Investigating the Bioavailability of Terrestrial Organic Matter to Marine Microorganisms

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Table of Contents

Abstract

Terrestrial organic matter is an important source of dissolved organic matter (DOM) to the global ocean, however, the fate and cycling of this DOM is poorly understood. While it is believed that this carbon pool is essential to marine heterotrophic bacteria in pelagic surface waters, several studies attribute variable levels of lability to this DOM fraction. This study attempts to characterize the bioavailability of terrestrial DOM to a marine isolate through carbon isotope analysis of microbially respired carbon dioxide (CO_2) using a novel bioreactor system. Potential contributions of background carbon, such as media off-gassing and vitamin additions, were evaluated and characterized to constrain isotopic measurements. Bioreactor incubations using Suwannee River DOM and *Vibrio sp.* 1A01 yielded rates of carbon respiration rates up to ~0.7 µg C L^{-1} min^{-1} and a maximum cell density of ~9.9 x 10^6 CFU/mL. The $\Delta^{14}C$ values of respired CO_2 fractions were -28.8‰ to -72.1‰, suggesting preferential degradation of older organic matter. The $\delta^{13}C_{CO2}$ values were -35.3‰ and -20.9‰, consistent with degradation of organic matter derived from modern macrophytes as well as aged C_3 and C_4 plants. The results of this study highlight the utility of carbon isotopes in tracing the origins of labile DOM and shed light on how marine microbes may interact with terrestrial DOM transported to the ocean.

Résumé

La matière organique terrestre est une source importante de matière organique dissoute (MOD) dans l'océan mondial, cependant, le devenir et le cycle de cette MOD sont mal compris. Alors que l'on pense que ce réservoir de carbone est essentiel aux bactéries hétérotrophes marines dans les eaux de surface pélagiques, un certain nombre d'études attribuent des niveaux variables de labilité à cette fraction de MOD. Cette étude tente de caractériser la biodisponibilité de la MOD terrestre à un isolat marin par l'analyse des isotopes du carbone du dioxyde de carbone (CO_2) respiré par des microbes à l'aide d'un nouveau système de bioréacteur. Les contributions potentielles du carbone de fond, telles que le dégazage des milieux et les ajouts de vitamines, ont été évaluées et caractérisées pour contraindre les mesures isotopiques. Incubations de bioréacteurs utilisant Suwannee River DOM et *Vibrio sp.* 1A01 a donné des taux de respiration du carbone jusqu'à ~0,7 µg C L^{-1} min^{-1} et une densité cellulaire maximale de ~9,9 x 10^6 CFU/mL. Les valeurs $\Delta^{14}C$ des fractions de CO_2 respirées étaient de -28,8‰ à -72,1‰, suggérant une dégradation préférentielle de la matière organique plus ancienne. Les valeurs de $\delta^{13}C_{CO2}$ étaient de -35,3‰ et -20,9‰, ce qui correspond à la dégradation de la matière organique dérivée des macrophytes modernes ainsi que des plantes C_3 et C_4 âgées. Les résultats de cette étude mettent en évidence l'utilité des isotopes du carbone pour retracer les origines de la MOD labile et mettent en lumière la façon dont les microbes marins peuvent interagir avec la MOD terrestre transportée vers l'océan.

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I acknowledge that this research took place at McGill University in the city of Montreal, Quebec, first named Tiohtià:ke, on the unceded lands of indigenous peoples. This land serves as a site of meeting and exchange amongst First Nations, including the Haudenosaunee and Anishinabeg nations. The Kanien'kehá:ka Nation has long been recognized as the custodians of the land and water on which Montreal has been colonized. I also acknowledge that this research uses material taken from the Suwannee River, Georgia, United States, which flows through the unceded lands of the Timucua and Muscogee Creek nations. The Suwannee River is considered a sacred and holy place to the Timucua nation. I honor and respect the long heritage of the peoples on whose land this research was conducted. Furthermore, I recognize the historical stewardship of these nations which preserved these lands before their colonization, and the brutality and pain that this colonization has caused - and continues to cause - the indigenous peoples residing here.

Contribution of Authors

All sections of this document were composed by the submitting student, Lindsey Potts. Feedback and edits were provided by Dr. Nagissa Mahmoudi acting as an academic advisor. Isotopic analysis of CO_2 samples was carried out by Dr. Xiaomei Xu at the Keck-Carbon Cycle AMS facility (University of California, Irvine) in collaboration with Dr. Brett Walker, (University of Ottawa). Data analysis and corrections were performed by the submitting student, Lindsey Potts, with feedback from Dr. Nagissa Mahmoudi. There were no further contributors to this document.

List of Tables

List of Figures

1. Literature Review

1.1. Marine dissolved organic matter (DOM)

The carbon sequestration potential of the ocean is one of the most important carbon balancing forces on Earth. Of the total carbon (C) stored in the ocean, 662 gigatonnes (Gt) are comprised of dissolved organic matter (DOM) molecules, representing one of the largest carbon reservoirs on the planet (Hedges 2002; Ogawa and Tanoue, 2003). Once transported to the deep ocean, DOM is considered relatively stable and may not reenter the surface carbon cycle for centuries or millennia (Hansell et al., 2009). As a result, there is particular interest in developing a mechanistic understanding of DOM cycling and its relationship to the broader carbon pool.

Organic matter is the most complex biomaterial on Earth and consists of thousands of different compounds derived from living organisms. Operationally, organic matter can be delineated into two fractions, DOM or particulate organic matter (POM), wherein the use of a 0.7 micron filter partitions DOM in the filtrate and POM remains on the filter. Both DOM and POM are considered a part of a size continuum, with either portion contributing to the concentration and composition of the other. As such, POM dissolution contributes to DOM and DOM flocculation contributes to POM (Hansell et al., 2009). Organic matter can be further defined by its relative availability for biological uptake and degradation. Labile DOM (LDOM) is considered the most bioavailable form of DOM and has a rapid turnover rate of minutes to hours, and consequently makes up a relatively small fraction of the bulk DOM pool (Hansell et al, 2009; Moran et al, 2015). Semi-labile DOM is degraded more slowly, it thus accumulates within the surface mixed layer of the ocean and persists from months to years (Hansell et al, 2009). Lastly, refractory DOM (RDOM) has an average radiocarbon age of 4,000 to 6000 years and accounts for an estimated 650 Gt of the overall marine DOM reservoir (Williams and Druffel,

1987; Ogawa and Tanoue, 2003). Given that the global ocean turns over every ~1500 years, this implies that most of the carbon sequestered in the deep ocean persists for several ocean mixing cycles. It is this "refractory" and stable DOM which comprises the ocean's substantial reservoir of reduced C and sequesters it for – in some instances – millennia.

Labile DOM represents the most readily available portion of C to microbes, therefore understanding the molecular composition of this pool could provide insight into which components are most readily cycled. However, identification of discrete molecules within the marine DOM reservoir represents a major obstacle for clarifying how C is microbially mediated. Owing to limitations in current methodologies, as well as the highly fragmented nature of the DOM pool, <10% of marine DOM exists in a form that can be recognized through current molecular analyses (Benner, 2002). The current understanding of the molecular composition of DOM is largely limited to broad categories. It is known that labile DOM can include amino acids, sugars, and organic acids (Ogawa and Tanoue, 2003), whereas semi-labile DOM is almost exclusively comprised of carbohydrates (Hansell et al, 2009). The composition of RDOM is largely unknown, as fragmentation of once whole DOM molecules during export into the deep ocean or sequential degradation over time leaves behind unidentifiable portions (Ogawa et al, 2001). Given the limited extent of DOM characterization, it is widely believed that DOM lability is dependent on a complex combination of its chemical composition, the metabolic pathways present in local microbial communities, and the physical and chemical properties of the surrounding environment (Lonborg and Alvarez-Salgado, 2009).

1.2. Transport of terrestrial organic matter to marine systems

Terrestrial organic matter is a significant source of carbon transported into marine environments. Annually, this flux represents approximately 0.25 to 0.5 Petagrams (Pg) of C moved from land to sea (Figure 1; Hedges et al., 1997). This export of terrestrial DOM by rivers constitutes ~33% of the total C – organic and inorganic C combined - transported into the ocean (Bianchi, 2011). The flux of DOM from land to ocean is considered well constrained, since it is a direct function of river discharge (Dai et al., 2012). Therefore, regional hydrology and climate play major roles in controlling terrestrial DOM delivery (Singh et al., 2019). The broader implication of this relationship indicates alterations in precipitation patterns from climate change and changes in hydrology from anthropogenic land use may have a profound impact on annual terrestrial DOM transport to the ocean (Wear et al., 2014).

