Picky Eaters: Carbon isotopic evidence for the uniform bioavailability of riverine dissolved organic matter to a model marine microorganism

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Key points

- Riverine dissolved organic matter (DOM) from distinct rivers shows similar bioavailability to marine bacterium.

- Radiocarbon values of microbially respired carbon dioxide during incubations reveal preferential utilization of modern carbon compounds.

- The fate of riverine DOM in coastal environments may depend on the metabolic potential of microorganisms that are present and active.

Abstract

Dissolved organic matter (DOM) is a key component of the global carbon cycle, with rivers delivering significant amounts of DOM to oceans. Urbanization and agricultural land-use alter the age and chemical composition of riverine DOM, which likely impact the downstream bioavailability of riverine DOM. Here, we use bioreactor incubations of a marine bacterium (*Pseudoalteromonas sp. 3D05*) to investigate DOM bioavailability from two distinct rivers: the Suwannee River (natural, non-urbanized), and the Upper Mississippi River Basin (anthropogenically influenced). We measured rates of microbial CO_2 production and radiocarbon ages (as $\Delta^{14}C$) to assess bioavailable DOM remineralization. We observed nearly identical cell densities and degradation patterns for both riverine DOM incubations. Respired DOM $\Delta^{14}C$ values were also similar and decreased over time indicative of preferential utilization of recently synthesized “modern” substrates. These findings reveal unexpected similarities in riverine DOM bioavailability, indicating similar short term biological reactivity despite large DOM compositional differences.

Plain Language Summary

Our study explores the relationship between anthropogenic activity and breakdown of dissolved organic matter (DOM) from rivers, which represents a vital link between land and ocean ecosystems. The composition of DOM in rivers is linked to the characteristics of the surrounding land. Urbanization and agricultural land-use change the age and chemical composition of the riverine DOM. Consequently, these alterations induced from human activity would be expected to impact the bioavailability of riverine DOM to microorganisms in coastal environments. We compared DOM sourced from a natural river system (Suwannee River) to DOM from a river impacted by anthropogenic activity (Upper Mississippi River Basin) to understand how the availability of DOM from these distinct rivers varies to marine microorganisms. We carried out laboratory experiments with a model marine bacterium and measured the respiration of carbon dioxide and associated isotopic signatures during the breakdown of riverine DOM. Surprisingly, we discovered striking similarities in the breakdown patterns of DOM from both rivers, despite their differing origins. This suggests that the impact of human activities on downstream transformation of DOM may not be as straightforward as previously assumed and underscore the need for a nuanced understanding of how microorganisms process DOM in coastal environments.

1. Introduction

Rivers act as vital conduits for the transfer of organic carbon from terrestrial to marine ecosystems. Annually, it is estimated that substantial amounts of carbon (~250 Tg C) are transported to the global ocean via riverine dissolved organic matter (DOM) (Hedges et al., 1997; Schlesinger & Melack, 1981). Despite this significant input, a large proportion of this riverine material is remineralized by microorganisms and/or photochemically altered in the coastal ocean (Mopper et al., 2015; Moran et al., 2000). As a result, less than 10% of the DOM present in the ocean originates from terrestrial sources (Meyers-Schulte & Hedges, 1986; Opsahl & Benner, 1997; Williams & Druffel, 1987). Thus, the transformation of riverine DOM within coastal environments plays a crucial role in regulating the exchange of carbon and nutrient flows between terrestrial and marine ecosystems.

Riverine DOM is derived from leaf litter, grass, soil, freshwater algae as well as anthropogenic inputs. Consequently, the composition of riverine DOM is shaped by a myriad of factors including river morphology, basin lithology, nutrient loading, microbial activity, land-use and agriculture (human impacts; (Parr et al., 2015; Williams et al., 2010). In fact, human activity has been shown to severely impact the input, composition, and dynamics of riverine DOM (Parr et al., 2015; Vaughn et al., 2021; Williams et al., 2010). High resolution mass spectrometry has revealed that rivers originating from natural forest water sheds contain DOM with higher concentrations of condensed aromatics and polyphenolics which reflect greater contributions from soils and vegetation (Coppola et al., 2015, 2018; Opsahl & Benner, 1997; Vaughn et al., 2021). In contrast, rivers impacted by urbanization and agricultural activities exhibit different molecular signatures in the DOM, including a higher relative abundance of protein-like and aliphatic compounds as more sulfur- or nitrogen-containing heteroatoms (Roebuck Jr et al., 2019; Vaughn et al., 2021, 2023). Most riverine DOM is modern (e.g. soil organic carbon, vegetation debris) whereas anthropogenic inputs (e.g. wastewater, agrochemicals) and lithogenic

OC contributions (e.g. shales) are ^{14}C-free which can lead to the re-introduction of aged carbon into the modern carbon cycle (Butman et al., 2012). Recent work suggests pre-aged DOM accounted for 3-9% of bulk riverine DOM exported from watersheds that were heavily impacted by human activity (Butman et al., 2015).

