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

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Abstract

Heterotrophic CO_2 fixation is a significant, yet underappreciated CO_2 flux in the global carbon cycle. In contrast to photosynthesis and chemolithoautotrophy – the main recognized autotrophic CO_2 fixation pathways - the importance of heterotrophic CO_2 fixation remains enigmatic. All heterotrophs – from microorganisms to humans – take up CO_2 and incorporate it into their biomass. Depending on the available growth substrates, heterotrophic CO_2 fixation contributes at least 2-8% and in the case of methanotrophs up to 50% of the carbon building up their biomass. Assuming a standing stock of global heterotrophic biomass of 47-85 Pg C, we estimate that up to 7 Pg C have been derived from heterotrophic CO_2 fixation and up to 20 Pg C yr^{-1} originating from heterotrophic CO_2 fixation are funneled into the global annual heterotrophic production of 34-245 Pg C yr^{-1}. These first estimates on the importance of heterotrophic fixation of inorganic carbon indicate that this carbon fixation pathway should be included in present and future global carbon budgets.

Key words: CO_2 fixation, heterotrophs, anaplerosis, carbon cycling

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1. Introduction

Fixation of CO_2 is a fundamental biosynthetic process in nature (Beer et al. 2010, Berg et al. 2007) providing the main source of metabolic energy on Earth (Giovannoni and Stingl 2005). At the same time, it acts as a sink for atmospheric CO_2, the most important greenhouse gas, which is responsible for more than 60% of the 'enhanced greenhouse effect' resulting in global warming (Beer et al. 2010, Berg 2011, Houghton 2007, Le Quéré et al. 2016).

While photosynthesis and chemosynthesis are the most important processes of carbon fixation, non-autotrophic carbon fixation, i.e., the carbon fixation mediated by heterotrophic organisms might also be relevant albeit not yet quantified. While heterotrophs are, per definition, organisms that respire organic compounds to gain energy and build up biomass, CO_2 fixation plays also an essential role in heterotrophic metabolism. The diversity of carboxylating enzymes in nature reaches far beyond autotrophy and virtually all heterotrophs harbor numerous enzymes fixing dissolved inorganic carbon. Even though the first carboxylase in heterotrophs was discovered already more than 80 years ago (Wood and Werkman 1936), the role of heterotrophs in carbon cycling has so far largely focused on the oxidation of organic substrate using oxygen or alternatives (e.g. nitrate, ferric iron, sulfate) as electron acceptor and the production of CO_2. Similar to the CO_2 fixation by autotrophs, "heterotrophic CO_2 fixation" might, however, constitutes a significant carbon flux in specific habitats and most likely in the global carbon cycle. The relevance of this process has not been quantified yet due to the lack of reliable estimates of heterotrophic CO_2 fixation for most organisms and the presumption that CO_2 fixation in natural environments is restricted to autotrophic organisms.

To fill this gap, we review the current knowledge on (i) the significance of heterotrophic CO_2 fixation for cellular metabolism, (ii) the CO_2 fixation in habitats dominated by heterotrophs, and (iii) merge both to estimate the contribution of heterotrophically fixed carbon on the global biomass and the annual heterotrophic CO_2 fixation rates.

2. Significance of heterotrophic CO_2 fixation for cellular metabolism

Currently, more than twenty carboxylases are known forming an integral part of the central and peripheral metabolic pathways of heterotrophic metabolism (Fig. 2), e.g., in the synthesis of fatty acids, biotin and purine, the assimilation of leucine, and in anaplerosis (Erb 2011, Sauer and Eikmanns 2005). Carboxylation in heterotrophs does not compensate for the dependence on organic matter, rather CO_2 fulfills the role of a "co-substrate" providing an effective and simple way to extend an existing organic carbon substrate by a single C1 unit as part of the secondary production (Erb 2011).

