mRNA, rRNA and DNA Quantitative Stable Isotope Probing with H218O Indicates Use of Old rRNA among Soil Thaumarchaeota

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mRNA, rRNA and DNA quantitative stable isotope probing with H$_2^{18}$O indicates use of old rRNA among soil Thaumarchaeota

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**ABSTRACT**

RNA is considered to be a short-lived molecule, indicative of cellular metabolic activity, whereas DNA is thought to turn over more slowly because living cells do not always grow and divide. To explore differences in the rates of synthesis of these nucleic acids, we used H$_2^{18}$O quantitative stable isotope probing (qSIP) to measure the incorporation of $^{18}$O into 16S rRNA, the 16S rDNA, amoA mRNA and the amoA gene of soil Thaumarchaeota. Incorporation of $^{18}$O into the thaumarchaeal amoA mRNA pool was faster than into the 16S rRNA pool, suggesting that Thaumarchaeota were metabolically active while using rRNA molecules that were likely synthesized prior to H$_2^{18}$O addition. Assimilation rates of $^{18}$O into 16S rDNA and amoA genes were similar, which was expected because both genes are present in the same thaumarchaeal genome. The Thaumarchaeota had significantly higher rRNA to tDNA ratios than bacteria, though the $^{18}$O isotopic signature of thaumarchaeal rRNA was lower than that of bacterial rRNA, further suggesting preservation of old non-labeled rRNA. Through qSIP of soil with H$_2^{18}$O, we showed that $^{18}$O incorporation into thaumarchaeal nucleic acids was generally low, indicating slower turnover rates compared to bacteria, and potentially suggesting thaumarchaeal capability for preservation and efficient reuse of biomolecules.

**1. Introduction**

It is axiomatic in biology that DNA replicates in proportion to cellular division (Marstorp and Witter, 1999). The production of ribosomal RNA (rRNA) is closely coupled to DNA replication because the dividing cells require high rates of protein synthesis (Ruimy et al., 1994), though new rRNA may also be synthesized to maintain a subset of proteins when a cell is not dividing (van Bodegom, 2007). Dormant cells generally have relatively low levels of rRNA because they synthesize fewer new proteins (Lennon and Jones, 2011). In pure cultures, rRNA can be relatively stable during exponential growth (Meselson et al., 1964), during stationary phase (Piir et al., 2011), as intact 70S ribosomes in prokaryotes (Zundel et al., 2009), or as 100S dimers (Wada, 1998). Alternatively, rRNA may be degraded in starved cells or at the transition point into stationary phase, when it can be used as a source of nutrients (Deutscher, 2003). As a cell transitions from dividing to a maintenance state, rRNA declines while the DNA concentration remains constant (Deutscher, 2003; Hsu et al., 1994). Messenger RNA (mRNA) turns over faster than rRNA in a cell (Brenner et al., 1961), and its instability is important for regulation of gene expression and rapid responses to environmental changes (Belasco and Higgins, 1988; Jain, 2002; Steege, 2000). Regulatory RNAs that control persistence of specific mRNAs are ubiquitous in microorganisms. While there are several mechanisms through which mRNA can be temporarily stabilized (Wong and Chang, 1986; Kushner, 2002; Hambraeus et al., 2003; Lee et al., 2003; Baker and Condon, 2004), degradation of mRNA by ribonucleases occurs continuously (Deutscher, 2006). Decay and synthesis of mRNA molecules are carefully controlled to optimize cell survival, growth, performance or metabolic activity.

Some studies (Malik et al., 2015) have examined the turnover of nucleic acids in soil but generally these studies are rare. Characterizing nucleic acids synthesis in soil is challenging because soil has many microhabitats, which can provide highly diverse physico-chemical conditions with notable effects on microbial growth and metabolism. Unlike a cell culture, an environmental sample will contain both older and newly formed nucleic acids. Upon cell death in the environment, the DNA can degrade, be assimilated, or adsorb to surrounding particles (Dlott et al., 2015; Morrissey et al., 2015). Ribosomal RNA is likely less...
persistent than DNA in environmental samples. Rates of mRNA synthesis in environmental samples are likely higher than rates of DNA synthesis, because some protein synthesis is related to cellular maintenance and activity, not growth. For these reasons, turnover of mRNA in environmental samples is presumed to be rapid, more rapid than turnover of DNA, though empirical tests of this hypothesis are rare. Here, we used quantitative stable isotope probing (qSIP) experiments with H$_2^{18}$O (Hungate et al., 2015) to measure synthesis rates of microbial nucleic acids in environmental samples. We quantified the $^{18}$O incorporation into nucleic acids of individual thaumarchaeal taxa and used it to estimate nucleic acid synthesis rates.

