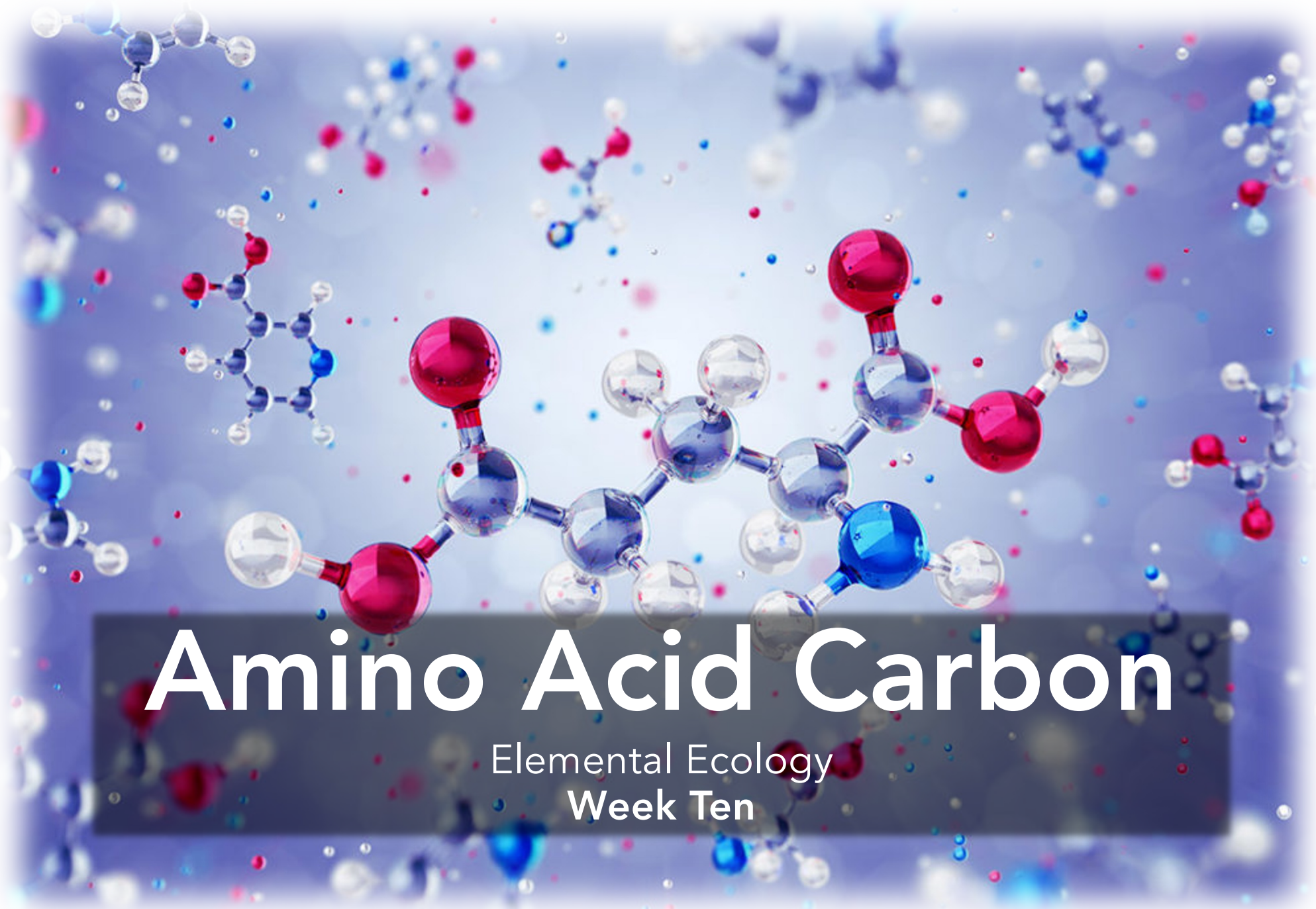
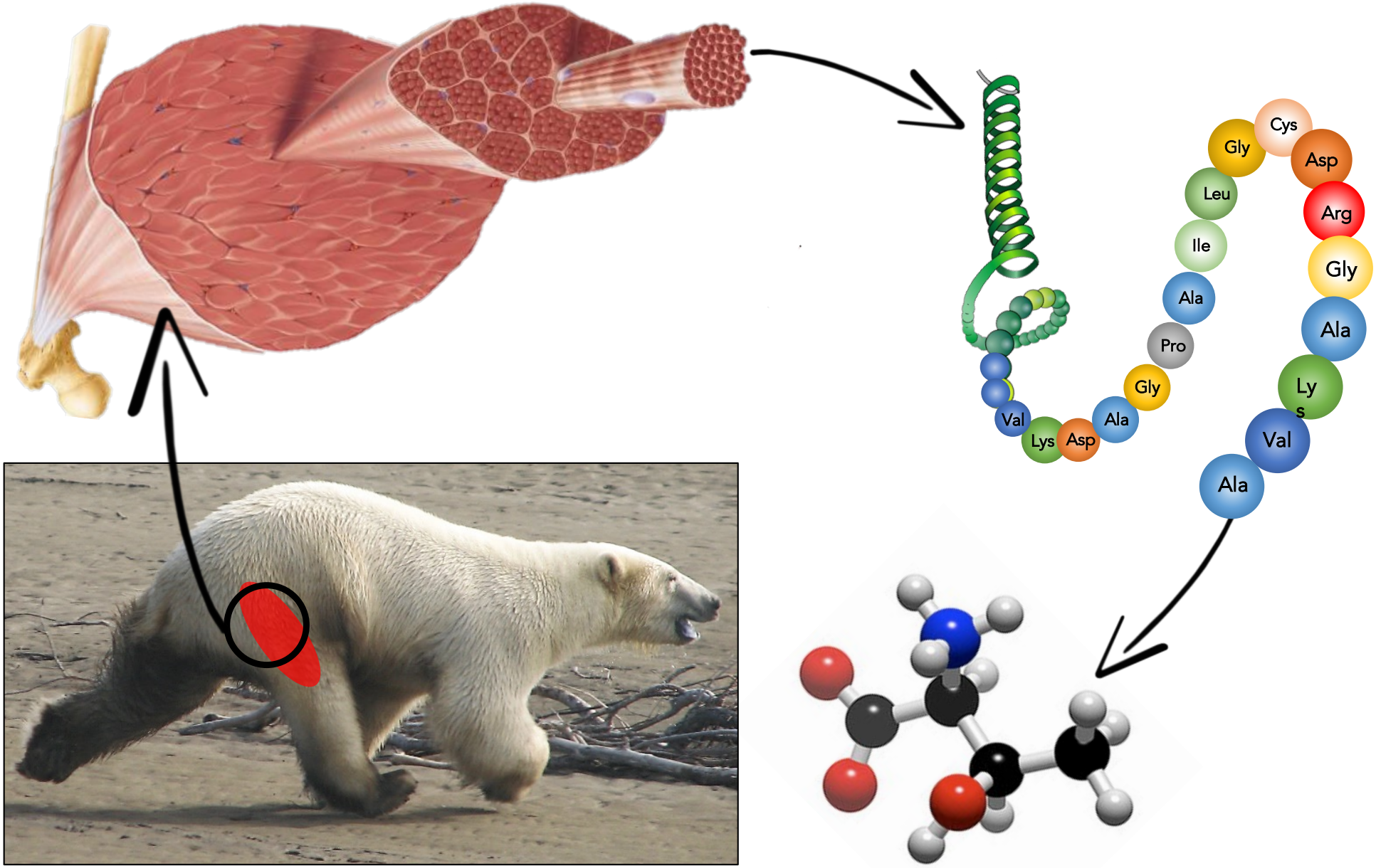


Amino Acid Carbon

Elemental Ecology

Week Ten

Moving beyond bulk tissues



Proteins have many functions

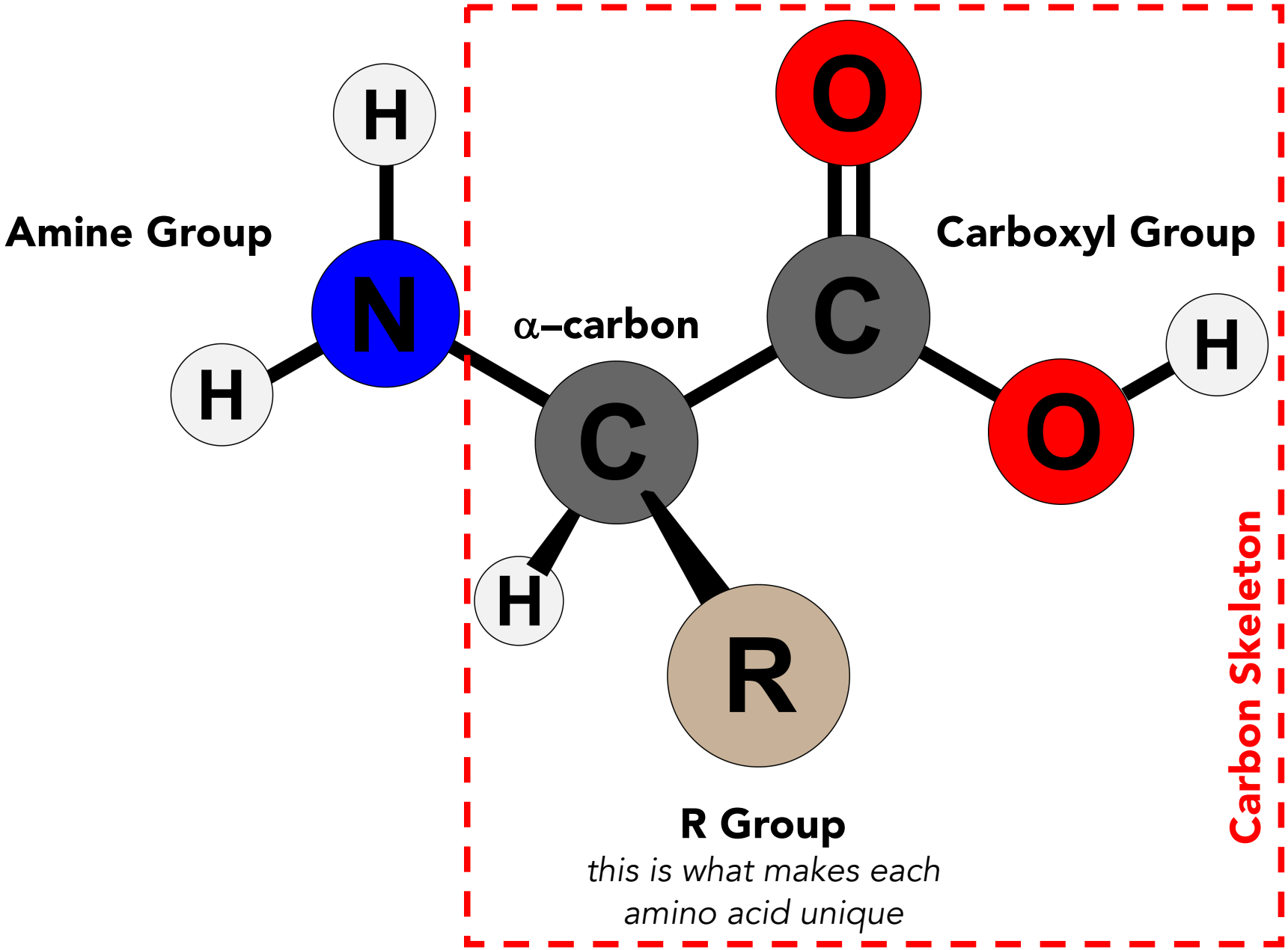
- Structure (e.g., hair)
- Catalysis (e.g., enzymes)
- Defense (e.g., antibodies)
- Movement (e.g., myosins)
- Signaling (e.g., receptor proteins)
- Transport (e.g., hemoglobin)

What is the main function of the proteins we sample from consumer tissues?

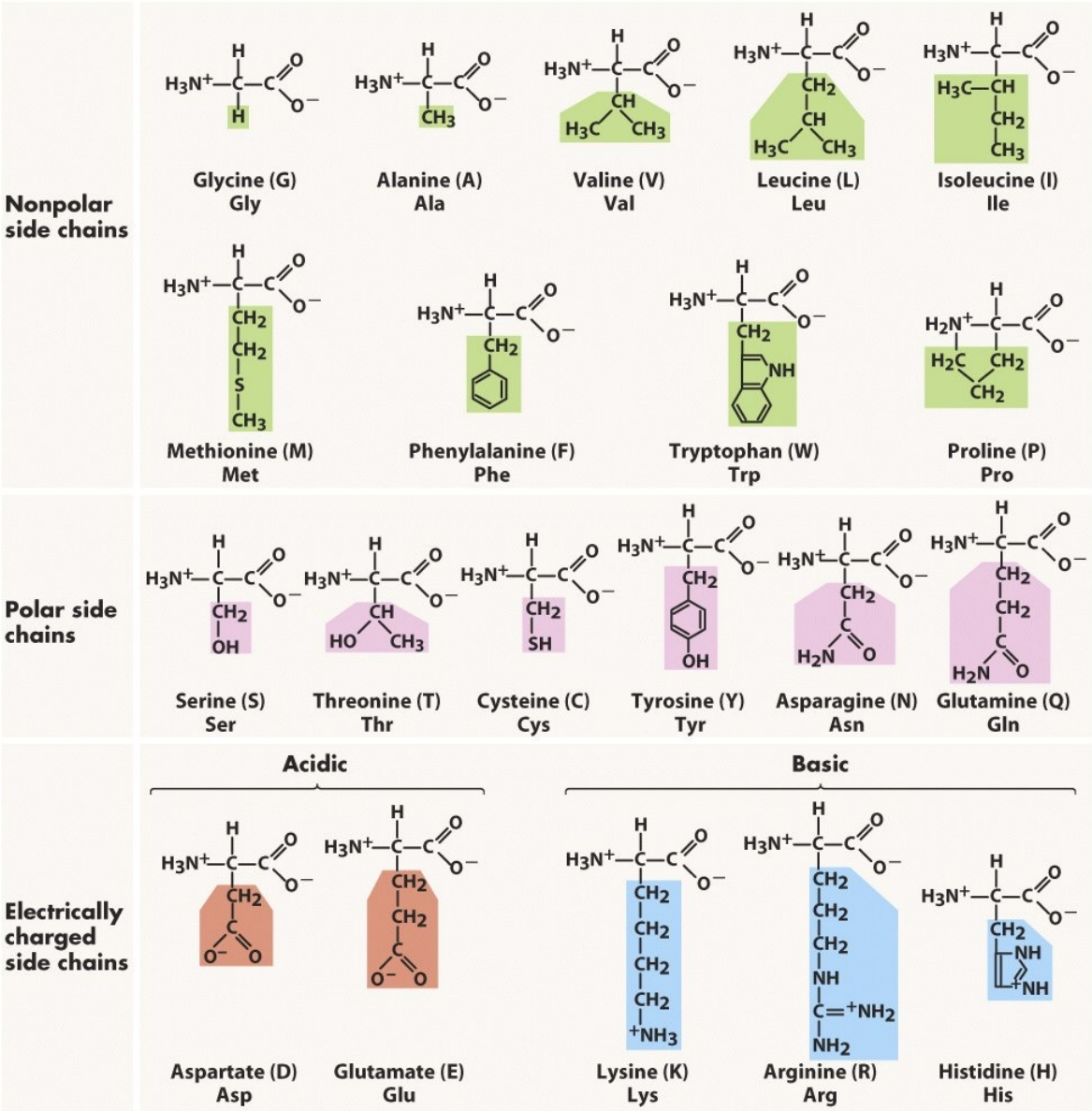
How about primary producer tissues?

Rubisco accounts for up to 50% of soluble proteins in C_3 leaves (or 20–30% of total leaf N)!

Back to Basics: Amino Acid Structure



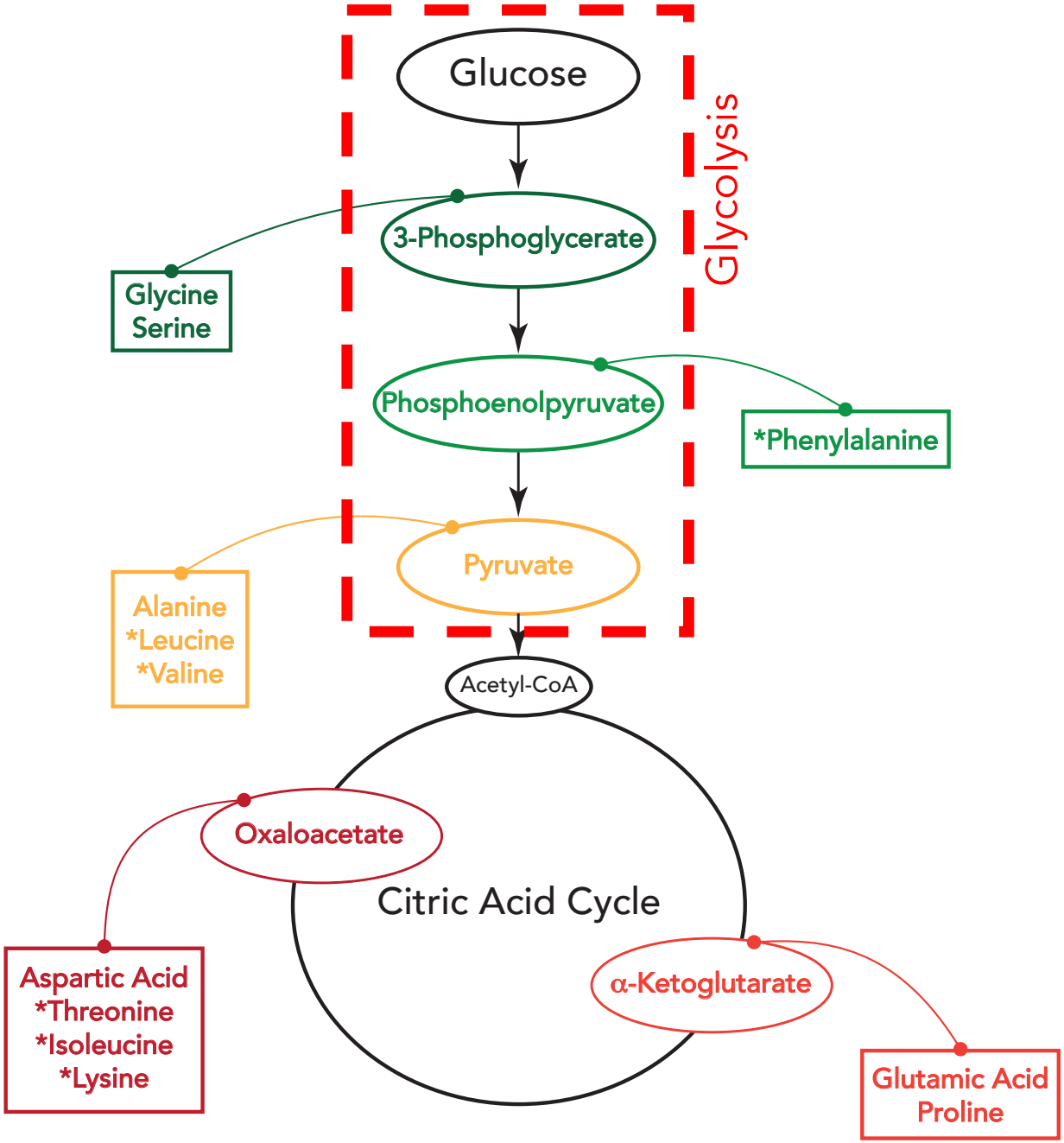
Amino Acids: The Building Blocks of Proteins



There are 20 *common* amino acids (and almost 500 other naturally occurring amino acids).

These amino acids have different chemical properties.

