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Role of organic components in regulating denitrification in the coastal water of Daya Bay, southern China

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Both dissolved and particulate organic materials have been proposed to be important factors in regulating heterotrophic denitrification in various aquatic environments. However, the specific pathways and mechanisms remain elusive. In this study, water column samples were collected from Daya Bay, southern China, to examine the relationships between potential denitrification and different organic components in the water column. Bulk dissolved organic carbon (DOC) was categorized into three major components including terrigenous fluorescent (tFDOC), autochthonous fluorescent (bFDOC) and non-fluorescent (nFDOC) fractions, while the bulk particulate organic carbon (POC) was divided into terrigenous (tPOC) and autochthonous (bPOC) fractions based on an isotope mixing model. Potential denitrification derived from *in situ* incubation experiments under anoxic conditions was evident (ranging from 6 to 107 nmol N₂ per L per h) and varied markedly among stations. When normalized to nitrate concentration, the denitrification rate (NDR) followed a positive trend with either the concentration or proportion of tFDOC, and a negative trend with the proportion of nFDOC, suggesting tFDOC was potentially favorable while nFDOC was unfavorable for denitrifying degradation. In comparison, the NDR showed a significant positive correlation with the proportion of bPOC in the bulk POC ($p = 0.01$), with a predictive power of >70%, indicating that the composition of POC has a substantial impact on potential denitrification. Furthermore, if both bPOC and suspended particulate matter (SPM) were considered as variables concurrently, the variability of NDR can be better predicted with a predictive power as high as 80%. Therefore, denitrifiers may preferentially utilize fresher and labile autochthonous POC instead of DOC especially in coastal waters where particles/colloids are abundant. Our results thus provide new insights for a better understanding of denitrification mechanisms in water columns and the importance of both suspended particles and POC components in regulating denitrification, especially in turbid and productive coastal environments.

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Environmental significance

Microbial denitrification is one of the major nitrogen (N) removal processes in various ecosystems. In coastal areas where increased anthropogenic loading of N is becoming a more and more serious environmental issue, denitrification plays a key role not only in regulating the N reservoir but also in relieving environmental eutrophication. For most natural heterotrophic denitrification, both dissolved and particulate organic matter are recognized to be important controllers, whereas the relative importance of these two organic pools has not been well studied. With the use of several state-of-the-art fluorescence and isotope tools, the relationships between water column denitrification and different organic fractions in the coastal China bay are explored in this study. The results reveal a more dominant role of particulate organic carbon (POC) in regulating potential denitrifying activity than that of dissolved organic carbon. They also demonstrate that the combination of marine autochthonous POC and suspended particulate matter contents will well predict the variation of denitrification (as high as 80% predictive power). These results shed light on the new understanding of denitrification pathways and mechanisms in coastal aquatic environments.

Introduction

Microbial denitrification, a step-wise anaerobic reduction converting nitrate (NO₃⁻) to dinitrogen gas (N₂) *via* intermediates,

including nitrite, nitric oxide and nitrous oxide,¹ is widespread among ecological habitats involved in nitrogen (N) transformation and has been recognized as a major sink of global N reservoir.^{2,3} Both NO₃⁻ availability and organic matter supply are considered as important environmental factors controlling denitrification,^{4,5} and the organic matter usually becomes limiting when the ambient NO₃⁻ is sufficient.^{6,7} During the past few decades, the potential denitrifying capacity has greatly

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enhanced because of the increased anthropogenic loading of N in aquatic ecosystems.⁸ As a result, organic matter plays an even more important role in water column denitrification.⁹ Thus, knowledge of the role of different organic matter components and phases and their influences on denitrification is indispensable to a better understanding of denitrification mechanisms and the coupled biogeochemical processes between carbon (C) and N in a water column, and the improvement of ecological management.

In general, organic matter affects denitrification mainly through both direct and indirect pathways. In the direct pathway, organic matter provides the electron donor for nitrate reduction and drives the denitrification.^{9,10} In addition, the input of organic matter to the environment facilitates oxygen consumption by aerobic respiration and creates suboxic (or anoxic) conditions conducive to denitrification.^{2,3} Indeed, a positive relationship between the organic carbon content and denitrification rate is commonly observed in aquatic environments. For example, recent studies in the oxygen-depleted waters of the Arabian Sea¹¹ and Baltic Sea¹² have demonstrated that treatment with bulk particulate organic matter (POM) and/or dissolved organic matter (DOM) would significantly enhance the incubated denitrifying activities, indicating the importance of organic matter to marine N budgets. Besides quantity, the quality of organic matter has also been recognized to play a critical role in regulating denitrification. A most recent study in coastal waters showed that autogenic POM from marine algal production could stimulate the denitrification rate more greatly than the POM from terrestrial sources.¹³ Even though DOM and POM both act as organic carbon sources to fuel environmental denitrification, the relative importance between these two phases is not clear. Little is known about the interrelation between denitrification and the composition of organic matter in marine environments although a few pioneering studies in land-based ecosystems have shown the coupled effects of DOM and POM on denitrification and the fact that the quantity and quality of DOM are related to POM.^{14,15}

Particle-associated denitrification as the N removal pathway in marine waters has received increasing attention over the past years. A model prediction even proposed that anaerobic N metabolism within particles would outweigh the water column denitrification rates on the global scale.¹⁶ O₂-depleted micro-environments inside particles may create suitable suboxic/anoxic centers for anaerobic respiration.¹⁷ From laboratory simulated incubations, it has been demonstrated that cyanobacterial aggregates¹⁸ and diatom aggregates¹⁹ produced in natural seawater can be hotspots for denitrifying activities. Moreover, *in situ* observations of chemical tracers in pelagic waters also provided hints for the occurrence of denitrification in sinking particles.²⁰ However, it is still unclear whether particle-associated denitrification can be significant in coastal areas, where particulate matter loading is usually high.

