

Nitrogen loads explain primary productivity in estuaries at the ecosystem scale

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Abstract

Increased nutrient loads stimulate estuary primary productivity and can alter the structure and function of biological communities within estuaries, particularly when producer groups respond differently to changes in nutrient availability. Here, the relative influence of riverine inputs of nitrogen and phosphorus were compared to determine their contribution to estuarine primary producers at large spatial scales. Indices of demersal (extent of macroalgae relative to other vegetation, total shallow water area vegetated) and planktonic (seasonally averaged chlorophyll concentration) primary producer communities were derived at whole-of-ecosystem scales in 14 estuaries dispersed across a longitudinal gradient using aerial imaging, underwater videography and in situ monitoring. A model selection framework was used to relate annual nutrient loads (total nitrogen [TN], dissolved inorganic nitrogen [DIN], total phosphorus [TP]), sediment loads (TSS), molar stoichiometric load ratios (TN_M: TP_M), and estuary water residence times to the demersal and planktonic indices. Dissolved inorganic nitrogen was the best predictor of the extent of macroalgae, total vegetation coverage, and the concentration of planktonic chlorophyll. Rapid increases in all three indices occurred at inorganic nitrogen loads of $\sim 5\text{--}10 \text{ Mg km}^{-2} \text{ yr}^{-1}$. There was some evidence that TN_M: TP_M, TP and TSS loads were informative model covariates. Relative to DIN loads, TP loads were a poor predictor of the macroalgal and planktonic indices. These findings underscore the critical role of catchment-derived nitrogen in contributing to producer communities at the whole-of-ecosystem scale and support the growing consensus that nitrogen loads (in addition to phosphorus) must be managed to effectively alleviate eutrophication in estuaries.

The eutrophication of estuaries by human actions is a global environmental problem (Howarth and Marino 2006) and a spectrum of ecological responses to eutrophication have been documented among estuary primary producers world-wide (Valiela et al. 1997; Nixon et al. 2001; Paerl et al. 2014). Seasonally ephemeral blooms of macroalgae and (or) phytoplankton are a consequence of eutrophication in many estuaries (Valiela et al. 1997; Conley et al. 2009) and are associated with extensive ecological and economic costs, such as reduced water quality, degraded seagrass beds, altered aquatic communities, and loss of cultural amenity (Cloern 2001; Nixon et al. 2001; Paerl et al. 2014). Increased nutrient loads, particularly nitrogen (N) and phosphorus (P), induce the growth of opportunistic producers in coastal ecosystems, but the relative importance of these nutrients indi-

vidually is uncertain (Elser et al. 2007). Mesocosm and patch-scale nutrient manipulation experiments suggest that bioavailable N is the primary limiting nutrient in estuaries (Nixon et al. 2001; Elser et al. 2007; Teichberg et al. 2010), but much of the uncertainty of the role of N as the limiting nutrient arises from the difficulties of conducting ecological studies at whole-of-ecosystem scales (Schindler and Hecky 2009). Wastewater treatment and management practices to control the delivery of P to aquatic ecosystems have been implemented since the 1970s (Finlay et al. 2013) and, given the high cost of adopting similar controls for N, clarifying the role of N as a driver of eutrophication in estuaries is critical and remains intensely debated (Conley et al. 2009; Schindler and Hecky 2009; Paerl et al. 2014).

The processes governing the availability of N and P to primary producers in estuarine waters differ in several important ways. External inputs of inorganic N as nitrates and

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nitrites (NO_3^- and NO_2^- ; hereafter, DIN) or ammonium (NH_4) are primarily derived from catchment sources delivered to the estuary by tributaries and from sources adjacent to the estuary through surface runoff and groundwater discharge (Valiela and Bowen 2002). Within the estuary, the bioavailability of DIN loads can be influenced by biogeochemical processes such as denitrification (Seitzinger 1988) and physical processes such as hydraulic loading and tidal flushing. These removal processes reduce the pool of catchment-derived DIN available to estuarine producers. Nitrogen fixation by cyanobacteria, an important source of new N in freshwater ecosystems (Schindler et al. 2008), is typically very low to absent in estuaries with mean salinities > 8 –10 (Howarth and Marino 2006).

Sources of bioavailable P include the catchment-derived and adjacent-to-estuary sources as for N, but also tidally mediated flux of low N : P waters from the coastal zone (Howarth et al. 2011) and the release of iron-bound P from the sediment to the water column arising from the preferential binding of iron to sulfides in estuarine sediments (Caraco et al. 1989). Denitrification in coastal waters can lead to coastal waters with low N : P nutrient ratios, and entrainment of this coastal water into estuaries during flood tide can provide an alternative source of P to estuarine producers in systems receiving relatively high catchment N loads (e.g., Hayn et al. 2014). In addition, the breakdown of organic matter in the sediment and changes in water-column oxygenation can lead to seasonal releases of P from the sediment. The annual incoming load of catchment-derived P can therefore be augmented by “new” P from adjacent coastal waters and “legacy” P recycled across the sediment–water interface during peak growth periods of primary producers in estuaries. These processes can alter the stoichiometry of bioavailable nutrients and can foster N-limiting conditions in many estuaries (Jensen et al. 1995; Cook et al. 2010; Hayn et al. 2014). Conversely, particulate-associated riverine P can be lost from the water column due to sedimentation, reducing the bioavailability of the riverine P load within the estuary.

We explore the relative importance of catchment derived N, P and N : P stoichiometry as drivers of estuarine productivity by analyzing ecosystem-scale patterns in submerged vegetation and pelagic chlorophyll over a spatial gradient of estuaries along a nutrient loading trajectory (Pickett 1989; Blois et al. 2013). Based on the cumulative evidence from small-scale experimental manipulations and synthetic reviews of multiple single system studies (e.g., Nixon et al. 2001; Hauxwell and Valiela 2004; Howarth and Marino 2006), we predicted that macroalgae coverage, total vegetation coverage and phytoplankton would increase with increasing N loads at the whole-of-ecosystem scale.

Materials and methods

Selection of study systems

Fourteen estuaries across the Australian state of Victoria (Fig. 1a; Table 1) were selected for habitat mapping based on

the consideration of riverine nutrient loading regimes (described in further detail below), general catchment land use characteristics, and estuary geomorphology. Representative estuaries were selected based on previous studies (e.g., Valiela et al. 1997; Nixon et al. 2001; Teichberg et al. 2010) that identified measurable changes in primary producer conditions associated with nutrient conditions. Catchment land use data were obtained from the National Environmental Stream Attributes database (Stein et al. 2014; v1.1.5, Geoscience Australia website: www.ga.gov.au) and the proportional area of the river catchments allocated to land uses that receive or generate fertilizers (e.g., residential areas, grazing pasture, horticulture) was used as an indicator of the likely intensity of catchment nutrient inputs. Estuary selection also included considerations of total area and estuary geomorphology to avoid potential scaling-effects arising from large-scale differences in hydrological conditions among estuaries.

