1 **ORT: A workflow linking genome-scale metabolic models with reactive transport codes**

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9 **Abstract**

10 **Motivation:** Nutrient and contaminant behavior in the subsurface are governed by multiple

11 coupled hydrobiogeochemical processes which occur across different temporal and spatial scales.

12 Accurate description of macroscopic system behavior requires accounting for the effects of

13 microscopic and especially microbial processes. Microbial processes mediate precipitation and

14 dissolution and change aqueous geochemistry, all of which impacts macroscopic system behavior.

15 As 'omics data describing microbial processes is increasingly affordable and available, novel

16 methods for using this data quickly and effectively for improved ecosystem models are needed.

17 **Results:** We propose a workflow ('Omics to Reactive Transport – ORT) for utilizing

18 metagenomic and environmental data to describe the effect of microbiological processes in

19 macroscopic reactive transport models. This workflow utilizes and couples two open-source

20 software packages: KBase (a software platform for systems biology) and PFLOTRAN (a reactive

21 transport modeling code). We describe the architecture of ORT and demonstrate an

22 implementation using metagenomic and geochemical data from a river system. Our demonstration

23   uses microbiological drivers of nitrification and denitrification to predict nitrogen cycling patterns

24   which agree with those provided with generalized stoichiometries. While our example uses data

25   from a single measurement, our workflow can be applied to spatiotemporal metagenomic datasets

26   to allow for iterative coupling between KBASE and PFLOTRAN.

27   **Availability and Implementation:** Interactive models available at

28   https://pflotranmodeling.paf.subsurfaceinsights.com/pflotran-simple-model/. Microbiological data

29   available at NCBI via BioProject ID PRJNA576070. ORT Python code available at

30   https://github.com/subsurfaceinsights/ort-kbase-to-pflotran. KBase narrative available at

31   https://narrative.kbase.us/narrative/71260 or static narrative (no login required) at

32   https://kbase.us/n/71260/258

33   **Contact:** rebecca.rubinstein@subsurfaceinsights.com or roelof.versteeg@subsurfaceinsights.com

34   **Supplementary information:** Supplementary data are available online.

35

36   **1   Introduction**

37   The critical zone (CZ) – the area between the top of the forest canopy and the bottom of

38   the groundwater table is essential in sustaining life (Guo and Lin, 2016). Being able to understand

39   and predict critical zone function is essential for both scientific and operational purposes. This

40   understanding and prediction requires the accurate representation of key hydrobiogeochemical

41   ecosystem processes which occur and interact in the critical zone. These ecosystem processes

42   operate at different scales and have different drivers, but at the same time are tightly

43   interconnected. For instance, while hydrological processes control the movement of water at

44   macroscopic scales and are driven by groundwater table gradients, precipitation, and

45   evapotranspiration whereas microbiological processes (Anantharaman *et al.*, 2016; Long *et al.*,

46   2016) occur at the microbe scale and are driven by microbial populations, soil properties, aqueous

47   geochemistry, and temperature. However, processes at these two scales influence one another in

48   many ways.

49   One well-established approach to obtaining an understanding of critical zone behavior is

50   through the use of reactive transport models (RTM) which can simulate coupled chemistry, flow,

51   and transport in hydrobiogeochemical systems. There are a variety of reactive transport codes (see

52   (C. I. Steefel *et al.*, 2015) for a review). These models are generally continuum scale models

53   which represent subsurface properties on grids, with grid volumes on the order of cubic meters. As

54   the earth is a porous media with grains and pores, such continuum scale models obviously do not

55   capture pore scale properties and dynamics. One fundamental challenge in numerical modeling is

56   thus how to link and couple processes and properties which happen at different scales (Battiato *et*

57   *al.*, 2011; Chu *et al.*, 2012, 2013; Carl I. Steefel *et al.*, 2015). Such linking is especially required

58   between macroscopic system behavior and microbial processes which change aqueous

59   geochemistry and mediate precipitation and dissolution.

60   With continued decrease in 'omics data analysis costs, one promising approach for this

61   linking is through the incorporation of site-specific microbiological data into RTM to represent

62   microbe-catalyzed biogeochemical more accurately than using generalized stoichiometries. The

63   feasibility of using the results of microbiological data analysis to parameterize RTM has been

64   shown previously. For instance, Scheibe et al. demonstrated the linking of genome scale models

65   with a reactive transport code (in their case, HYDROGEOCHEM) to improve incorporation of

66   microbiological processes on in situ uranium bioremediation (Scheibe *et al.*, 2009). Specifically,

67   they used a genome scale model of *Geobacter sulfurreducens* to populate a lookup table spanning

68   reasonable expected ranges for all combinations of three key system parameters. This was then

69   used to predict the effects of varying concentrations of three key growth factors (acetate, Fe(III),

70   and ammonium) on reduction of uranium (VI) at a systems level. More recently, Song et al.

