The role of substrate identity on microbial processing of low molecular weight organics in soil solution

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Abstract

Microbial processing of fresh carbon inputs is recognized as a key step in the formation of mineral-associated organic matter. Low molecular weight (LMW) compounds comprise a notable fraction of these inputs and are rapidly assimilated and metabolized by the microbial community. In this work, we employ ecophysiological studies of microbial isolates to better understand the role of substrate identity as a control on preferences, uptake kinetics, and carbon use efficiencies (CUE) across a gradient of phylogenetic differences (gram negative, gram positive, and fungal). Soil-extracted, solubilized organic matter (SESOM) derived from the Oa horizon of a hemlock-hardwood forest stand and synthetic media based off of this extract were chosen as liquid media for batch growth studies. A combination of exometabolomic techniques (1H NMR, UHPLC-MS) were used to quantify 35 LMW substrates in the original extract (0.4 – 195 µM), comprising 19.5% of total C and 39.9% of total N. Consumption of these substrates by microbial isolates accounted for a substantial amount of total C and N assimilated during growth, representing 43-75% and 58-74%, respectively. Time resolved sampling allowed modeling of sigmoidal uptake curves and the comparison of the midpoint of consumption (Th, hr) and 90% usage windows (ranging from 0.18 – 2.29 hr). Complementary experiments were conducted using synthetic media with all substrates at equimolar concentrations (25 µM) to better constrain the impact of initial concentration. We use stable isotope probing to determine CUE for five different LMW substrates of interest (glucose, acetate, formate, glycine, and valine). Ultimately, we are interested in whether unifying trends can be observed across the physiological gradient and how the metabolic transformations of these inputs may impact the organo-mineral formation process.

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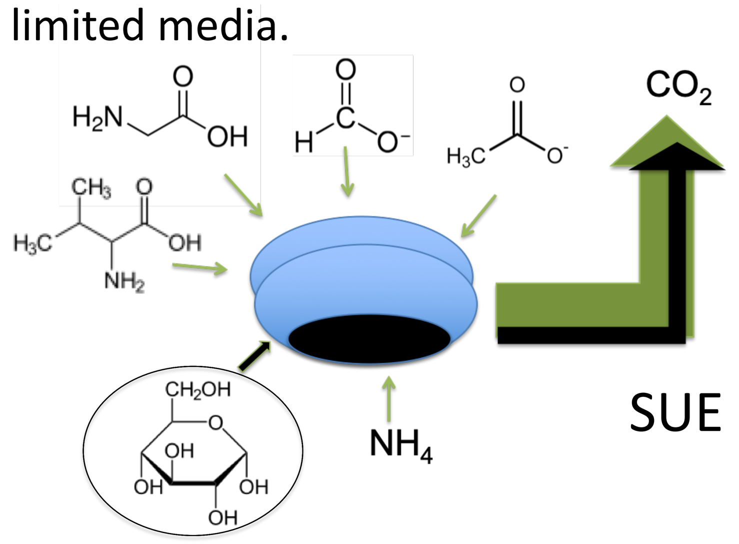
AGU American Geophysical Union

