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

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Introduction S1.

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

In the case of $\delta^{15}N_{POM}$, 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-*a* concentration, C: N ratio, and longitude (Fig. S1h–n). Compared to $\delta^{13}C_{POM}$, 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 $\delta^{15}N_{POM}$ 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 $\delta^{15}N_{POM}$ and environmental parameters were only observed for salinity and latitude ($p < 0.001$).

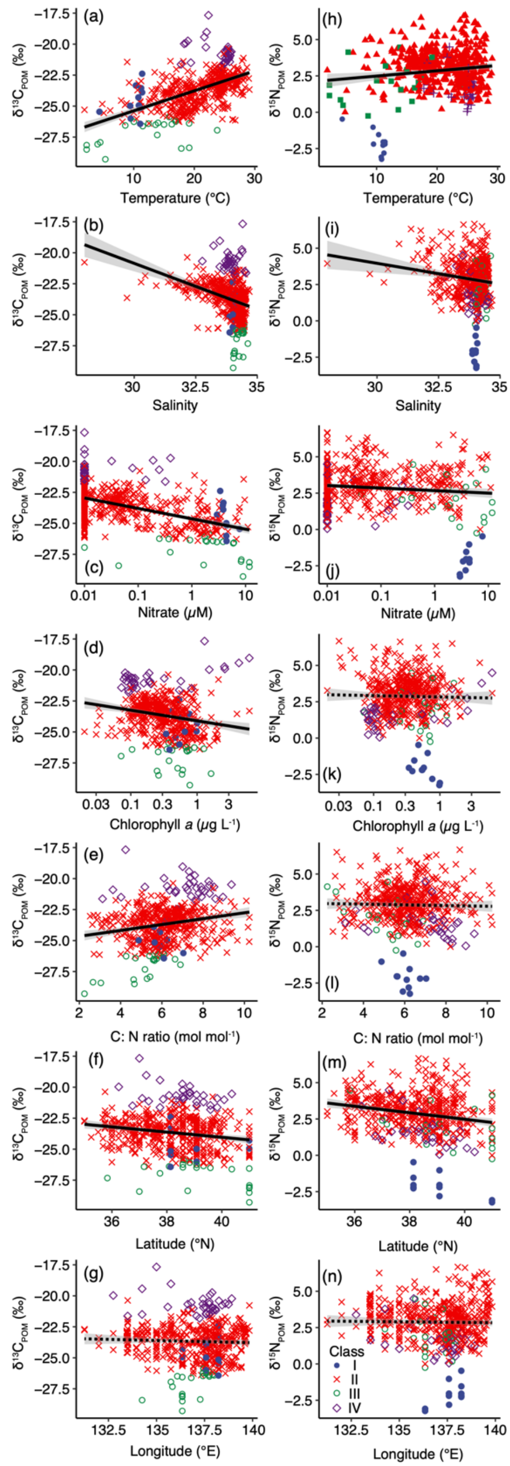

Fig. S1. Relationships between $\delta^{13}C_{POM}$ (a–g) or $\delta^{15}N_{POM}$ (h–n) and environmental parameters (temperature, salinity, nitrate concentration, chlorophyll-a concentration, C: N ratio, latitude, and longitude). The color and shape differences indicate classes divided based on $\delta^{13}C_{POM}$ and $\delta^{15}N_{POM}$. 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 $\delta^{15}N_{POM}$ and nitrate concentration were performed. The difference between the two is only the $\delta^{15}N_{NO3}$ range: the first one was set as 0–8.3‰ (Umezawa et al., 2014; Umezawa et al., 2021), and the second one was set it as 5–6‰, which was the representative $\delta^{15}N_{NO3}$ 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 (ε_{NO3}) is 3‰ (Sigman et al., 2009), the initial nitrate concentration ($[NO3_{ini}]$) to 0.05–10 µM, and the fraction of remaining nitrate (F_{NO3}) was set as 0–0.5. We assumed the open system and thus $\delta^{15}N_{POM}$ originated from the nitrate ($\delta^{15}N_{POM\text{-}NO3}$) was calculated from the equation of Sigman et al. (2009) ($\delta^{15}N_{POM\text{-}NO3} = \delta^{15}N_{NO3} - \varepsilon_{NO3} \times F_{NO3}$). 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 $\delta^{15}N_{POM}$. Thus, it was possible to regard that $\delta^{15}N_{POM}$ based on the regenerated production is as the same as $\delta^{15}N_{POM}$ based on the new production. To consider the regenerated production, the F_{NO3} 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_{N2}) was not reported, but that in the East China Sea was reported as 10–82% in summer (Liu et al., 2013). $\delta^{15}N_{POM}$ produced with nitrogen fixation ($\delta^{15}N_{POM\text{-}N2}$) was set at -2.1–0.8‰ (Minagawa and Wada, 1986). In hence, $\delta^{15}N_{POM}$ was calculated as the following equation.

