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

Amine Group

Carboxyl Group

Hydrogen Atom

α-carbon

Carbon Skeleton

R Group

this is what makes each amino acid unique
There are 20 common amino acids (and almost 500 other naturally occurring amino acids).

These amino acids have different chemical properties.
A GUIDE TO THE TWENTY COMMON AMINO ACIDS

Chart Key:
- ALIPHATIC
- AROMATIC
- ACIDIC
- BASIC
- HYDROXYLIC
- SULFUR-CONTAINING
- AMIDIC

Chemical Structure:
- NAME (single letter code)
- three letter code
- DNA colors

ALANINE (A)
GLYCINE (G)
ISOLEUCINE (I)
LEUCINE (L)
PROLINE (P)
VALINE (V)

PHENYLALANINE (F)
TRYPTOPHAN (W)
TYROSINE (Y)
ASPARTIC ACID (D)
GLUTAMIC ACID (E)
ARGININE (R)
HISTIDINE (H)

LYSINE (K)
SERINE (S)
THRONE (T)
CYSTEINE (C)
METHIONINE (M)
ASPARAGINE (N)
GLUTAMINE (Q)

Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.
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>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Aspartate</td>
<td>NE</td>
<td>0</td>
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<td>1</td>
</tr>
<tr>
<td>Tyrosine¹</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glutamine</td>
<td>NE</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Asparagine</td>
<td>NE</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Serine</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glycine</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Proline</td>
<td>NE</td>
<td>1</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Cysteine²</td>
<td>NE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Valine</td>
<td>E</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Threonine</td>
<td>E</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Leucine</td>
<td>E</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Methionine</td>
<td>E</td>
<td>7</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Lysine</td>
<td>E</td>
<td>3</td>
<td>4</td>
<td>4</td>
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<tr>
<td>Phenylalanine</td>
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<td>2</td>
</tr>
<tr>
<td>Arginine</td>
<td>E</td>
<td>7</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Histidine</td>
<td>E</td>
<td>6</td>
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<tr>
<td>Isoleucine</td>
<td>E</td>
<td>2</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>E</td>
<td>5</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**Non-essential amino acids**
- Animals can synthesize de novo

**Essential amino acids**
- Animals cannot synthesize de novo

**Conditionally essential**

*Note: ATP and NADP values are not shown in the table.*
Why bother?
Bulk tissues got some issues

#1 Applications of trophic discrimination factors involve many assumptions

#2 Shifting and/or overlapping primary producer isotopic baselines
$\Delta^{13} C$ (TDFs) for essential amino acids are negligible
Primary producer $\delta^{13}C$ values can vary widely

Also... one isotope can’t handle so many sources!
Bulk tissue $\delta^{13}$C values can’t help us distinguish between aquatic and terrestrial sources in the MRG.

Besser et al. in review.
Figure 4. Carbon isotope ratios of individual amino acids separated from bone collagen of C_3 and C_4 pigs. (—□—), C_4 pig no. 1; (—▲—), C_3 pig; (—■—), C_4 pig no. 2.

(Received 1 April 1990, revised manuscript accepted 1 September 1990)
Stable Carbon Isotope Analysis of Amino Acid Enantiomers by Conventional Isotope Ratio Mass Spectrometry and Combined Gas Chromatography/Isotope Ratio Mass Spectrometry

J. A. Silfer and M. H. Engel*
School of Geology and Geophysics, The University of Oklahoma, 100 E. Boyd Street, Norman, Oklahoma 73019

S. A. Macko
Department of Environmental Sciences, The University of Virginia, Charlottesville, Virginia 22903

E. J. Jumeau
VG Isotech Limited, Aston Way, Middlewich, Cheshire CW10 0HT, U.K.
Chemical Preparation: Amino Acid Derivatization

Amino Acid Structure

Amine Group

Carboxyl Group

Chemical Preparation: Amino Acid Derivatization
1. **Acid Hydrolysis** → 1–20 mg of tissue are hydrolyzed in 1–1.75 mL of 6 N HCl at 110°C for 20 hours

2. **Esterification of Carboxyl Terminus**
   Carboxyl termini are esterfied by adding 1 mL of a 4:1 solution of isopropanol:acetyl chloride and heating at 110°C for 60 minutes

