Response to Reviewer 2 comments

I must preface my response to Reviewer 2 with my general disappointment in their review. While Reviewer 2 does provide one useful comment, the majority of their comments are simply based on a series of non sequiturs. I have attempted, where possible, to improve or enhance problem descriptions that might have lead to the reviewer's erroneous statements, but many of their comments are based on quantitative errors of their own fabrication. It is unfortunate that Reviewer 2 concluded that the manuscript is fatally flawed based on their incorrect assessments, which I comment on below.

Response to Comments:
Reviewer 2 comments are in italics and numbered correspondingly to the review.

1...the actual system (control volume) under examination is not properly specified:...

I agree with the reviewer in this case that the system boundary was not defined as clearly as it could have been. In chemical thermodynamics, it is customary to ignore entropy of mixing terms because their contribution is so small that they do not affect the outcome of analyses. Consequently, the original manuscript implicitly incorporated this practitioner's intuition and did not formally incorporate the entropy mixing terms into the optimization. Instead, the entropy of mixing terms were calculated to demonstrate their minor contributions, but this caused some concern to Reviewer 2, because the 100,000 Monte Carlo simulations might have missed some special case.

For the sake of completeness, I have,

a. Added a formal entropy balance equation and description, and defined the system boundary in Fig. 1 for both global and local optimizations. The entropy balance equation clearly differentiates entropy import from internal entropy production.
b. Defined internal entropy production to include both reaction and mixing entropy terms. That is, "Definition 1" described by Review 2 is used. This was always the definition implied, with the demonstration that the entropy mixing terms were negligible based on the Monte Carlo simulations.
c. Defined internal entropy production for local optimization to include entropy of mixing terms associated with the box being optimized.
d. Changed notation slightly to emphasize internal entropy production as defined by $\dot{\sigma}_I$.
e. Recomputed internal entropy production solutions for both local and global optimizations.
f. Updated Tables 3, 4 and 5, and Figs. 6, 7 and 8 with the new simulation results.

As expected, the formal inclusion of the entropy of mixing terms in the optimization produced very minor changes in the global optimization solution and negligible changes in the local optimization solution. Paper results were not changed in any significant manner, nor were any of the conclusions changed by incorporating the minor entropy of mixing terms. However, other readers not familiar with typical assumptions in chemical thermodynamics might also be confused by the original presentation, which the new version of the manuscript avoids. Consequently, addressing this comment by Reviewer 2 has produced a clearer manuscript.

2. It is not at all clear why the author omits the entropy flux between boxes 1 and 2 from his models (14) or (15)

This is basically a restatement of Comment 1. As indicated above, the entropy of mixing fluxes have now been formally incorporated into the analyses. As expected, their contribution is minor, and their inclusion has no impact on the results or conclusions.

3. There are many inconsistencies and/or errors in the use of units in the manuscript, some quite serious

Considerable thought was given to the choice of units used in the manuscript, and to ensure consistency and ease of interpretation, units were explicitly given with each equation, so I am uncertain how Reviewer 2 arrived at his or hers erroneous conclusions.

Because the model system under study represents a marine system, a standard "water column" representation is used, so that each box's volume or mass is given on a per unit area basis. As this is common practice in oceanography, this presentation has not been changed. However, the per unit area approach is now described under model description to remove any doubts, and the modeled system is now defined as a water column. I suspect the water column approach was the cause for Reviewer 2's errors, but this should not have been difficult to deduce.

3(a, part 1) (p7) There appear to be two serious problems with the equation for EP in each compartment (8):

The total entropy in a box, per unit area, is **correctly** given as J m^{-2} °K^{-1} d^{-1}. The volume of each box is given by $h[i]A$, where area, A, is the same for all boxes and surfaces in the water column. The rate of entropy production per unit volume by reaction is given by $-r\Delta G/T$ (J m^{-3} °K^{-1} d^{-1}), so the total entropy production in box $[i]$ is $-r\Delta Gh[i]A/T$. Since area, A, is common through all terms in the equation, it can be removed, with the resulting entropy per unit area given correctly in the text as Eq. (8). The same reasoning applies to the entropy of mixing terms; they are correct as given.

