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Exploring the distance between nitrogen and phosphorus limitation in mesotrophic surface waters using a sensitive bioassay

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Abstract

The balance in microbial consumption of nitrogen and phosphorus was investigated in samples collected in two mesotrophic coastal environments: the Baltic Sea (Tvärminne field station) and the North Sea (Espegrend field station). For this, we have refined a bioassay based on the response in alkaline phosphatase activity (APA) over a matrix of combinations in nitrogen and phosphorus additions. This assay not only provides information on which element (N or P) is the primary limiting, but also gives a quantitative estimate for the excess of the secondary limiting element (P^* or N^*, respectively), as well as the ratio between N and P consumption over short time scales (days). As expected for a Baltic Sea late spring-early summer situation, the Tvärminne assays (n=5) indicated N-limitation with an average P^*=0.30±0.10 µM-P, when incubated for 4 days. For short incubations (1–2 days), the Espegrend assays indicated P-limitation, but the shape of the response surface changed with incubation time, resulting in a drift in parameter estimates toward N-limitation. Extrapolating back to zero incubation time gave P-limitation with N^*≈0.9 µM-N. The N:P ratio (molar) of nutrient consumption varied considerably between investigated locations; from 2.3±0.4 in the Tvärminne samples to 13±5 and 32±3 in two samples from Espegrend. Our assays included samples from mesocosm acidification experiments, but statistically significant effects of ocean acidification were not found by this method.

Keywords: alkaline phosphatase activity, bioassays, mesotrophic temperate seas, nutrient limitation, phytoplankton

1 Introduction

N to P balance is a core biogeochemical feature of aquatic systems as highlighted in Redfield´s classical question of whether it is the chemistry of seawater that has determined the stoichiometry of the marine organisms, or biology is the cause for the "normal" 16:1 (molar) ratio between N and P in seawater (Redfield et al., 1963). The

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issue of surface ocean nutrient limitation is as acute as ever (Moore et al., 2013), since it has bearings on phenomena ranging from the global carbon cycle, where it plays a key role in the dynamics of the ocean´s biological pump (Ducklow et al., 2001); via basin scale issues such as N deficiency in Arctic water of Pacific origin (Lehmann et al., 2005), P deficiency in Eastern Mediterranean deep waters (Krom et al., 1991) and the North Atlantic gyre (Mather et al., 2008); via regional issues such as the question of P and/or N removal from the Baltic Sea (Elmgren and Larsson, 2001; Granéli et al., 1990; Räike et al., 2003); to local ecosystem characteristics such as P-deficient brackish layer overlaying potentially more N-limited marine waters in the fjords of western Norway (Thingstad et al., 1993). The classical idea of predominantly N-limitation in marine systems (as opposed to predominantly P-limitation in limnic systems) (Hecky and Kilham, 1988) has also become considerably more nuanced, not only due to the cases mentioned above, but also with the identification of the High Nutrients Low Chlorophyll (HNLC) areas as being iron-limited (Franck et al., 2003), phosphorus and iron as co-limiting elements of nitrogen fixation in the tropical North Atlantic (Mills et al., 2004) and N_2 fixers having a competitive advantage in oligotrophic P-starved regions (Landolfi et al., 2015). While some of the mechanisms behind these apparent deviations from Redfield stoichiometry seem to be well understood, there are others which lack generally accepted explanations.

In deep waters with most of the bioavailable N and P converted to NO_3 and PO_4, the chemical determination of N or P in excess of the Redfield ratio may be relatively straight forward. In biogeochemistry this excess is referred to as N* and P* (Gruber and Sarmiento, 1997). In productive surface waters this is a more complex issue. Not only may both N and P accumulate in pools of DON and DOP with different grades of bioavailability, but organisms may also have flexible stoichiometry, and groups of organisms may differ in stoichiometry, theoretically allowing for the adjustment of the N:P ratio in nutrient consumption at both an individual and a community level (Bertilsson et al., 2003; Geider et al., 2002; Krauk et al., 2006). A potential solution to the chemically intractable problem of measuring a large suite of presumably bioavailable pools is to use a quantitative bioassay, i.e. to ask the organisms how much of limiting elements they can "see".

Microorganisms have evolved sophisticated physiological mechanisms to adapt to the different forms of nutrient limitation (Geider et al., 1997; Ivančić et al., 2012; Thingstad et al., 2005; Van Mooy et al., 2009). Activation of the genes found in the inducible Pho-operon (Torriani-Gorini, 1994) is an indicator of the organism responding to P deficiency. One of the enzymes induced as a part of this operon is the extracellular enzyme alkaline phosphatase, used to split the ester bound in phosphomonoesters (Hoppe, 2003). Since the alkaline phosphatase activity (APA) can easily and sensitively be determined fluorometrically (Perry, 1972), it is used as a convenient indicator of the Pho-operon being de-repressed, and therefore of P deficiency in aquatic systems. This method was further exploited by Thingstad and Mantoura (2005) in the oligotrophic Eastern Mediterranean, showing that the concentration of added PO_4 needed for APA to disappear in a P-limited system, or alternatively NH_4 needed to induce APA in an N-limited system, could be used as a bioassay to quantitatively estimate N^* or P^*. We here expand this technique by using a matrix-setup including simultaneous gradients in both PO_4 and NH_4 additions. This is applied to samples from the coastal waters of western Norway and the Baltic Sea, confirming that the assay gives informative results also in temperate, mesotrophic environments.

