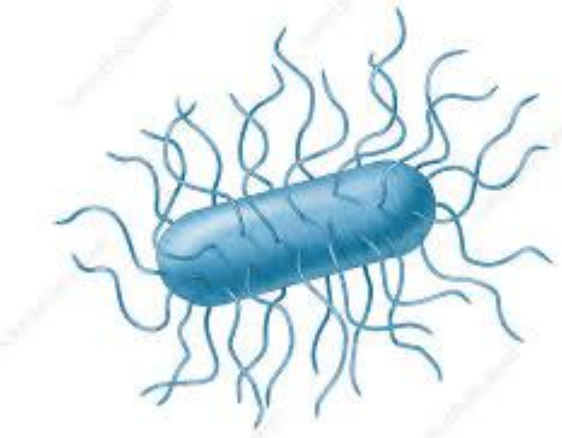


Amino Acid Hydrogen

Elemental Ecology

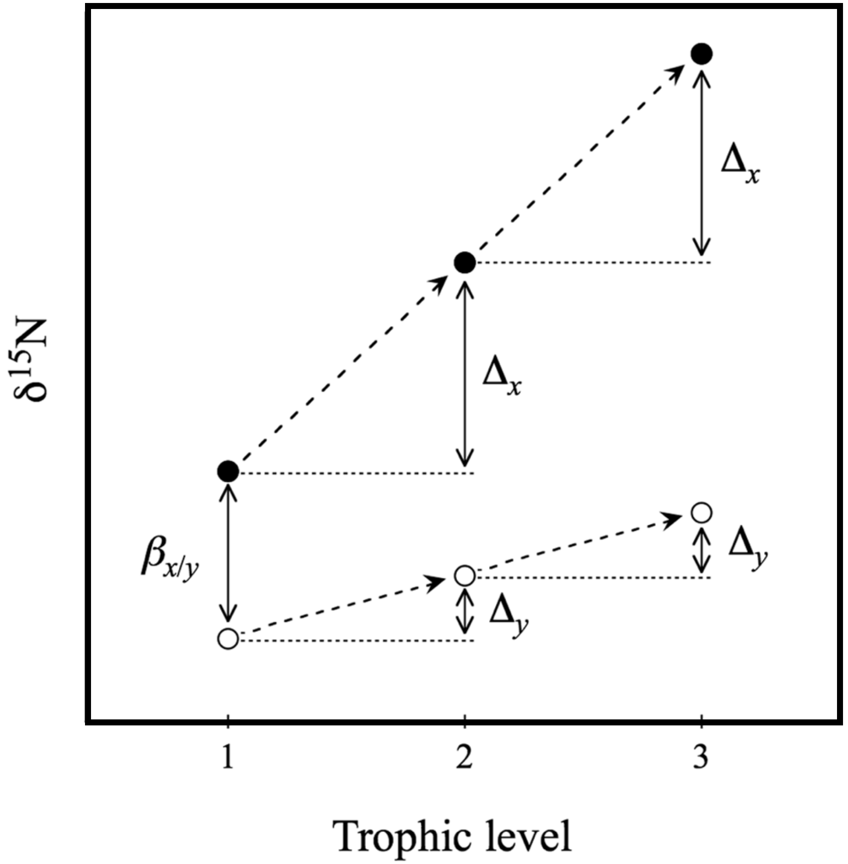
Week Thirteen

CARBON

NITROGEN

CARBON

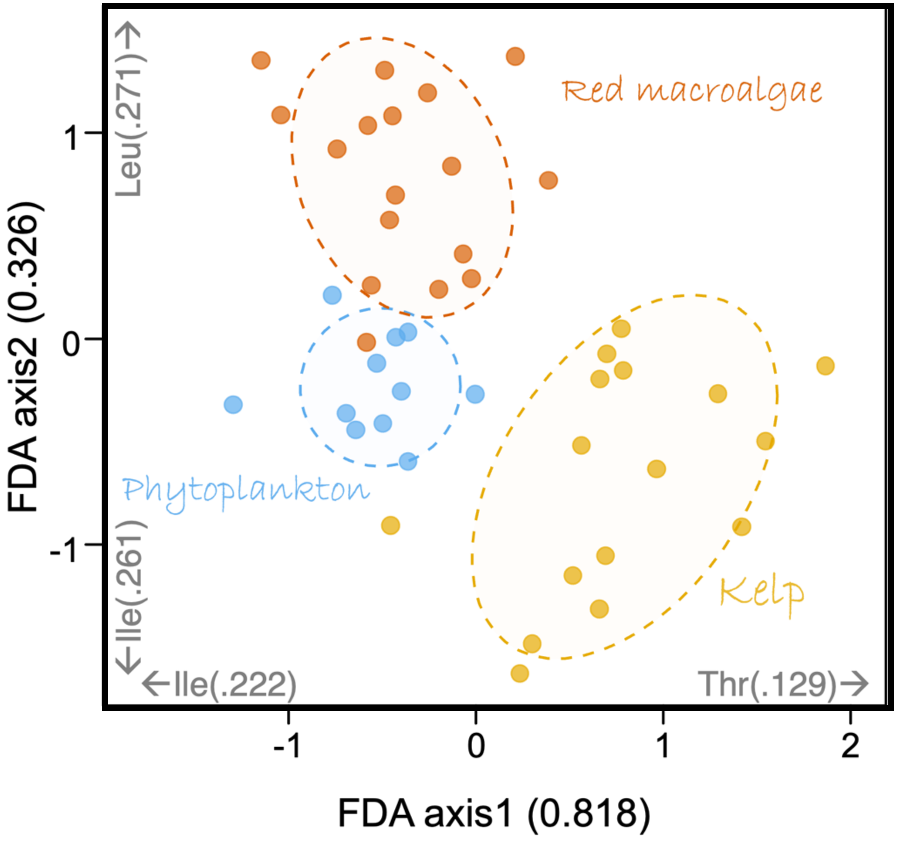
NITROGEN



Single sample trophic level estimations

Chikaraishi et al. 2015 Limn. Ocea. Meth.

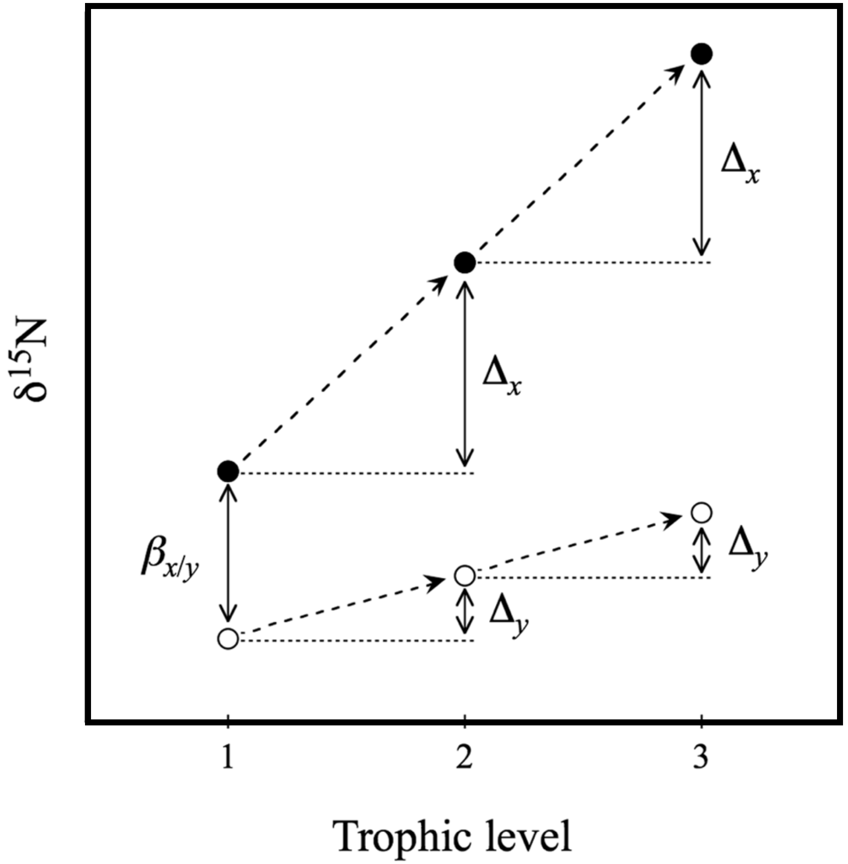
CARBON



Energy flow quantification through multivariate isotopic "fingerprinting"

Yiu et al.. 2025 Sci Advance

NITROGEN



Single sample trophic level estimations

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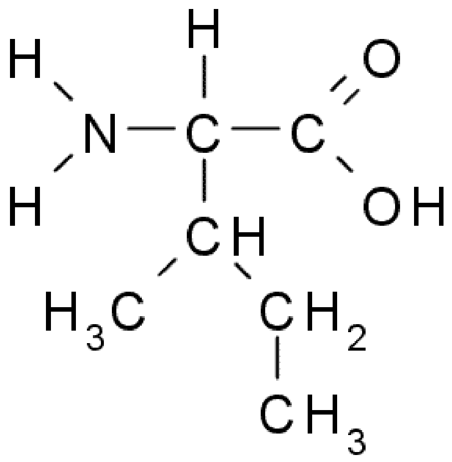
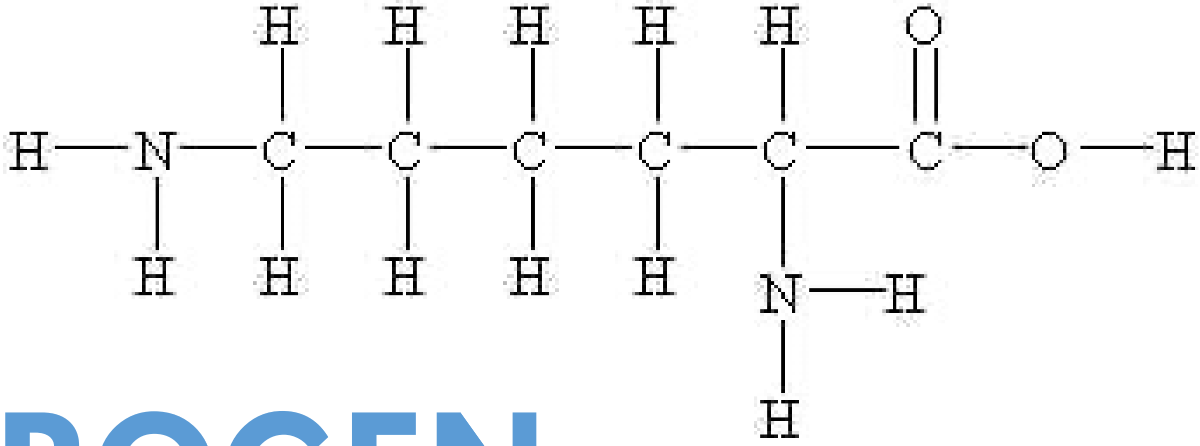
Valine

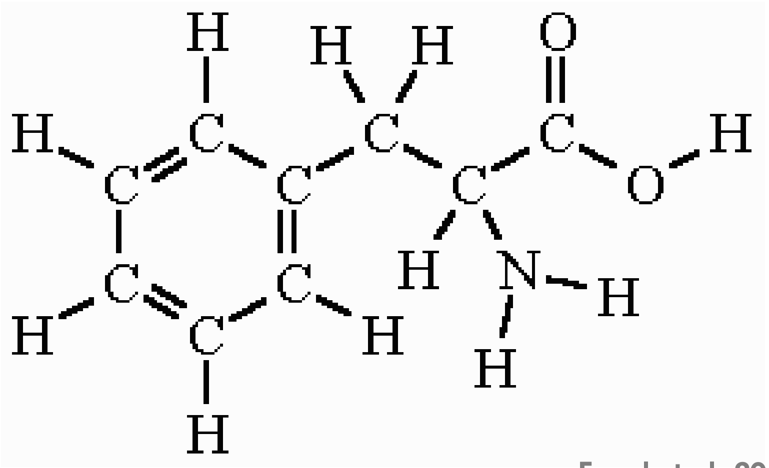


Lysine

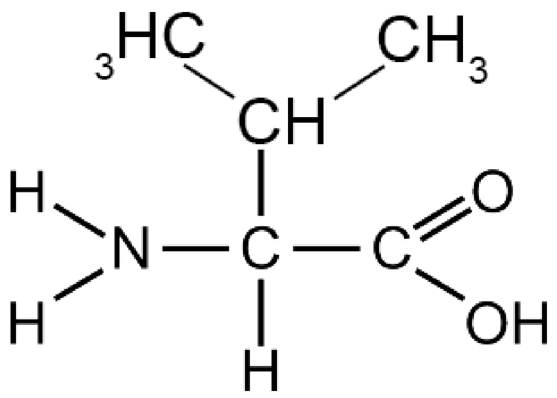


HYDROGEN

Phenylalanine

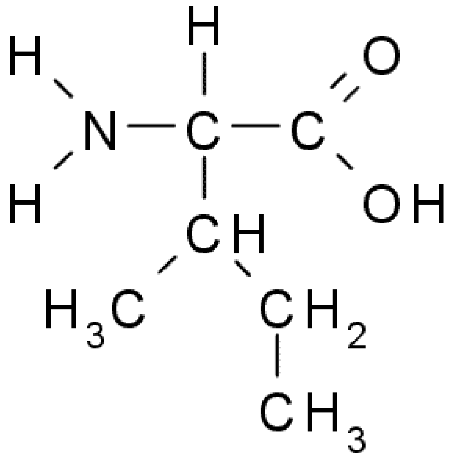


Isoleucine



Fogel et al. 2016 PNAS; Newsome et al. 2020 Oecologia; Morra et al. 2021 Front. Ecol. Evo; Smith et al. 2022 Front. MicroBio; Gharibi et al. 2022 J. Am Chem Soc; Mancuso et al. 2023 Rapid Comms MS; Silverman et al. 2024 Front. MicroBio

Valine



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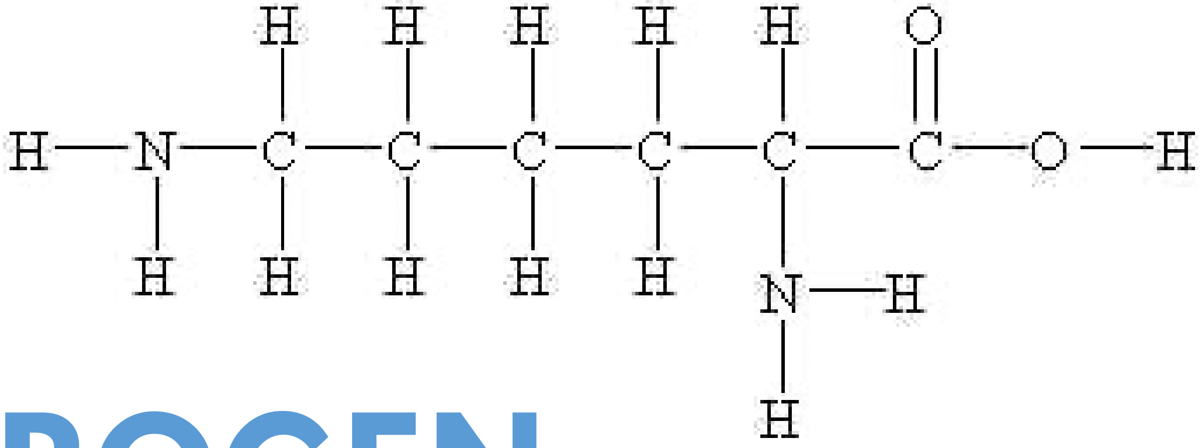

HYDROGEN

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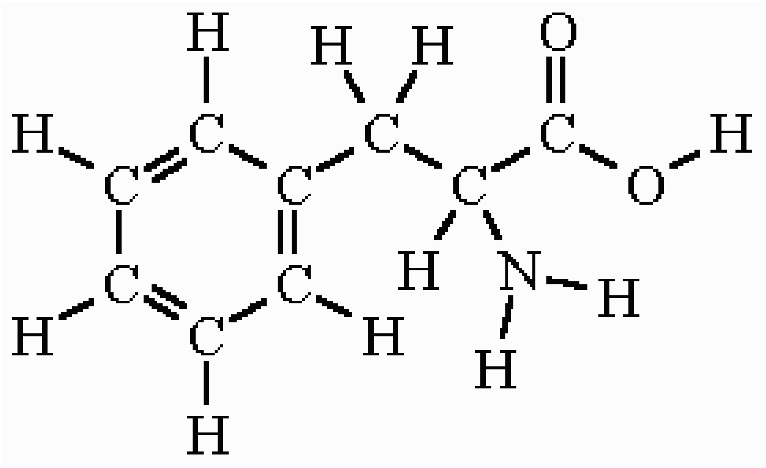
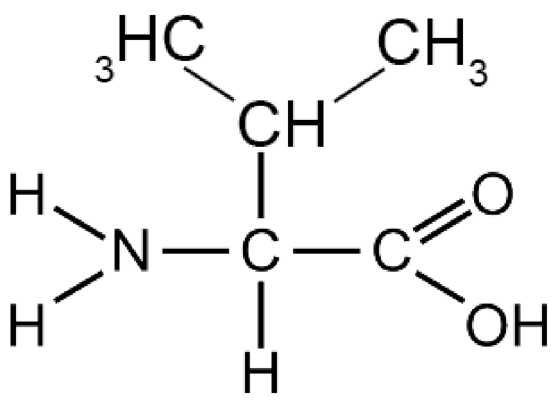


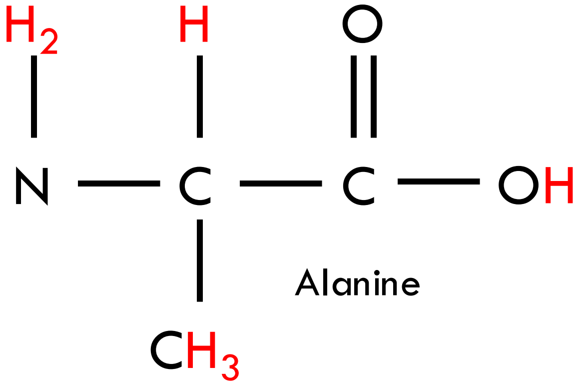
In bulk, protein-rich, tissues ~ 70% of hydrogen comes from food, compared to only 25% from water

What about individual amino acids?

