Towards resolving disparate accounts of the extent and magnitude of nitrogen fixation in the Eastern Tropical South Pacific oxygen deficient zone

Supplementary materials

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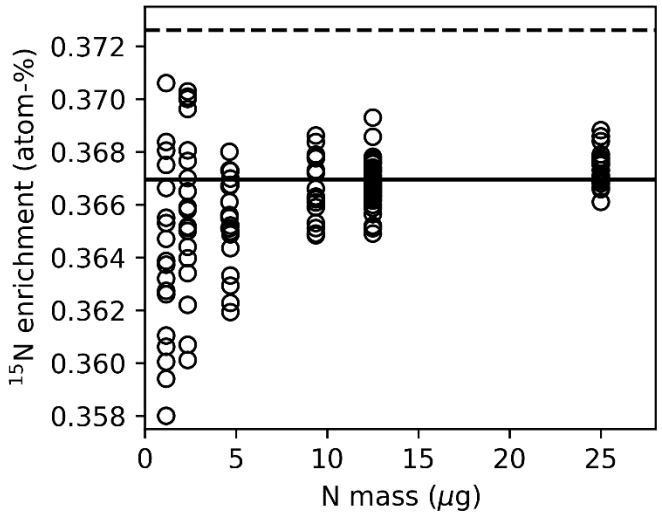
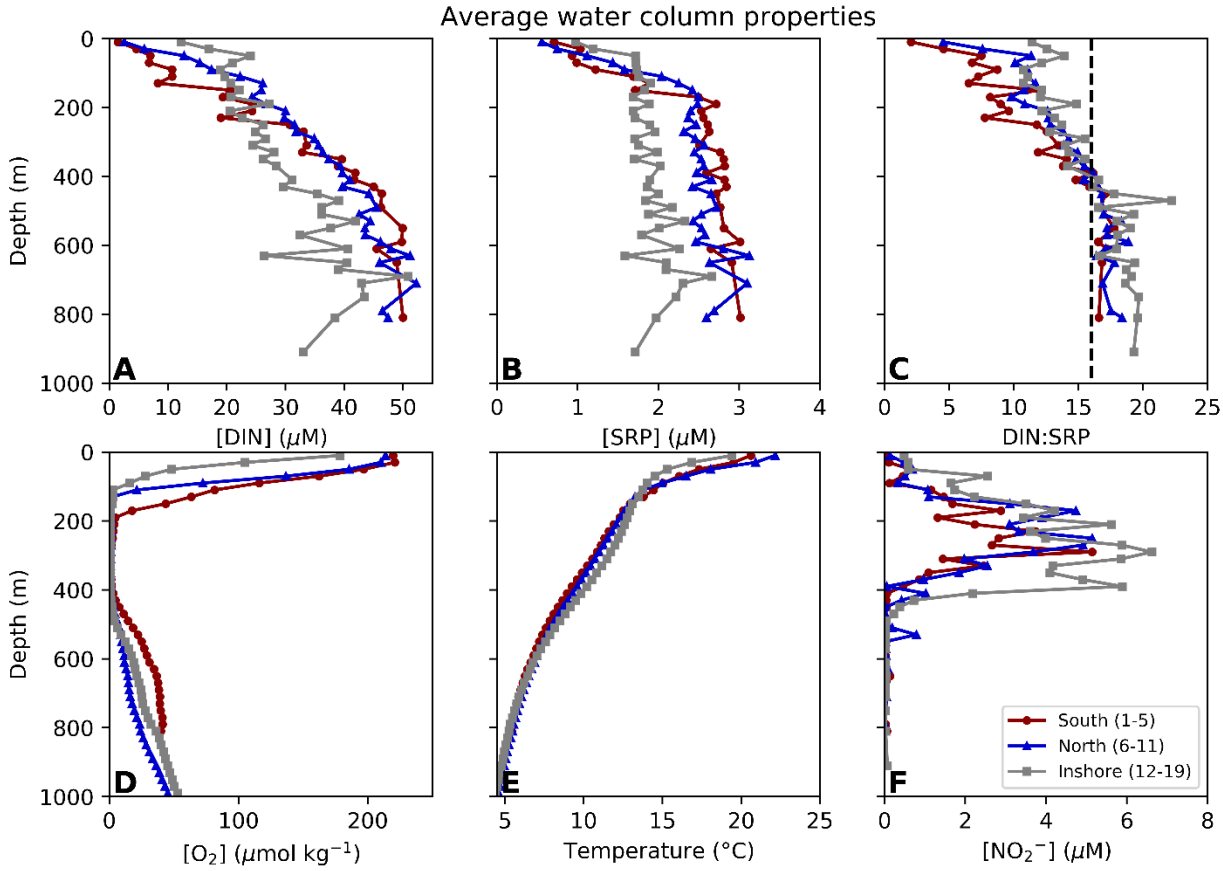
Running title: Oxygen deficient zone nitrogen fixation

Suppl. Text 1. There remains little consensus on how to quantify NFR uncertainty, which is important for any study aiming to assess differences among low rates (e.g., analyze low NFRs with respect to hydrographic characteristics). Simply taking the standard deviation of rates from replicate incubations does not constrain variability in the initial (and independent) ^{15}N-PN enrichment measurement, which may significantly influence whether rates are deemed detectable and the magnitude of rates when ^{15}N enrichment at the final time point is low. An alternative approach for calculating NFR error is to propagate the analytical error associated with the five component measurements in Eqn. 1—source pool (N_2) ^{15}N enrichment, initial and final target pool (PN) ^{15}N enrichment, PN concentration, and time—following traditional statistical approaches in analytical chemistry (Miller and Miller 1988). This value is sometimes applied as a "minimum quantifiable rate" (e.g., Gradoville et al. 2017) or alternate LOD (White et al. 2020). If using the bubble removal technique, analytical error must be propagated first for each incubation individually because source pool (N_2) enrichment can vary among replicate incubations, affecting final target pool (PN). In this study, N_2 enrichment was measured only once for each incubation due to the prohibitive cost of the analysis. Additionally, difficulties with achieving sufficient filter N mass often precluded replication of $A_{PN_{t=0}}$ measurements. In some cases, a mean value was applied (see above). Consequently, we could not accurately assess the analytical error of each measurement individually.

