

Translating microbial growth kinetics into quantitative responses and testable hypotheses using Kinbiont

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This work applies a contemporary AI paradigm to classic population dynamics and microbial growth kinetics. Specifically, an open-source tool called Kinbiont has been developed to integrate mathematical models of population dynamics with machine learning methods for data-driven discovery in microbiology. It is not a secret that mathematical modeling does not always stimulate research progress. Success crucially depends on the ability of the modeler to translate a vague and often contradictory biological ideas into definite mathematical structures. Then, inadequate hypotheses and interpretations can be safely discarded by comparing simulation against real-life observations. The key innovation of Kinbiont is its capability to generate testable hypotheses through a method known as **symbolic regression**. Unlike traditional regression methods that optimize parameters for predefined model structures (linear, exponential, or polynomial), symbolic regression employs machine learning to select/generate the most suitable model structures and then find the optimized parameters. Thus, Kinbiont aims to stimulate creative thinking and theoretical advancements in microbiology. However, one might wonder if it's too good to be true. Let us see how convincingly the manuscript justifies its ambitious title and far-reaching objectives. The manuscript is hard to read because it mixes several independent genres, i.e., reviews, experimental reports, and software self-promotion. The 'Methods' section is placed at the end, so we learn about the original experimental study (recording growth of *E. coli* auxotrophic strains) only *post factum*. I would publish it separately as an experimental paper. Other applications of Kinbiont have been demonstrated by using the published data, including so-called nonstandard growth kinetics of various prokaryotes (diauxia, bacteria infected by phages), growth curves in the presence of diverse toxic compounds, and heterogeneous populations consisting of active and dormant cells. I failed to find (could be my fault) any explicit example of generating novel kinetic models. Instead, the authors charged Kinbiont with "the most comprehensive collection of pre-built microbial dynamics models," which formed a selection pool.

It was disappointing to see that this 'comprehensive collection' only included the classic logistic equation (introduced by Belgian mathematician Verhulst as early as 1830) and its various modifications (e.g., adding shape adjustment parameters, sink and source terms, etc. - all empirical terms) or analogs (Gompertz, Richards, Weibull, Morgan, Bertalanffy, and others). What is the reason for disappointment? This collection brings us back to the first half of the 20th century when microbiologists did not use chemically defined media. Agar plates and static flasks with bullion were the only ways to grow microorganisms. Bacterial growth was viewed as a sequence of genetically predetermined stages analogous to the embryonic development of Metazoa. Biological kinetics was established at that time only in enzymology (Henri, Michaelis, Menten, Haldane) and introduced to microbiology by mid-century (Monod, Novick, Szilard, Pirt, Powel, Fredrickson...). Chemostat cultivation and chemostat theory discarded pre-determinism of microbial growth by establishing the nutrient concentration and other environmental factors as real drivers. Sure, we can still use the logistic equation that does not account for these critical factors, but its value is downgraded to the level of a purely empirical mathematical formulation that does not carry a mechanistic explanation of the growth. Literally, it states that population growth is restricted by the negative interactions between cells. Yes, it happens in some rare overcrowded habitats, but a more typical case (in lab practice, industry, and nature) is nutrient depletion and accumulation of toxic byproducts, including metabolic acidification or alkalization. The simplest kinetic models describing this mechanism are the Monod and Monod-Ierusalimsky models. They should use at least two or three dynamic variables: (i) cell concentration, (ii) limiting substrate concentration, and (iii) inhibitory byproduct concentration. All 30 models in the collection use only the first variable N . The last historical point is the turn of the 20/21 century: a principal difference between enzyme and microbial kinetics became perfectly clear. If enzyme catalytic properties (e.g., K_m , V_{max} , activation energy, etc.) remain constant over the observed reaction, microbes are subjected to self-regulating their cellular composition orchestrated through conditionally expressed synthesis of macromolecules (differential gene expression). Besides, spontaneous mutations at a longer time scale result in either

heterogeneity of the initially homogeneous population or a genetic sweep, complete replacement of WT with mutant line. Today, microbiology relies on omics data to record the listed processes specific to microbial kinetics and use essentially more realistic **genome-scale dynamic reconstructions**. These models are much more complex than the unstructured Monod-Ierusalimsky-Pirt models (needless to say, about oversimplistic logistic models). However, researchers are very inventive people constructing simplified but still fundamentally correct mathematical formulations adequately mimicking the self-regulatory behavior of microorganisms (cybernetic models, synthetic chemostat model). This is the most essential drawback of submission, giving support to the fatally outdated modeling style. Nevertheless, most probably, the software itself is adaptable and supports user-defined models. A real evaluation of Kinbiont can be achieved only when we start using it in practice. Other less global deficiencies of the manuscript include the following.

1. Non-conventional terminology. 'Total growth' is absurd as a definition of N_{max} , the maximum population density. The 'phage-bacteria co-cultures' is also an incorrect expression for the 'bacterial growth accompanied by phage lysis.' Respective models belong to the category 'prey-predator', and can be called 'host-parasite' models.
2. Error in Blackman equation (19), which should be a segmented function, first kinetic order at small substrate concentration and zero-order at high concentration
3. The authors generally consider segmentation a great advantage. However, it makes fitting more subjective and prevents a more desirable compact and uniform solution.
4. The experimental design for recording growth curves in a plate reader is faulty because the BioTek Synergy instrument does not prevent moisture condensation in the optical path. An alternative solution, such as Bioscreen, uses a temperature gradient across the plate lid.
5. Numeric differentiation of growth curves to calculate the specific growth rate, $SGR ((dN/dt)/(1/N))$, is always accompanied by a large error with BioTek and a time interval of 6 min. One manifestation is shown in Fig 2 (panel 2 top row), where the first SGR peak is obviously artefactual.
6. The time scale is given in min, while a more natural way is hours and days (for the slow growers). If you want to stick to the international physics standards, you should select seconds, producing even more zeros.

Conclusion. My critical comments should help the journal's Editorial Board to make a fair judgment about the fate of this submission. My personal and biased opinion is that the manuscript should be deeply revised, made more coherent, modest, and realistic (do not promise too much), and then published. It is vital to revive the interest of microbiologists in growth kinetics.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

This paper presents a new package called "Kinbiont" which implements automated fitting of various growth models to time series growth data. It covers most known models and also assists the user in finding the best fitting models. While similar approaches already exist, such as curveball in Python, Kinbiont is implemented in Julia, a programming language gaining popularity due to its high computing speed. Compared to other implementations in other programming languages, Kinbiont also offers the feature to segment a growth curve and automatically fit models to the segmented growth curves. This is an exciting feature since for example diauxic shifts are commonly observed in experimental data.

We have identified a few points that we think could improve the paper. Our first point is about the focus of the paper. We found the main text to be focusing too much on the machine learning aspects of the package. However, only a small part of the package on github's documentation focuses on machine learning assisted downstream analysis. This part covers the implementation of a decision tree and it was not shown clearly in the paper how this can be used to come up with new hypothesis. Indeed, in Figure 6, it's not very clear what new hypothesis has been generated. Our understanding was that the data is sorted in a more meaningful way to allow the user to better analyse it.

