Metabolomics-driven quantitative analysis of ammonia assimilation in *E. coli*

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Supplemental material

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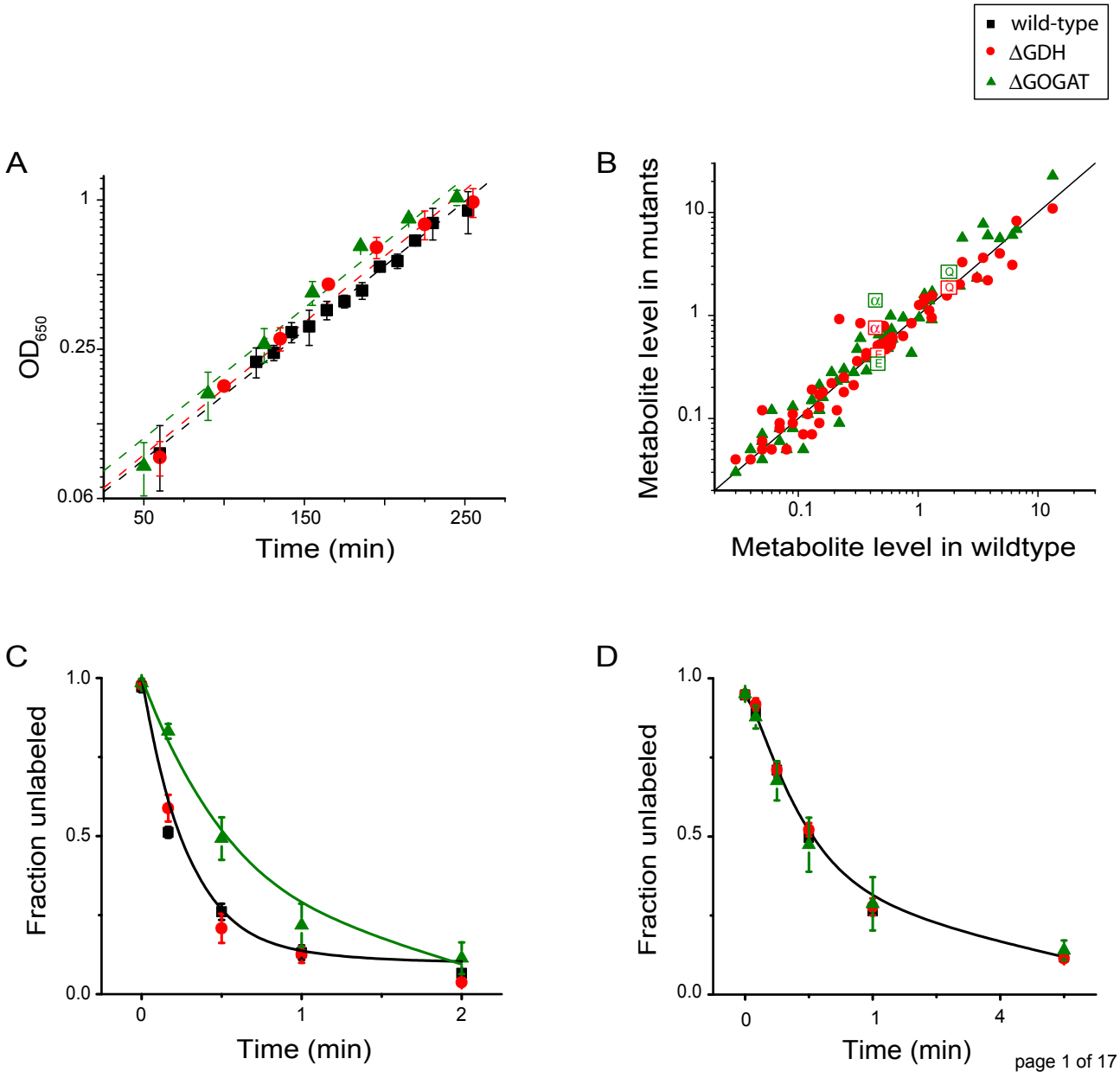
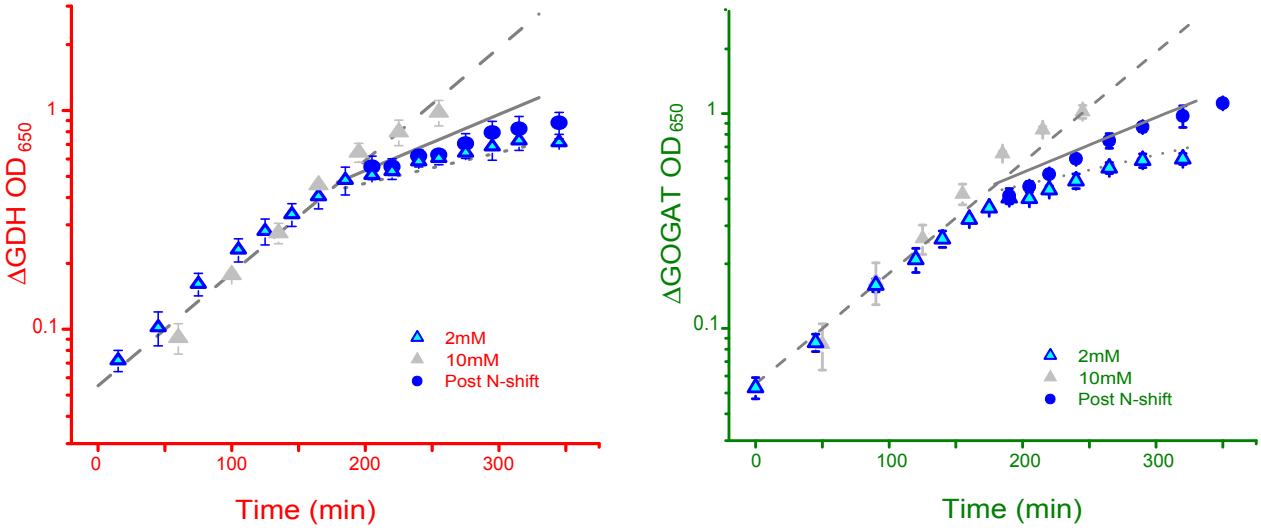
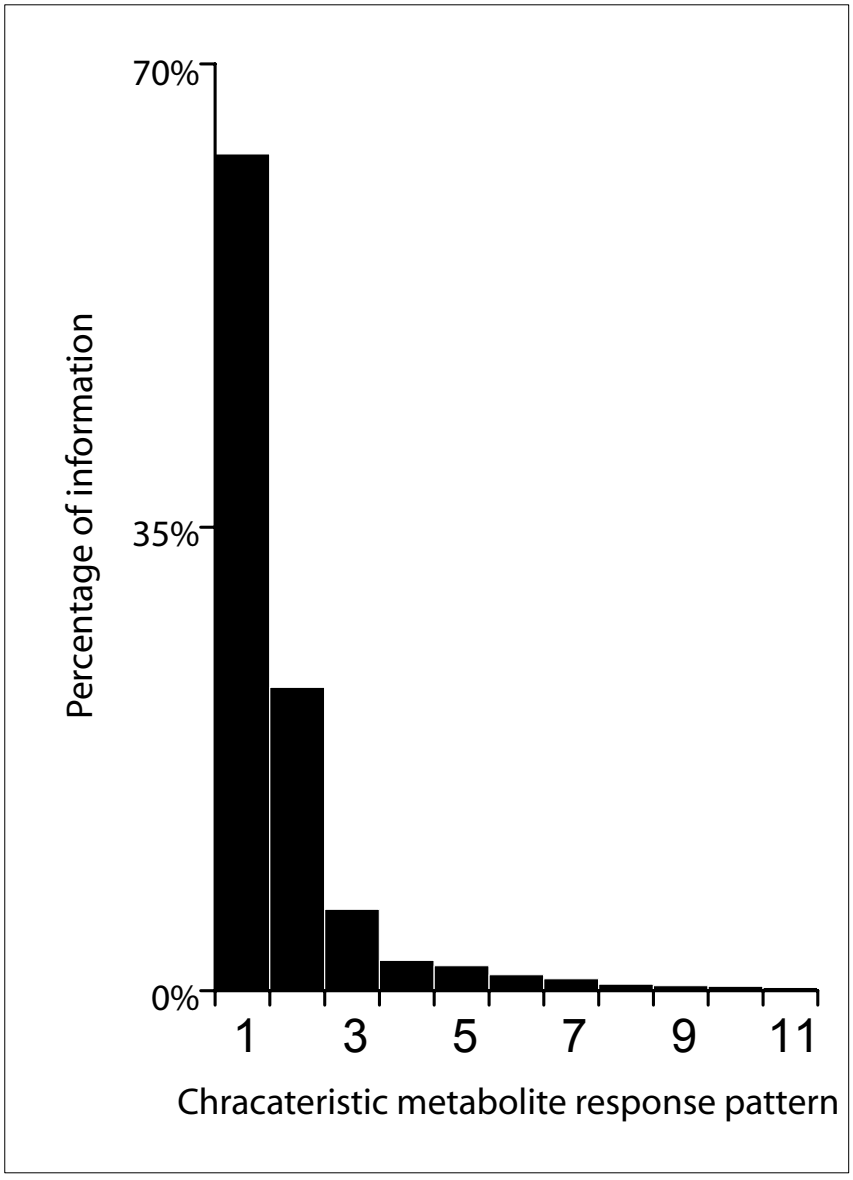
Supplementary Figure 1. Metabolite concentrations and fluxes in ΔGDH and ΔGOGAT *E. coli* grown with ample glucose and ammonium. (A) Growth curves of wild-type (■), ΔGDH (●), and ΔGOGAT (▲) *E. coli* on membrane filters sitting atop agarose plates loaded with minimal media with 10 mM ammonium. Data are mean ± SEM (N = 3 independent experiments) and dashed lines show fits to an exponential growth function. (B) Correlation of metabolite levels in ΔGDH (●) and ΔGOGAT (▲) to those in wild-type; R^2=0.92 for both strains. Boxes show the central nitrogen assimilation compounds α-ketoglutarate (α), glutamate (E), glutamine (Q). Data are means (n≥ 3 independent cultures). Metabolites were measured with LC-MS using ^{13}C cell extract as internal standard. Plotted were ^{12}C signal/^{13}C signal. (C) Kinetic flux profiling results for glutamine. The Y-axis indicates the fraction of unlabeled glutamine as a function of time after switching the cells to ^{15}N-ammonium (X-axis). The rate of decay is proportional to the glutamine flux. (D) Analogous kinetic flux profiling results for glutamate. In C and D, data are mean ± SEM (n≥ 3 for each strain), and curves represent fits generated by Origin (version 6.0, OriginLab Corporation, Northampton, MA) to the following exponential equation $X= (1-\alpha) \exp(-k_x t)+\alpha$, where X represent the fraction of unlabeled metabolite (See Yuan *et al* 2008 for derivation and further details). The color key is the same as in panel A.



