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Using eDNA to determine the source of organic carbon in seagrass meadows

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Abstract

Seagrasses sequester globally significant amounts of carbon (C), which is stored mainly in the sediment. Both C fixation by the seagrass (autochthonous) and the trapping of organic matter that is derived from outside the ecosystem (allochthonous) contribute to seagrass sediment organic C (OC). However, due to limitations in current methods we do not yet fully understand which sources of C are most important to blue C ecosystems. We used environmental-DNA (eDNA) to identify and estimate the contribution of floral sources to seagrass sediment OC sampled from three sites in Moreton Bay, Australia that differ in potential levels of autochthonous and allochthonous OC inputs. Using the plant barcode gene *rbcL* and a next-generation sequencing platform we identified 150 plant operational taxonomic units in the sediment samples. We found that seagrass DNA composed on average 88% of the eDNA pool in the sediment samples. Allochtonous sources were primarily mangrove, saltmarsh, and freshwater marsh species, but the proportional contribution of other sources varied among meadows. Carbon and nitrogen (N) stable isotope mixing models suggested a lower autochthonous contribution than the eDNA data, but had high ambiguity due to indistinguishable isotopic values among some of the sources. Our study shows that eDNA can be an effective tool for identifying sources to sediment OC in blue C ecosystems and can be used in conjunction with stable isotope analysis to reduce ambiguity. Identifying the sources of OC to the sediments of blue C ecosystems is crucial for building robust and accurate C budgets for these systems.

Vegetated coastal habitats including seagrass meadows, mangrove forests, and tidal marshes, are some of the most carbon (C)-rich ecosystems in the world, storing upwards of 25 billion tonnes of below-ground C (Donato et al. 2011; Fourqurean et al. 2012; Duarte et al. 2013). Unlike terrestrial soils, the sediment of vegetated coastal habitats accrete vertically over time, increasing their C stocks which are stored on the order of millennia (Mateo et al. 1997; McLeod et al. 2011). Carbon accumulation and preservation rates in vegetated coastal habitats are at high risk because of anthropogenic-driven changes to these ecosystems (Valiela et al. 2001; Orth et al. 2006; Waycott et al. 2009; Deegan et al. 2012), which result in loss of vegetated habitats and CO_2 emissions as C stored in their sediments is eroded and remineralized (Coverdale et al. 2014; Kauffman et al. 2014; Marbà et al. 2015). Estimated losses of coastal vegetated habitats are 0.5–3% annually, with a total loss of 25–50% of these ecosystems over the past 50 yr (Waycott et al. 2009; Pendleton et al. 2012). Because of the high organic C (OC) stocks and the high level of threats to vegetated coastal ecosystems it has been argued that they should be an important component of climate change mitigation efforts, which have been collectively called "blue C" strategies (Duarte et al. 2013).

In contrast to climate change mitigation strategies in the terrestrial environment (e.g., Reducing Emissions from

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Additional Supporting Information may be found in the online version of this article.

Deforestation and Forest Degradation, REDD+), where the OC resides largely in the biomass of the trees, in blue C ecosystems the OC is mostly within the sediment, which is contributed to both by the marine plants (autochthonous) and also by trapping of organic matter and sediment that is derived from outside the ecosystem (allochthonous). The external or allochthonous C can be derived from terrestrial flora, other marine habitats, and riverine and freshwater habitats, which is transferred to blue C ecosystems by water flows and air currents (Kennedy et al. 2010; Watanabe and Kuwae 2015) or animal vectors (Reef et al. 2014). Allochthonous C has been treated as a "deduction" and subtracted from the total C stock in C accounting methodologies that seek to value the C stock within blue C ecosystems (Emmett-Mattox et al. 2010 and VCS Methodology VM0033, 2015, for tidal wetland and seagrass restoration). Yet the contribution of allochtoanous C is variable (Kennedy et al. 2010; Samper-Villarreal et al. 2016) and there is uncertainty in the size of the allochthonous OC pool at any given site. Understanding the sources of the sediment OC is therefore important for estimating the value of C stocks in blue C ecosystems, but also for understanding the magnitude of C fluxes from different ecosystems (Bauer et al. 2013).

There are many techniques to determine the origin of OC in coastal habitats. These techniques include analyses of physical parameters (e.g., grain size, sediment color; Leithold and Hope 1999), biomarkers (e.g., glomalin, n-alkalenes; Prahl et al. 1994; Adame et al. 2012) and stable isotopes (δ^{13}C, δ^{15}N, δ^{34}S). While these methods can be very powerful for determining the origin of OC in blue C habitats, they need to be used in combination due to limitations in performance and overlapping of signals. Furthermore, interpretation can be qualitative rather than quantitative in nature. For example, although terrestrial plants have lower δ^{13}C values compared to marine plants (e.g., seagrasses; Hemminga and Mateo 1996; Fry et al. 2008), they are not different from δ^{13}C values of mangrove trees, which are also C3 plants (Ball 2009). Therefore, it is not possible to distinguish the contribution of terrestrial from that of mangrove forests using stable isotopes (Rodelli et al. 1984; Lamb et al. 2006). Furthermore, using biomarkers and isotopes do not usually allow for discovery of the taxonomic proportions of contributing OC sources. Thus, novel methods for discerning the identity of OC sources in blue C ecosystems are needed.

Free deoxyribonucleic acid (DNA) is released by all organisms into their environment and is found in plant remains, leaving behind a trace of their identity. The amalgamation of DNA left by different organisms in an environmental sample (e.g., sediment or water) is known as environmental DNA (eDNA). Although analysis of eDNA is a relatively new technique, it has been used broadly to identify the presence of species in an ecosystem and for dietary analyses (Yoccoz et al. 2012; Rees et al. 2014). To our knowledge, however, eDNA has not been used to determine sources of OC in sediments of blue C ecosystems. Like isotope analysis eDNA can provide proportional estimates of different sources of OC to the sediment. Unlike isotope analyses, eDNA can provide fine-scale detail as to which species or families of organisms comprise the bulk of the DNA in the sediment sample (Thomsen and Willerslev 2015), and because DNA is unique to a species, values do not overlap. The ability to identify sources of OC at a finer taxonomic-scale would allow for more detailed assessment of the components of OC in blue C ecosystems and an enhanced understanding of OC fluxes and budgets in the coastal zone.

