

*The following supplement accompanies the article*

# **Reconstructing $\delta^{13}\text{C}$ isoscapes of phytoplankton production in a coastal upwelling system with amino acid isotope values of littoral mussels**

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**Supplement.** Additional data are provided on literature  $\delta^{13}\text{C}$  values for both bulk and compound-specific isotope analysis of amino acids (CSI-AA) in phytoplankton, which were used to run statistical analyses yielding the corrections required for reconstructing phytoplankton source bulk values, as well as data used for inter-laboratory calibration of data between our laboratories, and all outputs from major statistical tests referenced in the text. Also included is a table of amino acid molar percent composition of mussels, and a summary of the major pathways for amino acid carbon skeleton biosynthesis in plants, to support discussion of major carbon sources.

Table S1. Linear discriminant analysis output for Fig. 4 in the main text. Posterior probabilities of the classifier samples from Larsen et al. (2013)

ID Species	Actual	Predicted (% probability)		
		Bacteria	Microalgae	Phaeophyceae
B1 <i>Burkholderia xenovorans</i>	Bacteria	100.00	0.0	0.0
B2 <i>Methylobacterium</i> sp.	Bacteria	100.00	0.0	0.0
B3 <i>Klebsiella</i> sp.	Bacteria	100.00	0.0	0.0
B4 <i>Rhodococcus</i> spp.	Bacteria	100.00	0.0	0.0
B5 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B6 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B7 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B8 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B9 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B10 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B11 Unidentified bacteria	Bacteria	100.00	0.0	0.0
B12 Unidentified bacteria	Bacteria	100.00	0.0	0.0
D1 <i>Achnanthes brevipes</i>	Microalgae	0.0	54.4	45.6
D2 <i>Amphora coffaeiformis</i>	Microalgae	0.0	71.9	28.1
C1 <i>Cyanothece</i> sp.	Microalgae	0.0	12.4	87.6
K1 <i>Dunaliella</i> sp.	Microalgae	0.0	62.9	37.1
H1 <i>Emilia huxleyi</i>	Microalgae	0.0	96.7	3.3
H3 <i>Isochrysis galbana</i>	Microalgae	0.0	97.0	3.0
D3 <i>Melosira varians</i>	Microalgae	0.0	77.7	22.2
C2 <i>Merismopedia punctata</i>	Microalgae	0.0	99.8	0.2
X1 <i>Ochromonas minima</i>	Microalgae	0.0	95.9	4.1
X2 <i>Ochromonas villosa</i>	Microalgae	0.0	95.5	4.5
D4 <i>Phaeodactylum tricornutum</i>	Microalgae	0.0	99.2	0.8
K2 <i>Prasinocladus marinus</i>	Microalgae	0.0	51.6	48.4
D5 <i>Stauroneis constricta</i>	Microalgae	0.0	71.7	28.3
N1 Composite natural sample	Microalgae	0.0	97.4	2.6
N2 Composite natural sample	Microalgae	0.0	98.9	1.1
N3 Composite natural sample	Microalgae	0.0	99.0	1.0
P1 <i>Macrocystis pyrifera</i>	Phaeophyceae	0.0	11.4	88.6
P2 <i>Macrocystis pyrifera</i>	Phaeophyceae	0.0	7.2	92.8
P3 <i>Macrocystis pyrifera</i>	Phaeophyceae	0.0	23.8	76.2
P4 <i>Macrocystis pyrifera</i>	Phaeophyceae	0.0	1.6	98.4
P5 <i>Macrocystis pyrifera</i>	Phaeophyceae	0.0	32.6	67.4
P6 <i>Scytosiphon</i> sp.	Phaeophyceae	0.0	60.3	39.7
P7 <i>Lamiria</i> sp.	Phaeophyceae	0.0	10.0	90.0
P8 <i>Silvetia</i> sp.	Phaeophyceae	0.0	0.9	99.1
P9 <i>Petrospongium</i> sp.	Phaeophyceae	0.0	11.3	88.7
P10 <i>Pelvetiopsis</i> sp.	Phaeophyceae	0.0	2.7	97.3
P11 <i>Ralfsia</i> sp.	Phaeophyceae	0.0	13.9	86.1
P12 <i>Cystoseira osmundacea</i>	Phaeophyceae	0.0	90.4	9.6