In our opinion, the paper should be rewritten with the focus on bringing a lot of established growth models to Julia instead of claiming that Kinbiont can be used to come up with new hypothesis. Another important novelty of this package that we think could be highlighted more is the segmented fits and it could be helpful to guide the user more through this procedure and also explain the referenced data better that they used for their figures. We also would have appreciated if it was explained why more changepoints were identified than biologically meaningful in Figure 3. The figure shows that having 2 changepoints is better than 1, but knowing that the experiment involved only one change in nutrient type, this seems like overfitting. The authors should discuss this point as a potential shortcoming of their approach. A third strong point of Kinbiont is the possibility for the user to define their own models in addition to the available ones. As far as we could tell, this feature was not mentioned in the main text and could be highlighted more.

The paper also emphasizes that Kinbiont can be used to identify new correlations between ecological or evolutionary parameters and parameters from the fitted models, but only showed this with one example. Of course it makes sense to test its ability to do so with a known phenomenon, but we found that this choice was not very clear. Perhaps it could be introduced as: "To test whether we can reproduce a known correlation between an environmental parameter and parameter inferred from our models, we repeated Monod's experiment [...]." And then explain that this was only tested for one example, but there is no reason why this wouldn't be helpful to detect other patterns., Monod's experiment that was carried out for this paper was not convincing that Kinbiont can be used to detect new correlations between environmental parameters and fitted model parameters. This reproduced correlation was known prior to the analysis and was probably also the only environmental parameter that was correlated with the specific growth rate.

Our second point is that Kinbiont requires improved documentation and perhaps some additional tutorials to make it accessible to less experienced users. With the current state, even for more experienced users it will be hard to apply Kinbiont to novel datasets due to specific data formats of annotation files. It would be really helpful if the analysis and the datasets used for this publication are available for the user through interactive notebooks. In addition, Kinbiont comes with a vast variety of functions and it would be greatly helpful to the reader if all those functions are visualized in a decision tree or some other illustration. This would clarify the fitting procedure and help the reader evaluate if this package is of any use appropriate for their applications.

Third, the discussion should contain an additional paragraph about limitations and pitfalls of this package and fitting in general. This is required in order that less experienced users are aware of the limitations of the output generated by Kinbiont.

In summary, this paper contributes to the field by bringing already existing features to a relatively new programming language that is gaining in popularity, and introducing a new feature of segmented fitting. For this contribution to be accessible and useful to less experienced members of the field, its documentation and tutorials need improvement. We also suggest to focus on the core features of the package and shift away from the narrative that Kinbiont can be used to create new hypotheses. This requires that the manuscript is restructured and partially rewritten.

Minor comments:

- Figure 1A: Color gradient of scale is the inverse of the color gradient of the well plate
- L97: Move sentence "We benchmarked [...]" to another paragraph.
- L248: Sentence should be rephrased
- L275-277: This is confusing and not clear how the data structure should look like. A supplementary figure explaining the input matrices would be helpful.
- Fig. 5, 6: these figures need more explanation. What are the dots in the bar plots, for example? Why are only a few chemicals shown in Fig. 5? Can you find a more intuitive way to describe the impurity score such that the reader doesn't need to dig further to understand the figure?
- It was unclear how Fig. 6 is automatically "detecting laws in microbiology". As outlined above, this should be toned down and instead it would be good to get a better explanation of what the decision tree analysis tells us.
- L. 287: "imputing" -> inputting

(Remarks on code availability)

The code is missing documentation to make it more usable (as outlined in the comments to authors).

Reviewer #3

(Remarks to the Author)

The manuscript introduces an open-source tool named Kinbiont for analyzing microbial growth kinetics using advanced machine learning techniques. While I am not an expert in model development, I perceive a robust methodology by the authors, such as the inclusion of a large number of closed-form and ODE models, as well as the use of change point detection algorithms and segmented fits for non-standard growth data. As a computational tool, this appears to be solid work.

However, one of my main concerns is the uncertainty in real-world applicability. While the paper demonstrates Kinbiont's performance on several controlled datasets, such as nutrient shifts, phage-bacteria interactions, and pollutant exposures, these scenarios are relatively well-structured and defined. It remains unclear how well Kinbiont can handle more realistically complex systems, such as those found in natural environments or industrial settings. These systems often involve multiple interacting species, dynamic nutrient fluxes, and less structured growth patterns. Notably, some of these environments, like wastewater engineering, are the ones most in need of accurate growth characterization rather than defined synthetic communities. For example, in wastewater treatment, models like ASM and ADM integrate very simple kinetics of different trophic groups of microbes. How would Kinbiont potentially apply to or improve upon these existing models?

Additionally, the manuscript lacks sufficient biological interpretation of the results. While the focus is clearly on the methodological advancements, it would be helpful to see a more detailed discussion on the biological implications derived from Kinbiont's application. Could Kinbiont provide insights into complex biotic-biotic and biotic-abiotic interactions, such as syntrophisms, competition, or parasitism?

Another comment is that the validation against existing tools is somewhat limited. A comparison against other well-known tools, such as Growthcurver or Grofit, on the same datasets is lacking. If existing tools are already solving the problem effectively, it is crucial to emphasize what specific advantages Kinbiont provides beyond these solutions.

Finally, as a reader who is not intimately familiar with nonlinear optimization and differential equations, a more detailed guide on choosing the appropriate kinetic model would be of great value. This addition would help users make informed decisions about which models are best suited for their particular datasets and conditions.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I believe the authors thoroughly revised their original submission. Practically every one of my critical suggestions has been adequately addressed. I am sure the revised manuscript can be published.

(Remarks on code availability)

Reviewer #2

(Remarks to the Author)

The authors have successfully improved the accessibility of Kinbiont, by improving the documentation, adding decision trees and improving figures to help the user to understand the methodology. Those changes will be of great help for users who are looking for a tool to process and fit models in Julia to their biological data. The features that Kinbiont provides are exciting and useful for the field, whether it is inference of growth parameters, identification of diauxic shift, or the disentanglement of confounding variables. We think that the authors could be more careful with how those capabilities are phrased and what the limitations are, as outlined in the attached document. We also suggest some further improvements for the new version of the manuscript as well as some minor comments.

(Remarks on code availability)

The installation is failing because `Pkg.add(Kinbiont)` is missing quotes in the documentation. It should be `Pkg.add("Kinbiont")`

Reviewer #3

(Remarks to the Author)

The authors addressed my comments.

(Remarks on code availability)

Reviewer #4

(Remarks to the Author)

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

(Remarks on code availability)

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Please find enclosed the revised version of our manuscript '*Translating microbial kinetics into quantitative responses and testable hypotheses using Kinbiont*'. We sincerely thank the reviewers for their constructive comments and suggestions, which have challenged us to improve both the tool and the paper. We are excited to resubmit this revised version of Kinbiont, which we hope will help rekindle interest in microbial growth kinetics across the life sciences.

A summary of the changes follows, and a point-by-point response to the reviewers' concerns is attached at the end:

- Clarified the manuscript structure, reformulated figures, and rewrote extensive sections to better reflect Kinbiont's core functionalities. All new text in the revised version is marked in blue.
- Included three new datasets to clarify and support methodological extensions in the revised version of Kinbiont: a co-culture study, (Jo, C., et al. 2023), a new chloramphenicol dose-response experiment performed in the lab, and an ethanol bioproduction dataset (Salazar, Yolocuauhtli., et al. 2023).
- Expanded Kinbiont's functionality to support arbitrary multi-dimensional systems, demonstrating this capability through an example application using the Monod-Ierusalimsky model for ethanol bioproduction.
- Added support for chemical reaction networks and cybernetic models for representing complex biochemical systems.
- Enhanced user documentation and online resources, including examples of varying complexity to make Kinbiont more accessible to the community ([Kinbiont · Kinbiont Documentation](#)).
- Improved reproducibility by releasing all relevant datasets, scripts, output files, and analysis notebooks used in the main text via [pinheiroGroup/Kinbiont_utilities](#).
- Clearly described and illustrated the segmented fitting approach. We added a new Extended Data Figure showing the schematics of the procedure, updated Figure 3 with Akaike information criterion (AIC) comparisons, and expanded the discussion to address overfitting and biological interpretability (such as nutrient consumption order).
- More clearly positioned symbolic regression and decision trees as tools for biological insight. For symbolic regression, we now explicitly show how models yield interpretable and testable rules linking experimental variables to kinetic parameters. For decision trees, we included a new dataset (see Extended Data Figure 6) to illustrate how this method generates falsifiable hypotheses.
- Corrected technical inaccuracies and standardized terminology, refining model equations and improving figure legends for greater clarity.