Efforts to make generalized characterizations of terrestrial organic matter transported by rivers are complicated by spatial and temporal factors that alter DOM composition. Overall, bulk transported DOM tends to be more heavily dominated by terrestrial rather than riverine inputs (McCallister et al., 2006; Raymond and Bauer, 2001a; Raymond and Bauer, 2001b; Raymond and Spencer, 2015). This DOM is progressively degraded by aquatic microbes during transport, resulting in the addition of somewhat diagenetically advanced and pre-aged DOM to coastal margins (Raymond and Bauer, 2001b). Terrestrial DOM predominately originates from C_3 plant sources such as litter, leachate, and root exudates (Hedges et al., 1997), where litter has been shown to contribute up to 85% of DOM in some rivers (McCallister et al., 2004). As a result, terrestrial DOM is often replete with aromatics, lignins, tannins, and humic substances and is considered to have enriched C contents relative to marine DOM (Seidel et al., 2014; Malcolm et al., 1995; Hopkinson and Smith, 2005). The relative abundance of compounds such as humic

substances lead many to conclude terrestrial DOM is largely recalcitrant (Blanchet et al., 2017, Moran and Hodson, 1990). However, in a phenomenon known as the 'size reactivity continuum', high molecular weight (HMW) compounds – which dominate the terrestrial DOM pool – are considered to be more labile than low molecular weight (LMW) compounds. This is owed to the fact that LMW DOM is comprised of more diagenetically advanced components, which are therefore less energetically favorable to access (Mannino and Harvey, 2000; McCallister et al., 2006; Young et al., 2005; Amon and Benner, 1996).

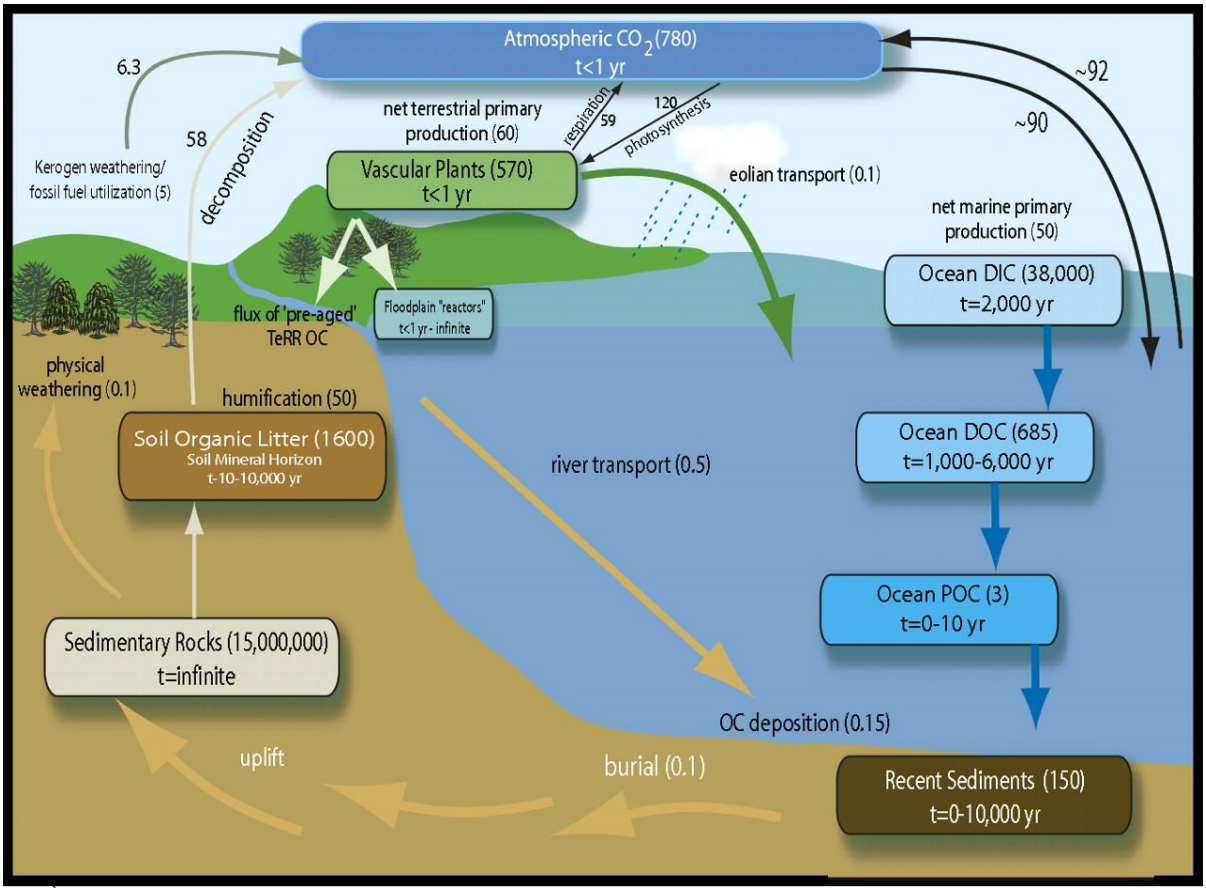

Figure 1. Transport of carbon between systems (in Pg C; Adapted from Bianchi, 2011)

Although the transport of terrestrial DOM constitutes a major addition of C to the ocean, stable isotope and biomarker (e.g., lignin, phenol) measurements suggest there is little terrestrial contribution to the overall marine DOM pool (Meyers-Schulte and Hedge, 1986; Opsahl and

Benner, 1997; Hernes and Benner, 2002; Hernes and Benner, 2006). This presents a contradiction wherein terrestrial DOM is considered largely refractory, yet must be almost completely removed or transformed within the continental margins. Current literature suggests that as little as 1% of DOM in the open ocean originates from terrestrial sources (Hernes and Benner, 2002; Hernes and Benner, 2006). This residual DOM, which escapes degradation or other forms of removal at the continental margins, can subsequently be entrained in deep ocean currents (Medeiros et al., 2015; Medeiros et al., 2016), where it may be stored on long timescales (centuries to millennia). Therefore, cycling of terrigenous DOM in the continental margins and its fate have broad implications on both the global C cycle and the C storage capacity of the deep ocean.

1.3. Heterotrophic consumption and degradation of terrestrial organic matter

Marine bacteria play a crucial role in consuming, transforming, and ultimately cycling, DOM. Their size relative to other microscopic marine life provides them an advantage, wherein bacteria can access nutrients at vanishingly low (nanomolar) concentrations, allowing them to transform DOM in even nutrient deficient regions (Azam et al, 1983; Kawasaki and Benner, 2006; Jiao et al, 2011). Heterotrophic bacteria are capable of consuming and degrading both DOM and POM from a wide variety of sources. However, the bioavailability of terrestrial DOM to marine bacteria is not well understood and widely debated. Estimates of bacterial degradation of terrestrial DOM pools range widely from as low as 3% derived from modelling (Raymond and Bauer, 2000) to as high as 30% using bottle incubations (Moran et al., 1999) and 60% using lignin as a biomarker (Fichot and Benner, 2014). In another study, terrestrial DOM was found to account for as much as 83% of C used to support coastal bacteria biomass production (McCallister et al., 2004). This substantial variation in observed DOM use, alongside the

apparent lack of terrestrial DOM in the open ocean, highlights the potentially wide variability in DOM quality and efficiency of microbial metabolic pathways to use this carbon pool.

Early studies focused primarily on the chemical composition of the labile DOM pool; however, the importance of microbial metabolic diversity in DOM degradation is a growing focal point. A study by Young et al. (2005), which used natural bacterial communities in bottle incubations and measured the change in cell density and the DOM pool, concluded that community assemblages and bacterial physiology played a significant role in DOM lability. A later study, which also used the bottle incubation approach, by Blanchet et al. (2017) came to a similar conclusion, noting that the modification of species dominance in native bacterial communities was stimulated by the addition of riverine DOM from other locations rather than a pure labile source. Further, it has been suggested that coastal and marine microbes are better suited to degradation of terrestrial DOM than riverine communities based on comparative incubations using fresh and marine microbial communities (Raymond and Spencer, 2015), highlighting the disparity in DOM degradation capacity across microbial communities. Potential reasons for this variation in efficiencies is due to the expression of extracellular enzymes, which marine bacteria produce more of than their aquatic counterparts (Raymond and Spencer, 2015). Broader implications of studies such as these indicate that the presence of certain metabolic pathways may be of greater importance to the efficient degradation of DOM than its strict chemical composition.

