

Environment constrains fitness advantages of division of labor in microbial consortia engineered for metabolite push or pull interactions

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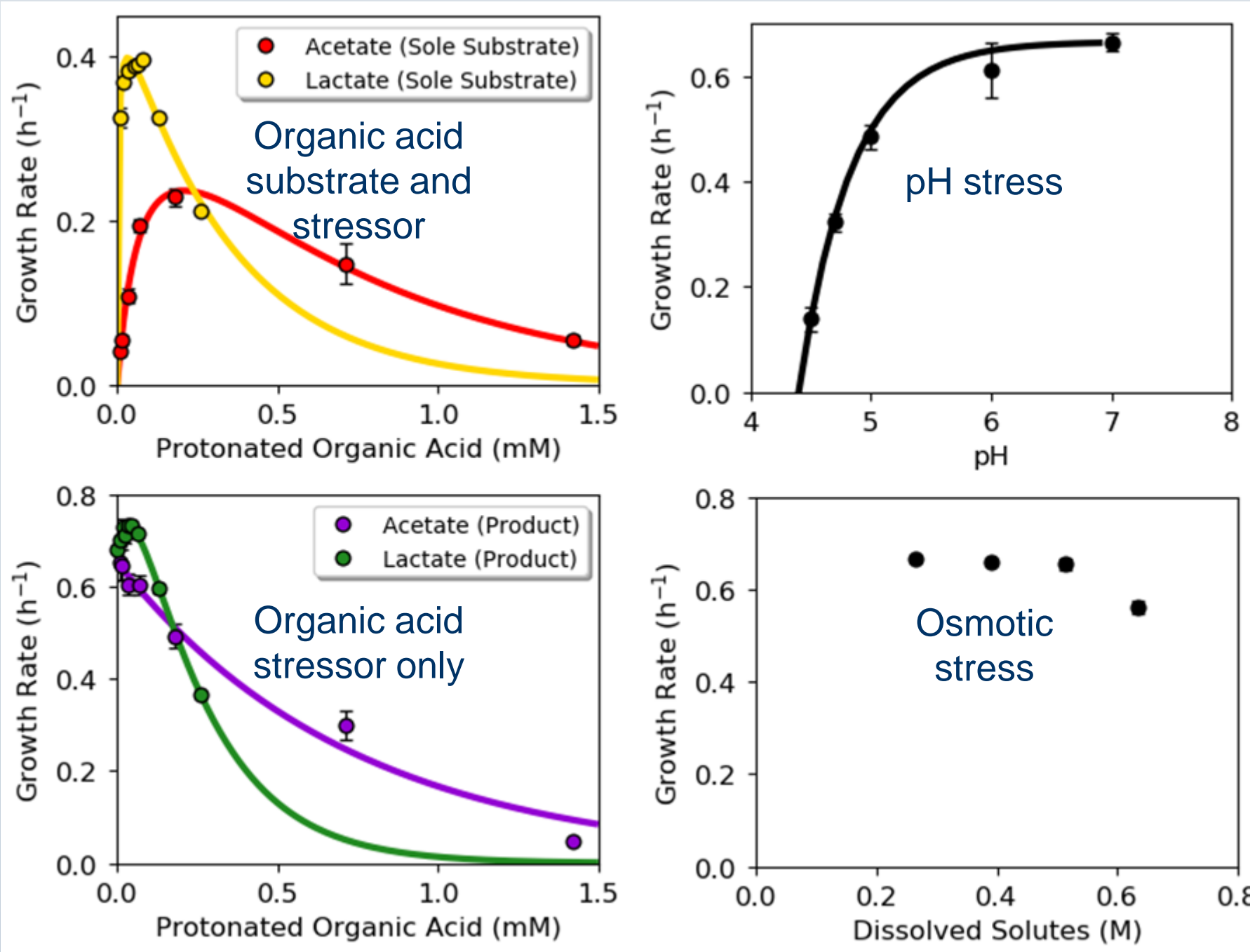
a National Science Foundation Engineering Research Center in the MSU College of Engineering

