Supplemental Figures, Tables and Experimental Procedures

Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics

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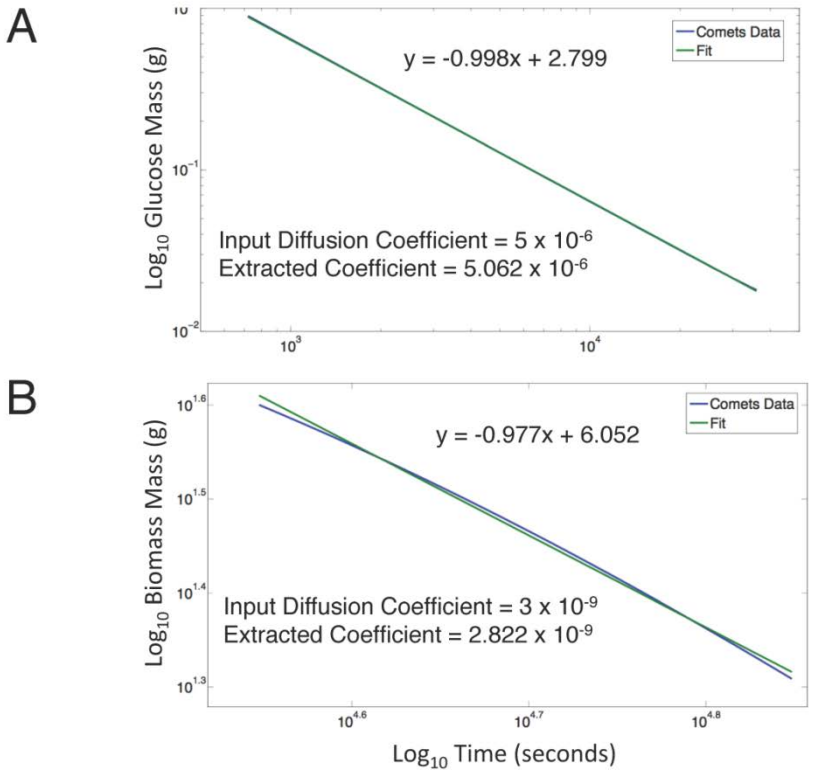

Figure S1 – Diffusion tests for glucose and biomass. The expected change in concentration over time is governed by

$$\Phi(r,t) = \frac{1}{4\pi Dt} e^{-r^2/4Dt}$$

where D is the desired diffusion coefficient, r is the radius of area with diffusing compound, and t is time. On a log-log (base 10) plot a straight line is expected with a slope of -1. Furthermore, a simulated diffusion coefficient can be calculated from

$$D = \frac{10^{-b}}{4\pi}(\Delta x)^2$$

and compared against the diffusion coefficient that was provided. Figure S1A shows glucose diffusion over 50 time steps from 0.2 to 10 hrs. The extracted slope has 0.2% error while the extracted diffusion coefficient has 1.2% error. Figure S1B shows biomass flow diffusion over 50 times steps from 10 hrs to 20 hrs. Since the biomass flow has a diffusion coefficient three orders of magnitude smaller, the delta function approximation breaks down for the same mass at a shorter time scale. However, on the longer time scale, the slope was within 2.4% and the diffusion coefficient was correct to 5.9%. For all subfigures, the initial condition was one pixel of 100g on a 100x100 grid of 0.2 cm^2 and a flux balance time step of 0.2 hours with 10 diffusion steps per flux balance time step.