71   developed an enzyme-based approach for simulating microbial reaction kinetics which captured

72   the overall behavior of a consortium rather than rely on individual taxa within the community and

73   coupled it with reactive transport simulations using PFLOTRAN's Reaction Sandbox (Song and

74   Liu, 2015; Song *et al.*, 2017; Hammond *et al.*, 2017). This approach is based on a mechanistic

75   understanding of microbial processes and thus can more accurately predict microbial response to

76   perturbations. However, this approach substantial experimental data, such as enzyme

77   concentrations and kinetics data, as well as advanced microbiological knowledge to implement.

78   These previous efforts have demonstrated the value and feasibility of accurately

79   representing microbial processes in RTM. This in turn opens up the potential to integrate

80   microbiological, geochemical, and physical subsurface properties and processes to predict

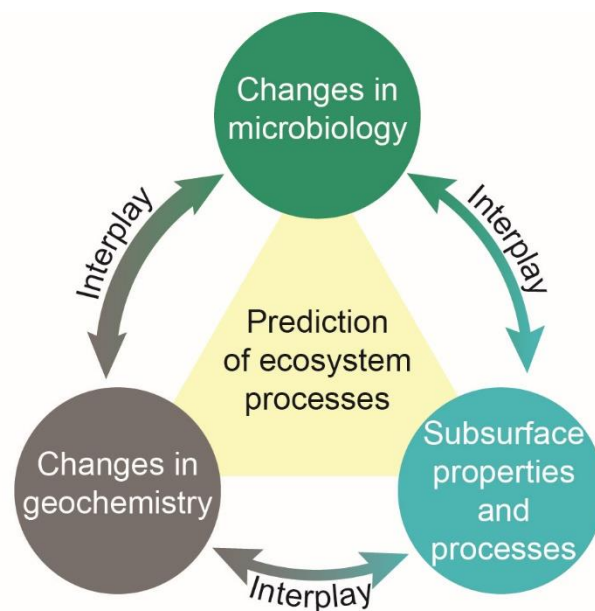81   ecosystem behavior and response (Fig. 1).

82

Fig. 1 – Accurately capturing the interplay between microbiology, geochemistry, and physical subsurface properties and processes is critical to understanding and predicting ecosystem processes.

86   However, each of the approaches described above requires substantial manual effort to

87   implement for a single site, which makes them challenging to scale. The challenge of scaling these

88   approaches limits the ability to rapidly develop models obtain the associated understanding for

89   many sites. An alternative approach (proposed and demonstrated here) is to an approach which

90   allows for automation.

91   Specifically, in our workflow we use KBase (a cloud-based software platform for systems

92   biology (Arkin *et al.*, 2018)), to automatically generate draft metabolic models from annotated

93   metagenome assembled genomes (MAGs) extracted from environmental samples. These

94   metabolic models can be used (still in KBase) to perform flux balance analysis (FBA) on different

95   media compositions. These media compositions are informed by metabolomics and other site-

96   specific chemistry data. The output of the FBA can be used in reactive transport models (such as

97   the reactive transport model PFLOTRAN (Mills *et al.*, 2009; Hammond and Lichtner, 2010;

98   Gardner *et al.*, 2015)), which we use in this work. PFLOTRAN is an open source, massively

99   parallel reactive transport code which supports multi-phase (e.g. aqueous, gaseous), multi-

100   component (multiple chemical species), and multi-scale (e.g. pore or macroscale) simulation of

101   contaminant transport in porous media, as well as includes a basic implementation of microbial

102   reactions modeled by Monod kinetics. One major benefit of PFLOTRAN is that users can

103   implement custom reactions or kinetics through the Reaction Sandbox (Hammond, 2017). Our

104   workflow, called 'Omics to Reactive Transport (ORT) (Fig. 2), thus captures both microbial

105   metabolisms based on environmental samples (using KBase) and macro-scale hydrologic and

106   geochemical processes (using PFLOTRAN). In the remainder of this paper we present the concept

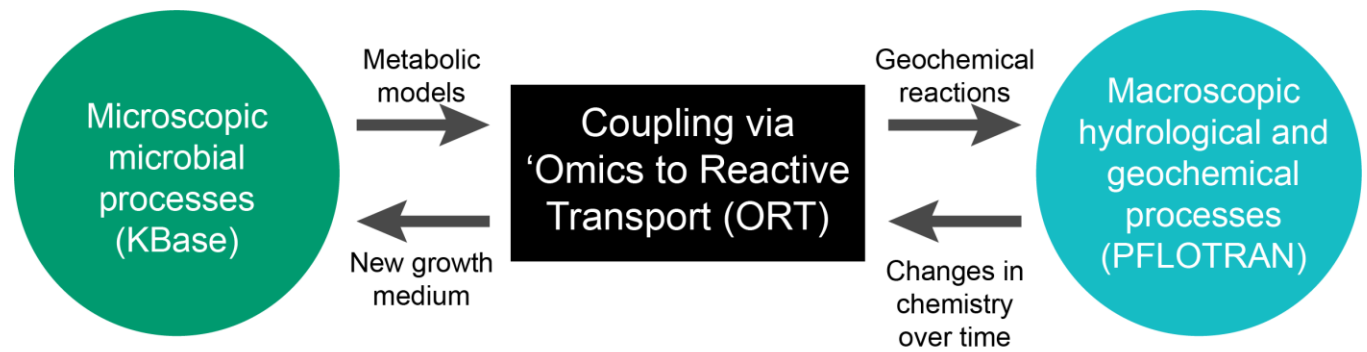107   and implementation of this workflow.