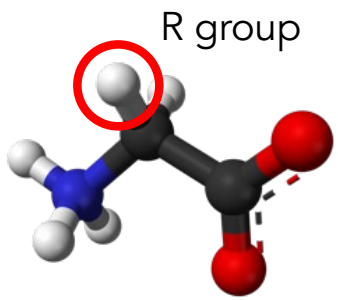
Amino Acid Biosynthetic Families



Amino Acids: The Building Blocks of Proteins

Non-Essential Amino Acids

- Simple
- Can be synthesized by all organisms using carbohydrates, lipids, and other amino acids
- Make up 60–70% of consumer proteinaceous tissues

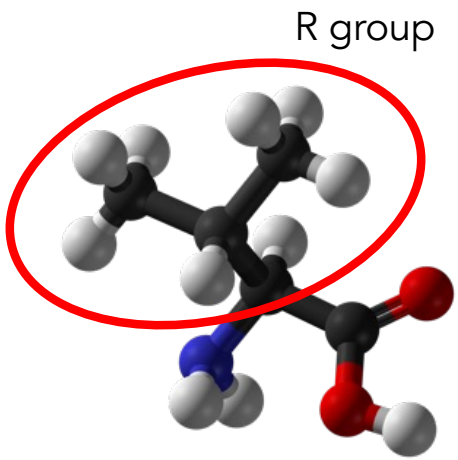


Glycine
non-essential amino acid

Ala, Arg, Asn, Asp, Cys,
Gln, Glu, Gly, Pro, Ser, Tyr

Essential Amino Acids

- More complex
- Can only be synthesized by plants, bacteria, and fungi
- Animals must acquire them in their diet OR from their symbiotic gut microbes
- Only account for 30–40% of consumer proteinaceous tissues

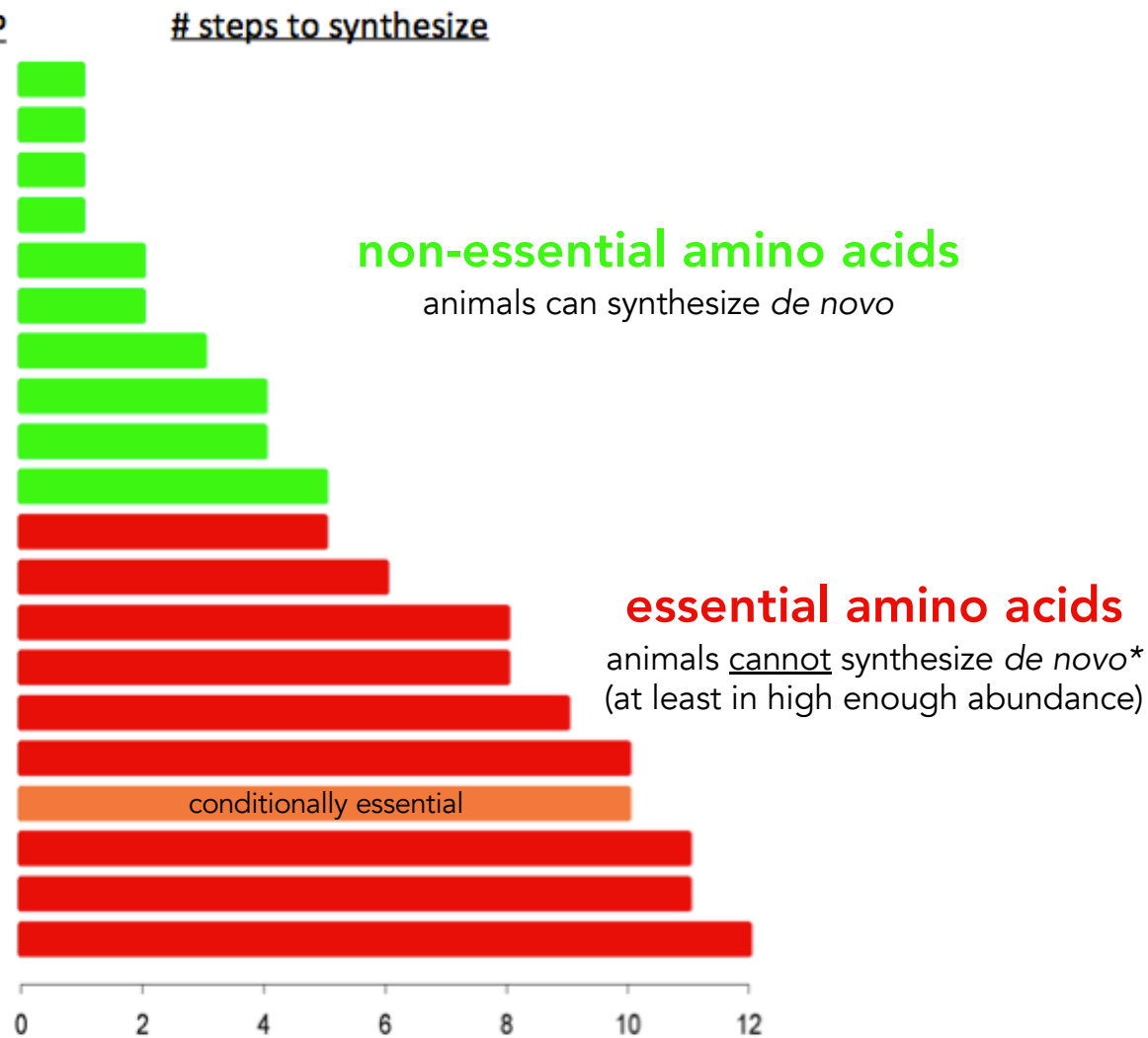


Valine
essential amino acid

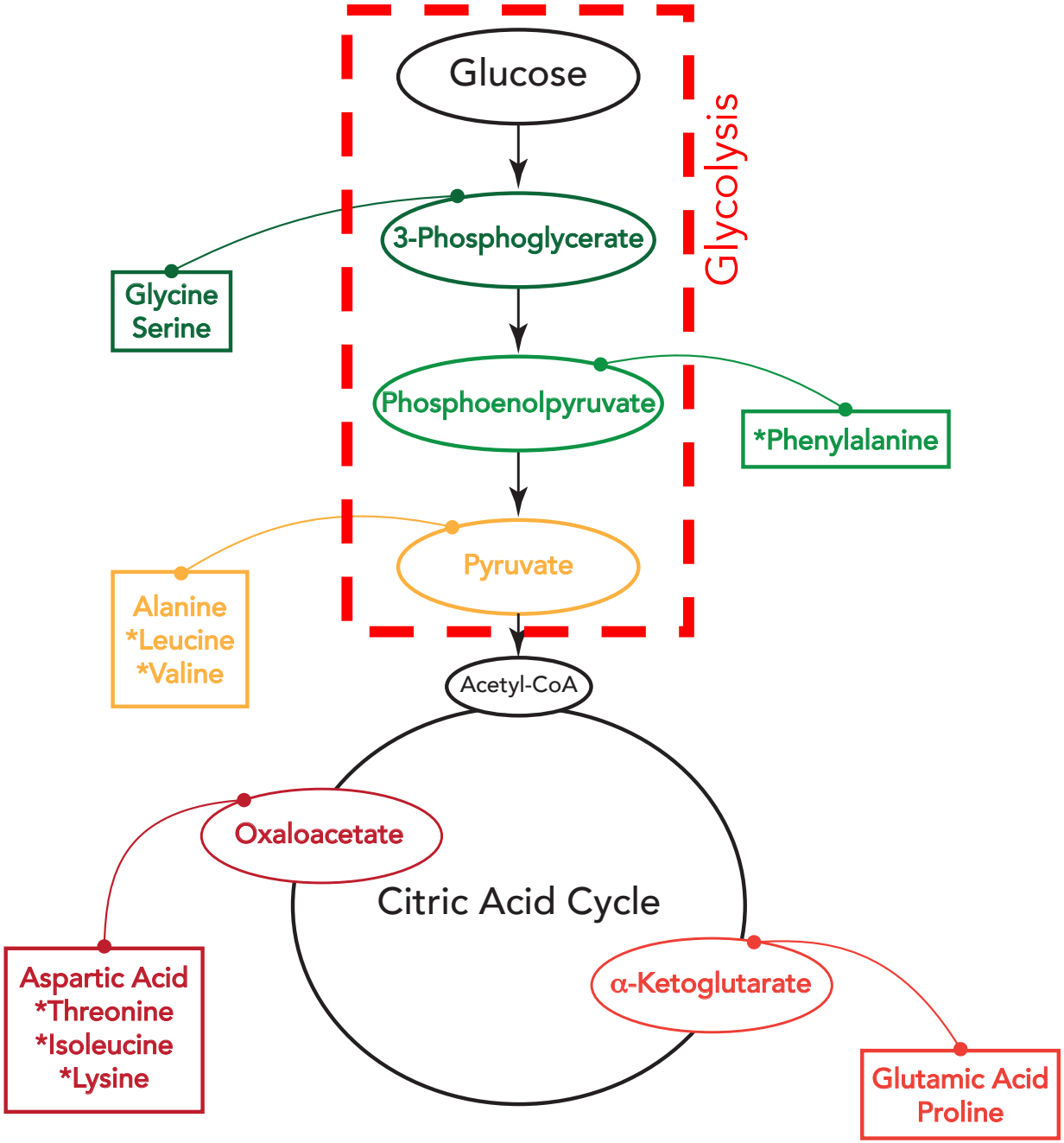
His, Ile, Leu, Lys,
Met, Phe, Thr, Try, Val

Non-essential amino acids are easier to synthesize, and animals have retained this ability

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



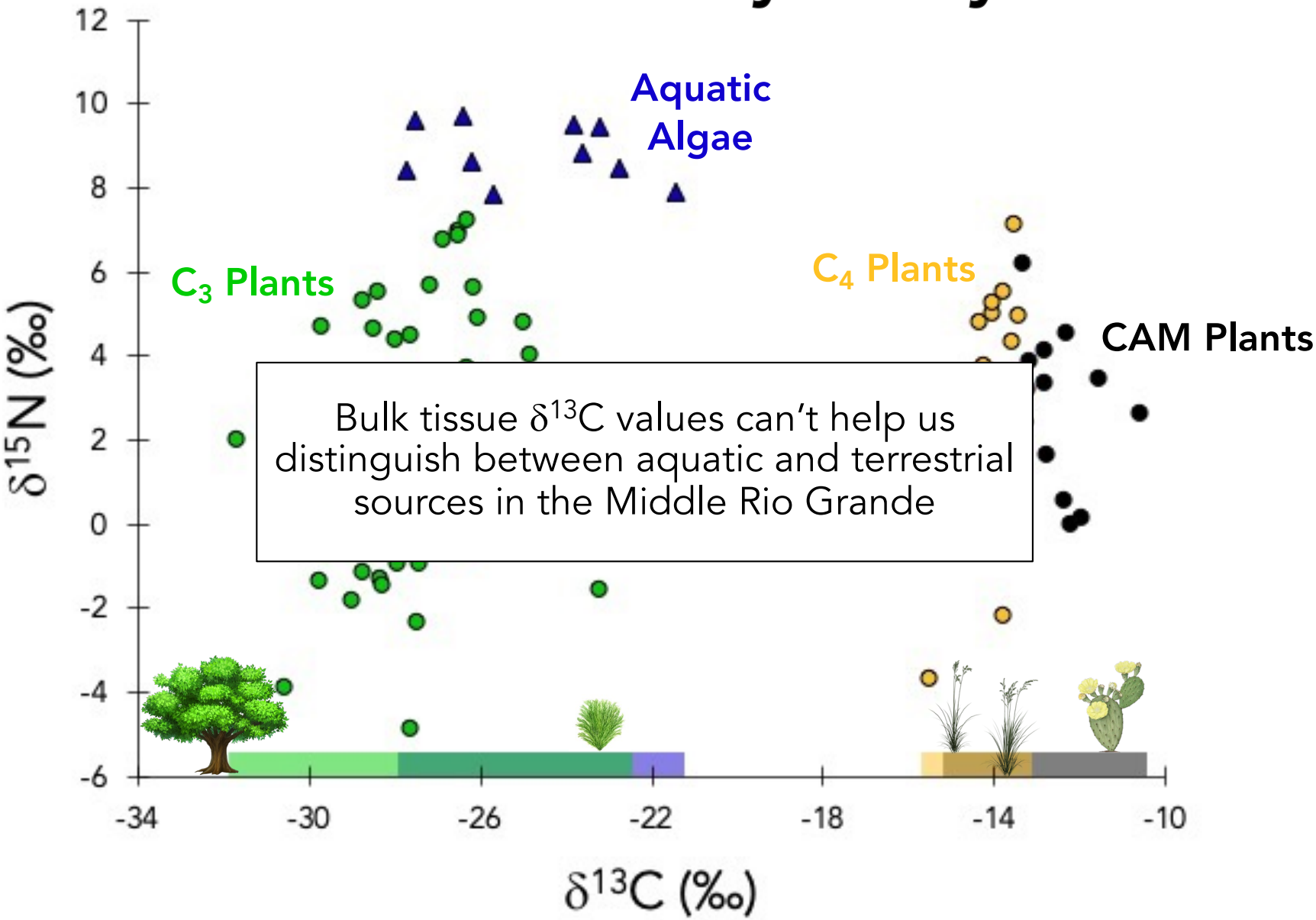
Amino Acid Biosynthetic Families



This seems like a lot of work…

Why bother?

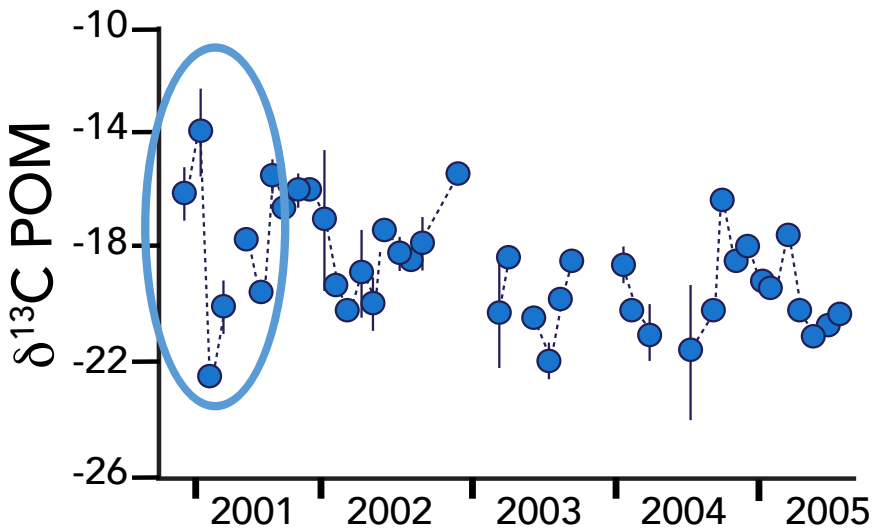
Primary producer bulk tissue $\delta^{13}C$ values can vary widely

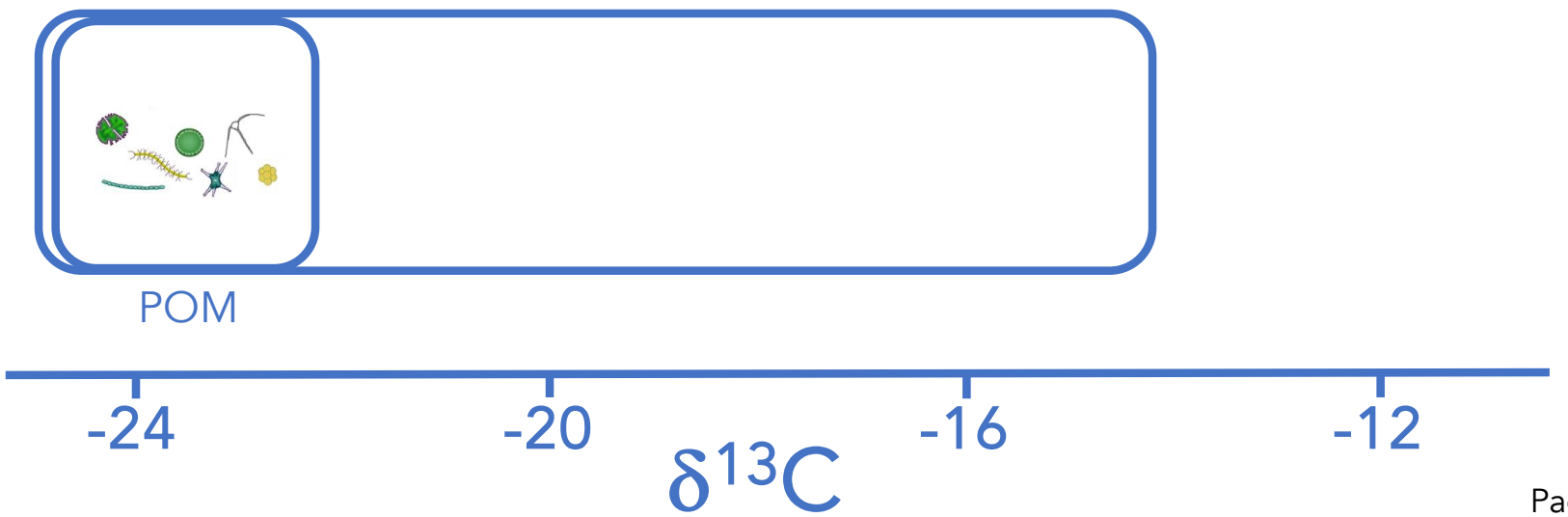


Bulk tissue $\delta^{13}C$ values can't help us distinguish between aquatic and terrestrial sources in the Middle Rio Grande

Besser et al. *in review*

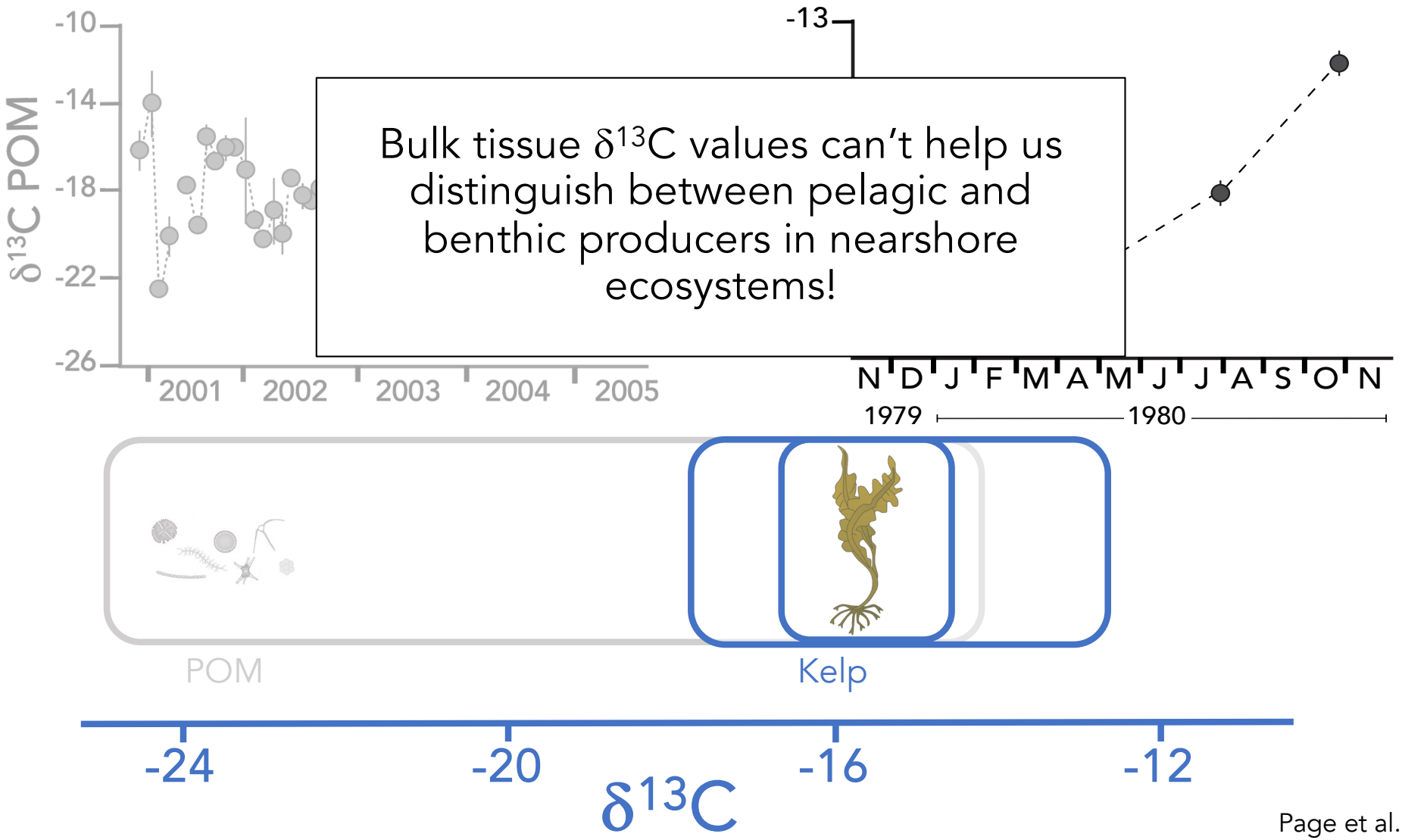
Primary producer bulk tissue $\delta^{13}C$ values can vary widely





Page et al. 2008
Dunton and Schell 1987

Primary producer bulk tissue $\delta^{13}C$ values can vary widely

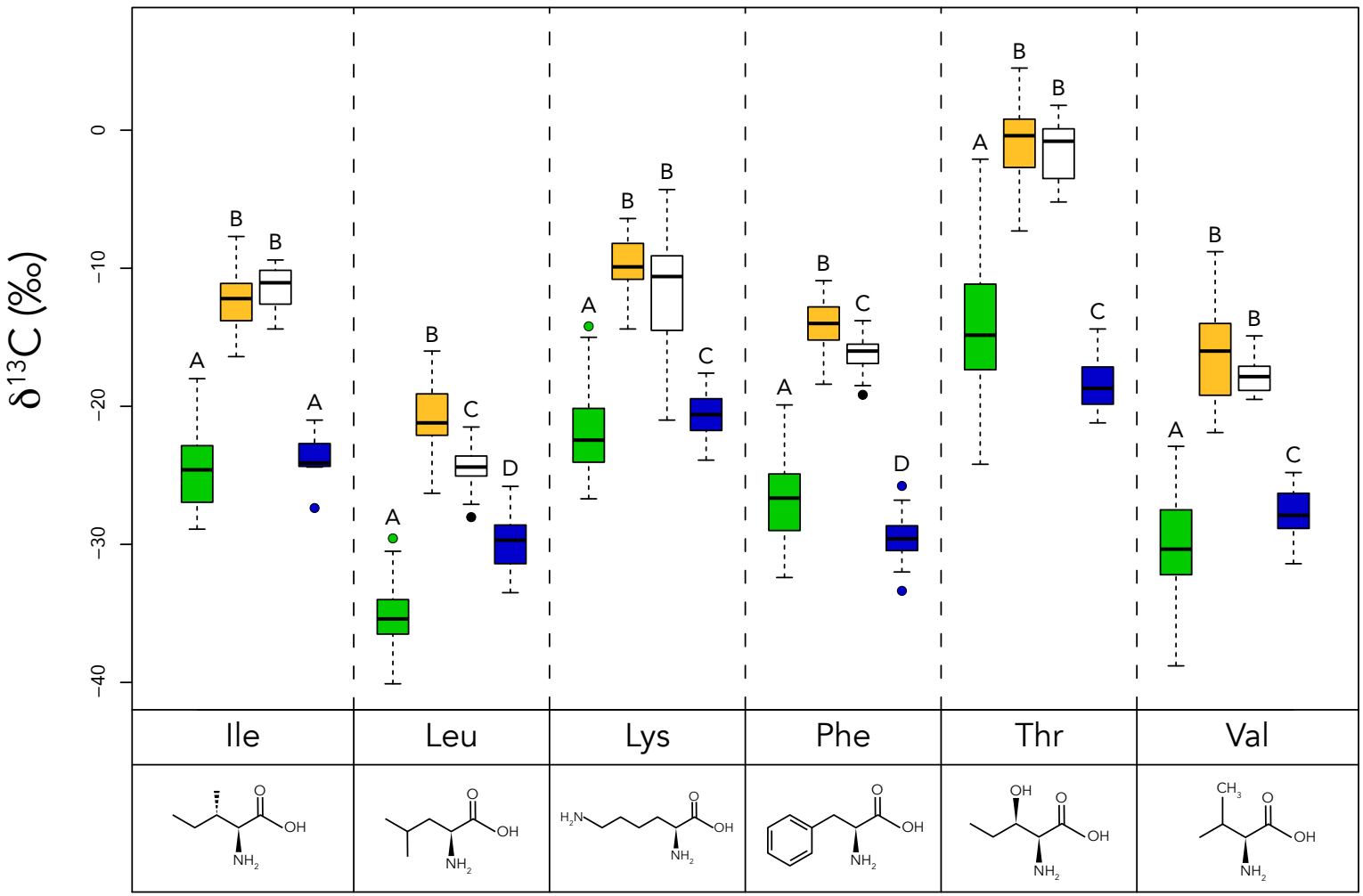


Bulk tissue $\delta^{13}C$ values can't help us distinguish between pelagic and benthic producers in nearshore ecosystems!

