**The answer to the criticisms and suggestions of reviewers # 3 and # 11**

**Anonymous Refere e #3**

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| **Suggestions for revision or reasons for rejection (will be published if the paper is accepted for final publication)** |
| **This is a significantly improved manuscript compared to the first version. I have a few final points to raise prior to final publication:** **In the abstract: “Thermodynamics calculations showed a high efficiency” What does efficiency mean here?**Offer redone: Nitrogen oxides are thermodynamically most favorable among possible oxidants of methane; however, even the activity of oxygen created by mineral buffers of iron in hydrothermal conditions is sufficient for methanotrophic acetogenesis.**line 51:“earth's crust periodically changed from -8 to +4 in Hadean and from -7 to +7 in the early”…What data says that the state of the Earth’s crust changed? What are the values indicated here? We are talking about oxygen fugacity correct? What is -8? Is it a log unit value? Please define.**This significant fluctuations in the crust redox state during this period of time indicate a pulsed regime of Earth degassing. Offer redone: Oxygen activity (log units) in the earth's crust periodically changed with regards to the magnetite - fayalite - quartz redox buffer from -8 to +4 in Hadean and from -7 to +7 in the early Archean**“Of all the magmatic formations of the world the alkaline magmatism is the deepest, andin its magma chambers hydrocarbon substances arises”A reference is needed**References added: Balashov, Y.A. and Glaznev, V.N.: Cycles of alkaline magmatism, Geochem. Int., 44, 274–285, [doi: 10.1134/S0016702906030050](https://doi.org/10.1134/S0016702906030050); 2006; Kolesnikov A., Kutcherov V., Goncharov A. Methane-derived hydrocarbons produced under upper-mantle conditions, Nature Geoscience, 2009, V. 2. P. 566–570. <https://doi.org/10.1038/ngeo591>; Marakushev and Marakushev, 2010.**Line 69:“apparently to be deep, forming at temperatures a”rephrase to “apparently is formed at depth, at temperatures of ca.”Insert:** apparently is formed at depth, at temperatures of ca.around line 80:“when the permeability of silicate shells across the Earth increased (during the expansion of silicate shells of the Earth due to the oscillatory nature of the geomagnetic field), there began a selective migration of hydrogen,”these silicate shells are unknown to me, please provide references and explanation. A change in the magnetic field changes the heat flux, which causes a melting or crystallization of the mantle substrate ([Labrosse](https://www.nature.com/articles/nature06355%22%20%5Cl%20%22auth-1) S., [Hernlund](https://www.nature.com/articles/nature06355#auth-2) J. W. and [Coltice](https://www.nature.com/articles/nature06355#auth-3) N., A crystallizing dense magma ocean at the base of the Earth’s mantle, *Nature,* 50, 866–869, [doi.org/10.1038/nature06355](https://doi.org/10.1038/nature06355), 2017), which in turn is associated with the expansion or compression of the silicate shells, respectively. Offer redone: However, during the process of the earth’s silicate shells (mantle and crust) extension (associated, in our opinion, with a Hadean to Paleoarchean geodynamo (Tarduno et al., 2015)), an increase of fluid permeability stimulates the selective migration of hydrogen (the most mobile component) from it.**...“methanogenesis pathway”There are two good new references that would be helpful to cite here:Reverse methanogenesis and respiration in methanotrophic archaeaPHA Timmers, CU Welte, JJ Koehorst, CM Plugge… - Archaea, 2017 - hindawi.com and Energy metabolism during anaerobic methane oxidation in ANME archaeaSE McGlynn - Microbes and environments, 2017 - jstage.jst.go.jp**References inserted: Timmers et al., 2017; McGlynn, 2017**line 378: “chemical potential of is indicated”potential of what? please specify.**Corrected: The transition between this facies with the change of oxygen chemical potential is indicated by a green arrow. |
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**Anonymous Referee #1**

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| **Suggestions for revision or reasons for rejection (will be published if the paper is accepted for final publication)** |
| **The manuscript is improved from the prior submission, but still suffers from a central problem: the proposal is thermodynamically possible, but little evidence is presented that it is kinetically feasible. In the absence of experiments or supporting chemical literature, one can only suppose it is highly unlikely to occur. The authors still overlook much of the prior relevant literature and the language still leaves the manuscript difficult to understand.**The solution of kinetic problems was not the main part of this work, but some possible ways of overcoming them are proposed. For example, activation of С-Н bond on some nickel centers with possible replacement of С-Н bond with С-S bond, as a biomimetic model of the active center of CoM reductase, or participation of oxygen radicals on the surface of minerals capable to methane transformation into methyl radical, as an intermediate product.Text edited by a native English speaker.