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Attachment Issues: Microbes, Minerals, and the Persistence of Soil Organic Matter

Md Shafiul Islam Rion

Thesis submitted
to the Davis College of Agriculture, Natural Resources, and Design
at West Virginia University

in partial fulfillment of the requirements for the degree of
Master of Science in
Applied and Environmental Microbiology

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Division of Plant and Soil Sciences

Morgantown, West Virginia
2023

Keywords: soil organic matter, necromass, mineralogy, microbial attachment
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ABSTRACT

Attachment Issues: Microbes, Minerals, and the Persistence of Soil Organic Matter

Md Shafiul Islam Rion

The remnants of microorganisms are now understood to account for the majority of organic matter in many mineral soils. Despite the significance of this microbial necromass for soil carbon storage, we know relatively little about how the traits of microorganisms interact with soil minerals to determine the stability of microbe derived carbon in soil. Soil minerals differ in their surface area and chemistry potentially influencing microbial attachment, biofilm formation, and the persistence of microbial necromass. To address this knowledge gap, we grew twelve bacterial species from four broad groups of varying cell wall morphology (Gram positive, Gram negative, filamentous actinobacteria, and capsule-forming bacteria) in ^{13}C-enriched minimal media with soil minerals (sand, clay, goethite-coated sand, and goethite-coated clay). The decomposition of heat-killed and dried necromass-mineral preparations was then traced in a 28-day soil microcosm experiment. Over the incubation period 20–80% of the necromass carbon was respired depending upon both cell wall morphology and mineral chemistry. In general, the necromass carbon from Gram-positive bacteria persisted longer than that of Gram-negative bacteria. Goethite coating on clay tended to reduce decomposition, especially for Gram-positive bacterial necromass (as only ~30% of the C was respired). This may be a consequence of anionic teichoic acids in the cell wall of Gram-positive bacteria adhering to positively charged iron oxides coating the clay mineral surface. Necromass decomposition was greatest for Gram-negative bacteria grown in the presence of sand (50–80% of the necromass C was respired) suggesting that these cells have difficulty forming stable attachments to sand surfaces. Taken together this work suggests that interactions between the surface chemistry of microbial cells and soil minerals may provide new insights into how microbes and minerals interact to influence soil organic matter persistence.

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1 INTRODUCTION

Atmospheric CO_2 concentration is increasing by 0.88 ppm annually (IPCC, 2007) and nearly one-third of this enhanced atmospheric CO_2 is coming from the soil organic carbon (SOC) loss owing to shifts in land use (Lal, 2004). Most of the C (~ 80%) in earth's terrestrial ecosystems is in soil (Lal, 2007) and the soil C pool is 3.1 times larger than the atmospheric C pool (Oelkers and Cole, 2008). As a result, C-exchange between this colossal reservoir and the atmosphere is decisive for atmospheric-C composition and its impact on climate (Bellamy et al., 2005; Mahecha et al., 2010). SOC is derived from various sources (e.g., dead plant parts, soil inhabiting microbes) and is a vital component of soil. Soil microbes generally uptake C by decomposing organic matter for their nutrition and respire C in the form of CO_2.

Scientific understanding of soil organic matter has undergone a remarkable paradigm shift. Previously, the larger recalcitrant compounds from dead plant parts were considered as the largest portion of soil organic matter. However, modern analysis techniques have revealed that much of soil organic matter consists of relatively simple compounds that may be stabilized through interactions with mineral surfaces (Cotrufo et al., 2013; Dungait et al., 2012; Schmidt et al., 2011). Hence, soil organic carbon persistence is more of an ecosystem property than a molecular property (Joseph et al., 2023; Schmidt et al., 2011; Wang et al., 2015).

Microbial necromass is composed of microbial dead cells, cell fragments, and extracellular compounds. An estimated 15-80% of soil carbon is microbial necromass and this necromass has a residence time similar to that of plant derived carbon (Angst et al., 2021; Liang et al., 2019; Schmidt et al., 2011). Despite being the crucial part of stable soil carbon, very few empirical studies have examined the decomposition of microbial necromass (Wang et al., 2020), as such it remains unclear if and to what extent, microbial necromass varies in its stability in soil. However, a pioneering study by Throckmorton et al. (2012) examined the decomposition of Gram positive, Gram negative, and fungal biomass in tropical and temperate soils. The type of microbial necromass had a limited impact on its stability in soil (Throckmorton et al., 2012). While valuable, past experiments (e.g., Buckeridge et al., 2022; Creamer et al., 2019; Throckmorton et al., 2012; Wang et al., 2020) have prepared necromass for decomposition

experiments from biomass generated in pure culture under conditions that fails to mimic necromass formation in soil. In soil, microbes grow on mineral surfaces and in biofilms (Jones and Bennett, 2017, 2014) and their necromass stays among mineral particles. Therefore, growing the microbes in liquid media without any attachments with minerals during their growth period does not represent natural microbial biomass and necromass generation. As such, studying the decomposition of microbial necromass, generated in the absence of minerals, may fail to capture important mechanisms of necromass stabilization in soil.

