Comments of Anonymous Referee #3 that need clarification and responses

1. "two methane molecules are used per cycle"..."it also seems highly unlikely",

Anonymous Referee #2: Would the authors consider modifying their hypothesis, keeping their original idea, but also involving CO_2 to offer the carboxyl radical?: Given the advice of Referee #2, we changed the proposed ancient MF cycle with the involvement of the CO_2 fixation reaction. The problem of introducing methane into the fumarate molecule remains and is being discussed.

Insertion I.

2. "better explain the diagrams": The diagram is described in more detail with the addition.

Insertion II.

3. "Why isn't Korzhinskii, D.S., 1959 cited?": The method of thermodynamic analysis of organic substances parageneses is described in (Marakushev, Belonogova, 2009), but the founder of the method is D.S. Korzhinskii, obviously, should be cited as done.

4. "Line 42 and 55: The process described as "impulse degassing" does not clarify the nature of this process and a description of it cannot be found in the reference (Touret, 2003)." : Sorry, it turned out that if methane is pulsed from the earth's core.

Insertion III.

Line 43: (Touret, 2003) points towards a metamorphic origin of the methane inclusions or most likely a hydrothermal vent system, but originating from mid-crustal depth at best, therefore not from the earth's core" :

Fluids are pulsed from the earth's core, while methane and other hydrocarbons are generated in deep magma chambers and ejected to the surface by volcanic processes, and in the process of mineral formation they are trapped in gas-liquid inclusions. The inclusion of hydrocarbons and sufficiently reduced organic compounds in Archean quartz (Touret, 2003, Schreiber et al., 2017) indicates a sufficiently reducing environment during the certain Archean periods. It was shown that prior to the Great Oxygenation Event the abiotic magnetite precipitation can be supported by massive reduction of CO_2 into CH_4 (Thibon et al., 2019. doi: 10.1016/j.epsl.2018.11.016).

There is evidence that the oxidation state of magma sources has changed (Aulbach et al., 2017), and that the atmosphere of Archean was enriched with methane (0.5%) and hydrogen (Tianet al., 2005; Zahnle et al., 2019. doi: 10.1016/j.gca.2018.09.017). The most important conclusion, however, is that the Hadean continental crust was significantly more reduced than its modern counterpart and experienced progressive oxidation ~3.6 billion years ago (Yang et al., 2014).

Insertion IV.

5. “Line 47: Significant concentrations of CH_4 should be substituted by estimated values. The upper limit of concentrations of abiogenic CH_4 has been given by Fiebig et al. 2007 as 50-80Mt/year, derived from serpentinization reactions in the ultramafic crust. These authors calculated that abiotic methane emissions would be around 1% of those today which are biologically driven. The above citations describe serpentinization reactions as the source of H_2 and none deal with H_2 degassing from the Fe-Ni core. How can the authors explain this?”：

Thanks for the comment, unfortunately, the link to Fiebig et al. 2007 are inconclusive, as well as at Haqq-Misra et al., 2008 and Dodd et al., 2017.

We believe that the bulk of methane is deep origin, and argues with reference to the discovered bubbles of hydrocarbons trapped in eclogite, a metamorphic rock that forms at high pressure at a depth of at least 80 km (Tao et al., 2018). It is shown the massive production of abiotic methane at ~40 km depth (Brovarone et al., 2017), as well as experiments on the synthesis of hydrocarbons and the inclusion of methane in diamond (Smit et al., 2016). Despite of the facts (eg, Mével, C. Serpentinization of abyssal peridotites at mid-ocean ridges. doi: 10.1016/j.crte.2003.08.006, 2003)], the origin of hydrothermal methane was until recently considered superficial (low-temperature serpentinization ~ 100° C). However, the bulk of even hydrothermal methane, is nevertheless deep, emerging at temperatures at ca.400°C under redox conditions characterizing intrusive rocks derived from sub-ridge melts (Wang et al., 2018). Paragraph edited.

Insertion V.

6. Line 51: Lost City Line 62: what is the meaning of silicate shells? What is the meaning of this statement and what are the references.