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2 Material and Methods

2.1 Study areas and sampling

The Baltic water was collected as integrated samples (depth 0–10 m) in Storfjärden near Tvärminne field station (59° 51.50' N, 23° 15.50' E) on 6 August 2012. The collection was performed 45-30 days after the first-last CO_2 treatments and 50 days after the mesocosm closure (Paul et al., 2015). Samples were collected from the fjord (417 µatm) and mesocosms M1 (365 µatm), M3 (1007 µatm), M6 (821 µatm) and M7 (497 µatm); where numbers in parentheses are average f(CO_2) over the period Day 1–Day 43. The mesocosms received no nutrient manipulations except the CO_2 treatments. Further details about location and the experiment can be found in Paul et al. (2015).
The samples from western Norway were collected during a similar mesocosm experiment in Raunefjorden close to Espegrend field station (60° 16.2' N, 5° 11.7' E). From one mesocosm (MR) an integrated (depth 0–20 m) sample (1165 µatm) was collected on 25 May 2015 corresponding to Day 22 after acidification treatment. The fjord sample was collected at nearby landlocked location Kviturspollen (60° 15.8' N, 5° 15' E) at the depth of 1 m using a Niskin sampler on 3 June 2015. Samples were pre-filtered through gauze of 112 µm mesh size to minimize the variability due to the occasional large zooplankton.

2.2 Matrices of nitrogen and phosphorus additions

Samples were distributed in 15 mL Falcon® polypropylene tubes (BD Biosciences®) organized in 10x10 or 8x8 columns x rows (Tvärminne and Espegrend, respectively). PO_4 (KH_2PO_4 10 µM) was added in final concentrations from 0 to 290 nM-P in steps of 32.2 nM (Tvärminne) and from 0 to 105 nM-P in steps of 15 nM (Espegrend). Each of the columns received additions of NH_4 (NH_4Cl 200 µM) in final concentrations from 0 to 964 nM-N in steps of 107 nM-N (Tvärminne) and from 0 to 2100 nM-N in steps of 300 nM-N (Espegrend). The tubes were incubated in light:dark (16 h:8 h) at 17–18°C (Tvärminne) and in light:dark (12 h:12 h) at 16.5°C (Espegrend), both at irradiance of 78 µmol photons m^{-2} s^{-1}. Incubation at Tvärminne lasted 4 days for all samples, whilst APA assays for Espegrend were repeated as given in each case.

2.3 Alkaline phosphatase activity

APA in the Baltic Sea was measured by Varian Cary Eclipse fluorometer, while APA in the coastal waters of the western Norway was measured using a PerkinElmer Enspire 2300 plate reader. Measurements of APA were done according to Perry (1972) but modified to the use of fluorescence plate reader by pipetting 200 µL subsamples from each Falcon tube into the wells containing the substrate. Results are expressed in relative fluorescence units (RFU). The need for intercalibration of different fluorometers was thus avoided.

2.4 Fitting the response surface

Thingstad and Mantoura (2005) fitted sigmoidal functions to the observed APA-responses; either a decreasing function parallel to the P-addition axis in the case of a P-limited system, or an increasing function parallel to the N-addition axis in the case of N-limitation. To avoid this pre-fitting choice of function, we here have instead started with

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the assumption that the *P,N*-plane is split into a P-limited and an N-limited part by the straight line $N = N_0 + rP$ where a negative value of the intercept N_0 corresponds to the excess-N (N*) present in a P-limited system and $-\frac{N_0}{r}$ is the amount of phosphate needed to shift the system to N-limitation. Conversely, a positive value of the intercept N_0 would correspond to the amount of N required to shift an N-limited system into P-limitation, while $\frac{N_0}{r}$ corresponds to the excess-P (P*) in this N-limited system. The shift from P- to N-limitation, and therefore the expression of APA in a point *P,N*, is assumed to be a function of the distance *Z* between this point and the line (Fig. 1). The sigmoidal function fitted is $APA_{est}(P,N) = \frac{A}{1+e^{sZ}} - B$. Here, the exponential function replaces the term $\left(\frac{Z}{Z_0}\right)^s$ adopted by Thingstad and Mantoura (2005) from standard toxicology. This standard expression is undefined for Z_0=0 and therefore not applicable with our approach. Visual inspection of residuals in graphs (see Fig. 2A, B) did not suggest systematic deviances between response surfaces fitted with this function and the observed data. Alternative fitting functions have therefore not been explored. With five parameters to fit (r, N_0, s, A, B), this leaves 95 and 59 degrees of freedom for the Tvärminne and Espegrend set-ups, respectively. The fitted surface APA_{est} has a maximum A-B obtained for co-ordinates combining low P with high N (large negative Z) and $APA_{est}=(A/2)$-B along the line $N=N_0+rP$ separating the P- and N-limited regions. The parameter s defines the steepness of transition between the two regions perpendicular to this line. B is the background APA_{est} found for high-P, low-N (large positive Z) co-ordinates. The fitting was done using the "fit" function in Matlab® with its default Levenberg-Marquardt algorithm providing the parameter estimates with 95% confidence intervals (c.i.) (code included in SI).