Interactions between microbial necromass and the surface chemistry of soil minerals are likely to influence necromass persistence (Dong et al., 2022; Dong and Lu, 2012; Moore et al., 2017; Shi et al., 2016). For example, bacteria and mineral surface interactions are critical factors for explaining soil organic carbon processes and persistence (Finley et al., 2022). Bacteria cells possess various types of extracellular structures (e.g., flagella, fimbriae, glycocalyx) and substances (e.g., polysaccharides, nucleic acids) that aid in the attachment to and colonization of surfaces (Lennon and Lehmkuhl, 2016; Van Houdt and Michiels, 2005). These organic extensions of bacterial cells are chemically variable and differences in their charge, polarity, and hydrophobicity likely determine how strongly they adhere to mineral surfaces in soil. Minerals are likewise highly variable with respect to their surface chemistry (e.g., surface charge, surface area, etc.). For instance, consider kaolinite clay and quartz sand, kaolinite has higher surface area and ion exchange capacity (cation exchange capacity, CEC ~ 3.3-15 meq/100g), whereas quartz has lower surface area and ion exchange capacity (CEC < 0.5 meq/100g). These distinct properties of minerals likely affect interaction of the minerals with microbial surfaces to determine how microbes attach to and grow on these surfaces ultimately impacting the persistence of microbial necromass C.

Mineralogy is well understood to influence the concentration and stability of organic carbon in soil. Soil type determines the storage of organic carbon - clay soil stores more organic carbon compared to sandy soil (Ahn et al., 2009; Shan et al., 2001; Yost and Hartemink, 2019). Organic matter in clay-rich soil is often physically protected by adsorption to clay particles and occlusion within aggregates. In contrast, soil microbes can easily access organic matter in sandy soils, fostering high decomposition rates and resulting in low soil organic carbon content. Further, certain mineral combinations, like low aluminous clay with high Fe-oxides are

particularly effective at stabilizing soil organic carbon (Kirsten et al., 2021). As soil weathers, iron oxides are formed, and can coat clay minerals like kaolinite and enhance the stabilization of soil organic matter by providing positive charges that interact with negatively charged groups of soil organic matter (Kirsten et al., 2021).

Bacteria are broadly classified as Gram positive or Gram negative depending on their cell wall morphology. Beside the thickness of the peptidoglycan layer, Gram positive and Gram negative bacteria differ in the molecular composition of their extracellular polymeric substances. Gram positive bacteria cells contain teichoic acids composed of glycerol phosphate units that are anionic in nature (Brock et al., 2003). In contrast, Gram negative bacteria cell walls contain lipopolysaccharides, which hold high negative charge (Beveridge, 1999; Wilhelm et al., 2021). Beyond these differences, some bacterial lineages have evolved distinct cellular surface chemistries that may alter their interactions with and attachments to soil mineral surfaces. For instance, *Mycobacteria* have unique cellular envelopes comprised of highly hydrophobic, long chain fatty acids (mycolic acids). Other bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa* have extra polysaccharide layers termed capsules outside their cell walls (Franklin et al., 2011; Rybtke et al., 2020; Sande and Whitfield, 2021; Whitfield et al., 2020). In addition to cell surface chemistry, cell morphology may alter bacterial interactions with and attachments to soil mineral surfaces. For instance, filamentous *Actinobacteria* exhibit an uncommon cell morphology for bacteria as they produce hyphae and mycelium (Amin et al., 2020; Barka et al., 2015; van der Meij et al., 2017). These mycelia might intermingle within mineral particles while growing and their necromass remains protected inside those particles. However, we are lacking sufficient insights of bacterial cell morphology interaction with soil minerals in terms of necromass persistence in soil.

The objective of this study was to decipher how bacterial cell morphology interacts with soil minerals to influence microbial necromass decomposition. We simulated necromass formation in soil by growing a diverse selection of bacteria (including Gram positive, Gram negative, capsule forming bacteria, and filamentous *Actinobacteria*) in media with commonly found soil minerals (kaolinite clay, quartz sand, goethite-coated kaolinite clay, and goethite-coated quartz sand). As anionic teichoic acids might aid in bacterial attachment and exposed cations on mineral surfaces our first hypothesis was that carbon from Gram positive bacteria

necromass would decompose more slowly than that of Gram negative bacteria necromass. Given that goethite (FeO_2H) increases the surface charge of minerals and could enhance the absorption of negatively charged necromass, we further hypothesized that goethite-coated minerals would have reduced necromass decomposition. To address these hypotheses, we measured the decomposition of necromass from 12 bacterial species grown in the presence of 4 common soil minerals.

2 MATERIALS AND METHODS

2.1 Experimental design

To address our objective and hypotheses, we conducted a microcosm experiment to determine the impact of microbial cell wall morphology and soil mineralogy on soil organic carbon sequestration. Specifically we assessed the decomposition of microbial necromass from twelve bacteria species to encompassing four broad classifications: (a) Gram negative bacteria (e.g., *Chryseobacterium gleum*, *Serratia marcescens*, and *Proteus vulgaris*), (b) Gram positive bacteria (e.g., *Arthrobacter oryzae*, *Corynebacterium glutamicum*, and *Micrococcus luteus*), (c) capsule forming bacteria (e.g., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium smegmatis*), and (d) filamentous *Actinobacteria* (e.g., *Streptomyces africanus*, *Actinomadura libanotica*, and *Cellulomonas humilata*). These bacteria were grown in the presence of four common soil minerals (e.g., kaolinite-clay, quartz-sand, goethite coated clay, and goethite coated sand) with ^{13}C enriched minimal media. These microbial necromass-mineral preparations were then added to soil and the decomposition of necromass carbon was monitored via the evolution of $^{13}CO_2$ in a laboratory microcosm experiment. There were a total of 64 different bacteria-mineral treatments and a control treatment (without any introduced bacteria and mineral) with 4 replicates per treatment.

