Supplementary information

Steady-State Growth under Inorganic Carbon Limitation Conditions Increases Energy Consumption for Maintenance and Enhances Nitrous Oxide Production in *Nitrosomonas europaea*.

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SUPPLEMENTAL MATERIALS AND METHODS

Chemostat experiments. Chemostat culture experiments to determine maintenance energy were carried out as outlined in the main text materials and methods with minor changes. To determine maintenance energy under ammonia (NH_3)-limited conditions, medium consisted of 5 mM $(NH_4)_2SO_4$ and mineral salts as described (1). Inorganic carbon (IC)-limited maintenance energy was calculated using culture conditions and data (see Fig. 2) from the main text.

Dry cell weight calculations. Assume that the specific growth rate, μ, for each organism is equal to the dilution rate, D; i.e., assume that the saturation constant for the limiting substrate, K_s, is small. Dry cell weight in chemostat (DCWc) from optical density at 600 nm (OD_{600}) was calculated from end point values for DCW and OD_{600} from fed-batch cultures (2). The conversion value calculated was 0.160 gDCW L^{-1} OD_{600}^{-1} for *Nitrosomonas europaea* (Eqn. S.1).

S.1 $$gDCWc = 0.16 \frac{gDCW}{L\ OD} * OD$$

Growth yield calculations. Calculation of the rate (q) of ammonia/ammonium (substrate) consumption was accomplished as outlined in Eqn. S.2.

S.2 $$q(substrate) = \frac{Dilution\ Rate(Outlet\ Concentration\ Substrate - Inlet\ Concentration\ Substrate)}{Cell\ Concentration\ in\ Chemostat}$$

Apparent growth yield expressed as cells produced per substrate consumed (gDCW [mmol NH_3]$^{-1}$) was calculated as outlined in Eqn. S.3 and S.4 where gDCWp is dry cell weight produced through cell growth.

S.3 $$Yield = \left|\frac{q_x}{q_{NH3}}\right|$$

S.4 $$Yield = \left| \frac{D * \frac{gDCWp}{gDCWc\,h}}{\frac{mol}{gDCWc\,h}} \right| = \frac{gDCWp}{mmol}$$

Maintenance energy calculations. Cellular maintenance energy (*m*) was calculated based on the specific growth rate assumptions outlined above and the growth yield using the Pirt equation (Eqn. S.5) with D substituted for μ (3-5). The intercept is the maintenance energy and the slope is the inverse growth yield in the absence of consumption of substrate for maintenance (Y_g, true growth yield).

S.5 $$q = \frac{D}{Y_G} + m$$

Table S1. Statistically significant changes in gene expression during IC limitation.

Gene number	Gene name	Role	Fold change[a]
C fixation and metabolism			
NE0050-0051	*sucC*, *sucD*	succinyl-CoA synthetase	-1.41 to -1.77
NE0325-0328	*pykA*, *pgk*, *cbbG*, *cbbT*	Gluconeogenesis, carbon fixation	1.39 to 1.82
NE0606	*cah*	carbonic anhydrase	-1.25 to -1.56
NE1730	*icd*	isocitrate dehydrogenase	1.78 to 2.19
NE1917-1922	*cbbOQSR*, *rbcL*	RuBisCO gene cluster	1.81 to 19.88
NE1926	*cynT*	carbonic anhydrase	1.42 to 1.45
Amino acid and nucleotide biosynthesis			
NE1319-1320	*leuA1*	leucine biosynthesis	2.03 to 2.29
NE1323	*ilvC*	valine/isoleucine biosynthesis	1.31 to 1.71
NE1661-1662	*carA, carB*	carbamoyl-phosphate synthase (arginine and pyrimidine biosynthesis)	1.44 to 2.01
N metabolism			
NE0448	*rh50*	ammonium uptake	-2.04 to -3.58
NE0924-0927	*aniA* (*nirK*) gene	nitrite reductase and	1.57 to 3.31