3 Results

Two examples of the fitted response surface, one from Tvärminne (Fjord) (Fig. 2A) and one from Espegrend (MR) (Fig. 2B) are shown to illustrate the difference in shape of the response in situations apparently N-limited (Tvärminne) and P-limited (Espegrend), with estimated P*=0.3 µM-P and N*=0.4 µM-N, respectively . All assays are summarized in Table 1.

For the two Espegrend samples, the change in shape of the response surface with incubation time was explored (Fig. 3). For both samples, N_0 increased with incubation time ($p \leq 0.05$, Table 2), i.e. the assay results drifted towards increasing N deficiency when using longer incubation times. In the sample MR, r and s decreased significantly over time (Table 2). Using linear regression, the parameter estimates can be extrapolated back to zero incubation time. With this technique the average P* for the Tvärminne samples, based on a single incubation time, was 0.3 µM-P, and the average N* for the two Espegrend samples, based on backward extrapolation, was 0.9 µM-N.

The assays from Tvärminne mesocosms include an f(CO_2) gradient. Linear regressions of N_0 (p=0.55), r (p=0.63) (Fig. 4) and s (p=0.19) (not presented) on f(CO_2) gave no indication of any statistically significant effect of the 45 days exposure of the systems to different CO_2-levels. Compared to a Redfield N:P value of 16, all the Tvärminne samples

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gave low *r* (2.3±0.5; mean over samples±sd), while the two Espegrend samples gave *r* of 13±2 (Kviturspollen) and 32±3 (MR) (mean±sd, both over incubation times).

4 Discussion

This study extends the demonstrated applicability of this type of assay from its previous use in warm oligotrophic waters (Thingstad and Mantoura, 2005) to mesotrophic temperate environments. We modified the technique so that no *a priori* assumptions are now required as to whether the system investigated is N- or P-deficient.

We explored the use of this modified assay in two environments with anticipated differences in ambient N:P stoichiometry. The Tvärminne mesocosm experiment was planned with the expectation of an N-limited spring-summer situation as characteristic in the Baltic Sea (Granéli et al., 1990; Rolff and Elfwing, 2015; Thomas et al., 2003), subsequently transiting from N-limitation towards an N- and P-co-limited situation as the result of "new" N being added through late summer blooming of diazotrophic cyanobacteria (Lignell et al., 2003). This bloom did not occur during the whole Tvärminne experiment and N-limitation at the time of sampling has been confirmed by Nausch et al. (2016) who studied the microbial P-cycle just before our experiment. The finding of positive N_0-estimates for all 5 samples (Table 1) is in line with this. The Tvärminne assays were performed after 4 days of incubation when the APA-responses emerged. The conclusion of N-limitation is therefore confounded by the potential drift in parameter estimates as observed for the Espegrend samples (Fig. 3). The drift obviously complicates the use of this assay since there may be no single incubation time that gives a "correct" set of parameter values. Since the drift seems to be reasonably linear for all parameters (Fig. 3), a promising option is to extrapolate the linear regressions back to time 0. In our case this gives negative N_0 values of -0.8 (-1.4,-0.2) and -1.0 (-2.4,0.4) for the samples from MR and Kviturspollen, respectively (intercept with 95% c.i.); suggesting initial P-limitation. This conclusion is in accordance with the expectation since the top layer of the fjords in western Norway has been shown to be P-deficient (Thingstad et al., 1993).

The mechanisms behind the drift in parameter estimates have not been studied further here. Two, not mutually exclusive, scenarios may, however, illustrate the options: 1) The microbial food web in the incubated tubes remineralizes P faster than N (Garber, 1984). The assay may then correctly reflect the succession of the limiting nutrient in the sense that the bioavailable pools in the tubes change with incubation time; 2) N added in excess of P in the upper P-limited part of the *P*,*N*-plane is used by the organisms to produce alkaline phosphatase (rather than biomass). This would lift the response surface for high values of added N and move the fitted line towards higher N_0, i.e. towards N-limitation. The use of extra N to produce exo-enzymes for acquisition of P from DOP has recently been argued for, but then with N-fixation as the N-source (Landolfi et al., 2015).

