Response to Anonymous Referee #1

We thank the reviewer for their very detailed and insightful comments and suggestions. Below, we specifically answer each of the issues raised and provide information on how we suggest changing the manuscript d accordingly. Accompanying this response letter, we provide a revised manuscript. In the revised manuscript we used blue font for the sections we suggest to change in response to the reviewers comment. Throughout this letter, we refer to page and line numbers of the revised manuscript.

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 varying 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?

The primary aim of the present study was to determine the presence and magnitude of diurnal variation in the coupling of photosynthetic electron transport and carbon fixation in iron-limited phytoplankton in the NE subarctic Pacific. Our results show significant uncoupling of the two rates over a diurnal cycle under conditions of iron limitation. This result is the key aspect of the paper, and its main contribution to the field. As pointed out by the reviewer, the new field data we present are the first of their kind, and should thus be of significant value to the community.

Our results further add to the large amount of experimental evidence which shows that a constant conversion factor cannot be used to derive rates of carbon fixation from FRRF derived rates of ETR. If, and how, the required conversion factor can be estimated with sufficient accuracy has become a major research question in the field (e.g. Lawrenz et al., 2013). Building on previously published work from the same oceanic region, we suggest that an observed empirical correlation of the derived conversion factor and NPQ_{NSV} holds promise to improve approaches aimed at modeling a variable conversion factor. However, this is not the focus of the

manuscript, and we fully recognize the potential limitations pointed out by the reviewer in relation to taxonomic and environmental variability. In the present manuscript, about half of the section describing the correlation between Kc and NPQ_{NSV} (section 4.4., pages 18824-16826) is dedicated to possible caveats, concluding with the sentence:

"Larger datasets, spanning multiple oceanic regions and phytoplankton assemblages of contrasting taxonomic composition and physiological state are needed to further investigate the correlation between NPQ_{NSV} and $\Phi_{e:C}/n_{PSII}$."*(section 4.4., pages 16826, lines 25-27)*

We feel that this is a very clear statement of exactly the kind of caveats raised by the reviewer. In the revised version of the manuscript we have taken great care to further emphasise the preliminary nature of the approach.

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)?

As the reviewer points out, the Oxborough approach to quantify [RCII] from a fluorescence based algorithm is problematic under conditions of iron limitation. Working in an iron-limited region of the ocean, we thus combined the two unknown parameters into one conversion factor out of necessity, as opposed to undervaluation of the benefits gained from knowing [RCII]. In response to an insightful suggestion by reviewer #3, the revised version now contains estimates of the relative diurnal change in [RCII] (and $1/n_{PSII}$), which we use to deduce the contribution of diurnal changes in K_c to the 'lumped' conversion factor (page 11, lines 292-316; page 14, lines 380-390; page 17, lines 487-488,; page 18 494-497).

We have also emphasised that our results may be difficult to extrapolate to high iron regions (page 22, lines 626-629). Yet, given that vast regions of the contemporary surface ocean (about 30%) are affected by iron limitation, our approach is likely applicable to a significant fraction of surface ocean environments (see additional comments below).

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?).

As discussed above, we now estimate relative changes in RCII, thus explicitly considering variability in this parameter. It is worth noting, however, that even with robust estimates of [RCII], derivation of carbon-based productivity rates still dependent on an estimate of K_c, which is subject to significant variability. Indeed, our results show that most of the variability in the overall conversion factor is due to changes in K_c, rather than [RCII]. We are not aware of any

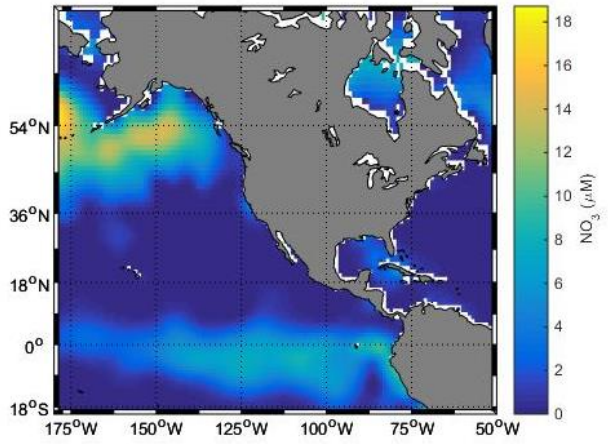
robust approach which could estimate K_c on its own. Our results provide a potential approach (which may not work under all conditions) to estimate K_c/n_{PSII} from FRRF data and thereby estimate carbon-based productivity from FRRF data alone.

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.