Isoleucine



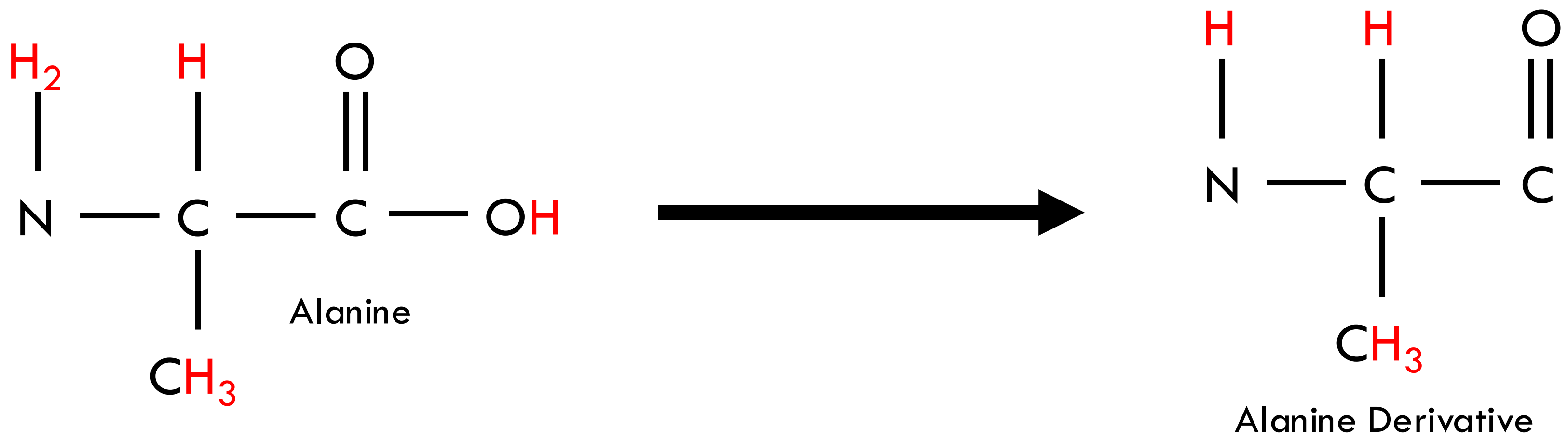
What hydrogen are we actually measuring?



Alanine

Fogel et al. 2016 PNAS

"Non-exchangeable" hydrogen

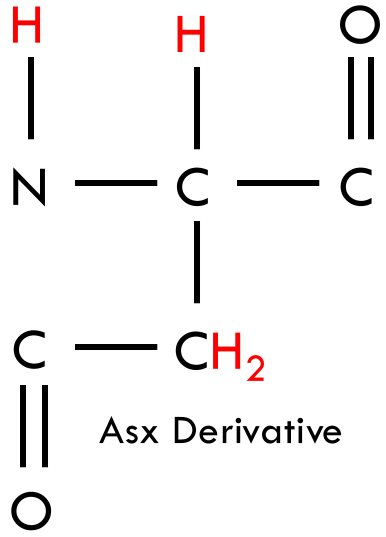


H bound to C (alpha and methyl) less likely to be replaced

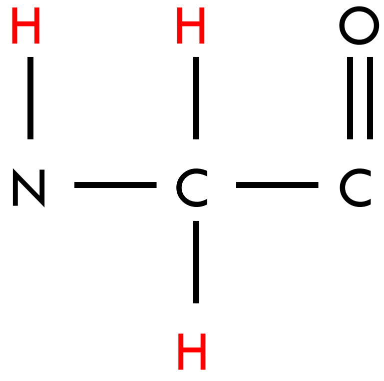
H bound to N or O: not as strongly bonded

Fogel et al. 2016 PNAS

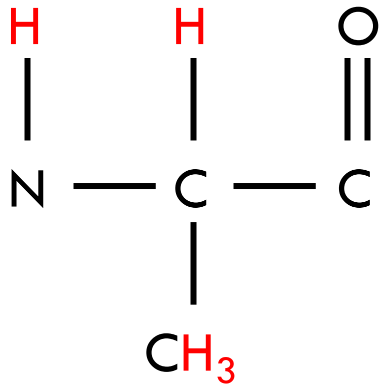
"Non-exchangeable" hydrogen



Asx Derivative



Gly Derivative



Alanine Derivative

H bound to C (alpha and methyl) less likely to be replaced

H bound to N or O: not as strongly bonded

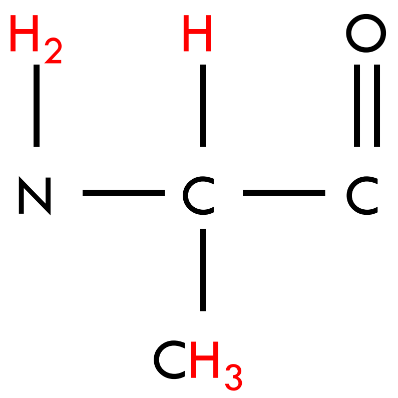
Fogel et al. 2016 PNAS

How is Hydrogen Swapped On and Off of Molecules? Enzymes

via dehydrogenases: transfers H to electron acceptor (e.g., NAD or NADP)

via hydrolases: use (body) water to break chemical bonds

via transaminases: replace keto group with NH_2



Isotope Rules:

Light isotope (^1H) has weaker bonds

Less energy to make bonds with light isotope (^1H)

How is Hydrogen Swapped On and Off of Molecules? Enzymes

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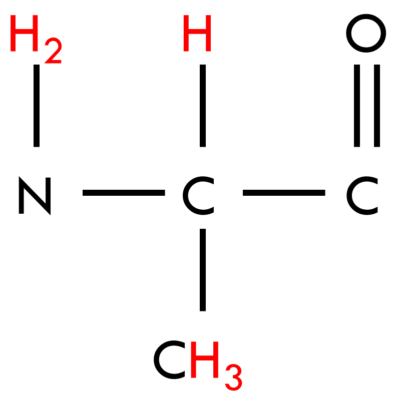


Isotope Rules:

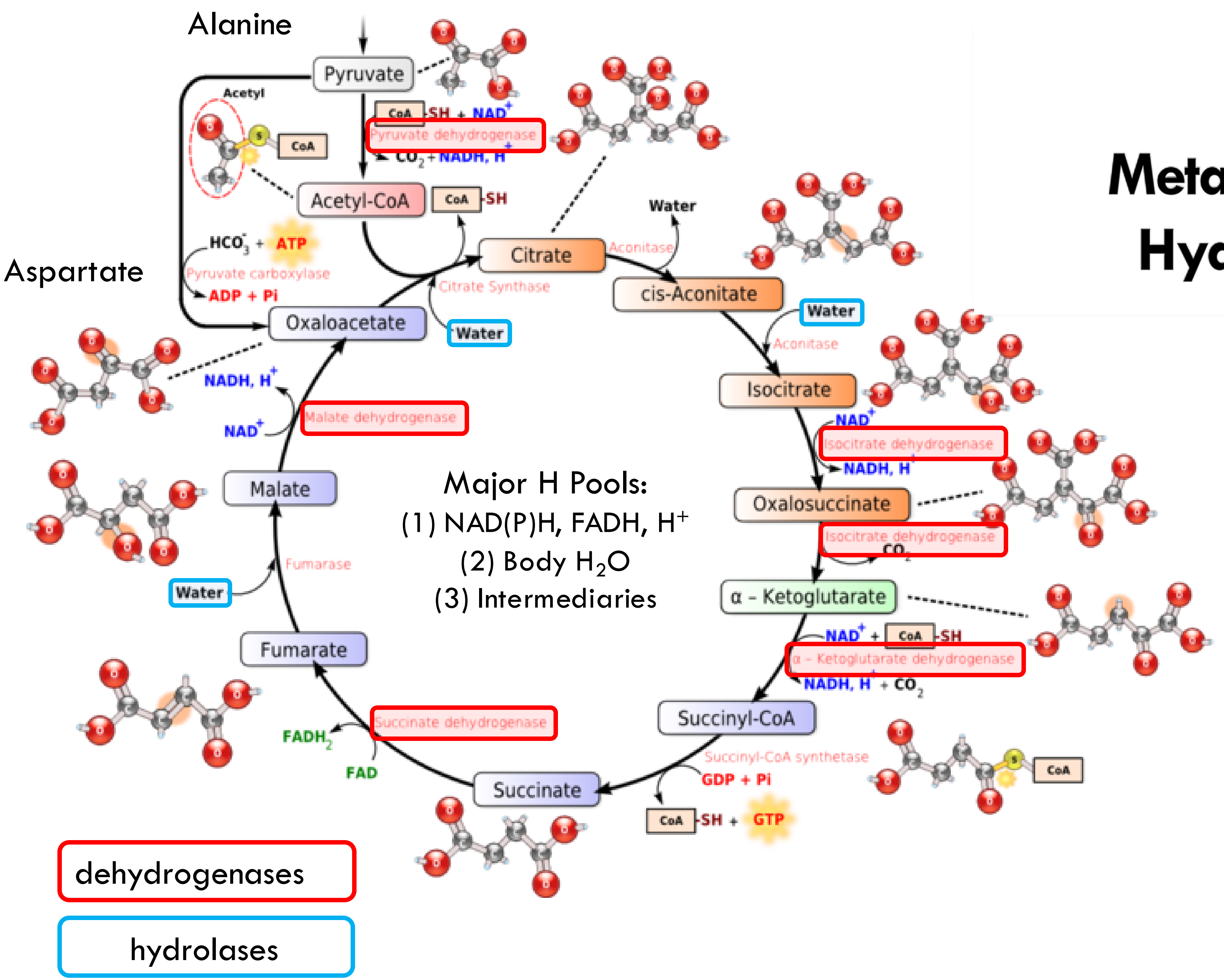
Light isotope (^1H) has weaker bonds

(removing H should increase compound δ^2H)

Less energy to make bonds with light isotope (^1H)

(adding H should decrease compound δ^2H)

Metabolism: Hydrogen

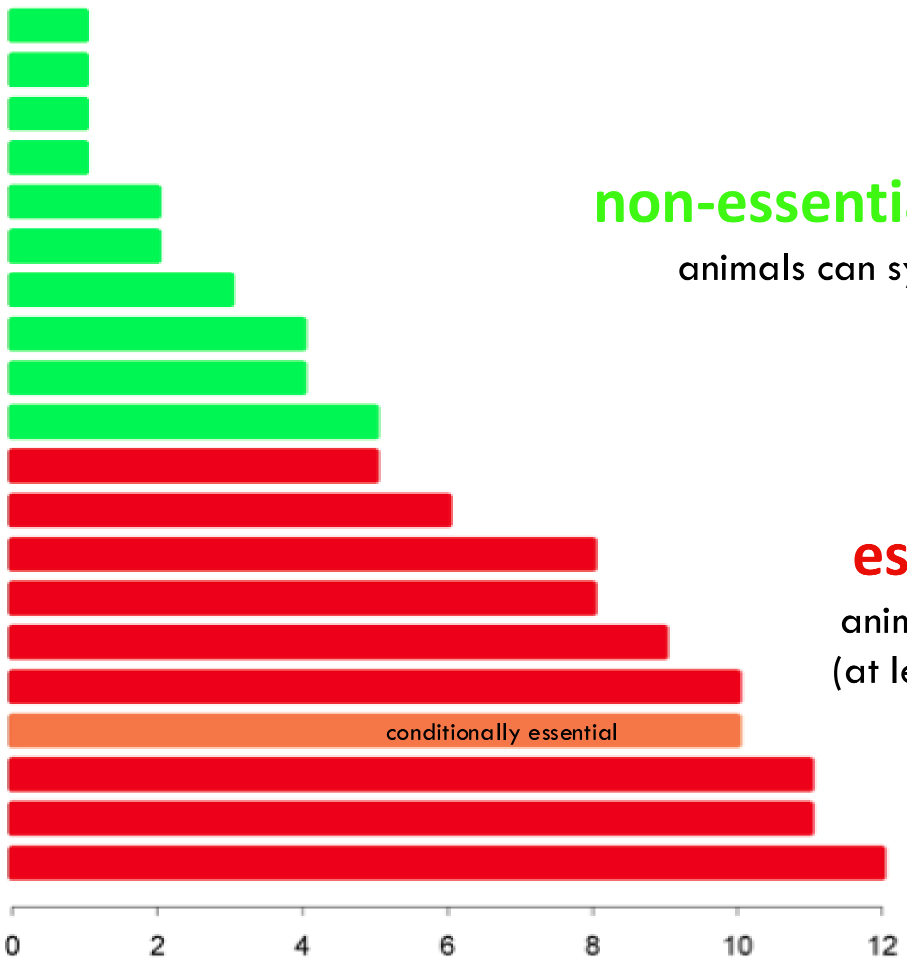


Glutamate
Proline
Arginine

AAs differ in the number of hydrogen-bearing reducing compounds (e.g., NADPH) required for synthesis

Amino acid	TYPE	ATP	NADP
Alanine	NE	0	1
Glutamate	NE	0	1
Aspartate	NE	0	1
Tyrosine[1]	NE	0	1
Glutamine	NE	1	1
Asparagine	NE	3	1
Serine	NE	0	1
Glycine	NE	0	1
Proline	NE	1	3
Cysteine[2]	NE	0	1
Valine	E	0	2
Threonine	E	2	3
Leucine	E	0	2
Methionine	E	7	8
Lysine	E	3	4
Phenylalanine	E	1	2
Arginine	E	7	4
Histidine	E	6	1
Isoleucine	E	2	5
Tryptophan	E	5	3

steps to synthesize



non-essential amino acids

animals can synthesize *de novo*

essential amino acids

animals cannot synthesize *de novo**
(at least in high enough abundance)

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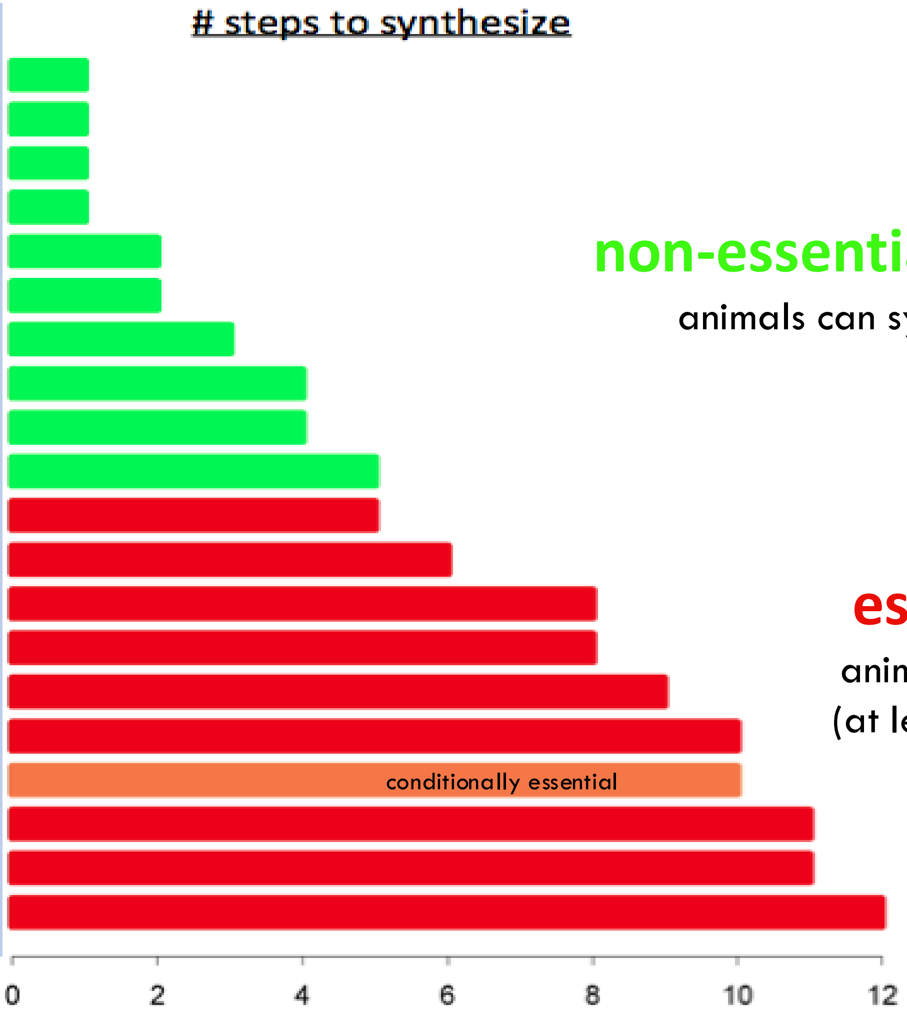
Asparagine	NE	3	1
Serine	NE	0	1
Glycine	NE	0	1
Proline	NE	1	3
Cysteine[2]	NE	0	1
Valine	E	0	2
Threonine	E	2	3
Leucine	E	0	2
Methionine	E	7	8
Lysine	E	3	4
Phenylalanine	E	1	2
Arginine	E	7	4
Histidine	E	6	1
Isoleucine	E	2	5
Tryptophan	E	5	3



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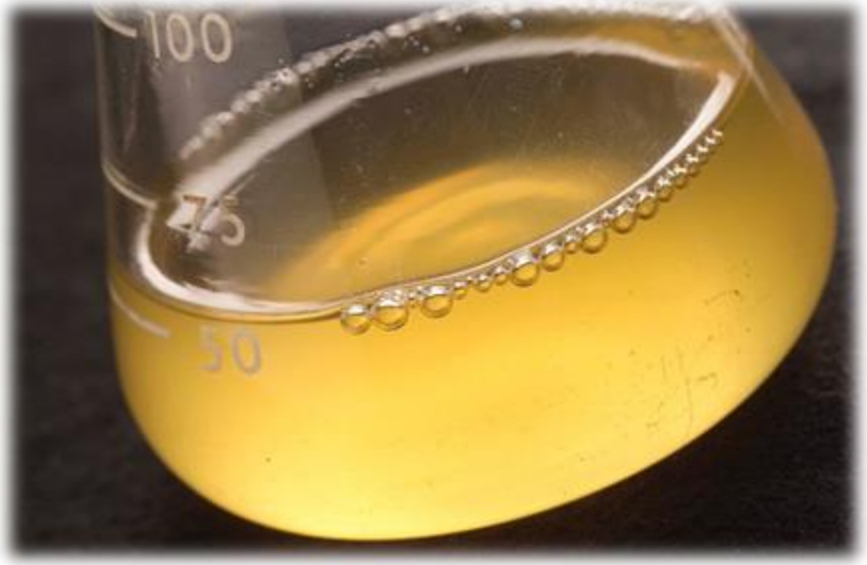
Our First Experiment with Amino Acid δ^2H: Prokaryotes



