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Constraining nitrogen sources to a seagrass-dominated coastal embayment by using an isotope mass balance approach

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ABSTRACT

Nitrogen (N) is often the key nutrient limiting primary production in coastal waters. Quantifying sources and sinks of N is therefore critical to understanding the factors that underpin the productivity of coastal ecosystems. Constraining nitrogen inputs can be difficult for some terms such as N fixation and marine exchange as a consequence of uncertainties associated with scaling and stochasticity. To help overcome these issues, we undertook a N budget incorporating an isotope and mass balance to constrain N sources in a large oligotrophic coastal embayment (Western Port, Australia). The total N input to Western Port was calculated to be 1400 Mg N $year^{-1}$, which is remarkably consistent with previous estimates of sedimentation rates within the system. Catchment inputs, N fixation, marine sources and atmospheric deposition comprised 44, 28, 28 and 13% of N inputs respectively. Retention of marine-derived N equated to ~3 and ~10% of total N and NO_x flushed through the system from the marine end-member. The relatively high contribution of N fixation compared with previous studies was most likely to be due to the high proportion of nutrient-limited intertidal sediments where N is mediated by seagrasses and sediment cyanobacteria.

Keywords: ^{15}N, denitrification, isotope, nitrogen, nitrogen budget, nitrogen fixation, seagrass, tidal flat.

Introduction

Nitrogen inputs to the coastal zone are one of the most pressing issues facing the biosphere (Steffen *et al.* 2015). Excessive nitrogen loads have led to algal blooms, hypoxia and seagrass loss in the coastal zone (Rabalais *et al.* 2002; Cook and Holland 2012; Cook *et al.* 2015). Anthropogenic sources of nitrogen enter via rivers, atmospheric deposition and sewage outfalls. In addition to anthropogenic sources, nitrogen can enter the coastal zone from offshore marine sources and nitrogen fixation. Nitrogen budgets are very commonly used to quantify the relative importance of these sources. For example, in Moreton Bay, Australia, it was found that nitrogen fixation was the dominant nitrogen input, being approximately double that of the catchment inputs (Eyre and Mc Kee 2002). By contrast, Chesapeake Bay nitrogen inputs are dominated by rivers, with nitrogen inputs by nitrogen fixation being considered negligible (Boynton *et al.* 1995), and in Golden and Tasman Bay in New Zealand, inputs were dominated by the marine source (Zeldis and Swaney 2018).

The reliability of nitrogen budgets will depend on how well constrained the input terms are. In reality, there will always be terms that are difficult to constrain depending on the geomorphology of the system and the availability of monitoring data. The accuracy of riverine inputs is dependent on the intensity of monitoring data during flow events (Harmel *et al.* 2009) and atmospheric deposition can have highly variable concentrations of nitrate depending on air source (Eriksson 1952). Marine exchange of nitrogen is particularly challenging to quantify, given the large, and hard to constrain, volumes of water exchanged within marine systems. As a consequence, it is generally

assumed that marine exchange is the difference between inputs and internal sinks (Boynton *et al.* 1995; Eyre and Mc Kee 2002), which is highly uncertain. Where a salinity gradient exists, salt budgets can be used to infer inputs from terrestrial and marine end-members (Gordon *et al.* 1996); however, this gives only a measure of an instantaneous input, leading to a high degree of uncertainty in scaling this over time.

Internal nitrogen addition and removal processes, including denitrification and nitrogen fixation, further add to the complexity of nitrogen budgets. Measurements of these process rates suffer from two key issues, including the accuracy of the rate measurement itself and scaling. With regard to the first issue, enclosure of the system may change metabolic and transport processes that can potentially change rates through the disruption of faunal and plant integrity. Furthermore, methods to measure denitrification and nitrogen fixation have several assumptions and potential shortcomings associated with tracer additions. For denitrification measured using the isotope pairing technique, it is assumed that the $^{15}NO_3^-$ added mixes homogenously into all denitrification zones, which might not always be the case in vegetated sediments (Risgaard-Petersen and Jensen 1997). In the case of nitrogen fixation, there are also assumptions about the conversion ratio of acetylene to ethylene when using the acetylene reduction assay, or the homogeneity of mixing of $^{15}N_2$ into the zones of nitrogen fixation when using the ^{15}N labelling approach (Capone and Kiene 1988; Karl *et al.* 2002)

With regard to the second issue, the extrapolation of nitrogen fixation and denitrification rates from discrete measurements to larger spatial scales, even in the case of local studies, can also account for the variability in the relative importance of these processes considered in previous studies. For instance, Mulholland (2007) found that a wide range of global rates of nitrogen fixation can be obtained, depending on whether the measurements are extrapolated from laboratory- or field-based experiments. Furthermore, the temporal and spatial variability of these processes means that repeated measurements over many different environments are required to get a truly representative estimation of these processes across time and space.

