10*(1), 5112. https://doi.org/10.1038/s41467-019-13119-z

Chen, X., Lin, J., Wang, P., Zhang, S., Liu, D., & Zhu, B. (2022). Resistant soil carbon is more vulnerable to priming effect than active soil carbon. *Soil Biology and Biochemistry*, *168*, 108619. https://doi.org/10.1016/j.soilbio.2022.108619

Conant, R. T., Ryan, M. G., Ågren, G. I., Birge, H. E., Davidson, E. A., Eliasson, P. E., et al. (2011). Temperature and soil organic matter decomposition rates - Synthesis of current knowledge and a way forward. *Global Change Biology*, *17*(11), 3392–3404. https://doi.org/10.1111/j.1365-2486.2011.02496.x

Dijkstra, F. A., Carrillo, Y., Pendall, E., & Morgan, J. A. (2013). Rhizosphere priming: A nutrient perspective. *Frontiers in Microbiology*, *0*(JUL), 216. https://doi.org/10.3389/FMICB.2013.00216

Dijkstra, F. A., & Cheng, W. (2007). Interactions between soil and tree roots accelerate long-term soil carbon decomposition. *Ecology Letters*, *10*(11), 1046–1053. https://doi.org/10.1111/J.1461-0248.2007.01095.X

Ditzler, C., Scheffe, K., & Monger, H. C., & United States Department of Agriculture (USDA). (2017). *Soil survey manual by soil science division staff*. Government Printing Office.

Farmer, V. C., Skjemstad, J. O., & Thompson, C. H. (1983). Genesis of humus B horizons in hydromorphic humus podzols. *Nature*, *304*(5924), 342–344. https://doi.org/10.1038/304342a0

Ferro-Vázquez, C., Nóvoa-Muñoz, J. C., Klaminder, J., Gómez-Armesto, A., & Martínez-Cortizas, A. (2020). Comparing podzolization under different bioclimatic conditions. *Geoderma*, *377*, 114581. https://doi.org/10.1016/j.geoderma.2020.114581

Finley, B. K., Dijkstra, P., Rasmussen, C., Schwartz, E., Mau, R. L., Liu, X.-J. A., et al. (2018). Soil mineral assemblage and substrate quality effects on microbial priming. *Geoderma*, *322*, 38–47. https://doi.org/10.1016/j.geoderma.2018.01.039

Fischer, H., Ingwersen, J., & Kuzyakov, Y. (2010). Microbial uptake of low-molecular-weight organic substances out-competes sorption in soil. *European Journal of Soil Science*, *61*(4), 504–513. https://doi.org/10.1111/J.1365-2389.2010.01244.X

Fontaine, S., Bardoux, G., Abbadie, L., & Mariotti, A. (2004). Carbon input to soil may decrease soil carbon content. *Ecology Letters*, *7*(4), 314–320. https://doi.org/10.1111/J.1461-0248.2004.00579.X

Fontaine, S., & Barot, S. (2005). Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecology Letters*, *8*(10), 1075–1087. https://doi.org/10.1111/J.1461-0248.2005.00813.X

Fontaine, S., Barot, S., Barré, P., Bdioui, N., Mary, B., & Rumpel, C. (2007). Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature*, *450*(7167), 277–280. https://doi.org/10.1038/nature06275

Gonzalez, Y. N., Bacon, A. R., & Harris, W. G. (2018). A billion tons of unaccounted for carbon in the southeastern United States. *Geophysical Research Letters*, *45*(15), 7580–7587. https://doi.org/10.1029/2018GL077540

Hamer, U., & Marschner, B. (2005). Priming effects in soils after combined and repeated substrate additions. *Geoderma*, *128*(1–2), 38–51. https://doi.org/10.1016/j.geoderma.2004.12.014

Henneron, L., Balesdent, J., Alvarez, G., Barré, P., Baudin, F., Basile-Doelsch, I., et al. (2022). Bioenergetic control of soil carbon dynamics across depth. *Nature Communications*, *13*(1), 7676. https://doi.org/10.1038/s41467-022-34951-w