Table S2. Mean  $\delta^{13}\text{C}$  values of bulk and compound-specific amino acids a range of cosmopolitan eukaryotic and prokaryotic photosynthetic species, grouped as essential and non-essential amino acids to determine the linear relationship between bulk and essential amino acid  $\delta^{13}\text{C}$  values in Fig. 5 in the main text. For individual amino acid values, the mean represents analytical variability (n = 3 injections) for a homogenized composite sample from all individuals sampled from each location. Data sources are from Larsen et al. (2013) and Lehman (2009). POM: particulate organic matter; lrg: large; nd: no data

Sample	Source	Bulk	Essential amino acids					Non-essential amino acids					
			Phe	Thr	Ile	Leu	Val	Asp	Glu	Pro	Ala	Ser	Gly
Eukaryotes													
<i>Achnanthes brevipes</i>	Larsen	-12.8	-18.1	-2.7	-10.2	-17.3	-13.8	-11.1	-11.4	nd	-9.7	nd	-11.2
<i>Amphora coffaeiformis</i>	Larsen	-10.5	-19.6	-2.0	-11.5	-18.7	-16.8	-9.1	-7.5	nd	-7.4	nd	-12.0
<i>Melosira varians</i>	Larsen	-14.6	-20.6	-7.0	-16.5	-22.1	-19.7	-16.2	-12.2	nd	-10.3	nd	-12.3
<i>Phaeodactylum tricorutum</i>	Larsen	-19.4	-23.8	-10.8	-16.1	-25.5	-22.9	-17.7	-18.9	nd	-16.0	nd	-12.2
<i>Stauroneis constricta</i>	Larsen	-9.1	-16.5	-3.1	-9.7	-19.6	-16.2	-4.2	-7.5	nd	-4.2	nd	-6.2
<i>Stauroneis constricta</i>	Larsen	nd	-16.0	-4.6	-10.4	-19.3	-15.5	-6.6	-7.4	nd	-1.3	nd	5.5
Diatoms Kiel Fjord 1	Larsen	nd	-27.0	-11.0	-21.0	-29.7	-26.4	-18.9	-20.3	nd	-17.4	nd	-21.7
Diatoms Kiel Fjord 2	Larsen	nd	-26.8	-11.2	-20.6	-29.3	-27.2	-18.2	-20.3	nd	-17.4	nd	-21.9
Diatoms Kiel Fjord 3	Larsen	nd	-26.9	-10.7	-19.8	-29.4	-27.1	-17.8	-20.4	nd	-17.4	nd	-21.8
<i>Amphidinium carterea</i>	Lehman	-23.7	-14.8	-10.4	-16.3	-26.1	-33.3	-12.2	-14.0	-7.2	-15.0	20.8	7.5
<i>Pseudo-nitzschia</i>	Lehman	-28.4	-31.5	-23.4	-26.2	-35.9	-40.6	-12.4	-17.2	-27.5	-18.9	7.9	-8.1
Dinoflagellate sp.	Lehman	-19.1	-21.4	-11.3	-15.5	-25.8	-30.0	-3.3	-5.0	-16.5	-17.9	14.6	10.0
Hawaii Net POM	Lehman	-19.0	-17.7	-4.6	-10.0	-14.4	-33.2	16.8	6.9	-11.2	-19.8	11.7	7.2
Prokaryotes													
<i>Cyanothece</i> (NO <sub>3</sub> )	Lehman	-32.4	-26.6	-32.6	-38.5	-36.9	-37.4	-28.2	-34.4	-63.9	-31.4	-15.0	-16.8
<i>Cyanothece</i> (N <sub>2</sub> )	Lehman	-38.7	-38.7	-30.7	-39.3	-43.7	-38.5	-28.9	-36.2	-35.5	-31.0	-28.4	-61.8
Raphidophyte	Lehman	-28.7	-26.9	-29.0	-27.8	-35.8	-39.6	-23.6	-28.4	-18.6	-28.3	-0.3	-5.4
<i>Synechococcus</i> lrg	Lehman	-32.0	-32.3	-36.9	-32.5	-35.2	-45.9	-26.1	-32.9	-22.9	-36.2	-17.0	-24.2
<i>Skeletonema</i>	Lehman	-35.6	-34.7	-37.4	-33.1	-41.4	-51.1	-28.5	-33.9	-23.8	-35.2	-8.1	-25.2
<i>Synechococcus</i> culture 1	Lehman	-20.1	-28.6	-10.1	-21.8	-28.7	-35.4	1.2	-8.3	-22.6	-19.6	4.4	-0.1
<i>Synechococcus</i> culture 2	Lehman	nd	-19.3	-11.8	-16.1	-26.1	-22.2	2.7	-9.3	-16.4	-6.7	1.5	5.3
<i>Trichodesmium</i> culture 1	Lehman	-14.7	-14.9	-9.4	-23.1	-23.1	-20.6	10.0	-1.3	-9.3	8.6	17.6	28.3
<i>Trichodesmium</i> culture 2	Lehman	nd	-15.0	-16.3	-22.2	-19.1	-23.7	-16.6	-16.5	-5.7	-15.8	9.2	-1.3
<i>Prochlorococcus</i>	Lehman	-15.5	-22.1	-9.5	-12.8	-21.5	-17.6	-0.5	-13.8	-12.4	-5.5	-6.8	-41.6