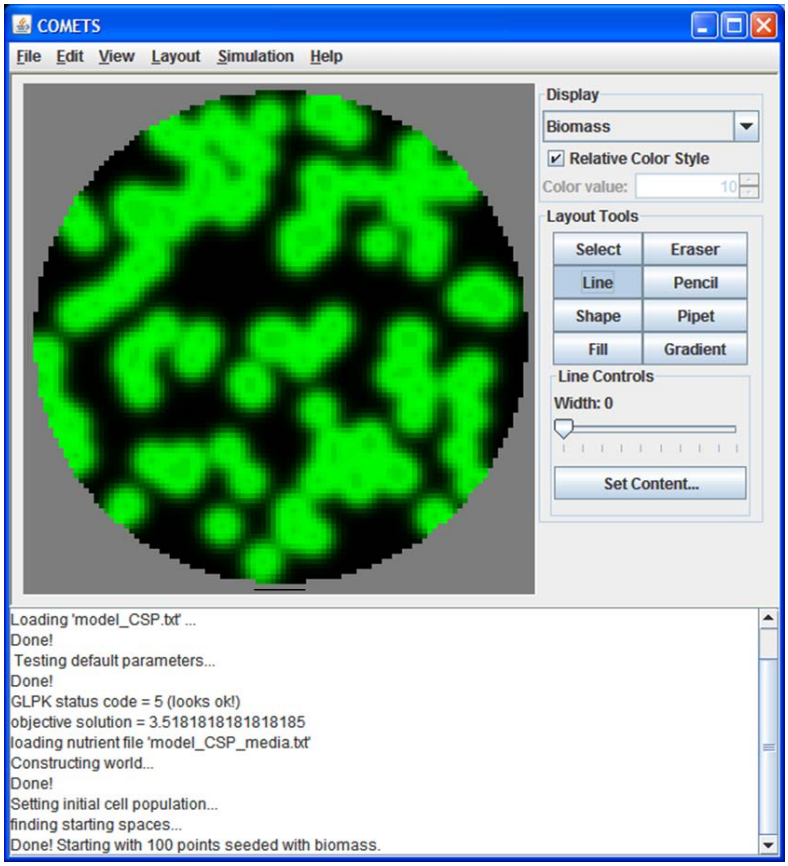

Figure S2 – A snapshot of the main graphic user interface of COMETS. In addition to visualizing the current spatial distribution of any species or metabolites, this interface makes it possible to upload, save or set initial conditions for *in silico* experiments. The initial conditions (abundance of organisms or metabolites) can be initialized at any individual discrete point in space, either using appropriate text files (see http://www.bu.edu/segrelab/comets/), or a phostoshop-like set of tools. Using these tools the user can literally "paint" the initial amounts of biomass for different species on the screen, as if this was a virtual Petri dish.

