Amino Acid Carbon
Elemental Ecology
Week Ten
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 C₃ leaves (or 20–30% of total leaf N)!
Back to Basics: Amino Acid Structure

R Group
this is what makes each amino acid unique

Amine Group

Carboxyl Group

α-carbon

Carbon Skeleton
Amino Acids: The Building Blocks of Proteins

<table>
<thead>
<tr>
<th>Nonpolar side chains</th>
<th>Polar side chains</th>
<th>Electrically charged side chains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine (G) Gly</td>
<td>Serine (S) Ser</td>
<td>Aspartate (D) Asp</td>
</tr>
<tr>
<td>Alanine (A) Ala</td>
<td>Threonine (T) Thr</td>
<td>Glutamate (E) Glu</td>
</tr>
<tr>
<td>Valine (V) Val</td>
<td>Cysteine (C) Cys</td>
<td>Lysine (K) Lys</td>
</tr>
<tr>
<td>Leucine (L) Leu</td>
<td>Tyrosine (Y) Tyr</td>
<td>Arginine (R) Arg</td>
</tr>
<tr>
<td>Isoleucine (I) Ile</td>
<td>Asparagine (N) Asn</td>
<td>Histidine (H) His</td>
</tr>
</tbody>
</table>

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

- Glucose
  - 3-Phosphoglycerate
    - Phosphoenolpyruvate
      - Pyruvate
      - Acetyl-CoA
      - Oxaloacetate
      - α-Ketoglutarate
      - Glutamic Acid
      - Proline

- Glycolysis
  - Glycine
  - Serine
  - Alanine
  - Leucine
  - Valine
  - Phenylalanine
  - Threonine
  - Isoleucine
  - Lysine

- Citric Acid Cycle
  - Aspartic Acid
    - Threonine
    - Isoleucine
    - Lysine
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

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

Glycine
- non-essential amino acid

Valine
- essential amino acid

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

His, Ile, Leu, Lys, Met, Phe, Thr, Try, Val
Non-essential amino acids are easier to synthesize, and animals have retained this ability.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>TYPE</th>
<th>ATP</th>
<th>NADP</th>
<th># steps to synthesize</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>NE</td>
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<tr>
<td>Glutamate</td>
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<td>0</td>
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<tr>
<td>Aspartate</td>
<td>NE</td>
<td>0</td>
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<tr>
<td>Tyrosine¹</td>
<td>NE</td>
<td>0</td>
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<tr>
<td>Glutamine</td>
<td>NE</td>
<td>1</td>
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<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>NE</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Serine</td>
<td>NE</td>
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<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>NE</td>
<td>1</td>
<td>3</td>
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<tr>
<td>Cysteine²</td>
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<td></td>
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<tr>
<td>Valine</td>
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<td></td>
</tr>
<tr>
<td>Threonine</td>
<td>E</td>
<td>2</td>
<td>3</td>
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<td>Leucine</td>
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<tr>
<td>Methionine</td>
<td>E</td>
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<td>8</td>
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<tr>
<td>Lysine</td>
<td>E</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Phenylalanine</td>
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<td>1</td>
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<tr>
<td>Arginine</td>
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</tr>
<tr>
<td>Histidine</td>
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<td>6</td>
<td>1</td>
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</tr>
<tr>
<td>Isoleucine</td>
<td>E</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Tryptophan</td>
<td>E</td>
<td>5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Non-essential amino acids: animals can synthesize de novo.

Essential amino acids: animals cannot synthesize de novo* (at least in high enough abundance).

Conditionally essential: Histidine
Amino Acid Biosynthetic Families

- Glucose
  - Glycine
  - Serine
- 3-Phosphoglycerate
- Phosphoenolpyruvate
- Pyruvate
  - Alanine
  - *Leucine
  - *Valine
- Acetyl-CoA
- Oxaloacetate
- Aspartic Acid
  - *Threonine
  - *Isoleucine
  - *Lysine
- α-Ketoglutarate
- Glutamic Acid
- Proline
- Citric Acid Cycle
- Glycolysis
- *Phenylalanine
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
Bulk tissue δ13C values can’t help us distinguish between pelagic and benthic producers in nearshore ecosystems!
"Only pack the essentials"
### Essential amino acids $\delta^{13}$C values vary among primary producer groups

