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Introduction

Marine Organic Matter

- The ocean's biological pump sequesters around 10Gt of carbon per year (roughly one third of anthropogenic emissions).
- The pump operates by exporting particulate organic matter (POM) via three pathways:

Passive sinking Transport by animals Physical mixing

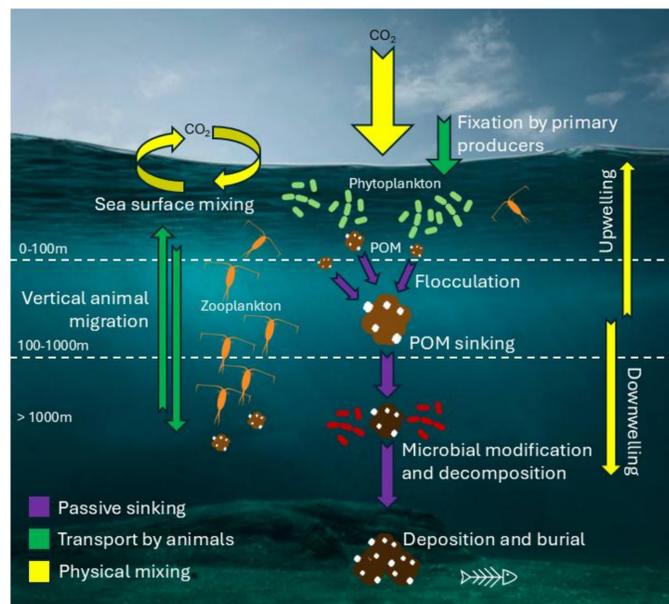
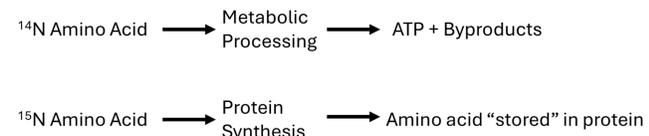


Figure 1 Diagram of the biological carbon pump showing the three main pathways for the export of POM to the deep ocean.

Microbes and POM

- Microbes modify and degrade POM as it sinks and after it has been deposited on the seafloor.
- Microbial degradation of POM produces dissolved organic matter (DOM)
 - DOM is a massive store of carbon in the ocean ~700gt
 - DOM persists in the environment for a LONG time ~6000yrs
- Microbes intake nitrogen and/or amino acids from POM to build protein.
 - Intake inorganic nitrogen to make amino acids (*de novo* synthesis)
 - Intake performed amino acids (Resynthesis)
- The biochemical pathways that microbes use to acquire nitrogen/amino acids from POM leave isotopic signatures in the POM and in the microbes themselves through a process called isotopic fractionation.



Hypothesis

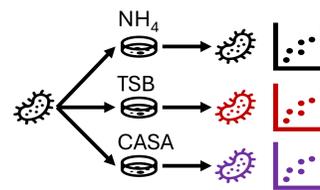
$\delta^{15}\text{N}_{\text{AA}}$ fractionation will systematically differ among *de novo* synthesis, resynthesis of preformed amino acids in peptides, and resynthesis of free amino acids.

Methods

Bacterial Feeding Trial and CSIA-AA

- Cultured eight species of heterotrophic bacteria on three sources of nitrogen
- Used compound-specific isotope analysis of amino acids (CSIA-AA) to measure the amount of ^{15}N relative to air in each amino acid in each species of bacteria and the substrate on which they were grown.

ID	Bacteria	Nitrogen Source	Abreviations
B1	<i>Kocuria rhizophila</i>	Ammonium Sulfate	NH ₄
B2	<i>Bacillus subtilis</i>	Tryptic Soy Broth	TSB
B3	<i>Bacillus licheniformes</i>	Casamino Acids	CASA
B4	<i>Sporosarcina ureae</i>		
B5	<i>Staphylococcus epidermidis</i>		
B6	<i>Stenotrophomas sp.</i>		
B7	<i>Escherichia coli</i>		
B8	<i>Pseudomonas fluorescens</i>		



Results and Discussion

Amino Acid Fractionation Patterns

- The $\delta^{15}\text{N}_{\text{AA}}$ patterns in heterotrophic bacteria performing *de novo* synthesis resembled the pattern found in phytoplankton (Figure 2a).
- The $\delta^{15}\text{N}_{\text{AA}}$ patterns in heterotrophic bacteria performing resynthesis resembled the pattern found in animals (Figure 2b and c).