Silicate shells are the lower, upper mantle and crust. Their periodic compression and expansion is called Earth's hydrogen respiration. References are provided.

7. Line 64: what is the meaning of “Oscillatory nature of the geomagnetic field”? what references support this idea?

We believe that the changes in the intensity of the hydrogen degassing of the earth’s core and the pulse of plume activity (Larson, Olson, 1991) is associated with impulses in the geomagnetic field (Alldredge, 1984; Aubert et al., 2010).

8. Line 86: what does “determine the alkaline slope in the development of magmatism” mean? What are the references and why is it relevant to this paper?

The evolution of the Earth over the past 4.6 billion years is determined by the impulsive degassing of its liquid core along the structures of the dislocation of its solid silicate shells (mantle and crust). In the regime of these structures expansion, which corresponds to a decrease in the depth of the magma chambers, hydrogen migrates from the upward-traveling fluids, so that the oxygen components predominate over the hydrogen components, creating the acidic carbon dioxide trend: $H_2 + 3CO = CO_2 + 2C + H_2O$. They deliver mainly CO_2 in the Earth's crust and this leads to the formation of carbonates. With such fluids the magmatism of normal alkalinity associated. With the transition to the regime of compression of dislocation structures, which is facilitated by an increase in the depth of the development of magmatic chambers, the migration of hydrogen from fluids becomes difficult, so that their primary hydrogen composition is restored. Then, the fluids obtain the carbon or hydrocarbon specificity, and with a temperature decreasing the methane are generated in them. In the environment of the predominance of hydrogen, the acidic components of fluids are decomposing: $5H_2 + H_2CO_3 = 3H_2O + CH_4$, which contributes to the development of alkaline slope in the magmatism with its specific chemical affinity for acidic components. Magmatic chambers of alkaline-basalt magmatism (increased content of alkalis, especially K_2O), unlike basalt-andesitic, are located at abyssal depth and are responsible for the synthesis of hydrocarbons, including methane. With increasing alkalinity (alkaline slope) in the fluid inclusions of igneous minerals invariably appear different hydrocarbons (Potter and Konnerup-Madsen, 2003; Nivin et al., 2005).

Insertion VI.

9. “Line 94: What have you demonstrated? I don’t see any demonstration.”:

Yes of course. Corrected as follows: However, we assume that in geodynamic regime II (CH_4 + H_2O paragenesis), carbon ancestral metabolism could use methane as a carbon source if the flow of free energy from the geochemical environment was coupled with biomass formation reactions.

10. “Line 97 – 101: This section is not clear and needs revision. How could it be that “different regimes of fluid degassing determined the emergence and development of various systems of ancient metabolism”?”

Sorry, the sentence is incorrect. Now so: Perhaps, these fluid degassing different regimes determined the ambient environment, which, in turn, provided an opportunity of emergence and development of various systems of ancient autotrophic metabolism.

11. “Line 120-122: This is incorrect, M. oxyfera was not shown to generate nitrite, but rather to consume it. Please read Haroon et al 2013 Nature.” :

Thank you, there is an error. The sentence will look like this: A similar relationship was later discovered in archaea species that functions in chemical conjunction with the bacterium

Candidatus Methylomirabilis oxyfera, which itself can independently couple AOM to denitrification, (Ettwig et al., 2010; Haroon et al 2013).

12. "Line 147. Citations are needed with reference to possibly being in the LUCA. Gunter Wachtershauser? Mike Russell? Eric Smith? Bill Martin?" :

Michael Russell with co-authors suggests the possibility of primacy of the methanotrophic acetogeneic pathway. The sentence is corrected, the reference is given.

13. "Line 155. There is no evidence that I know of about this "predominance of methane" – how can the authors make this claim? What are the citations?":

Well, we will not be so categorical: The LUCA era apparently proceeded in an environment with high CO_2 partial pressure, whereas the pre-LUCA period proceeded in a more reducing environment with a significant availability of methane.

