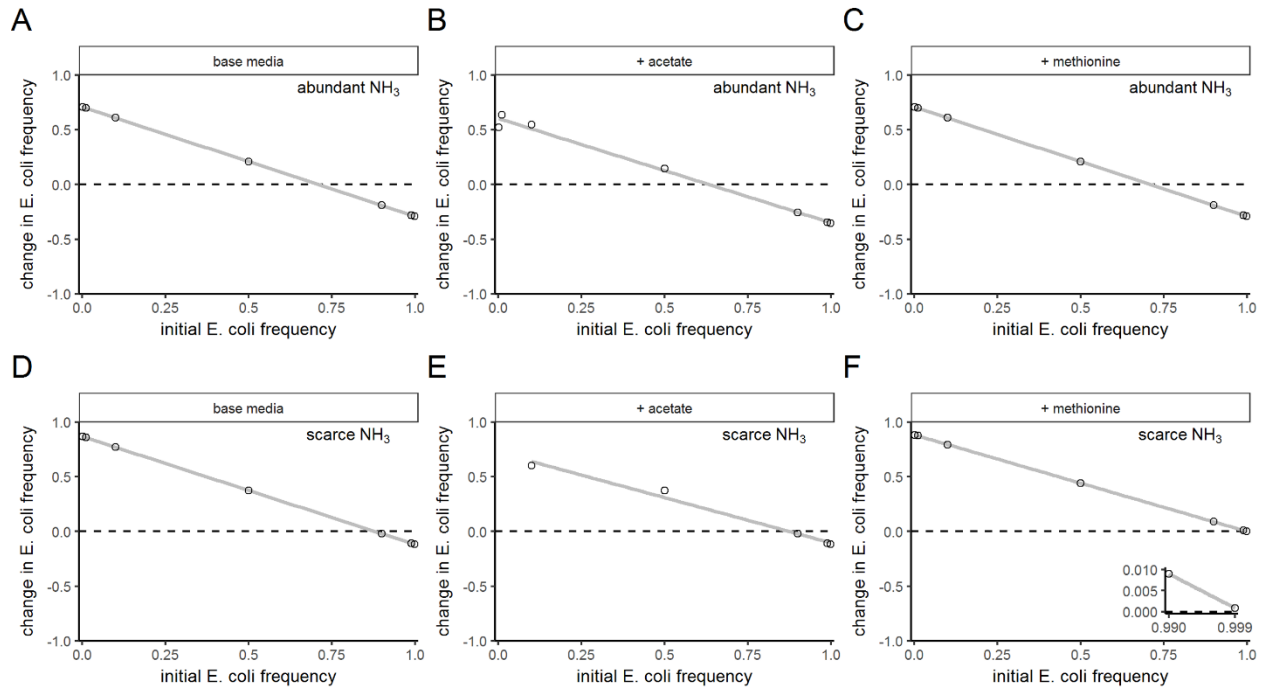


## Supplementary Information

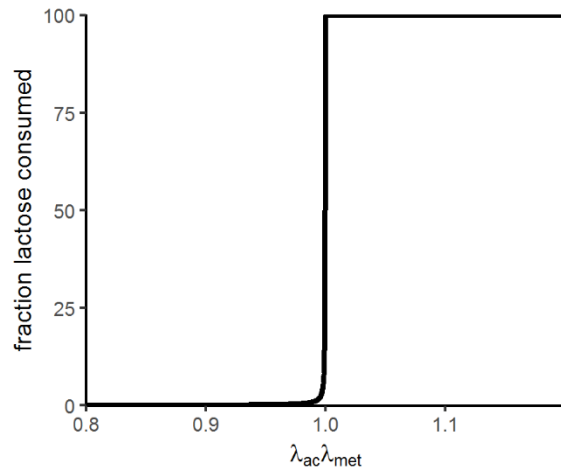
**Supplementary Table S1: Explanation of parameters in scaled resource-explicit model.** \*Analyses which altered these default values are stated in the results or in figure legends.

