# Supplemental Information to ‘Changing nutrient stoichiometry affects phytoplankton production, DOP build up and dinitrogen fixation – a mesocosm experiment in the eastern tropical North Atlantic‘

**Selected data.** The subset of data used for statistical modelling comprised 177 observations (see supplementary file S1\_Meyer\_et\_al\_2015.xlsx). Nine explanatory variables were retained according to variance inflation factor VIF evaluation, exhibiting VIF values between 1.01 and 1.64. Supplementary figure S1 provides a heat map-style visualization of correlations between selected (rows) and sorted-out (columns) explanatory variables. POP, POC, and PON were positively correlated with Day and Chl *a*; DOC was positively correlated with Day; NtoP\_d2 was negatively correlated with PO4\_d2 and DOP, but positively correlated with NO3\_d2; Vol\_initial was positively correlated with Run\_ID, DOP, and PO4\_d2. This means that, whenever one of the selected variables significantly contributes to a model, its effect also represents (at least in part) effects of the variables correlated with it. For example, a positive effect of NO3\_d2 in the statistical model implies a simultaneous positive effect of NtoP\_d2, while a positive effect of PO4\_d2 in the statistical model implies a simultaneous negative effect of NtoP\_d2.

**Selected models.** The stochastic part of all models was based on a Poisson distribution with a logarithmic link between the mean of the response variable and the predictor function. This variance structure was appropriate for the count data at hand, accounting both for a non-negative response and increasing variance with increasing count values. Its suitability was also confirmed by a considerably lower AIC value when compared to a model with the same fixed effects structure but standard Gaussian error distribution (data not shown). The chosen variance structure further comprised temporal autocorrelation modeled by an order-1 autocorrelation structure, as well as a random intercept by Treat\_ID and Meso\_ID.

Both the selected gene and transcript count models were highly significant (gene counts: *F*5,674 = 3516.3, *p* <10-4; transcript counts: *F*11,1022 = 116.8, *p* <10-4). Summaries of selected gene and transcript count models are given in supplementary files S2\_model\_genes.txt and S3\_ model\_transcripts.txt, respectively. Graphical representations of conditional effects can be found in Fig. S2-S4. Some terms were retained in the final models despite being insignificant, as they improved the model fit. Overall fitted temporal response patterns by Gene and Run\_ID are displayed in Fig. 8 and 9, respectively. The latter figures indicate that there might be a slight overdispersion in the counts, which is not well-captured by a Poisson distribution. We therefore also tried to fit the models with a negative binomial distribution (which could account for overdispersion), but models did not converge in that case.

For all *nifH* clusters but nifH\_Fil, conditional temporal responses (i.e., of the day effect alone with other effects partialled out) were distinctly different between Varied\_N and Varied\_P runs. Specifically, in the case of nifH\_AO, the smoother for Varied\_N conditions was only marginally significant (*p* ≈ .03) and predicted a slight transitory increase in gene counts between Day 5 and 6. On the other hand, under Varied\_P conditions, the smoother showed higher significance (*p* ≈ 1.7×10-6) and predicted a clear initial increase of gene counts with a maximum between Day 3 and 4, followed by a slight decrease to the mean of nifH\_AO counts.

The difference in temporal responses between runs was even more pronounced for nifH\_UA. While the model did not predict any changes of nifH\_UA counts over time under Varied\_N conditions, the response pattern within Varied\_P was complex: After a marked initial increase with a narrow maximum at Day 3, nifH\_UA counts exhibited a downward trend below their mean level until Day 5 before rising once more above the mean level.

The smoother of the covariate DOP differed only marginally from a straight line and thus implies a near-linear effect on the overall gene count distribution, with higher DOP concentrations resulting in lower count values. The influence of DON\_d2 on the estimated gene count was comparatively complex, showing a positive effect at intermediate concentrations and a negative to neutral effect at both low and high concentrations of DON (the predicted positive effect at very low DON concentrations presumably being a leverage effect of a single ill-fitting observation).