Page et al. 2008
Dunton and Schell 1987

"Only ~~pack~~ the essentials"

Essential amino acids $\delta^{13}C$ values vary among primary producer groups



C_3 Plants

C_4 Plants

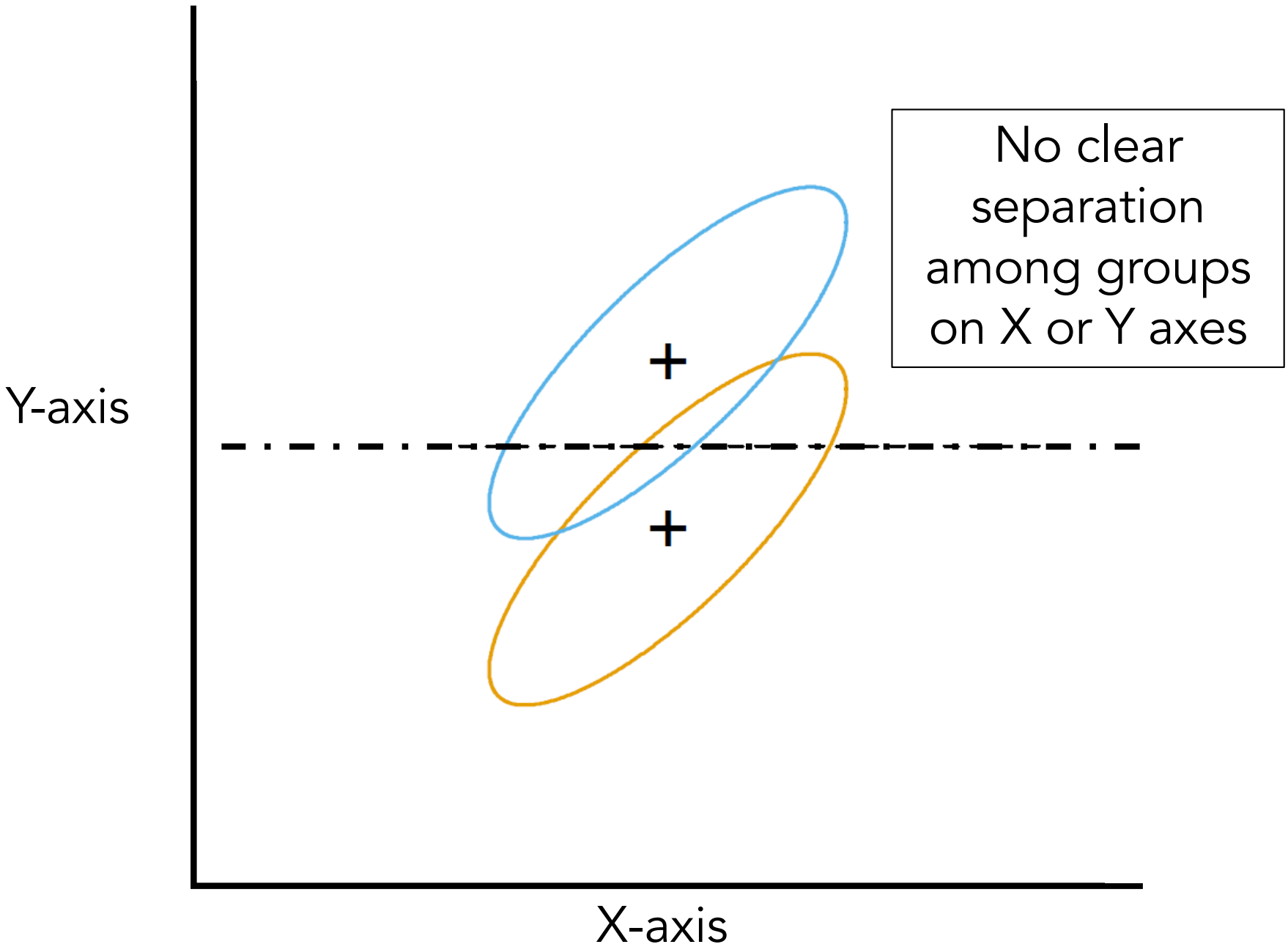
Aquatic Algae

CAM Plants

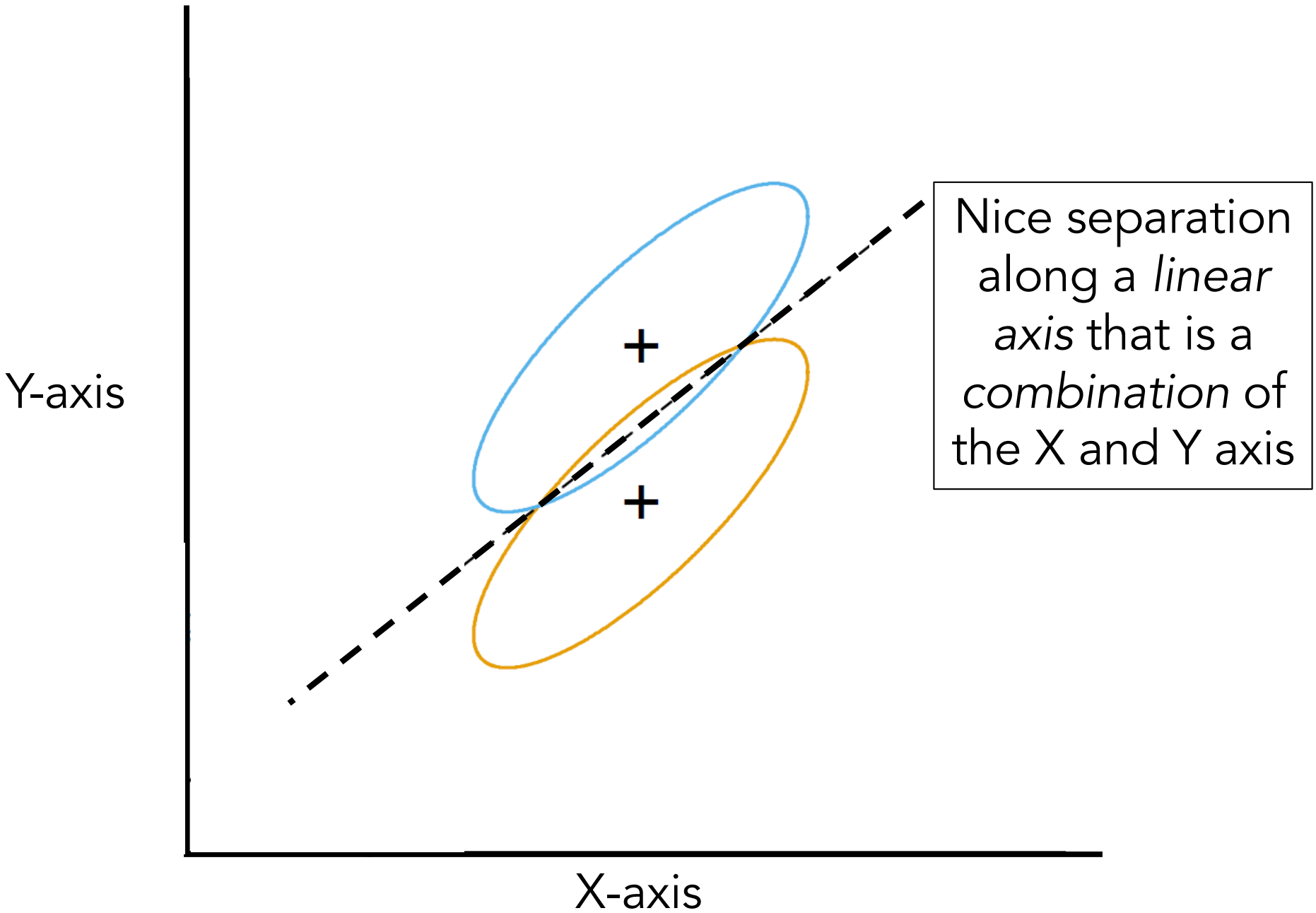
Besser et al. 2022 J. Ecology

Essential amino acid $\delta^{13}C$ "fingerprinting"

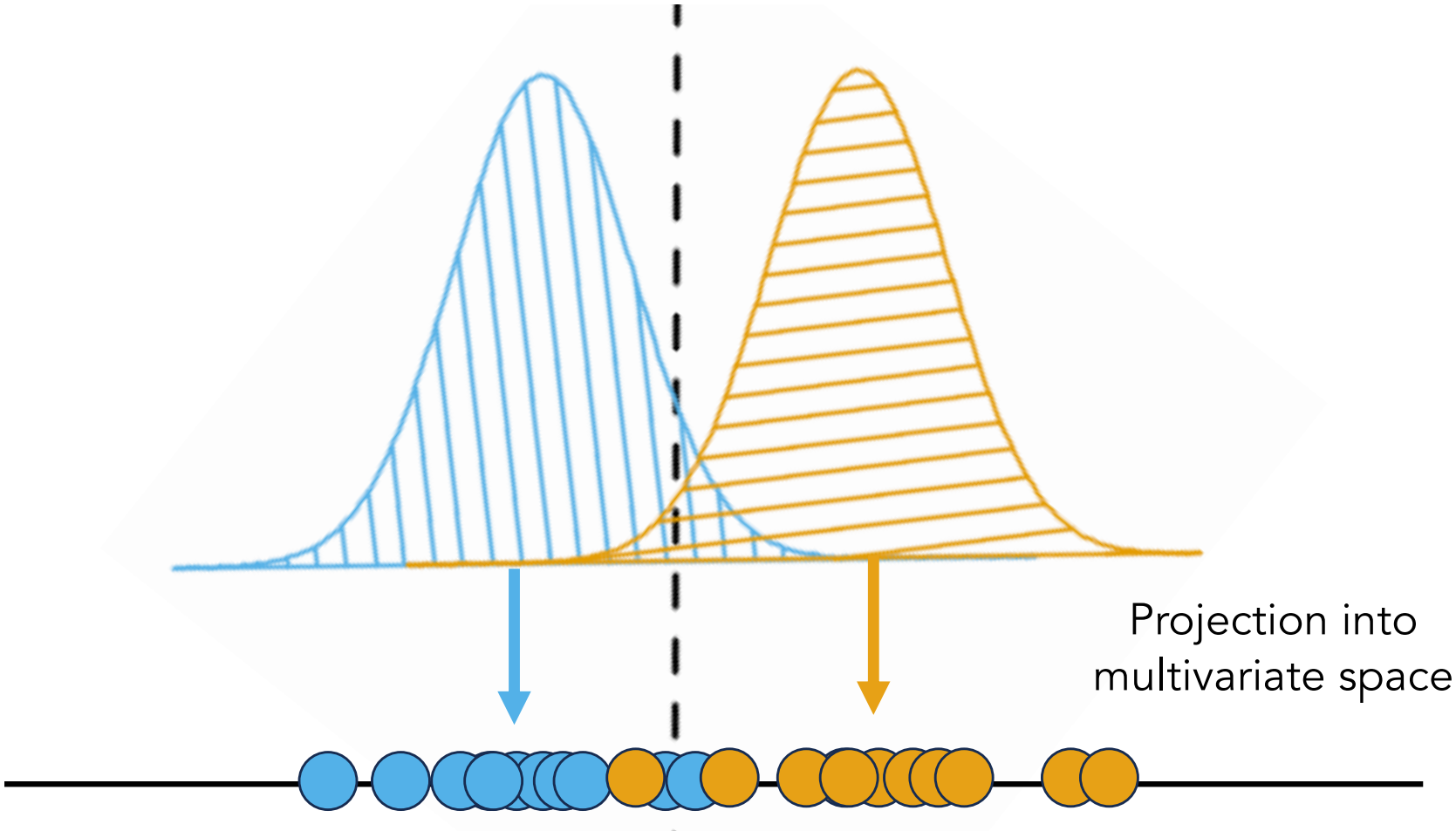
Essential amino acid $\delta^{13}C$ "fingerprinting"



Essential amino acid $\delta^{13}C$ "fingerprinting"



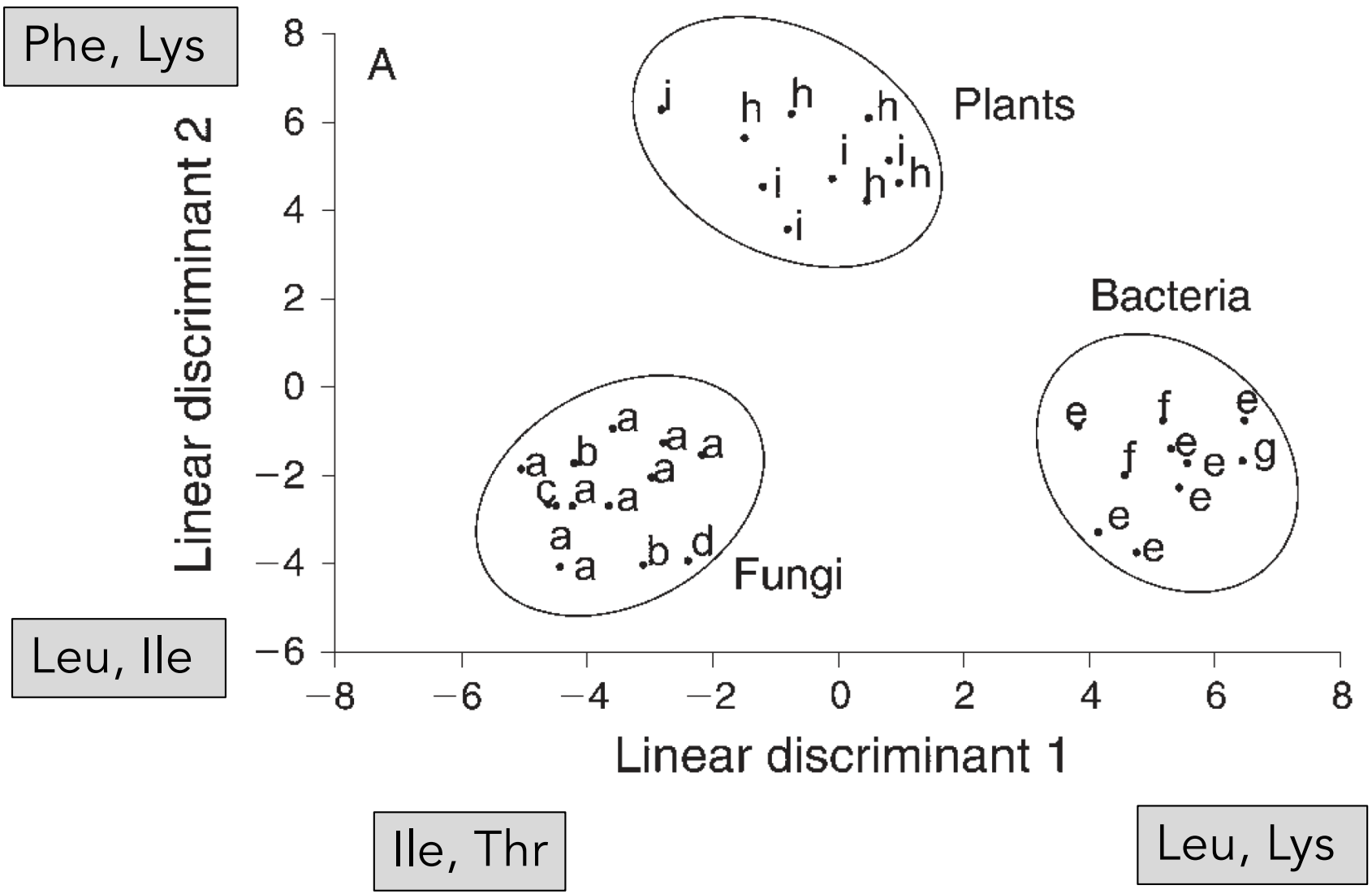
Essential amino acid $\delta^{13}C$ "fingerprinting"



Linear Discriminant 1

α(x-axis value) + β(y-axis value)

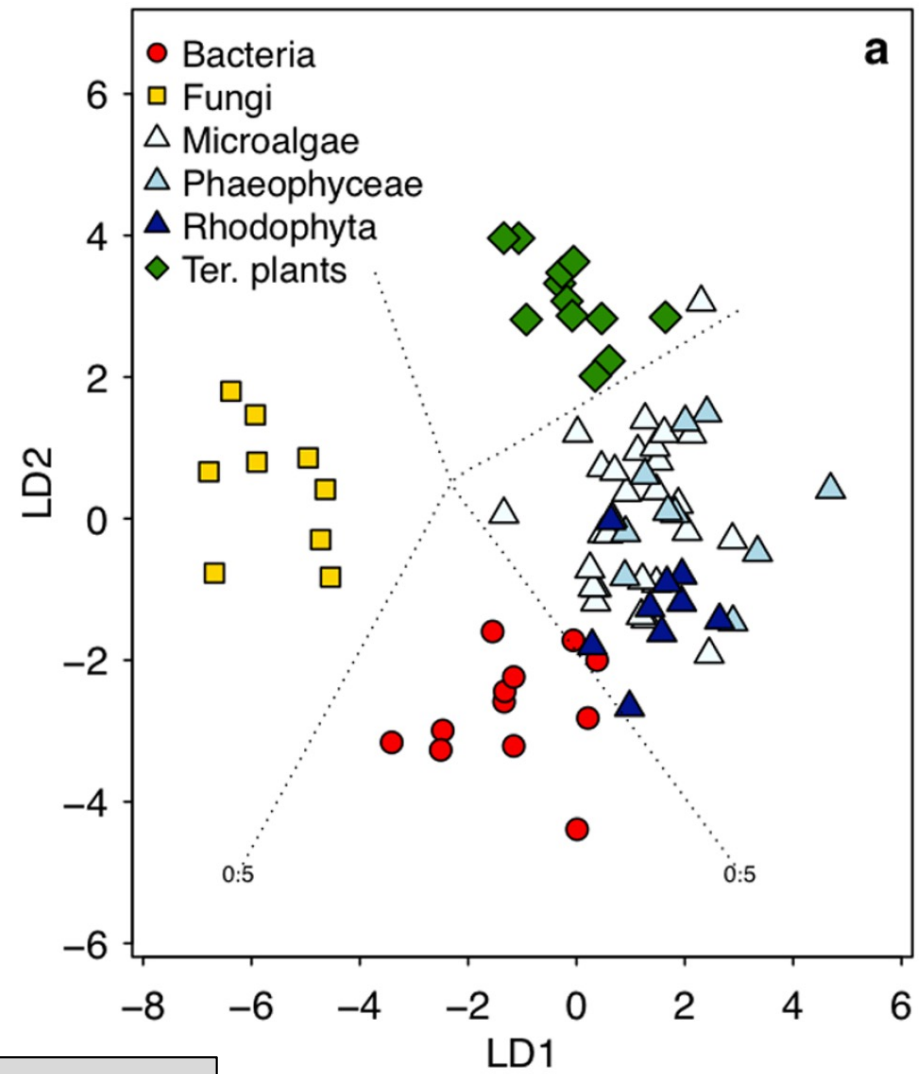
Plants, bacteria, and fungi have distinct EAA $\delta^{13}C$ fingerprints



Larsen et al. 2009

Plants, bacteria, fungi, *and* algae have distinct EAA $\delta^{13}C$ fingerprints