Ammonia oxidizing Thaumarchaeota (Könneke et al., 2005; Schleper et al., 2005; Venter et al., 2004) have a cosmopolitan distribution, are found in a wide range of habitats including aquatic (Kirchman et al., 2007; Teira et al., 2006; Yakimov et al., 2009) and terrestrial ecosystems (Treusch et al., 2005; Kemnitz et al., 2007), and have a complex chemolithoautotrophic physiology (Hatzenpichler, 2012). They have been found to outnumber ammonia-oxidizing bacteria (AOB) in many ecosystems (Leininger et al., 2006; Adair and Schwartz, 2008; Chen et al., 2008; Shen et al., 2008), suggesting an important role in the nitrogen cycle. They can be detected and quantified through measurements of 16S rRNA or amoA gene sequence abundance (Francis et al., 2005). By studying this group of organisms in a quantitative stable isotope probing experiment, it is possible to measure the turnover of DNA, rRNA and mRNA in individual microbial taxa. In the present study, we investigated transcription and replication of the 16S rDNA and the amoA gene of soil Thaumarchaeota through H$_2^{18}$O mRNA, rRNA and DNA-qSIP. Specifically, we measured the incorporation of $^{18}$O into newly synthesized 16S RNA and 16S rDNA as well as into the amoA gene and its transcripts. For Thaumarchaeota, amoA gene transcription indicates cellular activity related to ammonia oxidation, which is central to metabolism for this particular group of organisms. In contrast, 16S rRNA transcription is required for any prokaryotic cell to construct protein synthesis machinery. We hypothesized that replication of both genes would be highly correlated, but that transcription rates would differ.

2. Materials and methods

2.1. Sample collection

Soil samples were collected from three separate locations (N: 34° 57′21.879′′ W: 111° 45′14.859′′; N: 34° 57′21.289′′ W: 111° 262′45′14.189′′ and N: 34° 57′21.591′′ W: 111° 45′14.683′′) in a semi-arid ecosystem near Sedona, Arizona, USA in April 2014. The soil was characterized as a sandy loam (average pH of 6.95 ± 0.42, soil moisture content of 13.7 ± 1.7%, and concentrations of NO$_3$ -N and NH$_4$ -N of 4.11 ± 0.24% at the time of sampling, organic matter content of 120 μg/g, and density of 1.73 g/ml, for DNA) density gradients. Samples were separated into approximately 120–150 μl fractions and purified as described in Papp et al. (2018a and 2018b). Briefly, RNA fractions were incubated with 200 μl of isopropanol and 100 ng glycogen at −20 °C overnight, and DNA fractions were incubated with 200–300 μl of water, 300–450 μl of isopropanol and 100 ng of ultra-pure glycogen at room temperature overnight. Fractions were then precipitated with 70% ethanol and resuspended in 20–50 μl 1X TE buffer. RNA from purified fractions and from non-fractionated total RNA extracts was reverse transcribed into complementary DNA (cDNA) using Maxima H-Minus First Strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA) and random pentadecamer primers (Eurofins MWG Operon, Huntsville, AL). Extracted nucleic acids and corresponding fractions were stored at −80 °C until further processing.

2.3. Sequencing

Sequencing of 16S rRNA gene fragments in cDNA and DNA samples was performed on the Illumina MiSeq platform at the Environmental Genetics and Genomics Facility (EnGen) at Northern Arizona University using the 2 × 150 paired-end read chemistry and 515F (5′-GTGACCAAGCMGCCGCGGTAA-3′) and 806R (5′ – GGACTACVSGGGT-ATCTTAAT – 3′) primers. Sequencing data were analyzed using a Quantitative Insights into Microbial Ecology (QIME) -based (Caporaso et al., 2010) chained workflow (Krohn, 2016). A detailed description of sequencing data processing steps is available at https://github.com/alk224/akutils-v1.2 and in Papp et al. (2018a and 2018b).