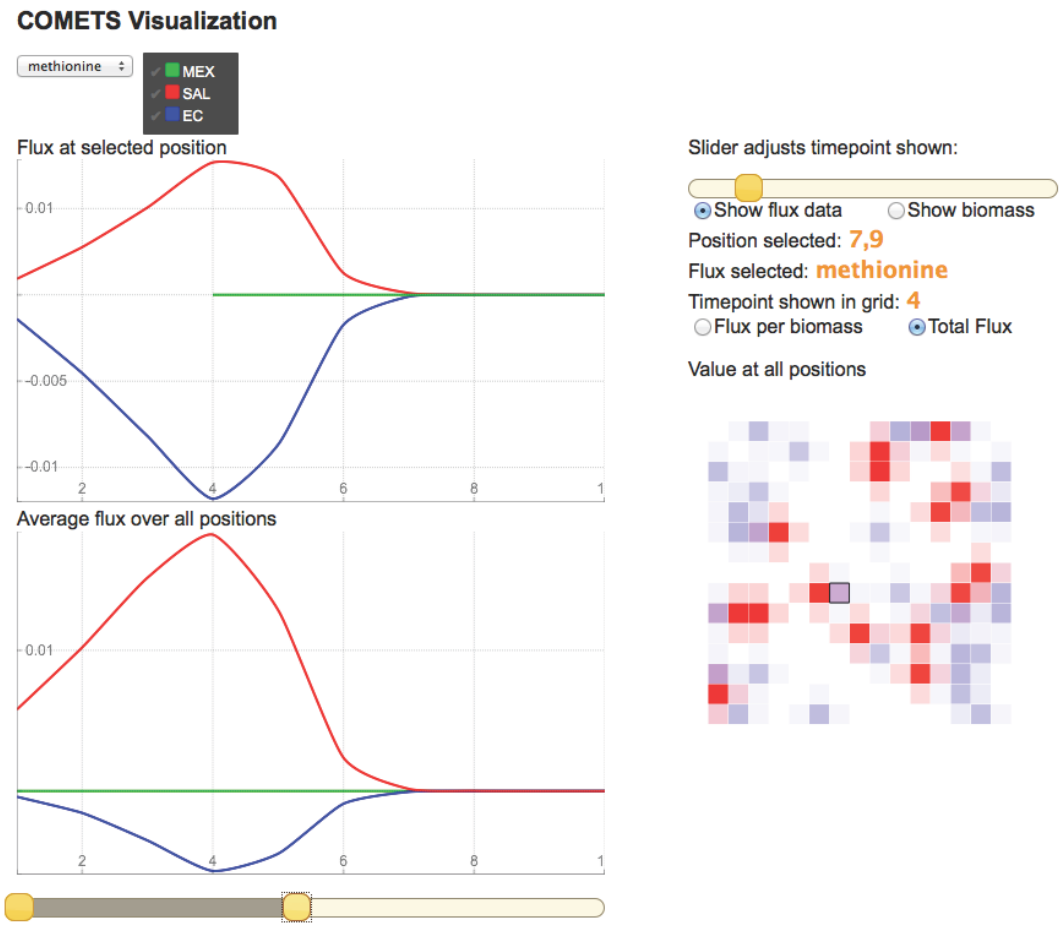

Figure S3 – The output data of a COMETS simulation can be very large, as it includes multiple variables (amount of biomass and complete flux vectors for different species, extracellular metabolite concentrations) for every discrete point in time and space. Thus, we have developed a set of scripts that allow users to explore this data in an efficient way, through an html-based visualization interface. The example shown here pertains to one of the simulations performed for the 3-species consortium. The corresponding interactive tool can be accessed at http://comets.bu.edu/viewer/.

