

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

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## Supplement 1. Additional figures

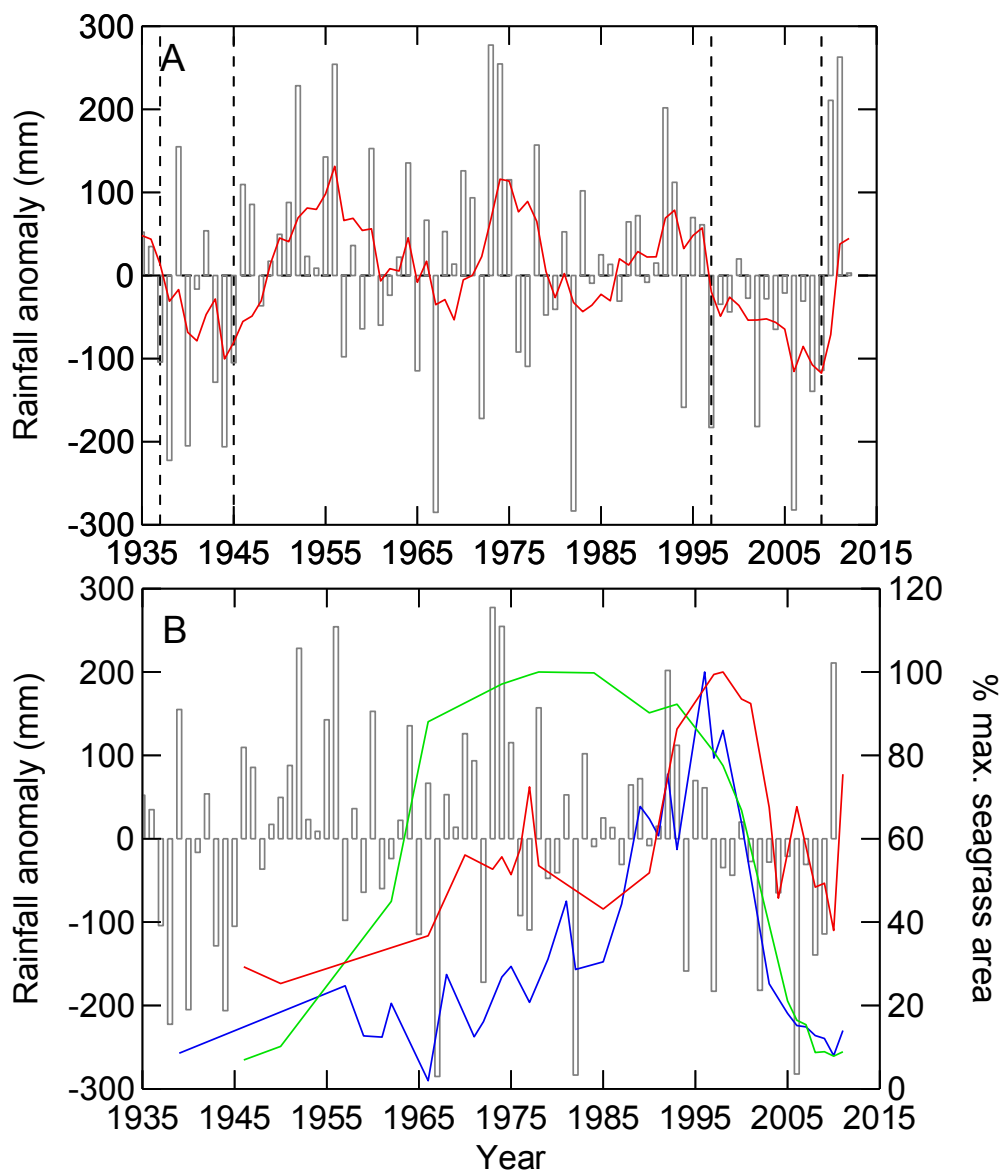


Fig. S1. Relationship between seagrass trends and climate: A) mean annual rainfall anomaly for Victoria 1935–2012 displaying 5-year moving average, and B) changes in % maximum seagrass cover at three locations: Blairgowrie (blue line), St Leonards (red line) and Bellarine Bank (green line). Horizontal lines indicate position of Second World War II (1937–1945) and millennium (1997–2009) droughts in southern Australia. Source: Jenkins et al. (2015)

