

# Linking nitrogen sources utilised by seagrass in a temperate marine embayment to patterns of seagrass change during drought

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**ABSTRACT:** Reductions in the extent of seagrass *Zostera nigricaulis* coverage in Port Phillip Bay (PPB), Australia, between 2000 and 2011 coincided with a prolonged period of drought (1997 to 2009) characterized by decreases in freshwater and nutrient inputs. This led us to hypothesize that patterns of seagrass expansion and decline in PPB may be linked to nutrient availability. Seagrasses in PPB can make use of a range of different nitrogen (N) sources depending on their relative availability. Accordingly, there is a need to identify the origin of the N utilised by seagrasses in order to understand how changes in the availability of nutrients from various sources may influence seagrass growth. This study used stable isotope analysis to estimate the contribution of different sources of N to seagrass growth in different parts of PPB. Source modelling indicated that regional patterns of N source utilisation matched changes in seagrass extent from 2000 to 2011. Regions in which seagrass declined contained a similar array of sources, including significant contributions from the catchment area, whereas regions where seagrass areas remained unchanged were largely dependent on a single N source (either fixation/recycled or sewage-derived). We propose that reductions in N from the catchment during the drought may have contributed to the decline of seagrasses in regions where N from the catchment is an important source. This finding is likely to have implications for the growth, distribution and resilience of *Z. nigricaulis* seagrass in PPB as well as in other parts of its range in southern Australia.

**KEY WORDS:** *Zostera nigricaulis* · Climate change · IsoSource model · Seagrass decline · Port Phillip Bay

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## INTRODUCTION

Seagrasses depend on nutrients, particularly nitrogen (N), for growth and survival (Touchette & Burkholder 2000). In coastal environments, seagrasses can obtain N from a variety of sources, including freshwater catchments (Udy & Bunn 2001), sewage (Costanzo et al. 2001) and atmospheric inputs (Welsh

2000), and internal recycling within the seagrass beds themselves (Romero et al. 2006). Seagrasses that depend on N from catchment inputs are likely to be strongly influenced by climate-related variability in freshwater flows. Annual changes in seagrass biomass in tropical northeast Australia have been correlated with river flows (Rasheed & Unsworth 2011), and nutrient limitation has been implicated in regu-

lating seagrass area in the tropics (Fourqurean et al. 1992, Udy et al. 1999). The occurrence of drought is predicted to increase in many parts of the world (Santoso et al. 2013, Cai et al. 2014), and this is likely to have significant impacts on the delivery of freshwater and nutrients to the coast, which will in turn influence the productivity of coastal systems (Justi et al. 2003). Seagrass ecosystems may be highly susceptible to such changes because they are situated in shallow coastal environments and are known to be directly affected by changes in coastal productivity and water quality (Ralph et al. 2006).

Historical trends in the seagrass area of Port Phillip Bay (PPB), southeastern Australia, indicate that nutrient availability may influence seagrass extent (Jenkins et al. 2015). Historical time-series derived from aerial photography show that the area of seagrass coverage at 3 locations in the southern part of PPB increased from 1960 to the mid-1990s, then declined rapidly from the late 1990s onwards (Ball et al. 2014). This decline coincided with a prolonged period of drought in southern Australia (1997 to 2009), characterised by decreases in freshwater and nutrient inputs into the bay (Hirst et al. 2012, Ball et al. 2014). Over a longer historical period, peaks and troughs in seagrass area have corresponded with wetter and drier periods, respectively, in Victoria's climate (see Fig. S1 in Supplement 1 at [www.int-res.com/articles/suppl/m549p079\\_supp.pdf](http://www.int-res.com/articles/suppl/m549p079_supp.pdf)) (Jenkins et al. 2015), which led us to hypothesize that patterns of seagrass expansion and decline in PPB may be linked to nutrient availability. Analysis of historical aerial photography revealed that seagrass area declined along the Bellarine Bank, Mornington Peninsula coast and around Mud Islands between 2000 and 2011 during the drought (Fig. S2 in Supplement 1). By comparison, the extent of seagrass coverage in Swan Bay, Corio Bay, and the western section of the Geelong Arm either remained unchanged or increased during the same period (Ball et al. 2014).

*Zostera nigricaulis* growth in parts of PPB has been shown to be N-limited (Bulthuis et al. 1992). The importance of N to seagrass growth is also likely to vary spatially, reflecting the proximity of seagrass beds to different sources, and the role that hydrodynamic processes plays in dispersing N throughout the water column and via food-webs. Accordingly, there is a need to identify the origin of the N utilised by seagrasses in order to understand how changes in the availability of nutrients from various sources may influence seagrass growth.