River flow (ML d^{-1}) from gauging stations and nutrient and total suspended solids (TSS) concentration data (mg L^{-1}) for each river system over the 13-yr interval from 2000 to 2012 were obtained by downloading archived data from the Department of Environment, Land, Water and Planning Water Measurement Information System website (data.water.vic.gov.au/monitoring.htm) or provided by Melbourne Water (melbournewater.com.au). We focused our analysis on total nitrogen (TN), oxidized dissolved forms of nitrogen (NO_3^- and NO_2^- , hereafter simply DIN), and total phosphorus (TP). Gauge and nutrient concentration sampling sites were selected to be as close to the head of the estuary as possible. River flow was measured daily; whereas, nutrient and TSS sampling intervals ranged from approximately biweekly ($n = 23$) to quarterly ($n = 3$ –4) with an average of $n = 12$ samples per river system per year (i.e., monthly sampling). Data were assigned to a 01 June–31 May hydrologic year rather than a calendar year to reflect the annual flow–nutrient cycle responsible for primary production dynamics in Victorian estuaries during the austral summer (Cook and Holland 2012). Annual loads (Mg yr^{-1}) of TN, DIN, TP and TSS were estimated from measured river flow and concentration data using a flow-stratified Kendall Ratio (Kendall et al. 1983) approach within a Monte-Carlo simulation-based spreadsheet routine (Tan et al. 2005). Annual loads for all variables were divided by estuary area to yield a standardized load of tonnes per unit area of estuary per year ($\text{Mg km}^{-2} \text{ yr}^{-1}$).

Field methods: habitat mapping and chlorophyll monitoring

Estuary vegetation mapping was conducted on each of the 14 study systems. Each estuary was overflown during the 2012 austral summer (January–February) and high resolution aerial photographs (24 megapixel) were collected along flight paths at an average altitude of 450 m and rate of 0.2 images s^{-1} . Image libraries from each estuary were examined and a

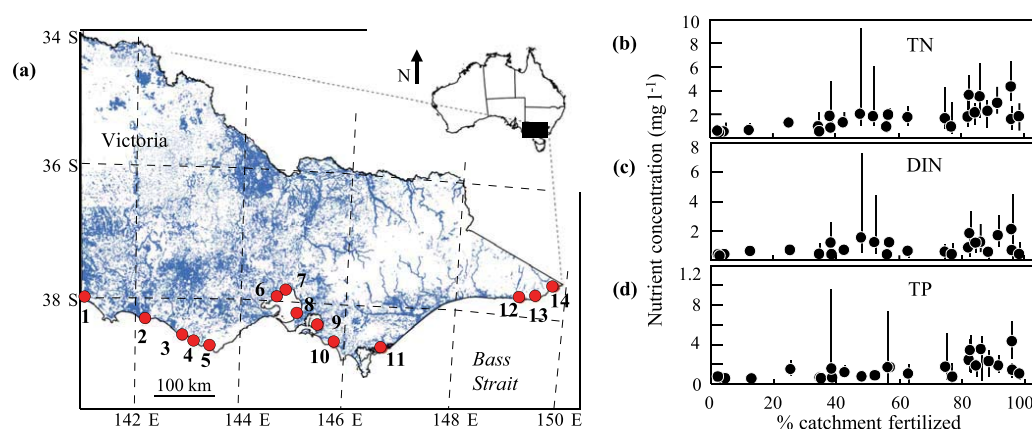


Fig. 1. Map showing estuaries ($n = 14$) selected for whole-of-ecosystem vegetation mapping in Australian state of Victoria (a). Mean riverine nutrient concentrations (mg L^{-1} ; error bars = min–max observed) of total nitrogen (TN, b), dissolved inorganic nitrogen oxides ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^-$, c), and total phosphorus (TP, d) from 2000 to 2012 shown for each estuary. Estuary numbers in (a) correspond to estuary identifications and properties in Table 1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

subset of “best” images, those that provided maximum underwater penetration depth and lowest reflective sun glare and wind disturbance, were selected. Selected images were stitched to create a smooth composite mosaic of each estuary, then rubbersheeted to the Victoria hydrographic reference layer (Geoscience Australia National Geoscience GIS dataset: www.ga.gov.au/nationaldatasets/gis/) using the ArcGIS (v.10.1, ESRI) software package. Upstream and downstream estuary boundaries were set using an established map of Victorian estuary extents based on geomorphological and physicochemical measurements (GIS polygon shapefile: Deakin University, Warrnambool, Victoria, Australia). Composite aerial images of estuaries were trimmed to include only water surface area within the extent of the estuary boundaries.

Field-based habitat surveys were conducted on $n = 8$ estuaries during the austral summer of 2011–2012 and on $n = 6$ estuaries during the summer of 2012–2013. Discrete drops of a georeferenced digital underwater video camera were used to map bottom cover in a generalized grid design in each estuary. Grid cell size varied depending on surface area of the estuary and heterogeneity of the bottom cover, but average edge distance between grid vertices was c. 20–100 m. During drops, the camera was lowered to the bottom, then slowly raised and lowered for 30 s within a range of 0.2–1 m to provide information on habitat type and heterogeneity within the field of vision. The camera and an LED diving light were affixed to a 3 m telescoping pole to allow fine vertical control and increased illumination at depth. Grab samples of vegetation were collected to provide additional verification of video classifications as well as physical specimens for taxonomic identification.

Video data were reviewed in the laboratory and bottom cover at each drop site was assigned to one or more of the following four primary habitat types: seagrass, macroalgae, bare sediment/unvegetated rocky reef, or channel habitat (>

2 m depth). Seagrass and macroalgae habitats were further classified as having sparse–medium (< 50%) or dense (50–100%) vegetation coverage. In the case of seagrass habitats with conspicuous epiphytic or intermingled macroalgae, the site was assigned to both habitat categories and each category was assigned a density classification. Spatial mapping was carried out in ArcGIS by constructing habitat raster maps (cell size = 2 m²) based on visual reconciliation of site-specific habitat classifications and photographic information from composite aerial images. Vegetated habitat areas were weighted by coverage classifications such that map cells assigned sparse–medium coverage were considered to contain 50% vegetation and dense coverage = 100% vegetation. For example, a 10 m² patch of medium seagrass was designated as having 5 m² of seagrass habitat and 5 m² of bare sediment. Total areas of each estuary and each coverage-weighted habitat class were calculated and exported for further analysis.

Surface (c. 0.2–0.5 m depth) chlorophyll concentrations ($\mu\text{g L}^{-1}$) were monitored on two successive outgoing tidal cycles on three separate occasions in a subset of $n = 8$ estuaries. Sampling occurred once for each estuary during the spring (September–October), early summer (November–December) and late summer (January–February) of 2011–2012. Chlorophyll measurements were taken adjacent to the main channel of the estuary with a calibrated Hydrolab water quality sonde (model DSX5). Concentration values were averaged for each occasion ($n = 3$ –16 observations) and across each of the three seasons to yield an integrated mean chlorophyll concentration in the surface waters of each estuary.

