Examining the radiocarbon age and bioavailability of riverine dissolved organic matter to a marine microbe

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August 2023

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

Abstract

The global carbon (C) cycle is a primary control on Earth's long-term climate dynamics. Dissolved organic matter (DOM) in aquatic ecosystems is one of the most actively cycled components of the global C cycle and is a vital source of C to microbes. It is suspected that the land surrounding rivers affects the bioavailability and composition of riverine DOM due to anthropogenic influences such as agriculture and urbanization. Altered watersheds impart a different DOM composition that may affect downstream bioavailability of C substrates to microbes. This thesis aims to address the bioavailability of riverine DOM from two distinct watersheds and the degradation of DOM in the marine coastal zone through laboratory incubations.

Chapter 1 of this thesis begins with background and a review of riverine DOM and coastal systems. Chapter 2 presents laboratory incubations that investigate the bioavailability of DOM from two distinct rivers. Composition of riverine DOM is dependent on the surrounding watershed that contributes different molecular signatures onto DOM which can affect age and bioavailability of DOM. Radiocarbon isotopic analyses are a powerful tool for investigating DOM because the isotopic ratio in the source C remains similar through the processes of consumption and respiration. Here, I incubated DOM from the Suwannee River and DOM from the Upper Mississippi River Basin with a marine microbe (*Pseudoalteromonas sp. 3D05*) to investigate riverine DOM bioavailability between these two river systems. These two river systems differ in their surrounding watersheds as the Suwannee River is forested while the Upper Mississippi is urbanized and farmed thereby receiving more anthropogenic inputs. We tracked cell density as well as microbial respiration rate and associated isotopic signatures ($\Delta^{14}C$) to evaluate the quantity and the age of remineralized C. We observed a singular respiration peak across all incubations within the first 14 hours of incubation and increases in cell density, indicative of microbial growth. We found a progressive depletion of microbially-respired CO_2 ages, suggestive of an age-reactivity

continuum in which younger compounds are preferentially consumed by microbes. Chapter 3 concludes with a summary, additional considerations, and insight for future research areas in this field.

Résumé

Le cycle global du carbone (C) exerce un contrôle primordial sur la dynamique climatique à long terme de la Terre. La matière organique dissoute (MOD) dans les écosystèmes aquatiques est l'une des composantes les plus actives du cycle global du C et constitue une source vitale de C pour les microbes. On soupçonne que les terres entourant les rivières affectent la biodisponibilité et la composition de la matière organique dissoute fluviale en raison d'influences anthropogéniques telles que l'agriculture et l'urbanisation. Les bassins hydrographiques modifiés confèrent une composition différente à la MOD qui peut affecter la biodisponibilité en aval des substrats de C pour les microbes. Cette thèse a pour but d'étudier la biodisponibilité de la MOD fluviale provenant de deux bassins versants distincts et la dégradation de la MOD dans la zone côtière marine par le biais d'incubations en laboratoire.

Le chapitre 1 de cette thèse commence par un historique et une revue de la MOD fluviale et des systèmes côtiers. Le chapitre 2 présente les incubations en laboratoire qui étudient la biodisponibilité de la MOD provenant de deux rivières distinctes. La composition de la MOD fluviale dépend du bassin versant environnant qui apporte différentes signatures moléculaires à la MOD, ce qui peut affecter l'âge et la biodisponibilité de la MOD. Les analyses isotopiques du radiocarbone sont un outil puissant pour étudier la MOD, car le rapport isotopique dans la source C reste similaire à travers les processus de consommation et de respiration. Ici, j'ai incubé de la MOD de la rivière Suwannee et de la MOD du bassin supérieur du Mississippi avec un microbe marin (*Pseudoalteromonas sp.* 3D05) afin d'étudier la biodisponibilité de la MOD fluviale entre ces deux systèmes fluviaux. Ces deux systèmes fluviaux diffèrent par les bassins versants qui les entourent : la rivière Suwannee est boisée tandis que le Mississippi supérieur est urbanisé et cultivé, recevant ainsi plus d'intrants anthropogéniques. Nous avons suivi la densité cellulaire ainsi que le

taux de respiration microbienne et les signatures isotopiques associées ($\Delta^{14}C$) pour évaluer la quantité et l'âge du C reminéralisé. Nous avons observé un pic de respiration singulier dans toutes les incubations au cours des 14 premières heures d'incubation et des augmentations de la densité cellulaire, indiquant une croissance microbienne. Nous avons constaté un épuisement progressif des âges du CO_2 respiré par les microbes, ce qui suggère un continuum âge-réactivité dans lequel les composés les plus jeunes sont préférentiellement consommés par les microbes. Le chapitre 3 se termine par un résumé, des considérations supplémentaires et un aperçu des futurs domaines de recherche dans ce domaine.

Acknowledgments

I would first like to thank my supervisor, Dr Nagissa Mahmoudi, for taking me on as a Master's student, providing funding for this research and introducing me to such a unique bioreactor. I thank her for her guidance as she taught me lab techniques, her patience as I learned the skill of scientific writing, and ultimately her help to make me a better research scientist. Thank you to my committee members, Dr. Brett Walker and Dr. Eric Galbraith, for your insight in navigating an ever-changing project. Special thanks to Dr. Xiaomei Xu for analyzing my isotopic samples in such a timely manner. Final thank you to the previous Earth and Planetary Sciences Department administrators Kristy Thornton and Anne Kosowski, for answering all questions and handling every variety of problem. Figures 2-7, 10, and 12 were made in BioRender.com.

I also would like to thank Dr. Kathryn Rico for all of her advice, wisdom and feedback even after she had left our lab, your help and encouragement has been invaluable. An extraordinary thank you and immense appreciation to Sam McNichol, you have been the best lab partner, friend, and support system during the completion of this degree. I am eternally grateful to have been introduced to this lab, university, and city with you next to me.

Special dedication to Peggy Carla Martinetti and Miss. D, both whom passed during the pursuit of this degree, you are infinitely missed.

Thank you to my parents for always supporting and encouraging me on any journey I decide to embark on. Gigi, you have been my long-distance soul sister and my life line these past two years. Thank you for endless facetimes, being the one to visit me, and our inexhaustible conversations, your friendship is priceless.

Andrés, thank you for making this city and life here feel so familiar. I am forever grateful for you support, your companionship while I worked odd hours, and for always watching a movie with me.

Contribution of Authors

The following thesis presents original research conducted by the submitting student at the Department of Earth and Planetary Sciences, under the supervision and academic mentoring of Dr. Nagissa Mahmoudi. The entirety of this document was written by the submitting student, Lea Baumser. Feedback, edits and suggestions were provided by Dr. Nagissa Mahmoudi. Figures 2 through 13 were created by the submitting student. Dr. Brett Walker (University of Ottawa) assisted in the initial study conception and design. All laboratory work, incubations, and data analysis were performed by the submitting student with feedback from Dr. Nagissa Mahmoudi. Isotopic analysis of CO_2 samples was performed by Dr. Xiaomei Xu at the Keck-Carbon Cycle AMS facility (University of California, Irvine).

List of Figures

Figure 11. Respiration rates (grey line; μg C L^{-1} min^{-1}) of *Pseudoalteromonas sp. 3DO5* with Suwannee River DOM incubation (A and B) and Upper Mississippi River DOM (C and D) over time (gray line) compared to average cell density over time (black squares) shown for initial incubation (A and C) and replicate (B and D). Error bars indicate standard deviations of the average (n=3).

Figure 12. Microbial respiration rates (grey line; μg C L^{-1} min^{-1}) and $\Delta^{14}C$ (red circles) signatures (‰) of CO_2 observed during incubation of *Pseudoalteromnas sp. 3D05* with 700 mg of (A) SR DOM or (B) 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.

Figure 13. Measured $\Delta^{14}C$ (‰) of microbially respired CO_2 during the course of incubations for SR DOM (A) and UMR DOM (B). Dashed line follows changes between collected respired CO_2 fractions while the solid line indicates the overall trend.

List of Tables

List of Abbreviations

C: Carbon

GCC: Global Carbon Cycle

OM: Organic Matter

DOC: Dissolved Organic Carbon

DOM: Dissolved Organic Matter

POM: Particulate Organic Matter

N: Nitrogen

ATP: Adenosine Triphosphate

LDOM: Labile Dissolved Organic Matter

RDOM: Recalcitrant Dissolved Organic Matter

CRAM: Carboxyl-Rich Alicyclic Molecules

HMW: High Molecular Weight

LMW: Low Molecular Weight

P: Phosphorus

S: Sulfur

UV/Vis: Ultra-Violet/ Visible

CDOM: Colored Dissolved Organic Matter

DIC: Dissolved Inorganic Matter

IHSS: International Humic Substance Society

SR: Suwannee River

CEX: Cation Exchange

UMR: Upper Mississippi River

T-R: Tibbles-Rawling

IsoCaRB: Isotopic Carbon Respirometer-Bioreactor

CFU: Colony Forming Units

LN_2: Liquid Nitrogen

AMS: Accelerator Mass Spectrometry

1. Literature Review

1.1. Global carbon cycle

1.1.1. The importance of organic matter cycling to global carbon fluxes

Biogeochemical processing and cycling of carbon (C) connect the land, oceans, and atmosphere. The global C cycle (GCC) exists in two main, yet broad cycles defined by their reservoir residence time. Generally, the slow cycle (i.e., long reservoir residence time; 10,000 years or longer) includes C in rocks and deep marine sediments and is by far the cycle with quantitatively more C (~15,000,000 Pg C). This cycle is involved with long term storage with implications for Earth climate systems. The fast cycle (i.e., short reservoir residence time) consists of major reservoirs such as the oceans (~38,700 Pg C), terrestrial soils (~1,950 Pg C), surface marine sediments (~1750 Pg C), terrestrial vegetation (~550 Pg C), and freshwaters (~1.7 Pg C) (Figure 1; Ciais et al., 2013; Kandasamy and Nath, 2016). The C in these reservoirs persists for anywhere between 0 and 10,000 years all with various biogeochemical processes within each reservoir and cycles connecting reservoirs to each other (Hansell, 2013; Trumbore et al., 1992; Galbraith et al., 2013). The GCC has crucial roles in long-term global climate mediation, the foundation of biological matter, and base energy sources making the intricacies of the GCC inherently imperative to understand.

Organic matter (OM) is one of the most actively cycled reservoirs of the fast GCC — connecting the biosphere, hydrosphere, lithosphere and atmosphere. OM moves from reservoir to reservoir through natural mechanisms (i.e., rivers, atmospheric deposits, soil erosion, sedimentation) and perturbed systems (i.e., sewage dumping, fertilizer run off, fossil fuel burning). One of the largest fluxes (~0.4 Pg C) of OM between the land and ocean is the river flux bringing terrestrial OM through inland waters and estuaries to coastal zones, continental shelves and eventually the deeps seas (Hedges et al., 1997; Ophsal and Benner, 1997). OM within the oceans represents one of the largest reservoirs of OM on Earth (Hedges, 1992; Ogawa and Tanoue, 2003).

The amount of C in marine OM (662 Pg C), in the form of dissolved organic carbon (DOC), is similar to the amount of C found within the atmosphere (~750 Pg C), and about two hundred times the C found in marine biomass (Siegenthaler and Sarmiento, 1993; Hansell et al., 2009). Together, this makes the oceans one of the most critical biogeochemical reactors and storage of C through OM processing on Earth.