Historically, riverine DOM has been considered recalcitrant, or less bioavailable, for microbial consumption due to its origins from soils and aquatic plants (Blanchet et al., 2017; Moran & Hodson, 1990). Extensive research over the two decades has demonstrated the capacity of microorganisms, particularly heterotrophs, to consume and remineralize DOM (Amon & Benner, 1996; McCallister et al., 2004; Moran et al., 2000; Raymond & Bauer, 2001a, 2001b). The specific mechanisms that mediate the bioavailability of DOM to microorganisms is still unclear. A multitude of chemical and physical properties have been shown to influence the bioavailability of DOM in aquatic environments including chemical structure/diversity, molecular size and sample history (fresh vs. aged organic material). Previous studies have found that DOM influenced by urban and agricultural activities typically exhibits a lower molecular weight and a higher proportion of labile components such as aliphatic and peptide-like compounds (Parr et al., 2015; Wagner et al., 2015; Wilson & Xenopoulos, 2009). In contrast, DOM from pristine river environments tends to have a greater concentration of aromatic compounds such as polyphenols which are thought to be more resistant to microbial degradation (Butman et al., 2012; Wagner et al., 2015). Consequently, chemical alterations induced from human activity would be expected to impact the bioavailability and downstream transformation of riverine DOM in coastal areas (Riedel et al., 2016). To the best of our knowledge, this has yet to be directly tested via controlled microbial incubations studies.

In this study, we use a novel bioreactor system (Isotopic Carbon Respirometer-Bioreactor; IsoCaRB, (Beaupré et al., 2016) to investigate the bioavailability and carbon isotopic composition of respired riverine DOM to a model marine isolate. We focus on two contrasting

different river systems: the Suwannee River system which is a natural, unaltered river and the Upper Mississippi River system that is highly altered and anthropogenically impacted. We discuss these results in the framework of riverine DOM bioavailability, respiration rates and the isotopic composition of labile DOM removed during each incubation.

2. Materials and Methods

2.1 Study locations and setting of riverine DOM

DOM Samples from Suwannee River (SR) and the Upper Mississippi River (UMR) were sourced from the International Humic Substances Society (IHSS). The SR is a blackwater river that runs southwestward from the Okenfenokee Swamp in Georgia to the Gulf of Mexico through Florida. This slow moving, river is characterized by high levels of terrestrially derived OM including high humic compound concentrations representative of low anthropogenic input (Cawley et al., 2013; Green et al., 2015). Suwannee River DOM (SR DOM; #2R101N) was collected in 2012 by the IHSS at the southernmost dam on the Suwannee River sill in Fargo, Georgia, a location chosen for its sparse human population (Figure 1; Green et al., 2015). SR DOM was extracted on-site with reverse osmosis systems, desalinated using cation exchange (CEX) and then subsequently, freeze-dried and homogenized for storage (Green et al., 2015). SR DOM underwent elemental analysis and was determined to contain ~50.7% C (IHSS; Table S1).

The Mississippi River is one of the largest river systems in the world, running southward and draining watersheds from about 30 states in the USA into the Gulf of Mexico. The Upper Mississippi River (UMR) system carved by glaciers thousands of years ago, is now highly altered by human actions converting the surrounding terrain into agricultural or urbanized land (Fremling et al., 1989; Vaughn et al., 2021). Since the 1950s, the UMR has experienced significant landcover transformations primarily due to the expansion of agricultural lands (Ramankutty & Foley, 1999; Schnitkey, 2013; Wright & Wimberly, 2013) and urbanization due

to a steady rise in population (Eathington, 2010). Upper Mississippi DOM (UMR DOM; #1R110N) was collected in 2013 in Minneapolis, Minnesota using combined reverse osmosis/electrodialysis system to process river water. Final concentrated samples were then desalinated and freeze dried. Elemental analysis indicates the UMR DOM contains ~49.98% C (IHSS). Compared to SR DOM, UMR DOM contains 1.85 times the nitrogen atoms and 1.47 times the sulfur atoms, highlighting the anthropogenic input to the DOM of the Upper Mississippi River system (Table S1; IHSS; Wagner et al., 2015).