Ishida, D. A., Montes, C. R., Lucas, Y., Pereira, O. J. R., Merdy, P., & Melfi, A. J. (2014). Genetic relationships between Ferralsols, podzols and white kaolin in Amazonia. *European Journal of Soil Science*, *65*(5), 706–717. https://doi.org/10.1111/ejss.12167

Jilling, A., Keiluweit, M., Gutknecht, J. L. M., & Grandy, A. S. (2021). Priming mechanisms providing plants and microbes access to mineral-associated organic matter. *Soil Biology and Biochemistry*, *158*, 108265. https://doi.org/10.1016/j.soilbio.2021.108265

Karhu, K., Hilasvuori, E., Fritze, H., Biasi, C., Nykänen, H., Liski, J., et al. (2016). Priming effect increases with depth in a boreal forest soil. *Soil Biology and Biochemistry*, *99*, 104–107. https://doi.org/10.1016/j.soilbio.2016.05.001

Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. (2015). Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change*, *5*(6), 588–595. https://doi.org/10.1038/nclimate2580

Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., & Nico, P. S. (2015). Chapter one - mineral–organic associations: Formation, properties, and relevance in soil environments. In D. L. Sparks (Ed.), *Advances in Agronomy* (Vol. 130, pp. 1–140). Academic Press. https://doi.org/10.1016/bs.agron.2014.10.005

Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. *Soil Biology and Biochemistry*, *42*(9), 1363–1371. https://doi.org/10.1016/J.SOILBIO.2010.04.003

Kuzyakov, Y., Friedel, J. K., & Stahr, K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, *32*(11–12), 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5

Langley, J. A., McKinley, D. C., Wolf, A. A., Hungate, B. A., Drake, B. G., & Megonigal, J. P. (2009). Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO2. *Soil Biology and Biochemistry*, *41*(1), 54–60. https://doi.org/10.1016/J.SOILBIO.2008.09.016

Manzoni, S., Taylor, P., Richter, A., Porporato, A., & Ågren, G. I. (2012). Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist*, *196*(1), 79–91. https://doi.org/10.1111/j.1469-8137.2012.04225.x

Mason-Jones, K., Schmücker, N., & Kuzyakov, Y. (2018). Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming. *Soil Biology and Biochemistry*, *124*, 38–46. https://doi.org/10.1016/j.soilbio.2018.05.024

Matus, F., Rumpel, C., Neculman, R., Panichini, M., & Mora, M. L. (2014). Soil carbon storage and stabilisation in andic soils: A review. *Catena*, *120*, 102–110. https://doi.org/10.1016/j.catena.2014.04.008

Mikutta, R., Kleber, M., Torn, M. S., & Jahn, R. (2006). Stabilization of soil organic matter: Association with minerals or chemical recalcitrance? *Biogeochemistry*, *77*(1), 25–56. https://doi.org/10.1007/s10533-005-0712-6

Mokma, D. L., & Buurman, P. (1982). *Podzols and podzolization in temperate regions*. Soil Science (Vol. 1, pp. 126). International Soil Museum. https://doi.org/10.1097/00010694-198707000-00014

Montes, C. R., Merdy, P., da Silva, W. T. L., Ishida, D., Melfi, A. J., Santin, R. C., & Lucas, Y. (2023). Mineralization of soil organic matter from equatorial giant podzols submitted to drier pedoclimate: A drainage topochronosequence study. *Catena*, *222*, 106837. https://doi.org/10.1016/j.catena.2022.106837

Moore, T. R., Trofymow, J. A., Prescott, C. E., Fyles, J., & Titus, B. D. (2006). Patterns of carbon, nitrogen and phosphorus dynamics in decomposing foliar litter in Canadian forests. *Ecosystems*, *9*(1), 46–62. https://doi.org/10.1007/S10021-004-0026-X