The *r* values representing the ratio of N- and P-consumption are comparable between all the Tvärminne samples (2.3±0.5, n=5 different samples), indicating good reproducibility of the assay for similar water samples. This low value compared to the Redfield value of 16 was, however, strikingly different from the Espegrend samples with a Redfield-like 13±2 (Kviturspollen) and a high 32±3 (MR), both over incubation times. A similar phenomenon was noted by Thingstad and Mantoura (2005) using this method to study in-out differences in a Lagrangian experiment where orthophosphate was added to the P-deficient surface system in the Eastern Mediterranean. While their P-limited out-

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sample gave an r=15±2, the inside system, when driven to N deficiency by the phosphate addition, gave r=3.0±0.2. Interestingly, we also here found the lower-than-Redfield r values in the probably N-limited samples from Tvärminne. From the limited number of determinations available, the linkage between N deficiency and low r values seems consistent, but the underlying mechanism is not immediately obvious. One could argue that, in a P-deficient system, the organisms present would be expected to have marked luxury consumption of any added P (Thingstad, 2005) (and *vice versa* for N) (Leonardos and Geider, 2004). As r represents the ratio between utilization of added N and added P, luxury uptake seems to lead to an expected effect on r opposite to that observed. One could speculate that organisms in N-deficient environments are selected for (or adapted to) low N:P requirements. Although this may be in qualitative agreement with our data, it seems doubtful that the large range in r (~2 to ~30) can be explained in this manner.

5 Conclusions

We have demonstrated the extension of the APA assay from the warm oligotrophic to the temperate, mesotrophic waters. This technique has the advantage over traditional nutrient-limitation bioassays because it determines not only the most limiting element, but also estimates the quantitative excess in bioavailable forms of the secondary limiting element (N^*, P^*); this without any requirement for a determination of the large variety of chemical and/or physical forms in which these excess nutrients exist. The assay is complicated by a drift in parameter estimates with incubation time. A backward extrapolation to zero incubation time appears promising.

Data availability

Original data are given in Supplementary Information (SI) for each assay. SI also contains the Matlab® program for fitting the response surfaces as shown in Fig. 2.

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References

Bertilsson, S., Berglund, O., Karl, D. M., and Chisholm, S. W.: Elemental composition of marine *Prochlorococcus* and *Synechococcus*: implications for the ecological stoichiometry of the sea, Limnol. Oceanogr., 48(5), 1721–1731, 2003.

Ducklow, H. W., Steinberg, D. K., and Buesseler, K. O.: Upper Ocean Carbon Export and the Biological Pump, Oceanography, 14(4), 50–58, 2001.

Biogeosciences Discuss., doi:10.5194/bg-2016-313, 2016
Manuscript under review for journal Biogeosciences
Published: 1 August 2016

Elmgren, R. and Larsson, U.: Nitrogen and the Baltic Sea: Managing Nitrogen in Relation to Phosphorus. Optimizing Nitrogen Management in Food and Energy Production and Environmental Protection, Proceedings of the 2nd International Nitrogen Conference on Science and Policy TheScientificWorld, 1(S2), 371–377. ISSN 1532-2246, DOI 10.1100/tsw.2001.291, 2001.

Franck, V. M., Bruland, K. W., Hutchins, D. A., and Brzezinski M. A.: Iron and zinc effects on silicic acid and nitrate uptake kinetics in three high-nutrient, low-chlorophyll (HNLC) regions, Mar. Ecol.-Prog. Ser., 252, 15–33, 2003.

Garber, J. H.: Laboratory Study of Nitrogen and Phosphorus Remineralization during the Decomposition of Coastal Plankton and Seston, Estuar. Coast. Shelf Sci., 18, 685–702, 1984.

Granéli, E., Wallström, K., Larsson, U., Granéli, W., and Elmgren, R.: Nutrient Limitation of Primary Production in the Baltic Sea Area, Ambio, 19(3), 142–151, 1990.

Gruber, N. and Sarmiento, J. L.: Global patterns of marine nitrogen fixation and denitrification, Global Biogeochem. Cy., 11, 235–266, 1997.

Geider, R. J. and La Roche, J.: Redfield revisited: variability of C : N : P in marine microalgae and its biochemical basis, Eur. J. Phycol., 37(1), 1–17, 2002.

Geider, R. J., MacIntyre, H.L., and Kana, T. M.: Dynamic model of phytoplankton growth and aclimation: responses of the balanced growth rate and the chlorophyll *a*: carbon ratio to light, nutrient-limitation and temperature, Mar. Ecol.-Prog. Ser., 148(1-3), 187–200, 1997.

Hecky, R. E. and Kilham, P.: Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment, Limnol. Oceanogr., 33(4-part 2), 196–822, 1988.

Hoppe, H.-G.: Phosphatase activity in the sea, Hydrobiologia, 493(1-3), 187–200, 2003.

Ivančić, I., Godrijan, J., Pfannkuchen, M., Marić, D., Gašparović, B., Đakovac, T., and Najdek, M.: Survival mechanisms of phytoplankton in conditions of stratification-induced deprivation of orthophosphate: Northern Adriatic case study, Limnol. Oceanogr., 57(6), 1721–1731, 2012.

Krauk, J. M., Villareal, T. A., Sohm, J. A., Montoya, J. P., and Capone, D. G.: Plasticity of N:P ratios in laboratory and field populations of *Trichodesmium* spp, Aquat. Microb. Ecol., 42(3), 243–253, 2006.

Krom, M. D., Kress, N., and Brenner, S.: Phosphorus limitation of primary production in the eastern Mediterranean Sea, Limnol. Oceanogr., 36(3), 424–432, 1991.