Fig. 2 - Omics to Reactive Transport (ORT) workflow couples microbe-scale and macroscale processes using the outputs of KBase and PFLOTRAN as inputs for each other.

In addition to the scientific value of this workflow, we want to highlight three operational attributes of interest. First, this workflow can be mostly automated, offering the potential of rapidly generating reactive transport models from microbiological data (detailed in Section 3) with a minimum of manual labor. Second, the resulting models can easily be shared and made accessible to other groups. For instance, we have provided two of the models we generated through a user-friendly web interface which allows end-users to interact with these models. Third, while in this paper we do not include results for this, our workflow lends itself well to an iterative approach. Specifically, it is well known that microbial processes will result in changes in geochemistry, which in turn will influence the microbial processes. In addition to this, macroscopically driven changes in saturation, temperature, and chemistry (e.g., resulting from stage-driven surface water/ground water interaction) will also influence microbial processes. The approach described here can be executed in an iterative manner to capture this two-way coupling between microscopic and macroscopic processes.

2 System & Methods

2.1 Workflow Concept

127   The ORT workflow was designed with automation in mind. Specifically, it is designed in a

128   modular manner with a well-defined start and end points and inputs and outputs, with each

129   component being fully automatable (Fig. 3). The inputs to this workflow are annotated genomes,

130   environmental chemistry, and a PFLOTRAN model template which incorporates physical site

131   data. This template (which would be customized to the specific site) would be something like "0D

132   batch reactor" or "2D model of unsaturated soil" (where "nD" indicates the number of spatial

133   dimensions accounted for in the model grid).

134   **In** this workflow we import annotated genomes (Shaffer et al., 2020) and site chemistry

135   (e.g., available carbon sources, electron acceptors, and micronutrients based on metabolome and

136   any other chemical analysis at the site, synthesized into a KBase media recipe) into KBase, then

137   use KBase apps to generate the overall reactions. Next, these reactions, as well as the site

138   chemistry, physical site data, and model template are used to build the actual PFLOTRAN model.

139   This model can then be used to simulate macroscopic system behavior.

140   In the iterative implementation, the PFLOTRAN model simulates changes in physical and

141   chemical conditions in space and time. We can then use the simulated chemistry as a new media

142   composition to be used by the KBase part of the workflow and repeat the process to generate and

143   retrieve new resulting overall reactions and substitute them into the PFLOTRAN input file.

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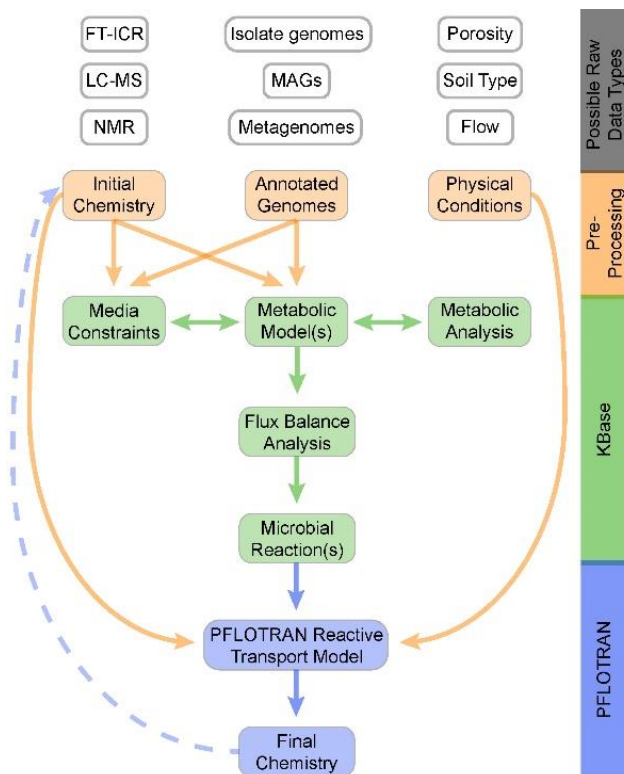
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Fig. 3 - Flowchart of the ORT workflow where orange boxes are workflow inputs based on site characterization which are pre-processed before use, green boxes are metabolic modeling steps carried out in KBase, and blue show the resulting RTM. The horizontally-aligned boxes and arrows in the KBase workflow represent robust curation steps (discussed in Section 4), and the dashed arrow indicates the iteration path wherein the PFLOTRAN-simulated chemistry is used as a new media condition in KBase.