1. Abstract

Fitness benefits from division of labor are well documented in microbial consortia, but the dependency of the benefits on environmental context is poorly understood. Two synthetic *Escherichia coli* consortia were built to test the relationships between exchanged organic acid, local environment, and opportunity costs of different metabolic strategies. Opportunity costs quantify benefits not realized due to selecting one phenotype over another. The consortia catabolized glucose and exchanged either acetic or lactic acid to create producer-consumer food webs. The organic acids had different inhibitory properties and different opportunity costs associated with their positions in central metabolism. The exchanged metabolites modulated different consortial dynamics. The acetic acid-exchanging (AAE) consortium had a 'push' interaction motif where acetic acid was secreted faster by the producer than the consumer imported it, while the lactic acid-exchanging (LAE) consortium had a 'pull' interaction motif where the consumer imported lactic acid at a comparable rate to its production. The LAE consortium outperformed wild type (WT) batch cultures under the environmental context of weakly buffered conditions, achieving a 55% increase in biomass titer, a 51% increase in biomass per proton yield, an 86% increase in substrate conversion, and the complete elimination of byproduct accumulation all relative to the WT. However, the LAE consortium had the tradeoff of a 42% lower specific growth rate. The AAE consortium did not outperform the WT in any considered performance metric. Performance advantages of the LAE consortium were sensitive to environment; increasing the medium buffering capacity negated the performance advantages compared to WT.

IMPORTANCE: Most naturally occurring microorganisms persist in consortia where metabolic interactions are common and often essential to ecosystem function. This study uses synthetic ecology to test how different cellular interaction motifs influence performance properties of consortia. Environmental context ultimately controlled the division of labor performance as shifts from weakly buffered to highly buffered conditions negated the benefits of the strategy. Understanding the limits of division of labor advances our understanding of natural community functioning which is central to nutrient cycling and provides design rules for assembling consortia used in applied bioprocessing.

2. Inhibition Kinetics

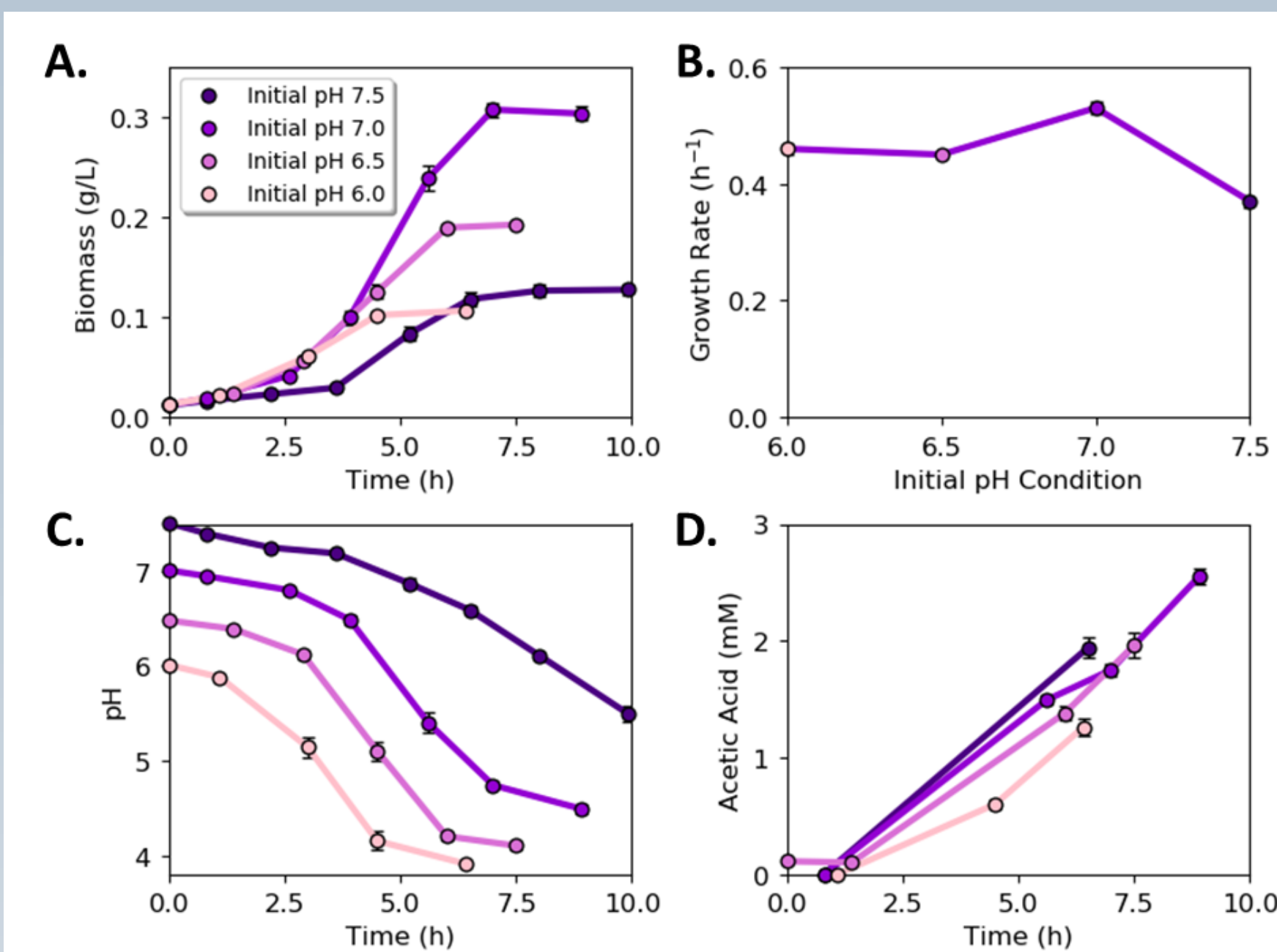
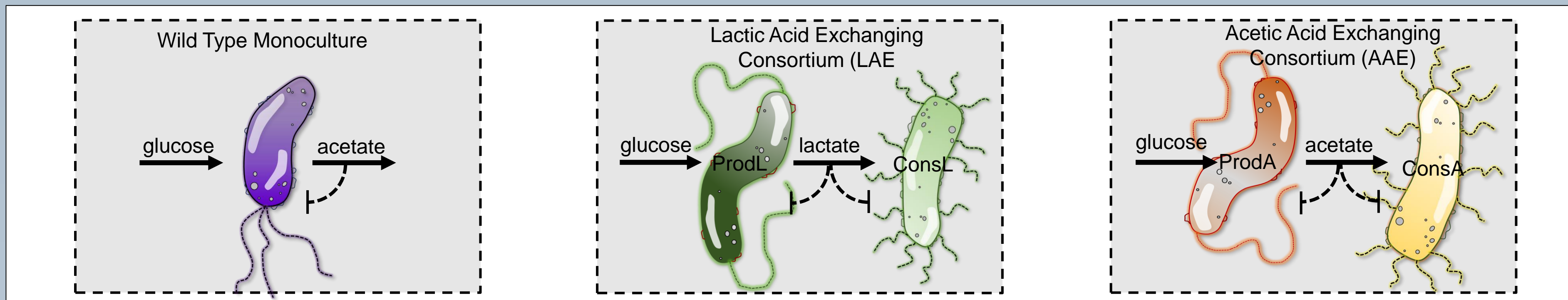