Sincerely,

Fabrizio Angaroni, Alberto Peruzzi, Edgar Z. Alvarenga, and Fernanda Pinheiro

Reviewer #1 (Remarks to the Author):

This work applies a contemporary AI paradigm to classic population dynamics and microbial growth kinetics. Specifically, an open-source tool called Kinbiont has been developed to integrate mathematical models of population dynamics with machine learning methods for data-driven discovery in microbiology. It is not a secret that mathematical modeling does not always stimulate research progress. Success crucially depends on the ability of the modeler to translate a vague and often contradictory biological ideas into definite mathematical structures. Then, inadequate hypotheses and interpretations can be safely discarded by comparing simulation against real-life

observations. The key innovation of Kinbiont is its capability to generate testable hypotheses through a method known as **symbolic regression**. Unlike traditional regression methods that optimize parameters for predefined model structures (linear, exponential, or polynomial), symbolic regression employs machine learning to select/generate the most suitable model structures and then find the optimized parameters. Thus, Kinbiont aims to stimulate creative thinking and theoretical advancements in microbiology. However, one might wonder if it's too good to be true. Let us see how convincingly the manuscript justifies its ambitious title and far-reaching objectives.

The manuscript is hard to read because it mixes several independent genres, i.e., reviews, experimental reports, and software self-promotion. The 'Methods' section is placed at the end, so we learn about the original experimental study (recording growth of E coli auxotrophic strains) only *post factum*. I would publish it separately as an experimental paper.

We thank the reviewer for their valuable comments, which greatly helped clarify and improve our manuscript. We appreciate their recognition of symbolic regression's potential to identify biologically meaningful response patterns directly from data—a view we deeply share. Kinbiont aims to bridge mathematical modeling and biological interpretation, providing systematic tools to explore biological hypotheses in microbial dynamics. Symbolic regression is a particularly promising approach to achieve this goal.

Regarding the manuscript structure, we place the Methods section at the end to follow journal guidelines. However, following your suggestion, we have expanded the Results section ("Kinbiont identifies mathematical models underlying microbial responses to environmental changes") to provide an earlier and clearer description of our experimental procedures, and we also created an "Experimental methods" section in the beginning of "Methods". We hope these modifications help clarify the experimental context, addressing your concerns.

We take your comment regarding the potential to publish the experimental data as a separate study as a big compliment. However, since our primary goal was to validate Kinbiont's capability for automated detection and analysis of microbial responses, (and time series data systematically generated to probe response data is not easily available in the literature), we opted to include original experimental data.

Regarding readability and structure, we acknowledge the complexity arising from integrating multiple datasets. We have substantially restructured the manuscript, incorporating feedback from other reviewers as well, to improve narrative flow and clarity. These revisions should clarify dataset distinctions and streamline the presentation.

Finally, we have carefully revised the manuscript to remove any content that might appear as software self-promotion (it was never our intention).

We believe these revisions significantly enhance clarity and readability, and we thank the reviewer again for the constructive feedback.

Other applications of Kinbiont have been demonstrated by using the published data, including so-called nonstandard growth kinetics of various prokaryotes (diauxia, bacteria infected by phages), growth curves in the presence of diverse toxic compounds, and heterogeneous populations consisting of active and dormant cells. I failed to find (could be my fault) any explicit example of generating novel kinetic models. Instead, the authors charged Kinbiont with "the most comprehensive collection of pre-built microbial dynamics models," which formed a selection pool.

We thank the referee for highlighting this important point. We have revised the manuscript to avoid overly bold statements such as "the most comprehensive." We would like to clarify that our primary goal with Kinbiont and this manuscript is not to explicitly propose novel kinetic models, but rather, to provide the microbial research community with a computational tool capable of fitting various dynamical models using state-of-the-art numerical methods and analyse the results with machine learning techniques. Kinbiont aims to efficiently test existing models, fit them to experimental data, and facilitate the exploration of new hypotheses. Depending on users' familiarity with mathematical modeling, they can choose from commonly used dynamical models already implemented in the library or define custom models expressed as ordinary differential equations or analytic functions. We believe that demonstrating Kinbiont's flexibility and broad applicability through diverse, published examples effectively supports this objective.

It was disappointing to see that this 'comprehensive collection' only included the classic logistic equation (introduced by Belgian mathematician Verhulst as early as 1830) and its various modifications (e.g., adding shape adjustment parameters, sink and source terms, etc. - all empirical terms) or analogs (Gompertz, Richards, Weibull, Morgan, Bertalanffy, and others). What is the reason for disappointment? This collection brings us back to the first half of the 20th century when microbiologists did not use chemically defined media. Agar plates and static flasks with bullion were the only ways to grow microorganisms. Bacterial growth was viewed as a sequence of genetically predetermined stages analogous to the embryonic development of Metazoa. Biological kinetics was established at that time only in enzymology (Henri, Michaelis, Menten, Haldane) and introduced to microbiology by mid-century (Monod, Novick, Szilard, Pirt, Powel, Fredrickson...). Chemostat cultivation and chemostat theory discarded pre-determinism of microbial growth by establishing the nutrient concentration and other environmental factors as real drivers. Sure, we can still use the logistic equation that does not account for these critical factors, but its value is downgraded to the level of a purely empirical mathematical formulation that does not carry a mechanistic explanation of the growth. Literally, it states that population growth is restricted by the negative interactions between cells. Yes, it happens in some rare overcrowded habitats, but a more typical case (in lab practice, industry, and nature) is nutrient depletion and accumulation of toxic byproducts, including metabolic acidification or alkalization. The simplest kinetic models describing this mechanism are the Monod and Monod-Ierusalimsky models. They should use at least two or three dynamic variables: (i) cell concentration, (ii) limiting substrate concentration, and (iii) inhibitory byproduct concentration. All 30 models in the collection use only the first variable N .

The last historical point is the turn of the 20/21 century: a principal difference between enzyme and microbial kinetics became perfectly clear. If enzyme catalytic properties (e.g., K_m , V_{max} , activation energy, etc.) remain constant over the observed reaction, microbes are subjected to self-regulating their cellular composition orchestrated through conditionally expressed synthesis of macromolecules (differential gene expression). Besides, spontaneous mutations at a longer time scale result in either heterogeneity of the initially homogeneous population or a genetic sweep, complete replacement of WT with mutant line. Today, microbiology relies on omics data to record the listed processes specific to microbial kinetics and use essentially more realistic **genome-scale dynamic reconstructions**. These models are much more complex than the unstructured Monod-Ierusalimsky-Pirt models (needless to say, about oversimplistic logistic models). However, researchers are very inventive people constructing simplified but still fundamentally correct mathematical formulations adequately mimicking the self-regulatory behavior of microorganisms (cybernetic models, synthetic chemostat model).