In this study, we used eDNA to identify and estimate the proportional contribution of OC sources to three spatially separated seagrass meadows in Moreton Bay, Australia. Furthermore, we compared our eDNA results to those determined by sediment δ^{13}C and δ^{15}N values. We hypothesized that OC in these habitats would come from multiple sources, including autochthonous and allochthonous sources. We expected the contribution of different plant communities (terrestrial, mangrove, seagrass, riverine, and freshwater) to the seagrass sediment eDNA pool to be associated with the exposure of the sampling site to inputs from these habitats. We expected eDNA to provide higher resolution in identifying OC sources than stable isotopes.

Materials

Study site

Moreton Bay represents an ideal place to investigate variation in the major sources of OC to seagrass ecosystems as it contains ~ 179 km^2 of seagrass meadows (Roelfsema et al. 2013) that vary in their exposure to marine, terrestrial, riverine, and mangrove/salt marsh organic matter. This is a subtropical shallow bay that covers 1523 km^2, and receives riverine inputs from five catchments totaling ~ 21,000 km^2 (Dennison and Abal 1999). Within this bay, three sites were selected to encompass differing potential levels of autochthonous and allochthonous OC inputs (Fig. 1). We sampled seagrass meadows at Lota Creek, close to the mainland, which are exposed to terrestrial inputs from Lota Creek including those from the surrounding suburban landscape. The shoreline includes both mangrove and saltmarsh habitats typical for the western side of the bay. In the eastern side of the bay, along the shore of North Stradbroke Island we sampled the seagrass meadow at Myora Springs, which is adjacent to a well-developed mangrove forest, freshwater swamps, and subtropical forest. A small creek runs from the upland areas which have low levels of human development to the shoreline. Finally, we sampled seagrasses on Amity Banks, which are sand banks to the north west of North Stradbroke Island. These banks are strongly influenced by the Pacific Ocean water that flows into the Bay through adjacent channels, and are relatively isolated from terrestrial influences (Fig. 1).

Sample collection

Sediment samples were collected from seagrass meadows at Lota Creek, Myora Springs, and Amity Banks. Ten samples of marine sediment were collected from three locations

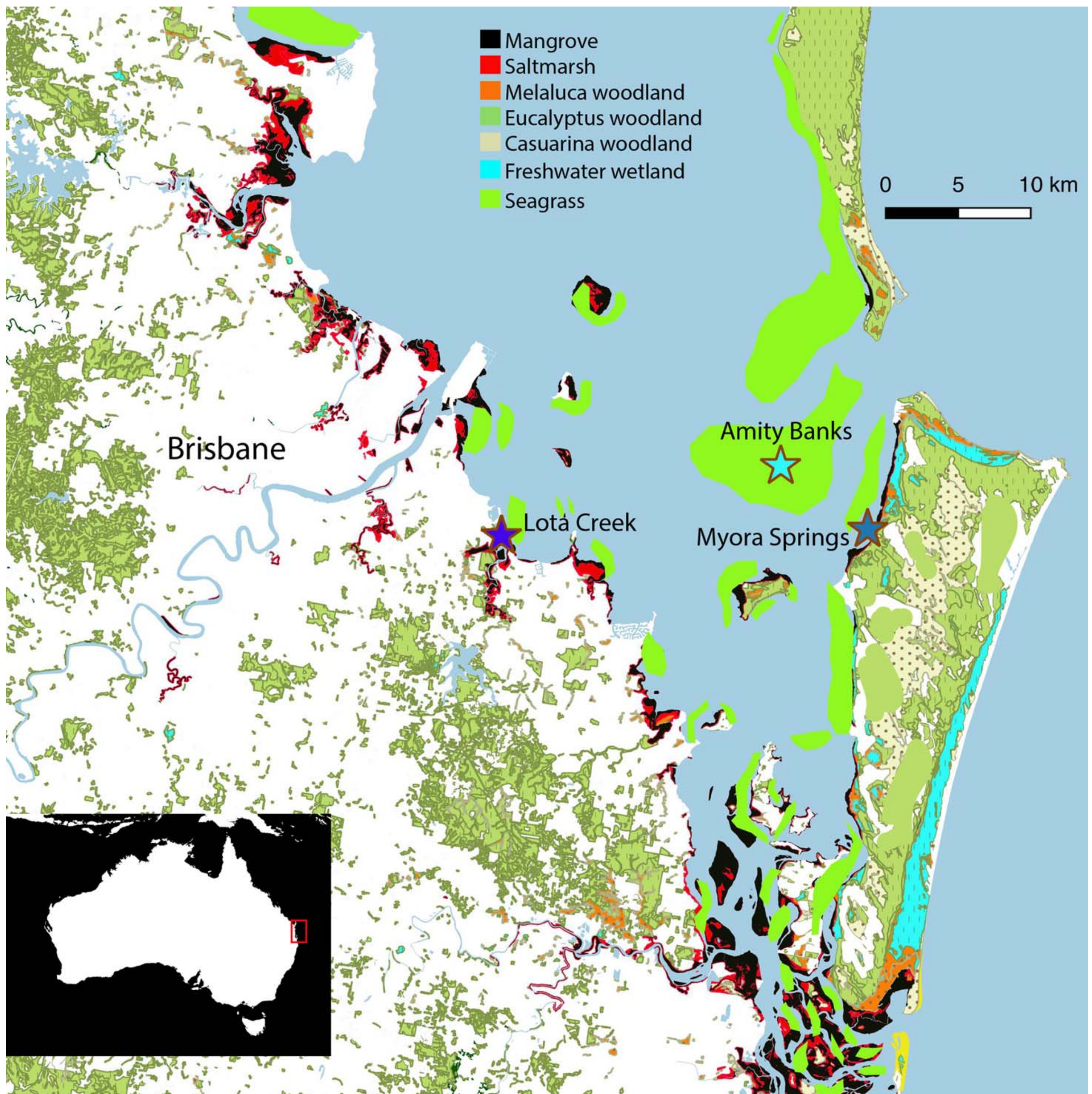

Fig. 1. Map of the sampling locations showing remnant vegetation types within the sampling site catchments. Remnant vegetation was mapped according to the regional ecosystem survey maps produced by the Queensland Herbarium in 2013. Sampling meadows are denoted by stars. [Color figure can be viewed at wileyonlinelibrary.com]