2.2 Cultivation of a model marine microbe

DOM incubations were carried out with a bacterial isolate (*Pseudoalteromonas sp.* 3D05) that was previously isolated from coastal ocean water samples (Canoe Beach, Nahant, MA; 42°25′11.5″ N, 70°54′26.0″ W) (Datta et al., 2016). This strain was selected based on its metabolic capability to degrade a wide spectrum of carbon substrates. Previous genomic analysis revealed that this marine isolate possesses multiple gene copies for extracellular enzymes involved in the hydrolysis of proteins, carbohydrates, and chitin (Mahmoudi et al., 2020). Cells were grown from frozen glycerol stocks that were thawed and added to 25 mL of marine broth 2216 (Difco #279110) in a combusted 125 mL flask and left to shake (145 RPM) at room temperature until log-phase. Subsequently, 500 µL of culture (1% inoculation) was transferred to 50 mL of modified Tibbles-Rawling (T-R) minimal media (Table S2) with glucosamine (0.5% w/v) as a carbon source in a 250 mL flask and left to shake until log phase was reached. Subsequently, cells were transferred to fresh modified T-R minimal media and grown until reaching mid-log phase. Cell density was monitored by measuring optical density (OD) 600 nm, based on a calibration curve between OD and colony forming units (CFUs). Once cultures had reached the desired cell density, a total of 50 mL of cells were harvested for injection into the IsoCaRB system. Prior to injection, cells were washed two times with modified T-R media containing no carbon sources. Briefly, the culture was centrifuged for 10 minutes at 3000 xg

(Beckman Coulter Allegra X-30R Centrifuge) and the supernatant was decanted. The resulting cell pellet was resuspended in 1 mL modified T-R media containing no carbon source and injected into the IsoCaRB system using a sterile 3 mL syringe (BD Biosciences # 309657) and a 20-gauge needle (BD PrecisionGlide^{TM}).

2.3. Bioreactor incubations

A series of incubations using SR DOM or UMR DOM was carried out using the IsoCaRB system. The IsoCaRB system is comprised of a gas delivery and purification system, a custom Pyrex culture vessel, an inline CO_2 detector and integrated LabVIEW data-logging program, custom CO_2 traps, and a vacuum extraction line. Microbially respired CO_2 is continuously collected as successive fractions in custom molecular sieve traps. Subsequently, CO_2 is recovered from the traps by baking (530°C for 30 min) under vacuum within 24 h of collection, then cryogenically purified, quantified, and stored in flame-sealed Pyrex tubes for isotopic analysis (see 2.4). Each experiment is allowed to proceed until CO_2 concentrations resume near-baseline values. Gaseous CO_2 concentration measurements are corrected for baseline drifts and then rescaled to agree with the higher-precision manometric yields obtained from the trapped CO_2 as previously described (Beaupré et al., 2016). The normalized CO_2 concentrations are then corrected for the confounding effects of mixing in the culture vessel headspace and decreasing media volume to constrain the rate of CO_2 generation per unit volume of growth medium (μg C L^{-1} min^{-1}), which serves as a proxy for the microbial CO_2 production rate. Details regarding the standard operating procedure for the IsoCaRB system, including sterilization and assembly, sample preparation, and CO_2 collection and purification are described in Beaupré et al. (2016).

For each experiment, 700 mg of SR DOM or UMR DOM and live cells were incubated at room temperature (~22°C) in the IsoCaRB system. Based on the elemental composition of samples, this resulted in a total DOM concentration of 14.8 mM and 14.6 mM in the UMR DOM and SR DOM incubations, respectively. The slurry was continuously stirred (90 rpm) under

aerobic conditions to provide an unlimited supply of O_2. A total of five CO_2 fractions for $\Delta^{14}C$ analysis was collected during incubation with UMR DOM and SR DOM (Table S3). In addition, the slurry was subsampled via the sampling port every 12 h to track the number of viable cells via plate counts. Approximately 100 µL of slurry was serially diluted to 10^{-5}. Subsequently, 100 µL was plated in triplicate onto MB2216 agar plates and spread with rattler beads (Zymo #S1001). Plates were allowed to grow at room temperature (~22°C), and colony forming units (CFU) were counted after 48 h to determine cell density at the time of sampling.

Abiotic production of CO_2 from the DOM samples resulting from off-gassing was quantified by carrying out a control incubation for both SR DOM and URM DOM samples in the absence of live cells. Approximately 700 mg of DOM (SR or UMR) and 2 l of modified T-B media were incubated in the IsoCaRB system. The DOM-media slurry was sparged for 72 hours in a manner identical to the incubations with live cells. After the completion of sparging, a molecular sieve trap was attached to the system to collect any CO_2 for the same duration of the incubations with live cells (~ 4 days). The resultant CO_2 fraction was quantified manometrically and sent for $\Delta^{14}C$ analysis. The $\Delta^{14}C$ values of microbially respired CO_2 that were observed during incubation with SR or UMR DOM were subsequently corrected using this respective value as described in Mahmoudi et al. (2017).