3. **Trifluoroacetylation of Amine Group**
   Amine groups are trifluoracetylated by adding 1 mL of a 1:1 solution of dichloromethane:trifluoroacetic anhydride (TFAA) and heating at 110°C for 10 minutes
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AA $\delta^{13}$C values are measured via
(Gas Chromatograph–Combustion–Isotope Ratio Mass Spectrometry)
GC–C–IRMS
AA $\delta^{13}$C Chromatographs

\[ \delta^{13}\text{C}_{\text{sample}} = \left[ \delta^{13}\text{C}_{\text{Dsample}} - \delta^{13}\text{C}_{\text{Dstd}} + (\delta^{13}\text{C}_{\text{std}} \times p_{\text{std}}) \right] / p_{\text{std}} \]

Besser et al. *in review*
$AA_{ESS} \delta^{13}C$ fingerprinting
Plants, bacteria, and fungi have distinct $AA_{ESS} \delta^{13}C$ fingerprints
Plants, bacteria, fungi, and algae have distinct $\text{AA}_{\text{ESS}} \delta^{13}\text{C}$ fingerprints
Looks like there may some differences in algal $AA_{ESS} \delta^{13}C$ fingerprints, too.
These first fingerprinting studies were conducted at a global scale.
### AA\textsubscript{ESS} \delta^{13}C: Producers

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Elliott Smith et al. *in review*
### $\text{AA}_{\text{ESS}} \delta^{13}\text{C}$ Fingerprinting: Expanding the Dataset

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Elliot Smith et al. *in review*
\( \text{AA}_{\text{ESS}} \delta^{13}\text{C} \) Fingerprinting: Expanding the Dataset

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### **AA$_{ESS}$ $\delta^{13}$C Fingerprinting: Expanding the Dataset**

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<tr>
<td>Particulate Organic Matter</td>
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</table>
$\delta^{13}C$ values for Ile, Leu, Lys, Phe, Thr, and Val
$\text{A}_{\text{ESS}} \delta^{13}\text{C} \text{ Fingerprinting: Expanding the Dataset}$

Elliott Smith et al. in review
Kelps reclassify as kelps 100% of the time

AA_{E_{ESS}} δ^{13}C Fingerprinting: Expanding the Dataset

Elliott Smith et al. in review
No temporal differences in kelp AA $\delta^{13}C$ fingerprints

Elliott Smith et al. *in review*
Red algae reclassify as red 88% of the time
$\text{AA}_{\text{ESS}} \delta^{13}\text{C Fingerprinting: Expanding the Dataset}$

POM reclassify as POM 71% of the time

Elliott Smith et al. in review
Ulva reclassify as Ulva only 47% of the time.
$\delta^{13}C$ values vary among primary producer groups

- C$_3$ Plants
- C$_4$ Plants
- Aquatic Algae
- CAM Plants
AA\textsubscript{ESS} $\delta^{13}$C fingerprints are unique among terrestrial and aquatic producers

Besser et al. \textit{in review}
AA_{ESS} \delta^{13}C fingerprints are unique among terrestrial and aquatic producers

99% successful reclassification rate

Besser et al. in review
Amino Acid Biosynthetic Families

- **Glucose**
  - *3-Phosphoglycerate*
  - **Phosphoenolpyruvate**
  - **Citric Acid Cycle**
    - **-Ketoglutarate**
    - **Oxaloacetate**
    - **α-Ketoglutarate**
    - **Glutamic Acid**
    - **Proline**

**Glycolysis**
- **Pyruvate Oxidation**
  - **Pyruvate**
  - **Acetyl-CoA**

**Initial product of CO₂ fixation in some C₄ plants**

**Involved in photorespiration**

**Precursor to many secondary metabolites in plants (e.g., lignin)**

**Heavily involved in central N metabolism**

- Glycine
- Serine
- Aspartic Acid
  - *Threonine*
  - *Isoleucine*
  - *Lysine*
- *Phenylalanine*
- *Leucine*
- *Valine*
- *Isoleucine*
Amino Acid Biosynthetic Families

**Pyruvate Family**
- Alanine
- Valine
- Leucine

**3-Phosphoglycerate Family**
- Serine
- Glycine
- Cysteine

**Phosphoenolpyruvate and Erythrose-4-P Family**
- Phenylalanine
- Tyrosine
- Typtophan

**α-Ketoglutarate Family**
- Glutamate
- Glutamine
- Proline
- Arginine
- Lysine (in fungi and protists)

**Oxalacetate Family**
- Aspartate
- Asparagine
- Methionine
- Threonine
- Isoleucine
- Lysine

**5-phosphoribosyl-1-pyrophosphate and ATP**
- Histidine
Isotopic discrimination ($\Delta^{13}C$) during de novo AA$_{ESS}$ synthesis varies among producer groups

Besser et al. in review