**Several missing references to key papers from the recent(ish) years:a) Abiotic methane formation: - Etiope, G., and B. Sherwood Lollar, Abiotic methane on Earth, Rev. Geophys., 51, 276–299 (2013) (doi:10.1002/rog.20011 and references therein)- Schulte, M., Blake, D., Hoehler, T. & McCollom, T. Serpentinization and its implications for life on the early Earth and Mars. Astrobiology 6, 364–76 (2006) (doi.org/10.1089/ast.2006.6.364)- Scott, H. P., Hemley, R. J. & Mao, H. Generation of methane in the Earth’s mantle: in situ high pressure–temperature measurements of carbonate reduction. Proc. Natl Acad. Sci. USA 101, 14023–14026 (2004) (doi.org/10.1073/pnas.0405930101).- McCollom, T. M. Laboratory simulations of abiotic hydrocarbon formation in Earth’s deep subsurface. Rev. Mineral. Geochem. 75, 467–494 (2013) (doi.org/10.2138/rmg.2013.75.15).- Kolesnikov, A., Kutcherov, V. G. & Goncharov, A. F. Methane-derived hydrocarbons produced under upper-mantle conditions. Nat. Geosci. 2, 566–570 (2009) (doi.org/10.1038/ngeo591).b) Methane as C1 building block:- Martin, W., Baross, J., Kelley, D. & Russell, M. J. Hydrothermal vents and the origin of life. Nature Reviews Microbiology 6, 805–814 (2008) (doi.org/10.1038/nrmicro1991)**Thanks for the advice, references are inserted.**L68-71: The sentence starting “Methane, which was considered […]” – is some verb missing? Needs to be rephrased.**Rephrased:Methane, which is usually considered to be relatively surface origin (low-temperature serpentinization ~100° C), apparently is formed at depth, at temperatures of ca. 400°. **L108-109: “The existing theories on the origin of autotrophic life identify carbon dioxide as the unique carbon source for metabolism” – this is not true, as even stated by the authors themselves 2 pages later (L155-157)! Also, how about theories involving e.g. formaldehyde or carbon monoxide?** Changed: The existing theories on the origin of autotrophic life **mainly** identify carbon dioxide as the unique carbon source for metabolism**L116-117: “[…] carbon fixation could occur in the form of methane or other hydrocarbons” – what kind of chemistry do the authors envision with “other hydrocarbons”?** As it follows from the composition diagram in the reduction regime (facies II), absolutely all hydrocarbons (betweenCH4 - C) can in principle be a carbon source for life.Refrased:In regime **II** (Fig. 1), methane and other hydrocarbons could be substrates of the emerging autotrophic metabolism.**L120-126: Do the authors agree with the notion that prebiotic chemistry was also vastly different from the chemistry used by life? If this is the case, it should be discussed/justified and appropriate literature cited.**We consider … that prebiotic chemistry was also vastly different from the chemistry used by life, however, it was its basis. Among several levels of the metabolism hierarchy (Braakman and Smith, 2013), we consider only the first (С–Н–О system) as the foundation that determines the direction of protometabolism development. The problem of the prebiotic pre-enzymatic metabolism organization is discussed in a number of works cited by us: Wachtershauser, 1988; Russell and Martin, 2004; Smith and Morowitz, 2004; Nitschke and Russell, 2013; Marakushev and Belonogova, 2015; Muchowska et al., 2017. **Table 1: What are the low and high temperatures (trend) denoted by L and H? Does this refer to 298K and 473K? Unclear.** Changed: Value of ΔG0T at 298 and 473 K indicates the advantage of the reactions at low (L) or high (H) temperatures.**L220-223: The sentence starting “The defined conservative sequences […]” – I’m not sure what the authors mean here. What are “conservative sequences” of the carbon fixation cycles mentioned before that would create “[…] the specific pathways of carbon fixation”. This is not logical at all.**I agree. The conservative sequence, of course, refers to the already identified persistent sequence of intermediates in the modern biochemical pathways. Although we assume their participation in the emergence of autotrophic metabolism, the word “conservative” removed. **Figure 2: Several issues:- The proposed methane fumarate cycle looks nice on paper but from a chemical perspective it is highly unlikely it would ever work without enzymes under hydrothermal conditions, and without any spatial/temporal separation of its intermediates. The fact that a reaction is thermodynamically downhill does not automatically mean it will happen due to kinetic constraints. Hydromethylation of fumarate with methane is one of the reactions that is quite unlikely to happen without a complex catalyst, let alone in water.** Our understanding of the emergence of methanotrophic metabolism is within the framework of hydrothermal theory for the origin of life. The problem of “spatial/temporal separation of its intermediates” exists for almost all proposed pre-cellular protometabolitic systems**.** We believe that there are different possibilities of organizing space without compartmentalization to solve this problem. They are mentioned by us in the text: Wächtershäuser, 1988; Russell and Martin, 2004; Kéraval et al., 2016. In principle, each cycle reaction has its own kinetic properties. We present data and assumptions about possible mechanisms (may be radical) of the hydromethylation reaction (fumarate addition), which could be the initial stage of the anaerobic methane oxidation. This is discussed.**- Citramalate – the formula shown is incorrect. Citramalate = 2-hydroxy-2-methylbutanedioic acid, and not 2-hydroxy-3-methylbutanedioic acid**Thank you. Structure of citramalate is corrected. **- The carboxylation of pyruvate to oxaloacetate is rather unlikely under the conditions proposed. It is thermodynamically unfavourable. Both ketoacids – and especially oxaloacetate – rapidly decarboxylate in the presence of transition metal ions. Assuming this reaction could happen, what is the energy source? in biochemistry this reaction uses ATP?