Title

Photosynthetic biohybrid coculture for tandem and tunable CO2 and N2 fixation

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Supplementary Information for

Photosynthetic biohybrid co-culture for tandem and tunable CO_2 and N_2 fixation

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This file includes:

Supplementary Text

Cell culture medium preparation recipes and notes:

Unless otherwise noted all the media is prepared in degassed H_2O. H_2O is degassed by purging N_2/Ar until boiling. 25% more ultrapure water is used to account for evaporation losses. In order to prevent precipitation of medium components during autoclaving, certain components are added after autoclaving (or boiling). These were separately autoclaved, or syringe filtered. The hungate technique was employed to maintain anoxic media and solutions.

Discussion on acetate to fixed N conversion efficiency:

SUPPLEMENTARY TABLES

	STOCK	BETAINE MEDIUM	YEAST MEDIUM	AUTOTROPHIC MEDIUM
BEFORE BOILING	NaCl, $MgSO_4$, NH_4Cl and $CaCl_2$ (5X) [1]	20 mL	20 mL	20 mL
	K_2HPO_4 and KH_2PO_4 (5X) [2]	20 mL	20 mL	20 mL
	$FeSO_4$ (500X) [3]	0.2 mL	0.2 mL	0.2 mL
	$NaHSeO_3$ (1000X) [4]	0.1 mL	0.1 mL	0.1 mL
	Trace Metal [5]	0.1 mL	0.1 mL	0.1 mL
	Tungstate (1X) [6]	0.1 mL	0.1 mL	0.1 mL
	Ultrapure water	55 mL	55 mL	55 mL
BEFORE AUTOCLAVE	Yeast	0.2 g	0.2 g	
	Casitone	0.2 g		
AFTER AUTOCLAVE	$NaHCO_3$ (8g/100mL)	5 mL	5 mL	5 mL
	Vitamin [7]	1 mL	1 mL	1 mL
	Reducing Reagent [8]	2 mL	2 mL	2 mL
	Betaine stock [9]	5 mL		
	Total:	**100 mL**	**100 mL**	**100 mL**

***S. ovata* Medium Supplementary Table 1**

	STOCK	CO-CULTURE MEDIUM
BEFORE AUTOCLAVE	NaCl, $MgSO_4$, and $CaCl_2$ (5X) [10]	20 mL
	$FeSO_4$ (500X) [3]	0.2 mL
	$NaHSeO_3$ (1000X) [4]	0.1 mL
	Trace Metal [5]	0.1 mL
	Tungstate (1X) [6]	0.1 mL
	Ultrapure water	54 mL
AFTER AUTOCLAVE	$NaHCO_3$ (8g/100mL)	5 mL
	Vitamin [7]	1 mL
	K_2HPO_4 and KH_2PO_4 [2]	20 mL
	Total:	**100 mL**

Co-culture Medium Supplementary Table 2

	STOCK	CO-CULTURE MEDIUM
BEFORE AUTOCLAVE	NaCl, $MgSO_4$, and $CaCl_2$ (5X) [11]	5 mL
	NaCl (5X) [12]	15 mL
	$FeSO_4$ (500X) [3]	0.2 mL
	$NaHSeO_3$ (1000X) [4]	0.1 mL
	Trace Metal [5]	0.1 mL
	Tungstate (1X) [6]	0.1 mL
	Ultrapure water	54 mL
AFTER AUTOCLAVE	$NaHCO_3$ (8g/100mL)	5 mL
	Vitamin [7]	1 mL
	K_2HPO_4, Na_2HPO_4 KH_2PO_4 and NaH_2PO_4 [13]	20 mL
	Total:	**100 mL**

Co-culture Electrolyte Table 3

	STOCK	PM MEDIUM	DIAZO/PHOTO-HETEROTROPHIC MEDIUM	DIAZO/PHOTO-AUTOTROPHIC MEDIUM
BEFORE AUTOCLAVE	0.5 M Na_2HPO_4	2.5 mL	2.5 mL	2.5 mL
	0.5 M KH_2PO_4	2.5 mL	2.5 mL	2.5 mL
	10% $(NH_4)_2SO_4$	1 mL		
	Concentrated Base [14]	0.1 mL	0.1 mL	0.1 mL
	0.1 M $Na_2S_2O_3$	0.1 mL	0.1 mL	
	PABA (2 mg/mL)	0.1 mL	0.1 mL	0.1 mL
	0.5 M $NaHCO_3$			5 mL
	Ultrapure water	92 mL	95 mL	88 mL
AFTER	1M Sodium Acetate	2 mL (20mM)	Variable	