This is the most essential drawback of submission, giving support to the fatally outdated modeling style. Nevertheless, most probably, the software itself is adaptable and supports user-defined models. A real evaluation of Kinbiont can be achieved only when we start using it in practice.

We greatly appreciate the reviewer's detailed and historically informed perspective and fully understand the disappointment expressed regarding the initial presentation's emphasis on classical, biomass-only models.

The decision to focus initially on biomass-only models – rather than those explicitly incorporating nutrient concentrations and inhibitory byproducts – was primarily motivated by the nature of available datasets. Most data we analyzed were collected using microplate readers, typically providing optical density (OD) measurements as proxies for biomass, without simultaneous information on substrate or byproduct concentrations. Without these additional measurements, models that include substrate or inhibitor dynamics become structurally unidentifiable and thus impossible to reliably fit.

However, we emphasize that Kinbiont is not restricted to these simplified, built-in models; rather, a central objective of Kinbiont is to offer a flexible extensible platform for microbial growth modeling. A point that we emphasized in this version of the manuscript is that Kinbiont supports arbitrary, user-defined systems of ordinary differential equations (ODEs), allowing the implementation and fitting of more complex models involving multiple dynamic variables. To illustrate this capability, we have expanded our manuscript to include a new analysis of multidimensional data from an ethanol bioproduction experiment (Salazar, Yolocuauhtli, et al. 2023; with data kindly provided by the authors), explicitly integrating biomass, substrate, and inhibitory byproduct dynamics using a modified, custom-implemented Monod–Ierusalimsky model. This application has been thoroughly documented in Kinbiont's Documentation to illustrate the procedure.

Moreover, we expanded modeling possibilities through the integration of Catalyst.jl, allowing users to define models in the form of chemical reaction networks, which Kinbiont can simulate and fit. For even more advanced modeling, we now provide dedicated functions supporting cybernetic models, simplifying the process for users who only need to define initial conditions and relevant parameters. While these models are not fitted to data, we have illustrated their usage with synthetic datasets in Kinbiont's documentation.

We thank the reviewer again for this remark, which motivated us to expand on Kinbiont's capabilities, considering improving our tool. We believe these changes implemented effectively address your concerns at the same time as they highlight the adaptability of Kinbiont for future methodological expansions.

Other less global deficiencies of the manuscript include the following.

1. Non-conventional terminology. 'Total growth' is absurd as a definition of N_{max} , the maximum population density. The 'phage–bacteria co-cultures' is also an incorrect expression for the 'bacterial growth accompanied by phage lysis.' Respective models belong to the category 'prey-predator', and can be called 'host-parasite' models.

Thanks for pointing this out. We have changed “bacteria-phage co-cultures” to “bacterial cultures infected with T4 phage” and “Total growth” to “Maximum biomass concentration” throughout the text and figures.

2. Error in Blackman equation (19), which should be a segmented function, first kinetic order at small substrate concentration and zero-order at high concentration

Thanks for pointing out this mistake, we have now fixed it.

3. The authors generally consider segmentation a great advantage. However, it makes fitting more subjective and prevents a more desirable compact and uniform solution.

We thank the referee for this comment. We acknowledge that the previous presentation of segmentation fitting may have been misleading. The idea behind segmentation is to divide the time series into phases to allow for a local analysis of the dynamics in specific segments. This approach is particularly useful when, as in the case of our diauxic data, nutrient and metabolite dynamics are not simultaneously measured with optical density, making it impossible to fit more detailed mechanistic models, such as cybernetic models.

Now, in Figure 3D, we show the corrected AIC values of the best-fitting models and illustrate how this representation changes depending on whether the data exhibit a two-phase growth behavior or not. Furthermore, by analyzing the growth rates of the different segments, we observe clear patterns in the response of growth rate across the segments, which reveal the order of sequential utilization of nutrients (Figure 3E).

Finally, to clarify the procedure, we have included a toy example in Extended Data Figure 3 that illustrates how the segmentation algorithm works. Other points regarding the segmentation-fitting approach are discussed in the response to reviewer 2.

4. The experimental design for recording growth curves in a plate reader is faulty because the BioTek Synergy instrument does not prevent moisture condensation in the optical path. An alternative solution, such as Bioscreen, uses a temperature gradient across the plate lid.

We thank the referee for drawing attention to the possibility of moisture condensation during plate- reader measurements. We agree that condensation can distort optical- density readings if it obstructs the light path. To verify that this was not an issue, we included at least two blank wells on every plate and recorded their full time series. The blank signals remained essentially flat, indicating no systematic optical interference during the measurement. Typical blank measurements are shown below:

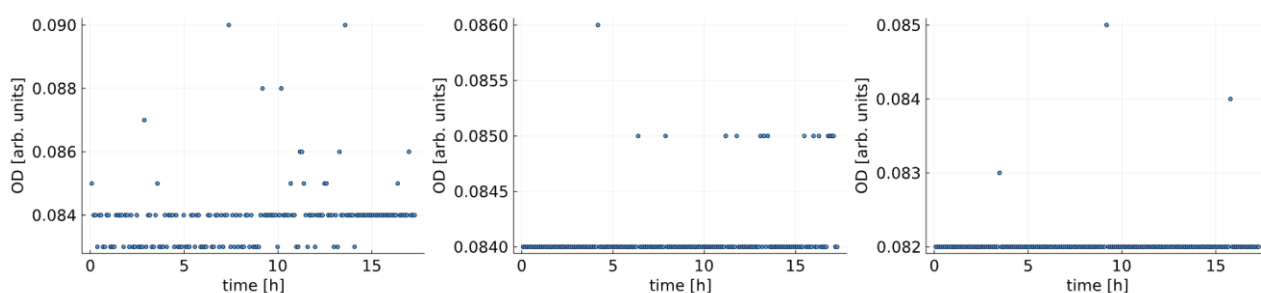


Figure 1: Sample blank measurements from different experiments and different wells.

We also calibrated Synergy H1 readings in two independent ways.

1. Against a ThermoFisher Genesys spectrophotometer, yielding an $R^2 = 0.99$ across the 0 - 0.7 OD range (the calibration data is provided in the GitHub repository).
2. Against biomass estimates from a CytoFLEX flow cytometer (cell count $\times$ forward- scatter area), giving an $R^2 = 0.86$ over the same range.

Both calibrations indicate that path-length artefacts are negligible. Deviations from linearity arise only at $OD \approx 0.8$, where multiple scattering begins to affect the spectrophotometer comparison. This artifact and the need for correction were already discussed in the previous version of the manuscript, and we keep it in this version.

Finally, while we understand that moisture condensation can be an issue when performing discontinuous kinetic procedures where plates are periodically taken from an incubator and inserted in the microplate reader, this is not our case. With a continuous kinetic procedure such as the one we use, the plate reaches equilibrium with the microplate reader temperature in a matter of a few minutes, therefore there are negligible moisture condensation effects from then onwards, consistent with our calibrations.

5. Numeric differentiation of growth curves to calculate the specific growth rate, $SGR ((dN/dt)/(1/N))$, is always accompanied by a large error with BioTek and a time interval of 6 min. One manifestation is shown in Fig 2 (panel 2 top row), where the first SGR peak is obviously artefactual.