2.4 Isotopic analysis of microbially respired CO_2

CO_2 fractions collected during incubations were sent to Keck-Carbon Cycle Accelerated Mass Spectrometry (AMS) facility at the University of California, Irvine for $\Delta^{14}C$ analysis. CO_2 was converted to graphite using the sealed-tube Zn method (Walker & Xu, 2019; Xu et al., 2007). Radiocarbon values (^{14}C) are reported in $\Delta^{14}C$ notation and corrected for year of measurement following the conventions set forth by Stuiver and Polach (1977). Prior to proceeding with the DOM incubations, *Pseudoalteromonas sp.* 3D05 was incubated in the IsoCaRB system with an isotopically characterized carbon substrate (glucosamine; $\Delta^{14}C$ = +33‰ ± 1.3). This incubation

was done in an identical manner to the DOM incubations. Analysis of the resulting CO_2 fraction from this test was determined to be $\Delta^{14}C$ = +30‰ ± 1.3, which confirmed the robustness of our experimental set up.

3. Results and Discussion

3.1 Microbial remineralization of DOM from distinct river systems

Anthropogenic activity can alter DOM composition (Jaffé et al., 2012; Lambert et al., 2015; Mattsson et al., 2009; Riedel et al., 2016; Wagner et al., 2015), but exactly how this may impact downstream processing by microorganisms in coastal and marine environments has yet to be tested. We experimentally tested how the bioavailability and remineralization of riverine DOM may vary to marine microorganisms due to constrasting watershed characteristics. The UMR has been significantly altered by human activities, with its surrounding areas converted into agricultural and urbanized land (Ramankutty & Foley, 1999; Schnitkey, 2013; Wright & Wimberly, 2013). Conversely, the SR is characterized by high levels of terrestrially derived organic matter and humic compounds, reflecting minimal anthropogenic impact. Interestingly, we observed nearly identical respiration patterns during bioreactor incubations with *Pseudoalteromonas sp.* 3D05 and SR DOM or UMR DOM. CO_2 production quickly increased to peak 0.7-0.9 μg C L^{-1} min within the first 10-16 hours of each incubation and then, progressively decreased to near baseline CO_2 levels within 4.5 days. Similarly, cell densities were comparable between both SR DOM and UMR DOM incubations. There was a steady increase in cell density during the initial half of the incubation, reaching a peak of 4.9-5.7 x 10^7 CFU/mL, follwed by a gradual decline for the remainder of the incubation period (Fig. 2). This decline in cell density is consistent with previous bioreactor work (Mahmoudi et al., 2020) and expected for a batch system (i.e. no replenishment of nutrients).

The rate of CO_2 production by microorganisms reflects of balance between catabolic and anabolic processes – this is often referred to as carbon use efficiency (CUE). When microorganisms transform organic matter a fraction of carbon is incorporated into biomass and the rest is respired as CO_2. The exact cellular mechanisms that underlie CUE remain unclear as it has been shown to vary with environmental factors (e.g. temperature), the supply and complexity of substrates (nutritional and elemental composition, as well as energy content), and on the biochemical pathway of degradation and assimilation (Keiblinger et al., 2010; Roels, 1980; Russell & Cook, 1995). For example, simple and easily metabolizable compounds may result in higher CUE as more assimilated carbon is directed toward biomass synthesis, leading to increased cell density, rather than CO_2 production. In contrast, the consumption of complex substrates that necessitates the production and secretion of extracellular enzymes would require more energy investment for degradation (Allison et al., 2014). This would result in lower CUE as a greater proportion of assimilated carbon is directed toward energy generation through respiration rather than biomass, resulting in a lower cell density. Our observations of nearly identical cell density and CO_2 respiration rates indicate that efficiency in which this bacterium assimilated carbon into biomass was similar irrespective of the pool of DOM. This implies that microorganisms accessed and consumed carbon substrates that were similar in their energy expenditure across both DOM pools.

The percent loss of DOM (based on the total quantity respired CO_2 from the total C pool; Table 1) showed striking similarity between incubations with SR and UMR DOM, with losses of 1.2 ± 0.01% and 1.3 ± 0.01%, respectively. Previous microbial incubations involving samples from ten different rivers observed DOM losses of 21% (±5%) over the span a year (Riedel et al., 2016). Given the shorter duration of our experiment (i.e. days), our observed DOM losses align with expectation for shorter incubation periods. For example, 28-day bottle incubations of Suwannee River samples resulted in a DOM loss of 3% (Textore et al., 2018). Nevertheless, the