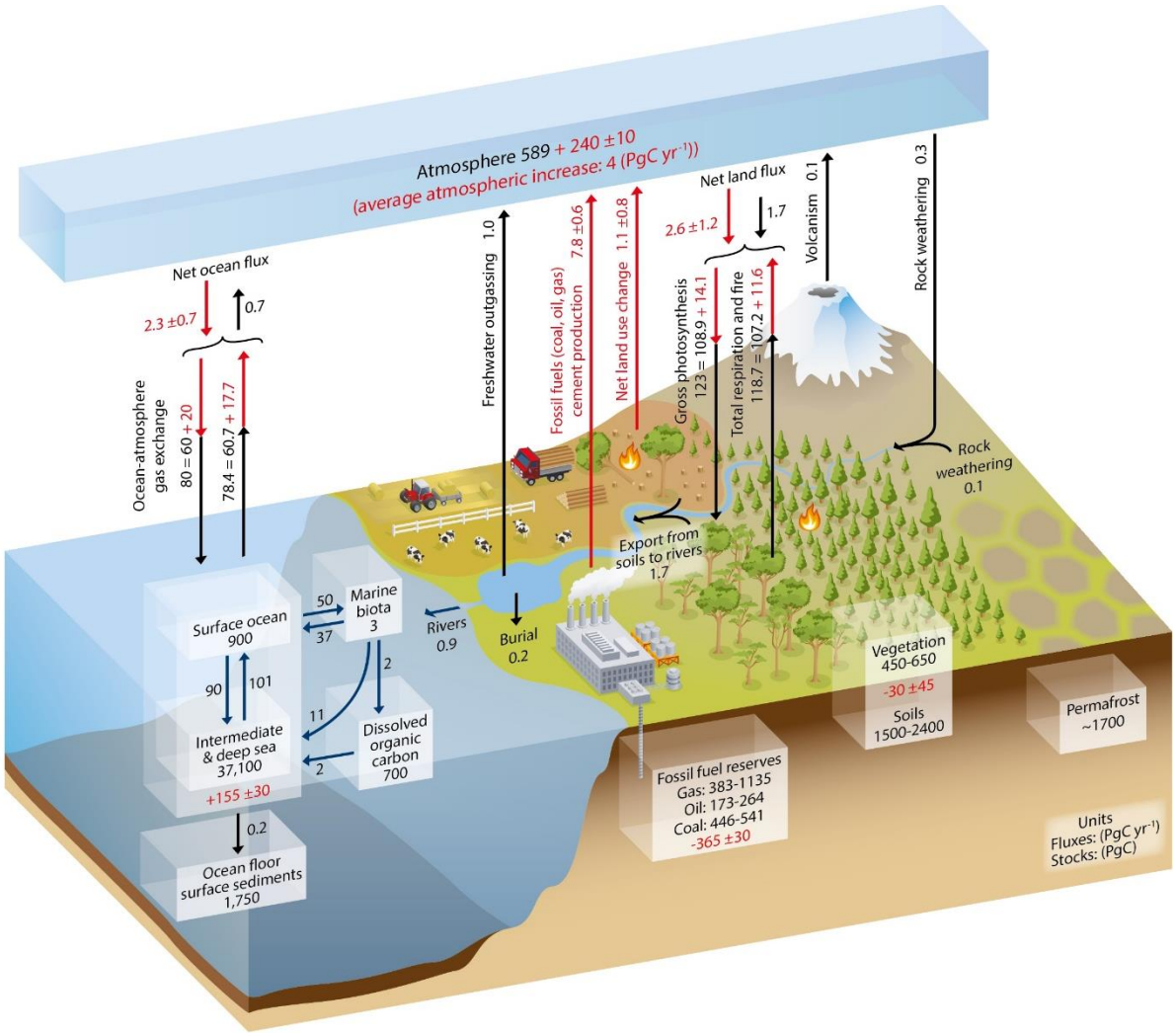

Figure 1. Schematic diagram of C cycling in costal zones alone the land-sea continuum showing fluxes and reservoirs, with anthropogenically influenced fluxes in red (Adapted from IPCC, 2013).

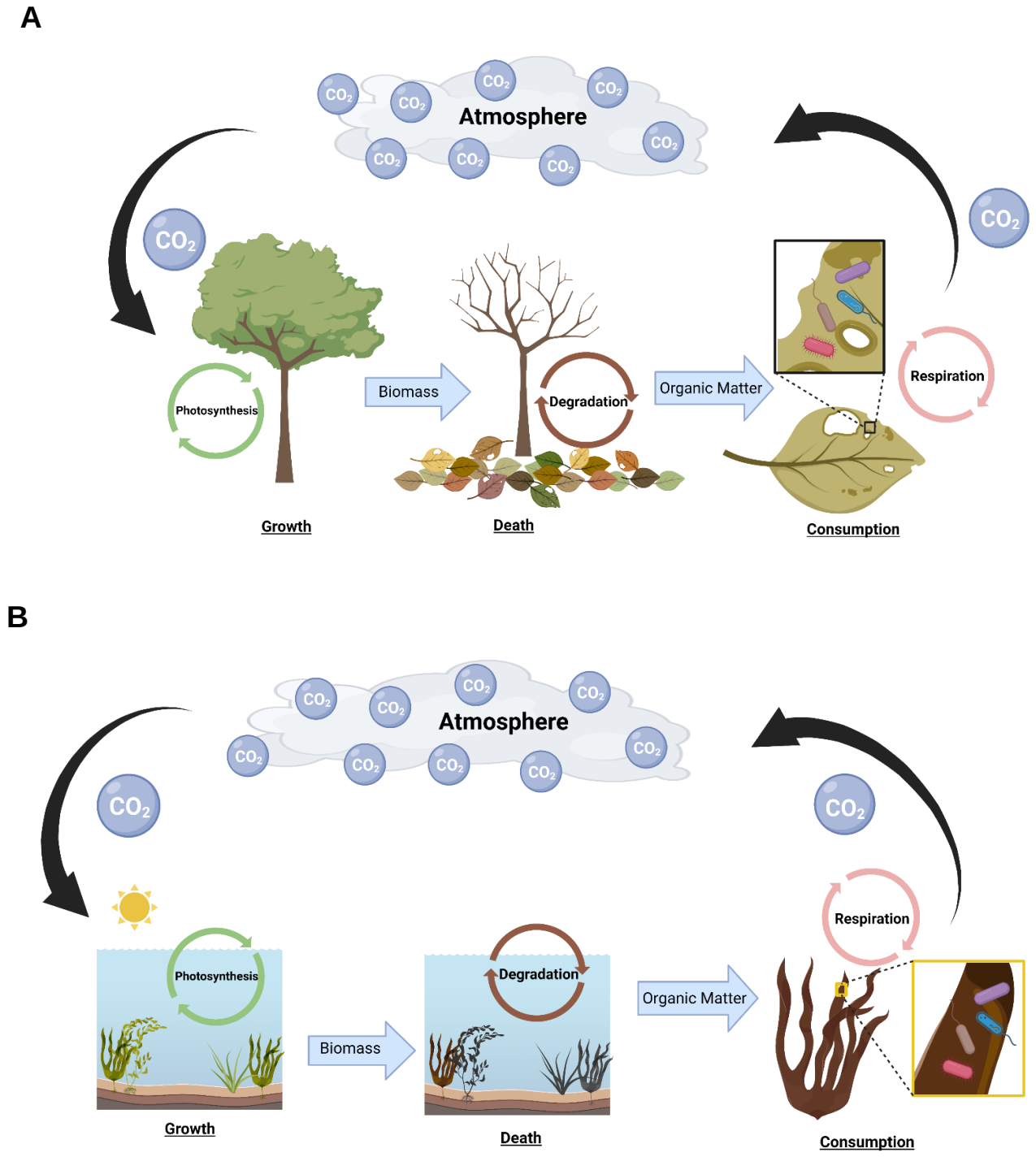

Figure 2. Simplified diagram depicting the flow of carbon dioxide (CO_2) from the atmosphere through the (A) terrestrial biosphere and (B) marine biosphere and its remineralization back to CO_2 in the atmosphere. C begins in the gaseous form of CO_2 and is converted to biomass through photosynthesis, then is consumed and remineralized back to CO_2 through the respiration of heterotrophic microorganisms.

1.1.2 The role of river systems and coastal zones in the global carbon cycle

Rivers were once thought to have the singular role of delivering OM to coastal zones (i.e., "pipes"), but have now been found to be biogeochemical reactors themselves (i.e., "processers") (Battin et al., 2023; Raymond et al., 2013; Hotchkiss et al., 2015). The inherent high microbial heterotrophy of rivers makes them a net source of CO_2 to the atmosphere, releasing approximately 2 Pg C per year globally (Battin et al., 2023; Liu et al., 2022; Raymond et al., 2013). Rivers bring an estimated ~0.4 Pg C per year of OM to coastal zones and oceans, effectively bridging the land-deep sea continuum (Hedges et al., 1997; Ophsal and Benner, 1997). C delivered to the oceans by rivers is transport limited meaning the main control on C delivery by rivers is riverine discharge, but temperature, precipitation and flood events also play roles in discharge and therefore, in the amount of C received by oceans (Meybeck, 1982; Beaulieu et al., 2012). OM concentrations in rivers vary globally, due to differences in sources, climate, morphology, seasons, and microbial populations, however all rivers reaching the ocean deliver OM to estuaries and coastal systems (Raymond et al., 2004; Ittekkot et al., 1985). Rivers meet the oceans at freshwater-saltwater interfaces with inherent changes to chemical, physical and biological properties of the water (i.e., salinity, light availability, microbial communities). Therefore, the C in river discharge is subjected to various fates throughout the journey to the coast and then again at the coast.

The coastal ocean makes up 7% of the global surface ocean and is comprised of ecosystems such as estuaries, river mouths, tidal wetlands, and the continental shelf (Bauer et al., 2013). These coastal systems disproportionally cycle more C than area they take up, but they were previously neglected; only in the past 10 years has the CO_2 budget of the coastal oceans been reported (IPCC, 2013). Previous work (Tsunogai et al., 1999) estimated that coastal systems were a sink of about 1 Pg C per year of CO_2 from the atmosphere. More recent work has further constrained the uncertainty and decreased the amount of CO_2 coastal systems sequester to about 0.5 Pg of C per year (Borges et al., 2005) and then further to approximately 0.2 Pg of C per year (Laruelle et al.,

2010; Dai et al., 2022; Roobaert et al., 2019). Polar and subpolar regions account for > 90% of atmospheric CO_2 removal in coastal zones, while tropical regions act as an atmospheric source of CO_2 (Gruber, 2015; Dai et al., 2022). Coastal ocean sediments may remove between 0.2 to 0.5 Pg of C per year (Muller-Kargen et al., 2005; Dunne et al., 2007). Estuaries, a component of coastal systems, are suspected to be C sources of atmospheric CO_2 due to high heterotrophy and release between 0.2 to 0.4 Pg C per year (Cai, 2011; Borges at al., 2005). Estuaries also bury about 0.1 Pg C per year of OM through the sinking of OM to the sediment floor (Regnier et al., 2013). Through these different ecological systems, and various methods to determine fluxes (i.e., models vs observational data) there exists a gap in our ability to quantitively or qualitatively characterize the C cycling in coastal zones. Further investigation on C cycling at these hotspots is vital to developing a complete and holistic understanding of the GCC.

1.2. Sources of organic matter to rivers

OM is one of the most complex mixtures on Earth, consisting of a heterogenous mix of a variety of compounds (Hedges et al; 2000). OM is operationally separated into two fractions dissolved OM (DOM) consisting of compounds that can pass through a 0.7-micron filter and particulate OM (POM) that cannot pass through a 0.7-micron filter. Sources of riverine OM from the surrounding organic rich terrestrial ecosystem are vast and dependent on the land surrounding the river, known as the watershed (Meybeck et al., 1988). Common sources from the biosphere include leaf litter and fragmented plant material from C_3 and C_4 vascular plants, leaching from soil, and rhizodeposition. Rhizodeposition is all material from plant roots, including exudates, insoluble compounds, and dead roots (Lynch and Whipps, 1990). Soils contribute to the OM pool of rivers through leaching and erosion of the OM in riparian soils along the path of the river (Hope et al., 1994; Ledesma et al., 2018). These sources of OM are considered to be natural, as they reflect

processes and degradation pathways that occur without anthropogenic influence to the environment.

As humans are altering more land than ever before with a population of 8 billion, ecosystems and C cycling are changing. The land-use alterations to the environment directly affect the sources of OM to river systems and subsequently the downstream coastal environment. Land-use changes from natural forested lands to urbanized land result in sources to nearby rivers such as sewer lines and septic drainage fields, petroleum products (i.e., oil and gas), pet waste, and imported vegetation and soil (Fork et al., 2018; Spiker and Rubin, 1975; Pennino et al., 2016). Additionally, land alterations to impervious surfaces (i.e., pavement), result in an increase export of OM to aquatic systems because the OM cannot interact with the covered soil (Fork et al., 2018; Hobbie et al., 2014).

