

A muddy time capsule: using sediment environmental DNA for the long-term monitoring of coastal vegetated ecosystems

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Abstract. Seagrass, saltmarsh and mangrove habitats are declining around the world as anthropogenic activity and climate change intensify. To be able to effectively restore and maintain healthy coastal-vegetation communities, we must understand how and why they have changed in the past. Identifying shifts in vegetation communities, and the environmental or human drivers of these, can inform successful management and restoration strategies. Unfortunately, long-term data (i.e. decades to hundreds of years) on coastal vegetated ecosystems that can discern community-level changes are mostly non-existent in the scientific record. We propose implementing DNA extracted from coastal sediments to provide an alternative approach to long-term ecological reconstruction for coastal vegetated ecosystems. This type of DNA is called ‘environmental DNA’ and has previously been used to generate long-term datasets for other vegetated systems but has not yet been applied to vegetation change in coastal settings. In this overview, we explore the idea of using sediment eDNA as a long-term monitoring tool for seagrass, saltmarsh and mangrove communities. We see real potential in this approach for reconstructing long-term ecological histories of coastal vegetated ecosystems, and advocate that further research be undertaken to develop appropriate methods for its use.

Additional keywords: climate change, eDNA, mangrove, saltmarsh, seagrass.

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Introduction

Seagrasses, saltmarshes and mangroves are plant communities that live along coastlines globally. Unfortunately, these environments are in decline worldwide, primarily as a result of human activities in coastal areas and, more recently, the effects of climate change (Alongi 2008; Gedan *et al.* 2009; Waycott *et al.* 2009). Protection and conservation of these coastal vegetation communities are extremely important because of the many valuable ecosystem services they provide (Costanza *et al.* 2014). Such services include providing breeding and migration habitats for marine life and birds, nutrient cycling and carbon storage, stabilising coastlines against erosion and sea-level rise, and supporting a range of industries, including fisheries and tourism (Barbier *et al.* 2011; Mcleod *et al.* 2011; Rogers *et al.* 2019). The specific ecosystem services provided by seagrasses, saltmarshes and mangroves are dependent on the distinctive species composition, morphology and habitat range of each community. Thus, coastal plant communities are highly diverse in their ecosystem service provision and all are essential to the survival of coastal ecosystems as a whole.

Our ability to protect coastal vegetated environments and prevent further decline rests on our understanding of how these communities have changed in the past in response to human stressors and environmental change. Long-term monitoring data that are high in resolution and span informative timescales (i.e. decades to hundreds of years), provide the ability to contextualise present conditions and predict future scenarios (Costanza *et al.* 2007). Change data that predate colonisation and industrialisation can help us understand ecological baselines and natural variability for coastal vegetated ecosystems, leading to an improved assessment of the impact of intensified human activities, the resilience of the system and the dynamics and time frames associated with recovery from disturbance (Bálint *et al.* 2018). Long-term data can also improve our understanding of invasion events and biotic interactions, further informing conservation policies and management efforts, and increasing their chances of success (Bálint *et al.* 2018). These important long-term change data are becoming increasingly sought after and, yet, are largely lacking for many ecosystems, including coastal environments (Boero *et al.* 2015).

Environmental DNA (eDNA), which is the DNA retained in environmental samples such as soil and water, can be extracted from coastal sediment cores and analysed to provide a new approach for obtaining long-term data on seagrass, saltmarsh and mangrove communities. eDNA from these coastal plant groups is preserved in sediment layers in various forms, including both active and dormant tissues, seeds, pollen and detritus (Fahner *et al.* 2016). This eDNA can be extracted and identified by matching the sequences obtained from the samples to those in an existing reference database. Sediment cores can be dated using radioisotopes, providing a core chronology where each layer has an estimated date that can be related to the coastal plant identification provided by eDNA analysis. The notion of using environmental DNA from sediment cores to reconstruct past vegetated environments is a well documented approach for gathering long-term data (Thomsen and Willerslev 2015; Bálint *et al.* 2018), but has not yet been applied to coastal vegetation systems. In this paper, we outline an environmental DNA ‘toolkit’ that can be adopted for assessing change in coastal plant communities, using samples from sediment cores. We highlight the benefits of this proposed approach over other existing monitoring methods, identify the emerging technologies that can support the analyses, and discuss how the resulting datasets will benefit the conservation, restoration and management of valuable coastal vegetated ecosystems.