14. "Line 176. Is there ANY evidence that methane can react with fumarate in this way? According to Beasley and Nanny 2012, the reaction is extremely difficult DOI: 10.1021/es3009503.":

Many details of how the microorganisms in anaerobic environments performed the methane oxidation are unknown. Fumarate addition has been widely proposed as an initial step in the anaerobic oxidation of both aromatic and aliphatic hydrocarbons (Musat, 2015). The reaction of methane with fumarate satisfies the "minimal energy requirements" for autotrophic growth (Beasley, Nunny, 2012) and we consider the possibility of its participation in nascent autotrophic metabolism.

Insertion VII:

15. "Line 185-189: The autocatalytic nature of intermediates of the citric acid cycle has never been shown and the notion that they are versatile (in terms of functions, assembly or as reaction intermediates?) requires references."

"Line 188: Which oxidants and where would they come from?":

"Line 203: Please indicate that the subscript numbers correspond to degrees K.":

"Line 219-220: Not clear what this sentence means.":

The autocatalytic nature of intermediates of the citric acid cycle derives from the branching point associated with citrate cleavage, cycle self-reproduction etc. References: Morowitz, 1999; Smith, Morowitz, 2004; Zubarev, et al., 2015. doi: 10.1038/srep08009; Meringer, Cleaves J., 2017. doi: 10.1038/s41598-017-17345-7 etc. In biomimetic models of rTCA, autocatalysis is not yet experimentally shown, although the natural cycle itself has autocatalytic properties. In connection with the modification of the MF-cycle structure and in view of these comments, the text is changed:

Text pp. 219-247 ported to p. 153, Page 201-209 text removed.

Insertion VIII: Insertion IX:

16. “Line 229: Why is sulfate/Sulfite considered? No organisms are known that only do this, they may reduce all the way to sulfide, or sulfite to sulfide, but this is not a reasonable redox couple.

The remark was accepted and we inserted the reduction of sulfate to sulfide ($CH_4 + 0.5H_2SO_4 = 0.5CH3COOH + H_2O + 0.5H_2S$) in the Table 1. The oxidized and reduced states of oxidant in the reaction are conditionally called the redox pairs.

17. Table 1: the authors state that the reactions are more energetic in the “ionized form” which I interpret to mean making acetate where the carboxy group is deprotonated. However, the reaction energies all seem fairly comparable except for that involving 4HNO2. What is the pKa of protonating NO2?”:

Methane oxidation reactions with HNO_2 and with HNO_3 are significantly different from reactions with NO. It is known that pK HNO_2 is 3.4, and HNO_3 is generally -1.64, however, the thermodyna mics of the reactions of protonated and ionized compounds in our work simply shows the temperature tendency of the reactions to proceed in an acidic or alkaline environment, respectively.

18. “Line 245: It is not possible to oxidize sulfate.”:

Sorry! Misprint.. Oxidation by sulfate …..

19. “Line 251: Paraphrase to make the meaning clear”:

Redox pairs nitrite-nitric oxide (HNO_2-NO), nitrogen oxide-nitrous oxide (NO-N_2O), and nitrogen oxide-molecular nitrogen (NO-N_2) are the most thermodynamically favorable combinations of methane oxidation process.

20. “Line 254: verb missing at the end of the subordinate clauses”:

Proposal corrected

21. “Line 258: example or reasoning behind the large energy barrier”:

We assume a possible participation of complexes of transition metals and their minerals as homogeneous or heterogeneous catalysts of the C-H bond activation in the methane molecule. In the field of alkane oxidation the enzymatic metal-oxo species promote C-H activation through a metallo-radical pathway.

Insertion X:

22. “Line 284: In prebiotic hypotheses of Russell and Wächtershäuser the reacting minerals, namely Mackinawite and Greigite, resemble the structure of the FeS-cluster catalyzing the reaction whereas here none of the mineral catalysts resemble the nitrogen coordinated Ni-centers in F_{430}”:

Primordial organo- and metallo-catalysis is generally a matter of separate discussion, but the sentence is changed: Nevertheless, these limitations are overcome in conditions of high methane and its oxidants concentration and in the presence of metallocatalysts (minerals and organocomplexes) similar to the active center of metalloenzymes, as the mononuclear and polynuclear non-heme iron and iron-nickel enzymes (Martin, Russell, 2007).

