Growth-independent cross-feeding modifies boundaries for coexistence in a bacterial mutualism

Running title: Growth-independent metabolism in a mutualism

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Significance statement.

Microbial mutualisms, such as those enforced by cross-feeding of metabolites, are widespread yet invariably face periods of nutrient limitation. Here we use experimental and theoretical approaches involving a synthetic community of two bacterial species to identify growth-independent maintenance metabolism as an important mechanism by which mutualistic cross-feeding can establish and persist in the face of nutrient limitation.
Summary

Nutrient cross-feeding can stabilize microbial mutualisms, including those important for carbon cycling in nutrient-limited anaerobic environments. It remains poorly understood how nutrient limitation within natural environments impacts mutualist growth, cross-feeding levels, and ultimately mutualism dynamics. We examined the effects of nutrient limitation within a mutualism using theoretical and experimental approaches with a synthetic anaerobic coculture pairing fermentative *Escherichia coli* and phototrophic *Rhodopseudomonas palustris*. In this coculture, *E. coli* and *R. palustris* resemble an anaerobic food web by cross-feeding essential carbon (organic acids) and nitrogen (ammonium), respectively. Organic acid cross-feeding stemming from *E. coli* fermentation can continue in a growth-independent manner during nutrient limitation, while ammonium cross-feeding by *R. palustris* is growth-dependent. When ammonium cross-feeding was limited, coculture trends changed yet coexistence persisted under both homogenous and heterogenous conditions. Theoretical modeling indicated that growth-independent fermentation was crucial to sustain cooperative growth under conditions of low nutrient exchange. In contrast to stabilization at low cell density, growth-independent fermentation inhibited mutualistic growth when the *E. coli* cell density was adequately high relative to that of *R. palustris*. Thus, growth-independent fermentation can conditionally stabilize or destabilize a mutualism, indicating the potential importance of growth-independent metabolism for nutrient-limited mutualistic communities.
Introduction

Mutualistic cross-feeding interactions between microbes crucially impact diverse processes ranging from human health (Flint et al., 2007; Ramsey and Whiteley, 2009; Hammer et al., 2014) to biogeochemical cycles (McInerney et al., 2010; Morris et al., 2013; Durham et al., 2015). Within most environments, microbial communities experience prolonged periods of nutrient limitation (Lever et al., 2015). In general, bacteria tolerate nutrient limitation by modulating their growth and metabolism (Lee et al., 1976; Wanner and Egli, 1990; Russell and Cook, 1995; Ferenci, 2001; Rittershaus et al., 2013). Sub-optimally growing and even non-growing cells continue to generate maintenance energy, that is energy required for any process that is not directly used for synthesizing and polymerizing biosynthetic precursors, and thereby survive by retaining partial metabolic activity (Wanner and Egli, 1990; Russell and Cook, 1995; Hoehler and Jørgensen, 2013; Rittershaus et al., 2013). We hereon refer to this growth-independent metabolic activity for cell maintenance as maintenance metabolism. While maintenance metabolism occurs during growth, it can make up the majority of metabolic activity in slow-growing and especially non-growing cells. As a consequence of maintenance metabolism being independent of growth, the overall metabolic activity of a cell does not decline proportionately to growth rate during nutrient limitation.

For fermentative microbes, the transformation of energy into ATP and a proton motive force is intimately associated with the excretion of fermentation products. During nutrient limitation, the maintenance metabolism of fermentative microbes is associated with product excretion, albeit at a lower rate than during growth. For example, in the absence of electron acceptors, and starved for essential elements (i.e., nitrogen or sulfur), *Escherichia coli* generates energy by fermenting glucose in a growth-independent manner (Wanner and Egli, 1990; LaSarre
et al., 2017). Fermentative microbes serve pivotal roles within natural anaerobic food webs, wherein their excreted products serve as nutrients for other microbes. It is possible that fermentation products associated with maintenance metabolism could serve to cross-feed other microbes and thereby influence the initiation and/or endurance of microbial mutualisms under growth-limiting conditions. Nonetheless, most microbial cross-feeding studies view nutrient release as being tightly coupled to growth. While mutualism flux balance models tend to include growth-independent maintenance parameters (Harcombe et al., 2014; Chubiz et al., 2015), most other mutualism models do not, and few studies have examined the impact of growth-independent cross-feeding on mutualism dynamics (Megee III et al., 1972; Shou et al., 2007; Stolyar et al., 2007). Studying mutualistic cross-feeding in natural environments can be challenging due to environmental and genetic stochasticity. Synthetic microbial communities, or cocultures, offer an alternative approach that mimics key aspects of natural communities while providing a greater degree of experimental control (Momeni et al., 2011; Ponomarova and Patil, 2015; Lindemann et al., 2016; Widder et al., 2016). We previously developed a bacterial coculture to facilitate the study of mutualistic cross-feeding in anaerobic environments (LaSarre et al., 2017) (Fig. 1). Our coculture resembles other fermenter-photoheterotroph cocultures, which have primarily been studied for converting plant-derived sugars into H₂ biofuel (Odom and Wall, 1983; Fang et al., 2006; Ding et al., 2009; Sun et al., 2010). However, unlike previous systems, our coculture enforces stable coexistence through bi-directional cross-feeding of essential nutrients. Specifically, *E. coli* ferments sugars to excreted organic acids, providing essential carbon and electrons for a genetically engineered *Rhodopseudomonas palustris* strain (Nx). *R. palustris* Nx has a NifA* mutation (McKinlay and Harwood, 2010) that results in NH₄⁺ excretion during N₂ fixation, providing essential nitrogen for *E. coli* (LaSarre et al., 2017). We
previously used our coculture to examine the effects of increased NH$_4^+$ cross-feeding on coculture dynamics (LaSarre et al., 2017). In that study, theoretical modeling suggested that coexistence would persist even at very low NH$_4^+$ excretion levels (LaSarre et al., 2017). This prediction prompted us to ask herein, how does this mutualism contend with limitation of cross-fed nutrients?

Using theoretical and experimental approaches, we show that fermentative maintenance metabolism is crucial for maintaining cooperative growth during limitation of cross-fed NH$_4^+$. Conversely, fermentative maintenance metabolism is detrimental to the mutualism under circumstances where the *E. coli* cell density far exceeds that of *R. palustris*, as the otherwise low maintenance metabolism rate is magnified and leads to an inhibitory acidification of the environment. Thus, growth-independent cross-feeding conditionally influences this mutualism in positive and negative manners and thereby sets both the lower and upper thresholds for cooperation.

**Results**

**Coexistence is maintained at reduced NH$_4^+$ cross-feeding levels.** Previously, we found that stable coexistence and reproducible trends in our mutualistic coculture were dependent on the transfer of NH$_4^+$ from *R. palustris* Nx to *E. coli* (LaSarre et al., 2017). Adding NH$_4^+$ to the medium broke the dependency of *E. coli* on *R. palustris* and resulted in *E. coli* domination due to its higher intrinsic growth rate relative to that of *R. palustris*. Thus, the NH$_4^+$ cross-feeding level controls the *E. coli* growth rate within the mutualism. We were intrigued that theoretical modeling predicted that mutualism coexistence would be maintained even at very low NH$_4^+$
To test this prediction, we sought to experimentally manipulate *R. palustris* NH$_4^+$ excretion. In our previous study, cocultures were grown under a 100% N$_2$ headspace with shaking to promote gas exchange and homogenous conditions (LaSarre *et al.*, 2017). Under these conditions, the NifA* mutation presumably results in a rate of N$_2$ fixation that exceeds the rate of biosynthesis, leading to excretion of excess NH$_4^+$. We therefore reasoned that limiting the N$_2$ supply in shaken cocultures could lower the rate of NH$_4^+$ production by nitrogenase, and consequently *R. palustris* Nx would retain a larger portion of the NH$_4^+$ for biosynthesis and excrete less. To limit the N$_2$ concentration, we injected N$_2$ into sealed coculture tubes with an argon-filled headspace to reach a final concentration of 18% N$_2$, a concentration we estimated would be close to limiting *R. palustris* growth in our coculture conditions, based on the elemental composition of *R. palustris* (McKinlay and Harwood, 2010). In agreement with our expectation, supernatants from shaking *R. palustris* monocultures with 18% N$_2$ contained half as much NH$_4^+$ compared to 100% N$_2$ monocultures (Fig. 2). We concluded that N$_2$ limitation was a suitable approach to manipulate NH$_4^+$ excretion levels.