	loci	electron transport	
NE0941-0945,	*amo* gene loci 1 and	ammonia oxidation	-1.33 to
NE2057-2064	2		-2.57
NE1411	*amoC3*	ammonia oxidation	1.00
NE0962, NE2044,	*hao1, hao2, hao3*	hydroxylamine oxidation	1.00
NE2339			
NE2003-2004	*norC, norB*	NO reductase	1.00 to
			-1.59
Electron Transport and Energy Transformation			
NE0143		nitrosocyanin	1.37[b]
NE0199-0206	*atp* gene loci	ATP synthase	1 to 2.30
NE0278		Heme biosynthesis	1.56 to 1.76
NE0314-316	*mnxG*	multi-copper oxidase gene	-1.26 to
		cluster	-6.38
NE0508-0510	SCO1/*senC*	cytochrome assembly	1.88 to 2.51[b]
NE0681-0684	SCO1/*senC*, *coxA2,*	cytochrome c oxidase	-1.47 to
	coxB		-2.13
NE0764-0765	*ccmA, ccmB*	cytochrome biogenesis	1.26 to 1.77
NE0959, NE2336	*cycX3, cycX1*	c554-associated	-1.37[c] to
		cytochromes	-1.41[c]
NE1215		short-chain	-1.46 to
		dehydrogenase/reductase	-1.84
NE1230-1245		oxidoreductase/unknown	-1.48 to

		gene cluster	-5.00
NE1313-1315	*ccp*	cytochrome c551 peroxidase gene cluster	-1.45 to -2.08
NE1764-1777	*nuo* gene loci	NADH dehydrogenase	1.00 to 1.97
NE1838, NE1868-1869	*ubiA*, *ubiB,* putative *ubiJ*	ubiquinone biosynthesis	1.56 to 2.07
NE1951		adenylosuccinate lyase	1.71 to 2.23
NE2130	*rnk*	regulator of nucleotide diphosphate kinases	1.98 to 2.06
NE2393-2397	*nqr* gene loci	Na+-translocating NADH-quinone reductase	-1.30 to -1.89
Carbon metabolism linked to photorespiration			
NE0221-0223		Folate biosynthesis	1.58 to 1.89
NE0362	*folD*	C1 pool folate metabolism	1.43 to 1.62
NE0607-0611	*gcv* gene loci	Glycine cleavage system	1.46 to 2.66
Fatty acid and phospholipid metabolism			
NE0741		4-cresol dehydrogenase	-1.56 to -1.96
NE1645, NE0612, NE1713, NE1321-1322	*plsX, plsC, cdsA, pssA, psd*	phosphatidylethanolamine biosynthesis	1.42 to 2.43
NE1646	*fabH*	3-oxoacyl-ACP synthase	1.37 to 1.41
NE2348-2353	*fadE1*, *ydiD*, *moeZ*	fatty acid degradation loci	-1.53 to

			-2.46
Replication			
NE0433	*dnaX*	DNA pol III γ/τ	1.37 to 1.43
NE0442	*holC*	DNA pol III χ	1.52 to 1.67
Signaling/ Transcription			
NE0533-536, NE0557		ECF sigma factors (sigma 70-type) and membrane-associated factors	-2.32 to -4.38
NE0584-0586	*rpoH*	Heat-shock sigma factor 32 and associated genes	1.42 to 2.57
NE0895-0896		hypothetical DNA-binding	1.53 to 2.37
NE1035	*rho*	termination factor ρ	2.07 to 2.58
NE1079, NE2435	*fecI*	ECF sigma factors	1.48 to 3.03
NE1287	*hfq*	small RNA regulator	-1.34 to -2.04
NE1313		DnaJ/RegA homolog	-1.45 to -2.08
NE1477	*hrcA*	heat-inducible repressor	1.35 to 1.53
NE1660	*greA*	transcription elongation	1.42 to 1.47
NE2138		ECF sigma factor	-2.48 to -3.46
NE2291		cAMP phosphodiesterase	1.67[b]
Transport			