Landolfi, A., Koeve, W., Dietze, H., Kähler, P., and Oschlies, A.: A new perspective on environmental controls of marine nitrogen fixation, Geophys. Res. Lett., 42, 4482–4489, doi:10.1002/2015GL063756, 2015.

Lehmann, M. F., Sigman, D. M., McCorkle, D. C., Brunelle, B. G., Hoffmann, S., Kienast, M., Cane, G., and Clement, J.: Origin of the deep Bering Sea nitrate deficit: Constraints from the nitrogen and oxygen isotopic composition of water column nitrate and benthic nitrate fluxes, Global Biogeochem. Cy., 19, DOI:10.1029/2005GB002508, 2005.

Leonardos, N. and Geider, R. J.: Effects of nitrate:phosphate supply ratio and irradiance on the C:N:P stoichiometry of *Chaetoceros muelleri*, Eur. J. Phycol., 39, 173–180, 2004.

Lignell, R., Seppälä, J., Kuuppo, P., Tamminen, T., Andersen, T., and Gismervik, I.: Beyond bulk properties: Responses of coastal summer plankton communities to nutrient enrichment in the Northern Baltic Sea, Limnol. Oceanogr., 48(1), 189–209, 2003.

Mather, R. L., Reynolds, S. E., Wolff, G. A., Williams, R. G., Torres-Valdes, S., Woodward, E. M. S., Landolfi, A., Pan, X., Sanders, R., and Achterberg, E. P.: Phosphorus cycling in the North and South Atlantic Ocean subtropical gyres, Nature Geosci., 1(7), 439–443, 2008.

Mills, M. M., Ridame, C., Davey, M., La Roche, J., and Geider, R. J.: Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic, Nature, 429, 292–294, 2004.

Moore, C. M., Mills, M. M., Arrigo, K. R., Berman-Frank, I., Bopp, L., Boyd, P.W., Galbraith, E. D., Geider, R. J., Guieu, C., Jaccard, S. L., Jickells, T. D., La Roche, J., Lenton, T. M., Mahowald, N. M., Maranon, E., Marinov, I., Moore, J. K., Nakatsuka, T., Oschlies, A., Saito, M. A., Thingstad, T. F., Tsuda, A., and Ulloa, O.: Processes and patterns of oceanic nutrient limitation, Nature Geosci., 6(9), 701–710, 2013.

Nausch, M., Bach, L., Czerny, J., Goldstein, J., Grossart, H. P., Hellemann, D., Hornick, T., Achterberg, E., Schultz, K., and Riebesell, U.: Effects of CO_2 perturbation on phosphorus pool sizes and uptake in a mesocosm experiment during a low productive summer season in the northern Baltic Sea, Biogeoscienses, 13, 3035–3050, doi:10.5194/bg-13-3035-2016, 2016.

Paul, A. J., Bach, L. T., Schulz, K. G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., and Riebesell, U.: Effect of elevated CO_2 on organic matter pools and fluxes in a summer Baltic Sea plankton community, Biogeosciences, 12(20), 6181–6203, 2015.

Perry, M. J.: Alkaline phosphatase activity in subtropical Central North Pacific waters using a sensitive fluorometric method, Mar. Biol., 15, 113–119, 1972.

Räike, A., Pietiläinen, O. P., Rekolainen, S., Kauppila, P., Pitkänen, H., Niemi, J., Raateland, A., and Vuorenmaa, J.: Trends of phosphorus, nitrogen and chlorophyll *a* concentrations in Finnish rivers and lakes in 1975–2000, Sci. Total Environ., 310, 47–59, 2003.

Redfield, A. C., Ketchum, B. H., and Richards, A.: The influence of organisms on the composition of sea water, In: Hill, M. M. (ed.), The sea, volume 2, New York, Wiley Interscience, 26–77, 1963.

Rolff, C. and Elfwing, T.: Increasing nitrogen limitation in the Bothnian Sea, potentially caused by inflow of phosphate-rich water from the Baltic Proper, Ambio, 44, 601–611, DOI 10.1007/s13280-015-0675-3, 2015.

Thingstad, T. F., Øvreås, L., Egge, J. K., Løvdal, T., and Heldal, M.: Use of non-limiting substrates to increase size; a generic strategy to simultaneously optimize uptake and minimize predation in pelagic osmotrophs?, Ecol. Lett., 8(7), 675–682, 2005.

Thingstad, T. F. and Mantoura, R. F. C.: Titating excess nitrogen content of phosphorous-deficient eastern Mediterranean surface water using alkaline phosphatase activity as a bio-indicator, Limnol. Oceanogr. Methods, 3, 94–100, 2005.

Thingstad, T. F.: Simulating the response to phosphate additions in the oligotrophic eastern Mediterranean using an idealized four-member microbial food web model, Deep Sea Res. Part II, 52, 3074–3089, 2005.

Thingstad, T. F., Skjoldal, E. F., and Bohne, R. A.: Phosphorus cycling and algal-bacterial competition in Sandsfjord, western Norway, Mar. Ecol.-Prog. Ser., 99(3), 239–259, 1993.