146 **2.2 Workflow Implementation**

147   The ORT workflow consists of Python scripts, KBase narratives, and PFLTORAN models.

148 In our implementation of ORT, KBase apps import genomes and chemical data into KBase and

149 use these as inputs for KBase metabolic modeling apps (process described in detail in Sections 2.4

150 and 2.5). After the completion of the KBase part of the workflow, the KBase API (application

151 programming interface) programmatically exports the KBase-predicted exchange fluxes from

152 KBase. These fluxes are translated by our Python script into an overall reaction string that

153 describes chemical uptake and secretion from each modeled organism, written in PFLOTRAN-

154   compatible naming conventions. The flux values are used as the stoichiometric coefficients for the

155   corresponding chemicals in the overall reaction used in PFLOTRAN, with positive fluxes

156   indicating reactants and negative fluxes indicating products. The summation of exchange fluxes is

157   not a chemical reaction in the traditional sense, but represents the chemical species removed from

158   and added to the system as a result of the microbial metabolism. Thus, this "pseudo-reaction"

159   provides the information needed by PFLOTRAN to simulate the resulting changes in chemical

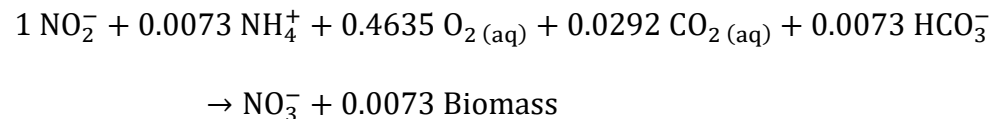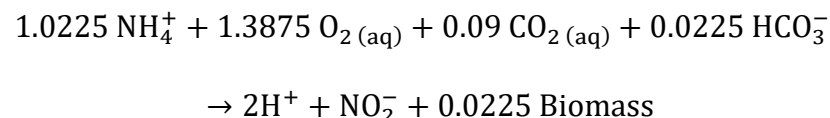160   concentrations.

161   The ORT Python script outputs a *.txt* file with the reaction strings and yield terms for use

162   in the MICROBIAL_REACTION card in PFLOTRAN as well as a set of *.dat* files which contain

163   compound names and details which need to be added to the PFLOTRAN geochemical database

164   (formatted for compatibility with the database). This step can either be done programmatically or

165   manually by substituting the content of these text files into a PFLOTRAN model input file (known

166   as an infile). This script bridges the disconnect between KBase and PFLOTRAN illustrated in Fig.

167   2.

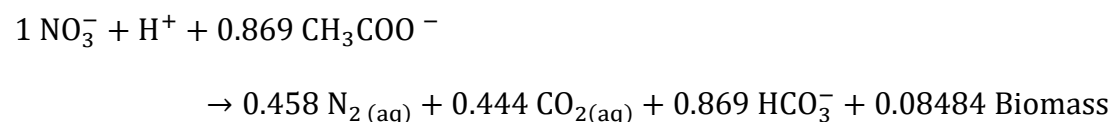168   **2.3 Test Case**

169   **2.3.1   System Description**

170   To evaluate the performance of our workflow, we used environmental samples from a

171   hyporheic zone in the Columbia River. In these zones, biological nitrogen cycling is known to

172   occur (Triska *et al.*, 1993; Zheng *et al.*, 2016). Biological nitrification and denitrification is a

173   classic, well-understood, and extensively studied system. We can calculate and compare models

174   which use traditional (textbook) stoichiometries for nitrification and denitrification to the model

175   generated from our workflow. In the remainder of this paper, we refer to these two models as the

176   "literature based model" and "genome derived model".

177   Nitrification is traditionally split into two sub-processes, ammonium oxidation (NH4 →

178   NO$_2$) and nitrite oxidation (NO$_2$ → NO$_3$), while denitrification is often represented as a complete

179   process (NO$_3$ → N$_2$), though in reality it is several sequential reactions. Within KBase, we could

180   implement separate models for each step for which genomes are available, but for comparison to

181   the traditional model we used a single model for complete denitrification in this test case. The

182   overall reactions used for the nitrification step were based on experimentally-determined

183   stoichiometries (Liu and Wang, 2012) determined by fitting data collected from bench-scale

184   reactors to traditional half-cell reactions (Rittmann and McCarty, 2012), as given by the following

185   reactions:

186    $1.0225\ NH_4^+ + 1.3875\ O_{2\ (aq)} + 0.09\ CO_{2\ (aq)} + 0.0225\ HCO_3^-$

187         $\rightarrow 2H^+ + NO_2^- + 0.0225\ Biomass$

188

189    $1\ NO_2^- + 0.0073\ NH_4^+ + 0.4635\ O_{2\ (aq)} + 0.0292\ CO_{2\ (aq)} + 0.0073\ HCO_3^-$

190         $\rightarrow NO_3^- + 0.0073\ Biomass$

191   The complete denitrification process stoichiometry was derived from half-cell reactions

192   (Rittmann and McCarty, 2012), scaled to one unit nitrate utilization for comparability with the first

