

As I understand the process we are not supposed to supply a modified version of the manuscript and so for many of the changes we have to just say what we would do.

General

This paper proposes a method to estimate the oxidative ratio (OR) combining stoichiometry, thermodynamics, and elemental budgets, which is a novel approach. They apply this method to data they had gained in previous studies from a blanket bog in north England. They find an OR of 0.88 for this site, which is much lower than previously found and conclude that this could lead to an underestimation of the terrestrial carbon sink of about 12%. They also conclude that 32% of the O₂ consumption is due to oxidation of NH₄⁺. The general feeling is that the method proposed could be a valuable attribution and would be good to propose a way to use this to come to an estimate of the global OR. However, I have great concerns about the validation and the scaling of the method in this paper (see below).

Thank you for understanding the intent of the paper.

In general I would say the method, and thus the paper, has much potential, but I would not recommend publishing it unless major changes are made in the validation and scaling of the method.

We hope that below we answer how we would address your concerns if we were supplying a revised version of the manuscript at this point.

Concerns

There is an uncertainty analyses done based on the elemental analyses and Eg but they haven't reported this in the paper. So, it's not clear what the uncertainty of the OR is! Thereby, there are many more uncertainties. For instance, the C-budget (equation x), where each component has its uncertainty (I find 22% carbon stored as peat also unrealistically high).

This is a C budget established by other studies of this site and not just by flux measurements (Worrall et al., 2009; 2012), but also by energetic and stoichiometric constraints (Worrall et al., 2022); and further that the C budget estimated from the flux measurements bracketed the long term C accumulation rate estimated from carbon-age model (Worrall et al., 2022). Below we agree that we can add this detail to the paper.

I wonder whether the concern here arises from the way we express the C budget – not as an absolute value (eg. tonnes C/ha/yr) but as proportion of the input.

There are different possibilities of TEA, what would have happened if a different electron acceptor dominated?

We do explicitly consider a range of TEAs, for example in the text:

“With respect to the problem of estimating OR these include the budgets of redox active elements: nitrogen (e.g. Hemond, 1983, Worrall et al., 2012), sulphur (Novak et al., 2005; Blodau et al., 2007; Boothroyd et al., 2021), and iron (Boothroyd et al., 2021).”

and ...

“Additionally, the budgets for nitrogen, sulphur, and iron for this study site have been 272 documented in previous research (Worrall et al. 2012, Boothroyd et al., 2021). These 273 established budgets can be utilized to validate and refine the predictions generated by the new 274 methodology in this study.”

Further, in Equations (xi) to (xvi) we are using N or S as a TEA.

However, we would be happy to extend this to make it clear, for example to make clear that we can assume no role for Fe and Mn in this case:

“While this assessment has focused on O, N, S, and C as terminal electron acceptors, iron (Fe) and manganese (Mn) can also play a critical role. Routine monitoring showed a depth-dependent increase in pore water Fe, indicating its reduction and mobilization in deeper layers. Although Boothroyd et al. (2021) noted a decline in atmospheric Fe deposition (ranging from 8.7 to 129 kg Fe km⁻² yr⁻¹), pore water concentrations remain significantly higher than precipitation levels, which dropped to a median of 0.00 mg l⁻¹ by 2012. Given that the Cottage Hill Sike (CHS) maintains a high average concentration of Fe (0.52 mg l⁻¹) and a consistent export flux (0.38–1.39 tonnes Fe km⁻² yr⁻¹), this catchment acts as a net source of iron. Manganese was never detected in the catchment. Therefore, we have assumed that Fe and Mn play no substantive role”

Denitrification and nitrogen deposition rates, what are the possible ranges for that? I was also wondering about the methanogenesis, this can also be done via the hydrogenotrophic pathway, did you consider that as well?

A partial understanding of the variation in N deposition for this study site is known (Worrall et al., 2012). The total N deposition for the site varied between 0.64 and 4.11 tonnes N/km²/yr between 1993 and 2007 without significant decline over that period and that this total N deposition would be dominated by flux of N₂. We do give this information in the Results:

“Across the reactions considered, 2.9 moles N as NO₃ and 1.3 moles of N as NH₄ are required, while 3.4 moles of N as NH₄ are produced (assuming Equation x). To meet this N requirement would mean that 2.1 moles of NH₄ must be converted to NO₃ which in turn means that 3.2 moles of O₂ will be consumed. The complete conversion of NH₄ to NO₃ would still require 0.8 moles of N as NO₃ which would be supplied from atmospheric deposition, i.e. the recycling of N predicted above is not sufficient for the required supply of NO₃. Worrall et al. (2012) suggest that atmospheric deposition would supply between 0.1 and 0.46 moles N as NO₃, but they also note that the deposition at the study site was not significantly changing. However, there would be sufficient supply of N if atmospheric NH₄ deposition could be oxidised. By accounting for O₂ consumption of this additional N from NH₄ deposition means, in total, 11.5 moles of O₂ are consumed, and thus yields a mean OR = 0.88.”