2.4. Arch-amoA qPCR

Standards for the archaean amoA qPCR assay were made with DNA purified from qSIP fractions. The archaean amoA gene was amplified in 20 μl reactions containing 2 μl of template and 7.32 μl of RNase-free water, 1X Phusion Mastermix (Water, 10X RedJuice (40% 1M Tris, pH 8.5/phenol red and 60% glycerol), 5X HF Buffer, 10 mM dNTPs, Phusion HS1 polymerase). 1.5X MgCl$_2$ and 0.2 μM of a modified archaean amoA primer: Arch-amoaAFadaptor (5′-caacaggaagacggcatacgagstaATGTTGCTGCTTAGAGC-3′) and Arch-amoaARadaptor (5′-caacaggaagacggcatacgatGGCGGCATCCATTCTGTA TGT-3′). These primers were modified by attaching the 5′caacaggaagacggcatacgat′ 3′ adapter sequence to the 5′end of the archamoA primer to ensure that the amoA primer binding sites were not at the ends of the standard templates. Amplification was carried out in a 96-well plate with cycling conditions starting at 95 °C for a 2-min denaturation step, followed by 30 cycles of 30 s at 95 °C, 1 min at 55 °C, and 1 min at 72 °C. After 30 cycles there was a final extension for 3 min at 72 °C followed by an indefinite hold at 4 °C. A subset of 18 PCR products were randomly selected and visualized by gel electrophoresis. All 96 products were subsequently pooled into a 5 ml tube and purified with magnetic beads (0.1% carbonyl-modified Sera-Mag Magnetic Speedbeads, Thermo Fisher Scientific, Freemont, CA) in 18% PEG following a standard bead cleanup protocol with two ethanol washes (DeAngelis et al., 1995), and eluted into 200 μl Tris-Cl (pH 8.0). These PCR products were visualized by gel electrophoresis, and purified using Qiaquick Gel Extraction Kit (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. Purified products were again visualized by gel-electrophoresis and quantified with Quant-it PicoGreen double-stranded DNA assay kit (Life Technologies, Grand Island, NY) on a BioTek HT Plate reader (BioTek, Vinooski, VT). Serial dilutions, ranging from 10$^6$ to 10$^5$ gene copies per μl, were used as the standard curve for assay optimization. They were analyzed in triplicate using a quantitative PCR assay performed on a CFX384 Touch Real-Time PCR Detection System (Biorad, Hercules, CA, USA). Each 10-μl reaction contained 1 μl of template (i.e. diluted standard), or no template control (NTC), 1X Phusion Mastermix, 1X EvaGreen dye (Biotium), 1.5X MgCl$_2$ and 0.2 μM of each primer: Arch-amoaAF (5′-
2.5. Incorporation of \(^{18}\)O into nucleic acids

Fraction density within a sample was corrected based on \(^{18}\)O- IRMS data (Table S1) as described in detail in Papp et al. (2018a). Briefly, nucleic acids were quantified, diluted with a salmon sperm DNA solution to 200\(\mu\)g oxygen and pipetted into silver capsules (Costech Analytical Technologies Inc., Valencia, CA) in a tray, which was set on a heat block at 50 °C for water to evaporate. Capsules were then closed, weighted, and sent to the Stable Isotope Facility at University of California, Davis, for isotopic analysis. The corrected densities were used for further calculations. Calculations of (1) taxon-specific weighted average densities (WAD), (2) weighted averages density shifts (WAD\(_f\)) and (3) atom percent excess \(^{18}\)O (APE \(^{18}\)O) of nucleic acids, including amoA mRNA, amoA gene, 16S rRNA, and 16S rDNA, were carried out manually. Subsequently, they were also confirmed in R (R Core Team, 2014), using a code available at https://bitbucket.org/QuantitativeSIP/qsip_repo, and described in Hungate et al. (2015).