Humans also turn land from forested, wetlands into agriculture sites to grow food to feed the ever-growing population. The main source of OM from agricultural sites is from the use of fertilizer adding synthetic nitrogen (N) to the environment. The fertilizer can wash into nearby rivers through precipitation or irrigation. Additionally, fertilizer can create an indirect addition of C to soil OM from easier growth due to increased root interactions. The fertilizer helps the crops grow, and these crops input more vegetative OM into the soil which can end up in aquatic systems (Vaugh et al., 2021). The increased OM tends to not increase concentration of OM compared to forested rivers, but instead compensates for the lost leaf litter derived OM (Vaughn et al., 2021). Therefore, although the quantity of OM does not change, the composition of it does as there is a difference in sources, leading to an OM shift from humic substances to more bioavailable compounds. Through land-use changes to support the growing world population, humans are altering the sources of OM to nearby rivers which has downstream implications for coastal and marine environments.

Coastal environments are at the interface of freshwater and marine systems. Freshwaters from rivers bring OM inputs, mostly terrestrial, while marine waters bring different OM inputs. Most of the OM in the marine waters is from primary production, such as the photosynthetic activity of phytoplankton released as exudates and biomass (Hansell, 2013; Kawasaki and Benner, 2006). The mixing of OM from marine waters and rivers can create a priming effect for the microbial communities, a process in which the input of a bioavailable C source alters the mineralization of more recalcitrant OM (Lohnis, 1926; Bingemen et al., 1953; Bianchi, 2011). Additionally, submarine groundwater discharge may bring a large portion of DOM to coastal environments through underwater porin exchange. Water with DOM percolates through soil and land and discharges straight into the coastal environment, without interacting with the atmosphere. Coastal regions are sites of extreme heterogeneity as multiple ecosystems meet to bring OM from a variety of sources.

1.3. Transformation and fate of riverine DOM in coastal systems

OM that reaches the coastal ocean is either sedimented out of the water column or degraded (biotically or abiotically). Sedimentation occurs when POM sinks in the water to the sediment floor and will become incorporated into continental shelf sediments. DOM in the water column can become POM, through flocculation or adsorption to be able to sink. DOM flocculates through ionic and charge differences that attract compounds together to create POM (Asmala et al, 2016; Sholkovits, 1976). A recent study has found a doubling in sedimentation across North American coasts despite human altered river systems (i.e., dams; Rodriguez et al., 2020). A fraction POM sinks and sediments upon arrival in coastal systems, the rest is either degraded or exported to the open ocean (Regnier et al., 2013).

Microbes — particularly heterotrophs (i.e., microbes that consume organic C to sustain metabolic processes such as growth and respiration) — play a substantial role in the transformation

and cycling of C. When OM is consumed by heterotrophic organisms, the compounds will either be incorporated into biomass (i.e., growth), re-oxidized and respired as CO_2 (i.e., respiration), or excreted from the cell as a transformed C molecule (i.e., exudation). However, some organic molecules are so large that microbes need to externally degrade them until the molecules are small enough (under 600 Da) to be transported into the cell through porins along the cell membrane (Weiss et al., 1991). Some heterotrophic microbes secrete extracellular enzymes from the intracellular environment, which shuttle past the cell membrane through transmembrane porins and into the extracellular environment (Arnosti, 2011). These enzymes are highly selective in which molecules in OM that they hydrolyze (i.e., proteins, carbohydrates, etc.) and how they do it, bonding to a specific location on the molecules and cleaving a specific bond. Extracellular enzymes are vital to these heterotrophic microbes as the vital primary step in consumption unlocking necessary nutrients that would otherwise be unavailable to these microbes (Balmonte et al., 2019).

Once a molecule is hydrolyzed by enzymes to smaller substrates, a microbe can consume the compound for respiration (Arnosti, 2011). Respiration is an important step in the mineralization of OM from its reduced state to fully oxidized CO_2. Respiration is required by bacterial cells to create energy in the form of adenosine triphosphate (ATP), a necessary molecule for other metabolic reactions that require energy. Respiration occurs in the plasma membrane when a cell has consumed a C substrate and passive diffusion has brought O_2 gas into the intracellular environment (Cohen, 2014). The waste products of H_2O and CO_2 are diffused back out of the bacterium, while the ATP remains for use in energy intensive metabolic reactions. The dissolved waste CO_2 will either be used by another organism (i.e., phytoplankton) or the molecule can dissolve out of the water into the atmosphere (Regnier et al., 2013). Respiration is the molecular pathway bacteria are able to oxidize and re-mineralize DOM to CO_2, effectively connecting and closing the C loop.

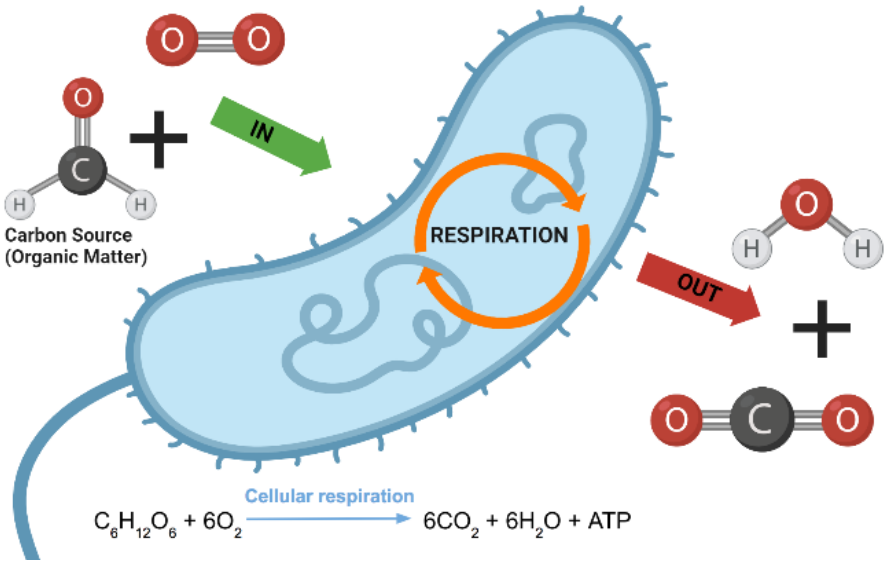

Figure 3. Simplified diagram showing how heterotrophic bacteria use oxygen and consume a C from a source and respire water and CO_2 out. Respiration creates energy in the form of adenosine triphosphate (ATP) for the cell.

Abiotic degradation of OM can occur through multiple chemical and physical processes; however, the principal process is photodegradation from sunlight. When the rays of light are absorbed by the compounds within OM the compounds can be completely oxidized into CO_2 in a process known as photo-remineralization or partially degraded into smaller compounds (Figure 4; Miller and Zepp, 1995; Mopper and Kieber, 2002). The resulting compounds from partial degradation can be more bioavailable to microbes than the parent compound. When terrestrial OM enters the coastal environment the increase in salinity has been found to affect the photochemical processing by decreasing the reactivity of OM to light, making it less susceptible to photodegradation (Minor et al. 2006). Therefore, abiotic and biotic degradation can work in tandem to process OM to remineralization or to sequestration (Kieber et al., 1989; 1990; Mopper and Kieber 2000).

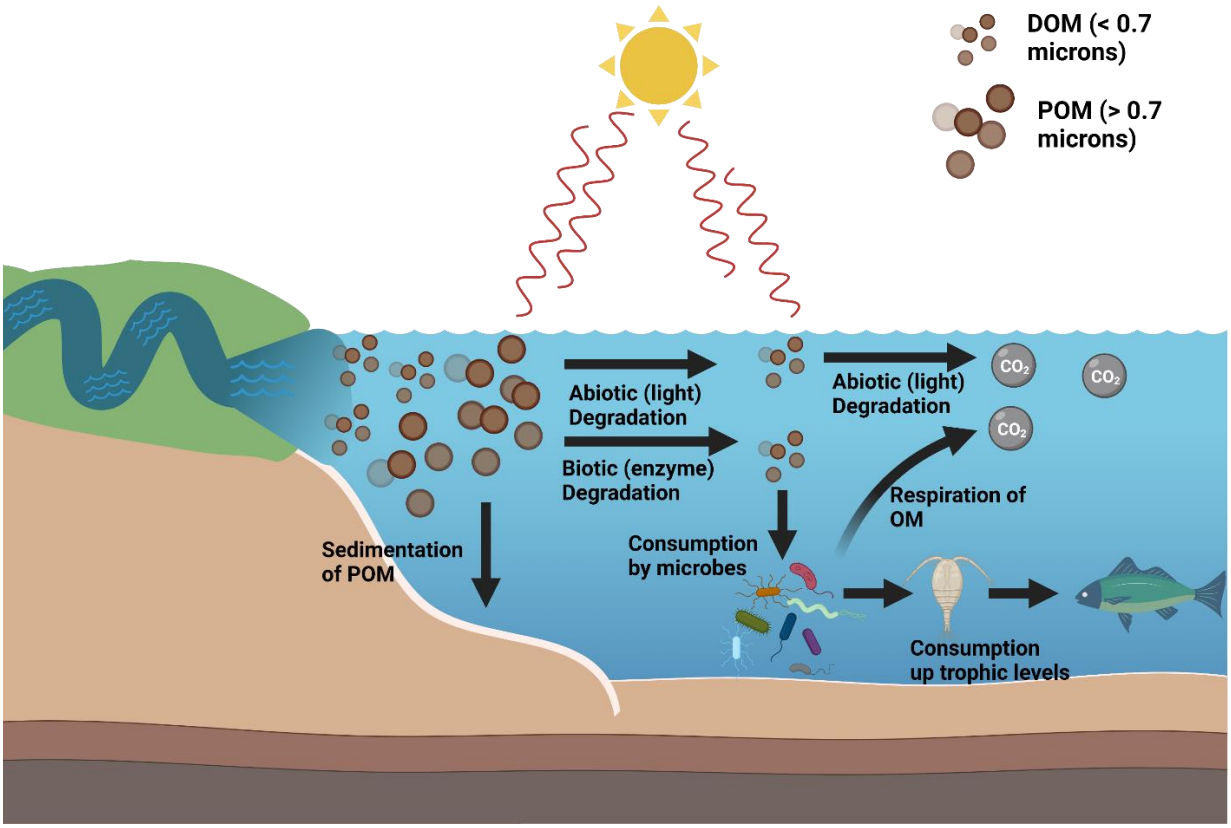

Figure 4. Simplified coastal C cycle depicting primary degradation pathways of riverine exported DOM and POM into the marine environment.

1.4. Composition and characteristics of riverine DOM

Two of the most important and researched aspects of DOM are the quantity and quality of DOM because these parameters represent the greatest contributions to the downstream biogeochemical cycling. The quantity of DOM is based on its concentration and can vary across temporal and spatial scales. Quantity is affected by abiotic inputs (e.g., soil erosion or wastewater additions) and the biotic addition (e.g., exudation) and processing of inputs (e.g., biomass). Quality of DOM is defined in terms of bioavailability or, reactivity to microbes that consume the DOM for metabolic processes. DOM that is more labile, deemed labile DOM (LDOM), is higher quality because it is easier for microbes to consume and degrade. While difficult-to-degrade DOM, called recalcitrant or refractory DOM (RDOM), is considered low quality DOM.

The quantity of DOM has intuitive controls input and output, yet there are still many differences between rivers due to land-use and location. Both urban and agriculture land-use have reduced tree cover resulting in increased sunlight and therefore, an increase in temperature. With more sunlight reaching the water, light-limited abiotic photodegradation can occur. Additionally, the high temperature leads to increased heterotrophic activity and DOM decomposition (Nagy et al., 2018). Together these two processes explain why agricultural rivers tend to have a lower concentration of DOM than their urbanized counterpart, yet a comparable concentration to natural watersheds (Vaughn et al., 2021). These processes do exist in urban land use, however urban areas tend to have significantly more sources of DOM than agricultural and continue to have significantly higher DOM concentrations compared to unaltered land and agricultural land (Vaughn et al., 2021).