We agree that laboratory experiments are needed to further investigate the correlation between K_c/n_{PSII} and NPQ_{NSV}. However, these need to include a wide range of species and light as well as nutrient conditions and are not within the scope of the present manuscript.

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.

We are not advocating the relationship as a broad predictor of K_c. Yet, the data presented in this paper, in addition to that of Schuback et al. (2015), do show that the relationship appears to hold up well for the iron-limited subarctic Pacific. We disagree that this oceanic region represents a 'very specific environmental niche'. As shown in the Figure below, iron limited waters (defined by areas with excess summer time macro nutrients) cover large swath of the Eastern Subarctic Ocean. We can conservatively estimate the area to cover ~ 1,500 x 1,500 km, which is equivalent to more than 2 million km^2. This surface area far exceeds that of many other regions (e.g. North Sea) that have received significant research attention, and would not likely be considered as 'specific environmental niches'.



As mentioned above, we have revised the manuscript in order to emphasise the main focus of the present study (the uncoupling of ETR and carbon fixation over a diurnal cycle). We do share the reviewers' reservations about the applicability of a NPQ_{NSV}-based conversion factor across contrasting environmental and taxonomic regimes and we have revised the manuscript to further emphasise this (page 1, line 31; page 22, lines 621-623). We do, however, stand by our claim that the observed empirical correlation holds promise to improve approaches aimed at modeling the conversion factor and in turn improve estimates of carbon-based primary productivity from FRRF measurements.

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.

We revised the manuscript to tone down the potential of the observed correlation and address potential limitations of the approach. As mentioned above, we agree that lab studies are certainly needed. But, in order to fully validate the approach, one would require lab studies with many different species, different iron, light, nutrient levels etc. We are actually pursuing these in our group, but we need a comprehensive suite of measurements, and more than could be put into this paper.

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)

We were trying to convey the fact that active chlorophyll a fluorescence based methods are recent in relation to "traditional" incubation based methods including 14C-uptake studies. We have changed "More recently" to "Over the past two decades" (page 2, line 39).

(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).

We have changed the nomenclature throughout the manuscript, and have explicitly noted the change in terminology with respect to our recent study (page 2, lines 52-53).

(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).

We have added information on the range of variability encountered as 1.15 – 54.2 mol e^- mol C^{-1} for K_c (Lawrenz et al., 2013) and approx. 200 – 950 mol chl a mol $RCII^{-1}$ for $1/n_{PSII}$ (Suggett et al., 2010).Using these ranges, the conversion factor K_c/n_{PSII} could vary from 230 - 51490 mol e^- mol C^{-1} mol chla mol $RCII^{-1}$ (page 11, lines 284-287).

The recent meta-analysis by Lawrenz et al., 2013 found a mean value of 11.8 mol e^- mol C^{-1} for K_c , while 500 mol chla mol $RCII^{-1}$ is commonly assumed for $1/n_{PSII}$ (Kolber and Falkowski, 1993). Using these values, the conversion factor K_c/n_{PSII} would be 5900 mol e^- mol C^{-1} mol chla mol $RCII^{-1}$, which is consistent with the range encountered in our study (2700 – 9200 mol e^- mol C^{-1} mol chla mol $RCII^{-1}$). These theoretical calculations were discussed in the original manuscript (page 16820, lines 19-23), and can be found in the revised version on (page 18, lines 490-493).

(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.

We agree with the reviewer and have rewritten the sentence (page 3, lines 59-62).

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.

The section has been rewritten (page 3, lines 62-69).

(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.

The whole paragraph has been removed from the introduction of the revised manuscript, and we now mention the likely diurnal hysteresis in ETR specifically in the discussion (page 17, lines 482-486).

(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.

We have changed the manuscript accordingly, referencing the study by Suggett et al., 2006 (page 3, lines 8-83).

(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).

The time period for which the PAR data was extrapolated (bases on continuously logged SW radiation data from the NOAA buoy) was 14:00 – 17:00. This has been clarified in the revised manuscript (page 5, lines 114).

(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).

We agree and, in light of the results, would love to have pigment data for all time-points. However, it was simply not feasible to collect samples at all time-points due to logistical constraints (all of the measurements and sampling were conducted by two people). The revised manuscript acknowledges that higher resolution data would have been desirable (page 14, lines 394-395; page 15, line 413).

(16811 LN1) Because the authors are working with low biomass samples (0.2ug/l chla) 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.