Daya Bay is one of the largest bays on the coast of southern China. The average concentration of dissolved inorganic nitrogen (DIN) across the bay was usually lower than 5 $\mu\text{mol L}^{-1}$ due to the lack of major river inputs.²¹ As a result, organic matter in the bay was mostly derived from autochthonous

sources.^{22,23} Over the past 30 years, however, rapid economic development and intensive human activities have led to the release of excess DIN and allochthonous organic matter into the bay,^{24,25} causing a major impact on the ecological environment.²¹ In addition, a previous study has confirmed the presence of denitrification in the sediments of Daya Bay.²⁶ Although the water column is well oxygenated throughout the year,²¹ the dynamic hydrological settings lead to a tight pelagic–benthic coupling in the bay (*e.g.*, sediment resuspension),²⁷ probably allowing the existence of potential denitrification within the water column. From another perspective, the natural oxygenated bay water can inhibit the appearance of reducers such as sulfides, reduced manganese or iron, and methane, which are usually detected in anoxic zones of marine systems and serve as alternative electron donors for lithotrophic or chemolithoautotrophic denitrification.¹² Thus, natural reduced organic carbon (both DOC and POC) even in apparent oxygenated water columns can serve as electron donors and promote denitrification. However, little is known about the potential denitrification processes resulting from natural organic matter in oxygenated water columns in marine environments. Knowledge is needed regarding the quantitative relationship between denitrification and organic matter composition, and the impacts on the nitrogen cycle under increased DIN and organic matter input in coastal environments.

In this study, potential denitrifying activity in a water column was determined by incubation with ¹⁵N labeling under oxygen degassing treatment, and the source and composition of DOM and POM were characterized by fluorescence spectroscopy and stable isotope analysis, respectively, to quantify the relationship between the denitrification rate and the composition of DOM and POM from different sources. We further compare the relative importance of DOM and POM in regulating potential denitrification and elucidate the coupling effect of DOM, POM and suspended particulate matter (SPM) on coastal denitrification. Our hypothesis is that fresher POM plays a more dominant role than DOM and the reactive organic component in SPM is a key factor in facilitating water column denitrification in coastal environments.

Materials and methods

Geographic and hydrographic description

Daya Bay is a semi-enclosed bay adjacent to the northern South China Sea (23.52–24.83 °N and 113.50–114.83 °E) with an area of 600 km² and an average water depth of about 10 m.²⁸ It is surrounded by highly urbanized cities such as Shenzhen to the west and Huizhou to the northeast. Daya Bay is also an important aquaculture area with two largest coves, Aotou and Dapeng Cove, situated in the northwest and southwest of the bay, respectively (Fig. 1). Most of the bay water originates from the South China Sea with several small rivers discharging into the western part seasonally.²¹ Tidal current in Daya Bay is dominated by an irregular semidiurnal tide and generally forms an anticlockwise gyre in the bay.²⁷ According to previous studies, warm water in summer supports high primary production²³ and intense benthic denitrification²⁶ in Daya Bay.

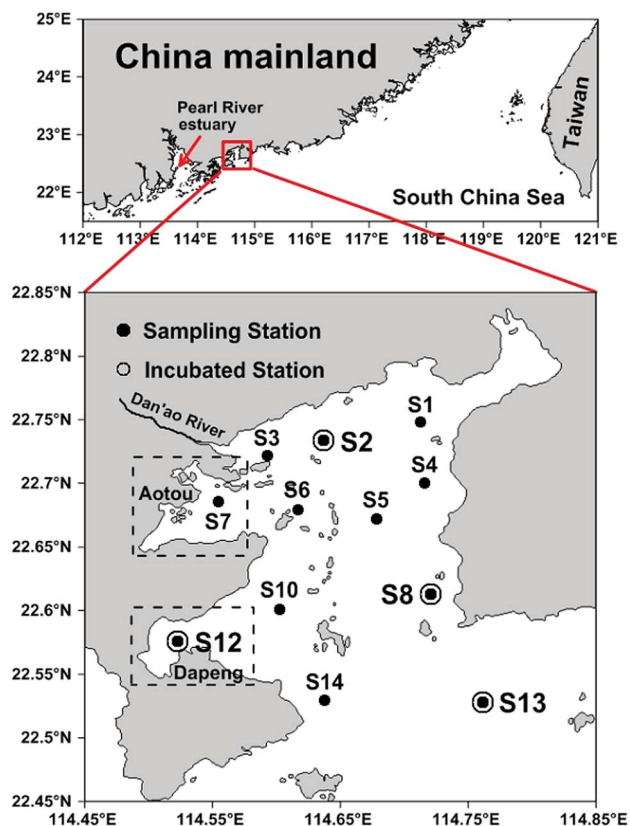


Fig. 1 Sampling locations in Daya Bay. The upper panel shows the geographical environment around Daya Bay. In the lower panel, all sampling stations are shown as black dots, and the stations for incubation are marked as open circles. The rectangles with dashed lines represent the approximate areas of two aquaculture zones, Aotou Cove and Dapeng Cove.

A high precipitation induced large terrigenous riverine input also occurs in this season.^{29,30}

Sample collection

A total of 12 stations were sampled during July 2015, among which Stations S2, S8, S12 and S13 were selected for denitrifying incubations (Fig. 1). Seawater samples were collected in 5 L GO-FLO bottles from the surface and bottom layers. Water temperature and salinity were recorded *in situ* using a YSI 6600 multi-probe sensor (Yellow Springs Instrument Co., USA). In addition, DIN, dissolved oxygen (DO), chlorophyll *a* (*Chl-a*), DOM, POM and SPM were concurrently analyzed.

Analysis of DIN, *Chl-a* and DO

For the measurements of *Chl-a* and DIN, including nitrate [NO_3^-], nitrite [NO_2^-], and ammonium [NH_4^+], water samples were filtered through Whatman GF/F membranes and frozen immediately at -20°C in the dark. Concentrations of NO_3^- , NO_2^- and NH_4^+ were determined using a Lachat QuickChem 8500 autoanalyzer (Lachat Instruments, Loveland, CO, USA) using the standard colorimetric methods.³¹ *Chl-a* concentration was measured by fluorescence using a Turner fluorometer after

extracting with 90% acetone.³² Samples for DO measurement were directly transferred into a 120 mL glass bottle on deck and then DO was determined using the standard Winkler titration method immediately.³¹

DOM measurement and characterization

DOM samples were collected in 60 mL acid-cleaned (1 mol L^{-1} HCl for 24 h) and pre-combusted (450°C for 5 h) amber borosilicate glass vials and kept in the dark at -20°C until analysis.³³ Dissolved organic carbon (DOC) was determined by the high-temperature combustion method using a total organic carbon analyzer (Shimadzu TOC-V_{CPH}). DOC working standards were periodically measured as a sample to ensure data quality. Ultrapure water ($18.2\text{ M}\Omega$) was used as a blank, and its DOC concentration was lower than $6\text{ }\mu\text{mol C per L}$. The accuracy of DOC measurement was better than 2%.