To examine the degree of N$_2$ limitation that would support coexistence, we grew cocultures with a range of N$_2$ concentrations and monitored H$_2$ yields and growth rates. We used H$_2$ yield as a proxy for N$_2$ limitation because nitrogenase produces more H$_2$ and less NH$_4^+$ as N$_2$ becomes limiting (Hoffman *et al.*, 2013). When nitrogenase is saturated with N$_2$, one mole of H$_2$ is obligately produced for every mole of N$_2$ converted into 2 NH$_4^+$ (Eq 1). In the absence of N$_2$, nitrogenase continues to oxidize electron carriers and acts as an ATP-powered hydrogenase,
producing H$_2$ as the sole product (Eq 2). Under sub-saturating N$_2$ conditions, the stoichiometric amounts of NH$_4^+$ and H$_2$ produced would be somewhere between Eqs. 1 and 2.

\[
\text{Eq 1. } N_2 + 10H^+ + 8e^- + 16 \text{ ATP} \rightarrow 2NH_4^+ + H_2 + 16 \text{ ADP}
\]

\[
\text{Eq 2. } 8H^+ + 8e^- + 16 \text{ ATP} \rightarrow 4H_2 + 16 \text{ ADP}
\]

Thus, progressively decreased N$_2$ availability should result in less NH$_4^+$ excretion and more H$_2$ production. Unlike NH$_4^+$, H$_2$ accumulates in coculture, making it a convenient compound to assess whether *R. palustris* cells in coculture are experiencing sub-saturating N$_2$ conditions. N$_2$-limited cocultures were incubated horizontally with shaking to promote a homogeneous environment. As expected, the coculture H$_2$ yield increased as N$_2$ concentration decreased, but the yield plateaued at N$_2$ concentrations below 15% (Fig. 3A). Below 15% N$_2$, *R. palustris* might redirect electrons away from H$_2$ production towards other products like polyhydroxybutyrate, as has been observed as part of a nitrogen-starvation response in *R. palustris* (McKinlay et al., 2014). N$_2$ limitation was also evident from the coculture growth rate, which decreased as the N$_2$ concentration decreased (Fig. 3B; Supporting Information Fig. S2A). Notably, cocultures still grew at the lowest concentration of N$_2$ tested (6% headspace N$_2$; Fig. 3B), indicating that sufficient NH$_4^+$ was cross-fed to permit mutualistic growth.

Moving forward, we focused on 18% N$_2$ to characterize how lower NH$_4^+$ cross-feeding affected coculture dynamics. We chose 18% N$_2$ as a concentration where nitrogen-limitation impacted coculture trends but without causing the observed H$_2$ yield plateau (Fig. 3). Previous model simulations predicted that decreasing NH$_4^+$ cross-feeding would result in a decrease in the *E. coli* population within the coculture (Supporting Information Fig. S1A). In agreement with this, we observed that *E. coli* made up 5% of the population in the cocultures with 18% N$_2$, which was significantly lower than the 9% *E. coli* frequency observed in cocultures with 100%
N₂ (Fig. 4A) (LaSarre et al., 2017). To assess coculture reproducibility, we performed serial transfers of cocultures with 18% N₂. Growth yield, H₂ yield, and growth rates were all reproducible across serial transfers (Fig. 4), indicating that coexistence was stable despite the lower level of NH₄⁺ cross-feeding.

**Coexistence is maintained in heterogeneous environments that decrease NH₄⁺ cross-feeding.** Spatial structuring can impact microbial mutualistic interactions, and is even required for coexistence in some cases (Kim et al., 2008; Harcombe, 2010; Summers et al., 2010; Hom and Murray, 2014). In other cases, well-mixed environments sufficiently promote cooperative relationships (Hillesland and Stahl, 2010; Mee et al., 2014; Pande et al., 2014). We hypothesized that the homogeneous environment in our shaking cocultures might dampen the impact of low NH₄⁺ cross-feeding levels. Thus, we examined whether a heterogeneous environment would affect coexistence within N₂-limited cocultures.

One way to induce a heterogeneous environment is by incubating in static conditions, wherein cocultures are not agitated. Static incubation results in settling of cells to the bottom of the culture tube in both cocultures and *R. palustris* monocultures, which we expected would result in a gradient of N₂ availability (Supporting Information Fig. S3). As N₂ diffuses into the medium, *R. palustris* cells closer to surface would be first to encounter and utilize the N₂, which would diminish the N₂ available to *R. palustris* cells towards the bottom of the tube. The N₂-limited cells at the bottom of the tube would therefore produce less NH₄⁺ and more H₂, leading to less NH₄⁺ excretion by the *R. palustris* population overall (Supporting Information Fig. S3). Confirming this hypothesis, static *R. palustris* Nx monocultures with 100% N₂ grew to a similar density as when shaken, but showed less NH₄⁺ excretion, similar to what was observed in
monocultures shaken with 18% N\textsubscript{2} (Fig. 2). This trend was exacerbated in static \textit{R. palustris} Nx monocultures with only 18% N\textsubscript{2}, which also showed lower cell densities (Fig. 2), likely due to a greater redirection of electrons to H\textsubscript{2} rather than to biosynthesis, the major \textit{R. palustris} electron sink when nitrogen is abundant (McKinlay \textit{et al.}, 2014).

To determine how heterogeneous environments affected coculture trends under N\textsubscript{2} limitation, we performed serial transfers of cocultures grown under static conditions with either 100% or 18% N\textsubscript{2} in the headspace, every 2 or 4 weeks, respectively. These longer incubation times were necessary to achieve similar final cell densities between shaking and static environments. Static cocultures with 100% N\textsubscript{2} had higher H\textsubscript{2} yields than shaken cocultures with 100% N\textsubscript{2} (Fig. 5A). This was expected given that an \textit{R. palustris} subpopulation was experiencing N\textsubscript{2} limitation (Eq 1 vs 2). Supplying only 18% N\textsubscript{2} in static cocultures amplified this trend further (Fig. 5A). In agreement with prior simulations (Supporting Information Fig. S1A), \textit{R. palustris} growth yields remained similar or increased in response to N\textsubscript{2} limitation whereas \textit{E. coli} growth yields decreased (Fig. 5B). Coexistence was maintained over serial transfers regardless of N\textsubscript{2} availability (Fig. 5B). Collectively, these data demonstrate the robustness of our coculture to low NH\textsubscript{4}\textsuperscript{+} cross-feeding levels in both homogenous and heterogeneous environments.

**Fermentation products from maintenance metabolism are crucial for coexistence at low cross-feeding levels.** In coculture, \textit{R. palustris} growth yields depend on carbon acquisition from \textit{E. coli}. The relative consistency of \textit{R. palustris} final cell densities despite lower \textit{E. coli} cell densities during N\textsubscript{2} limitation (Fig. 5) led us to hypothesize that fermentation associated with \textit{E. coli} maintenance metabolism could be sustaining \textit{R. palustris} during N\textsubscript{2} limitation. By this hypothesis, NH\textsubscript{4}\textsuperscript{+}-limited \textit{E. coli} would grow at a slower rate but would continue to use
fermentation for maintenance energy; consequently, *R. palustris* would receive a slower but continuous supply of organic acids for growth and N\textsubscript{2} fixation. Ultimately, *E. coli* would assimilate less glucose, as a larger proportion would be used for maintenance, whereas *R. palustris* would receive a similar or even greater amount of carbon from *E. coli*. This hypothesis in turn implies that fermentative maintenance metabolism by *E. coli* is important for sustaining *R. palustris* metabolism and thereby coculture viability during N\textsubscript{2} limitation.

To empirically test whether fermentative maintenance metabolism can support *R. palustris* growth, we first examined the most extreme condition by completely preventing *E. coli* growth. To prevent *E. coli* growth we made an *E. coli* histidine auxotroph (ΔHisB). Our coculture medium does not contain amino acids, so *E. coli* ΔHisB cannot grow regardless of NH\textsubscript{4}\textsuperscript{+} cross-feeding. In cocultures with *E. coli* ΔHisB, glucose was slowly consumed, the *E. coli* ΔHisB cell density declined, and the *R. palustris* cell density increased (Fig. 6A). These results suggested that fermentative maintenance metabolism supported *R. palustris* growth. However, metabolism of cellular material released by lysed cells can be important for maintaining microbial populations through periods of starvation (Finkel, 2006; Rozen et al., 2009). Therefore, we also considered cell lysis of *E. coli* ΔHisB as a carbon source for *R. palustris*. We estimated how much carbon could be released from *E. coli* cell lysis based on the observed decline in CFUs and elemental compositions of each bacterium (see Methods). Our estimates suggest that all of the carbon from dead *E. coli* cells could only account for ~0.4% of the carbon required for the amount of *R. palustris* growth observed (Fig. 6B). In contrast, there was more than enough carbon from the glucose consumed in coculture to account for all the *R. palustris* biomass observed (Fig. 6B). We therefore concluded that fermentative maintenance metabolism can contribute to the cross-feeding of carbon within the mutualism.
Based on the above result we reasoned that fermentative maintenance metabolism was also likely contributing to coexistence in N₂-limited cocultures with wild-type *E. coli*. However, gauging the impact of fermentative maintenance metabolism during partial starvation conditions is experimentally infeasible as the essential nature of maintenance metabolism means it cannot be genetically eliminated. We therefore turned to modeling to assess the importance of fermentative maintenance metabolism during N₂ limitation that permits slow *E. coli* growth. We first modified our previous model (LaSarre *et al.*, 2017) to account for the effects of N₂ limitation on the shift from NH₄⁺ to H₂ production by *R. palustris* (SyFFoN_v2; Supporting Information Methods, Fig. S4-6, and Table S2). The model does not take into account ATP availability for the nitrogenase reactions (Eq.1 and 2); we assumed that ATP is readily available during N₂ limitation since *R. palustris* produces high concentrations of H₂ when completely starved for nitrogen but provided with light (McKinlay *et al.*, 2014). We then adjusted SyFFoN_v2 parameters to simulate growth rate and metabolite yield data observed at various N₂ concentrations (Fig. 3 and Supporting Information Fig. S2 and S4-6). In doing so we found that parameters based on *E. coli* monoculture data (LaSarre *et al.*, 2017) could not accurately simulate coculture growth rates observed at low N₂ concentrations. Rather, *E. coli* maintenance metabolism had to be increased by up to two-orders of magnitude to more accurately simulate empirical growth rates (Fig. 3B and Supporting Information Table S2). The need for these changes to more accurately simulate observed trends suggests that *R. palustris* consumption of fermentation products pulls *E. coli* fermentation by minimizing end-product inhibition, analogous to what has been observed in other fermentative cross-feeding systems (Iannotti *et al.*, 1973; Hillesland and Stahl, 2010). SyFFoN_v2 accurately predicted H₂ yields (Fig. 3A), normalized growth rates (Fig. 3B), and product yields (Supporting Information Fig. S2) between
15% and 100 % N\textsubscript{2}. At N\textsubscript{2} levels below 15%, \textit{R. palustris} likely undergoes a starvation response resulting in physiological effects that our model does not predict. We also verified that SyFFoN\_v2 could reproduce trends from our previous study (LaSarre et al., 2017), namely the effects of added NH\textsubscript{4}\textsuperscript{+} (Supporting Information Fig. S7) and varying the \textit{R. palustris} NH\textsubscript{4}\textsuperscript{+} excretion levels (Supporting Information Fig. S1A).