Experimental inhibition data for wildtype monocultures grown on M9 medium. Data is mean of three biological replicates with error bars representing standard deviation.

Table 1. Selected expressions and parameters used to model organic acid growth and inhibition kinetics for wildtype *E. coli*.

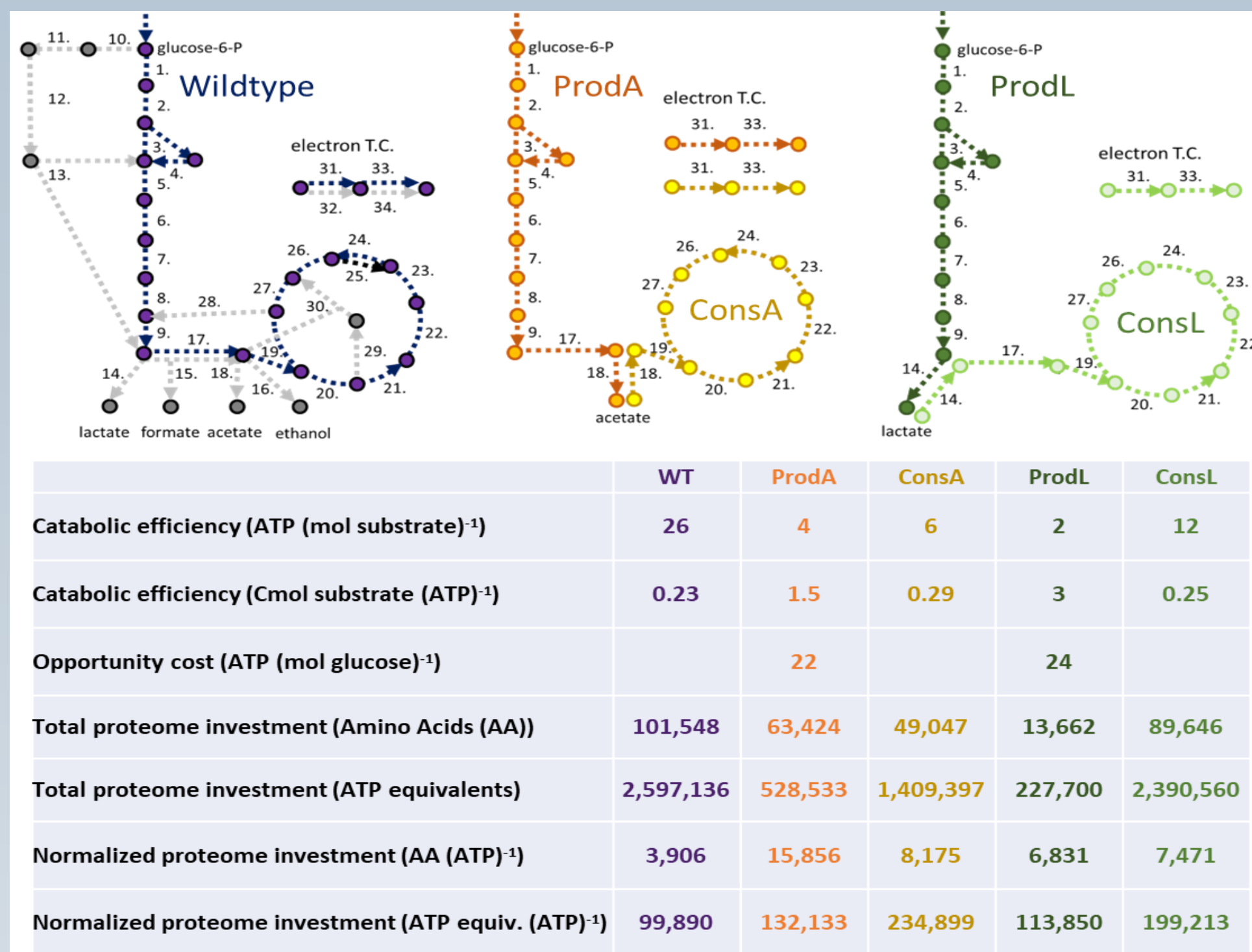
Condition	Best-fit Expression	Parameters
Acetic acid as sole substrate	$\mu = \mu_m \left(\frac{A}{K_A + A} \right) e^{-\frac{A}{K_I}}$ where μ is growth rate (h^{-1}) and A is concentration of protonated acetate (mM)	$\mu_m = 0.4 \text{ h}^{-1}$, $K_A = 0.0723 \text{ mM}$, $K_I = 0.760 \text{ mM}$, $R^2 = 0.98$
Lactic acid as sole substrate	$\mu = \mu_m \left(\frac{L}{K_L + L} \right) e^{-\frac{L}{K_I}}$ where μ is growth rate (h^{-1}) and L is concentration of protonated lactate (mM)	$\mu_m = 0.5 \text{ h}^{-1}$, $K_L = 0.0038 \text{ mM}$, $K_I = 0.317 \text{ mM}$, $R^2 = 0.93$
Acetic acid with glucose	$\mu = \mu_m \left(\frac{G}{K_G + G} \right) e^{-\frac{A}{K_I}}$ where μ is growth rate (h^{-1}), G is concentration of glucose, and A is concentration of protonated acetate (mM)	$\mu_m = 0.65 \text{ h}^{-1}$, $K_G = 0.005 \text{ mM}$, $K = 1.35 \text{ mM}^{-1}$, $R^2 = 0.97$
Lactic acid with glucose	$\mu = \mu_m \left[\left(\frac{G}{K_G + G} \right) + \left(\frac{L}{K_L + L} \right) \right] e^{-\alpha L}$ where μ is growth rate (h^{-1}), G is concentration of glucose (set to 56 mM), and L is concentration of protonated lactate (mM)	$\mu_m = 0.65 \text{ h}^{-1}$, $K_G = 0.005 \text{ mM}$, $K_L = 0.0743 \text{ mM}$, $\alpha = 4.44 \text{ mmol}^{-1}$, $R^2 = 0.98$
pH	$\mu = \mu_m \left(1 - \frac{H}{H_c} \right)$ where μ is growth rate (h^{-1}), and H is concentration of protons (M)	$\mu_m = 0.665 \text{ h}^{-1}$, and $H_c = 10^{-4.4} \text{ M}$ (critical threshold above which growth is not possible)

