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Fingerprinting Blue Carbon: Rationale and Tools to Determine the Source of Organic Carbon in Marine Depositional Environments

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Blue carbon is the organic carbon in oceanic and coastal ecosystems that is captured on centennial to millennial timescales. Maintaining and increasing blue carbon is an integral component of strategies to mitigate global warming. Marine vegetated ecosystems (especially seagrass meadows, mangrove forests, and tidal marshes) are blue carbon hotspots and their degradation and loss worldwide have reduced organic carbon stocks and increased CO_2 emissions. Carbon markets, and conservation and restoration schemes aimed at enhancing blue carbon sequestration and avoiding greenhouse gas emissions, will be aided by knowing the provenance and fate of blue carbon. We review and critique current methods and the potential of nascent methods to track the provenance and fate of organic carbon, including: bulk isotopes, compound-specific isotopes, biomarkers, molecular properties, and environmental DNA (eDNA). We find that most studies to date have used bulk isotopes to determine provenance, but this approach often cannot distinguish the contribution of different primary producers to organic carbon in depositional marine environments. Based on our assessment, we recommend application of multiple complementary methods. In particular, the use of carbon and nitrogen isotopes of lipids along with eDNA have a great potential to identify the source and quantify the contribution of different primary producers to sedimentary organic carbon in marine ecosystems. Despite the promising potential of these new techniques, further research is needed to validate them. This critical overview can inform future research to help underpin methodologies for the implementation of blue carbon focused climate change mitigation schemes.

Keywords: blue carbon, carbon accounting, environmental DNA, isotopes, organic carbon, sequestration

INTRODUCTION

Blue carbon ecosystems (i.e., tidal marshes, mangrove and seagrass meadows) constitute hotspots of carbon cycling and are among the largest carbon sinks in the biosphere (Nellemann et al., 2009; Duarte et al., 2013). Among the multiple ecosystem services that coastal vegetated ecosystems provide, the potential to sequester and retain large carbon stocks over millennial timescales has generated interest among scientists and policy makers (Nellemann et al., 2009; Fourqurean et al., 2012; Duarte et al., 2013). Blue carbon strategies describe a range of activities for preventing or mitigating carbon dioxide (CO_2) emissions through the conservation and restoration of coastal vegetated ecosystems (Wylie et al., 2016), which rank among the most threatened ecosystems on Earth (Duarte et al., 2013).

The accumulation of carbon stocks is the result of higher accretion rates than decomposition and erosion rates of C-containing materials (detritus and sediment). There are several reasons why blue carbon ecosystems accumulate organic matter and are hotspots of carbon sequestration. First, they are highly productive ecosystems converting CO_2 into plant biomass. Second, above-ground macrophyte biomass enhances deposition through altering water flow, and the below-ground biomass reduces erosion and adds organic matter to anoxic soils. These characteristics result in the net accumulation of both living and dead organic material produced within the ecosystem (*autochthonous*), and/or from external sources (*allochthonous*) (Kennedy et al., 2010; Saintilan et al., 2013). Third, soils within blue carbon ecosystems have low oxygen concentrations which reduce decomposition, thereby contributing to the accumulation and preservation of organic carbon (C_{org}) in the marine environment (Nellemann et al., 2009; Mcleod et al., 2011; Duarte et al., 2013). Although soil and sediment can have distinct definitions, we use them interchangeably to facilitate comprehension among different disciplines.

The value of blue carbon ecosystems in sequestering C_{org} has intensified conservation interests as a measure to mitigate climate change and offset CO_2 emissions. However, while both autochthonous and allochthonous sources contribute to the soil C_{org} pool in blue carbon ecosystems (Kennedy et al., 2010), the presence of allochthonous C_{org} complicates carbon accounting exercises, because of the risk of duplicating carbon sequestration gains that may have already been accounted for where allochthonous carbon from terrestrial environments is concerned. In contrast, carbon derived from seaweed (Krause-Jensen and Duarte, 2016; Krause-Jensen et al., 2018), epiphytes or plankton, also included in the allochthonous inventory (Kennedy et al., 2010), are not accounted elsewhere and could be included in reports of carbon inventories from blue carbon habitats.