23. "Line 288: Why is it highly possible? I'm not convinced":

The high methane partial pressure naturally must shift the equilibrium of the methane assimilation process towards the reaction products.

24. "I recommend the authors try to better explain their diagrams"; "Line 307: What is "nonvariant equilibria"; "Please clarify this depiction of chemical potential."

In a k component system, each association composed of k + 2 phases is nonvariant according to the Gibbs phases rule and the certain point corresponds to it in the P – T diagram. If one of phases disappears, the association of k + 1 phases becomes monovariant and, hence, it should correspond to a curve starting from a nonvariant point in the P – T diagram. Thus, in the general case, there are k + 1 phases involved in each monovariant reaction and k + 2 monovariant curves should start from a nonvariant point to form a so-called Schreinemakers beam (Korzhinskii, 1959). Each field between two curves of a beam corresponds to temperature and pressure conditions, in which no more than k phases simultaneously are in stable association in the given system. These fields are called facies of these phases. The Schreinemakers method is a geometric approach used to determine the relationships of reaction curves, which intersect at a nonvariant point in multicomponent systems. Explanations added, **Insertion II.**

25. "Line 382: What is the reasoning behind this statement? It could be that early life was all heterotrophic and that autotrophy was late, so I'm not sure we need to believe in a greater variety of early carbon fixation schemes":

The reversibility of rTCA cycle reactions (Mall et al., 2018; doi: 10.1126/science.aao2410) indicates the possibility of the almost simultaneous occurrence of both autotrophic and heterotrophic metabolism. In itself, the existence of a reversible and therefore differently directed acetyl-CoA pathway path points to an obvious opportunity of the early methanotrophy appearance. Most of our knowledge of anaerobic methanotrophic metabolism has been obtained using highly enriched or pure cultures grown in the laboratory. However, many methanotrophs are currently unisolated microorganisms. Only in the last 15 years, two new CO_2-fixing cycles (3-Hydroxypropionate/4-Hydroxybutyrate and Reductive Dicarboxylate/4-Hydroxybutyrate) have been discovered, which indicates a lack of knowledge of carbon-fixing pathways in modern prokaryotes.

Inset: incommensurably replaced by appreciably

26. "Figure 1: The composition ratio of the H-C-O compounds does not contribute to a deeper understanding of the underlying properties of the geodynamic regimes/ reaction conditions/ catalysts that perform these reactions":

The phase diagram of the compositions determines the regions of stability of the parageneses of substances (in this case, simple substances). It shows that at the high concentrations of hydrogen (H_2) the formation of methane occurs, and the acid components of the fluids is destroys: $3H_2+CO = H_2O+CH_4$, $4H_2+H_2CO_3 = 3H_2O+CH_4$, and this determines the alkaline slope in development of the magmatism. The decreasing of the hydrogen partial pressure results in more acidic fluids: $H_2+2CO = H_2O+0.5CO_2+1.5C$ and $H_2O+CO_2 = H_2CO_3$. That is, the composition of fluids is determined by different geodynamic regimes. At the high concentrations of hydrogen (H2) the formation of methane occurs, and the acid components (in) of the fluids is destroys: $3H_2+CO = H_2O+CH_4$, $4H_2+H_2CO_3 = 3H_2O+CH_4$, and this determines the alkaline slope in the development of the magmatism. The decreasing of the hydrogen partial pressure results in more acidic fluids: $H_2+2CO = H_2O+0.5CO_2+1.5C$ and $H_2O+CO_2 = H_2CO_3$. That is, the composition of fluids is determined by different geodynamic regimes.

27. "Figure 2: Is an enzymatic reaction known that can insert a hydroxyl group into the C2 position? Mechanistically this seems too difficult":

Figure 2 is changed because really this reaction is impossible.