However, the reviewer is correct that such an approach does not mention denitrification. However, the N requirement does not change, i.e. even if some N is lost to N₂ and N₂O, the N requirement to meet the budget remains the same it would only mean that more of the N deposition would be required. I have gone back to the data as published by Worrall et al. (2012) and assumed that the total N budget was closed by denitrification to either N₂ or N₂O (Previously this study had assumed literature values for these and that the total N budget was closed by N accumulation). If the budget is closed by denitrification then 0.9 tonnes N/km²/yr are lost to N₂ + N₂O, this would mean that an additional 0.39 to 0.61 moles N would be required, currently we assume that between 0.34 and 0.7 moles N are supplied from NH₄ deposition, Allowing for denitrification would mean that 1.03 ± 0.02 moles of N as NH₄ is required, however, the median NH₄ deposition would supply 1.1 moles of N as NH₄ and another 0.04 moles N available from DON deposition. So I would suggest that I extend the results section to include this:

“Across the reactions considered, 2.9 moles N as NO₃ and 1.3 moles of N as NH₄ are required, while 3.4 moles of N as NH₄ are produced (assuming Equation x). To meet this N requirement would mean that 2.1 moles of NH₄ must be converted to NO₃ which in turn means that 3.2 moles of O₂ will be consumed. The complete conversion of NH₄ to NO₃ would still require 0.8 moles of N as NO₃ which would be supplied from atmospheric deposition, i.e. the recycling of N predicted above is not sufficient for the required supply of NO₃. Worrall et al. (2012) suggest that atmospheric deposition would supply between 0.1 and 0.46 moles N as NO₃, but they also note that the deposition at the study site was not significantly changing. There is also the probability that some of the N will be lost through denitrification to N₂ or N₂O. Recalculating the total N budget from Worrall et al. (2012) suggest that a further 0.5 moles N would need to be supplied from atmospheric deposition – a median of 1.03 moles of N would be required from atmospheric deposition not already supplied by NO₃ in deposition. However, over the period of the observation reported for this study catchment in Worrall et al. (2012) there would be sufficient supply of N if atmospheric NH₄ deposition could be oxidised. By accounting for O₂ consumption of this additional N from NH₄ deposition means, in total, 11.5 moles of O₂ are consumed, and thus yields a mean OR = 0.88.”

The idea of different methanogenic pathways is a good one, i.e. hydrogenotropic production of CH₄. However, the approach here is not

sensitive to the pathway as you can see in Equation (ix) the consideration of the C substrate and product and not on the energetics of the specific TEA. The inclusion of O,N,S and C as TEAs arises from the need of the energetics and the stoichiometry. Equally, we do explain that after the formation of surface peat the nature of the change to deep peat does not require any consumption of oxygen, therefore the method is independent of the path or mechanism – in the Results we do note that:

“If we consider 100 moles C fixed as glucose, we can then propagate the progress of C released and O₂ consumed (Table 2 and summarised in Fig. 3). Because the reaction of C from surface peat onwards does not consume O, the total amount of O₂ consumed by organic C processing is not dependent upon the pathway after surface peat formation (i.e. is independent of production of DOC or CH₄ or of deep peat). For every 100 moles of C fixed in photosynthesis 7.8 moles of O₂ are consumed, therefore 92.2 moles of O₂ are released, i.e. an OR = 0.92.”

So therefore I suggest we extend this paragraph to make it clear that a range of mechanisms are possible:

“If we consider 100 moles C fixed as glucose, we can then propagate the progress of C released and O₂ consumed (Table 2 and summarised in Fig. 3). Because the reaction of C from surface peat onwards does not consume O, the total amount of O₂ consumed by organic C processing is not dependent upon the pathway after surface peat formation (i.e. is independent of production of DOC or CH₄ or of deep peat). Equally, Equation (xvii) does not expect any particular mechanism of methane production rather it relies on the relative stoichiometric and energetics of the available substrates and products. Equation (xvii) uses the peat soil organic matter as a substrate and its relative C_{ox} compared to CH₄ means that all of it has to be consumed. An alternative substrate would be the DOM, but the C_{ox} of soil water DOM is lower than that of peat soil (Table 1), i.e. making it a less favourable substrate than currently considered in Equation (xvii). Two alternatives might be considered, firstly, the DOM flushing to surface waters in this catchment has a higher C_{ox} than peat soil SOM making it a more favourable substrate, but how much of the DOM generated in surface layers is transported to depth in the profile is not known. Secondly, hydrogenotrophic methane production (Heimann et al., 2010) would require an inorganic carbon substrate and to what extent that is present in the catotelm of this study site is unknown. Therefore, given Equation (xvii), for every 100 moles of C fixed in photosynthesis 7.8 moles of O₂ are consumed, therefore 92.2 moles of O₂ are released, i.e. an OR = 0.92.”