similarity in total DOM loss between the SR and UMR DOM incubations is both surprising and unexpected. Considering that both incubations had the same experimental conditions (e.g. temperature, incubation time, genetically identical bacterial cells, cell density, nutrient conditions, etc.), DOM composition was assumed to be the only variable determining the riverine DOM bioavailability. Our findings suggest that the bioavailability of both DOM pools did not differ substantially. Past DOM chemical characterization studies have found that urban- and agriculturally-impacted DOM contains lower molecular weight, higher relative aliphatic and peptide-like compounds and lower aromatic compounds in comparison to pristine river environments (Parr et al., 2015; Vaughn et al., 2021; Wagner et al., 2015). For example, a recent study by Vaughn and co-workers (2023) conducted 28-day incubations using streams draining from three dominant landcovers (forested, agriculture and urban) in the Upper Mississippi River Basin. They observed greater DOM loss in riverine samples collected from urbanized (10 ± 4.4%) and/or agricultural (7.6 ± 3.1%) landscapes compared to more pristine, forested landscapes (5.6 ± 3.2%). If we assume anthropogenically influenced DOM is more bioavailable than natural riverine DOM (e.g. terrestrial plants and organic-rich soils) (Butman et al., 2012; D'Andrilli et al., 2015; Riedel et al., 2016; Textor et al., 2018; Wagner et al., 2015), then we would expect differences in the amount of DOM respired.

The bioavailability of DOM to microorganisms is dictated by complex interplay of chemical, physical, and biological factors that ultimately determine the rate and extent of its degradation and utilization in aquatic environments. Our results may imply two plausible interpretations. Firstly, they suggest a similar pool of bioavailable DOM—both in quantity and quality—across distinct river systems, which diverges from prior studies linking DOM reactivity to catchment properties (Butman et al., 2012; Riedel et al., 2016; Vaughn et al., 2023; Williams et al., 2010). Moreover, the reactive portion in DOM has been shown to be molecularly very dissimilar between rivers such that each system has a unique group of compounds that is labile or

produced (Riedel et al., 2016). Alternatively, our results suggest that the marine bacterium employed in our study might exhibit limitations in its capacity to degrade certain DOM substrates selectively. Microbes possess variable enzymatic capabilities that directly impacts their ability to break down specific molecular classes of DOM (Allison et al., 2014). Despite potential differences in the chemical composition of DOM between the two rivers, the marine bacterium in our incubations might be constrained to consuming similar compounds from both DOM pools. This aligns with the evolving hypothesis that the diversity and abundance of specific microbial species in a given environment impacts the degradation and remineralization of organic matter (Carlson et al., 2004; Glassman et al., 2018; Mahmoudi et al., 2020). While prior incubation studies have observed significant differences in DOM loss between natural and anthropogenically influenced river samples (Riedel et al., 2016; Vaughn et al., 2023), these studies employed the native river water as the inoculum which likely contain disparate microbial communities. As a result, the differences in DOM loss might be attributed to disparities in the enzymatic capabilities of the microbial populations in each river sample. Taken together, our results suggest that the remineralization of riverine DOM may be heavily influenced by the degradation pathways of the active microbial communities, rather than by variations in DOM composition originating from different watersheds.

3.2 $\Delta^{14}C$ signatures of microbially respired CO_2

Unraveling the relationships between the characteristics of DOM and its susceptibility to microbial utilization is complex and challenging to address. Natural abundance ^{14}C analysis is a robust tool for resolving the sources and ages of natural organic matter consumed and assimilated by microorganisms (Mahmoudi et al., 2013a, 2013b; Mailloux et al., 2013; Mindorff et al., 2023; Pearson et al., 2008; Petsch et al., 2001; Wakeham et al., 2006). This approach is grounded in the fact that heterotrophic microorganisms carry the same ^{14}C signatures as their carbon sources. Thus, the ^{14}C signatures of both microbial cellular components (e.g., membrane

lipids) and respired CO_2 can be used to infer microbial utilization of isotopically-distinct carbon sources (Hayes, 2001). We employed this approach to evaluate the ^{14}C age of microbially respired CO_2 during our incubations thereby allowing us to directly compare the bioavailability of DOM to its ^{14}C age. The ^{14}C age of DOM is thought to be an important factor in terms how easily microorganisms can access and utilize the organic compounds. Older organic material is thought to have undergone various transformations that make it less accessible to microorganisms while younger material is composed of recently photosynthesized compounds that are more readily degradable compounds.

The observed $\Delta^{14}C$ values of respired DOM CO_2 ranged from +34 to +14‰ for SR DOM and from +24 to +14‰ for UMR DOM. During both incubations, respired DOM $\Delta^{14}C$ values became more negative over time – by precisely 10‰. Recent work suggests northern hemisphere CO_2 $\Delta^{14}C$ values were between +30 and +40‰ in 2012 and 2013 when SR DOM and UMR DOM were collected (Hua et al., 2022). This indicates preferential utilization of recently synthesized "modern" riverine DOM by microorganisms in our incubations. These $\Delta^{14}C$ findings are consistent with previous time series incubations (Raymond & Bauer, 2001c) and biomarker approaches (Cherrier et al., 1999) that have observed microorganisms preferentially consuming DOM that is younger than the bulk DOM pool during the degradation.