Grew *E. coli* in water that varied in δ^2H from -55 ‰ to +1070‰

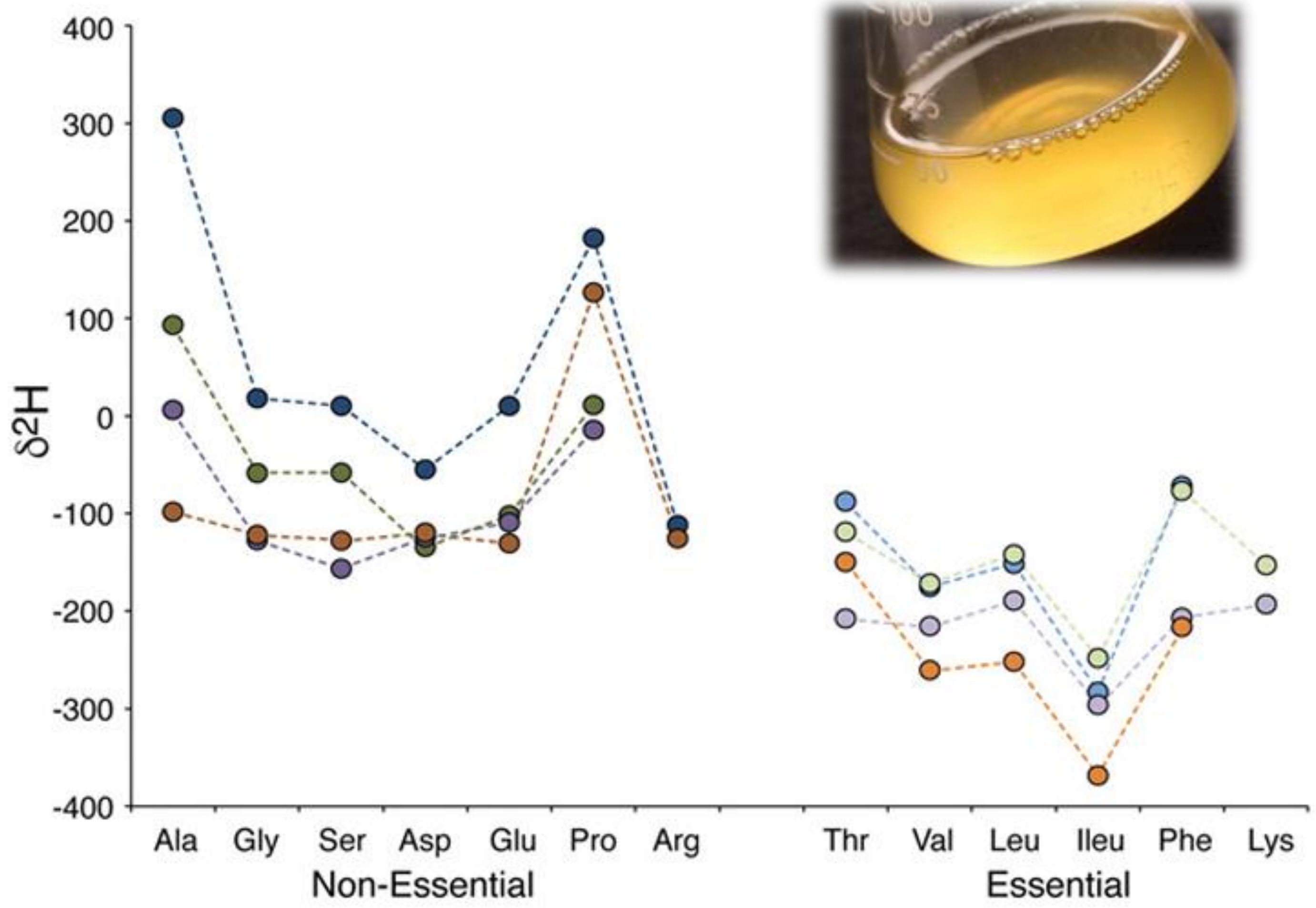
(4 Treatments)

2 "Diet" Treatments:

Glucose+NH_3 OR Tryptone (protein-rich)

Fogel et al. 2016 PNAS

Our First Experiment with Amino Acid δ^2H: Prokaryotes



Highlights

Fogel et al. 2016 PNAS

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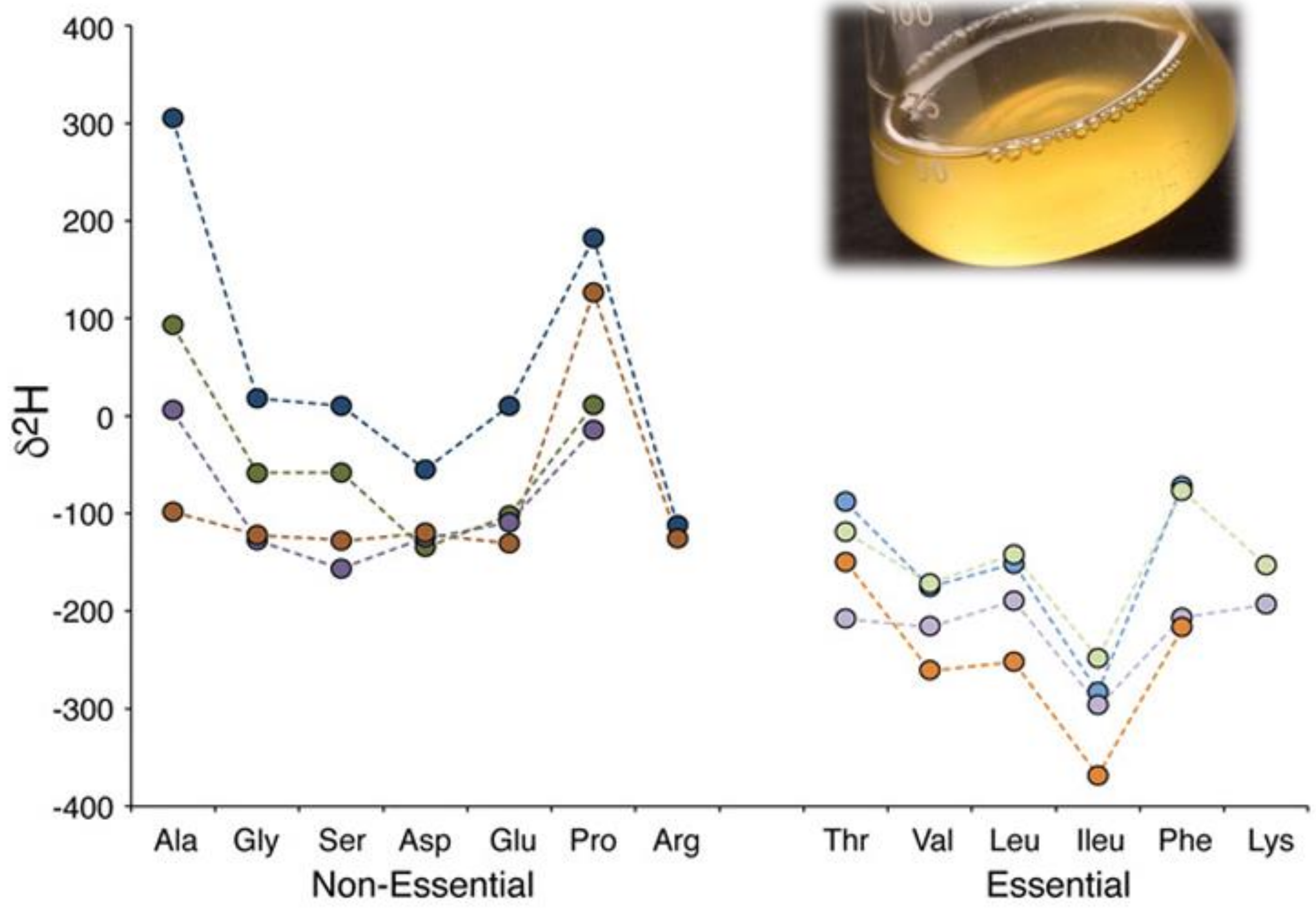
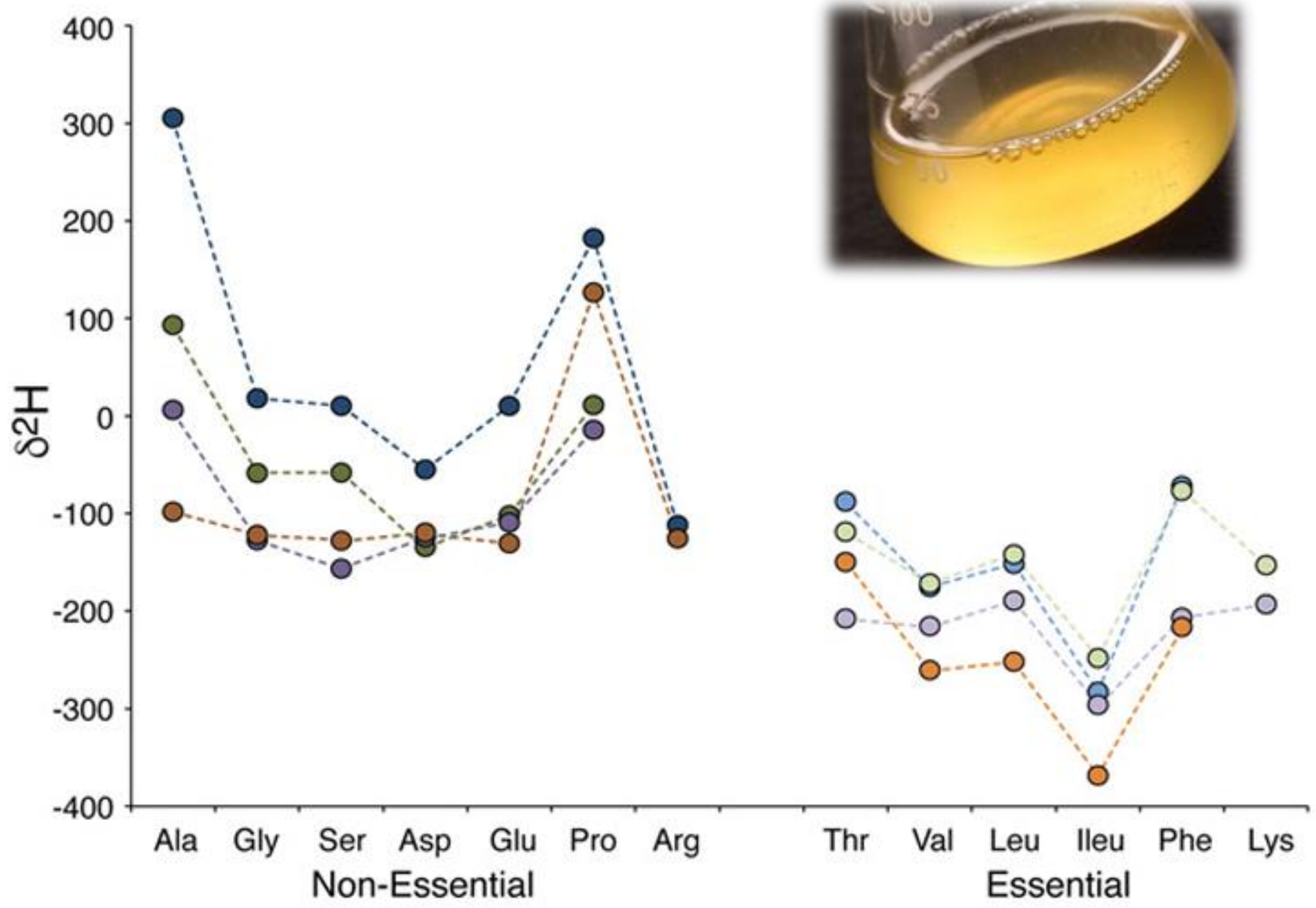


Highlights

- **<u>Huge</u>** range in AA δ^2H values (>300‰)

Fogel et al. 2016 PNAS

Our First Experiment with Amino Acid δ^2H: Prokaryotes



Highlights

- **<u>Huge</u>** range in AA δ^2H values (>300‰)
- Essential AAs are generally lower in δ^2H values than non-essential AAs

Fogel et al. 2016 PNAS

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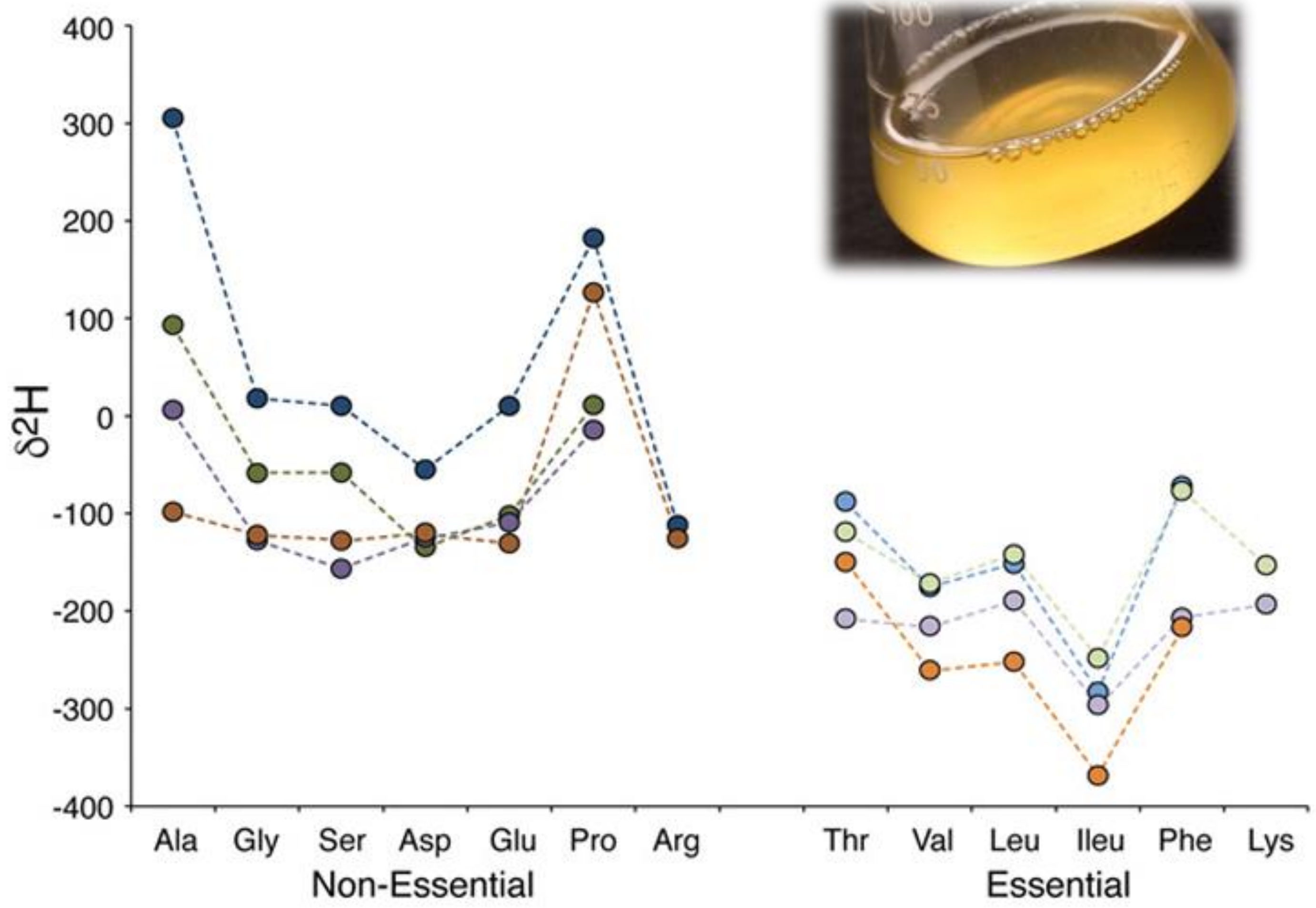


Highlights

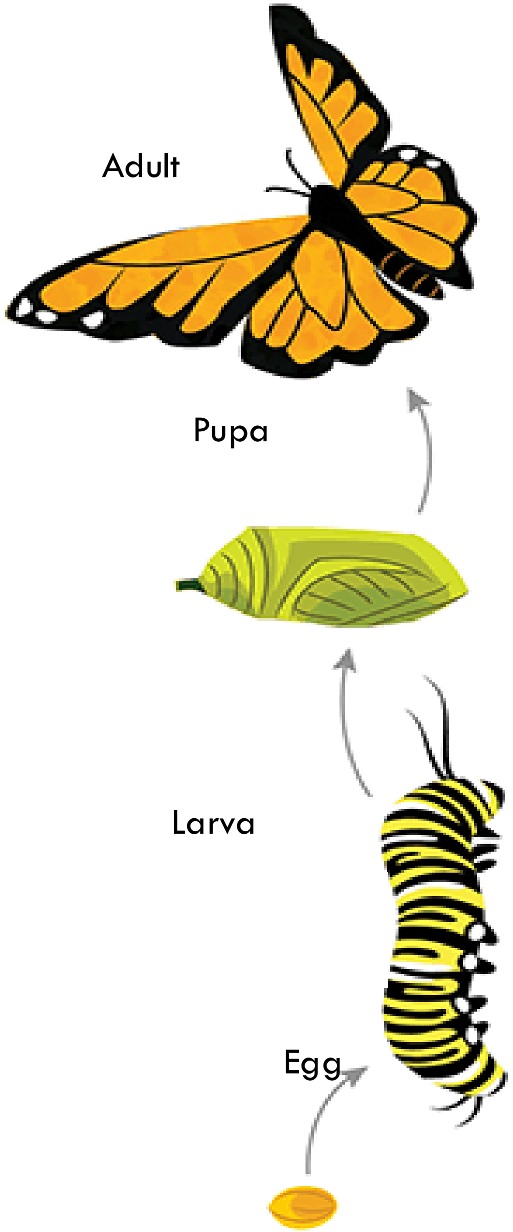
- **<u>Huge</u>** range in AA δ^2H values (>300‰)
- Essential AAs are generally lower in δ^2H values than non-essential AAs
- Evidence of direct routing of media protein into nearly all AAs

Fogel et al. 2016 PNAS

Does Amino Acid δ^2H Vary with Trophic Level and Metamorphosis?