To characterize the chemical composition of DOM, 3-D fluorescence spectra were measured using a Cary Eclipse Spectrofluorometer (Varian, Australia) with a 1 cm path-length quartz cuvette. The fluorescence spectra were scanned in 5 nm increments over the excitation (Ex) wavelength range of 200–450 nm and scanned in 2 nm increments over the emission (Em) wavelength range of 230–600 nm. Parallel Factor Analysis (PARAFAC) was then performed to resolve the excitation–emission matrix (EEM) *via* MATLAB software (MathWorks R2015b) and DOMFluor toolbox processing.³⁴ Based on the EEM-PARAFAC analysis, three major fluorescent components were identified for the bulk DOM pool. Component 1 (C1) was characterized by its double Ex/Em peaks at 260/460 nm and 355/460 nm, corresponding to a typical terrigenous UV humic-like substance.^{34,35} Component 2 (C2) exhibited two peaks with Ex/Em wavelengths of 285/494 nm and 385/494 nm, similar to the characteristics of terrestrial UVA humic-like or fulvic moieties.^{34–36} Component 3 (C3) was characterized by a primary and secondary Ex/Em maximum at 240/390 nm and 310/390 nm, respectively, which are attributed to the autochthonous protein-like constituents.^{35,36} The PARAFAC results were verified using the consistent error distribution at the scanned Ex/Em wavelengths with the four- or five-component modeling.

Interestingly, there is a significant linear relationship between the intensity of each fluorescent component (in Quinine Sulfate Units, Q.S.U.) and DOC, *i.e.*,

$$\text{C1 (Q.S.U.)} = 0.064 \times \text{DOC} - 5.06 \quad (r^2 = 0.86, p < 0.0001, n = 26) \quad (1)$$

$$\text{C2 (Q.S.U.)} = 0.042 \times \text{DOC} - 3.39 \quad (r^2 = 0.92, p < 0.0001, n = 26) \quad (2)$$

$$\text{C3 (Q.S.U.)} = 0.030 \times \text{DOC} - 2.45 \quad (r^2 = 0.76, p < 0.0001, n = 26) \quad (3)$$

The fitting relationships show that when the fluorescence intensity reaches zero, the non-fluorescent DOC corresponding to eqn (1), (2) and (3) was 79, 81 and 82 $\mu\text{mol L}^{-1}$, respectively, with an average of $81 \pm 1\text{ }\mu\text{mol L}^{-1}$. Therefore, fluorescent DOC can be estimated to be:

$$\text{FDOC}_i = (\text{DOC} - 81) \times \frac{Q_i}{\sum_1^3 Q_i} \quad (4)$$

where FDOC_i and Q_i are the DOC concentration (in $\mu\text{mol C per L}$) and fluorescence intensity of each fluorescent component, respectively. Since C1 and C2 are both from terrestrial sources and C3 is derived from marine autochthonous sources, here the sum of FDOCs in C1 and C2 is defined as terrestrial fluorescent DOC (tFDOC), and the FDOC in C3 is defined as biological fluorescent DOC (bFDOC).

POM measurements and characterization

Suspended particulate matter was sampled by filtering 3 to 5 L of seawater through a pre-weighed and pre-combusted (400°C , 4 h) 47 mm GF/F membrane. After rinsing with ultrapure water and drying at 60°C , the membrane containing the particles was weighed to obtain the SPM content. One quarter of the membrane was used and acid-fumed to remove inorganic carbon for subsequent measurements of particulate organic carbon (POC) and its isotopic composition ($\delta^{13}\text{C}_{\text{POC}}$). Half of the remaining membrane was untreated to measure particulate nitrogen (PN) and its isotopic composition ($\delta^{15}\text{N}_{\text{PN}}$). POC and PN contents as well as $\delta^{13}\text{C}_{\text{POC}}$ and $\delta^{15}\text{N}_{\text{PN}}$ were determined using an isotopic ratio mass spectrometer (Delta^{plus} XP, Thermo Finnigan) coupled to an elemental analyzer (Flash EA 1112 series, Thermo Finnigan). The isotopic reference standards for C and N are PeeDee Belemnite and atmospheric N_2 , respectively. To monitor instrument performance and data quality, certified standard samples IAEA-C8 ($\delta^{13}\text{C} = -18.3\text{‰}$) and USGS40 ($\delta^{13}\text{C} = -26.4\text{‰}$) were used for $\delta^{13}\text{C}$, and IAEA-N2 ($\delta^{15}\text{N} = 20.3\text{‰}$) and IAEA-N3 ($\delta^{15}\text{N} = 4.7\text{‰}$) were used for $\delta^{15}\text{N}$. The detection limit of both POC and PN is 0.1 μmol , and the analytical precision of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ is $\pm 0.2\text{‰}$.²⁹

Sources of POM were distinguished by isotopic compositions to explore the effect of organic composition on denitrification. In the first approximation, POM in Daya Bay during the summer included three major sources, *i.e.*, terrestrial, estuarine aquagenic, and marine autochthonous.²⁹ A three end-member mixing model and their $\delta^{13}\text{C}_{\text{POC}}$ and $\delta^{15}\text{N}_{\text{PN}}$ signatures were used to quantify the contribution of each component based on the following equations:

$$f_{\text{ter}} + f_{\text{est}} + f_{\text{mar}} = 1 \quad (5)$$

$$\delta^{13}\text{C}_{\text{ter}} \times f_{\text{ter}} + \delta^{13}\text{C}_{\text{est}} \times f_{\text{est}} + \delta^{13}\text{C}_{\text{mar}} \times f_{\text{mar}} = \delta^{13}\text{C}_{\text{d}} \quad (6)$$

$$\delta^{15}\text{N}_{\text{ter}} \times f_{\text{ter}} + \delta^{15}\text{N}_{\text{est}} \times f_{\text{est}} + \delta^{15}\text{N}_{\text{mar}} \times f_{\text{mar}} = \delta^{15}\text{N}_{\text{d}} \quad (7)$$