AUTOCLAVE	NiCl			1 µM
	Total:	**100 mL**	**100 mL**	**100mL**

***R. palustris* Medium Supplementary Table 4**

[1] NaCl 11.25 g/L, $MgSO_4{*}7H_2O$ 2.5 g/L, NH_4Cl 2.5 g/L and $CaCl_2$ 1.25 g/L

[2] K_2HPO_4 1.74 g/L and KH_2PO_4 1.135 g/L

[3] $FeSO_4$ $7H_2O$ 1 g/L

[4] $NaHSeO_3$ $5H_2O$ 10^{-4} mol/L

[5] Trace Metal

Component	**Amount**
HCl (25%)	10 mL
$FeCl_2$ $4H_2O$	1.5 g
$ZnCl_2$	0.07 g
$MnCl_2$ $4H_2O$	0.1 g
H_3BO_3	0.006 g
$CoCl_2$ $2H_2O$	0.19 g
$NiCl_2$ $6H_2O$	0.002 g
Na_2MoO_4 $2H_2O$	0.036 g
Ultrapure water	990 mL
Total	**1000 mL**

[6] $NaWO_4$ $2H_2O$ 4 g/L

[7] Vitamin

Component	**Amount**
Biotin	2 g
Folic acid	2 g
Pyridozine-HCl	10 g
Thiamine-HCl	5 g
Riboflavin	5 g
Nicotinic acid	5 g
Ca-D-pantothenate	5 g
Vitamin B_{12}	0.1 g
p-Aminobenzoic acid	5 g
a-Lipoic acid	5 g
Ultrapure water	1000 mL
Total	**1000 mL**

[8] Reducing Reagent

Component	**Amount**
L-Cysteine-HCl H_2O	1.5 g
Na_2S $9H_2O$	1.5 g
Ultrapure water	100 mL
Total	**100 mL**

[9] Betaine H_2O 1.34 g/L

[10] NaCl 11.25 g/L, $MgSO_4{*}7H_2O$ 2.5 g/L, and $CaCl_2$ 1.25 g/L

[11] NaCl 4.518 g/L, $MgSO_4{*}7H_2O$ 2.5 g/L, and $CaCl_2$ 1.25 g/L

[12]NaCl 4.518 g/L

[13] K_2HPO_4 1.74 g/L KH_2PO_4 1.135 g/L, $Na_2HPO_4{*}7H_2O$ 10.725 g/L, $NaH_2PO_4{*}H_2O$ 4.69 g/L