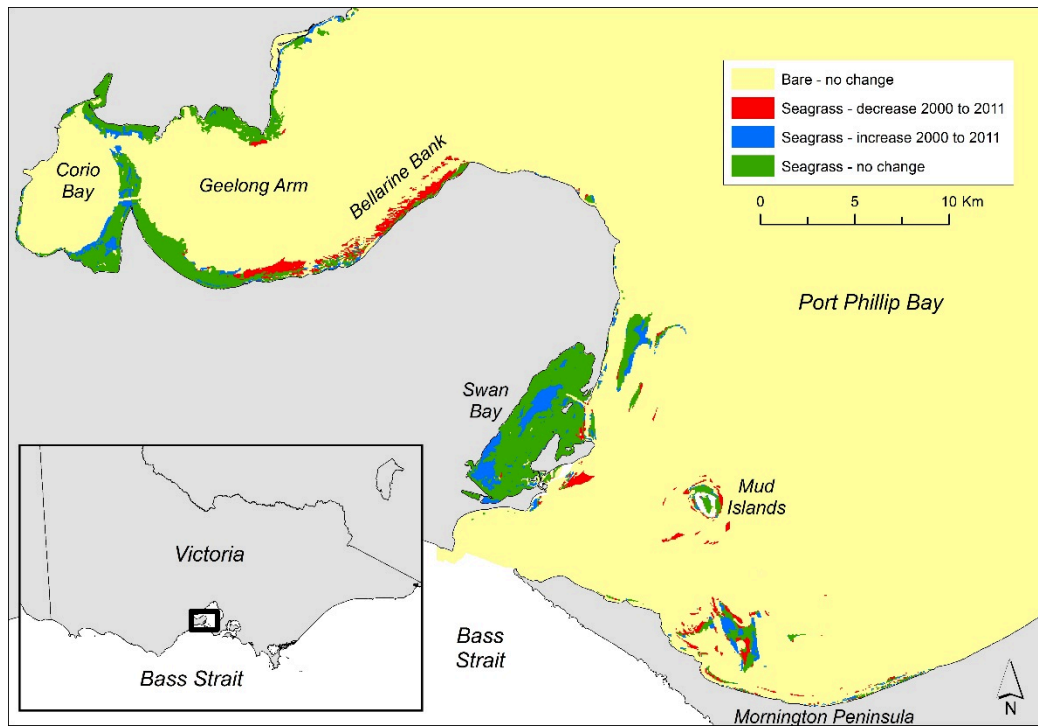


Fig. S2. Change in seagrass area between 2000 and 2011 in southern and western Port Phillip Bay. Based on work undertaken in Ball et al. (2014) and Hirst et al. (2012).