We are sorry for this misunderstanding. We would like to clarify that the panels in the previous version of Figure 2 were generated using synthetic data with artificial noise, intended solely for illustrative purposes. In particular, the example in question was a toy simulation, not based on real experimental data.

To avoid any confusion, we have now updated the figures and explicitly stated in the captions of Figures 1 and 2 that these are synthetic examples created to demonstrate the functionality of the software.

6. The time scale is given in min, while a more natural way is hours and days (for the slow growers). If you want to stick to the international physics standards, you should select seconds, producing even more zeros.

We have changed the unit of measurements in the plots from minutes to hours and report growth rates, and symbolic regression results to hours.

Conclusion. My critical comments should help the journal's Editorial Board to make a fair judgment about the fate of this submission. My personal and biased opinion is that the manuscript should be deeply revised, made more coherent, modest, and realistic (do not promise too much), and then published. It is vital to revive the interest of microbiologists in growth kinetics.

We thank the reviewer for comments that improved both Kinbiont and the manuscript. In response, we have reduced overstated claims and streamlined the presentation. Models in the earlier version focused on biomass- only data and a small set of predefined models; the revised text highlights that users can now supply their own models—ODE systems or chemical- reaction networks—alongside the built- in library commonly used for microplate data. Although this manuscript does not include

reaction- network or cybernetic fits to real data, the online documentation now provides step- by- step tutorials and example notebooks for those models.

We believe these improvements better communicate Kinbiont's strengths and limitations clearly, accurately, and realistically, aligning closely with the reviewer's constructive recommendations.

Reviewer #2:

This paper presents a new package called "Kinbiont" which implements automated fitting of various growth models to time series growth data. It covers most known models and also assists the user in finding the best fitting models. While similar approaches already exist, such as curveball in Python, Kinbiont is implemented in Julia, a programming language gaining popularity due to its high computing speed. Compared to other implementations in other programming languages, Kinbiont also offers the feature to segment a growth curve and automatically fit models to the segmented growth curves. This is an exciting feature since for example diauxic shifts are commonly observed in experimental data.

We thank Reviewer 2 for highlighting the segmentation feature as particularly exciting and especially useful for diauxic data. In response, we have added an example that shows how growth rate estimates obtained from segmented fits reveal sequential nutrient use (new Fig. 3E and text).

We have identified a few points that we think could improve the paper. Our first point is about the focus of the paper. We found the main text to be focusing too much on the machine learning aspects of the package. However, only a small part of the package on github's documentation focuses on machine learning assisted downstream analysis.

We thank the reviewer for pointing out this gap in the documentation. In response, we have deployed a new website: [Kinbiont · Kinbiont Documentation](#) .

To address the limited documentation on applications of machine learning methods we have expanded the examples and approaches as follows:

- We added a dedicated webpage with proof-of-concept and synthetic examples in the documentation ([Examples of ML analysis · Kinbiont Documentation](#)).
- We expanded the example script to include new cases with both real and synthetic data ([Kinbiont.jl/Example scripts at main · pinheiroGroup/Kinbiont.jl](#)).
- We provided a precompiled notebook showing the output of some these scripts ([Kinbiont.jl/Notebook examples at main · pinheiroGroup/Kinbiont.jl](#)).
- We added a compact version of the ML analysis of the main text as notebook at ([Kinbiont utilities/Notebooks at main · pinheiroGroup/Kinbiont utilities](#)).
- We conducted a new experiment and employed symbolic regression to characterize antibiotic dose response (Figure 4D). All data and scripts can be found at [Kinbiont utilities/Automatic Law detection at main · pinheiroGroup/Kinbiont utilities](#)
- We incorporated a new real dataset from: (Jo, C., et al, 2023) related to ecological interactions of different strains (Extended Data 6). We specify the motivation behind including this new dataset below.

This part covers the implementation of a decision tree and it was not shown clearly in the paper how this can be used to come up with new hypothesis. Indeed, in Figure 6, it's not very clear what new

hypothesis has been generated. Our understanding was that the data is sorted in a more meaningful way to allow the user to better analyse it.

We acknowledge that the previous manuscript version did not clearly explain how decision trees graphically represent falsifiable rules extracted from data. To address this, we included an illustrative example in Extended Data Figure 6, demonstrating how decision trees encode a set of testable propositions. In particular, the figure shows how a decision tree can be interpreted as a structured summary of experimental conditions linked to specific growth parameter outcomes. Rather than merely summarizing experimental data, the decision tree automatically selects relevant features, discarding variables with minimal or no impact. This capability becomes essential for efficiently analyzing large datasets, where manual inspection would be impractical.

This procedure informed Figure 6, where we extracted and visually represented a concise set of statistically relevant rules. Figure 6E shows one such rule: combined treatment of Oxytetracycline and Imidacloprid significantly lowers the maximum biomass density (N_{max}) more than either drug alone.

Figure 5 also contains hypotheses derived from the usage of decision trees. In fact, observing that yield is affected by different chemicals compared to those influencing growth rate is an insight that emerges directly from the importance score and can be validated in a subsequent experiment. In summary, the tree automatically selects the relevant experimental variables, allowing users to generate specific follow-up experiments without manual screening of thousands of curves.

In our opinion, the paper should be rewritten with the focus on bringing a lot of established growth models to Julia instead of claiming that Kinbiont can be used to come up with new hypothesis.

We have revised to both emphasize Kinbiont's strength as a platform for implementing and fitting established growth models using Julia and clarify the utility of symbolic regression and decision trees to analyze response. The new version of the manuscript includes extensively restructured Figures 1 and 2, an expanded discussion and interpretation of segmented fits and symbolic regression results, and a new case study (the dataset of Jo, C., et al., 2023, mentioned above; to specific illustrate application and interpretation of decision trees results).

Another important novelty of this package that we think could be highlighted more is the segmented fits and it could be helpful to guide the user more through this procedure and also explain the referenced data better that they used for their figures. We also would have appreciated if it was explained why more changepoints were identified than biologically meaningful in Figure 3. The figure shows that having 2 changepoints is better than 1, but knowing that the experiment involved only one change in nutrient type, this seems like overfitting. The authors should discuss this point as a potential shortcoming of their approach.

We appreciate the reviewer's comment regarding the segmented fitting procedure. In response, we have expanded the segmented-fit description and documentation, clarified dataset descriptions, and added a schematic illustration of the workflow (Extended Data Fig. 3).

Fig.3D now plots corrected AIC versus number of changepoints: two segments improve the fit for some diauxic curves, whereas a third segment is penalized, confirming that the model avoids overfitting. We also note that even growth curves without actual biological changepoints (e.g., single substrate experiment) might benefit from analysis with one change point because stationary-phase often deviates from the ideal flat profile described by most models. This is illustrated in Figure 4A

and associated Supplementary Figures 7 and 8. In this respect, the segmentation approach can also be used to exclude atypical stationary-phase data from downstream analyses.

Furthermore, to strengthen biological interpretation in the context of the segmented-fit approach, the diauxic growth dataset analysis now includes estimates for the growth rates from the first and second growth phases (whenever identified) in Figure 3E. The result indicates substrate utilization preferences, in the form of sequential consumption of available substrates (this strain will first consume all Alanine, despite reaching higher growth rates in Glutamate) as is now discussed in the text as well.

A third strong point of Kinbiont is the possibility for the user to define their own models in addition to the available ones. As far as we could tell, this feature was not mentioned in the main text and could be highlighted more.