2.5.1. Weighted average density shifts of nucleic acids

All calculations and equations used for determining weighted average density (WAD) shifts of microbial nucleic acids (16S rRNA and rDNA) were previously published in Papp et al. (2018a). The same equations were used for determining the WAD shifts of 16S rRNA and rDNA for the thaumarchaeal taxa. We calculated WAD of thaumarchaeal amoA gene from a given taxon as the weighted average across density fractions. For a given fraction, we multiplied the relative abundance of that thaumarchaeal taxon (density fractions. For a given fraction, we multiplied the relative abundance of that thaumarchaeal taxon \((\text{density fractions})\) by the number of amoA copies detected via qPCR in that fraction \((\text{amoA copy number})\):

\[
\text{ABS amoA copy} = \text{rel. ab}_\text{amoA} \times \text{amoA copy number}
\]

yielding an estimate of the absolute abundance of amoA copies for a given taxon in a given fraction \((\text{ABS amoA copy})\). The WAD for a given thaumarchaeal taxon \((\text{WAD}_\text{amoA})\) was then calculated as the sum of products of density times the absolute amoA copy number abundance in each fraction, divided by the total abundance across all fractions:

\[
\text{WAD}_\text{amoA} = \frac{\sum_{k=1}^{K} \text{ABS amoA copy} \times \text{density}}{\sum_{k=1}^{K} \text{ABS amoA copy}}
\]

where:

- \(k\) = fraction of a nucleic acid sample
- \(K\) = total number of fractions from a nucleic acid sample
- density = density of a nucleic acid in each fraction

To obtain a WAD shift, we took the difference between WAD of nucleic acids from a sample incubated with H\(_2\)\(^{18}\)O and the paired sample incubated with water containing natural abundance levels of \(^{18}\)O. The WAD shift reflects the increase in density of nucleic acids following incubation with isotopically labeled water. The procedure for estimating amoA mRNA was analogous but the amoA copy number was derived from fractions of complementary DNA (cDNA).

2.5.2. Atom percent excess \(^{18}\)O of nucleic acids

All calculations and equations used for determining the atom percent excess \(^{18}\)O (APE \(^{18}\)O) values of microbial nucleic acids (16S rRNA and rDNA) were also previously published in Papp et al. (2018a) and in Hungate et al. (2015). Some of these equations are provided in this section as well for clarity. Specifically, we converted WAD shifts of thaumarchaeal nucleic acids into atom percent excess \(^{18}\)O (APE \(^{18}\)O) values, which show the nucleic acids’ isotopic enrichment above natural abundance of the \(^{18}\)O isotope (0.2 atom%). We used equation (5) from Papp et al. (2018a) (equation (3) below) to convert WAD of thaumarchaeal RNA (16S rRNA or amoA mRNA) into atom percent \(^{18}\)O values:

\[
\text{WAD} = 0.0744 \times \text{atom percent }^{18}\text{O of RNA} + 1.7803
\]

This equation was obtained by using the known molecular weight of the rRNA molecule at different \(^{18}\)O isotopic contents (natural abundance and 100% \(^{18}\)O content), verified by empirical measurements of the density of RNA at natural abundance \(^{18}\)O. We also used equation (6) from Papp et al. (2018a) (equation (4) below) to convert WAD of thaumarchaeal DNA (16S rDNA or amoA gene) into atom percent \(^{18}\)O values:

\[
\text{WAD} = 0.0644 \times \text{atom percent }^{18}\text{O of DNA} + 1.6946
\]

This equation was generated with results from a culture experiment of growing E. coli (strain HB101) at 5 different \(^{18}\)O enrichment levels (natural abundance, 5, 25, 50 and 70% atom fraction \(^{18}\)O). DNA was extracted from these cultures which was centrifuged to determine its density, and analyzed by IRMS to determine its \(^{18}\)O signature. The equation is fully described and explained in Hungate et al. (2015). To calculate the atom percent excess \(^{18}\)O (APE \(^{18}\)O), the isotopic values of nucleic acids extracted from non-labeled incubations were subtracted from the isotopic values of nucleic acids extracted from the labeled treatment. We obtained three replicate APE \(^{18}\)O values for each thaumarchaeal taxon at each time point.