This study aimed to estimate the importance of different sources of N to seagrass growth in various

regions of PPB using stable isotopes of N. We focussed on N rather than phosphorus (P), because levels of P in the water column are high relative to those of N; the ratio of dissolved inorganic N to dissolved inorganic P (DIN:DIP) is ~0.4 to 4, and thus P is unlikely to be a limiting nutrient in PPB (Longmore et al. 1996). Stable isotope analysis is a powerful tool, and useful for linking the N found in marine ecosystems to terrestrial or other sources. N originating from a particular source has a signature that is retained and reflected in the ratio of elemental isotopes present (i.e.  $^{15}\text{N}/^{14}\text{N}$  or  $\delta^{15}\text{N}$ ). The contribution of different sources can be inferred by comparing signatures in recipient components to those of the sources (Robinson 2001). Nitrogen is taken up by seagrasses beds in the forms of ammonium, nitrate and  $\text{N}_2$ , each of which may have different isotopic signatures depending on their origins and the transformations that they have undergone prior to uptake. Anthropogenic sources typically have distinctive signatures that can be used to trace the influence of human impacts in aquatic ecosystems (Costanzo et al. 2005, Pitt et al. 2009). We specifically examined whether regions where seagrass extent declined during the drought were more dependent on N from the catchment than other regions within PPB. We explored this question by estimating the relative importance of different N sources for seagrass growth in different parts of PPB.

## MATERIALS AND METHODS

### Study location

Port Phillip Bay (38° 09' S, 144° 52' E) is a large marine embayment located on the southeastern coast of Australia. The bay covers an area of approximately 1930 km<sup>2</sup> with a coastline approximately 264 km in length. The bay is composed of a central, deeper basin adjacent to a shallower western arm (the Geelong Arm). Half of the bay's area is less than 8 m deep, with the deepest point being only 24 m in depth. An extensive region of shallow sand banks (the 'Great Sands') stretches across the entrance to the bay in the south, attenuating both oceanic waves and tides. A smaller, extremely shallow (<2 m depth) bay (Swan Bay) connects to PPB in the southwest.

Sub-tidal, meadow-forming seagrasses in PPB are dominated by a single species: *Zostera nigricaulis* (formerly *Heterozostera tasmanica*; Kuo 2005, Jacobs & Les 2009). This species is widely distributed along the southern coast of Australia, from Western Aus-

tralia to New South Wales, and occurs in semi-exposed locations to depths of 10 m (Womersley 1984). In PPB, *Z. nigricaulis* seagrass meadows occur predominantly in the western and southern regions of the bay in shallow, relatively well-protected waters (Fig. 1). Seagrass distribution in PPB is principally constrained by depth/light and wave exposure.

Over 4 million people live in the catchment areas that drain into PPB; most in the city of Melbourne, situated on the lower reaches of the Yarra River at the northern end of the bay. Over 70% of catchment freshwater inputs emanate from the Yarra River (Harris et al. 1996). The largest source of N into PPB is the discharge of treated sewage effluent from the Western Treatment Plant (WTP) located at Werribee on the northwest coast of PPB (Fig. 1). In the mid-1990s, the WTP was estimated to contribute about 55% of all N inputs into PPB (Harris et al. 1996), although this contribution has declined to about 45% due to improved wastewater treatment (Spooner et al. 2011, Barbee et al. 2015). Other sources of N include inputs from the Yarra River catchment, smaller rivers and creeks, atmospheric inputs (wet and dry deposition of  $\text{NO}_x$  and N-fixation) and seasonal influxes from Bass Strait (Harris et al. 1996). The WTP and catchments contribute over 85% of the total N load entering PPB

(Harris et al. 1996), although the contribution for both these sources declined during the drought (see Fig. S3 in Supplement 1 at [www.int-res.com/articles/suppl/m549p079\\_supp.pdf](http://www.int-res.com/articles/suppl/m549p079_supp.pdf)). From 1997 to 2009, southern Australia experienced the longest and most severe drought on record (Ummenhofer et al. 2009). Freshwater river flows from the Yarra River declined substantially from 1997 onwards, and this coincided with reduced N loads in the bay. N inputs into PPB from the catchment declined by 56% between 1991–1997 and 1998–2009 (Jenkins et al. 2015).

### Contribution of different N sources to seagrass growth

The contribution that different sources of N make to *Z. nigricaulis* growth was examined using  $\delta^{15}\text{N}$  from seagrass tissues collected from across PPB (Fig. 1); leaf and root material were collected from 145 locations in 11 regions where seagrass predominantly occurs (Fig. 1). These tissues may provide different source signals integrated over varying time scales due to differences in turnover rates (Hemminga et al. 1999). Root material is typically longer-lived and may be indicative of nutrient conditions

over longer time periods than the shorter-lived leaf tissue. Samples were collected by snorkel/SCUBA divers or using an anchor (operated from a vessel) between 31 May and 15 July 2011. A smaller subset of sites from 4 regions indicative of different  $\delta^{15}\text{N}$  signals were re-sampled during winter 2012 (5 July to 2 August) and summer 2013 (26 February to 6 March) to determine if there was seasonal variation in these values. The geographical position and depth of each sample was recorded in the field using GPS and an echo sounder.