A kind of sensitivity analyses about which elements influence the OR the most would have been useful as well.

Not quite sure what is meant by this? Only two elements control the OR in this catchment C and N. We do show in this study that with respect to oxygen that Both C and N are required and that 32% of the O consumption is due to recycling of NH₄. We could, for example, extend the results underneath Equation (xv) to include what happens to S:

“The oxidation states of the substrate and product suggest the need for an oxidant. Given the release of N, sulphate (SO₄) is likely the terminal electron acceptor. So for the pathway from surface peat to deep peat for every 100 moles of carbon (C) transitioning through this pathway, 83 moles of C are lost as CO₂, 3.9 moles of S are consumed as SO₄, and 1.4 moles of N are produced as NH₄.”

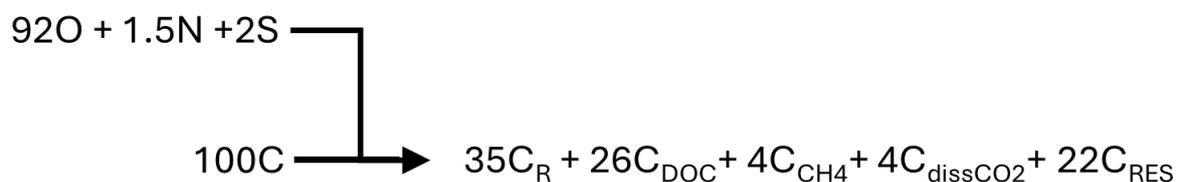
We can add this to Table 2 and we can include S in the overall calculation and summary, but it does not add to O₂ consumption.

“For every 100 moles of C fixed in photosynthesis 7.8 moles of O₂ are consumed, therefore 92.2 moles of O₂ are released, i.e. an OR = 0.92.

Across the reactions considered, 2.9 moles N as NO₃ and 1.3 moles of N as NH₄ are required, while 3.4 moles of N as NH₄ are produced (assuming Equation x). To meet this N requirement would mean that 2.1 moles of NH₄ must be converted to NO₃ which in turn means that 3.2 moles of O₂ will be consumed. The complete conversion of NH₄ to NO₃ would still require 0.8 moles of N as NO₃ which would be supplied from atmospheric deposition, i.e. the recycling of N predicted above is not sufficient for the required supply of NO₃. Worrall et al. (2012) suggest that atmospheric deposition would supply between 0.1 and 0.46 moles N as NO₃, but they also note that the deposition at the study site was not significantly changing. There is also the probability that some of the N will be lost through denitification to N₂ or N₂O. Recalculating the total N budget from Worrall et al. (2012) suggest that a further 0.5 moles N would need to be supplied from atmospheric deposition – a median of 1.03 moles of N would be required from atmospheric deposition not already supplied by NO₃ in deposition. However, over the period of the observation reported for this study catchment in Worrall et al. (2012) there would be sufficient supply of N if atmospheric NH₄ deposition could be oxidised. By accounting for O₂ consumption of this additional N from NH₄ deposition means, in total, 11.5 moles of O₂ are consumed, and thus yields a mean OR = 0.88.

Given the budget of the study site then or every 100 moles of C fixed in photosynthesis 2 moles of S are consumed, but that does not lead to any further O₂ consumption.”

Further, we could alter Equation (x) to reflect this input of TEAs:



This new version of Equation (x) could be added to the end of the results section and shows that in reality the value of OR is not that sensitive to changes in N and S. Firstly, because a considerable proportion of N is recycled and that S as SO₄ is a very efficient TEA because it transition to H₂S releases 8 electrons.

There is also no validation done on the method. It is probably hard and expensive to do above-ground OR measurements, but isotopic validation or nitrate and sulphate concentrations measurements could have been done. Another option would be to apply this method where there are measurements from OR available.

Actually, this study is the validation of the previous studies. In a previous study we have used an entirely different technique. In Worrall et al. (2017) thermogravimetric analysis of we showed that the OR of this peatland was between 0.96 and 0.99. In that study we did not consider an energy transfer efficiency term. This study then used a different approach and showed that the value was closer to 0.88, but in the Discussion we do show that the early study and this study get the same result if the energy transfer term was considered, i.e. these two studies validate each other. We do also consider other studies, using other approaches, and their measurements.

We would be happy to emphasize this role of this study compared to the previous studies.

