Palaeoecophylostoichiometrics
Searching for the Elemental Composition of the Last Universal Common Ancestor

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Summary: Extant life on Earth shares a common ancestor that probably existed $\sim$4 billion years ago. We describe a novel approach to study the root of the phylogenetic tree of life by measuring the elemental abundances of living organisms and their environment.

We discuss the conservation of different elemental ratios across different taxa. It may be possible to use stoichiometric and phylogenetic relationships between taxa to investigate how changes in the distribution of elements in environments such as the oceans, may have affected the evolution of life over the past $\sim$4 billion years – particularly during the great oxidation event $\sim$2.4 billion years ago.

We outline some strategies to identify modern analogues of geological sites where the common ancestor may have existed and where life first evolved from its pre-biotic origins on Earth and possibly elsewhere in the universe.

Introduction

Darwin's book [1] contained a single figure with hypothetical branches of descent for organisms. Today, Darwin's branches have grown into the universal tree of life, where all extant and many extinct organisms, have found a place [4]–[6]. At the root of the tree, is the Last Universal Common Ancestor (LUCA) (Figure 1) [7], [8].

Biogeochemists are beginning to connect the major patterns of evolutionary diversification seen in the phylogenetic tree with environmental events in Earth's history [9], [10]. The rise in O_2 $\sim$2.4 billion years ago [11], was coupled to changes in the biogeochemical cycles of elements like iron, which was oxidised from soluble Fe^{2+} to form insoluble Fe^{3+} precipitates, reducing the amount of free soluble iron available to life in the oceans. Changes in oxygen levels and metal concentrations in the oceans between $\sim$2.4 - 1.8 billion years ago would have led to changes in the distribution of metal ions inside cells (the metallome). Metals play a role in almost all metabolic pathways (providing structure and catalytic activity to enzymes) and life would have adapted to the changing conditions leading to new metabolic pathways [12]–[16].

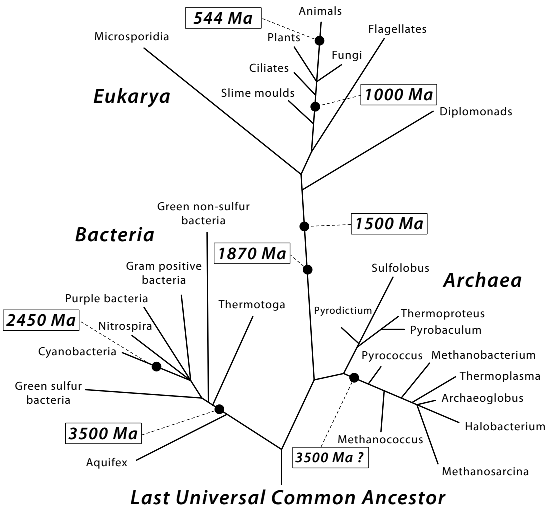

Fig. 1: Phylogenetic relationships of extant organisms, as inferred from sequence comparisons of 16S ribosomal RNA genes. The boxed dates indicate the minimum age of selected branches, based on deductions from molecular biomarkers, isotopic signatures of biogenic materials, body fossils and molecular clocks. Adapted from [9] and based on [8].

In comparison to techniques available just a few decades ago, it is possible to measure the elemental composition (the stoichiometry) of life forms and their environments much more easily and to much higher precision. Most of the stable elements in nature can be measured to approximately parts per billion concentrations, using less than a few grams of material.

Our approach, termed *Palaeoecophylostoichiometrics*[1], is based on quantifying the stoichiometry of extant bacteria and archaea, known from phylogenetic studies to be most closely related to LUCA. It could reveal new insights into the links between changing paleo-ecologies and early evolution of life and may help to identify the geochemistry of the site for the origin of life.

Stoichiometry of life

Although life is made of elements from its environment and exhibits similar elemental abundances to that of its environment, it is at disequilibrium with the environment. Some elements like carbon, nitrogen and phosphorus are more abundant in life than in the environment, while elements like sodium and potassium are less abundant in life compared to the environment. In [17], we present the view that life may use the disequilibrium in the elemental abundances relative to the environment for its metabolic reactions.