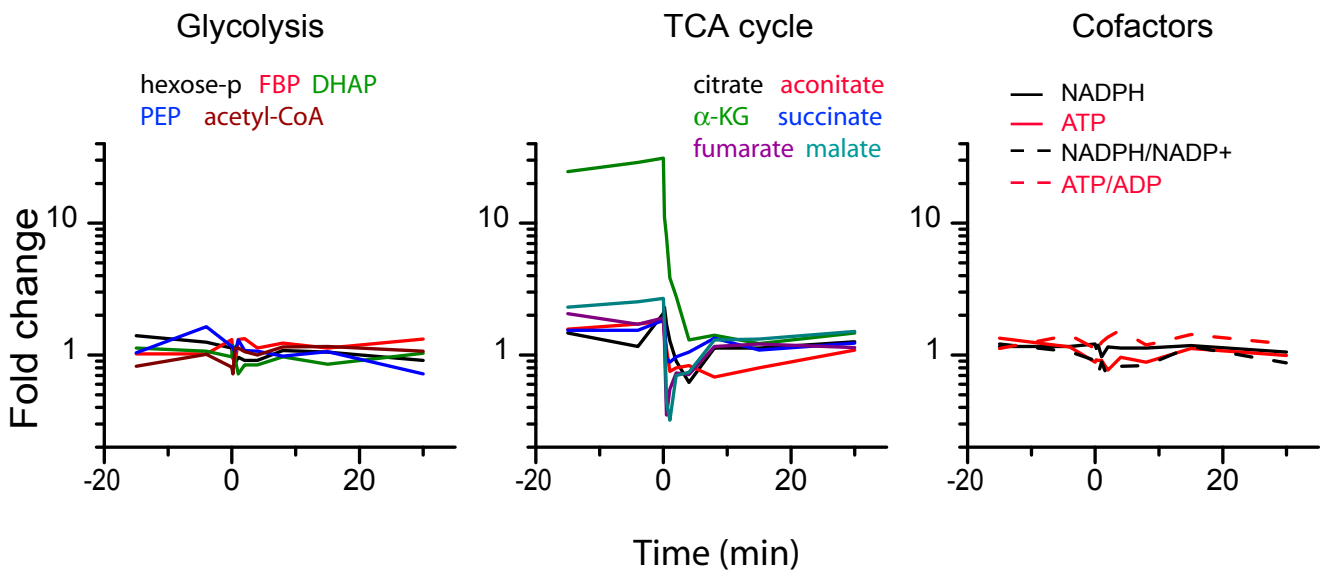
Supplementary Figure 2. Growth of ΔGDH (left) and ΔGOGAT (right) on plates with 10 mM ammonia (gray), 2mM ammonia (cyan), and after N-upshift (blue). Data are represented as mean ± SEM (n≥2 independent experiments) The gray lines are fits to wildtype growth curves as shown in Fig. 2.