within each meadow using 10 mL syringes, for a total of 30 sediment samples per site (see Supporting Information Table S2 for site descriptions). The top 1 cm of the sediment was sampled during low tide, at a maximum water depth of 1.0–2.0 m. We sampled only the top 1 cm of sediment as we were interested in recent (< 10 yr) sources of organic matter as opposed to historical sources because current sources are more important for C accounting and because we could create a customised reference database of potential sources of plant eDNA from vegetation maps. Sediment samples

Table 1. Nested PCR design of the *rbcL* gene using primers described in Little et al. (2014).

PCR 1: primers Z1aF and R604 (~ 600bp):	
Z1aF:	ATGTCACCACCAACAGAGACTAAAGC
R604:	CTGRGAGTTMACGTTTTCATCATC
PCR 2: primers F52_tag and rcblB_tag (tag refers to the addition of Nextera adapters as specified below underlined) (~ 350bp)	
F52_tag:	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTGGATTCAAAGCTGGTGTTA
rcblB_tag:	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACCYTCTTCAAAAAGGTC

were frozen within 1 h of collection. Biomass of potential OC sources was also collected from the seagrass meadows (seagrass fragments and algal sources) and from North Stradbroke Island and Lota Creek (mangrove leaves and saltmarsh plant fragments).

Environmental DNA

DNA was extracted from 10 marine sediment samples from each of the three locations and the plant samples of potential OC sources. DNA was extracted to assess traces of higher plant species (both marine and non-marine plants) in the seagrass sediments and to determine the proportional contribution of DNA from each taxa to the sediment sample. DNA was isolated from the sediment samples following the method described in (Reef et al. 2009). Briefly, samples were ground in liquid nitrogen and purified using a sequential phenol : chloroform treatment. All DNA was diluted 1 : 10 from original extractions. Successful DNA extraction and amplification of the target gene *rbcL*, was achieved for all samples and source material. Weaker amplification was detected for the mangrove *Rhizophora stylosa*.

We used a region of the 5′ half of the plastid gene *rbcL* as the DNA barcode in this study. *rbcL* is an agreed land plant DNA barcode by the Consortium for the Barcode of Life. The barcode offers high universality, although poor discrimination among species for some plant groups, and is well characterized, with an extensive GenBank reference sequence library (CBOL Plant Working Group 2009). Due to possible DNA degradation in the environmental sample, we used the mini-barcode approach described in (Little 2014), amplifying smaller fragments of the gene rather than the full-length sequence, which might not be reliably amplified in degraded samples.

Polymerase chain reactions (PCR) were prepared in 10 μL reactions using Invitrogen Platinum TAQ by adding 7.43 μL ddH_2O, 1.0 μL PCR buffer, 0.4 μL $MgSO_4$, 0.5 μL dNTP, 0.25 μL of both forward and the reverse primers (Table 1), and 0.07 μL of TAQ. Template for the first PCR (1) was 1 μL of gDNA and amplicons from this first reaction were used as a template (without dilution) by adding 1 μL to the second PCR (2) irrespective of whether they were visible on agarose gels. Cycling conditions were the same for both reactions: 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 52°C for 30 s, 72°C for 1 min and a final extension of 10 min at 72°C.

Sequencing of the amplified transcripts was done on the Illumina platform at the Australian Genome Research Facility, using paired end reads. Image analysis was performed in real time by the MiSeq Control Software (MCS) v2.5.0.5 and Real Time Analysis (RTA) v1.18.54, running on the instrument computer. RTA performs real-time base calling on the MiSeq instrument computer. The Illuminabcl2fastq 1.8.4 pipeline was used to generate the sequence data. The samples yielded a total of 5.853.291 paired end reads (2.93 Gbp). MegaBLAST analysis was done on the genomics sequence data using against a custom reference database of the *rbcL* gene. The customised reference database was extracted from the GenBank list of *rbcL* sequences using *makeblastdb* with a FASTA file of sequences of the *rbcL* gene (or parts of) of plant species (or congenerics if species information was not available) known to occur within the region based on the Queensland Department of Science, Information Technology and Innovation (DSITIA) Wildlife Online database.

Stable isotopes

Sediment and biomass of potential OC sources were analyzed for C%, N%, δ^{13}C, and δ^{15}N in an elemental-analyser isotope ratio mass spectrometer (EA-IRMS, Sercon System, Griffith University). Samples were analyzed before and after being treated with hydrochloric acid (10%) to estimate inorganic C content; in all samples, inorganic C was less than 5% of the total. Analytical errors (SD) were 0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N. Carbon sources within the sediment were assessed with biogeochemical source plots and mixing models (Monacci et al. 2011).

Data analysis

Stratigraphic analysis was conducted using the R package *analogue* (Simpson and Oksanen 2015). Multivariate analysis was conducted using R package *vegan* (Oksanen et al. 2015). Differences in species composition among sites were analyzed using Bray–Curtis distances and PERMANOVA. SIMPER analysis was applied to identify species that were responsible for differences in plant species (OTUs) among sites. We conducted the multivariate analysis also on functional plant groups (seagrass, terrestrial, freshwater, mangrove, and saltmarsh) rather than individual species.

Stable isotope mixing models were run using the Bayesian stable isotope mixing model SIAR (R package *siar*, Parnell et al. 2010) using two isotopes (^{13}C and ^{15}N) and four sources (seagrass, mangrove/C3 vegetation, C3 saltmarsh, and C4 saltmarsh). We ran 200,000 iterations, thinned by 15 and with an initial discard of the first 50,000. We assessed the ability of the model to distinguish between the different sources by examining the correlations among the posterior distributions in each model. We determined that the model was not able to differentiate between the C4 saltmarsh plant *Sporobolus* sp. and seagrass (correlations ranged between −0.95 and −0.98), so these two sources were pooled in our analyses.