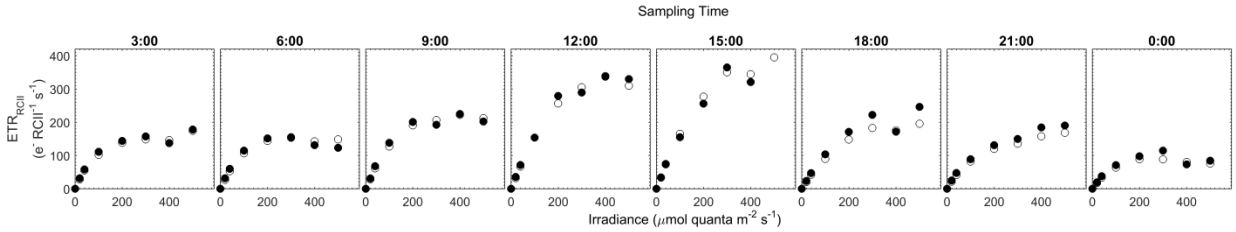
If we just consider the last acquisition (average of 20 sequences) at each light level, the signal to noise ratio (S/N), calculated as the ratio of signal mean to signal standard deviation, is very high (approx. 400) for all parameters acquired during the dark and low background light levels of our light curves. As the reviewer points out, the S/N ratio of the derived parameters decreases at high background irradiances where the S/N for F' and F_m' is still above 200, but as low as 5 for σ'. The significant decrease in the S/N for σ' is to be expected, due to loss in variable fluorescence and therefore recovery of fit parameters. However, given that deriving ETR using calculations which include σ' (low S/N) as well as calculations which only use σ measured in the dark-regulated state (high S/N) give us very similar results (see answer to comment below), we are confident that σ' can be reliably extracted from 20 sequences.

It is furthermore worth stressing that all derived parameters used for the ETR calculation are the mean of the last three fits, each of which is the average of 20 sequences.

(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.

The two alternative approaches to calculate ETR described above are ETR=E x σ' x Fq'/Fv' and ETR=E x σ x (Fq'/Fm')/(Fv/Fm). The main difference between these approaches relates to how (and where in the equations) one accounts for the effects of non-photochemical quenching on measured ChlF yields. The two approaches are equivalent under conditions where non-photochemical quenching is caused by thermal dissipation of absorbed energy in the light harvesting antenna and sigma'=sigma x (Fq'/Fm')/(Fv/Fm) (Gorbunov et al., 2001).

As shown in the figure below, these two equations give very similar results. For all ETR_{RCII} values used in this study (n=71) the difference between values calculated in both ways ranged from 1 % to 16 % with a mean coefficient of variance of 6%. This information is now included in the manuscript (page 8, lines 216-221).



● $ETR=E \times \sigma'_{PSII} \times F_q'/F_v'$

○ $ETR=E \times \sigma_{PSII} \times (F_q'/F_m')/ (F_v/F_m)$

(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.

Blank corrections were performed for each sample and values for each wavelength automatically subtracted during the sample run. We added a description of the exact procedure for blank-correction in the revised methods section (page 7, lines 181-182).

(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

We have used these units to be consistent with the editorial requirements (SI units) of the journal.

(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.

Less than 2 hr incubations would have potentially been too short to observe a meaningful signal, given the low growth rate, low biomass and small volume used. Since the diurnal sampling time-points were 3 hr apart, a 3.5 hr incubation time was necessary to spike and set up samples from one TP and then filter samples from the previous TP. In the revised methods

section, we have now added some text (and two supporting references; Halsey et al., 2011; Pei and Laws, 2013) stating that our approach may approximate a rate closer to net rather than to gross carbon fixation (page 9, lines 244-246).

(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.

The inherent assumption to the approach of Oxbrough et al. is that the ratio of the rate constants of photochemistry (kp) and fluorescence (kf) stay within a narrow range. This is not the case under iron limitation, where kp decreases while kf increases (e.g. Vassiliev et al., 1995). Indeed, the original paper by Oxborough et al. cautioned that the approach relied on assumptions which might not hold under nutrient limitation, especially if the limiting nutrient is iron (Oxborough et al., 2012). This potential caveat was recently repeated by Robinson et al., 2014, who say:

"The calculation of [RCII] using the relationship between the minimum fluorescence parameter (Fo) and [RCII] as determined by Oxborough et al. (2012) may be sensitive to nutrient stress (C.M. Moore pers. comm.) which results in the enhanced uncoupling of chlorophyll complexes and PSII reaction centres (…) ."