We thank the reviewers for this suggestion. The Results (Sections 1 - 2) now state explicitly that users can define their models as any ODE system (of any dimension) or reaction networks (which Kinbiont automatically converts into ODEs). In addition, we completely revised Figure 2, added a new example of this functionality on experimental data in Figure 3B and associated Supplementary Figure 5.

The paper also emphasizes that Kinbiont can be used to identify new correlations between ecological or evolutionary parameters and parameters from the fitted models, but only showed this with one example. Of course it makes sense to test its ability to do so with a known phenomenon, but we found that this choice was not very clear. Perhaps it could be introduced as: "To test whether we can reproduce a known correlation between an environmental parameter and parameter inferred from our models, we repeated Monod's experiment [...]." And then explain that this was only tested for one example, but there is no reason why this wouldn't be helpful to detect other patterns., Monod's experiment that was carried out for this paper was not convincing that Kinbiont can be used to detect new correlations between environmental parameters and fitted model parameters. This reproduced correlation was known prior to the analysis and was probably also the only environmental parameter that was correlated with the specific growth rate.

We thank the reviewer for this feedback and we agree that the original presentation of symbolic regression on the variation of Monod's experiment could have been clearer in its intent.

We clarify that the pattern observed in the amino acid limitation experiment (Figure 4B, C) was not known *a priori*. The trend emerged from the analysis, and its biological plausibility was assessed only *a posteriori*. Our intention was to demonstrate Kinbiont's potential in exploratory data analysis, where patterns not previously described in the literature may emerge from model fitting. To further illustrate the discovery of new relationships, we added the analysis of a newly generated chloramphenicol dose-response experiment and explicit interpretation of the parameters in the functional forms identified in both cases.

It is important to note that symbolic regression goes beyond simple correlation analysis, in that it explicitly searches the space of mathematical expressions to identify functional relationships that explain the observed data. Unlike statistical correlation, it yields equations that can be interpreted mechanistically and tested under experimental conditions not included in the training dataset. Furthermore, symbolic regression inherently includes feature selection, meaning that if multiple environmental conditions are tested, irrelevant variables tend to be excluded from the resulting

models. While we have not yet demonstrated this specific use case in the current manuscript, it represents a promising direction for future applications of Kinbiont.

Our second point is that Kinbiont requires improved documentation and perhaps some additional tutorials to make it accessible to less experienced users. With the current state, even for more experienced users it will be hard to apply Kinbiont to novel datasets due to specific data formats of annotation files. It would be really helpful if the analysis and the datasets used for this publication are available for the user through interactive notebooks. In addition, Kinbiont comes with a vast variety of functions and it would be greatly helpful to the reader if all those functions are visualized in a decision tree or some other illustration. This would clarify the fitting procedure and help the reader evaluate if this package is of any use appropriate for their applications.

We appreciate the reviewer's concerns regarding Kinbiont's usability of Kinbiont and documentation, and we have addressed them as follows:

- We have launched a redesigned documentation site that now includes a step-by-step workflow diagram for the fitting procedure (also shown in Extended Data Fig. 2). By mapping common analysis goals to relevant commands, this workflow should help users navigate Kinbiont's many functions.
- We have added a library of example datasets, fully executed notebooks, and all analysis scripts and data used in this manuscript. These resources allow users to reproduce every figure or adapt the code to new datasets.
- Because annotation formats differ across instruments, we added a detailed [Data formatting and outputs · Kinbiont Documentation](#) page that walks users through Kinbiont's annotation requirements and conversion utilities. While a universal microplate standard would be ideal, it does not yet exist.

We hope these additions make Kinbiont accessible to both new and experienced users.

Third, the discussion should contain an additional paragraph about limitations and pitfalls of this package and fitting in general. This is required in order that less experienced users are aware of the limitations of the output generated by Kinbiont.

We added limitations to the discussion, in particular in the paragraphs between line 323 and line 341.

In summary, this paper contributes to the field by bringing already existing features to a relatively new programming language that is gaining in popularity, and introducing a new feature of segmented fitting. For this contribution to be accessible and useful to less experienced members of the field, its documentation and tutorials need improvement. We also suggest to focus on the core features of the package and shift away from the narrative that Kinbiont can be used to create new hypotheses. This requires that the manuscript is restructured and partially rewritten.

We thank the reviewer for their appreciation of our work. We have substantially revised the manuscript to emphasize Kinbiont's core functionalities, making a broad set of established growth-curve models and analyses tailored to microbial kinetics data available in the Julia ecosystem, and we now describe the segmented-fitting routine as a distinct new feature (revised sections in Results, new Figures 1 and 2, revised Figure 3, and Extended Data Figure 3). At the same time, we retain – and strengthen – the discussion of hypothesis generation by adding two new case studies that show how interpretable machine learning modules link fitted parameters to biological questions (new analysis of the chloramphenicol dose-response dataset, and Extended Data Fig. 6). To make the

package accessible to less-experienced users, we have expanded the documentation with a workflow diagram, example datasets, and executed notebooks, all available in [Kinbiont · Kinbiont Documentation](#). These changes respond to the reviewer's request for clearer focus, improved tutorials, and a more balanced presentation of Kinbiont's capabilities.

Minor comments:

- Figure 1A: Color gradient of scale is the inverse of the color gradient of the well plate

We completely reworked Figure 1.

- L97: Move sentence "We benchmarked [...]" to another paragraph.
- L248: Sentence should be rephrased
- L275-277: This is confusing and not clear how the data structure should look like. A supplementary figure explaining the input matrices would be helpful.

We improved the description of data input, we improved the GitHub pages and the examples.

- Fig. 5, 6: these figures need more explanation. What are the dots in the bar plots, for example? Why are only a few chemicals shown in Fig. 5? Can you find a more intuitive way to describe the impurity score such that the reader doesn't need to dig further to understand the figure?

The chemicals highlighted in Fig. 5 are those with the strongest influence on the measured variables. Dots show the values of inferred parameters and we have clarified the meaning in the figure caption. We have also added a brief explanation of the Gini impurity score in the main text.

- It was unclear how Fig. 6 is automatically "detecting laws in microbiology". As outlined above, this should be toned down and instead it would be good to get a better explanation of what the decision tree analysis tells us.

We expanded the description and added a second, simpler example (Extended Data Fig. 6) to make the point more clearly.

- L. 287: "imputing" -> inputting

We thank the reviewer for the suggestion. However, in this case, we intended to use the term "*imputing*" in the statistical sense—i.e., assigning or estimating values for missing or unmeasured data. Therefore, we believe "*imputing*" is the appropriate term in this context.

Reviewer #2 (Remarks on code availability):

The code is missing documentation to make it more usable (as outlined in the comments to authors).

We thank the reviewer for pointing out the need for better documentation. Since the initial submission we have revised the user materials:

1. New documentation site with step-by-step workflow pages.

2. Example repository containing ready- to- run scripts and notebooks for both real and synthetic datasets.
3. Replication archive with all raw data and analysis scripts used in the manuscript.
4. Inline documentation: Every function in Kinbiont provides its description when used with Julia's help.
5. Compiled notebooks that reproduce all main- text figures are available in the project GitHub.

Reviewer #3 :

The manuscript introduces an open-source tool named Kinbiont for analyzing microbial growth kinetics using advanced machine learning techniques. While I am not an expert in model development, I perceive a robust methodology by the authors, such as the inclusion of a large number of closed-form and ODE models, as well as the use of change point detection algorithms and segmented fits for non-standard growth data. As a computational tool, this appears to be solid work.