The use of stable isotopes offers a potential solution to the problems described above that use a mass balance only approach. The most easily constrained systems are those with limited nitrogen end-members such as the open ocean (Mahaffey *et al.* 2003). This approach has also been used in more complex coastal systems (Liu *et al.* 1996; Voss *et al.* 2005; Radtke *et al.* 2012; Korth *et al.* 2014; Woodland and Cook 2014); however, the focus has been on constraining nitrogen fixation in pelagic systems where blooms of cyanobacteria are known to occur. Currently, there have been no studies using isotope mass balance approaches to constrain nitrogen budgets in coastal embayments. These systems are more complex than open ocean systems that have been previously studied; however, if the isotope end-members are well constrained, then adding isotopes into the mass balance can help constrain the nitrogen budget. Riverine nitrogen isotope signatures are highly variable depending on the land-use types, with $\delta^{15}N$ values increasing from ~4 to >20‰ as land-use intensity increases from forested to agricultural catchments (Wong *et al.* 2018). Atmospheric deposition is typically <0‰, although this can be highly variable (Proemse *et al.* 2013). The marine $\delta^{15}N$ end-member typically ranges between 6 and 10‰ in the surface oceans globally (Rafter *et al.* 2019). Newly fixed nitrogen typically has a value of ~−2–0‰ (Owens 1988). The efficacy of adding $\delta^{15}N$ into a nitrogen budget will therefore depend on the nature of a system. Systems likely to benefit most from the addition of $\delta^{15}N$ onto the nitrogen budget are those where the putative dominant inputs have a distinct isotopic separation (for example, nitrogen fixation and rivers with a high $\delta^{15}N$ or sewage). For example in the case of Moreton Bay (Eyre and Mc Kee 2002), $\delta^{15}N$ would aid in validating the estimated high nitrogen fixation term relative to marine and catchment sources.

Here, we undertook a mass and stable isotope balance to create a nitrogen budget for a shallow embayment with a large marine exchange, Western Port, Australia. The aim was to better constrain marine nitrogen inputs to the system by using a novel isotope mass balance approach.

Materials and methods

Study site

The combined mass and isotope balance described in this study was undertaken across Western Port, a macrotidal shallow marine embayment of ~650 km^2, 55 km southeast of Melbourne. The major tributaries flowing into the bay are Bass River, Bunyip River, Lang Lang River, Toomuc Creek and Watsons Creek, and there are two separate openings to the ocean located on either side of Phillip Island (Fig. 1, Table 1).

Atmospheric deposition

All budget mass and isotopic input terms are summarised in Table 2. Long-term rainfall data for the period 2006–2016 were obtained for Melbourne and were then extrapolated to the entire area of Western Port (https://www.melbournewater.com.au/water/rainfall-and-river-levels#/, accessed February 2017). On the basis of the previous work of Lansdown (2009) and Wong *et al.* (2014), it was assumed that the concentrations of NH_4^+ and NO_x in the rainfall were 14 ± 1.4 and 14 ± 0.7 μmol L^{-1} respectively, resulting in an estimated total input of ~186 ± 20 tonnes (Mg) of nitrogen per year. Similarly, it was assumed that the end-members for the atmospheric deposition of NH_4^+ and NO_x were 1.0 ± 2.0 and −1.4 ± 2.9‰ respectively, on the basis of the previous work of Lansdown (2009) and Wong *et al.* (2014).