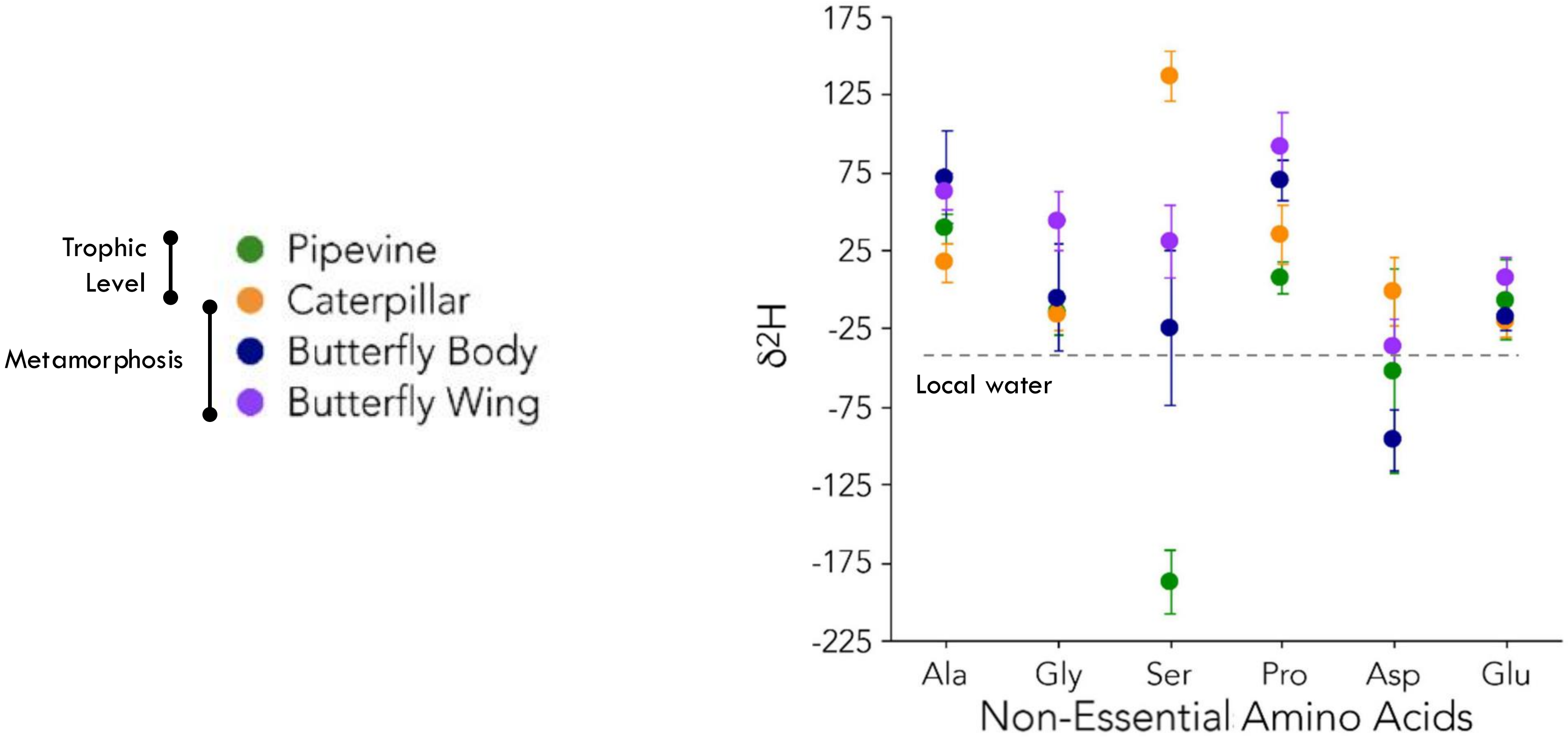
Pipevine Swallowtails (*Battus philenor*)

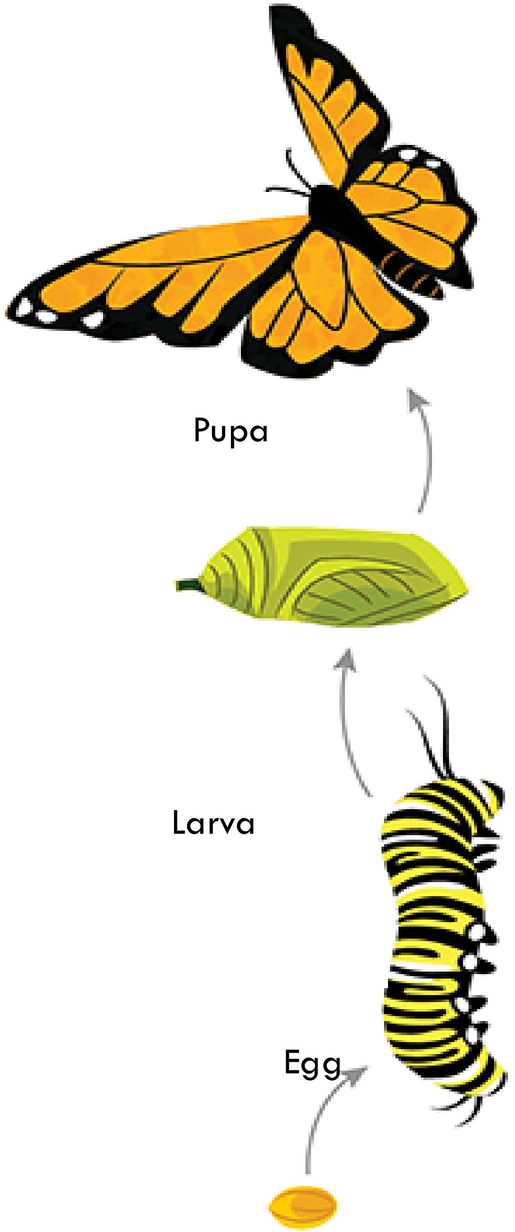
Pipevine
(*Aristolochia*)



Morra et al 2021. Frontiers Eco. Evol.

Amino Acid δ^2H Values Change With Trophic Level and Metamorphosis





Morra et al 2021. Frontiers Eco. Evol.

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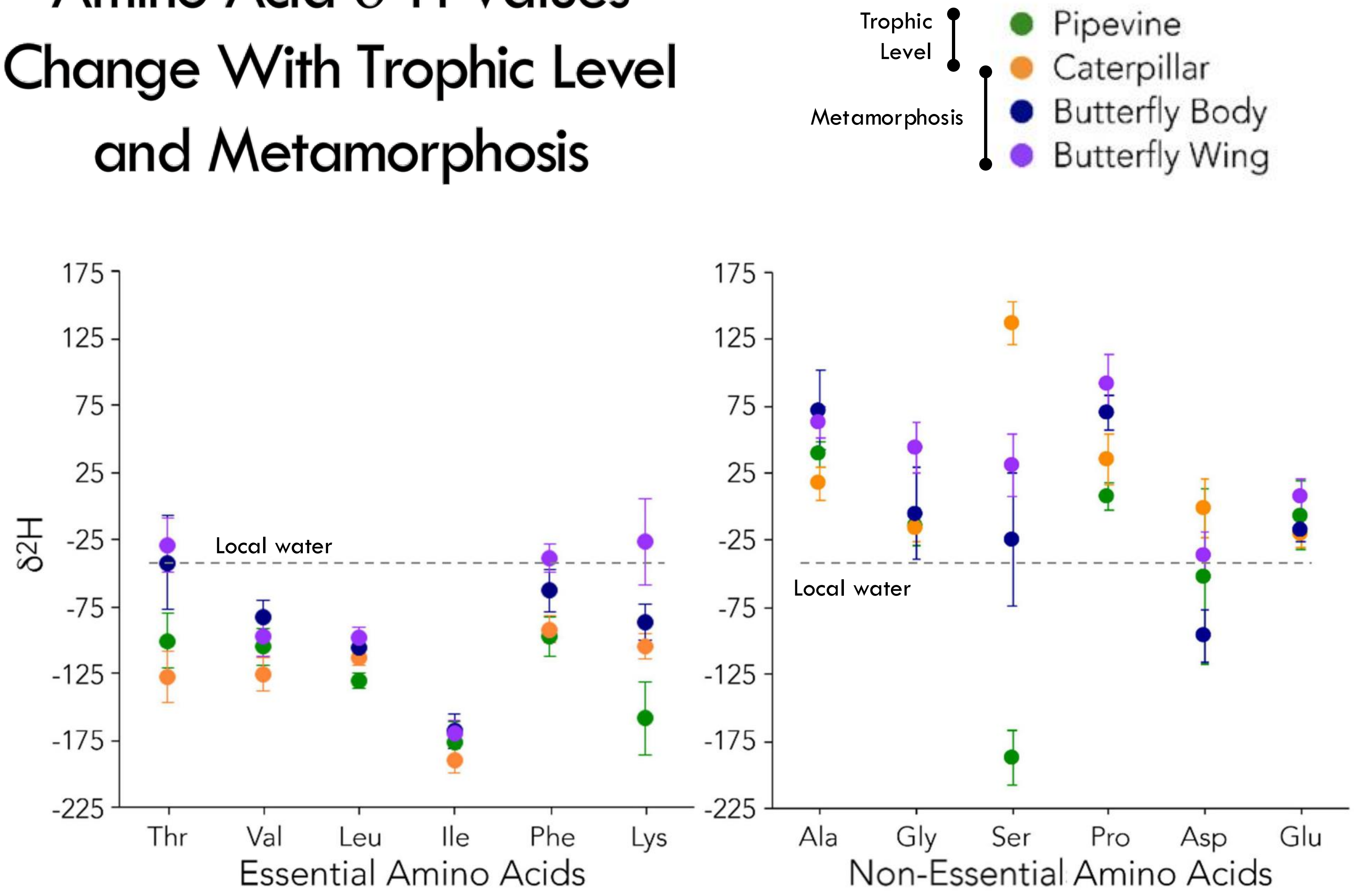
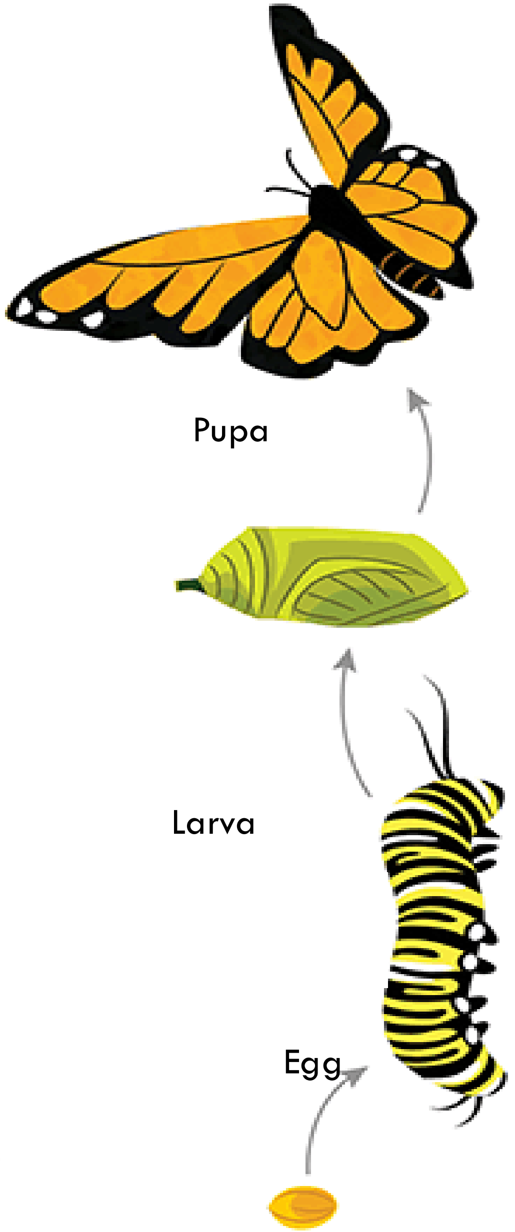
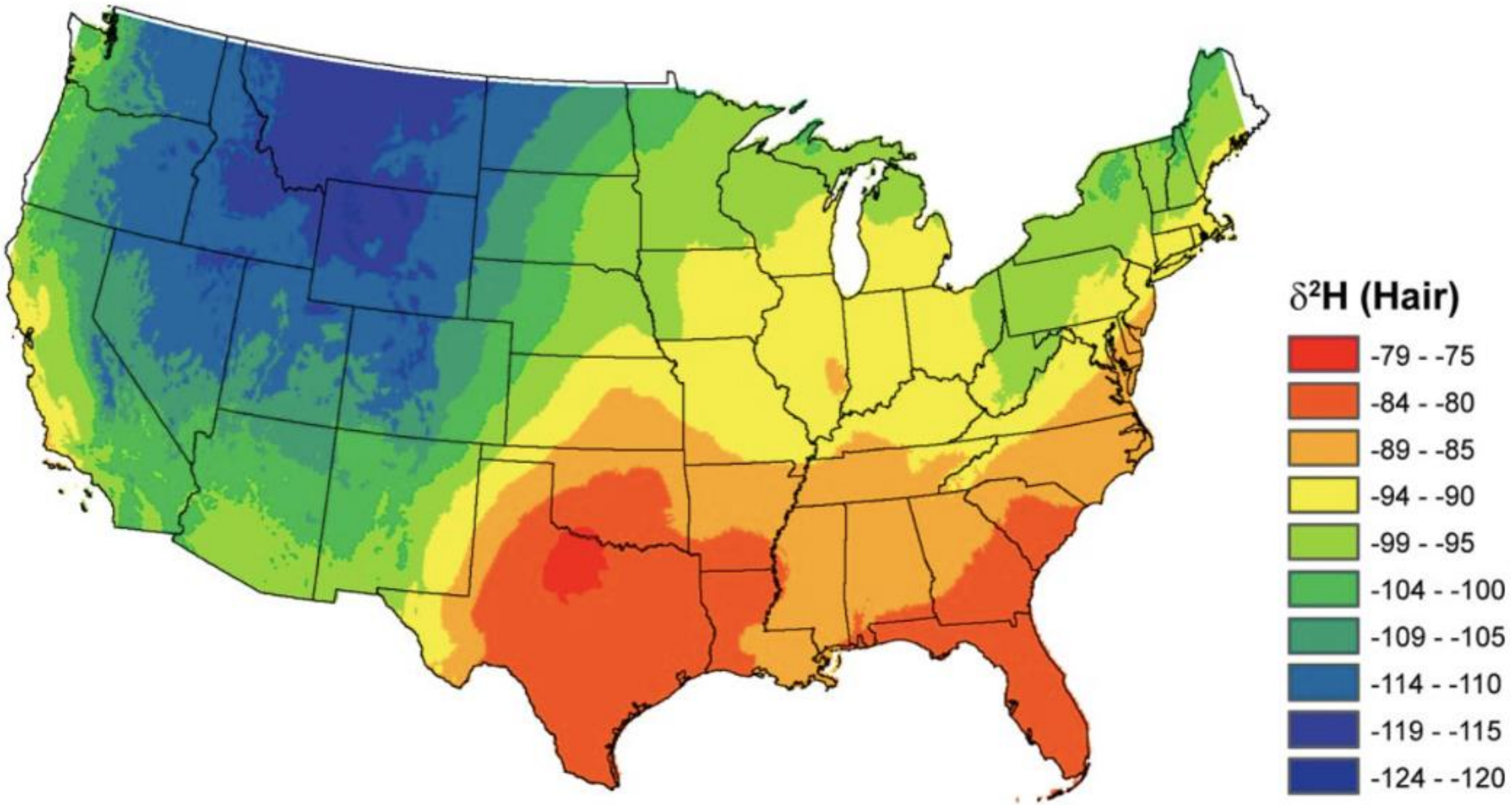




Morra et al 2021. Frontiers Eco. Evol.