28. "Figure 3: axis labeled in Russian. Please clarify this depiction of chemical potential. Does it mean that above the line, the reaction proceeds right to left, and below the line, the reaction proceeds left to right? What does the vertical line with fumerate, succinate, and acetate correspond to?":

Figure 3 corrected: The chemical potential practically reflects the activity of O2 in solution: $\mu_{O2} = RT\ln\alpha_{O2} = 19{,}12T\lg\alpha_{O2}$. A line is an equilibrium between substances located on its different sides. An increase in the chemical potential of oxygen leads to the facies CO_2, and a decrease to the facies CH_4. The vertical line with fumerate, succinate, and acetate divides the areas of thermodynamic stability (facies) of succinate on the one hand and fumarate + acetate on the other at a temperature of 549 K, regardless of μ_{O2}.

Insertion I: In the field of alkane oxidation, enzymatic metal-oxo species, promote C–H activation through a metallo-radical pathway. This involves hydrogen radical abstraction from the alkane by the oxo species, followed by rapid rebound of the radical species onto the metal hydroxo intermediate [Roudesly et al., 2017, doi: 10.1016/j.molcata.2016.06.020]. The calculation of the potential energy surface showed the thermodynamic possibility of anaerobic oxidation of methane via fumarate addition, in a reaction catalyzed by the glycyl radical [Beasley, Nanny, 2012]. The reaction mechanism fumarate + $CH_4 \rightarrow$ 2-methylsuccinate, fig. 2, seems to be similar to the radical mechanism of breaking the C–H bond with the formation of the

C–C bond, catalyzed by benzylsuccinate synthase (Buckel, Golding, 2006, doi: 10.1146/annurev.micro.60.080805.142216), during microbiological fixation of toluene by fumarate. Radicals of amino acids and dipeptides may be the possible catalysts of methane activation with the formation of methyl radical as an attacking agent. According to [Weiss et al., 2016, 10.1038/NMICROBIOL.2016.116**]**, LUCA metabolism had an excess of radical reaction mechanisms, which, in our opinion, could also participate in the reaction of fixation of CH_4 in the cycle, overcoming the activation barriers of kinetically adverse reaction steps. Moreover, these limitations are overcome in conditions of high substrate concentration and in the presence of metallo-catalysts similar to the active center of metalloenzymes, as the mononuclear and polynuclear non-heme iron and iron-nickel enzymes.

Insertion II: The chemical potentials (μ_i) of components representing its partial energy, the value μ_i is expressed through activity α_i or fugacity f_i as follows: $\mu_i = (\mu^0_i)_{\mathrm{T,p}} + RT\ln\alpha_i = (\mu^0_i)_{\mathrm{T,p}} + RT\ln f_i$. Here numerical values depend on conventional standard states. For activity, the state of pure crystaline substance or unit molal concentration is usually considered as a standard state at given temperature and pressure. In this state $\alpha_i = 1$ and, hence, $\mu_i = (\mu^0_i)_{\mathrm{T,p}}$. According to Gibbs' phase rule, at arbitrary pressure, the nonvariant equilibria in the diagram (points) consist of four phases, and the three-phase equilibria (lines) divide the divariant stability fields (facies) of the two-phase equilibria.

Insertion III: On Earth, the methane and other hydrocarbons are generated in magma chambers and are carried by fluids to the surface in volcanic processes, and during the formation minerals are trapped in the gas-liquid inclusions.