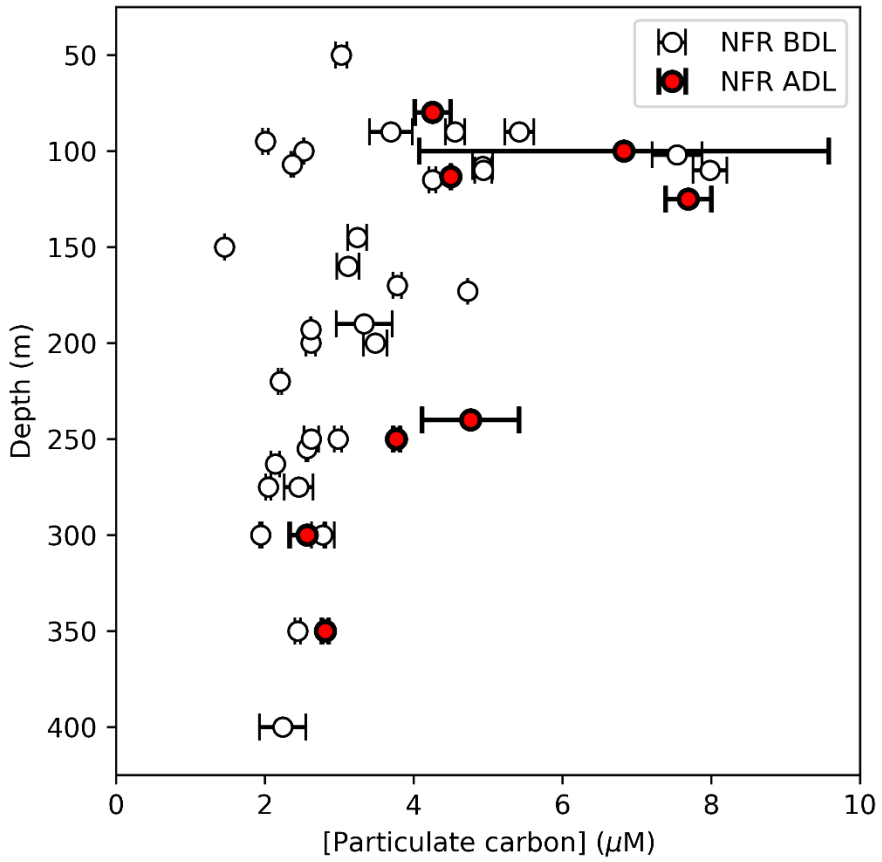
Suppl. Text 2. Non-diazotrophic N cycling processes may theoretically affect $A_{PN_{t=f}}$ (White et al. 2020). We conducted control incubations (no $^{15}N_2$ addition) at three depths at station 1 and several locations around the ETNP ODZ on a cruise in 2017 (Suppl. Table 3). $A_{PN_{t=f}}$ was greater than $A_{PN_{t=0}}$ at five of seven locations with a mean change in enrichment of 0.0009 atom-%. The difference between control incubation $A_{PN_{t=f}}$ and $A_{PN_{t=0}}$ never exceeded the minimum detectable difference in enrichment (3σ, n = 7 12.5 µg standards), meaning that it did not represent a detectable change. However, given the potential significance of small changes in enrichment to the detection/calculation of low NFRs, we advise that future studies attempting to detect low NFRs consider conducting incubation controls more extensively.



Suppl. Fig. 1. Isotope ratio mass spectrometer linearity at low mass. Open dots represent low mass (≤ 25 µg N) ammonium sulfate standards from all standard curves (run daily) measured during sample analysis. Solid and dashed lines represent, respectively, the standard enrichment (0.367 atom-%) and the standard enrichment plus the mean detectable difference ($A_{PNt=f} - A_{PNt=0}$) i.e., the mean LOD (the standard deviation of all 12.5 µg standards analyzed alongside samples multiplied by 3).



Suppl. Fig. 2. Profiles of DIN concentration (A), SRP concentration (B), DIN:SRP (C), O_2 concentration (D), temperature (E), and NO_2^- concentration (F) averaged at 20 m intervals for southern (1-5), northern (6-11) and inshore (12-19) stations. The black dashed line in panel C marks the canonical Redfield ratio (16:1).



Suppl. Fig. 3. Particulate carbon concentration within suboxic (<20 µmol O_2 kg^{-1}) waters. Measurements highlighted in red represent depths where N_2 fixation rates (NFRs) were above the detection limit (ADL). Open circles represent depths where NFRs were below the detection limit (BDL).

Suppl. Table 1. Mean N_2 fixation rates and corresponding hydrographic parameters. See associated "Read Me" file for complete parameter descriptions.

Suppl. Table 2. Results from control incubations conducted within and around the Eastern Tropical South (ETSP) and North Pacific (ETNP) oxygen deficient zones. See associated "Read Me" file for complete parameter descriptions. ETNP values are associated with data presented in Selden et al. (2019).