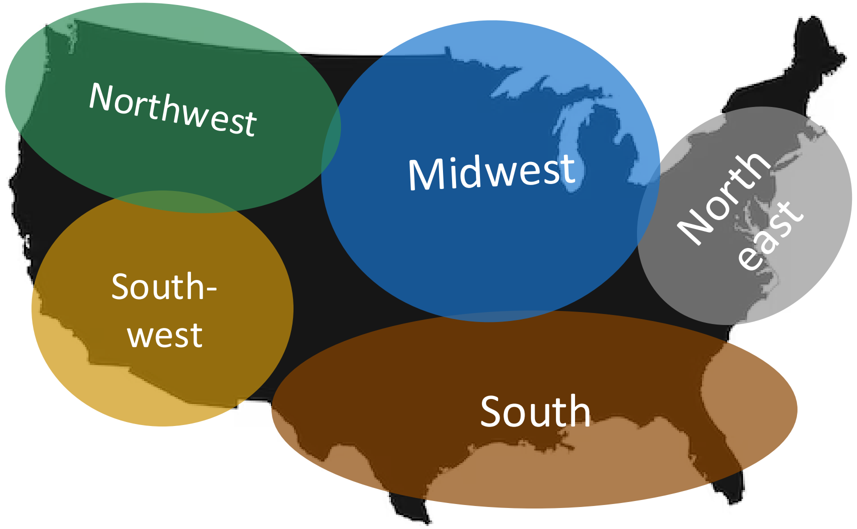
Geographic Assignment of Humans via Amino Acid δ^2H

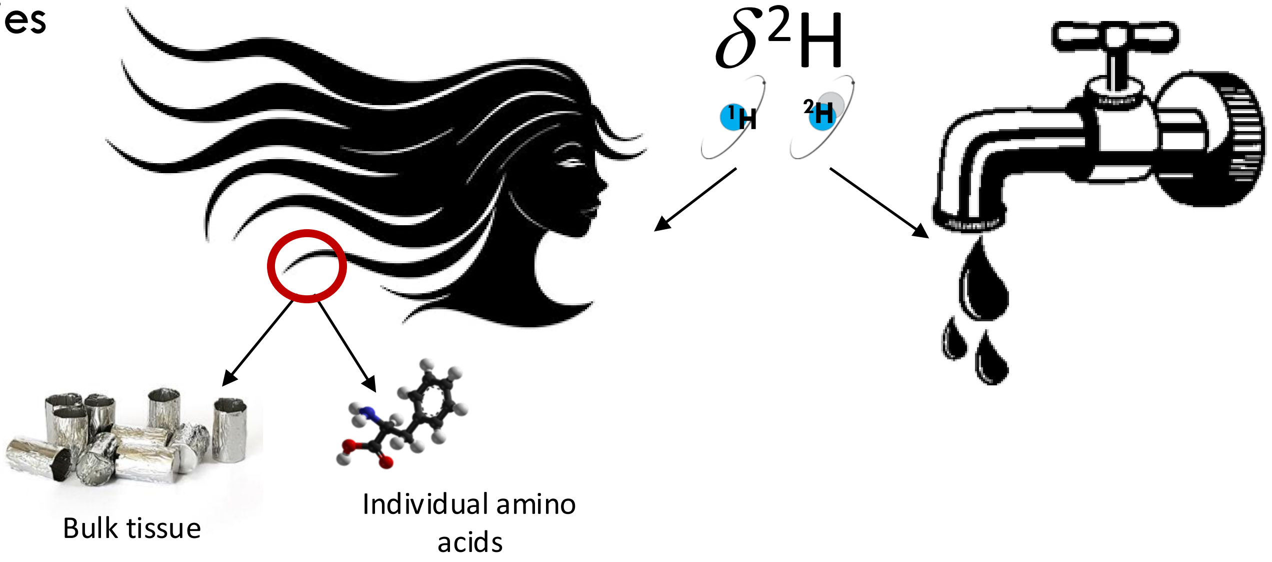


Ehleringer et al. 2008

Geographic Assignment of Humans via Amino Acid δ^2H

- 152 samples total:
  - Hair and fingernails
- Metadata:
  - δ^2H of local waters: measured paired tap water samples using a Picarro at UNM-CSI
  - Compiled data on local population densities at the county level using 2024 U.S. census data

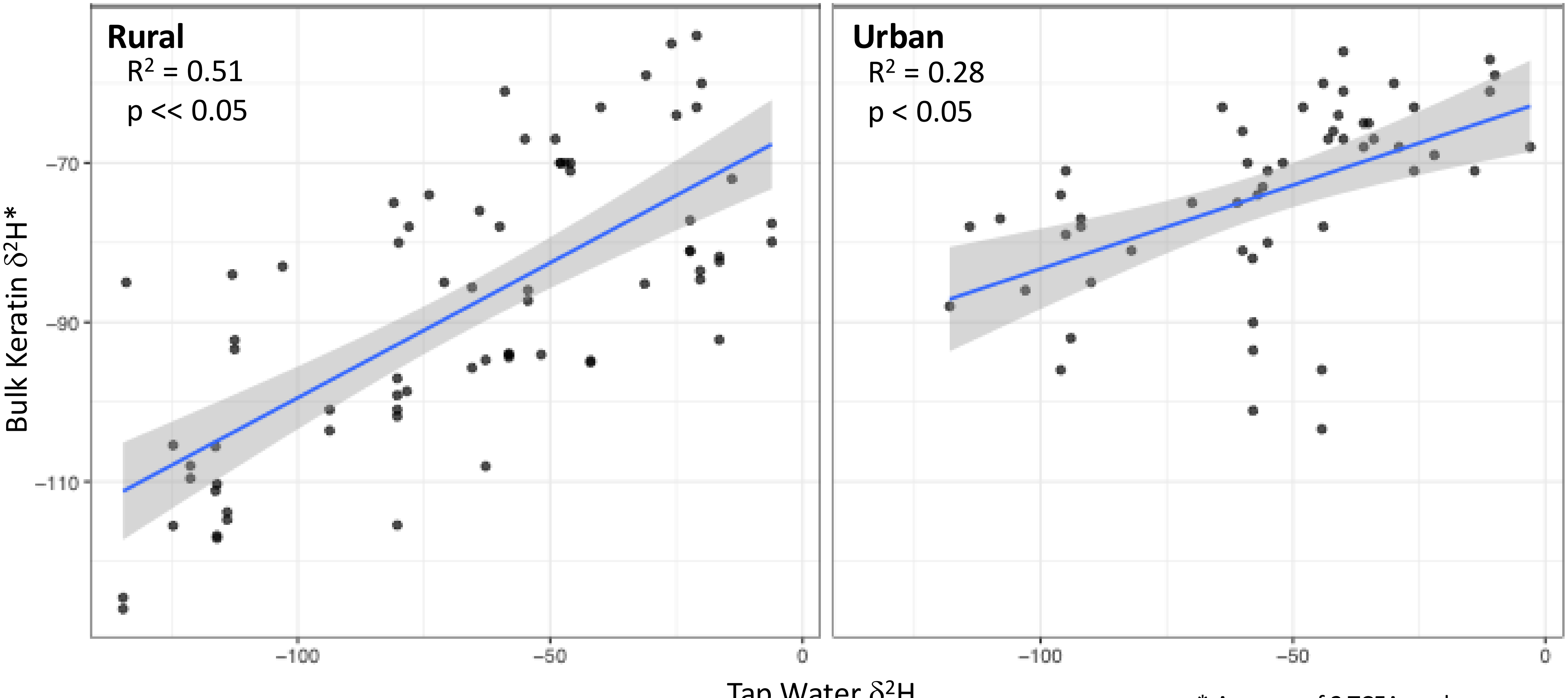




Dr. Christy Mancuso

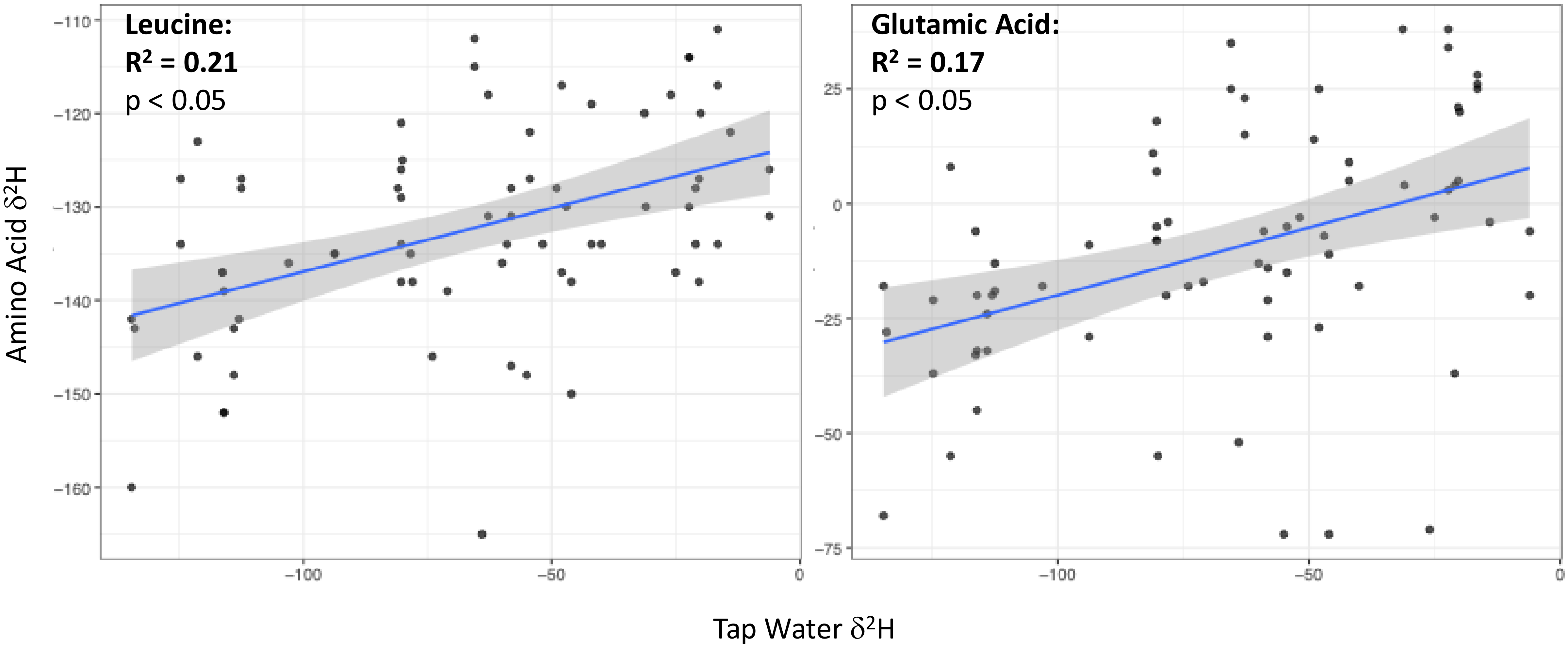
People living in rural areas have a stronger relationship between **bulk hair** δ^2H and **local tap water** δ^2H than those living in urban areas

Rural here defined as fewer than 120 people/km^2; defined at the county level

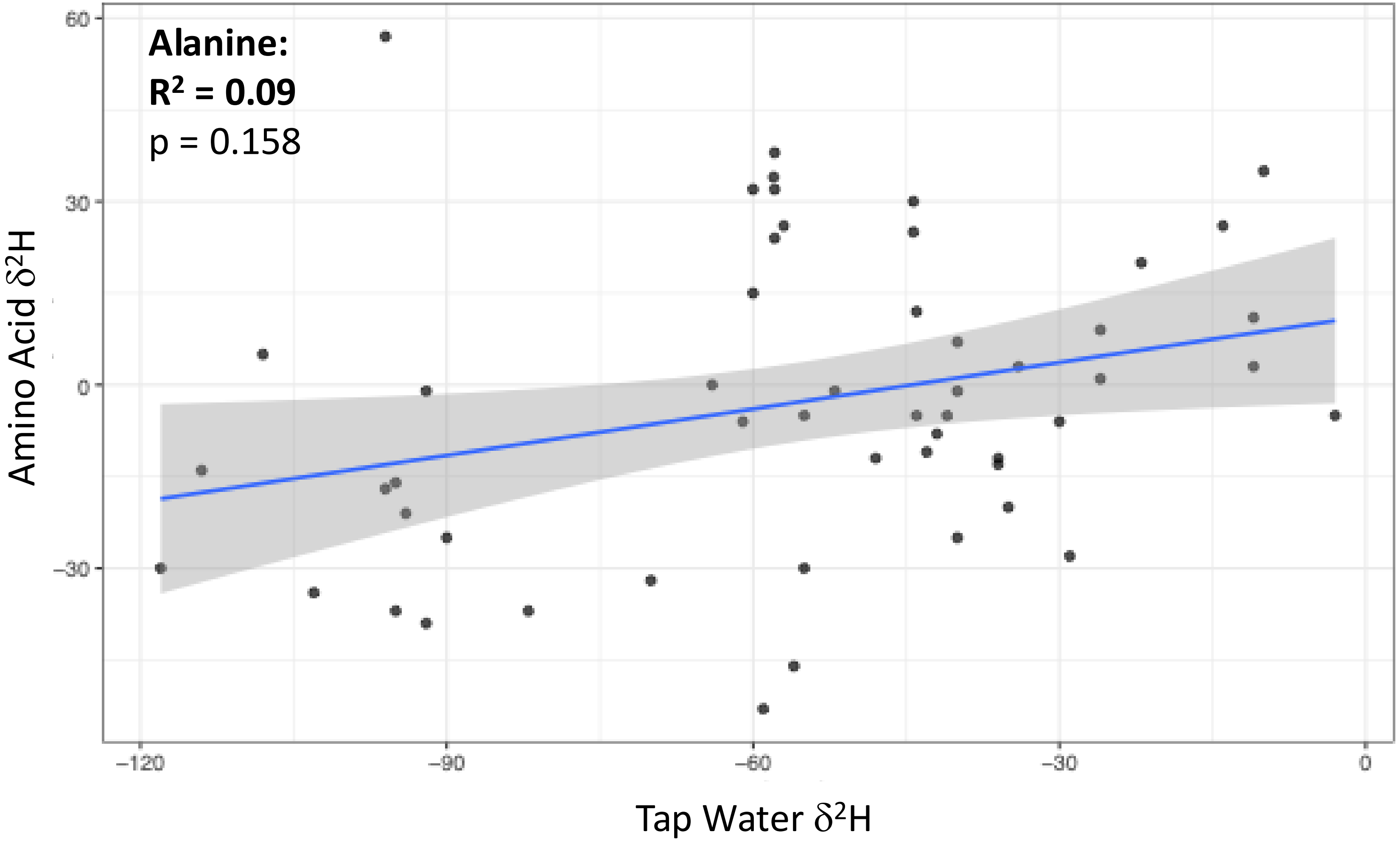


* Average of 2 TCEA analyses per sample

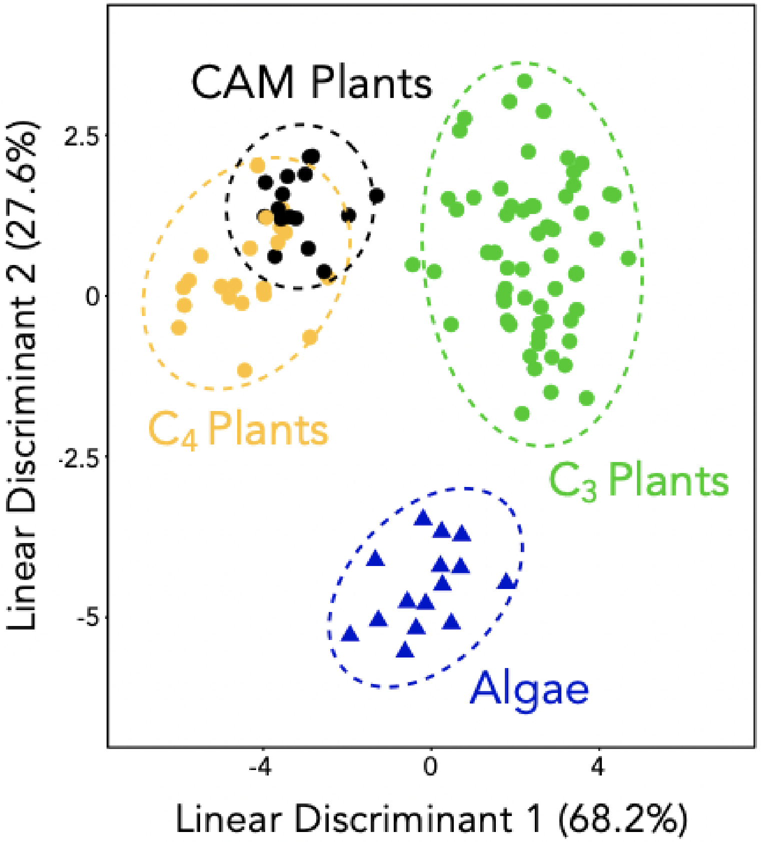
People living in **rural areas** show correlation between **amino acid** δ^2H and **local tap water** δ^2H

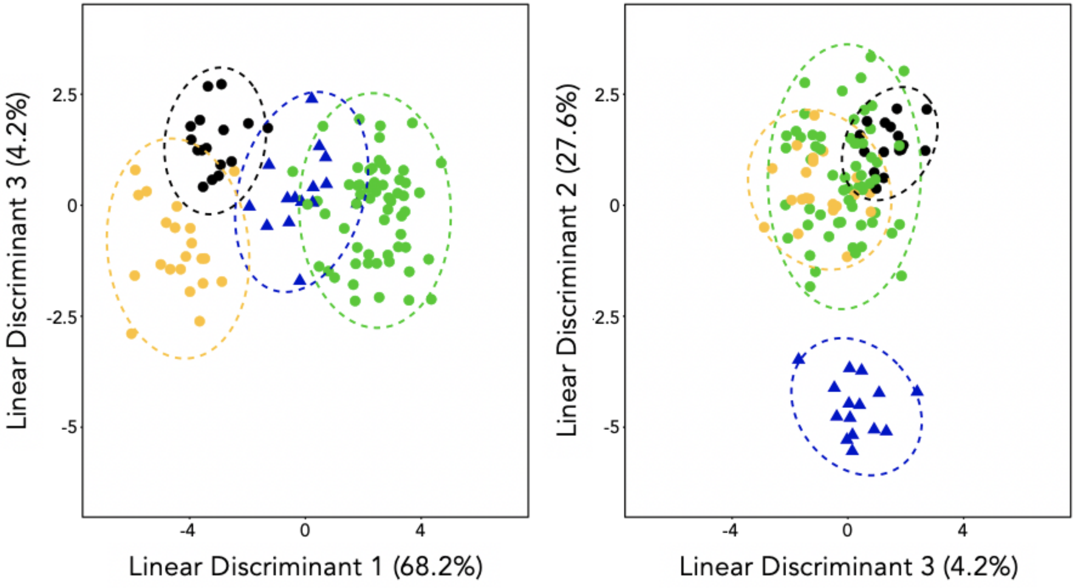


People living in **urban areas** have no significant relationships between **amino acid** δ^2H and **local tap water** δ^2H

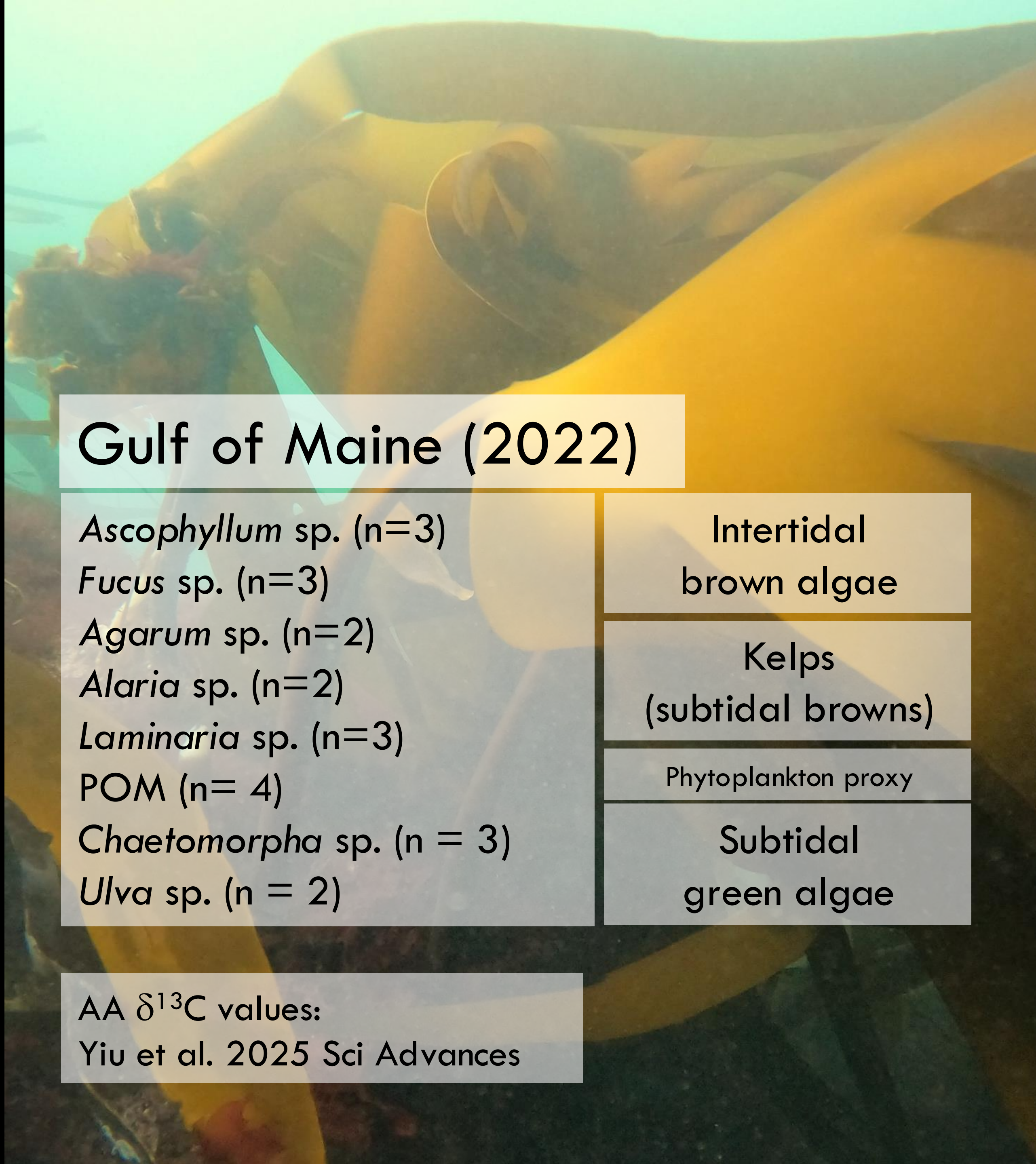


Essential Amino Acid δ^2H Fingerprints?