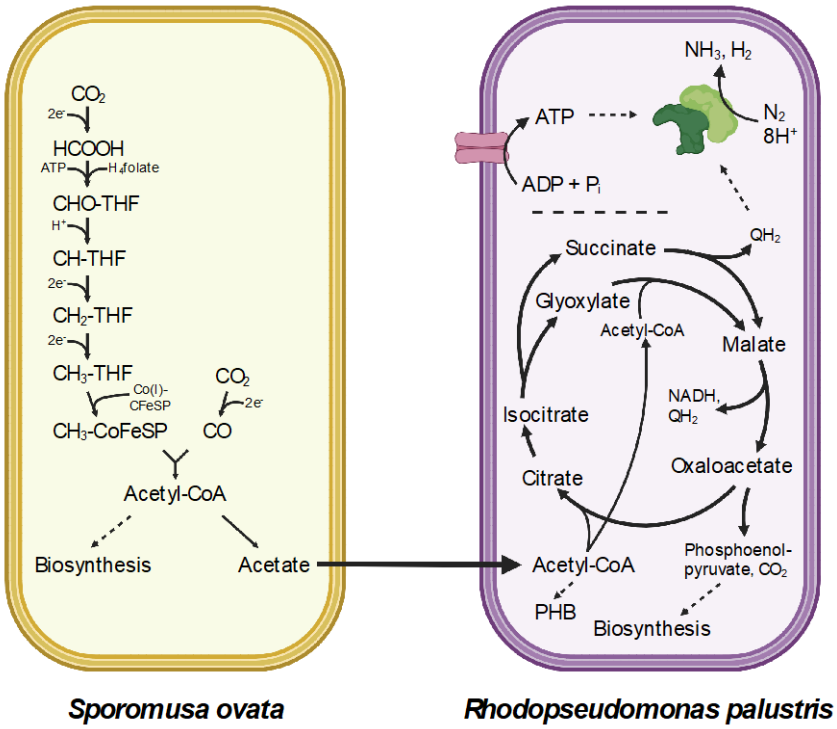
[14] Concentrated Base

Component	**Amount**
Nitrilotriacetic acid (free acid)	20 g
$MgSO_4$ anhydrous	28.9 g
$CaCl_2$ $2H_2O$	6.67 g
$(NH_4)_6Mo_7O_{24}$ $4H_2O$	0.0185 g
$FeSO_4$ $7H_2O$	0.198 g
Ultrapure water	900 mL
Metal 44 [15]	100 mL
Total:	**1000 mL**

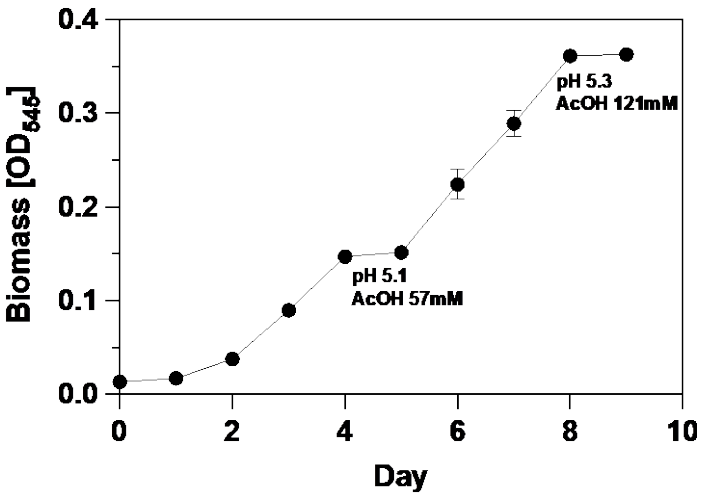
[15] Metal 44

Component	**Amount**
EDTA (free acid)	2.5 g
$ZnSO_4$ $7H_2O$	10.95 g
$FeSO_4$ $7H_2O$	5 g
$MnSO_4$ H_2O	1.54 g
$CuSO_4$ $5H_2O$	0.392 g
$Co(NO_3)_2$ $6H_2O$	0.25 g
$Na_2B_4O_7$ 10 H_2O	0.177 g
Ultrapure water	1000 ml
Total:	**1000 mL**