To examine how fermentative maintenance metabolism influenced this mutualism, we used SyFFoN\_v2 to simulate the effect of N\textsubscript{2} limitation on population dynamics in the presence or absence of \textit{E. coli} maintenance metabolism (Fig. 7). With \textit{E. coli} maintenance metabolism included, the model predicted that mutualistic growth would be sustained even as N\textsubscript{2} concentrations approached zero. \textit{E. coli} final cell densities were predicted to decline as N\textsubscript{2} levels fall below \(~30\%\) while \textit{R. palustris} final cell densities would decline as N\textsubscript{2} levels fall below \(~20\%\) (Fig. 7A). In the absence of \textit{E. coli} maintenance metabolism, simulations predicted a truncated range of N\textsubscript{2} concentrations that would support coculture growth (Fig. 7B). In fact, the simulations suggested that \textit{E. coli} maintenance metabolism is necessary at N\textsubscript{2} concentrations where we observed reproducible coculture growth trends (Fig. 7). The model predicted similar trends when NH\textsubscript{4}\textsuperscript{+} excretion levels were varied in place of N\textsubscript{2} availability (Supporting Information Fig. S1).

A closer inspection of simulated cross-feeding levels revealed why coculture growth at low N\textsubscript{2} concentrations (or low NH\textsubscript{4}\textsuperscript{+} cross-feeding levels) required fermentative maintenance metabolism. At high N\textsubscript{2} levels (100% N\textsubscript{2}), growth-coupled fermentation alone is sufficient to support coculture growth, as any increase in populations results in progressively more metabolites exchanged over time (Supporting Information Fig. S8A). However, near the transitional N\textsubscript{2} concentration where coculture growth is predicted to fail in the absence of
fermentative maintenance metabolism (28% N2), metabolite excretion levels decrease as populations grow, resulting in continuously less essential resources for subsequent generations despite available glucose; in other words, cross-feeding spirals into a cycle of diminishing returns (Supporting Information Fig. S8B). Our data indicate that fermentative maintenance metabolism can circumvent diminishing returns. Fermentation products will always be produced, and thus R. palustris will eventually grow to a density that collectively excretes sufficient NH4+ to allow for E. coli growth. Indeed, when E. coli maintenance metabolism is included at 28% N2, growth-independent cross-feeding by E. coli stimulates sufficient reciprocal NH4+ excretion to sustain coculture growth (Supporting Information Fig. S8C). These simulations strongly suggest that fermentative maintenance metabolism permits cooperative growth at low NH4+ excretion levels that would otherwise be insufficient.

**E. coli maintenance metabolism prevents cooperative growth at high E. coli cell densities.**

On a per cell basis, fermentation product excretion due to maintenance metabolism is considerably slower than that associated with growth (Russell and Cook, 1995). However, we reasoned that a high E. coli cell density could amplify this low rate such that organic acid production would be substantial at a population level. We previously demonstrated that dose-dependent toxicity governs mutualism dynamics in our coculture; specifically, organic acids play a beneficial role as a carbon source for R. palustris, but a detrimental role when they accumulate enough to acidify the medium (LaSarre et al., 2017). Thus, we hypothesized that if E. coli cell densities were sufficiently high, the collective fermentation rate attributed to maintenance metabolism alone might destabilize the mutualism by producing organic acids faster than the smaller R. palustris population could consume them, resulting in growth-inhibiting acidification.
To test this hypothesis, we first simulated coculture growth from different initial species densities using SyFFoN_v2. The model correctly predicted that a common equilibrium would be reached from a wide range of initial *E. coli* densities (Fig. 8A) (LaSarre *et al.*, 2017). However, in agreement with our hypothesis, the model also predicted a maximum initial *E. coli* density that would allow cooperative growth (Fig. 8A). An upper limit was experimentally verified, albeit at a lower *E. coli* density than what was predicted (Fig. 8A). At an initial *E. coli* density of \( \sim 2 \times 10^9 \) CFU/ml, the pH reached acidic levels known to prevent *R. palustris* growth and metabolism (Fig. 8A) (LaSarre *et al.*, 2017). As a result, neither species’ population increased (Fig. 8A). These results contradicted predictions when maintenance metabolism was omitted from the model, as there was no predicted initial *E. coli* density that would prevent cooperative growth (Fig. 8B).

While SyFFoN_v2 qualitatively predicted that the maintenance metabolism from high initial *E. coli* cell density would lead to growth-inhibiting acidification, it was quantitatively inaccurate (Fig. 8A). This prediction used maintenance metabolism rates that were based on data from *E. coli* cells that had been adapted to non-growing conditions for 1 day. However, fermentation rates during days 1-7 were 2- to 10-fold slower than those observed over days 0-1; thus, the inaccuracy could stem from an underestimation of the early maintenance fermentation rate (Supporting Information Fig. S9). To explore this possibility, we calculated the level of organic acids that would accumulate in 24 h for a given initial *E. coli* cell density using fermentation rates determined during the first day of *E. coli* cell suspensions (Supporting Information Fig. S9). These organic acid levels were then entered as initial values into SyFFoN_v2 to account for organic acid production during the first 24 h, and batch cultures were then simulated with all the other parameter values the same as used for Fig. 8A. The simulations
indicate that when the initial *E. coli* cell density is low, a rapid early rate of organic acid production leads to negligible organic acid accumulation and thus the population trends are unaffected (Fig. 8C). However, when the initial *E. coli* cell density is high, a rapid early rate of organic acid production can lead to substantial organic acid accumulation and better approximate the upper *E. coli* cell density that would prevent coculture growth (Fig 8C). A potentially compounding factor to help explain the discrepancy between simulated and observed inhibitory initial *E. coli* levels is that once inhibitory acid levels are reached, cell death will likely occur; SyFFoN_v2 does not simulate cell death.

SyFFoN_v2 simulations also indicated that it is the initial *E. coli* cell density rather than the initial species ratio that determines if coculture growth will be prevented through dose-dependent toxicity; the inhibitory effect of high initial *E. coli* cell densities could be offset by a high initial *R. palustris* cell density enabling organic acid consumption at a rate sufficient to hamper accumulation (Fig. 8D). However, simulations suggest that an initial *R. palustris* concentration of $10^{10}$ cells / ml would be required to fully offset the acidification from an initial *E. coli* cell density of $10^9$ cells / ml, mainly because NH$_4^+$ cross-feeding by *R. palustris* would stimulate *E. coli* growth and thereby accelerate fermentation and organic acid accumulation. Thus, while *E. coli* maintenance metabolism is a stabilizing factor at low NH$_4^+$ exchange levels, it can also serve to destabilize the mutualism at high *E. coli* densities.

**Discussion**

In this study, we demonstrated that cross-feeding stemming from maintenance metabolism can circumstantially impede or promote mutualism. We found that mutualism destabilization by maintenance metabolism depends on dose-dependent toxicity of a cross-fed
nutrient. Specifically, destabilization occurs when a maintenance metabolism leads to the accumulation of cross-fed nutrients to levels that inhibit growth of the partner species. Destabilization of a natural mutualism linked to maintenance metabolism would require specific conditions. In our system, organic acid toxicity is relatively low, in part due to the buffered medium. Thus, an extremely high initial \textit{E. coli} cell density was required before fermentative maintenance metabolism could inhibit cooperative growth via culture acidification. However, inhibitory effects stemming from maintenance metabolism could occur at cell densities relevant to natural systems in a less well-buffered system or if the toxicity of the cross-fed metabolite was intrinsically high. For example, notoriously toxic compounds like cyanide (Harris and Knowles, 1983) and antibiotics (Dantas \textit{et al}., 2008; Barnhill \textit{et al}., 2010) can serve as nutrients for some bacteria as long as concentrations remain low.

While the likelihood of mutualism destabilization linked to maintenance metabolism is difficult to gauge, promotion of cross-feeding relationships by maintenance metabolism is likely widespread. Vast areas of the Earth’s biosphere are limited for key nutrients (Lever \textit{et al}., 2015), and it is well appreciated that nutrient limitation can promote cross-feeding in natural environments (Hom and Murray, 2014; Seth and Taga, 2014). However, it is poorly understood how established mutualisms respond to perturbations that limit cross-feeding itself. It is thought that exchange rates within obligate mutualisms must be sufficient to support sustained growth of both species in order to avoid eventual extinction (Shou \textit{et al}., 2007). Our results demonstrate that cross-feeding associated with maintenance metabolism can ease this requirement. In our system, fermentation product excretion from \textit{E. coli} maintenance metabolism can preserve the mutualism amid unfavorable NH$_4^+$ exchange levels by continually cross-feeding organic acids. This persistent cross-feeding stimulates \textit{R. palustris} growth and NH$_4^+$ excretion, thereby lifting

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both species out of starvation. In other words, maintenance metabolism allows cross-feeding to persist and facilitates cooperative success over an extended range of excretion levels compared to metabolites whose excretion depends on growth. Given that the majority of microbes in natural environments are in a state of dormancy or low metabolic activity (Hoehler and Jørgensen, 2013; Lever et al., 2015; Jørgensen and Marshall, 2016), we postulate that metabolite release is more likely to be growth-independent. As such, maintenance metabolism could better serve to initiate and maintain mutualisms in natural environments. Separately, although fermentative maintenance metabolism promoted partner growth under our study conditions, it is imaginable that mutualistic cross-feeding could purely support maintenance energy requirements in some cases, thereby promoting survival until nutrient availability improves.