We thank the reviewer for the positive assessment of Kinbiont's methodology. The additional examples, documentation, and manuscript revisions incorporated in this version should reinforce that robustness.

However, one of my main concerns is the uncertainty in real-world applicability. While the paper demonstrates Kinbiont's performance on several controlled datasets, such as nutrient shifts, phage-bacteria interactions, and pollutant exposures, these scenarios are relatively well-structured and defined. It remains unclear how well Kinbiont can handle more realistically complex systems, such as those found in natural environments or industrial settings. These systems often involve multiple interacting species, dynamic nutrient fluxes, and less structured growth patterns. Notably, some of these environments, like wastewater engineering, are the ones most in need of accurate growth characterization rather than defined synthetic communities. For example, in wastewater treatment, models like ASM and ADM integrate very simple kinetics of different trophic groups of microbes. How would Kinbiont potentially apply to or improve upon these existing models?

We appreciate the reviewer's concern about applying Kinbiont to complex, real- world systems. Kinbiont is presented as a proof of concept that demonstrates how closed- form, ODE systems, and machine- learning approaches can be combined in a single workflow.

Although we validated the pipeline on well- structured datasets to ensure reproducibility, the software is not limited to laboratory conditions. Users can import any system of differential equations—including multi- species or substrate- explicit models—and fit them when multi- channel data are available (e.g., separate readouts for different trophic groups or nutrients).

Kinbiont's machine- learning modules can also operate independently of kinetic fitting: given environmental variables, they can learn direct mappings to user- supplied response metrics. This design makes it possible to adapt the pipeline to settings such as wastewater treatment, where simplified ASM/ADM kinetics are now standard.

We agree that data availability is the main constraint. As high- resolution, multi- channel measurements become more common in industrial and environmental processes, tailored modules could be developed to further extend Kinbiont's capabilities in those domains.

Additionally, the manuscript lacks sufficient biological interpretation of the results. While the focus is clearly on the methodological advancements, it would be helpful to see a more detailed discussion on the biological implications derived from Kinbiont's application. Could Kinbiont provide insights into complex biotic-biotic and biotic-abiotic interactions, such as syntrophisms, competition, or parasitism?

We appreciate the reviewer's suggestion to strengthen the biological interpretation. Although the manuscript's primary goal was methodological, we have added several elements that show how Kinbiont can generate biological insight, and we believe these additions substantially strengthen the manuscript:

- Extended Data Fig. 6 presents a *Drosophila* microbiome coculture dataset (Jo et al., 2023) and demonstrates that growth dynamics of one strain can reveal potential interactions with a partner strain.
- The segmented- fitting section now explains how changepoints identify nutrient- utilization phases.
- A new antibiotic dose-response example has been included (Fig 4 D).
- The symbolic- regression section has been expanded to discuss the functional relationships uncovered and their biological implications.

While these examples come from controlled laboratory datasets, the same pipeline can be applied to complex ecological or industrial data when paired with metadata or environmental measurements. We hope these additions clarify Kinbiont's value as both a modelling platform and a generator of biological hypotheses.

Another comment is that the validation against existing tools is somewhat limited. A comparison against other well-known tools, such as *Growthcurver* or *Grofit*, on the same datasets is lacking. If existing tools are already solving the problem effectively, it is crucial to emphasize what specific advantages Kinbiont provides beyond these solutions.

We appreciate the reviewer's concern regarding the validation of Kinbiont's fitting procedures against existing tools such as *Growthcurver* or *Grofit*. Kinbiont, however, is intended as a full modelling pipeline rather than a single- model fitting utility, so a strict one- to- one benchmark is less informative than it might first appear.

If the question is confined to curve- fitting computational time or accuracy, unlike many existing tools that use a fixed set of models and optimization routines, Kinbiont can be customized from the viewpoint of optimization algorithms, numerical solvers, numerical precision, and tolerance. This yields full control fitting results, to the point where it is possible to reproduce the implementation of other packages using the corresponding backend solvers (Julia, Python, Fortran, C++).

Meaningful cross- tool benchmarks would also require that every package be run on the same mathematical model and input format—conditions rarely met in practice. Comprehensive computational time comparisons of numerical solvers and optimisers are already available (e.g., Fig. 3 in *Julia for Biologists* (Roesch E. 2023)); replicating them here would add length without new insight.

What Kinbiont adds is the ability to swap models, solvers, and optimisation routines in a single framework, apply automatic segmentation, and pass on fitted parameters directly to downstream machine- learning analyses, with flexibility being the main focus of the manuscript.

Finally, as a reader who is not intimately familiar with nonlinear optimization and differential equations, a more detailed guide on choosing the appropriate kinetic model would be of great value. This addition would help users make informed decisions about which models are best suited for their particular datasets and conditions.

We appreciate this suggestion. To help users choose a kinetic model, we have added:

1. A workflow diagram (Extended Data Fig. 3 and GitHub examples) that walks through model selection step by step.
2. A “Mathematical Models” table in the documentation that lists every built-in model together with its main properties.

When a clear choice is not obvious, Kinbiont can run model selection, using Akaike information criteria, and obtain the best option between the input list. This automated check cannot replace expert judgment in every case but provides practical guidance for most datasets.

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We are grateful for your contribution through the Nature Communications co-review initiative. Your comments helped us refine both the manuscript and the Kinbiont documentation, and we appreciate the time you invested in the review process.

REVIEWER COMMENTS

Reviewer #2:

We thank the authors for the revision of their paper and the extensive responses to our comments. We acknowledge and appreciate the efforts displayed by the authors to improve their previous submitted manuscript. Below, you can find our comments to the response in red. Since this manuscript was thoroughly revised, we provide some new comments and suggestions for improvements for this version of the manuscript, followed by minor comments.

We are grateful for the reviewer's careful re-reading of the revised manuscript and for the additional suggestions. Below we provide a detailed, point-by-point response (reviewer comments in red; our replies in green), together with the corresponding changes in the text.

Curveball is still missing as a reference and we think it is a major contributor to the field of growth curve fitting and should therefore be mentioned. We thank the authors for adding Fig. 3E and for explaining the Figures better in the text.

We have added a reference to Curveball (Ram, Y., et al., PNAS 2019).

However, we are still missing an explanation why more changepoints are identified by Kinbiont than biologically obvious. Fig. 3E shows that this identification comes from a slight increase in the growth curve (dashed line). The text misses to interpret if this slight increase is significant enough to justify adding additional parameters to the fitting.

Kinbiont compares every segmentation candidate with the corrected Akaike Information Criterion (AICc), which balances goodness-of-fit against model complexity. An additional segment—and therefore an extra set of parameters—is retained only if the reduction in residual error is large enough to offset the built-in penalty that AICc applies to each new parameter. Hence, when Kinbiont keeps a second changepoint the improvement in fit is, by definition, greater than the AICc penalty.

We have rephrased the discussion of segmented fits in the *Acinetobacter* dataset to include clarifications. The text now reads as “As demonstrated in Fig. 5D, Kinbiont reproduces the observed *Acinetobacter* growth curves from Ref. [18] across all tested conditions. For each curve we retain the fit with the lowest corrected Akaike information criterion (AICc; Fig. 5D, left panel). Because AICc penalizes additional parameters, this criterion selects the model–segmentation combination that offers the best trade-off between goodness-of-fit and complexity, countering overfitting.”

We thank the authors for improving the documentation as this will be very helpful for the end users. We think that the provided examples and guidelines for choosing the right models are sufficient for inexperienced users to apply Kinbiont to their experimental data.