Table S3. Coefficients of linear discriminant (LD) for each essential amino acid and proportion trace output for Fig. 4 in the main text

Amino acid	LD 1	LD 2
Isoleucine	0.151	-0.372
Leucine	-0.989	0.170
Phenylalanine	0.023	0.115
Threonine	0.128	0.203
Valine	0.739	0.0632
Trace	0.920	0.080

Table S4. Predicting classification of the biosynthetic origin of essential amino acids from mussel samples (probability in %) output for Fig. 4 in the main text. Site abbreviations are defined in Table 1 in the main text. Uppercase abbreviations are sites from our study; lowercase abbreviations are sites from Larsen et al. (2013)

Site ID	Bacteria	Microalgae	Phaeophyceae
gav	0.1	99.2	0.9
sc	0.1	98.2	1.7
HMPO	0.0	99.4	0.6
MCPR	0.0	92.4	7.6
PSB	0.0	93.6	6.4
PCL	0.0	95.2	4.8
HMB	1.5	98.5	0.0
DAV	0.0	92.9	7.1
ML	0.0	96.2	3.8
MC	0.0	25.1	74.9
TOP	0.0	96.9	3.1
OCE	8.2	91.7	0.1
LAJ	0.0	92.1	7.9

Table S5. Inter-laboratory calibration of essential amino acid  $\delta^{13}\text{C}$  values. To ensure that animal and primary producer data were comparable for predicting the biosynthetic origin of essential amino acids (EAAs) using the linear discriminant model of Larsen et al. (2013),  $\delta^{13}\text{C}_{\text{EAA}}$  values (‰) in muscle tissue from 3 California mussel samples (see Table 1 in the main text for sample identities) were measured at both the University of California, Santa Cruz, CA, USA ('Vokhshoori'), and the University of Kiel, Germany ('Larsen') to generate calibration  $\delta^{13}\text{C}_{\text{EAA}}$  values for the mussel dataset