**Figures:**

Figure S1: Heat map showing the correlation between selected (rows) and sorted-out (columns) explanatory variables of A: all parameters, B: significant parameters.

Figure S2: Selected conditional responses (i.e. of the effect of interest alone and other effects partialled out) of nifH transcript counts to selected terms of the predictive model. Terms are (A) transcripts\_Het\_I in Varied\_N by Day, (B) transcripts\_Het\_I in Varied\_P by Day, (C) transcripts\_Het\_II in Varied\_N by Day, (D) transcripts\_Het\_II in Varied\_P by Day, (E) Chla, (F) DOP, (G) NO3\_d2, (H) SiO\_d2. Dashes at the bottom of the plots (“rag plot”) denote presence of values. Responses at the scale of the linear predictor (here: ln(counts)) are centered by reference level and shown as solid lines, their 95% confidence intervals as grey shades or dashed lines, respectively. Reference levels are group means by nif cluster for temporal effects, and the global mean otherwise. With smoothers (A-F), reference levels are marked by a dashed line.

Figure S3: Conditional responses (i.e., of the effect of interest alone and other effects partialled out) of nifH gene counts to selected terms of the predictive model. Terms are (A) nifH\_Fil in Varied\_N by Day, (B) nifH\_Fil in Varied\_P by Day, (C) nifH\_CR in Varied\_N by Day, (D) nifH\_CR in Varied\_P by Day, (E) nifH\_AO in Varied\_N by Day, (F) nifH\_AO in Varied\_P by Day, (G) nifH\_UA in Varied\_N by Day, (H) nifH\_UA in Varied\_P by Day, (I) DOP, (J) PO4\_d2, (K) DON\_d2, (L) Gene, (M) Run\_ID. Dashes at the bottom of the plots (“rag plot”) denote presence of values. Responses at the scale of the linear predictor (here: ln(counts)) are centered by reference level and shown as solid lines, their 95% confidence intervals as grey shades or dashed lines, respectively. Reference levels are group means by nif cluster for temporal effects, and the global mean otherwise. With temporal smoothers, reference levels are marked by a dashed line.

Figure S4: Conditional responses (i.e., of the effect of interest alone and other effects partialled out) of nifH transcript counts to selected terms of the predictive model. Terms are (A) transcripts\_Fil in Varied\_N by Day, (B) transcripts\_Fil in Varied\_P by Day, (C) transcripts\_CR in Varied\_N by Day, (D) transcripts\_CR in Varied\_P by Day, (E) transcripts\_AO in Varied\_N by Day, (F) transcripts\_AO in Varied\_P by Day, (G) transcripts\_UA in Varied\_N by Day, (H) transcripts\_UA in Varied\_P by Day, (I) Gene, (J) Run\_ID, (K) DON\_d2, (L) PO4\_d2. Dashes at the bottom of the plots (“rag plot”) denote presence of values. Responses at the scale of the linear predictor (here: ln(counts)) are centered by reference level and shown as solid lines, their 95% confidence intervals as grey shades or dashed lines, respectively. Reference levels are group means by nif cluster for temporal effects, and the global mean otherwise. With smoothers, reference levels are marked by a dashed line.