Parton, W., Silver, W. L., Burke, I. C., Grassens, L., Harmon, M. E., Currie, W. S., et al. (2007). Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science*, *315*(5810), 361–364. https://doi.org/10.1126/science.1134853

Rasmussen, C., Southard, R. J., & Horwath, W. R. (2007). Soil mineralogy affects conifer forest soil carbon source utilization and microbial priming. *Soil Science Society of America Journal*, *71*(4), 1141–1150. https://doi.org/10.2136/sssaj2006.0375

R Core Team. (2024). R: A language and environment for statistical computing [Software]. R Foundation for Statistical Computing. Retrieved from https://www.R-project.org

Rumpel, C., & Kögel-Knabner, I. (2011). Deep soil organic matter—A key but poorly understood component of terrestrial C cycle. *Plant and Soil*, *338*(1), 143–158. https://doi.org/10.1007/S11104-010-0391-5

Schimel, J., & Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: A theoretical model. *Soil Biology and Biochemistry*, *35*(4), 549–563. https://doi.org/10.1016/S0038-0717(03)00015-4

Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: Challenges of a changing paradigm. *Ecology*, *85*(3), 591–602. https://doi.org/10.1890/03-8002

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., et al. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49–56. https://doi.org/10.1038/nature10386

Schwartz, D. (1988). *Histoire d'un paysage: le lousséké: paléoenvironnements quaternaires et podzolisation sur sables Batéké: quarante derniers millénaires, région de Brazzaville* (p. 285). R.P. du Congo. Paris: ORSTOM.

Silva, E. A., Gomes, J. B. V., Araújo Filho, J. C. D., Vidal-Torrado, P., Cooper, M., & Curi, N. (2012). Morphology, mineralogy and micro-morphology of soils associated to summit depressions of the Northeastern Brazilian Coastal Plains. *Ciencia E Agrotecnologia*, *36*(5), 507–517. https://doi.org/10.1590/S1413-70542012000500003

Soong, J. L., Fuchslueger, L., Marañón-Jimenez, S., Torn, M. S., Janssens, I. A., Penuelas, J., & Richter, A. (2020). Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biology*, *26*(4), 1953–1961. https://doi.org/10.1111/gcb.14962

Thiessen, S., Gleixner, G., Wutzler, T., & Reichstein, M. (2013). Both priming and temperature sensitivity of soil organic matter decomposition depend on microbial biomass – An incubation study. *Soil Biology and Biochemistry*, *57*, 739–748. https://doi.org/10.1016/j.soilbio.2012.10.029

Torn, M. S., Trumbore, S. E., Chadwick, O. A., Vitousek, P. M., & Hendricks, D. M. (1997). Mineral control of soil organic carbon storage and turnover. *Nature*, *389*(6647), 170–173. https://doi.org/10.1038/38260

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987a). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, *19*(6), 703–707. https://doi.org/10.1016/0038-0717(87)90052-6

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987b). Microbial biomass measurements in forest soils: The use of the chloroform fumigation-incubation method in strongly acid soils. *Soil Biology and Biochemistry*, *19*(6), 697–702. https://doi.org/10.1016/0038-0717(87)90051-4

Wickham, H. (2016). Ggplot2: Elegant graphics for data analysis [Software]. Springer-Verlag. Retrieved from https://ggplot2.tidyverse.org

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., et al. (2019). Welcome to the Tidyverse [Software]. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686

Zechmeister-Boltenstern, S., Keiblinger, K. M., Mooshammer, M., Penuelas, J., Richter, A., Sardans, J., & Wanek, W. (2015). The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. *Ecological Monographs*, *85*(2), 133–155. https://doi.org/10.1890/14-0777.1

Zhang, X., Song, Z., Zhang, D., Wu, Y., Van Zwieten, L., Sun, S., et al. (2023). Soil properties and anthropogenic influences control the distribution of soil organic carbon in grasslands of northern China. *Land Degradation and Development*, *35*(1), 33–45. https://doi.org/10.1002/ldr.4895