In our study, the observed $\Delta^{14}C$ signatures of respired CO_2 are consistent with utilization of modern carbon compounds that are likely derived from recently photosynthesized sources (e.g. algae, plants, etc.). In the case of UMR DOM, these $\Delta^{14}C$ values were very similar to the bulk DOM $\Delta^{14}C$ value (29‰ ± 3), suggesting that microorganisms consumed the freshest material within this DOM pool. However, the $\Delta^{14}C$ signatures of respired CO_2 during the SR DOM were more negative (by ~20‰) compared to the bulk DOM $\Delta^{14}C$ value (+54 ± 3‰). This implies the presence of younger organic compounds, plant-derived compounds, in SR DOM that might be less accessible to *Pseudoalteromonas sp. 3D05*. SR receives substantial inputs of

terrestrially derived organic matter which is enriched lignin-derived polyphenols. *Pseudoalteromonas sp. 3D05* lacks genes for known enzymes (e.g. dioxygenases, xylosidase, cellobiase) that are needed to break down newly synthesized plant and terrestrial inputs. Thus, it is likely forced to consume compounds that may be slightly older in ^{14}C-age (lower $\Delta^{14}C$) but may be easier to access based on its enzymatic repertoire.

4. Summary and Implications

The anticipated increase in precipitation and flood events with climate change may amplify the export of anthropogenically-influenced DOM exported to marine ecosystems (Bianchi et al., 2013; Parr et al., 2015). Our DOM bioreactor experiments, allow for the direct comparison of anthropogenically influenced vs. natural river DOM microbial remineralization in coastal ocean conditions. Our monoculture experiments reveal that the majority of terrestrial DOM can readily escape remineralization on short timescales, irrespective of variations in DOM composition stemming from different watersheds. Thus, our findings suggest that the remineralization of riverine DOM in coastal environments may largely depend on the metabolic potential of the microbial community is both present and active. This is in combination with environmental factors that might also facilitate access to labile DOM (e.g. photochemical oxidation, Moran & Zepp, 1997). In addition, our results further corroborate the concept of an age-reactivity continuum (e.g. Walker et al., 2016), wherein microorganisms initially consume the younger, more bioavailable compounds before progressing to metabolize progressively older DOM compounds. Future work examining the molecular level information (i.e., molecular-level formula, functional groups, and structures) of the DOM in our experiments will help shed light on mechanisms underlying this process. Subsequent studies could investigate bioavailability of riverine DOM using a broader range of model microorganisms, potentially working in synergy to enhance the transformation and removal of DOM.

Acknowledgements

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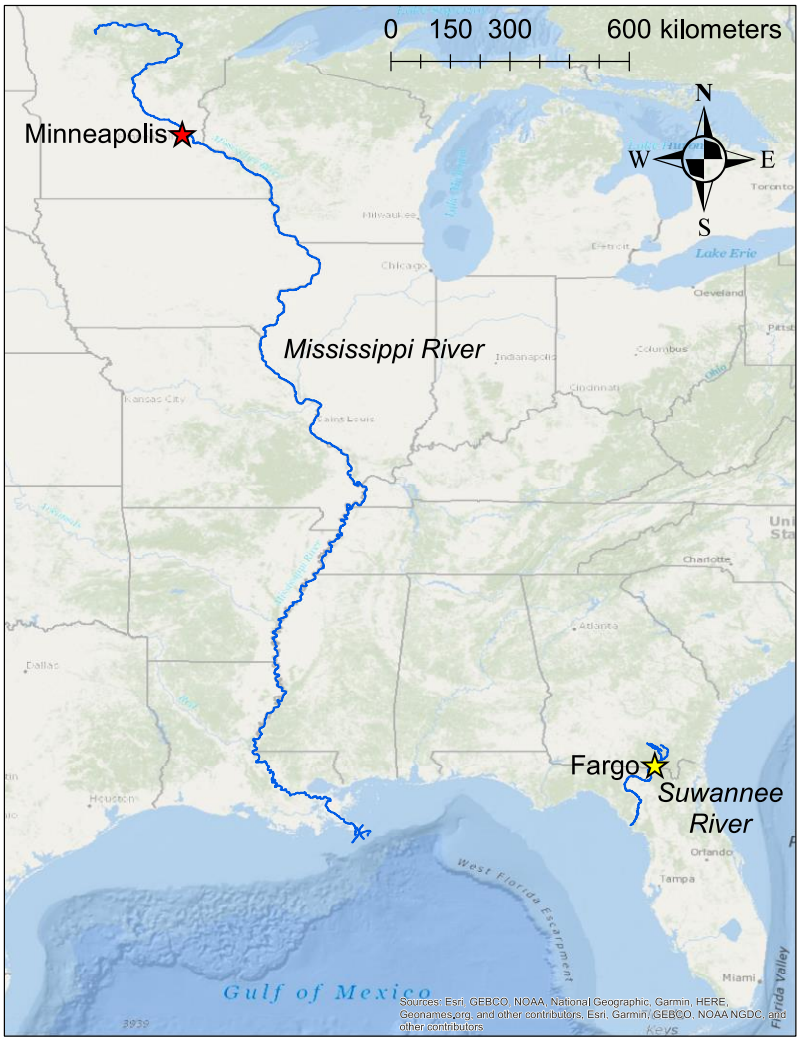
Open Research