3. Organic Acid Exchanging Synthetic Consortia



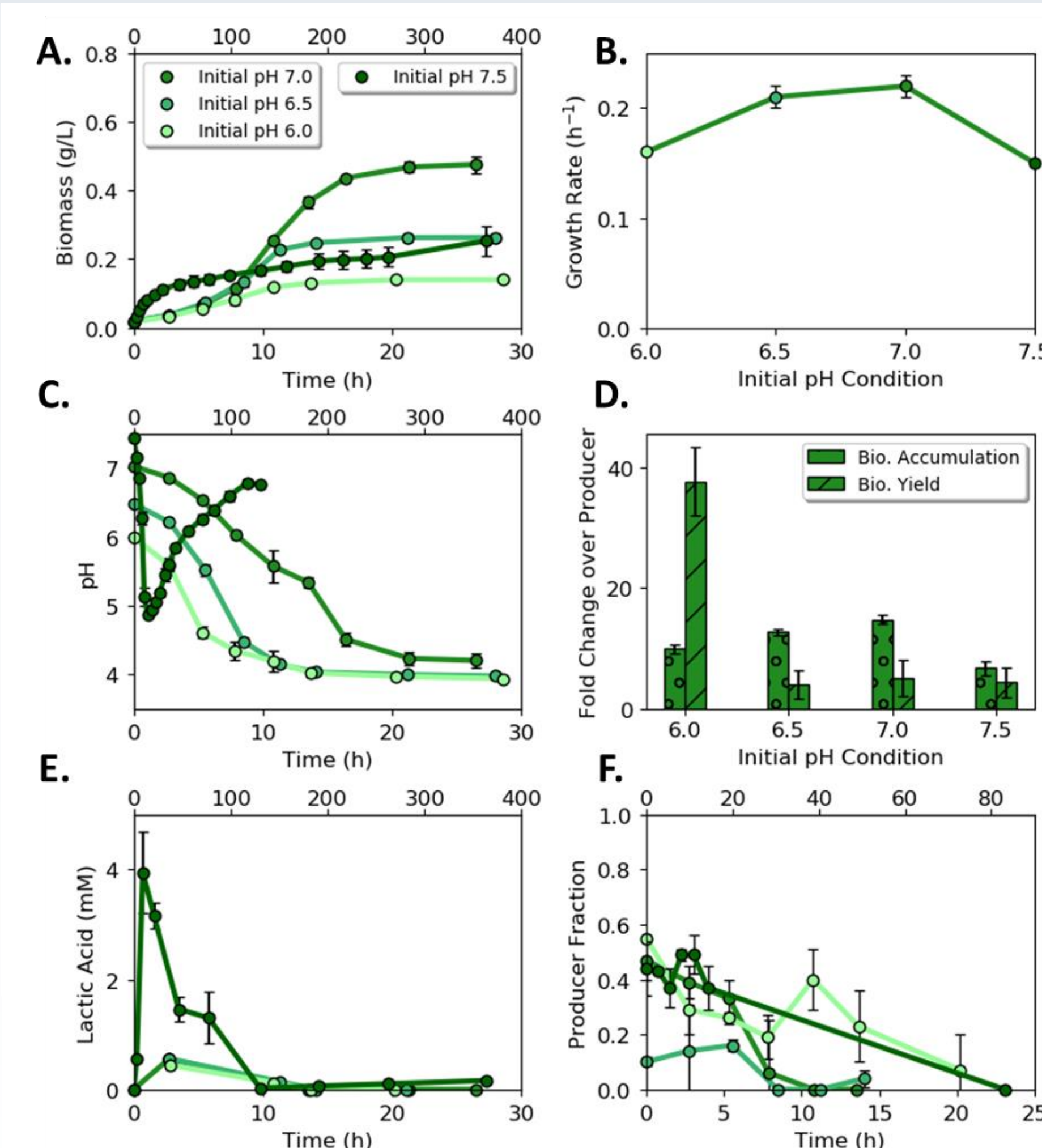
Wild type culture data as a function of initial medium pH. Data is mean of three biological replicates with error bars representing standard deviation.

In silico analysis of consortia metabolic properties and resource investment.