Parameter or state variable	Units	Default or initial value*	Name	Biological Interpretation
$\lambda_x$	Cells / cell	1.5	production	The value indicates how many cells can be supported by growth of one focal cell. E.g. $\lambda_{met} = 5$ indicates that every time S grows one cell, 5 E cells' worth of methionine are excreted by S into the environment
$k_x$	Cell equivalents	0.001	Monod half-saturation constant	The value of $k_x$ determines the resource concentration where growth rate is half-maximum.
$\mu_{Smax}$	(none)	0.5	Relative growth rate	A scaling term that describes proportionally how large S's growth rate is relative to E's
E	Cells	198 at start	<i>E. coli</i> population size	Number of E cells
S	Cells	198 at start	<i>S. enterica</i> population size	Number of S cells
lcts	Cell equivalents	10,000	lactose	This number equals how many E cells could be grown assuming lcts is limiting.
met	Cell equivalents	(variable)	methionine	This number equals how many E cells could be grown assuming met is limiting.
ac	Cell equivalents	(variable)	acetate	This number equals how many S cells could be grown assuming ac is limiting.
NH <sub>3</sub>	Cell equivalents	(variable)	ammonia	This number equals how many E cells or S cells could be grown assuming NH <sub>3</sub> is limiting.

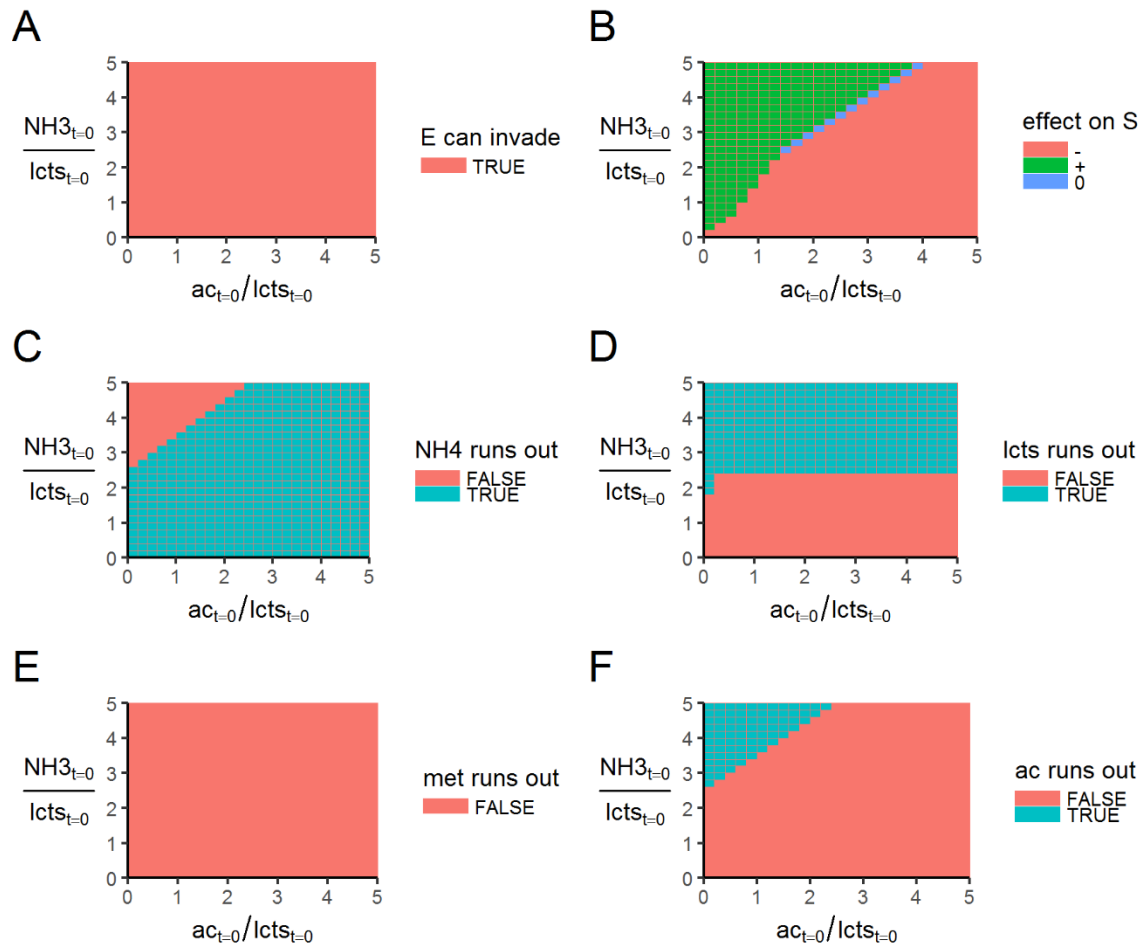
## Supplemental Figures



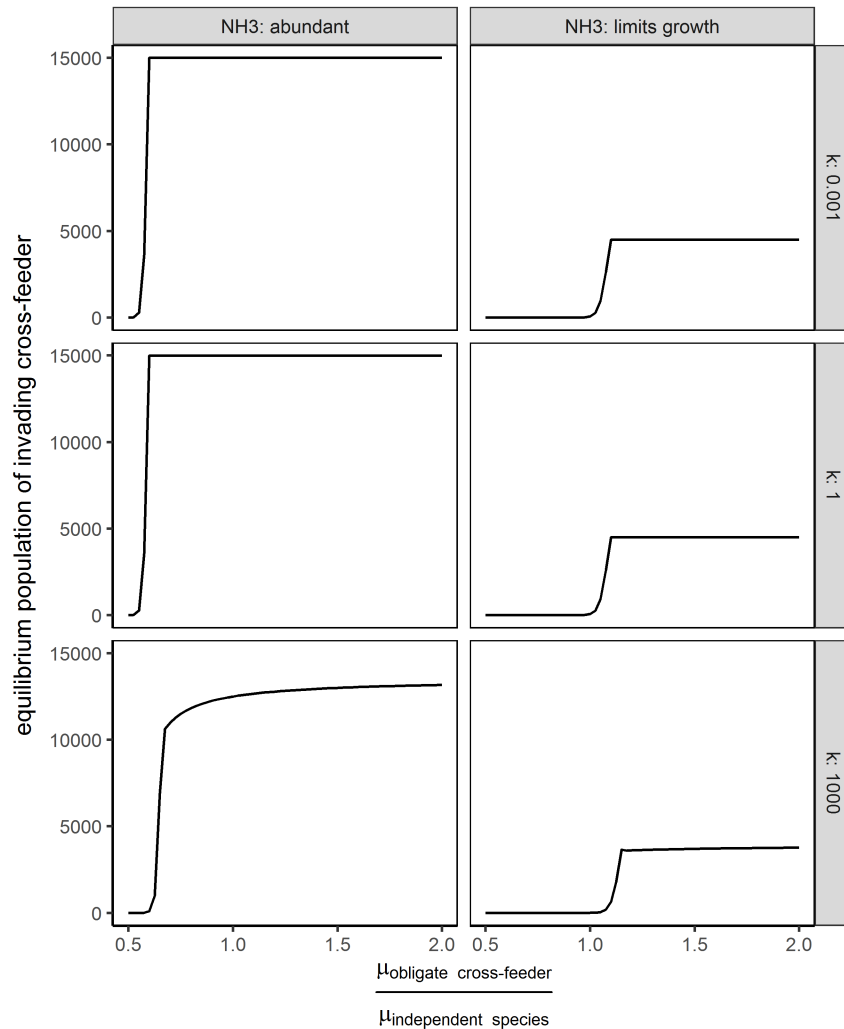
**Figure S1. The scaled ecological model can recapitulate the coexistence patterns observed in experiments and genome-scale metabolic models.** Invasion-from-rare simulations were conducted using the scaled model with parameters similar to our experimental system, in which E is the less generous partner ( $\lambda_{ac} = 0.4$ ) compared to S ( $\lambda_{met} = 7.3$ ) and S grows at approximately half the maximum growth rate of E ( $\mu_{Smax} = 0.5$ ). All panels show the change in frequency of E over the initial E frequency.  $lcts_{t=0} = 10000$  for all simulations.  $NH_{3t=0} = 15520$  (non-limiting) in (A-C) but is limiting ( $NH_{3t=0} = 705$ ) in (D-F). In (A,D),  $met_{t=0} = ac_{t=0} = 1$ , to “jumpstart” the mutualism. In (B,E)  $met_{t=0} = 1$  and  $ac_{t=0} = 4110$  (making S independent). In (C,F)  $met_{t=0} = 10000$  and  $ac_{t=0} = 1$  (making E independent). The inset in (F) shows that E increases in frequency even when abundant, meaning S is not able to coexist with E.