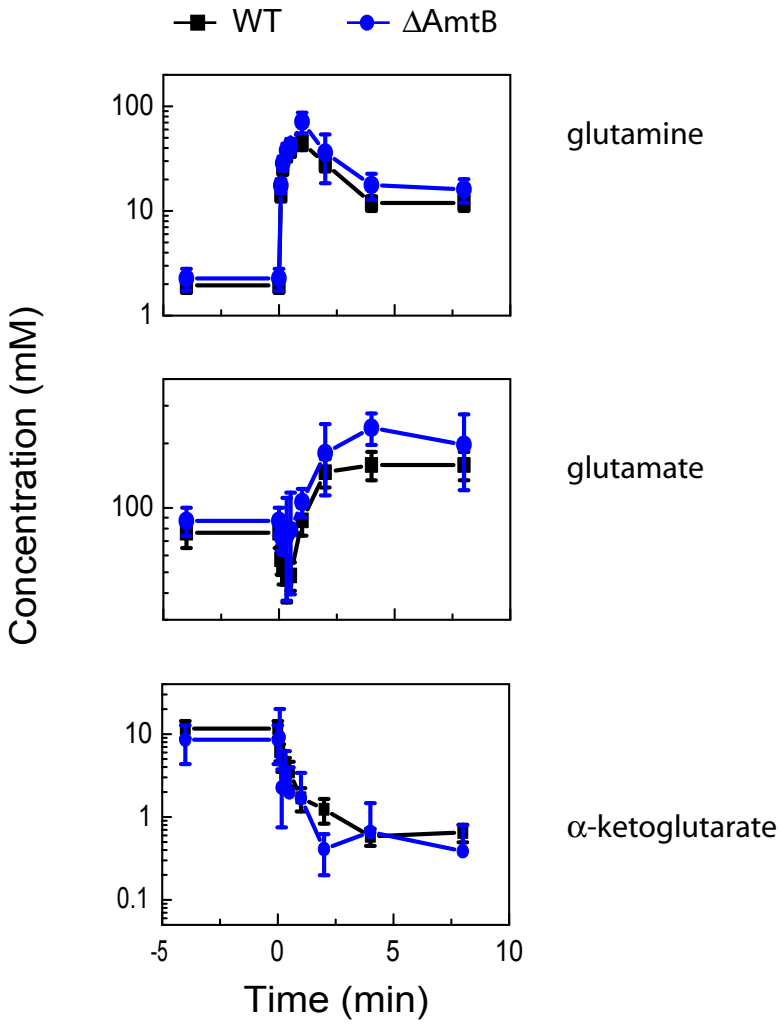
Supplementary Figure 3. Percentage of information in metabolome response matrix (Fig. 3) that is captured by characteristic metabolite response patterns identified by SVD.



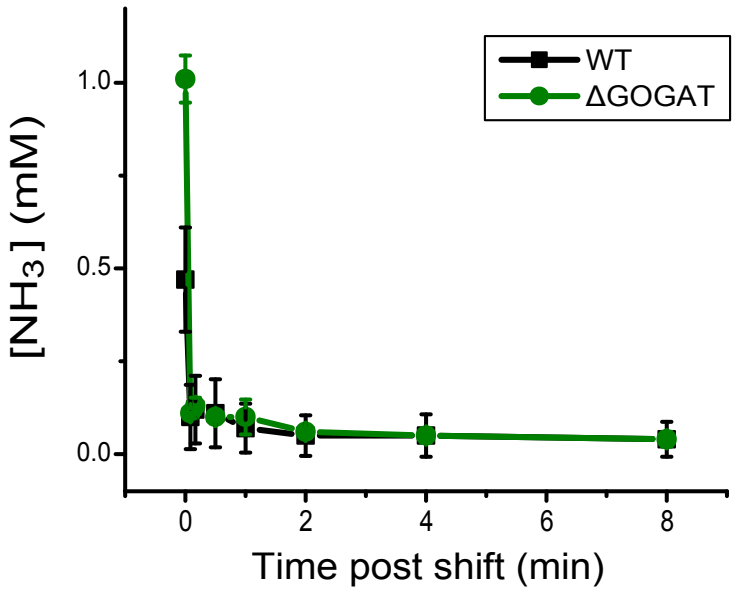
Supplementary Figure 4. Response of glycolytic intermediates (left), TCA cycle intermediates (middle) and key cofactors (right) to N-upshift. Fold change is relative to exponentially growing *E.coli*, and the data are the same as those used for Fig. 3.