2.2 Bacteria culture with minerals

Fine quartz sand (from Wards Science, Rochester, NY) was ground in a mixer mill and wet sieved (20 μm) to get uniform particle size comparable to that of fine clay (2 μm). Subsamples of fine sand and kaolinite clay (Kga-2; Source Clays Repository, Purdue University, West Lafayette, Indiana) were coated with goethite (FeO_2H) following the procedure mentioned

in Scheidegger et al. (1993). Briefly, goethite (0.3 g/L) was suspended in dilute HNO_3 (pH 2.5) and shaken in a rotary shaker (25°C, 120 rpm) for 24 hours. The pH of the goethite mixture was then adjusted to 7.9 (i.e., coating is strongest when pH is between 7 and 8) prior to suspending clay or sand (1.8 g/mL) in it. Then the mixture was agitated for an additional 24 hours prior to centrifugation and drying the coated minerals at 60°C for 2 days. Each bacterial species was inoculated from pure culture into 0.05 excess atom fraction ^{13}C-glucose supplemented M9-minimal liquid media prepared with each of the minerals (1 g/100 mL of media) in sterile serum vials. The vials were then sealed and placed in a shaking incubator on their side at 25°C and 100 rpm for 7 days to permit microbial growth. With 12 bacterial species and 4 minerals this resulted in a total of 48 cultures.

2.3 Necromass preparation and incubation

After 7 days cultures were submerged in a preheated (80°C) hot water bath for 40 minutes to kill the live bacteria cells to generate necromass. The heat-killed cultures were permitted to cool at room temperature (25°C) before centrifuging at 2500 rpm for 15 minutes. Then the supernatant was decanted, the necromass-mineral preparations were dried at 70°C, and then ground with a sterilized spatula. Subsamples of necromass-mineral preparations (~30 mg) were analyzed on a Carlo Erba NC2500 elemental analyzer interfaced with a Thermo Delta V+ isotope ratio mass spectrometer (IRMS) for determination of the $\delta^{13}C$ and %C at the Central Appalachian Stable Isotope Facility (Frostburg, Maryland).

2.4 Mineral aggregation test

Settling rates of the necromass-mineral preparations were determined using the spectroscopy technique described in Lenter et al. (2002) to assess the formation of microbe-mineral aggregates during microbial growth. In brief, prior to drying each heat killed necromass-mineral was shaken to a uniform suspension and 3 mL of solution was collected. Then a 500 µL aliquot was combined with 2.5 mL of DI water in a cuvette. The change in absorption at 600 nm was measured at 5 minute intervals for 1 hr. Before each round data of recording, the spectrophotometer was zeroed using sterilized DI water.

2.5 *Decomposition microcosm experiment*

For the mesocosm study, soil (0-10 cm) was collected from four randomly established 1x1 m plots within a 4 acre pasture at WVU JW Ruby Research Farm, Reedsville, West Virginia. The soil samples were maintained as distinct field replicates and subsamples from each plot received each treatment. This soil was selected because its near neutral pH (6.45-6.84) should minimize the chelation of the Fe-oxides present in goethite coated minerals. After collection the soil was immediately transported to the laboratory in Morgantown, West Virginia. Soil pH and water holding capacity (WHC) were measured as described in Dane and Topp (2020). Field moist soils were sieved (2mm-mesh) and 10 g subsamples were added to Mason jars (350 ml) with septa inserted in the lid. Then each microcosm received 150 mg of dried, ground necromass-mineral preparation and was mixed with a sanitized spatula. Mesocosms were incubated for 28 days in the dark and soil moisture was maintained at 60% WHC. The controls did not receive any substrate (here, necromass-mineral preparations).

2.6 *Microbial respiration measurement*

Decomposition of added necromass in each microcosm was measured via analysis of headspace CO_2 concentration and delta $^{13}CO_2$ similar to Kane et al. (2023). Head space gas samples were collected 0, 2, 7, 14, 21, and 28 days after necromass addition and analyzed on a LI−COR 6400XT (for total CO_2 concentration) and Picarro isotope analysis system (for delta $^{13}CO_2$) sensu Kane et al. (2023). After headspace analysis, mesocosm lids were removed for 10 minutes to refresh the headspace. The atom percent of ^{13}C in the jar headspace was used to calculate the total amount of substrate C (carbon from added necromass-mineral preparation) respiration from per g dry soil. Then cumulative amount of C respired from the substrate over the 28 days period was determined by the sum of individual respiration measured on different sampling days over the 28-days incubation period as described in Morrissey et al. (2017) and the percent respiration from the initial amount of substrate C added was calculated.

2.7 *Data analyses*

All the statistical analyses were performed in RStudio (RStudio Team, 2022) running R 4.1.2 (R Core Team, 2020) using the – *stats* (R Core Team, 2021) and *lme4* (Bates et al., 2015)

packages. Two-way nested model analysis of variance (ANOVA) was performed to find the effects of soil minerals and bacterial morphology on the percent of substrate C respiration from the added substrate, and bacterial species identity was nested within bacterial morphology ($\alpha = 0.05$). The microbial respiration data, combining both the soil minerals treatment and bacterial morphology treatments, were visualized in an heatmap with hierarchical cluster analysis, constructed using the - *pheatmap* (Kolde, 2012) and *heatmaply* (Galili et al., 2018) packages. All other graphs were prepared using the *ggplot2* (Wickham, 2016) package.