Phe

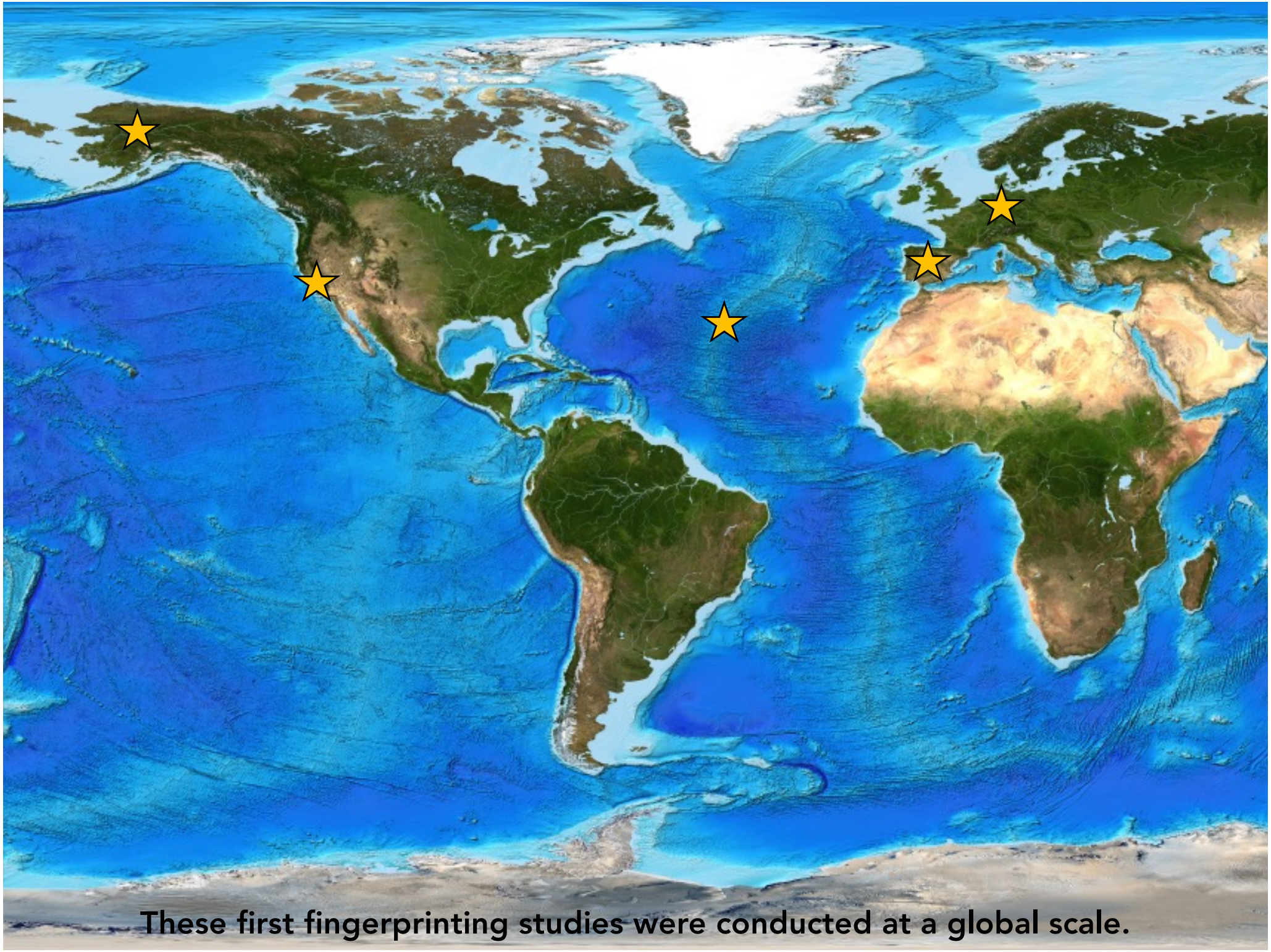


Leu

Val, Phe

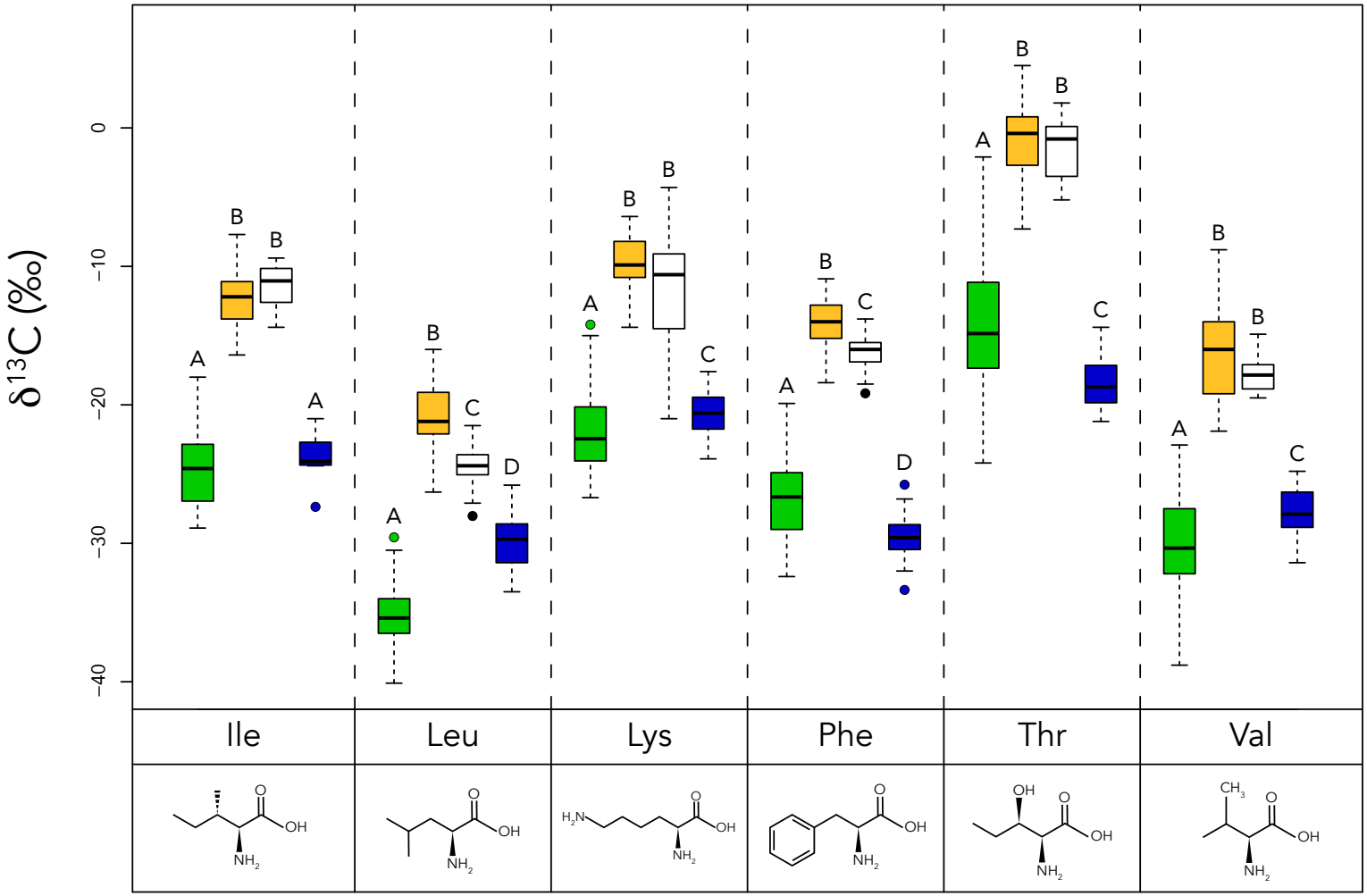
Lys

Larsen et al. 2013



These first fingerprinting studies were conducted at a global scale.

What about our New Mexico producers?



C_3 Plants

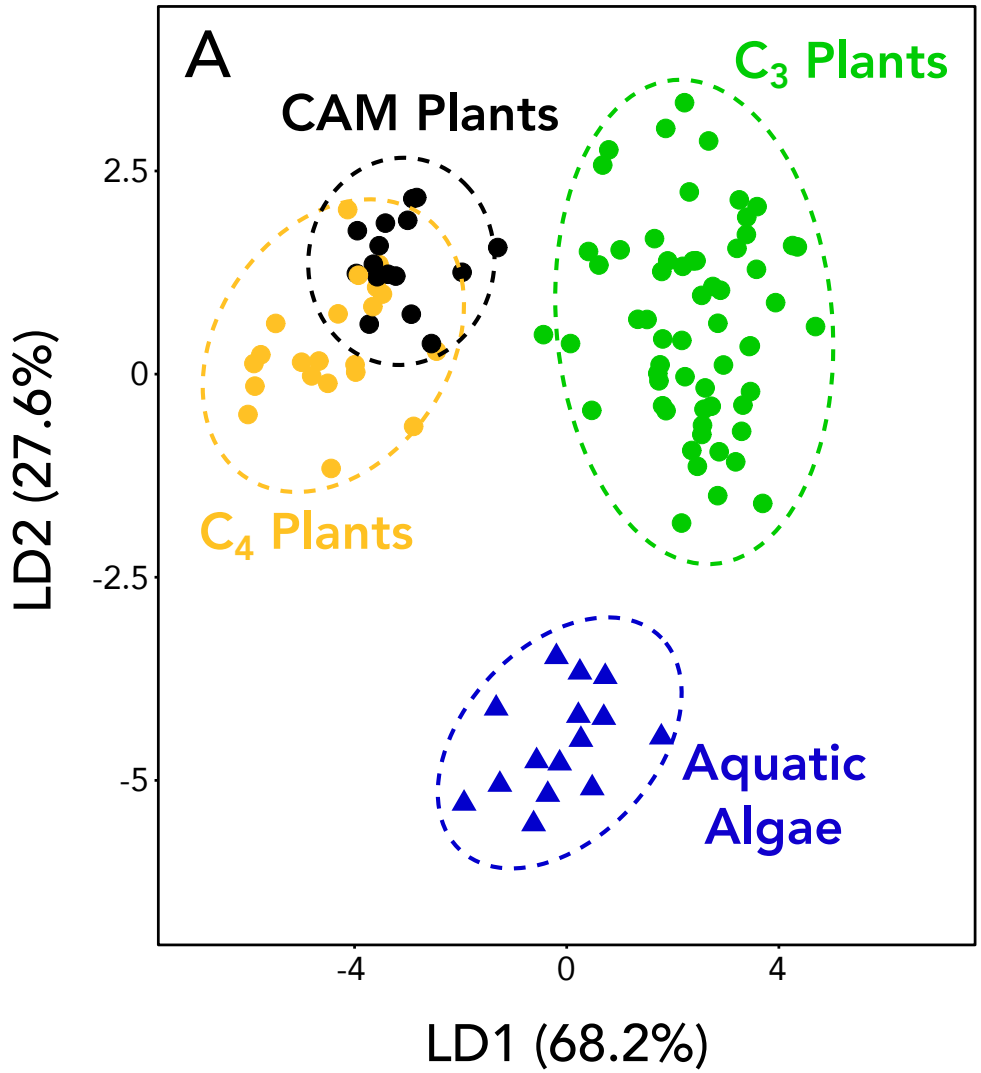
C_4 Plants

Aquatic Algae

CAM Plants

Besser et al. 2022 J. Ecology

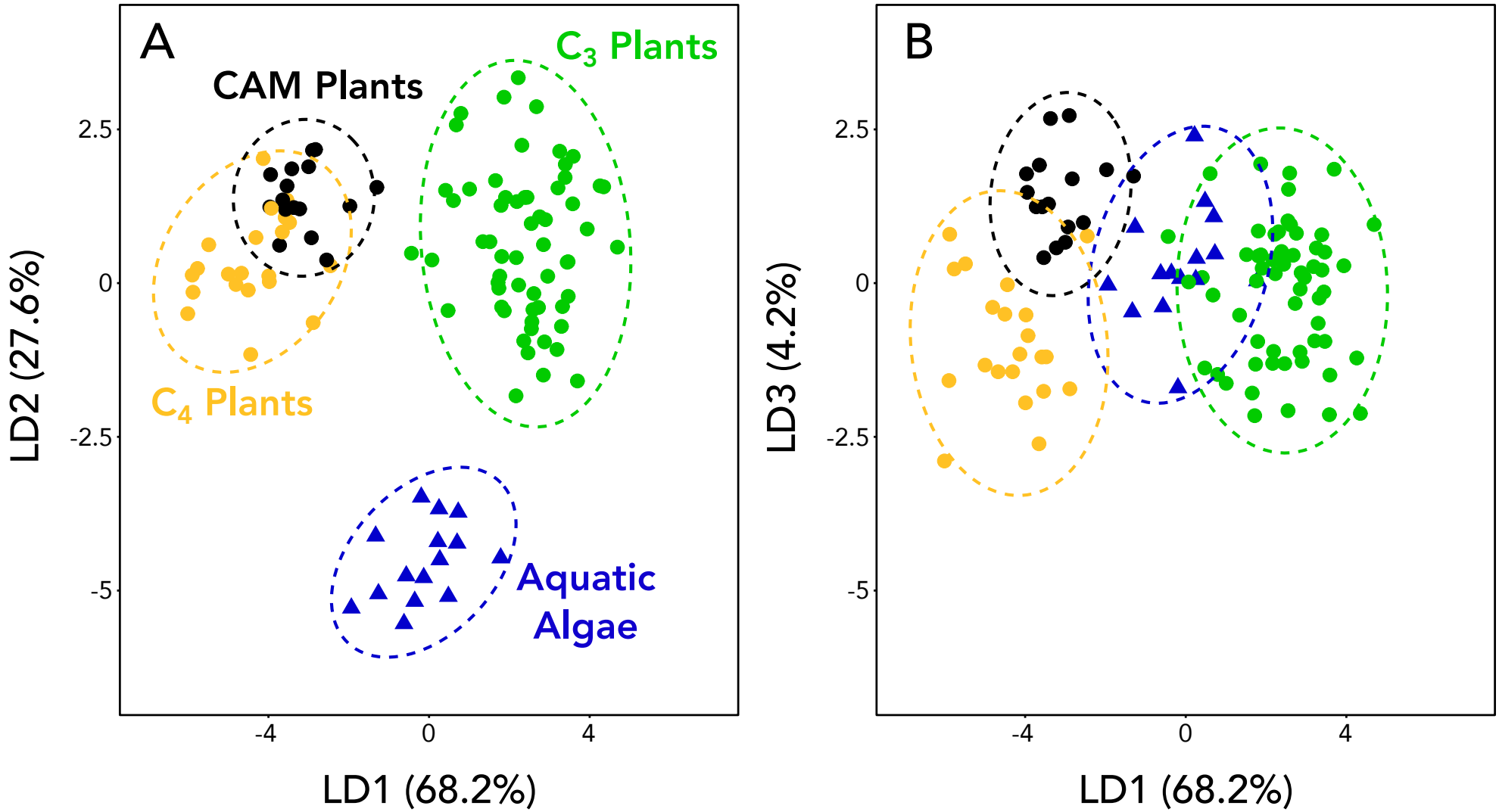
EAA $\delta^{13}C$ fingerprints are unique among terrestrial and aquatic producers



Besser et al. 2022 J. Ecology

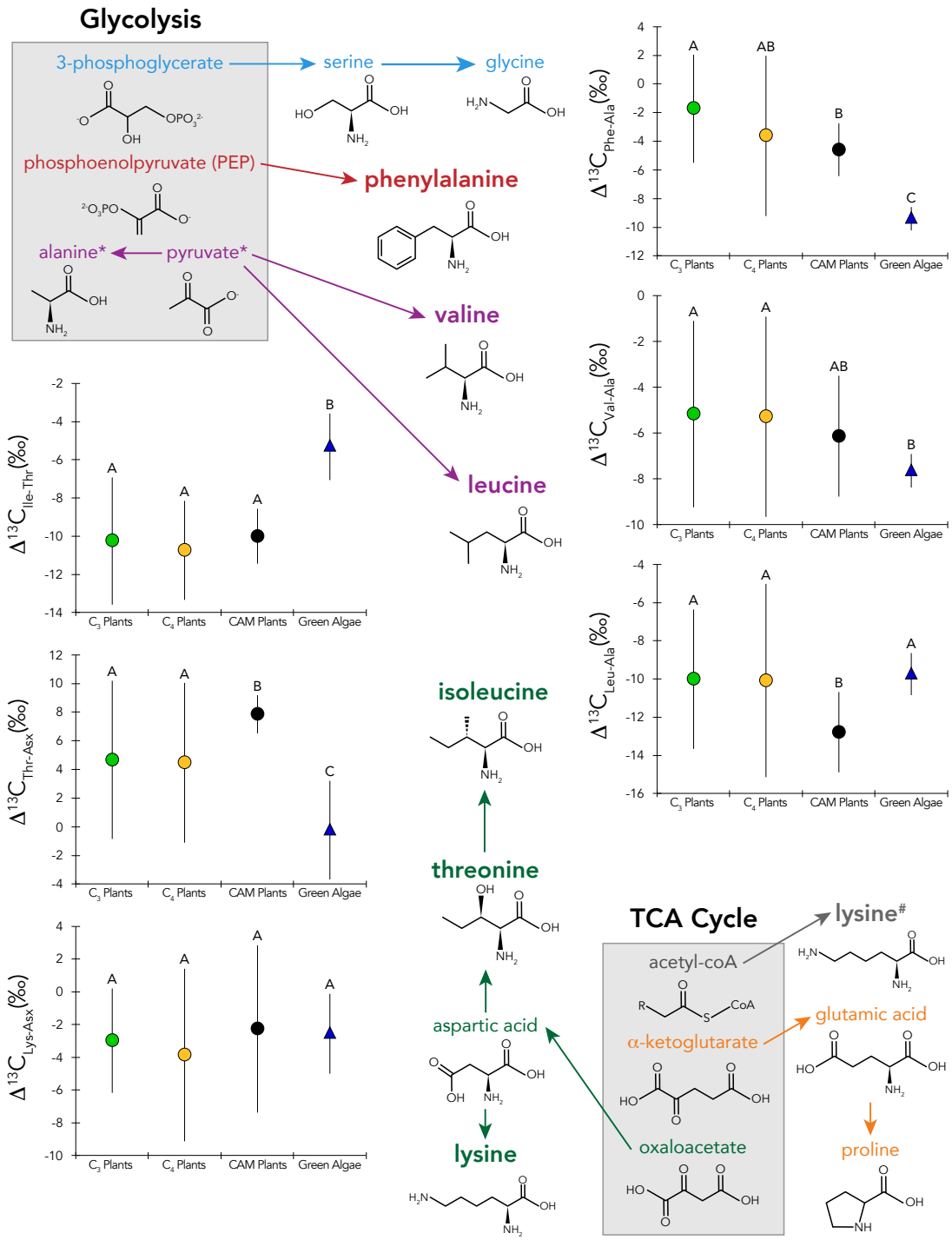
AA_{ESS} $\delta^{13}C$ fingerprints are unique among terrestrial and aquatic producers

99% successful reclassification rate



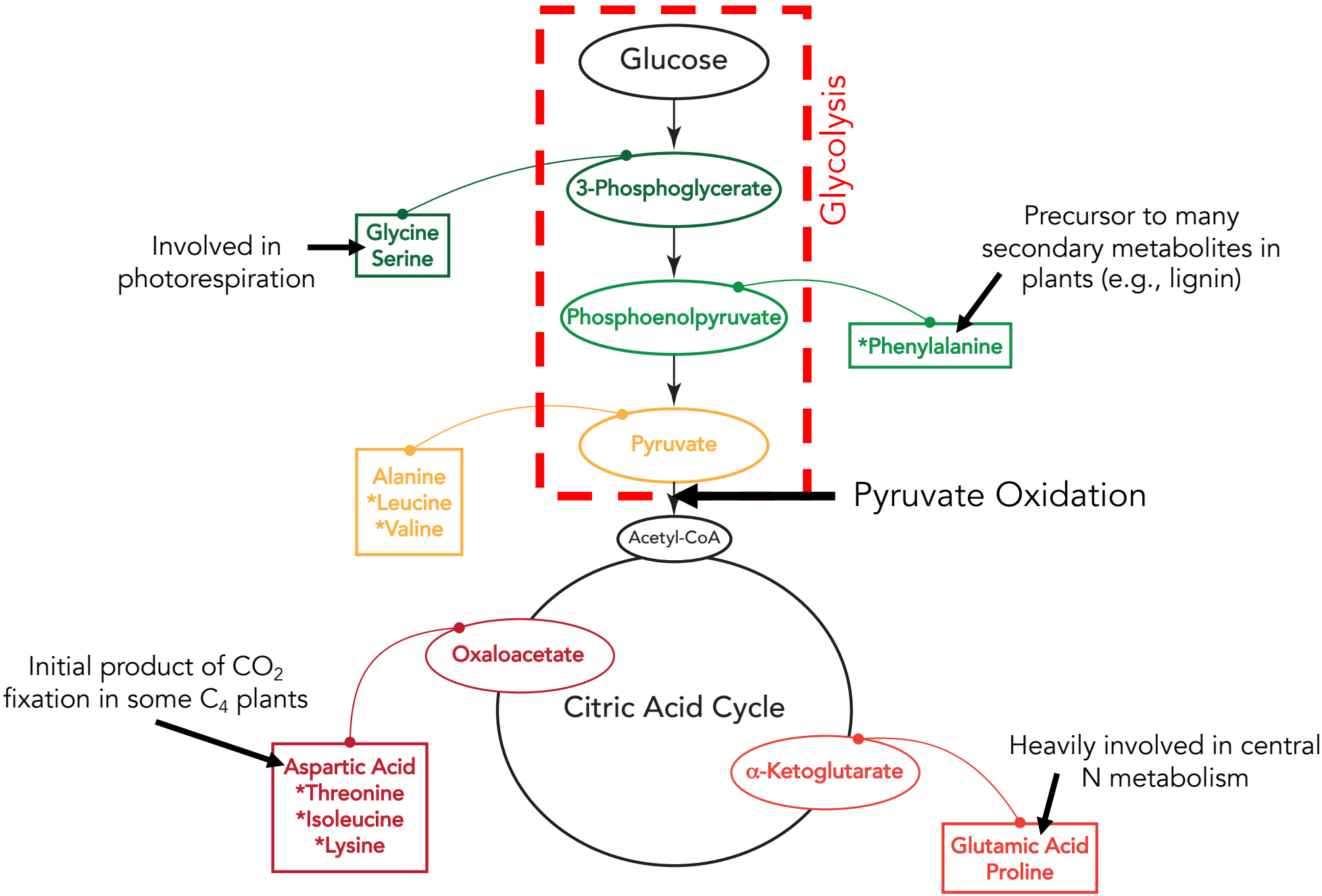
Besser et al. 2022 J. Ecology

Isotopic discrimination ($\Delta^{13}C$) during *de novo* EAA synthesis varies among producer groups