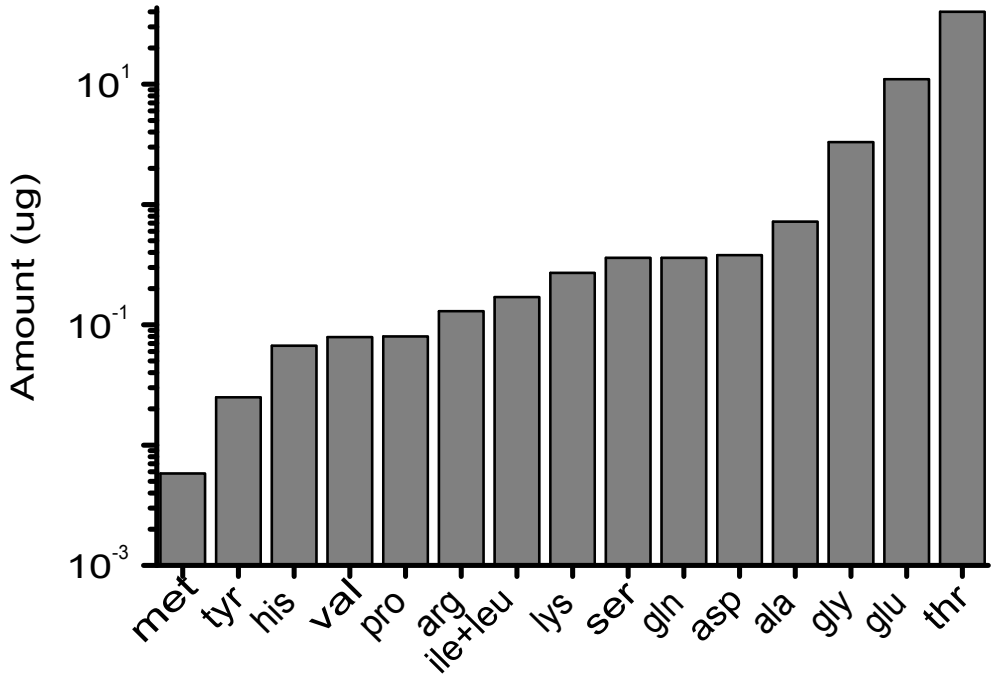
Supplementary Figure 5. ΔAmtB (●) displays similar responses to N-upshift as WT (■). Data are represented as mean ± SEM (n≥ 2 independent experiments,see method for details) . Lines directly join adjacent points.



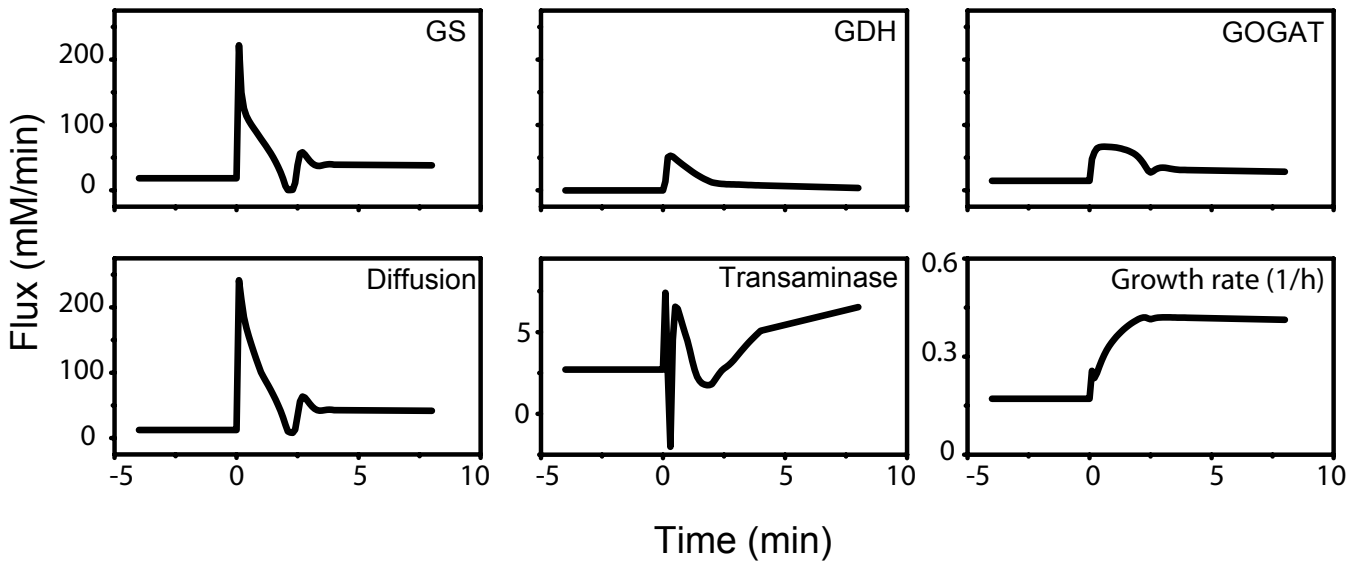
Supplementary Figure 6. Consumption of the trace residual ammonium on the filters when N-limited *E.coli* were shifted to plates with zero ammonium. Data are mean ± SEM (n=3 independent measurements).



Supplementary Figure 7. Amino acids leaked by ΔAT/AR strain upon N-upshift. Amounts shown were leaked in a 30 min period post N-upshift.



Supplementary Figure 8. Model simulation of flux dynamics in wild-type during 13-fold N-upshift.



Supplementary Table 1. Measured fluxes of glutamate and glutamine in wild-type, ΔGDH and ΔGOGAT.

	Strain (doubling time)	**k** (min^{-1})	**Conc.** (mM)	**Measured flux** (mM min^{-1})
Glutamate*	WT (58 min)	1.0± 0.1	96	96 ±10
	ΔGDH (56 min)	1.0± 0.1	86	86 ±15
	ΔGOGAT (57 min)	1.0± 0.1	69	70 ±8
Glutamine	WT	14.3±6.2	3.8	54 ±24
	ΔGDH	14.3±6.2	4.0	57 ±26
	ΔGOGAT	2.5±0.2	5.3	13 ±2

*Although the rate constant (k) of ^{15}N incorporation from ^{15}N-ammonium into glutamate did not change in either mutant compared to wildtype strain, the concentration of glutamate decreased slightly in both mutants (p=0.002 for ΔGOGAT, n.s. for ΔGDH), resulting in reduced glutamate flux (flux is the product of the rate constant k and corresponding metabolite concentration). The significantly reduced flux in ΔGOGAT is consistent with reduced glutamate consumption by the GS to feed the GS/GOGAT cycle . The rate constants (k) and errors (Δ*k*) were obtained by fitting the decay of fraction unlabeled data (Supplementary Fig. 1 C, D) to appropriate equations (see Yuan et al. 2008) using Origin 6.0 (Origin lab). Metabolite concentrations in wild-type are as reported (Bennett *et al.* 2009). Metabolite concentrations in mutants were calculated from corresponding concentations in wild-type and the ratio between mutants and wild-type (Supp. Fig. 1 B). Error of measured fluxes (Δ*F*) were calculated from error of metabolite concentrations (Δ*C*) and rate constants (Δ*k*) using following equation:

$$\left(\Delta F/F\right)^2 = \left(\Delta k/k\right)^2 + \left(\Delta C/C\right)^2$$

Supplementary Table 2: Metabolite projections onto the top two characteristic response patterns identified by SVD. For the characteristic response patterns, see Fig. 3.