Organic acids, and other fermentation products, are important metabolic intermediates in anaerobic food webs (McInerney et al., 2008; Jørgensen and Marshall, 2016). Fermentative maintenance metabolism could therefore play an important role under nutrient-limiting conditions by sustaining mutualistic relationships with acetogens, methanogens, photoheterotrophs, and anaerobically respiring microbes that rely on fermentation products for carbon and electrons. However, contributions of maintenance metabolism to mutualisms need not be restricted to fermentation nor to natural environments. Generation of maintenance energy is essential. Thus, mutualistic relationships encompassing diverse lifestyles could conceivably be preserved at low metabolic rates, provided that the limiting nutrient(s) still permits the excretion of factors required to sustain partner viability. Forcing a partner into a primary state of maintenance metabolism by limiting cross-feeding could also conceivably be a selfish strategy by which a partner could capitalize on resources that are limiting to both partners without severing essential mutualistic ties. Understanding maintenance metabolism-linked cross-feeding
could also benefit industrial bioprocesses, which commonly use growth-limiting conditions to boost product yields. Indeed, growth-independent cross-feeding helped sustain our coculture during N2-limiting conditions under which the highest H2 yields were observed (Fig. 4 and 5). Applications of microbial consortia for industrial processes is gaining interest (Sabra et al., 2010) but the effects of nutrient limitation have yet to be investigated. Clearly, the role of maintenance metabolism in fostering microbial cooperation deserves closer appraisal in both natural and applied systems.

**Experimental Procedures**

**Strains, plasmids, and growth conditions.** Strains are listed in Supporting Information Table S1. *E. coli* and *R. palustris* were cultivated on Luria-Burtani (LB) agar or defined mineral (PM) (Kim and Harwood, 1991) agar with 10 mM succinate, respectively. For determining colony forming units (CFU), LB agar or PM agar minus (NH4)2SO4 were used for *E. coli* and *R. palustris*, respectively. Cultures were grown in 10-mL of defined M9-derived coculture medium (MDC) (LaSarre et al., 2017) in 27-mL anaerobic test tubes. The medium was made anaerobic by bubbling with N2, sealed with rubber stoppers and aluminum crimps, and then autoclaved. After autoclaving, MDC was supplemented with cation solution (1 % v/v; 100 mM MgSO4 and 10 mM CaCl2) and glucose (25 mM). For defined N2 concentrations, the medium was bubbled with argon and after autoclaving defined volumes of N2 were injected through a 0.2 micron syringe filter. All cultures were grown at 30°C either laying horizontally under a 60 W incandescent bulb with shaking at 150 rpm (shaking conditions) or upright without agitation (static conditions). Static cultures were only mixed for sampling upon inoculation and at the termination of an experiment. Thus, growth rates were not measured under static conditions.
Starter cultures were inoculated with 200 µL MDC containing a suspension of a single colonies of each species. Test cocultures were inoculated using a 1% inoculum from starter cocultures except for cocultures with the *E. coli* ΔHisB strain. For these cocultures, *E. coli* ΔHisB and *R. palustris* Nx starter monocultures were grown to equivalent cell densities, washed twice with MDC, and inoculated at a 1:1 ratio for a total of 1% inoculum. For serial transfers, cocultures were incubated for either one week (shaking), two weeks (100% N₂; static), or four weeks (18% N₂; static) before transferring a 1% stationary phase inoculum to fresh medium.

**Generation of the *E. coli* ΔHisB mutant.** P1 transduction was used to introduce ΔhisB::Km from the Keio strain JW2004-1 (Baba *et al.*, 2006) into MG1655 as described (Thomason *et al.*, 2007). The ΔhisB::Km mutation was confirmed by PCR and histidine auxotrophy was verified by a requirement for histidine for growth in M9 medium.

**Analytical procedures.** Cell density was assayed by optical density at 660 nm (OD₆₆₀) using a Genesys 20 visible spectrophotometer (Thermo-Fisher, Waltham, MA, USA). Growth curve readings were taken in culture tubes without sampling. Specific growth rates were determined by fitting an exponential trend line to measurements between 0.1-1.0 OD₆₆₀ where there is linear correlation between cell density and OD₆₆₀. Final OD₆₆₀ measurements (i.e., the maximum OD₆₆₀ value corresponding to the cessation of growth) were taken in cuvettes wherein samples were diluted into the linear range as necessary. To compare cell densities between growth conditions, CFUs were converted into growth yields (CFUs per µmol glucose consumed), as N₂ limitation prevented complete glucose consumption during the assay period. H₂ and N₂ were quantified using a Shimadzu (Kyoto, Japan) gas chromatograph with a thermal conductivity detector as described (Huang *et al.*, 2010). Glucose, organic acids, and ethanol were quantified using a
Shimadzu high-performance liquid chromatograph as described (McKinlay et al., 2005). NH$_4^+$ was quantified using an indophenol colorimetric assay as described (LaSarre et al., 2017).

**Calculations of carbon release due to lysis of *E. coli ΔHisB***. To estimate carbon released due to *E. coli* lysis and carbon assimilated by *R. palustris*, CFU/ml values were first used to estimate OD values using a conversion factor of $5 \times 10^8$ CFU/ml/OD for both species based on experimental standard curves. OD values were then converted into mg of dry cell weight (DCW)/L and then to mM carbon using published conversion factors and molecular weights from elemental compositions for *E. coli* (351 mg/L/OD, CH$_{1.77}$O$_{0.40}$N$_{0.24}$) (Neidhardt, 1987; Stockar and Liu, 1999) and *R. palustris* (625 mg/L/OD, CH$_{1.8}$N$_{0.18}$O$_{0.38}$) (McKinlay and Harwood, 2010). Dividing these values of *E. coli* carbon by *R. palustris* carbon gives the fraction of *R. palustris* growth that could be supported by *E. coli* lysis. This fraction represents an upper bound, as it is based on the liberal assumption that *R. palustris* can assimilate all carbon from a lysed *E. coli* cell.

**SyFFoN_v2 model**. SyFFoN_v2 was modified from a previous version of the model (LaSarre et al., 2017) as described in the Supporting Information. Default parameter values and descriptions of functions are in Supporting Information Table S2 and Fig. S4-6). SyFFoN_v2 runs in R studio and is available for download at: [https://github.com/McKinlab/Coculture-Mutualism](https://github.com/McKinlab/Coculture-Mutualism).

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References


Figure Legends

**Fig. 1. Bi-directional anaerobic cross-feeding between fermentative *E. coli* and phototrophic *R. palustris* Nx.** *E. coli* anaerobically ferments glucose into fermentation products, including the organic acids acetate, lactate, and succinate, which provide essential carbon for *R. palustris* Nx. In return *R. palustris* Nx uses light energy to fix N\(_2\) and excrete NH\(_4^+\), which provides *E. coli* with essential nitrogen. Formate and ethanol, produced by *E. coli*, and CO\(_2\) and H\(_2\), produced by both species, accumulate. *E. coli* fermentation can be growth-independent. Filled bubbles indicate compounds externally added to the coculture.