Methods: modeling approach

Nonlinear maximum-likelihood model fitting and an information theoretic model selection criterion (Akaike's

Table 1. Physical (area), catchment (Fert, % catchment land uses involving fertilizer application or generation), hydrological (T_f = estuary water residence time, d), loading (annual areal load: total nitrogen, TN; dissolved inorganic nitrogen oxides, DIN = $\text{NO}_2^- + \text{NO}_3^-$; total phosphorus, TP, total suspended solids, TSS, molar ratio of TN to TP, $\text{TN}_M : \text{TP}_M$), demersal vegetation (MA: TV = areal coverage of macroalgae/areal coverage of total vegetation; TA: TV = total vegetation coverage/total estuary area ≤ 2 m), and mean surface chlorophyll concentration (CHL, $\mu\text{g L}^{-1} \pm 1$ SE) conditions in 14 estuaries during 2012–2013 in South Eastern Australia. Map identification (ID) numbers correspond to sites in Fig. 1.

Estuary	Area (m^2)	Fert	T_f	Annual loading ($\text{Mg km}^{-2} \text{yr}^{-1}$)				$\text{TN}_M : \text{TP}_M$	MA : TV	TA : TV	CHL ($\mu\text{g L}^{-1}$)	Map ID
				TN	DIN	TP	TSS					
Glenelg River	5.11×10^6	63.0	1.2	145.2	54	5.9	2,475.6	54.4	0.93	0.45		1
Moyne River	1.50×10^6	98.4	1.5	75.4	31.9	2.7	271.4	61.6	0.83	0.70	6.1 ± 3.1	2
Curdies Inlet	3.38×10^6	86.1	2.0	59.5	17.0	5.5	942.8	23.8	0.93	0.97	5.9 ± 1.5	3
Gellibrand River	2.93×10^5	25.5	0.6	1,142.3	459.4	79.9	13,617.5	31.6	0.71	0.17		4
Aire River	8.60×10^5	12.8	2.4	15.9	11.7	0.6	211.5	61.1	0.20	0.17	4.5 ± 1.6	5
Werribee River	4.21×10^5	56.4	1.9	120.2	37.4	12.8	1,279.3	20.9	0.89	0.79	5.9 ± 1.0	6
Kororoit Creek	6.38×10^4	82.3	1.6	259.6	127.8	21.9	3,069.3	26.3	0.77	0.48	5.9 ± 2.7	7
Patterson River	7.50×10^5	57.1	1.4	93.6	26.0	8.4	1,114.5	24.7	0.75	0.32		8
Bass River	2.08×10^5	91.7	1.1	942.4	377.4	84	8,111.9	24.8	0.64	0.29	9.5 ± 3.5	9
Anderson Inlet	1.26×10^7	84.6	3.0	61.3	31.5	4.8	1,166.1	28.5	0.90	0.13		10
Tarra River	1.24×10^6	38.5	2.9	54.5	34.2	2.2	987.3	54.2	0.58	0.78	11.9 ± 6.8	11
Tamboon Inlet	8.06×10^6	2.0	4.6	3.0	1.0	0.1	1,019.6	48.1	0.04	0.01		12
Wingan Inlet	1.36×10^6	0.5	2.1	53.8	2.0	2.8	1,575.8	43.1	<0.01	0.01	1.9 ± 0.3	13
Mallacoota Inlet	3.31×10^7	4.7	4.2	1.8	0.3	0.1	17.5	43.6	0.02	0.67		14

information criterion corrected for small sample size, AICc) were used to analyze the response of the estuarine vegetation community to nutrient loads. Predictor variables in models included annual loads of TN, DIN, TP, and TSS, the stoichiometric ratio of molar TN: TP loads ($\text{TN}_M : \text{TP}_M$), and estuary water residence time. Estuary water residence time (T_f) was calculated using the tidal prism method. Tidal prism is calculated as the sum of the volume of freshwater flow entering the estuary over half a tidal cycle (m^3) and the volume of ocean water delivered to the estuary on the flood tide (m^3). The tidal prism approach assumes complete tidal exchange and a well-mixed estuary at high tide (Sheldon and Alber 2006). All loading variables were strongly intercorrelated (Pearson's correlation coefficient $r \geq 0.92$, Table 2); however, these high r values were primarily attributable to two influential estuaries (Bass River and Gellibrand River, Table 1) with high nutrient and TSS annual loads.

We developed two demersal vegetation response metrics based on aspects of vegetation type and spatial coverage in each estuary. These were the ratio of macroalgae coverage (m^2) to the total vegetated area (m^2) within an estuary (MA: TV), and the ratio of total vegetation coverage (m^2) to the total area (m^2) of shallow habitat < 2 m depth within an estuary (TV: TA). The MA: TV index is a measure of the relative dominance of seasonal macroalgae during the summer in each estuary and, as such, is an indicator of a well-established nutrient load response in estuaries (Valiela et al.

1997). The TA: TV index is a measure of total productivity summed across ephemeral macroalgae and interannual sea-grass taxa. Both indices are dimensionless and are standardized for differences in total area, potential growth area (i.e., depth $< c. 2$ m), and (for the MA: TV index) total productivity among estuaries. We used mean chlorophyll concentration as the response of pelagic primary producers (phytoplankton) to nutrient loading regimes.

The response of the MA: TV and TV: TA indices to local nutrient, TSS and flushing regimes was analyzed using an additive model structure:

$$\begin{aligned} \text{Response} &= f_i(\text{asymptotic term}) + f_j(\text{covariate}) \\ f_i &= \alpha_n (1 - \exp^{-\beta_n x_n^n}) \\ f_j &= \phi_k(l_k) \end{aligned}$$

where x_n represents one of the three annual nutrient loads (TN_{load} , DIN_{load} , TP_{load}); l_k represents one of the five potential covariate terms (TN_{load} , DIN_{load} , TP_{load} , TSS_{load} , $\text{TN}_{\text{mol}} : \text{TP}_{\text{mol}}$, T_f), and α_n , β_n , and ϕ are shape parameters solved by dual quasi-Newton optimization. A subset of 16 plausible univariate and bivariate models was defined prior to the model selection analysis to reduce the likelihood of obtaining spurious results. Each model variant included one nonlinear term (f_i) and up to one linear covariate (f_j). Covariate identity was not allowed to duplicate the identity of the nutrient in the nonlinear model term. This model structure

Table 2. Matrix of Pearson's correlation coefficient (r) values among: total nitrogen (TN), dissolved inorganic oxides of nitrogen ($\text{DIN} = \text{NO}_2^- + \text{NO}_3^-$), total phosphorus (TP), and total suspended solids (TSS) annual areal load ($\text{Mg km}^{-2} \text{yr}^{-1}$); molar stoichiometric ratio of TN to TP annual areal load ($\text{TN}_M : \text{TP}_M$), and water residence time (d, T_f) for $n = 14$ estuaries during 2012–2013 in South Eastern Australia. Parenthetical values = interannual r values based on annual loads from 2000 to 2012.