The quality of DOM can be difficult to determine due to the variety of factors involved, such as compounds present, abiotic influences (i.e., light), and microbial taxa present. For example, carboxyl-rich alicyclic molecules (CRAM) and CRAM-like compounds within DOM have been historically deemed refractory, yet recently have been found to be the preferred C substrate for specific microbial lineages with special enzymes (Liu et al., 2020). However, there are some patterns that researchers have found to make general conclusions on the quality of DOM to microbes. Similar to the size division between DOM and POM, DOM can be further size classified by size into high molecular weight (HMW) DOM (> 1kDa) and low molecular weight (LMW) DOM (<1kDa) classes (Amon and Benner, 1996). The finding that HMW DOM is the preferential substrate for bacterial consumption resulted in the size-reactivity continuum which states that the size and bioavailability of OM are directly correlated (Amon and Benner 1994; 1996; Walker et al., 2016; Santschi et al., 1995); this is to say that HMW DOM is generally higher quality than LMW DOM.

Another control on quality of DOM to microbes is the inherent chemical make-up of the compounds. Proteins and protein-like compounds in DOM are typically consumed first due to high reactivity and high bioavailability and constitute higher quality DOM (Balcarczyk et al., 2009; Wiebe and Smith, 1977). Therefore, the concentration of protein and protein-like compounds in DOM is a good predictor to bulk DOM bioavailability (Fellman et al., 2009; Williams et al., 2010). Additionally, the presence of humic substances and specific aromatics (CRAM and CRAM-like) that are too difficult to biologically degrade indicate a generally low-quality DOM (Hertkorn et al. 2006).

Together these patterns of quantity and quality become especially interesting when applied to in-situ environments that have a presence or absence of human interference. DOM from urbanized rivers has been found to be higher quality due to the increased presence of N and sulfur (S) containing C compounds and is associated with a higher degree of microbial degradation (Reid et al., 2020; Gong et al., 2022; Vaughn et al., 2023). Additionally, autochthonous DOM (i.e., microbially derived) is considered high quality due to the high concentration of amino acids and sugars (Cherrier et al., 1996; Williams et al., 2010). These two pools contrast forested, unaltered watersheds that have low quality DOM from the high terrigenous inputs with molecular complexity, such as humic compounds and N-free biomacromolecules (e.g., lignin, cutin) that are resistant to degradation (Hedges et al., 1997). The innate complexity of DOM makes synthesis of current findings vital, as well as, a need for further research studying the interconnectedness of biogeochemical cycling and bioavailability of DOM.

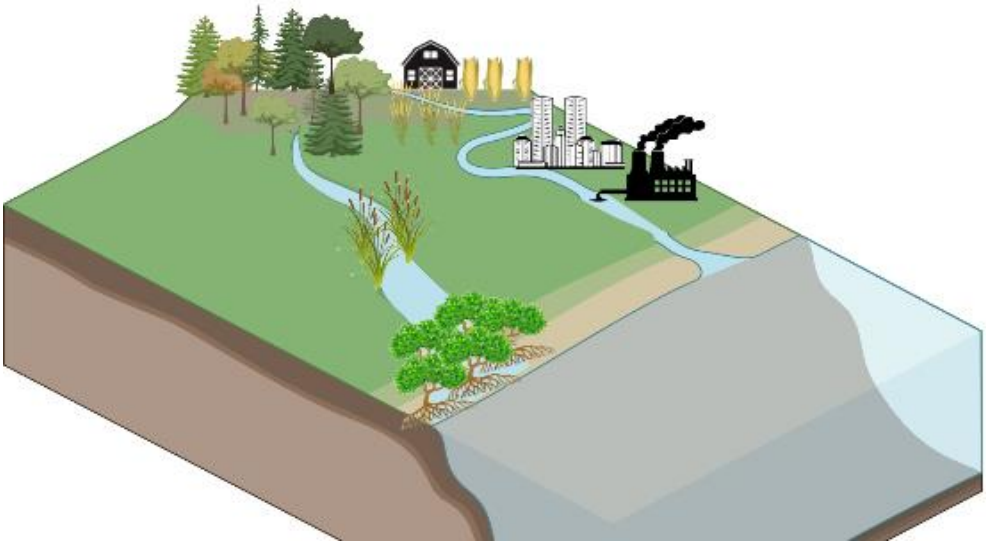

Figure 5. Schematic of rivers with different land uses in the water shed leading to different sources of OM within the river with deposition in a coastal zone. The river on the left represents a more natural system, surrounded by forest and wetlands, while the river on the right is influenced by the surrounding city, factory and agricultural farm. OM in the river from a more natural system would be expected to have a higher concentration of vegetative-derived OM (i.e., leaf litter) compared to OM in the river from an urbanized area. OM from an anthropogenically influenced system would include terrestrial OM, but also be more diverse, with sources such as fertilizer run-off and wastewater affecting OM composition making it more bioavailable to microbes.

1.5. Analytical approaches to understanding DOM cycling

Due to advancing technology in recent years, the analytical techniques for studying DOM have quickly progressed. Analytical techniques allow for quantification and qualification of concentration and composition, two vital factors in determining the quality of DOM. The need for analytical methods stems from C relationship with global climate, analytical methods produce data that can feed global system models to inform and predict changes in climate.

Fourier transform-ion cyclotron resonance mass spectrometry has the capability to differentiate thousands of molecular formulae in a single sample (Vaughn et al., 2023; Ware et al., 2022). This method of analysis allows for broad characterization and determination of the abundance of heteroatoms and abundance of formulae. Formulae are often presented as CHO, CHON, CHONS, and CHOS with CHO formulae considered the most refractory in comparison (Vaughn et al., 2023; Koch et al., 2005) This method also allows for clarity into molecular richness,

or the measure of unique molecular formulae (Vaughn et al., 2021). This number can determine the variety of molecules present and determine if a sample is more or less complex, however this method presents limitations in ascertaining concentrations of these formulae.

Another analytical method for quantifying and qualifying DOM, relies on the use of a spectrometer. A fraction of DOM can absorb and remit light in the ultraviolet-visible (UV/Vis) wavelength range known as colored DOM (CDOM; Blough and Del Vecchio, 2002). The inherent spectroscopic characteristics of CDOM can reflect sources (i.e., terrestrial, marine, and microbial) and concentration of CDOM through absorbance (Pain et al., 2019). The power of spectroscopy also allows for the determination of degree of degradation (biotically and abiotically), and even molecular properties such as aromaticity and molecular weight, which can draw conclusions to the bioavailability of the DOM (Blough and Del Vecchio, 2002). The major drawback to the use of spectrometer on CDOM is that it is not representative of bulk DOM, as not all DOM has the chromophore region that absorbs and re-emits light, therefore results and conclusions are only partial of the entirety of DOM present in an in-situ sample (Blough and Del Vecchio, 2002).

1.5.2. Radiocarbon

Radiocarbon can provide more definitive answers regarding the age of DOM, and thereby further clarify DOM sources. Due to the known half-life of radiocarbon (^{14}C; half-life = 5,730 ± 40 years), radiocarbon isotopic ratios ($\Delta^{14}C$) can be used to determine the age of the C compound, providing insight into the residence time of these compounds in the water column (Godwin, 1962; Williams and Druffel, 1987). A dual C isotopic approach is a fairly recent method to determining DOM source, requiring more work to be done for a comprehensive understanding to be achieved.

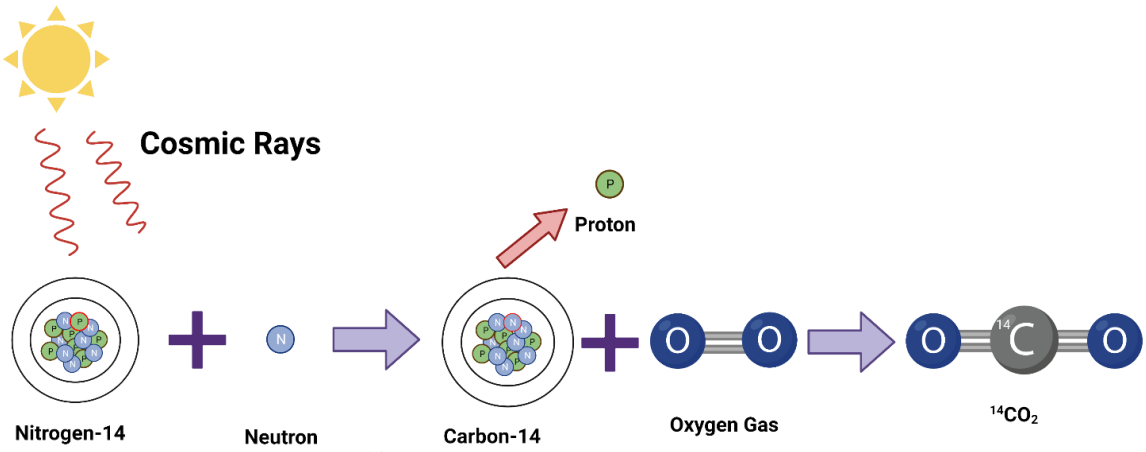

Figure 6. Creation of CO_2 with ^{14}C atom. Cosmic rays induce a reaction between nitrogen and a neutron that results in the expulsion of a proton and the incorporation of the neutron (red circle). The newly made ^{14}C atom then bonds with the O_2 gas present in the atmosphere to form CO_2.

The ^{14}C atom is created in the atmosphere when a nitrogen atom with 7 protons, loses a proton, but gains a neutron using the incoming cosmic rays (Figure 6). The loss of a proton changes the elemental status of that atom to C, as elements are defined by the number of protons present. After the ^{14}C atom is created and bonds with O_2 to become a $^{14}CO_2$ molecule, it diffuses into the aquatic systems or is used by plants where it undergoes photosynthesis and the eventual creation into biomass (Beaupré, 2015). Overtime the radiocarbon within biomass degrades at a known rate (i.e., half-life), and the $\Delta^{14}C$ signature of DOM can be used to assess when the compound was synthesized (i.e., age). Modern $\Delta^{14}C$ values match the ratio currently found in the atmosphere which is currently at approximately 50‰, while after about 50,000 years, compounds are too depleted in radiocarbon and are considered radiocarbon dead (-1000‰). When $\Delta^{14}C$ values are extremely distinct, differences in source can be shown, for example an extremely depleted value of -800‰ in deep water indicates chemoautotrophic DOM, reduced from extremely old, mantle originating DIC (McCarthy et al., 2011). In general, terrestrial originating DOM has modern radiocarbon values (22‰ to 46‰), in rivers due to the atmospheric C being the common substrate for photosynthesis (Schiff et al., 1990; Richter et al., 1999; Marwick et al., 2015). While marine

DOM has an extreme range of 100‰ to -350‰ depending on depth, size, and source of the compound (Walker et al., 2016; Druffel et al., 1992; Druffel and Bauer, 2000).

Radiocarbon values can have consistent trend through an environment. For example, these values decrease with depth in soil, as more time has elapsed in older soil for the radiocarbon to decay. Therefore, when DOM from coastal erosion is transported to the marine environment through rivers, there is an isotopically older signature of this DOM (Richter et al., 1999; Raymond and Spencer, 2015). Radiocarbon values also decrease with depth in the marine environment, leaving older compounds deeper in the water column (Bauer et al., 1992).