Characteristic vector 1		Characteristic vector 2	
α-ketoglutarate	9.01	Glutamine	5.27
Phenylpyruvate	7.82	Tyrosine	2.83
Tyrosine	6.03	Asparagine	2.74
Phenylalanine	5.43	Tryptophan	2.49
Glutamine	4.54	Phenylalanine	1.43
Tryptophan	4.13	Aspartate	1.41
2,3-Dihydroxybenzoic acid	3.56	Glycine	1.38
(Iso)leucine	2.10	Alanine	1.29
Asparagine	1.86	Glutamate	0.91
dCTP	1.77	Lysine	0.73
Histidine	1.68	Dihydrooroate	0.60
Citrate	1.45	Glucono-lactone	0.56
Lysine	1.33	Serine	0.51
Glutathione-Reduced	1.20	Acetyl-CoA	0.50
dATP	1.20	dCTP	0.45
Succinate	1.10	Threonine	0.42
Arginine	1.04	Glucosamine	0.37
Valine	0.86	FBP	0.24
Threonine	0.70	Methionine	0.20
Aconitate	0.64	$NADP^+$	0.15
S-Adenosyl-Methionine	0.64	UDPGA	0.08
Phosphoenolpyruvate	0.61	Valine	0.02
Malate	0.55	3-Phospho-glycerate	0.02
ATP	0.55	ATP	-0.03
$NADP^+$	0.49	Glutathione-Reduced	-0.09
FBP	0.44	UDPAG	-0.10
UDPGA	0.43	6-Phosphogluconic acid	-0.11
Carbamoyl-Aspartate	0.43	Glutathione-Oxidized	-0.12
Adenine	0.27	NAD^+	-0.13
Glucono-lactone	0.23	Phosphoenolpyruvate	-0.23
3-Phospho-glycerate	0.16	dATP	-0.31
Hexose-Phosphate	0.15	Carbamoyl-Aspartate	-0.35
Riboflavin	0.14	Myo-inositol	-0.35
Alanine	0.05	UDP-D-glucose	-0.38
Glutathione-Oxidized	0.05	NADPH	-0.38
Fumarate	0.00	ADP	-0.42
Serine	0.00	Pentose-phosphate	-0.47
NADPH	-0.15	Adenine	-0.48
Acetyl-CoA	-0.18	Hexose-Phosphate	-0.49
DHAP	-0.18	DHAP	-0.51

Erythrose-4-phosphate	-0.28	Proline	-0.51
Myo-inositol	-0.30	PRPP	-0.59
ADP	-0.30	Erythrose-4-phosphate	-0.63
NAD^+	-0.30	(Iso)leucine	-0.64
UDPAG	-0.41	2-Deoxyribose- 1(5)-Phosphate	-0.65
UDP-D-glucose	-0.43	Arginine	-0.66
Glucosamine	-0.56	Orotate	-0.72
Glutamate	-0.56	Citrate	-0.76
PRPP	-0.64	AMP	-0.81
Pentose-phosphate	-0.83	Succinate	-0.81
Dihydrooroate	-0.92	Riboflavin	-1.29
Methionine	-1.02	Aconitate	-1.48
Proline	-1.03	Phenylpyruvate	-1.50
AMP	-1.23	2,3-Dihydroxybenzoic Acid	-1.86
6-Phosphogluconic Acid	-1.35	Fumarate	-1.88
Glycine	-1.37	Histidine	-1.93
Orotate	-1.88	Malate	-2.49
Aspartate	-2.36	S-Adenosyl-Methionine	-2.82
2-Deoxyribose-1(5)-Phosphate	-2.64	α-ketoglutarate	-3.92

Supplementary Table 3. Parameter values for the kinetic model.

Literature parameters			
Enzyme	Parameter	Value (mM)	Reference
Glutamine synthetase (GS)	K_m, NH_3	0.1	Alibhai 94
	K_m, Glutamate	5.5	
Glutamate synthase (GOGAT)	K_m, α-Ketoglutarate	7.0×10^{-3}	Miller 72
	K_i, Glutamate	28	
	K_i, Aspartate	0.35	
Glutamate dehydrogenase (GDH)	K_m, NH_3	1.5	
	K_m, α-Ketoglutarate	0.64	Sakamoto 75
	K_i, Glutamate	1.3	
Aspartate aminotransferase (AST)	K_m, Glutamate	0.9	Powell 78
	K_m, Oxaloacetate	0.58	
	K_i, Aspartate	0.45	
	K_i, α-Ketoglutarate	0.59	Deu 02
Directly measured parameters			
Fold GS underexpression in ΔGOGAT		0.5	
Fold GDH overexpression in ΔGOGAT		3	
Parameters selected based on data in Fig. 4			
Enzyme	Parameter	Value	
Glutamine synthetase (GS)	V_{max}, unadenylated	9.12×10^{3} mM/min	
	V_{max}, adenylated	6.10 mM/min	
	K_i, Glutamine	5.96×10^{-2} mM	
Glutamate synthase (GOGAT)	V_{max}	74.8 mM/min	
	K_m, Glutamine	0.795 mM	
Glutamate dehydrogenase (GDH)	V_{max}	645 mM/min	
Aspartate aminotransferase (AST)	V_{max}	3.79×10^{3} mM/min	
Adenyltransferase/adenyl-removing enzyme (ATAR)	V_{max}, AT	0.887 (fraction of GS)/min	
	K_m, GS	1.19×10^{-5} (fraction of GS)	
	K_m, PII	3.65×10^{-4} (fraction of PII)	
	K_i, α-Ketoglutarate	9.8×10^{-3} mM	
	K_m/K_i, Glutamine	12.2 mM	
	V_{max}, AR	8.09 (fraction of GS)/min	
	K_m, GSAMP	6.97 (fraction of GS)	
	K_m, PIIUMP	1.95×10^{-3} (fraction of PII)	
Uridyltransferase/uridyl-removing enzyme (UTUR)	V_{max}, UT	0.251 (fraction of PII)/min	
	K_m, PII	1.91×10^{-2} (fraction of PII)	
	K_m/K_i, Glutamine	0.016 mM	
	V_{max}, UR	1.09×10^{-4} (fraction of PII)/min	
	K_m, PIIUMP	7.55×10^{-3} (fraction of PII)	
Ammonia membrane diffusion constant (k_{diff})		$24.6\ min^{-1}$	