**Figure S2. The product of the cross-fed nutrient production parameters must be greater than one to sustain mutualism.** Results of simulations with excess  $\text{NH}_3$  and 10 units of acetate initially present to “jumpstart” the mutualism. The x-axis shows the product of the two production rate parameters. The y-axis shows the percent of the lactose that was consumed. When this y value is  $< 100\%$ , production of cross-fed nutrients is too low to sustain growth, and met and acetate run out. When this value is  $> 100\%$ , production of cross-fed nutrients sustains growth and lactose limits the system. In these simulations,  $\mu_{E,max} = 2 \mu_{S,max}$ .

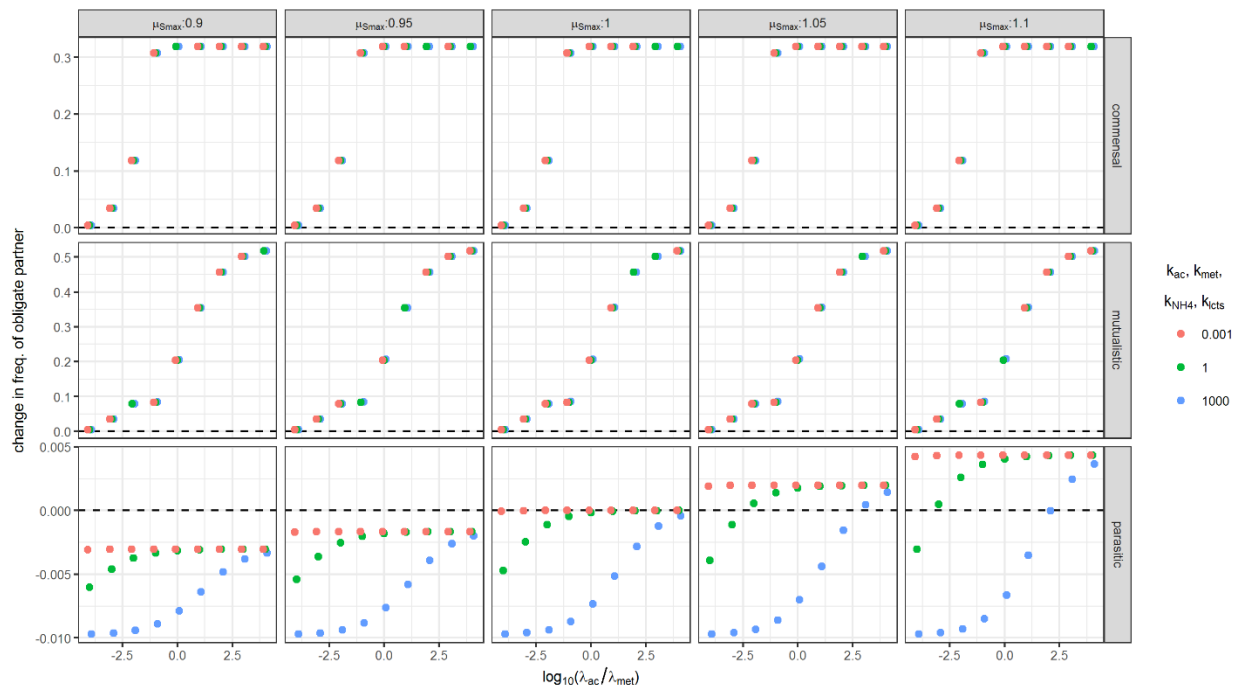


**Figure S3. Simulated effect of initial acetate and  $NH_3$  concentrations on the ability of a faster-growing obligate cross-feeding species to coexist with its nutrient supplier.** Results of simulations across many initial values of  $NH_3$  and  $ac$ . In all simulations,  $S_{t=0} = 198$ ,  $E_{t=0} = 2$ ,  $lcts_{t=0} = 10000$ ,  $\mu_{Smax} = 0.5$ , and  $\lambda_{ac} = \lambda_{met} = 1.5$ . **(A)** Whether E can increase in frequency and invade from rare. **(B)** The net effect of E's presence on S, calculated by comparing S's yield in the presence / absence of E. The net effect of S on E due to E's dependence on met, which is absent in the environment. **(C-F)** show whether different nutrients run out in the simulations (**(C)** =  $NH_3$ , **(D)** =  $lcts$ , **(E)** = met, **(F)** =  $ac$ ).