Results

The nested PCR protocol yielded reliable *rbcL* contigs for all 90 sediment and 10 potential OC sources. MegaBLAST analysis was used against the customised reference database of plant species known to occur within the region. We identified 150 plant operational taxonomic units (OTUs) within the seagrass sediment samples. Sequences from five OTUs were present in all samples: the mangroves *Avicennia marina* and *R. stylosa*, the seagrasses *Halodule* sp. and *Zostera muelleri*, and the freshwater wetland plant *Philydrum lanuginosum*. Of the list of identified OTUs 78% occurred in more than two samples. On average, 31 (± 9 SD) unique barcodes were represented in each sample. Taxa richness was higher at Lota Creek (ANOVA, $F_{(2,87)} = 7.4$, $p = 0.001$) as was species diversity measured as Shannon H (ANOVA, $F_{(2,87)} = 10.4$, $p < 0.001$) compared to the other two sites.

DNA from seagrass plants were overwhelmingly the most abundant contigs within each sample, making up on average 88% (± 10.3) of the DNA pool. The relative abundance of seagrass plant DNA was lower than 50% in only two samples (Amity Banks sample #26 and Myora Springs sample #5). Allochthonous OTUs consisted mainly of mangrove vegetation and varied among collection sites (Fig. 3A).

A bayesian isotope mixing model based on δ^{13}C and δ^{15}N values in the samples, with mangrove/terrestrial C3 vegetation, C3 saltmarsh, C4 saltmarsh and seagrass vegetation as the sources determined the contribution of seagrass to the OC pool to be 36%, 60%, and 68% for Lotta Creek, Myora Springs, and Amity Banks meadows respectively. C4 saltmarsh vegetation (*Sporobolus* sp.) could not be differentiated from seagrass by our mixing model. In the mixing model, C4 saltmarsh contribution to the OC pool was determined as 21% for the isolated Amity Banks meadow, and 27% and 32% for Myora Springs and Lota Creek respectively. Our eDNA data shows that contigs of the C4 saltmarsh *Sprobolus* sp., are very rare in sediment samples from Myora Springs and Amity Banks (making up 0.02% of the contig pool, Fig. 2). At these sites, *Sprobolus* sp. is unlikely to make a significant contribution to the seagrass OC pool. A simplified stable isotope model, where C4 saltmarshes were removed as a source of OC and thus ambiguity reduced, concluded that seagrasses are the major contributor to the OC pool in these two meadows (87–90%, Fig. 3B). At Lota Creek, eDNA data shows higher abundance of *Sporobolus* sp. contigs (2.3% of the pool), thus there is likely to be a contribution of C4 saltmarshes to the OC pool. Due to the inability of the stable isotope mixing model to resolve the seagrass and C4 saltmarsh sources ($r = -0.95$), the solution provided for Lota Creek (Fig. 3B) was ambiguous.

Multivariate analysis to compare species composition among sites was conducted using the 50 most abundant OTUs. The species composition differed significantly among sites (PERMANOVA, $F_{(2,89)} = 13.04$, $p = 0.001$, Fig. 4). An analysis based on functional vegetation groups rather than species resulted in similar findings (PERMANOVA, $F_{(2,89)} = 5.39$, $p = 0.001$). Differences in seagrass species contributions to the DNA pool as well as relative differences in the contribution of mangroves and salt marsh vegetation were the highest contributors to the dissimilarity observed among sites (SIMPER, average dissimilarity Table 2).

Mean OC concentration of the seagrass sediments for the three sites was 0.30% ± 0.01% and was similar among sites (0.29%, 0.32%, and 0.30% for Lota Creek, Myora Springs, and Amity Banks, respectively). Mean inorganic carbon concentrations were low with a mean value of 0.02% ± 0.02%. Sediment N concentrations were also similar among sites with a mean of 0.01% ± 0.006% (0.02, 0.02, and 0.01 for each site, respectively).

Plotting N : C ratios against δ^{13}C values of the different sediment samples (Fig. 5) reveals differences among sites, but for all sites it is apparent that most of the OC derives from seagrasses. The relative contributions of the different vegetation groups indicated by the isotopic mixing model was supported by the eDNA results (Fig. 3A,B).

Discussion

The Environmental DNA (eDNA) barcoding approach to characterize plant communities has been successfully applied in many ecological settings (e.g., honeybee pollen pellets) (Galimberti et al. 2014) and forest biodiversity of contemporary (Yoccoz et al. 2012) and ancient forests (Anderson-Carpenter et al. 2011). By comparing our DNA metabarcoding data to established stable isotope methods, our study confirms that using tagged primers targeting the rbcL gene and next generation sequencing technology, we can characterize the plant derived organic matter (e.g., plant fragments, pollen, and extracellular DNA) in the surface sediments of seagrass meadows and with a greater resolution than traditional stable isotope methods alone. The rbcL primers used in this study successfully amplified all of the seagrass species present in the region [adding to the genomic data collected for Indian seagrass species (Lucas et al. 2012)], although species level identification using rbcL was not always possible for