Microbes in rivers preferentially degrade young, more labile compounds, corroborated through a decrease in the ^{14}C value of upriver DOM to downriver DOM from in situ measurements, and more concretely through long-term, dark incubations where DOM age increased from the initial DOM to the final DOM (Raymond and Bauer, 2001a). In incubations of a natural bacterial community with DOM from the York River, ^{14}C value of the DOM decreased over the course, indicating that ^{14}C-enriched (young) substrates were preferentially utilized and degraded (Raymond and Bauer, 2001a). It has been hypothesized that older compounds correspond to recalcitrant qualities, because otherwise these compounds would have already been degraded. However, there is evidence that old compounds do not necessarily indicate refractory qualities as one study found that estuarine bacteria were using older, more radiocarbon depleted DOM from wastewater and marine phytoplankton, when younger DOM was also present and available (Griffith and Raymond, 2011). Another study from various Australia rivers shows a correlation between bioavailability of DOM and an older bulk DOM age (Fellman et al., 2014). These disparate results seem to be from differences in the individual rivers and the sources of DOM to them, but they highlight the need for further studies on riverine DOM bioavailability to microbes.

1.6. This study

The aim of this study was to observe and investigate the biological reactivity of DOM from two distinct rivers in a coastal system. To do so, I incubated a model marine isolate in the laboratory with DOM from the Suwannee River (SR) and the Upper Mississippi River (UMR) and measured the concentration and radiocarbon age of respired CO_2. SR acts a proxy for an undisturbed, natural watershed influencing riverine DOM dynamics, while the densely populated and highly urbanized and farmed watershed of the UMR represents anthropogenic impacts on downstream DOM reactivity. This study takes a novel approach of investigating DOM consumption by directly measuring the age of respired CO_2 rather than the more traditional method of measuring the bulk age of the leftover DOM pool. The results of this study provide novel insights into the interplay between the age and bioavailability of riverine DOM, shedding light on the intricate mechanisms governing microbial C cycling in and across aquatic environments.

2. Examining the radiocarbon age and bioavailability of riverine dissolved organic matter to a marine microbe

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2.1. Abstract

Dissolved organic matter (DOM) is one of the most actively cycled components of the global carbon cycle, with rivers delivering a significant amount of DOM to oceans. There still exists a gap in our understanding of the bioavailability of DOM to microorganisms and ultimately what factors dictate the rate and extent of mineralization. Rivers impacted by urbanization and agricultural land-use impose different molecular signatures on DOM compared to their natural counterparts, such as increased heteroatoms and increased inorganic nutrient concentrations. These changes are thought to alter the chemical composition of the DOM as well as the overall bioavailability of DOM. This study investigated the bioavailability of DOM from a natural, unaltered river system (Suwannee River) against DOM from a highly altered, anthropogenically-influenced river system (Upper Mississippi River Basin) using bioreactor incubations with a model marine isolate (*Pseudoalteromonas sp. 3D05*). We tracked microbial respiration rate and associated isotopic signatures ($\Delta^{14}C$) as well as cell density to evaluate the age of C substrate that was remineralized. We observed a singular respiration peak across all incubations (0.7-0.9 μg C L^{-1} min^{-1}) within the first 14 hours of incubation and similar cell counts (4.9-5.7 x 10^7 CFU/mL) peaking within the first 48 hours of incubation. Isotopic values of respired CO_2 from both incubations indicated a modern radiocarbon age and suggested a preference for younger C substrates. In addition, the total quantity of CO_2 remineralized across incubations was nearly

identical indicating that anthropogenically influenced watersheds may not significantly alter the downstream bioavailability of DOM.

2.2. Introduction

Rivers act as vital conduits in the carbon (C) transfer between terrestrial and marine ecosystems by facilitating the export of organic matter (OM) from land to the oceans (Hedges et al., 1997; Schlesinger and Melack, 1981). These water bodies are responsible for delivering a significant amount of C within OM (~0.4 Pg C) to the oceans. However, isotopic ($\delta^{13}C$, $\Delta^{14}C$) and biomolecular (lignin, phenol) analyses of marine DOM suggest that less than 10% of the C present in the open ocean originates from terrestrial sources (Williams and Druffel, 1987; Meyers-Schulte and Hedges, 1986; Opsahl and Benner, 1997). Historically, the riverine OM brought to the coast has been considered recalcitrant—or, less bioavailable—for microbial consumption due to its origins from soils and aquatic plants (Blanchet et al., 2017; Moran and Hodson, 1990). Furthermore, as riverine OM travels downstream, it undergoes continuous biological degradation, leading to the preferential removal of labile compounds by microbes and the eventual entry of recalcitrant components into marine systems (Moran and Hodson 1990; Raymond and Bauer, 2001a). Extensive research has demonstrated that coastal and estuarine microbial communities possess the capacity to transform and degrade riverine OM (Raymond and Baur, 2001a; 2001b; McCallister et al., 2004; Moran et al., 2000; Amon and Benner, 1996). Thus, the microbial degradation of riverine OM in coastal systems represents a critical role in mediating the transfer of C between terrestrial and aquatic biospheres.

Multiple factors intricately regulate the quantity and quality of riverine DOM including river morphology, microbial activity, and land characteristics surrounding the river (Williams et al., 2010; Parr et al., 2015). These bodies of water receive OM from diverse sources, including natural contributors such as leaf litter and soil, as well as human-influenced inputs like wastewater

and fertilizer run-off. As climate change progresses, the anticipated rise in precipitation and flood events may amplify the export of anthropogenically-influenced DOM exported to marine ecosystems (Bianchi et al., 2013; Parr et al., 2015). Rivers impacted by urbanization and agricultural land-use exhibit different molecular signatures in the DOM, such as increased heteroatoms and increased inorganic nutrients concentrations (i.e., nitrogen, sulfur and phosphorus) compared to their pristine counterparts (Vaughn et al., 2021). These increased nutrient levels contribute to enhanced C consumption and biological degradation, consequently altering the downstream DOM composition to one that resembles autochthonous, microbially-derived DOM (Vaughn et al., 2021). Conversely, rivers originating from natural forest water sheds contain DOM with higher concentrations of humic substances, aromatic C, and lignin (Vaughn et al., 2021; Opsahl and Benner, 1997). The chemical alterations induced from anthropogenic activities in transported DOM from rivers to the marine environment has the potential to impact microbial processing in coastal zones and significantly affect global C storage.

There is ongoing debate regarding whether isotopically old compounds are inherently less bioavailable to microorganisms (McCallister et al., 2004; Mayorga et al., 2005; Raymond and Bauer, 2001a; Fellman et al., 2014). Long term dark incubations of heterotrophic microbes and DOM from the York River observed that as DOM concentration decreased, the $\Delta^{14}C$ age of non-consumed bulk DOM increased (older) (Raymond and Bauer, 2001a), suggesting that microbes were preferentially consuming younger compounds. In contrast, McCallister et al. (2004) found that the microbial communities at the York coastal sites were preferentially assimilating $\Delta^{14}C$ depleted OM sources at specific temporal times, challenging the belief that age and bioavailability are inversely related. The contradicting nature of age to microbial C uptake has only been further highlighted in more recent literature. For example, DOM samples from an Amazonian River show preferential microbial use of young DOM and ^{14}C depletion downstream, while Australian rivers exhibit depleted isotopic $\Delta^{14}C$ measurements of DOM correlated with an increased bioavailable

fraction of DOM indicating that old may not mean recalcitrant (Mayorga et al., 2005; Fellman et al., 2014).

This study investigated the bioavailability of DOM from a natural, unaltered river system against DOM from a highly altered, anthropogenically-influenced river system. We explored how the effects of an anthropogenically altered watershed can affect the bioavailability of DOM by carrying out incubations using a novel bioreactor (Isotopic Carbon Respirometer-Bioreactor system) that allowed us to measure the concentration and isotopic signature of microbially respired CO_2. We carried out comparative incubations with a model marine isolate (*Pseudoalteromonas sp. 3D05*) and DOM isolated from the Suwannee River and the Upper Mississippi River Basin, both river systems in the USA. We measured and tracked microbial respiration rate (i.e., CO_2) and associated isotopic signatures ($\Delta^{14}C$) to assess the age of DOM utilized by microbes over the course of the incubation. We observed similar amounts of C degraded between two different riverine DOM samples with similar peak cell counts appearing ~12 hours into all incubations. Isotopic signatures of respired CO_2 were primarily modern throughout the incubation, but did decrease slightly over time indicative of preferential utilization of younger pools of C. The results of this work provide unique insight into the reactivity and remineralization of riverine DOM in coastal, marine environments.

2.3. Methods

2.3.1. Dissolved organic matter

Riverine DOM samples from Suwannee River and the Mississippi River were sourced from the International Humic Substances Society (IHSS). The Suwannee River (SR) is a blackwater river that runs southwestward from the Okenfenokee Swamp in Georgia, to the Gulf of Mexico through Florida. This slow moving, darkly colored river is characterized by high levels of

terrestrial-derived OM (rather than microbially derived) and high humic compound concentrations representative of low anthropogenic input. Additionally, the SR has a low pH (3.7), a DOM concentration of 82.7 mg C L^{-1} and low amounts of inorganic salts (Cawley et al., 2013; Green et al., 2015). Suwannee River DOM (SR DOM; catalog number 2R101N) was collected in 2012 by the IHSS at the southernmost dam on the Suwannee River sill, a location chosen for its sparse human population (Figure 7; 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).

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). Upper Mississippi DOM (UMR DOM; catalog number 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 1; IHSS; Wagner et al., 2015).

Figure 7. Map of the United States depicting location of rivers and DOM collection sites. Yellow star corresponds to the collection site for UMR DOM along the Mississippi River and white star shows location of collection site for SR DOM along the Suwannee River. (Map modified from USGS streamer).

Table 1. Elemental compositions of Suwanee River and Upper Mississippi riverine samples used in this study that were procured from International Humic Substance Society (IHSS).

	Elemental Composition (%)
Suwannee River DOM	
Carbon	50.70
Nitrogen	1.27
Sulfur	1.78
Upper Mississippi River DOM	
Carbon	49.98
Nitrogen	2.36
Sulfur	2.62

2.3.2. Microbial cultivation

The model bacterial isolate, *Pseudoalteromonas sp. 3D05,* was chosen for its ability to degrade complex organic matter (Enke et al., 2018; Mahmoudi et al., 2020). Cells were grown from -80°C 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 in room temperature (~22°C) at

145 RPM until log-phase growth (approximately 5 hours). Subsequently, 500 µL of culture was transferred to 50 mL of modified Tibbles-Rawling (T-R) minimal media with glucosamine (0.5% w/v) in a 250 mL flask and left to shake at 145 rpm until log phase growth was reached. In order to allow the cells to become more accustomed to the limited nutrients, this 1% inoculation was repeated in fresh modified T-R minimal media and cells were grown until reaching a density of 1.5 x 10^9 cells/mL, ~optical density (OD) of 0.6 to 0.7 for *Pseudoalteromonas sp. 3D05*. Cell density was determined using a calibration curve correlating OD at a wavelength of 600 nm measured through a spectrophotometer and colony forming units per mL (CFUs/mL; Figure 8).

Prior to injection into the bioreactor, cells were washed twice to remove traces of glucosamine as a C contaminant. A total of 50 mL of the log-phase culture were centrifuged for 10 minutes at 3000 xg (Beckman Coulter Allegra X-30R Centrifuge). The supernatant was decanted and the cell pellet was resuspended in modified T-R media with no C source and centrifuged again for 8 minutes at 3000 xg. After decanting again, the cell pellet was then resuspended in 1 mL of modified T-R media with no C source. The 1 mL cell slurry was injected into the Isotopic Carbon Respirometer-Bioreactor (IsoCaRB) system using a sterile 3 mL syringe (BD Biosciences # 309657) and a 20-gauge needle (BD PrecisionGlide™).