Every organism will have an elemental composition that is dependent on its metabolic activity. If the organism's oxygen carrying protein in the blood is hemoglobin that uses iron (as in humans), instead of hemocyanin that uses copper (as in horseshoe crabs [18]), the organism will probably have a higher abundance of iron than an organism using hemocyanin.

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[1] *Palaeoecophylostoichiometrics* is a modification of 'Paleoecostoichiophylogenomics' introduced by Elser in [21] to describe an emerging sub-discipline that links biogeochemical and genomic studies.

The elemental abundances in an organism will also be affected to some extent by the abundances in the environment. Individuals living in an environment with high concentrations of mercury will probably exhibit increased abundances of mercury than individuals living in environments with low mercury concentrations. Due to slight non-specificity in the mechanism of selectivity between metal ions, elements like lead and strontium can be accumulated in the body as a result of calcium intake, even though they may not be utilised by life. Such environmental effects will introduce noise to any signal that is due to the metabolism of the organism. However, we hypothesise that despite such environment-dependent variations, if the environmental factors are quantified and controlled, there will be signals in the metallome that can be used to distinguish between the physiology and evolutionary history of different taxa. These signals could be based on the relative abundances of elements within the organism and differences between these abundances compared to the abundances found in the environment (such as the growth medium).

Redfield found in the Western Atlantic that stoichiometric ratios of both phytoplankton and the surrounding water column were in ratios of C_{106}:N_{16}:P_1, by atoms [19]. The similarity in the ratios of phytoplankton and the water column indicated a balanced flow of C, N and P in and out of the environment.

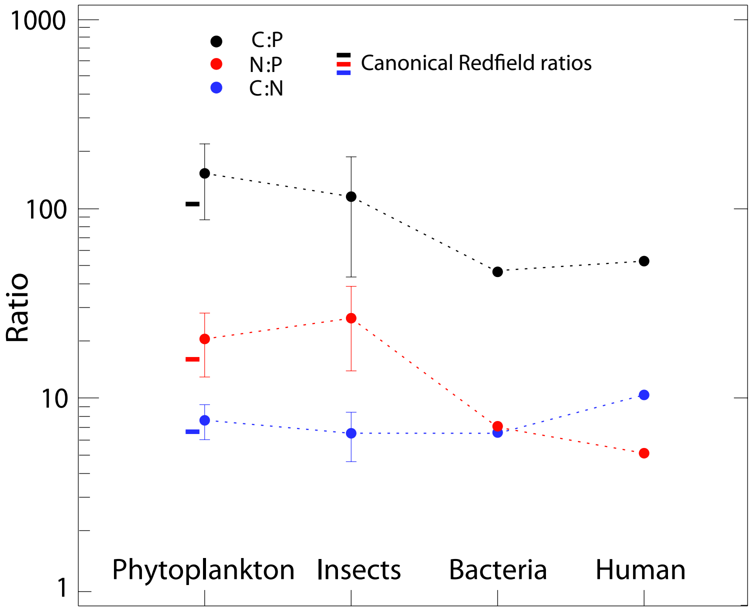

Fig. 2: The C:N:P ratios are an example of a stoichiometric signal. Variations from the canonical Redfield ratios [19] for phytoplankton reflect differences in the metabolic pathways, environments and evolutionary histories of each taxa. Ratios given are by number of atoms and the uncertainties correspond to standard deviations. "Phytoplankton" represents marine phytoplankton from surface waters at coastal and off-shore sampling sites around North America [20]. Insects, represent whole-body measurements of different species of land insects [21]. Bacteria abundances are representative of 7-40 species depending on the element [22]. Human abundances are from a study involving about 150 individuals [23]. The uncertainty in measurements for bacteria and humans have not been quantified by the authors. We have used data for phytoplankton and insects from [20] and [21] as they were two of the most comprehensive data sets which have uncertainties associated with them. Elemental abundances for other taxa, especially in Bacteria and Archaea have not been well quantified.