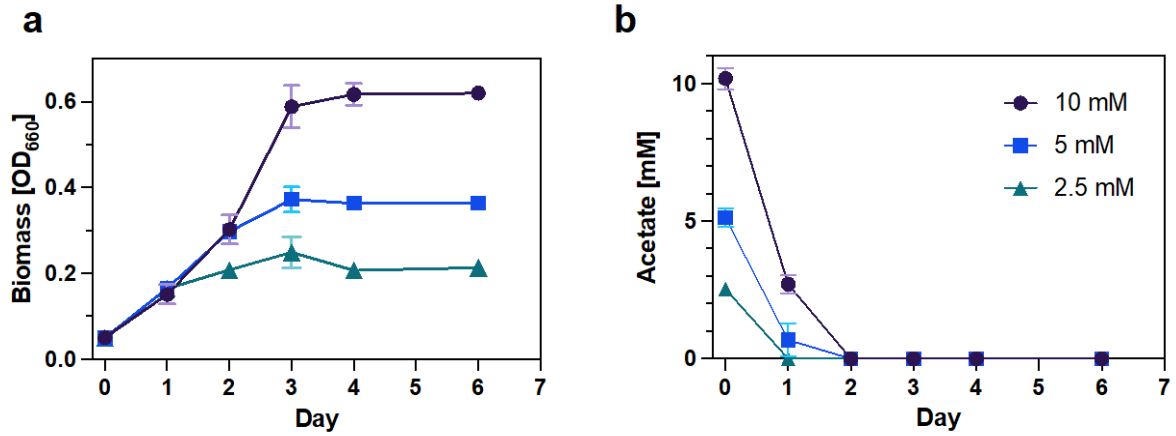
Supplementary Figures



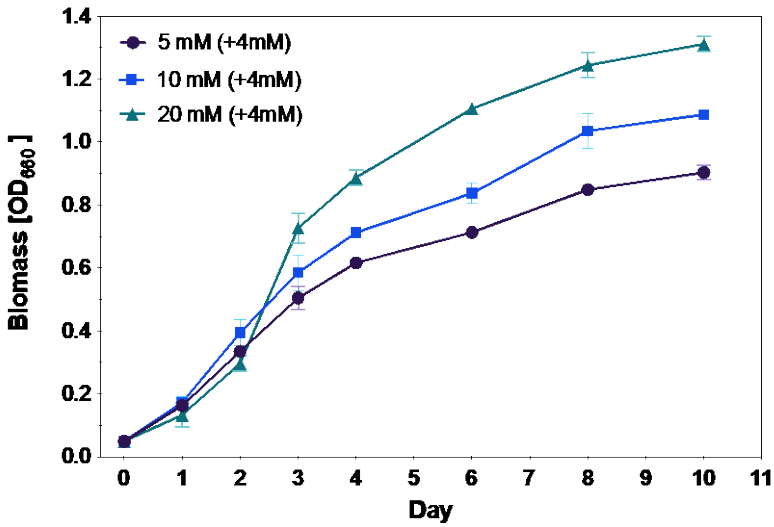
Supplementary Figure 1 | Overview of internal metabolic pathways in *S. ovata* and *R. palustris nif*A*. *S. ovata* converts CO_2 to acetate via the Wood-Ljungdahl pathway in which 1 CO_2 is converted to formate to be adhered onto tetrahydrofolate and finally combined with an iron coronoid protein as a methyl group. The methyl group is joined with CO to form Acetyl-CoA which is either converted to acetate (releasing ATP) is used in protein biosynthesis. *R. palustris nif*A* initially transforms acetate to Acetyl-CoA which is then used in the tricarboxylic acid cycle with a glyoxylate shunt to generate reducing equivalents (to be used in cellular processes like N_2 hydrogenation) and produce metabolic intermediates for protein biosynthesis.



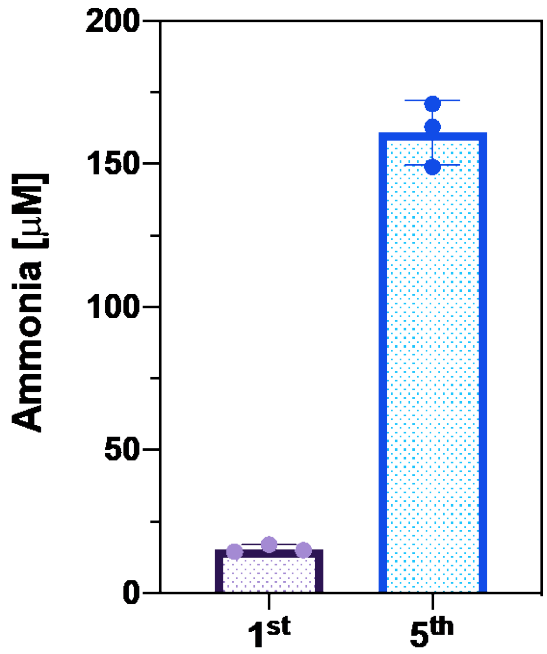
Supplementary Figure 2 | Autotrophic *S. ovata* culture shows dependency of culture growth with medium pH. Autotrophic medium has a pH of 6.8 pre-inoculation, which decreases to ~5.2 with an acetate (AcOH) concentration of ~60mM. Culture growth plateaus once medium is acidified, but growth can be recovered by stabilizing medium pH to 6.8. Culture plateaus once again with medium acidification. Error bars represent one standard deviation of three independent measurements.