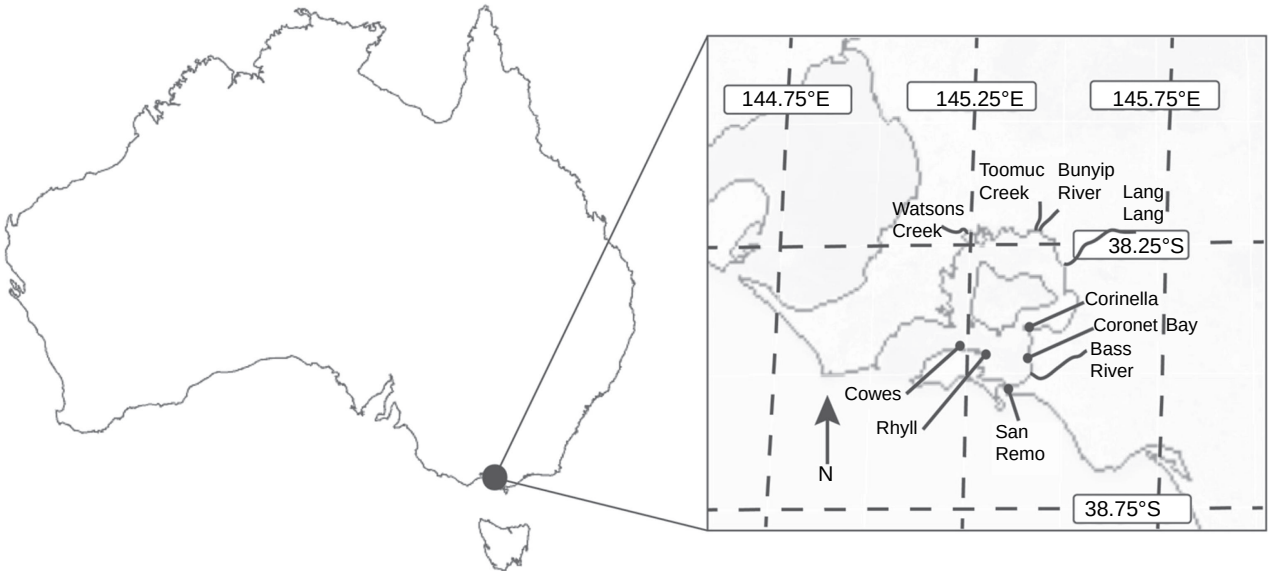

Fig. 1. Location of study sites in Western Port, Victoria, Australia. Used with permission from Russell *et al.* (2016) (©Wiley 2016).

Table 1. Sample locations for oceanic and riverine inputs.

Site	Coordinates	Terrestrial or oceanic
Bass River	−38.467045°, 145.515672°	Terrestrial
Bunyip River	−38.196927°, 145.477834°	Terrestrial
Lang Lang River	−38.255695°, 145.548879°	Terrestrial
Toomuc Creek	−38.178193°, 145.441891°	Terrestrial
Watsons Creek	−38.229754°, 145.235564°	Terrestrial
Cowes	−38.446132°, 145.239837°	Oceanic
San Remo	−38.520262°, 145.364816°	Oceanic

Table 2. Summary of the final budget and isotopic end-members or fractionation factors used in this study.

Parameter	Average loading (Mg N year^{-1} ± s.d.)	Reference	Average end-member or fractionation factor (‰ ± s.d.)	Reference
Riverine TN	612 ± 30	This study	9.2 ± 2.8	This study
Nitrogen fixation	434 ± 40	Russell *et al.* (2016)	0.0 ± 1.0	Owens (1988)
Rainfall – NH_4^+	92 ± 13	This study	1.0 ± 2.0	Lansdown (2009)
Rainfall – NO_x	94 ± 9	This study	−1.4 ± 2.9	Lansdown (2009) Wong *et al.* (2014)
Oceanic	400 (Q1 = 200, Q3 = 700)	This study	6.7 ± 1.1	This study
Sinks				
Denitrification	228 ± 10	Russell *et al.* (2016)	3.5 ± 2.0	Brandes and Devol (2002)
Sediment (export and burial)	1400 (Q1 = 1200, Q3 = 1700)		4.0 ± 1.1	This study

Note that the oceanic import and sediment export burial terms have the median and interquartile range presented due to highly skewed distributions.

Nitrogen fixation

The production of bioavailable nitrogen through nitrogen fixation was estimated to be 434 ± 40 Mg N $year^{-1}$ on the basis of work by Russell *et al.* (2016). This value was obtained by extrapolating rates from vegetated and non-vegetated core incubations collected at Corinella, Coronet Bay and Rhyll (Fig. 1) throughout the austral winter–summer period (July 2014–March 2015). On the basis of the available literature, it was assumed that the isotopic end-member for nitrogen fixation was 0.0 ± 1.0‰ (Owens 1988).