where $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and f refer to $\delta^{13}\text{C}_{\text{POC}}$, $\delta^{15}\text{N}_{\text{PN}}$ and the proportion of each end-member, respectively. The subscripts ter, est and mar represent terrestrial, estuarine and marine sources, respectively, and $\delta^{13}\text{C}_{\text{d}}$ and $\delta^{15}\text{N}_{\text{d}}$ denote the determined isotopic composition. As chosen in Mu *et al.* (2017),²⁹ the end-member $\delta^{13}\text{C}_{\text{POC}}$ and $\delta^{15}\text{N}_{\text{PN}}$ values were -24.5‰ and 0.7‰ for terrestrial, -29.0‰ and 15.0‰ for estuarine, and -16.9‰ and 9.0‰ for marine autochthonous POM, respectively. The POC content of each source was calculated as follows:

$$\text{POC}_i = \text{POC} \times f_i \quad (8)$$

where POC_i and f_i denote the organic carbon content and proportion of each source in the bulk POC, respectively. To simplify, POM contents from both terrestrial and estuarine are combined as terrigenous, while marine autochthonous POM is *in situ* produced biogenic POM. Accordingly, the POC from terrestrial and estuarine sources was defined as tPOC, and the marine autochthonous POC was defined as bPOC.

Measurements of potential denitrifying activity

Apparent denitrification rate (ADR). Potential ADR was determined by ^{15}N -labeled incubation under DO-degassed conditions at selected stations. Similar to the procedure described by Zeng *et al.* (2018),¹³ a batch of 9 mL bulk water from each depth was collected in a series of 12 mL Exetainer vials (Labco, UK) for time-series incubation. The water samples were purged with ultrapure helium gas (99.999%) for 10 min to remove background N_2 and O_2 before the ^{15}N amendments, by which the anaerobic potential was activated. $^{15}\text{NO}_3^-$ and $^{15}\text{NH}_4^+$ were added separately up to a final concentration of $1 \mu\text{mol L}^{-1}$ to synchronously measure denitrification and anaerobic ammonium oxidation (anammox), another important microbial N_2 production route.³⁷ Samples were incubated in the dark at *in situ* temperature. The time-series points were set at 0, 12, 24, 36, and 48 h. At each termination point, 40 μL of saturated HgCl_2 solution was injected to prevent microbial activity. The incubation was conducted in duplicate for samples from each depth. Sample vials after incubation were kept in a water bath at room temperature ($\sim 20^\circ\text{C}$) to avoid N_2 contamination from the atmosphere until analysis.

When back to the laboratory, the samples were sonicated at 40°C for 40 min to equilibrate N_2 between the headspace and the solution. Concentrations of ^{15}N -labeled N_2 species ($^{29}\text{N}_2$ and $^{30}\text{N}_2$) were measured using a GasBench II system in combination with IRMS (Delta^{plus} XP, Thermo Finnigan) with a standard deviation of less than 0.1%.¹³

In the $^{15}\text{NO}_3^-$ amended incubation, ^{15}N -labelled N_2 (*i.e.*, $^{29}\text{N}_2$ and $^{30}\text{N}_2$) generally began to accumulate in a linear manner after 12 h with a consistent pattern of time-series, indicating that denitrification was mainly responsible for nitrogen removal.^{13,38} In contrast, the incubation with $^{15}\text{NH}_4^+$ showed no production of both $^{29}\text{N}_2$ and $^{30}\text{N}_2$, implying the absence of anammox. Therefore, the ADR with $^{15}\text{NO}_3^-$ -amendment can be obtained as:

$$\text{ADR} = \frac{R_{29}}{2 \times F_{\text{N}} \times (1 - F_{\text{N}})} \quad (9)$$

or

$$\text{ADR} = \frac{R_{30}}{F_{\text{N}}^2} \quad (10)$$

where R_{29} and R_{30} denote the production rate of $^{29}\text{N}_2$ and $^{30}\text{N}_2$, respectively, and F_{N} denotes ^{15}N abundance in the bulk nitrate pool. R_{29} (or R_{30}) was calculated *via* linear regression of $^{29}\text{N}_2$ (or $^{30}\text{N}_2$) concentration against incubation time. The average ADR calculated using R_{29} and R_{30} was used in this study.

NO₃⁻-normalized denitrification rate (NDR). The response of the ADR to varying NO₃⁻ concentrations was evaluated using the surface sample from Station S2 and the bottom sample from Station S8. The ambient NO₃⁻ concentration of the surface water at Station S2 (5.73 μmol L⁻¹) was about 4 times that of the bottom water at Station S8 (1.50 μmol L⁻¹). Prior to incubation, the gradient of NO₃⁻ level in the vial was manipulated by the addition of ¹⁵NO₃⁻ up to a final concentration of 1 μmol L⁻¹, 5 μmol L⁻¹, and 10 μmol L⁻¹, respectively. The measurement of the denitrification rate was carried out under the same conditions and using the same procedures as above.

The response patterns of ADR with NO₃⁻ concentration were different in the two samples. The ADR increased obviously with the increase of NO₃⁻ concentration in the bottom sample from Station S8, while the ADR declined slightly with the increase of NO₃⁻ concentration in the surface samples from Station S2 (Fig. 2). When the samples of the two layers were integrated, the combined data fit well the Michaelis–Menten equation, and the half-saturation constant (*K_m*) was deduced to be 2.3 μmol L⁻¹ (Fig. 2), which is rather similar to the typical values of natural denitrification.^{39,40} This means that when the ambient NO₃⁻ is as high as 6.5 μmol L⁻¹, its effect on denitrifying activity is almost saturated. In order to better discuss the effect of organic matter on denitrification, a linear regression was used to normalize the ADR to a NO₃⁻ concentration of 6.5 μmol L⁻¹ by assuming that the denitrification rate follows a first-order relationship when NO₃⁻ concentration is lower than *K_m*.

For samples from Stations S8 and S13, where the NO₃⁻ concentrations were below *K_m* (*i.e.*, 2.3 μmol L⁻¹), the NDR was obtained as follows:

$$\text{NDR} = \text{ADR} \times \frac{6.5}{[\text{NO}_3^-]_{\text{bulk}}} \quad (11)$$

where [NO₃⁻]_{bulk} represents the bulk NO₃⁻ concentration within the incubation system. Whereas for samples from Stations S2 and S12, where the NO₃⁻ concentrations were close

to 6.5 μmol L⁻¹, we ignored normalization and considered the measured ADR as saturated.