Insertion IV: Inclusion of hydrocarbons and reduced organic compounds in Archean quartz (Touret, 2003, Schreiber et al., 2017) indicates a sufficiently reducing environment at this time. There is evidence that Archean atmosphere was enriched in hydrogen and methane (Tianet al, 2005; Zahnle et al, 2019), and the oxidation state of magma sources apparently has changed (Aulbach et al, 2017.). According trace-elements data of igneous zircons of crustal origin (mainly Ce-based oxybarometer), it was shown that the Hadean continental crust was significantly more reduced than its modern counterpart and experienced progressive oxidation ~3.6 billions years ago [Yang et al., 2014]. Regarding quartz - fayalite - magnetite redox buffer, the state of the earth's crust periodically changed from -8 to 4 in Haden and from -7 to 7 in the early Archaea. Significant fluctuations in the redox state of Archean and Haden zircons indicate a pulsed regime of earth degassing during this period of time, which, in our opinion, is related to impulses in the geomagnetic field (Alldredge, 1984; Larson, Olson, 1991; Aubert et al., 2010). Degassing impulses created ascending fluid flow (plumes) that formed magma chambers. Thus, the evolution of the Earth over a period of 4.6 billion years is determined by the impulsive degassing of its liquid core along the structures of the dislocation of its solid silicate shells (mantle and crust).

Insertion V: Of all the magmatic formations of the world alkaline magmatism is the deepest, and in its magma chambers hydrocarbon substances arises. Thermodynamic calculations show the preference of deep formation of hydrocarbons, which, rising in fluids to the surface of the Earth, transform into methane (Marakushev, Marakushev, 2006). This is confirmed by the massive production of abiotic methane at ~40 km depth (Brovarone et al., 2017) and discovered bubbles of hydrocarbons trapped in eclogite, a metamorphic rock that forms at high pressure at a depth of at least 80 (Tao et al., 2018). The gas-liquid inclusions of methane in diamond and P,T experiments on the synthesis of hydrocarbons (Smit et al., 2016) also prove its deep origin. Methane, which was considered to be of relative surface origin (low-temperature serpentinization ~100° C), apparently to be deep, forming at temperatures at ca. 400 ° C under redox conditions characterizing intrusive rocks derived from sub-ridge melts (**Mével** 2003;

Wang et al., 2018). Thus, deep, alkaline - basalt magmatism (elevated alkali content, especially K_2O), in contrast to basalt-andesitic magmatism, is mainly responsible for methane degassing on the Earth's surface. The high content of potassium in high-silica Hadean crust (Boehnkea et al., 2018) indicates the depth of magamatism and its hydrocarbon specificity.

Insertion VI: Of all the magmatic formations of the world the alkaline magmatism is the deepest, and in its magma chambers hydrocarbon substances arises. Thermodynamic calculations show the preference of deep formation and stability of hydrocarbons, which, rising in fluids to the surface of the Earth (decreasing temperature and pressure), are transformed to methane (Marakushev and Marakushev, 2006).

Insertion VII: Cycle is initiated by the reaction of fumarate + methane → 2-methylsuccinate. In the hydration and dehydrogenation or anaerobic oxidation reactions, 2-methylsuccinate is converted to citramalate, which is disproportionated to acetate and pyruvate with cleavage of carbon-carbon bond. Pyruvate is an important "hub" metabolite that is a precursor for amino acids, carbohydrates, cofactors, and lipids in extant metabolic network. The following carbon assimilation reaction in the form of CO2 with the formation of oxaloacetate is a biomimetic analogue of the reductive tricarboxylic acid (rTCA) cycle reaction. An α-carboxylation of pyruvate is a critical anabolic pathway in modern biochemistry, which resupplies rTCA cycle intermediates. Oxaloacetate is transformed into fumarate in the reactions of the citrate cycle intermediates. The nonenzymatic flow of some reaction sequences of the rTCA cycle, such as oxaloacetate → malate → fumarate → succinate has been experimentally confirmed [Muchowska et al., 2017]. The resulting fumarate assimilates methane and begins a new MF cycle, in one turnover of which an acetate molecule is formed from methane and carbon dioxide molecules: $CH_4+CO_2 = CH_3COOH$, Table. 1. Transformation of fumarate into 2-methylsuccinate introduces into the cycle five-carbon intermediates, such as citramalate and mesaconate, functioning, for example, in the reductive 3-hydroxypropionate CO_2 fixation cycle.