3 (a, part 2): There also appears to be a unit imbalance in (8), unless ΔGri is given in units of J m^{-3} instead of the usual units J mol^{-1}. It would be preferable to give ΔGri in standard form, and explicitly show the dependencies on density (concentration) and molecular mass.

I am a little baffled here. The units of ΔG_{r_i} **are** in J $mmol^{-1}$ (or equivalently, kJ mol^{-1}). I can only assume Reviewer 2 has unexplainably assigned reaction rates, r, to units of d^{-1}; however, reaction rates are **explicitly** stated in the text to be in units of mmol m^{-3} d^{-1}, which is also clearly evident by simple inspection of Eqs. (6) or (7).

3 (b, part 1): (p10) The mass balances (13) also have two problems:

Again, the reviewer is incorrect. The total mass of constituent k in box [i] is given by $C_k[i]Ah[i]$, so the change in mass over time is $Ah[i]\,{dC_k[i]}/{dt}$ in mmol d^{-1}, where A and $h[i]$ have been removed from the differential because they are time independent. The rate of mass flow (in mmol d^{-1}) into a box is given by $AF_{i-1,i}(C_k)$. Dividing both terms by the volume causes A to drop out of the equations, so that the mass balance equation (13) can be expressed as concentrations, which are the state variables of interest. The mass balance equations are correctly written, and their derivation is quite trivial. Instead of questioning their own interpretation, Reviewer 2 has jumped to the conclusion that the author is unable to construct a simple mass balance.

3(b, part 2): Since the units of parameters ν_i and hence r_i are specified as d^{-1}...

I do not understand how the reviewer could possibly arrive at their erroneous statement that r_i has the units of d^{-1}. The units of r_i are **explicitly** stated in the text, and simple inspection of equations (6) and (7) would also support their units to be in mmol m^{-3} d^{-1}. The units cannot be made more explicit. The units of Λ_k are clearly mole of species per mole of reaction (which makes them dimensionless), as are all the reaction stoichiometric coefficients; what else could they be, and why would Reviewer 2 assume they are not?

3 (c) (p 12) Although the author does not yet do this (see comment 2), the EP flux terms (12) cannot simply be added to the EP productions within each box (8)...

As indicated above, all units are completely consistent, so the flux terms can be added to the entropy production terms. To remove any confusion, internal entropy production nomenclature has been changed from ${dS^{r_i}}/{dt}$ and ${dS_k^{F_{i,j}}}/{dt}$ to $\dot{\sigma}_{r_i}$ and $\dot{\sigma}_{F_{i,j}(k)}$ to represent internal entropy production from reactions and mixing fluxes, respectively.

3(d) The author deviates from standard SI or CGS chemical units...

SI units are used throughout the text; however, I utilize standard prefixes so that variables are of order 1. For instance, concentrations in marine systems are in the μM range, so it is most appropriate to use mmol m^{-3} for the units for concentration. This also leads to the gas constant being expressed in J mmol^{-1} °K^{-1}, unless numerical multipliers are included in the equations, which I view as unappealing. I do not think it is asking too much from the reader to convert from mmol to mol.

4. (p5) The chemical reactions (1) and (2) are not in stoichiometric balance...

The reviewer has arbitrarily made up his or her own definition for the stoichiometry of biological structure, $\mathbb{S}$, then states the chemical reactions are not balanced? Such iterative guessing to solve for an unknown is unfamiliar to me. Clearly, the stoichiometry of $\mathbb{S}$ **must** be $CH_2O(NH_3)_\rho$ in order for the equations to balance. While this is rather obvious, the composition is now explicitly stated in the text.

…The stoichiometry of the biological structures should be discussed and justified…

It would defeat the objective of the manuscript to rigorously specify the composition of biological structure for just one class of organisms. The objective is to view biology from the perspective of general catalysts that organize in such a manner to maximize internal entropy production based on which reactions are catalyzed. The details of biological structure stoichiometry are unimportant. What does matter is the observation that the reaction free energy associated with biological structure synthesis from sugar and ammonia (and other necessary elements) is very close to zero. This is described in some detail on pg. 9 in the paragraph beginning "Living organisms..."