Results in [24] suggest that bulk elemental compositions reflect phylogenetic differences in two phytoplankton plastid superfamilies, those that use chlorophyll-*b* and those that use chlorophyll-*c* as the major photosynthetic pigment [25]. While both plastid families share common metabolic functions, the C:N:P stoichiometries of different phytoplankton reflect different ancestral pre-symbiotic host cell phenotypes, while the abundances of trace elements like iron and copper probably reflect differences in the evolution of the acquired plastids in different ocean redox states since the Proterozoic era.

We hypothesise that some stoichiometric signals will be conserved between different taxa. Stoichiometric signals are likely to be more similar within low taxonomic orders such as species and genera and less similar between higher taxonomic orders such as phyla, kingdom and domain. In order for a stoichiometric signal to be useful, the level of conservation of the stoichiometric signal must be such that it is sufficiently distinct for particular taxa. Indeed, if the signal remained exactly the same in every taxa, it would not be possible to distinguish between two taxa. In contrast, if the abundances were randomly distributed then it would be impossible to relate different taxa by stoichiometric signals.

Looking back in time

There are a number of features in life on Earth that have been recognised as being conserved over timescales of millions to billions of years. One widely used feature of life on Earth that is used to represent evolutionary descent, in the form of a phylogenetic tree (Figure 1), is the 16S ribosomal RNA gene (16S rRNA). The 16S rRNA's highly conserved sequence is attributed to its essential signalling and structural role in protein synthesis from a genetic code. Phylogenetic trees that are based on other conserved molecules such as transfer RNAs and proteins, are topologically similar to 16S rRNA trees [26]–[30].

Genetic and proteomic conservation translates to conservation of metabolic pathways [31]. Therefore, the stoichiometry of elements that make up proteins in metabolic pathways will be conserved to varying degrees across taxa. We expect that stoichiometric relationships between taxa will, to some extent, reflect phylogenetic relationships established by genomic and proteomic studies [32]–[37]. Figure 3 shows that the relative proportions of the chemical elements in organisms like humans and bacteria, that represent two different domains of life (Eukarya and Bacteria), are similar [17], [38], [39].

In some cases, elemental composition may be more conserved than biomarkers such as lipids or genetic markers such as the 16S rRNA sequence. For example, until recently viruses were not considered part of most phylogenetic trees as they do not contain 16S rRNA [6], [27], [40]–[43]. However, their DNA or RNA based genes, proteins and lipids are made of the same chemical elements that are used by other life forms.

Rooted phylogenetic trees can have their branch lengths (distance between two nodes) calibrated for inferring the extent of divergence of species from their common ancestor. The shorter the branch length of an extant species from the root of the tree, represented by LUCA, the more it is likely to resemble LUCA. By studying elemental abundances in bacteria and archaea that have short branch lengths from LUCA, it may be possible to infer the elemental compositon of LUCA and its environment [44], [45].

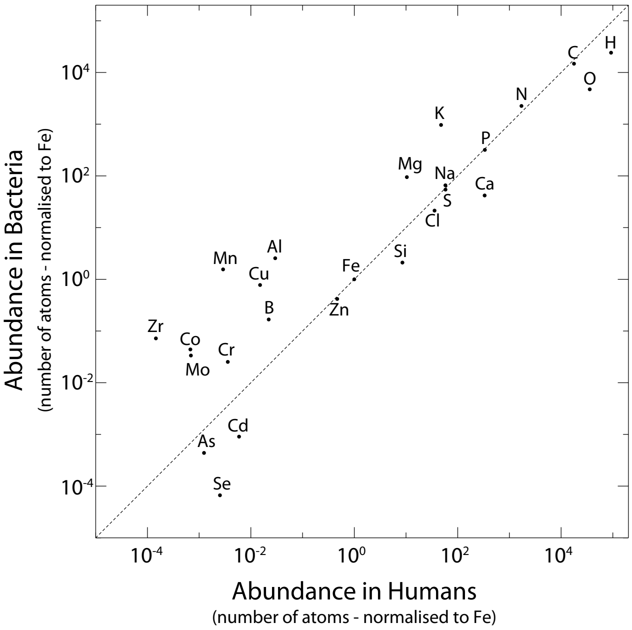

Fig. 3: A positive correlation between elemental abundances (by number of atoms) in the representatives of two domains of life, Bacteria (bacteria [22]) and Eukarya (humans [23]). Uncertainties were not quantified by the authors. The abundances are normalised to iron.