1.4. Isotopic approaches to measuring microbial DOM cycling

1.4.1. Applications of isotopic analyses to microbial degradation of DOM

Methodological applications of isotopes to investigate biogeochemical cycling have been employed for decades (Benner, 2004). Without the ability to measure isotopes, the earliest studies investigating DOM degradation relied on simplistic approaches such as measurements of absolute concentrations of DOM and biological O_2 demand over time, resulting in mischaracterizations of the DOM pool and the relative importance of microbes (e.g., microbes are not important in the ocean; Keys et al., 1935). The addition of isotopic analyses to investigate microbial cycling of DOM has been a boon, fundamentally transforming how microorganisms and DOM are framed (Anderson et al., 2015). However, traditional bottle incubations investigating the interactions between microbes and DOM are still limited to isotopic changes in microbial biomass and DOM (Raymond and Bauer, 2001a). These bulk measurements can provide broad generalizations about the DOM pool, allowing for distinction between modern and aged C, as well as marine, aquatic, or terrestrial C (Raymond and Bauer, 2001a; Raymond and Bauer, 2001b). While this begins to provide insight into the origin of DOM in the marine pool, it does not provide specific information regarding which portions of DOM is being degraded by microbes. This is owed to the nature of measuring changes in bulk DOM, wherein movement towards a more depleted or enriched isotopic value can infer what is being removed, but is indirect and relies on various assumptions about the composition of the initial DOM pool.

Improvements over time in methodology and technology have granted a greater understanding of the complexities of DOM cycling due to specificity in isotopic variations of constituents in the DOM pool. For example, isotopic changes between source pools and the biomass $\delta^{13}C$ of specific compounds such as monosaccharides, lipids and other organic

molecules have been explored (Van Dogen et al., 2002; Teece and Fogel, 2007). How these components are isotopically altered during degradation can provide important insight when interpreting bulk DOM data from in situ samples. That being said, stable carbon isotope analyses alone only provide a partial picture of the nature of DOM degraded by microbes. Further information can be attained by the analysis of radiocarbon, which elaborates on the age of utilized DOM. The use of radiocarbon ($^{14}C/^{12}C$) analyses in natural abundance samples was often impossible due to mass requirements of such samples. Strides to decrease the mass needed to perform sensitive isotope analyses, such as $\Delta^{14}C$, has allowed for novel approaches to microbial incubations with OM (Khosh et al., 2010). Previous studies utilizing isotopic measurements for investigating microbial degradation of DOM have been limited to direct in situ measurements (McCallister et al., 2004; Raymond and Bauer, 2001a). The implementation of bioreactor incubations using microbial communities or isolates have not been extensively explored, with only a handful of studies using this approach for POM (Mahmoudi et al., 2019). Therefore, the incorporation of stable and radiocarbon isotopes in controlled incubations represents a novel method of investigating microbial DOM degradation. Direct measurements of respiration or biomass using these isotopic measurements may provide greater insight into the nature of microbial degradation of DOM.

1.4.2. Stable carbon and radiocarbon analyses

Natural abundance stable carbon (^{13}C) and radiocarbon (^{14}C) isotopic analyses have become powerful tools for understanding the sources and ages of natural organic matter consumed by microbes (Pearson et al., 2008; Mahmoudi et al., 2013). This approach is derived from the observation that respiration from heterotrophic microorganisms reflects the same $\Delta^{14}C$ and nearly identical $\delta^{13}C$ signatures as their carbon sources (Blair et al., 1985; Hayes, 2001).

Thus, the carbon isotopic signature of microbially respired CO_2 provides a direct measure of substrate utilization of isotopically-distinct sources.

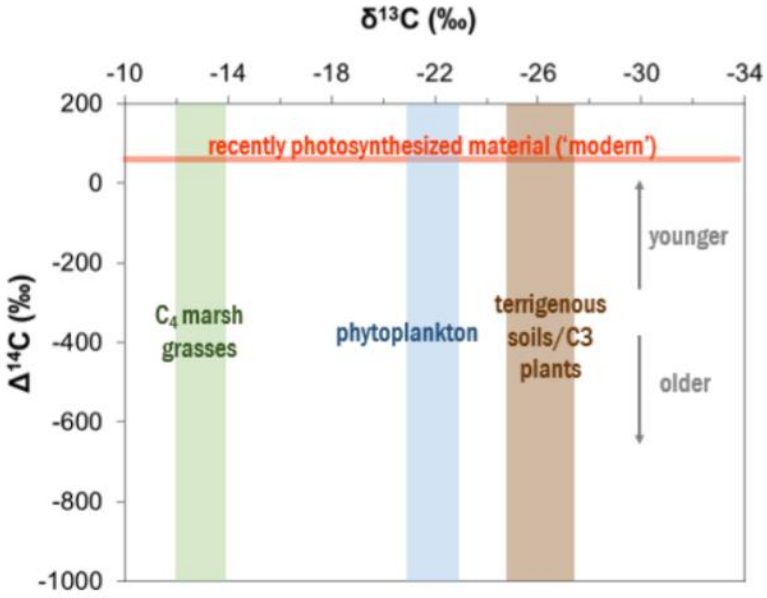

Figure 2. Comparison of $\delta^{13}C$ and $\Delta^{14}C$ values of C_4 plants (green), phytoplankton (blue), and soil or C_3 plants (brown); wherein progressively negative $\Delta^{14}C$ values indicate older C.

Organic matter in the environment consists of a wide spectrum of $\Delta^{14}C$ and $\delta^{13}C$ signatures, which can be exploited to identify sources and ages of DOM used by microbes. Due to their utility in many applications, significant efforts have been made to characterize and understand the isotopic composition of major sources of organic matter (Figure 2). Major DOM reservoirs which microbes may degrade include both terrestrial and aquatic plants, phytoplankton, and petrogenic byproducts, each having their own distinct isotopic ranges. Terrestrial plants can be separated into two groups on the basis of photosynthetic pathways: C_3 and C_4. While C_3 plants have a generally light isotopic composition, C_4 plants – found frequently in water limited regions - are much more isotopically enriched (Hayes, 2001). The result is a significant separation of observed values between the two, where C_3 plants range between -30‰

to -20‰ and C_4 plants from -14‰ to -10‰ (Fry and Sherr, 1989); to simplify their potential contributions, widely accepted averages (-27‰ and -12‰ respectively) are frequently used when evaluating relative contributions of C from potential sources (Fry and Sherr, 1989; Ehleringer et al., 1993).

Unlike terrestrial plants, aquatic plants' stable isotope range is dependent on whether their leaves are in contact with the atmosphere, as well as the $\delta^{13}C$ value of the dissolved inorganic carbon (DIC) pool (Osmond et al., 1981; Keeley and Sandquist, 1992). Submerged macrophytes are entirely influenced by DIC due to their lack of contact with the atmosphere, whereas emergent macrophytes use both atmospheric CO_2 and DIC for photosynthesis. Therefore, submerged macrophytes can have significantly depleted $\delta^{13}C$ values, as low as -44‰, whereas values of emergent macrophytes are close to those found in C_3 plants (Osmond et al., 1981; Keeley and Sandquist, 1992; France, 1996; Mendonca et al., 2013, Chappuis et al., 2017). Although $\delta^{13}C$ is a useful measurement of distinct C sources, there exists non-trivial overlap of some major sources of OM. DOM produced by phytoplankton, for instance, overlaps C_3 plants (-22‰), owing in part to use of the same photosynthetic pathway (Fry and Sherr, 1989; Hayes, 2001). Finally, DOM from petrogenic byproducts is depleted in $\delta^{13}C$, but has a range wherein the values overlap terrestrial plant C (-34‰ to -24‰) (Vieth and Wilkes, 2010). This overlap of stable carbon values of $\delta^{13}C$, necessitate the use of multiple isotopes to better resolve DOM consumed and degraded by microbes.

In some instances, radiocarbon is used in conjunction with stable carbon isotopes to provide better clarity on the nature of microbially degraded OM. Radiocarbon will decay over time such that older compounds will contain less ^{14}C; therefore, ratios of $^{14}C/^{12}C$ or radiocarbon signatures ($\Delta^{14}C$) can be used to assess the age of organic compounds and their residence time in

the environment. Recently photosynthesized material will have a "modern" ^{14}C value that is consistent with atmospheric CO_2 values (~+50‰). Thus, organic matter from terrestrial and aquatic plants reflects young $\Delta^{14}C$ values (Schiff et al., 1990; Richter et al., 1999). Older compounds contain little ^{14}C, resulting in depleted values. For example, the ancient nature of fossil fuels makes them radiocarbon dead ($\Delta^{14}C$=-1000‰), and represent a uniquely distinct isotopic signal compared to other sources DOM.

In terrestrial soil there is a gradient of $\Delta^{14}C$ values which become aged with depth according to time since burial and diagenetic state (Richter et al., 1999). This is an important consideration depending on the mode of transport of terrestrial DOM to rivers, as erosion of old sediment will have an isotopically aged signature (Raymond and Spencer, 2015). Aged DOM with terrestrial stable isotope values may originate from soil if hydrological characteristics promote deep soil erosion. Similarly, DOM from marine phytoplankton is characterized by an apparent older $\Delta^{14}C$ value due to the aged isotopic signature from marine DIC as a result of the marine reservoir effect (Beaupre, 2015). Therefore, marine DOM from C recently fixed by marine phytoplankton will appear more aged compared to DOM from other photosynthetic material (McNichol and Aluwihare, 2007; Griffith and Raymond, 2010; Beaupre, 2015).