2.6. rRNA to rDNA ratio calculations

Ratios of rRNA to rDNA were calculated for Thaumarcheota by dividing the relative abundance of RNA in each non-fractionated sample by the relative abundance of rDNA in the same sample. This was done for each replicate at each time point, yielding a total of 9 ratios per treatment. The replicate ratios were then averaged. There were two reasons for averaging the ratios: (1) there was no statistically significant difference over time \((p = 0.404)\), and (2) we did not intend to investigate how the ratios changed over time.

2.7. Model of nucleic acid turnover

Details about turnover of microbial rRNA are provided in (Papp et al., 2018b). Briefly, Excel’s Solver was used to determine the turnover rate of thaumarchaeal 16S rRNA and amoA mRNA that yielded the minimum sum of squared deviations from the observed data. We assumed that 50% of oxygen atoms in the thaumarchaeal nucleic acids originated from water and 50% from organic substrates (Chaney et al., 1972) and used findings from Ostle et al. (2003) and Yuan and Shen (1975) to obtain the starting turnover rates (20% per day, and 25% per hour respectively). After calculating the expected atom percent \(^{18}\)O values of rRNA and mRNA at each of the reported rates (20% per day, and 25% per hour), we compared them to our observed values (on day 1, 4 and 8) and used Excel’s Solver function to find the optimal turnover rate for each RNA type.

2.8. Statistical analyses

Statistical analyses were performed in SPSS (IBM SPSS Statistics 24), with statistical significance set to \(\alpha = 0.05\). Normality was tested with the Shapiro-Wilk test, homogeneity of variances was tested with Levene’s test, and sphericity was tested with Mauchly’s test. Additionally, we used a two-way mixed ANOVA to test for a significant interaction.
between APE $^{18}$O of the different nucleic acid types (i.e. 16S rRNA, 16S rDNA, amoA mRNA, and amoA gene) and time. We also used a Spearman’s rank order correlation test to assess the strength of correlations between (I) APE $^{18}$O of 16S rDNA and amoA gene, (II) APE $^{18}$O of amoA mRNA and 16S rRNA, (III) APE $^{18}$O of 16S rRNA and 16S rDNA, and (IV) APE $^{18}$O of 16S rDNA and 16S rRNA or amoA mRNA. Regressions were carried out using a model II regression analysis performed in R to test whether the slope of each correlation was significantly different from 1. We acknowledge that taxa within each replicate are not independent, and therefore, from the standpoint of inference from experimental design, the taxa are pseudoreplicates (taxa within replicate samples). However, the goal of our analyses was not to make inferences about differences between samples, or between groups of samples. The level of pseudoreplication therefore does not affect our conclusions. Combining all taxa within replicates would furthermore result in a significant loss of biological meaning caused by a considerable and particularly interesting variation among the individual taxa. Populations of different taxa are precisely the fundamental groups within fundamental biological and evolutionary units, where we would expect to see with great resolution a decoupling of DNA and mRNA synthesis - if it were to occur. Therefore, regressions in this manuscript show individual taxa within each replicate to illustrate general trends in nucleic acid synthesis with the highest resolution. The regression slopes were bootstrapped, which does not violate assumptions of the approach.

2.9. Accession numbers

All sequences have been deposited in the NCBI Sequence Read Archive (SRA) under accession numbers SAMN07960499 to SAMN07960874, SAMN07965143 to SAMN07965605, and SAMN07968111 to SAMN07968486. Data can directly be accessed at https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP123236.

3. Results

3.1. Incorporation of $^{18}$O into nucleic acids

The amount of $^{18}$O incorporated into thaumarchaeal nucleic acids, expressed as atom percent excess $^{18}$O (APE $^{18}$O), significantly differed over time and between 16S rRNA, the 16S rDNA, amoA mRNA, and the amoA gene ($p < 0.005$). amoA mRNA contained more $^{18}$O than thaumarchaeal 16S rRNA ($p < 0.005$) and, with the exception of day 4, the amoA gene (Fig. 1, Figure S2, Supplemental material S2). As expected, the 16S rDNA and amoA genes had similar $^{18}$O enrichment on each day ($p > 0.1$). The $^{18}$O isotopic composition of 16S rRNA and amoA mRNA increased over time ($p < 0.05$, Fig. 1). The $^{18}$O signature of thaumarchaeal 16S rDNA and amoA genes also increased over time with a slope significantly greater than zero for both genes (slope = 0.014 for 16S rDNA, and slope = 0.012 for amoA gene, Fig. 1, Supplemental material S2).