3 RESULTS

3.1 Respiration from different necromass-associated minerals

Mineral composition, bacterial morphology, as well as the interaction between these factors, influenced the decomposition of microbial necromass (Table 1, Figure 1) and together could explain ~36% of the variation in necromass decomposition. Necromass derived from individual bacterial species differed in their decomposition patterns as species identity explained an additional 38% of the variation in necromass decomposition. Bacterial necromass decomposition was lowest when prepared with FeOx-coated clay for all bacteria except *Proteus vulgaris* and *Streptomyces africanus.* Gram negative bacterial necromass decomposed most readily in the presence of sand, while Gram positive bacterial necromass decomposition was greatest in the presence of FeOx-coated sand. There were no consistent effects of mineral composition on the decomposition of capsule forming bacteria or filamentous *Actinobacteria*. For instance, necromass of the capsule forming bacterium *Escherichia coli* lost highest percent of C from FeOx-coated clay, whereas another capsule forming species, *Mycobacterium smegmatis*, lost the maximum amount of necromass C from FeOx-coated sand.

3.2 Effect of FeOx-coating on minerals

The presence of FeOx on sand or clay altered decomposition of necromass for roughly half of the bacterial species (Figure 2). The addition of FeOx to clay surfaces reduced the necromass decomposition for five of the seven Gram positive bacteria by ~ 39.75% on average. In contrast, FeOx had the opposite effect on Gram positive necromass decomposition when added to sand. In general, Gram positive necromass decomposition rates were higher for FeOx-

coated sand than uncoated sand. However, *Streptomyces africanus* necromass C decomposition was not influenced by FeOx mineral coating.

Despite choosing twelve bacteria species with four different types of cell morphology, all the bacteria can be classified in two broad groups: either Gram positive or Gram negative. Distinct trends were observed in the decomposition of Gram negative bacteria necromass. Specifically, necromass C mineralization was reduced with FeOx addition to both clay and sand for all Gram negative bacteria except *Proteus vulgaris*. This FeOx-coating of minerals reduced the average respiration of Gram negative necromass C from clay and sand by 11.51% and 30.51%, respectively. Overall, the effect of FeOx-coating on necromass decomposition differed between Gram positive and Gram negative bacteria.

3.3 Trend of necromass C respiration across the species

The capsule forming bacteria we studied included both Gram positive (e.g., *Mycobacterium smegmatis*) and Gram negative (e.g., *Escherichia coli* and *Pseudomonas aeruginosa*) species, while the filamentous bacteria were all Gram positive. In general, Gram positive and Gram negative bacteria necromass followed a common pattern of decomposition across the four minerals. Accordingly, the hierarchical cluster analysis mostly grouped Gram positive and Gram negative bacteria together (Figure 3). Gram positive bacterial necromass C exhibited low decomposition in FeOx-coated clay relative to the other three minerals. Gram negative bacterial necromass C had the greatest decomposition with sand and the least decomposition when grown with FeOx-coated clay. In the presence of sand, the decomposition of Gram negative necromass C was negatively correlated with (adjusted R^2 = 0.72; p-value = 0.04) the sedimentation rate of the necromass-mineral preparation (Figure 4).

4 DISCUSSION

To determine if and how interactions between bacterial cell wall morphology and soil minerals influence necromass decomposition we examined the decomposition of necromass from morphologically distinct bacterial species growth in the presence of minerals with differing surface chemistry. We found that both Gram positive and Gram negative bacteria necromass C decomposes the least when grown with FeOx-coated clay. However, FeOx-coating on sand decreases the decomposition of Gram negative necromass but increases the decomposition of Gram positive necromass, demonstrating interactions between bacterial morphology and soil mineralogy that may influence soil organic matter accumulation.

4.1 Bacteria necromass attachment in minerals

Many Gram positive bacteria have anionic teichoic acids embedded in their cell wall (Brock et al., 2003). Kaolinite crystals are negatively charged due to the isomorphous substitution of Al^{3+} for Si^{4+} and broken bonds of Si-O-Si and Al-O-Al in the crystal structure. These broken bonds and isomorphous substitutions form hydroxyl ions and oxygen atoms with a net negative charge (Moore and Reynolds Jr., 1997; Zhu et al., 2016). Therefore, when exposed to FeOx the positively charged Fe-ions attach to negatively charged kaolinite. Positively charged Fe on the kaolinite might interact with the anionic phosphate groups of teichoic acids facilitating the adsorption of Gram positive necromass to the mineral surface and protecting it from decomposition. This could explain the reduced decomposition we observed for Gram positive bacterial necromass in the presence of FeOx-coated clay.

Though Gram negative bacteria lack teichoic acids, and they possess negatively charged lipopolysaccharides on the cell surface. These negative charges could associate with Fe-ions from goethite and enhance the attachment of bacteria cells to FeOx-coated minerals decreasing the decomposition of bacterial necromass. Indeed, di- or trivalent cations have been shown to enhance the adsorption of negatively charged extracellular polysaccharides (EPS) to negatively charged clay minerals (Santoro and Stotzky, 1967). Our results support this possibility as FeOx-coating on clay reduced necromass decomposition for some Gram negative bacteria (Figure 2).