$$\delta^{15}N_{POM} = (1 - f_{N2}) \times (\delta^{15}N_{NO3} - \varepsilon_{NO3} \times F_{NO3}) + f_{N2} \times \delta^{15}N_{POM\text{-}N2} \quad (1)$$

Except with ε_{NO3}, these values varied randomly between the setting ranges, and 500 data points were prepared to identify each linear relationship between $\delta^{15}N_{POM}$ and remnant nitrate concentration (= $[NO3_{ini}] \times F_{NO3}$). To identify the proportions of the appearance of a significant relationship between the $\delta^{15}N_{POM}$ and nitrate concentration, we conducted each simulation 1000 times. The one representative results of the first simulation ($\delta^{15}N_{NO3}$ range: 0–8.3‰) denote that the relationship between $\delta^{15}N_{POM}$ 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 $\delta^{15}N_{POM}$ and nitrate concentration (Fig. S2b). On the other hand, in the second simulation ($\delta^{15}N_{NO3}$ range: 5–6‰), the relationship between $\delta^{15}N_{POM}$ 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 $\delta^{15}N_{POM}$ and nitrate concentration was always significant ($p < 0.05$, Fig. S2d).

This f_{N2} based on Liu et al. (2013) may be much higher in the actual contribution of nitrogen fixation to $\delta^{15}N_{POM}$, and when f_{N2} was set as low, the relationship between $\delta^{15}N_{POM}$ and nitrate concentration became stronger than high f_{N2} case (Fig. S2e). Thus, we set f_{N2} 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 $\delta^{15}N_{POM}$ and nitrate concentration was observed approximately 30% of the trials (Fig. S2f). This indicated that a wide range of $\delta^{15}N_{NO3}$ values contributed to an unclear relationship between $\delta^{15}N_{POM}$ and nitrate concentration, which is observed in our study conducted in the SOJ.

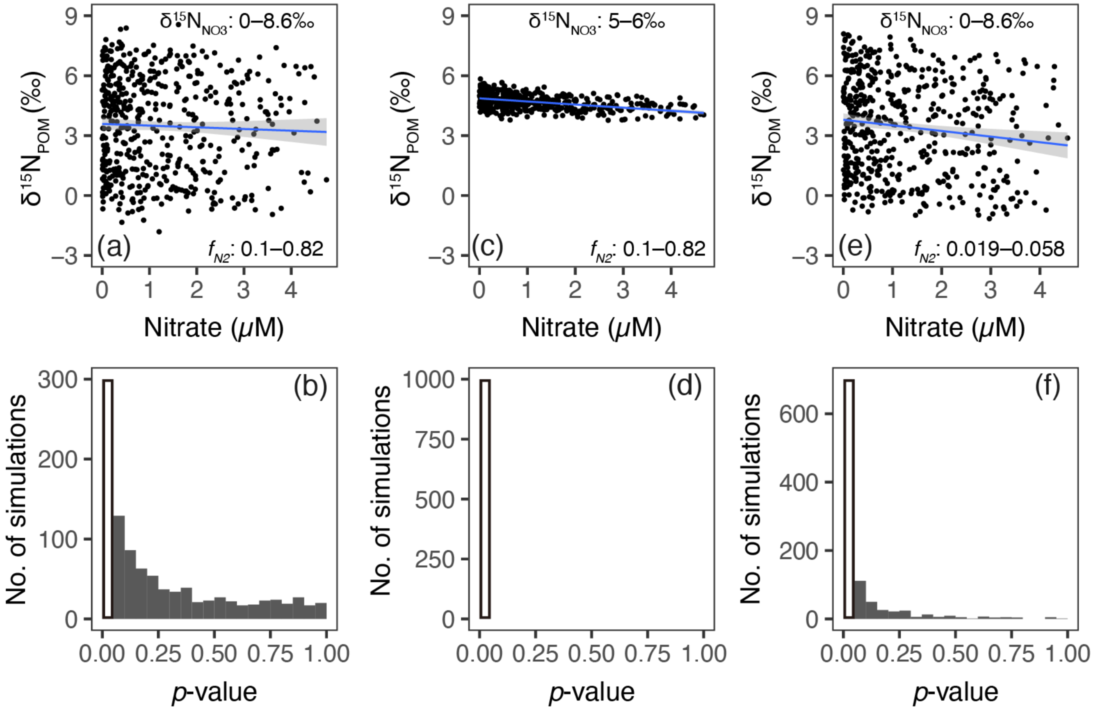

Fig. S2. The results of the simulations when the $\delta^{15}N_{NO3}$ varies 0 – 8.3‰ and the contribution of nitrogen fixation (f_{N2}) was 10–82% (a and b), those $\delta^{15}N_{NO3}$ varies 5 – 6‰ and f_{N2} was 10–82% (c and d), and those $\delta^{15}N_{NO3}$ varies 0 – 8.3‰ and f_{N2} was 1.9–5.8% (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 ≥0.05 (significant), and the closed bars denote *p*-values were >0.05.

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