	DIN	TN	TP	TSS	$\text{TN}_M : \text{TP}_M$
TN	0.99 (0.97)				
TP	0.98 (0.78)	0.98 (0.87)			
TSS	0.95 (0.71)	0.96 (0.76)	0.92 (0.72)		
$\text{TN}_M : \text{TP}_M$	−0.35	−0.36	−0.43	−0.34	
T_f	−0.58	−0.61	−0.58	−0.60	0.28

allowed four functional responses of the vegetation indices: a positive asymptotic response to TN_{load} , DIN_{load} or TP_{load} ; and linear positive or negative responses (or no response) to the covariates TN_{load} , DIN_{load} , TP_{load} , TSS_{load} , $\text{TN}_M : \text{TP}_M$ and residence time. The response of phytoplankton was also analyzed as a nonlinear curve using the same asymptotic model. Due to the lower sample size of the phytoplankton dataset, covariates were not included in the set of plausible models; therefore, only three nonlinear model variants (one each for TN_{load} , DIN_{load} , TP_{load}) were considered for the phytoplankton.

Model weights calculated from AIC values corrected for small sample size (AIC_c) were used to identify optimal model variants. Summed variable weights (w_{Var}) were treated as variable probabilities for the phytoplankton model results (Link and Barker 2006); however, odds ratios were first used to adjust for unequal prior probabilities of variables in the demersal vegetation models. Unequal variable occurrence in the subset of plausible models implicitly alters the probability of observing a significant result for each variable. This can be seen by calculating the prior probabilities for each of the variables included the subset of models. Correcting variable probabilities (= odds ratio/[1 + odds ratio]) reduces the likelihood of identifying more evidence for variables that occur in more model variants. Modeling was conducted in SAS (v 9.3).

Results

Mean nutrient concentrations from riverine monitoring stations directly upstream of estuaries ranged from 0.31 mg L^{-1} to 3.52 mg L^{-1} TN, 0.03 – 1.54 mg L^{-1} DIN, and 0.02 – 0.33 mg L^{-1} TP (Fig. 1b–d). The upstream catchments of these estuaries differ markedly in the prevalence of land uses that involve direct (e.g., horticultural application) or indirect (e.g., intensive animal farming) additions of fertilizer, ranging from 0.5% to 98.4% of total catchment surface area. Mean annual nutrient concentrations were positively correlated with the percent of catchment area receiving fertilizer applications (all pairwise Pearson's $r \geq 0.67$, $p < 0.05$; Fig. 1b–d). During the 2012–2013 austral hydrological year, annual loads within these estuaries spanned 1–2 orders of magnitude, ranging 1.8 – $1142 \text{ Mg km}^{-2} \text{yr}^{-1}$ for TN_{load} ,

0.3 – $459 \text{ Mg km}^{-2} \text{yr}^{-1}$ for DIN_{load} , and 0.1 – $84 \text{ Mg km}^{-2} \text{yr}^{-1}$ for TP_{load} , (Fig. 2).

Maps of subsurface vegetation cover derived from overflights and underwater videography showed that nearly all estuaries supported both seagrass and macroalgal taxa during summer. Seagrass beds covered 1–33% of shallow estuary areas ($\leq 2 \text{ m}$ depth) and were primarily composed of *Zostera* spp. (includes *Z. muelleri* and *Z. nigracaulis*) or *Ruppia* spp. Macroalgal communities included several genera (e.g., *Ulva*, *Enteromorpha*, *Hypnea*, *Gracilaria*) closely associated with estuarine eutrophication (Valiela et al. 1997). Macroalgae coverage ranged 0–93% of shallow estuary areas and was present in all but one estuary. Unvegetated shallow areas were more common in estuaries with lower macroalgae coverage, ranging from 78% in estuaries with $< 10\%$ macroalgae habitat to 47% in estuaries with $> 10\%$ macroalgae habitat. Values of the MA: TV index ranged from 0 to 0.93 and, similar to in-river nutrient concentrations, were positively related to the extent of fertilizing land uses within river catchments (Pearson's $r = 0.85$, $p < 0.001$). The TV: TA index ranged 0.01–0.97 and also correlated positively with the extent of fertilizing land uses in upstream river catchments ($r = 0.59$, $p = 0.03$). There was no relationship, linear or otherwise, between relative seagrass coverage (i.e., area of seagrass coverage divided by area of total shallow water habitat) and TN_{load} (Pearson's $r = -0.10 \pm 0.14$ [SE]; Spearman rank correlation coefficient $r_s = -0.03 \pm 0.29$), DIN_{load} ($r = -0.07 \pm 0.16$, $r_s = 0.14 \pm 0.28$), or TP_{load} ($r = -0.10 \pm 0.16$, $r_s = -0.16 \pm 0.32$).

Chlorophyll concentrations during ebb tide cycles were highly variable among estuaries and across sampling occasions. Across estuaries, mean chlorophyll concentrations ranged from a minimum of $1.9 \pm 0.3 \mu\text{g L}^{-1}$ (SE) to a maximum of $11.9 \pm 6.6 \mu\text{g L}^{-1}$, with mean concentrations among the remaining six estuaries falling between $4.5 (\pm 1.6)$ and $9.5 (\pm 3.4) \mu\text{g L}^{-1}$. Temporal variability was also apparent—within estuary differences between subsequent sampling occasions averaged $5.5 \mu\text{g L}^{-1}$ and ranged 0.7 – $16.6 \mu\text{g L}^{-1}$. There was not a consistent temporal pattern in relative chlorophyll concentration changes among estuaries.

Modeling results based on the response of the demersal vegetation indices revealed statistical support for several