**Fig. 2. Limiting N\(_2\) in monoculture results in decreased NH\(_4^+\) excretion.** Final cell densities (A) and supernatant NH\(_4^+\) levels (B) in stationary-phase *R. palustris* CGA4004 (Parent) and CGA4005 (Nx) monocultures. *R. palustris* was cultured in MDC with 5 mM acetate, with a headspace of 100% N\(_2\) or 18% N\(_2\), and incubated either horizontally with shaking or upright without agitation (static). Error bars indicate SD, n=3-6. Different letters indicate statistical differences, p < 0.0001, determined by one-way ANOVA (DFn, DFd = 4, 16; (A) F = 50; (B) F = 269) with Tukey’s multiple comparisons post test.
Fig. 3. Cocultures maintain coexistence despite lower \( \text{NH}_4^+ \) cross-feeding. \( \text{H}_2 \) yields (A) and normalized growth rates (B) of cocultures grown with various \( \text{N}_2 \) concentrations (% of gas in 17 ml of headspace) under shaking conditions. Circles indicate empirical data. Lines indicate SyFFoN_v2 model predictions. (A) Shaded region indicates low \( \text{N}_2 \) concentrations where empirical trends do not match model predictions. (B) Empirical and simulated growth rates are normalized to the corresponding average measured or simulated growth rate of cocultures with 100% \( \text{N}_2 \) (parameter N = 70 mM).
Fig. 4. N₂-limited cocultures exhibit stable coexistence and reproducible trends through serial transfers in a well-mixed environment. Species growth yields and final *E. coli* percentages (A), H₂ yields (B), and growth rates (C) from three serial transfers of shaken cocultures grown with 18% N₂. Transfer 1 was inoculated from a stationary phase starter coculture grown under 100% N₂ with shaking. Cocultures were serially transferred every 7 days. Error bars indicate 95% CI, n=4. Shaded horizontal bars indicate 95% CI for shaken cocultures grown with 100% N₂ (LaSarre et al., 2017). (A) Growth yields are compared for each species rather than final cell densities to account for incomplete glucose consumption after 1 week in cocultures with 18% N₂. Final *E. coli* (Ec) percentages are the mean ± SD, n=4. *, statistical difference from *E. coli* percentages in shaken cocultures serially transferred with 100% N₂ (LaSarre et al., 2017), p < 0.001, determined using one-way ANOVA (DFn, DFd = 3, 12; F = 13) with Tukey’s multiple comparison post test.
Fig. 5. N₂-limited cocultures exhibit stable coexistence and reproducible trends through serial transfers in a heterogeneous environment. H₂ yields (A), and individual species growth yields and final E. coli percentages (B) from three serial transfers of static cocultures grown with either 100% or 18% N₂. Transfer 1 was inoculated from a stationary phase starter coculture grown under 100% N₂ with shaking. Static cocultures with 100% N₂ and 18% N₂ were serially transferred every 2 and 4 weeks, respectively. Error bars indicate 95% CI, n=4. Shaded horizontal bars indicate 95% CI for shaken cocultures grown with 100% N₂ (LaSarre et al., 2017). (A) Different letters indicate statistical differences, p <0.01, determined by one-way ANOVA (DFn, DFn = 5, 18; F = 7) with Tukey’s multiple comparisons post test. (B) Final E. coli (Ec) percentages are the mean ± SD, n=4; *statistical difference from E. coli percentages in shaken cocultures serially transferred with 100% N₂ (LaSarre et al., 2017), p < 0.0001, determined using one-way ANOVA (DFn, DFn = 3, 12; (100%) F = 34; (18%) F= 56) with Tukey’s multiple comparison post test.
Fig. 6. Fermentative maintenance metabolism facilitates carbon cross-feeding. (A) Timecourse of *E. coli ΔHisB* and *R. palustris* cell densities and glucose consumption in coculture. *E. coli ΔHisB* and *R. palustris* were inoculated at equivalent CFUs/mL. Most error bars are too small to see. Error bars indicate SD, n=3. (B) Estimated carbon for glucose consumption, *E. coli* lysis, and *R. palustris* growth based on the data in (A). Conversion of CFU/ml to mM carbon is described in the Methods. Error bars indicate SD, n=3. Numerical values are averages ± SD.
Fig. 7. Fermentative maintenance metabolism permits coexistence at low NH$_4^+$ cross-feeding levels. Simulated cell densities (lines) from cocultures grown with different N$_2$ concentrations when maintenance metabolism is included (A) or omitted (B) from the model. Ec$_{\text{sim}}$ and Rp$_{\text{sim}}$, initial simulated *E. coli* (Ec) and *R. palustris* (Rp) cell densities; Ec$_{\text{f sim}}$ and Rp$_{\text{f sim}}$, final simulated *E. coli* and *R. palustris* cell densities. Symbols are empirical CFU/mL data for *E. coli* (Ec$_{\text{f emp}}$) and *R. palustris* (Rp$_{\text{f emp}}$) from 7 day samples from shaken cocultures with 6%, 18%, 40% or 100% N$_2$. The same empirical data is overlaid on both panels (n=3).
Figure 8. Fermentative maintenance metabolism prevents coculture growth at high *E. coli* cell densities. (A-C) Simulated changes in *E. coli* (*Ec*ₘᵢₙ) and *R. palustris* (*Rp*ₘᵢₙ) cell densities (lines) in cocultures grown with 100% N₂ with different initial *E. coli* cell densities when maintenance metabolism is included (A, C) or omitted (B) from the model. The simulated initial *R. palustris* cell density was 10⁷ cells/ml. (A, C) These two simulations were identical except that (C) included initial consumable organic acids [C] and formate [f] levels that were calculated by multiplying the initial *E. coli* cell density by the 0 – 1 day maintenance fermentation rate shown in Supporting Information Fig. S9 x 24 h. Symbols are empirical CFU/mL data for *E. coli* (*Ec*ₑₘₚ) and *R. palustris* (*Rp*ₑₘₚ) from cocultures with different initial *E. coli* cell densities and an average initial *R. palustris* density of 2.4 x 10⁷ ± 0.3 x 10⁷ CFU/ml. Experimental cell densities were determined 10 d after coculture inoculation. The measured coculture pH after 10 d is also shown. (D) Simulated *E. coli* and *R. palustris* growth when cocultures grown with 100% N₂ are initiated from different initial cell densities. Species ratios are shown under the cell densities.
Supporting Information

Growth-independent cross-feeding modifies boundaries for coexistence in a bacterial mutualism

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Methods

Mathematical modeling. A Monod model describing bi-directional cross-feeding in batch cultures, called SyFFoN_v2 (Syntrophy between Fermenter and Fixer of Nitrogen), was modified from our previous model (LaSarre et al., 2017) as follows: (i) a sigmoidal function, rather than a Monod function, was used to control the transition to growth-independent fermentation \([\frac{10}{10+1.09^{(1000*\text{uEc})}}]\); (ii) sigmoidal functions were used to transition from \(\text{NH}_4^+\) excretion \((1-(40/(40+1.29^N)))\) to \(\text{H}_2\) production \((40/(40+1.29^N))\) by \(R.\text{palustris}\) as \(\text{N}_2\) becomes limiting; (iii) a sigmoidal function was used to simulate the inhibiting effects of accumulated organic acids on both growth and metabolism for both species \((\frac{b_x}{b_x+10^{(f+C)}})\); (iv) a sigmoidal function was used to dampen growth-independent fermentation rates when consumable organic acids (lactate, succinate, and acetate) accumulate \((r_x*(100/(100+6^{C})) + \text{r}_x\text{ _mono})\) and simulate the slow growth-independent fermentation observed in \(E.\text{coli}\) monocultures (LaSarre et al., 2017), compared to faster growth-independent fermentation in coculture; (v) \(R.\text{palustris}\) \(\text{H}_2\) production was coupled to consumable organic acid depletion, assuming that 0.5 \(\text{CO}_2\) are produced per \(\text{H}_2\) (McKinlay et al., 2014); (vi) the \(R.\text{palustris}\) \(\text{Km}\) for \(\text{N}_2\) (\(K_N\)) was given a value of 6 mM, based on the change in growth rate at limiting \(\text{N}_2\) concentrations in coculture; (vii) product formation parameters (\(R\) and \(r\)) were increased to more accurately simulate observed growth rates in coculture; (viii) the \(E.\text{coli}\) acid resistance parameter \((b_{Ec})\) was increased relative to that for \(R.\text{palustris}\) \((b_{Rp})\) based on terminal pH values observed in \(E.\text{coli}\) monocultures versus...
cocultures. The effects of these modifications can be visualized in Figure S4. Equations are listed below with default values in Supplementary Table 2. SyFFoN_v2 runs in R studio and is available for download at: https://github.com/McKinlab/Coculture-Mutualism.

Equations 1 and 2 were used to describe *E. coli* and *R. palustris* growth rates:

**Eq. 1:** *E. coli* growth rate; \( \mu_{Ec} = \mu_{Ec_{MAX}} \cdot \frac{G}{(K_G+G)} \cdot \frac{A}{(K_A+A)} \cdot \frac{b_{Ec}}{(b_{Ec}+10^{(f_C)})} \)

**Eq. 2:** *R. palustris* growth rate; \( \mu_{Rp} = \mu_{Rp_{MAX}} \cdot \frac{C}{(K_C+C)} \cdot \frac{N}{(K_N+N)} \cdot \frac{b_{Rp}}{(b_{Rp}+10^{(f_C)})} \)

Equations 3-12 were used to describe temporal changes in cell densities and extracellular compounds. Numerical constants in product excretion equations are used to account for molar stoichiometric conversions. Numerical constants used in sigmoidal functions are based on those values that resulted in simulations resembling empirical trends. All R and r parameters are expressed in terms of glucose consumed except for \( R_A \), which is the amount of \( NH_4^+ \) produced per *R. palustris* cell (Supplementary Table 2).

