Reviews and syntheses: Heterotrophic fixation of inorganic carbon – significant but invisible flux in environmental carbon cycling

Supplementary Information

Alexander Braun[1], Marina Spona-Friedl[1], Maria Avramov[1], Martin Elsner[1,2], Federico Baltar[3], Thomas Reinthaler[3], Gerhard J. Herndl[3,4] & Christian Griebler[1,3]*

[1] Helmholtz Zentrum München, Institute of Groundwater Ecology, Ingolstaedter Landstrasse 1, D-85764 Neuherberg, Germany

[2] Technical University of Munich, Department of Analytical Chemistry and Water Chemistry, Munich, Germany

[3] University of Vienna, Department of Functional and Evolutionary Ecology, Althanstrasse 14, 1090 Vienna, Austria

[4] Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research, Utrecht University, PO Box 59, 1790 AB Den Burg, The Netherlands

* Author for correspondence: christian.griebler@univie.ac.at

Table SI-1: Global standing stock of organic carbon in living biomass and contribution from anaplerotic CO_2 fixation (only anaplerosis is considered here; other mechanisms of heterotrophic CO_2 fixation were neglected). In heterotrophs, a conservative estimate of 1-5% of the cell carbon is assumed to originate from inorganic carbon fixation (see references in text).

Continental habitats	**Carbon biomass [Pg C]**	**Carbon in biomass derived from anaplerotic CO_2 fixation [Pg C]**	**References for carbon biomass**
Terrestrial animals	0.6	0.006 – 0.03	(Bar-On et al. 2018)
Soil fungi	12	0.12 – 0.6	(Bar-On et al. 2018)
Terrestrial protists	1.6	0.016 – 0.8	(Bar-On et al. 2018)
Soil prokaryotes (upper 100 cm of soil)	23.2	0.23 – 1.16	(Xu, Thornton and Post 2013)
Continental subsurface prokaryotes	2.4 – 12.6*	0.024 – 0.63	(Magnabosco et al. 2018)
Heterotrophic prokaryotes in freshwater and saline inland surface waters	0.013**	0.00013 – 0.00065	(Whitman et al. 1998)
Marine and oceanic habitats			
Marine Animals	2	0.02 – 0.1	(Bar-On et al. 2018)
Marine protists	2	0.02 – 0.1	(Bar-On et al. 2018)
Marine fungi	0.3	0.003 – 0.015	(Bar-On et al. 2018)
Marine planktonic heterotrophic prokaryotes	1.4 – 3.5***	0.014 – 0.175	(Whitman et al. 1998)
Subseafloor sedimentary prokaryotes	1.5 – 22	0.015 – 1.1	(Kallmeyer et al. 2012, Schippers et al. 2005)
Prokaryotes of the oceanic crust	0.5 – 5	0.005 – 0.25	(Bar-On et al. 2018)
Total heterotrophic carbon biomass	**47 – 85**	**0.47 – 4.96**	

* Cell abundances (2 – 6 x 10^{29} cells) from Magnabosco et al. (2018) were converted into cell carbon using the carbon conversion factors 12 fg C $cell^{-1}$ and 21 fg C $cell^{-1}$ (Wilhartitz et al. 2009, Griebler et al. 2002) for the minimum and maximum values of the range, respectively. In favor of a conservative estimate, quite low carbon conversion factors were used (at the lower end of the carbon content values for freshwater prokaryotic cells reported in literature).

** Cell abundance (2.3 x 10^{26} cells) from Whitman et al. (1998) were converted into cell carbon using a carbon conversion factor of 57 fg C $cell^{-1}$, which is the arithmetic mean of the minimum and maximum of a range of values (6 to 107 fg C $cell^{-1}$) reported for freshwater lakes and rivers of different trophic states in literature (Pedrós-Alió and Brock 1982, Bjørnsen 1986, Simon 1987, Lever et al. 2015).