Data analysis and statistics

Correlation analyses were performed using the SigmaPlot 12.5 software package, and the results were accepted only when the residuals passed the Shapiro–Wilk normality test and the constant variance test. On the basis of the *t*-test, denitrification rates were adopted only if the slope value of linear regression was significantly greater than zero (*p* < 0.05). The detection limit for the denitrification rate was 0.68 nmol N₂ per L per h. The *K_m* value in the Michaelis–Menten equation was estimated from non-linear least squares fitting. One-way analysis of variance (ANOVA) with the Tukey's HSD test was applied to evaluate statistical differences of environmental parameters among stations using SPSS 16.0.

Results

Hydro-chemical parameters

During the sampling period in summer, the seawater temperature ranged from 24.5 °C to 31.1 °C, and increased monotonically from the outer bay to the inner bay (Fig. 3a). The lowest salinity (as low as 19.4) was recorded in the surface water of Station S3 near the river mouth. The salinity outside the bay was higher than 33.0, reflecting the impact of the shelf water from the northern South China Sea (Fig. 3b).²¹ Oxidized nitrogen (NO₃⁻ + NO₂⁻, abbreviated as NO_x⁻) accounted for the majority of DIN (~65%). Both NO_x⁻ and NH₄⁺ increased toward the inner bay, and the highest concentration was found in the surface water of Station S3 with the lowest salinity, showing the footprint of riverine input (Fig. 3c and d). The variation pattern of *Chl-a* was similar to that of the nitrogen species, except that the highest value of *Chl-a* was found in the surface water of Station S6 (Fig. 3e), where an intense algal bloom occurred.³⁰ The SPM contents ranged from 0.70 to 14.16 mg L⁻¹, with higher contents in the bottom water (Fig. 3f), showing a benthic SPM source from sediment resuspension. The water column was well oxygenated during sampling with DO concentrations ranging from 150 to 330 μmol L⁻¹ for all stations. The average DO concentrations in surface and bottom water were 307 μmol L⁻¹ and 191 μmol L⁻¹, respectively.

DOC and its sources

DOC accounted for the majority of the total organic carbon (TOC) pool in the bay, with concentrations ranging from 80 to 232 μmol L⁻¹ (ave. 122 ± 39 μmol L⁻¹), equivalent to 58–94% of TOC (*i.e.*, TOC = DOC + POC). The distribution of DOC showed a sharp decrease from the inner bay to the outer bay, and the highest concentration was found in the surface water at Station S3, indicating the input from river discharge (Fig. 4a). The FDOC accounted for near-zero to 65% of the bulk DOC, of which tFDOC was the main fluorescent fraction, accounting for an average of 83 ± 7% and 24 ± 13% of the total FDOC and bulk DOC, respectively (Table 1). A significant negative correlation between tFDOC and salinity (*p* < 0.001) was observed (Fig. 5a),



Fig. 2 Response of apparent denitrification rate (ADR) with manipulated NO₃⁻ concentration at Stations S2 and S8. The solid line is fitted according to the Michaelis–Menten kinetics. *K_m* represents the half-saturation constant of NO₃⁻ demand.



Fig. 3 Spatial distributions of hydro-chemical parameters in the surface layer (the upper plots) and bottom layer (the lower plots) in Daya Bay: (a) temperature ($^{\circ}\text{C}$); (b) salinity; (c) $\text{NO}_3^- + \text{NO}_2^-$ ($\mu\text{mol L}^{-1}$); (d) NH_4^+ ($\mu\text{mol L}^{-1}$); (e) *Chl-a* ($\mu\text{g L}^{-1}$); (f) SPM (mg L^{-1}).

further demonstrating predominant terrestrial sources for tFDOC. The bFDOC concentration ranged from 0 to $17.6 \mu\text{mol L}^{-1}$, accounting for ~ 0 – 13% of the bulk DOC (Table 1). A positive correlation between bFDOC and *Chl-a* ($p = 0.001$) was observed (Fig. 5b), attesting the marine autochthonous source of bFDOC. Both tFDOC and bFDOC followed the same distribution pattern as the bulk DOC in the bay, which was characterized by a decrease from the inner bay to the outer bay (Fig. 4b and c). Among the four stations where denitrification measurements were conducted, the bulk DOC, tFDOC and bFDOC concentrations were higher at Stations S2 and S12, while those at Stations S8 and S13 were lower (Table 1).

POC and its sources

The POC concentration varied greatly from 6.6 to $148.3 \mu\text{mol L}^{-1}$ (average $37.6 \pm 32.3 \mu\text{mol L}^{-1}$) and decreased from the inner bay to the outer bay, which was similar to the DOC distribution (Fig. 4d). Unlike DOC, which was mainly from terrestrial sources, POC was mainly contributed by *in situ* biogenic sources with bPOC accounting for $68 \pm 15\%$ of the bulk POC pool (Table 1). It is worth noting that both the concentration and proportion of bPOC correlated fairly well with those of *Chl-a* (Fig. 6), indicating that bPOC was predominantly from algal production. The high proportion of bPOC was



Fig. 4 Spatial distributions of the bulk DOC and POC and their different components in the surface layer (the upper plots) and bottom layer (the lower plots) in Daya Bay: (a) bulk DOC ($\mu\text{mol C per L}$); (b) tFDOC from terrestrial sources ($\mu\text{mol C per L}$); (c) bFDOC from marine biogenic sources ($\mu\text{mol C per L}$); (d) bulk POC ($\mu\text{mol C per L}$); (e) tPOC from terrestrial sources ($\mu\text{mol C per L}$); (f) bPOC from marine biogenic sources ($\mu\text{mol C per L}$).