**Eq. 3:** Glucose; \( \frac{dG}{dt} = -\mu_{Ec} \cdot Ec \cdot Y_G - \mu_{Ec} \cdot Ec \cdot (R_c+R_f+R_e+R_{CO2}) - \)

\[
Ec \cdot \left( \frac{G}{(K_G+G)} \right) \cdot \frac{10}{(10+1.09 \cdot 10^{(1000 \cdot \mu_{Ec})})} \cdot \frac{b_{Ec}}{(b_{Ec}+10^{(f_C)})} \cdot \frac{100}{(100+6^C)} \cdot \)

\[
(r_c+r_f+r_e+r_{CO2}) + r_{C\_mono} + r_{f\_mono} + r_{e\_mono} + r_{CO2\_ mono} \)

**Eq. 4:** \( \frac{dN}{dt} = -\mu_{Rp} \cdot Rp \cdot 0.5 \cdot R_A \cdot (40/(40+1.29^N)) - \mu_{Rp} \cdot Rp \cdot Y_N \)

**Eq. 5:** Consumable organic acids; \( \frac{dC}{dt} = Ec \cdot 2 \cdot (\mu_{Ec} \cdot R_c +

\[
\left( \frac{G}{(K_G+G)} \right) \cdot \frac{10}{(10+1.09 \cdot 10^{(1000 \cdot \mu_{Ec})})} \cdot \frac{b_{Ec}}{(b_{Ec}+10^{(f_C)})} \cdot \frac{r_c}{(100/(100 + 6^C))} + r_{C\_mono} \right) -

\[
(\mu_{Rp} \cdot Rp \cdot Y_c - 0.25 \cdot Rp \cdot (\mu_{Rp} \cdot Rh_{Rp} + r_{Hp} \cdot \left( \frac{C}{(K_C+C)} \right) \cdot \frac{40}{(40+1.29^N)}) \cdot \frac{b_{Rp}}{(b_{Rp}+10^{(f_C)})}) \)

**Eq. 6:** Formate; \( \frac{df}{dt} = Ec \cdot 6 \cdot (\mu_{Ec} \cdot R_f + \left( \frac{G}{(K_G+G)} \right) \cdot \frac{10}{(10+1.09 \cdot 10^{(1000 \cdot \mu_{Ec})})} \cdot

\[
\left( \frac{b_{Ec}}{(b_{Ec}+10^{(f_C)})} \right) \cdot \frac{r_f}{(100/(100+6^C))} + r_{f\_mono} \)
Eq. 7: \( \text{NH}_4^+; \) \( \frac{dA}{dt} = R_p \mu R_p \cdot R_A \cdot (1 - (40/(40 + 1.29^N))) - \mu E_c \cdot E_c / Y_A \)

Eq. 8: \( \text{E. coli}; \) \( \frac{dE_c}{dt} = \mu E_c \cdot E_c \)

Eq. 9: \( \text{R. palustris}; \) \( \frac{dR_p}{dt} = \mu R_p \cdot R_p \)

Eq. 10: Ethanol; \( \frac{dE}{dt} = E_c \cdot 3 \cdot (\mu E_c \cdot R_e + (G/(K_G + G)) \cdot (10/(10 + 1.09^{1000\mu E_c})) \cdot \left( b_{Ec} / (b_{Ec} + 10^{(f_1 C)}) \right) \cdot \left( r_e \cdot (100/(100 + 6^C)) + r_{e\text{-mono}} \right) \)

Eq. 11: \( \text{CO}_2; \) \( \frac{dC}{dt} = E_c \cdot 6 \cdot (\mu E_c \cdot R_{CO_2} + (G/(K_G + G)) \cdot (10/(10 + 1.09^{1000\mu E_c})) \cdot (b_{Ec} / (b_{Ec} + 10^{(f_1 C)})) \cdot \left( r_{CO_2} \cdot (100/(100 + 6^C)) + r_{CO_2\text{-mono}} \right) + R_p \cdot 0.5 \cdot (\mu R_p \cdot R_{H_R} + R_{H_P} \cdot (C/(K_C + C)) \cdot (40/(40 + 1.29^N)) \cdot (b_{Rp} / (b_{Rp} + 10^{(f_1 C)})) \)

Eq. 12: \( \text{H}_2; \) \( \frac{dH}{dt} = R_p \cdot (\mu R_p \cdot R_{HR} + R_{HP} \cdot (C/(K_C + C)) \cdot (40/(40 + 1.29^N)) \cdot (b_{Rp} / (b_{Rp} + 10^{(f_1 C)})) \cdot \left( b_{Ec} / (b_{Ec} + 10^{(f_1 C)})) \cdot (r_{H} \cdot (100/(100 + 6^C)) + r_{H\text{-mono}} \right) \)

Where,

\( \mu \) is the specific growth rate of the indicated species (h\(^{-1}\)).

\( \mu_{\text{MAX}} \) is the maximum specific growth rate of the indicated species (h\(^{-1}\)).

\( G, A, C, N, f, e, H \) and \( \text{CO}_2 \) are the concentrations (mM) of glucose, \( \text{NH}_4^+ \), consumable organic acids, \( \text{N}_2 \), formate, ethanol, \( \text{H}_2 \), and \( \text{CO}_2 \), respectively. All gasses are assumed to be fully dissolved. Consumable organic acids are those that \( \text{R. palustris} \) can consume, namely, lactate (3 carbons), acetate (2 carbons), and succinate (4 carbons). All consumable organic acids were simulated to have three carbons for convenience. Only net accumulation of formate, ethanol, \( \text{CO}_2 \) and \( \text{H}_2 \) are described in accordance with observed trends.

\( K \) is the half saturation constant for the indicated substrate (mM).

\( E_c \) and \( R_p \) are the cell densities (cells/ml) of \( \text{E. coli} \) and \( \text{R. palustris} \), respectively.
b is the ability of a species to resist the inhibiting effects of acid (mM). Default values were chosen based on levels of formate and other organic acids observed to inhibit growth and metabolism in cocultures and E. coli monocultures.

Y is the E. coli or R. palustris cell yield from the indicated substrate (cells / µmol glucose). Y values were determined in MDC with the indicated substrate as the limiting nutrient.

R is the fraction of glucose converted into the indicated compound per E. coli cell during growth (µmol of glucose / E. coli cell), except for R_A. Values were adjusted to accurately simulate product yields measured in cocultures and in MDC with and without added NH_4Cl.

R_A is the ratio of NH_4^+ produced per R. palustris cell during growth (µmol / R. palustris cell). The default value was based on that which accurately simulated empirical trends.

r is the growth-independent rate of glucose converted into the indicated compound (µmol / cell / h). Default values are based on those which accurately simulated empirical trends in coculture.

r_mono is the growth-independent rate of glucose converted into the indicated compound by E. coli when consumable organic acids accumulate. Default values are based on linear regression of products accumulated over time in nitrogen-free cell suspensions of E. coli (LaSarre et al., 2017).
Figure S1. SyFFoN_v2 predicts that coexistence at low \(NH_4^+\) cross-feeding levels requires \textit{E. coli} maintenance metabolism. Simulated effect of the \textit{R. palustris} \(NH_4^+\) excretion level on growth and organic acid accumulation in 100\% \(N_2\)-supplied cocultures in the presence (A) or absence (B) of \textit{E. coli} maintenance metabolism. 1X is the default \(NH_4^+\) excretion level (0.15 fmol \(NH_4^+\) / cell) and is thought to represent that excreted by \textit{R. palustris} \(Nx\) based on model approximation of empirical trends. OAc{s, consumable organic acids (lactate, acetate, and succinate); For, formate; Ec\textsubscript{i} and Rp\textsubscript{i}, initial \textit{E. coli} (Ec) and \textit{R. palustris} (Rp) cell densities; Ec\textsubscript{f} and Rp\textsubscript{f}, final \textit{E. coli} and \textit{R. palustris} cell densities. (A) Trends from SyFFoN_v2 are consistent with trends from a previous version of the model (LaSarre et al., 2017).

Figure S2. SyFFoN_v2 predictions of growth rates (A), formate yields (B), and ethanol yields (C) at various \(N_2\) concentrations. Circles indicate empirical data from shaken cocultures. Lines indicate model predictions. Shaded regions indicate low \(N_2\) concentrations where empirical trends do not match model predictions.
Figure S3. Cell settling in static cocultures is assumed to lead to zone of N₂ limitation that favors H₂ production over NH₄⁺ production. With saturating N₂, nitrogenase produces 2 moles of NH₄⁺ and one mole of H₂ for every mole of N₂ fixed (top equation). When N₂ is absent, nitrogenase only produces H₂ (bottom equation). Under sub-saturating N₂ conditions, the amounts of NH₄⁺ and H₂ produced would be somewhere between the two equations.
\[
\mu_X = \frac{\mu_X \text{Max} \cdot S_1 / (K_{S_1} + S_1)}{\mu_X \text{Max} \cdot \ldots \cdot S_1 / (K_{S_1} + S_1)}
\]

Where,
\( \mu_X \) is the growth rate of species X (h\(^{-1}\))
\( \mu_X \text{Max} \) is the maximum growth rate of species X (h\(^{-1}\))
\( S_1 \) is the concentration of a given substrate (mM)
\( K_{S_1} \) is the Km value for a given substrate (mM)

\[
\mu_X = \mu_X \text{Max} \cdot \ldots \cdot b_X / (b_X \cdot 10^{(f + C)})
\]

Where,
\( \mu_X \) is the growth rate of species X (h\(^{-1}\))
\( \mu_X \text{Max} \) is the maximum growth rate of species X (h\(^{-1}\))
\( b_X \) is an arbitrary value used to indicate the ability of species X to resist inhibition by acid
\( f \) is the concentration of formate (mM)
\( C \) is the concentration of consumable organic acids (acetate, lactate, and succinate; mM)

\[
dP/dt = r_P \cdot \ldots \cdot 10(10 + 1.09 \cdot 10^{(1000 \cdot \mu_Ec)})
\]

Where,
\( dP/dt \) is the change in product concentration per unit time (mM/h)
\( r_P \) is the maximum growth-independent rate of product formation in coculture
\( \mu_Ec \) is the growth rate of E. coli (h\(^{-1}\))

\[
dP/dt = r_P \cdot \ldots \cdot (100 \cdot (100 + 6^C) \cdot r_{P\text{-mono}})
\]

Where,
\( dP/dt \) is the change in product concentration per unit time (mM/h)
\( r_P \) is the maximum growth-independent rate of product formation in coculture
\( r_{P\text{-mono}} \) is the maximum growth-independent rate of product formation in E. coli cell suspensions

\[
dA/dt = \text{Rp} \cdot \mu_{E}\text{p} \cdot \ldots \cdot 1 - (40(40 + 1.29^n))
\]

Where,
\( dP/dt \) is the change in product concentration per unit time (mM/h)
\( r_P \) is the maximum growth-independent rate of product formation in coculture
\( r_{P\text{-mono}} \) is the maximum growth-independent rate of product formation in coculture
Figure S4. The effect of SyFFoN_v2 functions on growth rate and product formation rates. 