Long-term monitoring of coastal vegetated environments; existing methods and an eDNA approach

Existing methods to document long-term change

Current approaches to collecting long-term data on seagrass, saltmarsh and mangrove environments include remote sensing, field-based observations, recovery of fossils and indirect estimates of vegetation composition based on molecular proxies and stable isotopes (Powell and Steele 1995). Remote sensing and field observations make it possible to evaluate change over metres to kilometres spatially; however, multiple surveys over time are needed to generate a time-series of records for change detection, and continued observations can be costly and time consuming (Danovaro *et al.* 2016). Additional field data can be generated at a lower cost when they are re-purposed as in Foster *et al.* (2017), where they applied underwater photography initially taken for estimating sediment accumulation, to the presence/absence of seagrass communities in South Australia. Despite the usefulness of these approaches, field observations date back only to when humans began recording their observations, and remote-sensing data are available only since the implementation of satellite-mounted instruments, with global, high-resolution data being limited to the past four decades.

In contrast, direct observation of plant communities using fossils enables assessment over geological timescales, but in a very site specific (spatially limited) and typically fragmentary manner. Indirect observations of past coastal vegetation can be made through chemical analysis of coastal sediments (using lipid biomarkers and stable isotopes). These data provide broad inference of climate and ecosystem changes that can be evaluated over decades to thousands of years (Ellegaard *et al.* 2006; Johnson *et al.* 2007; Cohen *et al.* 2012). However, this approach provides limited information regarding community composition

and taxonomic resolution, which is a significant limitation when attempting to reconstruct the ecological history of a coastal site (Duffy *et al.* 2019). Owing to either a temporal limitation, limited taxonomic resolution or a general lack of data, current methods for monitoring long-term change are difficult to apply to answer many of the questions we have around change in coastal vegetated environments.

Why an eDNA approach?

We propose that long-term data on the dynamics of coastal vegetated ecosystems can be improved by analysing environmental DNA buried in the sediment layers under these communities, to identify when and how plant communities have changed over time. These changes can be dated (with radioisotope approaches), which will allow the ecological history of the site to be reconstructed over periods of hundreds to thousands of years. This chronology of vegetation dynamics can then be related to other datasets, such as, for example, on management actions, human activities or environmental change. This approach will allow a longer-term view of coastal plant-community baselines and how they have historically responded to changes in their environment. Any correlations that can be identified between vegetation-community change and environmental change can provide evidence to predict the response of the ecosystem to future changes. A long-term dataset on vegetation-community dynamics will also allow a better understanding of the natural state of coastal vegetation ecosystems, acting as a potential baseline for conservation, restoration and management strategies (Fig. 1).

Several studies have already applied an eDNA reconstruction approach to terrestrial plant communities. Giguet-Covex *et al.* (2014) related plant dynamics to livestock farming over millennia, correlating farming practices to vegetation changes; Pansu *et al.* (2015) identified vegetation shifts from forest to pastoral plant assemblages, which were found to be due to human modification such as burning, logging and grazing. Additionally, Ficetola *et al.* (2018) found that plant-community change was rapid after the introduction of rabbits to a remote island. It is unclear why there has been a lack of eDNA studies in coastal environments, because the environmental properties of coastal sediments, such as high salt concentrations, protection from UV, low temperature and the absence of oxygen, are all known to favour DNA preservation (Giguet-Covex *et al.* 2019). However, there is uncertainty around using eDNA recovered from coastal sediments, owing to the influence of tidal flow. Additionally, seawater flushing (horizontal) and water seepage (vertical) through sediment layers may influence the reliability of reconstructed ecological chronologies. For example, there is the risk that seawater can seep vertically through layers of sediment, transporting fragments of DNA from one layer to the next, thus smearing the signal of a vegetation community across multiple sediment layers (i.e. time periods).