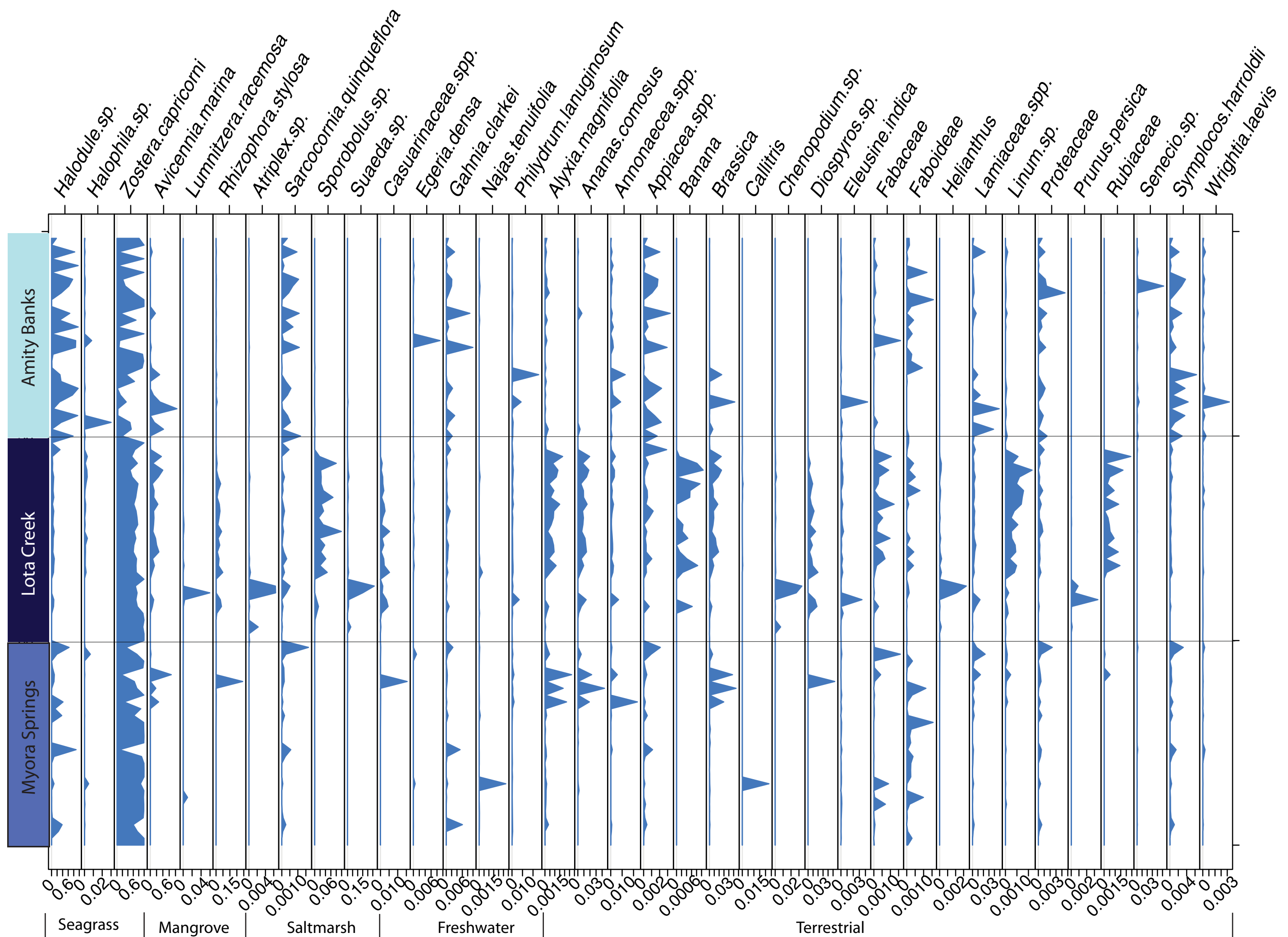

Fig. 2. A stratigraphic diagram showing the relative abundance of the 50 most abundant OTUs (operational taxonomic units) in each of the 90 sediment samples from the three seagrass meadows. The seagrass meadows are illustrated by a legend to the left of the figure. Note that the scale on the *x*-axis varies among species. [Color figure can be viewed at wileyonlinelibrary.com]

the genus *Halophila* (Nguyen et al. 2015). Our single species amplifications were also successful for most of the saltmarsh and mangrove species in the region with the exception of *R. stylosa*, where amplification was inconsistent.

Our eDNA results suggest that seagrass was the primary contributor to sedimentary C at all three sites, regardless of location and proximity to ecosystems other than seagrass (e.g., saltmarsh, mangrove, open ocean, or terrestrial). These results were contrary to our original prediction that contributions of different C sources would be dependent on proximity and connectivity to other source habitats (e.g., saltmarsh, terrestrial, and mangrove habitats) (Gonneea et al. 2004; Kennedy et al. 2010). Surprisingly, mangrove OC contributed very little to the seagrass sediment OC, despite mangroves fringing two of our sites and the slower decomposition rates of mangrove leaves compared to seagrass leaves (Holmer and Olsen Bachmann 2002).

Stable isotope analyses corroborated eDNA findings for all sites except Lota Creek. Here, stable isotope analyses suggested that > 50% of the seagrass sediment OC was contributed by mangroves and saltmarsh vegetation. This discrepancy between eDNA and stable isotope analyses for Lota Creek could have been caused by the high level of variability in the isotope values of Lota Creek seagrass sediments leading to higher levels of variability in the SIAR model predictions and/or because the isotopic values for seagrass could not be distinguished from those of C4 saltmarsh plants (*Sporobolus* sp.), in which case the SIAR model determines both sources to be contributors.

Our data shows that > 85% of the DNA amplicons found in the seagrass sediment samples belonged to seagrass species occurring in the community. The contribution of allochthonous plant material to the DNA pool was small (< 15%) and corresponded to the terrestrial vegetation communities