(A) Growth rate approaches zero as substrate supply is exhausted according to a Monod model (whole-cell Michaelis-Menten kinetics). The function, \( \frac{S1}{(K_{S1}+S1)} \), is used in SyFFoN_v2 equations 1 and 2 to control growth rates in response to substrate concentration. The function was also used in equations 3, 5, 6, 10, 11, and 12 to control growth-independent product excretion rates in response to substrate concentration. The function was not applied to growth-dependent product excretion rates since these are already dependent on growth rate which in turn is dependent on substrate concentration. Km values were taken from the literature or assumed as indicated in Supplementary Table 2. The Km value for \( N_2 \) was determined experimentally from \( R. palustris \) exponential growth rates at different \( N_2 \) concentrations (Figure S5).

(B) Growth rate declines sharply at a threshold organic acid concentration according to the sigmoidal function, \( \frac{b_X}{(b_X+10^{(r-C)})} \). The curve was modeled to resemble that of an acid titration curve in buffer. The function is used in equations 1 and 2 to control growth rate in response to acid accumulation. The function was also used in equations 3, 5, 6, 10, 11, and 12 to control growth-independent product excretion rates in response to acid accumulation. The function was not applied to growth-dependent product excretion rates since these are already dependent on growth rate which in turn is dependent on organic acid concentration. Values for ‘b’ were determined by manually varying values until simulated growth inhibition occurred at organic acid levels resembling those observed in cocultures and \( E. coli \) monocultures with excess glucose, such that growth halted due to acid accumulation.

(C) \( E. coli \) growth-independent fermentation rates (\( \mu \text{mol} \) / cell / h) increase as the \( E. coli \) growth rate decreases according to the sigmoidal function, \( \frac{10}{(10+1.09^{1000+\mu E_c})} \). The function is used in equations 3, 5, 6, 10, 11, and 12 to transition to growth-independent product formation rates when \( E. coli \) growth slows. Product formation rates associated with maintenance metabolism (r) were modeled to increase as \( E. coli \) growth slows rather than being kept constant since product formation rates observed in non-growing \( E. coli \) cell suspensions with glucose could not explain the rate of \( R. palustris \) growth and formate accumulation in cocultures. Consumption of organic acids by \( R. palustris \) likely increases these growth-independent fermentation rates by removing inhibitory effects of end-products. The values of ‘10’ and ‘1.09’ were arrived at after manually trying several arbitrary values until simulated growth inhibition occurred at organic acid levels resembling those observed in cocultures and \( E. coli \) monocultures with excess glucose, such that growth halted due to acid accumulation.

(D) \( E. coli \) growth-dependent fermentation rates (\( \mu \text{mol} \) / cell / h) decrease to a rate observed in non-growing \( E. coli \) cell suspensions as consumable organic acid concentrations increase according to the sigmoidal function, \( \frac{100}{(100+6^C)+r_{P_{\text{mono}}}} \). The function is used in equations 3, 5, 6, 10, 11, and 12 to transition to growth-independent product formation rates observed in \( E. coli \) cell suspensions when consumable organic acids accumulate. This function is necessary to ensure that the model does not use the high growth-independent fermentation rates observed in coculture when consumable organic acids accumulate (e.g., when the \( NH_4^+ \) availability is low and the \( E. coli \) density is high, or in nitrogen-starved \( E. coli \) cell suspensions). The function uses C (consumable organic acids) instead of (C+f) (consumable organic acids and formate), to avoid incorrectly halting formate production in cocultures even when C is fully consumed. The values of ‘100’ and ‘6’ were arrived at after manually trying several arbitrary values to control the levels of organic acid accumulation similar to what is observed in nitrogen-starved \( E. coli \) cell suspensions (see Figure S6B and C).
(E) As N₂ availability increases, the *R. palustris* nitrogenase activity shifts from producing only H₂ to a mixture of H₂ and NH₄⁺ according to:

- No N₂: \(8\text{H}^\circ + 8\text{e}^- + 16 \text{ATP} \rightarrow 4\text{H}_2 + 16 \text{ADP}\)
- Saturating N₂: \(\text{N}_2 + 10\text{H}^\circ + 8\text{e}^- + 16 \text{ATP} \rightarrow 2\text{NH}_4^+ + \text{H}_2 + 16 \text{ADP}\)

In SyFFoN_v2 this shift is controlled by two functions. One function, \(1-(\frac{40}{40+1.29^{\text{N}_2}})\) (green) is used in equation 4 to increase NH₄⁺ production with increasing N₂ concentration. The other function, \(\frac{40}{40+1.29^{\text{N}_2}}\) (red) is used in equation 12 to decrease H₂ production with increasing N₂ concentration. The values of ‘40’ and ‘1.29’ were arrived at after manually trying several arbitrary values to control the shape of the curves and give final product yields similar to those observed in Figure 3 and Figure S2 (see also Figure S6D).

**Figure S5. Determination of *R. palustris* whole cell Km value for N₂ in coculture.** A Michaelis-Menten curve was fit to coculture growth rate data determined at various N₂ concentrations using Graphpad Prism v.6.0h. Symbol error bars are SD.
Figure S6. Effect of arbitrarily chosen function values on simulated trends.

(A) Altering the arbitrary values of 10 (left panel) and 1.09 (right panel) in the function \( \frac{X}{X+1.09(1000 + \mu_{Ec})} \) affects the simulated time required for cocultures to reach stationary phase (i.e., when the optical density is observed to plateau). The example shown is for simulated coculture times with 18% N\(_2\) for which experimental cocultures were observed to reach stationary phase in approximately 145 h (dotted line).
(B) The function \( \frac{100}{(100+6^C)} + r_{P_{\text{mono}}} \) is necessary to dampen product excretion rates associated with \( E. coli \) maintenance metabolism \( (r) \) when consumable organic acids accumulate, such as in simulations of nitrogen-starved \( E. coli \) cell suspensions (left panel; compare with trends in Figure S9) or when \( E. coli \) cell densities in coculture are high but \( \text{NH}_4^+ \) availability is low. Without this function organic acids would accumulate in simulations of nitrogen-starved \( E. coli \) cell suspensions at an unrealistically high rate (right panel).

(C) Altering the arbitrary values of 100 (left panel) and 6 (right panel) in the function \( \frac{100}{(100+6^C)} + r_{P_{\text{mono}}} \) affects the levels of metabolite accumulation in nitrogen-starved \( E. coli \) cell suspensions by 14 d. Target concentrations of 7 mM consumable organic acids and 5.5 mM formate were chosen based on approximate levels observed in experimental nitrogen-starved \( E. coli \) cell suspensions at 14 d (Figure S9).

(D) Altering the arbitrary values of 1.29 (left panel) and 40 (right panel) in the function \( \frac{40}{(40+1.29^N)} \) affects product yields over different \( N_2 \) concentrations. The example shown overlays simulated \( H_2 \) yields (red and blue lines) on Figure 3A when different arbitrary values are used, with green dots representing experimentally determined \( H_2 \) yields and the black line representing simulated values when the values 40 and 1.29 were used in the function. Use of different arbitrary values similarly affected other simulated product yields and growth yields by shifting trends for away from observed values.
Figure S7. SyFFoN_v2 simulations of batch cocultures with and without externally added NH$_4^+$. (A, B) Simulated growth and metabolic profiles of cocultures supplied with NH$_4^+$ (parameter A = 15mM) (A) or 100% N$_2$ alone (B). Ec, E. coli; Rp, R. palustris; OAcs, consumable organic acids (lactate, acetate, and succinate). Trends from SyFFoN_v2 are consistent with trends from a previous version of the model (LaSarre et al., 2017).

Figure S8. E. coli maintenance metabolism is predicted to permit coculture growth at low N$_2$ concentrations by circumventing diminishing returns. Simulated exchange of NH$_4^+$ (green) and consumable organic acids (blue) during growth of E. coli (orange lines, triangles) and R. palustris (purple lines, squares) populations when maintenance metabolism is omitted (A, B) or included (C). Cross-fed NH$_4^+$ and organic acid values for the indicated time points (symbols) are the sum of those free in the medium plus those assimilated in the exchange (µM).
Figure S9. Estimation of *E. coli* fermentation rates associated with maintenance metabolism in cell suspensions lacking nitrogen. (A-C) Growth and metabolic trends in nitrogen-starved *E. coli* cell suspensions. Each panel is data from a separate biological replicate. (D) Average specific product formation rates (± SD) in *E. coli* cell suspensions for the indicated time frames, determined from the data in A-C. Fold changes in specific formation rates are shown for days 1–7 relative to days 0–1. Figure S8A is the same data that appeared in Supplementary Figure 1 in LaSarre *et al.*, 2017.
Table S1. Strains, plasmids and primers used in this study.