Supplementary Table 4A. Thermodynamics of the GOGAT reaction

Reaction	Glutamine+α-ketoglutarate+NADPH➔2 Glutamate +$NADP^+$							
	Glutamine (mM)	α-KG (mM)	Glutamate (mM)	$NADP^+$/ NADPH	K_{eq}	Q (reaction quotient)	ΔG (KJ/mol)	Fold forward driven (K_{eq}/Q)
Pre-shift [a]	1.95	11.7	76.6	0.33 [c]	390000 [d]	85.9	-21.7	4540
Post-shift [b]	10	0.6	160			1421.2	-14.5	274

a. The steady state before N-upshift (i.e., t ≤ 0 min in Fig. 3 & 4)
b. The steady state post N-upshift (i.e., t ≥ 8 min in Fig. 3 & 4)
c. Penfound, T. & Foster, J. Biosynthesis and recycling of NAD. In *Escherichia coli* and *Salmonella*: cellular and molecular biology (Neidhardt EA, ed.) (1996).
d: K_{eq} of GOGAT was calculated from the K_{eq}'s of GS, GDH and ATP hydrolysis. The source of all K_{eq}'s was the NIST database of Thermodynamics of Enzyme-Catalyzed Reactions (http://xpdb.nist.gov/enzyme_thermodynamics/)

Supplementary Table 4B. GOGAT active site competition allows flux regulation. Values are the average occupancy of the GOGAT active site by glutamine as calculated in Eq. 2, in the presence of the competing species listed.

	GLN occupancy (nitrogen limited)	GLN occupancy (post up-shift)	FOLD CHANGE
No competition	84	98	1.2
Glu competition	60	89	1.5
Glu + Asp competition [e], (K_{GLN}=0.3 mM)	40	67	1.7
Glu + Asp competition [f], (K_{GLN}=0.8 mM)	25	51	2.0

e. Calculated using literature reported K_m of glutamine (0.3 mM).
f. Calculated using adjusted K_m of glutamine (0.8 mM).

Supplementary Table 4C**.** Contribution of kinetic and mass action terms to elasticity coefficient (EC, $\partial \ln V / \partial \ln C$) of reaction rate with respect to each metabolite[g], calculated without aspartate as a competitor (rows 3 and 4) and with (rows 5 and 6) using literature K_{GLN}, and with aspartate as a competitor as well as using adjusted K_{GLN}.

	Glutamine			α-ketoglutarate			Glutamate			Aspartate
	kinetic	mass action	SUM (EC)	kinetic	mass action	SUM(EC)	kinetic	mass action	SUM (EC)	kinetic
Pre-shift [e], (K_{GLN}=0.3 mM)	-0.64	1.00	**0.37**	-1.00	1.00	0.00	-0.27	-0.0013	-0.27	Not included
Post-shift [e], (K_{GLN}=0.3 mM)	-0.83	1.01	**0.18**	-0.93	1.01	0.08	-0.20	-0.022	-0.23	
Pre-shift [e], (K_{GLN}=0.3 mM)	-0.42	1.00	**0.58**	-1.00	1.00	0.00	-0.18	-0.0013	-0.18	-0.34
Post-shift [e], (K_{GLN}=0.3 mM)	-0.58	1.01	**0.43**	-0.93	1.01	0.08	-0.16	-0.022	-0.18	-0.30
Pre-shift [f], (K_{GLN}=0.8 mM)	-0.21	1.00	**0.79**	-1.00	1.00	0.00	-0.24	-0.0013	-0.24	-0.46
Post-shift [f], (K_{GLN}=0.8 mM)	-0.35	1.01	**0.67**	-0.9274	1.01	0.08	-0.22	-0.022	-0.24	-0.47

g. Calculated according to Hofmeyr, J S. Journal of Bioenergetics and Biomembranes, Vol. 27, No. 5, 1995

Supplementary Table 5A. Reaction rate formulations employed in the model.

For detailed analysis of rate equations of this form, see Rohwer et al., 2006.

$$v_{GOG} = \frac{\frac{V_{\max}}{K_{GLN}K_{\alpha KG}}\left([GLN]\cdot[\alpha KG] - \frac{[GLU]^2}{K_{eq}}\right)}{\left(1+\frac{[GLN]}{K_{GLN}}+\frac{[GLU]}{K_{GLU}}+\frac{[ASP]}{K_{ASP}}\right)\left(1+\frac{[\alpha KG]}{K_{\alpha KG}}+\frac{[GLU]}{K_{GLU}}\right)}$$

$$v_{GS} = \frac{\frac{V_{\max}}{K_{GLU}K_{NH3}}\left([GLU]\cdot[NH3_{\text{int}}] - \frac{[GLN]}{K_{eq}}\right)}{\left(1+\frac{[GLU]}{K_{GLU}}+\frac{[GLN]}{K_{GLN}}\right)\left(1+\frac{[NH3_{\text{int}}]}{K_{NH3}}\right)}$$

$$v_{GDH} = \frac{\frac{V_{\max}}{K_{\alpha KG}K_{NH3}}\left([\alpha KG]\cdot[NH3_{\text{int}}] - \frac{[GLU]}{K_{eq}}\right)}{\left(1+\frac{[\alpha KG]}{K_{\alpha KG}}+\frac{[GLU]}{K_{GLU}}\right)\left(1+\frac{[NH3_{\text{int}}]}{K_{NH3}}\right)}$$