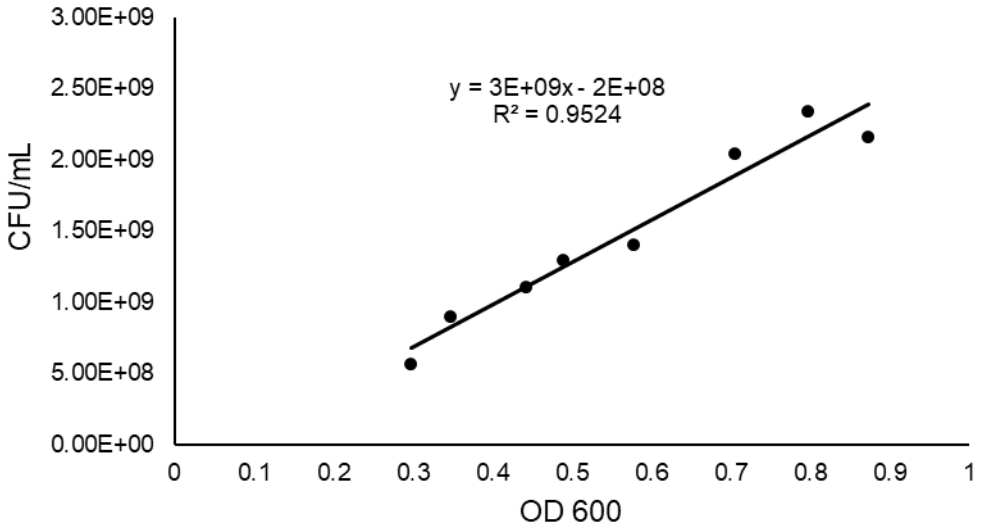

Figure 8. Optical density (600nm) vs colony forming units (CFU/per mL) of *Pseudoalteromonas sp. 3D05* in modified Tibbles-Rawling (TB) media with glucosamine as a C source.

Table 2. Components added to make 2 L of modified Tibbles-Rawling minimal media.

Component	Amount
Part 1	
NaCl	51.9 g
$MgSO_4 \cdot 7H_2O$	6 g
$MgCl_2 \cdot 6H_2O$	4 g
$CaCl_2 \cdot 2H_2O$	0.24 g
Tris (1M; pH 8.0)	50 mL
NH_4Cl (1M)	20 mL
MilliQ water	850 mL
Part 2	
K_2HPO_4	1.6 g
KH_2PO_4	0.4 g
MilliQ water	850 mL
Additives	
$FeSO_4$ solution	2 mL
Na_2MoO_4 solution	2 mL
Trace metals (1000X)	2 mL
MilliQ water (total)	Fill to 2,000 mL (approx. 250 mL)

2.3.3. Incubation in the IsoCaRB system

Two liters of modified T-R minimal media (Table 2), and approximately 700 mg of SR DOM or UMR DOM were added to the combusted custom Chemglass (Pyrex) culture vessel with a stir bar. SR DOM incubations contained ~355 mg of C or ~178 mg/L of C, while UMR DOM incubations contained ~350 mg of C or ~175 mg/L of C. The slurry was spun at 90 rpm and sparged with 100% He for 48 hours at a flow rate of ~250 mL/min and then sparged for an additional 24 hours with 20% O_2: 80% He at a flow rate of ~100 mL/min. Gases remained at this proportion for the entirety of the incubation to mimic atmospheric conditions. The culture vessel was covered completely in aluminum foil to decrease the potential of UV/Vis light penetration and alteration to DOM.

After 72 hours of sparging, the microbial cultural was injected into the system through the injection port. Respired CO_2 was measured and tracked on an infrared CO_2 analyzer (Sable Systems CA-10) and a custom LabVIEW program (National Instruments) (Figure 9). Custom

molecular sieve traps were used to obtain the respired CO_2 for processing on the vacuum line. Molecular sieve CO_2 traps were changed every 12 - 36 hours. Replicate incubations (A and B) were performed with the SR DOM and UMR DOM. A total of 5 CO_2 fractions were collected per incubation with the exception of incubation B of SR DOM which had 6 traps due to method development. Lastly, ~ 40 mL subsamples were removed every 12 hours to track cell density.

2.3.4. Blank test in the IsoCaRB system

Blank tests were performed for each DOM sample to quantify the contribution of "background C " as a result of off-gassing rather than biological activity. The blank test incubations contained 700 mg of DOM (SR or UMR) and 2 L modified T-B media. 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 C for the same duration (~ 4.5 days) of the blank test. Therefore, each blank test resulted in one fraction of C collected that was quantified manometrically and sent for isotopic analysis.

Table 3. Summary of microbially-respired CO_2 fractions and approximate collection time.

Fraction #	Collection Time (days)	Collection Duration (hours)
1	0.0-0.5	12
2	0.5-1.0	12
3	1.0-2.0	24
4	2.0-3.0	24
5	3.0-4.5	36

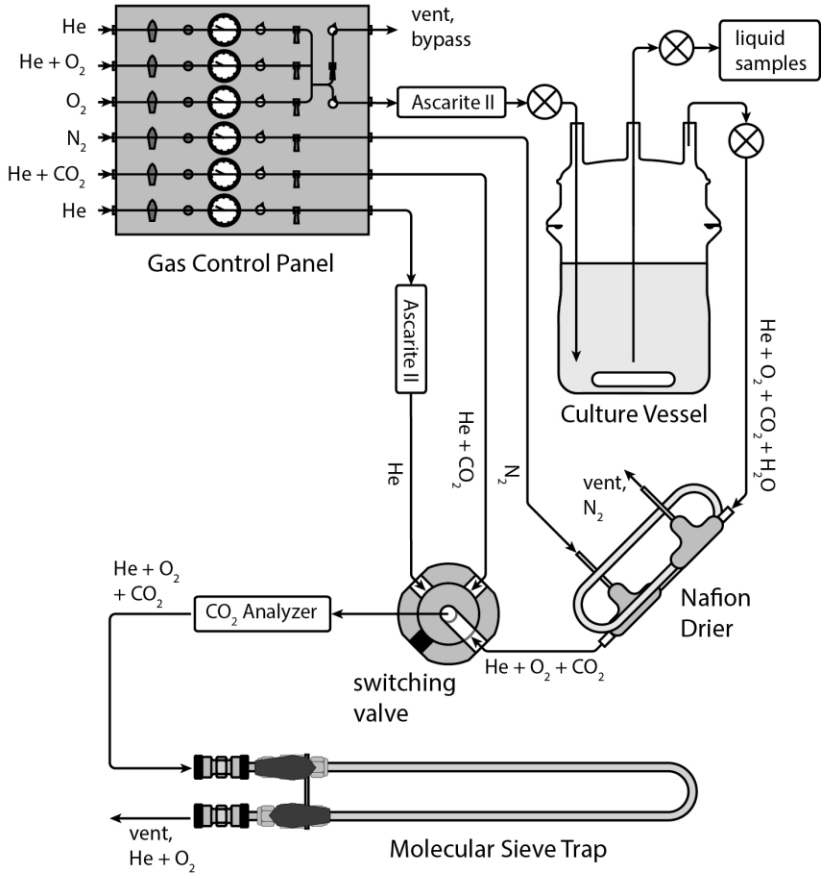

Figure 9. Diagram of the IsoCaRB system showing the gas delivery and purification system, 3 L culture vessel, an inline CO_2 detector (CA-10 analyzer), and custom CO_2 traps (molecular sieve traps). Ascarite is used to remove any CO_2 from contaminated He and N_2 is used to dry the wet, respired CO_2 gas due to respiration of H_2O. Cells are injected through a Teflon septum and growth medium can be sampled from the culture vessel through a stainless-steel tube.

2.3.5. Cell density

Approximately 100 µL of incubation slurry was serially diluted to 10^{-5}. Subsequently, 100 µL was plated in triplicate onto marine broth 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 to determine cell density at the time of sampling. Subsamples for cell density taken every 12 hours for the duration of each incubation.

2.3.6. Extraction and purification of respired CO_2

Molecular sieve traps loaded with respired CO_2 were baked in an oven at 530°C under vacuum for 30 minutes to extract the CO_2. When heated, the CO_2 is released from the molecular

sieve beads and travels through the vacuum line. The vacuum line is equipped with two cold traps (E and F; Figure 10) that consist of a glass dewar flask filled with powdered dry ice mixed with minima ethyl alcohol (~72°C). When the sample passes through these slush traps the water is deposited out as ice. The CO_2 is collected in a cryogenic cold trap (D; Figure 10) which is submerged in a glass dewar flask filled with liquid nitrogen (LN_2; -196°C), the lower temperature causes the sample to trap out. After the 30-minute bake, the CO_2 trap is then switched to a water trap for 5 minutes to allow the CO_2 to thaw into a gaseous phase while maintaining any water still in the sample to be frozen. The CO_2 sample is then moved through the line to a manometer for quantification (C; Figure 10). Finally, the sample is moved to a combusted, glass collection tube for flame sealing (B; Figure 10).

The tubes of gaseous CO_2 were then sent to the Keck-Carbon Cycle Accelerated Mass Spectrometry (AMS) facility at the University of California, Irvine ^{14}C measurement. Each sample was reduced to graphite (Vogel et al., 1987) to measure the radiocarbon value ($\Delta^{14}C$) using an AMS and corrected for graphite and media contributions. Respired CO_2 was sent for isotopic analyses from a single incubation (incubation A) for each DOM sample in an effort to reduce costs.

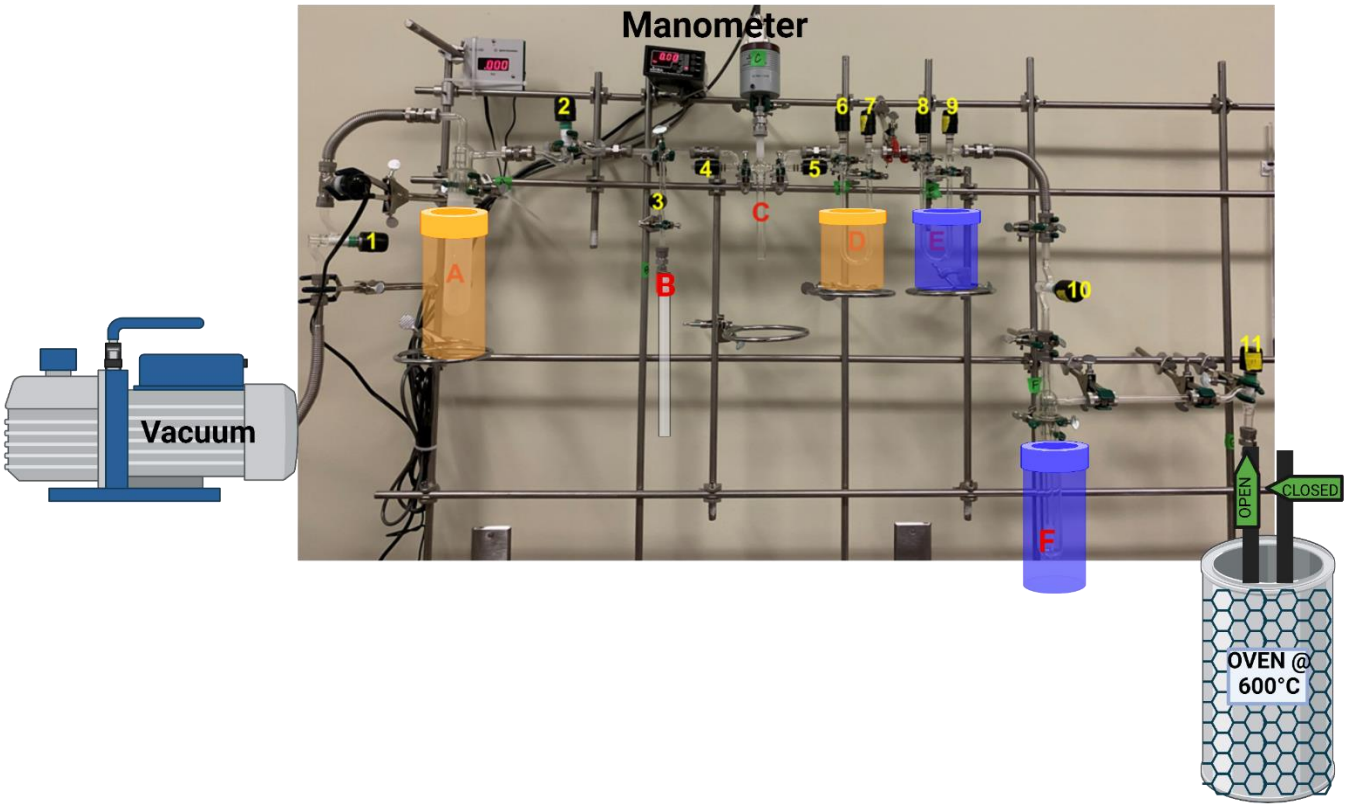

Figure 10. Diagram of the vacuum line indicating location of slush traps composed of powdered dry ice and ethyl alcohol, estimated temperature -78°C (blue cylinders) and liquid nitrogen traps, temperature -196°C (yellow cylinders).