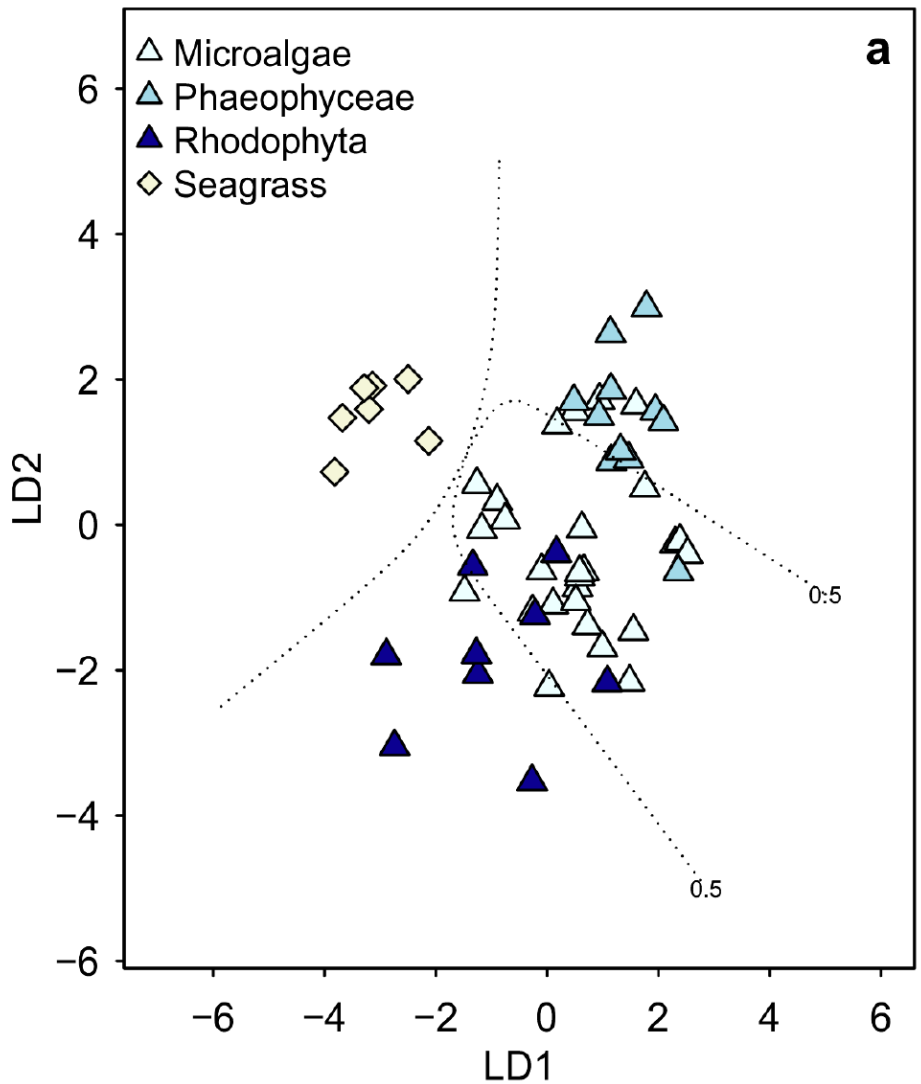


Besser et al. 2022 J. Ecology

Amino Acid Biosynthetic Families



Looks like there may some differences in algal AA_{ESS} $\delta^{13}C$ fingerprints, too



Larsen et al. 2013

Building an isotopic library of marine producers

Chlorophyta (Green Algae)

Ulva

Rhodophyta (Red Algae)

Neorhodomela

Plocamium

Rhodymenia

Chondrus

Ochrophyta (Kelps)

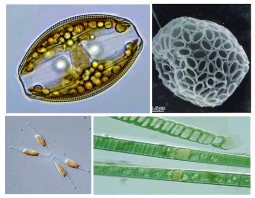
Saccharina

Lessonia

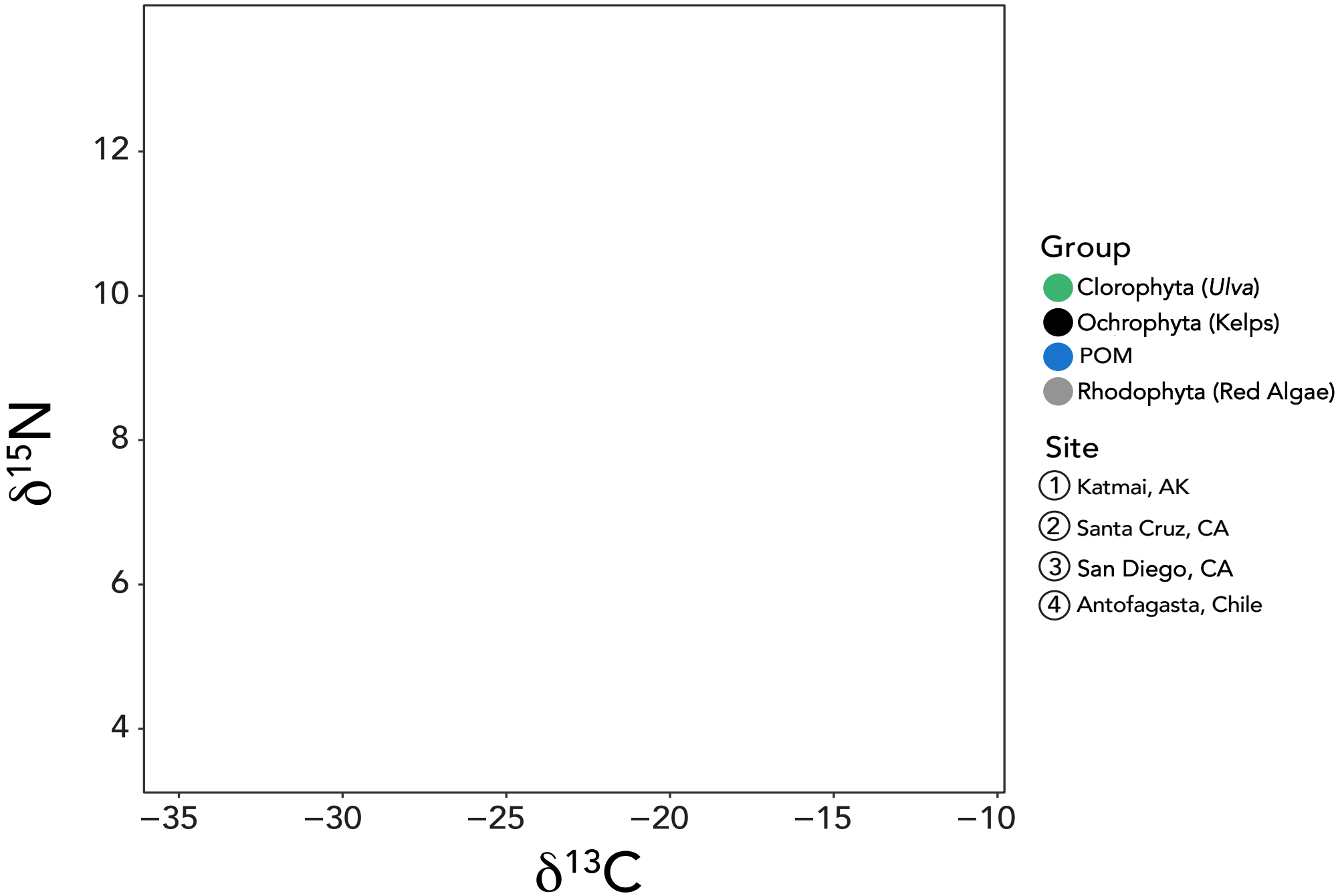
Macrocystis

Microalgae (POM)

Phytoplankton Cultures

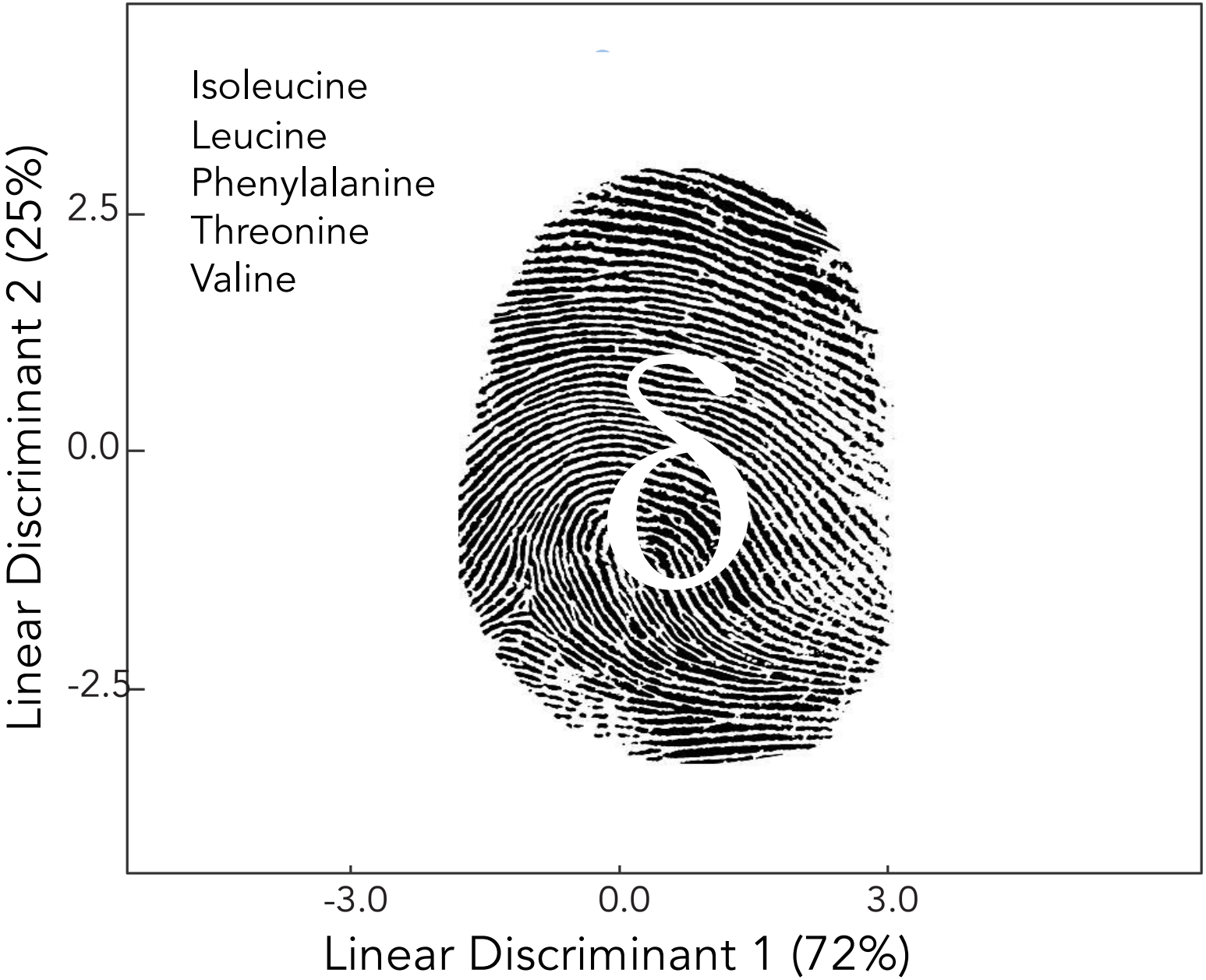


Elliott Smith et al. 2022.
Func. Ecol.

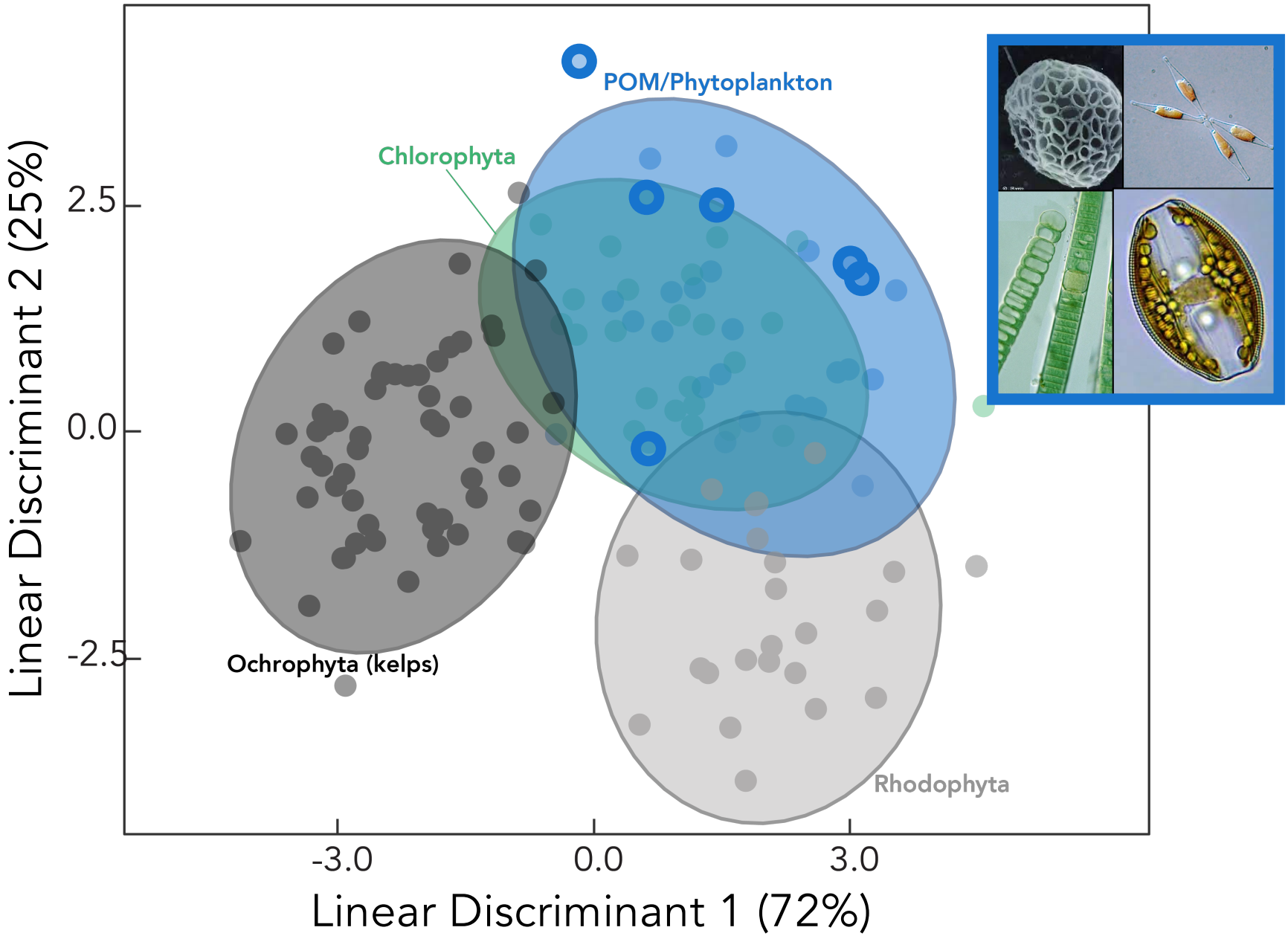


Elliott Smith et al. 2022. *Func. Ecol.*

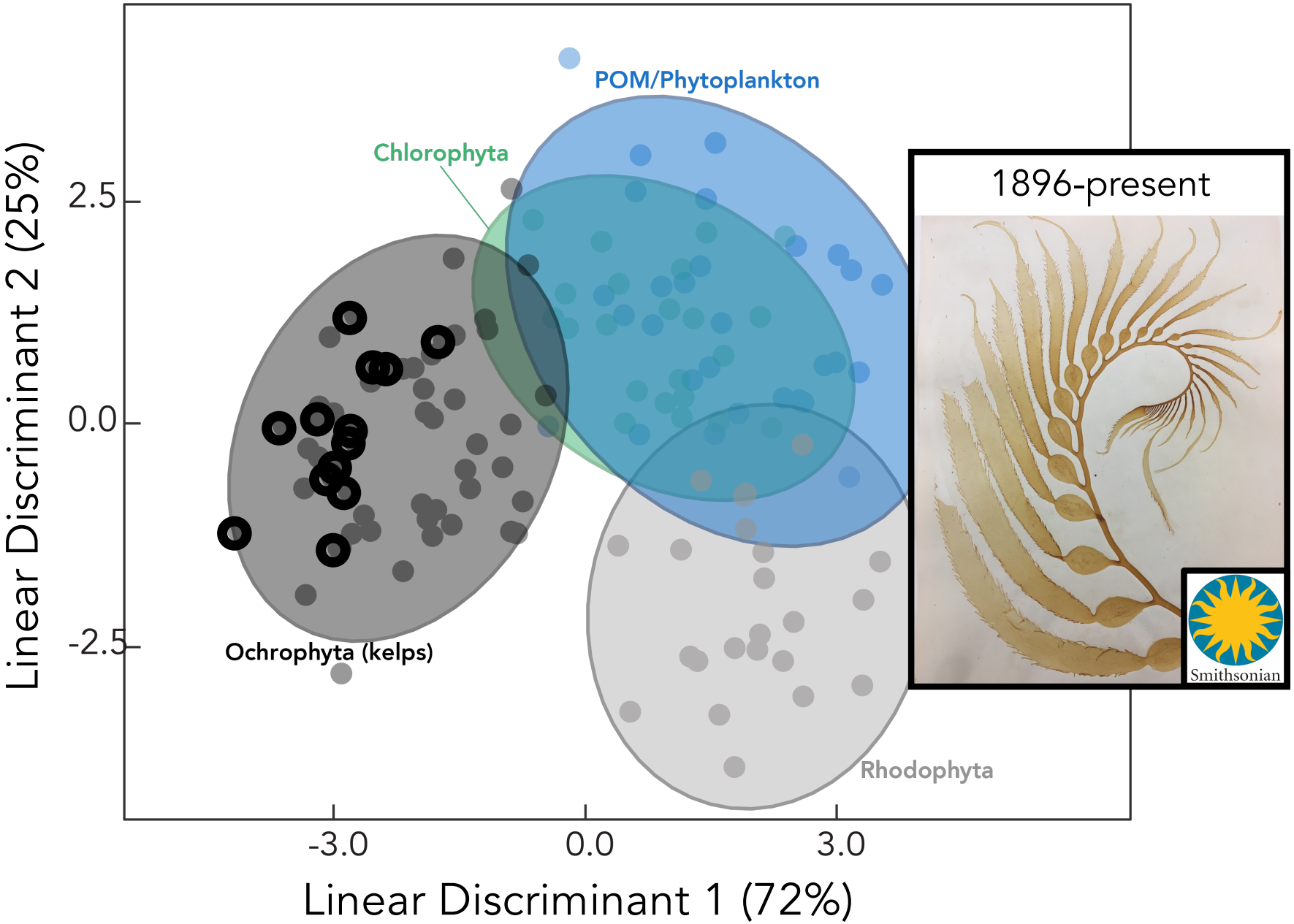
Essential amino acid $\delta^{13}C$ "fingerprints" of marine producers



Particulate organic matter (POM) as a proxy for phytoplankton



Historical and modern EAA $\delta^{13}C$ fingerprints are identical



Why bother?
Bulk tissues got some issues

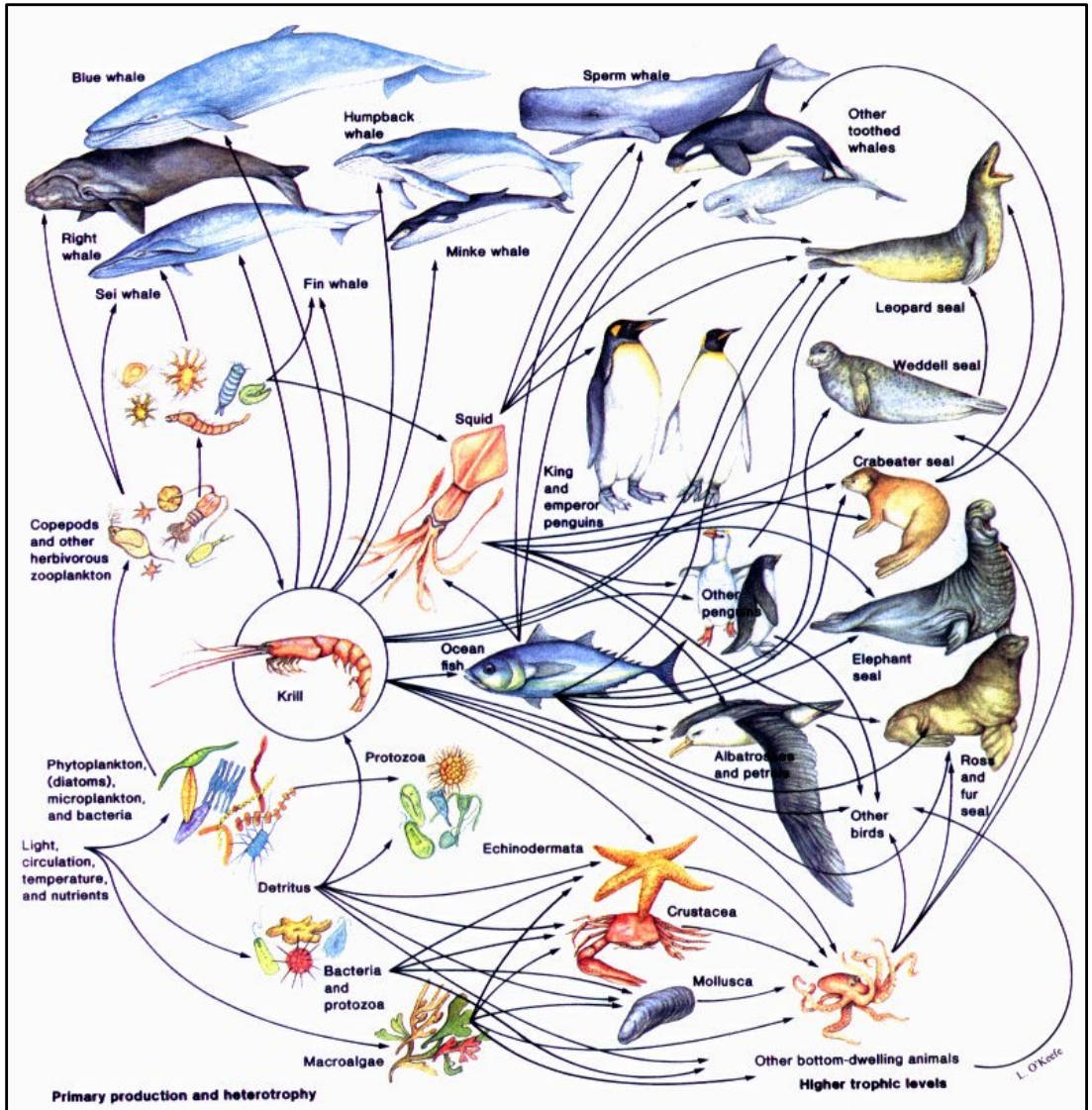


FIGURE 15.3 Food Webs. An Antarctic food web. Small crustaceans called krill support nearly all life in Antarctica. Krill are eaten by 6 species of baleen whales, 20 species of squid, over 100 species of fish, 35 species of birds, and 7 species of seals. Krill feed on algae, protozoa, other small crustaceans, and various larvae.