Knowing the origin of C_{org} (often termed "provenance" within carbon accounting settings) in marine depositional environments is important to decipher biogeochemical cycles and underpin management as it indicates: (1) the key producers of organic matter accumulated within blue carbon ecosystems and other marine depositional environments; (2) the degree of connectivity within and among marine and terrestrial ecosystems; (3) the ultimate fate of the large C_{org} flux exported from vegetated coastal habitats; and (4) the potential shifts in ecosystem functioning under global change threats.

Knowledge of carbon sources and fluxes among terrestrial and marine ecosystems is useful for managers and policy-makers. This includes the determination of whether local site management is suitable to enhance or maintain blue carbon ecosystems, or whether activities that are off-site, for example those occurring in adjoining watersheds or habitats, are needed to achieve ecosystem management goals. For instance, epiphytic macroalgae are often abundant in seagrass meadows and associated with mangrove areal roots, yet the contribution of algae to soil C_{org} stocks, food webs or its export to adjacent habitats is debated (Howard et al., 2017). Additionally, carbon from macroalgae (Krause-Jensen and Duarte, 2016) and seagrass (Duarte and Krause-Jensen, 2017) growing in coastal habitats can be found in deep ocean environments, and there is the possibility that carbon from mangroves and tidal marshes is also exported to the deep ocean.

Thus, there is a need to elucidate both the sources of C_{org} in blue carbon soils as well as the contribution of these habitats and other primary producers to carbon sequestration in depositional environments (Krause-Jensen and Duarte, 2016; Duarte and Krause-Jensen, 2017). Understanding connectivity, in terms of carbon flows across marine ecosystems, is key to support blue carbon accounting as well as the successful management of aquatic ecosystems within land- and seascapes (Smale et al., 2018).

The purpose of this paper is to synthesize existing information on C_{org} provenance in marine systems and the methods used, and then determine what techniques could be used to improve our understanding of blue carbon sources. Although we focus on well-studied vegetated habitats, depositional environments in the open ocean can also provide an important global sink of blue carbon, including carbon from planktonic sources and carbon exported from blue carbon ecosystems reaching the deep sea (Duarte and Krause-Jensen, 2017). Export of carbon from the surface ocean to oceanic sinks is enhanced by oceanic fronts (Stukel et al., 2017), and marine canyons also concentrate carbon fluxes from coastal vegetated systems to oceanic sinks (Krause-Jensen and Duarte, 2016). Embedding deep ocean sinks into blue carbon frameworks is currently hindered by, among other things, difficulty in identifying the sources of carbon to those sinks (Krause-Jensen et al., 2018). The methods we discuss are also applicable to trace the C_{org} provenance in the deep ocean and possibly to track oceanic fronts (Khare and Chaturvedi, 2012).

Overview of Blue Carbon Provenance

Bulk properties of soils such as C and N elemental (%) and stable isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition have been widely tested and used for quantifying sources of organic matter in mangrove, tidal marsh and seagrass soils (Kennedy et al., 2010; Greiner et al., 2016). These tracers have been used to differentiate between terrestrial and marine sources of organic matter (Fry and Sherr, 1989), among marine sources with distinct isotopic ratios such as seagrasses, seston, and macroalgae (e.g., Kennedy et al., 2010; Greiner et al., 2016) and between C3 and C4 vegetation (Smith and Epstein, 1970). Apportioning

of organic matter sources using C and N concentration and isotopes depends on: (1) accurate knowledge of potential source values; (2) sources with significantly different values; and (3) the assumption of little or no alteration of source values during decomposition (Fourqurean and Schrlau, 2003; Bouillon et al., 2008). Yet, sources of organic matter in coastal ecosystems can be complex with variable and overlapping isotopic values among plant species, tissues, microhabitats, seasons and growth cycle, which complicates the use of bulk C and N isotopic ratios to discern C_{org} sources (Marchand et al., 2003; Blair and Aller, 2012). For example, it is difficult to distinguish the contribution of mangrove, tidal marsh and other terrestrial plants to soil C_{org} using $\delta^{13}C$ because these sources have similar isotopic values (Saintilan et al., 2013). Thus, there is a need for methods to complement the information provided by C and N isotopic values (Cloern et al., 2002). Recent studies have highlighted that more specific markers, such as eDNA and compound-specific isotopes, could help reduce uncertainty when determining the sources of C_{org} (Reef et al., 2017). Therefore, the analyses of additional proxies that are reviewed here, have the potential to greatly enhance our understanding of the fluxes of C_{org} in marine systems (see **Table 1** for summary).