2.4. Results

Bioreactor incubations of SR DOM and UMR DOM with *Pseudoalteromonas sp. 3D05* displayed similar respiration patterns with a single distinct peak of produced CO_2 for all incubations. CO_2 production quickly increased to peak within the first 10-16 hours of each incubation and then, progressively decreased to near baseline CO_2 levels within 4.5 days. This is consistent with previous incubations of this same isolate with sterilized marine sediment (Mahmoudi et al., 2020).

Similar CO_2 production rates were observed during incubation with SR DOM and UMR DOM, with a peak rate of 0.7-0.9 µg C L^{-1} min^{-1} indicating that thebioavailabilty of these two C pools was similar. Correspondingly, the total quantity of respired C collected across incubations was similar both between replicates and sources of DOM (Table 4) and ranged from 3.9 to 4.5 mg.

Over two-thirds of the respired C was collected in the first 24 hours (fractions 1 and 2) of the incubation coinciding with the appearance and subsequent decline of the single peak.

All radiocarbon ages of respired CO_2 from successive fractions (fractions 1 through 5) were deemed modern and therefore are C from recently photosynthesized sources, such as plant material within riverine DOM (Table 6; Czimczik and Welker, 2010). The SR DOM incubation values ranged from 33‰ to 13‰ (Table 6) and exhibited a general depletion over time indicating progressively older C utilization, with a 20‰ decline from fraction 1 to fraction 4 (Figure 12A). The $\Delta^{14}C$ values of respired CO_2 for the UMR DOM incubation ranged from 27‰ to 12‰. (Table 6, Figure 12B). From fraction 1 to 2 there was an enrichment by 4 ‰, followed by a 10‰ depletion from fraction 2 to 3. Then, fraction 4 was enriched again by 3‰, with a final depletion of 8‰ from fraction 4 to 5.

Cell counts increased for the first 48 hours peaking at 4.9-5.7 x 10^7 CFU/mL (Figure 11). Following the growth phase, cell densities decreased for the remainder of the incubation to 4.5 x 10^6 - 1.4 x10^7 CFU/mL (Table 5). The appearance of the cell count peak was offset and lagged behind the respired C peak, peaking around 48 hours.

Table 4. Total collected mass from the incubations with *Pseudoalteromonas sp. 3D05* and SR or UMR DOM. Additionally total collected mass of blank incubations with SR or UMR DOM in the absence of live cells.

	Total mass collected (µg C)
Suwannee River DOM	
Incubation A	4514 ± 11
Incubation B	3920 ± 10
Blank	197 ± 1
Upper Mississippi River DOM	
Incubation A	4378 ± 11
Incubation B	4203 ± 10
Blank	161 ± 1

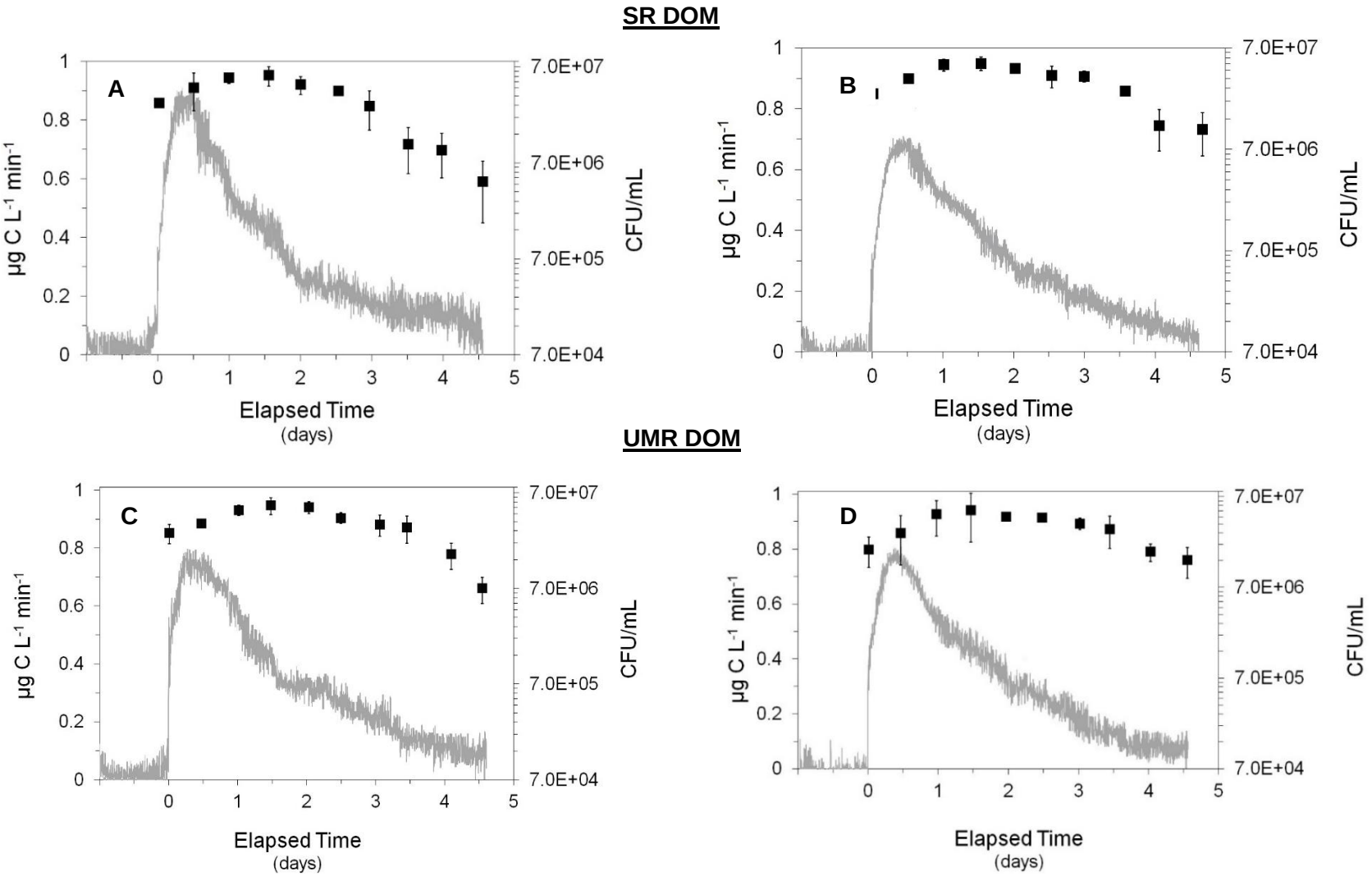

Figure 11. Respiration rates (grey line; µg C L^{-1} min^{-1}) of *Pseudoalteromonas sp. 3DO5* with Suwannee River DOM incubation (A and B) and Upper Mississippi River DOM (C and D) over time (gray line) compared to average cell density over time (black squares) shown for initial incubation (A and C) and replicate (B and D). Error bars indicate standard deviations of the average (n=3).

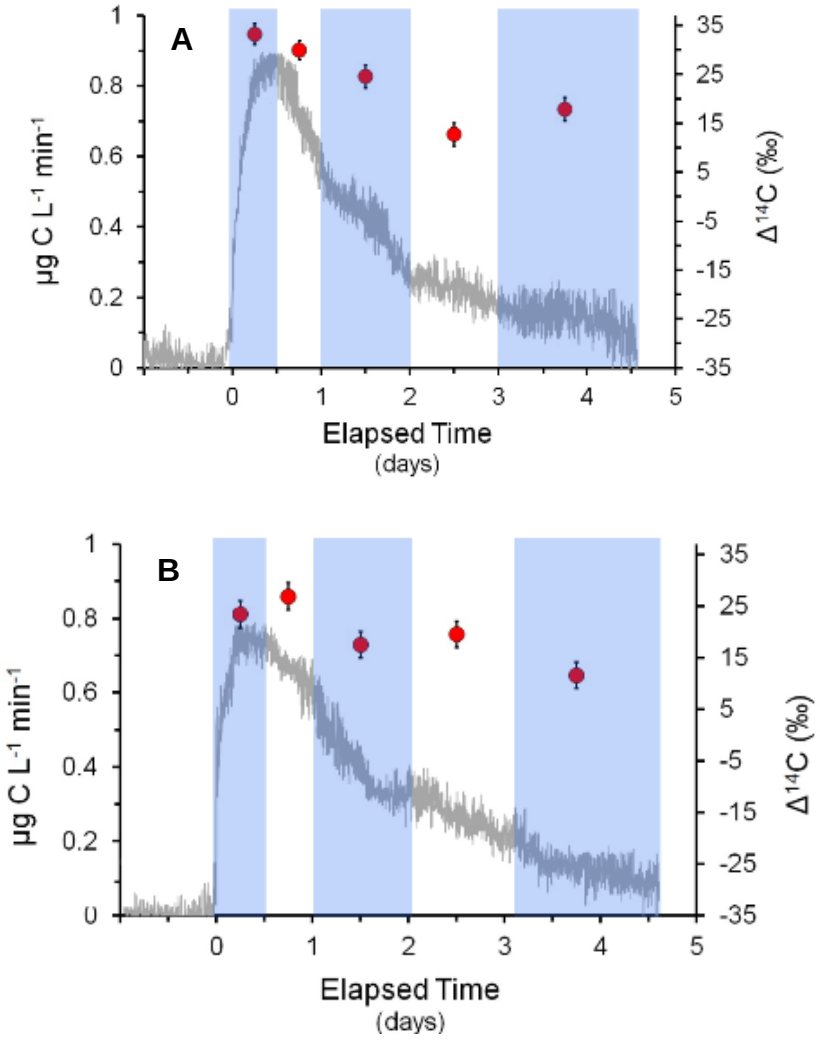

Figure 12. Microbial respiration rates (grey line; μg C L^{-1} min^{-1}) and $\Delta^{14}C$ (red circles) signatures (‰) of CO_2 observed during incubation of *Pseudoalteromnas sp. 3D05* with 700 mg of (A) SR DOM or (B) UMR DOM. The width of each blue box spans the time interval during which each CO_2 fraction was collected for isotopic analysis, with the corresponding data point plotted at the mid-point for each fraction.

Table 6. Summary of respired CO_2 from incubations of *Pseudoalteromonas sp. 3D05* with SR and UMR DOM. Individual fractions and their collection times, duration, mass of CO_2 and $\Delta^{14}C$ (‰) values. --- indicates no value since they were not measured.