193   two reactions:

194    $1\ NO_3^- + H^+ + 0.869\ CH_3COO^-$

195         $\rightarrow 0.458\ N_{2\ (aq)} + 0.444\ CO_{2(aq)} + 0.869\ HCO_3^- + 0.08484\ Biomass$

196   In both cases, the chemical species represented are limited to classical compositions, which

197   in some cases may serve as analogs for a range of compounds. These stoichiometries are not

198   associated with any specific microbes or metabolic pathways, but rather represent the exchange

199   fluxes observed. While this approach is very effective for process design, it does not offer much

200   insight into the microbiology of a system, and may obscure finer-scale dynamics – such as less

201 obvious resources that may become limiting or change how the microbes process available

202 macronutrients, particularly in systems with complex carbon sources.

203   The rates determined through batch kinetics tests (Liu and Wang, 2012) were used for

204 ammonium oxidation and nitrite oxidation and the denitrification rate was based on rates reported

205 in the literature (Raboni *et al.*, 2014). The same rates (shown in Table 1) were used for both the

206 literature-based and genome-based models (described in Section 2.5 - 2.66) in order to directly

207 compare the effects of the different stoichiometries. In future enhancements, we anticipate that

208 reaction rates could be used as tunable parameters to fit these models to system-specific

209 experimental data.

210 Table 1 – Baseline reaction rates used in nitrogen-cycling models

Process	Rate (mol/L·s)
Ammonium Oxidation	1.0×10^{-7}
Nitrite Oxidation	8.51×10^{-8}
Nitrate Reduction	2.34×10^{-8}

2.3.2   Leveraging Existing Multiomics Data

218   This study made use of multiomics data from previously published work. Sediment was

219 collected and DNA extracted as previously described (Graham et al., 2017). To identify the

220 metabolites available to microorganisms in these river sediments, we performed 1H Nuclear

221 Magnetic Resonance (NMR) spectroscopy on 17 paired sediment pore water samples which also

222 had microbial DA extracted, as described previously (Tfaily *et al.*, 2019). Briefly, sediment

223 samples were mixed with water in a 1:1 ratio and then diluted by 10% (vol/vol) with 5 mM 2,2-

224 dimethyl-2-silapentane-5-sulfonate-d6 as an internal standard. The 1D 1H NMR spectra of all

225 samples were processed, assigned, and analyzed using Chenomx NMR Suite 8.3 with

226 quantification based on spectral intensities relative to the internal standard as described. To obtain

227 a representative bulk summary of the metabolite environment in these sediments, the

228 concentration of 31 of the NMR identified metabolites was averaged across the 17 sediment

229 samples, and this data was used as the chemical data input in our ORT workflow (data available in

230 Supplementary Table S1).

231 Purified genomic DNA was sent to the Joint Genome Institute (JGI, n=33) under

232 JGI/EMSL proposal 1781 and to the Genomics Shared Resource facility at The Ohio State

233 University (OSU, n=10), producing 43 metagenomes from 34 sediment samples with an average

234 sequencing depth of 3.84 (JGI) 25 Gbp (OSU) per sample. JGI and OSU sequencing was

235 performed as previously described in Graham et al (Graham *et al.*, 2018) and Borton et al (Borton

236 *et al.*, 2018) respectively. Raw reads were processed, assembled, and binned as outlined in

237 previous publications (Shaffer *et al.*, 2020) or via the Wrighton Lab GitHub Page

238 (https://github.com/TheWrightonLab). The genomes are available on NCBI via BioProject ID

239 PRJNA576070.

240 From the sediments, we obtained metagenome assembled genomes (MAGs) from which

241 we selected four genomes that represented key parts of the nitrogen cycle. For each stage of the

242 cycle, the most complete genomes capable of filling those roles were selected. To represent

243 nitrification, we chose the most complete genome representatives of the ammonium oxidizing

244 archaea classified by GTDB-Tk (version 1.3.0, as of 1-21-21) as a member of the family

245 Nitrososphaeraceae within the genus TA-21 (previously within the Phylum Thaumarchaeota) and

246 nitrite oxidizing bacterial member of the Nitrospiraceae for nitrification. Given that the expression

247 and activity of nitrite reductase encoded in Nitrososphaeraceae (previously Thaumarchaeota) is

248 poorly understood at this time (Kuypers et al., 2018), we did not incorporate the production of

249 nitric oxide by Nitrososphaeraceae, and focused only on nitrite outputs from ammonification. To

250 represent denitrification, we selected two Gammaproteobacterial MAGs, both classified within the

251 family Steroidobacteraceae. Note that neither of these genomes encoded a gene to produce N_2 gas,

252   but the reaction to convert nitrous oxide to nitrogen gas was added to the metabolic models during

253   gapfilling (see Section 2.5). We selected only four genomes to maintain the simplicity of this

254   proof of concept, but the approach could incorporate as many as are needed to capture system

255   behavior. Each nitrogen-cycling genome was annotated using DRAM (Distilled and Refined

256   Annotation of Metabolism (Shaffer et al., 2020)) with default parameters. The raw annotations

257   containing an inventory of all database annotations for every gene from each input genome are

258   included in the online Supplementary Materials. These genomes and their annotations were

259   uploaded to KBase (Section 2.5) and were the basis for the KBase-derived model (Section 2.6).