A recent publication by Silsbe et al. (2015) includes data for cultures grown without added iron, though no specific information is presented on the extent of iron limitation in these cultures. In the assessment of their data, Silsbe et al. note that:

"…, both Tp-Fe and Tw-Fe cultures grown in the absence of iron predicted higher [RCII] than measured. Consequently these cultures yielded lower K_R values than other cultures. This key finding is consistent with the concept that iron limited phytoplankton may accumulate a store of non-energetically coupled chlorophyll-binding complexes that increases the quantum yield of fluorescence (Φ_f) relative to iron replete phytoplankton (Behrenfeld and Milligan 2013; Macey et al. 2014). As K_R is proportional to Φ_P/Φ_f, an increase in Φ_f would diminish KR as observed in this study. Omission of these iron-deplete cultures generally increased the mean K_R value for each instrument and reduced its variance…"

In the revised manuscript we changed the wording to be less ambiguous about why we did not apply the Oxborough approach. We furthermore added a whole section in which we applied a simplified version of the Oxborough approach to quantify relative diurnal changes in $1/n_{PSII}$ (page 11, lines 292-316; page 14, lines 380-390; page 17, lines 487-488,; page 18 494-497).

(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").

We changed the abbreviations used in the text to P_{max}-ETR_{RCII} and P_{max}-C as well as α-ETR_{RCII} and α-C.

(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.

We did not mean to imply mid day as the strict noon hour, and have clarified the sentence (page 14, lines 399-401).

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

We clarified, that, like throughout the manuscript, in situ irradiance refers to irradiance at 5 m depth (page 15, line 425).

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

Corrected.

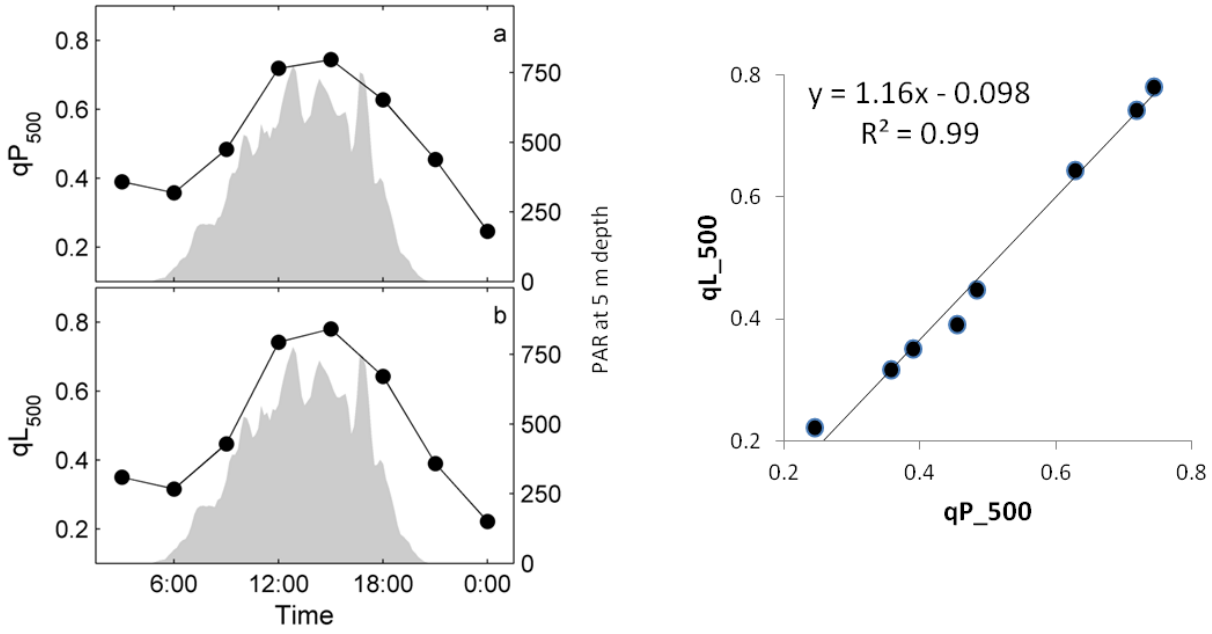
(16818 LN12) The low Fv/Fm values are entirely attributable to iron limitation? The question of blank subtraction raises its head here otherwise.

As discussed above, blank corrections were performed for each sample (page 7, lines 181-182).

(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)

The original manuscript actually described the term qP in the method section (page 16811, line 20), where we explicitly state that $F_q'/F_v' = qP$. We reasoned that using both, F_q'/F_v' and qP, while stating that they are the same, would give the reader the ability to see how the parameter is derive as well as making it easier to relate our findings to many earlier studies, which use qP. In the revised manuscript we use the term F_q'/F_v' throughout.