Amino acid	OCE		PCL		PSB		Calibration values for mussel dataset
	Vokhshoori	Larsen	Vokhshoori	Larsen	Vokhshoori	Larsen	
Threonine	-19.6	-12.3	-17.1	-11.4	-12.7	-10.5	$-5.0 \pm 2.6$
Valine	-37.1	-24.4	-34.7	-23.5	-35.6	-22.4	$-12.4 \pm 1.1$
Leucine	-29.3	-26.1	-30.1	-26.1	-29.1	-25.2	$-3.8 \pm 0.5$
Isoleucine	-15	-18.9	-16.2	-19.2	-13.6	-17.4	$3.6 \pm 0.5$
Phenylalanine	-26.4	-24.7	-27	-24.6	-23.1	-23.4	$-1.3 \pm 1.4$

Table S6. Predicting classification of mussel samples (probability in %) output for Fig. S1. Site abbreviations are defined in Table 1 in the main text. Uppercase abbreviations are sites from our study; lowercase abbreviations are sites from Larsen et al. (2013)

Site ID	Microalgae	Phaeophyceae
gav	99.3	0.7
sc	98.4	1.6
HMPO	99.5	0.5
MCPR	88.9	6.0
PSB	88.9	11.1
PCL	97.7	2.3
HMB	100	0.0
DAV	97.9	2.1
ML	96.8	3.2
MC	39.2	60.8
TOP	98.6	1.4
OCE	99.9	0.1
LAJ	96.1	3.9

Table S7. Coefficients of linear discriminant (LD) for each essential amino acid output for Fig. S1

Amino acid	LD
Isoleucine	-0.237
Leucine	-2.000
Phenylalanine	0.223
Threonine	0.220
Valine	0.213

Table S8. Posterior probabilities of the classifier samples output for Fig. S1

ID	Actual	Predicted (% probability)	
		Microalgae	Phaeophyceae
D2	Microalga	75.5	24.5
C1	Microalga	22.7	77.3
K1	Microalga	55.0	45.0
H1	Microalga	94.1	5.9
H3	Microalga	92.9	7.1
D3	Microalga	78.1	21.9
C2	Microalga	99.9	0.1
X1	Microalga	97.8	2.2
X2	Microalga	97.2	2.8
D4	Microalga	99.1	0.9
K2	Microalga	47.7	52.3
D5	Microalga	58.1	41.9
N1	Microalga	97.6	2.4
N2	Microalga	98.8	1.2
N3	Microalga	98.9	1.1
P1	Phaeophyceae	10.2	89.8
P2	Phaeophyceae	7.8	92.2
P3	Phaeophyceae	28.9	71.1
P4	Phaeophyceae	2.7	97.3
P5	Phaeophyceae	42.1	57.9
P6	Phaeophyceae	53.5	46.5
P7	Phaeophyceae	5.8	94.2
P8	Phaeophyceae	0.8	99.2
P9	Phaeophyceae	10.0	90.0
P10	Phaeophyceae	3.9	96.1
P11	Phaeophyceae	9.3	90.7
P12	Phaeophyceae	84.7	15.3

Table S9. Mole percent composition of individual amino acids hydrolyzed. ‘Sum total’ is the total mole percent from amino acid peaks detected by HPLC methods, and ‘Sum measured AAs’ is from isotope measurements from GC-IRMS

Amino acid	Mole percent
Aspartic acid/Serine	16.1
Glycine	5.7
Glutamic acid	14.2
Alanine	7.1
Proline	2.7
Threonine	8.8
Valine	3.7
Isoleucine	4.1
Leucine	8.2
Phenylalanine	2.7
Methionine	2.5
Histidine	5.7
Lysine	5.9
Arginine	6.4
Unknown 1	3.2
Unknown 2	3.0
Sum total	100.0
Sum measured AAs	73.3