Table 1 Concentrations of DOC and its components, POC and its components, SPM, and nitrate, and potential denitrification rates in Daya Bay

Station	Bottom depth (m)	Sampling depth (m)	DOC ^a (μmol C per L)			POC ^b (μmol C per L)			SPM (mg L ⁻¹)	NO ₃ ⁻ (μmol L ⁻¹)	ADR (nmol N ₂ per L per h)	NDR ^c (nmol N ₂ per L per h)
			Bulk	tFDOC	bFDOC	Bulk	tPOC	bPOC				
S2	7.0	0	136	39 (29%)	18 (13%)	36.28	9.39 (26%)	26.89 (74%)	5.58	5.73	62.4 ± 5.4	62.4 ± 5.4
		6	113	27 (24%)	6 (5%)	47.47	3.10 (7%)	44.37 (93%)	6.21	7.16	107.1 ± 35.5	107.1 ± 35.5
S8	8.4	0	102	20 (20%)	2 (2%)	11.39	3.55 (31%)	7.84 (69%)	1.39	0.76	16.5 ± 5.3	57.2 ± 8.6
		7.5	93	12 (12%)	2 (2%)	20.72	8.90 (43%)	11.82 (57%)	3.09	1.50	6.3 ± 1.2	15.7 ± 3.0
S12	8.0	0	174	76 (44%)	16 (10%)	34.12	10.07 (30%)	24.05 (70%)	2.25	8.26	u.d.	u.d.
		7	104	20 (19%)	3 (3%)	51.86	19.84 (38%)	32.02 (62%)	9.29	4.03	32.1 ± 3.9	32.1 ± 3.9
S13	19.6	0	94	13 (13%)	1 (1%)	9.86	4.99 (51%)	4.87 (49%)	1.67	0.84	15.0 ± 9.2	50.1 ± 14.1
		19	80	0 (0%)	0 (0%)	18.03	9.35 (52%)	8.68 (48%)	5.02	2.57	15.7 ± 0.7	27.7 ± 1.3

^a The values in parentheses represent the proportion of the identified component in bulk DOC. ^b The values in parentheses represent the proportion of the identified component in bulk POC. ^c u.d. denotes under detection.

consistent with the earlier recognition that marine biological production was the major source of POM in Daya Bay.²² The spatial distribution of POM from different sources indicated that the contents of tPOC and bPOC in the inner bay were higher than those in the outer bay, and the tPOC and bPOC contents in surface water were generally lower than those of bottom water (Fig. 4e and f). The concentrations of bulk POC and bPOC were significantly different among the four incubation stations ($p < 0.05$). The bPOC concentrations at Stations S2 and S12 were, on average, 3.8 times higher than those at Stations S8 and S13, while the tPOC was less variable except for the higher concentrations observed at Station S12 (Table 1).

Variability of denitrifying activity

Except for the surface at Station S12, the ADRs at the four incubation stations ranged from 6.3 ± 1.2 to 107.1 ± 35.5 nmol N₂ per L per h with an average of 36.4 nmol N₂ per L per h (Table 1). These ADRs were at the upper end of the reported denitrification rates in typical marine suboxic waters,^{39–41} indicating an intense potential denitrification in the bay. The highest denitrifying activity was observed at Station S2, and its ADR was about 6 times higher than that at other stations. After normalizing to the NO₃⁻ concentration, the NDRs at Stations S2 and

S12 were close to the ADRs, while the NDRs at Stations S8 and S13 were 2–3 times greater than the ADRs (Table 1). There was no significant correlation between the NDR and bulk DOC or bulk POC ($p > 0.2$), indicating that the quantity of organic matter alone could not explain the denitrifying variability in Daya Bay, and its control factors were more complicated.

Discussion

Effect of DOM from different sources on denitrification

When NO₃⁻ is abundant in the environment, the availability of organic matter usually becomes a limitation to denitrifying activity.^{6,7,42} In most heterotrophic bacteria, organic substrates are directly utilized in dissolved form through transmembrane transport.⁴³ Thus, DOC is reasonably assumed to be the primary carbon source for denitrification. In a recent study at the oxic–anoxic interface in the Baltic Sea, it was proposed that DOC might be the main driver of water column denitrification.¹² However, the bulk DOC concentration cannot adequately explain the potential denitrification changes in Daya Bay. In fact, the lack of positive stimulation of denitrification by DOC was sometimes observed in land-based aquatic ecosystems, where the ambient DOC loading was relatively high.^{44,45}



Fig. 5 The relationship between tFDOC and salinity (a) and between bFDOC and Chl-a (b) in Daya Bay.



Fig. 6 The relationship between concentration of bPOC and *Chl-a* (a) and between proportion of bPOC and *Chl-a* (b) in Daya Bay.

According to the classic stoichiometry of the denitrification reaction,² the amount of organic carbon required for complete nitrogen removal was estimated to be only 7.0 $\mu\text{mol C per L}$ in Daya Bay, which is less than 10% of the bulk DOC. In addition, as reported for the anoxic Baltic Sea, the half-saturation constant for DOC demand of denitrification was only $<1 \mu\text{mol L}^{-1}$, suggesting high affinity of heterotrophic denitrification for DOC.¹² This means that the supply of DOC in our experiments was sufficient for denitrification, and the bulk DOC concentration might not be the primary factor limiting denitrification.

DOC quality, not just quantity, is increasingly recognized as an important factor in regulating denitrification. For example, laboratory incubations with substrate additions have shown that simple organic molecules such as acetate and glucose can greatly stimulate denitrifying activity in a wide range of habitats, including river sediments,^{5,46} hyporheic zone of land streams⁷ and typical marine oxygen-depleted waters.^{11,12} However, the composition of natural DOM is far more complex than the ones used in experiments. Based on fluorescence spectra and PARAFAC modeling, the DOM in our study area was divided into a fluorescent fraction and non-fluorescent fraction, of which the former includes terrigenous and marine autochthonous moieties. In general, organic matter from terrestrial sources is usually rich in lignin or humic substances and thus is more recalcitrant and energetically less favorable to break down compared with the moieties from algal autochthonous production.^{35,47} However, in our study, the NDR broadly positively correlated with the concentration or proportion of tFDOC although not significant ($r = 0.64$, $p = 0.06$) (Fig. 7a and b), which is much better than the relationship between the NDR and bFDOC ($r = 0.44$, $p > 0.2$). It seems that tFDOC may be more beneficial for denitrifying degradation than bFDOC in the bulk DOC pool. Similarly, previous studies also showed that typical terrestrial organic matter, especially aromatic, humic or fulvic substances, can maintain or even stimulate the denitrifying activity of certain bacterial strains (e.g., Nozawa and Maruyama, 1988; Pfenning and McMahon, 1996).^{46,48} Actually, the terrestrial DOM is not necessarily refractory. In a study from boreal lakes, it was revealed that the absolute amount of the labile fraction in a terrestrial DOC pool was even larger than that in an

autochthonous algal DOC pool, and the former part fueled not only short-term but also long-term microbial carbon consumption in ecosystems.⁴⁹