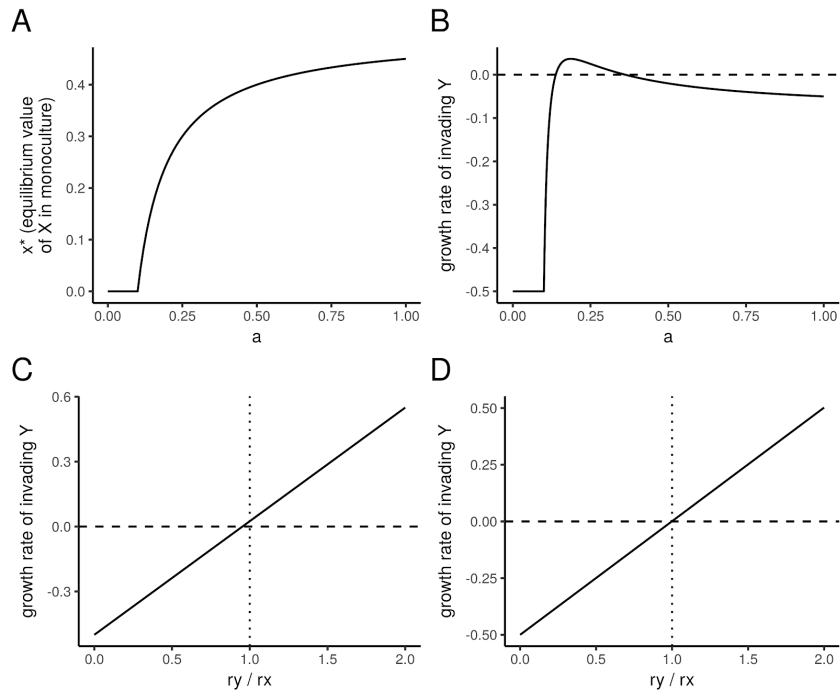


**Figure S4: Growth rate and a shared limiting nutrient determine whether an obligate species can coexist in continuous culture.** To extend the generality of our results, we ran simulations similar to the scaled ecological model main text simulations, but using a chemostat model instead of a batch culture model. The equations were the same as in the scaled model, except that chemostat terms were added to cause continuous dilution of all state variables as well as continuous resource replenishment from a reservoir. Using lactose as an example, the resource equations gained two terms, which change the equation from  $dl_{cts} / dt = -\mu_E l_{cts}$  to  $dl_{cts} / dt = -\mu_E l_{cts} - D * l_{cts} + D * l_{cts_{reservoir}}$ .  $D$  is the dilution constant (/hr), and  $l_{cts_{reservoir}}$  is the concentration of lactose in the reservoir (in cell equivalents). Species equations were similarly modified, except the reservoir concentrations = 0.  $D = 0.5$  / hr for all simulations. Our simulations had two starting conditions: abundant  $NH_3$  (left) and a low ammonia concentration that resulted in  $NH_3$  limiting the growth of the ecosystem (right). The initial values for the abundant  $NH_3$  case were:  $l_{cts} = 10000$  cell equivalents,  $met = 12500$  cell equivalents,  $ac = 0$  cell equivalents,  $NH_3 = 50000$  cell equivalents. When  $NH_3$  was limiting,  $NH_3 = 7500$  cell equivalents. The reservoir concentrations equaled these initial values. As in the main text,  $\lambda_{met} = \lambda_{ac} = 1.5$ . Initial

population sizes were independent (E) = 198 cells and obligate (S) = 2 cells. The half-saturation constants  $k$ , which applied to all resources / species for a given simulation, are shown in the figure. We then tested many values of  $\mu_{\text{obligate}}$ , and measured the equilibrium concentrations of the obligate, which are the plotted values. Similar to the main text, when  $\text{NH}_3$  was abundant, the obligate could grow slower than the independent and still coexist. However, when  $\text{NH}_3$  limited system productivity, the growth rate of the obligate must be higher than the independent's growth rate to persist. Higher  $k$  values made it more difficult to survive the dilution pressure in general, but didn't qualitatively alter the influence of growth rate and  $\text{NH}_3$  limitation.