Recent Developments in Tracing Carbon Provenance

Bulk Hydrogen, Oxygen and Sulfur Isotopes

In addition to $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios, studies tracing the origin of organic matter in marine and freshwater ecosystems have measured $\delta^{18}O$, $\delta^{2}H$, and $\delta^{34}S$ (Peterson and Fry, 1987). The emerging use of $\delta^{2}H$ in marine soils has potential to provide evidence for the source of organic matter. $\delta^{2}H$ values have been used to measure resource use by aquatic consumers demonstrating the utility of this method to potentially be used to track C_{org} provenance. For example, a combination of $\delta^{2}H$ and C and N isotopes was used to determine clams' consumption of organic matter derived from macroalgae and microalgae (Hondula and Pace, 2014). Discriminating the diet of clams was possible because microalgae, macroalgae, seagrass and wetland macrophytes differ in $\delta^{2}H$ (Hondula and Pace, 2014). In addition, Duarte et al. (2018) recently showed that Red Sea seagrass and macroalgae have distinct $\delta^{2}H$ signatures and that the combination of $\delta^{2}H$ and $\delta^{13}C$ holds promise to discriminate these carbon sources in C_{org} sediment stocks. Several factors such as photosynthesis, lipid content, isotopic discrimination during water uptake, biochemical and biophysical processes, and environmental seasonality allow differentiation of $\delta^{2}H$ values in primary producers (Hondula et al., 2014; Ladd and Sachs, 2015; Adame et al., 2016).

A combination of $\delta^{18}O$ with $\delta^{2}H$ could be used to determine the provenance of C_{org} in sediment and although using this combination has not been used for this to our knowledge, the following examples demonstrate its relevance for tracking C_{org} provenance. While $\delta^{18}O$ is not altered upon water uptake by plants (Roden et al., 2000), it is fractionated during photosynthesis leading to variation of $\delta^{18}O$ values of plant biomass (Barbour et al., 2007). Variation of $\delta^{18}O$ in mangrove woody biomass has been linked to variation in rainfall (Verheyden et al., 2004) and salinity (Ish-Shalom-Gordon et al., 1992). Yet, direct watershed comparison of $\delta^{18}O$ of biomass from co-occurring terrestrial and aquatic species are needed to validate this technique. Moreover, the isotopic values of $\delta^{2}H$ and $\delta^{18}O$ of water in mangrove stem water are distinct from those of adjacent terrestrial plants (Wei et al., 2013) and vary with rainfall and among species (Santini et al., 2015; Lovelock et al., 2017), but direct comparison of isotopic values in water with biomass from the same plants have not yet been made.

There are complexities in using $\delta^{18}O$ and $\delta^{2}H$ ratios to identify unique carbon sources. First, isotopic composition of primary producers is not universal and second, the source materials are required to determine provenance on a case-by-case basis. Some complications such as the presence of inorganic hydrogen and the exchange of hydrogen after soil collection, may be overcome with the use of stringent extraction and drying methods (Chesson et al., 2009; Meier-Augenstein et al., 2013; Ruppenthal et al., 2013; Soto et al., 2017). However, other complications remain to be solved including measuring only a small fraction of total organic matter due to incomplete extraction (<80% of the total organic matter; Ruppenthal et al., 2013), accounting for source specific element ratios (i.e., C/H and C/O), isolating non-exchangeable H, and determining the effect of bacterial degradation on $\delta^{2}H$ and $\delta^{18}O$. While these isotopic tracers are successfully used to study animal movement (Rubenstein and Hobson, 2004) and could assess food web interactions (Zanden et al., 2016), there is little or no information on how bulk values may change during decomposition. In summary, $\delta^{2}H$ along with $\delta^{18}O$ may be used to determine contribution of different primary producers to organic matter in soils or possibly even organic matter derived from mangrove trees in different environments. However, their use to discriminate sources of C_{org} in blue carbon sediments has not yet been attempted and the complications mentioned should be addressed to determine the accuracy of these methods.