The genome for the bacterial isolate used in this study can be found on National Center for Biotechnology Information under BioProject ID PRJNA414740 under accession number PDUS00000000 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA414740).



368
369 **Figure 1.** Map of the United States depicting location of rivers and collection sites for DOM.
370 Red star corresponds to the collection site for Upper Mississippi River DOM and yellow star
371 shows collection site for the Suwannee River DOM.

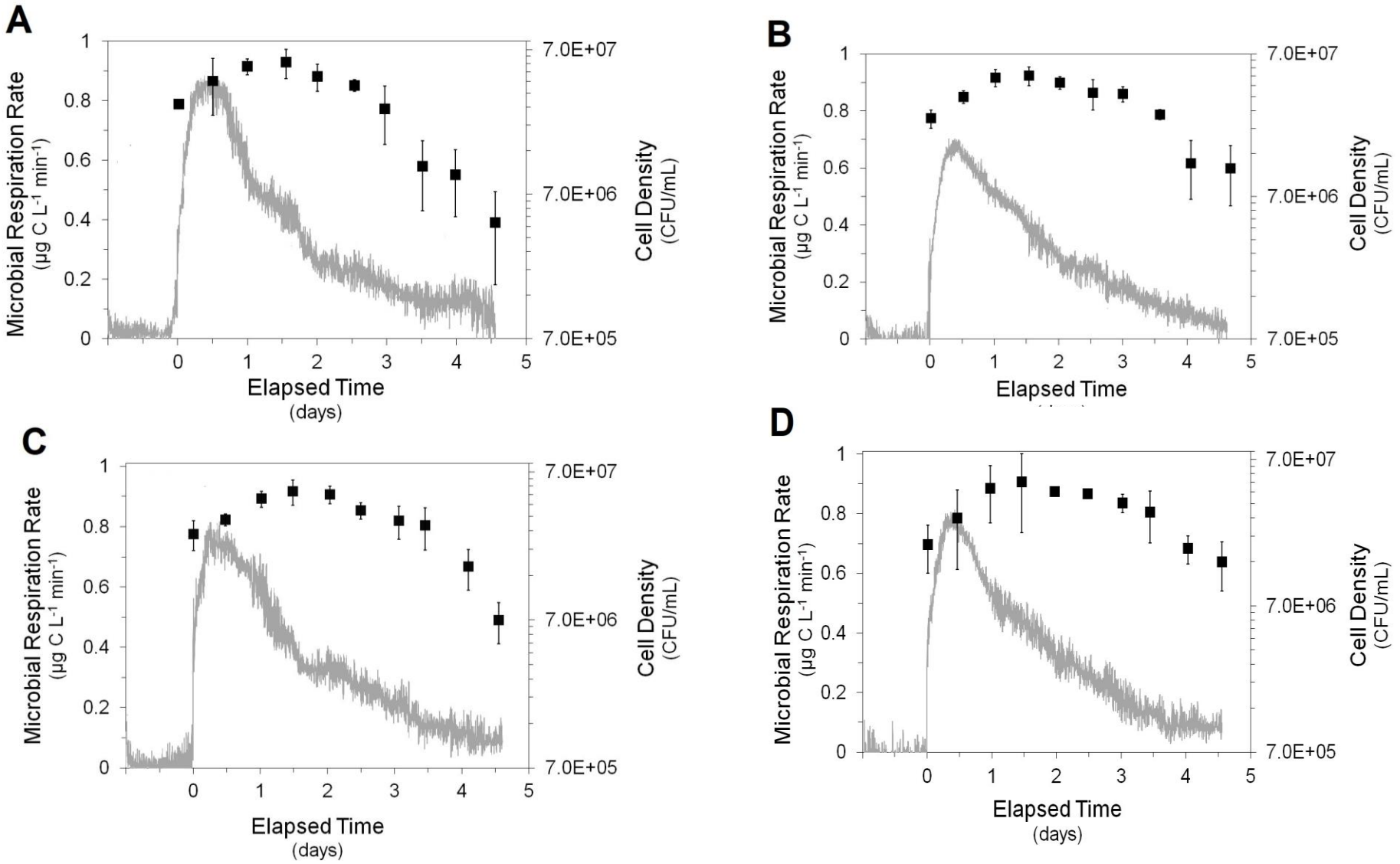


Figure 2. Microbial respiration rates (grey line) and cell densities (squares) measured during replicate incubations of *Pseudoalteromonas sp. 