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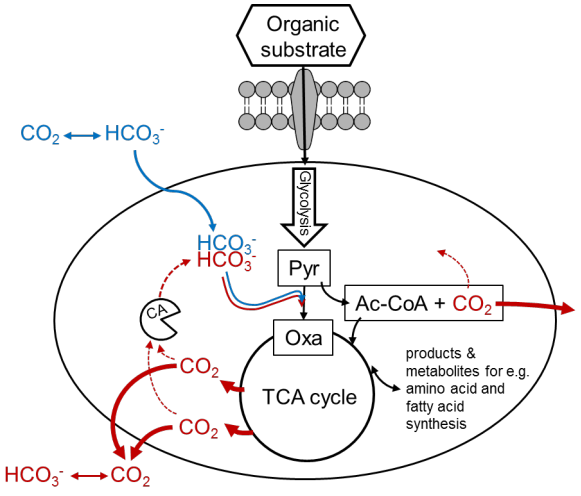
69 The most important CO_2 fixation pathway in all organisms is anaplerosis. The replenishment
70 of metabolites continuously withdrawn from the citric acid (TCA) cycle via the anaplerotic
71 reaction of pyruvate carboxylase entails an assimilation of CO_2 corresponding to 25% of the
72 initial substrate's carbon content. Moreover, TCA metabolites are used as building blocks for
73 macromolecular compounds, e.g. almost half of all amino acids in prokaryotes are directly
74 synthesized from oxaloacetate and α-ketoglutarate (Fuchs 1999). In mammals, 4 and 10% of
75 the carbon in proteins and carbohydrates, respectively, originate from heterotrophic CO_2
76 fixation (Kleiber, Smith and Black 1952).

77



78

79 **Figure 1:** Flow of organic and inorganic carbon in a heterotrophic prokaryotic cell with focus on organic carbon
80 oxidation (e.g. glucose) and anaplerotic fixation of inorganic carbon. Intracellular inorganic carbon produced,
81 released or re-fixed is marked in red. Extracellular inorganic carbon taken up by the cell for anaplerotic fixation
82 is marked in blue. Arrow size points at the relative contribution to fluxes. Ac-CoA = acetyl coenzyme-A, CA =
83 carbonic anhydrase, HCO3- = bicarbonate, Oxa = oxaloacetate, Pyr = pyruvate, TCA cycle = tricarboxylic acid
84 cycle.

85 Heterotrophic CO_2 fixation via anaplerosis in prokaryotes generally contributes around 1 to
86 8% to the carbon biomass (Romanenko 1964, Doronina and Trotsenko 1984, Roslev et al.
87 2004, Hesselsoe et al. 2005). The advantage that CO_2 is readily available to the cell either as
88 atmospheric gas or, more commonly, in its hydrated form HCO_3^-, obviously outcompetes the
89 disadvantage that carboxylation is generally an endergonic reaction (Faber, Fessner and
90 Turner 2015). This thermodynamic obstacle may be less important when carboxylation
91 supports the assimilation of organic substrates that are more reduced than the organism's
92 biomass, resulting in carbon-limited but excess-energy conditions (Battley 2013, von Stockar
93 et al. 2006). In this case, in addition to anaplerosis further carboxylation reactions are
94 induced (Fig. 2) to add oxidized C (from CO_2) to the reduced organic substrate for adjusting

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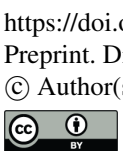

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the degree of reduction to that of the biomass (Fig. 3). For example, the assimilation of leucine and propionate into biomass entails carboxylation of the initial C-6 and C-3 carbon bodies, respectively, and thus triggers an assimilation of inorganic carbon that corresponds to 17% and 33% of the initial substrate's carbon content, respectively (Erb 2011). In aerobic methane oxidation, the full oxidation potential of one molecule of CO_2 is needed to adjust the high degree of reduction of methane to that of biomass during its assimilation. Consequently, methanotrophs derive up to 50% of their carbon biomass from CO_2 (Strong, Xie and Clarke 2015, Battley 2013). These figures highlight the fundamental role of CO_2 fixation in heterotrophs in specific habitats.