3.2. Correlations between isotopic signature of thaumarchaeal nucleic acids

Incorporation of $^{18}$O into thaumarchaeal amoA genes was strongly, and positively, correlated to incorporation of $^{18}$O into 16S rDNA ($p(37) = 0.953, p < 0.005$, Fig. 2). The equation describing the overall relationship between the two variables showed that the rates of incorporation of $^{18}$O into both genes were similar (Fig. 2, Supplemental material S2). Correlation between the isotopic signature of thaumarchaeal amoA mRNA and 16S rRNA was also strong and positive ($p(34) = 0.860, p < 0.005$). The relationship, depicted in Fig. 3, showed that rate of assimilation of $^{18}$O isotopes into amoA mRNA was greater than into 16S rRNA (Fig. 3, Supplemental material S2). Additionally, the rate of amoA mRNA turnover was higher (6.78% per day) compared to the rate of 16S rRNA turnover (4.61% per day). Lastly, incorporation of $^{18}$O into 16S rDNA of Thaumarchaeota was correlated with incorporation of $^{18}$O into their 16S rRNA (Figure S1A) and amoA mRNA (Figure S1B). Model II regression analysis showed that the slopes of both regressions were significantly smaller than one (slope = 0.40 for 16S rRNA vs 16S rDNA, and slope = 0.45 for amoA mRNA vs 16S rRNA).

**Fig. 2.** Correlation between the APE $^{18}$O of thatharchaeal amoA gene and 16S rDNA (all individual time points are shown). Symbols represent individual thaumarchaeal taxa in replicate soil samples. Filled symbols show positive APE $^{18}$O values, and open symbols show negative values, which were not included in the analysis. The green line represents a 1:1 line where the APE $^{18}$O of both genes are equal. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Fig. 3.** Correlation between the APE $^{18}$O of thaumarchaeal amoA mRNA and 16S rRNA using all individual incubation time points. Symbols show individual taxa in replicate soil samples. Positive APE $^{18}$O values are represented by black symbols, and values of – zero by open symbols, the latter were not used in the analyses. The green line represents a 1:1 ratio, where the isotopic content of mRNA and rRNA is equal. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
4. Discussion

4.1. 18O isotopic content of thaumarchaeal nucleic acids

The natural abundance isotopic composition of newly synthesized nucleic acids within a cell, whether DNA, mRNA, or rRNA, should be similar because they all originate from interchangeable nucleotide pools. Ribonucleotide reductase catalyzes the conversion of ribonucleotides to deoxyribonucleotides (Herrick and Sclavi, 2007; Reichard, 1993), so therefore it is likely that both newly made ribonucleotides and deoxyribonucleotides have a similar 18O content since 18O water was added in very high amounts and fractionation processes could be ignored. Even if fractionation led to differences in natural abundance 18O composition between types of nucleic acids, a large change in natural abundance (e.g., 50%) corresponds to a very small difference in tracer concentrations (± 0.01 atom % 18O). The observed significant differences in the 18O content of the bulk DNA, mRNA, or rRNA, following the addition of 18O-water can thus only be explained by the fraction of the nucleotide pool composed of older, non-labeled molecules. Additionally, the 18O content of a nucleic acid extract is also affected by the amount of newly synthesized molecules, the turnover rate, the synthesis strategy (de-novo vs recycling of nucleotides, discussed further), and the nucleic acid pool size.