Thomas, H., Pempkowiak, J., Wulff, F., and Nagel, K.: Autotrophy, nitrogen accumulation and nitrogen limitation in the Baltic Sea: A paradox or a buffer for eutrophication?, Geophys. Res. Lett., 30(21), DOI: 10.1029/2003GL017937, 2003.

Biogeosciences Discuss., doi:10.5194/bg-2016-313, 2016
Manuscript under review for journal Biogeosciences
Published: 1 August 2016

Torriani-Gorini, A.: The Pho regulon of Escherichia coli, In: Torriani-Gorini, A., Yagil, E., and Silver, S. (eds.), Phosphate in Microorganisms: Cellular and Molecular Biology, Washington DC, ASM Press, 1–4, 1994.

Van Mooy, B., Fredricks, H. F., Pedler, B. E., Dyhrman, S. T., Karl, D. M., Kobližek, M., Lomas, M. W., Mincer, T. J., Moore, L. R., Moutin, T., Rappe, M. S., and Webb, E. A.: Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity, Nature, 458(7234), 69–72, 2009.

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Manuscript under review for journal Biogeosciences
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Table 1: Estimates (with 95% c.i.) of the intercept (N_0) and the slope (r) of the line $N=N_0+rP$ separating the N- and P-limited regions as illustrated in Fig. 1. s represents the steepness of transition from N- to P-limitation, perpendicular to the line. R^2-values are for the fitted response surfaces.

	N_0 (µM-N)	r (µM-N:µM-P)	s (µM^{-1})	R^2
Tvärminne (Baltic Sea): (incubation time 4 days)				
Fjord	0.79 (0.75, 0.83)	2.5 (2.2, 2.8)	29 (23, 35)	0.674
M1	0.34 (0.30, 0.38)	2.9 (2.7, 3.2)	43 (31, 56)	0.622
M3	0.76 (0.69, 0.83)	2.2 (1.9, 2.5)	19 (15, 24)	0.664
M6	0.64 (0.56, 0.72)	2.2 (1.9, 2.6)	19 (13, 25)	0.569
M7	0.70 (0.66, 0.75)	1.6 (1.4, 1.8)	20 (16, 25)	0.635
Mesocosm Raunefjorden				
Incubation time (days):				
1	-0.41 (-0.60, -0.22)	40 (36, 44)	64 (52, 76)	0.965
2	-0.63 (-1.02, -0.25)	34 (28, 40)	50 (31, 70)	0.764
3	-0.21 (-0.47, 0.06)	31 (27, 36)	48 (33, 63)	0.843
4	-0.13 (-0.34, 0.08)	31 (27, 34)	47 (35, 59)	0.933
4.5	-0.13 (-0.30, 0.04)	30 (27, 33)	51 (40, 63)	0.951
5	0.42 (0.15, 0.69)	26 (23, 29)	36 (25, 47)	0.907
Kviturspollen				
2	-0.09 (-0.26, 0.08)	9.9 (8.5, 11.4)	25 (17, 33)	0.940
3	0.58 (0.46, 0.70)	12 (11, 13)	20 (15, 25)	0.972
4	1.83 (0.72, 2.95)	14 (13, 16)	15 (8, 22)	0.946
5	1.65 (1.32, 1.99)	15 (14, 17)	25 (20, 30)	0.963
7	2.84 (0.10, 5.59)	14 (11, 16)	18 (9, 27)	0.855

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Table 2. Linear regressions of parameter estimates against incubation time for the Espegrend samples (see Fig. 3). Extrapolation to zero time is given (Day 0).

	Intercept (Day 0)	Slope of linear regression	p (H_0: slope≠0)	R^2
		MR		
N_0	-0.8 (-1.4,-0.2)	0.19 (0.01,0.37)	0.05	0.674
r	41 (36,46)	-2.8 (-4.1,-1.5)	0.004	0.896
s	65 (48,81)	-4.7 (-9.4,0.0)	0.05	0.656
		Kviturspollen		
N_0	-1.0 (-2.4,0.4)	0.6 (0.3,0.9)	0.01	0.917
r	10 (3,16)	0.8 (-0.7,2.3)	*0.2*	0.479
s	24 (6,42)	-0.8 (-4.7,3.1)	*0.6*	0.121