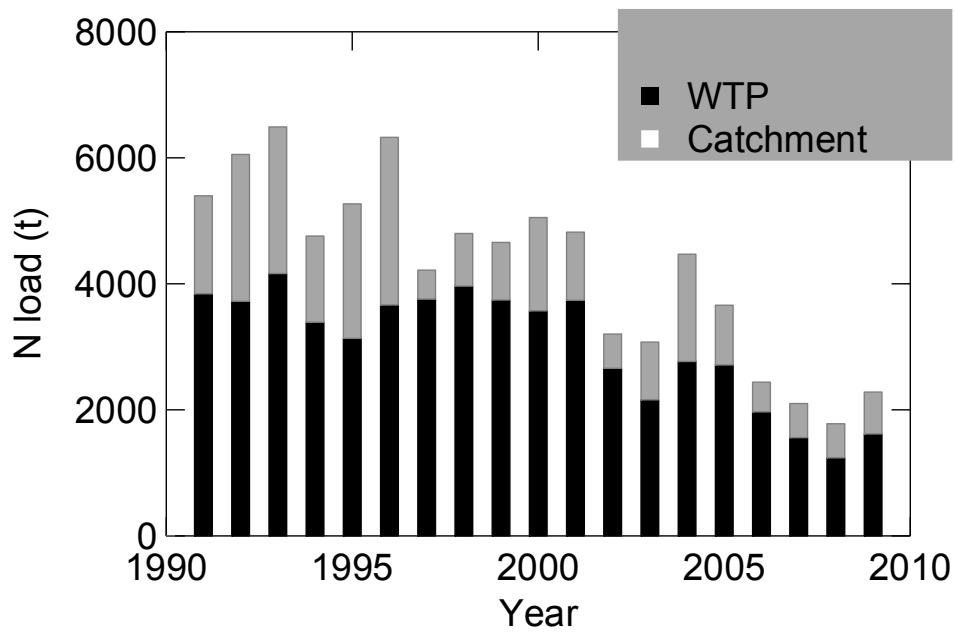


Fig. S3. Annual nitrogen load (tonnes) discharged into Port Phillip Bay from Western Treatment Plant (WTP) and bay's freshwater catchments from 1991–2009.