260

261   **2.4 Pre-Processing**

262   Prior to executing the workflow, we need to gather and preprocess data and make several

263   decisions such as selecting a model template. In this section, we describe the data preprocessing

264   steps in generic terms, as the same steps will be required for any system. To begin our workflow,

265   user inputs were organized and prepared, which consisted of three broad steps:

266   (1) Qualitative assessment – to balance model complexity and utility, the system definition

267   phase began with a qualitative description of the system in terms of model type (batch,

268   chemostat, continuously stirred tank reactor, etc.), important processes (such as nitrification

269   or sulfur reduction, depending on the system), and parameters of interest (pH, specific

270   chemical species, etc.) that can guide model development. This step includes evaluating if

271   there is any "missing" data, which might render the model inaccurate or impossible, and

272   would need to be estimated in order to produce a viable system (for example, concentrations

273   of biologically necessary compounds that were not measured). These are identified through a

274   combination of subject matter knowledge and comparison with KBase default media recipes.

275   Note that this does not entail delineation of every process and parameter involved in the

276   system, but rather selection of those important to the specific research or application. The

277   goal of this step is to develop a conceptual model of the system of interest, which may be

278   augmented and refined as needed to accommodate new data. Because many of these models

279   will be similar (e.g. 0D batch models), we can build a library of model templates which can

280   be readily reused.

281   (2) Data Gathering - data describing the site may be drawn from a variety of sources, including

282   direct sampling at the site and public resources such as weather stations or national

283   databases. Biological data could come in the form of annotated genomes or metagenomes

284   collected from the site, or genomes for key microbes as determined using 16S rRNA gene

285   data or literature review could be drawn from public databases. Chemical data could include

286   traditional geochemical analysis as well as metabolomics and metaproteomics to provide a

287   more detailed picture of the chemical profile at the site. Physical data could include

288   temperature, soil porosity, or other parameters of that nature that would be included in the

289   PFLOTRAN input file to produce a more site-specific model.

290   (3) Translation to KBase and PFLOTRAN - the data produced by the various analyses above are

291   not necessarily in formats that may be directly imported to KBase and/or PFLOTRAN.

292   Therefore, the final step in this phase was to translate these data to forms that can be used by

293   the relevant tools (KBase or PFLOTRAN). Aside from managing file formats (see the

294   KBase documentation for details), one major consideration was accounting for any un-

295   measured chemical species identified in the first step of the preparation phase that needed to

296   be added to the KBase media composition to make it biologically viable or usable by the

297   metabolic models generated in KBase. Additions were limited to chemical species or

298   compounds known (or reasonably expected) to be present and were added in sufficient

299   concentration that they would not be growth-limiting. The primary check for the presence

300   assumption was that the experimental data indicated that the microbes used were both

301   present and involved in nitrogen cycling at that site. We did not investigate the assumption

302   that these compounds were non-limiting, as this is outside the scope of this work.

303   **2.5 KBase Metabolic Modeling**

304   Once pre-processing was complete, we can start the ORT workflow. Genomes were uploaded to

305   KBase as paired FASTA and GFF3 text files using the "Import GFF3/FASTA file as Genome

306   from Staging Area" app and then annotated with RASTtk using the "Annotate Microbial Genome"

307   app in KBase. Additional custom annotations from DRAM were uploaded as flat text files using

308   the beta version of "Import Annotations from Staging" app. If using DRAM annotations,

309   preprocessing may be carried out using the provided script at

310   https://github.com/subsurfaceinsights/ort-kbase-to-pflotran. Notably, both RASTtk and DRAM are

311   available as apps in KBase, allowing users to functionally annotate genomes without high memory

312   computational resources. However, note that the DRAM app in KBase differs from the version

313   used in this example narrative (Shaffer *et al.*, 2020) as the KBase DRAM app annotates using

314   KOfam instead of KEGG genes and does not currently include EC reaction identifiers, so end

315   results may differ from the included narrative. Chemical data was uploaded as flat text files using

316   the "Import Media file (TSV/Excel) from Staging Area". The use of pre-processed flat text files as

317   inputs to the workflow significantly simplifies the process compared to using raw data, especially

318   for genomes, and these can be generated automatically using scripts such as the one developed for

319   the DRAM outputs. This first step brought all of our data in the KBase workspace in an integrated

320   manner.

321   After this step, we used all this data as inputs to the "Build Metabolic Model" app, and the

322   generated models were used in conjunction with the media objects as inputs to the "Run Flux

323   Balance Analysis" (FBA) app. Going forward, we refer to this pairing as "growing a model,"

324   meaning we ran the analysis to determine if biomass growth was possible under the given

325   chemical conditions. The output from the FBA app included the reaction and exchange fluxes for

326   each model grown on the corresponding media.