Overall, the decomposition of necromass C was higher for sand and FeOx-coated sand than for clay or FeOx-coated clay (Figure 4). The FeOx-coated sand provided the least protection to Gram positive necromass C, hence made them readily available to the decomposers. Kaolinite has comparatively higher surface area than quartz, which might enhance reactive surface area and permits the adsorption of more positive ions followed by more bacteria cells. Several studies reported that large surface area and net negative charge stimulates bacteria growth on clay minerals, which facilitates surface adhesion (Cuadros, 2017; Mueller, 2015). Additionally, the CEC of minerals can affect the adsorption of organic matter (Satterberg et al., 2003) and some studies have reported the superiority of kaolinite in organic matter adsorption over other minerals like smectite (Feng et al., 2005; Zhou et al., 1994). Quartz has a lower CEC than kaolinite. As such, the greater CEC of kaolinite may have facilitated bacterial attachment and adsorption of the resultant necromass, reducing necromass decomposition.

4.2 FeOx-coating affected necromass C decomposition

The FeOx-coating altered substrate C respiration from coated minerals relative to their uncoated parent mineral (Figure 2). Saidy et al. (2013) reported that goethite coating enhanced dissolved organic carbon sorption capacity of kaolinite. Chen et al. (2009) also found that degradation of adsorbed carbaryl was the lowest in goethite, followed by kaolinite and montmorillonite. In addition to influencing organic matter adsorption, FeOx may also have an impact on bacterial attachment and colonization. For instance, Fe content has been found to facilitate bacterial colonization, biomass formation, and the composition of bacterial communities (Gleeson et al., 2006; Phillips-Lander et al., 2014). Ten of the twelve bacteria species had lower necromass C decomposition when clay was coated with FeOx (Figure 2). This decrease in decomposition suggests FeOx on the clay surface allowed bacterial cells to attach more readily during their growth in the minimal media.

For sand, FeOx-coating reduced the necromass decomposition for most Gram negative bacteria but increased the decomposition of Gram positive bacterial necromass (Figure 2). As quartz has a low surface charge, FeOx complexes may not have attached to the surface of the sand particles. If the FeOx remained suspended in the media during bacterial growth this may

have reduced the ability of Gram positive bacteria to attach to and grow on the sand, thus reducing mineral protection of the resulting microbial necromass.

Other mechanism, like microbe-mineral aggregate formation may have influenced necromass decomposition. The Gram negative bacteria necromass decomposition in the presence of sand was correlated with the sedimentation rate of the necromass mineral preparation (Figure 4). As smaller particles settle more slowly (Gee and Or, 2002; Loveland and Whalley, 2000; Merkus, 2009), the accelerated sedimentation rates of some necromass mineral preparations likely reflect the formation of microbe-mineral aggregates with greater particle sizes (Dong et al., 2022; Liu et al., 2021; Sparks, 2012). Aggregation may cause some bacterial necromass to become trapped inside clusters of sand particles. While the dried necromass-mineral preparations were lightly ground we did not ensure a uniform particle size in the necromass-mineral powder. Consequently, the negative relationship between sedimentation rate and decomposition we observed for Gram negative bacteria in the presence of sand (Figure 4) suggests aggregate formation by bacteria may have resulted in the occlusion of microbial necromass, reducing rates of decomposition.

4.3 Defying the trend, Mycobacterium and Streptomyces

Clustering of Gram positive *Mycobacterium smegmatis* and *Streptomyces africanus* with Gram negative bacteria (Figure 3) was also observed. The cell wall morphology of these two Gram positive bacteria is inconsistent with the typical Gram positive bacteria. Despite being Gram positive, the decomposition of necromass from *Mycobacterium smegmatis* was similar to that of Gram negative bacteria necromass across all four minerals (Figure 3). This might have occurred because of *Mycobacterium smegmatis*'s atypical cell wall structure. Despite being a Gram positive bacterium, *Mycobacterium smegmatis* possesses a thin peptidoglycan layer and has an extensive outer membrane of long, branched fatty acids named mycolic acids (Singh et al., 2016). These two cell wall properties likely make the necromass derived from *Mycobacterium smegmatis* distinct from that of typical Gram positive bacteria and perhaps more similar to that of Gram negative bacteria.

Another discrepancy was observed in case of *Streptomyces africanus*. This species did not show any impact of FeOx-coating and decomposed more rapidly on sand than on clay. The mycelium produced by this filamentous *Actinobacteria*, might have played the pivotal role for such necromass decomposition pattern. Streptomycetes species form vegetative hyphae in liquid-rich environments to attach to different surfaces like soil particles, organic debris (Petráčková et al., 2013). Additionally, vegetative hyphae of streptomycetes generally have hydrophilic cell surfaces while the aerial hyphae are mostly hydrophobic (Del Sol et al., 2007; Elliot et al., 2003) making them distinct from most Gram positive bacteria.

Streptomyces with smooth vegetative hyphae can attach to silica surfaces using extracellular matrix secretion (Del Sol et al., 2007). These unique characteristics of Streptomyces may enable these bacteria to firmly attach to mineral surfaces even in the absence of FeOx.

4.4 Future directions

Future work leveraging scanning electron microscopy could provide crucial insights into how the microbial necromass is attached to mineral surfaces. Additionally investigating more bacteria representative of a wide range of cellular morphologies could enhance our understanding of microbial necromass persistence period in soil. In addition, the changes in soil microbial community upon introduction of any specific group of bacteria necromass might prove helpful in soil-microbe interaction research. Besides, exploring more soil minerals with known bacterial necromass can help identify the contribution of any specific group of bacteria that is essential to restore soil carbon in any soil.