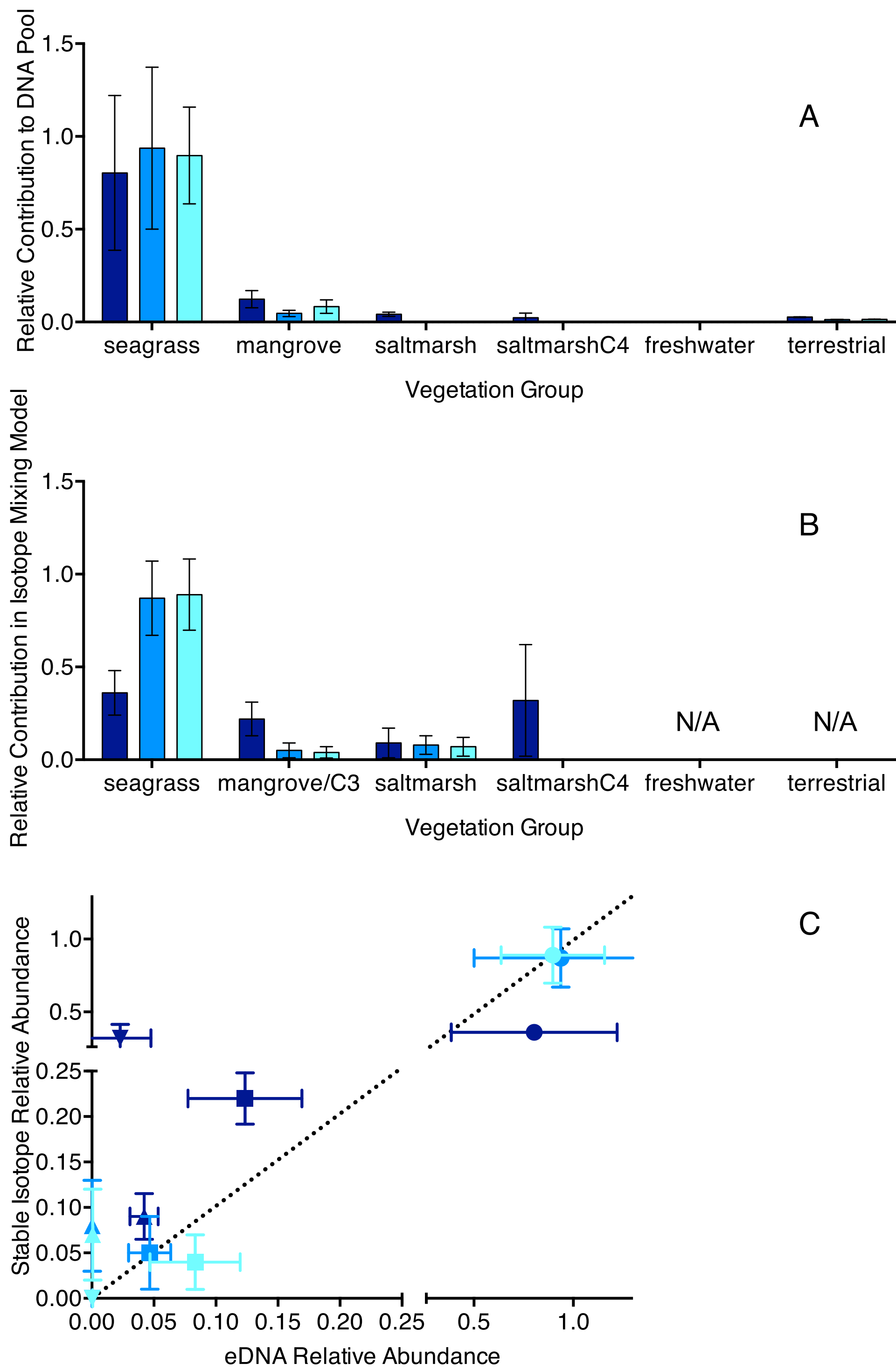

Fig. 3. The mean (± SD) relative contribution of the different vegetation groups to the organic material in shallow seagrass cores at three meadows in Moreton Bay, QLD. Contributions to (**A**) the eDNA pool and (**B**) the isotope mixing model. We were not able to distinguish between mangrove, terrestrial, and freshwater vegetation using ^{13}C and ^{15}N stable isotopes. (**C**) The mean (± SE) relative contribution of the different vegetation groups (circle = seagrass, square = mangrove, triangle = saltmarsh, inverted triangle = C4 saltmarsh), to the organic material at each meadow as determined by eDNA and the isotope mixing model. The dotted line is X = Y. Dark blue = Lota Creek, Blue = Myora Springs and Light Blue = Amity Banks. [Color figure can be viewed at wileyonlinelibrary.com]

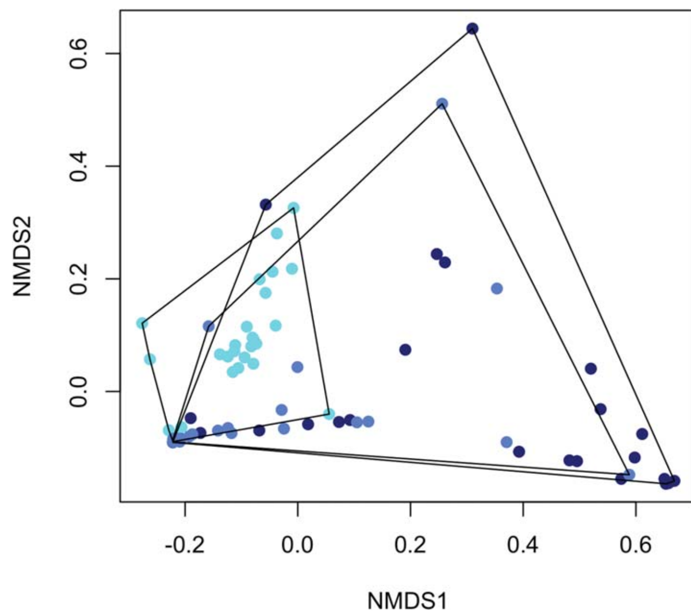

Fig. 4. NMDS of Bray–Curtis similarities of operational taxonomic units (OTU) relative abundances from the three different seagrass meadows sampled. Dark blue = Lota Creek, Blue = Myora Springs and Light Blue = Amity Banks. Stress = 0.05. Grouping by meadow is statistically significant (PERMANOVA, $p = 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

adjacent to the meadows (Table 2). It is possible that lower contributions of allochthonous carbon was the result of greater degradation of these sources' DNA compared to seagrass due to greater transport time from their site of origin. eDNA degradation is a complex process that is influenced by the physical, chemical, and biological micoenvironment, and currently it is not well understood (Pedersen et al. 2015). In fresh and sea water, the half-life of eDNA is only several hours to several weeks (Dejean et al. 2011; Thomsen et al. 2012). However, DNA that adsorbs to sediments (especially sand and clays) or complexes with humic substances is 100–1000 times more resistant to nuclease degradation (Romanowski et al. 1991; Pedersen et al. 2015), and plant DNA > 400 kyr old has been amplified in soils (Willerslev et al. 2003). Furthermore, studies in lotic habitats have found that eDNA from animals was still detectable even after it was transported > 10 km from the site of origin (Deiner and Altermatt 2014).