Seagrass samples were sorted into leaves and rhizomes (including roots), rinsed with distilled water and frozen at  $-20^\circ\text{C}$ . Epiphytic biota was removed from the leaf tissue prior to chemical analysis. Samples were then freeze-dried and ground to powder in a ball mill. Isotopic analysis was performed using an ANCA GSL2 elemental analyser interfaced to a Hydra 20-22 continuous-flow isotope ratio mass spectrometer (Sercon), with a

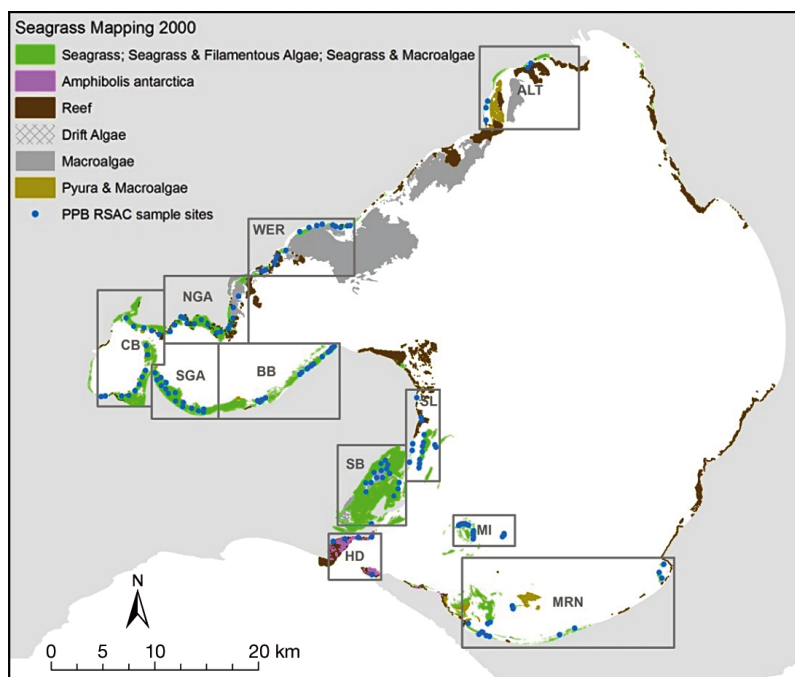


Fig. 1. Port Phillip Bay (PPB), Victoria (Australia), showing seagrass regions and sites sampled in this study. ALT: Altona; WER: Werribee; NGA: northern Geelong Arm; CB: Corio Bay; SGA: southern Geelong Arm; BB: Bellarine Bank; SL: St. Leonards; SB: Swan Bay; HD: PPB Heads; MI: Mud Islands; MRN: Mornington Peninsula

precision of 0.5  $\mu\text{g}$  for N ( $n = 5$ ). Ratios of  $^{15}\text{N}/^{14}\text{N}$  (i.e.  $\delta^{15}\text{N}$ ) are expressed as the relative difference in ‰ notation between the sample and a recognised international standard for N (atmospheric  $\text{N}_2$ :  $R_{\text{Air}} = 0.0036765$ ). The precision for isotopic analysis was 0.55‰.

Differences between regions and tissues were analysed using a split-plot ANOVA (SYSTAT v.12 software) treating seagrass tissues as the 'plot' split into leaf and rhizome tissues for samples collected from each site. Tissue type and region were treated as fixed factors. Assumptions of linearity, normality and homogeneity of variances were assessed through examination of residuals, and variables were normalised using transformations where appropriate. Post hoc multiple comparisons between regions were analysed using Tukey's HSD tests.

IsoSource v.1.3.1 software (Phillips & Gregg 2003) was used to partition source contributions for seagrasses in each of the 11 regions. IsoSource calculated the range of mixtures that could account for the mean  $\delta^{15}\text{N}$  signal in seagrass leaf tissues in each region, based on 4 potential sources: the WTP, Yarra River, Bass Strait, and N-fixation (Table 1). Nitrogen enters the seagrass ecosystems via a range of pathways, but principally as DIN or via sedimentation of particulate organic matter (POM) that contains variable amounts of organic N (Hemminga et al. 1991). POM is likely to be an important source of N in seagrass ecosystems, in part because seagrass canopies act as particle traps, facilitating sedimentation (Koch et al. 2006). A third source, N-fixation, may also be important in some seagrass ecosystems (Welsh 2000). A significant proportion of N entering seagrass ecosystems is subsequently recycled via decomposition of leaf material, which regenerates nutrients, returning them to a soluble form (Mateo & Romero 1997). Nutrients released in dissolved forms can then be re-used by seagrasses or other primary producers,

incorporated by bacteria and then re-mineralized following ingestion by detritivores, or bound to refractory compounds in detritus that can be either exported or stored as organic nutrients in the sediments. The majority of N used by *Z. nigricaulis* in PPB is a mixture of N from the water column (allochthonous in origin) or particulate and fixed N (autochthonous). Sediment is likely to be the dominant source of immediate uptake, as N concentrations in the sediment ( $>10 \mu\text{M l}^{-1}$ ) are typically an order of magnitude greater than that in the water column ( $<1 \mu\text{M l}^{-1}$ ) (Cook et al. 2015).