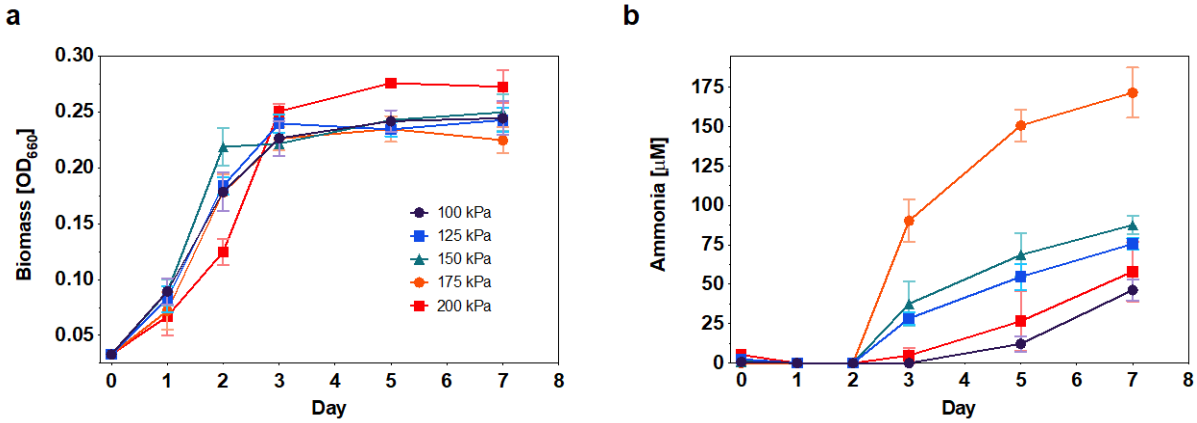
Supplementary Figure 3 | *R. palustris nif*A* cultures supplemented with 2.5mM, 5mM and 10mM acetate. a) Final biomass yield is proportional to the acetate provided. b) Tracking the acetate concentration reveals that the acetate is largely consumed by the mid-exponential phase. Error bars represent one standard deviation of three independent measurements.



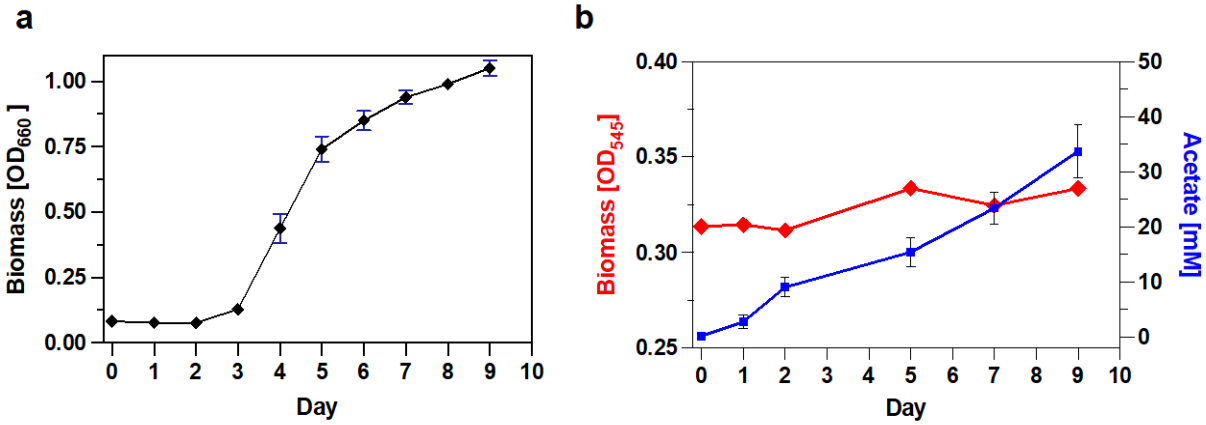
Supplementary Figure 4 | *R. palustris nif*A* cultures are supplemented with 5mM, 10mM and 20mM acetate initially and an additionally 4mM acetate is provided on days 2, 4 and 6. This indicates that further *R. palustris nif*A* culture growth may be induced by additional acetate provision. Error bars represent one standard deviation of three independent measurements.