18O incorporation into amoA mRNA was greater than into the amoA gene at the beginning and at the end of our experiment, likely because mRNA synthesis rates were greater than DNA replication rates. This is consistent with the observations of Dumont et al. (2011) throughout a 4-day incubation with 13C-labeled methane, whereby the isotopic signature of pmoA mRNA of methanotrophic communities was greater than the 13C isotopic signature of pmoA genes. Since growth is not a prerequisite for metabolic activity (Blazewicz et al., 2013), greater 18O content in microbial mRNA relative to the corresponding gene was expected. Surprisingly, the overall isotopic enrichment of thaumarchaeal amoA mRNA was relatively low (~20% after 8 days of incubation with H2 18O, turnover rate 6.78% per day). This suggested that turnover rate of mRNA may be slower in soil than in pure cultures on media with ideal growth conditions (such as 37°C temperature, Hambraeus et al., 2003) and high cellular activity. In these cultures, studies have measured short half-lives of bacterial mRNAs, ranging anywhere from ~30 s to ~30 min (Belasco and Higgins, 1988; Hambraeus et al., 2003; Rauhut and Klug, 1999; Wong and Chang, 1986). At such a fast turnover rate, we hypothesized that many oxygen atoms in amoA transcripts would be replaced with 18O. We did not expect the mRNA to be 100% labeled with 18O because microorganisms assimilate oxygen into ribonucleotides from both water and other organic or inorganic compounds. In the case of Thaumarchaeota, oxygen is likely to come from water and potentially from fixed carbohydrates as they mostly use inorganic HCO3− as a carbon source (Hatzenpichler, 2012; Könneke et al., 2014). Oxygen in the fixed carbohydrates may partially come from water since water is used to produce Acetyl-CoA, a precursor for gluconeogenesis (Berg et al., 2010). Thus, newly synthesized nucleic acids of the Thaumarchaeota could potentially be more labeled with 18O than nucleic acids of heterotrophs, but further research is needed to elucidate the rate of 18O incorporation into newly fixed carbohydrates among autotrophs. Additionally, many ribonucleotides within a cell are likely recycled, and therefore new mRNA sequences will still contain many 18O atoms, which likely contributed to the low 18O content of thaumarchaeal amoA mRNA.

4.2. Chemolithoautotrophic metabolism

The thaumarchaeal amoA gene encodes a subunit of the ammonia monooxygenase enzyme (AMO) (Francis et al., 2005), which, in Thaumarchaeota, is responsible for energy generation (Hatzenpichler, 2012). AMO catalyzes the oxidation of ammonia to hydroxylamine,
which is the first and rate-limiting step of nitrification (Jia and Conrad, 2009; Leininger et al., 2006; Mertens et al., 2009; Nicol and Schleper, 2006). Preserving amoA transcripts for longer periods of time could increase the number of translated AMOs per amoA template and directly save energy associated with transcription. Ammonia oxidizing bacteria (AOB) are already known for their high in vivo stability of amo mRNA, proteins, and ribosomes (Koops et al., 2003; Bock and Wagner, 2006). Our study suggests that soil ammonia oxidizing archaea (AOA) exhibit a similar physiology. Incomplete mRNA labeling was also observed in German agricultural soils (Pratscher et al., 2011). In their study, up to 50% of the amoA transcripts had densities below 1.80 g/ml even after 12 weeks of incubation with 13CO2 and 100 μgNg−1 dry weight soil. This indicated that the transcripts were not fully labeled with the heavy isotope (Pratscher et al., 2011). The detection of 18O-labeled amoA mRNA in our study suggested that soil Thaumarchaea were active in nitrogen cycling (Gubry-Rangin et al., 2010; Huang et al., 2012; Stopniček et al., 2010; Yao et al., 2011), but relating 18O content of amoA mRNAs to ammonia oxidation rates was beyond the scope of this study.