**Figure S5: The interaction between production of cross-fed nutrients and the half-saturation constant on coexistence of an obligate cross-feeder.** This figure is related to Figure 5B, with additional data showing how  $k$  can affect coexistence. The  $k$  value was used for all resources for all species in a given simulation. The data here where  $k = 0.001$  and the regime is parasitic (bottom row) are the same data as in Fig. 5B. The rows show different initial values which correspond to the starred locations in Figure 4A. The x-axis is the relative “generosity,” or the ratio of the production terms. The panels (left-to-right) are in order of increasing relative growth rate of the obligate species, and the colors are the half-saturation parameter. The y-axis shows the change in frequency of the invading obligate cross-feeder, from an initial frequency of 0.01. In other words, a positive change indicates successful invasion and therefore coexistence, whereas a negative change indicates extinction. In the commensal (top) and mutualistic (middle) regimes,  $k$  does not influence the change in the obligate species’ frequency. Neither does the generosity affect coexistence, although it does affect the final frequency. In contrast, in the parasitic regime,  $k$  and generosity interact. As described in the main text, when  $k$  is very small, generosity does not matter because the obligate species is usually growing at its maximum growth rate. In contrast, when  $k$  is larger, the obligate species will grow more slowly when  $ac$  concentrations are low, making it more difficult to invade. This results in situations like the blue dots in the bottom-right panel, where despite having a higher maximum growth rate, the obligate species cannot invade when the independent species has low generosity and the half-saturation constant is non-negligible.



**Figure S6: Support for the FFG hypothesis using the phenomenological model of Hoek et al. (2016).** Here, we modify a recently published phenomenological model which examined the effect of simultaneously feeding both species in an obligate mutualism. We change the model slightly to feed only one at a time, then determine the influence of growth rate on whether the obligate can survive. We chose this model specifically because it has two simultaneous interactions occurring: a direct mutualistic effect that each species can have on the other's growth rate, and a negative effect the species have due to a shared carrying capacity, which we interpret, following Hoek et al. (2016), as competition for shared nutrients, like  $\text{NH}_3$ . The model is:

$$\begin{aligned} dX &= r_x * X * ((Y + a) / (Y + a + k)) * (1 - X - Y) - d * X \\ dY &= r_y * Y * (X / (X + k)) * (1 - X - Y) - d * Y \end{aligned}$$

X and Y are the two species,  $r_x$  and  $r_y$  are their maximum growth rates, and  $k$  is the half-saturation constant which here influences how the presence of the other species affects the mutualist's growth.  $a$  is the nutrient abiotically, and continuously, supplied to X.  $d$  is the dilution rate. Like Hoek et al. (2016), we set  $d = 0.5$  and  $r_x = 1.0$ . We set  $k = 0.15$ . The maximum population size is 1.

For these figures, we analytically determined the ability of Y to invade a steady-state community of X. This involved finding the steady-state concentration of X by setting  $dX = 0$ , which yields:

$$X^* = [((r_x * a) / (a + k)) - d] / [(r_x * a) / (a + k)]$$



We then plug this  $X^*$  into  $dY/Y$  to calculate the per-capita growth rate of  $Y$ . We also assume  $Y = 0$  for the competition term, since it is invading and therefore has a negligible population. If  $dY > 0$ ,  $Y$  can invade the system and coexist. Consistent with Hoek et al. (2016), this model predicts that  $X$  requires a minimum resource supply  $a$  to survive at all (panel A). If  $Y$  has the same growth rate as  $X$ , then  $Y$  can invade when  $X$  has a moderate resource supply (panel B). For example, it can invade when  $a = 0.25$ , because the growth rate is  $> 0$ . We therefore set  $a = 0.25$ , and re-assess  $dY / Y$  for multiple values of  $r_y$ , to learn how the relative growth rates influence coexistence. We see that when the independent grows faster than the obligate (when  $r_y / y_x < 1$ ), the obligate cannot invade, because its growth rate is  $< 0$  (panel C). If we set  $k = 0.001$ , to make it negligible as in most of the resource-explicit simulations in the main text, then the growth rate ratio where coexistence can occur occurs almost exactly where the growth rates are equal (panel D).