It is possible to use branch lengths as a proxy for divergence times if the rate of evolutionary change is known [46]. The rate is often measured by the number of substitutions in a conserved genetic sequence like 16S rRNA but amino acid sequences for proteins can also be used. However, this is not always possible as molecular clocks are not constant across taxa and over long timescales [47], [48]. The issue can be resolved to some extent by calibration against fossil and biogeochemical records [9], [49]–[51]. By studying bacteria and archaea near the root of phylogenetic trees, it may be possible to relate evolutionary changes in the metallome of life to changes in the environment.

Analyses of the elemental composition of taxa with respect to branching order from the root of the phylogenetic tree can be used to determine if there is any identifiable trend of life using a larger suite of elements as a function of time. Those species that are deeply rooted in the tree may utilise fewer elements in their metabolic pathways compared to species that have branched out further from the root. Figure 4 shows a suggestive model that represents the total number of elements in use across all life forms on Earth, as a function of time.

Life may have begun with a basic set of elements and then progressively increased its elemental ‘toolkit’. Initially, life may have been based on abundant elements like carbon, hydrogen and oxygen and ions like calcium or boron to stabilise its structures [53], and catalytic metals like iron [54], [55]. This increase may be in response to changes in the bioavailability of elements.

Alternatively, just as a novice chef starts baking a cake with simple ingredients like flour, eggs and sugar, life may have started with the most abundant (or bioavailable) elements like carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. Later, life began to accessorise with more elements like bromine and selenium – like an experienced chef who has learnt to use vanilla, cinnamon and the rest of the spice rack.

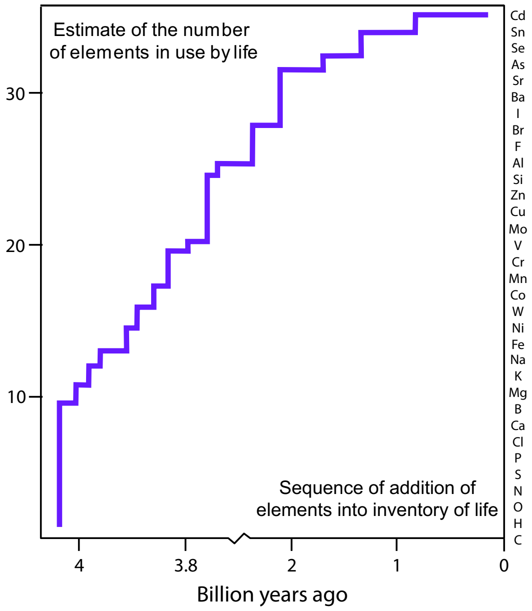

Fig. 4: A possible sequence of increasing diversity of elements used by life based on estimates of abundances in oceans and discussion in [36]. The order of the elements on the right-hand-side y-axis is speculative but plausible.

Mediated by processes such as endo-symbiosis [56] and horizontal gene-transfer [57], recipes would have been exchanged between different life forms at different times over the past 4 billion years, resulting in usage of new elements and new metabolic pathways. Not shown in Figure 4, there may have been instances of a transient decrease in the number of elements in use by life, due to factors such as extinction of particular taxa or large decrease in the bioavailability of an element, even if for short timescales. Whether our research or others will be able to resolve such transient decrease in the number of elements used by life remains to be seen.