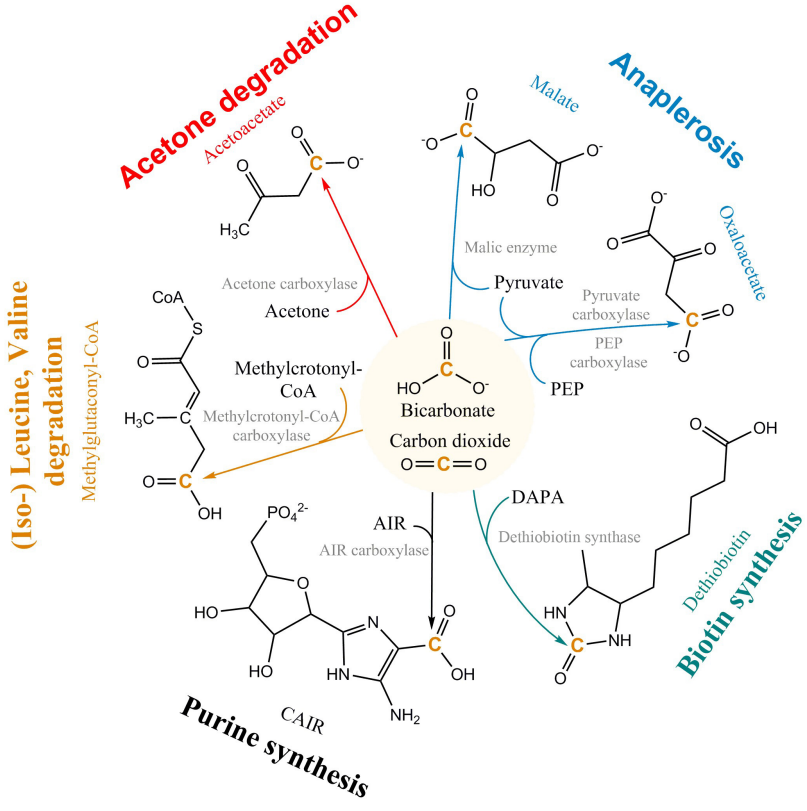

Figure 2: Selected heterotrophic CO_2 fixation reactions and pathways. PEP: phosphoenolpyruvate, DAPA: 7,8-diaminononanoate, AIR: 1-(5'-phosphoribosyl)-5-aminoimidazole, CAIR: 1-(5-phospho-D-ribosyl)-5-amino-4-imidazolecarboxylate, CoA: Coenzyme-A.

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The heterotrophic fixation of CO_2 is generally considered a back-reaction, i.e., part of the originally produced CO_2 from respiration is re-assimilated. Consequently, heterotrophic fixation of inorganic carbon does not necessarily lead to a net carbon biomass production. However, if microbes oxidize geogenic methane new biomass is generated.

Growth stimulation has been found in heterotrophic marine bacteria harboring proteorhodopsin, linked to an overexpression of the glyoxylate shunt genes and CO_2 fixation (Palovaara et al., 2014). In stable isotope labelling experiments with *Bacillus subtilis*, a gram-positive heterotrophic bacterium widespread in the environment, the interdependency of pathways and rates of CO_2-fixation on the concurrent utilization of organic substrate(s) was explored. Over the course of the experiments *B. subtilis* assimilated up to 6% of biomass carbon from the external $H^{13}CO_3$ pool when growing on glucose (Spona-Friedl et al. 2020). Growth on lactate and malate revealed a contribution to biomass production from inorganic carbon of 3% and 2%, respectively. Heterotrophic CO_2-fixation took place even in the absence of cell growth during the stationary phase.