5 CONCLUSION

In contrast to prior works (e.g., Buckeridge et al., 2022; Creamer et al., 2019; Wang et al., 2020), this study simulated necromass formation more accurately, by growing the bacteria in the liquid culture with minerals and then heat killing. Our results demonstrate that mineral composition greatly influences necromass decomposition. Notably FeOx-coating has enhanced necromass binding capability in kaolinite for most Gram positive and Gram negative bacteria; however, FeOx-coating of sand enhanced the decomposition of Gram positive necromass C. In contrast, Gram positive necromass C was more persistent than Gram negative necromass C when

generated with clay, sand, and FeOx-coated clay. As such our results demonstrate that necromass mineral attachment for organic carbon stabilization is not only mineral specific, but also bacterial cell wall morphology specific. These results suggest that microbial biodiversity, and resulting variation in cell morphology can interact with soil mineralogy to determine soil carbon persistence. With further study, microbe-mineral interactions may enhance our ability to model and manage necromass carbon in soils.

6 FIGURES

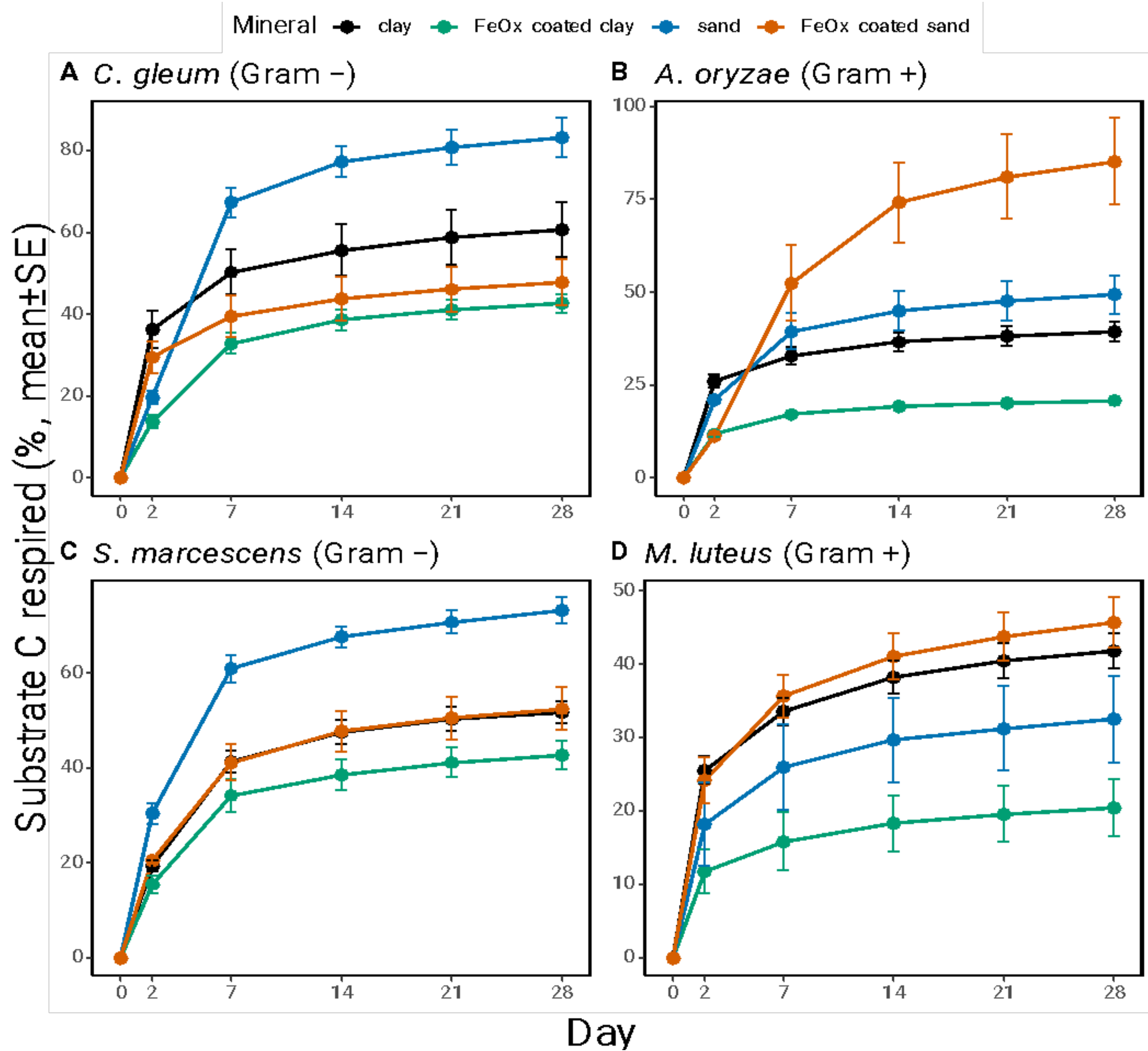

Figure 1. Decomposition of microbial necromass C substrate of four bacteria species including the Gram negative *Cryseobacterium gleum* (A) and *Serratia marcescens* (C); and Gram positive – *Arthrobacter oryzae* (B) and *Micrococcus luteus* (D) prepared with kaolinite-clay, FeOx-coated kaolinite clay, quartz sand, and FeOx-coated quartz sand. All the data points represent the cumulative percent C respiration (mean±SE) from the added necromass.