Our stable isotope data also suggests that the main contributors to C storage in seagrass sediments are the seagrass plants themselves. This is in contrast to previous calculations by Kennedy et al. (2010) who, using stable isotope mixing models, found much lower mean autochthonous contributions to the OC pool (~ 50%) for seagrass locations worldwide based on stable isotope analysis, although there was high level of variation about the 50% with the 25th of 75th percentile equivalent to 33% and 62% respectively (Kennedy et al. 2010). Furthermore, Samper-Villarreal et al. (2016) also found variations of a 50/50 ratio contribution from allochthonous and autochthonous sources for several seagrass sites in Moreton Bay, including Myora and Amity sites sampled in our study. However, both Samper-Villarreal et al. (2016) and Kennedy et al. (2010) conducted stable isotope analyses using bulk sediment samples collected 5–10 cm deep, while we only sampled the top 1 cm of the sediment, where more live seagrass material is present. On average, seagrasses accumulate ~ 1–3 mm yr^{-1} of sediment (Saunders et al. 2013), suggesting that these previous studies represent a historical average of C sources over the past ~ 30 yr (with less focus on recent seagrass growth, which occurs near the surface), and our study represents modern sources over the past ~ 3–10 yr. As a result, differences in our finding may be due to several factors related to the integrated depth of the sediments sampled. First, the relative contributions of different sources of C to Moreton Bay could have changed over the past 30 yr. Second, finer resolution information about sources of C could be lost when integrated over longer time-periods. Finally, although seagrasses are the dominant C source to sediment C in seagrasses of Moreton Bay over short time scales (~ 3 yr), its chemical composition may result in large components of the seagrass organic matter being processed, leaving behind only recalcitrant OC derived from seagrass tissues with the remaining 50% coming from recalcitrant allochthonous sources. However, understanding how source contributions change over time was beyond the scope of this study, and future

Table 2. SIMPER analysis results showing the five most influential species in discriminating among sites. The full results can be found in the Supporting Information (Table S1).

	Species	Contribution	SD	Cumulative contribution
Amity Banks vs. Lota Creek				
1	*Z. muelleri*	0.21	0.13	0.40
2	*Halodule* sp.	0.19	0.17	0.77
3	*A. marina*	0.07	0.08	0.90
4	*Sporobolus* sp.	0.01	0.01	0.93
5	*Suaeda* sp.	0.01	0.03	0.94
Amity Banks vs. Myora Springs				
1	*Z. muelleri*	0.23	0.17	0.46
2	*Halodule* sp.	0.20	0.17	0.85
3	*A. marina*	0.05	0.10	0.96
4	*R. stylosa*	0.00	0.02	0.97
5	*Lamiaceae* spp.	0.00	0.01	0.98
Lota Creek vs. Myora Springs				
1	*Z. muelleri*	0.13	0.09	0.42
2	*Halodule* sp.	0.07	0.09	0.63
3	*A. marina*	0.06	0.07	0.83
4	*Sporobolus* sp.	0.01	0.01	0.87
5	*R. stylosa*	0.01	0.02	0.90