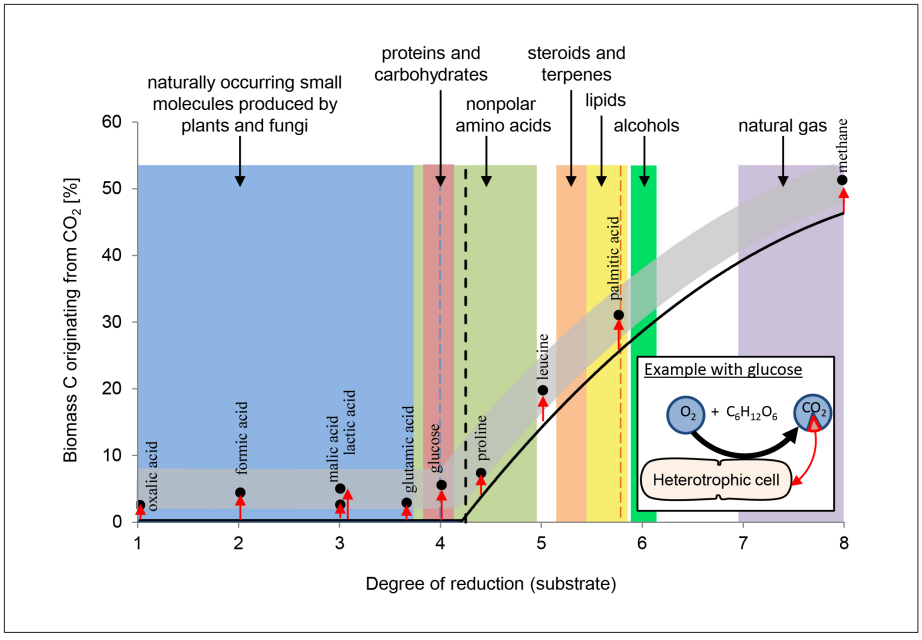

Figure 3: Aerobic heterotrophs gain energy from the oxidation of reduced organic compounds. As a result, oxygen is consumed and CO_2 is released into the environment. Not all of the CO_2 is released – part of it is also recycled in the cell and used in anaplerosis, as well as in other heterotrophic CO_2 fixation pathways. The amount of CO_2 that is fixed into biomass varies, depending on the degree of reduction of the utilized substrate (see explanation in text). Anaplerosis makes up for 1-8% of the biomass carbon (grey shaded area). Red arrows indicate the contribution of carbon from CO_2 fixation to be expected when growing on the individual substrate. The dashed line depicts the degree of reduction of the cell's biomass (for further explanation see text).

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In an ecological context, the amount of inorganic carbon fixed by heterotrophs, either from an endogenous or exogenous source, is directly related to their biomass production and respiration (Spona-Friedl et al. 2020). The ratio between carbon biomass production and the total organic carbon assimilated (commonly estimated as the sum of C-biomass production and the amount of C respired) represents the carbon use efficiency or also coined growth efficiency of heterotrophic organisms. Generally, the more reduced an organic substrate is, the less CO_2 is released (Fig. 3). Respiration in aquatic systems is frequently determined via the consumption of dissolved oxygen (Robinson and Williams 2005) and potentially underestimates the carbon use efficiency of heterotrophs. Depending on the substrate, the respiration quotient ($\Delta CO_2/-\Delta O_2$) varies between 0.7 – 1.3 (Robinson 2019) leading to an error between 20 and 40% with regard to CO_2 production from respiration. Moreover, the respiration ratio also varies because of other oxygen consuming processes potentially taking place simultaneously (e.g. nitrification) (Robinson 2019). For instance, it is 138 O_2 for 106 CO_2 for ideal Redfield type organic matter, and 150 O_2 for 106 CO_2 for more realistic marine organic matter (Fraga et al. 1998; Paulmier et al. 2009). Collectively, with respect to C cycling, heterotrophic CO_2 fixation and the carbon flux from the inorganic pool into heterotrophic biomass can be regarded as a process more important than hitherto assumed.

3. CO_2 fixation in habitats dominated by heterotrophs

In contrast to sunlit habitats, where autotrophs make up a significant portion of the total biomass and photosynthesis is of major importance in carbon cycling, heterotrophs (and chemolithoautotrophs) represent the only biota in the “dark habitats”, i.e., soils, subsurface environments and the deep sea. These dark environments exceed their photic counterparts in both, volume and biomass. In the case of oceans, the deep sea (below 200 m) exceeds the sunlit surface layer by a factor of 18 in volume and, remarkably, by a factor of two in biomass (Arístegui et al. 2009). Therefore, the so-called “dark CO_2 fixation” does not only occur in specific 'hot spots' on the seafloor (hydrothermal vents, cold seeps and mud volcanoes), or in anoxic waters, but throughout the whole oxygenated 'dark' water column (Reinthaler et al., 2010, Yakimov et al., 2014). Yet, heterotrophic CO_2 fixation does not occur only in the dark environments since heterotrophs are also found in the photic zone. This is particularly relevant in the ocean because the photic zone is where the highest biomass concentrations are found. Recently, it has been estimated that the inclusion of dark CO_2 fixation (integrated over the euphotic layer, 0-150 m depth) would increase oceanic primary production estimates by 2.5–22 % (Baltar et al., 2019).