Early literature suggested that DOM lability and age were directly related, stating that the older the DOM source, the less labile it was. In other words, old DOM was considered stable and to be largely unusable by most – if not all – microbes. However, the decoupling of age and lability has now become apparent. Extremely labile DOM of petrogenic origins or released from thawing permafrost has illustrated that physical characteristics of the environment play a significant role in preventing degradation, and that age is not necessarily indicative of OM lability (Raymond and Spencer, 2015). Moreover, the introduction of anthropogenic C from

fossil fuels in the form of aerosols and changes in land use by agriculture release substantial pre-aged, yet labile, C into the environment (Raymond and Spencer, 2015).

As an accompaniment to $\delta^{13}C$ values, radiocarbon can show the relative age – and provide further hints on the source – of degraded DOM. Applications of a dual isotope approach to measuring changes in DOM facilitated by microbes include mass balances and mixing models (Raymond and Bauer, 2001a; Mahmoudi et al., 2019). These approaches can be applied on a broad range of scales, from basin wide estimates to incubations. During this process, accepted values are used in order to constrain relative contributions of each isotopic end member. For example, as described above, marine phytoplankton are typically assigned a value of -22‰ $\delta^{13}C$ and terrestrial plants a value of -27‰. These approaches highlight why the painstaking characterization of major C pools are essential to evaluating the importance of each source to a given process – such as microbial degradation – or region.

2. Introduction

2.1. Background

Terrestrial organic matter represents an important source of carbon to the ocean and plays a fundamental role in supporting coastal microbial communities (Hopkinson and Smith, 2005). Much of this organic carbon (C) is in the form of dissolved organic matter (DOM) and is delivered to the ocean through riverine inputs which are estimated to contribute ~0.2 Pg of C per year to the global ocean (Hedges et al., 1997; Seitzinger and Harrison, 2005; Meybeck 1982; Dai et al., 2012; Hedges et al., 1997). This transport of DOM is predominately controlled by hydrological events, such as droughts or flooding (Singh et al., 2019; Asmala et al., 2021), indicating that the terrestrial DOM flux to the ocean will be sensitive to alteration of precipitation patterns from climate change (Wear et al., 2014; Raymond et al., 2016).

Although terrestrial DOM is a substantial contributor to the marine carbon cycle, direct measurement of isotopic ($\delta^{13}C$) and biomolecular (e.g., lignin, phenol) tracers have shown that very little terrestrial DOM exists in the open ocean (Meyers-Schulte and Hedge, 1986; Opsahl and Benner, 1997). Some studies using biomarkers and mass balances suggest terrestrial DOM comprises only ~1-3% of bulk DOM in the open ocean (Hernes and Benner, 2002; Hernes and Benner, 2006). This implies that the majority of terrestrially sourced DOM must be transformed or removed almost completely in surface waters before being exported (Fichot and Benner, 2014). Though the exact quantity of terrestrial DOM that is removed in a given region is dependent on a number of physical factors, most crucially residence times, microbial degradation may play an important role in its removal.

Heterotrophic bacteria play a key role in consuming and degrading DOM in the ocean. Evidence from direct measurements of stable isotopes ($\delta^{15}N$, $\delta^{13}C$) from bacterial nucleic acids

has shown that in some locations, terrestrially sourced DOM can support as much as 83% of coastal bacterial biomass production (McCallister et al., 2004). While there is sufficient evidence that DOM is removed in the coastal margins of the ocean (Hernes and Benner, 2002), there is limited understanding as to how it is degraded. This is of particular interest, as much terrestrial DOM is considered to be refractory and – therefore – not accessible by microbes (Blanchet et al., 2017; Moran and Hodson, 1990). The bioavailability of terrestrial DOM may be dependent on several variables including (1) inorganic and trace nutrient requirements (Amon and Benner, 1996), (2) temperature dependence of metabolic and enzymatic reactions (Raymond and Bauer, 2000), (3) bacterial physiology and community (Young et al., 2005), and (4) chemical composition.

Isotopic analyses have been shown to be an effective method to understand the lability of natural organic matter to microorganisms (Beaupre, 2015; Benner, 2004). Stable and radiocarbon measurements have the potential to infer the sources of carbon that support microbial populations, since microbially respired CO_2 reflects the isotopic signature of their C source (Hayes, 2001). Thus, we can evaluate the isotopic fingerprint of respired CO_2 to determine what C sources are being degraded and consumed by active microbial populations. For example, the isotopic signature of photosynthetically fixed carbon varies between terrestrial C_3 (-27‰) and C_4 (-12‰) plants (Fry and Sherr, 1989), as well as marine phytoplankton (-22‰) (Coleman and Fry, 1991). Additionally, $\Delta^{14}C$ values provide further information regarding age and origin based on the partitioning of modern and aged DOM into particular reservoirs. As with $\delta^{13}C$ values, marine and terrestrial DOM values tend to have relative distinctions, where bulk terrestrial DOM is observed to be modern and marine DOM appears aged due to the reservoir effect (Beaupre,

2015). Therefore, the coupling of these two isotopic measurements can act as a powerful tool to resolve the lability of terrestrial DOM in the ocean.

2.2. Study objectives

This study aimed to assess the bioavailability of terrestrial DOM to marine microbes using a bioreactor (IsoCaRB system) to measure and collect microbially respired CO_2 for $\delta^{13}C$ and $\Delta^{14}C$ analysis. We carried out a series of experiments in which we incubated a model marine bacterium (*Vibrio splendidus* 1A01) along with isolated DOM from Suwannee River (Georgia, United States). In contrast to traditional bottle incubations which rely on indirect proxies – such as DOM concentrations or biological oxygen demand – our approach allows us to directly measure DOM degradation and determine the source and age of substrates that were consumed and respired.

3. Methodology

3.1. Dissolved organic matter

Suwannee River DOM (SRDOM) was obtained from the International Humic Substances Society (IHSS; catalog number 2R101N). This material was collected in 2012 by the IHSS at the headwaters of the river where the Okefenokee Swamp drains (Figure 3). The river itself is characterized by high levels of organic matter, low pH, and low amounts of inorganics and anthropogenic input (Malcolm et al., 1995). SRDOM is considered high in terrestrial organic matter (Cawley et al., 2014). Extraction procedure for this DOM is described by Green et al. (2015); to summarize – approximately 36,890 l of water were filtered and concentrated using reverse osmosis, this water was then desalted by CEX, and the purified sample was freeze dried and homogenized. According to elemental analysis, the final sample contains ~50.7% C (IHSS). Approximately 700 mg of SRDOM was added to each incubation, resulting in ~354.9 mg of C or 177.45 mg l^{-1} per incubation. This concentration was chosen to allow for a high enough yield of CO_2 for continuous concentration measurement and isotopic analysis, but was low enough to be cost efficient and with the aim to eventually modify this method for other, less readily accessible DOM (e.g., marine DOM). Prior to incubation, DOM sterility was tested by plating a small aliquot dissolved in Tibbles-Rawling (T-R) minimal media. After 72 hours, the plates were checked for colony growth and no colonies were observed.

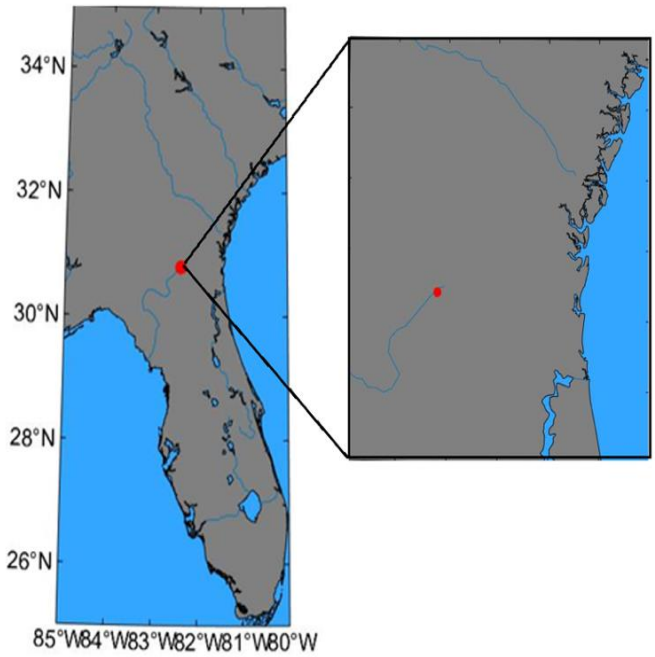

Figure 3. Location of SRDOM sampling site at the headwaters of the Suwannee River, Georgia, US.