327

328   **2.6 PFLOTRAN Reactive Transport Modeling**

329   We used our workflow to download the FBA exchange flux values using the KBase API and

330   translate them from KBase objects with ModelSEED (Henry *et al.*, 2010) compound IDs to flat text

331   files with reaction strings written using PFLOTRAN naming conventions. We then used either the

332   KBase-derived reaction strings and biomass yield values or the literature-based stoichiometries

333   introduced in Section 2.3 to fill in the MICROBIAL_REACTION card in our 0D model template.

334   All parameters except the reactions and yield terms were held the same for both the literature-based

335   model and the genome-derived model.

336   **3   Model behavior and General behaviors and trends**

337   Both models exhibited sequential ammonium and nitrite oxidation followed by nitrate

338   reduction, ultimately producing dissolved nitrogen gas (Fig. 4). Despite using the same reaction

339   rates, inhibition constants, and initial nutrient concentrations, the overall progress of the system is

340   noticeably different. The genome-derived model exhausts the available ammonium within 1.5 hours

341   of the simulation start, while the literature based model does not exhaust ammonium until a little

342   more than 3.5 hours into the simulation. Nitrite concentration peaks earlier and at a lower level for

343   the genome-derived model (~18 µM at approximately 1 hr) than the literature based model (~51 µM

344   slightly before 3 hrs). Similarly, nitrate peaks at approximately 4 µM after 1.5 hrs for the genome-

345   derived model but peaks at 40 µM at the 6 hr mark for the literature based model. In the 6 hour

346   period shown in Fig. 4, the genome derived model has exhausted ammonium, nitrite, and nitrate,

347   while the literature based model is still processing nitrite and nitrate. This variance is expected since

348   we are comparing generic reactions (with generic substrate utilization and biomass production

349   reactions) to site-specific reactions based on the most dominant taxa found at our study site.

350   One important difference was that the microbiologically-explicit, genome-based

351   stoichiometry provided much greater detail on the chemistry, particularly with respect to carbon

352   catabolism (Fig. 4 and Fig S1). Specifically, the literature-based models relied entirely on either

353   carbon dioxide (nitrification) or acetate (denitrification), however, because we provided additional

354   carbon compounds detected from our bulk sediment metabolome, the site models used 15 to 23

355   unique additional carbon sources, such as betaine, leucine, and choline (see Supplementary Table

356   1). This greater detail allows us to evaluate more precisely the potential chemical drivers or

357   limiters of a system which would be entirely overlooked with traditional representations, which

358   presents the opportunity to probe and improve our conceptual and mechanistic understanding of

359   these systems and individual metabolisms.
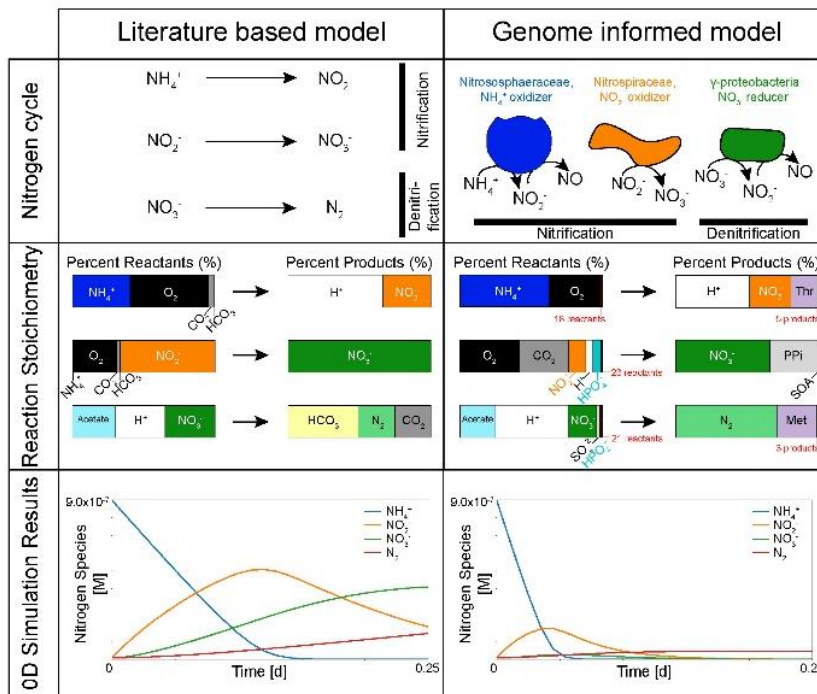
Fig. 4 - Using our Omics to Reactive Transport (ORT) workflow allows us to not only tailor a model to a specific environmental site and system, but also provides much finer insight into the changes in chemistry driven by microbial processes. The top frame shows the steps captured by the literature-based and genome-informed models respectively. The middle frame shows graphical representations of the two sets of reaction stoichiometries. Abbreviations used in the site-specific model frame are Met for Methionine, Thr for Threonine, and SAO for S-Adenosyl-4-methylthio-2-oxobutanoate, which are compounds predicted by KBase as an output which is not part of standard literature representations. The bottom frame shows the results of using each set of reactions in a 0D PFLOTRAN simulation of nitrogen cycling.