## Supplemental Experimental Procedures

### *Scaling of the General Ecological Model*

#### 1. The original model

First, the growth rates functions, which specify biomass growth of each species as a function of that species' population size and the concentrations of their required nutrients.

$$\mu_E = \mu_E(E, \mu_{E\max}, lcts, met, NH_3) = (E \mu_{E\max}) \min [(lcts / (lcts + k_{lcts})), (met / (met + k_{met})), (NH_3 / (NH_3 + k_{NH_3}))]$$

$$\mu_S = \mu_S(S, \mu_{S\max}, ac, NH_3) = (S \mu_{S\max}) \min [(ac / (ac + k_{ac})), (NH_3 / (NH_3 + k_{NH_3}))]$$

In the above equations, the  $k_x$  parameters signify the concentration of  $x$  at which growth is half-maximum. The  $\mu_{x\max}$  parameters signify the maximum possible growth rate (/hr) species  $x$  can attain.

The above growth rate functions drive each species' growth:

$$dE / dt = \mu_E$$

$$dS / dt = \mu_S$$

Resources are either consumed during growth or produced during growth:

$$dlcts / dt = -\gamma_{lcts}\mu_E$$

$$dNH_3 / dt = -\gamma_{NH_3}\mu_E - \gamma_{NH_3}\mu_S$$

$$dmet / dt = -\gamma_{met}\mu_E + \lambda_{met}\mu_S$$

$$dac / dt = \lambda_{ac}\mu_E - \gamma_{ac}\mu_S$$

In the resource equations, the  $\gamma_x$  parameters signify the number of units of resource  $x$  which are *consumed* during growth of one unit of the relevant species, and the  $\lambda_x$  parameters signify the number of units of resource  $x$  which are *produced* during growth of one unit of the relevant species.

The species  $E$  and  $S$  are in units of cells, the resources are in units of grams, and therefore the  $\gamma$ s and  $\lambda$ s are in units of grams per cell.

#### 2. The scaling

First, we scale the resources from units of grams / cell, to equivalent cell units, by dividing the resources by the associated consumption parameter  $\gamma$ :

$$ac^* = ac / \gamma_{ac}, \quad met^* = met / \gamma_{met}, \quad lcts^* = lcts / \gamma_{lcts}, \quad NH_3^* = NH_3 / \gamma_{NH_3}$$

Next, we scale the production parameters ( $\lambda$ s) by the consumption parameters ( $\gamma$ s), which allows us to consider production in terms of the number of cells of one species which can be supported by the growth of one cell of the other species:

$$\lambda_{ac}^* = \lambda_{ac} / \gamma_{ac}, \quad \lambda_{met}^* = \lambda_{met} / \gamma_{met}$$

These scaled production parameters are in units of cells / cell, i.e. are unitless. For intuition, if  $\lambda_{ac}^* = 2$ , then for each cell of E grown, enough acetate is produced to support growth of 2 S cells.

Next, the half-saturation parameters ( $k$ ) must be scaled to keep the units consistent. This is also done by scaling by the consumption parameters ( $\gamma$ s), such that the scaled half-saturation parameters describe how many cells' worth of resources are present when growth is at half-maximum:

$$k_{lcts}^* = k_{lcts} / \gamma_{lcts}, \quad k_{ac}^* = k_{ac} / \gamma_{ac}, \quad k_{met}^* = k_{met} / \gamma_{met}, \quad k_{NH_3}^* = k_{NH_3} / \gamma_{NH_3}$$

Using the chain rule and substitutions of the above scaled parameters, we can remove the consumption terms from our resource equations, for example resulting in the ammonia equation now specified as:

$$dNH_3^* / dt = -\mu_E - \mu_S$$

The growth rate functions ( $\mu_x$ ) were similarly adjusted, to take in scaled resource variables and half-saturation parameters.

The final scaling we do is to scale time by E's maximum growth rate. We do this because we are interested in the effect of relative growth rates between E and S, not their absolute growth rates, which only change the time scale but not the species ratios. We scale time by doing:

$$t^* = t \mu_{E_{max}}$$

And then scale S's maximum growth rate by E's:

$$\mu_{S_{max}}^* = \mu_{S_{max}} / \mu_{E_{max}}$$

With these scalings we can remove E's maximum growth rate and are only left with the relative growth rate  $\mu_{S_{max}}^*$  in S's growth equation. For clarity, in the main text, we omit the asterisks from variable names.