3.2. Microbial cultivation

The model bacteria strain *Vibrio splendidus* 1A01 was chosen for its significant abundance in coastal systems (Le Roux et al., 2009) and its ability to degrade complex substrates (Enke et al., 2018). Cultures were prepared from an isolate stock frozen at -80˚C in glycerol solution. Stock solution was added to 20 mL of Marine Broth (Difco 2216) in a 125 mL flask and placed on a shaker table set to 150 RPM. After the culture had achieved mid-log phase (5 hours), it was transferred to 50 mL of T-R minimal media (2% inoculation) amended with glucosamine (TCI Chemicals) as a carbon source (0.5% w/v) in a 250 mL flask. The newly inoculated flasks of media were also placed on a shaker table set to 150 RPM and grown to mid-log phase (9 hours). A final aliquot was transferred to a new set of 50 mL of T-R media (2% inoculation) until it reached the desired cell density of 4 x 10^8 cells/mL (16 hours). Cell density

was determined using a calibration curve relating optical density and colony forming units per mL (CFUs/mL).

Bacterial cells were washed with carbon-free media prior to injection into the system. To achieve this, 50 mL was transferred into Falcon tubes and centrifuged for 10 minutes at 3000 x g (Beckman Coulter Allegra X-30R Centrifuge). The overlying solution was decanted, and the pellet resuspended with T-R minimal media containing no carbon source. The cells were again centrifuged at 3000 x g for 8 minutes. The final pellet was resuspended with 1 mL of T-R minimal media containing no carbon source and injected into the IsoCaRB system using a sterile 3 mL syringe (BD Biosciences) with a 20-gauge needle (BD PrecisionGlide).

3.3. Incubation in the IsoCaRB System

Incubations were carried out in the IsoCaRB system using the protocol as described by Beaupre et al. (2016). For each experiment, approximately 2 l of T-R minimal media and 700mg of SRDOM were added to the sterilized custom Pyrex culture vessel (Figure 4). The vessel was purged of atmospheric CO_2 by sparging the media with ultra-high purity He for 48 hours, after which gases were adjusted to create a 20% O_2 concentration in the vessel and further sparging occurred for an additional 24 hours to reduce the vessel background from off-gassing by the media and sample DOM. Inflowing gases remained at this O_2/He ratio during the incubation so the internal conditions were similar to atmospheric concentrations of O_2.

On completion of the sparging, the bacterial culture was injected into the system to begin the incubation. Respired CO_2 was measured and logged continuously using an infrared CO_2 analyzer (Sable Systems CA-10) paired with a custom LabVIEW program (National Instruments) (Figure 4). Custom molecular sieve traps were used to capture CO_2 and were

removed after accruing approximately 1000 µg of C as estimated by LabVIEW, resulting in 3 traps collected for the initial incubation and 2 for its replicate. Incubations were allowed to continue until measured CO_2 levels reached apparent baseline levels again, indicating no new CO_2 production from respiration. A total of two incubations were run for a duration of three days each, excluding sparging times.

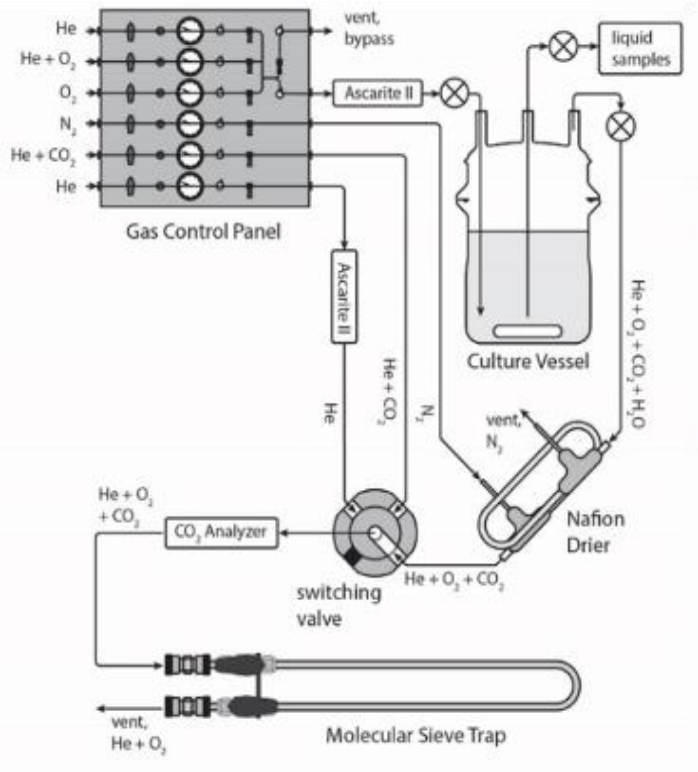

Figure 4. The IsoCaRB system containing: a gas delivery and purification system, 3 l culture vessel, an inline CO_2 detector, and custom CO_2 traps. Growth medium can be sampled from the culture vessel through a stainless-steel tube.

3.4. Collection and analysis of carbon isotopes

The molecular sieve traps used to collect respired CO_2 were sent to University of California, Irvine's Keck Carbon Cycle AMS Facility for processing and analysis. There, they were baked at 500˚C for 30 minutes to recover the stored carbon, which yielded between 40 to

90% of C estimated by LabVIEW. This C was then turned into graphite as described in Vogel et al. (1987) and modified by Xu et al. (2007), samples were then analyzed for their $\Delta^{14}C$ isotopic composition by accelerator mass spectrometer (AMS). Prior to graphitization, an aliquot was removed for the purpose of $\delta^{13}C$ analysis by GC-IRMS. All isotopic values were corrected for background contamination from both the system and the sample DOM (total ~80.5 µg C/day, $\delta^{13}C$ = -29.5‰, $\Delta^{14}C$ = 12.2‰) (Table 1). Stable isotope values are reported against VPDB and radiocarbon values are reported in $\Delta^{14}C$ as outlined in Stuiver and Polach (1977).

3.5. Tracking cell density

Cell density was tracked throughout the incubation via cell counts using Marine Broth agar plates. Approximately 1mL of subsample was collected using the sampling port and serially diluted. Timepoints taken within the first 48 hours were diluted by 10^4, all timepoints afterwards had lower dilutions of 10^3 due to low cell density; from the diluted sample, 100µL was plated in triplicate using rattler beads (Zymo #S1001). Plates were grown at room temperature and colony forming units (CFU) were counted after 48 hours had elapsed since plating.

The overall degradation of SRDOM can be normalized for cross literature comparisons, even given differences in methodology, using a decay constant. This constant uses the change in concentration of DOM over time. For this study, we estimated final DOM concentrations based off of total respired CO_2 and biomass determined by cell counts as described above, assuming those were the only two losses of carbon. The following equation was used to calculate the decay constant:

$$Eq.\ (1)\quad k = \frac{\ln\left(\frac{C_f}{C_0}\right)}{\Delta t}$$

where k is the first order decay constant, C_f and C_0 are final and initial concentrations, respectively, and Δt is the change in time between first and last timepoints.

3.6. Tests to evaluate and characterize blank contributions

Prior to experimentation with *Vibrio sp.* 1A01, a thorough evaluation of potential contaminant sources of C was carried out. This was essential not only for the correction of isotopic measurements as described previously, but to also confirm the validity of our experimental measurements. For the following tests, the preparation of the IsoCaRB system and the processing of collected C occurred as described above except for the addition of the isolate. First, the media contribution was evaluated by adding 2 l of T-R minimal media to the IsoCaRB system and running the system for ~93 hours. Next, a repeat test was done with the addition of ~700 mg of SRDOM to T-R media run for ~93 hours. Finally, carbon isotope analysis of the cell pellet used to inoculate the system, the vitamins added to the media, and the glucosamine used to grow the bacteria prior to its introduction to the IsoCaRB system was carried out. Stable isotope values were obtained by EA-GC-IRMS and radiocarbon values were by analyzed by AMS. Bulk blank contributions measured by the molecular sieve traps were used to correct measured sample values via a simple mass balance formula derived from Hayes (2004):

$$Eq.\ (2)\ m_{measured}\delta_{measured} = m_{sample}\delta_{sample} + m_{blank}\delta_{blank}$$

$$Eq.\ (3)\ f_{measured}\delta_{measured} = f_{sample}\delta_{sample} + f_{blank}\delta_{blank}$$

Assuming:

$$f_{measured} = f_{sample} + f_{blank} = 1$$

Which was further derived to solve for δ_{sample}:

$$Eq.\ (4)\ \frac{\delta_{measured}-\delta_{blank}f_{blank}}{1-f_{blank}} = \delta_{sample}$$

where $f_{measured}$, $f_{sample,}$ and f_{blank} are the fractional contributions of the measured, sample, and blank respectively; the terms $\delta_{measured}$, δ_{sample}, and δ_{blank}, are the $\delta^{13}C$ or $\Delta^{14}C$ values; and $m_{measured}$, m_{sample}, and m_{blank} are the mass contributions of each term. The fraction of sample and blank were found by determining the rate of µg C hr^{-1} contributed by the T-R minimal media with SRDOM test, calculating the total µg C produced by the bulk blank for the duration the trap's collection, and then dividing the blank by the µg C recovered from the molecular sieve trap during sample analysis.