The authors could have tried to apply the method to different ecosystems. Peatlands only cover 3% of the world's land surface, and blanket bogs are even a rarity among them. They are different in the way they store carbon and how nutrients cycle in the system. So to see if the global carbon uptake would really change by applying a different method, you need to scale this to other systems. Or otherwise, don't make a statement about the implications of the found OR on a global scale.

We have actually done this in a separate study where we have taken data from across the globe (some of the data contributing to the studies quoted in this study - for example Clay and Worrall 2015b) but also bringing data from new studies. In that approach we use global ecosystems and global soil taxa proportions and create a weighted sum to give a global OR.

The paper is also a bit hard to read because they refer to other papers but don't give a short well understandable summary. For a better understanding, I would highly recommend extending the descriptions of the methods and assume less inside information from your reader. I've made some suggestions below.

I am happy to do this and followed the suggestions above and below.

Detailed comments

Please remember that at this stage we not supposed to return a corrected manuscript rather we are to return answers to the questions.

L23: Mention what kind of site (peatland, blanket bog)

Happy to do so, and so the sentence could read:

“The study shows that for the study site, a blanket bog in northern England, although the majority of the O_2 is consumed in the processing of organic-C (68%), 32% of O_2 consumption is due to oxidation of NH_4 .”

L88: Explain this a bit more, what ranges or units and how is it determined

C_{ox} is an oxidation state and so does have units but for organic carbon it does have a range, hence the sentence could read:

“The C_{ox} is the oxidation state of the C in the analysed organic matter and is the nominal oxidation state of carbon (NOSC, LaRowe and van Cappellan, 2011) and has a range from +4 to -4, with CO_2 being the most oxidised form and carbon in CH_4 being the most reduced.”

L126: Lann = Laan

Happy to correct.

L138: These are not recent studies, see e.g. Evans et al. 2021, but there are many more!

Happy to improve this. Indeed there is an even larger compilation carbon budgets than Evans et al. (2021),

“More recently, it has been possible to measure the range of contemporary carbon fluxes in and out of a peat ecosystem where the sum of these fluxes is the C budget (also known as C balance) (e.g. Worrall et al., 2003, Billett et al., 2004, Roulet et al., 2007, Nilsson et al., 2008) and compilation of carbon budgets (eg. Evans et al., 2021, Liu et al., 2024).”

L145-147: Explain a bit better why this is not sufficient

Good idea, and the difference will be the role of other terminal electron acceptors, indeed as shown in this study there is critical role for the inorganic N cycle. I would change the text to read:

“However, this estimation of O accumulation rate is not sufficient to solve Equation (i) and so not sufficient to calculate an OR for this ecosystem as there maybe a role for other redox active elements such nitrogen.”

L208-209: It's not so clear what is meant with how surface peat can become deep peat with these conversions. Could you explain this a bit better?

Yes, can add further detail.

L220: terminal electron acceptor (TEA)

Yes, I can add that detail.

L229-231: Explain where these data come from (citation) and where you can find the ranges.

I have tended to add this detail to the results section, but happy to clarify here and new text would read:

“The triplicate measurements of the composition of the aboveground biomass, below ground biomass, litter, peat soil and DOM were used to give the median and the interquartile range (IQR) of all elemental analysis – these are values reported in Worrall et al. (2022).”

L 259: give a short description of how the carbon budget was established (flux measurements for 18 months e.g.).

Yes, I can add that detail, and would now say:

“The carbon budget for the study site was established from flux measurements reported in Worrall et al. (2009), with updates incorporated from the N budget presented in Worrall et al. (2012). Subsequently, the range of the C budget reported from flux measurements was confirmed by long term carbon accumulation rate measured with radiocarbon dates (Worrall et al. (2022).”

L263 (eq X): match the uncertainties with the numbers above.

Yes, I can correct this.

L346: What about the hydrogenotrophic pathway?

Good question I have detailed that above.

L381: make clear that the organic matter fluxes are estimated.

The method presented whether the fluxes are estimated or calculated and so I see no clarity brought by this, but since I think makes no difference I am happy to add this detail, sentence would now read:

“The study has proposed an independent method for finding the OR of an ecosystem via understanding the element analysis of estimated organic matter fluxes and reservoirs in an environment constrained by stoichiometric and energy balances. “

L480-482: You cannot extrapolate this OR to a global scale

See previous answer.

We did find an additional mistake in line 331.

“So for the pathway from surface peat to deep peat for every 100 moles of carbon (C) transitioning through this pathway, 83 moles of C are lost as CO₂, 16 moles as CH₄, and 1.4 moles of N are produced as NH₄.”

It should read:

“So for this pathway from surface peat to deep peat for every 100 moles of carbon (C) transitioning through this pathway, 83 moles of C are retained as deep peat, 16 moles as CO₂, 3.9 moles of S are lost as H₂S, and 1.4 moles of N are produced as NH₄.”