In the absence of solar radiation, particularly in the dark ocean, CO_2 fixation rates of up to ~125mg C m^{-3} d^{-1} have been measured, which is as much as 30% (on a per volume basis) of the phototrophic CO_2 fixation taking place in ocean surface waters (Zopfi et al. 2001, Detmer

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et al. 1993, Casamayor et al. 2001, Baltar et al. 2010). We recently showed that the ratio between dark/light CO_2 fixation in surface oceanic waters is usually around 0.1 but it increases with depth reaching a ratio of 1 at 120-160 m depth (Baltar et al., 2019).

Part of the dark CO_2 fixation has been attributed to chemolithoautotrophic archaea (Wuchter et al. 2003, Ingalls et al. 2006) obtaining the energy required for the endergonic carboxylation through the oxidation of reduced inorganic compounds, such as ammonia or hydrogen sulfide (Swan et al. 2011; Zhang et al. 2020). A total annual chemolithotrophic CO_2 fixation rate of 0.77Pg C was calculated for the oceans (Middelburg 2011). The observed fluxes of the reduced compounds available as energy sources, however, seem largely insufficient to explain the relatively high dark CO_2 fixation rates (Overbeck 1979, Tuttle and Jannasch 1979, Baltar et al. 2010, Reinthaler et al. 2010, Herndl and Reinthaler 2013). In some cases, the supply rates of the reduced compounds used as an energy source explain less than 40% of the observed dark CO_2 fixation rates (Zopfi et al. 2001). Recently, chemoautotrophic nitrification was estimated to explain <13% of the dark CO_2 fixation (integrated over the euphotic zone) with the rest coming from either heterotrophic DIC fixation or other chemoautotrophic fixation (Baltar et al., 2019).

The potential energy sources for the unexplained proportion of the dark CO_2 fixation remain enigmatic. Possible explanations could be either an underestimation of the supply rates of reduced inorganic compounds or the uptake of CO_2 by heterotrophic organisms (Zopfi et al. 2001, Baltar et al. 2019). In the surface ocean in particular, DIC incorporation via anaplerotic reactions might play an important role in compensating metabolic imbalances in marine bacteria under oligotrophic conditions, contributing > 30 % of the carbon incorporated into biomass (González et al. 2008; Palovaara et al., 2014). Evidence for the latter comes from experiments with Arctic seawater, which exhibited high bicarbonate incorporation rates (0.5–2.5 µg C L^{-1} d^{-1}) correlating with heterotrophic bacterial production. Using different molecular tools, DIC uptake was attributed mainly to heterotrophic *Gamma-* and *Betaproteobacteria* rather than to typical chemoautotrophs (Alonso-Sáez et al. 2010), thus showing that chemolithoauthotrophs were not the main drivers of CO_2 fixation in this habitat. Further evidence comes from the genome of *Polaribacter* sp. MED152, a representative of Bacteroidetes, which typically comprise about 10–20% of the prokaryotic abundance in seawater. A unique combination of membrane transporters and carboxylases in these organisms indicates the importance of anaplerosis besides other DIC fixation pathways (González et al. 2008). If the heterotrophic metabolism of bacteria is suddenly intensified (e.g., after an input of organic matter), dark DIC fixation rates and the expression of transcripts associated with key anaplerotic enzymes increase proportionally (Baltar et al., 2016). Based on these lines of evidence, we argue that heterotrophic CO_2 fixation is an important process which needs to be considered when interpreting dark CO_2 fixation rates.

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4. Global estimates of heterotrophic CO_2 fixation

4.1. Carbon biomass stock originating from heterotrophic CO_2 fixation

Earth's total living biomass is estimated to amount to about 499 – 738 Pg C, of which approx. 451 – 653 Pg C is photoautotrophic biomass (Bar-On et al. 2018). Heterotrophic biomass contributes 47 – 85 Pg C (Table 1). Therein, the estimates of heterotrophic biomass of the terrestrial subsurface have huge uncertainties (Whitman, Coleman and Wiebe 1998, McMahon and Parnell 2014, Bar-On, Phillips and Milo 2018). Nevertheless, assuming that a minimum of 2-8% of this biomass C originates from anaplerotic CO_2 fixation, and ignoring further pathways and processes of heterotrophic fixation of DIC, we conclude that at least 0.9 – 7 Pg of DIC are temporarily sequestered in living biomass by anaplerosis.