4. Results

4.1. Blank characterization and tests

To constrain potential sources of background carbon, we carried out a series of blank tests prior to performing incubations with *Vibrio sp.* 1A01 (Table 1). First, 2 l of T-R minimal media only was incubated for a ~93 hours. A total of 39 µg C over 4 days (~9.96 µg C d^{-1}) was collected from this 'media only' blank test and determined to have a $\Delta^{14}C$ = -309.5‰ (Table 1). We suspect that the source of this carbon is the vitamin mixture added to the media which was found have a very old $\Delta^{14}C$ value (-920.54 ‰) (Table 1). However, it is important to note that the total input of carbon from the vitamin mixture (~ 480 µg C) is very small proportional to the carbon present in added SRDOM (~355 mg).

A second blank test was carried out by incubating 700 mg of SRDOM along with 2 l of T-R minimal media for 93 hours (Table 1). A total of 322 µg (~83 µg C d^{-1}) of C was collected and determined to have a $\Delta^{14}C$ value of 12.2‰ and $\delta^{13}C$ value of -29.5‰ (Table 1). This blank test representing off-gassing from both the T-R minimal media and SRDOM was used for blank corrections on isotopic values of CO_2 during incubation with *Vibrio sp.* 1A01. While this blank carbon was higher than we had anticipated, confidence in the isotopic measurements of our experiment is high given the efforts to characterize background sources of carbon and their respective isotopic values.

Lastly, we examined the extent to which any respiration of C may be derived from recycling of cellular biomass or metabolic waste rather than the added carbon source. We determined that the initial *Vibrio sp.* 1A01 cell pellet that is injected into the IsoCaRB system has a $\Delta^{14}C$ value of -457.19 ‰, reflecting the old carbon present in the T-R minimal media

(Table 1). Subsequently, we incubated *Vibrio sp.* 1A01 with 10 mg (5 mg l^{-1}) of a simple sugar with a known isotopic value (glucosamine, $\Delta^{14}C$ =33.4‰; Table 1). During this incubation, we collected CO_2 for only the first 10 hours (total of 250 μg C) when we assumed that turnover of the initial cellular biomass would likely occur. Indeed, we found that $\Delta^{14}C$ values of this respired carbon was -125.5‰ despite the glucosamine being modern ($\Delta^{14}C$ =33.4‰) indicating that there was a small contribution of respiration from older carbon substrates (Table 1). In the context of this study, it is impossible to determine the extent to which remineralization of this older material may occur in the presence of a large pool of DOM. Based on our starting cell density, we determined that there was a total of 483 μg of C in the form of *Vibrio sp.* 1A01 biomass, assuming 50 fg C $cell^{-1}$ (Loferer-Krossbacher et al., 1998), in our IsoCaRB incubations which is significantly smaller than the pool of SRDOM.

Table 1. Summary of blank tests. Characterization of the stable and radiocarbon isotopes, collection duration (when applicable), and total mass collected.

Sample	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	Collection Duration (hrs)	Mass (μg C)
Glucosamine (pure)	-24.4 ± 0.1	33.4 ± 2.1	-	80
Vitamin Mixture	-32.3 ± 0.1	-920.54 ± 0.7	-	483
Respired Glucosamine	-	-125.5 ± 1.5	10	249
SRDOM Blank	-29.5 ± 0.2	12.2 ±1.6	93	336
Media Blank	-	-309.5 ± 4.1	93	39
Freeze Dried Cells	-	-457.19 ± 3.7	-	51

4.2. Respiration rates and cell density

Incubation of *Vibrio sp.* 1A01 with SRDOM revealed a respiration pattern with a single distinct peak consistent with previous incubations done with this bacterial isolate (Mahmoudi et al., 2019). Most of the respiration occurred rapidly within the first 24 hours of incubation, with

maximum respiration rates of ~ 0.7 µg C L^{-1} min^{-1} within the first 10 hours (Figure 5a). Following this initial rapid increase of CO_2, respiration gradually declined such that by 24 hours the observed respiration rate was substantially lower. By the end of 48 hours, CO_2 production was elevated only ~0.02 µg C L^{-1} min^{-1} above background, and 72 hours showed a complete decrease back to baseline rates.

Subsamples were collected through the incubation to track cell density. Bacterial cell density ranged from 7.0 x 10^5 CFU/mL to 9.9 x 10^6 CFU/mL and mirrored respiration patterns with a slight time offset (Figure 5a). Peak respiration and peak cellular density were decoupled, with the latter lagging behind by approximately 2 hours. The decrease in respiration corresponded with a substantial decrease in cell density in the culture, which appeared to plateau at its lowest value around ~7x10^5 CFU/mL (Figure 5a).

The replicate incubation was performed with identical parameters as initial incubation, including SRDOM added and sampling times relative to elapsed time of the incubation. Peak rates reached an observed maximum of ~0.95 µg C L^{-1} min^{-1} within the first 12 hours of incubation (Figure 5b). Respiration subsequently decreased and had somewhat elevated rates of ~ 0.08 µg C L^{-1} min^{-1} (Figure 5b). By the end of 72 hours, respiration rates had functionally returned to baseline.

As with the first incubation, the replicate incubation's growth patterns indicated maximum respiration and cell density were decoupled. Maximum observed cell density occurred 10 hours after inoculation and was ~1.1 x 10^7 CFU/mL (Figure 5b). Cell density then trended

downwards over time, with minimum cell density measured after 72 hours at ~1.3 x 10^6 CFU/mL (Figure 5b).