Insertion VIII: The autocatalytic nature of the cycle derives from the branching point associated with citramalate cleavage, and can be shown by the example of doubling the intermediate as in the reaction: $C_4H_6O_5$ (malate) + $1{,}5CH_4+2{,}5CO_2 = 2C_4H_6O_5$ (malate). This type of autotrophic metabolism, as in the case of the acetyl-CoA pathway, can be defined as carboxy-methanotrophic acetogenesis. The problem of the most energetically unfavorable reaction of 2-methylsuccinate transformation into citramalate ($\Delta G^0_{298} = 96.57$ kJ), **Table 2a,** can be solved by using oxidants, such as oxides of nitrogen and iron (Fig. 2, inset). Nitric oxide (NO) is the strongest oxidant, but the reaction with Fe_2O_3 is also favorable at physiological temperatures.

Insertion IX: The most favorable reaction $CH_4 + 2NO = 0{,}5CH_3COOH + H_2O + N_2$ (Table 1) can be represented as a model of methanotrophic acetogenesis, the chemical mechanism of which is part of the reverse acetyl-CoA pathway. The second part of this path is the reaction of CO_2 reduction: $CO_2 + 2H_2 = 0{,}5CH_3COOH + H_2O$. In sum, this is a very thermodynamically favorable pathway of carbon fixation in the form CH_4 и CO_2: $CH_4 + CO_2 + 2NO + 2H_2 = CH_3COOH + N_2 + 2H_2O$. Somewhat different stoichiometry of acetogenesis was observed in the Archean Methanosarcina acetivorans, when methane oxidation was associated with the reduction of iron (III) [Soo et al, 2016]. A reaction is proposed in which four methane molecules are oxidized and two CO_2 molecules are reduced to form three acetate molecules. Increasing the ratio of CH_4 to CO_2 ($4CH_4 + 2CO_2 + 24Fe_2O_3 + 4H_2 = 3CH_3COOH + 6H_2O + 16Fe_3O_4$) makes the process of anaerobic acetogenesis more thermodynamically favorable (Table. 1).

Insertion X: In the field of alkane oxidation, enzymatic metal-oxo species, promote C–H activation through a metallo-radical pathway. This involves hydrogen radical abstraction from the alkane by the oxo species, followed by rapid rebound of the radical species onto the metal hydroxo intermediate [Roudesly et al., 2017]. The calculation of the potential energy surface showed the thermodynamic possibility of anaerobic oxidation of methane via fumarate addition, in a reaction catalyzed by the glycyl radical [Beasley and Nanny, 2012]. The reaction mechanism fumarate + $CH_4 \rightarrow$ 2-methylsuccinate, fig. 2, seems to be similar to the radical mechanism of breaking the C – H bond with the formation of the C– C bond, catalyzed by benzylsuccinate synthase (Buckel and Golding, 2006; Austin et al., 2011) during microbiological fixation of toluene by fumarate. Radicals of amino acids and dipeptides may be the possible catalysts of methane activation with the formation of methyl radical as an attacking agent. According to [Weiss et al., 2016], LUCA metabolism had an excess of radical reaction mechanisms, which, in our opinion, could also participate in the reaction of CH_4 fixation in the cycle, overcoming the activation barriers of kinetically unfavorable reaction steps.

Insertion XI: Figure 2. The scheme of the proposed methane-fumarate (MF) cycle. Carbon from methane is introduced into the fumarate (indicated in red), and from CO_2 into the pyruvate (blue) with the formation of a C–C bond. The inset shows options for the oxidative transformation of 2-methylsuccinate to citramalate with hematite (Fe_2O_3) and nitric oxide (NO) as oxidants. The chemical potential of hydrogen in the environment determines the equilibrium shift in the reactions succinate ↔ fumarate and 2-methyl succinate ↔ mesaconate.