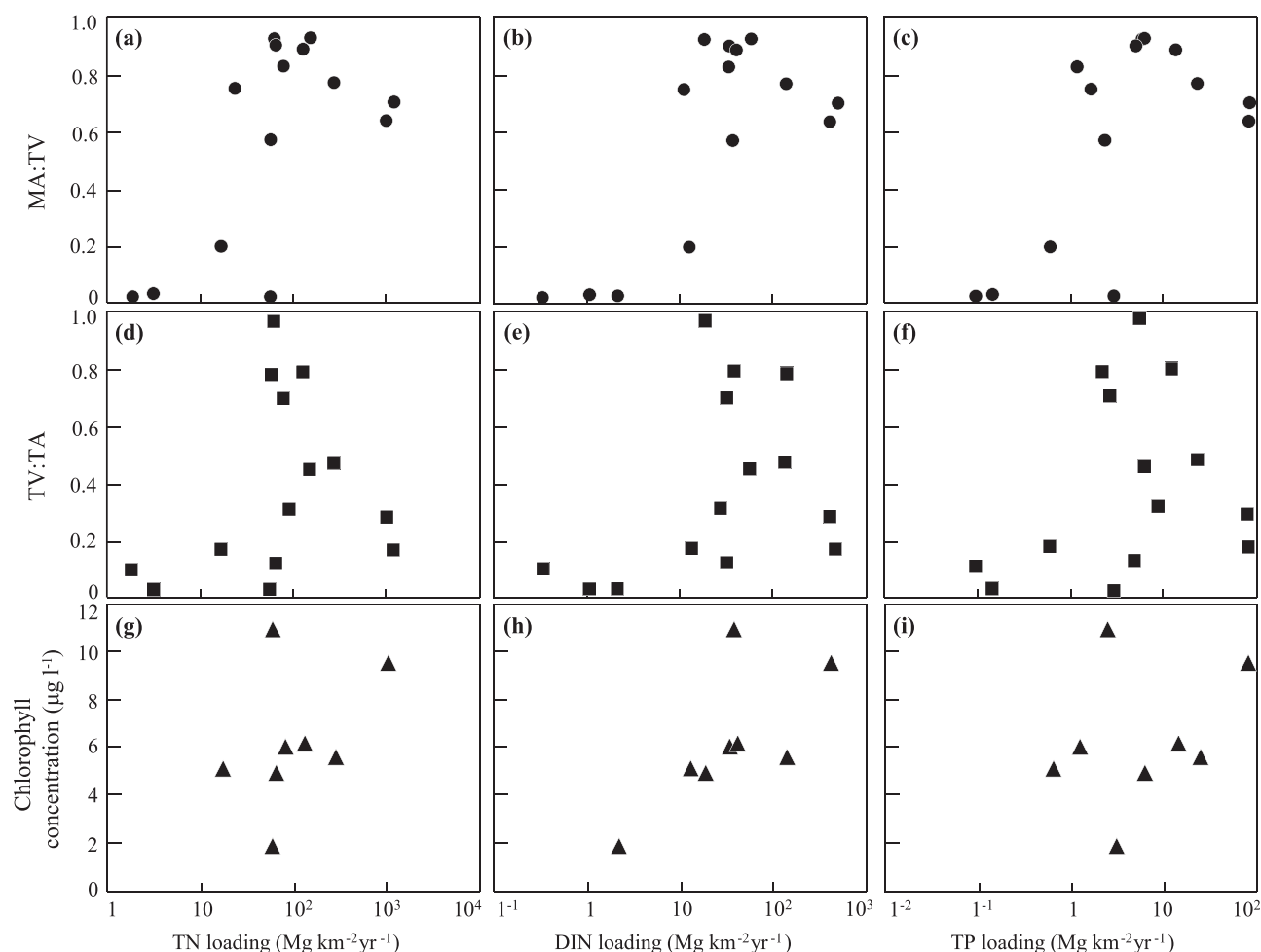


Fig. 2. Annual areal loads ($\text{Mg km}^{-2} \text{ yr}^{-1}$) of total nitrogen (TN), dissolved inorganic nitrogen oxides ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^-$), and total phosphorus (TP) plotted against estuarine macroalgal index (MA: TV, panels a–c; $n = 14$), total vegetation index (TV : TA, panels d–f, $n = 14$), and chlorophyll concentration ($\mu\text{g L}^{-1}$, panels g–i; $n = 8$) in Victorian estuaries from 2012.

model variants, most of which included DIN load as the primary explanatory variable (Table 3). Summed AICc variable weights provided strong evidence ($w_{\text{var}} = 98\%$) that the asymptotic DIN load term was a predictor of the MA : TV index and some evidence that the linear covariates TN_M : TP_M ($w_{\text{var}} = 66\%$, Fig. 3c) and TP ($w_{\text{var}} = 60\%$) were also important (Table 4). Modelling results from the TV: TA analysis were less definitive and despite evidence that DIN load was the single best predictor of TV: TA ($w_{\text{var}} = 77\%$), TN- and TP-based models performed comparably (Table 3). There was some evidence to suggest that TSS ($w_{\text{var}} = 63\%$, Fig. 3d) and TP ($w_{\text{var}} = 58\%$) were informative covariates (Table 4). For both MA: TV and TV: TA, single term models including only DIN load yielded a model structure that maximized model performance (MA: TV $r^2 = 0.80$, TV: TA $r^2 = 0.33$; Fig. 3a,b) while minimizing model complexity (Table 3). There was little evidence that estuary residence time ($w_{\text{var}} \leq 54\%$) was influential in models of either demersal vegetation index. Results from the phytoplankton response modeling

were qualitatively identical to those of the demersal vegetation response (Table 3). The best predictor of chlorophyll concentration was the asymptotic DIN load term ($w_{\text{var}} = 94\%$; Fig. 3e). Performances of the TN and TP load model variants were poor ($w_{\text{var}} = 0.10$) relative to the DIN load model (Table 4).

Discussion

Overall, we found that several whole-of-ecosystem scale indicators of primary producer assemblage composition and total productivity could be explained by catchment derived nutrient loads. Our findings indicate that riverine DIN load was the best predictor of seasonally ephemeral primary productivity (i.e., macroalgae, MA: TV; total chlorophyll concentration, CHL) in these estuaries. Based on the fundamental relationship between nutrient supply and plant growth and given the relatively poor model performance of the TN- and TP-based models, supply of DIN appears to set