Our result was somewhat inconsistent with that of Barnes *et al.* (2012),⁴² whose study depicted a negative correlation between denitrification and the percentage of terrigenous quinone-type components, and a positive correlation between denitrification and the percentage of biogenic amino acids, in the sediments of Boulder Creek watershed. On the one hand, the inconsistency could be probably caused by the distinct environmental media between stream sediments and marine waters, in which denitrification rates were measured. On the other hand, different correlations between studies may also result from a difference in DOM properties. In Barnes *et al.*'s (2012) study,⁴² their sampling areas ranged from alpine to plains with relatively low vegetation coverage and the ecosystem was primitive. Unlike the Boulder Creek watershed, a variety of highly productive habitats exist along the coast of Daya Bay, especially mangroves and intertidal mudflats.²¹ Furthermore, a large amount of anthropogenic organic input has had a great impact on the Daya Bay ecosystem.²⁵ It is well documented that the land use and vegetation cover markedly influence the quantity and quality of DOM delivered into the adjacent watersheds or aquatic systems.⁵⁰ For example, the DOM originating from soils with higher organic share is recognized to be more labile for microbial degradation.⁵¹ In addition, the source and quality of DOM would have an impact on the patterns of microbial metabolism. It was proposed that humic DOC from forest headstreams generally supports bacterial growth instead of their respiration in freshwater systems.⁵² Therefore it is reasonable to speculate that the terrestrial DOM entering Daya Bay may be more degradable or favorable for microbial respiration than that entering the Boulder Creek watershed, thus favoring denitrification in Daya Bay. With regard to autochthonous components, they are mainly recognized as protein-like substances (or amino acids) and are generally considered to be bio-favorable. The lack of correlation between the NDR and bFDOC in Daya Bay can be attributed to the low concentration of biogenic components. For example, the bFDOC only accounts for a minor fraction (average 5%) of the bulk DOC in



Fig. 7 The relationship between NDR and DOM components from different sources in Daya Bay: (a) NDR versus tFDOC; (b) NDR versus proportion of tFDOC; (c) NDR versus proportion of nFDOC.

seawater samples from the four NDR stations (Table 1). Due to the deficiency of autochthonous DOM, its regulation on denitrifying activity was limited. It should be pointed out that photochemical processes can oxidize DOC to CO₂ and consume O₂ in aqueous environments.⁵³ Thus, it is possible that the tFDOC follows these reactions and creates a low O₂ condition to stimulate denitrifying activity instead of its degradation for denitrification. However, dissolved O₂ concentrations in the water column during our cruise were generally higher than 150 μmol L⁻¹ (refer to the Results section), which is far exceeding the typical O₂ threshold of 2 μmol L⁻¹ for denitrification.³⁷ In other words, the photochemical oxygen consumption of tFDOC, if any, may only have little effect on enhancing denitrification potentials in Daya Bay. On the other hand, some other research studies even suggested that dissolved O₂ at nanomolar levels suppresses denitrifying N₂ production and the related gene expressions.⁵⁴

In our study, a negative correlation, although not significant ($r = -0.60, p = 0.07$), between the NDR and the proportion of non-fluorescent DOC (nFDOC) was observed concurrently (Fig. 7c). It implies that nFDOC may be less degradable for denitrifying bacteria relative to tFDOC and bFDOC. Even though the chemical properties and biodegradability of the non-fluorescent DOM fraction remain poorly characterized, black carbon, produced from incomplete combustion of fossil fuel and biomass and primarily composed of condensed aromatics,⁵⁵ is recognized as one of the most chemically recalcitrant fractions of organic carbon in aquatic environments.⁵⁶ The riverine transport significantly contributes black carbon from terrestrial sources to the oceans.^{57,58} It has been shown that dissolved black carbon in aquatic systems is very susceptible to partial photooxidation and its fluorescence is greatly reduced under light exposure.⁵⁹ Therefore, the partially photooxidized dissolved black carbon should probably account for the non-fluorescent DOM in our study area. However, dissolved black carbon in marine ecosystems usually represents a minor proportion of bulk DOC.⁶⁰ In a recent study, it has been shown that dissolved black carbon in coastal waters of China only accounted for less than 5% of DOC pools.⁶¹ If this fraction is used for a first-order calculation, the maximum concentration of dissolved black carbon in Daya Bay is estimated to be 12 μmol C per L, which is far from explaining the identified nFDOC concentration of 81 μmol

L⁻¹. Although there are still many unknowns for the non-fluorescent DOM, our results show for the first time that non-fluorescent DOM has an impact on regulating denitrification in coastal environments.

Effect of POM from different sources on denitrification

A negative trend between the NDR and terrigenous POC (tPOC) concentration ($r = -0.55, p = 0.10$) and a positive trend between the NDR and autochthonous POC (bPOC) concentration ($r = 0.63, p = 0.06$) were observed (Fig. 8a and b), although both of them lack statistical significance. In comparison, there was a significant and positive correlation between the NDR and the bPOC fraction in the bulk POC ($r = 0.86, p = 0.01$) (Fig. 8c), similar to what was observed recently in the Beibu Gulf of southern China.¹³ These relationships suggest that marine autochthonous POM not only favors denitrification reactions, but also plays a dominant role in regulating denitrifying activity in coastal environments. Similar findings have been reported for other terrestrial ecosystems. For example, Dodla *et al.* (2008)⁶² observed in wetland soils that the measured denitrification rate increased linearly with increasing labile polysaccharide content, but was negatively correlated with the percentage of recalcitrant phenolic carbon in the total organic carbon pool. In addition, POM with low C/N ratio induced higher denitrifying activity in wetland and stream sediments,^{14,63} pointing to the effectiveness of labile POM. In contrast, terrigenous POM is usually a mixture of plant debris and degraded organic matter, characterized by high contents of lignin or cellulose components with a higher C/N ratio, and thus is more resistant to biodegradation⁴⁷ and less effective in promoting denitrification.