Tracing the evolution of life

Williams and da Silva [58] have suggested that the major changes from the first life form(s) to present life forms were linked to the changing chemistry of the external environment. For example, the development of enclosing membranes provided a stable physical and chemical internal environment for sustaining metabolic reactions of the earliest prokaryotic life form(s).

Most origin of life hypotheses require a redox coupled energy source as the earliest metabolisms. An abundant source of a redox potential accessible to life on the Hadean and early Archean Earth, $\sim$4.5 to $\sim$3.5 billion years ago, are crustal minerals or the redox gradients at hydrothermal vents [52]. These sites most likely had a host of organic molecules such as amino-acids and nucleotides that formed *in situ* or were brought in by chondritic and cometary material and could have formed auto-catalytic polypeptides and nucleaic acids that developed into the information and metabolic system of the first of the single-celled organisms [55]. On depletion of the initially easily available free energy sources, other metabolisms evolved and some life forms developed the ability to perform oxygenic photosynthesis [59].

Oxygenation of the atmosphere ~2.4 billion years ago [11] by efficient oxygenic photosynthetic organisms, led to the oxidation of metals in the environment. The resulting oxidised metals would be incompatible with a reducing cytoplasm, that would have reflected the reducing chemistry of the primitive oceans and atmosphere from which it formed. Isolation of the incompatible pathways within internal compartments through the gradual introduction of vesicles in cells from localised invagination or endosymbiotic events may have resulted in eukaryotic life forms with organelles and a nucleus [59]–[61].

Changes in the environmental abundances would affect the elemental composition of and probably the usage of elements in different taxa that over time would lead to the evolution of new metabolic pathways. If the concentration of a metal used by life decreases in the environment such that an alternative metal is more competitive in terms of bioavailability, reaction rate or reaction efficiency, then life will probably co-opt the alternative metal in a modified metabolic pathway.

For example, the rise in atmospheric oxygen concentration in the period ~2.4 to ~0.8 billion years ago has been linked to changes in the chemical components of the oceans (see Figure 5). This period also corresponds to an increase in number of enzymes that use metals such as copper and molybdenum [32], [36]. As soluble Fe^{2+} was oxidised to Fe^{3+} (which forms insoluble precipitates of $Fe(OH)_3$), the availability of free or complexed ions of biologically active iron would have decreased substantially in the oceans in the period ~2.4 to ~0.8 billion years ago. The oxygenation also oxidised another biologically active metal, copper from its Cu^{+} to Cu^{2+} state.

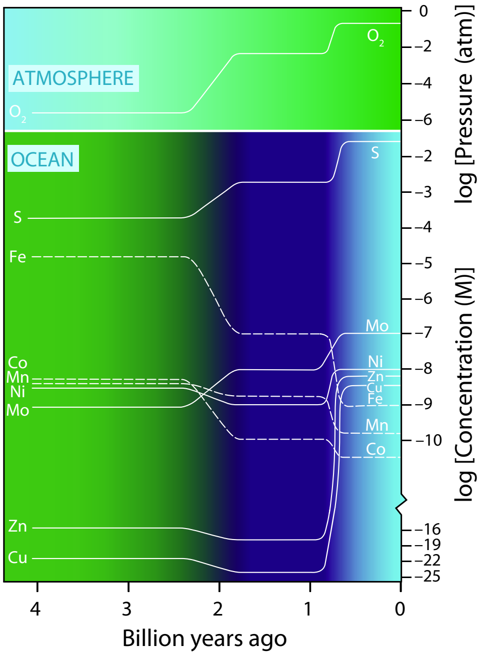

Fig. 5: The increase in O_2 concentration of the atmosphere (top) affected the concentration of metals and other ions in seawater (bottom). Modified from Anbar et al. [33] and based on geochemical models and inferences from ancient sediments. Dashed lines are for elements with falling concentrations. Color gradations indicate a transition from anoxic oceans before ~2.4 billion years ago (green) to H_2S-rich oceans between ~1.8 billion and ~800 million years ago (purple), subsequently giving way to complete ocean oxygenation (light blue). [M: moles per litre; atm: atmosphere]

This change in the environment may have triggered the development of new metabolic pathways which harnessed the electrochemical potential of the higher oxidation state of copper for roles previously performed by iron [58].