4.2. Carbon flux related to heterotrophic CO_2 fixation

In terms of annual global heterotrophic production rates, oceans and the terrestrial subsurface (including soils) are the main habitats of heterotrophic CO_2 fixation (Cole et al. 2002; Magnabosco et al. 2018) (Table 2). We calculated a global heterotrophic C production of 34 – 245 Pg C yr^{-1}, which would translate into 0.7 to 20 Pg of DIC bound by heterotrophic CO_2 fixation each year. Interestingly, these numbers are consistent with the recently calculated contribution of CO_2 fixation for the integrated epipelagic ocean of ca. 1.2– 11 Pg C yr−1 (Baltar et al., 2019). This is a significant carbon flux amounting to 1-20% of the global net amount of carbon produced annually by photoautotrophs (NPP: 90 – 110 Pg C yr^{-1}).

Our estimates, as already mentioned, are subject to a high uncertainty, which, on the one hand, results from the dependency of the extent of heterotrophic CO_2 fixation on the organic carbon oxidized and, on the other hand, on the predominant environmental conditions. Moreover, data on terrestrial and marine subsurface environments, although huge in dimension, are scares. Here, no detailed information on the abundance, growth (yield) and metabolic activity of microbial communities is available, particularly with increasing depth. Most of the deeper subsurface environments, even when harboring considerable living biomass, do not participate in the global carbon cycle on a short term (years to decades), but rather in centennial to geological timescales. Nevertheless, in order to provide a first estimate and to be able to roughly evaluate the relevance of heterotrophic CO_2 fixation for all habitats of high uncertainty (e.g. the continental subsurface) we adopted a conservative approach to avoid overestimation in our calculations (see Tables 1 and 2 for explanations).

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Table 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, 2-8% of the cell carbon is assumed to originate from inorganic carbon fixation (see references in text), for photoautotrophs a contribution of 1-5% of carbon biomass from anaplerotic CO_2 fixation is assumed (Melzer and O'Leary 1987).

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	0.01 – 0.05	(Bar-On et al. 2018)
Soil fungi	12	0.2 – 1	(Bar-On et al. 2018)
Terrestrial protists	1.6	0.03 – 0.1	(Bar-On et al. 2018)
Soil prokaryotes (upper 100 cm of soil)	23.2	0.5 – 1.9	(Xu, Thornton and Post 2013)
Continental subsurface prokaryotes	2.4 – 12.6*	0.05 – 1	(Magnabosco et al. 2018)
Heterotrophic prokaryotes in freshwater and saline inland surface waters	0.013**	0.0003 – 0.001	(Whitman et al. 1998)
Marine and oceanic habitats			
Marine Animals	2	0.04 – 0.2	(Bar-On et al. 2018)
Marine protists	2	0.04 – 0.2	(Bar-On et al. 2018)
Marine fungi	0.3	0.01 – 0.02	(Bar-On et al. 2018)
Marine planktonic heterotrophic prokaryotes	1.4 – 3.5***	0.03 – 0.3	(Whitman et al. 1998)
Subseafloor sedimentary prokaryotes	1.5 – 22	0.03 – 1.8	(Kallmeyer et al. 2012, Schippers et al. 2005)
Prokaryotes of the oceanic crust	0.5 – 5	0.01 – 0.4	(Bar-On et al. 2018)
Total heterotrophic carbon biomass	**47 – 85**	**0.9 – 6.8**	
For comparison:			
Plants (terrestrial)	450 – 650	4.5 – 32.5	(Watson et al. 2000, Prentice et al. 2001, Ciais et al. 2013, Bar-On et al. 2018)
Plants (marine)	0.4 – 1.8	0.004 – 0.09	(Schlesinger 1997, Whitman et al. 1998, Groombridge and Jenkins 2000)
Phytoplankton (marine)	1	0.01 – 0.05	(Falkowski, Barber and Smetacek 1998)
Total photoautotrophic carbon biomass	**451-653**	**4.5 – 32.6**	
Total carbon biomass on Earth	**499-738**	**5.5 – 39.4**	

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* Cell abundances ($2 – 6 \times 10^{29}$ 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 (2.3×10^{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.