**I agree, the reaction is really very difficult. In modern biochemistry (pyruvate carboxylase), activation of two substrates of CO2 and pyruvate is required at once. On the other hand, pyruvate carboxylation is a very ancient process and, apparently, this mechanism was already functioning in LUCA (Lombard and Moreira, 2011, doi: [10.1186/1471-2148-11-232](https://doi.org/10.1186/1471-2148-11-232)). The demand for this reaction in early biochemistry is obvious. We can also mention the possibility of pyruvate carboxylation without ATP, but with the participation of the activated thioester bond (Braakman and Smith, 2013). The values of the free energy of this reaction closed to zero, indicate that it is practically reversible and becomes more favorable with decreasing temperature.**- The authors argued on the previous pages for the abundance of methane and low quantities of CO2 on the early Earth. Is this argumentation consistent with the author’s proposal of CO2 being used for carboxylations?** As it happened in the archaean WL pathway, the methanotrophy probably should be combined with the carboxylation process (CO2 fixation). For example, the Soo et al, 2016 have shown that in this pathway the process of acetogenesis is carried out with the fixation of CH4 and CO2 in a 2: 1 ratio. Therefore nature (even in the conditions of predominant methane degassing) had to look for all possible sources of carbon dioxide.**L285: It appears that the authors proposed the reactions to be occurring under hydrothermal conditions. How compatible is this with NO used as oxidant? Fe2O3 is insoluble in water (unless strong acid is added) and will be kinetically inert as oxidant under most hydrothermal conditions (this does not mean that its surface could not be useful for chemistry).**In early Earth’s atmosphere nitrogen oxides was proved as electron acceptors for life’s emergence (Wong et al., 2017, doi:10.1089/ast.2016.1473), however the problem of the existence of electron acceptors in the ancient hydrothermal conditions exists and is being actively discussed, for example [Russell et al., 2017]. From the diagram, Fig. 3, it follows that under hydrothermal conditions the chemical potential of O2, created even by quartz- magnetite-fayalite redox buffer, is sufficient to create conditions for the oxidation of methane.**L327-329: The sentence starting “Radicals of amino acids and dipeptides may be the possible catalysts for methane activation [..]” - given that the authors propose an autotrophic origin of life through carbon fixation, where do the amino acids come from? This is not logical.**Inserted sentences: The formation of pyruvate and oxaloacetate in MF cycle, Fig. 2, indicates a very likely formation of amino acids in simple aqueous synthesis, for example: С3Н4О3 (pyruvate)+NH3 = C3H7O3N (serine), ΔG0298  = -10,10 kJ or pyruvate+NH3 +H2 = C3H7NO2(alanine)+H2O, ΔG0298 = **-**124,8 kJ. Barge et al., 2019 show that pyruvate can form the alanine in hydrothermal systems in the presence of mixed-valence iron oxyhydroxides. Moreover, the generation of reactive oxygen species H2O2 and OH• from minerals and H2O in anaerobic environments (eg. Xian et al., 2019) creates the possibility of various radical mechanisms for the oxidation of substrates in a hydrothermal environment.**L329: The authors mention protometabolism occurring on the surface of minerals. Is the proposed cycle also supposed to occur on the mineral surface (or in bulk solution)? This is not clear from the writing.**The MF cycle presumably functioned on the minerals surface, and this was considered in the last text edition. It is also mentioned that perhaps some kind of “mineral matrix” like the soil (Kéraval et al., 2016) can create an environment, where the first ancestral metabolisms may have occurred before they were incorporated in the first cell.**L424: “Generally accepted […] solely in the form of CO2” is not the best choice of words, as there are several hypotheses regarding what the carbon building blocks could have been, with CO2 being perhaps the most popular one. The hypothesis about methane being one has been known for years. Also, methane may be of an abiotic origin, but it is an organic compound (not “inorganic”).** To the best of our knowledge, the well-founded hypothesis of the origin of early methanotrophic metabolism was first expressed in (Nitschke and Russell, 2013).**L425-427: This sentence needs to be rephrased, it is hard to understand in its current form.**Offer changed: It is widely accepted that autotrophic metabolism is the fixation of inorganic carbon solely in the form of CO2, but the origin of methane, both on the ancient lifeless Earth, and on the planets and satellites (for example, on Titan) is clearly inorganic; therefore, carbon fixation from methane is also a manifestation of autotrophic metabolism (formation of organic compounds from inorganic precursors).**L428: Why did the CO2 degassing suddenly (?) start to dominate?**Offer changed: When approximately ~ 3.6 billion years (Yang et al., 2014), the CO2 degassing regime became dominant on our planet, relic methanotrophy systems were forced to die out or be thrown into unusual and extreme ecological niches. |

We express our gratitude to the editor and anonymous reviewers for important critical comments and helpful suggestions which have been taken into consideration.