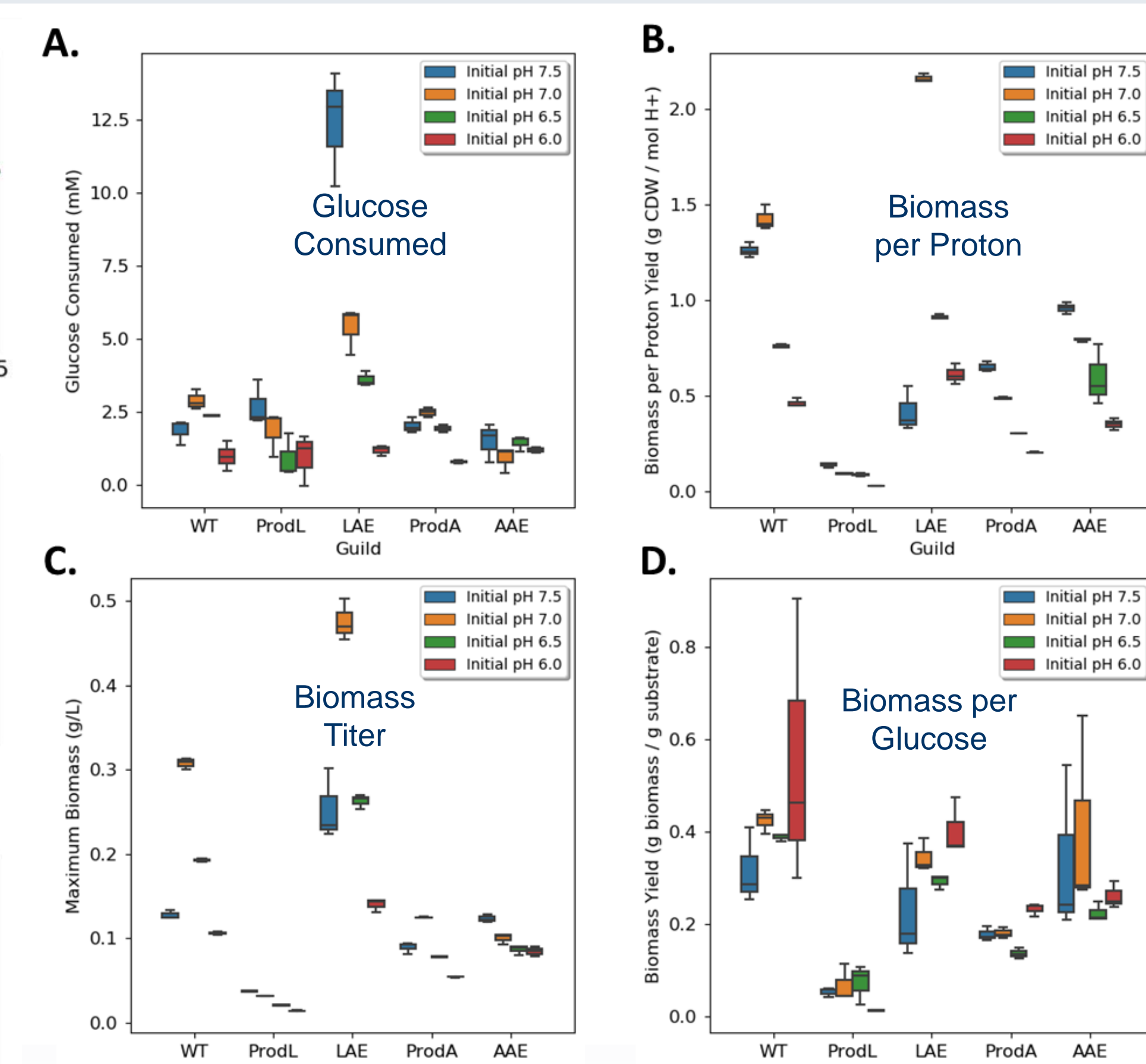


	Generalist (WT)	Acetate Producer (ProdA)	Lactate Producer (ProdL)	Consumer (ConsA/ConsL)
Carbon source	Glucose ($n=4$)	Glucose ($n=3$)	Glucose ($n=9$)	Acetic acid ($n=2$)/ Lactic acid ($n=9$)
Maximum growth rate	$0.65 \text{ h}^{-1} \pm 0.01$	$0.54 \text{ h}^{-1} \pm 0.007$	$0.24 \text{ h}^{-1} \pm 0.003$	$0.14 \text{ h}^{-1} \pm 0.003 / 0.41 \text{ h}^{-1} \pm 0.01$
Biomass yield	$0.43 \text{ g biomass per g glucose} \pm 0.02$	$0.20 \text{ g biomass per g glucose} \pm 0.01$	$0.05 \text{ g biomass per g glucose} \pm 0.001$	$0.24 \text{ g biomass per g acetic acid} \pm 0.00 / 0.48 \text{ g biomass per g lactic acid} \pm 0.01$
Organic byproduct yield	$0.11 \text{ g acetic acid per g glucose} \pm 0.01$	$0.34 \text{ g acetic acid per g glucose} \pm 0.004$	$0.86 \text{ g lactic acid per g glucose} \pm 0.01$	NA

