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Supplement of
Multiple nitrogen sources for primary production inferred from $\delta^{13}$C and $\delta^{15}$N in the southern Sea of Japan

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Introduction S1.
The linear relationships between $\delta^{13}\text{C}_{\text{POM}}$ or $\delta^{15}\text{N}_{\text{POM}}$ and temperature, salinity, log-transformed nitrate concentration, log-transformed chlorophyll-$\alpha$ concentration, C: N ratio, latitude, and longitude were not shown in the main text because $\delta^{13}\text{C}_{\text{POM}}$ or $\delta^{15}\text{N}_{\text{POM}}$ did not show the normal distributions. However, the relationships were the basic information, and thus we showed them in the supporting information.

Text S1.
When the classification of $\delta^{13}\text{C}_{\text{POM}}$ and $\delta^{15}\text{N}_{\text{POM}}$ was ignored, the linear relationships between $\delta^{13}\text{C}_{\text{POM}}$ and temperature, salinity, log-transformed nitrate concentration, log-transformed chlorophyll-$\alpha$ concentration, C: N ratio, and latitude were significant ($p < 0.001$, DF = 505). However, the relationship between $\delta^{13}\text{C}_{\text{POM}}$ and longitude was insignificant ($p = 0.33$, DF = 505) (Fig. S1a–g). The most robust relationship was observed between $\delta^{13}\text{C}_{\text{POM}}$ and temperature ($r^2 = 0.314$, $F$-value = 231, $p < 0.001$), with $\delta^{13}\text{C}_{\text{POM}}$ increasing with warming (Fig. S1a). The second most robust relationship was observed between $\delta^{13}\text{C}_{\text{POM}}$ and log-transformed nitrate concentration ($r^2 = 0.252$, $F$-value = 171, $p < 0.001$), $\delta^{13}\text{C}_{\text{POM}}$ high in low-nitrate water (Fig. S1c). A weak ($r^2 < 0.05$) but significant relationship was observed between $\delta^{13}\text{C}_{\text{POM}}$ and log-transformed chlorophyll-$\alpha$ concentration, C: N ratio, and latitude (Fig. S1d–f). The relationship between $\delta^{13}\text{C}_{\text{POM}}$ and nitrate concentration, which was not log-transformed, was also significantly negative ($r^2 = 0.172$; $F$ = 106).

In the case of $\delta^{15}\text{N}_{\text{POM}}$, significant linear relationships were observed between temperature, salinity, log-transformed nitrate concentration, and latitude ($p < 0.002$, DF = 505) but not between log-transformed chlorophyll-$\alpha$ concentration, C: N ratio, and longitude (Fig. S1h–n).

Compared to $\delta^{13}\text{C}_{\text{POM}}$, the relationships were weak; the detection coefficient ($r^2$) was always < 0.05. The relationship with logarithm-transformed nitrate concentration was negative ($r^2 = 0.011$, $F$-value = 6.42). Moreover, this relationship was similar when nitrate concentration was not transformed ($r^2 = 0.023$, $F$-value = 13.2). Positive and negative relationships were observed for temperature ($r^2 = 0.016$, $F$-value = 9.45) and salinity ($r^2 = 0.02$, $F$-value = 11.5).

These relationships have ignored the classification; class I, whose $\delta^{15}\text{N}_{\text{POM}}$ was significantly lower than the other classes (II–IV), appeared as outliers in the relationships. When data classified into class I was removed, significant relationships between $\delta^{15}\text{N}_{\text{POM}}$ and environmental parameters were only observed for salinity and latitude ($p < 0.001$).

Fig. S1. Relationships between $\delta^{13}\text{C}_{\text{POM}}$ (a–g) or $\delta^{15}\text{N}_{\text{POM}}$ (h–n) and environmental parameters (temperature, salinity, nitrate concentration, chlorophyll-$\alpha$ concentration, C: N ratio, latitude, and longitude). The color and shape differences indicate classes divided based on $\delta^{13}\text{C}_{\text{POM}}$ and $\delta^{15}\text{N}_{\text{POM}}$. Solid lines with shadows indicate significant regression lines with 95% confidence intervals, whereas dotted lines indicate insignificant regression lines.
**Introduction S2.**
Simulation results are not shown in the main text. The simulations were conducted with random numbers, and thus the results were different among the simulations.