NE0827, NE1453-1454		ABC-type gene clusters	1.61 to 2.18
NE0869	*exbD/tolR*	biopolymer transport	-1.43 to -1.61
NE1196		ABC-type transporter	-1.44 to -1.86
NE1529-1532, NE1535-37		TonB-dependent receptor gene clusters	-1.64 to -5.75
NE1541-42		putative hemin transport	2.04 to 5.60[b]
NE1834	*trkA*	K+ transporter	1.50 to 1.70
NE1906		monovalent cation/H+ antiporter	1.85 to 2.05
NE2059		copper export	-1.56 to -1.69
NE2290		type II secretion protein E	1.82[b]
Ribosomal			
NE0196-0197, NE0400-0407, NE1292-1293, NE1483-1484 NE1825	*rpsRFUJSCI, rnpA, rpmHA, rplCDWBVUMY*	Ribosome-associated proteins	1.23 to 2.89
NE0399	*tuf2_1*	Elongation factor Tu	1.51 to 2.15
NE0955	*rplT*	Ribosome-associated	-1.42 to

		proteins	-1.48
NE1672	*rim*	16S rRNA-processing	1.38 to 1.56
Protein synthesis and stress response			
NE0027-0031,	*groES, groEL, tig,*	chaperonins, protein	1.28 to 5.39
NE0035	*clpP*	export/processing	
NE0084		thioredoxin	-1.27 to
			-1.46
NE0367		TrxA homolog	-1.45 to
		(thioredoxin)	-1.74
NE0813	*sspB*	stringent starvation protein	1.38 to 1.42
		B	
NE1201		UspA homolog	-1.29 to
			-1.51
NE1312	*cspD2_1*	cold shock protein	1.73 to 1.81
NE1712	*dxr*	1-deoxy-D-xylulose 5-	1.44 to 1.95
		phosphate	
		reductoisomerase	
		(terpenoid biosynthesis)	
NE1948	*dnaJ*	heat shock chaperonin	1.51 to 1.74
NE1950	*grpE*	heat shock chaperonin	1.64 to 1.66
NE2074-2076		HSP20 family gene cluster	-1.37 to
			-2.61
NE2131	*phoB*	phosphate regulon	1.80[b]

NE2292	*yxiE*	universal stress protein	1.59[b]
NE2553-2554	*typA*	GTP-binding, stress-associated gene cluster	1.61 to 2.32
Hypothetical			
NE0155, NE0230, NE0251, NE0271[b], NE0453, NE0585, NE0895, NE1821, NE1839		mobile element or phage-associated	1.56 to 3.09
NE0255-0256, NE0588, NE0745, NE1135, NE1179, NE1230, NE1350, NE1353, NE1363, NE1379-1380, NE1790, NE2151-2154, NE2409, NE2413, NE2441, NE2513		mobile element or phage-associated	-1.33 to -12.36
NE1445-1452	*ycf16*	NifU domain and Fe-S cluster assembly gene cluster	-1.58 to -2.15
NE1542		conserved hypothetical	5.60[b]

NE1545	pirin domain	-5.56 to -8.00
NE1546	putative oxidoreductase	-2.80 to -3.08
NE2218	conserved hypothetical	8.00[b]
NE2544-2545	hypothetical, potential DNA-binding motif	1.66 to 3.56

[a]Fold change is the difference in mRNA transcripts between the control (replete inorganic C) and the treatment (1.0 mM or 0.2 mM Na_2CO_3) ($p \leq 0.05$). A value of 1.00 indicates no statistically significant change in at least one of the listed genes.

[b]Significant fold change only observed in 0.2 mM Na_2CO_3 treatment.

[c]Significant fold change only observed in 1.0 mM Na_2CO_3 treatment

Table S2. Comparison of fold change expression between replete and IC-limited treatments analyzed by qPCR and mRNA-Seq.

Gene; name	1.0 mM Na_2CO_3 treatment fold change		0.2 mM Na_2CO_3 treatment fold change	
	mRNA-Seq	qPCR	mRNA-Seq	qPCR
NE0328; *cbbT*	1.82	2.64	1.40	1.23
NE0448; *rh50*	-3.58	-3.31	-2.03	-1.96
NE0533; Sigma-70	-4.17	-2.49	-4.38	-4.75
NE1238; oxygenase	-3.01	-1.35	-1.61	-2.92
NE1918; *cbbO*	15.66	15.97	12.58	9.21
NE1919; *cbbQ*	19.88	15.46	14.15	11.98
NE1921; *rbcL*	8.12	9.49	7.42	5.45

Table S3. Genes and primers used to corroborate gene expression expression.