#1 Shifting and/or overlapping primary producer isotopic baselines

Why bother?
Bulk tissues got some issues

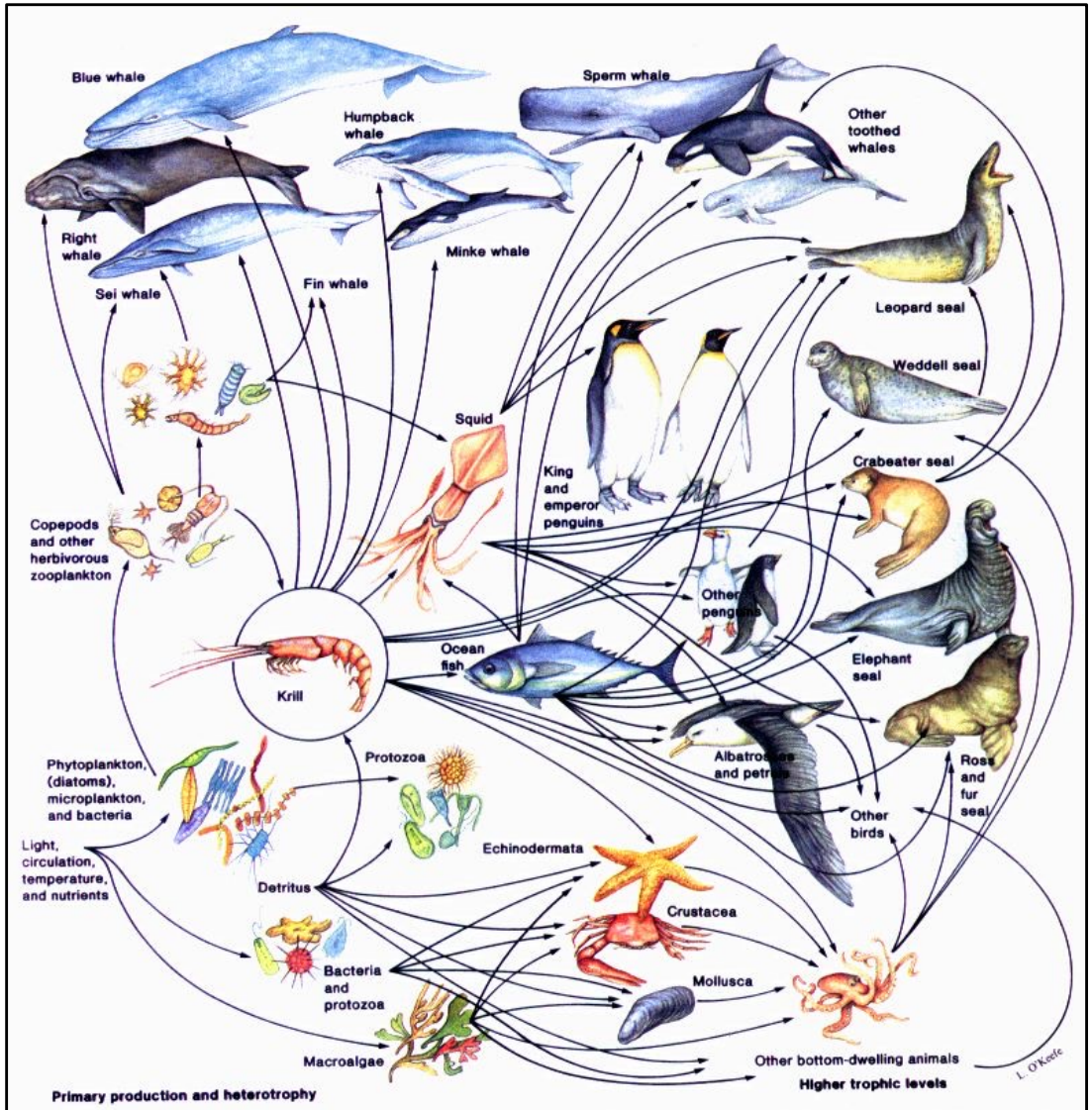


FIGURE 15.3 **Food Webs.** An Antarctic food web. Small crustaceans called krill support nearly all life in Antarctica. Krill are eaten by 6 species of baleen whales, 20 species of squid, over 100 species of fish, 35 species of birds, and 7 species of seals. Krill feed on algae, protozoa, other small crustaceans, and various larvae.

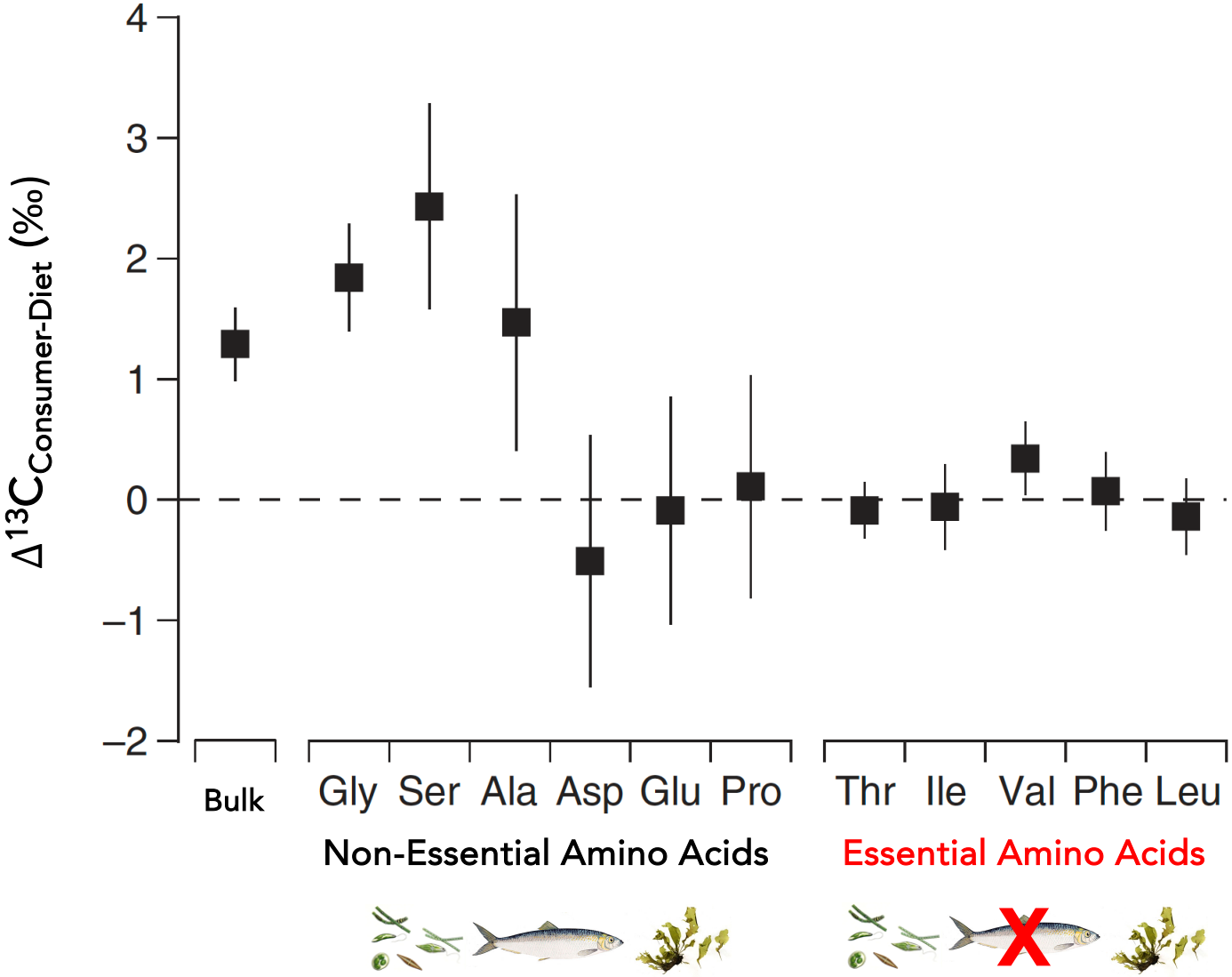
#1 Shifting and/or overlapping primary producer isotopic baselines

#2 Applications of trophic discrimination factors involve many assumptions

$\Delta^{13}C_{Consumer - Diet}$ values for essential amino acids are (sometimes) negligible

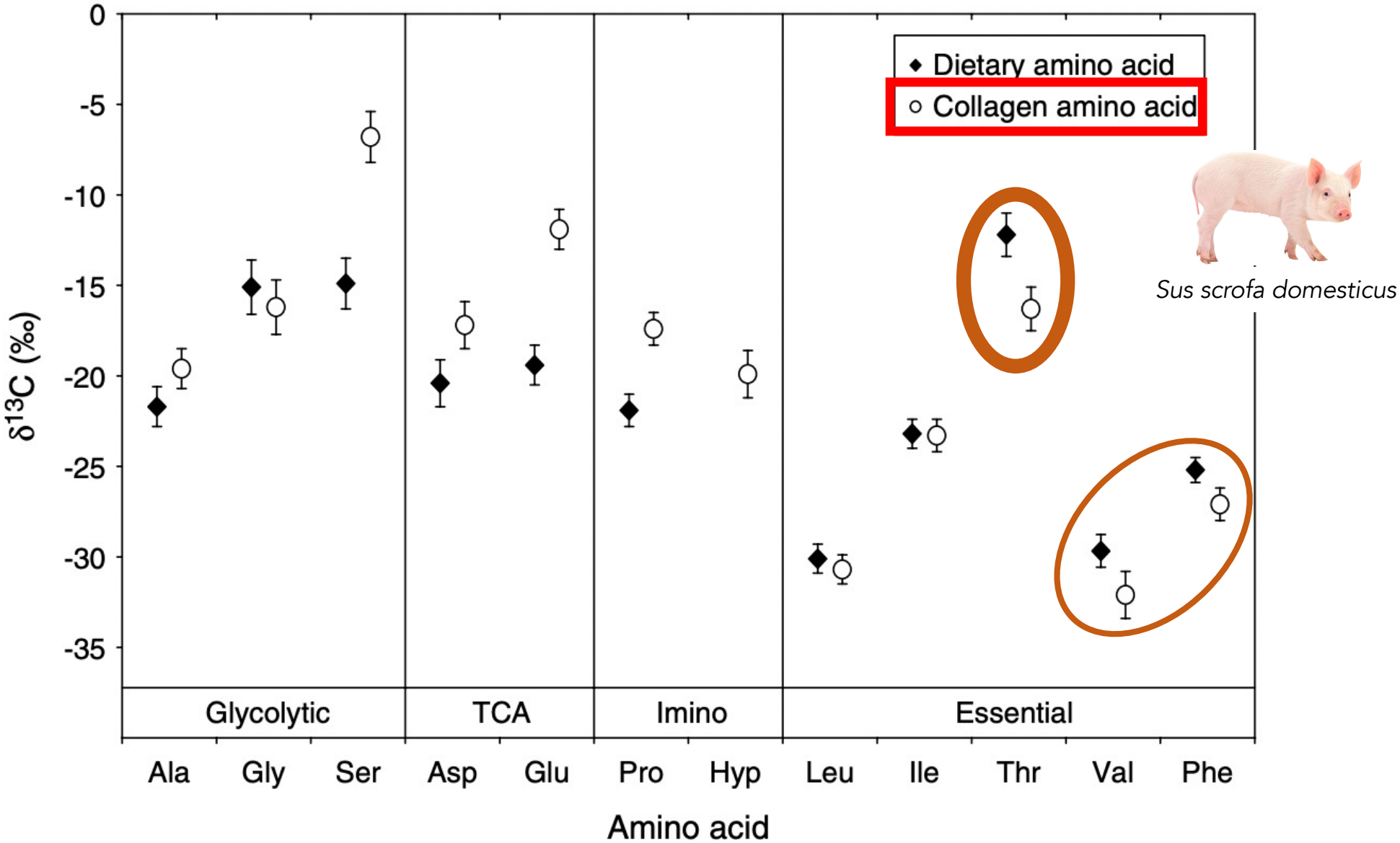
consumer

diet



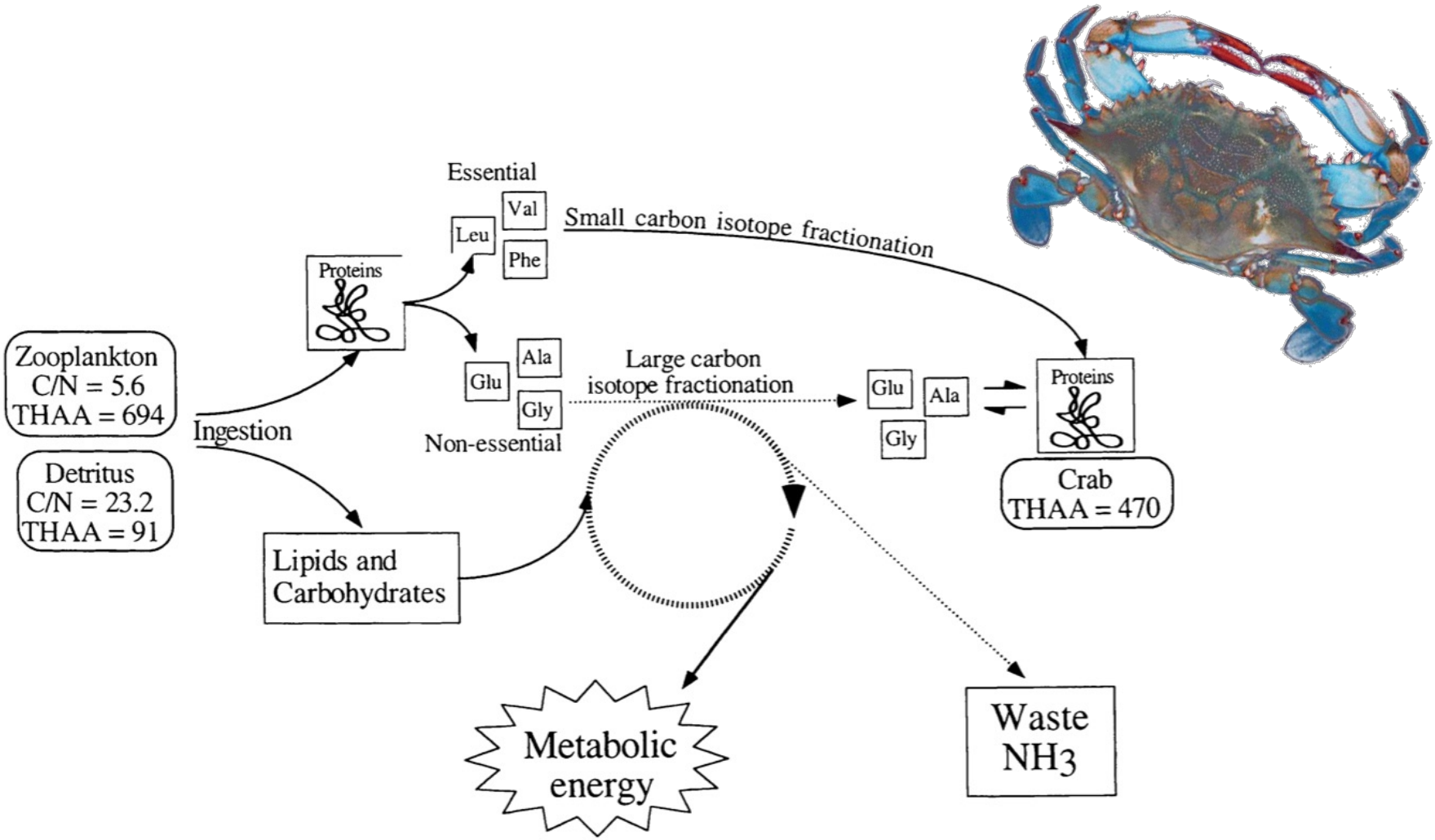
McMahon et al. 2015 Ecol. Evol

$\Delta^{13}C_{Consumer - Diet}$ values in an omnivorous mammal



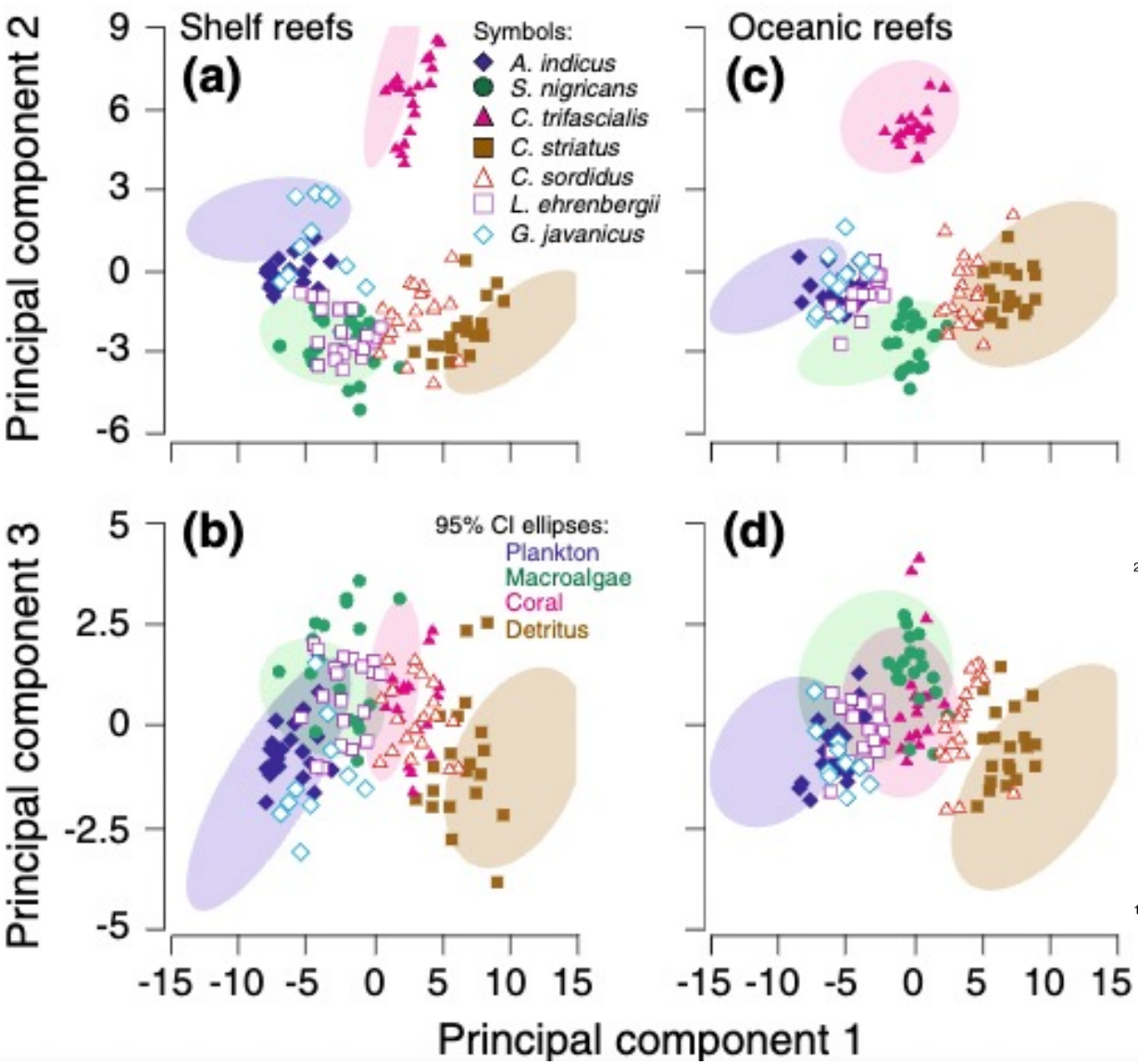
Howland et al. 2003 *J. Int. Osteology*

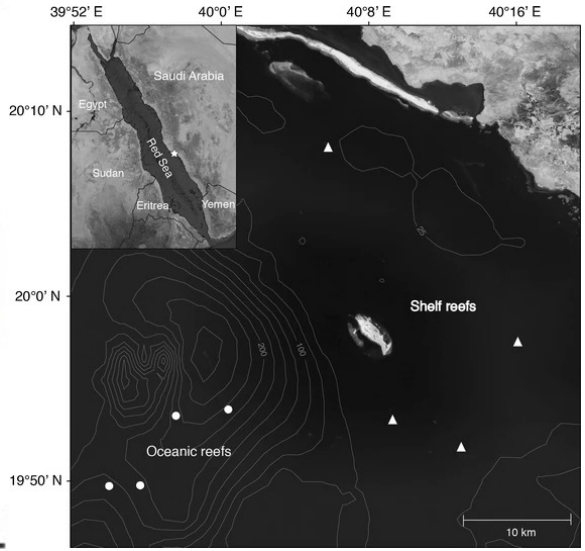
$\Delta^{13}C_{Consumer - Diet}$ values of amino acids may depend on diet in some consumers