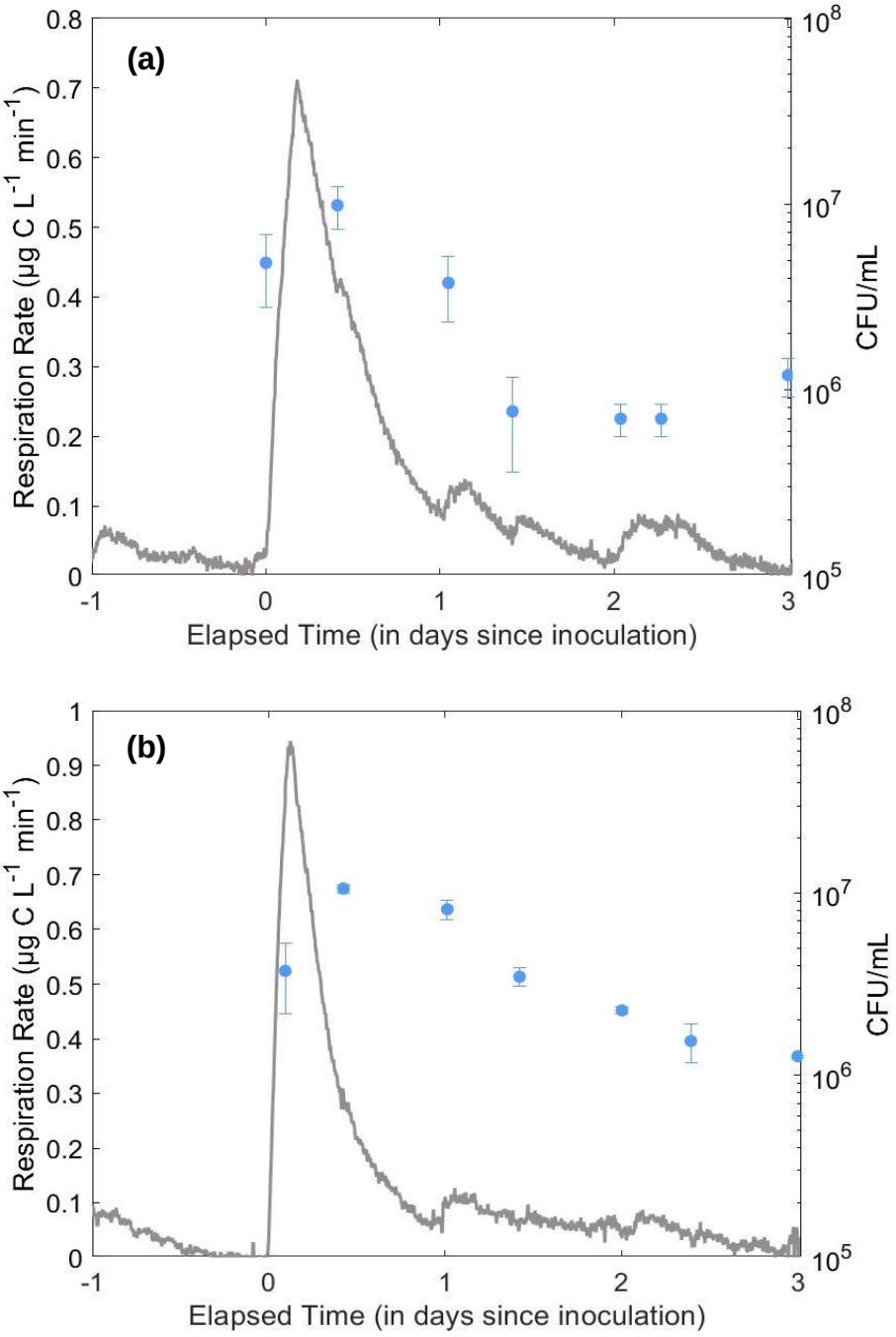

Figure 5. Respiration rates of *Vibrio sp.* 1A01 with Suwannee River DOM incubation over time (gray line) compared to average cell density over time (blue circles) shown for initial incubation (a) and replicate (b). Error bars indicate standard deviations of the average (n=3).

4.3. Carbon isotopes

Over the course of the incubation, CO_2 fractions were collected for $\delta^{13}C$ and $\Delta^{14}C$ analysis to understand the age and source of labile DOM. The first fraction was captured in the first 10 hours of incubation and encompasses the onset of CO_2 production and its maximum. A total of 928 µg C was recovered, and analysis of $\Delta^{14}C_{CO2}$ values showed carbon respired within the first 10 hours of the incubation was -28.8‰ (Figure 6a; Table 2). The second fraction, which lasted 38 hours and incorporated the remainder of the initial respiration peak, as well as the decline down to baseline, had an older carbon signature with a $\Delta^{14}C$ = -72.1‰ (Figure 6a). The third fraction was discarded due to high blank contribution (44.6%) and low C yield (190 µg C) (Table 2). These results show that for both fractions, a less than modern source of carbon is being utilized for respiration, and progressively older carbon is being degraded over time.

Table 2. Summary of microbially-respired CO_2 collected during incubation of *Vibrio sp.* 1A01 with SRDOM. Individual fractions and their collection durations, total mass collected, $\delta^{13}C$ and $\Delta^{14}C$ values, and percent of blank carbon contribution.

Sample	Collection Duration (hr)	Mass Collected (µg)	$\delta^{13}C$ (‰)	$\Delta^{14}C$ (‰)	Blank (%)
Fraction 1	10	928	-35.3 ± 0.1	-28.8 ± 2.0	4.4
Fraction 2	38	450	-20.9 ± 0.1	-72.1 ± 1.9	30.1
Fraction 3	24	190	-	-	44.6

For each fraction, a respective $\delta^{13}C$ value was also analyzed. Isotopic analysis of fraction one showed a $\delta^{13}C$ value of -35.3‰ (± 0.1) (Figure 6b). The second fraction was more enriched with a $\delta^{13}C$ value of -20.9‰ (± 0.1) (Figure 6b). As above, the third fraction was not analyzed due to its large blank and low C yield (Table 2). Isotopically light material appears to be favored initially for respiration and, over time, heavier sources are incorporated. Overall, this would

suggest that more modern and lighter carbon sources are used first before older and heavier components.

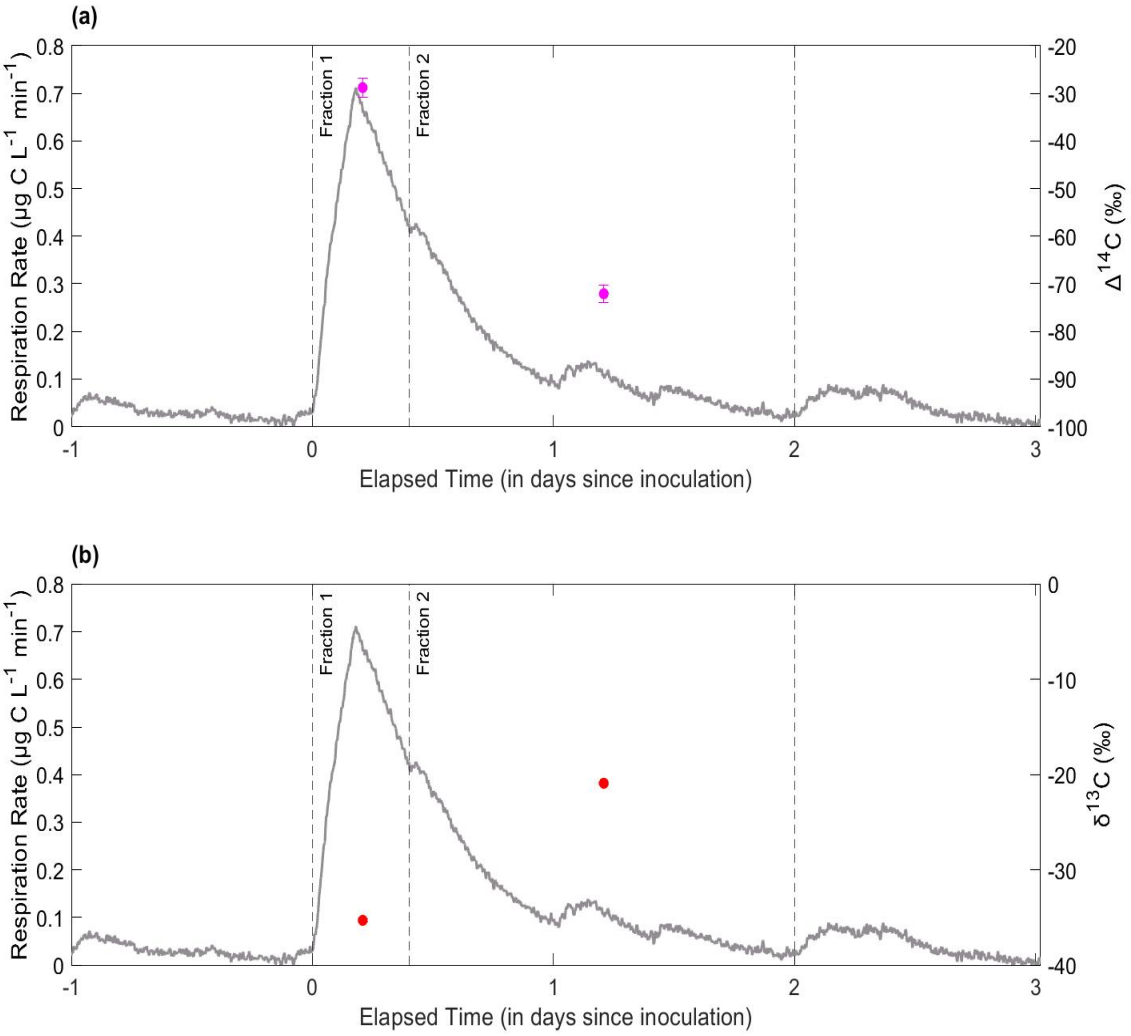

Figure 6. Respiration rates of *Vibrio sp.* 1A01 over time (gray line); (a) showing change in ^{14}C values (pink circles); (b) showing change in $\delta^{13}C$ values (red circles); width of dashed vertical black lines indicate duration of fraction 1 and 2 collection, respectively. Error bars are the standard deviation of averaged analytical replicates (n=3).

5. Discussion

5.1. Microbial degradation of DOM

In this study we utilized a novel bioreactor system to investigate the microbial degradation of terrestrially derived DOM by a marine isolate. Our results demonstrate that *Vibrio sp.* 1A01 were able to access and consume a fraction of SRDOM as their sole energy and carbon source for growth and respiration, much of which took place during the first 24 hours of incubation. Likewise, others have noted similar growth patterns during bottle incubations of seawater communities and riverine DOM in which the maximum cell density occurred within the first 24 hours (Young et al., 2005; Blanchet et al., 2017). This suggests that SRDOM has a labile portion which can be rapidly consumed by bacteria, consistent with observations and modelling data that show labile DOM is mineralized within minutes or hours in the ocean (Hansell et al., 2009). Importantly, our approach differed from what may be considered "traditional" bottle incubations in that it occurred in a controlled bioreactor with a single isolate, therefore many of the results discussed within are not necessarily directly comparable to other incubations with DOM and microbes. The observed patterns in growth and respiration in our study indicate that terrestrial DOM, which is often thought to be much more recalcitrant than marine-derived DOM, also has the potential to be consumed and mineralized within hours by marine bacteria.

