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Interactive comment on "Diurnal variation in the coupling of photosynthetic electron transport and carbon fixation in iron-limited phytoplankton in the NE subarctic Pacific" *by* N. Schuback et al.

Anonymous Referee #1

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This paper aims to further our understanding of the how electron transport rates (ETRs) are coupled to carbon fixation (i.e. CO2 uptake rates), by examining the diurnal variability of the electron rate for carbon fixation (Kc) in the field. The authors directly compare ETR measurements against 14c-uptake for the first time throughout a diel cycle, a relevant goal with the potential to improve our capacity to derive primary productivity estimates from FRRf fluorometers. Similarly, the observed relationship between non-photochemical quenching (NPQNSV) and Kc may provide supporting evidence for their previous findings (Schuback et al. 2015) that this parameter may hold value as a predictor for this conversion factor.

In spite of the positives, I believe that the viability of NPQNSV to predict Kc under vary-

ing environmental/taxonomic scenarios needs more attention from the authors; as it stands this paper does not really add a huge amount of value to their previous study for this reason. Given that taxonomic groups likely have different capacities for NPQ (and therefore potentially varying levels of reliance upon alternative electron pathways to relive excitation pressure upon PSII) it needs to be considered how the dominance of particular groups may influence the NPQ signature relative to Kc. In other words, how widely do the authors expect their findings to hold across waters where taxonomy is changing. Additionally, the authors have a reasonable argument that deriving [RCII] from a fluorescence-based algorithm (Oxborough, 2012) is problematic due to iron-limitation (and this is potentially due to a change in the quantum yield of fluorescence) so does /will their approach to reconcile Kc from NPQNSV still perform under iron-replete conditions (or indeed other environmental conditions that alter the quantum yield of fluorescence)? By divorcing themselves from the requirement to quantify [RCII], this approach rests heavily on the ability to empirically relate NPQNSV and Kc in order to derive ecologically-relevant productivity rates from ETRs , so this needs to be robustly tested under different scenarios to determine its validity (can we really not consider the variability of npsii?). My main concern here is that not quantifying [RCII] the authors potentially advocate a "backwards step" for the field of active fluorometry (until the reliability of NPQNSV as a predictor of Kc is robustly evaluated) where the variability of npsii is not quantified. For this reason, the paper (and the robustness of the messages, and hence impact, the authors are trying to convey) would immensely benefit from an additional side-experiment using cultures (iron-replete preferably from a selection of taxa) to see how robust this approach is.

Overall the approach to examine the diurnal variability of Kc does add to our existing understanding of the coupling of ETR to C-uptake (but would benefit from more commentary to discuss/infer upon the mechanisms that act to decouple the rates during periods of saturating light). I remain less convinced about their advocing NPQNSV as a broad predictor of Kc due to the fact that it has been tested under a very specific environmental niche. I would encourage the authors to provide additional lab-based

data to support this, or alternatively to critically evaluate the potential conditions where this relationship may break-down, in order that studies following on from this work can begin to systematically test this.

Specific Comments (16805 LN8) – I'm not convinced that the 1993 or 2004 papers cited are "more recently" (either amend the nature of this sentence or the references used to justify)

(16805 LN23) – Hancke et al. (2015, PLoS One) recently proposed the symbol Kc is more appropriate to describe the "electron requirement for carbon fixation" following the (correct) logic that the symbol Phi widely denotes a quantum yield of a process, rather than a quantum requirement. The authors could also consider adopting this nomenclature to standardise terminology (which can often be confusing for non-specialists).

(16805 LN24) – "Plasticity in both parameters can be observed" this sentence needs to be supported by appropriate references and perhaps the range of variability encountered with each parameter (and hence what the scale of assuming 1/nPSII x Phiec amounts to).

(16806 LN2-9) - The main concern with this paragraph is that is implies these past studies have examined this "conversion factor", ATP/NADPH requirements as well as assimilation efficiencies – I don't think this is the case and perhaps the authors need to consider more appropriate references or clarify exactly how these references support this statement. Also, a slightly better description than "backpressure" is needed here – it is not explicit as to what the authors are referring to as an accumulation of electrons within the electron transport chain (and/or subsequent effects upon intracellular reductant/ADP-ATP ratios etc). Perhaps also clarify that this "backpressure" is undesirable.