4. Advantages of Consortium Relative to Wildtype

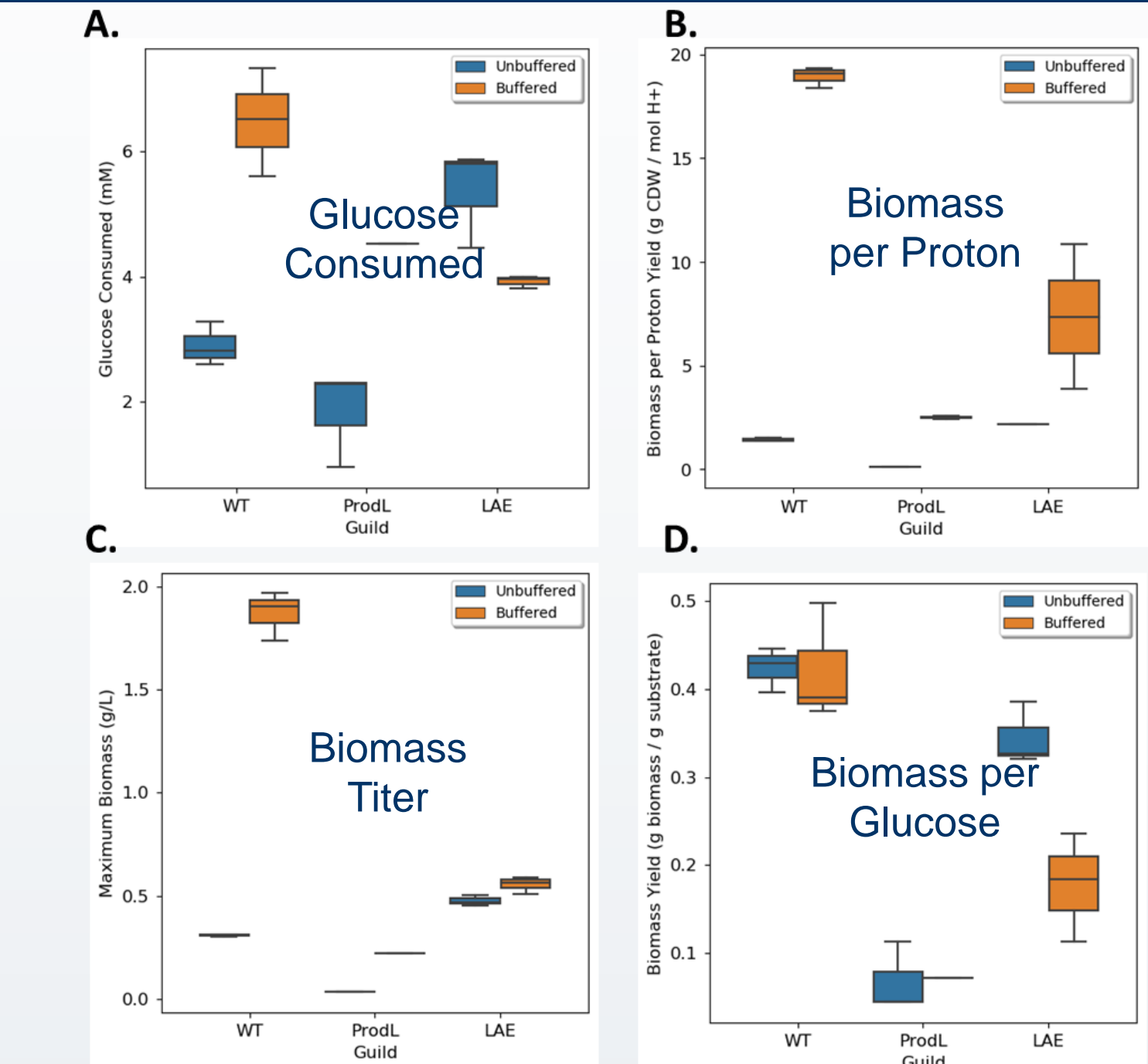


Lactic acid exchanging (LAE) consortium as a function of initial pH. Data is mean of three biological replicates with error bars representing standard deviation.