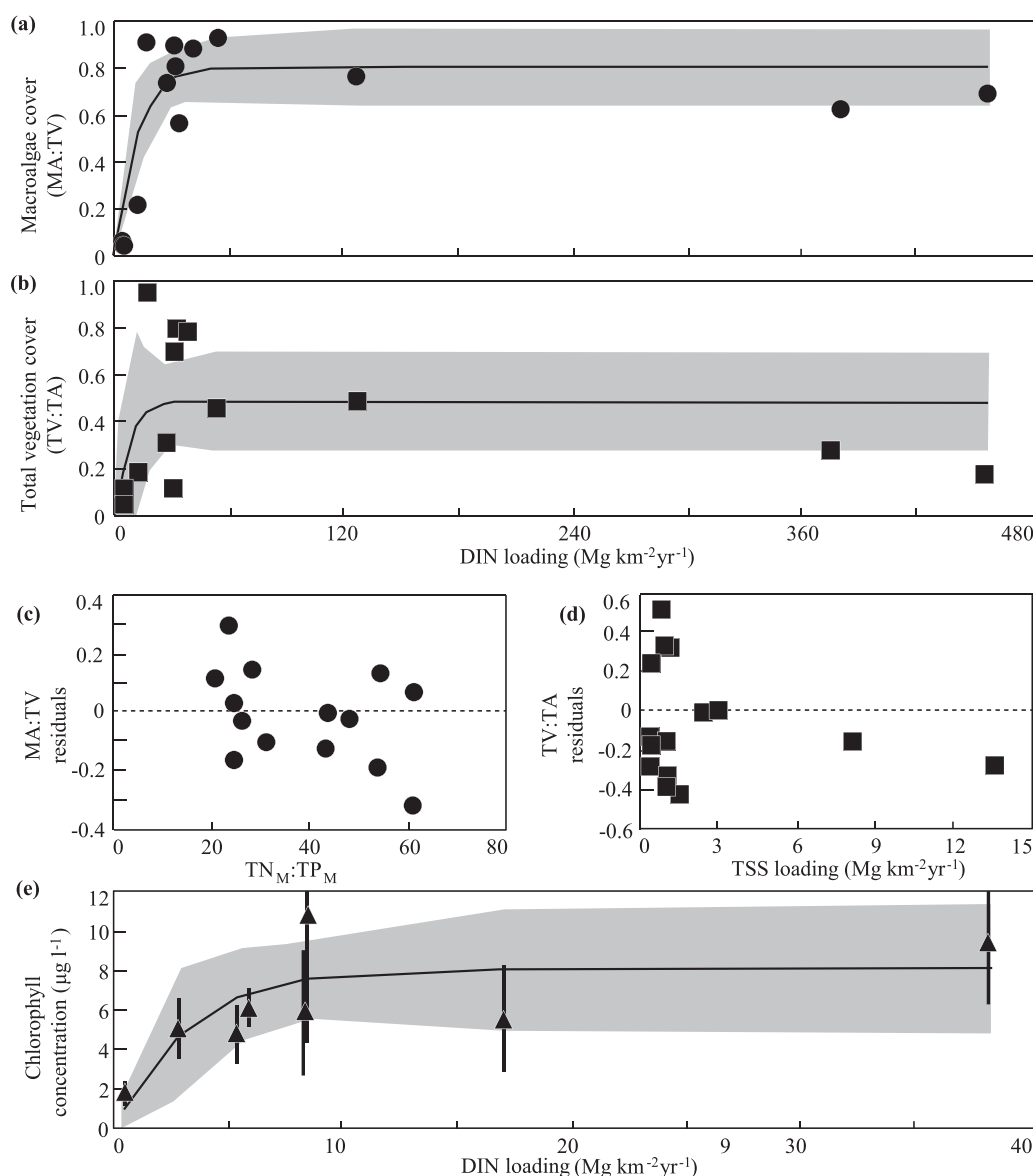


Fig. 3. Modeled predicted response (solid line) and 95% confidence interval range (grey area) of estuarine macroalgal index (MA: TV; $n = 14$; a) and total vegetation index (TA: TV; $n = 14$, b) to annual areal loads ($\text{Mg km}^{-2} \text{yr}^{-1}$) of dissolved inorganic nitrogen oxides ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^-$). Residuals from nonlinear regression of MA: TV (c) plotted against molar ratio of total nitrogen to total phosphorus annual areal loads ($\text{TN}:\text{TP}$ [molar]), and TV: TA (d) plotted against annual areal loads of total suspended solids (TSS). Modeled predicted response and 95% confidence interval range of chlorophyll concentration ($\mu\text{g L}^{-1}$; ± 1 SEM; $n = 8$) to annual areal loads of DIN shown in (e).

the overall scope for potential production of both macroalgae and phytoplankton in these estuaries. The evidence that DIN is acting as a fundamental control of ephemeral estuarine producers in Victorian estuaries is supported by previous experimental and observational work in estuaries around the world (D'elia et al. 1986; Valiela et al. 1997; Howarth and Marino 2006; Teichberg et al. 2010).

Results from the total vegetation (i.e., TV: TA) modeling were less definitive and overall model performance was lower. Despite incrementally better model performance by the DIN models, models that included TN and TP as nonlin-

ear predictors performed similarly. The poor explanatory power of the models likely arises from the inclusion of both seagrass and macroalgal areal extents in the calculation of the TV: TA index. Unlike seasonally ephemeral macroalgae taxa, positive feedback processes (e.g., reduced turbidity, enhanced nutrient mineralization) within seagrass beds can mitigate changes in water quality conditions over periods of years to decades (Gurbisz and Kemp 2014). When rapid shifts in seagrass coverage do occur, these threshold responses can be preconditioned by extended periods of chronic change to water quality (Gurbisz and Kemp 2014).

Additionally, large blooms of ephemeral macroalgae reduce light availability at depth, can alter biogeochemical conditions at the sediment–water interface, and have been shown

Table 3. Results from model selection analysis relating the ratio of macroalgae area to total vegetated area (MA: TV), total vegetated area to shallow area < 2m (TV: TA), and mean surface chlorophyll concentration (CHL, $\mu\text{g L}^{-1}$) to nutrient (total nitrogen TN, $\text{DIN} = \text{NO}_2^- + \text{NO}_3^-$, TP) and total suspended solids (TSS) annual areal loads ($\text{Mg km}^{-2} \text{yr}^{-1}$), nutrient molar stoichiometry (TN_M : TP_M), and estuary water residence time (T_f). Model variants include a nonlinear component (Nonlinear) and some include a linear covariate (Linear). Difference in AICc model selection criterion between each variant and the lowest AICc value (ΔAICc), and model probability weights (w_i ; probability of each variant given the other variants) provided for each model variant. Only the five best performing model variants (i.e., highest w_i) from MA: TV and TA: TV analysis shown.

Response	Model components		ΔAICc	w_i
	Nonlinear	Linear		
MA:TV	DIN		0	0.42
	DIN	TP	1.8	0.18
	DIN	TN_M : TP_M	1.9	0.16
	DIN	TSS	2.2	0.14
	DIN	T_f	3.8	0.06
TV:TA	DIN		0	0.23
	DIN	TSS	0.4	0.18
	DIN	TP	1.1	0.13
	TN		2.0	0.08
	TP		2.0	0.08
CHL	DIN		0	0.91
	TP		6.2	0.04
	TN		6.0	0.05

Table 4. Variable probability weights (w_{var} ; probability that a given variable is included in the best model variant) for nonlinear and linear model components from model selection analysis relating the ratio of macroalgae area to total vegetated area (MA: TV) and mean surface chlorophyll concentration (CHL, $\mu\text{g L}^{-1}$) to annual nutrient loads (TN, $\text{DIN} = \text{NO}_2^- + \text{NO}_3^-$, TP; $\text{Mg km}^{-2} \text{yr}^{-1}$), nutrient molar stoichiometry (TN_M : TP_M), and estuarine water residence time (T_f). Odds ratios (OR, posterior odds/prior odds; given parenthetically) were used to adjust w_{var} for MA: TV models to account for differences in the number of model variants that included each variable.