There are two pathways for POM to regulate denitrification in water columns. One is to directly affect the number of particle-associated denitrifiers (*e.g.*, Liu *et al.*, 2013),⁶⁴ and the other is to affect the supply of organic matter for the growth of ambient free-living denitrifiers (*e.g.*, Stelzer *et al.*, 2015).¹⁴ In aquatic systems, bacteria preferentially adhere to suspended particles or aggregates,⁶⁵ where suboxic or anoxic micro-sites usually exist and favor anaerobic respiration.^{17,18} It was proposed that SPM and aggregates in seawater could be hotspots for denitrification and may contribute significantly to global N removal.^{16,38,64} In order to better reveal the coupled effect of



Fig. 8 The relationship between NDR and POM components from different sources in Daya Bay: (a) NDR versus tPOC; (b) NDR versus bPOC; (c) NDR versus proportion of bPOC; (d) NDR versus bPOC/SPM ratio.

particulate matter on denitrifying activity, the bPOC concentration was normalized to SPM content to gauge the POM quality in terms of $\mu\text{mol C}$ per mg particles. Compared with the relationship between the NDR and non-normalized bPOC, the significance of the positive correlation between the NDR and SPM-normalized bPOC (bPOC/SPM) was significantly improved, and the bPOC/SPM parameter can explain about 70% of the variability of the NDR (Fig. 8d). In other words, the component of POM or the relative organic carbon content in SPM is a more effective factor in controlling denitrification in coastal environments.

In addition to DOM and POM, the denitrification rate correlated to single cell activity and bacterial abundance. For example, the denitrifier amount per gram of particles in the turbid river water positively correlated with the organic carbon content in suspended particles.⁶⁴ Obviously, particulate matter with high organic carbon should facilitate the attachment of denitrifying bacteria and subsequent denitrification.

If multiple linear regressions were applied using both bPOC (in $\mu\text{mol C}$ per L) and SPM (in mg L^{-1}) as independent variables, the NDR can be well predicted in Daya Bay with a better predictive power ($r^2 = 80\%$; $p = 0.02$):

$$\text{NDR} = 45.1 + 2.77 \times \text{bPOC} - 10.6 \times \text{SPM} \quad (12)$$

It can be seen from the regression that the NDR exhibits a positive correlation with bPOC and a negative correlation with SPM with both of their coefficients being significant ($p \leq 0.05$). Therefore, high SPM but low bPOC will not enhance denitrifying activity. In other words, it is the quality of SPM that is essential for promoting denitrification. A recent study has found that the bottom nepheloid layer in coastal marine environments may be a potential hotspot for N removal, and sediment resuspension is likely the key mechanism promoting denitrification.¹³ In Daya Bay, the SPM contents in the bottom layers were higher than those in the surface layers, indicating the prevalence of sediment resuspension. Resuspension of sediments provides large amounts of particles and likely denitrifying bacteria into the overlying water column, which may increase the abundance of particle-associated denitrifiers and denitrifying activity.^{13,64} However, denitrification was observed to be inhibited by the abundant suspended particles derived from sediment resuspension (eqn (12)). Our hypothesis is that lithogenic materials introduced by sediment resuspension could dilute the organic matter content in the SPM, and reduce the energy source for the denitrification reaction. Therefore, both POC composition (especially the autochthonous component) and SPM are important factors in regulating coastal denitrifying activity, although in different directions, in highly



Fig. 9 Conceptual diagram showing the role of organic matter in regulating denitrification in Daya Bay.

turbid and productive ecosystems. These two parameters should be incorporated into future modeling and taken into consideration in future studies.

The relative role of DOM vs. POM in regulating denitrification

As discussed above, POM plays a more dominant role than DOM in promoting denitrifying activity in coastal environments. However, DOM has been shown to be the dominant organic matter pool and outweighs POM in seawater (e.g., Guo *et al.*, 1995).⁶⁶ Our observations that denitrifiers preferentially utilize POM over DOM by extracellular enzymes are seemingly contradictory. One plausible reason is that POM has a larger labile carbon pool than DOM. Indeed, bPOC was the major biogenic organic pool with an average concentration ($27.3 \mu\text{mol L}^{-1}$) three times as high as that of bFDOC (an average of $8 \mu\text{mol L}^{-1}$) in Daya Bay (Table 1). We also found a positive linear relationship between bFDOC and bPOC ($r = 0.71$, $p < 0.0002$, $n = 22$) throughout the bay, indicating that bPOC is a major source of bFDOC, consistent with the carbon flow direction from particulate to colloidal to dissolved phases observed in marine environments.^{67,68} Additionally, fitting eqn (12) with the concentration or proportion of DOM components did not give rise to a significant regression. Thus, both DOM and its components did not seem to be relevant parameters for predicting the spatial distribution of denitrification in coastal waters. This is consistent with previous studies documenting that in estuarine and coastal systems, DOM typically contains more aged-carbon and higher C/N ratio, while the carbon in

POM is often characterized as that of modern age and with lower C/N ratio.^{67,69} This implied that DOM contains more reworked or refractory organic materials while POM is fresher and likely more labile, which could enhance denitrification in the water column (Fig. 9).

Conclusions

In this study, the relative importance of DOM vs. POM and the influence of different organic matter components on aquatic denitrification were investigated. Our results indicate that in coastal environments the composition of POM has a substantial impact on potential denitrification activity, while DOM and its components have little effect on it. Based on the results of multiple linear regressions, we further found that combining both SPM and autochthonous POC abundance can better predict the variability of denitrification in the study area. Therefore, both bPOC and SPM should be taken into account as variables when modelling denitrifying N removal, especially in highly turbid and productive aquatic systems. Although the water column in the study area was dominantly oxygenated and the measured denitrification rates were merely potential, our novel findings here provide new insight into the coupled effects of DOM–POM–SPM on coastal denitrification and the controlling mechanisms of marine nitrogen cycling. Further studies are needed to elucidate seasonal changes in the relationship between the denitrification rate and natural organic matter composition and to test our hypothesis. In addition, similar investigations should be conducted in typically oxygen-depleted

marine waters to better understand the effects of organic matter composition on denitrification.

Author contributions

M. Chen and J. Zeng co-designed the study. J. Zeng and L. Fan were responsible for carrying out ^{15}N -labelling incubations on board and the measurement of ^{15}N - N_2 production in laboratory. H. Lin was responsible for DOM sampling and analysis. X. Mu was responsible for POM sampling and analysis. M. Zheng and Y. Qiu contributed the experimental tools. J. Zeng, M. Chen and L. Guo co-wrote the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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