4.3. Presence of old rRNA

We found that Thaumarchaea had higher 18O content in their amoA mRNA than 16S rRNA, indicating that old thaumarchaeal rRNA was present in the cells (Deutscher, 2006, 2003; Jain, 2002). We expected the 16O content of newly made mRNA and rRNA to be similar because both are transcribed by the same RNA polymerase (Ebright, 2000; Darst, 2001) and because, within a cell, ribonucleotides used for synthesis of mRNA and rRNA derive from the same nucleotide pool. Thus, if all RNA molecules had very high turnover rates and were recently synthesized, the APE 16O of amoA and 16S ribosomal RNA should be very similar, which was not what we observed. The difference in APE 18O of the two transcripts was most likely due to the presence of a greater amount of older non-labeled rRNA than mRNA. This suggests that mRNA turns over faster than rRNA. The latter, more stable, can thus be reused for maintenance and reduce cell’s overall energy expenditure. Ribosome storage has been previously documented by, for example, Wada (1998) who observed formation of 100S dimers, which was proposed to be a mechanism for excess ribosome storage during the stationary phase of growth. Large amounts of stored ribosomes have also been observed in dormant cyanobacteria (Sukenik et al., 2012). Some thaumarchaeal cells may have been in stationary phase and have contained stored ribosomes, thus contributing to overall low isotope content measurements. Yet we observed that the nucleic acids of all taxa were enriched in 16O. Such nucleic acid labeling indicated that at least some individuals within a population were metabolically active and growing.

4.4. Genome replication

As expected, the 16S rDNA and amoA genes had very similar isotopic contents. Both genes reside on a single chromosome within a thaumarchaeal cell, and as the cell divided, both became similarly enriched. Our finding of a strong correlation between the APE 16O of the 16S rDNA and amoA genes with a slope of 1 confirmed that genome replication was uniform, resulting in all genes being labeled with isotopic tracers at the same rate. It also demonstrated that qSIP can measure the rate of genome replication, thus providing a unique opportunity to measure microbial growth rates in situ.

Our experiment simulated a rain event. Studies have shown that microbial respiration and growth rates increase after rewetting (Bloem et al., 1992; Iovieno and Bååth, 2008; Pesaro et al., 2004), but the total microbial biomass can decrease (Fierer and Schimel, 2002; Gordon et al., 2008). In H2O18O stable isotope studies, no major changes in the absolute size of bacterial or archaeal populations were found after rewetting because growth was counter-balanced by death (Angel and Conrad, 2013; Blazewicz et al., 2014; Koch et al., 2018). Water addition may have stimulated metabolic activity, including growth, of taxa that might otherwise have stayed dormant in our soil. Water addition increased the soil moisture content from 4% to 20% approximately. Nevertheless, since even desert ecosystems receive precipitation, our experiment remains representative of naturally occurring events and provides valuable information about microbial dynamics following these events.

4.5. Metabolic activity

RNA to DNA ratios are often used as an indicator of microbial metabolic activity. The assumption is that the higher the ratio the higher the activity level because more ribosomes are needed to sustain rapid metabolism. We were thus interested to see how well the ratios compared to qSIP. Through qSIP, we found that Thaumarchaeota incorporated low amounts of 16O into their rRNA, suggesting that synthesis of new rRNA molecules was rather low compared to bacterial taxa. However, we measured high rRNA to DNA ratios of the soil Thaumarchaeota, which is traditionally interpreted as an indication of high metabolic activity (Roszak and Colwell, 1987; DeAngelis et al., 2010; Baldrian et al., 2012; Dlott et al., 2015). This was in disagreement with result from qSIP but can be explained by the thaumarchaeal capability to preserve and use older ribosomes. The Thaumarchaeota were relatively abundant in the rRNA library but the level of 16O incorporation into their rRNA was low. These two findings suggested that a substantial fraction of their rRNA was synthesized prior to H218O addition and was thus at least 8 days old. Our results suggest that ribosomal RNA can be relatively long lived in the environment, whether used by metabolically active bacteria and archaea or perhaps intact and preserved but no longer part of living cells.

Declarations of interest

None.

Acknowledgements

This research was supported by award 1142096 from the National Science Foundation, division of solar programs, NSF DEB-1241094, the Department of Energy’s Biological Systems Science Division, Program in Genomic Science (DE-SC0010579, and DE-SC0016207), and by the IGERT Fellowship. Additionally, the authors would like to thank Paul Dijkstra, Matthew Bowker, Rebecca Mau, Michaela Hayer, Ben Koch, and Lela Andrews from Northern Arizona University and Joshua Sackett from Desert Research Institute for their help and suggestions. The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.12.016.

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