In response to the reviewers concern about different approaches to estimate the degree of RCII closure from ChlF yields, we below show two versions of Fig. 6d (Fig 5d in the revised manuscript).. The top panel shows the original figure, where the fraction of open RCII (i.e. the first stable electron acceptor Q_A oxidized) is estimated as F_q'/F_v' (= qP). This approach assumes zero energy transfer between closed and open RCII ('puddle' model). The bottom panel shows the same data, where the fraction of open RCII is estimated as $F_q'/F_v' \times F_o'/F'$ (= qL). This approach assumes perfect energy transfer between closed and open RCII ('lake' model).



As shown in the figures above, both approaches give very similar results, and lead to the exact same interpretation of the data.

(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.

We agree with the reviewer and changed the wording of this section (page 16, lines 437-444).

(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.

We understand the reviewers concern and have rewritten the section to stress that our study does not include any direct verification of the cell physiological processes we suggest to be responsible for the observed decoupling between ETR_{RCII} and carbon fixation. We have also added a section about potential caveats, as suggest (page 19, lines 535-536).

(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 will 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.

We did not mean to imply that iron limitation is the only reason for the low F_v/F_m. Rather; we wanted to give evidence for the iron-limited state of the phytoplankton assemblage sampled. We changed the wording of the section (page 20, lines 568-571).

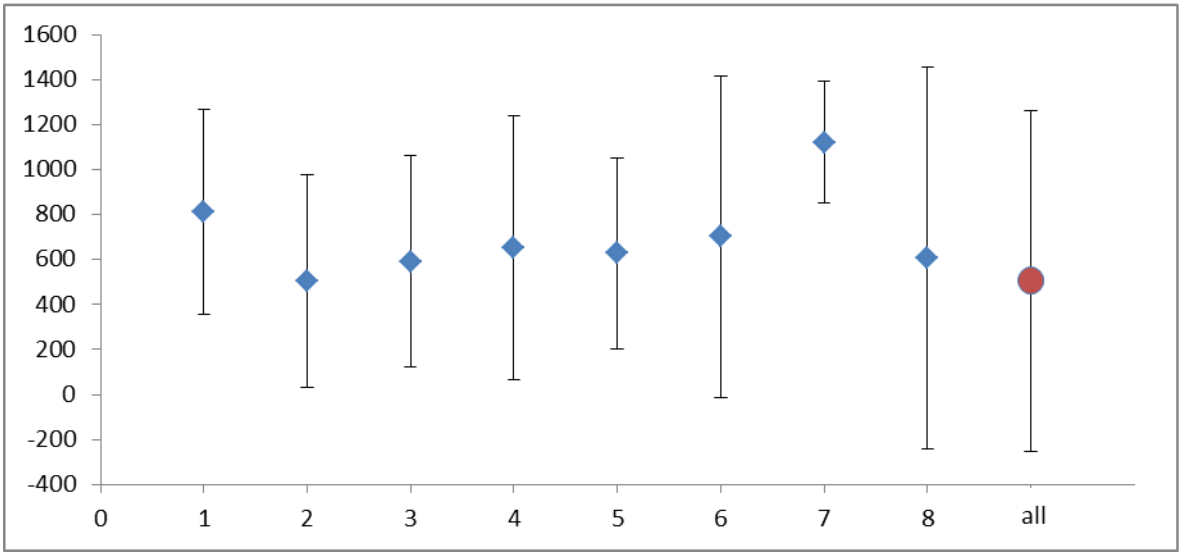
This aside, we can rule out the blank issue (as blanks correction was indeed performed), and chlororespiration should have been minimal as samples were incubated under low light before measurements.

(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.

The reviewer's suggestion is very good, and we show below the linear regression slopes and their standard errors of estimate (SEE) for each TP over the diurnal cycle. The figure shows that the slopes are, in fact, not statistically different from each other. We therefore removed the section where we discuss the differences in regression of different TP from the discussion, and replotted Fig 7 accordingly.

TP	1	2	3	4	5	6	7	8	all
time	3:00	6:00	9:00	12:00	15:00	18:00	21:00	0:00	
slope	811	505	593	653	628	701	1122	607	506
intercept	524	743	570	721	329	1199	378	1373	1887
SEE	457	471	470	586	423	714	273	849	758



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.

In our original discussion, we discussed, at some length, a variety of limitations of this analysis. In response to the reviewer's comments, we have further revised the section to (hopefully) make it even clearer that our results present a novel and preliminary observation that requires significant follow up with future studies (page 1, line 31; page 22 lines 621-623; page 23, lines 649-652). We see the work as a first step, which requires validation before any broad conclusions can be reached.

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).

This is a good suggestion, and we have now combined these figures.

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.

The lines have been removed.

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

A panel showing 1/τ has been included in Fig. 6.

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

See answer to comment above.

References

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