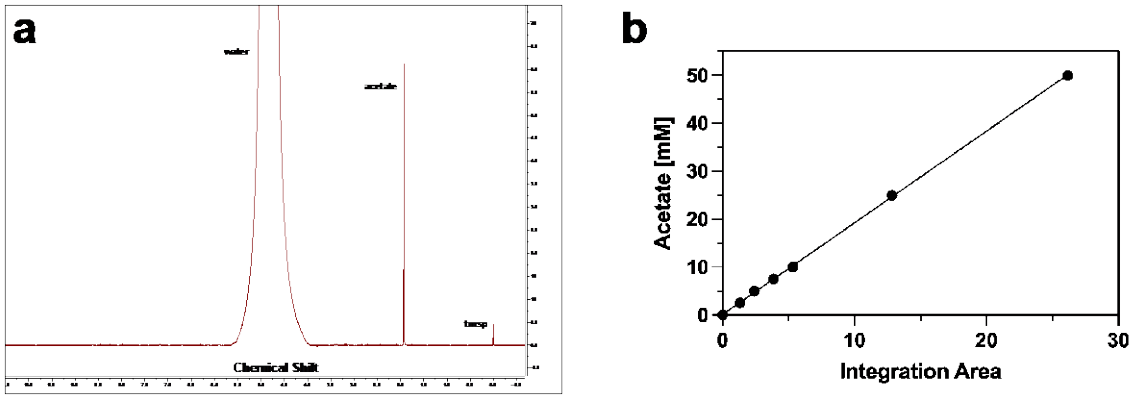
Supplementary Figure 5 | *R. palustris nif*A* cultures grown diazotrophically in 1st cycle from frozen stock and 5th cycle from frozen stock. Ammonia secretion in *R. palustris nif*A* may be increased by recursively selecting for cultures with the highest ammonia content. Additionally, cultures soon after the frozen stock stage may not be as metabolically active. Error bars represent one standard deviation of three independent measurements.



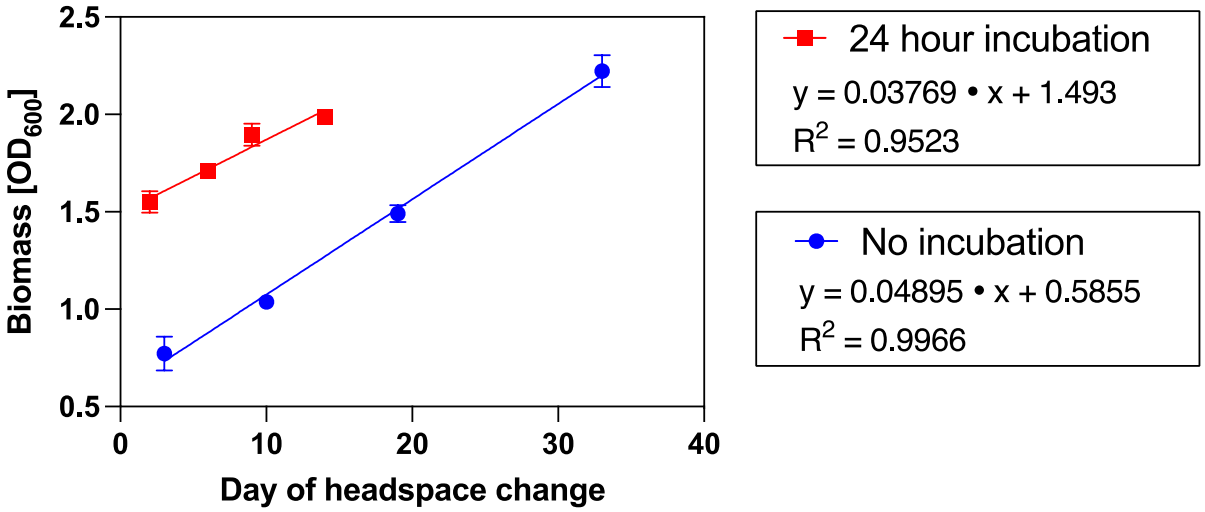
Supplementary Figure 6 | a) Diazotrophic, photoheterotrophic *R. palustris nif*A* cultures grown under increasing headspace pressures. Cultures were supplemented with 2.5mM acetate and kept under pure N_2 headspace. b) Extracellular ammonia increases with increasing headspace pressure up to 175kPa. Error bars represent one standard deviation of three independent measurements.