H_0 null hypothesis

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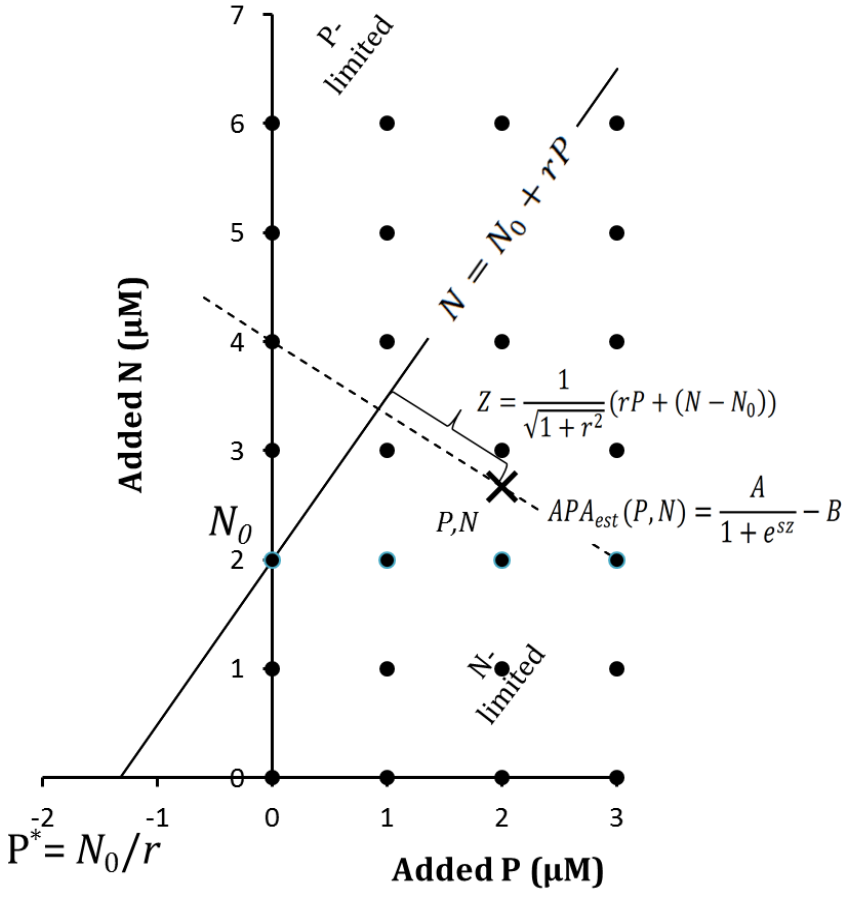

Figure 1. Illustration of the fitting algorithm used. With APA measured over a (here) 4x7 matrix of combinations in additions of P and N (black circles), the objective is to find the line that splits the *P*,*N*-plane in an upper P-limited region with high APA and a lower N-limited region with low APA. This is done by least square fitting of the surface $APA_{est} = \frac{A}{1+e^{sZ}} - B$ to the APA-values measured in each grid point. $APA_{est}(P,N)$ is a sigmoidal function of the distance $Z = \frac{1}{\sqrt{1+r^2}}(rP + (N - N_0))$ from the point *P*,*N* (marked X) to the line. The situation illustrated represents an N-limited system with the positive *N*-axis intercept (N_0) and excess-P (P^*) represented by the negative *P*-axis intercept N_0/r.

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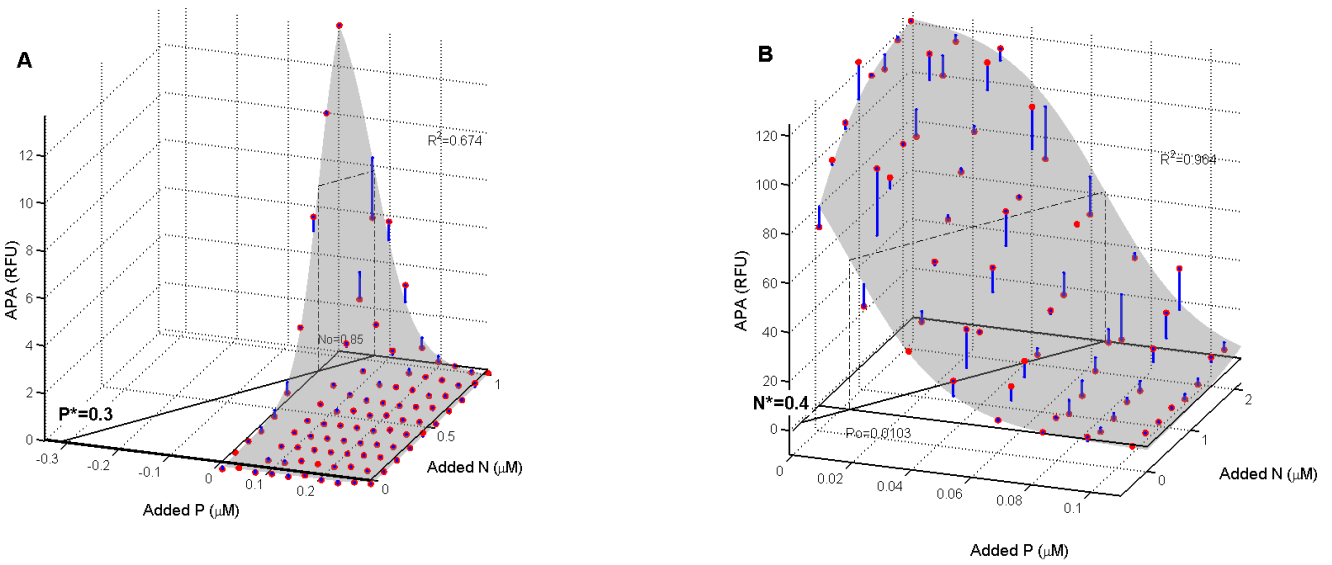

2
3 Figure 2. Measured APA values (red), fitted response surface (grey) and residuals (blue) for assays: (A) Fjord from Tvärminne and (B)
4 MR from Espegrend (Day 1); illustrating situations interpreted as N-limited with P^*=0.3 μM-P and a P-limited with N^*=0.4 μM-N,
5 respectively.