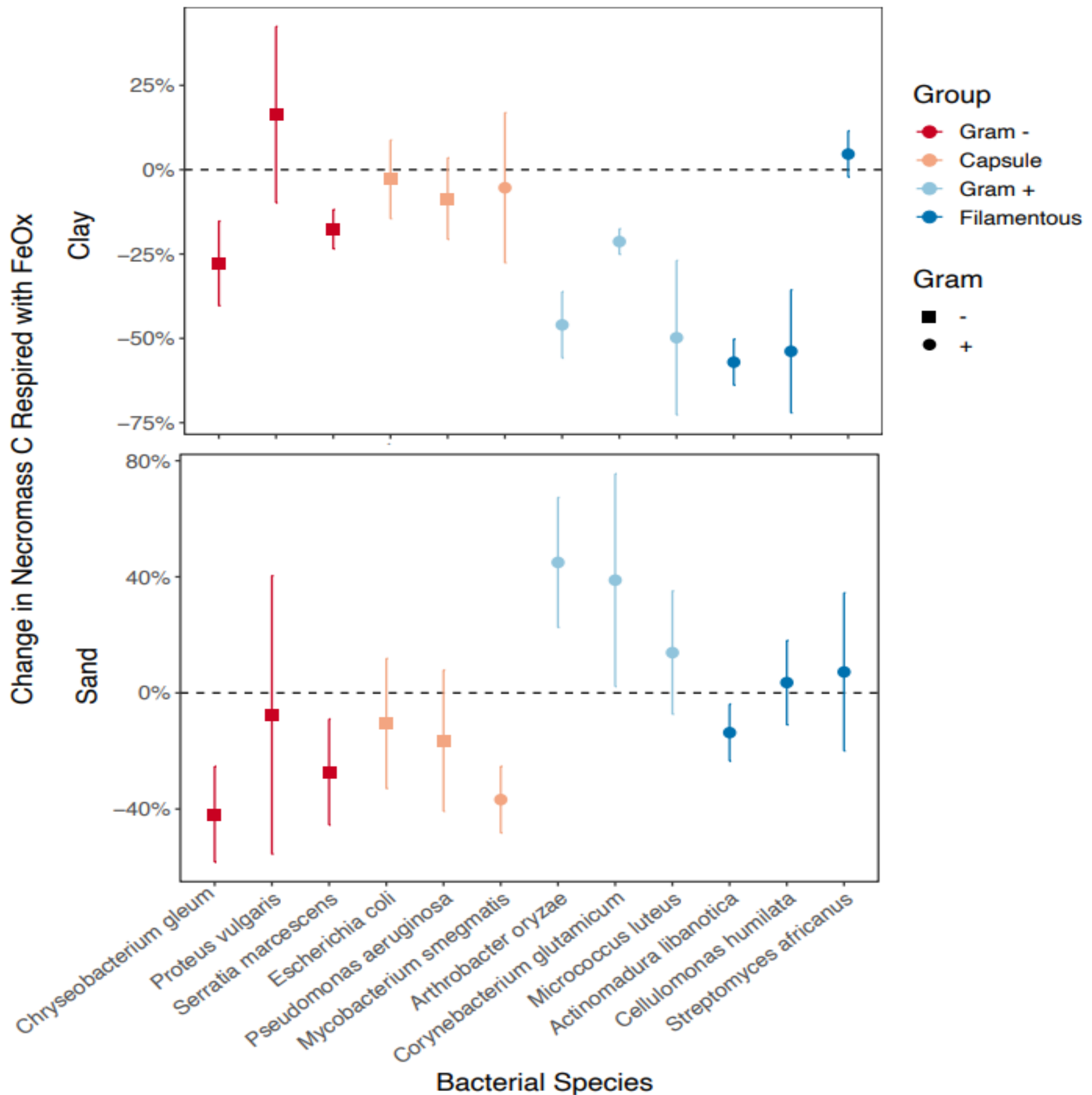

Figure 2. Change in necromass C respired with FeOx-coating on kaolinite clay and quartz sand after 28 days of decomposition. All the data points represent the percent change in the cumulative necromass C respiration (mean±SE). Color indicates the morphological group (Gram negative bacteria, capsule forming bacteria, Gram positive bacteria, and filamentous *Actinobacteria*) while shape identifies species as Gram positive or Gram negative.