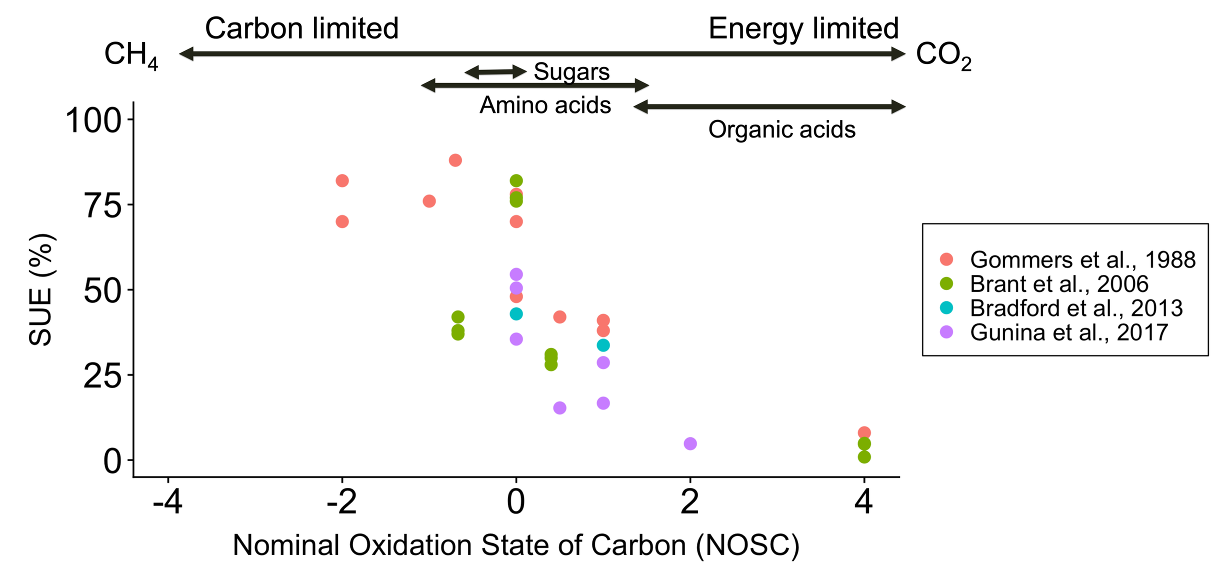
Introduction

Microbial processing of fresh carbon inputs is recognized as a key step in the formation of mineral-associated organic matter. Low molecular weight compounds comprise a notable fraction of these inputs and are rapidly assimilated and metabolized by the microbial community. In this work, we employ ecophysiological studies of individual microbial strains to better understand if inherent energy content (denoted as nominal oxidation state of carbon – NOSC) is a useful predictor of uptake preference, rate, and substrate use efficiency (SUE) across a gradient of phylogenetic differences. All strains were isolated on soil extract derived from a hemlock-hardwood Oa horizon before comparison in defined, C-limited media.



$$CUE = (MB)/(MB + R_T)$$

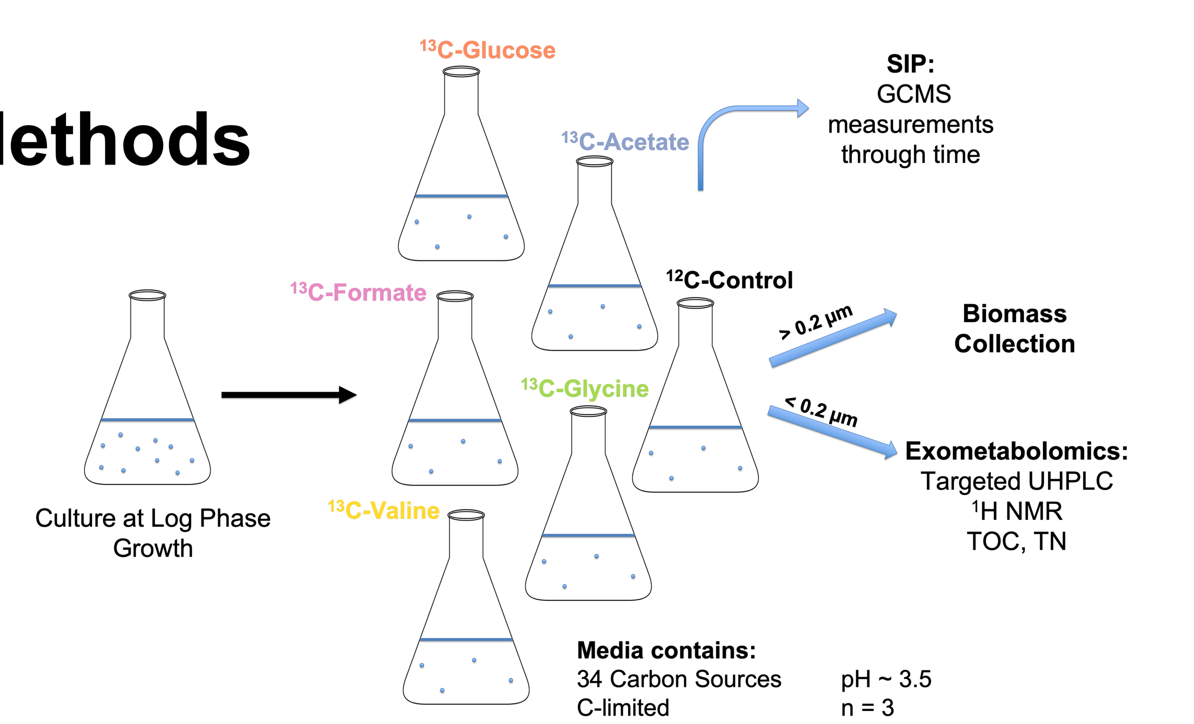
$$SUE = (^{13}C\text{-}MB)/(^{13}C\text{-}MB + {}^{13}C\text{-}CO_2)$$