Insertion XII: We propose a simplified model of the methane-fumarate (MF) cycle, Fig. 2, originated in the reductive Archean hydrothermal systems, at a high partial pressure of endogenous methane (facies II, Figure 1). Cycle is initiated by the reaction of fumarate + methane → 2-methylsuccinate. In the hydration and dehydrogenation or anaerobic oxidation reactions, 2-methylsuccinate is converted to citramalate, which is disproportionate to acetate and pyruvate with cleavage of carbon-carbon bond. Pyruvate is an important "hub" metabolite that is a precursor for amino acids, carbohydrates, cofactors, and lipids in extant metabolic network. The following carbon assimilation reaction in the form of CO2 with the formation of oxaloacetate is a biomimetic analogue of the reverse tricarbonic acid cycle cycle (rTCA) reaction. An α-carboxylation of pyruvate is a critical anabolic pathway in modern biochemistry, which resupplies rTCA cycle intermediates. Oxaloacetate is transformed into fumarate in the reactions of the citrate cycle intermediates. The nonenzymatic flow of some sequence of reactions of the cycle, such as oxaloacetate → malate → fumarate → succinate (rTCA cycle module) has been experimentally confirmed [Muchowska et al., 2017]. The resulting fumarate assimilates methane and begins a new MF cycle, in one turnover of which an acetate molecule is formed from methane and carbon dioxide molecules.: $CH_4+CO_2 = CH_3COOH$, Table. 1. Transformation of fumarate into 2-methylsuccinate introduces into the cycle five-carbon intermediates, such as citramalate and mesaconate, functioning, for example, in the 3-hydroxypropionate CO_2 fixation cycle. The autocatalytic nature of the cycle derives from the branching point associated with citramalate cleavage and can be shown by the example of doubling the intermediate as in the reaction: $C_4H_6O_5$ (malate) + $1,5CH_4+2,5CO_2 = 2C_4H_6O_5$ (malate). This type of autotrophic metabolism, as in the case of the acetyl-CoA pathway, can be defined as carboxy-methanotrophic acetogenesis.

The problem of the most energetically unfavorable reaction of 2-methylsuccinate transformation into citramalate (ΔG^0_{298} = 96.57 kJ), Table 2a can be solved by using oxidants, such as oxides of nitrogen and iron (Fig. 2, inset). NO is a much stronger oxidant, however, and the reaction with Fe_2O_3 becomes thermodynamically more favorable with increased temperature. Anaerobic methane oxidizing branch of cycle represents the transformation of fumarate to fumarate, pyruvate and acetate, the calculation of the free energies of which with various inorganic oxidants is given in Table 2b.

Clarification to Comments of Anonymous Referees #1and #2 and Reply

A number of questions raised by Referees # 1and # 2 are answered by previous comments to the them and in responses to Referee # 3 comments. Corrections made to the text. The question raised by Referees about the mechanism of activation of a very stable methane C–H bond is briefly discussed in Manuscript insert I.

Briefly consecrated a problem raised by Sébastien Fontaine, how first metabolisms might occur in absence of cellular organization?

Insert XIII:

Our understanding of the emergence of methanotrophic metabolism is within the framework of the hydrothermal theory for the origin of life with all its advantages (continuous flow of energy and matter, the temperature gradient, great possibilities of homogeneous and heterogeneous metal catalysis). Before the occurrence of cellular structures, the primary autotrophic metabolism on the surface of minerals created the space of competing autocatalytic carbon fixation cycles. The accumulation of "biomass" probably led to the emergence of heterotrophic protometabolism and the creation of a certain matrix of the organo-mineral system in which a cascade of proto-biochemical redox reactions could occur, such as in the modern soils (Kéraval et al., 2016). Regardless of the specific mechanism of the functioning of the precellular autotrophic metabolism ("reductive surface pyrite pulled world" (Wächtershäuser, 1988), "hydrothermal reactor" (Russell and Martin, 2004,), "organo-mineral matrix" (Kéraval et al., 2016), and others) its origin and development was subject to the laws of aqueous thermodynamics.

Since the time of Urey and Miller experiments, the discussion about the redox conditions of the early Earth has continued. We consider it sufficiently reductive for the emergence of a "hydrocarbon metabolism" and present the existing arguments for this.

While maintaining the general idea, the article was edited taking into account the comments and suggestions of associate Editor and Referees # 1, # 2, and # 3.