Riverine inputs

The catchments and tributaries that surround Western Port also make a significant contribution of nitrogen input to the bay, with nitrogen entering in various forms, including total and dissolved nitrogen (in particular nitrate, NO_3^-). Nutrient concentrations for both total nitrogen (TN) and NO_3^- in addition to flow data for the major tributaries were available for the period 1990–2013 from the Department of Environment, Land, Water & Planning (http://data.water.vic.gov.au/monitoring.htm, accessed January 2017). Loading calculations were made using the GUMLEAF program ('Generator Uncertainty Measures and Load Estimates using Alternative Formulae', ver. 0.1 α, University of Melbourne, see https://environmetrics.net/resources/software/; Tan *et al.* 2005) in conjunction with the flow regime-stratified Kendall's ratio estimator (Tan *et al.* 2005). Loads were calculated using daily flow data in combination with water-quality samples for each tributary collected, on average, in monthly intervals, and the average concentrations of NO_x and TN were 6.48 and 7.9 mg L^{-1} respectively, and it was estimated that yearly input of total nitrogen was ~612 ± 30 Mg N $year^{-1}$ (Russell *et al.* 2016).

The isotopic signatures ($\delta^{15}N$) of the catchment-derived NO_3^- flowing into the bay were taken from a previous study (Wong *et al.* 2018). During the present study, water samples were collected from the main tributaries flowing into Western Port between April 2014 and May 2015. These samples were collected from the most downstream freshwater section of each river or creek (Table 1), to ensure that all samples collected were terrestrially derived and not influenced by tidal exchange. Samples were filtered through 0.22-μm Sartorius Minisart syringe filters and frozen until analysis. The approach of McIlvin and Altabet (2005) was used to determine the isotopic signature of ^{15}N–NO_3^-. Initially, cadmium was used to reduce NO_3^- to NO_2^-, afterwards sodium azide in an acetic acid buffer was used to further reduce the NO_2^- to N_2O. The N_2O produced was analysed on a Hydra 20-22 continuous-flow isotope ratio mass spectrometer (CF-IRMS; Sercon Ltd, UK) interfaced to a cryoprep system (Sercon Ltd, UK); the precision of the stable isotope analysis was ±0.3‰ (s.d.; n = 5) for NO_3^- concentration >5 μmol L^{-1}.

The isotopic signature of the total nitrogen load was estimated using average isotopic signature of particulate (SPN) and dissolved (NO_3^-) nitrogen weighted for their respective loads. Samples of SPN (n = 30; November 2015–October 2016) were collected under high and low flow conditions to ensure that the riverine isotopic signature estimated was representative of the annual input of riverine nitrogen into Western Port. Only concentrations and isotopes of NO_x and particulate nitrogen were considered when calculating the total nitrogen loads. The fractions of NH_4^+ and dissolved organic nitrogen were ignored, on the basis that these fractions generally comprised <5 and <10% of total nitrogen respectively. Pre-ashed Whatmann 25 mm GF/F paper was used to determine the isotopic signature of the particulate nitrogen. These filters were then dried to a constant weight at 60°C for 48 h, before being analysed using an ANCA GSL2 elemental analyser interfaced to a Hydra 20–22 continuous-flow isotope ratio mass-spectrometer (Sercon Ltd, UK). Stable isotope data for nitrogen were reported in delta notation (Eqn 1) relative to the ^{15}N:^{14}N ratio of air, the precision of the stable isotope analysis was ±0.2‰ (s.d.; n = 5) and the precision of the elemental analysis was ± 0.5 μg (s.d.; n = 5).

$$\delta^{15}N = \left(\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{N_2\text{-Air}}} - 1 \right) \times 1000 \quad (1)$$

On the basis of a loading of ~220 ± 30 Mg N $year^{-1}$ and an isotopic signature of ~12.6 ± 7.0‰ for riverine NO_3^- and a loading of ~390 ± 40 Mg N $year^{-1}$ and an isotopic signature of ~7.3 ± 1.4‰ for particulate nitrogen, it was estimated that the overall isotopic signature of the total riverine nitrogen input into Western Port was ~9.2 ± 2.8‰ (Eqn 2).

$$\delta^{15}N_{\text{Riverine TN}} = \frac{m_{\text{Riverine NO}_x}}{m_{\text{Riverine TN}}} \times \delta^{15}N_{\text{Riverine NO}_x} + \frac{m_{\text{Riverine SPN}}}{m_{\text{Riverine TN}}} \times \delta^{15}N_{\text{Riverine SPN}} \quad (2)$$