Significant Differences in Patterns

- The mean Casamino Acid cultures, when normalized to glutamic acid, showed an enrichment in $\delta^{15}\text{N}$ relative to the NH₄⁺ cultures in:
 - Leucine
 - Isoleucine
 - Phenylalanine
 - Valine
- The mean NH₄⁺ cultures, when normalized to the substrate, showed depletion in $\delta^{15}\text{N}$ relative to the other cultures in:
 - Asparagine
 - Alanine
 - Leucine
 - Lysine
 - Serine

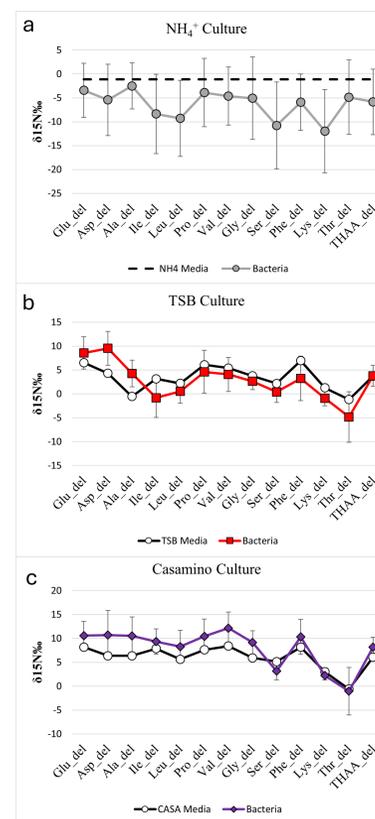


Figure 2 Mean $\delta^{15}\text{N}_{\text{AA}}$ patterns of bacteria grown on three different nitrogen sources.

Results and Discussion

PCA

- $\delta^{15}\text{N}_{\text{AA}}$ patterns formed three distinct clusters (Figure 3):
 - De novo* synthesis
 - Resynthesis
 - Bacillus subtilis*

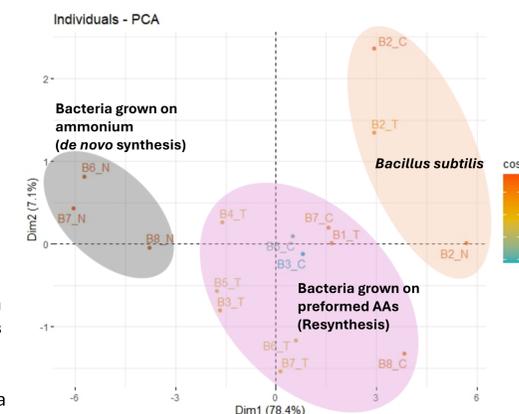


Figure 3 Principal component analysis of $\delta^{15}\text{N}_{\text{AA}}$ of all bacteria species normalized to the $\delta^{15}\text{N}_{\text{AA}}$ of the substrate in all three treatments, where BX_N is bacteria grown in the ammonium medium, BX_T is bacteria grown in the TSB medium, and BX_C is bacteria grown in the casamino acid medium.

Why is *Bacillus subtilis* different?

- B. subtilis* form a biofilm of extracellular polymers containing protein.
- The $\delta^{15}\text{N}_{\text{AA}}$ in the bacteria AND the extracellular polymers were measured together.

NEW Heterotrophic Bacteria End-member

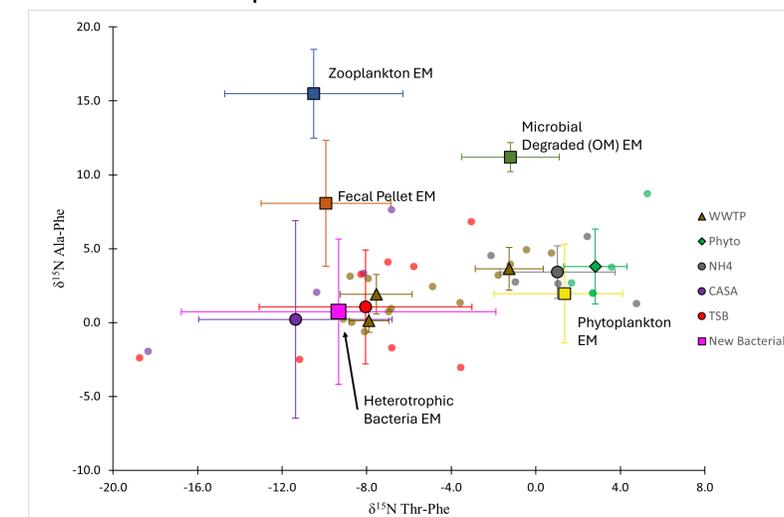


Figure 4 Doherty plot of the bacteria from this study, POM samples from three waste-water treatment plants in the Halifax Regional Municipality (Noddle et al. 2023), phytoplankton from the Dalhousie Aquatron facility, Doherty end-members (Doherty et al. 2022), and the proposed heterotrophic bacterial end-member. Individual data points are represented by small borderless circles, means of each group are represented by larger bordered shapes with error bars showing the standard deviation.

Conclusions

- $\delta^{15}\text{N}_{\text{AA}}$ patterns differ between bacteria performing *de novo* AA synthesis and those performing AA resynthesis.
- $\delta^{15}\text{N}_{\text{AA}}$ patterns do not differ between bacteria that intake free amino acids and those that intake amino acids from peptides.
- Identified a new heterotrophic bacteria end-member to be used in marine degraded organic matter and sediment mixing models and analysis.

Acknowledgements

I would like to thank my supervisor, Dr. Owen Sherwood, for all his support and guidance throughout my research, Claire Normandeau for bulk stable isotope analysis, and Bev Rogerson and Hongxia Liu for their assistance in bacterial culturing. Funding was provided through the NSERC Faye Sobey Undergraduate Research award to OAS.