We acknowledge that movement of genetic material could potentially hinder the effective use of eDNA in reconstructing coastal vegetation dynamics over time. However, previous literature has pointed to eDNA being representative of localised vegetation in habitats with water movement. Shackleton *et al.*

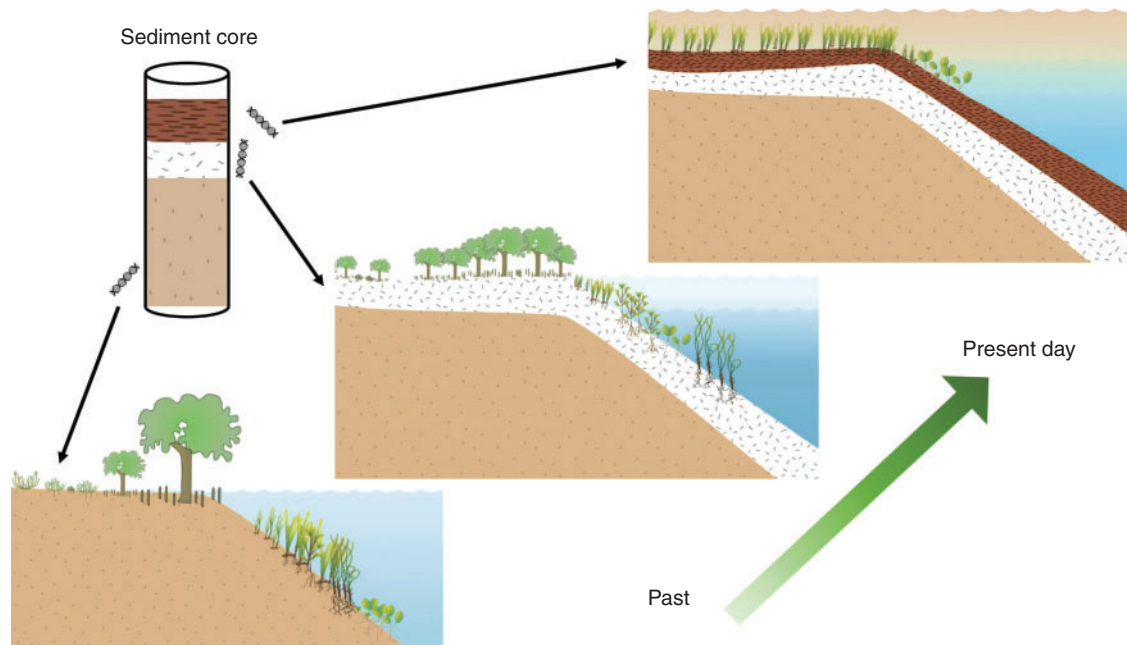


Fig. 1. Diagram depicting the information that can be gathered from an environmental DNA analysis on coastal sediments. There is a clear change from saltmarsh to mangrove to seagrass from the past to the present day. Additionally, the seagrass community has changed at the species level, which can be detected via eDNA analysis methods of high resolution, i.e. hybridisation capture.

(2019) were able to successfully use eDNA to reconstruct spatial patterns in plant communities within floodplains and Jeunen *et al.* (2019) found that eDNA surveys detected taxa that were spatially discrete in a coastal setting, noting that localised species generated local signals. Additionally, eDNA extracted from the soil at a specific location has been proven to reliably reflect aboveground plant species in a terrestrial environment (Yoccoz *et al.* 2012). For long-term data, eDNA complements and confirms results of pollen analysis dating back to the early Holocene in a lake system (Paus *et al.* 2015) as well as macrofossils and pollen in a late glacial lake (Parducci *et al.* 2019). Because of this evidence, we feel that eDNA analysis should certainly be trialled in coastal vegetated environments because the potential benefits to management would be considerable (See section ‘Using eDNA data to improve outcomes for coastal vegetated ecosystems’).

Common methods applied to environmental DNA analysis

Metabarcoding of environmental DNA from sediments is a common approach to generating data on plant communities in soil (Zinger *et al.* 2019). DNA metabarcoding allows the identification of multiple species from a single environmental sample (Taberlet *et al.* 2012). This method was made possible because of the advances in DNA sequencing technologies and was further refined through the use of next-generation sequencing (NGS) that can read DNA from multiple samples in parallel. When using this approach, DNA is extracted from a sediment sample and then either one or multiple universal barcode regions are selected and amplified by primers via polymerase chain reaction (PCR) and then sequenced using NGS. With metabarcoding, the DNA barcode locus used must have

gene regions that are short enough to allow adequate amplification from degraded DNA fragments, which are generally found in environmental samples. These fragments are shorter than DNA recovered from fresh plant tissue, that is, live plants (Epp *et al.* 2012). This means that the choice of barcode is essential so as to recover all the taxa present in the sediment sample. A commonly used universal locus for detecting plant DNA in sediments is the P6 loop of the *trnL* intron (Taberlet *et al.* 2007). This is a short barcode, which is, therefore, more likely to be detected in sediments than is a longer barcode region. However, although a shorter barcode has a greater likelihood of recovering plant species present in the sediment, the reliance on this single short barcode means that taxonomic resolution will likely be only to family or order level and, in rare instances, genus level. Therefore, depending on the questions to be answered, that is, whether broader or finer taxonomic resolution is required, will determine whether metabarcoding is the appropriate choice, or whether alternative methods are required.