Measuring sulfur isotopes also has the potential to discriminate sources of C_{org} in marine depositional environments. Exposure of plant roots to sulfide in marine soils affects their sulfur isotopic values due to incorporation of ^{34}S-depleted sulfides (leading to lower $\delta^{34}S$ values), which does not occur in non-rooted primary producers such as macroalgae (Peterson and Fry, 1987). When used in combination with other tracers, sulfur stable isotopes improve the elucidation of sources of organic matter. For instance, Moncreiff and Sullivan (2001) used $\delta^{34}S$, in combination with $\delta^{13}C$ and $\delta^{15}N$, to resolve the contribution of seagrass and epiphytic algae to the diet of marine consumers. Connolly et al. (2004) reviewed estuarine and marine food web studies to conclude that the use of $\delta^{34}S$ isotopes, in combination with $\delta^{13}C$, yields a high probability of distinguishing the contribution of different producers to aquatic food webs. However, whereas $\delta^{34}S$ has been used extensively to resolve food sources in food web studies, the use of this isotope ratio to discriminate sources of C_{org} in marine soils is more complicated. Soils containing sulfide mineral are common when anoxic conditions pertain in the sediment and these minerals are about 40% depleted in ^{34}S compared to seawater sulfate. Isotopic analysis for $\delta^{34}S$ uses roughly the same technology as

TABLE 1 | Summary of the advantages, and potential limitations for techniques to discern the flow of organic carbon in marine ecosystems.

Method	Advantages	Disadvantages	Discern source identity	Discern source contribution	Cost per sample ($US)
Bulk C and N isotopes	Wealth of information on sources and limitations	Limitations in discerning multiple sources	medium	medium	∼10
Bulk H and O isotopes	Discern contribution of terrestrial and mangrove carbon sources and possibly different plant tissues	Changes in hydrogen isotope after collection and presence of inorganic hydrogen	unknown	unknown	∼25
Bulk Sulfur isotopes	Showed utility in food web studies	Not enough known about transformation in soils	unknown	unknown	∼20
Biomarkers (e.g., lipids)	Good for discerning between terrestrial and marine organic matter	Can be difficult to apportion to specific sources	medium	medium	∼50–300
Compound-specific isotopes (e.g., lipid isotopes and amino acid isotopes)	Larger specificity and stability than bulk org. matter	Not enough known about transformation in soils	medium	high	∼200
eDNA	Identify source to species	Breadth and accuracy of macrophyte primers need to be tested	high	medium*	∼30

**Experiments still need to be conducted to test both the variation in DNA degradation among species and compared to other organic carbon components.*

for $\delta^{13}C$ and $\delta^{15}N$, but it is recommended that non-acidified samples are run to maintain the integrity of the soil $\delta^{34}S$ for sulfur isotope analysis (Connolly and Schlacher, 2013), and if bulk soil is analyzed the resultant $\delta^{34}S$ values is a measure of both inorganic and organic sulfur isotopic composition which can be much more isotopically depleted than any of the potential C_{org} sources (Oreska et al., 2018). Although this represents an untapped research opportunity, a first step would be to employ methodology that separates inorganic from organic sulfur in the soils prior to analysis. In addition, it remains to be investigated how the incorporation of reduced sulfur into organic matter affects the $\delta^{34}S$ of C_{org} during diagenesis.

Molecular Properties of Bulk Organic Matter

There are multiple methods to characterize the chemical composition of organic carbon in soils (Derrien et al., 2017). The most common sources of organic matter in blue carbon ecosystems (vascular plants, macroalgae, phytoplankton, fungi, bacteria, zooplankton, etc.) can have different biopolymer chemical composition (e.g., polysaccharides, proteins, lignin, chitin, peptidoglycan). Differences in biopolymer identity and abundance can be analyzed with infrared spectroscopy (IR) to characterize marine C_{org} (Benner et al., 1992; Trevathan-Tackett et al., 2017). IR is a rapid, non-destructive and cost-efficient method and is therefore a very useful tool for bulk chemical characterization of blue carbon. However, a disadvantage of using IR is that some sources can have similar biopolymer fingerprints. Another complementary method to characterize the composition of C_{org} is nuclear magnetic resonance spectroscopy (solid-state ^{13}C NMR). Broad morphological, physical and chemical characteristics of organic matter fractions can be determined with ^{13}C NMR, including dissolved and particulate C_{org}, and recalcitrant organic matter. Although ^{13}C NMR has been used to describe C_{org} in terrestrial soils (Baldock et al., 2004), the use in marine systems maybe more complex because the marine C_{org} often undergoes greater levels of processing before deposition compared to C_{org} in terrestrial systems (Baldock et al., 2004; Hayes et al., 2017; Kelleway et al., 2017; Macreadie et al., 2017). An area for future research is to develop molecular mixing models with the application of chemometric approaches (Doucet et al., 2008) based on both IR and NMR results to characterize blue carbon sources.