Model component	Variable	w_{var} (OR)		
		MA: TV	TV: TA	CHL
Nonlinear	DIN	0.98 (58.7)	0.77 (3.29)	0.94 (16.4)
	TN	0.03 (<0.1)	0.35 (0.53)	0.10 (0.11)
	TP	0.05 (<0.1)	0.30 (0.43)	0.10 (0.11)
Linear	TN_M : TP_M	0.66 (1.97)	0.19 (0.23)	
	TSS	0.43 (0.74)	0.63 (1.71)	
	T_f	0.54 (1.17)	0.19 (0.24)	
	DIN	0.05 (<0.1)	0.30 (0.43)	
	TN	0.06 (<0.1)	0.35 (0.53)	
	TP	0.60 (1.52)	0.58 (1.37)	

to have a negative influence on seagrass recruitment, growth and shoot density (Hauxwell et al. 2001). Dissimilar temporal scales in nutrient-response dynamics as well as the potential for negative feedbacks between seagrass and macroalgae taxa could both be contributing to the poor explanatory power of our nutrient load models for the TV: TA index.

For demersal vegetation, nitrogen loads corresponded to a highly dynamic region of the nutrient-vegetation response spectrum in which primary producer communities shifted from being seagrass-dominated in low loading systems to being macroalgae-dominated in the high loading systems (Valiela and Bowen 2002; Hauxwell and Valiela 2004). We observed this seagrass-to-macroalgae community transition at DIN loads of $\sim 5\text{--}20 \text{ Mg km}^{-2} \text{yr}^{-1}$. The close agreement in the nitrogen-vegetation response between our estuaries and previous studies (Fig. 4a), even with the mix of different temporal and spatial scales of those previous studies (Nixon et al. 2001; Valiela and Bowen 2002), suggests that our results are robust and offers further support for the generality of these patterns. In the Victorian estuaries, these patterns primarily arose from increases in the extent of macroalgal coverage rather than declines in seagrass extent, and the ability of seagrasses to compensate (at least partially) for increased N-demand during periods of rapid growth (i.e., via assimilation of newly fixed N in the rhizosphere) might explain the lack of a clear relationship between nutrient loads and relative seagrass coverage. Alternatively, seagrasses in these estuaries could be responding to environmental variables that were not considered in this study (e.g., salinity, water temperature, light regime). Compositional data were not available for the phytoplankton community, but the concurrent increases in chlorophyll concentration and MA: TV ratio over approximately the same range of DIN loads

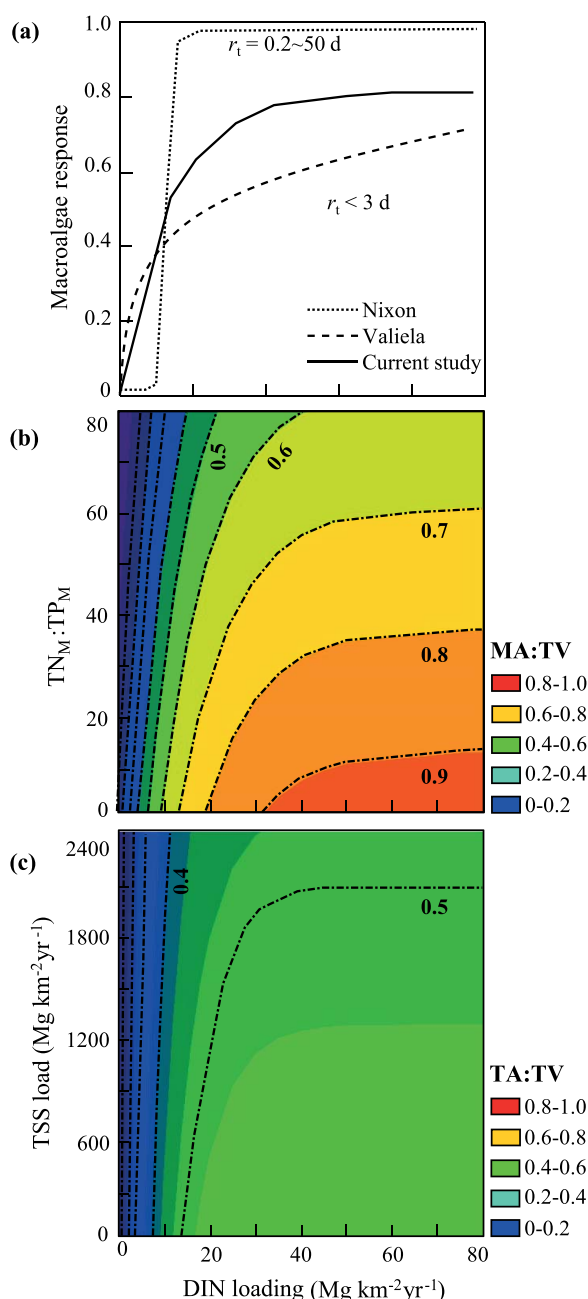


Fig. 4. Observed (this study) and expected shifts in contribution of macroalgae to estuarine vegetation assemblage values with increasing dissolved inorganic nitrogen load ($\text{DIN} = \text{NO}_3^- + \text{NO}_2^-$ loading; $\text{Mg km}^{-2} \text{yr}^{-1}$) for estuaries (a). Modeled response surfaces (with contours) of the estuarine macroalgal index (MA:TV) to a gradient of DIN annual areal loading (abscissa) and molar ratio of total nitrogen to total phosphorus annual areal loads ($\text{TN}_M : \text{TP}_M$; ordinate), and the total vegetation index (TV:TA) to a gradient of DIN loading (abscissa) and total suspended solids annual areal loads (TSS; ordinate) based on ecosystem-level measurements of vegetation coverage in Victorian estuaries. Expected shifts with long or short water residence times (r_t) based on previous studies (Nixon et al. 2001; Valiela and Bowen 2002; panel a). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

underscores the potential for systemic responses of estuarine primary production to changes in nitrogen availability.

Despite the overall pattern of N limitation observed here, estuaries can shift from N to P limitation if N becomes sufficiently abundant relative to P availability (Howarth et al. 2011). Stoichiometric N : P ratios in plant tissues often are close to 30: 1 for marine macroalgae (Lapointe 1997; Lapointe et al. 2005) or 16: 1 for phytoplankton (Redfield 1958; Guildford and Hecky 2000), and ambient nutrient concentrations above (or below) these reference conditions can be evidence of P (or N) limitation. The negative relationship between $\text{TN}_M : \text{TP}_M$ load stoichiometry and the MA:TV index suggests that within the scope for growth set by the size of the bioavailable N pool, the riverine supply of P can act as a “fine-tuning” nutrient responsible for controlling the production of macroalgae by modulating the composition of the available nutrient pool (Fig. 4b). Increased P per unit N yielded higher-than-predicted values of the MA:TV index, suggesting that local macroalgae P-demand may outpace P availability when the N : P stoichiometry of riverine loads is high. When $\text{TN}_M : \text{TP}_M$ load ratios were $< 30: 1$, the observed contribution of macroalgae to total estuarine vegetation averaged 7% higher than predicted based on DIN loading alone (see Fig. 3c). Our results indicate that catchment-derived P, either alone or in combination as a ratio (i.e., $\text{TN}_M : \text{TP}_M$), was less effective at explaining variance in the MA:TV index than the coincident N load.