As the availability of metals such as manganese, zinc and cobalt increased in the environment, new metabolic pathways using these metals arose in both prokaryotes and eukaryotes [35]–[37], [61], [64]. An example is the metalloenzyme superoxide dismutase, which is a detoxifying agent to remove the O^{2-} anion, a by-product of metabolic reactions involving one electron reduction of O_2. Most eukaryotes use a form of the enzyme with copper and zinc metal centres at the active site. However, most prokaryotic organisms and mitochondria contained in eukaryotes[2] contain a form with manganese and or iron. The concentration of iron and manganese decreased after the oxygenation $\sim$2.4 billion years ago and it may explain the emergence of copper- and zinc- based superoxide dismutases in eukaryotes [63].

Since, the elemental abundances in an organism reflects to some extent the elemental abundances in its environment [17], it may be possible to suggest geological sites where LUCA could have existed and help identify modern analogues of geological sites where the origins of life on Earth could have taken place (Figure 6). For example, if LUCA is inferred to have contained and utilised boron, it could add support to the borate-minerals-stabilising-ribose model [53], necessary for efficient synthesis of RNA in the transition from prebiotic to biotic life.

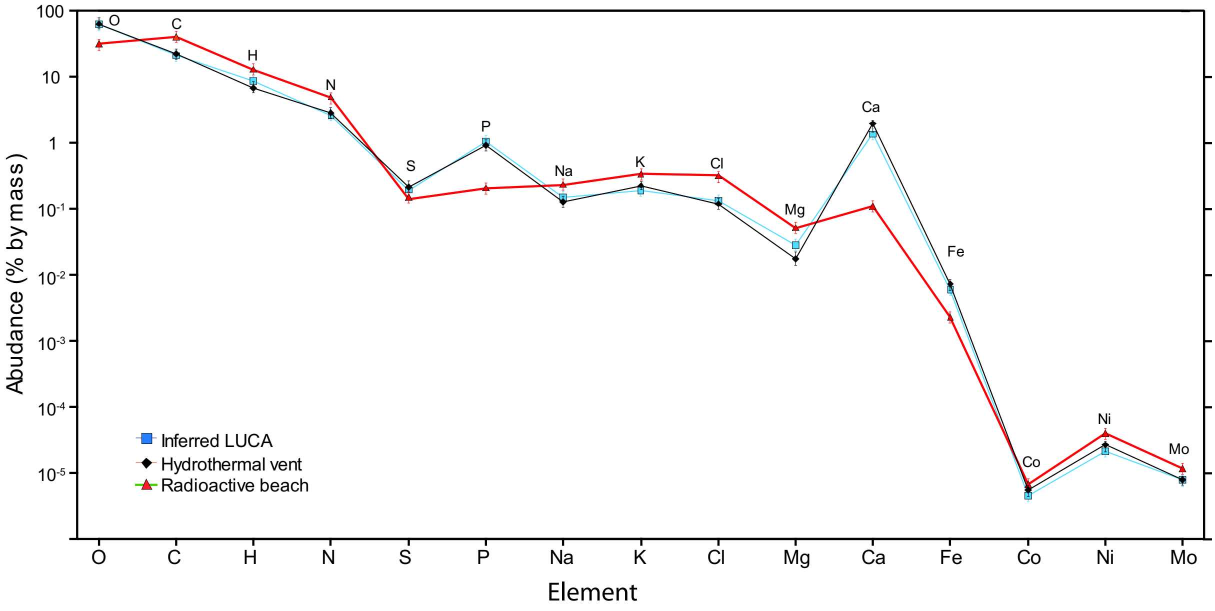

Fig. 6: An environment for LUCA and the origin of life, may be determined by comparing abundances in LUCA and two candidates sites. Abundances and uncertainties are hypothetical. The abundances for a hydrothermal vent show stronger correlation to abundances in LUCA than the correlation between the radioactive beach and LUCA. The result would suggest that hydrothermal vents are more likely than radioactive beaches to represent the geochemistry of the site for the origin of life [54], [55], [69].