Besser et al. in review



Samples

Gulf of Maine (2022)

Ascophyllum sp. (n=3) *Fucus* sp. (n=3)	Intertidal brown algae
Agarum sp. (n=2) *Alaria* sp. (n=2) *Laminaria* sp. (n=3)	Kelps (subtidal browns)
POM (n= 4)	Phytoplankton proxy
Chaetomorpha sp. (n = 3) *Ulva* sp. (n = 2)	Subtidal green algae

AA $\delta^{13}C$ values:
Yiu et al. 2025 Sci Advances

Samples

New Mexico (2018)

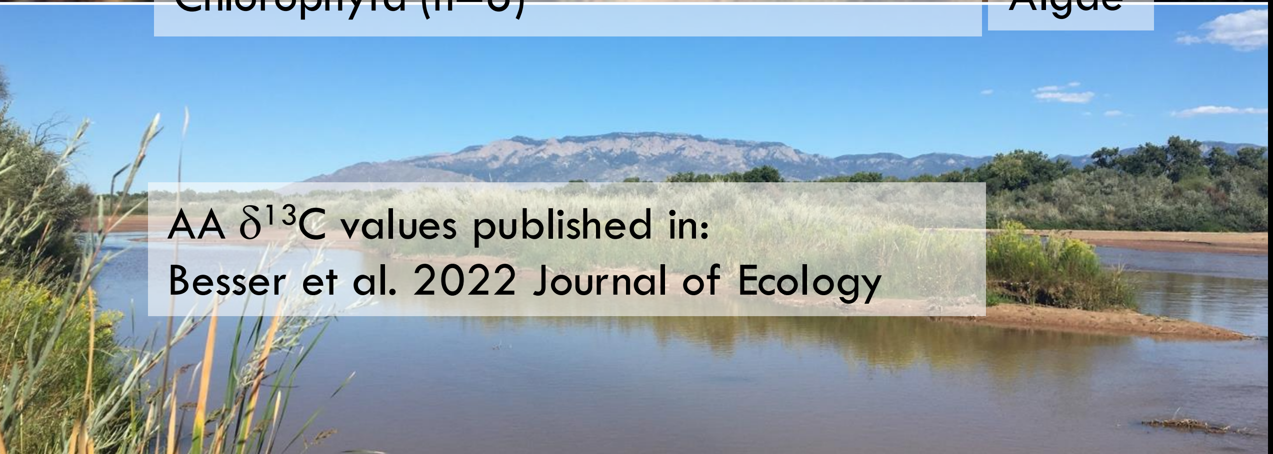
Gutierrezia sarothrae (n=2)
Populus deltoides wislizenii (n=3)
Sphaeralcea sp. (n=3)
Bouteloua eriopoda (n=3)
Muhlenbergia sp. (n=3)
Opuntia sp. (n=3)
Chlorophyta (n=6)

C_3 Plants

C_4 Plants

CAM Plant

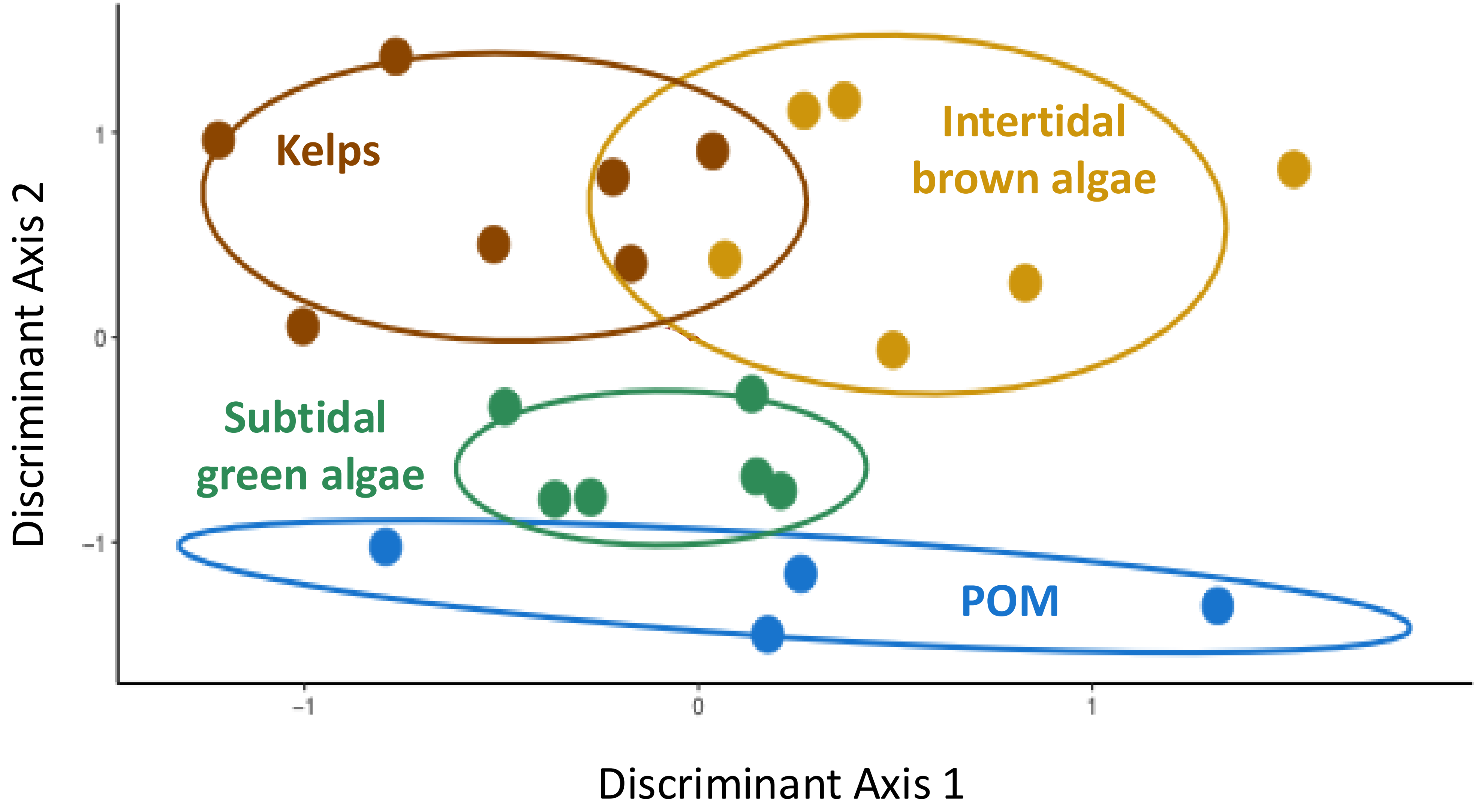
Algae

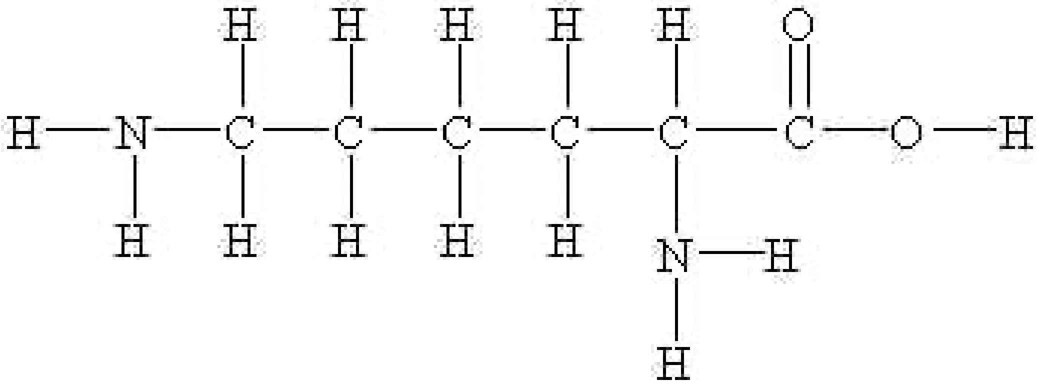


AA $\delta^{13}C$ values published in:
Besser et al. 2022 Journal of Ecology

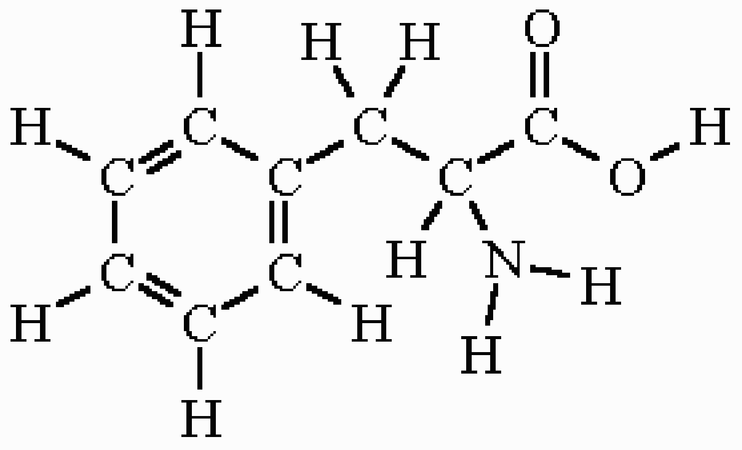
AA δ^2H “fingerprints” of primary producers

Gulf of Maine

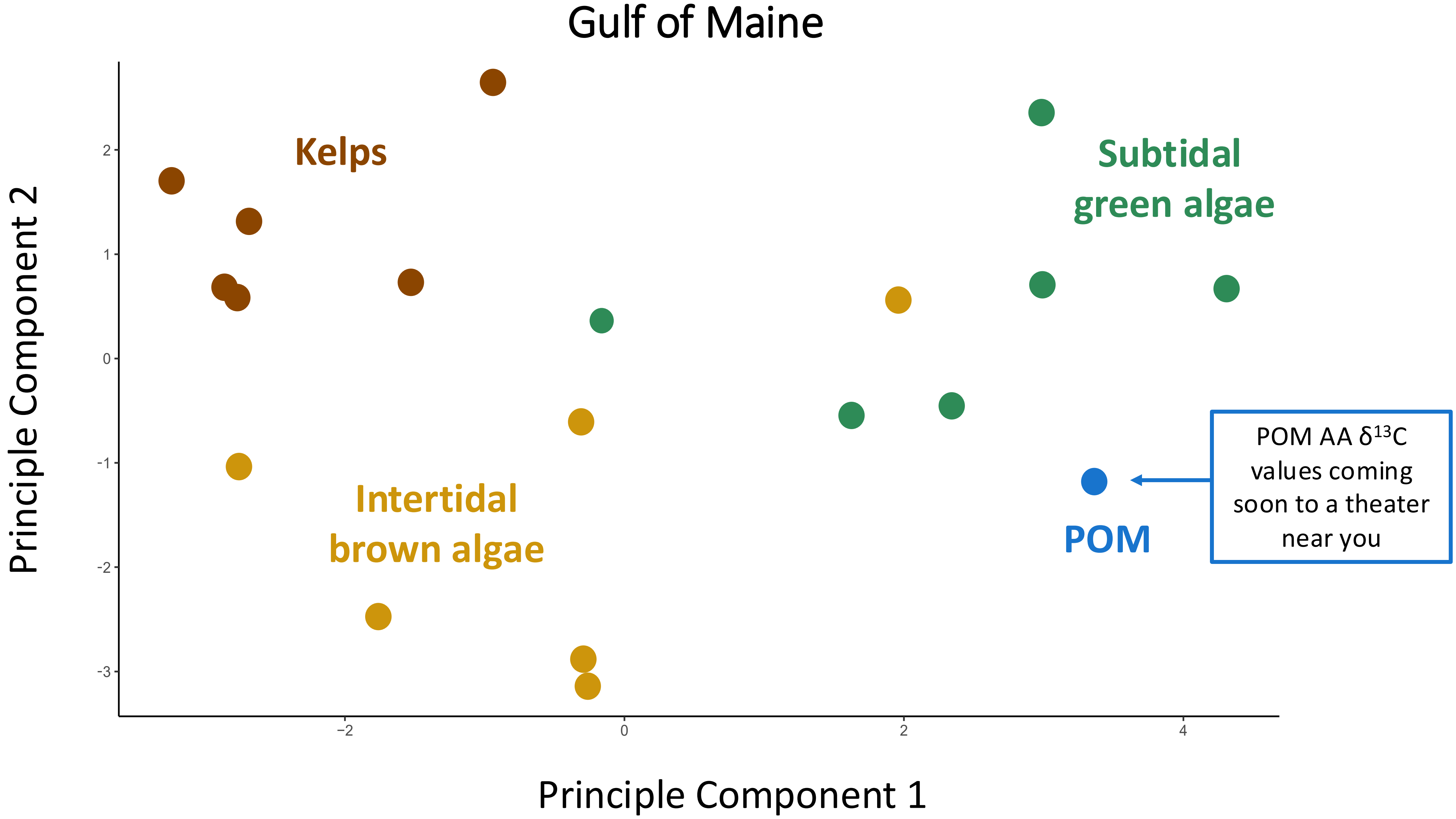




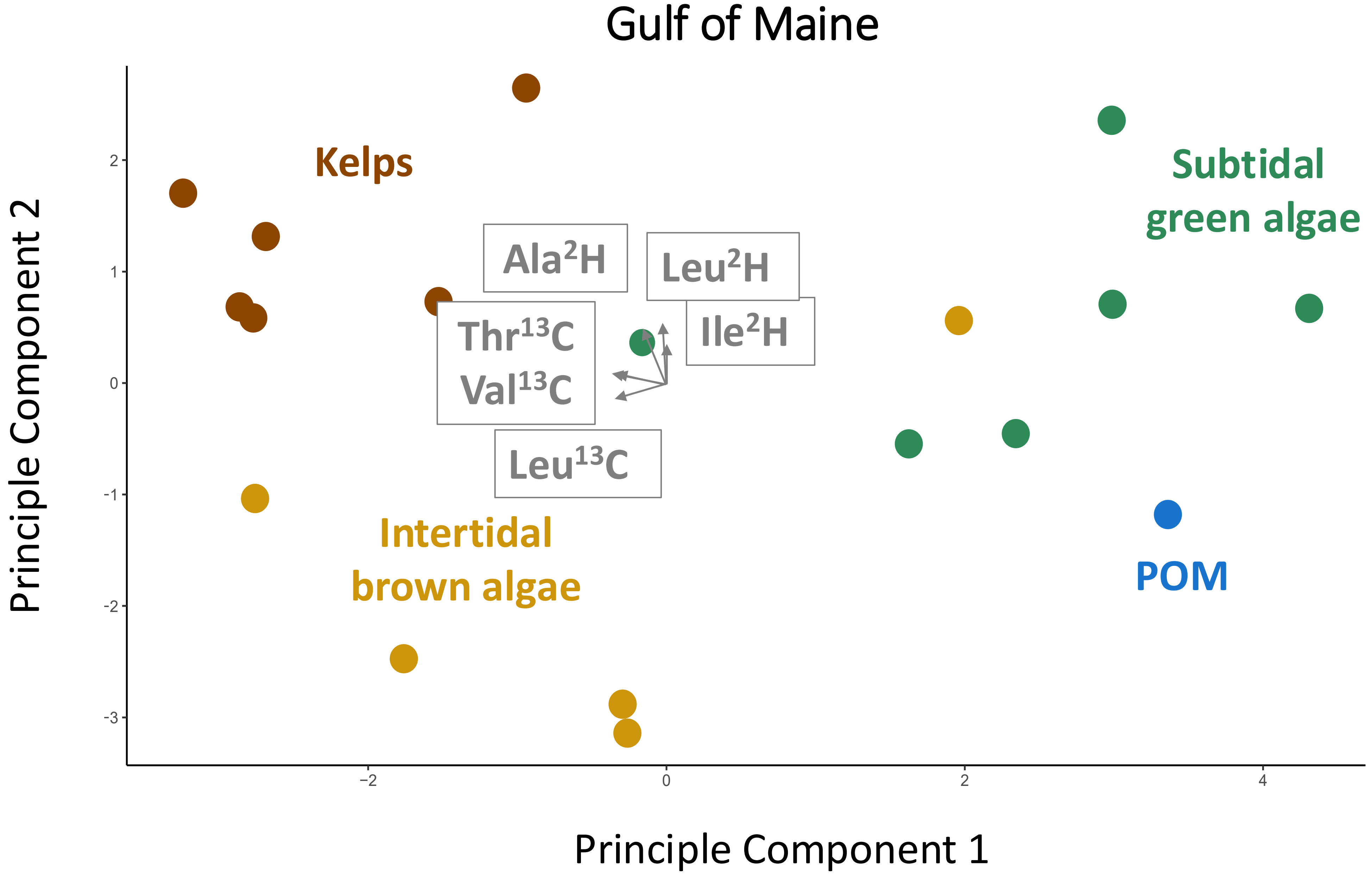
What about ‘fingerprints” using AA δ^2H and $\delta^{13}C$ values?