5. (p6) Concerning the biological growth curve...

It appears Reviewer 2 has not been able to grasp the abstraction of the biological reactions given by (1) and (2). It is rather obvious that ε_i control the extent of respiration associated with biological structures 1 and 2. The specific uptake rate given by (4) specifies the specific rate of substrate consumption. A fraction ε_i of that substrate is converted to biological structure, which gives the familiar specific growth rate given by (5), while $(1-\varepsilon_i)$ is respired. The principle control variables in the model are ε_1 and ε_2 that define the rate of respiration. Specifying a fixed respiration rate would completely undermine the entire analysis. Respiration rate can vary from less than 30% to greater than 90%? Which value would Reviewer 2 arbitrarily choose? It is not an organic chemistry constraint that dictates respiration rate, but is rather controlled by evolution. However, if living systems organize to maximize internal entropy production, then a tradeoff between synthesis of biological catalyst (which does not dissipate free energy) versus free energy dissipation by oxidation of reduced organic carbon must be struck. The ε_i control variables via reactions (1) and (2) are a means to get at this important tradeoff between entropy production versus catalyst synthesis.

Carrying capacity? I do not believe I have ever seen any modern description of microbial growth kinetics that includes a carrying capacity term. Carrying capacity is largely a throwback to Lotka-Volterra models that somehow seem to maintain their relevancy to some mathematicians and physicists. The approach has some relevancy in terrestrial macro ecology where 2D surfaces limit organism densities, but carrying capacity has no place in modeling microorganisms whose densities do not come anywhere close to filling the space they occupy. Even at densities six orders of magnitude greater than observed would not come close to the carrying capacity.

Equation (4), like all growth equations, is empirically based. As described in the text, the $(1-\varepsilon^2)$ term approximates an Arrhenius-like connection that limits reaction rate as the reaction approaches reversibility as $\varepsilon \rightarrow 1$. The important aspect of (4) is that it replaces complex growth models that contain numerous organism-specific parameters (i.e., half saturation constant, maximum growth rate, growth efficiency, etc.), with one adjustable control parameter, ε. However, the equation is still able to reproduce the extremes in growth kinetics observed between *E. coli* growing in the lab under optimal conditions to bacteria growing under the most extreme oligotrophic conditions. Furthermore, it is formulated to produce growth kinetics expected from the competitive exclusion principle. The purpose of (4) is to be able to capture,

crudely, the growth kinetics of *all* microorganisms, as it is assumed any organism can occupy either box. The justification to (4) is given simply by Fig. 2; Eq. (4) reasonably represents the growth kinetics of a multitude of microorganisms, and is a significant contribution to modeling microbial biogeochemistry in its own right.

I agree that there are many ideas embedded in (4) that would be extremely interesting to explore; however, exploring them would go beyond the scope of the current manuscript, and is why the statement, "Of course, other mathematical functions could be used in place of Eq. (4), but this is not our primary interest for this manuscript, but it is an interesting topic that warrants further research." is given on page 8.

6. (p8) I do not understand the changes in Gibbs free energy (9) and (10), in particular the presence of the two leading terms on the right. ***Surely the ΔGri's are completely expressed by the RT ln(..) terms?****...*

I find this statement particularly disturbing, as it indicates Reviewer 2 lacks an elementary understanding of chemical thermodynamics, which is the focus of the manuscript! No, ΔG_r is NOT completely expressed by the RT ln() terms. Please see any elementary chemistry or thermodynamics textbook. The leading terms on the right-hand-side of (9) and (10) are needed to calculated the standard Gibbs free energy of reaction as a function of ε. This is described in some detail in the paragraph that precedes (9) and (10).

Due to the above serious problems, I did not examine the findings in §3. I would like to do so, once I have more confidence in the author's theoretical framework and methodology used.

Given the plethora of serious errors in Reviewer 2's analysis, and their lack of familiarity with chemical thermodynamics evident in their Comment 6, I have no confidence in Reviewer 2's ability to complete the review of the manuscript.

Response to Minor Comments

7. It is not stated explicitly that each compartment in Fig. 1 is considered a "well mixed tank" or "completely mixed flow reactor" (CMFR).