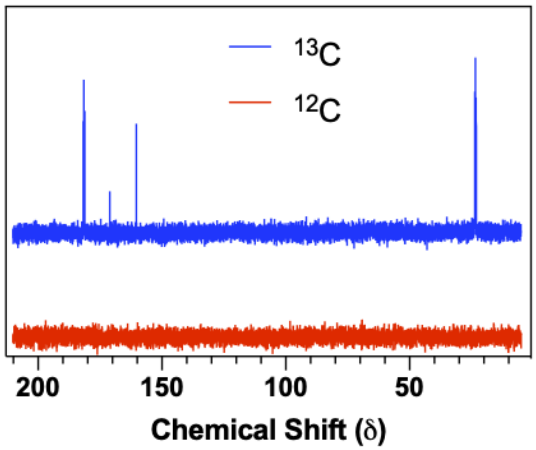
Supplementary Figure 7 | *R. palustris nif*A* and *S. ovata* grown individually to verify compatibility of co-culture medium. a) *R. palustris nif*A* culture supplemented with 25mM acetate and b) *S. ovata* culture supplemented with 100uM ammonia. Error bars represent one standard deviation of three independent measurements.



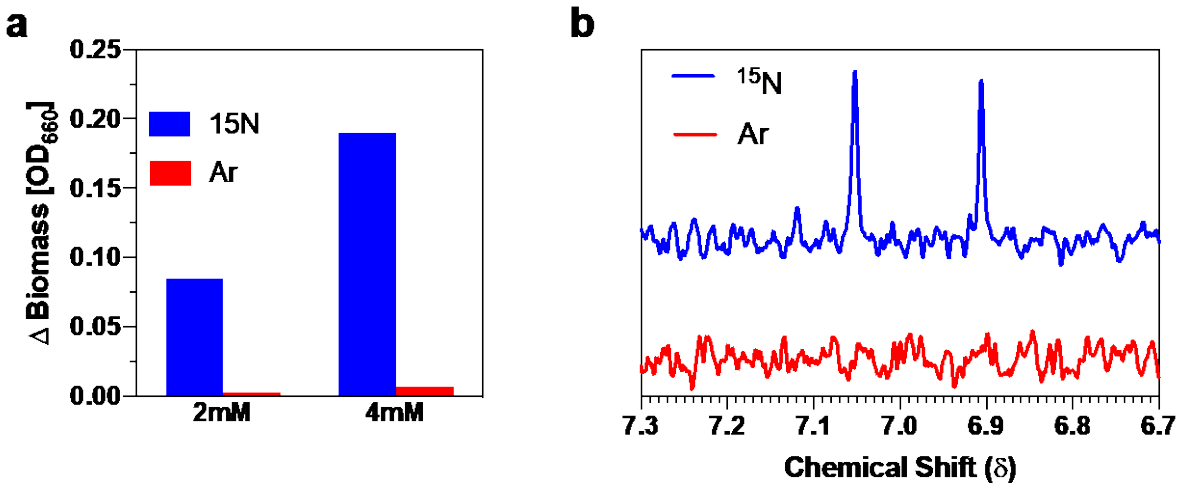
Supplementary Figure 8 | a) Representative ^{1}H-NMR spectrum of the medium from an autotrophic *S. ovata* culture. Protons from the methyl group of acetate are detected at 1.92 ppm. sodium 3- (trimethylsilyl)-2,2',3,3'-tetradeuteropropionate (tmsp) is used as an internal standard. b) Calibration curve for acetate quantification with ^{1}H-NMR (R^2=0.999).