All possible combinations of each source contribution (0 to 100%) were examined in 1% increments. Combinations that summed to the observed seagrass isotopic ratio within a tolerance of  $\pm 0.1\%$  were considered to be feasible solutions, from which a range of potential source contributions were determined. In isolation, the range of feasible solutions do not provide any information on the likelihood of how 'real' each solution is (Phillips & Gregg 2003). Source modelling assumes that fractionation is low and relatively uniform across the bay (see Cook et al. 2015). In N-limited systems such as PPB, primary producers tend to show little fractionation during DIN uptake and typically reflect the stable isotope composition of the DIN assimilated during growth (Peterson & Fry 1987, Derse et al. 2007).

## RESULTS

### Contribution of different N sources to seagrass growth

#### Regional pattern

$\delta^{15}\text{N}$  values in seagrass tissues displayed high regional variation across PPB, but did not vary between leaf and rhizome tissues and displayed similar within-region patterns (Table 2, Fig. 2).  $\delta^{15}\text{N}$  values for seagrass tissues ranged from  $-4.7\%$  at Swan Bay to  $+19.6\%$  for seagrass collected off Altona. Post hoc tests indicated that values of  $\delta^{15}\text{N}$  in the Altona and Werribee regions were significantly higher ( $p < 0.001$ ) than all other regions and  $\delta^{15}\text{N}$  in Swan Bay was significantly lower ( $p < 0.001$ ) than all other regions.  $\delta^{15}\text{N}$  values for Altona and Werribee regions were high (means = 17.2 and 15.5‰, respectively) and seagrass tissues from Swan Bay (mean = 0.7‰) had very low  $\delta^{15}\text{N}$  values. The remaining regions had  $\delta^{15}\text{N}$  values in the range of 5 to 8‰, and were indistinguishable from one another.

Table 1. Nitrogen stable isotope values ( $\delta^{15}\text{N}$ ) assigned to sources in Port Phillip Bay. WTP: Western Treatment Plant

Source	Mean $\delta^{15}\text{N}$	Source details
WTP	22.8	WTP outfall monitoring for 6 mo in 2010–2011 (Nicholson et al. 2011)
Yarra River	9.7	Measurement in Nov 2011 (P. L. M. Cook unpubl. data)
Bass Strait	5	Global oceanic average (Dahnke & Thamdrup 2013)
N-fixation	0	Owens (1988)

Table 2. Split-plot ANOVA testing for differences  $\delta^{15}\text{N}$  between seagrass tissues and regions

Source	df	MS	F	p
Region	10	180.7	44.258	<0.001
Tissue type	1	2.35	3.649	0.058
Region $\times$ tissue	10	1.067	1.653	0.099
Sample (region)	126			
Tissue $\times$ sample (region)	124			

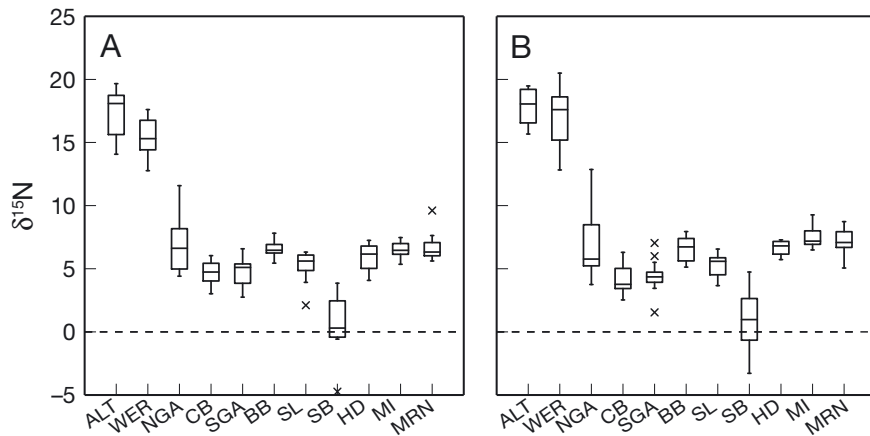


Fig. 2. Nitrogen stable isotope ( $\delta^{15}\text{N}$ ) signals of for (A) leaf and (B) rhizome tissues collected from 11 regions in Port Phillip Bay (region abbreviations are listed in Fig. 1). Horizontal lines are medians, boxes are inter-quartile ranges, and vertical bars are ranges excluding outliers ( $\times$  = outlier)