We appreciate the reviewer's positive feedback and are pleased that the expanded documentation and example workflows are now meeting the needs of less-experienced users.

The revisions help the reader to understand how the decision tree can be helpful in identifying confounding variables. This is indeed useful to design potential follow up experiments to better understand complex datasets. However, in the paragraph from L249-L252, it is not clear how targeted interventions can be designed based on the output from Kinbiont. It is also not obvious why "optimizing microbial growth" is desirable in the context of the referenced study. It would be helpful if the paper were to illustrate what follow up experiments would be designed based on the output of Kinbiont.

We rephrased the paragraph to "More broadly, by efficiently identifying which perturbations impact individual growth parameters, this framework can be used to guide targeted interventions that steer microbial behavior toward specific goals -- for example, slowing the growth rate or reducing biomass density."

Then, in the end of next section (decision tree analysis with interactions) we elaborated on how the methodology presented here can be used to concretely design follow-up experiments.

"As one concrete example, our decision-tree analysis (Fig. 8E) ranks the stressor pair Oxytetracycline and Imidacloprid as giving the largest drop in maximum biomass for *N. soli*, whereas Oxytetracycline and Tebuconazole most strongly slows the growth rate. Because killing by many antibiotics shows linear relationships with growth rate (Bren *et al.* 2023), an informed next step would be a four-condition time-kill assay: (i) Imidacloprid alone, (ii) Tebuconazole alone, (iii) Oxytetracycline and Imidacloprid (largest biomass drop), and (iv) Oxytetracycline and Tebuconazole (largest growth-rate drop). Comparing (iii) and (iv) distinguishes whether biomass suppression or growth rate suppression accelerates bacterial clearance. In this way Kinbiont narrows dozens of potential drug combinations to a small set that merits immediate experimental validation."

See comment above. It is still unclear why two changepoints were identified.

Kinbiont identified two change points in some conditions because according to the AICc criterion, the model-segmentation combination in those cases offered the best trade-off between goodness-of-fit and complexity.

We thank the authors for considering our comment and highlighting this feature.

We still think that the authors slightly overstate the capabilities of Kinbiont. For example L213 says "providing a proof-of-concept for the automated discovery of empirical laws describing responses to ecological perturbations". However, a selection of models are hard-coded in

Kinbiont and Kinbiont ranks the provided models successfully. Calling this automated discovery of empirical laws is a bit of stretch.

We respectfully disagree. Symbolic-regression “law discovery” is independent of the hard-coded kinetic models: while it works on the parameters inferred with kinetic models from the time series, symbolic regression candidate equations do not draw on our built-in library. During this step no functional forms are supplied; the algorithm explores an expression space (addition, multiplication, division, exponentiation, ...) to evolve compact algebraic relationships that minimize error while penalizing complexity. The Monod-type saturation and Langmuir-type inhibition laws recovered in the current Fig. 6 emerge solely from this data-driven search.

The updated documentation greatly improved the accessibility of the tool.

We thank the reviewer for the appreciation of our work on documentation.

The manuscript is still lacking a clear statement about the pitfalls of Kinbiont, especially on the potential risks of over-fitting.

We sincerely thank the reviewer for this suggestion, we added new sentences regarding the possible pitfalls as model identification (L331-333) and overfitting in the segmentation fits (L338-342) . See below.

The authors successfully improved the accessibility of Kinbiont, by improving the documentation, adding decision trees and improving figures to help the user to understand the methodology. Those changes will be of great help for users who are looking for a tool to process and fit models in Julia to their biological data. The features that Kinbiont provides are exciting and useful for the field, whether it is inference of growth parameters, identification of diauxic shift, or the disentanglement of confounding variables. We think that the authors could be more careful with how those capabilities are phrased as already outlined in our responses above.

We sincerely thank the reviewer again for their valuable suggestions, which have challenged us to improve both the tool and the paper.

Minor comments:

Below we suggest some further improvements for the new version of the manuscript as well as some minor comments.

1. The (i), (ii), (iii) listing is confusing because it is not consistent with the listing used in the abstract.

We have changed the enumeration style to be consistent with the abstract.

2. The legend of Fig. 2 needs clarification. Currently, it is hard to understand which sub-legend belongs to which panel.

We have revised the caption of Fig. 2 to bring the panel references to the beginning of each sentence.

3. In our opinion, the discussion is too focused on an outlook which is hard to understand. We strongly suggest to instead discuss the limitations and pitfalls of Kinbiont, as this section is still missing.

In the previous revised version of the paper we added 2 paragraphs commenting on the limitations of Kinbiont. We expanded that part of the discussion to highlight two concrete pitfalls of the Kinbiont framework: (i) Loss-landscape flatness in over-parametrized models—explaining how nearly flat optima can yield non-unique parameter sets and hamper biological interpretation; and (ii) Over-segmentation when an excessively large library of candidate segment models is supplied—noting that too many plausible sub-models can drive the algorithm to insert spurious change-points.

The revised text now reads “...model selection. In over-parametrized models, some data and noise combinations can leave specific parameters unresolved because multiple parameter sets fit equally well [41] weakening robustness and limiting biological interpretation.”, ... “Another specific challenge concerns segmented fitting: there is no universal rule for picking the optimal number and placement of segments across individual curves or replicate datasets, and supplying an overly large library of candidate segment models can drive the algorithm toward over-segmentation, producing more break-points than are biologically warranted.”

4. The installation is failing because `Pkg.add(Kinbiont)` is missing quotes in the documentation. It should be `Pkg.add("Kinbiont")`

Thank you for pointing out this mistake is now fixed.

L33: We are still not completely convinced by the testable hypotheses statement. Maybe change it to “Doing so requires a fundamental mechanistic understanding”.

We appreciate the reviewer’s suggestion but feel the original wording is more accurate for the goal of the manuscript. A *fundamental mechanistic understanding* is certainly desirable, yet in many data-rich areas of microbiology (e.g. large chemical screens, mixed-species communities) such understanding is still incomplete. Our intention is to emphasize that, regardless of how much mechanistic detail is currently known, progress depends on generating quantitative, falsifiable predictions that can be probed in follow-up experiments. The phrase “models that generate testable hypotheses” captures this requirement without implying that full mechanistic detail is already in hand. We therefore retain the original sentence.

L38: Unclear what “Phenotypic variables” are

The phenotypic variables were mentioned in L36 and include, e.g., growth rate, lag duration, or yield.

L38: Remove last sentence

We respectfully disagree to remove the last sentence as it conveys an important difficulty in modeling biological systems, that in many cases, we don’t even know what are the variables we should write equations for, and how to relate models to data collected in experiments.

L50: “Kinbiont can fit virtually any system of differential equations or analytic function”. We would be careful with this statement as it implies that Kinbiont is unaware of over-fitting. You probably do not want to fit any ODE to your biological data.

Our intent was to highlight flexibility, not to recommend indiscriminate model choice. Our point is that the Kinbiont framework lets users implement and fit any custom model they may require. Although highly parameter-rich models can over-fit, Kinbiont tries to mitigate this through built-in model-selection tools (e.g. AICc) and parameter constraints. Thus, while one would not choose an arbitrary model, the software can accommodate newly developed models when biologically warranted and evaluate them against alternative models.

L67: “Stable documentation”. We do not think that documentation can be stable or not since it is plain text.

We removed the word stable.

L86: Fig. 2 is referenced before Fig. 1

The first reference of Fig.1 is in L70 and for Fig.2 L84.