3DO5* with Suwannee River DOM incubation (A and B) and Upper Mississippi River DOM (C and D). Error bars indicate standard deviations of the average (n=3).

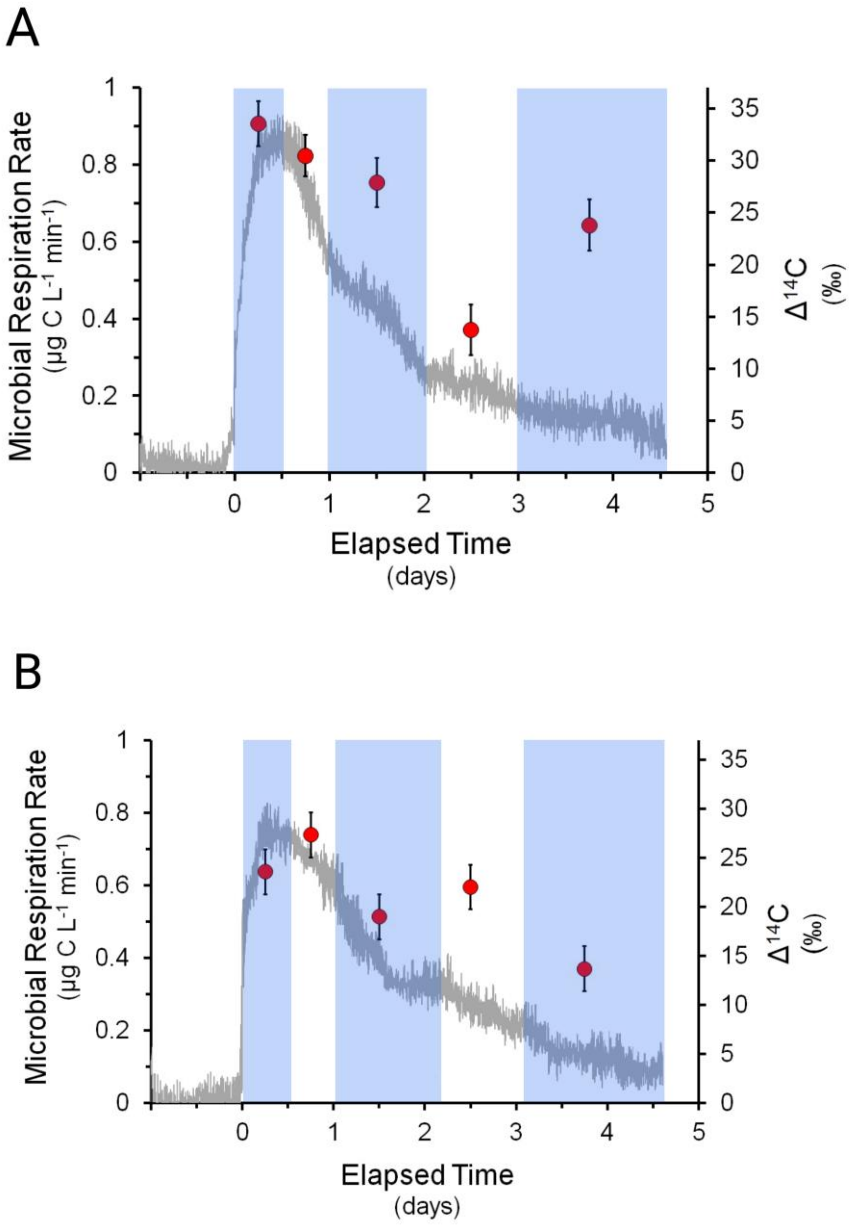


Figure 3. Microbial respiration rates (grey line) and $\Delta^{14}C$ (red circles) signatures of respired CO_2 observed during incubation of *Pseudoalteromonas sp. 3D05* with (A) Suwannee River DOM (SRDOM); and (B) Upper Mississippi River DOM (UMR DOM). The width of each blue box spans the time interval during which each CO_2 fraction was collected for isotopic analysis, with the corresponding data point plotted at the mid-point for each fraction.

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