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[2] Mitochondria contained in eukaryotes are believed to have been derived from endosymbiosis between a eukaryote and a prokaryote (α-proteobacterium) approximately $\sim$1.7 to $\sim$2 billion years ago [56], [62].

The Moon-forming impact $\sim$4.5 billion years ago would have sterilised Earth of any extant life at that time [67]. LUCA probably existed $\sim 4.2 \pm 0.4$ billion years ago [68]. Therefore, any inferences about the environment of LUCA will at best relate to a few hundred million years after the Moon forming impact.

We are not attempting to examine past extinct life but rather inferring the evolutionary history of extant taxa. Metabolic pathways in extant taxa are often conserved in the sense that they reflect the pathways in use by the taxa when the taxa first appeared on Earth. However, particular lineages may have gone extinct or metabolic pathways may not be sufficiently conserved within taxa due to responses to environmental changes over billions of years. In future analysis, we will establish the extent to which extant basal organisms preserve a record of the metabolisms in use up to $\sim$4 billion years ago.

Although the geochemistry of past environments over the past $\sim$4 billion years is not well established, there are some constraints on elemental abundances in the oceans, especially in the proterozoic era ($2500 - 550$ million years ago) based on the geological record and biogeochemical evidence [33], [34], [36]. It is also possible to correlate metal availability in the environment to the evolution of metabolic pathways as in [32].

We have not established the extent to which environmental abundances affect the elemental composition of and usage of elements in different taxa. In future analyses we will investigate if there are signals that are large enough to be detected over the noise introduced by fluctuations in the abundances in the environment.

Sun → Earth → Crust → Life

All atoms that are or have been part of living matter on Earth have either been produced during big bang nucleosynthesis [72] or in different processes of the stellar nucleosynthetic pathways that take place in stars [73]. Our Sun was formed out of a collapsing molecular cloud that was polluted by earlier stellar processes and the proto-planetary disk that was made of the remaining dust eventually gave rise to terrestrial planets like the Earth.

The composition of solar system objects including other planets and asteroids reflects to a large extent the composition of the Sun, except that relative to the Sun, all are depleted in the most volatile elements hydrogen, helium and the noble gases. When the Sun formed from the solar nebula, $4.5672\,(\pm\,0.0006)$ billion years ago [74], the solar wind caused the depletion of volatile elements such as hydrogen and helium from the region of the solar nebula where terrestrial planets formed. Rocky planets like Earth, accreted from the fractionated nebular condensate whose composition (particularly C1 chondrites) closely resembles the solar composition, except for the abundances of volatile elements [75].

Input from chondritic material and a late-veneer of volatile elements due to the impacts of comets and other objects from beyond the snow-line led to a crust which exhibits elemental abundances more like solar abundances, depleted in volatile elements, than the abundances of the bulk Earth. Life on Earth is based on elements such as hydrogen, oxygen, carbon and nitrogen which were added to the crust in form of water, carbon dioxide and ammonia ice in the chondritic and cometary material.

Today, life on Earth is primarily composed of the chemical elements oxygen, carbon, hydrogen and nitrogen, which in humans and bacteria make up $96.8 \pm 0.1\%$ of the mass. Apart from these bulk elements, phosphorus and sulphur together make up $1.0 \pm 0.3\%$ of the mass. The remaining $2.2 \pm 0.2\%$ of the mass is dominated by potassium, sodium, calcium, magnesium and chlorine, while $0.03 \pm 0.01\%$ is attributed to trace elements such as iron, copper and zinc.

Life on Earth is based on the most abundant elements in its environment. Life does not reside in the mantle or the core of the Earth and so its elemental abundances are more reflective of abundances in the upper crust (specifically the biosphere) than abundances in the bulk Earth.