Table S1: Nominal and measured nutrient concentrations after the addition of nitrate or phosphate to the mesocosms in both experimental runs.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Run** | **Treat ID** | **NO3- nom**  **[µmol L-1]** | **PO43+ nom**  **[µmol L-1]** | **SiO4 nom**  **[µmol L-1]** | **N : P nom** | **NO3-**  **[µmol L-1]** | **PO43+**  **[µmol L-1]** | **SiO4**  **[µmol L-1]** | **N : P** |
| 1 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 11.52 | 0.73 | 15.22 | 15.78 |
| 1 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 10.97 | 0.68 | 14.97 | 16.11 |
| 1 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 10.63 | 0.52 | 15.04 | 20.47 |
| 1 | 6.35N/1.10P | 6.35 | 1.1 | 15 | 5.76 | 5.66 | 1.00 | 15.06 | 5.66 |
| 1 | 12.0N/1.25P | 12 | 1.25 | 15 | 9.6 | 10.74 | 1.14 | 15.01 | 9.39 |
| 1 | 12.0N/1.25P | 12 | 1.25 | 15 | 9.6 | 11.16 | 1.12 | 15.33 | 9.95 |
| 1 | 12.0N/1.25P | 12 | 1.25 | 15 | 9.6 | 10.89 | 1.09 | 15.13 | 9.97 |
| 1 | 12.0N/1.75P | 12 | 1.75 | 15 | 6.86 | 10.55 | 1.57 | 14.97 | 6.74 |
| 1 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 10.82 | 0.61 | 15.10 | 17.64 |
| 1 | 12.0N/1.75P | 12 | 1.75 | 15 | 6.86 | 10.82 | 1.58 | 14.90 | 6.86 |
| 1 | 12.0N/1.75P | 12 | 1.75 | 15 | 6.86 | 11.07 | 1.53 | 15.01 | 7.24 |
| 1 | 12.0N/0.25P | 12 | 0.25 | 15 | 48 | 11.16 | 0.15 | 15.12 | 76.78 |
| 1 | 12.0N/0.25P | 12 | 0.25 | 15 | 48 | 11.18 | 0.16 | 15.00 | 69.80 |
| 1 | 17.65N/1.10P | 17.65 | 1.1 | 15 | 16 | 16.90 | 1.01 | 15.27 | 16.75 |
| 1 | 12.0N/0.25P | 12 | 0.25 | 15 | 48 | 11.33 | 0.15 | 15.15 | 75.77 |
| 2 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 12.58 | 0.47 | 14.51 | 27.00 |
| 2 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 12.36 | 0.51 | 14.18 | 24.32 |
| 2 | 12.0N/0.75P | 12 | 0.75 | 15 | 16 | 12.61 | 0.51 | 14.34 | 24.72 |
| 2 | 6.35N/0.40P | 6.35 | 0.4 | 15 | 15.99 | 6.91 | 0.18 | 14.63 | 39.35 |
| 2 | 17.65N/1.10P | 17.65 | 1.1 | 15 | 16.05 | 18.43 | 0.79 | 14.47 | 23.45 |
| 2 | 20.0N/0.75P | 20 | 0.75 | 15 | 26.67 | 20.57 | 0.47 | 15.09 | 43.92 |
| 2 | 20.0N/0.75P | 20 | 0.75 | 15 | 26.67 | 20.60 | 0.45 | 14.16 | 45.92 |
| 2 | 20.0N/0.75P | 20 | 0.75 | 15 | 26.67 | 21.90 | 0.45 | 15.18 | 48.81 |
| 2 | 4.00N/0.75P | 4 | 0.75 | 15 | 5.33 | 4.62 | 0.45 | 15.33 | 10.38 |
| 2 | 17.65N/0.40P | 17.65 | 0.4 | 15 | 44.46 | 18.47 | 0.22 | 15.36 | 84.31 |
| 2 | 4.00N/0.75P | 4 | 0.75 | 15 | 5.33 | 4.49 | 0.47 | 14.92 | 9.59 |
| 2 | 4.00N/0.75P | 4 | 0.75 | 15 | 5.33 | 3.99 | 0.49 | 15.68 | 8.17 |
| 2 | 2.00N/0.75P | 2 | 0.75 | 15 | 2.67 | 2.06 | 0.46 | 16.39 | 4.52 |
| 2 | 6.00N/1.03P | 6.00 | 1.03 | 15 | 5.77 | 6.69 | 0.78 | 15.46 | 8.55 |
| 2 | 2.00N/0.75P | 2 | 0.75 | 15 | 2.67 | 1.87 | 0.56 | 17.64 | 3.33 |
| 2 | 2.00N/0.75P | 2 | 0.75 | 15 | 2.67 | 2.71 | 0.48 | 15.04 | 5.60 |