Gas chromatography coupled with mass spectrometry (GC-MS) can also be applied to blue carbon fingerprinting. Similarly, pyrolysis has been used to discern allochthonous and autochthonous organic matter in mangrove soils (Marchand et al., 2008). GC-MS can identify the thermal or chemical degradation of macromolecules. For example, lignin composition differs between angiosperms vs. gymnosperms and between woody tissues vs. non-woody tissues (Haddad and Martens, 1987). Thus, lignin can be analyzed by GC-MS after cupric oxide oxidation, which can provide information on the abundance and source of plant material. Lignin can also be analyzed by thermal degradation using analytical pyrolysis which can then be analyzed with GC-MS (Py-GC-MS; Carr et al., 2010; Zhang et al., 2016). Due to the invasive nature of pyrolytic breakdown, Py-GC-MS is quantitatively weak but has the advantage that it also provides information on other macromolecular materials, such as polysaccharides, proteins, chitin (in zooplankton and fungi), peptidoglycan (in bacteria), chlorophyll (in phytoplankton), charred organic matter (an important type of recalcitrant organic matter) and others (e.g., cutin, suberin, algaenan, and tannin; Carr et al., 2010 and references therein). Such information can be useful not only as a molecular screening method to identify sources, but can also be used to compliment or validate less complex data from IR, elemental or isotopic analysis. Analysis of the molecular properties of bulk organic matter does necessitate specific and advanced analytical methodologies such as IR, NMR, GC-MS, and PY-GC-MS.

Biomarkers (Targeted Compounds)

Biomarkers, such as *n*-alkanes and phenolic compounds, have been proposed as taxonomic fingerprints and the biomarker profile of some marine primary producers has been characterized (Zidorn, 2016; Gachet et al., 2017). Lipids can provide convenient biomarkers to trace the source and fate of C_{org} and have great potential as a molecular biomarker for coastal systems (Derrien et al., 2017). For example, *n*-alkanes lipids together with stable C and N isotopic compositions, have been used to quantify the source of C_{org} along estuarine gradients (Jaffé et al., 2001; He et al., 2014). In addition, n-alkanes can indicate the relative proportion of terrestrial and marine sources of organic matter (Silliman et al., 1996; Ortiz et al., 2013) and can differentiate the source of organic matter within coastal systems among terrestrial sources, emergent aquatic plants, and submerged macrophytes (Sikes et al., 2009). Other compounds with the potential to fingerprinting C_{org} include proteins, such as glomalin which has been used to indicate terrestrial-derived carbon in blue carbon soils (Adame et al., 2012; López-Merino et al., 2015; Wang et al., 2018). In general, biogeochemical plasticity of biomarkers can exist within species based on their geographical distribution and through time (Derrien et al., 2017). Similar to molecular properties of bulk organic matter, biomarker analysis requires advanced analytical methodologies, for example liquid chromatography (LC, HPLC), GC and MS. Biogeochemical plasticity of biomarkers can exist within species based on their geographical distribution and through time (Derrien et al., 2017). A recent review provides a good overview of using biomarkers to trace organic matter (Derrien et al., 2017). To date, few studies have used this approach to fingerprint sources of C_{org} in blue carbon soils, and some studies have used biomarkers in combination with compound-specific isotopes (Apostolopoulou et al., 2015).