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Table 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 *	0.05 – 6.1	(Cole, Findlay and Pace 1988, del Giorgio and Duarte 2002)
Oceanic subseafloor	0.1 – 9.8 **	0.002 – 0.8	(Schippers et al. 2005)
Continental habitats			
Aquifers and unsaturated subsurface	0.12 – 26.3 †	0.002 – 2.1	(Magnabosco et al. 2018, Griebler et al. 2014)
Soils	31.3 – 133.2 ††	0.6 – 10.7	(Prentice et al. 2001, Manzoni et al. 2012, Hashimoto et al. 2015, Potter and Klooster 1998)
Total heterotrophic C-biomass production	**34 – 245**	**0.7 – 20**	

* 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 361419000 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 quite 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 4829 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 2-8% of the annually produced carbon biomass of heterotrophs originate from anaplerotic CO_2 fixation (see ref. in the text). A fraction of 2% was applied to the minimum, and 8% to the maximum value of the C-biomass production ranges in this table, respectively.

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5. Conclusions

Current models of carbon cycling and carbon sequestration do not account for heterotrophic CO_2 fixation (Le Quéré et al. 2009, Randerson et al. 1997, Gruber et al. 2004). Despite the uncertainties in the data on heterotrophic biomass and production rates for some habitats (e.g. the terrestrial subsurface), the numbers presented here represent the first attempt to quantify the global contribution and relevance of heterotrophic CO_2 fixation to carbon cycling. Our results indicate that heterotrophs significantly contribute to global CO_2 fixation – especially (although not restricted to) in dark habitats. In specific environments, this may explain the mismatch between autotrophic C input, consumption, and sequestration that is exemplarily observed in marine systems (Baltar et al. 2009, Burd et al. 2010, Reinthaler et al. 2010, Morán, Pérez and Fernández 2007, Hoppe et al. 2002, Tait and Schiel 2013). Particularly in aphotic habitats (which outnumber the photic habitats in both size and volume) such as the dark ocean, subseafloor sediments, soils, as well as the sediments and rocks of the terrestrial subsurface (Miltner et al. 2004, Miltner et al. 2005, Yakimov et al. 2014, Wegener et al. 2012), carbon cycling needs to be re-evaluated taking into account anaplerotic CO_2 fixation and other inorganic carbon uptake pathways in heterotrophs. In subseafloor sediments, wetlands and marshes, as well as in other habitats where methane oxidation is a key process, a large fraction (10-50%) of heterotrophic biomass potentially originates from heterotrophic CO_2 fixation. Recently, a time-series study showed a tendency towards higher ratios of dark to light DIC fixation in the top half of the euphotic layer (0– 65 m) in the years 2012-2019 than in the preceding years (data started in 1989), which was linked to oceanographic changes (i.e., a deepening of the mixed zone) (Baltar et al., 2019). Moreover, the metabolic theory of ecology (MTE) posits that heterotrophic metabolism increases more than gross primary production in the ocean in response to warming (see Baltar et al., 2019b and reference therein), which might also make heterotrophy DIC fixation relatively more important in a warmer ocean. In the light of global warming leading to an extensive thawing of permafrost soils and providing new habitats for methanotrophs, these processes are expected to become more important in the future. Hence, the potential contribution of heterotrophic CO_2 fixation under climate change conditions clearly deserves further investigations.

Author contributions

A.B., M.E. and C.G. conceived the idea for the manuscript. A.B., G.J.H. and C.G. wrote the manuscript. M.S.F., M.E., M.A. F.B. and T.R. substantially commented on and edited the manuscript. M.A., M.S.F. and C.G. did the literature search on available global carbon data. C.G. and M.A. performed the estimation of heterotrophic CO_2 fixation on a global scale.

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