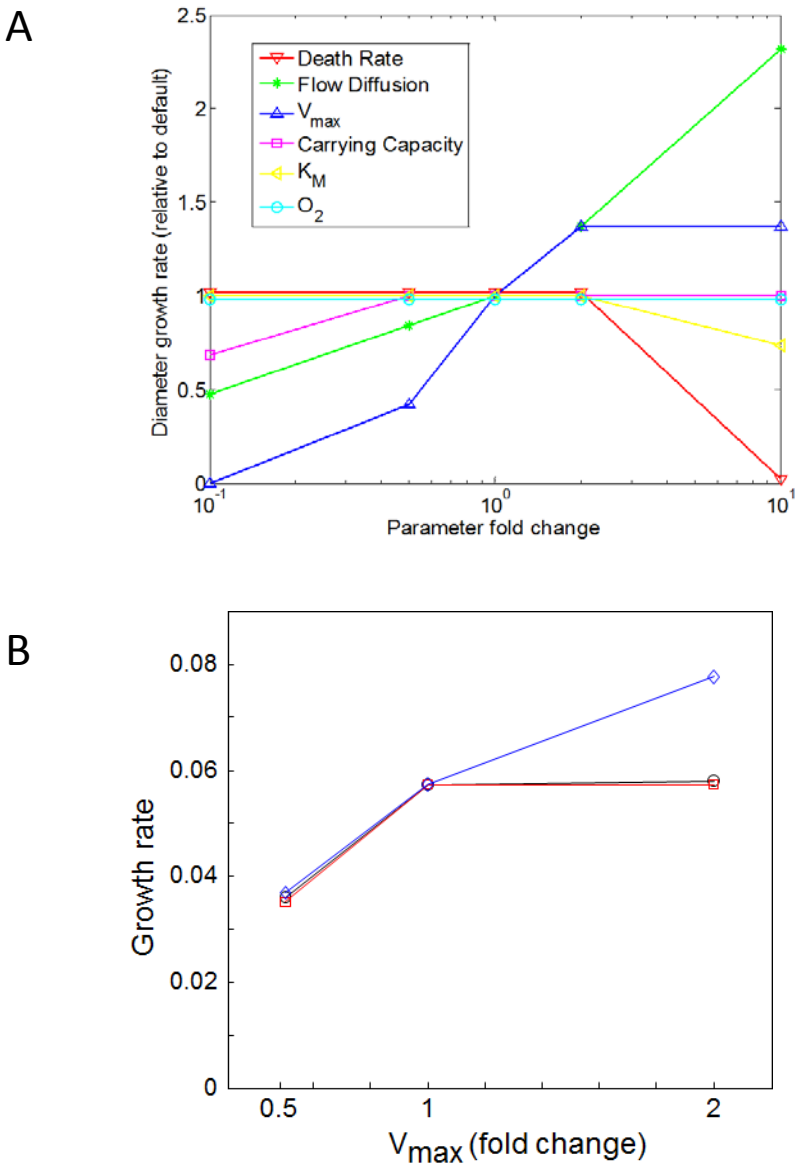

Figure S4 – Parameter sensitivity for the rate of *E. coli* colony expansion on glucose. **A** Lines connect the predicted rate of colony expansion as each parameter was individually multiplied by the listed perturbation factor. Unperturbed parameters were maintained at the values listed in Table 1. Changes of 2-fold had little impact on results across parameters. A 10-fold increase in diffusion led to less than a 2.5-fold change in rate of colony expansion. Colonies did not expand if V_{max} was dropped to 1 (while K_m remained at 0.01), or if death rate was increased to 10%. **B** Sensitivity of the initial growth rate of the total biomass as a function of the exchange parameter V_{max} for glucose (black), nitrogen (red) and oxygen (blue) uptake. The growth rates were calculated as the slope of the semilog plot of the total biomass vs. time. The figure indicates that the values of V_{max} for glucose and nitrogen are at their maximums beyond which the model is insensitive to their change. This is not the case for oxygen.