Compound-Specific Isotopes (Amino Acids, Carbohydrates, Lipids)

Compound-specific stable isotopes of organic matter can enable the molecular specificity and isotopic value of compounds to be exploited concomitantly to trace the origin and fate of organic matter (Evershed et al., 2007; Chikaraishi, 2014). Many types of amino acids, carbohydrates, and lipids have been used for isotopic fingerprinting and to trace C_{org} sources through food webs (De Troch et al., 2012; Larsen et al., 2013), with the finding that they seem to be considerably more stable and specific than that of the bulk organic matter (Larsen et al., 2015). The $\delta^{13}C$ and $\delta^{2}H$ values of sterols in living algae are consistent with those found in marine sediments, suggesting that the isotopic compositions of algal sterols are well-preserved in sediments and therefore could be used as tracers of C_{org} origin (Chikaraishi, 2006). Lastly, isotopes of amino acids, have also been identified as a powerful tracer of material origin, because environmental conditions have a minimal effect on $\delta^{13}C$ patterns of different amino acids in seagrass (*Posidonia oceanica*) and giant kelp (*Macrocystis pyrifera*) (Larsen et al., 2013). Thus, patterns of $\delta^{13}C$ among individual amino acids have a much greater potential than bulk $\delta^{13}C$ to distinguish between C_{org} derived from algae, seagrass, terrestrial plants, bacteria and fungi (Larsen et al., 2013). Over time, sedimentary diagenesis may lead to increased contribution of bacterial sources (Larsen et al., 2015), but the method still seems a promising complementary approach to overcome some of the limitations of bulk isotope analysis in estuaries and other complex environments with mixed aquatic and terrestrial inputs for determining the origin of organic matter. A few studies have used both bulk and compound-specific isotopes to track changes in the provenance of blue carbon including stable isotopes within higher plant leaf wax lipids (Johnson et al., 2007) or n-alkanes (Tanner et al., 2010).

Environmental DNA

Fingerprinting marine organisms through environmental DNA (eDNA) has recently become a widely used technique to determine the presence and abundance of individual macroorganisms (Rees et al., 2014; Thomsen and Willerslev, 2015). Most of the marine research using eDNA has targeted macrofauna, while minimal attention has been given to macroflora (Thomsen and Willerslev, 2015; Goldberg et al., 2016), with only one paper to our knowledge on eDNA of macrophytes in marine sediments (Reef et al., 2017). Given that approximately 3% of cellular C_{org} is DNA (Landenmark et al., 2015) and that eDNA can identify individual species, this approach has great potential for determining the provenance of C_{org} in soils of blue carbon ecosystems. In aquatic environments, phytoplankton identified from eDNA isolated from sediments in deep water were representative of the pelagic community (Corinaldesi et al., 2011; Capo et al., 2015). However, eDNA analysis may underrepresent phytoplankton that lack hard structures (Boere et al., 2011b), and underrepresent phytoplankton compared to terrestrial plants (Boere et al., 2011a), due to differential preservation of their DNA. Currently, the sole study using eDNA to measure the provenance of blue carbon found that seagrass meadows had a greater input of autochthonous C_{org} based on eDNA than when sources of C_{org} where estimated using $\delta^{13}C$ and $\delta^{15}N$ (Reef et al., 2017). This discrepancy between methods highlights the need to experimentally test the relationship between bulk organic carbon sources and sequenced eDNA during diagenesis at different timescales.

The basic steps in analyzing eDNA using metabarcoding include isolating DNA from sediment, replicating target DNA sequences through polymerase chain reaction (PCR), determining the base pairs of the replicated sequences using next generation sequencing, and matching the sequences to known taxa. The relationship between eDNA and natural abundance is better when focusing on single taxon or species using quantitative PCR or related techniques (Yates et al., 2019), as compared to using PCR and metabarcoding which may not have a relationship between initial DNA concentration and final sequences but have the benefit of uniquely identifying many species in a single sample. To relate the number of DNA sequences to the abundance of organisms based on metabarcoding, knowledge of the factors that affect eDNA quantity in sediment and PCR bias (i.e., the preferential replication of some DNA sequences relative to others) should be identified and measured (Yoccoz et al., 2012). When using eDNA reads as an indicator of blue