Recent eDNA-method developments

Hybridisation capture is a new approach that can be applied to analysing eDNA from sediment cores, which can potentially improve on metabarcoding. Instead of applying a single universal locus per PCR, many loci are targeted via the design of custom, short RNA probes called ‘baits’ (Fig. 2). This reduces the reliance on a single PCR step which can lead to biases related to primers preferentially binding to particular species or the absence of primer binding sites. To design baits, target species genomes are selected, and these are then used to generate very specific target loci (Lemmon *et al.* 2012). If multiple baits are designed, and they capture at the family or order level

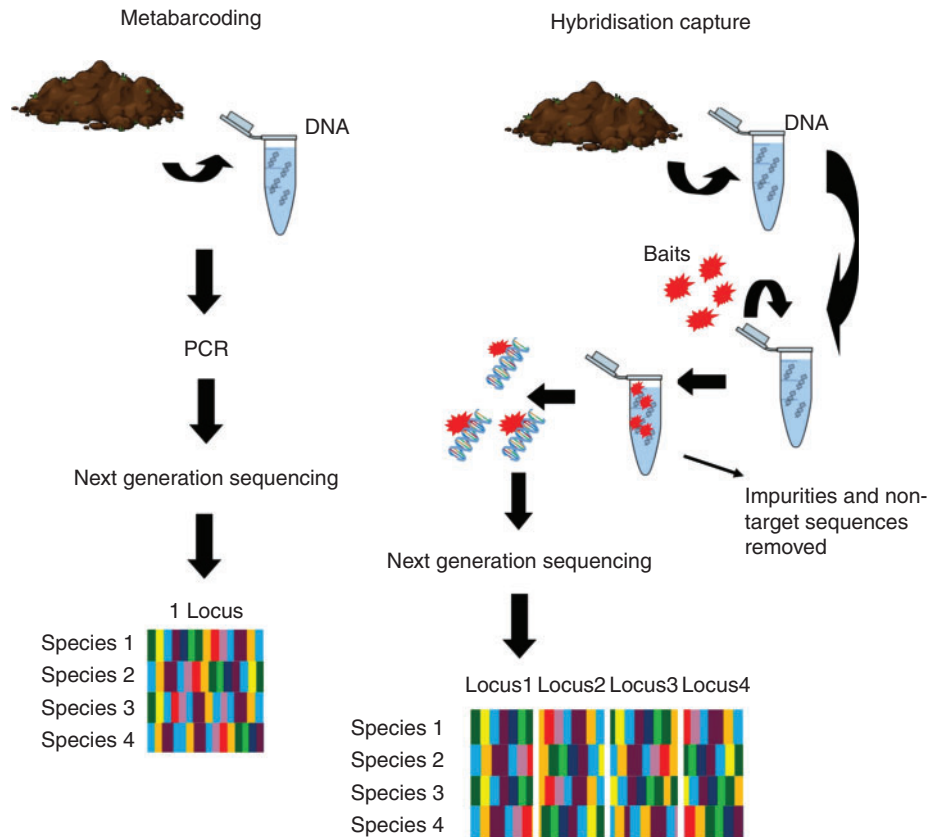


Fig. 2. Flow diagram showing the different DNA analysis approaches used for species identification from sediment samples: metabarcoding and hybridisation capture.

(Hart *et al.* 2016), then multiple species can be targeted using the same bait set. Recently, a bait set was designed by Waycott *et al.* (M. Waycott, E. Biffin, K. Van-Dijk, unpubl. data) to capture across all flowering plants in a single capture, that is, the baits are not designed to be species specific but are designed to capture multiple species. This not only decreases costs but also increases the chance of detecting all taxa present in a sediment sample. Once DNA has been extracted from the sediment, the hybridisation capture protocol includes assigning unique identification codes to samples, which is a series of unique bases attached to the ends of the reads. This means that multiple samples can be pooled and they can be separated in the analysis step, this is ideal because it further reduces costs of this approach. Baits are then added to these pooled samples and the bait–DNA combination is removed from solution by using magnetic beads, thus leaving behind only unwanted sequences and impurities, which can then be discarded. The targeted sequences that have been removed are then amplified via PCR and sequenced using NGS. Because of the degradation of DNA in sediments, single target loci can be difficult or impossible to recover for every species. By targeting many loci instead of one, there is a greater probability of detecting the eDNA left behind by all individual coastal plants present in the sediment. The discriminatory ability of multiple loci also allows greater taxonomic resolution to be achieved when identifying eDNA sequences recovered from sediments. Overall, we suggest that hybridisation capture could provide a

highly effective tool for analysing eDNA from coastal sediments, where it is important to document all taxa present.