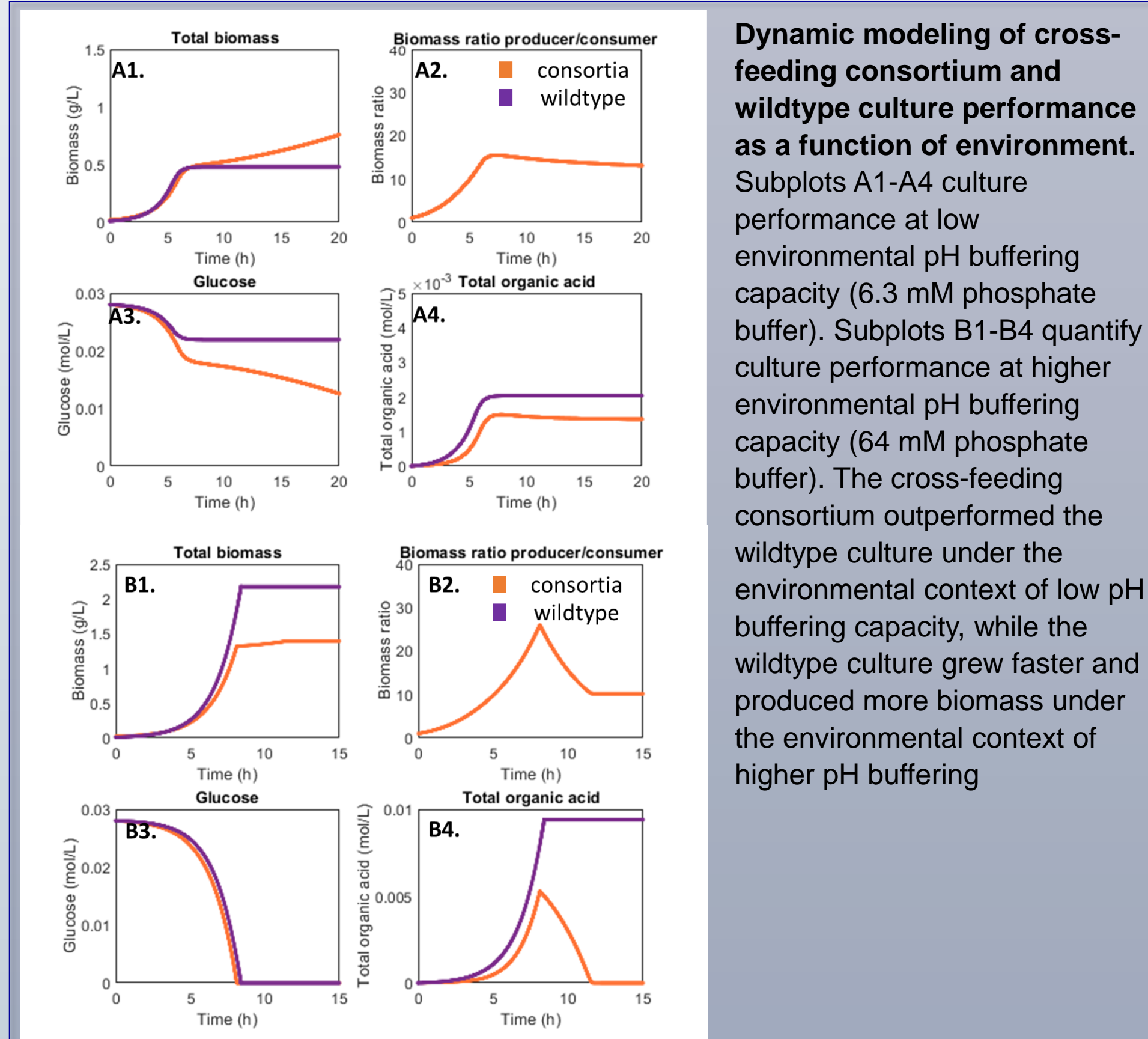
Summary of performance metrics for organic acid exchanging consortia compared to producer monocultures and wild type monocultures. **A)** Total glucose consumed during batch growth. **B)** Biomass (g cdw) produced per mole H⁺ accumulated in medium. **C)** Final biomass concentration (g cdw L⁻¹) at stationary phase. **D)** Biomass yield on glucose (g cdw (g glucose)⁻¹). **The WT grew faster than the LAE consortium.** cdw = cell dry weight.

5. Buffer Vs. Unbuffered Growth



Performance metrics for lactic acid exchanging (LAE) consortium compared to wild type generalist in highly buffered M9 medium. **A)** Total glucose consumed during batch growth. **B)** Biomass (g cdw) produced per mole H⁺ accumulated in medium. **C)** Final biomass concentration (g cdw L⁻¹) at stationary phase. **D)** Biomass yield on glucose (g cdw (g glucose)⁻¹). cdw = cell dry weight.

6. ODE Model for Consortia Design



Dynamic modeling of cross-feeding consortium and wildtype culture performance as a function of environment. Subplots A1-A4 culture performance at low environmental pH buffering capacity (6.3 mM phosphate buffer). Subplots B1-B4 quantify culture performance at higher environmental pH buffering capacity (64 mM phosphate buffer). The cross-feeding consortium outperformed the wildtype culture under the environmental context of low pH buffering capacity, while the wildtype culture grew faster and produced more biomass under the environmental context of higher pH buffering.

7. Conclusions

The presented work used synthetic consortia to test hypotheses governing microbial interactions mediated by push or pull metabolite exchange, quantified the inhibitory properties of the exchanged metabolites, calculated the opportunity costs associated with different phenotypes, and demonstrated the powerful role of environmental context on consortia performance. Ultimately, environment constrains whether division of labor strategies can enhance or decrease the fitness of participants.

8. Reference, Data, and Code

DOI: <https://doi.org/10.1128/msystems.00051-22>

Python code and data can be found at:

github.com/rosspcarlson/becketal-syntheticconsortia.git.

9. Acknowledgements

The study was supported by NSF awards DMS 1361240 and DGE 0654336, NIH award U01EB019416, the Interagency Modeling and Analysis Group (IMAG) and the MultiScale Modeling (MSM) Consortium.

10. Contact Information

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