360

361   Instead of generic bacterial enzymatic reactions, we can determine which site specific bacterial –

362   or archaeal – reactions are drivers in the system. Instead of pre-set stoichiometries, our 'Omics to

363   Reactive Transport workflow uses chemistry determined based on metabolomics – using these

364   data to describe the initial chemistry rather than generic or simplified chemistry. For example,

365   even with the same rate constants, we can see that the genome-informed model utilizes a higher

366 proportion of ammonium in the first step of nitrification, resulting in more rapid depletion of

367 ammonium in the system and earlier generation of nitrite. As a result, subsequent steps begin

368 earlier, resulting in an overall accelerated process. At the same time, both versions exhibit the

369 expected cycling of ammonium to nitrite to nitrate and finally to nitrogen gas. Since PFLOTRAN

370 relies on user-defined chemistry (as opposed to automatically generating reactions), this allowed

371 us to incorporate more realistic, mechanism-driven reactions.

372 The genome-based model also allows for greater chemical breadth. The nitrogen cycling

373 reactions are modulated by a wider range of carbon sources. Additionally, the by-products of this

374 carbon and nitrogen metabolism also resulted in more complex chemical outputs in some cases,

375 such as L-Threonine or L-Methionine. These inferred reactions could be further refined by using

376 gene expression data (e.g., metatranscriptomics or metaproteomics data) to calibrate the models

377 (by way of reaction rates, saturation constants, etc.) to a particular set of environmental conditions.

378 Again, this presents an opportunity to test and enhance our understanding of the metabolic

379 processes involved.

380 Readers can explore and interact with both of these models (without sign-in) through

381 Subsurface Insights' web-based PFLOTRAN interface at

382 https://pflotranmodeling.paf.subsurfaceinsights.com/pflotran-simple-model/. For the literature-

383 based model, we have made the input concentrations of ammonium, bicarbonate, and acetate

384 accessible to web users using sliders. For the Hanford 300 Area-specific version of the model, we

385 have made accessible the reaction rate for each of the steps modeled. There is no limit to the

386 number of parameters that may be exposed this way, but for the sake of a user-friendly and un-

387 cluttered demonstration, we limited our selections to three per model. We selected the parameters

388 we did both because the effects of varying them are significant and to highlight the power and

389 flexibility provided by this approach.

4   Discussion

We demonstrated an Omics to Reactive Transport (ORT) workflow for creating site specific reactive transport models that include local chemical and biological content. The ORT workflow was applied to a well-understood system, and the results agree generally with expected behavior in a nitrogen cycling system. We interpret the differences in magnitude and timing to be due to the difference between generic, simplified reactions and metabolism-informed reactions, as KBase-derived stoichiometries made it possible to capture microbial metabolism in much greater detail than conventional approaches allow.

While the model predictions are borne out by comparison to traditional models, we would need extensive new data which currently is not available to comprehensively validate our modeling results. Specifically, we would need high resolution time series data. Such data was not available in this effort, but is a component of ongoing work, and is in general becoming increasingly available as technology improves and cost per sample decreases. Given similar data types, the same workflow could be applied to build and tune a model for other sites.

Much of the future work on this workflow will be focused on enhancing and expanding automation and on making it more robust in several ways. One capability which would be highly beneficial to our workflow is automated metabolic model curation. In our effort, curation was carried out manually using two different approaches: metabolism-based and media-based. The former is labor intensive and requires substantial subject-matter expertise to carry out. The latter is more straightforward and relies on a more general system understanding, but still requires manual iteration to obtain reasonable results. Partially or fully automated model curation will eventually be needed for full automation. This is a topic of active effort by both the KBase core team and other groups, and we will leverage their efforts. Additional work will be in expanding PFLOTRAN models to include processes such as temperature mediated biological processes and

414 material recycling. While these are currently not part of the core PFLOTRAN capabilities, these

415 can be implemented using the PFLOTRAN sandbox.

416   While previous researchers have demonstrated the feasibility of coupling genome-scale

417 metabolic models with reactive transport simulations, our work is different in some fundamental

418 ways. First, our workflow, lends itself to automation and rapid model generation from 'omics data.

419 As 'omics data becomes increasingly affordable, the ability to rapidly translate this data into

420 information on its the implications for macroscopic system behavior will be needed, and our

421 workflow provides a path towards that. Second, our workflow lends itself to easy incorporation of

422 more realistic microbial reaction kinetics (e.g., based on temperature or soil conditions). Third, our

423 workflow lends itself to iteration, which allows us to couple microscopic and macroscopic

424 processes in either direction. Finally, our workflow provides an easy way to couple two powerful

425 and complex software packages which typically are used by scientist in different domains, and

426 allows these scientists a path to generate 'omics informed reactive transport models.

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