It is clear from the model that the boxes must be homogeneous; otherwise, there would be need to be equations describing the heterogeneity of the system. It is also clearly not a CMFR, (i.e., chemostat) as the governing transport equations are purely diffusive; no advective transport is being considered. Nevertheless, a statement has been added in Section 2.1 that states each box is homogeneous and isothermal.

8. (pp5-7) The symbols r1, r2 are used to name the chemical reactions (1) and (2), and then later are used for their rate constants (6) and (7).

The names r_1 and r_2 have been removed, and the reactions are now referred to reaction (1) and reaction (2).

9. (pp7-8) The chemical rate equations and Gibbs free energies should, of course, depend on the chemical activities of each species, not their concentrations;…

As stated in the text, Gibbs standard free energies of reaction have been calculated to approximately account for activities based on ionic strength, so that concentrations can be directly used in (9) and (10) instead of activities. A reference to a textbook (Alberty 2003) is given for the interested reader (or Reviewer 2). To include such details in this manuscript would simply be a distraction.

…Arguably, these [activities] should also be included in the biochemical growth rate equations (4)….

No, I disagree. Equations (4), (6) or (7) should not be based on activities. As stated above, these are empirically-based equations and are not derived from thermodynamics. I have never seen anyone formulate such Monod-like equations based on activities. Furthermore, in all cases concentrations are a very good approximation to activities, as marine systems are extremely dilute and constituents even in the μM concentration range are considered high.

10. (p7) The extra ε_i term in (5) appears to be omitted from the equations (7). Surely they depend on ε_i^3? It would be preferable to specify the actual first-order rate equations, so that the reader can understand the distinction between ν_i and μ_i .

No, the reviewer is incorrect. These are very standard and elementary equations. As stated in the text, ν_i is the specific uptake rate of substrate, so when multiplied by biomass concentration ($C_{\mathbb{S}_i}$), one obtains the rate of substrate consumption (in mmol m^{-3} d^{-1}), which is the overall reaction rate given by (6) or (7). While this is very clear, I have nevertheless expanded (6) and (7) to

$$r_1 = \nu_1 C_{\mathbb{S}_1} = \nu^* \varepsilon_1^2 (1-\varepsilon_1^2) \left(\frac{C_{CH_2O}}{C_{CH_2O} + \kappa^* \varepsilon_1^4} \right) \left(\frac{C_{NH_3}}{C_{NH_3} + \kappa^* \varepsilon_1^4} \right) C_{\mathbb{S}_1}$$

$$r_2 = \nu_2 C_{\mathbb{S}_2} = \nu^* \varepsilon_2^2 (1-\varepsilon_2^2) \left(\frac{C_{\mathbb{S}_T}}{C_{\mathbb{S}_T} + \kappa^* \varepsilon_2^4} \right) C_{\mathbb{S}_2}$$

The substrate update rate for reaction *i* is $\nu_i C_{\mathbb{S}_i}$; however, only ε_i of the substrate leads to biological structure synthesis, the rest is respired. Hence, the rate of biomass synthesis is $\varepsilon_i \nu_i C_{\mathbb{S}_i}$; dividing this by the concentration of biological structure gives the specific growth rate, $\mu_i = \varepsilon_i \nu_i$, as given by (5) in the text. Anyone familiar in modeling microbial systems should not have any difficulty in understanding this without the trivial details outlined here.

11. (p9) The β term in (11) is omitted from (12).

Typo corrected.

12. (p11) The author mentions the possibility of optimisation for the transient problem. Please note that there is no theory or even any viable hypothesis for a transient MEP principle, only some evidence for the existence of MEP at steady state.

While I agree there is no agreed upon theory of MEP for transient systems, there is nevertheless a proposed hypothesis with a proposed computational approach. This work has been published, and I see no reason not to reference it.

Author Final Remarks

The manuscript has been written with the assumption that the reader has a reasonable understanding of chemical thermodynamics and has some familiarity in biogeochemistry and the associated modeling thereof. However, the model has been made extremely simple, and any reader competent in quantitative analysis should have no problem understanding the equations with a minimum of effort.