AA $\delta^{13}C$ and $\delta^{2}H$ "fingerprints" of primary producers

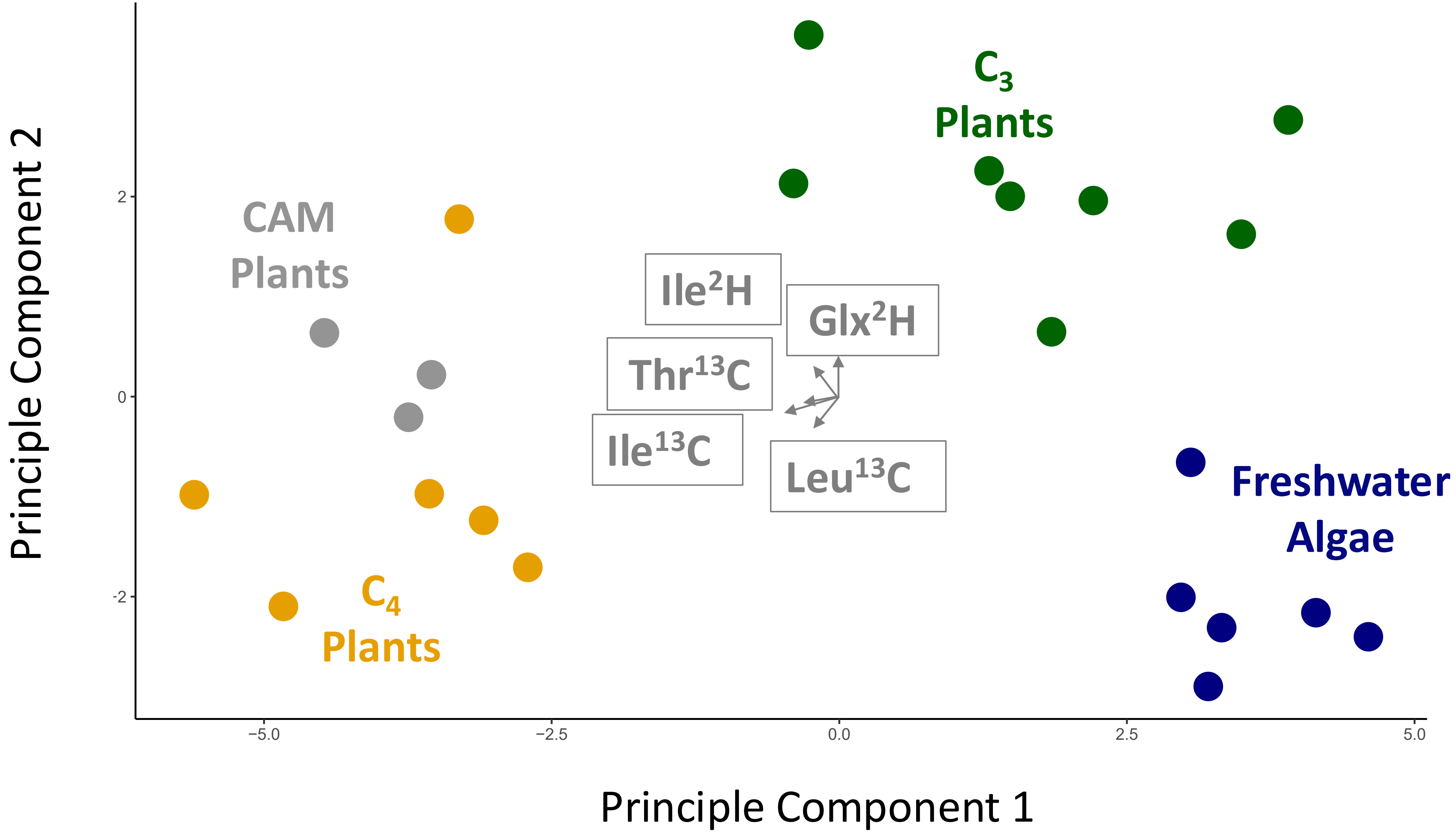


AA δ13C and δ2H "fingerprints" of primary producers



AA $\delta^{13}C$ and $\delta^{2}H$ "fingerprints" of primary producers

New Mexico



Take Home Message(s): Amino Acid δ^2H

For δ^2H, amino acids (AA) are classified as non-essential and essential and generally mirror patterns seen in $\delta^{13}C$.

Experiments on bacteria and mice show that essential AA δ^2H are faithful tracers of dietary protein δ^2H values, while non-essential AA δ^2H values are more influenced by carbohydrates and (drinking) water.

δ^2H analysis of non-essential AA (Ala) may provide a method for assessing region of origin (and movement/migration) at higher resolution than bulk tissue analysis for humans where diet is more controlled.

This approach may allow for tracing sources of water and food in a single tissue sample.

Amino acid δ^2H analysis is a promising but relatively unexplored proxy.
(empty niche waiting to be filled)

BONUS SLIDES

How About a Eukaryote?

Ingredient	Macromolecule	Diet 1	Diet 2	Diet 3	δ^2H (SD)	[H] (SD)	δ^{13}C (SD)	[C] (SD)	δ^{15}N (SD)	[N] (SD)
Casein	Protein	0.05	0.20	0.35	-107 ± 4.2	6.0 ± 0.02	-24.8 ± 0.2	48.4 ± 0.32	5.5 ± 0.3	14.1 ± 0.35
Sucrose	Carbohydrates	0.45	0.30	0.15	-14 ± 4.1	6.3 ± 0.04	-11.7 ± 0.8	41.9 ± 0.15	–	–
Corn Meal	Carbohydrates	0.15	0.15	0.15	-18 ± 4.4	5.8 ± 0.05	-10.8 ± 0.2	47.2 ± 0.05	1.5 ± 0.8	0.8 ± 0.05
Corn Oil	Lipids	0.02	0.02	0.02	-154 ± 4.6	9.6 ± 0.21	-15.5 ± 0.2	73.9 ± 2.65	–	–
Cellulose	Binder	0.25	0.25	0.25	-25 ± 4.0	5.8 ± 0.02	-25.6 ± 0.2	41.9 ± 0.41	–	–
Fortified Salt	Salt	0.04	0.04	0.04	–	–	–	–	–	–
Brewer's Yeast	Yeast	0.02	0.02	0.02	-66 ± 4.1	5.8 ± 0.05	-21.4 ± 0.3	44.0 ± 0.30	3.2 ± 0.2	7.3 ± 0.15
Vitamin Mix	Vitamins	0.01	0.01	0.01	1 ± 4.4	6.3 ± 0.05	-12.9 ± 0.3	39.9 ± 0.38	–	–
	Protein:Carb Ratio	5:60	20:45	35:30						
	Bulk δ^2H	-26.0 ± 3.0	-41.0 ± 3.0	-56.0 ± 3.0						
	Bulk δ^{13}C	-16.2 ± 0.2	-18.3 ± 0.2	-20.3 ± 0.2						
	Bulk δ^{15}N	2.6 ± 0.7	3.8 ± 0.6	4.3 ± 0.5						