Denitrification

In earlier work by Brandes and Devol (2002), the contribution of denitrification to nitrogen isotope and mass balances was separated into sedimentary- and water column-derived denitrification. However, because the concentration of dissolved inorganic nitrogen in the water column was extremely low (Russell *et al.* 2016), and no anoxia has ever been recorded in the well mixed water column, it was assumed that water column-derived denitrification represented an insignificant sink, and was ignored. Prior core-incubation experiments by Russell *et al.* (2016) estimated that the yearly removal of bioavailable nitrogen through denitrification was 228 ± 10 Mg N $year^{-1}$ across the entirety of Western Port. In the present study, the isotopic value arising from sedimentary denitrification was assumed to be 3.5 ± 2.0‰, on the basis of the global estimates of Brandes and Devol (2002).

The globally estimated fractionation factor for sedimentary denitrification used here is similar to those that have been determined on smaller spatial scales, with Alkhatib *et al.* (2012) calculating a fractionation factor of 4.6 ± 2‰ along the Saint Lawrence Estuary in Canada.

Baywide $\delta^{15}N$ values

To determine the average actual isotopic value of sediment nitrogen, against which the model output would be validated, intact sediment samples were collected from sites across Western Port (Fig. 2, Supplementary Table S1) by using Perspex tubes to a depth of ~5 cm. These samples were dried to a constant weight at 60°C for 48 h and then homogenised using a mortar and pestle. Up to 135 mg of the sample was used for analysis of sediment nitrogen content and isotopic analysis, by using the CF-IRMS described above (n = 119; February 2012–November 2016).

Marine isotope end-member

Samples of particulate nitrogen were obtained from Cowes and San Remo during the incoming and outgoing tides (Table 2) by using pre-ashed Whatmann 25 mm GF/F paper (n = 43–47; October 2015–January 2017). These filters were processed and analysed using the CF-IRMS, as described previously.

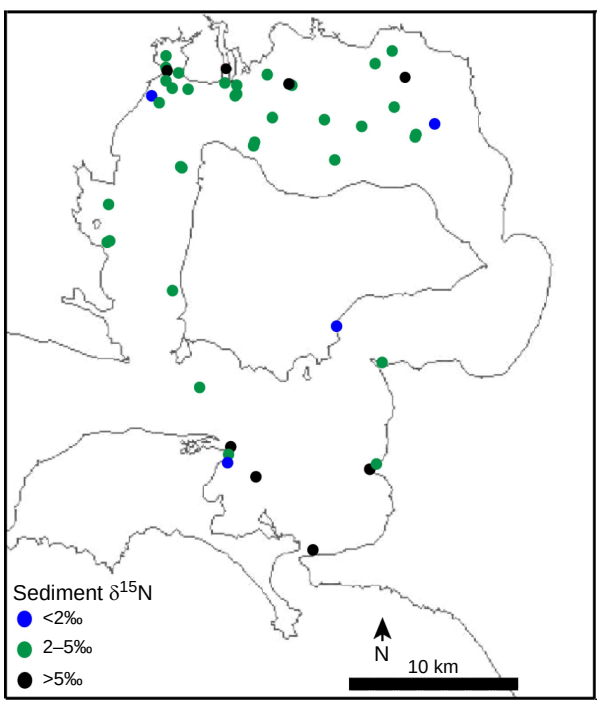

Fig. 2. Location of discrete sediment samples taken from Western Port, Victoria, Australia. The coloured dots represent the isotopic signature of the sediment ($\delta^{15}N$). Note: multiple discrete samples have been taken from some sites; hence, the number of sites does not match the number of discrete samples obtained (n = 119). The full list of discrete samples and corresponding isotope signatures is provided in Supplementary Table S1.

Isotope mass balance to estimate marine import and burial

So as to estimate burial and marine import, the following mass balance was set up:

$$m_{sed} = m_{oce} + m_{sou} - m_{dni} \quad (3)$$

in which $m_{sou} = m_{riv} + m_{fix} + m_{NO_3} + m_{NH_4}$, where m_{sed} is the mass of sediment N created in Western Port, m_{oce} is mass of N from oceanic sources, m_{dni} is mass denitrified, m_{riv} is riverine-derived N (particulate + dissolved), m_{fix} is N fixation, m_{NO_3} and m_{NH_4} are atmospheric deposition of NO_3^- and NH_4^+. It should be noted that m_{sed}, once created, can either be buried or exported from Western Port.