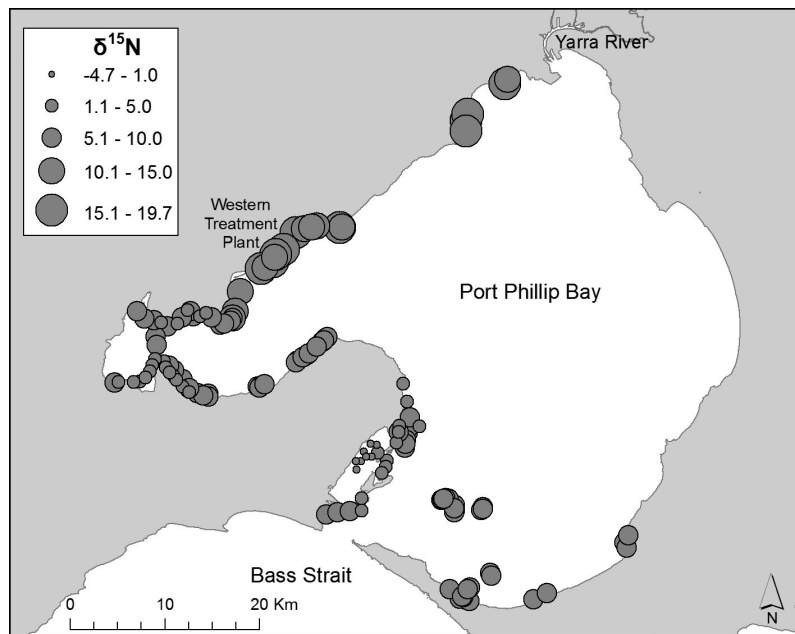


Fig. 3. Nitrogen stable isotope ( $\delta^{15}\text{N}$ ) signals of for leaf tissues collected from 145 locations across Port Phillip Bay

As leaf and rhizome tissues were not significantly different, spatial analysis of  $\delta^{15}\text{N}$  regional patterns was limited to leaf tissues (Fig. 3). High  $\delta^{15}\text{N}$  values occurred along the northwest coast, and there was a clear gradient in values from north to south, with values appreciably lower west of Point Wilson in the northern Geelong Arm region (Fig. 3).  $\delta^{15}\text{N}$  values in Corio Bay and the southern part of the Geelong Arm were low compared to the southern part of PPB, particularly in seagrasses collected from the southeast corner of Corio Bay and in the shallow waters of the southern Geelong Arm, where values ranged from 2.5 to 4.0‰ (Fig. 3). Seagrass tissues in Swan Bay exhibited very low  $\delta^{15}\text{N}$  values. The most depleted tissues (including negative values) occurred in the central part of Swan Bay, whereas seagrasses collected from the entrance to Swan Bay were 2 to 3‰ higher, although still substantially lower than the remainder of the southern part of PPB. Seagrasses in the southern part of PPB (excluding Swan Bay) had values ranging from 5 to 8‰ (Fig. 3).

### Temporal pattern

$\delta^{15}\text{N}$  values were similar in summer and winter for all regions re-sampled, except Werribee (generalized linear model [GLM] region  $\times$  season;  $F_{1,22} = 9.8$ ,  $p < 0.001$ ) (Fig. 4). During summer,  $\delta^{15}\text{N}$  signals for seagrasses sampled from Werribee were higher than winter (Tukey’s HSD;  $p < 0.001$ ) (Fig. 4).

### Source identification using $\delta^{15}\text{N}$ signals

With the exception of seagrasses from Altona, Werribee, and Swan Bay, the IsoSource model indicated that seagrasses utilised N from a range of possible sources (Fig. 5). Because the mixing model analysis was based on a single isotope ratio (i.e.  $\delta^{15}\text{N}$ ) there were many plausible combinations of sources, and this is reflected in the range of possible contributions shown

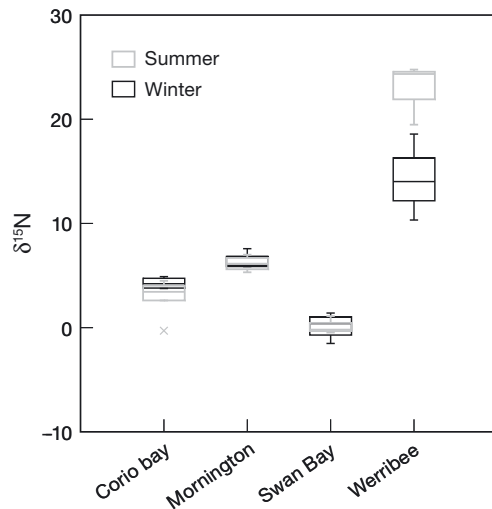


Fig. 4. Nitrogen stable isotope ( $\delta^{15}\text{N}$ ) signals for seagrasses collected from 4 regions in Port Phillip Bay during summer and winter 2012: Corio Bay, Mornington Peninsula, Swan Bay and Werribee. Horizontal lines are medians, boxes are inter-quartile ranges, and vertical bars are ranges excluding outliers ( $\times$  = outlier)

in Fig. 5. The clearest solutions were found for the Swan Bay, Altona and Werribee regions, which exhibited the smallest range of plausible sources (Fig. 5).