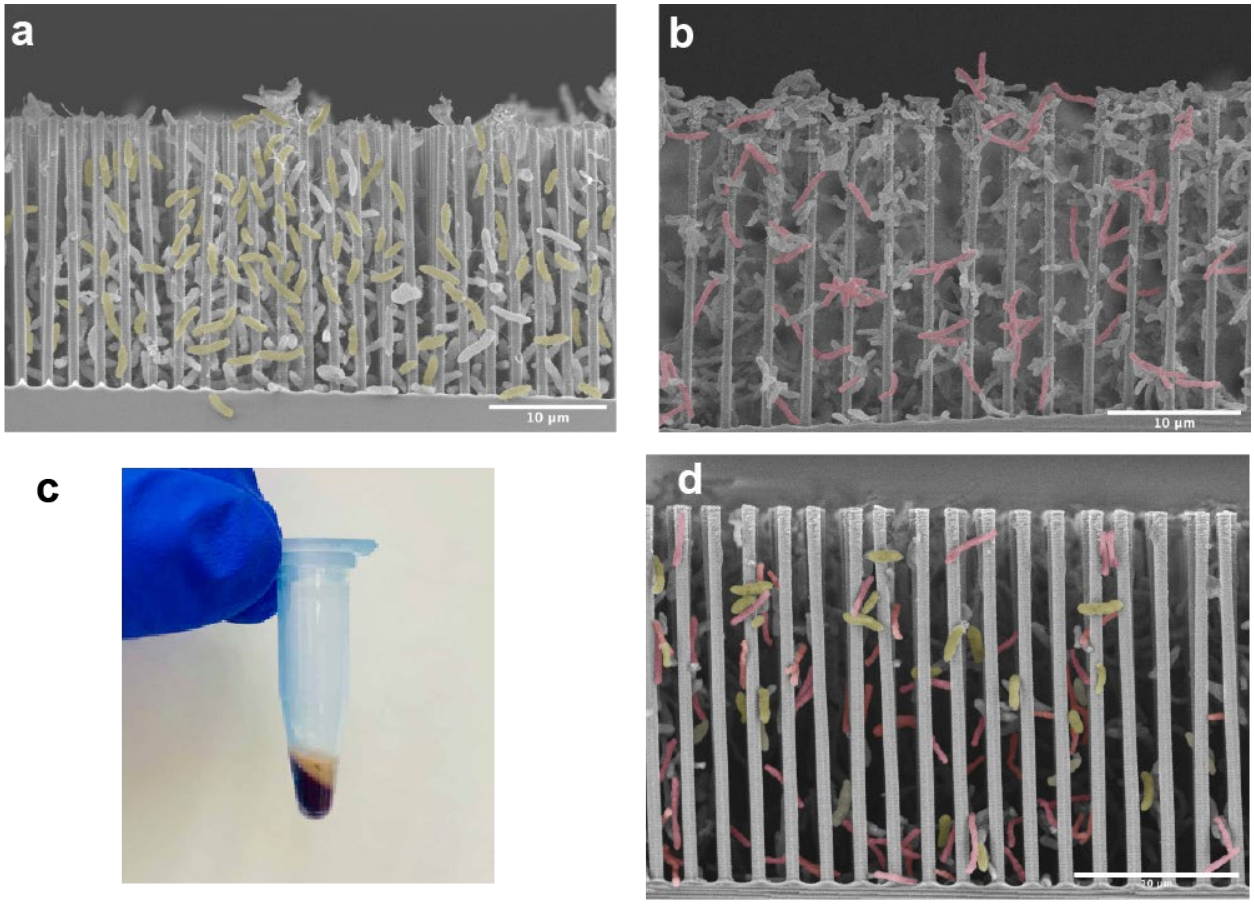
Supplementary Figure 9 | *R. palustris nif*A* and *S. ovata* co-culture growth dynamic. Blue plot corresponds to co-cultures where *R. palustris nif*A* and *S. ovata* were inoculated simultaneously while the red plot corresponds to the co-cultures where *S. ovata* was incubated individually for 24 hours prior to the addition of *R. palustris nif*A*. Plot points denote the biomass yield on the day on which the headspace was exchanged to pure N_2. Linear regression fit indicates a linear relation between biomass yields in different co-cultures conditions. Error bars represent one standard deviation of three independent measurements.



Supplementary Figure 10 | Carbon NMR spectrum depicting the produced isotope labeled acetate from ^{13}C labeled experiment (blue) and the unlabeled experiment (red) without any isotope labeled acetate.



Supplementary Figure 11 | *R. palustris nif*A* cultures with $^{15}N_2$ and Ar headspaces supplemented with 2mM and 4mM acetate. A) Total biomass yield for each condition. B) ^{1}H-NMR spectrum with $^{15}NH_3$ (blue) and no NH_3 for cultures grown with Ar (red).



Supplementary Figure 12 | Scanning electron micrographs of pure a) *S. ovata* and b) *R. palustris nif*A* cultures on silicon nanowire arrays. c) *S. ovata* and *R. palustris nif*A* co-culture ultracentrifuged (12,000 RPM) showing separation of individual bacterial strains visible by color (*S. ovata* is yellow/beige and *R. palustris* is dark pink). The approximate final cell ratio is 2:3 *S. ovata* to *R. palustris nif*A*, having been seeded at a 6:1 *S. ovata* to *R. palustris nif*A* cell ratio. d) Scanning electron micrograph of *S. ovata* and *R. palustris nif*A* co-culture after a routine electrochemical experiment. *S. ovata* and *R. palustris* are false-colored yellow and pink respectively.