(16806 LN13) - I guess another way of looking at this is that 14C P versus E data has a "classic" diurnal "hysteresis" to it. The question is whether ETRs also are affected in the same way (and /or to the same extent). I was not convinced that ETRRCII would be

(effectively) independent of time of day (no hysteresis) since systems can easily build NPQ, RCII deactivation etc, which would cause much less efficient systems in the afternoon (increasing E) than afternoon (decreasing E). This sentence needs proper thought, clarification and appropriate support from past studies.

(16806 LN19) - Agreed, but it may be useful to state that at best past FRRf studies have integrated ETR and C-uptake over entire diel scales (Suggett et al. 2006, Limnology & Oceanography) and thus the potential time-dependency remains unresolved.

(16808 LN6) – The authors state that 3 hours of PAR data is lost, which is understandable (if unfortunate) however I think it would be useful to clarify which 3-hour time period is missing from the dataset and has been extrapolated (for consideration when interpreting results).

(16809 LN8) – Perhaps justify why was pigment analysis was only performed at 4 time points? – Noon pigment samples would have been useful to look at photoprotective pigments rather than have a 6 hour gap (9am – 3pm).

(16811 LN1) Because the authors are working with low biomass samples (0.2ug/l chl-a) is the averaging of 20 sequences adequate to reliably extract fluorescence parameters? (particularly at higher PAR levels). I know the Soliense is a capable instrument with high sensitivity so just a line or two confirming the authors have considered this would be useful.

(16811 LN17) - It might help to justify why the authors use sigma' x Fq'/Fv' in eq 3 for the non-photochemical quenching/photochemical quenching components as opposed to sigma x Fq'/Fm'? The two approaches may give different ETRRCII if not all NPQ is coming out of the antennae (e.g. RCII-bed quenching), which may be important under diel conditions where RCIIs are deactivating (e.g. Gorbunov et al. 2001 Limnology & Oceanography) – could this be why the ETR and 14C decouple under the diel scenario and perhaps an artefact of the ETR algorithm used. This may need some additional data analysis to rule out.

(16811-16812) - Similarly, I see no mention of subtraction of background fluorescence, which could entirely influence the outcome on the derived fluorescence parameters (Cullen & Davis 2003) and in turn the Fq'/Fv' retrieval – was this performed? Given the low biomass this step could have an important impact upon derived fluorescence parameters, and contribute to the low Fv/Fm values recorded (if not performed), which would them carry through to Fo' and in turn Fq'/Fv'. The authors will need to carefully consider whether a lack of blank correction at each time/depth is contributing to the decoupling of ETR and 14C uptake over time. .

(16811 LN23) – I think it makes more sense to specify that you are converting units of angstroms to m2 rather than simply just 10-20 m2 to m2

(16812 LN10) - It would be good to provide justification for the incubation time and briefly discuss, as an incubation of this length falls closer to NPP along the continuum of GPP – NPP compared to the (shorter) ETR measurements.

(16814 LN11) - It was not clear in this sentence "it is unlikely to give accurate results under conditions of iron limitation" why the following citationns were used – have they explicitly tested the algorithm of Oxbrough to derive [RCII]? If not, why do the authors suspect the references provide the evidence that the [RCII] algorithm would not apply. Some serious justification and clarification is needed here.

(16814 LN16) I think that if the authors differentiated parameters by using the nomenclature Pmax to refer to maximum carbon uptake rates, and ETRmax (which is more in line with convention and specific to the measurements from which the parameter is derived) when referring to maximum electron transport it would avoid any possible confusion between the terms (e.g Pmax of ETR) (this issue also applies to the previous paragraph where deriving "Pmax").

(16817 LN22) I feel that the authors are definitely stating that PPC is highest at noon, when in fact this timepoint was not sampled, and the data between 9am-3pm has been extrapolated. This could be phrased better to include the potential element of

uncertainty in this statement.

(16818 LN7) Typo – should read "in-situ 5m irradiance".