The process of formation of a star like the Sun, out of a collapsing molecular cloud polluted by heavy elements from earlier generation of stars, is observed wherever there are molecular clouds. The associated process of terrestrial planet formation is probably common in the universe and it is likely that the elemental abundances of the surfaces of extrasolar habitable planets will also be similar to cosmic abundances as represented by the Sun.

Since, the abundance of most elements in life forms and their environments on Earth follow cosmic abundances (Figure 7), perhaps extraterrestrial life will also exhibit elemental abundances similar to those found in life on Earth [38]. Alternatively, if extraterrestrial life is to be found on planets and moons with environments where elemental abundances are different to those found on Earth, it may be possible to predict the elemental abundances and metabolic processes of the extraterrestrial life based on the stoichiometric principles of life on Earth.

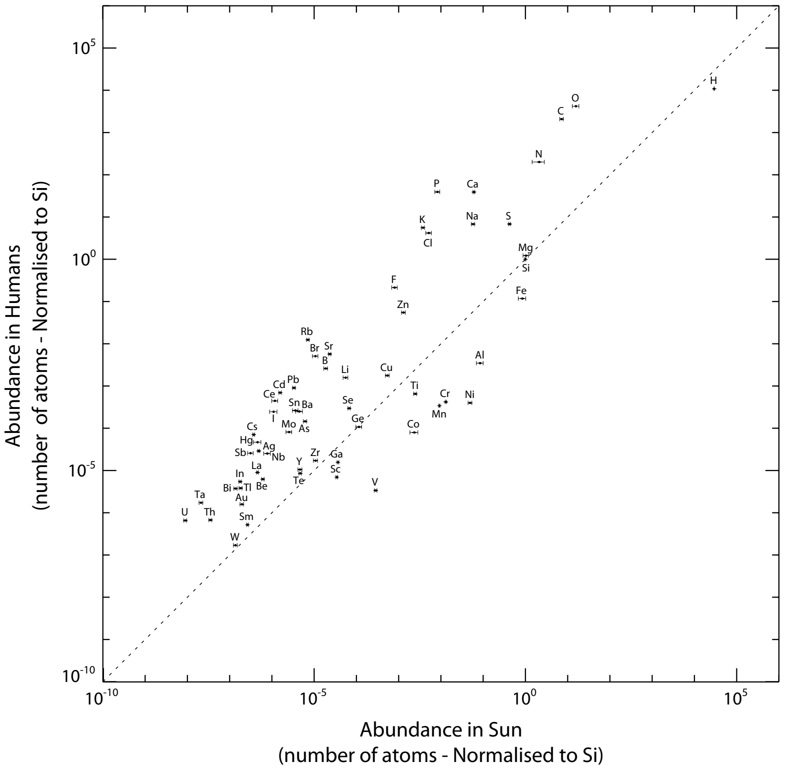

Fig. 7: A positive correlation between elemental abundances (by number of atoms) in life, as represented by humans [23], and the Sun [75]. The abundances are normalised to silicon.

Our approach to studying the origins and evolution of life by measuring the elemental abundances of extant taxa and their environment will requires a sufficiently large data set looking at a number of taxa with relatively small uncertainties associated with the measurements. A major shortcoming of existing data in the literature was that measurements were made many decades ago, before the availability of high precision analyses offered by analytical techniques today. It is possible that the errors associated with previous measurements of abundances of some elements could be in the range of a few orders of magnitude.

Results from authors such as Sedykh et al. [70], who used Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) techniques and Heldal et al. [71], who used X-ray microanalysis to measure elemental abundances of about 10 elements in cyanobacterial species, suggest that once culturing and analytical methods have been standardised, it will be possible to quantify elemental abundances to a precision of 5-10%, in a high-throughput method.

If the effect of environmental variations on elemental composition of taxa is sufficiently small then we may be able identify elemental ratios that are conversed across different taxa and correlate changes in the bioavailability of elements in environments with evolution of metabolic pathways.

Palaeoecophylostoichiometrics could help us understand LUCA and its environment and the principles that we learn about life on Earth could be applicable elsewhere in the universe.

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