Then, the isotope balance for the sediment in Western Port can be written as follows:

$$\delta^{15}N_{sed} = \frac{m_{oce}\cdot\delta^{15}N_{oce} + \Sigma(m_{sou}\cdot\delta^{15}N_{sou}) - m_{dni}\cdot\epsilon_{dni}}{m_{oce} + m_{sou}} \quad (4)$$

where $\delta^{15}N$ is the average nitrogen isotope signature of each compartment and ε is the fractionation factor for denitrification. This equation can be rearranged so that

$$m_{oce} = \frac{\Sigma(m_{sou}\cdot\delta^{15}N_{sou}) - \Sigma(m_{sou}\cdot\delta^{15}N_{sed}) - m_{dni}\cdot\epsilon_{dni}}{\delta^{15}N_{sed} - \delta^{15}N_{oce}}$$

and m_{sed} can be calculated using Eqn 3.

This model assumes Western Port is well mixed on annual timescales which is reasonable given the macrotidal and well mixed nature of the bay.

To propagate the uncertainty in the known parameters onto estimates of m_{oce} and m_{sed}, we solved Eqn 3 and 4 in R and calculated m_{oce} and m_{sed} for 5000 independent random draws from a truncated mean ± 1 s.d. of the known parameter values. From the resulting 5000 solutions for m_{oce} and m_{sed}, a total of 2324 solutions returned a negative and therefore infeasible m_{oce} flux; these were removed from the solution set. The results for the combined isotope and mass balance are based on the remaining 2676 solutions.

Data analysis

Differences in isotopic and nitrogen composition of particulate material from Cowes and San Remo were tested for significance using two-factor ANOVA in R (ver. 3.2.2, R Foundation for Statistical Computing, Vienna, Austria). The Tukey's HSD *post hoc* test was used in the event that significant responses were returned for the two-factor ANOVA. Data were evaluated graphically by using plots of residuals and boxplots to test for the homogeneity and

normality of variances Quinn and Keough (2002). For all analyses, $P < 0.05$ was chosen as the level of significance for the rejection of the null hypothesis.

Results

Inputs to Western Port were dominated by catchment inputs (612 Mg N year^{-1}), followed by nitrogen fixation (430 Mg N year^{-1}, Table 2). The calculated combined δ^{15}N of the catchment inputs was 9.2‰. Atmospheric deposition was approximately half of nitrogen fixation at 186 Mg N year^{-1}. Both the isotopic signature and the nitrogen content of the particulate nitrogen in the water column showed little variation over the tidal cycle. Samples from Cowes had particulate nitrogen isotopic signatures of between 5.4 and 6.8‰ and a nitrogen concentration of between 50 and 56 μg N L^{-1} (Fig. 2). Whereas at San Remo, the particulate nitrogen isotopic signatures were between 6.4 and 7.0‰, with a nitrogen concentration of between 29 and 33 μg N L^{-1} (Fig. 2). On average, it was determined that the isotopic signature of the particulate material was 6.7 ± 1.1‰ across both sites for all sampling periods (Table 2). There was no significant difference in the nitrogen content or δ^{15}N of particulate nitrogen on the incoming and outgoing tides ($P > 0.05$; two-way ANOVA, with tide and site as factors).

Sediment from various locations around Western Port had an average δ^{15}N of 4.0 ± 1.1‰ (Fig. 3, Table 2). Little variation in the signature was observed around the majority of the bay, with the highest and lowest values being evenly distributed across the north and south of the bay (Supplementary Table S1).

A histogram of the isotope mass balance runs shows that a median oceanic import of 400 Mg N year^{-1} into Western Port is most likely. The isotope mass balance suggests a most likely burial of 1400 Mg N year^{-1} (Table 2, Fig. 4).