Fantle et al. 1999 Oecologia

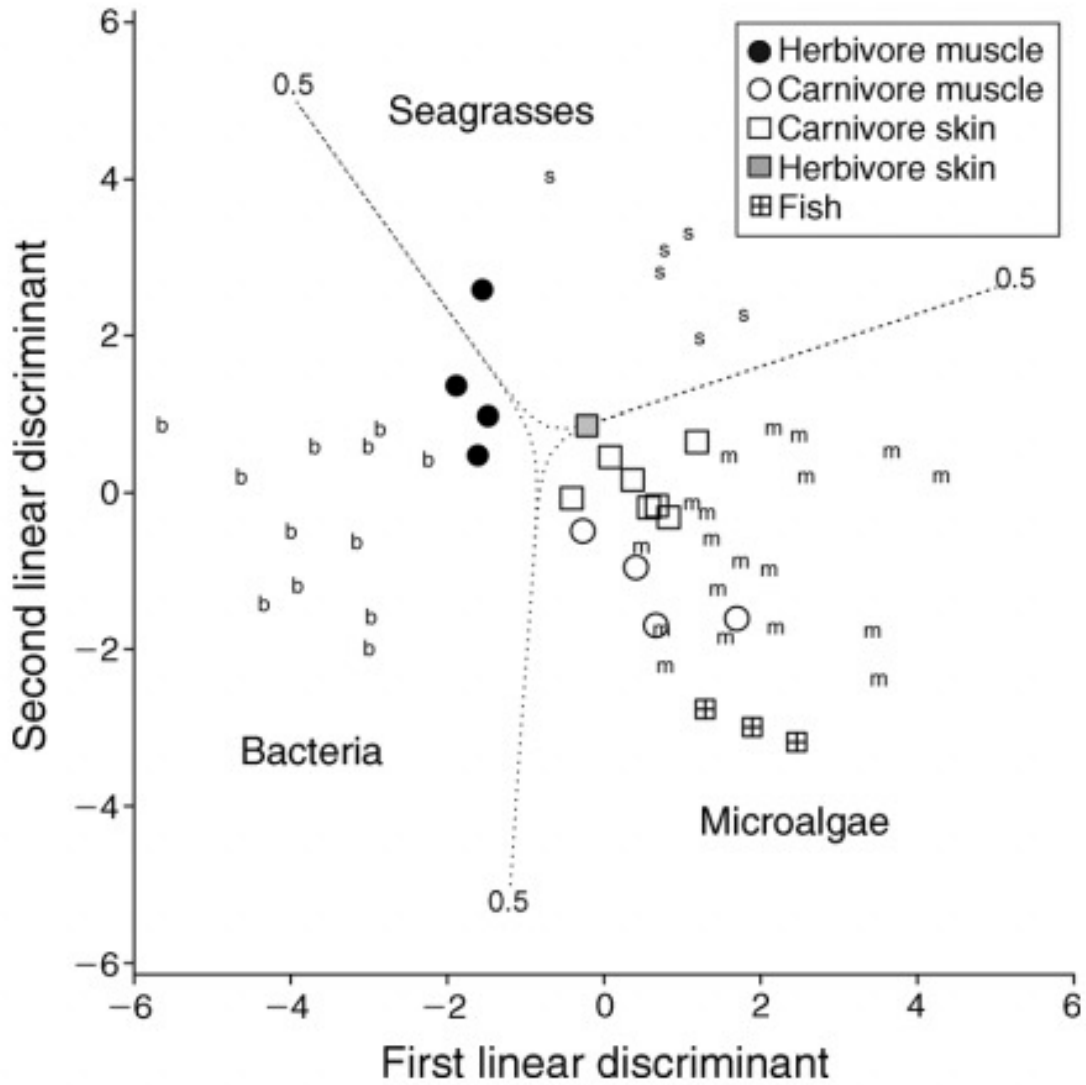
Marine ecology – coral reef fishes





McMahon et al. 2016 Oecologia

Marine ecology – sea turtles



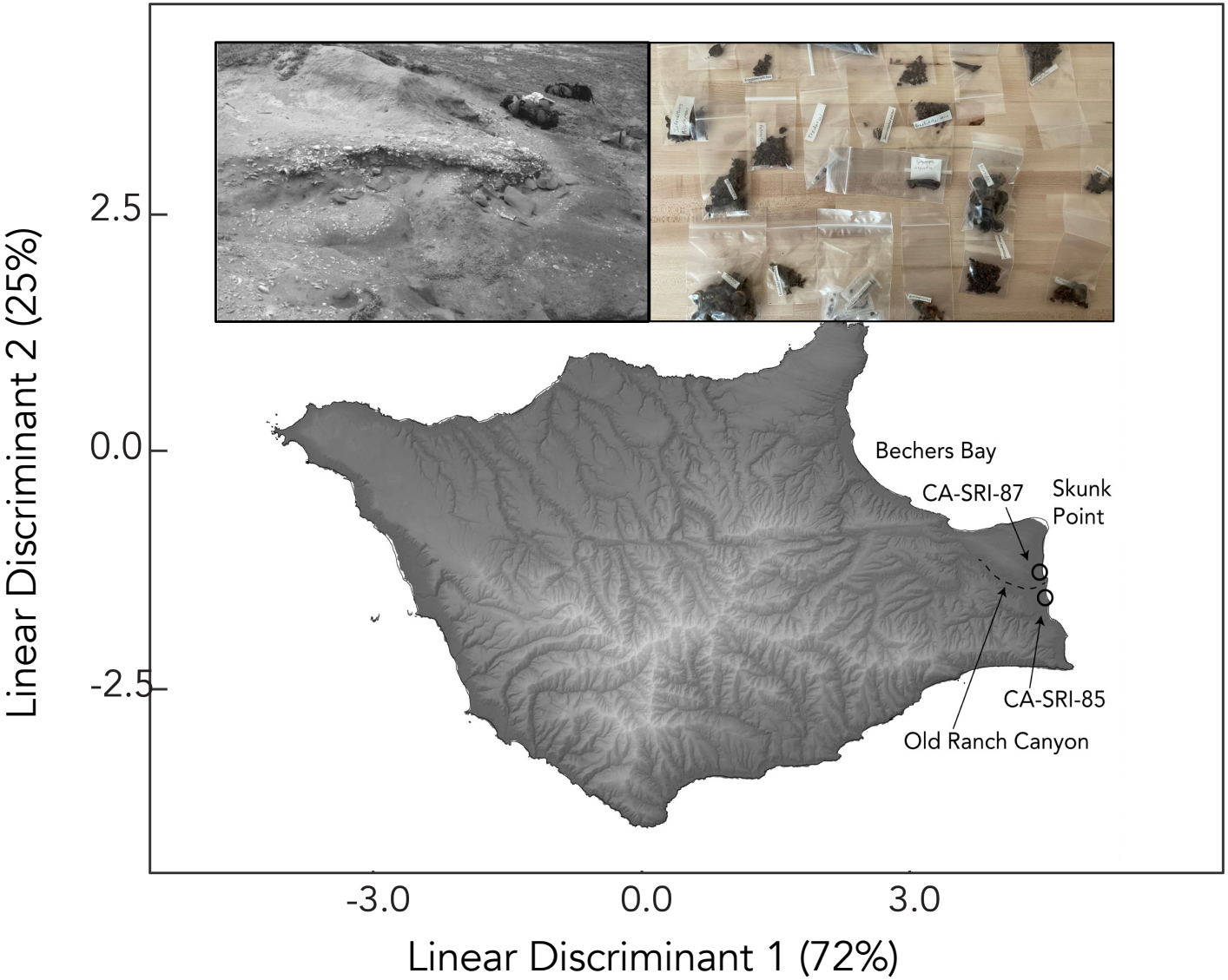
Chelonia mydas

Lepidochelys olivacea

Caretta caretta

Arthur et al. 2014 Ecology

Historical marine ecology & zooarchaeology



Pelagic Species

1. Clupeidae
2. *Sardinops sagax*
3. *Seriola lalandi*
4. *Trachurus symmetricus*
5. *Sphyraena argentea*

Mixed Substrate Species

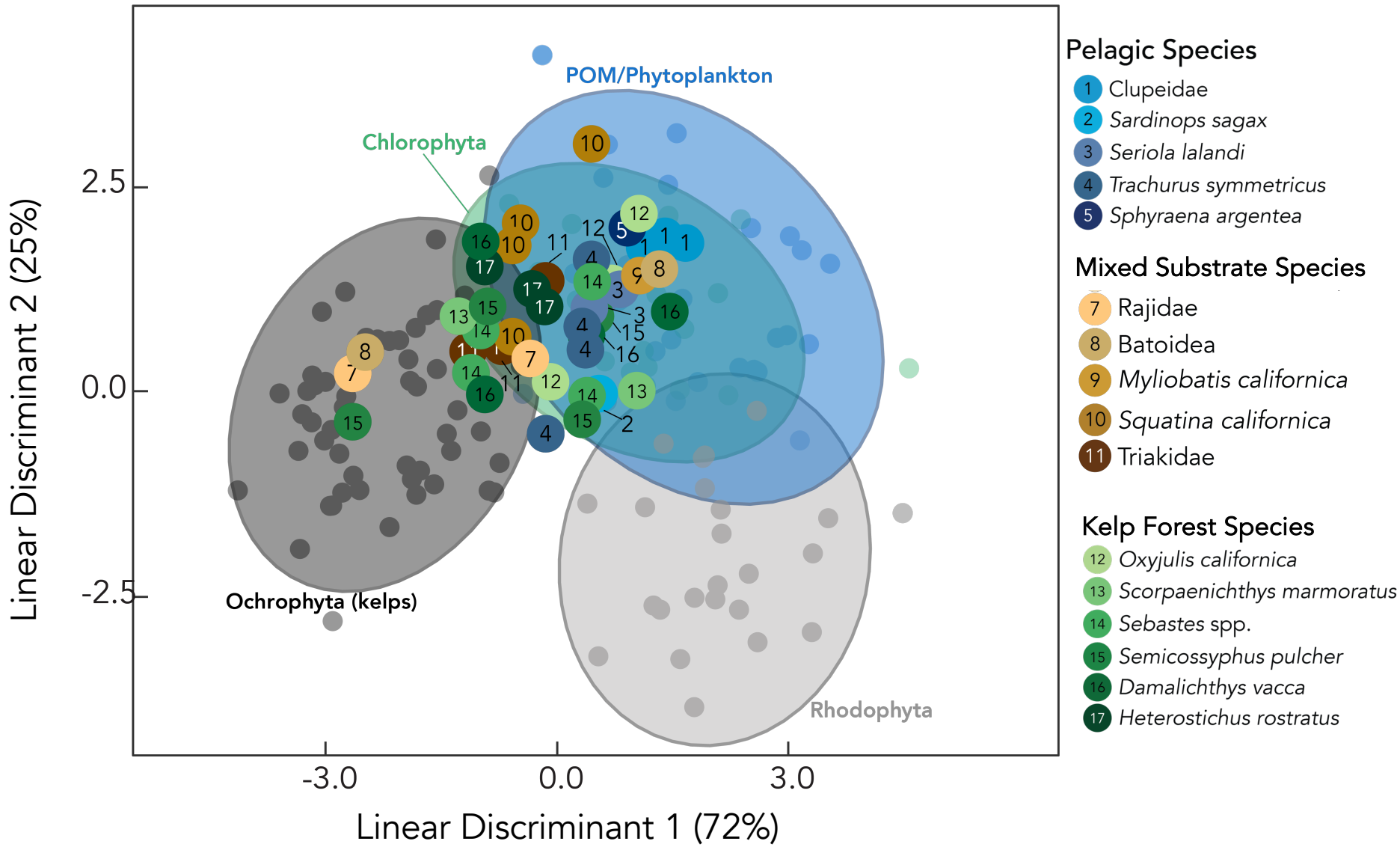
7. Rajidae
8. Batoidea
9. *Myliobatis californica*
10. *Squatina californica*
11. Triakidae

Kelp Forest Species

12. *Oxyjulis californica*
13. *Scorpaenichthys marmoratus*
14. *Sebastes* spp.
15. *Semicossyphus pulcher*
16. *Damalichthys vacca*
17. *Heterostichus rostratus*

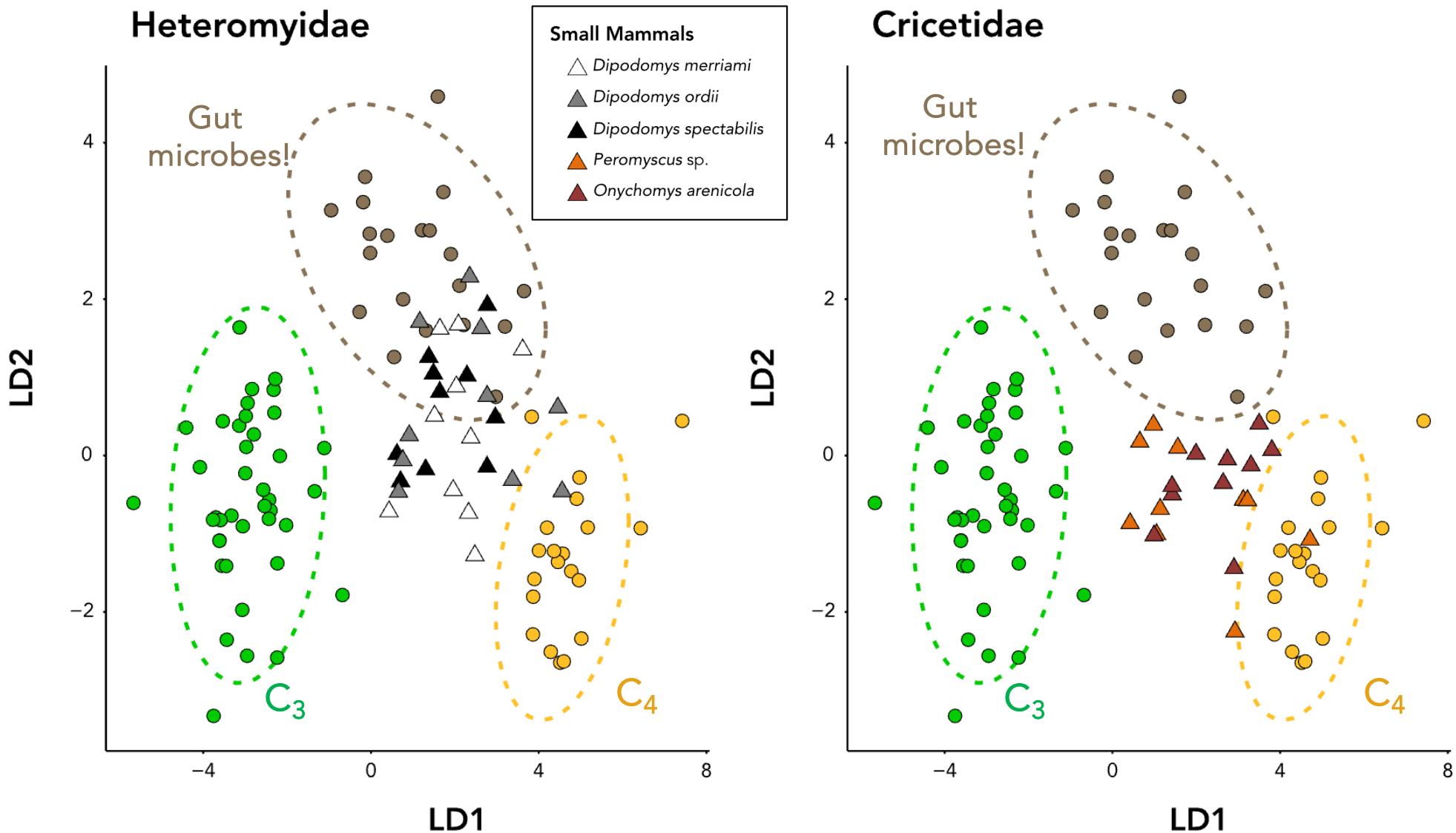
Elliott Smith et al. 2023. *The Holocene*

Historical marine ecology & zooarchaeology



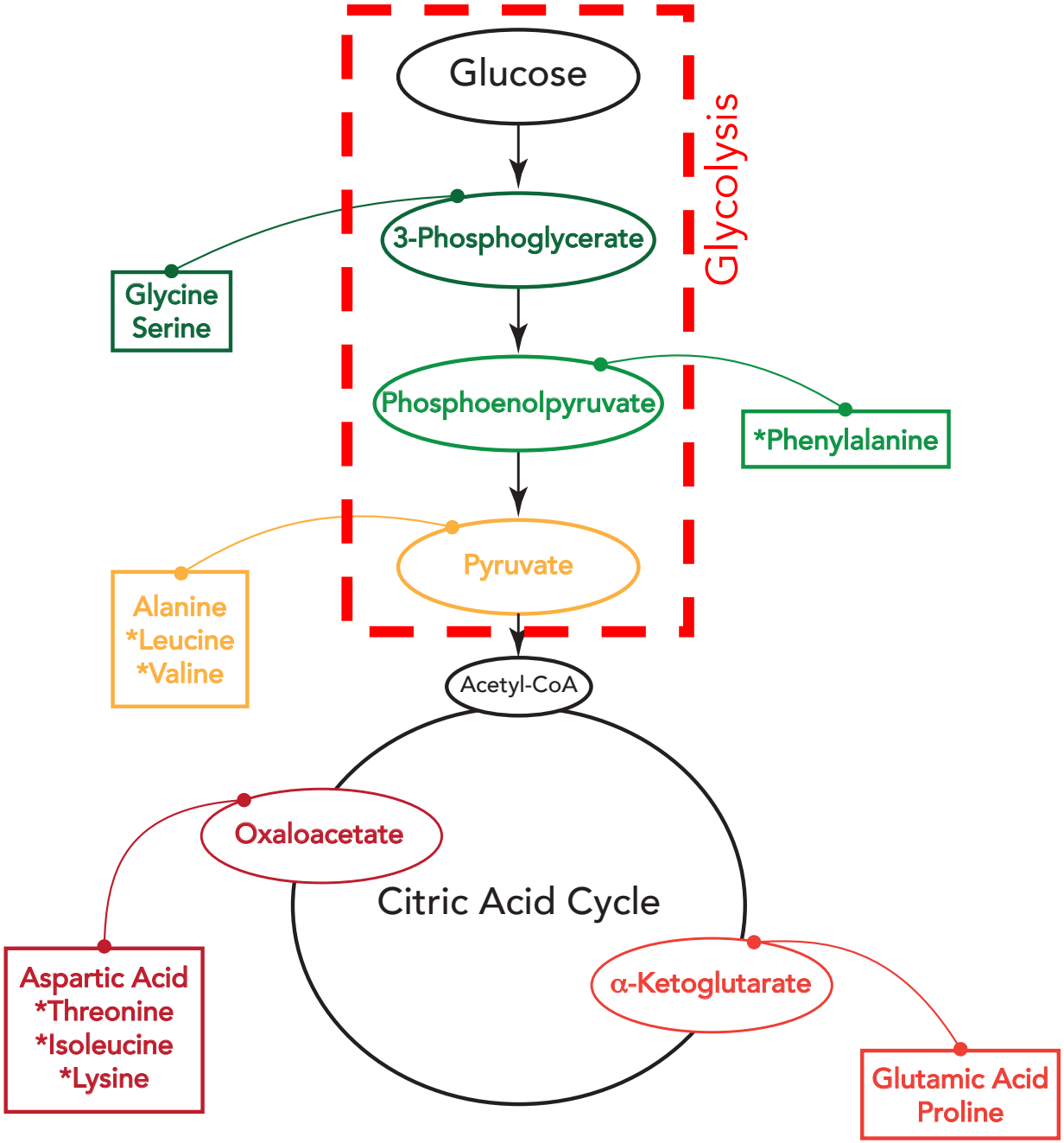
Elliott Smith et al. 2023. *The Holocene*

Use of essential amino acids from dietary sources and gut microbes

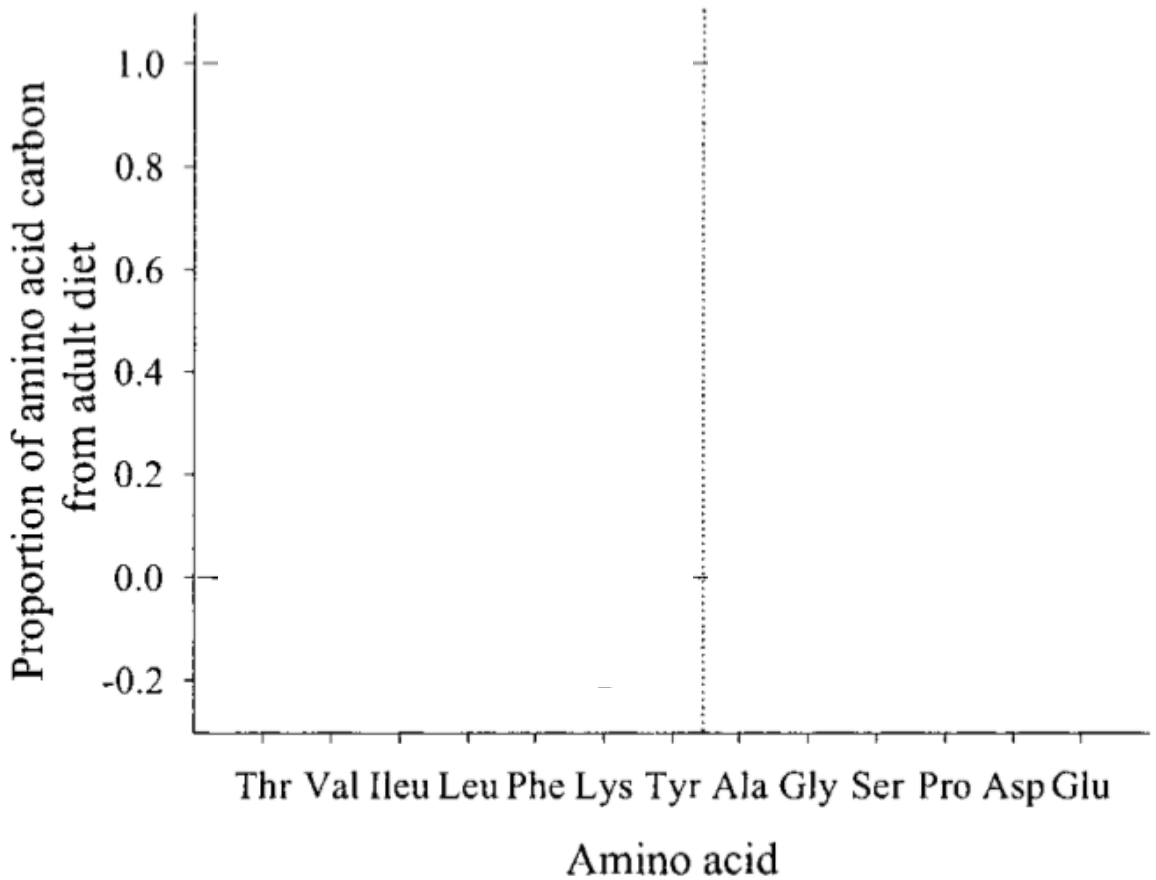


Besser et al. 2023 Ecology Letters

Amino Acid Biosynthetic Families

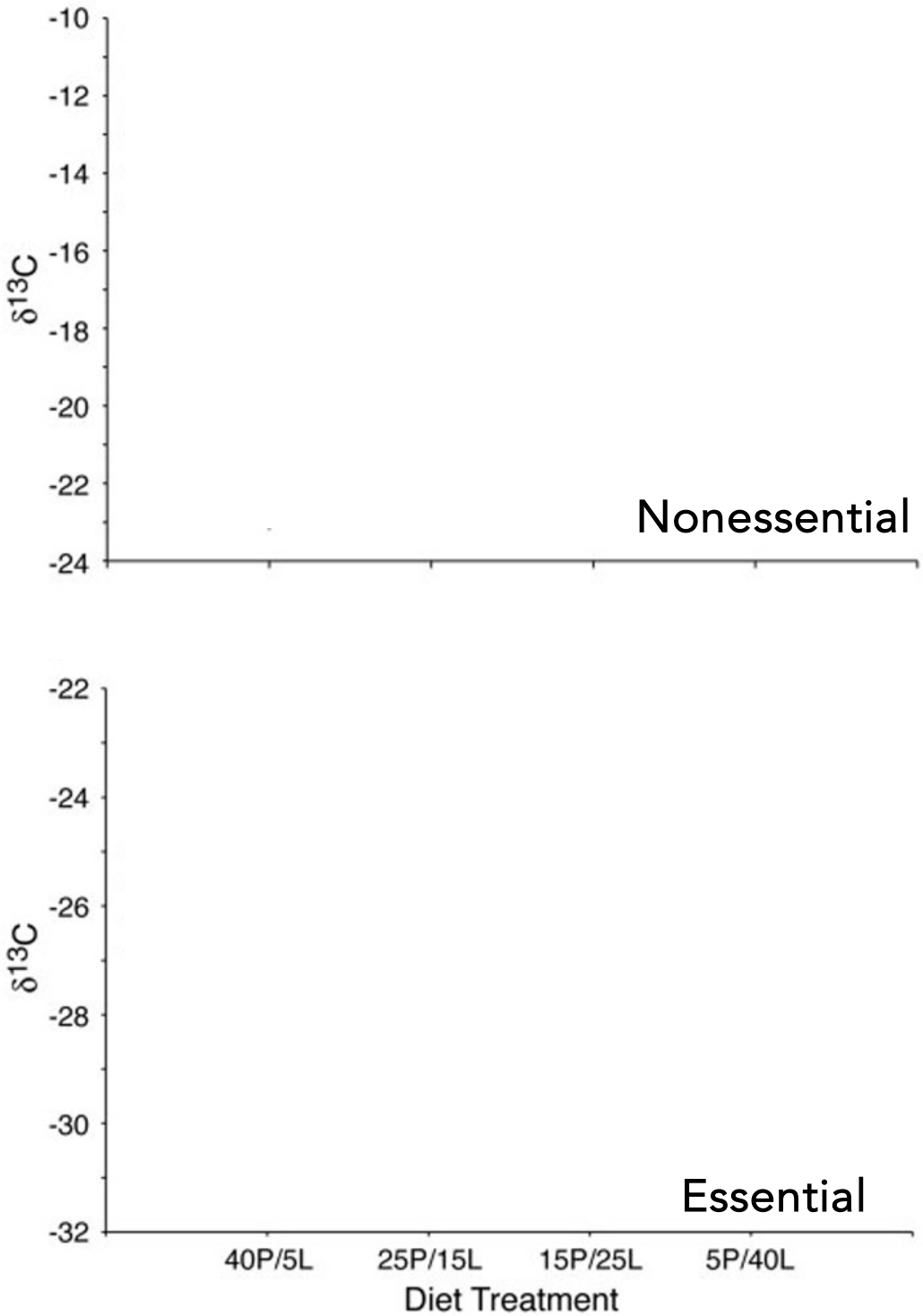


Tracing the use of larval resources and de novo synthesis of non-essentials in a nectar-feeding insect