Assuming assimilation and respiration were the only losses of carbon during the incubation, the total utilization of carbon over the course of the experiment was ~1.9 mg (total respiration = 1.2 mg; assimilation = 0.7 mg), representing <1% of the DOM pool. Previously reported ranges for microbial use of DOM are between 1.7% and 17.7%, therefore our observed value is comparatively low (Moran et al., 1999; McCallister et al., 2006; Raymond and Bauer, 2000). However, these studies were conducted on timescales that were significantly longer with

initial concentrations of DOM ~3-times lower. Average decay constants reported in these studies and calculated for this study are identical (-2 x 10^{-3} d^{-1}). This agreement indicates that despite methodological differences, our study follows observed trends in DOM use.

There are two prevailing hypotheses that address the persistence of DOM in the ocean. The first notion is that DOM is recalcitrant due to its inherent chemical structure which makes it resistant to microbial degradation. An alternative explanation, referred to as the "dilution hypothesis", suggests that all DOM is labile but exists in concentrations below the levels matching the energetic investment required for their uptake and degradation (Arrieta et al., 2015). Our incubation had a DOM concentration of 14.7 mM, which is double the concentration measured in the Suwannee River (~7 mM)(Green et al., 2015), and significantly higher than most natural riverine concentrations (μM) (Raymond and Bauer, 2001b). Despite this, we did not observe respiration or DOM utilization patterns that deviated significantly from studies which used *in situ* concentrations and native bacterial assemblages (Moran et al., 1999; McCallister et al., 2006; Raymond and Bauer, 2000). Furthermore, incubation of the same bacterial isolate in the IsoCaRB with identical parameters using POM, in the form of sterilized sediments, resulted in a significantly larger quantity of C respired (total of 2.3 mg; Mahmoudi et al., 2019), indicating that the limitation in our incubations was the lability of provided C and not nutrients. Therefore, results of the current study suggest that the relative lability of DOM is not dependent on concentration and may be due to the inherent chemical structure of compounds found within this carbon pool.

5.2. Sources and trends of respired CO_2

Previous studies analyzing the bulk $\delta^{13}C$ of terrestrial DOM flux to the ocean from rivers found that the dominant C input originated from C_3 vascular plants (Hedges et al., 1997). These

plants have a distinct $\delta^{13}C$ value of between -28‰ to -25‰ and $\Delta^{14}C$ values reflect modern carbon from atmospheric CO_2 used in photosynthesis (Hayes, 2001; Fry and Sherr 1989; Schiff et al., 1990; Richter et al., 1999). Similarly, bulk measurement of SRDOM reflect modern origins (Coppola et al., 2015). Surprisingly, our first CO_2 fraction that encompassed the initial 10 hours of the incubation was found to have a $\delta^{13}C$ value of -35.3‰. This depleted $\delta^{13}C$ value is consistent with aquatic macrophytes, which are known to have a large isotopic variability for which the reported range encompasses -44‰ to -11‰ (Osmond et al., 1981; France, 1996; Mendonca et al., 2013, Chappuis et al., 2017). Submerged aquatic macrophytes, specifically, have isotopic values more heavily influenced by the dissolved inorganic carbon pool and water chemistry, than they are atmospheric CO_2 (Keeley and Sandquist, 1992; Mendonca et al., 2013). As a result, the isotopic signature of these plants is sensitive to *in situ* pH values, wherein low pH results in lighter values of biomass C (Chappuis et al., 2017). Suwannee River is a notably acidic environment, as anaerobic degradation of peat from its source in the Okefenokee Swamp results in a pH of ~4 at the sampling site where SRDOM is isolated (Green et al., 2015; Malcolm et al., 1995). Therefore, the signature of a $\delta^{13}C$ depleted macrophyte endmember in respired CO_2 is in line with established trends in aquatic plant physiology. Conversely, the second fraction's $\delta^{13}C_{CO2}$ (-20.9‰) is more enriched than the range of strictly C_3 plant contribution, it is therefore likely that this C originated from a mixture of C_3 and C_4 plants.

Temporal trends observed in both the stable and radiocarbon isotopes in respired CO_2 show progressively older C used throughout the incubation, and initial $\delta^{13}C$ derived from aquatic macrophytes. Previous studies using bottle incubations with fresh, brackish, and seawater samples with native bacterial assemblages showed a preferential degradation of younger DOM (Raymond and Bauer, 2001a). It is therefore unsurprising that initial respired C reflects a

younger source than what is found in the second fraction. However, considering the availability of modern terrestrial C in SRDOM, it is unexpected that the bacteria would exclusively use aged C. The isotopic value of the later timepoint suggests a shift to aged terrestrial matter from C_3 and C_4 plants. Our isotopic values indicate that the initial reactive pool of DOM is dominated by C from aquatic macrophytes, and a mixture of modern and aged fractions throughout. As labile DOM becomes scarce, an older $\delta^{13}C$ terrestrial plant source is consumed.

6. Conclusion

6.1. Summary of research and findings

The objective of this study was to investigate the lability of terrestrial DOM to a marine isolate using stable and radiocarbon isotopes. To do so, we conducted extensive characterizations and constrained potential sources of background C. This entailed multiple preliminary test incubations during which CO_2 was collected for isotopic analysis, as well as the isotopic analysis of potential alternative systemic C sources such as the vitamins, glucosamine, and cell biomass. After achieving a suitable understanding of potential methodological error, we conducted replicate incubations with SRDOM and a model marine microorganism. The results from both incubations showed near identical patterns in respiration rates and cell growth, which verified the reproducibility of our tests. The isotopic values we reported were derived from aged carbon with a $\Delta^{14}C$ value less than modern, and a $\delta^{13}C$ signature that suggested a contribution from both aquatic macrophytes and terrestrial vascular plants. While our unique approach precluded us from direct comparison to most measurements reported in the literature, there was meaningful overlap between our observed trends in cell growth and calculated decay constant and what has been previously reported. The culmination of our findings indicates that SRDOM has a small (<1%), labile fraction of DOM that is rapidly mineralized by microbes, leaving behind an apparently refractory pool. This study highlighted the utility of C isotopes in tracing the origins of labile fractions of DOM and how marine microbes may interact with terrestrial organic matter transported to the ocean.

6.2. Additional considerations

This study provides a unique insight into the microbial degradation and cycling of terrestrial DOM. Traditional laboratory incubations are performed in bottles and measure DOM consumption through indirect proxies such as O_2 demand or absolute changes in concentration, in contrast, this study utilized a novel bioreactor system to measure direct CO_2 production resulting from the consumption and respiration of DOM. While this approach provides the advantage of more constrained measurements and the direct analysis of respiration, there are also several limitations. One such limitation is the quantity of DOM required to carry out such large-scale incubations. Additions of DOM to the cultures in our study were three-fold higher than natural concentrations in even the most DOM rich rivers. As a result, this approach is not feasible for most natural DOM samples, which is isolated by filtering thousands of liters, (Green et al., 2015). In addition, our study used a single marine isolate, which allowed for a species-specific exploration of DOM cycling. However, the use of a single bacteria strain eliminates the role of community dynamics on DOM degradation processes. Literature suggests that these interactions play a more substantial control on DOM degradation rates and DOM lability than individual microbial physiology or DOM molecular composition on their own (Young et al., 2005). Although *Vibrio sp.* 1A01 was chosen due to its ubiquitous nature in oceanic and coastal systems, the lack of a natural assemblage potentially oversimplifies and overlooks interactions which could affect the lability the DOM pool. While our study provided a well-constrained approach to microbial DOM degradation measurements, these limitations are important to consider in the context of the results and within the broader literature.

6.3. Future research

In this text, we presented results from a highly constrained bioreactor system which allowed for the isotopic analysis of respiration from microbial DOM degradation. Taking this study as a foundational proof-of-concept reveals there are several future directions that harness the novelty of the IsoCaRB system to shed light on the production, consumption and cycling of DOM. While this study relied on C isotopes, replicate incubations using molecular analyses, such as NMR, or -omics, such as metabolomics, could highlight specific transformations of DOM fractions and the potential capacity of *Vibrio sp.* 1A01 to access certain C substrates. Additional incubations with other isolates and media ranging from fresh, brackish, and saline would provide insight on cross species and ecosystem variability in DOM lability. Alternatively, iterations of studies found in current literature using natural bacteria communities, or combining isolates into co-cultures, can further investigate the importance of community interactions in DOM degradation. Finally, an ambitious approach could be taken to scale down and eliminate systemic contributions of C to allow for the use of natural concentrations or smaller volumes of collected water samples similar to traditional bottle incubations. Regardless of which approach is taken, there are a multitude of potential research avenues that could provide additional clarity to DOM degradation.

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