Although we focus here on terrestrially derived sources of nutrients delivered through river inflow, the entrainment of coastal ocean water during tidal exchange, the release of iron-bound P from estuarine sediments, and nitrogen fixation can all alter the relative availability of each nutrient for estuarine producers (Howarth et al. 1988; Conley et al. 2002, 2009; Howarth et al. 2011). Tidal exchange between estuaries and nearshore coastal waters with low N : P stoichiometry can support N-limitation in estuaries by supplying bioavailable P, but relatively little N, to primary producers (Howarth et al. 2011). For example, recent work in a Cape Cod lagoon (Hayn et al. 2014; Howarth et al. 2014) documented sustained summertime N-limitation among primary producers—a condition that was coupled with the simultaneous retention of the watershed nitrogen load and the net import of P from coastal waters outside the lagoon. Similarly, increases in organic carbon deposition to estuary sediments can foster reducing conditions at the sediment–water interface, leading to the accumulation of sulfides and the subsequent release of iron-bound P from sediments within the estuary (Conley 1999; Conley et al. 2009).

Nitrogen fixation is an important source of N for producers in freshwater ecosystems and can also be important to estuarine producers when and where it occurs (Howarth et al. 1988; Welsh 2000; Woodland and Cook 2013). Rates of nitrogen fixation by cyanobacteria are typically quite low in estuaries with salinities in excess of 8–10 ppt (Howarth et al.

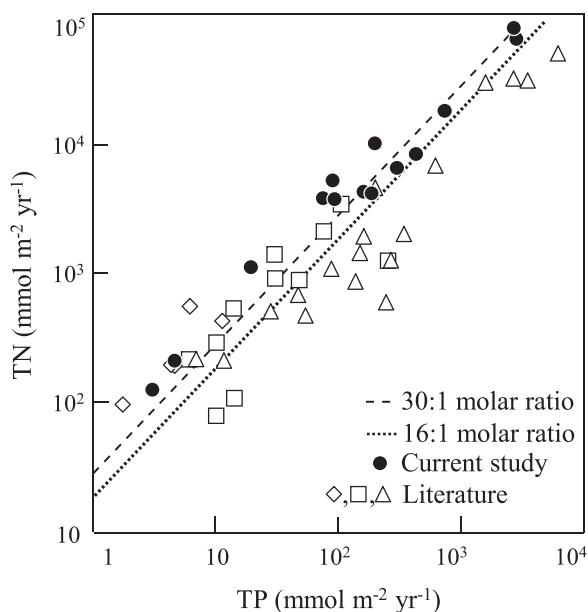


Fig. 5. Total nitrogen (TN) annual loading ($\text{mmol m}^{-2} \text{yr}^{-1}$) plotted against total phosphorus (TP) annual loading ($\text{mmol m}^{-2} \text{yr}^{-1}$) in Victorian estuaries (filled circles, this study), Baltic Sea regions (open diamonds, Elmgren and Larsson 2001), and global distribution of estuarine and coastal ecosystems (open squares, Boynton et al. 1995), (open triangles, Nixon et al. 1986). Reference molar TN: TP ratios of 16: 1 and 30: 1 given by dotted and dashed lines, respectively.

2011) and N stable isotope values ($\delta^{15}\text{N}$) of suspended particulate organic matter (ranging 7.2–17.0‰) collected from the subset of eight estuaries with chlorophyll concentration measurements support the assumption that fixed N did not contribute substantially to phytoplankton production. Previous studies documenting large fluxes of fixed N into planktonic food webs documented SPOM $\delta^{15}\text{N}$ values, which were lighter than those measured here (e.g., Rolff 2000; Gu 2009; Woodland et al. 2013). Additionally, a recent study found that N-fixation contributed < 15% of the N demand for seagrass in beds from the same region of Australia in which the current study was conducted (Cook et al. 2015). Taken together, the potential for P augmentation from tidal exchange or sediment release and the absence of evidence for N-fixation suggests that nonriverine nutrient sources could have a role in preventing P-limitation among primary producers at riverine load ratios above taxonomic N : P optima in these estuaries.

Ecosystem-scale evaluations of the relative importance of these nutrients are critical to establish the integrated effects of N and P at ecologically relevant scales and our results highlight the potential role of N supply to estuarine production and eutrophication dynamics. Overall, our results indicate that estuaries receiving high N loads with low N : P stoichiometry are the most likely to suffer from intense seasonal blooms of macroalgae and (or) phytoplankton. We did not observe seagrass decline in estuaries receiving the high-

est nutrient loads; however, there was some evidence to suggest a negative relationship between TSS load and total vegetation coverage at very high TSS loads. In estuaries with river catchments characterized by P-limited terrestrial geochemistry or land uses that generate relatively large N inputs (e.g., horticulture, pasturage, water treatment facilities), the resultant riverine loads should lead to higher-than-average N : P nutrient ratios. The estuaries in this study show relatively high N : P load ratios, approximately centered around the 30: 1 ratio (Fig. 5). The resulting stoichiometry of these nutrient loads should yield a dynamic situation in which conditions for macroalgae growth can shift from N- to P-limiting through space or time, and this could explain some of the residual variability in our modelling. It is also worth noting that areal loads to these estuaries are relatively high on a global scale—10 of the 14 estuaries receive TN loads of 1000–9500 kg per square km of estuary per year (Fig. 5). In Fig. 5 it can be seen that the estuaries from this study span the full range of TN loading ($\text{mmol m}^{-2} \text{yr}^{-1}$) values reported in Nixon et al. (1986), Boynton et al. (1995), and Elmgren and Larsson (2001). In fact, TN loads reported here actually exceed the highest loading values reported in those studies, yet even the most eutrophied estuaries in this study retained significant demersal vegetation. The prevalence of demersal vegetation at high nutrient loads is likely due to the low water residence times of the estuaries in this study ($T_f \leq 4.6$ d) and the fact that those estuaries with the highest residence times also had the lowest nutrient loads.

The conceptual model of coastal eutrophication is evolving toward a more balanced consideration of both N and P as drivers of estuarine eutrophication processes (Conley 1999; Howarth and Marino 2006; Conley et al. 2009; Paerl et al. 2014). Here, we provide direct observational evidence that the relative abundance of macroalgae, a common indicator of estuarine eutrophication, and the concentration of planktonic chlorophyll increases primarily in concert with concurrent dissolved inorganic N loads. It appears that riverine P loads, in combination with alternative sources of P (e.g., tidal exchange, sediment release), are sufficient to support the biological demand of macroalgae in these ecosystems. In-estuary physical and biogeochemical processes such as flushing rates, particle settling, sediment storage and denitrification are also critical components of the nutrient load-producer response. The remarkably consistent response observed in this study as well as its consistency with previous studies on other continents suggests that these processes are relatively constant and that knowledge of riverine nutrient loads alone can provide good predictive power for several common indicators of estuarine eutrophication. Our results underscore the importance of N loading *in addition to* P loading and support the growing consensus that a multi-nutrient strategy is required to manage the health of coastal ecosystems.

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