## Supplement 2. Examination of nutrient status of seagrasses in PPB

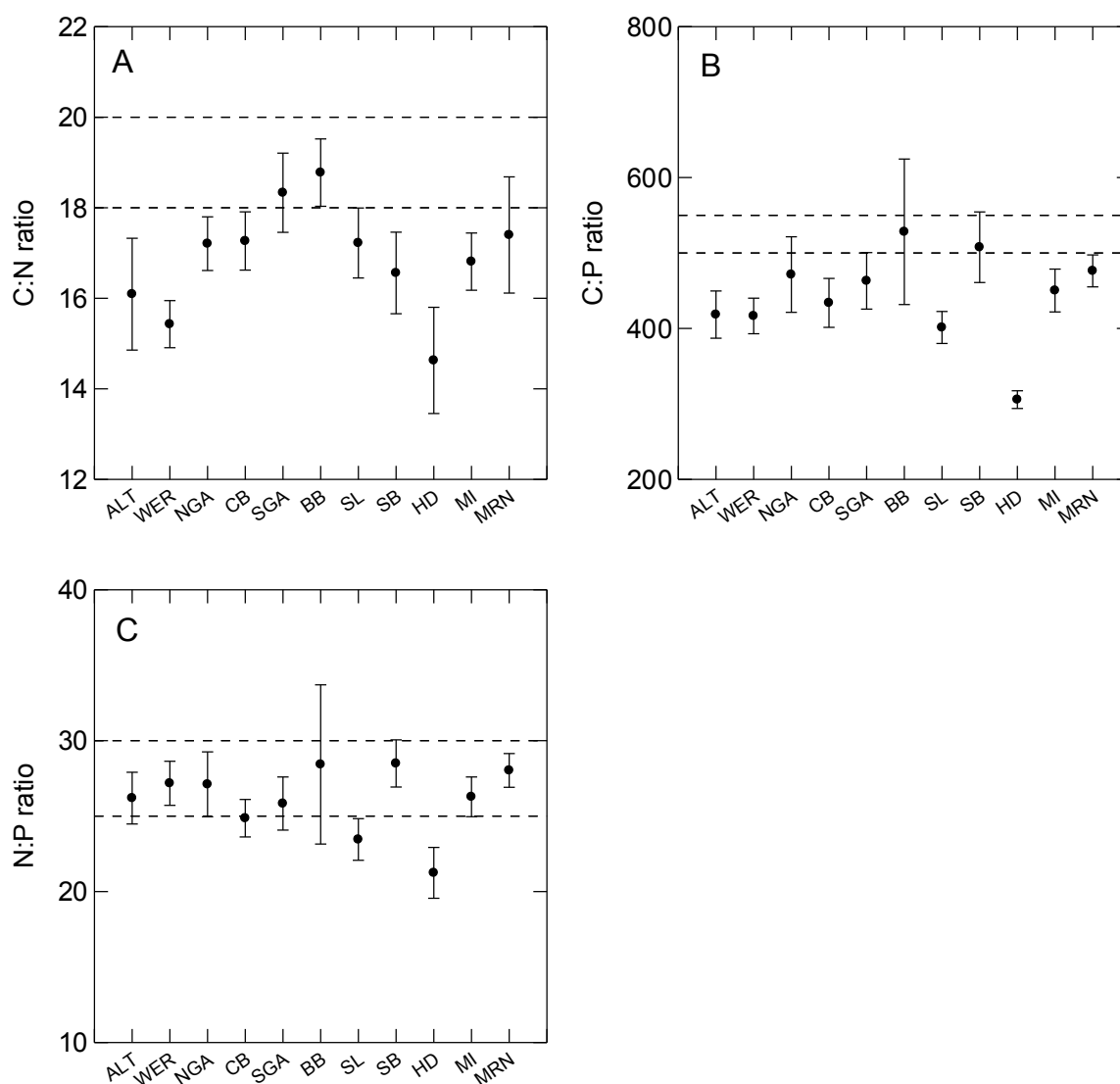


Fig. S4. Mean ( $\pm$  se) for A) C:N, B) C:P and C) N:P elemental ratios for seagrass leaf tissues sampled for 11 regions across PPB. Horizontal limits (dashed lines) display seagrass Redfield Ratio limits derived from Duarte (1990).

Total N and C seagrass content were analysed using an ANCA GSL2 elemental analyser (Sercon Ltd., UK). The precision of the elemental analysis was  $0.5 \mu\text{g}$  for both C and N ( $n = 5$ ). Total phosphorus content was analysed using a modification of the digestion procedure of Nicholls (1975). Weighed samples were evaporated to dryness in the presence of excess sulphuric acid. The organic material was oxidised with hydrogen peroxide, and organic phosphorus converted to orthophosphate. The digestate was neutralised and analysed for orthophosphate concentration by segmented flow analysis, based on the colorimetric method of Murphy and Riley (1962). Instrument performance was monitored by the frequent analysis of blanks and internal standards. Digestion recovery was checked by the analysis of NBS reference material #1571 (orchard leaves) which has a certified P content of  $0.21 \pm 0.01\%$ .

Molar ratios of C:N:P for leaf tissues were derived from the % N, C and P content. Ratios are expressed as the number of units of the most abundant element to one unit of the least abundant element (i.e. C:N, C:P and N:P). Molar ratios of C:N, C:P and N:P atoms were used to explore N and/or P limitation of seagrass tissues in different regions of PPB using the seagrass Redfield Ratio (SRR) (Duarte 1990, C:N:P 474:24:1). Plants that are strongly N or P limited tend to have tissues depleted in N or P relative to carbon content and therefore have high C:N or C:P ratios (Duarte 1990, Fourqurean et al. 2007). Higher N:P ratios tend to be indicative of tissues that are P limited, whereas low N:P ratios are indicative of N limitation (Fourqurean et al. 2007).

#### C:N:P ratios of seagrass tissues in PPB

There is little evidence that seagrass leaf tissues from any of the 11 regions sampled were N-limited using seagrass Redfield Ratios (SRR) as a guide (Supplementary Figure 4). C:N and C:P (Figure 7B) ratios were comparable to, or lower, than the SRR, indicating a high N and P to C ratios. N:P ratios for leaf tissues were consistent with the SRRs and indicated neither N- or P-limitation (Figure 7C). C:N:P ratios were largely uniform across PPB, regardless of the N sources utilised within different regions (Figure 7).