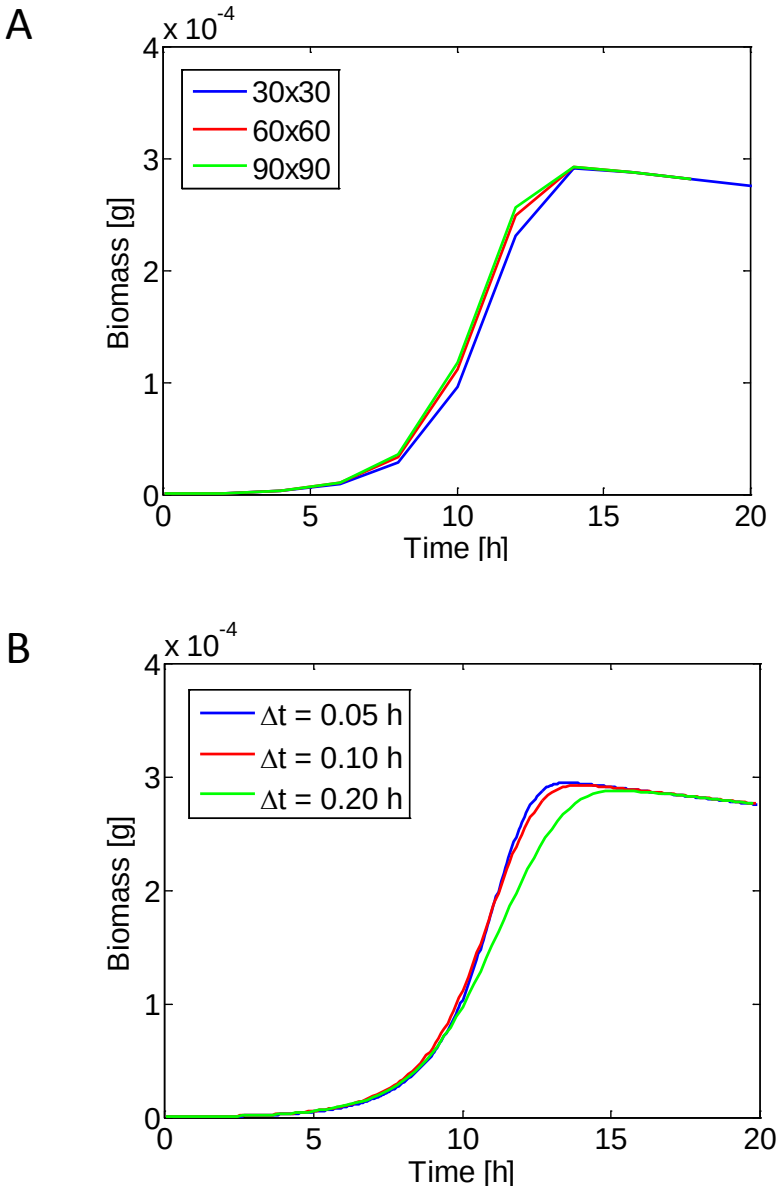

Figure S5 – Sensitivity of COMETS to spatial and time scales. **A** The total biomass growth curves display minor changes for increasing size of the finite spatial grid, and scale monotonically. The linear size of the simulation square was 0.6 cm in all three cases shown here. The growth curves correspond to simulations with the spatial area divided into grid of 30 by 30(blue), 60 by 60(red) and 90 by 90(green) squares, each with uniform biomass. The figure indicates that the difference between the curves becomes smaller with increasing grid size and would converge for an infinitely fine grid. **B** The total biomass growth curves display minor changes for decreasing finite simulation time step, and scale monotonically. The total simulation time was 20 hours. The growth curves correspond to simulations with discrete unit time step of 0.05 hours (blue), 0.1 hours (red) and 0.2 hours (green). The figure indicates that the difference between the growth curves becomes smaller with decreasing unit time step and would converge for an infinitesimally small time step.