<table>
<thead>
<tr>
<th></th>
<th>Ile</th>
<th>Leu</th>
<th>Lys</th>
<th>Phe</th>
<th>Thr</th>
<th>Val</th>
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</thead>
<tbody>
<tr>
<td>$\delta^{13}$C (%)</td>
<td></td>
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</tbody>
</table>

**Legend**
- **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”

No clear separation among groups on X or Y axes
Essential amino acid $\delta^{13}C$ “fingerprinting”

Y-axis

X-axis

Nice separation along a linear axis that is a combination of the X and Y axis
Essential amino acid $\delta^{13}C$ “fingerprinting”

Linear Discriminant 1

$\alpha(x\text{-axis value}) + \beta(y\text{-axis value})$

Projection into multivariate space
Plants, bacteria, and fungi have distinct EAA δ¹³C fingerprints.
Plants, bacteria, fungi, and algae have distinct EAA $\delta^{13}$C fingerprints.
These first fingerprinting studies were conducted at a global scale.
What about our New Mexico producers?

<table>
<thead>
<tr>
<th></th>
<th>Ile</th>
<th>Leu</th>
<th>Lys</th>
<th>Phe</th>
<th>Thr</th>
<th>Val</th>
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</thead>
<tbody>
<tr>
<td>C3 Plants</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4 Plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aquatic Algae</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CAM Plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Besser et al. 2022 J. Ecology
EAA $\delta^{13}C$ fingerprints are unique among terrestrial and aquatic producers.

Besser et al. 2022 J. Ecology
$\text{AA}_{\text{ESS}} \delta^{13}\text{C}$ fingerprints are unique among terrestrial and aquatic producers

99% successful reclassification rate

(A) CAM Plants

$\text{C}_3$ Plants

$\text{C}_4$ Plants

Aquatic Algae

(B) LD1 (68.2%)

LD2 (27.6%)

LD3 (4.2%)

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

- **Glucose**
  - 3-Phosphoglycerate
  - Phosphoenolpyruvate

Glycolysis

- Pyruvate Oxidation
  - Pyruvate
  - Acetyl-CoA

Citric Acid Cycle

- Oxaloacetate
- α-Ketoglutarate
- Glutamic Acid
- Proline

- Glycine
- Serine
- Aspartic Acid
- Threonine
- Isoleucine
- Lysine
- Alanine
- Leucine
- Valine
- Phenylalanine

- Initial product of CO₂ fixation in some C₄ plants
- Heavily involved in central N metabolism
- Precursor to many secondary metabolites in plants (e.g., lignin)
- Involved in photorespiration
Looks like there may some differences in algal $AA_{ESS} \delta^{13}C$ fingerprints, too.
Building an isotopic library of marine producers

**Chlorophyta (Green Algae)**
- *Ulva*

**Rhodophyta (Red Algae)**
- *Neorhodomela*
- *Plocamium*
- *Rhodymenia*
- *Chondrus*

**Ochrophyta (Kelps)**
- *Saccharina*
- *Lessonia*
- *Macrocytis*

**Microalgae (POM)**
- Phytoplankton Cultures

Elliott Smith et al. 2022.
*Func. Ecol.*
Group
- Clorophyta (*Ulva*)
- Ochrophyta (*Kelps*)
- POM
- Rhodophyta (Red Algae)

Site
1. Katmai, AK
2. Santa Cruz, CA
3. San Diego, CA
4. Antofagasta, Chile

Essential amino acid δ^{13}C “fingerprints” of marine producers

- Isoleucine
- Leucine
- Phenylalanine
- Threonine
- Valine

Linear Discriminant 1 (72%)
Linear Discriminant 2 (25%)
Particulate organic matter (POM) as a proxy for phytoplankton

Linear Discriminant 1 (72%)
Linear Discriminant 2 (25%)
Historical and modern EAA $\delta^{13}$C fingerprints are identical.
Why bother?
Bulk tissues got some issues

#1 Shifting and/or overlapping primary producer isotopic baselines
Why bother?
Bulk tissues got some issues

#1 Shifting and/or overlapping primary producer isotopic baselines

#2 Applications of trophic discrimination factors involve many assumptions
$\Delta^{13}C_{\text{Consumer}} - \Delta^{13}C_{\text{Diet}}$ values for essential amino acids are (sometimes) negligible.
$\Delta^{13}C_{\text{Consumer} - \text{Diet}}$ values in an omnivorous mammal