Discussion

Numerous budgets have now been made to examine nitrogen sources in coastal embayments and estuaries (Boynton *et al.* 1995; McKee *et al.* 2000; Zeldis and Swaney 2018); however, none has attempted to add additional constraints on these end-members by using nitrogen stable isotopes. This is the first example of a budget of a coastal embayment

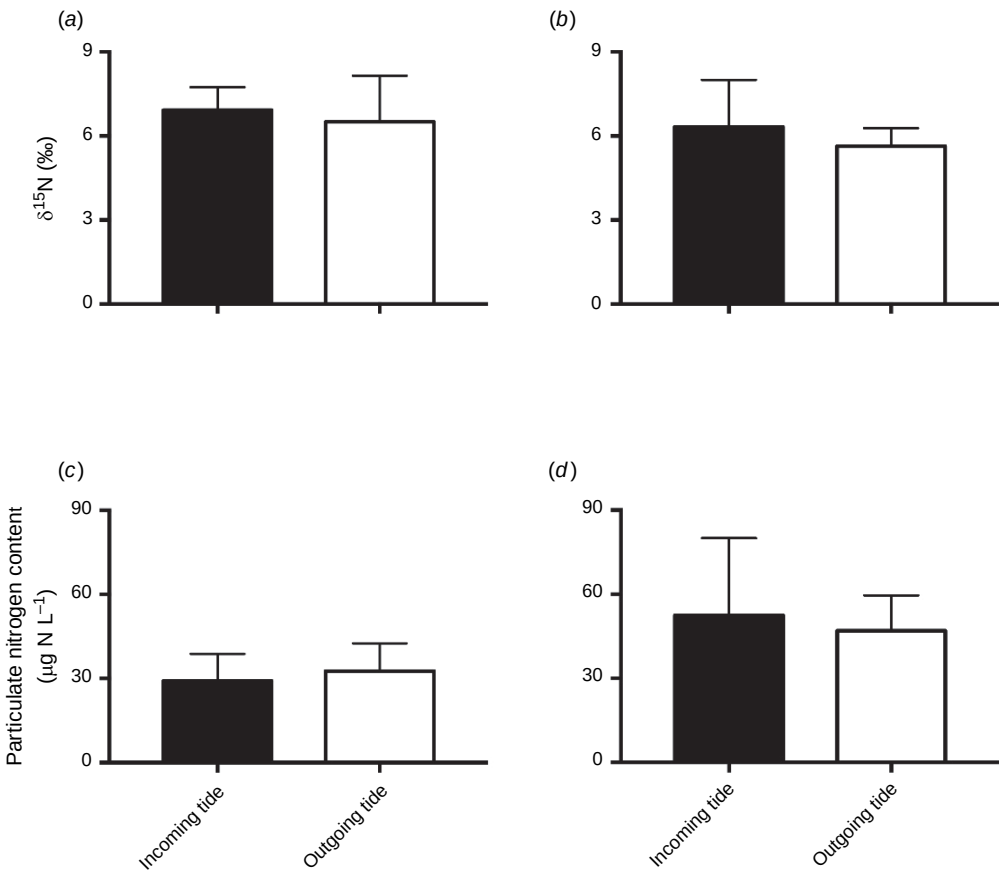

Fig. 3. Comparison of particulate δ^{15}N for the incoming and outgoing tides at (*a*) San Remo and (*b*) Cowes, and the particulate nitrogen concentration for the incoming and outgoing tides at (*c*) San Remo and (*d*) Cowes. All values are mean ± s.d.

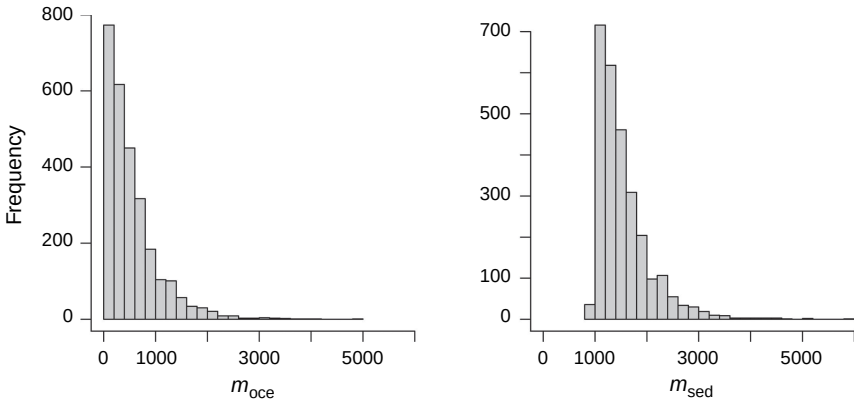

Fig. 4. Frequency of model solutions for different masses of imported marine nitrogen (m_{oce}) and nitrogen burial rates (m_{sed}).

to use $\delta^{15}N$ to constrain different nitrogen sources. Using this approach, we were able to better constrain nitrogen fixation and marine exchange, which are difficult to measure and scale up.