$$v_{AST} = \frac{\frac{V_{\max}}{K_{GLU}K_{OA}}\left([GLU]\cdot[OA] - \frac{[\alpha KG][ASP]}{K_{eq}}\right)}{\left(1+\frac{[GLU]}{K_{GLU}}+\frac{[\alpha KG]}{K_{\alpha KG}}\right)\left(1+\frac{[OA]}{K_{OA}}+\frac{[ASP]}{K_{ASP}}\right)}$$

$$v_{AT} = V_{\max}\left(\frac{[GS]}{K_{GS}+[GS]}\right)\left(\frac{[PII]}{K_{PII}\left(1+\frac{[KG]}{K_{KG}}\right)+[PII]}\right)\left(\frac{[GLN]}{K_{GLN}+[GLN]}\right)$$

$$v_{AR} = \frac{V_{\max}\left(\frac{[GSAMP]}{K_{GSAMP}+[GSAMP]}\right)\left(\frac{[PIIUMP]}{K_{PIIUMP}+[PIIUMP]}\right)}{\left(1+\frac{[GLN]}{K_{GLN}}\right)}$$

$$v_{UT} = \frac{V_{\max}\left(\frac{[PII]}{K_{PII}+[PII]}\right)}{\left(1+\frac{[GLN]}{K_{GLN}}\right)}$$

$$v_{UR} = V_{\max}\left(\frac{[PIIUMP]}{K_{PIIUMP}+[PIIUMP]}\right)\left(\frac{[GLN]}{K_{GLN}+[GLN]}\right)$$

$$\tau = \tau_0\left(1+\left(\frac{K_{GLN}}{[GLN]}\right)^2+\left(\frac{K_{GLU}}{[GLU]}\right)^2\right)$$

Following formulation was used for GOGAT reaction in the initial modeling attempt, **before competition by aspartate was included**. This equation was not employed in the final model.

$$v_{GOG} = \frac{\frac{V_{\max}}{K_{GLN}K_{\alpha KG}}\left([GLN]\cdot[\alpha KG]-\frac{[GLU]^2}{K_{eq}}\right)}{\left(1+\frac{[GLN]}{K_{GLN}}+\frac{[GLU]}{K_{GLU}}\right)\left(1+\frac{[\alpha KG]}{K_{\alpha KG}}+\frac{[GLU]}{K_{GLU}}\right)}$$

Reference:

Rohwer, J.M., Hanekom, A.J., Crous, C., Snoep, J.L. and Hofmeyr, J.H. (2006) Evaluation of a simplified generic bi-substrate rate equation for computational systems biology. *Syst Biol (Stevenage)*, **153**, 338-341.

Supplementary Table 5B**.** Differential equations employed in the model. $V_{GLU,N}$, $V_{GLU,F}$, $V_{GLN,N}$, $V_{GLN,F}$, $V_{ASP,F}$ represent reactions leading to biomass generation and therefore are proportional to τ . Subscript N or T indicates the reaction consumes the amino group only (e.g., transamination to produce other amino acids or nucleotides) or the entire molecule respectively (e.g., protein synthesis).

$V_{GLN,N}$ = 882.0 mM/τ ; $V_{GLN,F}$ = 107.9 mM/τ ; $V_{GLU,N}$ = 3046.0/τ ; $V_{GLU,F}$ = 347.0/τ ; $V_{ASP,F}$ = 658.0/τ

$$\frac{d[GLU]}{dt} = 2\cdot v_{GOG} + v_{GDH} - v_{GS} - v_{AST} - v_{GLU,N} - v_{GLU,F} + v_{GLN,N}$$
$$\frac{d[GLN]}{dt} = v_{GS} - v_{GOG} - v_{GLN,N} - v_{GLN,F}$$
$$\frac{d[ASP]}{dt} = v_{AST} - v_{ASP,F}$$
$$\frac{d[GS]}{dt} = v_{AR} - v_{AT}$$
$$\frac{d[PII]}{dt} = v_{UR} - v_{UT}$$
$$\frac{d[NH3_{\text{int}}]}{dt} = k_{diff}\left([NH3_{ext}]-[NH3_{\text{int}}]\right) - v_{GS} - v_{GDH}$$

Supplementary table 6A**.** Thermodynamics of the aspartate aminotransferase reaction.

Reactions involved [a]	Glutamate+oxaloacetate ⇔aspartate+α-ketoglutarate (K_{eq1}=6.7) Malate+NAD^+⇔oxaloacetate +NADH (K_{eq2}=0.00025)								
Overall rxn	Glutamate+malate+NAD^+⇔aspartate+ α-KG+NADH								
	Glutamate (mM)	Malate (mM)	Aspartate (mM)	α-KG (mM)	NADH/ NAD^+	K_{eq}(overall) =K_{eq1}*K_{eq2}	Q (reaction quotient)	ΔG (KJ/mol)	Fold forward driven (K_{eq}/Q)
Pre-shift [b]	76.6	3.6	1.83	11.6	0.02 [d]	1.7×10^{-3}	1.5×10^{-3}	-0.25	1.1
Post-shift [c]	160	1.3	6.0	0.60			2.7×10^{-4}	-4.7	6.3

a. The source of Keq's is the NIST database of Thermodynamics of Enzyme-Catalyzed Reactions (http://xpdb.nist.gov/enzyme_thermodynamics/)
b. The steady state before N-upshift (i.e., t ≤ 0 min in Fig. 3 & 4)
c. The steady state post N-upshift (i.e., t ≥ 8 min in Fig. 3 & 4)
d. Bennett, B. D.; Kimball, E.; Gao, M.; Osterhout, R.; Van Dien, S. J.; Rabinowitz, J. D., Absolute metabolite concentrations and implied enzyme active site occupancy in *Escherichia coli*. Nature Chemical biology 2009, in press. The best estimate of NAD^+ / NADH ratio from Bennett was 31. However, the 95% confidence limits were broad (18—51) and the value of 31 resulted in a slightly positive ΔG for the AST reaction during N-limitation, which is not possible. Accordingly, a somewhat higher value of 50 was used here. Note that, for the purpose of this work, a shift in the NAD^+/NADH ratio is equivalent to changing the ΔG' for the MDH reaction. The shift of NAD^+ / NADH ratio from 31 to 50 is equivalent to an error in ΔG' measurement of 1.2 kJ/mol, which is within error range reported for ΔG' for this reaction.