Mauriel
Rodriguez Curras

Dietary protein (casein) varied from 5% to 35%; low δ^2H: -108‰

Dietary carbohydrates varied from 30% to 60%; high δ^2H: -16‰

Drinking water δ^2H varied from -95‰ to -50‰ to +5‰

Dietary fat was low and did not vary among diet treatments

Rodriguez Curras et al. 2018

Bulk Tissue δ^2H Results

P_{Water}: 15–26%

P_{Carbs}: 10–35%

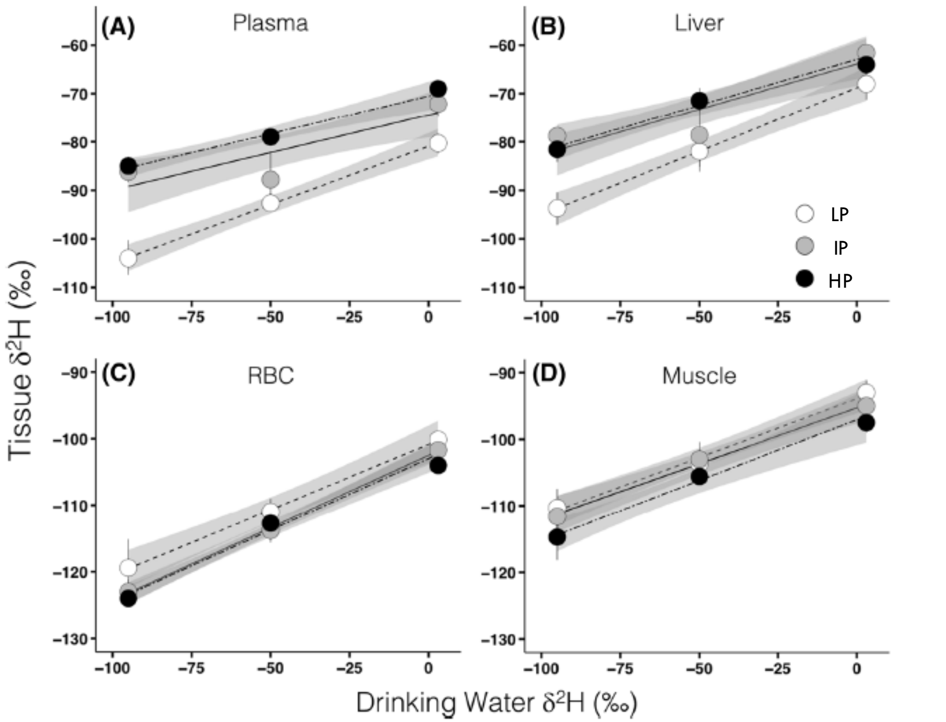
$P_{Protein}$: 41–69%

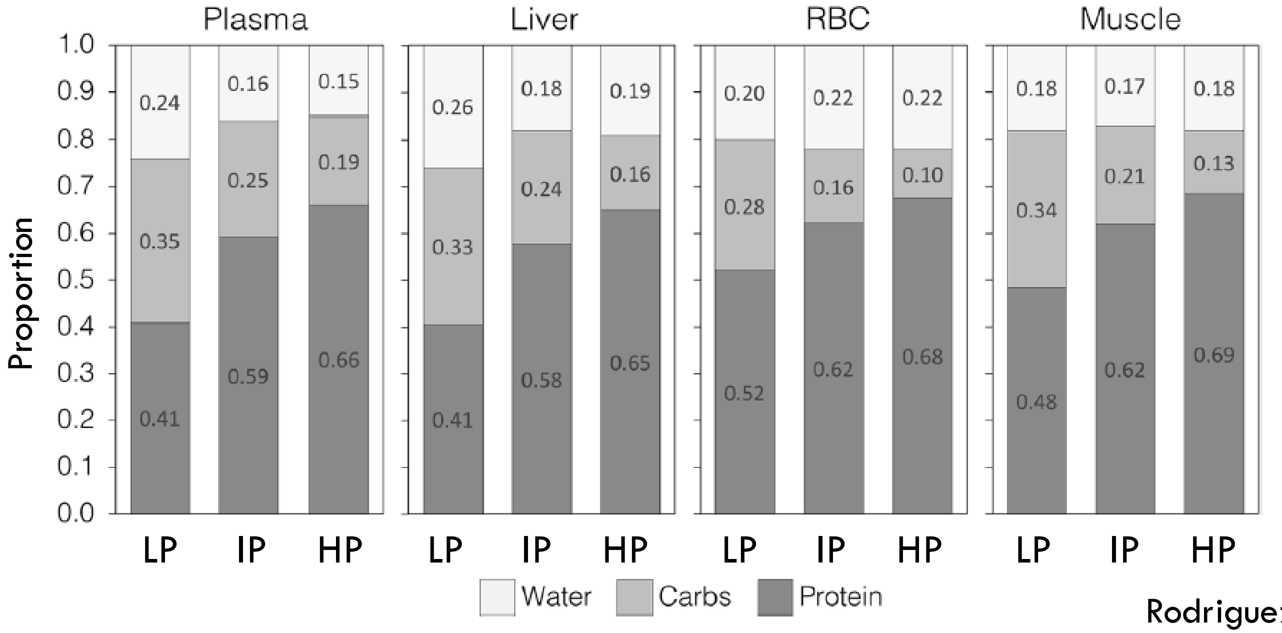
Larger P_{carbs} in low protein diet

Larger $P_{protein}$ in high protein diet

More AA *de novo* synthesis in low protein diet

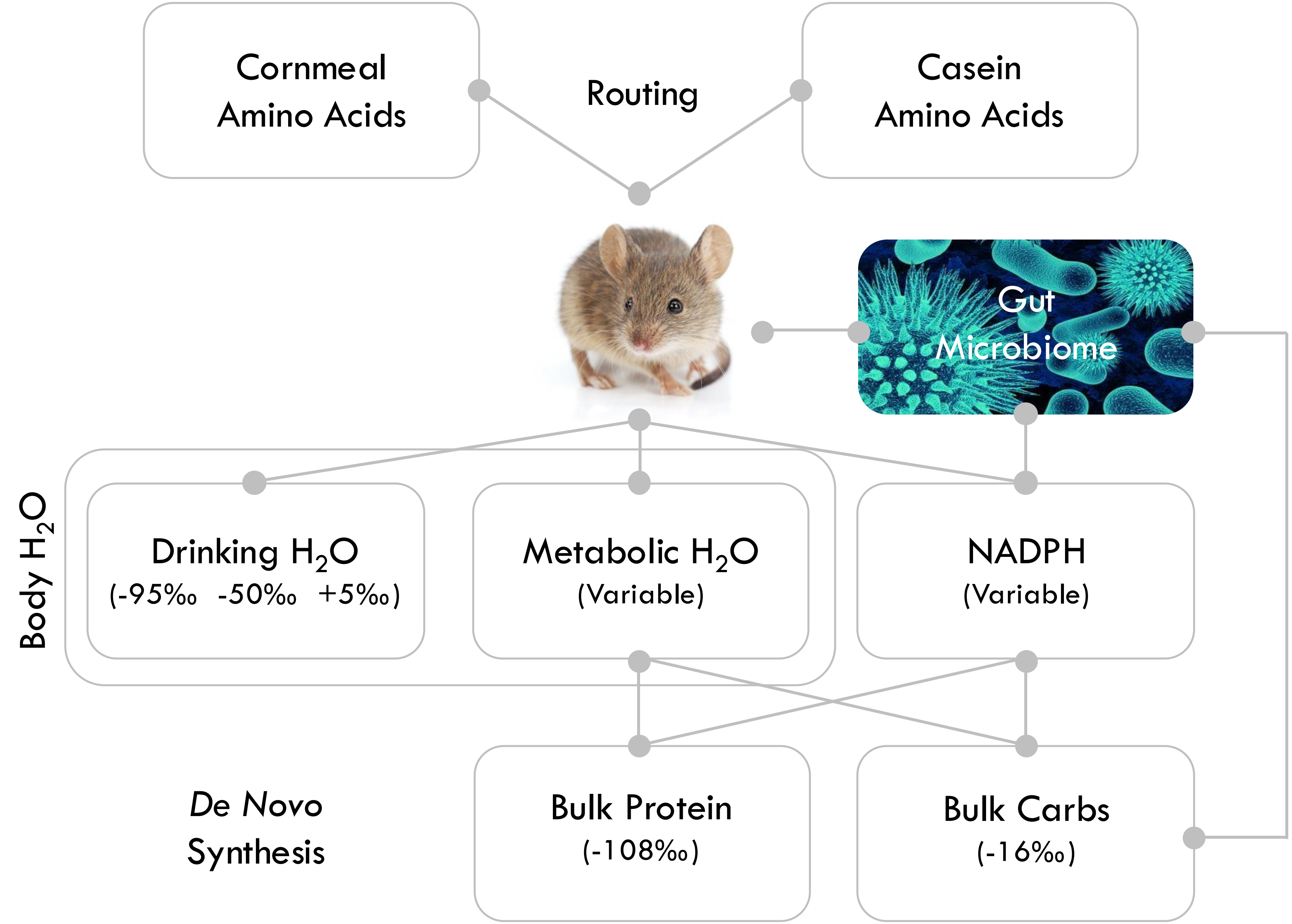
More AA routing in high protein diet





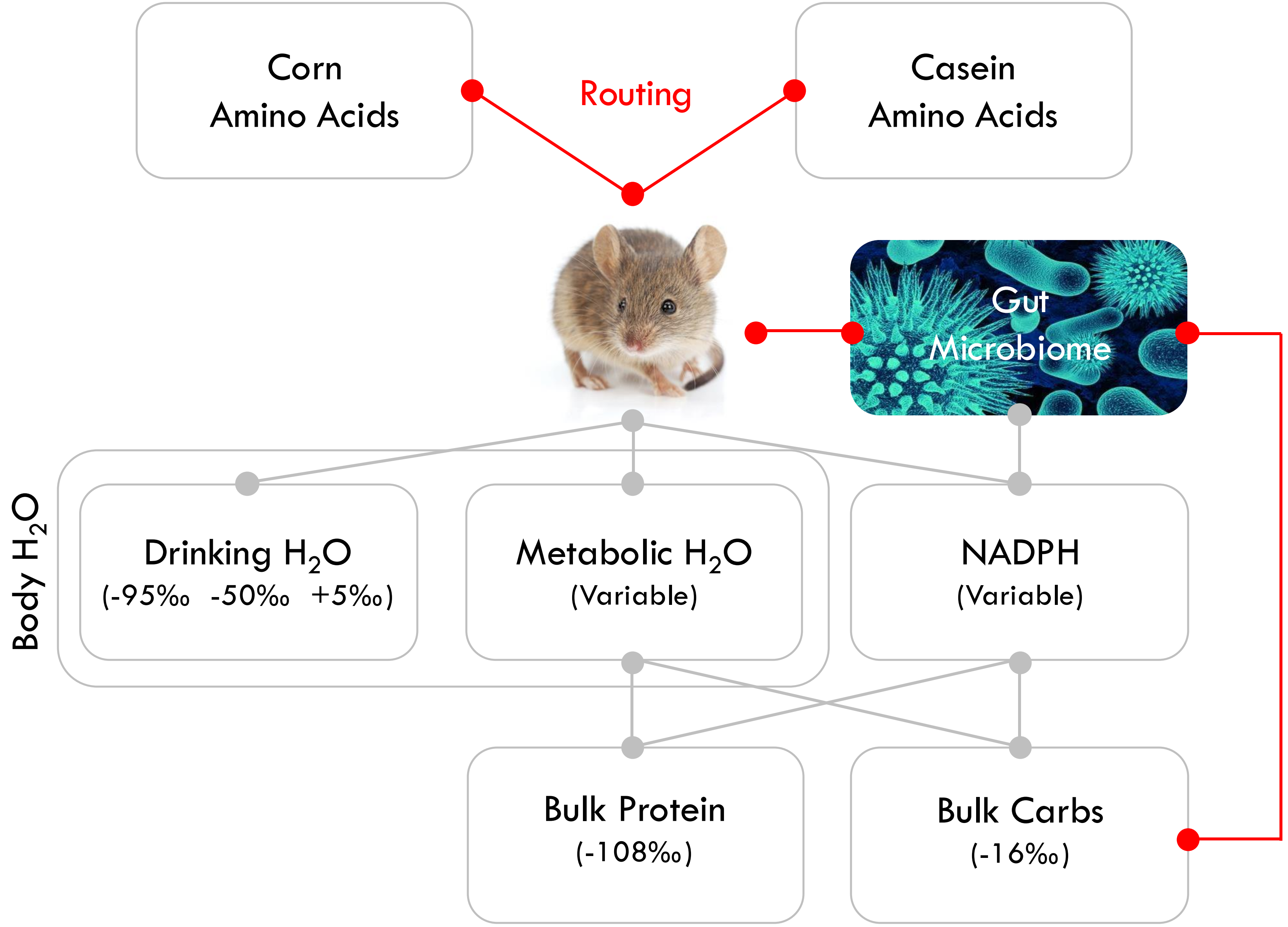
Rodriguez Curras et al. 2018

Our (Working) Model



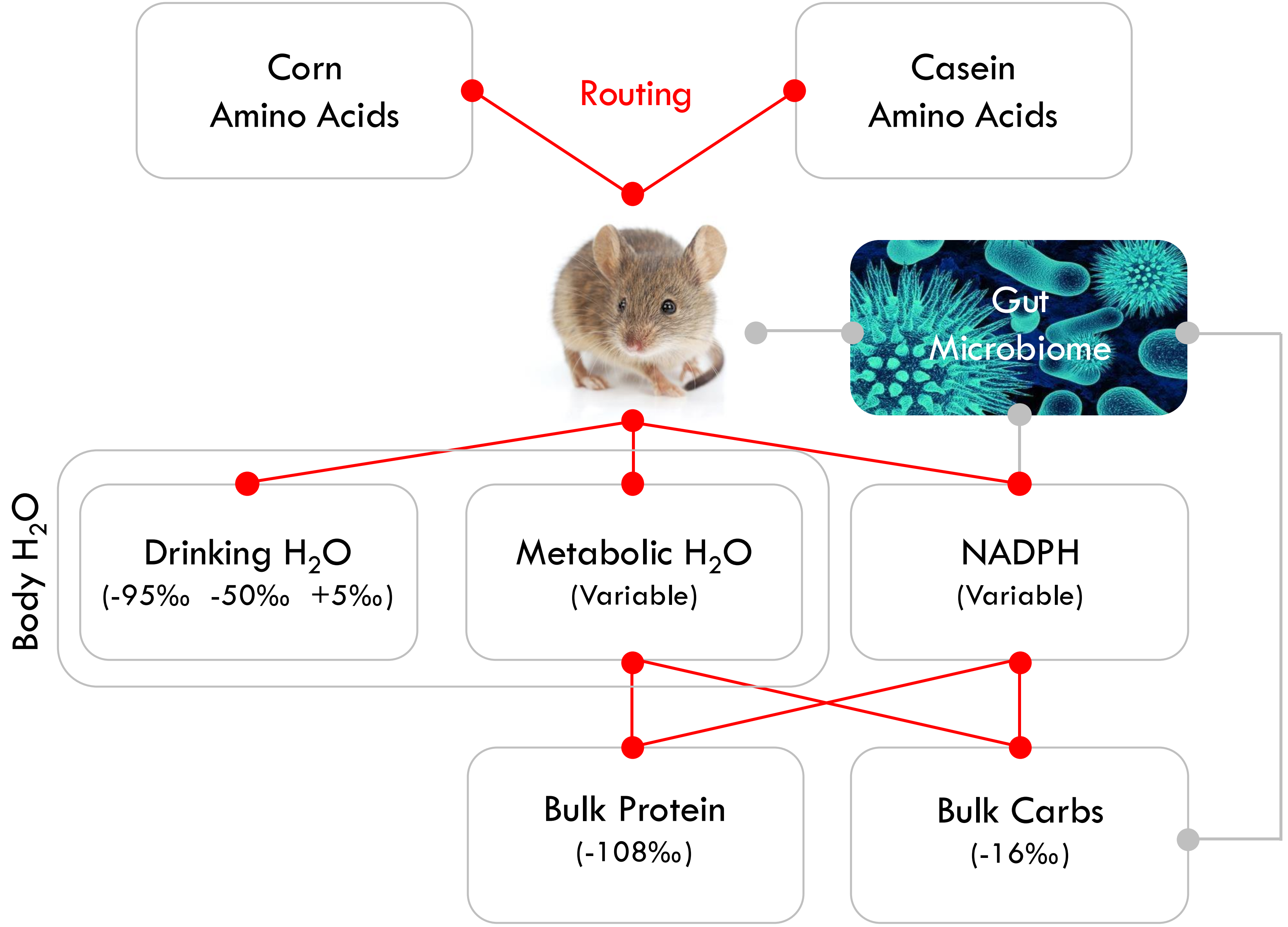
Newsome et al. 2020

Essential Amino Acids



Newsome et al. 2020

Non-Essential Amino Acids



Newsome et al. 2020

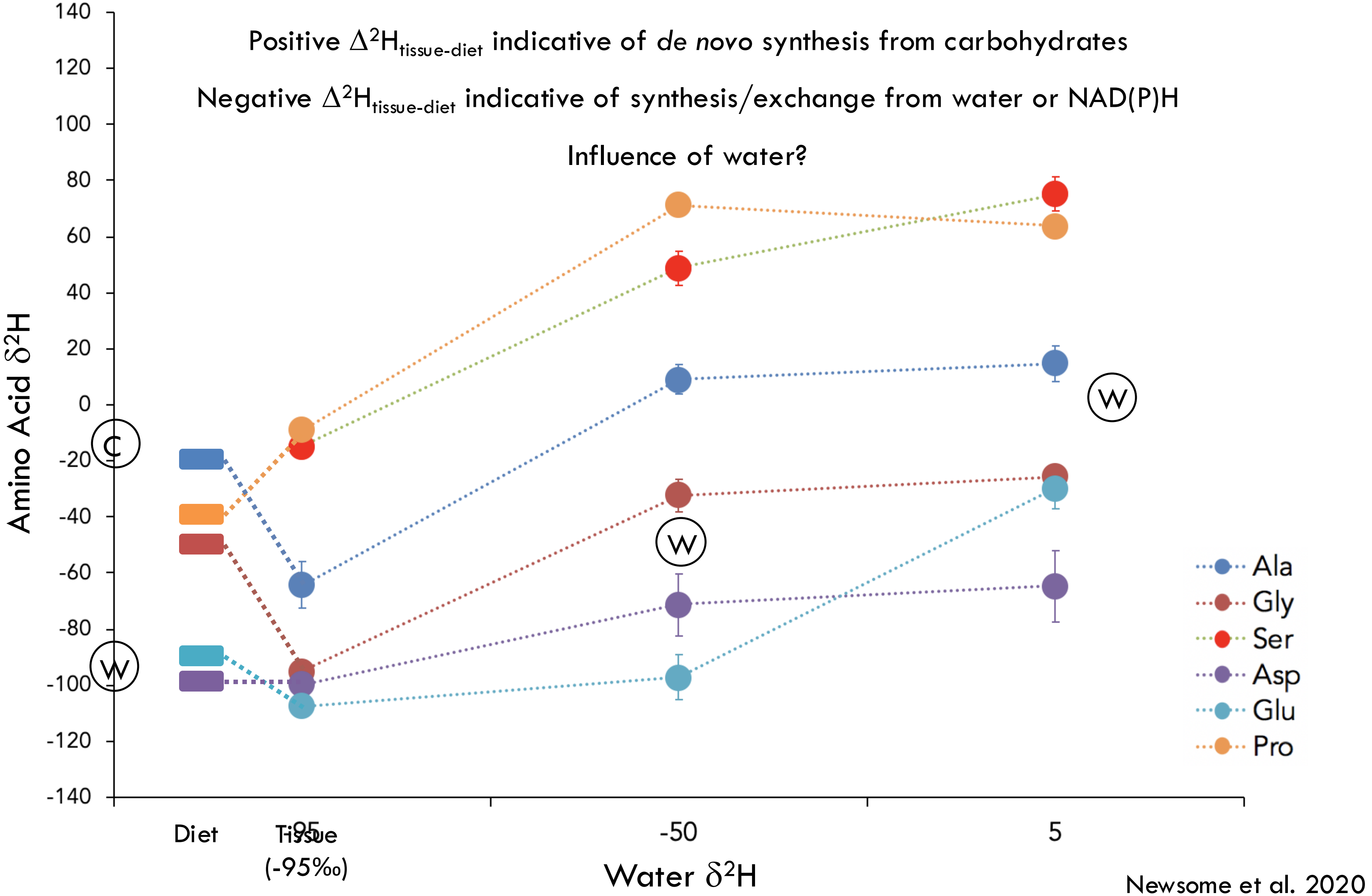
Example

No $\Delta^2H_{tissue-diet}$ indicative of direct routing

Positive $\Delta^2H_{tissue-diet}$ indicative of *de novo* synthesis from carbohydrates

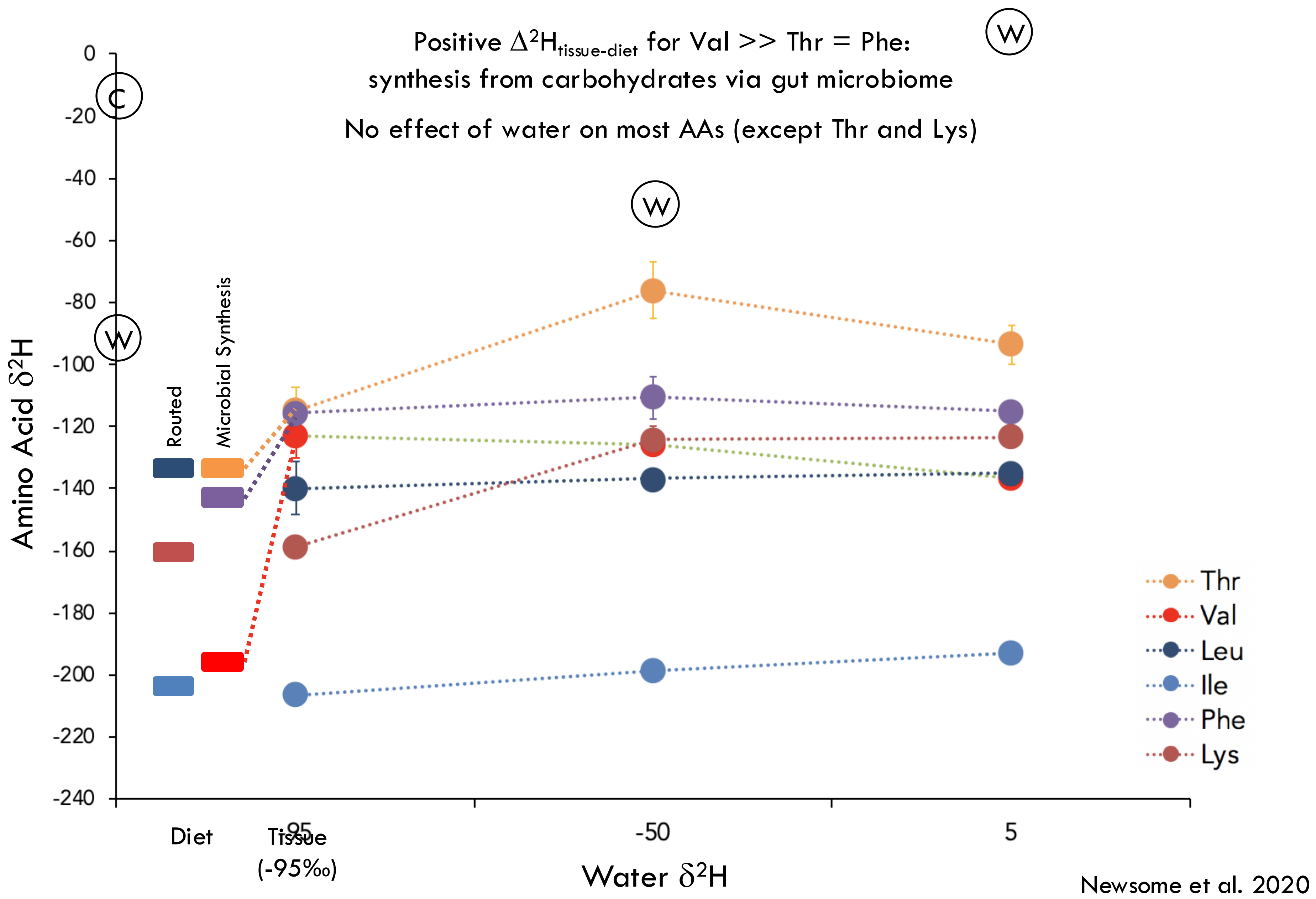
Negative $\Delta^2H_{tissue-diet}$ indicative of synthesis/exchange from water or NAD(P)H

Influence of water?



Newsome et al. 2020

Essential Amino Acids (Low Protein Diet)



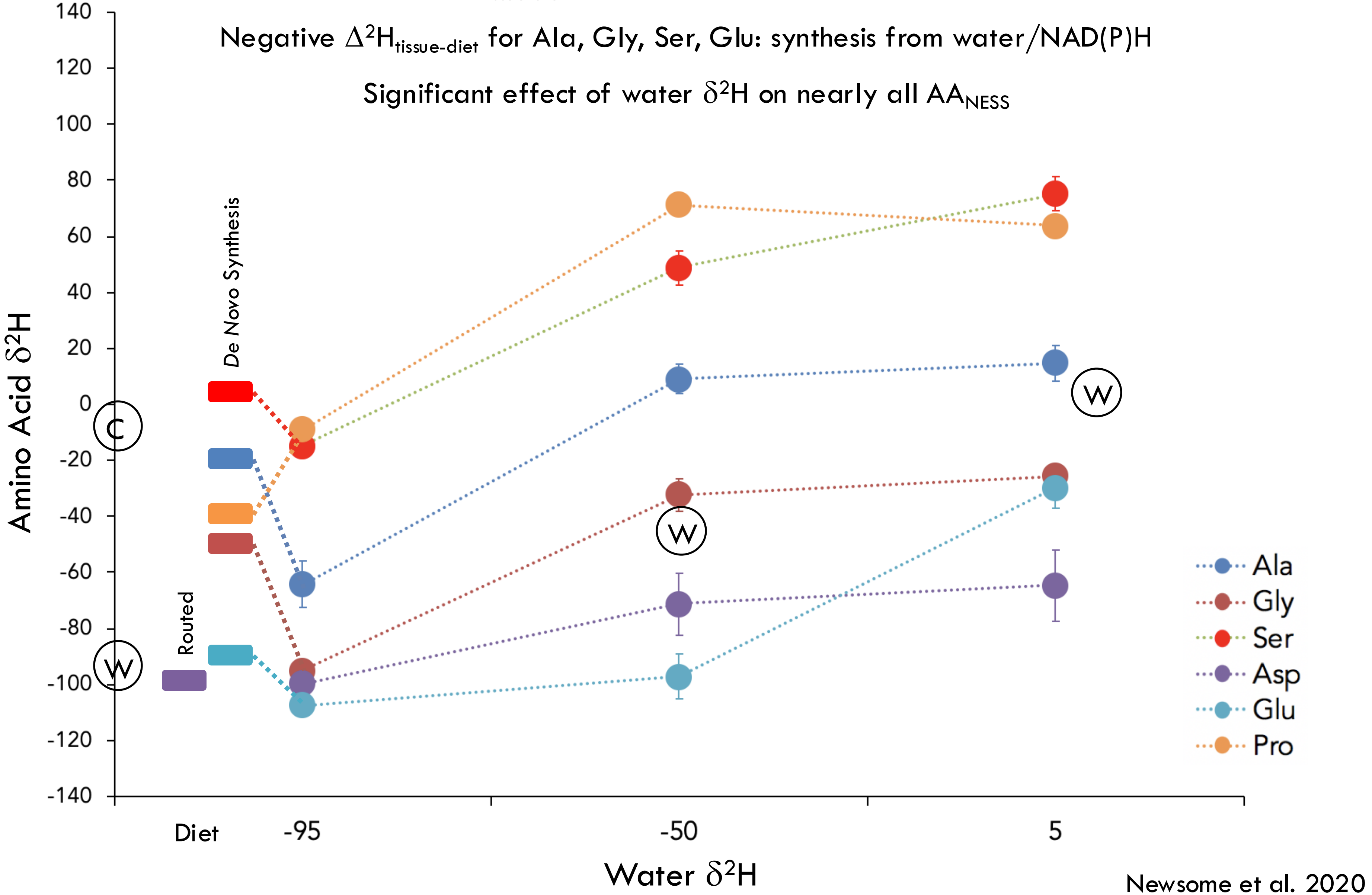
Newsome et al. 2020

Non-Essential Amino Acids (Low Protein Diet)

Positive $\Delta^2H_{tissue\text{-}diet}$ for Pro: synthesis from carbohydrates

Negative $\Delta^2H_{tissue\text{-}diet}$ for Ala, Gly, Ser, Glu: synthesis from water/NAD(P)H

Significant effect of water δ^2H on nearly all AA_{NESS}



Newsome et al. 2020

Why the Plateau in Non-Essential δ^2H in Low Protein Diet?

Carbohydrates (34%) are ~2X more important than water (18%) for *de novo* synthesis
(Rodriguez Curras et al. 2018)