(16818 LN28) Typo – should read de-epoxidation (not de-epoxilation)

(16818 LN12) The low Fv/Fm values are entirely attributable to iron limitation? The question of blank subtraction raises its head here otherwise.

(16818 LN18) Why introduce the term "qP" here (when Fq/Fv' is used earlier); also, why qP and not some other measure of the degree of RCII closure (see Oxborough et al. 2012)

(16818 LN28) I'm not sure the authors can robustly defend the statement "As the first study to investigate diurnal pattern of cellular energy allocation" – it's the first study to examine the empirical connection between ETR and 14C (net)uptake) but it does not look at cellular energy allocation!!!! Energy allocation is only subsequently 'inferred' through discussion/speculation via the patterns.

(16822 LN10) – "In conclusion, we suggest that the observed changes in the conversion factor". OK, potential methodological artefacts aside (see points above, and as I said these need to be really robustly considered to ensure that the diel story holds), this entire section is a theoretical 'journey' with a laundry list of physiological pathways to explain how cells operate and therefore could possibly account for the diel decoupling. I'm not sure the value this has without any real physiological evidence per se. As such, I strongly recommend this entirely speculative section be toned down but also that 'caveat' text put in place upfront to state that this is purely speculative at this stage - possible diel coupling could be envisaged through increasing re-balancing of energy and/or reductant; for example. . .however, the nature and extent of operation of these various pathways and the exact nature with which diel coupling operates remains to be verified. This is important since depending on the environment or taxa under investigation one might imagine that these processes operate more strongly/weakly and hence

ETRRCII and 14C uptake more distantly/closely coupled.

(16823 LN17) – Blank issues again? Also, any evidence of chlororespiration (important under Fe limitation according to Behrenfeld and others) – this would be evident form the light response curves for Fq'/Fm' – this wil also need to be discounted. Also, Suggett et al. (2009) MEPS notes that Fv/Fm can be as low as 0.35-0.4 for small flagellates under nutrient replete conditions – this wil be the case where photoprotective pigments act to really drag Fv/Fm down. The bottom line is that there's a whole suite of variables that need to be discounted before Fe limitation alone is left as the smoking gun.

(16824 LN22) – I liked Fig. 7 BUT there's an obvious (and necessary) analytical step missing – the coupling between the two variables appears to follow different trajectories for each different time bin; it would really help to run calculate (linear) regression slopes for each time point (and intercompare statistically these for the different time points). This would objectively inform the authors if the coupling is drifting in a certain direction overtime and just whether time matters. By eye, a single linear regression for the whole data set would imply that diel variance is not important (i.e. the variance across the data set is too large to pull out any time differences) – the point being that time of day is clearly important BUT that NPQNSV can generally account for this? The authors allude to this in the discussion (16825 LN20) but this is not supported statistically and the reader has to take a large leap of faith.

More generally with this entire section, it reads as though the issue is done and dusted, i.e. NSV should "always" explain the relation between ETRRCII and 14C uptake (and hence that non-photochemical quenching always reflects how energy/reductant is utilised/rebalanced, according to the discussion by the authors, which it is unlikely to be – what about N-assimilation for example?); also, that at present the authors have only explored this approach for a single Fe limited region/community but would they expect it to hold for other taxa/communities where NPQ and physiological process differ and for different types of environmental limitation. Some word of caution are needed to tie back their findings to the (currently) limited scope of the data set.

Figure 3&4 – These figures could be combined – Ek could be added as a panel to figure 4 (as it stands I don't see that the Ek data alone warrants an independent figure when it could easily be included at the bottom of fig 3).

Figure 5 – I am not convinced about the need for the lines extrapolating between each timepoint – it only serves to visually fill in the gaps between samples (a lengthy 6 hours gap), which really should have been addressed at the time of sampling. The overall trend would still be apparent without this.

Figure 6 –Tau should be included here, (after all, why not? – Fv/Fm and Sigma are here and I think Tau would provide an extra level of information in understanding how ETR and downstream processes (i.e. C-fixation) are linked

Figure 7 – Whilst the overall correlation looks reasonably good, the different "trajectories" that seem to be apparent and need better consideration, see comment above.

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