Objectives

-Understand isolate substrate preferences and uptake kinetics
-Measure overall carbon use efficiency (CUE) of each isolate
-Test the SUE of 5 different compounds (Glc - glucose, Ace - acetate, Form - formate, Gly - glycine, Val - valine) ranging in NOSC (-0.8 – 2)

Methods



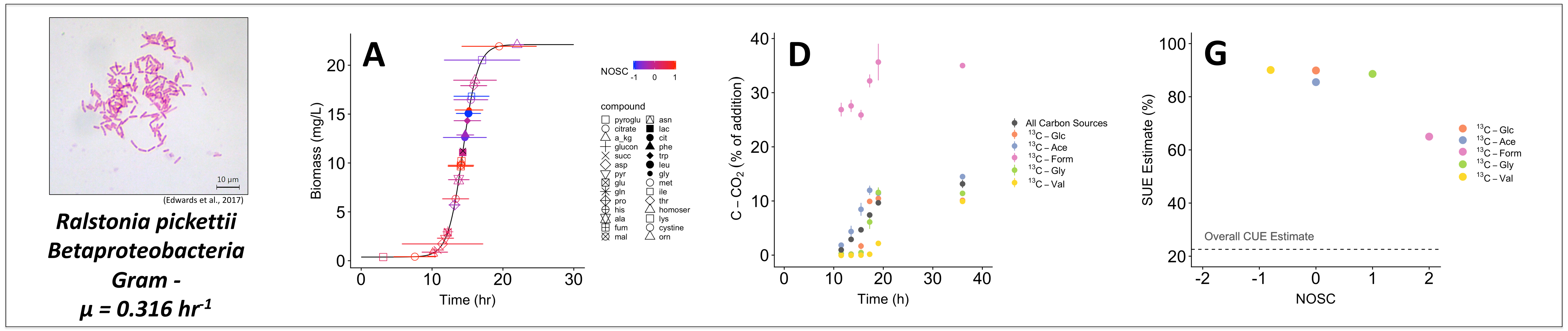
Results

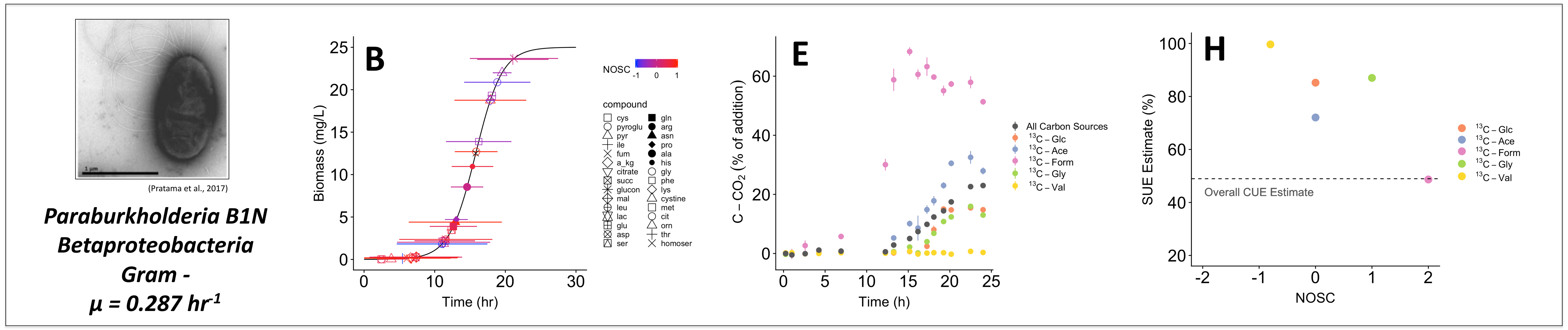
Behrends Plots (A-C). Substrate uptake overlain on each isolate's growth curve. Bars represent 90% usage windows centered around the midpoint of consumption. Compounds in legend are colored by NOSC and displayed in order of uptake.

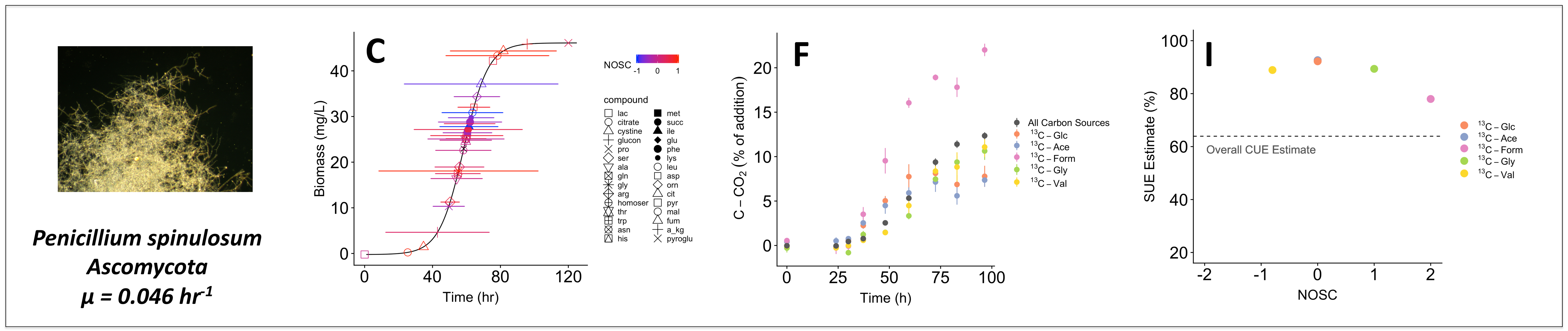
Respiration time-series plots (D-F) of all isotopically labeled substrate additions as well as total respiration. Isotopic values are control subtracted and and all points are displayed in terms of the amount of C added from each source. Bars represent standard error (n = 3).

Substrate Use Efficiency (SUE) estimates (G-I) of labeled substrates at stationary phase. All estimates are made assuming 100% assimilation and therefore are upper estimates. These will be lowered using biomass label data when available.

Decreasing Growth Rate







Conclusions

-Overall CUE increases with decreasing growth rate (G-I).
-There were no readily apparent trends between uptake preference and substrate NOSC (A-C). *Ralstonia pickettii* most aligned with hypothesis that oxidized compounds (higher NOSC) are preferentially used first (A).
-Bacterial isolates respired more oxidized (higher NOSC) SIP substrates earlier and faster (D,E), though this was not observed in *Penicillium spinulosum* (F).
-Trends in SUE as a function of NOSC seem to be more pronounced in faster growing bacterial isolates (G, H).

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