Supplementary Table 6B**.** Aspartate aminotransferase active site competition. The 5-carbon unit binding site of AST can bind glutamate or α -ketoglutarate; the 4-carbon unit binding site can bind aspartate or oxaloacetate.

		OCCUPANCY pre-perturbation	OCCUPANCY post-perturbation	FOLD CHANGE
5-carbon site	Glutamate	79	99	1.2
	α-ketoglutarate	20	0.6	0.03
4-carbon site	Aspartate	76	92	1.2
	Oxaloacetate	2	0.6	0.3

Supplementary Table 6C**.** Contribution of kinetic and mass action terms to elasticity coefficient (EC, $\partial \ln V / \partial \ln C$) of reaction rate with respect to each metabolite[e].

	Glutamate			Oxaloacetate			Aspartate			α-ketoglutarate		
	kinetic	mass action	SUM (EC)	kinetic	mass action	SUM (EC)	kinetic	mass action	SUM (EC)	kinetic	mass action	SUM (EC)
Pre-shift	-0.80	12.4	11.6	-0.0151	12.4	12.4	-0.79	-11.4	-12.2	-0.19	-11.4	-11.6
Post-shift	-0.99	1.26	0.27	-0.0020	1.26	1.26	-0.93	-0.26	-1.19	-0.0057	-0.26	-0.27

e. Calculated according to Hofmeyr, J S. Journal of Bioenergetics and Biomembranes, Vol. 27, No. 5, 1995

Supplementary Table 7. Response coefficient* of metabolite pools and fluxes to model parameters calculated by metabolic control analysis. Background is color coded to denote positive (yellow) or negative (blue) coefficient, and color intenty correlates with the mangnitude of the coefficient.

	GLU pool	GLN pool	GS Flux	GOGAT Flux	Growth rate
GOGAT V_{max}	9.3×10^{-1}	-4.16×10^{-1}	1.21×10^{-2}	1.02×10^{-2}	-1.54×10^{-3}
GOGAT K_i Glu	2.42×10^{-1}	-1.10×10^{-1}	4.22×10^{-3}	2.04×10^{-3}	-2.17×10^{-3}
GOGAT K_m Gln	-7.5×10^{-1}	3.37×10^{-1}	-1.10×10^{-2}	-6.43×10^{-3}	2.22×10^{-3}
GOGAT K_m α-KG	-2.19×10^{-3}	0	9.5×10^{-4}	-1.67×10^{-3}	-8.49×10^{-4}
GOGAT K_i Asp	4.21×10^{-1}	-1.90×10^{-1}	6.22×10^{-3}	3.42×10^{-3}	-1.78×10^{-3}
GS V_{max}	-5.11×10^{-3}	3.06×10^{-3}	5.68×10^{-3}	6.52×10^{-3}	6.82×10^{-4}
GSAMP V_{max}	0	0	4.34×10^{-5}	0	0
GS K_m Glu	3.65×10^{-3}	0	-5.28×10^{-3}	-3.66×10^{-3}	1.42×10^{-3}
GS K_m NH_3	5.11×10^{-3}	-3.06×10^{-3}	-5.27×10^{-3}	-6.52×10^{-3}	-6.82×10^{-4}
GS K_i Gln	-3.65×10^{-3}	0	5.05×10^{-3}	2.31×10^{-3}	-1.42×10^{-3}
GDH V_{max}	-5.11×10^{-3}	0	6.14×10^{-3}	4.05×10^{-3}	-1.98×10^{-3}
GDH K_m α-KG	3.65×10^{-3}	0	-4.58×10^{-3}	-3.66×10^{-3}	1.42×10^{-3}
GDH K_m NH_3	5.11×10^{-3}	0	-6.14×10^{-3}	-4.05×10^{-3}	1.98×10^{-3}
GDH K_i Glu	-3.65×10^{-3}	0	4.58×10^{-3}	3.66×10^{-3}	-1.42×10^{-3}
V_{max} AT	7.3×10^{-4}	0	-4.37×10^{-4}	-1.93×10^{-4}	2.83×10^{-4}
K_m AT PII	-7.3×10^{-4}	0	-1.01×10^{-3}	1.93×10^{-4}	-2.83×10^{-4}
AT K_m GS	0	0	0	0	0
AT/AR K_m Gln	-7.3×10^{-4}	0	4.37×10^{-4}	1.93×10^{-4}	-2.83×10^{-4}
AT K_i α-KG	7.3×10^{-4}	0	3.18×10^{-4}	-1.93×10^{-4}	2.83×10^{-4}
AR V_{max}	-7.3×10^{-4}	0	9.69×10^{-4}	1.93×10^{-4}	-2.83×10^{-4}
AR K_m PIIUMP	0	0	6.93×10^{-4}	0	0
AR K_m GSAMP	7.30×10^{-4}	0	9.50×10^{-5}	-1.93×10^{-4}	2.83×10^{-4}
UT V_{max}	0	0	5.08×10^{-5}	0	0
UT K_m PII	0	0	0	0	0
UT/UR K_m Gln	0	0	5.08×10^{-5}	0	0
UR V_{max}	0	0	0	0	0
UR K_m PIIUMP	0	0	0	0	0
AST V_{max}	-2.41×10^{-2}	1.22×10^{-2}	-1.88×10^{-3}	8.74×10^{-5}	1.31×10^{-3}
AST K_m OA	2.41×10^{-2}	-1.22×10^{-2}	2.57×10^{-3}	-8.74×10^{-5}	-1.31×10^{-3}
AST K_m Glu	5.11×10^{-3}	0	-1.99×10^{-3}	1.35×10^{-3}	1.98×10^{-3}
AST K_i Asp	-1.83×10^{-2}	6.12×10^{-3}	2.22×10^{-3}	-1.01×10^{-3}	-1.75×10^{-3}
AST K_i α-KG	-5.11×10^{-3}	0	1.99×10^{-3}	-1.35×10^{-3}	-1.98×10^{-3}
K_{diff} (ammonia)	$-3.36\times10-^{2}$	1.16	9.85×10^{-1}	9.85×10^{-1}	9.94×10^{-1}

*: Response coefficient R(y,p) is calculated as following:
R(y,p) = (dy/dp)*(p/y) = (dy/y)/(dp/p)
where y is a system variable (pool size or flux, listed in the 1st row) and p is a parameter (K and V listed in the first column).