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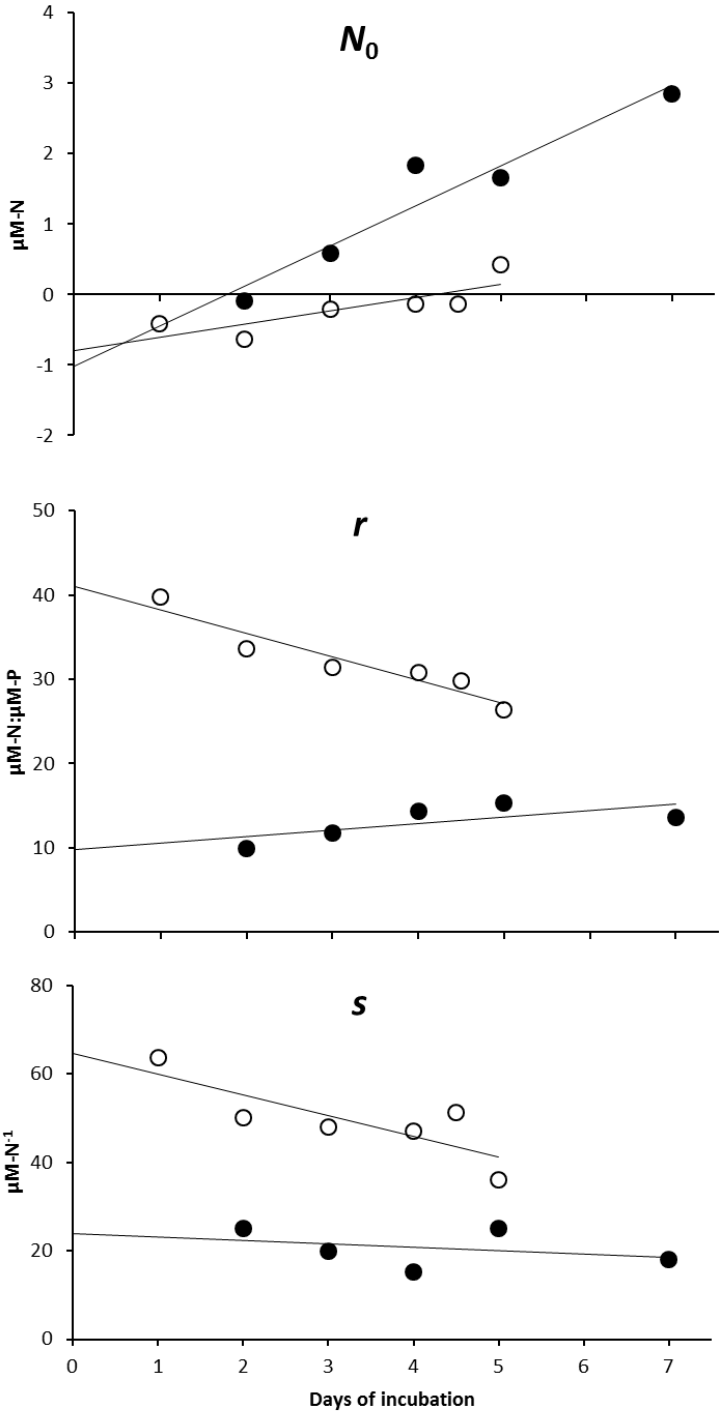

Figure 3. Change in parameter estimates with incubation time for the two samples from western Norway. Kviturspollen has filled symbols, mesocosm MR has open symbols.

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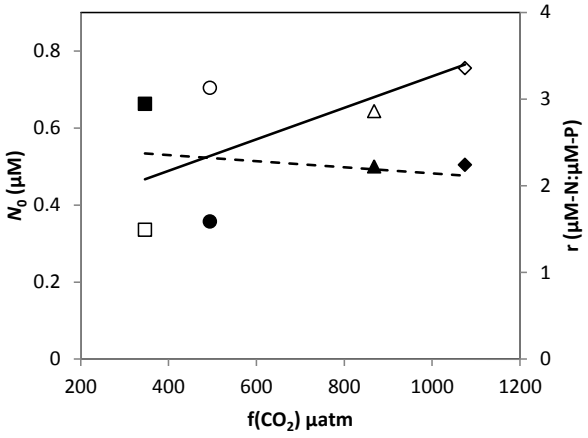

Figure 4. Scatterplots between f(CO_2) and estimates of N_0 (open symbols, solid regression line) and r (closed symbols, dotted regression line) for Tvärminne mesocosms M1 (squares), M3 (diamonds), M6 (triangles) and M7 (circles). Regression slopes are not significant (p=0.27 and 0.79, respectively).