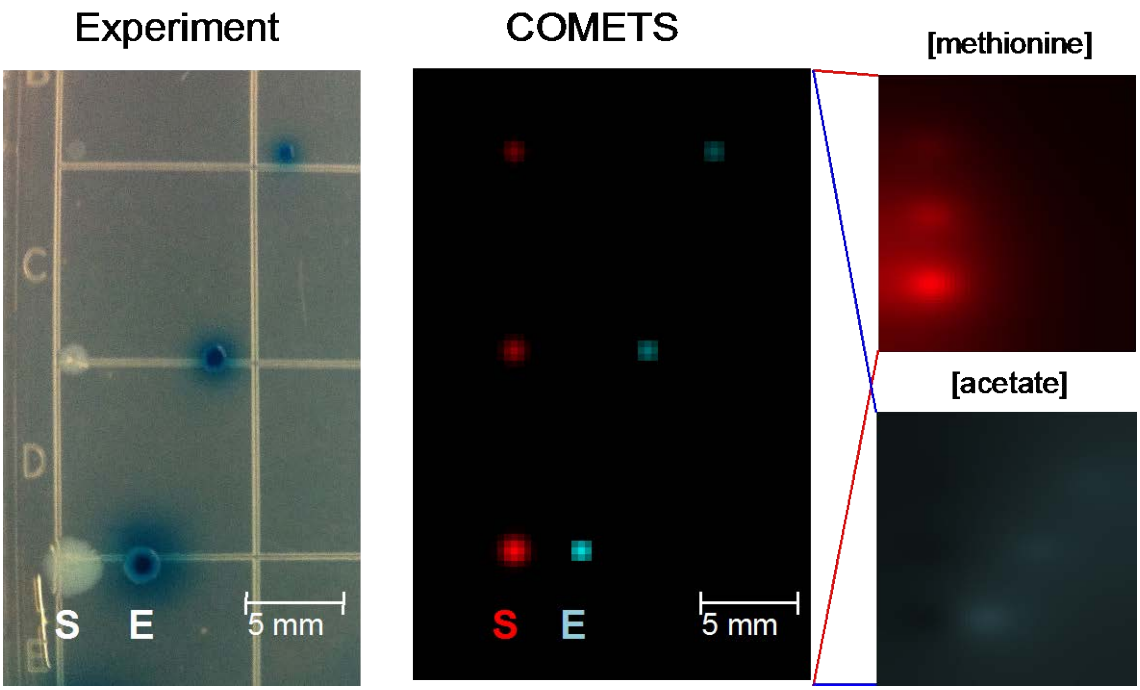

Figure S6 - Comparison of predictions and experimental results for the growth impact of increasing distance between mutualistic *S. enterica* (white colonies observed, red colonies predicted), and *E. coli* (blue colonies observed, and predicted – the blue on observed plates is the result of X-gal). Predicted metabolite gradients are displayed as insets for methionine (red) and acetate (blue).

Table S1 – Metabolite concentrations used to simulate minimal medium in COMETS. Lactose (lcts) was replaced with alternative carbon sources for individual *E. coli* colony simulations (glc = 3.5e-6, lac-L = 7.1e-6, or ac = 1.1e-5). Oxygen was lowered to maintain the same concentration with smaller pixel size (i.e. if box length (L)= 0.2 mm then o2 = 1e-5). For the 3-species consortium methylamine was added (mea = 2.0e-6).

Compound	mM per box
ca2[e]	1000.0
cl[e]	1000.0
cobalt2[e]	1000.0
cu2[e]	1000.0
fe2[e]	1000.0
fe3[e]	1000.0
k[e]	1000.0
lcts[e]	1.2e-5
mg2[e]	1000.0
mn2[e]	1000.0
mobd[e]	1000.0
nh4[e]	1000.0
ni2[e]	1000.0
o2[e]	6.2e-5
pi[e]	1000.0
so4[e]	1000.0
zn2[e]	1000.0

Table S2 - Metal Mix used in Hypho minimal media for lab experiments

Metal	1000x concentration (mM)
$ZnSO_4$	0.60
$CaCl_2$	9.98
$MnCl_2$	0.51
$(NH_4)_6Mo_7O_{24}$	1.00
$CuSO_4$	0.50
$CoCl_2$	1.00
Na_2WO_4	0.17
$FeSO_4$	8.88

Supplemental Experimental Procedures

Time scales

COMETS exploits a natural separation of time scales between growth and the diffusion of small molecules to efficiently model the complex dynamics in a computationally tractable manner that is physically consistent. The typical time scale associated with growth, t_{growth}, is set by cell doubling times and is of order 10^3-10^4 seconds. Importantly, the time step for simulating the diffusion of small molecules, t_D ~10 seconds, is chosen to be two to three orders of magnitude smaller than the doubling time. The diffusion time scale, $t_{D,}$ can be thought of as the time it takes a small molecule to diffuse one lattice spacing. For this reason, fixing t_D associates a length scale l with each point in our lattice. This length scale is related to the diffusion constant, D, of small metabolites and is given by $l^2 \sim Dt_D$. For small metabolites, $D \sim 10^{-5}$ cm^2/s, implying that we have a spatial resolution of about 10^{-2} cm per grid point.

COMETS performance

A COMETS simulation of a 100 by 100 grid, partially filled with biomass from a single species requires solving of the order of 1000 FBA problems per time cycle. Considering a time step of 0.1 h, COMETS performs approximately a 10^4 FBA cycles for 1h of real time simulation. On 32 CPUs (using a multi-thread version of COMETS), this will take approximately a minute.