carbon provenance, the factors that may alter the relationship between DNA and C_{org} should also be understood. Potential artifacts that deserve attention when using eDNA to track blue carbon include: (1) DNA may degrade at a faster rate than other C_{org} components, such as carbohydrates and other macromolecules (Volkman, 2006), so there is the potential for eDNA to underestimate allochthonous C_{org} and species with relatively less resistant cellular structure (e.g., algae vs. vascular plants); (2) primers need to be tested to ensure that the species thought to contribute to the C_{org} in soil are amplified because primer amplification is imperfect (Deagle et al., 2014); (3) the initial and amplified number of sequences may not be related when using PCR (Acinas et al., 2005); and (4) accurate fingerprinting requires that sequences for the putative source primary producers be deposited in reference data banks, which is not always the case. Recent studies have used mock samples to assess whether PCR amplicons are related to initial DNA concentration, finding that this relationship does exist for most species (Thomsen et al., 2016). Given that eDNA within aquatic sediments have been used to track changes over millennia in the surrounding terrestrial and aquatic autotroph communities (Capo et al., 2015; Sjögren et al., 2016) and C_{org} contributions (Coolen et al., 2007; Boere et al., 2011a), there is great potential for using eDNA to track the provenance and fate of C_{org} within blue carbon ecosystems (Reef et al., 2017). The potential resolution of eDNA to detail carbon contribution to species level, makes this approach unparalleled by any other approach used to fingerprint the sources of C_{org} in blue carbon soils. However, the limitations mentioned when using PCR should be addressed before inferring a relationship between metabarcoding results and contribution of C_{org}.

Common Methodological Issues

The disparate techniques described here share limitations that can likely be addressed to improve our ability to determine the provenance of sequestered C_{org} in the marine environment (**Table 1**). First, all the techniques assume conserved relationships between the source of specific markers and those in sedimentary C_{org} pools, yet changes in the markers through space and time may occur, hence these changes need to be quantified. Thus, some of these methods should be considered qualitative or semi-quantitative until this assumption has been tested. Second, all methods are based on known standards, such as a comprehensive library of known DNA sequences, isotopic values of source materials or biomarker profiles, and it requires a community effort to develop and expand these standards so as to enable the full power of these techniques. Third, these methods often depend on large databases and/or necessitate computationally intensive programs to match samples with standards. For example, mixing models are often used for stable isotopes but have multiple limitations (Fry, 2013), and advances have been made such as Bayesian methods that can include additional information to reduce uncertainty in undetermined systems (Moore and Semmens, 2008). Mixing models for isotope ratios of specific compounds are less developed than for bulk isotope ratios and need further refinement to validate their use. Finally, methods are constantly being developed and specialized to determine C_{org} properties and many are associated with specialized equipment that can be esoteric and too expensive for many researchers (see **Table 1** for estimated cost). However, as is the case with DNA sequencing, many methods have and will become available to a broader scientific community as technology improves and costs continue to decrease, a consequence of the increasingly large number of researchers using these techniques.

CONCLUSION

We reviewed and critiqued multiple methods that have the potential to improve our understanding of the provenance and fate of C_{org} within and among coastal ecosystems. Our goal is to encourage research aimed at improving the assessment of the C_{org} origin in blue carbon ecosystems which is needed to decipher biogeochemical cycles and underpin management such as blue carbon initiatives. This advance would include assessment of species of macroalgae, microphytobenthos and epifauna, which can have high production rates, but their contribution to blue carbon has been illusive because of current limitations in tracking their origin and fate. In addition, recent research has suggested that coastal primary producers, such as macroalgae and seagrass, could significantly contribute to C_{org} sequestration beyond their habitat, including in the deep ocean (Krause-Jensen and Duarte, 2016; Duarte and Krause-Jensen, 2017), but direct measures of their contribution are limited at best. The ability to accurately determine the identity and contribution of primary producers to soil C_{org} will likely depend on a combination of the previously discussed methods that minimize the associated limitations. In our opinion, the methodologies with the greatest potential to determine the provenance of soil C_{org} are the C and N stable isotopes of lipids to determine the quantity of discerned taxa in combination with eDNA to identify the species that contribute to the isotopic composition. The ability to mitigate the negative effects of elevated atmospheric CO_2 can be aided by management schemes that maintain existing carbon storage and promote carbon sequestration. The marine environment, and blue carbon ecosystems in particular, are hotspots of carbon storage. Enhanced knowledge of the sources and fate of C_{org} stored in marine sediments is important for both managing coastal carbon stocks and understanding carbon cycling.

AUTHOR CONTRIBUTIONS

NG and CD conceived the idea of the manuscript. All authors contributed to the writing and revising of the manuscript.

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