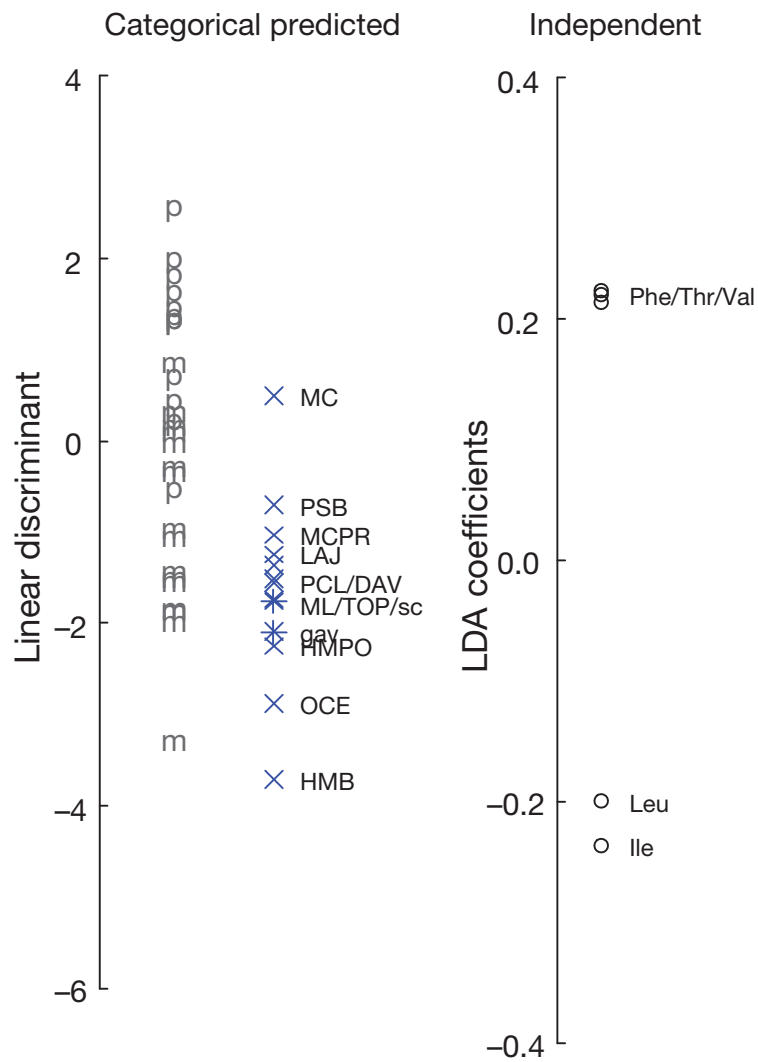


Fig. S1. Linear discriminant analysis (DA) plot based on  $\delta^{13}\text{C}$  values of 5 essential amino acids (EAA: Ile, Leu, Phe, Thr, Val) from 2 categorical variables, viz. marine microalgae (m, n = 16) and Phaeophyceae (p, n = 12; including *Macrocystis* kelp) from Larsen et al. (2013) to predict the biosynthetic origins of EAAs among mussels *Mytilus californianus*. All mussel samples except MC classify with microalgae; see Table S4 for linear discriminant scores. At right are the DA coefficients of the independent variables; Leu and Ile have contrasting coefficients compared to Phe, Thr, and Val. Amino acid abbreviations are as defined in ‘Materials and methods’ in the main article. Site abbreviations are defined in Table 1 in the main text. Uppercase abbreviations are sites from our study; lowercase abbreviations are sites from Larsen et al. (2013)