Nitrogen fixation

The mean sediment $\delta^{15}N$ of Western Port was 4.0 ± 1.1‰, which is relatively isotopically depleted compared with catchment (9.2 ± 2.8‰) and marine (6.7 ± 1.1‰) sources, suggesting another major source of nitrogen. Although atmospheric sources are also isotopically depleted ($\delta^{15}N$ = −1.4 ± 2.9‰), this was a minor source of nitrogen and is well constrained. The most likely source of isotopically depleted nitrogen is nitrogen fixation, for which we have previously measured high rates within the system. Using a combined isotope and mass balance approach, we are able to provide strong additional evidence to support our previous conclusions on the importance of nitrogen fixation within Western Port (Russell *et al.* 2016).

In many previous studies, the importance of nitrogen fixation to the total inputs of various marine studies has been found to vary from between 3 and 60% (Huber 1986; Howarth *et al.* 1988; Larsson *et al.* 2001; Radtke *et al.* 2012; Korth *et al.* 2014; Woodland and Cook 2014). The studies that found the highest contribution of nitrogen fixation to the total input of nitrogen typically had cyanobacterial blooms or cyanobacterial mats present. Within Western Port, the contribution of nitrogen fixation to the total input of nitrogen was 28%, placing it in the middle of the range of previous studies; however, they are some of the highest for systems without widespread cyanobacterial blooms and mats. Previous work by Russell *et al.* (2016) has shown that this nitrogen fixation was occurring as a result of both sulfate-reducing bacteria and 'freely associated' cyanobacterial assemblages. We suggest that a key driver of the high rates of nitrogen fixation relative to other inputs of nitrogen are a direct result of relatively oligotrophic conditions that exist within Western Port. It been shown previously that the catchment-derived inputs of nitrogen to Western Port are low on both a local and global scale (Woodland *et al.* 2015), and the marine waters in this location are also highly nutrient limited (Gibbs *et al.* 1986), resulting in nitrogen limitation in seagrass meadows found in Western Port (Russell *et al.* 2016).

Marine and sediment inputs

The isotope mass balance suggested that a median value of 400 Mg N $year^{-1}$ is imported into Western Port from marine sources. The total N inputs to Western Port from the marine end-members can be estimated on the basis of the daily water exchange of 0.8 km^3 (Anon. 2011) and the measured total N concentration of ~50 N μg L^{-1}. This equates a total annual N exchange of 15 000 Mg N $year^{-1}$ and a retention rate of 3%. We did not measure NO_x concentrations in this study; however, the annual average NO_x concentration in Bass Strait adjacent to Western Port is ~14 μg L^{-1} (Gibbs *et al.* 1986), which equates to an exchange of 4000 Mg N $year^{-1}$ and a retention rate of 10%. Therefore, the retention rate of N within Western Port from marine sources is relatively low, which is likely a consequence of the low residence time of water (days) in the south of the system where most of water is exchanged (Anon. 2011).

The veracity of the sediment production term in the budget can be assessed by comparison with previously published sedimentation rates (Hancock *et al.* 2001). These previously reported sedimentation rates range between 2 and 5 mm $year^{-1}$ with one extreme value of up to 1.6 cm $year^{-1}$. If we scale this up to the intertidal area of Western Port of ~214 km^2 (1/3 of 650 km^2), assuming a sediment dry mass of 0.5 kg L^{-1} and a sediment nitrogen content of 0.32% (based on sediment organic C/6.7 reported in Sharp *et al.* 2013), then this gives a nitrogen burial rate of 700–1700 Mg N $year^{-1}$, which is in good agreement with the sediment nitrogen production rate of ~1400 Mg N $year^{-1}$ estimated from the model.

Conclusions

This study has described the development of a combined mass and isotope balance to constrain the sources of

nitrogen to Western Port. The total nitrogen input to Western Port was calculated to be 1400 Mg N $year^{-1}$, which is remarkably consistent with previous estimates of sedimentation rates within the system. Catchment inputs, nitrogen fixation, marine sources and atmospheric deposition comprised 44, 28, 28 and 13% of nitrogen inputs respectively. Denitrification removed ~16% of total nitrogen inputs.

Supplementary material

Supplementary material is available online.

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Data availability. The data used in the mass balance presented here are shown in Table 2 and Supplementary Table S1. The raw data underpinning these data are available from the authors upon request.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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