Lepidoptera
(hawkmoth)

O'Brien et al. 2002. *PNAS*



Physiological flexibility and use of dietary lipid carbon for *de novo* synthesis of amino acids

Mus musculus

Newsome et al. 2014 Int. Comp. Bio

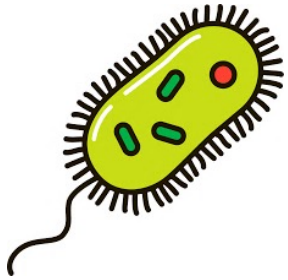
Table 1 Composition (weight percent) and $\delta^{13}C$ values (‰) of dietary ingredients and bulk diets of the four experimental diet-treatments of varying protein (P) and lipid (L) contents

Ingredient	40P:5L diet	30P:15L diet	20P:25L diet	5P:40L diet	$\delta^{13}C \pm SD$
Brewer's yeast	2.4	2.4	2.4	2.4	-23.5 ± 0.8
USP fortification salt	4.2	4.2	4.2	4.2	–
Vitamin fortification mixture	1.2	1.2	1.2	1.2	-12.0 ± 0.1
Corn oil	1.2	1.2	1.2	1.2	-11.0 ± 0.1
Cellulose	9.6	9.6	9.6	9.6	-11.0 ± 0.1
Casein	40.4	30.1	20.4	5.4	-27.0 ± 0.1
Lipid	4.8	15.1	25.1	39.8	-14.5 ± 0.1
Sucrose	36.1	36.1	35.9	36.1	-11.6 ± 0.1
Bulk diet $\delta^{13}C$ (‰)	−24.8	−23.3	−22.1	−19.3	–

Newsome et al. 2014 Int. Comp. Bio

What about carbohydrates… and gut microbes?

Ingredient	$\delta^{13}C$ (‰)	Diet #1 (%)	Diet #2 (%)	Diet #3 (%)	Diet #4 (%)
Protein	-26.5±0.2	5	10	20	40
Cornmeal	-12.5±0.2	40	20	10	5
Sucrose	-11.5±0.2	35	35	35	35
Cellulose	-25.5±0.2	10	25	25	10
Lipids	-11.5±0.2	1	1	1	1



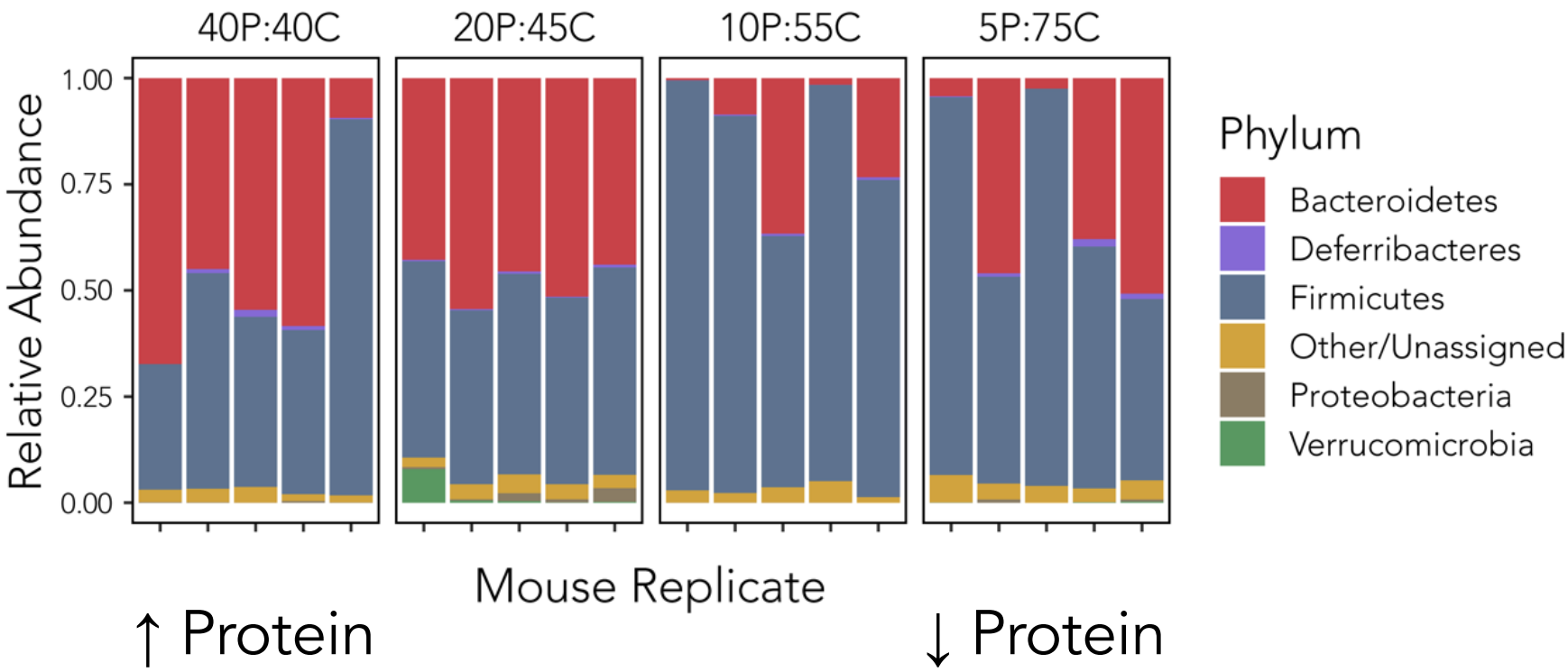
Mice (10/treatment) fed diets for 4 months, culled, and tissues & guts collected
Tissues (Isotopes): muscle, liver, blood plasma, red blood cells, hair
Guts (Genetics): duodenum, small intestine, ceca, large intestine

Mus musculus

Newsome et al. 2020 Proc B.

Relative Abundance by Diet Treatment: Mice Ceca

Higher Firmicutes:Bacteroidetes with decreasing dietary protein content.



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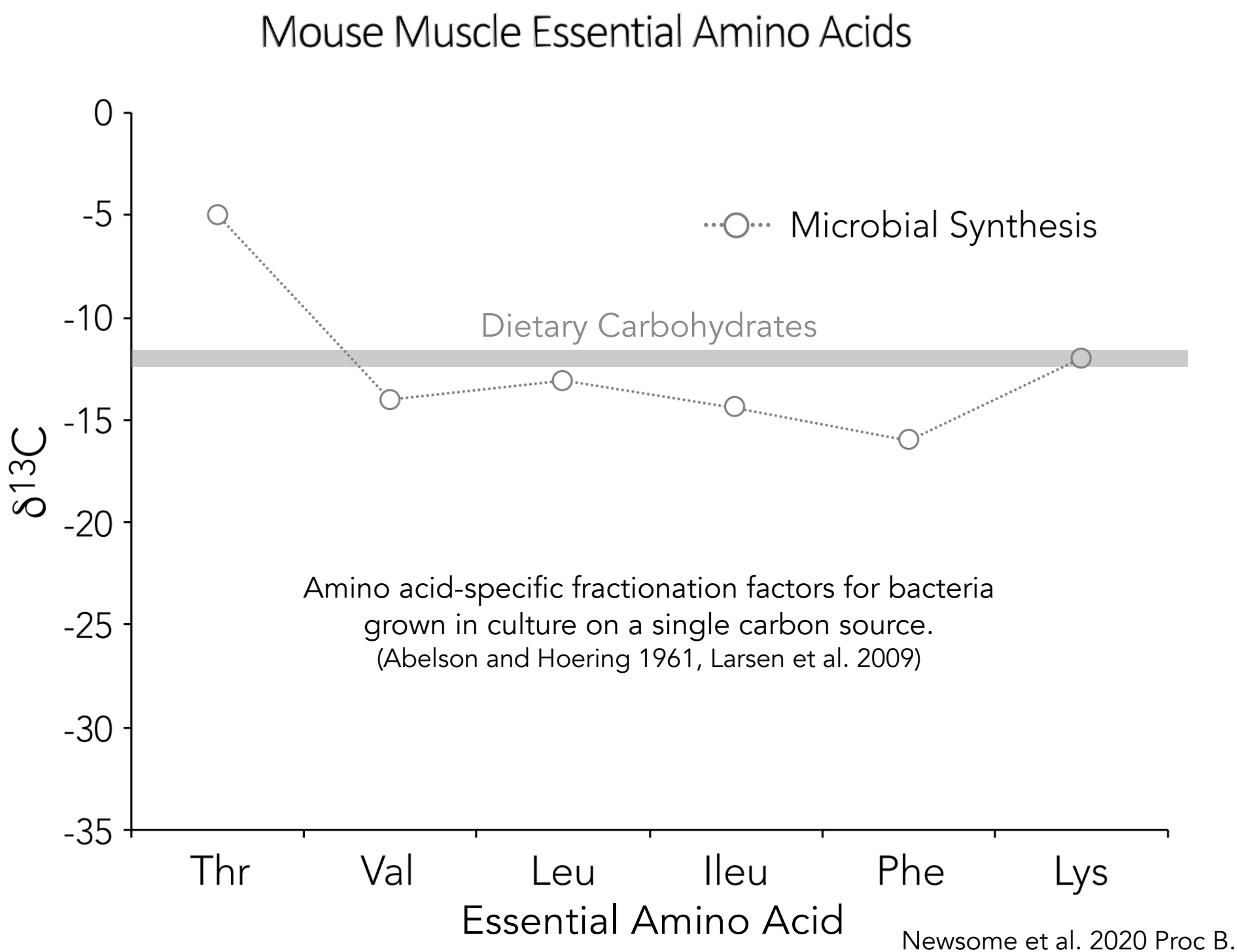

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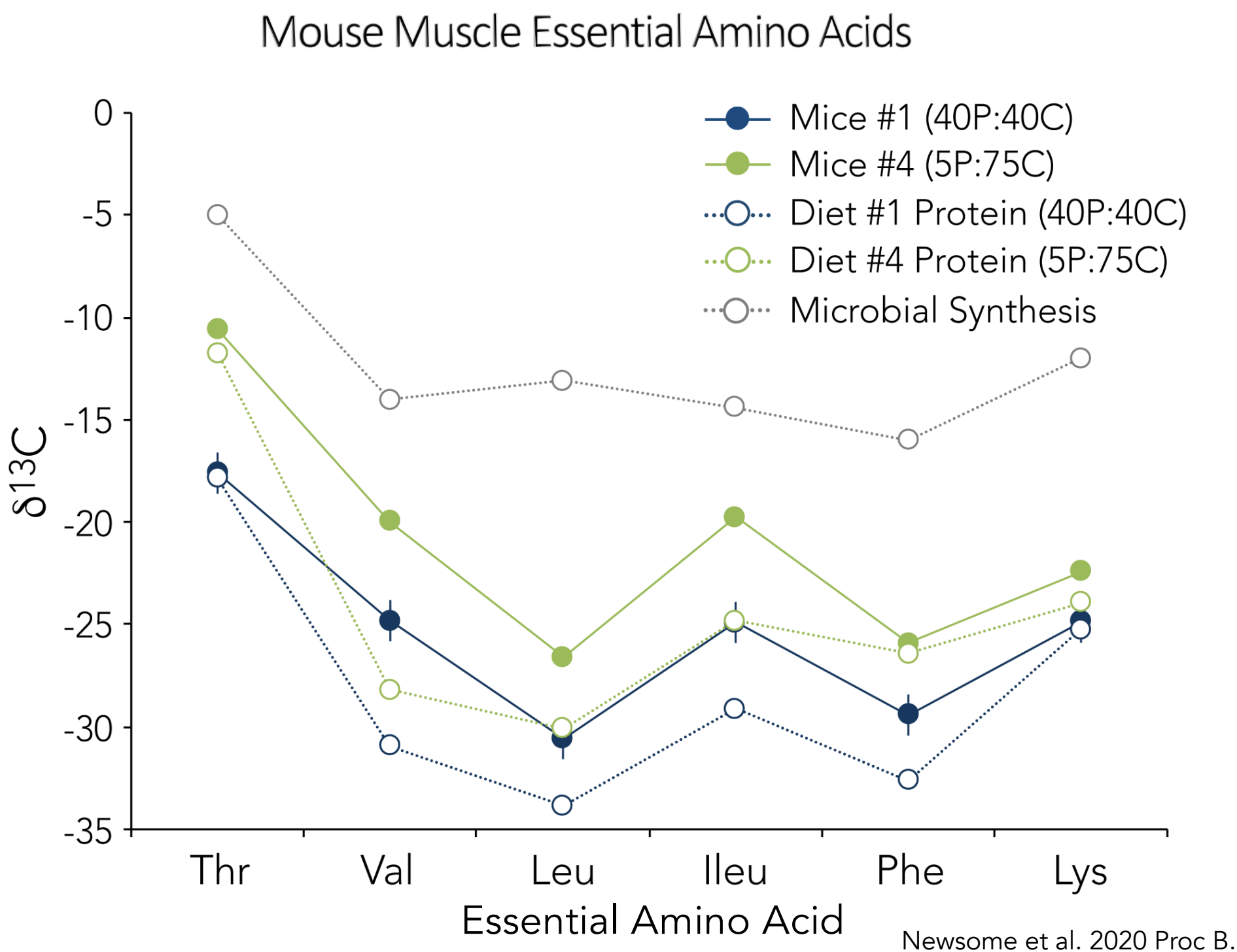

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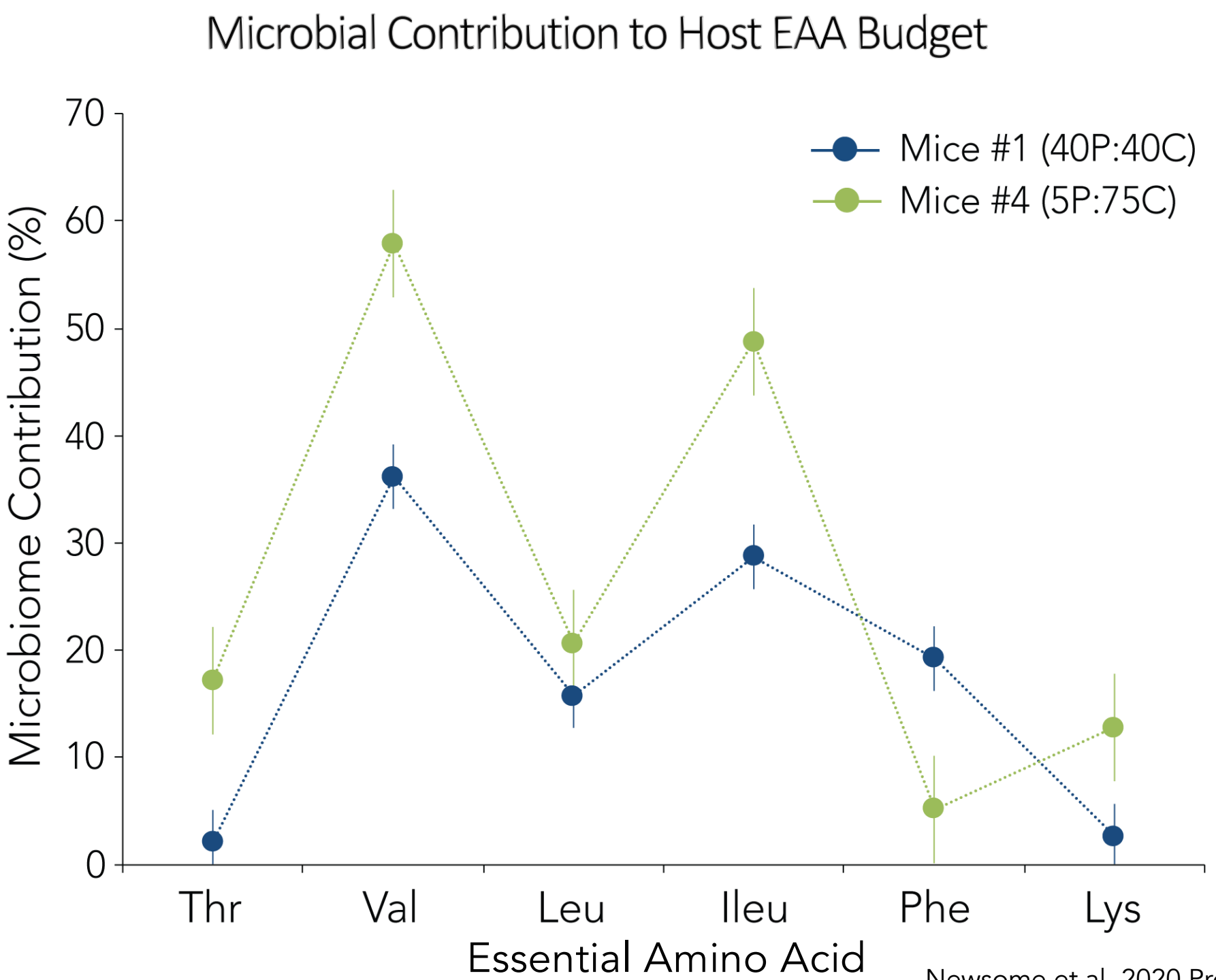

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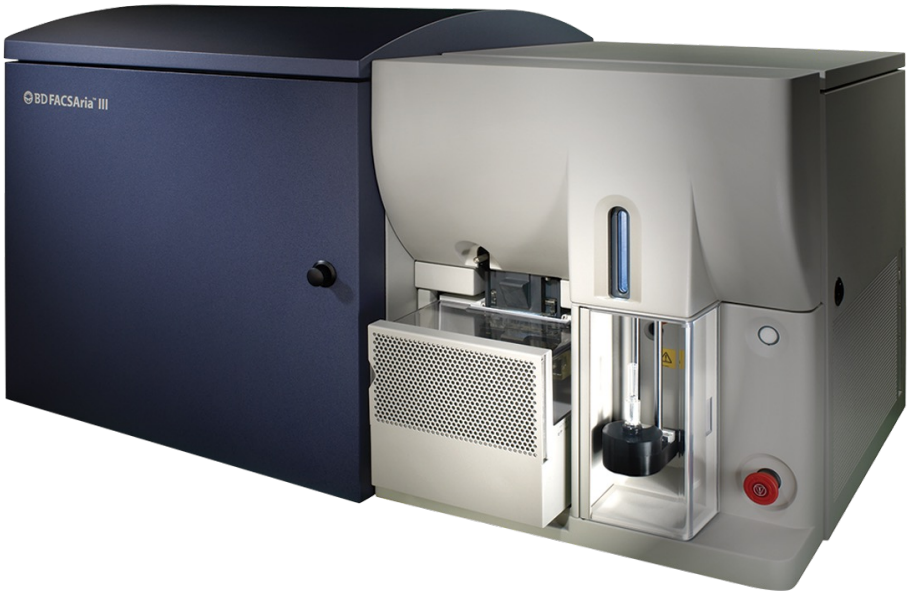
Mus musculus

Fluorescence-Activated Cell Sorting



Dr. Christy Mancuso

Conner Mertz

Peromyscus maniculatus