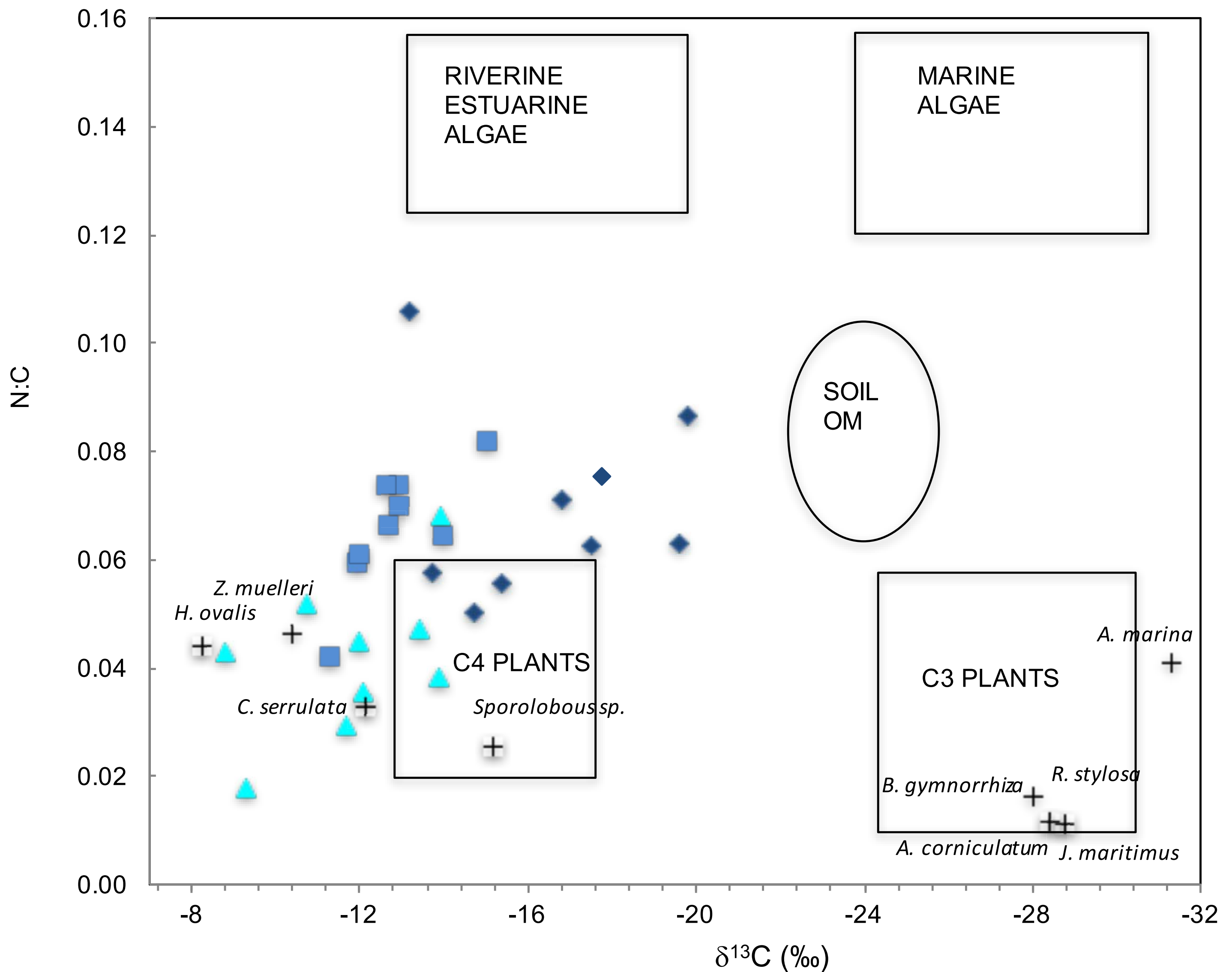

Fig. 5. Plot of N : C (mol : mol) vs. δ^{13}C, where ranges of possible carbon sources have been drawn from published data synthetized in Monacci et al. (2011). Symbols represent sediment samples from Lota Creek (dark blue diamonds), Myora Springs (blue squares), and Amity Banks (light blue triangles). Crosses denote end-members or sources for mangroves: *A. marina*, *R. stylosa*, *Bruguiera gymnorrhyza* and *Aegiceras corniculatum*; for marshes: *Juncus maritimus* and *Sporolobous* sp.; and for seagrass: *Z. muelleri*, *Cymodocea serrulata*, and *Halophila ovalis*. [Color figure can be viewed at wileyonlinelibrary.com]

research efforts on this topic will need to determine whether eDNA is an appropriate method for investigating temporal differences in source contributions.

An additional hypothesis for the discrepancies between our results and previous studies could also be due to limitations in the ability of stable isotope analyses to distinguish among contributing organic matter sources. As discussed, the number of potential sources of OC in Moreton Bay is large with stable isotope values of ^{13}C and ^{15}N of seagrass overlapping with other potential sources, such as C4 vegetation and epiphytic algae (Connolly and Waltham 2015), which can lead to uncertainties and underestimation of seagrass contributions in mixing model solutions (Fry 2013). The low taxonomic detail in these previous stable isotope models compared to our use of eDNA could be an additional reason why we found significantly higher autochotonous contributions to seagrass sediment organic matter than those using isotopic modelling.

We identified some limitations in the use of eDNA for determining contributions of different organic matter sources to seagrass sedimentary OC. First, the *rbcL* gene encodes the large subunit of Rubisco in both land plants and algae. However, the effectiveness of *rbcL* in taxonomic discrimination has been tested only for land plants with the universal primers designed for land plant sequences. Thus, these primers did not amplify algal DNA fragments that could have

been in the sediment samples. Although several studies have suggested that marine algae contribute little to sediment organic matter, likely as a result of its labile nature (Trevathan-Tackett et al. 2015), we could not estimate algal contributions to our samples using eDNA. A new DNA barcode, the internal transcribed spacer (ITS1/ITS2) has recently been suggested as a possible barcode for the identification of all eukaryotic plant species (Chen et al. 2010), and its use in future studies could help elucidate contributions of algae and phytoplankton to marine sediment organic matter with more certainty. Second, our study shows that eDNA can be a successful tool for reconstructing relatively recent sources of organic matter to seagrass sediment. Further work is still needed to understand how degradation of DNA through time could affect its use for deeper, older sediments. However, seagrass, and other blue C ecosystems have environmental conditions (e.g., anoxic sediments) that may be conducive for conserving eDNA in sediments for millennia and thus this technique may have application for detecting long term changes in contribution of different organic matter sources into marine sediments.

Unlike stable carbon isotope analyses, eDNA does not quantitatively measure the bulk C pool, it measures the frequencies of DNA amplicons, and the relative contribution of sources is determined based on those frequencies. Quantifying C source contributions using eDNA can be challenging as tissue degradation can mask a sources signal over time, leading to underestimation of its contribution. However, studies in terrestrial soils have demonstrated that soil DNA frequencies can accurately reflect the abundance of extant plants communities (e.g., Yoccoz et al. 2012). In our study, we found strong similarities between the relative contribution of different vegetation groups to the soil carbon pool estimated using eDNA and stable isotope analyses (Fig. 3C). Future studies are needed to understand the relationships among the total DNA concentration of the soil DNA pool, soil DNA frequencies, and organic C concentrations of the soil, and how these relationships change as a result of plant tissue degradation. Until these relationships are thoroughly examined, eDNA should not replace stable isotope analyses in quantitative carbon concentration determination, but rather be used in combination with them. Reconstructing the source plant communities using eDNA can provide a means to simplify the number of sources (and thus ambiguity) in isotope mixing models by determining the presence or absence of source species or functional groups in the sample and to improve the taxonomic resolution.

Identifying the major sources of organic matter to the sediments of blue C ecosystems is crucial for building robust and accurate C budgets. To date, however, our understanding of the major contributors of organic matter to blue C sediments has been hampered by limitations in stable isotope analyses, particularly its inability to separate mangroves, salt marshes, and terrestrial vegetation from one another because of overlapping isotopic values. Our study shows that eDNA can be an effective, high resolution (in some cases down to species level) tool for identifying major sources to sediment organic matter in blue C ecosystems. Furthermore, through the use of eDNA we showed that the limitations associated with traditional stable isotope methods, at least for Moreton Bay seagrass, may be leading to an underestimation of autochothonous contributions to sediment organic matter. If we want to protect the blue C ecosystems through carbon financial mechanisms, it is essential that we continue to improve methods for identifying major sources of organic matter to these ecosystems—eDNA appears to be a promising avenue for this endeavor.

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Conflict of Interest

None declared.

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