Gene name	Left primer	Right primer
NE0328;	NE0328_L:	NE0328_R:
cbbT	ACAGGACAAACCCACACTGA	CTGCAGCAATTTCGTCATTT
NE0448;	NE0448_L:	NE0448_R:
rh50	GTTGTGTTTCAGCAGTTGGG	GCATGGCCAGAATGTTTATG
NE0533;	NE0533_L:	NE0533_R:
Sigma-70	CTGATGCTGATCGATGGACT	CTGATCGAGACTTGCATCGT
NE1238;	NE1238_L:	NE1238_R:
oxygenase	CCTCATCTGCAATCATCCAC	GAAGCTGGTCAATTGCTTCA
NE1918;	NE1918_L:	NE1918_R:
cbbO	GTGCAGCTTGCTCATGAAAT	ATCGCTCGAAATCAAATTCC
NE1919;	NE1919_L:	NE1919_R:
cbbQ	GGCGCGTAATCTTAAAGGTC	TACGACAAGCTGCATGAACA
NE1921;	NE1921_L:	NE1921_R:
rbcL	TGGAAGCAATCCATAAGGC	GCTCTTTGGCATATTCAGCA

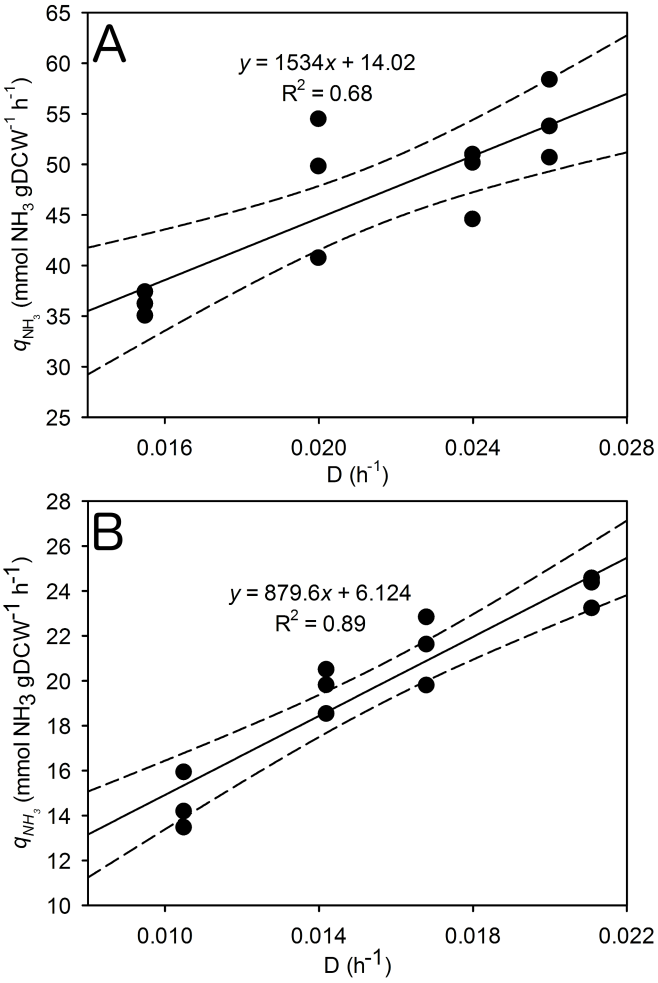

Figure S1. Maintenance energy during IC-limited (**A**) and NH_3-limited (**B**) growth. Maintenance energy is calculated based on the *y* intercept of the linear trend observed when comparing the rate of NH_3 consumption (q_{NH3}, mM NH_3 consumed $gDCW^{-1}$ h^{-1}; *y*-axis) to dilution rate (D, h^{-1}; *x*-axis). The straight black line is the regression of q_{NH3} compared to D and dotted lines indicate the 95% confidence band. The regression for IC-limited growth (**A**) was $y = 1534x + 14.02$, $R^2 = 0.68$, and the predicted maintenance energy was 14.0 mmol NH_3 $gDCW^{-1}$ h^{-1}. The regression for NH_3-limited growth (**B**) was $y = 879.6x + 6.124$, $R^2 = 0.89$, and the predicted maintenance energy was 6.12 mmol NH_3 $gDCW^{-1}$ h^{-1}. Outlier replicates that deviated ≥ 20% from the mean were removed from analysis.

SUPPLEMENTAL REFERENCES

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