<table>
<thead>
<tr>
<th>R. palustris strains: designation in paper</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CGA009 Wild-type strain; spontaneous Cm(^r) derivative of CGA001</td>
<td>(Larimer et al., 2004)</td>
</tr>
<tr>
<td>CGA4004 CGA009 ΔhupS Δrpa2750; Parent</td>
<td>(LaSarre et al., 2017)</td>
</tr>
<tr>
<td>CGA4005 CGA4004 nifA(^*); Nx</td>
<td>(LaSarre et al., 2017)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>E. coli strains</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>MG1655 Wild-type K12 obtained from the Coli Genetic Stock Center (# 7740, MG1655(Seq)), WT</td>
<td>(Blattner et al., 1997)</td>
</tr>
<tr>
<td>JW2004-1 Δ(araD-araB)567, ΔlacZ4787::rrnB-3, λ(^−), ΔhisB720::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514</td>
<td>(Baba et al., 2006)</td>
</tr>
<tr>
<td>MG1655 ΔHisB MGI655 ΔhisB720::Km</td>
<td>This study</td>
</tr>
</tbody>
</table>
### Table S2. Default parameter values used in the model unless stated otherwise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description (Units); Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{E_c}$MAX</td>
<td>0.2800</td>
<td><em>E. coli</em> max growth rate (h$^{-1}$); Monoculture</td>
</tr>
<tr>
<td>$\mu_{R_p}$MAX</td>
<td>0.0772</td>
<td><em>R. palustris</em> max growth rate (h$^{-1}$); Monoculture</td>
</tr>
<tr>
<td>G</td>
<td>25</td>
<td>Glucose (mM)</td>
</tr>
<tr>
<td>A</td>
<td>0.00005</td>
<td>$\text{NH}_4^+$ (mM); from initial (NH$_4$)$_2\text{MoO}_2\cdot4\text{H}_2\text{O}$ concentration</td>
</tr>
<tr>
<td>C</td>
<td>0</td>
<td>Consumable organic acids (those that <em>R. palustris</em> was observed to consume at low pH; mM)</td>
</tr>
<tr>
<td>N</td>
<td>70</td>
<td>$\text{N}_2$ (assumed to be fully dissolved; mM)</td>
</tr>
<tr>
<td>f</td>
<td>0</td>
<td>Formate (mM)</td>
</tr>
<tr>
<td>e</td>
<td>0</td>
<td>Ethanol (mM)</td>
</tr>
<tr>
<td>CO2</td>
<td>0</td>
<td>Carbon dioxide (mM)</td>
</tr>
<tr>
<td>$K_G$</td>
<td>0.02</td>
<td><em>E. coli</em> affinity (Michaelis-Menten constant (Km)) for glucose (mM); (Buhr et al., 1992)</td>
</tr>
<tr>
<td>$K_C$</td>
<td>0.01</td>
<td><em>R. palustris</em> affinity (Km) for consumable organic acids (mM); Assumed</td>
</tr>
<tr>
<td>$K_A$</td>
<td>0.01</td>
<td><em>E. coli</em> affinity for $\text{NH}_4^+$ (mM); (Khademi et al., 2004)</td>
</tr>
<tr>
<td>$K_N$</td>
<td>6</td>
<td><em>R. palustris</em> affinity (Km) for $\text{N}_2$ (mM); determined by fitting a Michaelis-Menten model to coculture growth rates versus $\text{N}_2$ concentration (Figure S5).</td>
</tr>
<tr>
<td>$E_c$</td>
<td>$0.4 \times 10^7$</td>
<td><em>E. coli</em> cell density (cells / ml)</td>
</tr>
<tr>
<td>$R_p$</td>
<td>$3.6 \times 10^7$</td>
<td><em>R. palustris</em> cell density (cells / ml)</td>
</tr>
<tr>
<td>$b_{E_c}$</td>
<td>$10^{41}$</td>
<td>Resistance of <em>E. coli</em> to low pH (mM)$^a$</td>
</tr>
<tr>
<td>$b_{R_p}$</td>
<td>$10^{32}$</td>
<td>Resistance of <em>R. palustris</em> to low pH (mM)$^a$</td>
</tr>
<tr>
<td>$Y_G$</td>
<td>$8 \times 10^7$</td>
<td>Glucose-limited <em>E. coli</em> growth yield (cells / µmol glucose); Determined by dividing the concentration of glucose consumed at early stationary phase in glucose-limited monocultures by the concentration of glucose at early stationary phase in glucose-limited monocultures</td>
</tr>
<tr>
<td>$Y_A$</td>
<td>$1 \times 10^9$</td>
<td>$\text{NH}_4^+$-limited <em>E. coli</em> growth yield (cells / µmol $\text{NH}_4^+$); Determined by dividing the concentration of $\text{NH}_4^+$ used at early stationary phase in $\text{NH}_4^+$-limited $E. coli$ monocultures by the concentration of $\text{NH}_4^+$ used at early stationary phase in glucose-limited <em>E. coli</em> monocultures</td>
</tr>
<tr>
<td>$Y_C$</td>
<td>$2.5 \times 10^6$</td>
<td>Organic acid-limited <em>R. palustris</em> growth yield (cells / µmol organic acid); Determined by dividing the CFU/ml by the concentration of acetate used at early stationary phase in acetate-limited <em>R. palustris</em> monocultures</td>
</tr>
<tr>
<td>$Y_N$</td>
<td>$5 \times 10^8$</td>
<td>$\text{N}_2$-limited <em>R. palustris</em> growth yield cells / µmol $\text{N}_2$; Determined by dividing the concentration of $\text{N}_2$ used at early stationary phase in $\text{N}_2$-limited <em>R. palustris</em> monocultures by the concentration of $\text{N}_2$ used at early stationary phase in glucose-limited <em>R. palustris</em> monocultures</td>
</tr>
<tr>
<td>$R_C$</td>
<td>$1.9 \times 10^{-8}$</td>
<td>Fraction of glucose converted to organic acids (µmol glucose / cell)$^a$</td>
</tr>
<tr>
<td>$R_f$</td>
<td>$8 \times 10^{-9}$</td>
<td>Fraction of glucose converted to formate (µmol glucose / cell)$^a$</td>
</tr>
<tr>
<td>$R_e$</td>
<td>$4.5 \times 10^{-9}$</td>
<td>Fraction of glucose converted to ethanol (µmol glucose / cell)$^a$</td>
</tr>
<tr>
<td>$R_{CO2}$</td>
<td>$5 \times 10^{-10}$</td>
<td>Fraction of glucose converted to CO$_2$ (µmol glucose / cell)$^a$</td>
</tr>
<tr>
<td>$R_{HRp}$</td>
<td>$2 \times 10^{-9}$</td>
<td><em>R. palustris</em> H$_2$ production (µmol H$_2$ / <em>R. palustris</em> cell)$^a$</td>
</tr>
<tr>
<td>$R_{HEc}$</td>
<td>$5 \times 10^{-9}$</td>
<td><em>E. coli</em> H$_2$ production (µmol H$_2$ / <em>E. coli</em> cell)$^a$</td>
</tr>
<tr>
<td>$R_A$</td>
<td>$0.15 \times 10^{-9}$</td>
<td><em>R. palustris</em> NH$_4^+$ production (µmol NH$_4^+$ / cell)$^a$</td>
</tr>
<tr>
<td>$r_C$</td>
<td>$300 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to consumable organic acids (µmol glucose / cell / h)$^a$</td>
</tr>
<tr>
<td>$r_f$</td>
<td>$47 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to formate H$_2$ (µmol glucose / cell / h)$^a$</td>
</tr>
<tr>
<td>$r_e$</td>
<td>$15 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to ethanol H$_2$ (µmol glucose / cell / h)$^a$</td>
</tr>
<tr>
<td>$r_{\text{CO}_2}$</td>
<td>$2 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to CO$_2$ (µmol glucose / cell / h)$^a$</td>
</tr>
<tr>
<td>$r_{\text{H}}$</td>
<td>$2 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of H$_2$ production (µmol H$_2$ / cell / h)$^a$</td>
</tr>
<tr>
<td>$r_{\text{C}_{\text{mono}}}$</td>
<td>$1.2 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to consumable organic acids when consumable organic acids accumulate (µmol glucose / cell / h)$^b$</td>
</tr>
<tr>
<td>$r_{\text{T}_{\text{mono}}}$</td>
<td>$0.83 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to formate when consumable organic acids accumulate (µmol glucose / cell / h)$^b$</td>
</tr>
<tr>
<td>$r_{\text{H}_{\text{mono}}}$</td>
<td>$0.5 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to ethanol when consumable organic acids accumulate (µmol glucose / cell / h)$^b$</td>
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<tr>
<td>$r_{\text{CO}<em>2</em>{\text{mono}}}$</td>
<td>$1.3 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to CO$_2$ when consumable organic acids accumulate (µmol glucose / cell / h)$^b$</td>
</tr>
<tr>
<td>$r_{\text{H}_{\text{mono}}}$</td>
<td>$0.83 \times 10^{-11}$</td>
<td><em>E. coli</em> specific growth-independent rate of glucose conversion to H$_2$ when consumable organic acids accumulate (µmol glucose / cell / h)$^b$</td>
</tr>
<tr>
<td>$r_{\text{HP}}$</td>
<td>$27 \times 10^{-11}$</td>
<td><em>R. palustris</em> specific growth-independent rate of H$_2$ production (µmol H$_2$ / cell / h)$^a$</td>
</tr>
</tbody>
</table>

$^a$ Values were manually varied until simulated trends resembled empirical trends observed in both monoculture and coculture (i.e., growth inhibition at a given cumulative concentration of formate and other organic acids). Growth-independent fermentation rates were increased to account for *R. palustris*’s pull on *E. coli* metabolism by consuming organic acids.  

$^b$ Determined from rates of product formation from glucose measured between days 1 and 7 of *E. coli* cell suspensions, wherein growth was prevented by omitting all nitrogen sources. 

Representative plots of fermentation product accumulation under these conditions is available in Figure S8.

**Supplementary References**