![Diagram](image.png)

Howland et al. 2003 J. Int. Osteology
$\Delta^{13}C_{\text{Consumer-Diet}}$ values of amino acids may depend on diet in some consumers.
Marine ecology – coral reef fishes

McMahon et al. 2016 Oecologia
Marine ecology – sea turtles

Arthur et al. 2014 Ecology

Chelonia mydas

Lepidochelys olivacea

Caretta caretta
Historical marine ecology & zooarchaeology

Linear Discriminant 1 (72%)
Linear Discriminant 2 (25%)

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

Mixed Substrate Species
6. Rajidae
7. Batoidea
8. Myliobatis californica
9. Squatina californica
10. Triakidae

Kelp Forest Species
11. Oxyjulis californica
12. Scorpaenichthys marmoratus
14. Semicossyphus pulcher
15. Dalmichthys vacca
16. Heterostichus rostratus

Elliott Smith et al. 2023. *The Holocene*
Historical marine ecology & zooarchaeology

Linear Discriminant 1 (72%)
Linear Discriminant 2 (25%)

Pelagic Species
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14. Semicossyphus pulcher
15. Damalichthys vacca
16. Heterostichus rostratus

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

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- Phosphoenolpyruvate
- Pyruvate
- Acetyl-CoA
- Oxaloacetate
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- Glutamic Acid
- Proline

- Glycine
- Serine
- Alanine
- Leucine
- Valine
- *Phenylalanine
- *Threonine
- *Isoleucine
- *Lysine

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

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>
<thead>
<tr>
<th>Ingredient</th>
<th>40P:5L diet</th>
<th>30P:15L diet</th>
<th>20P:25L diet</th>
<th>5P:40L diet</th>
<th>$\delta^{13}\text{C} \pm \text{SD}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewer’s yeast</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>2.4</td>
<td>$-23.5 \pm 0.8$</td>
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<tr>
<td>USP fortification salt</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td>4.2</td>
<td>–</td>
</tr>
<tr>
<td>Vitamin fortification mixture</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>$-12.0 \pm 0.1$</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>$-11.0 \pm 0.1$</td>
</tr>
<tr>
<td>Cellulose</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
<td>9.6</td>
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<tr>
<td>Casein</td>
<td>40.4</td>
<td>30.1</td>
<td>20.4</td>
<td>5.4</td>
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<tr>
<td><strong>Lipid</strong></td>
<td><strong>4.8</strong></td>
<td><strong>15.1</strong></td>
<td><strong>25.1</strong></td>
<td><strong>39.8</strong></td>
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<tr>
<td>Sucrose</td>
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<td>36.1</td>
<td>35.9</td>
<td>36.1</td>
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<tr>
<td>Bulk diet $\delta^{13}\text{C}$</td>
<td>$-24.8$</td>
<td>$-23.3$</td>
<td>$-22.1$</td>
<td>$-19.3$</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1: Composition (weight percent) and $\delta^{13}\text{C}$ values (‰) of dietary ingredients and bulk diets of the four experimental diet-treatments of varying protein (P) and lipid (L) contents.
What about carbohydrates… and gut microbes?

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

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

Newsome et al. 2020 Proc B.
Higher **Firmicutes**: **Bacteroidetes** with decreasing dietary protein content.

Newsome et al. 2020 Proc B.
Dietary Carbohydrates

Amino acid-specific fractionation factors for bacteria grown in culture on a single carbon source.

(Abelson and Hoering 1961, Larsen et al. 2009)

Mouse Muscle Essential Amino Acids

δ¹³C

Amino acid-specific fractionation factors for bacteria grown in culture on a single carbon source.

(Abelson and Hoering 1961, Larsen et al. 2009)

Newsome et al. 2020 Proc B.
Mouse Muscle Essential Amino Acids

- Mice #1 (40P:40C)
- Mice #4 (5P:75C)
- Diet #1 Protein (40P:40C)
- Diet #4 Protein (5P:75C)
- Microbial Synthesis

δ¹³C vs. Essential Amino Acid

Newsome et al. 2020 Proc B.
Microbial Contribution to Host EAA Budget

- Mice #1 (40P:40C)
- Mice #4 (5P:75C)

Newsome et al. 2020 Proc B.
Dr. Christy Mancuso

Mus musculus

Conner Mertz

Peromyscus maniculatus

Fluorescence-Activated Cell Sorting