The source model suggested that Bass Strait, Yarra River and sediment-derived N were potentially all important sources of N for seagrasses in the northern Geelong Arm, Corio Bay, southern Geelong Arm,

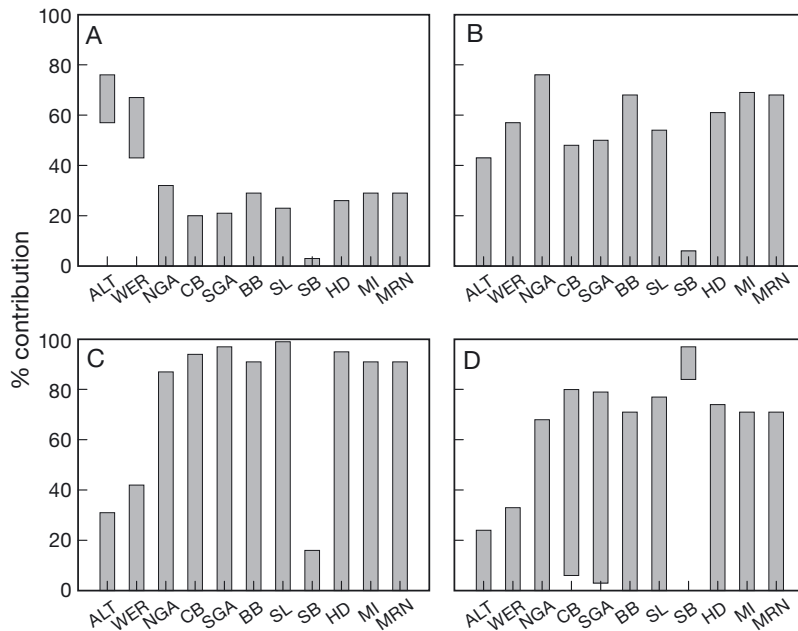


Fig. 5. Range (min.–max.) of plausible contributions of 4 identified N sources into Port Phillip Bay: (A) Western Treatment Plant (WTP), (B) Yarra River, (C) Bass Strait, and (D) sediment N in each region (region abbreviations are listed in Fig. 1)

Bellarine Bank, St. Leonards, Port Phillip Heads, Mud Islands and Mornington regions (Fig. 5). In general, N from the WTP provided the smallest contribution of N utilised by seagrasses in these regions, but was the principal source of N in the Altona and Werribee regions. Seagrass in Swan Bay obtained N from within the sediments, which is ultimately derived from N-fixation (Fig. 5).

## DISCUSSION

### Spatial pattern of $\delta^{15}\text{N}$ signals

At a large spatial scale,  $\delta^{15}\text{N}$  signals displayed a clear spatial pattern reflecting the regional importance of a range of N sources within PPB. The  $\delta^{15}\text{N}$  signals of seagrass tissues fell into 3 broad categories: high (13 to 20‰), low (–1 to 5‰), and background levels typical of most of the bay (5 to 8‰). Seagrass tissues sampled along the northern coast of PPB and adjacent to the WTP exhibited high levels of  $\delta^{15}\text{N}$ . This signal is characteristic of sewage-derived N originating from the WTP (mean  $\delta^{15}\text{N}$  signal = 22.4‰; Nicholson et al. 2011) and is highly enriched in  $^{15}\text{N}$  due to isotopic fractionation that occurs during denitrification and possibly ammonia volatilisation in waste water treatment plants (Cole et al. 2004, Granger et al. 2008). This distinctive signal suggests that the importance of this source for seagrass growth in PPB can be readily traced by measuring  $\delta^{15}\text{N}$  in seagrass tissues. However, its spatial influence appears largely restricted to the north coast, despite the size of this source in terms of total load to the bay. Further west along the coast,  $\delta^{15}\text{N}$  signals return to background levels typical of Corio Bay and the western end of the Geelong Arm. There is also little trace of this signal amongst seagrass tissues sampled directly south of the WTP on the Bellarine Peninsula. Elevated  $\delta^{15}\text{N}$  signals found in seagrass tissues in the Altona region to the north of the WTP may also partly reflect uptake of N from another enriched source, the Altona sewage treatment plant (source  $\delta^{15}\text{N}$  unknown).

Low  $\delta^{15}\text{N}$  signals were characteristic of seagrass tissues sampled from Swan Bay, but also parts of Corio Bay

and the southern Geelong Arm. These protected locations support dense, extensive seagrass beds dominated by organically rich fine sediments. Low  $\delta^{15}\text{N}$  values are typically associated with plants that derive a high proportion of their N from compounds fixed directly from the atmosphere (Owens 1988). The exact cause of the low  $\delta^{15}\text{N}$  is not clear. We suggest the 2 most likely explanations are N-fixation and recycling within the sediment. There is currently a lack of information on the relative importance of these processes in producing isotopically light  $^{15}\text{N}$ . We broadly classify this source of N as being largely autochthonous and within this context we refer to this nitrogen pool as 'sediment N'.