	Fraction #	Collection Times (days)	Collection Duration (hr)	Mass CO_2 (µg C)	$\Delta^{14}C$ (‰)
Suwannee River DOM					
Incubation A	1	0.0-0.5	12.0	1697 ± 4	33 ± 2
	2	0.5-1.0	12.0	1680 ± 4	30 ± 2
	3	1.0-2.0	24.0	450 ± 1	25 ± 2
	4	2.0-3.0	24.0	370 ± 1	13 ± 2
	5	3.0-4.6	37.4	317 ± 1	18 ± 2
Incubation B	1	0.0-0.5	12.4	1422 ± 3	---
	2	0.5-1.0	11.9	1378 ± 3	---
	3	1.0-2.0	24.2	412 ± 1	---
	4	2.0-3.0	23.5	257 ± 1	---
	5	3.0-4.0	25.0	221 ± 1	---
	6	4.0-4.7	14.9	230 ± 1	---
Upper Mississippi River DOM					
Incubation A	1	0.0-0.5	12.0	1581 ± 4	23 ± 2
	2	0.5-1.0	12.4	1592 ± 4	27 ± 2
	3	1.0-2.0	24.3	496 ± 1	17 ± 2
	4	2.0-3.1	24.7	398 ± 1	20 ± 2
	5	3.1-4.6	36.1	310 ± 1	12 ± 2
Incubation B	1	0.0-0.5	11.6	908 ± 2	---
	2	0.5-1.0	12.5	2100 ± 5	---
	3	1.0-2.0	24.2	454 ± 1	---
	4	2.0-3.0	23.8	432 ± 1	---
	5	3.0-4.6	37.4	309 ± 1	---

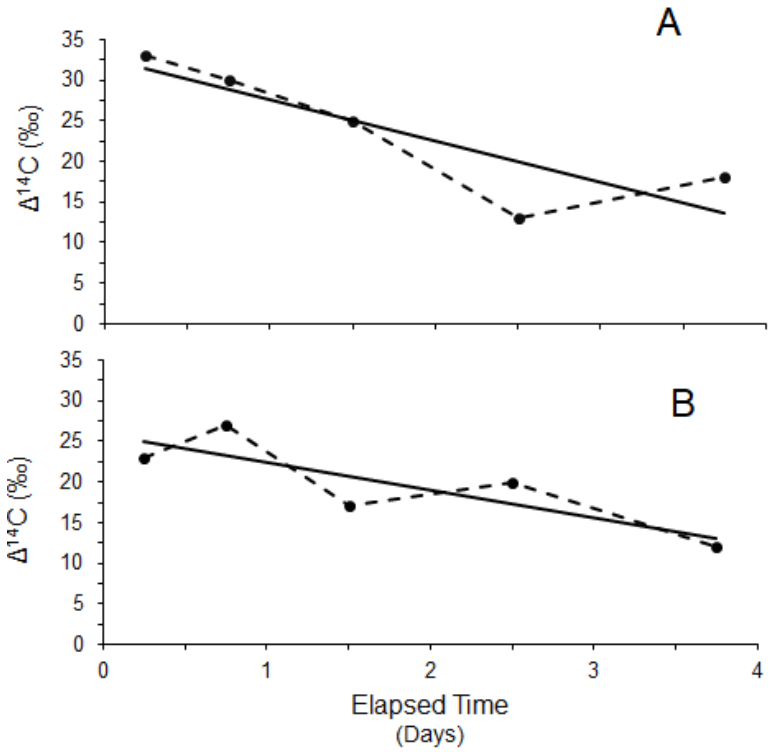

Figure 13. Measured $\Delta^{14}C$ (‰) of microbially respired CO_2 during the course of incubations for SR DOM (A) and UMR DOM (B). Dashed line follows changes between collected respired CO_2 fractions while the solid line indicates the overall trend.

2.5. Discussion

Understanding the mechanisms that underlie microbial degradation of DOM is vital for developing a comprehensive understanding of the global C cycling. Here, we incubated a marine microbe and riverine DOM from two distinct sources to investigate whether an anthropogenically altered watershed can affect the downstream bioavailability of DOM to microbes. Unlike traditional bottle incubations, we utilized a novel system that enabled us to resolve the sequential degradation of DOM over time through analysis of consecutive CO_2 fractions. We observed similar degradation patterns in terms of total respired C, and ^{14}C age of respired CO_2 between DOM isolated from two different river systems. These results suggest that biotic degradation of

riverine DOM in coastal environments may be universal, regardless of differences in DOM composition due to watersheds.

Respiration rates across all incubations were similar and consistent with previous incubations of different riverine DOM, indicating that microbes were respiring and growing at approximately expected rates (Moran et al., 1999). The total quantity respired CO_2 (from the total C pool; Table 1) was nearly identical for SR (~1.2%) and UMR DOM (~1.3%) indicating that the bioavailability of both DOM pools did not differ substantially. This is similar to previous incubation work that estimated between 0.8% and 17.7% of DOM utilization by microbes in aquatic environments (Moran et al., 1999; McCallister et al., 2006; Raymond and Bauer, 2000). Although our results are on the lower end of that range, the incubations from previous studies were conducted on much longer timescales (i.e., months) with natural microbial assemblages.

The similar bioavailability of SR and UMR DOM is surprising because historically, anthropogenically influenced DOM (i.e., UMR DOM) has been found to be more degradable and more labile (i.e., shorter residence time) than natural watershed counterparts (Butman et al., 2012; Vaughn et al., 2023). Anthropogenically influenced DOM tends to have increased concentrations of heteroatoms, specifically N (Wagner at al., 2015). Studies comparing C: N ratios to bioavailable DOM have found that increasing N content of riverine DOM can be a predicator of bioavailable DOM (Benner, 2003; Hunt et al., 2000; Sun et al., 1997). In many aquatic environments, N is a limiting nutrient, therefore compounds within DOM containing N (i.e., proteins and amino acids) are “mined” specifically for N (Benner, 2003). UMR DOM has approximately twice the N content as SR DOM and therefore, it was expected that significantly more UMR DOM would be consumed by the bacteria (Table 1; IHSS). Our experiments used an artificial media with added N and no limiting nutrients suggesting that when N availability is accounted for N content of DOM may not

be an indicator for bioavailable DOM, as a similar amount of C was consumed from DOM from both rivers.

Resolving the sequential consumption of C pools by microbes can help elucidate the degradation process by revealing trends in C preferences. The recalcitrant fraction of DOM may be difficult to degrade for inherent chemical or physical factors (Hedges et al., 2001; Rothman and Forney, 2007; Amon and Benner; 1994 Mahmoudi et al., 2020). Through incubations of riverine DOM and a natural bacterial community, Raymond and Bauer highlighted an apparent age-reactivity continuum within riverine DOM, in which radiocarbon deplete (i.e., younger) compounds are likely more labile than the older compounds present (Raymond and Bauer 2001a; 2001b). This was further corroborated in 2005 when by isotopic data which suggested that most of the CO_2 outgassed by the Amazon River due to microbial respiration was isotopically younger than the bulk OM pool (Mayorga et al., 2005). These findings applied to the marine environment may explain why marine DOM is aged 4,000-6,000 years, due to microbial degradation selectively consuming younger compounds first and old DOM remaining (William and Druffel, 1987). The results from this study further this concept by showing that this concept holds true in the case of riverine DOM in coastal systems based on our isotopic signatures which showed preferential consumption of younger compounds.

The $\Delta^{14}C$ signatures of respired CO_2 across all incubations were determined to be of modern origin, with an age of within the last 10 years which precisely matches measured ages of other DOM from USA rivers (Raymond and Bauer et al., 2001b). Marwick et al. (2015), proposed predictions of decadal aged riverine DOM exported to coastal areas ($\Delta^{14}C = +22$ to $+46‰$), also matching our results. Results from the SR incubation show a clear depletion overtime, indicating that younger pools of C were more labile to *Pseudoalteromonas sp.* 3D05. This result corroborates

an age-reactivity continuum and shows that this continuum can occur in short times spans (~12 hours) of the degradation process. In the SR incubation, *Pseudoalteromonas sp.* 3D05 consumed compounds with radiocarbon ages of 33‰ to 13‰, yet SR DOM has a bulk radiocarbon age of 45‰ (Coppola et al., 2015), indicating that there are substrates that are in fact more modern than those that were respired during our incubation. These more enriched compounds are more likely to be terrestrially derived plant matter that contribute to modern radiocarbon age from recent photosynthetic activity but may be less bioavailable to this microbe.

There is also general depletion in $\Delta^{14}C$ from fraction 1 to 5 of the UMR DOM incubation, however the fractions displayed a continuous pattern with likely same aged compounds consumed for the duration of the incubation (Figure 13). This could be due to quantitatively more molecular formulas with a same age because anthropogenically influenced rivers have DOM with more individual molecular formulae or higher molecular richness (Vaughn et al., 2021). The general ageing of respired compounds from the onset to the end of the incubation does indicate an age-reactivity continuum for riverine DOM. The different trends in $\Delta^{14}C$ between the two incubations may suggest that early DOM degradation in coastal environments is nuanced and may vary only slightly from locations.

Although these incubations could have been left to continue even when respiration rate approached baseline, it would be expected that the microbes would switch from consuming the provided DOM to preferentially consuming the more bioavailable, autochthonous DOM produced by the surrounding dead cells. Therefore, incubation length was determined and cut off to avoid this process and focus on riverine DOM consumption solely.

We looked at the very early stages of DOM degradations by running much shorter incubations (~ 4.5 days) creating an inherent a focus on labile DOM. Although the overall

degradation of both rivers was extremely similar, the differences on hourly timescales may be relevant during pulse-shunt events that occur due to flooding, snow melt and storm surges (Raymond et al., 2016). The pulse-shunt conceptional model refers to the increased DOM concentration and the faster water velocities that occur during various environmental events (Raymond et al., 2016). These events are known to bring quantitatively more terrestrial DOM through rapid transport to coastal areas that can create altered DOM degradation patterns (Bianchi et al., 2013; Raymond et al., 2016). Due to climate change and increasing storm severity, flooding and pulse-shunt events are expected to occur more often in the near future, highlighting the need to understand how they alter the short and long- term C cycles.

3. General Conclusion

3.1. Summary of findings and implications

The purpose of this study was to compare the degradation of DOM from two distinct rivers in the marine environment. These rivers varied in their watershed, or surrounding land use, with one being more anthropogenically influenced and the other maintaining its pristine conditions. The UMR Basin has been affected by growing population requiring the watershed to be converted from natural conditions to urbanized and crop land. The SR has not been as affected, making these two rivers excellent sources to compare the bioavailability of DOM. To investigate this and general coastal DOM degradation, we incubated the riverine DOM with an artificial marine media and a coastal marine microbe. Overall, degradation rates and respired CO_2 quantity and age were nearly identical indicating that bioavailability of DOM may not vary due to watershed use. Additionally, the results of this study further corroborate the age-reactivity continuum in which microbes first consume the young, most bioavailable compounds and then consume progressively older compounds.

3.2. Additional considerations

Our unique approach to studying DOM degradation allows for direct measurement and capture of respired CO_2 over time, while the more traditional method of bottle incubations relies on indirect proxies for DOM studying such as bulk DOM changes in age and concentration or O_2 requirements. We utilized a species-specific investigation into riverine DOM cycling, which neglects community composition dynamics that play a role in DOM degradation processes (Young et al., 2005). A more accurate view into in-situ DOM degradation would require the use of natural microbial communities and populations to study the interplay of various microbial species. *Pseudoalteromonas sp. 3D05* was chosen as a model organism because it can degrade polymers and is found in the coastal ocean. However, the use of a different coastal marine microbe would

likely result in different patterns of observed DOM degradation. This is because bioavailability is dependent on the microbes that are present and a C substrate may be accessible to one species, but not be to another (Mahmoudi et al., 2020).

3.3. Future research

Continuation of this line of research using this method is vital to unlocking the mystery of DOM in the oceans. Further studies should include DOM from other rivers to continue comparisons across river types and should harness a dual isotopic approach ($\delta^{13}C$ and $\Delta^{14}C$). The use of both isotopes allows for a mass balance end-member model to be used to determine the likely sources and percentage of each source consumed within fractions. Additionally, incubations with other isolates of microbes could provide information between taxa and community differences in C consumption of DOM from the same source. Marine DOM would be a difficult to obtain, yet highly interesting C source to use in the bioreactor because of its role in long-term C sequestration in the global C cycle.

Besides altering DOM source, recreating conditions more similar to in-situ conditions may give a more realistic look into DOM degradation. Using filtered water samples and natural assemblages may provide a new perspective into degradation with the addition of factors from the in-situ environment. Additionally, this approach would test the controversial dilution-limit hypothesis (Arrieta et al., 2015), that claims that the recalcitrant fraction of DOM is unable to be degraded by microbes because it is present in low concentrations. Natural systems have a much lower DOM concentration than the incubations presented here. We utilized a higher DOM concentration to ensure sufficient C was consumed for isotopic tests.

Finally, the DOM itself can be altered then incubated and compared to investigate various environmental conditions. For example, DOM can be exposed to UV/Vis radiation to further the

photochemical studies on DOM and include the novelty of consecutive isotopic fractions. Conversely, DOM can be treated with enzymes to study how enzymatic cleavage changes bulk DOM bioavailability to microbes and if ages are significantly altered over time.

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