**Text S2.**
Here, two types of simulation of the relationship between the $\delta^{15}$NPOM and nitrate concentration were performed. The difference between the two is only the $\delta^{15}$NNO$_3$ range: the first one was set as 0–8.3‰ (Umezawa et al., 2014; Umezawa et al., 2021), and the second one was set as 5–6‰, which was the representative $\delta^{15}$NNO$_3$ in the water originating from the Kuroshio (Umezawa et al., 2014; Umezawa et al., 2021). The other parameter was the same between the two simulations, that is, the kinetic isotope effects of nitrate assimilation ($\varepsilon$NO$_3$) is 3‰ (Sigman et al., 2009), the initial nitrate concentration ([NO$_3$$_{ini}$]) to 0.05–10 μM, and the fraction of remaining nitrate ($F$NO$_3$) was set as 0–0.5. We assumed the open system and thus $\delta^{15}$NPOM originated from the nitrate ($\delta^{15}$NPOM-NO$_3$) was calculated from the equation of Sigman et al. (2009) ($\delta^{15}$NPOM-NO$_3$ = $\delta^{15}$NNO$_3$ – $\varepsilon$NO$_3$ × $F$NO$_3$). Regenerated nitrogen such as ammonium also supports primary production, but the kinetic isotope effect of nutrient recycling is not clear (Sigman et al., 2009), and $\delta^{15}$N of ammonium depends on $\delta^{15}$NPOM. Thus, it was possible to regard that $\delta^{15}$NPOM based on the regenerated production is as the same as $\delta^{15}$NPOM based on the new production. To consider the regenerated production, the $F$NO$_3$ was set as <0.5. Here, nitrate is not only the nitrogen supporting new production. Nitrogen fixation is also an important process in the SOJ and contributed ~3.8% to primary production (Sato et al., 2021). In the SOJ, the contribution of nitrogen fixation to new production ($f$N$_2$) was not reported, but that in the East China Sea was reported as 10–82% in summer (Liu et al., 2013). $\delta^{15}$NPOM produced with nitrogen fixation ($\delta^{15}$NPOM-N2) was set at -2.1–0.8‰ (Minagawa and Wada, 1986). In hence, $\delta^{15}$NPOM was calculated as the following equation.

\[ \delta^{15}\text{NPOM} = (1-f\text{N}_2) \times (\delta^{15}\text{NNO}_3 - \varepsilon\text{NO}_3 \times F\text{NO}_3) + f\text{N}_2 \times \delta^{15}\text{NPOM-N2} \]

(1)

Except with $\varepsilon$NO$_3$, these values varied randomly between the setting ranges, and 500 data points were prepared to identify each linear relationship between $\delta^{15}$NPOM and remnant nitrate concentration (= [NO$_3$$_{ini}$] × $F$NO$_3$). To identify the proportions of the appearance of a significant relationship between the $\delta^{15}$NPOM and nitrate concentration, we conducted each simulation 1000 times. The one representative results of the first simulation ($\delta^{15}$NNO$_3$ range: 0–8.3‰) denote that the relationship between $\delta^{15}$NPOM and nitrate concentration was weak and insignificant ($n = 500$, $p = 0.789$, Fig. S2a). Since the data point was set at random, approximately seven of ten times showed an insignificant ($p > 0.05$) relationship between $\delta^{15}$NPOM and nitrate concentration (Fig. S2b). On the other hand, in the second simulation ($\delta^{15}$NNO$_3$ range: 5–6‰), the relationship between $\delta^{15}$NPOM and nitrate concentration showed a significant negative relationship ($n = 500$, $p < 10^{-16}$, Fig. S2c). While the nitrogen fixation contribution was the same in the first simulation, the relationship between $\delta^{15}$NPOM and nitrate concentration was always significant ($p < 0.05$, Fig. S2d).

This $f$N$_2$ based on Liu et al. (2013) may be much higher in the actual contribution of nitrogen fixation to $\delta^{15}$NPOM, and when $f$N$_2$ was set as low, the relationship between $\delta^{15}$NPOM and nitrate concentration became stronger than high $f$N$_2$ case (Fig. S2e). Thus, we set $f$N$_2$ as 1.9–5.8% which were corresponding to the contribution of nitrogen fixation to primary production (Liu et al., 2013). Even in this case, an insignificant relationship between $\delta^{15}$NPOM and nitrate concentration was observed approximately 30% of the trials (Fig. S2f). This indicated that a wide range of $\delta^{15}$NNO$_3$ values contributed to an unclear relationship between $\delta^{15}$NPOM and nitrate concentration, which is observed in our study conducted in the SOJ.
The results of the simulations when the $\delta^{15}$N$_{NO_3}$ varies 0 – 8.3‰ and the contribution of nitrogen fixation ($f_{N_2}$) was 10–82% (a and b), those $\delta^{15}$N$_{NO_3}$ varies 5 – 6‰ and $f_{N_2}$ was 10–82% (c and d), and those $\delta^{15}$N$_{NO_3}$ varies 0 – 8.3‰ and $f_{N_2}$ was 1.9–5.8% (e and f). (a), (c) and (e) denote the representative result of their relationship of each simulation, and (b), (d) and (f) were the histograms of the $p$-values of simulations repeated 1000 times. The open bar in (b), (d) and (f) denote the $p$-values were $\geq 0.05$ (significant), and the closed bars denote $p$-values were $>0.05$.

**Fig. S2.**

**References**


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