*** Cell abundances were converted into cell carbon using the carbon conversion factors 12 fg C $cell^{-1}$ and 30 fg C $cell^{-1}$ (Fukuda et al. 1998) for the minimum and maximum values, respectively.

Table SI-2: Annual global heterotrophic carbon biomass production and contribution from heterotrophic CO_2 fixation (via anaplerosis).

	Annual heterotrophic C-biomass production [Pg C yr^{-1}]	**Anaplerotically fixed carbon [Pg C yr^{-1}] §**	
Marine and oceanic habitats			
Marine and freshwater	2.4 – 76 *	0.024 – 3.8	(Cole, Findlay and Pace 1988, del Giorgio and Duarte 2002)
Oceanic subseafloor	0.1 – 9.8 **	0.001 – 0.49	(Schippers et al. 2005)
Continental habitats			
Aquifers and unsaturated subsurface	0.12 – 26.3 †	0.0012 – 1.315	(Magnabosco et al. 2018, Griebler et al. 2014)
Soils	31.3 – 133.2 ††	0.313 – 6.66	(Prentice et al. 2001, Manzoni et al. 2012, Hashimoto et al. 2015, Potter and Klooster 1998)
Total heterotrophic C-biomass production	**34 – 245**	**0.34 – 12.3**	

* Bacterial carbon production (BCP) rates from 54 marine and freshwater studies (Cole et al. 1988) were converted from [mg C m^{-2}d^{-1}] into [Pg C yr^{-1}] and extrapolated to global scale using a world water surface area of 361,419,000 km^2 (http://www.worldatlas.com/aatlas/infopage/oceans.htm).

** The total number of living cells [1.3 x 10^{29}] was divided by the turnover time of subseafloor bacteria [0.25-22 yrs], multiplied by the mean carbon content per cell [19 fg C], and converted from [fg C] to [Pg C]. All data as given in Schippers et al. (2005).

† The range of bacterial carbon production rates [fg C L^{-1} yr^{-1}] from 14 groundwater wells (sampled in spring and autumn) located in an oligotrophic porous aquifer in the Bavarian Alps (close to Mittenwald in Southern Germany) was divided by the corresponding bacterial abundance [cells L^{-1}] to obtain BCP rates per cell (data from Griebler et al. 2014). The minimum and the maximum values of these cell-specific BCP rates were then multiplied by the minimum and the maximum estimated total number of prokaryotes in the continental subsurface [2-6 x 10^{29} cells] from Magnabosco et al. (2018), respectively, and carbon mass units were converted from [fg] to [Pg]. Note: since comprehensive, global data on microbial carbon production in aquifers are currently still missing, the level of uncertainty of this estimate is high. Therefore, in order to avoid overestimation, and in favor of obtaining a most conservative estimate, we selected out of the available data only those production rates, which were determined in pristine, highly oligotrophic environments. If all other data from the dataset in Griebler et al. (2014), in total 88 wells throughout Germany, sampled twice, as well as the data from four other available studies with sites in the USA, Austria and Denmark (Thorn and Ventullo 1988, Kazumi and Capone 1994, Albrechtsen and Winding 1992, Wilhartitz et al. 2009) were to be included, a much higher estimate of the global annual heterotrophic carbon biomass production in aquifers would be obtained, ranging from 0.06 to 4,829 Pg C yr^{-1}, and corresponding to 0.001 – 386 Pg C yr^{-1} of anaplerotically fixed carbon each year.

†† Global terrestrial heterotrophic respiration in soils [55 Pg C yr^{-1}] from Prentice et al. (2001) was extrapolated to carbon biomass production assuming that respiration accounts for 30-62% of the total carbon consumed

(corresponding to a carbon use efficiency (CUE) of 38-70%) in the course of organic matter decomposition in different types of soils (Manzoni et al. 2012).

[§] It was assumed that 1-5% of the annually produced carbon biomass of heterotrophs originate from anaplerotic CO_2 fixation (see ref. in the text of the main MS). A fraction of 1% was applied to the minimum, and 5% to the maximum value of the C-biomass production ranges in this table, respectively.

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