N-fixation by bacteria associated with seagrass roots has been shown to be an important source of N in tropical systems which are characteristically oligotrophic, but is thought to be less important in temperate systems (Welsh 2000). In PPB, direct measurements have indicated that N-fixation is only a minor source of N for seagrasses (i.e. <15% of total N required) and that the  $\delta^{15}\text{N}$  signal of leaf tissues is unrelated to the instantaneous contribution provided by N-fixation (Cook et al. 2015). Even though a small fraction of the instantaneous N demand is met by N-fixation, the ability of seagrass beds to conserve nutrients (Barrón et al. 2006, McGlathery et al. 2007) suggests that over many years there will be an accumulated pool that is recycled within the sediment. The isotopically light signal for Swan Bay (including some negative  $\delta^{15}\text{N}$  values) may be explained by fractionation of N isotopes within the porewater during decomposition and assimilation. Cook et al. (2015) suggested that some fractionation was occurring within sediment porewater based on a consistent observed  $\delta^{15}\text{N}$  offset of 2.4‰ between seagrass and macroalgae collected across PPB; although this offset does not explain the regional differences observed. Agricultural run-off was dismissed as the source of this signal because stream flows into Swan Bay are low and highly intermittent, and daily tidal flushing dominates the nutrient flux to Swan Bay.

The majority of seagrasses surveyed in PPB had  $\delta^{15}\text{N}$  signals ranging from 5 to 8‰. This includes seagrasses from the Mornington Peninsula, Mud Islands, St. Leonards and Bellarine Bank regions. Source modelling indicated that seagrasses in these regions potentially utilised N from a range of sources, including the Yarra River catchment, Bass Strait and through N-fixation. The importance of these N sources for seagrasses in the southern and western parts of PPB is consistent with the way in which N enters PPB and is transported via currents throughout

the bay (O'Leary et al. 1999, Walker 1999). The largest external sources of N into PPB are the WTP and the Yarra River; N inputs from the WTP are principally forced onshore, and north-easterly alongshore, by the prevailing southwest winds that drive the dominant currents in this part of PPB (Lee et al. 2012). Seagrasses in the southern part of PPB are therefore largely geographically removed from the spatial influence of the WTP, and this conclusion is consistent with the absence of a distinctive 'WTP' signal in the tissues of seagrass beyond the north coast of PPB. The shape and extent of the Yarra River plume is highly dependent on river flows and wind speed and direction. During periods of high river flows and westerly winds the Yarra River plume is directed along the eastern shore of PPB, delivering N to the eastern, central and southern regions of the bay (O'Leary et al. 1999). Southern PPB also receives seasonal (autumn-winter) influxes of nitrates from Bass Strait delivered by tidal exchange with the Strait (Harris et al. 1996). Although the overall size of this source is small in comparison to the WTP and catchments (Harris et al. 1996), its relative importance to seagrasses growing adjacent to the entrance to PPB may be high.

### Temporal pattern of $\delta^{15}\text{N}$ signals

Seagrass tissues in PPB were characterised by high spatial variation in  $\delta^{15}\text{N}$  signals, but relatively low temporal variation. Seasonal variation of  $\delta^{15}\text{N}$  signals in *Zostera nigricaulis* tissues was examined for 4 regions having distinctive regional signals: Corio Bay, Mornington, Swan Bay and off the Werribee coastline. With the exception of the Werribee region, regional  $\delta^{15}\text{N}$  signals in leaf tissues did not vary among seasons, suggesting there was negligible seasonal variation in the sources of N utilised by seagrasses in these regions. In the Werribee region,  $\delta^{15}\text{N}$  signals in leaf tissues were higher in summer than winter, reflecting variation in the source signal for treated N originating from the WTP. Leaf and rhizome tissue were expected to integrate N source signals over varying shorter time scales due to differences in turnover rates between leaf and root/rhizome material (Hemminga et al. 1999); however,  $\delta^{15}\text{N}$  signals of leaf and rhizome tissue were similar across all regions. Seagrasses utilise N in the form of ammonium, nitrate and  $\text{N}_2$  taken up through both the roots and leaves (Touchette & Burkholder 2000). The absence of a difference between leaf and rhizome  $\delta^{15}\text{N}$  signals indicates that leaf and rhizome tissues utilise similar N sources, either via direct

uptake from the environment, or via translocation between tissues.

### Relative importance of different N sources in PPB

One limitation of this study was our inability to calculate precise estimates of the contribution of different N sources to seagrass growth in many parts of PPB. This limitation stemmed from an insufficient number of isotopes (only  $\delta^{15}\text{N}$ ) within the mixing model with which to generate a unique attribution of the proportion of each source incorporated within the seagrass leaf tissue (Phillips & Gregg 2003), and insufficient information on the variability of source signatures (Parnell et al. 2010). Despite the shortcomings of this approach, a number of general statements can be made about the relative importance of N sources for seagrass growth in PPB based on the results of the mixing model analysis and the way in which different sources are dispersed within the bay. First, the WTP was the major source of N utilised by seagrasses in the Altona and Werribee regions, but a relatively minor source for all other regions. The importance of this source is therefore largely restricted to the north coast of PPB, where the prevailing local currents limit the dispersal of N to the coastline adjacent to the WTP (Lee et al. 2012). Second, sediment N was the major source of N utilised by seagrasses in Swan Bay and is also likely to be an important source in other protected locations in PPB where sediments are dominated by fine, organically rich sediments. Finally, seagrasses in all other regions obtained N from a range of sources (the catchment, Bass Strait, and sediment N), rather than depending on any single source.