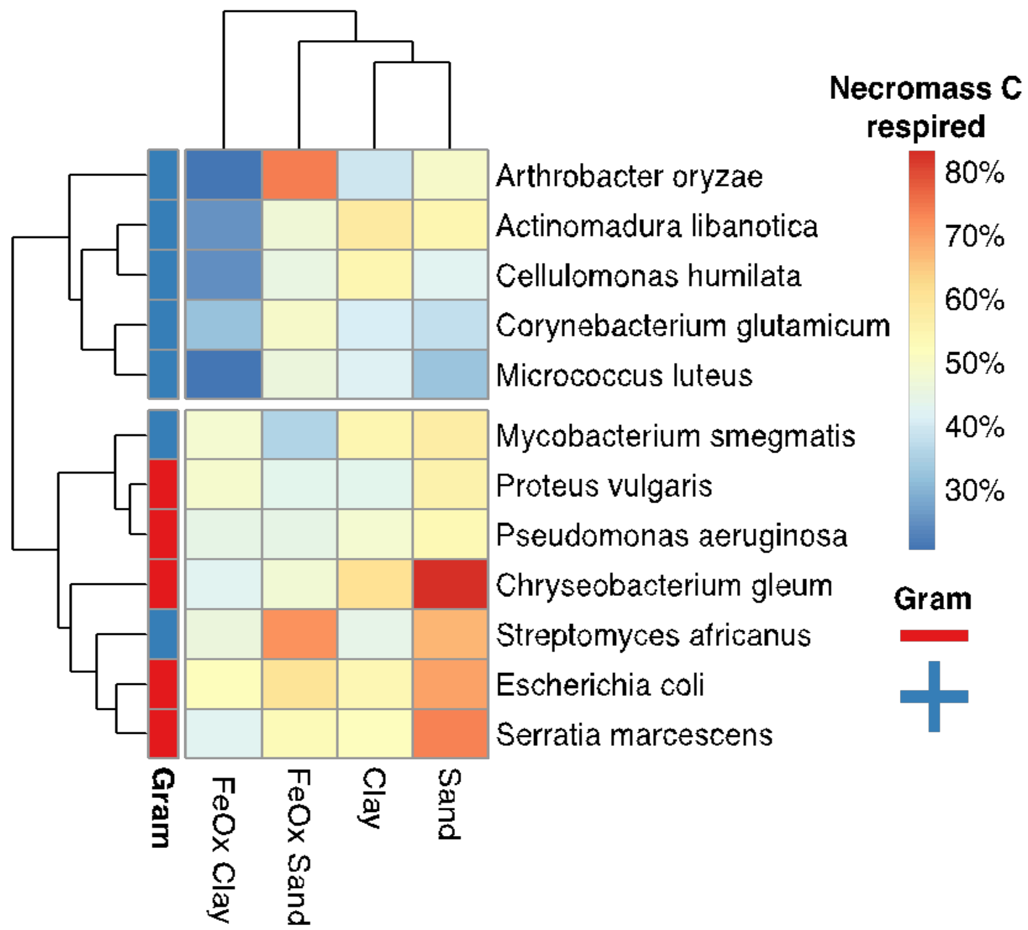

Figure 3. Heatmap of the cumulative necromass C respiration (%) after 28-days in a microcosm experiment with 12 bacteria species (in right Y-axis) grown with 4 minerals (in X-axis). Bacteria species are clustered according to their necromass C decomposition across the minerals.

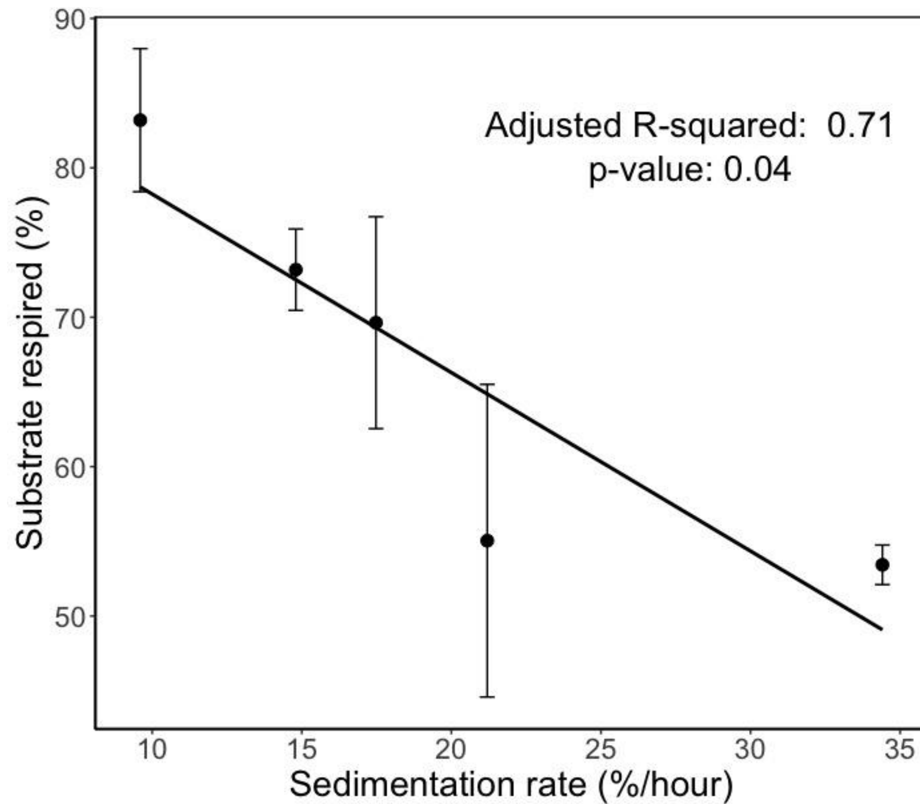

Figure 4. Correlation between sedimentation rate (percent change per hour) of liquid necromass-mineral preparations, after heat killing the bacteria in liquid media, and percent of the initial substrate necromass C respiration (Y-axis) for Gram negative bacteria grown in the presence of sand. All the data points represent the cumulative percent C respiration (mean±SE) from the added necromass.

7 TABLES

Table 1. The influence of mineral type, cell morphology, and species identity on the percent of necromass C respired as determined by two-way Nested ANOVA model. *** represents p value <0.001.

Factor	**F-value**	**% Explained**
Mineral	40.35***	21.00
Morphology	24.29***	12.65
Mineral x Morphology	9.71***	15.17
Mineral x Morphology :Bacterial Species	4.74***	26.34

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