



Supplement of

Brief communication: Stream microbes preferentially respire young carbon within the ancient glacier dissolved organic carbon pool

Amy D. Holt et al.

Correspondence to: Amy D. Holt (adh19d@fsu.edu)

The copyright of individual parts of the supplement might differ from the article licence.

Sect. S1 Description of C₃ and C₄ Vegetation

Plants can be divided into two main groups based on photosynthetic pathway (e.g., Hobbie and Werner, 2003; Langdale., 2011 and references there in). The C₃ photosynthetic pathway produces a 3-carbon acid as a result of carbon fixation using the Calvin cycle, whereas C₄ plants produce a 4-carbon containing compound following the Hatch-Slack pathway (Hobbie and Werner., 2003). C₃ plants make up the majority (~95% of biomass) of the world's plants and comprise trees, shrubs, and flowering plants. C₃ plants are typically found in moist, temperate to cold climates (Still et al., 2003). C₄ plants (~5% of biomass) are typically found in hot, dry regions and include most tropical species of grass and agricultural crops like maize and sugar cane (Still et al., 2003). The C₄ photosynthetic pathway is a more recent (~30 Mya) adaptation to conserving water and reducing photorespiration in hot, arid climates (Langdale., 2011). Differences in photorespiration pathways between C₃ and C₄ plants result in variances in isotopic fractionation (i.e., differences in ¹³C:¹²C ratios), meaning that the contribution of carbon from the two plant groups can be distinguished in environmental samples, including of dissolved organic carbon (e.g., Khon., 2010; Drake et al., 2020). C₄ plants (δ¹³C -14 to -10 ‰; Cerling et al., 1993, 1997) are enriched in ¹³C in comparison to C₃ plants (δ¹³C -37 to -20 ‰; Khon., 2010).

Table S1. Number of formulae and relative abundance (RA) weighted mass, H/C and O/C ratio, nominal oxidation state of carbon (NOSC; Riedel et al., 2012), and modified aromaticity index (AI_{mod}; Koch and Dittmar 2016), together with the percent RA of each compound and heteroatom class

	Eagle Glacier	Herbert Glacier	Mendenhall Glacier	Mendenhall Supraglacial
Formulae (#)	15,537	18,793	17,709	16,077
Mass (Da)	495.1	544.4	510.2	493.9
H/C	1.27	1.27	1.26	1.32
O/C	0.46	0.48	0.48	0.45
NOSC	-0.3	-0.27	-0.25	-0.37
AI _{mod}	0.24	0.22	0.23	0.21
Highly Unsaturated and				
Phenolic (% RA)	68.7	73.0	70.6	58.4
Aliphatics (% RA)	22.6	21.0	21.5	34.2
Polyphenolic (% RA)	7.0	5.3	6.6	5.9
Condensed Aromatic (%RA)	1.7	0.6	1.1	1.4
CHO (% RA)	79.5	75.5	75.9	74.6
CHON (% RA)	16.7	19.2	18.6	15.4
CHOS (% RA)	3.8	5.3	5.4	9
CHONS (% RA)	0	0	0.1	1

Table S2: Quantities of CO₂ produced during respiratory carbon recovery bioincubations

	Eagle Glacier Outflow	Herbert Glacier Outflow	Mendenhall Glacier Outflow	Mendenhall Supraglacial Stream
CO ₂ (mg C)	0.3	0.3	0.3	0.2

References

- Cerling, T. E., Wang, Y., and Quade, J.: Expansion of C4 ecosystems as an indicator of global ecological change in the late Miocene, *Nature*, 361, 344-345, 1993.
- Cerling, T. E., Harris, J. M., MacFadden, B. J., Leakey, M. G., Quade, J., Eisenmann, V., and Ehleringer, J. R.: Global vegetation change through the Miocene/Pliocene boundary, *Nature*, 389, 153-158, 1997.
- Drake, T. W., Wagner, S., Stubbins, A., Wabakanghanzi, J. N., Dinga, J. B., Six, J., and Spencer, R. G.: Du Feu à l'Eau: Source and Flux of Dissolved Black Carbon From the Congo River, *Global Biogeochemical Cycles*, 34, e2020GB006560, 2020.
- Hobbie, E. and Werner, R. A.: Intramolecular, compound-specific, and bulk carbon isotope patterns in C3 and C4 plants: a review and synthesis, *New Phytologist*, 161, 371-385, 2004.
- Koch, B. and Dittmar, T.: From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter, *Rapid Commun. Mass Sp.*, 30, 250–250, 2016.
- Langdale, J. A.: C4 cycles: past, present, and future research on C4 photosynthesis, *The Plant Cell*, 23, 3879-3892, 2011.
- Riedel, T., Biester, H., and Dittmar, T.: Molecular fractionation of dissolved organic matter with metal salts, *Environmental Science & Technology*, 46, 4419-4426, 2012.
- Still, C. J., Berry, J. A., Collatz, G. J., and DeFries, R. S.: Global distribution of C3 and C4 vegetation: carbon cycle implications, *Global biogeochemical cycles*, 17, 6-1-6-14, 2003