Is hybridisation capture feasible and what information can we obtain?

A hybridisation-capture approach for genomic analysis of multiple species in a single sample has already shown promising results and prompted consideration of how this information can fill knowledge gaps in other disciplines. Recent work by Foster *et al.* (N. R. Foster, J. M. Young, K. Van-Dijk, E. Biffin, A. R. Jones, B. M. Gillanders, M. Waycott, unpubl. data) has demonstrated that hybridisation capture can consistently identify individual coastal plant species in a mock sample of up to 10 different species. This was then tested on modern sediments, that is, only ~20 years before present, where the results confirmed that a hybridisation-capture approach is able to recover species-level information from eDNA in sediments. Further testing will need to be undertaken to determine the temporal limitations of this approach; however, the current results have suggested that this method can recover high-resolution information for individual plant species within an environmental sample. Such information can be applied to determining sources of organic carbon in coastal plant environments as in Reef *et al.* (2017). Extrapolating these data to long-term trends will provide insight into which vegetation communities were responsible for the historic sequestration of stored organic carbon in coastal

sediments (Geraldi *et al.* 2019), which can then inform the way these environments are managed. The species-specific genetic data uncovered with this approach can also be applied to population-level variability within species because there is a higher likelihood of detecting within-species variability because multiple genes are targeted. Within-species variability can provide information on recruitment and effective population size, which are useful metrics for understanding vegetation community dynamics. In addition, population-genetic applications are widely used in management of natural systems (Adams *et al.* 2019). Ongoing advances in the application of eDNA, such as the suggested hybridisation capture or even genome skimming, that avoid the inherent biases of PCR-based methods, are enabling more reliable eDNA-based population genetic data to be generated. Work is ongoing to overcome remaining challenges, including testing to remove confounding factors (e.g. degraded DNA leading to misidentification of sequence variation within a species; Adams *et al.* 2019). Evidently, a hybridisation-capture approach is not only feasible but can supplement data in other environmental research areas that are otherwise difficult to obtain.

Additional considerations when applying an eDNA approach to coastal systems

The application of eDNA for monitoring coastal vegetation change is certainly feasible; however, a major limiting factor of this approach is the availability of reference sequences for coastal plant communities. A taxonomically verified and high-quality reference sequence database is required to identify DNA sequences extracted from coastal sediment samples. Although unknown sequences and assignment of sequences to a higher order can still be useful, sequence data are far more useful when they can be accurately classified to a species or genus (Reef *et al.* 2017; Cristescu and Hebert 2018; Dormontt *et al.* 2018).

So as to construct a reference database, species of interest need to be properly vouchered with correct taxonomic identification and deposited in Herbaria (Dormontt *et al.* 2018). A lack of vouchered specimens and their associated DNA sequences significantly impairs progress (Thomsen and Willerslev 2015) and reduces our ability to identify sequences, which affects the accuracy and utility of the results (Jørgensen *et al.* 2012). False positives may be mistakenly generated where coastal plant species are believed to have been present because they have a partial match to a sequence in an incomplete reference database. Conversely, a coastal plant sequence may be generated but not have a match in the reference database, leading to it be mistakenly discarded. While there is software available to decrease the likelihood of a false classification, a complete reference library will still greatly improve the accurate assignment of eDNA sequences to known species.

Reference libraries for plant communities are being constructed, such as the database on arctic flora initiated by Sonstebø *et al.* (2010), which was then expanded by (Boessenkool *et al.* 2014). However, coastal plant reference databases are yet to be constructed or made widely available (Duffy *et al.* 2019); therefore, this is an area of research that needs to be addressed before implementing eDNA as a management tool. Additionally, reference sequences must also be of the

same loci as chosen for amplification of the DNA from sediments, that is, either a universal locus in the case of metabarcoding or multiple loci in the case of hybridisation capture. Fortunately, if the latter is chosen, it is a faster and more cost-effective approach and will generate significantly more reference data because of the fact that samples can be pooled, and many loci are targeted instead of one.