### Evidence of N-limitation

There was no evidence that *Z. nigricaulis* was either N- or P-limited when this study was conducted following the end of the drought in winter 2011 (Fig. S1 in Supplement 1). C:N:P ratios for seagrass leaf tissues displayed little regional variation and were consistent with established Redfield elemental ratios for seagrass (Duarte 1990, Fourqurean et al. 2007) (see Fig. S4 and text in Supplement 2 at [www.int-res.com/articles/suppl/m549p079\\_supp.pdf](http://www.int-res.com/articles/suppl/m549p079_supp.pdf)). Seagrasses in the southern part of the PPB were no more N- or P-limited than seagrasses growing adjacent to the WTP. Unfortunately, this does not represent a rigorous test of the hypothesis that seagrass tissues in some parts of PPB become nutrient-limited during the drought because

this survey was undertaken following the end of the drought in southern Australia from 1997 to 2009, and during one of the wettest years on record (see Fig. S1). Seagrass tissues surveyed during winter 2011 are therefore likely to reflect the greater availability of N from the catchment and other terrestrial sources during this wetter climatic period.

### Link between seagrass decline and N sources

Regional patterns of N source utilisation corresponded to changes in seagrass extent between 2000 and 2011. *Z. nigricaulis* in regions where seagrass cover declined between 2000 and 2011 (including the Bellarine Bank, Mornington coast and Mud Islands) used a similar array of sources. Consequently, a reduction in N inputs from Yarra River during the drought is likely to impact the pool of N available to seagrasses in these regions. Dispersion modelling of nutrient inputs into PPB also indicated these regions occur in areas of relatively low nutrient status (Jenkins et al. 2015). By comparison, *Z. nigricaulis* in regions where the extent of seagrass remained relatively stable between 2000 and 2011 (such as Swan Bay, Corio Bay and the southern Geelong Arm) were largely dependent on a single source, sediment N, and were much less dependent on N originating from the catchment. These regions tend to be isolated from the WTP and Yarra River, both in terms of distance from source and the way in which nutrients are dispersed and circulated within the bay. It is unclear how the dispersal of N from the WTP and Yarra River catchment may have changed during the drought. Limited hydrodynamic modelling suggests that the influence of the WTP plume moved further offshore due to a weakening in the strength of prevailing southwesterly winds during this period (Jenkins et al. 2015). In contrast, the influence of the Yarra River and other catchments may have become more episodic as the number of significant flood (pulse) events declined during the drought.

It is counterintuitive that dependence on a single source may lead to greater stability in terms of seagrass area within PPB. However, this stability will largely depend on which N sources are utilised and how variable these sources are through time. N inputs from the catchment and the WTP declined significantly during the drought (Barbee et al. 2015, Jenkins et al. 2015), but it is less clear how variable the other N sources considered in this study were. It is therefore conceivable that reductions in N from the catchment may have contributed to the decline in



seagrasses at a number of locations in PPB because these locations coincided with regions where N from the Yarra River is likely to be an important source. This is not a definitive test of whether reductions in N from the catchment caused the declines between 2000 and 2011; however, this finding is consistent with the hypothesis and is worthy of further testing.

Southeastern Australia's future climate is expected to become drier on average as a consequence of anthropogenic global warming (Christensen et al. 2013). Projected decreases in rainfall coupled with increased temperature and evaporation rates are expected to reduce run-off across southeastern Australia by 20 to 36% by 2060 (Poloczanska et al. 2007). Increasing intensification of the El Niño–Southern Oscillation in the western Pacific is also expected to result in higher incidence of drought in eastern Australia (Power et al. 2013, Santoso et al. 2013). It is highly likely that, under such a scenario, the relative importance of catchment inputs of N for seagrass growth will diminish as flows from the catchment decline and become more variable over time as southeastern Australia experiences longer and more frequent periods of drought. This is likely to have consequences for the growth, distribution and resilience of *Z. nigricaulis* seagrass habitats in PPB. Experimental enrichment of the sediments by Bulthuis et al. (1992) and Jenkins et al. (2015) indicate seagrasses in the southern region of PPB were N-limited. This is a region directly influenced by pulses of N from the Yarra plume during flood events. It is conceivable that, as part of a drying climate, seagrasses in this region may become further N-limited.

Few studies have examined the link between climatic variability (particularly drought), nutrient availability and changes to seagrass extent. Drought-related declines of *Thalassia testudinum* were linked to increasing salinity in Florida Bay (Zieman et al. 1999), whilst the northern expansion of *Halophila* spp. in the Indian River lagoon, Florida, during drought conditions coincided with increased water clarity and warmer winters (Virnstein & Hall 2009) rather than changes in nutrient availability. The results of this study suggest that climate-driven variation in nutrient availability may also influence seagrass extent, particularly in oligotrophic regions where rainfall patterns are highly variable, and is likely to become more variable under future climate scenarios. This study also suggests that further work should be undertaken to examine the relationships between seagrasses and the sources of nutrients they utilise, and the way in which these relationships may be influenced by future global climate change.

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