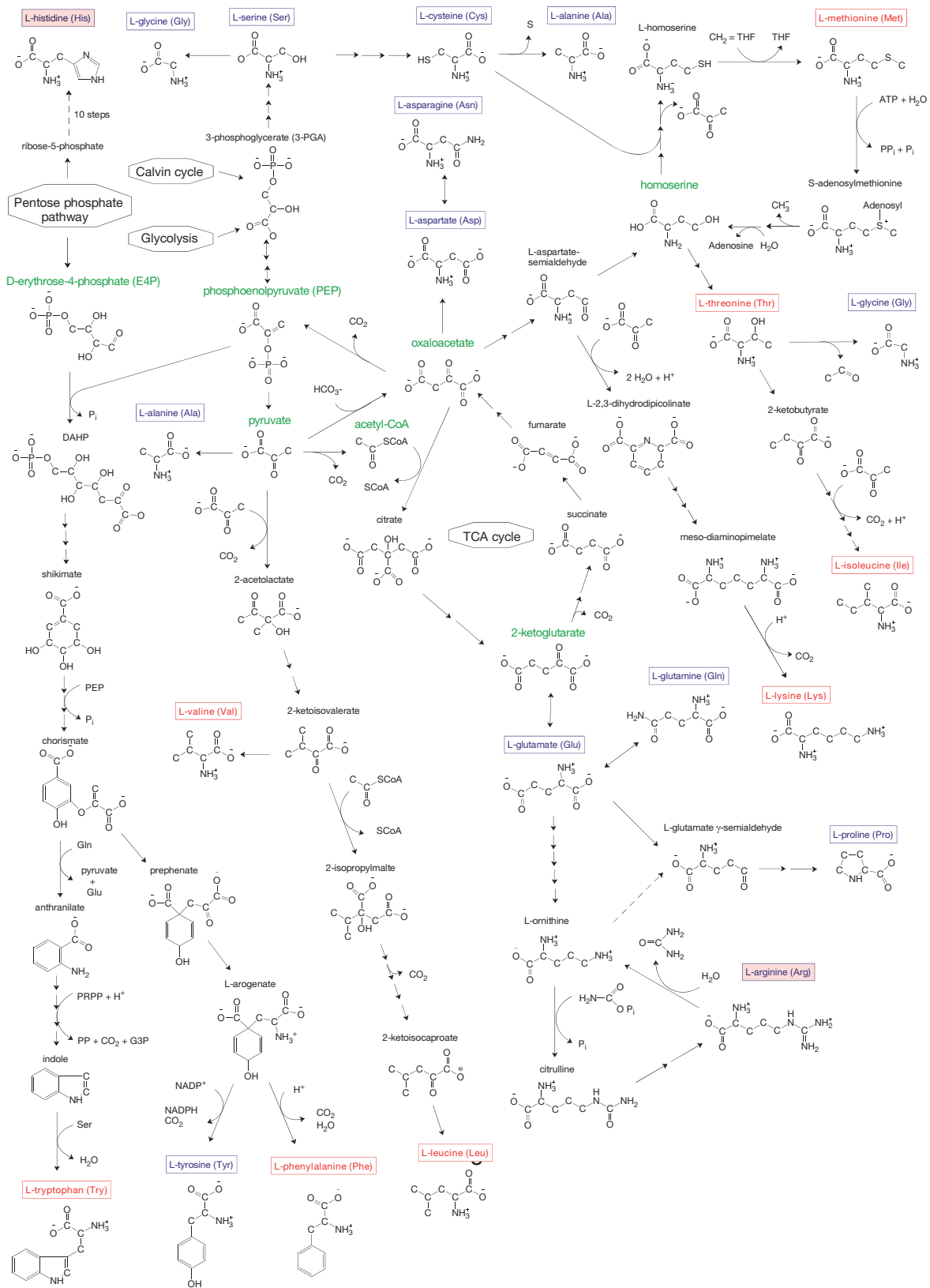


Fig. S2. Generalized model of the major pathways for amino acid carbon skeleton biosynthesis in plants. Modified from the PlantCyc Pathway database of the Plant Metabolic Network (<http://pmn.plantcyc.org>). Each arrow indicates a biosynthetic step. Red type indicates amino acids that are essential for all animals, shaded rectangles indicate those that are essential for most animals, blue type are those that are nonessential, and green type shows the most prominent metabolic precursors. DAHP: 3-deoxy-D-arabino-heptulosonic acid-7-phosphate. TCA: tricarboxylic acid



## LITERATURE CITED

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