Using eDNA data to improve outcomes for coastal vegetated ecosystems

Effective management and conservation of coastal vegetated ecosystems requires a long-term outlook that is difficult to achieve with existing monitoring methods, but critical for understanding ecosystem dynamics (Duffy *et al.* 2019; McAfee *et al.* 2019). For example, Watson *et al.* (2011) used pollen and isotope evidence from dated cores to establish a long-term ecological history for Elkhorn Slough tidal marsh in California. Reconstruction of marsh community composition over a period of 5000 years considerably altered the perspective within which recent changes (in the past few decades) have been interpreted (Jackson *et al.* 2001). Using long-term datasets to establish ecological baselines, and understand the bounds of natural variability, greatly improves our ability to predict the likely ecosystem response to future changes, on the basis of its response to past change (Fordham *et al.* 2016; Nogués-Bravo *et al.* 2018), with higher temporal and taxonomic resolution datasets providing greater gains (Duffy *et al.* 2019; García 2019). The proposed eDNA approach for reconstructing coastal vegetation ecosystem dynamics will allow generation of well resolved taxonomic data time series (i.e. species presence and community composition) over hundreds to thousands of years at approximately decadal temporal resolution for specific sample locations.

The foundational information that can be obtained from such long time series include the following: identification of historically stable vegetation communities at specific locations, which may be used as a target for native vegetation restoration; identification and timing of invasive species introductions, and the subsequent responses of native vegetation communities to invasions; detection of rare, uncommon or cryptic species that are difficult to monitor by using other methods; and biotic responses of coastal vegetation communities to stressors such as coastal habitat modification and sea-level rise. As such, long-term eDNA-based ecological reconstructions of coastal vegetation can provide scientific understanding to underpin policy and management decisions. Below we have outlined a few key examples of potential applications of these data.

Sediment core samples from multiple locations could be analysed to generate data on the spatial and temporal patterns of coastal vegetation species' range shifts, local extirpations, evolutionary adaptations and extinctions in response to climate change over periods of potentially thousands of years (depending on the sediment age profile). The same datasets could also be used to assess the responses of different taxa to human activities, including understanding how cumulative impacts may push coastal vegetation communities towards tipping points and result in significantly altered species compositions. It may also be possible to use these well resolved

eDNA-based time series to assess the success or failure of previous management actions (e.g. attempts at restoration) and inform adaptive management approaches; to better understand the time frames required to restore ecosystems to their baseline state; and ultimately to assess whether the conditions required for restoration of the stable baseline community are realistically achievable under current and future climatic conditions (Breed *et al.* 2016).

In addition to the above practical applications, eDNA-based reconstruction of coastal vegetation environmental histories can make a difference for public engagement and the public perception of coastal wetland ecosystems. As noted by McAfee *et al.* (2019), using ecological reconstructions to create a historical context for proposed restoration projects can generate confidence by clearly demonstrating tangible targets, and may also positively affect the intrinsic value that stakeholders attribute to an ecosystem.

Conclusions

Ongoing climate change and negative impacts from human activities in the coastal zone threaten the survival of coastal vegetation communities and the enormous ecosystem services they provide. We need new approaches that can generate accurate, long-term data on the presence and species composition of these ecosystems over long time periods (up to thousands of years), because this provides vital information for conservation plans, future predictions, ecosystem processes and successful management policies. Environmental DNA is an emerging toolkit that is already being applied to monitoring of vegetation and aquatic species in other environmental settings and has great potential to be implemented in coastal vegetated environments. We advocate for novel methods such as hybridisation capture to continue to be trialled on dated sediments sampled from coastal vegetated environments, because this will only improve the quantity and quality of the taxonomic information generated. There is great potential for this proposed novel approach to generate previously unobtainable ecological histories for coastal vegetated ecosystems. Armed with such datasets, we can better understand their long-term dynamics, engage stakeholders, inform successful management and ultimately achieve positive outcomes for these valuable ecosystems into the future.

Conflicts of interest

N. R. Foster and M. Waycott are Guest Editors of the Translating Seagrass Science into Action issue and B. M. Gillanders is an Associate Editor with *Marine and Freshwater Research*. Despite these relationships, they took no part in the review and acceptance of this or any other manuscript in this issue that they authored. The authors declare that they have no further conflicts of interest.

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