



# Quantitative stable isotope probing with H<sub>2</sub><sup>18</sup>O reveals that most bacterial taxa in soil synthesize new ribosomal RNA

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## Abstract

Most soil bacterial taxa are thought to be dormant, or inactive, yet the extent to which they synthesize new rRNA is poorly understood. We analyzed <sup>18</sup>O composition of RNA extracted from soil incubated with H<sub>2</sub><sup>18</sup>O and used quantitative stable isotope probing to characterize rRNA synthesis among microbial taxa. RNA was not fully labeled with <sup>18</sup>O, peaking at a mean of 23.6 ± 6.8 atom percent excess (APE) <sup>18</sup>O after eight days of incubation, suggesting some ribonucleotides in soil were more than eight days old. Microbial taxa varied in the degree they incorporated <sup>18</sup>O into their rRNA over time and there was no correlation between the APE <sup>18</sup>O of bacterial rRNA and their rRNA to DNA ratios, suggesting that the ratios were not appropriate to measure ribonucleotide synthesis. Our study indicates that, on average, 94% of soil taxa produced new rRNA and therefore were metabolically active.

## Introduction

Most bacteria in soil are thought to be dormant [1, 2], while only small active fractions control ecosystem processes [3]. Active bacterial cells have higher metabolic rates than dormant cells, leading to higher protein and rRNA synthesis. In contrast, dormant bacteria have very-low metabolic

activity [4]. RNA concentrations likely decrease as most metabolic processes, including RNA synthesis, are halted, while DNA concentrations may remain stable because dormant cells are alive.

The relative abundances of ribosomal RNA (rRNA) and DNA extracted from environmental samples are commonly used as indicators of microbial metabolic activity [5]. However, rRNA to DNA ratios among taxa in communities substantially vary, often unrelated to metabolic activity, suggesting RNA alone may not be reliable indicator of active populations [6].

Stable isotope probing (SIP) can assess microbial activity independent of rRNA to DNA ratios. SIP with <sup>18</sup>O-labeled water is powerful for assessing growth and activity of microbial communities because water is a universal substrate for nucleic acid synthesis [7]. In this study, we incubated 2 grams of soil with 400 μl of sterile 95 atom % H<sub>2</sub><sup>18</sup>O or with 400 μl of sterile, natural abundance H<sub>2</sub><sup>18</sup>O, for 1, 4, and 8 days (*N* = 18), and extracted total RNA following each incubation. Newly synthesized <sup>18</sup>O-containing RNA has higher buoyant density than old RNA, and can be separated through isopycnic ultracentrifugation on a CsTFA density gradient. We fractionated the ultracentrifuged RNA, purified the fractions and sequenced a fragment of the 16S rRNA gene from complementary DNA (cDNA) as described in Supplement S1. Sequencing data were analyzed using a QIIME 1.7 based [8]

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Subject Categories: Microbial population and community ecology, Microbial ecology and functional diversity of natural habitats

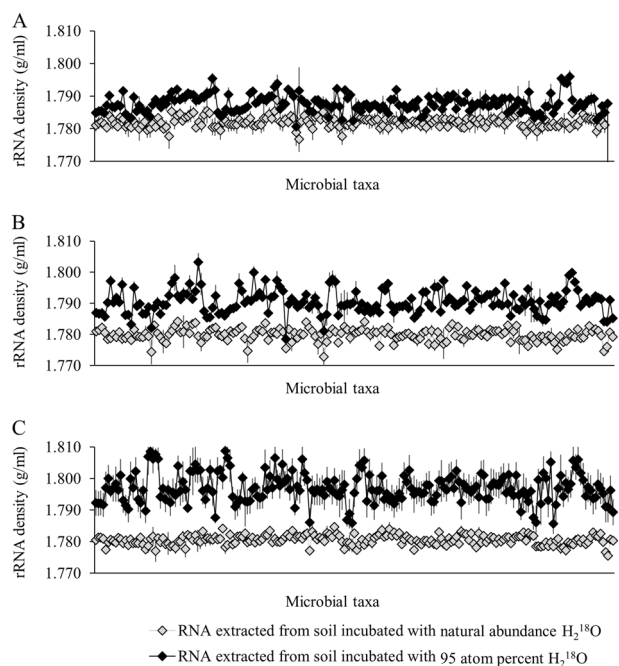
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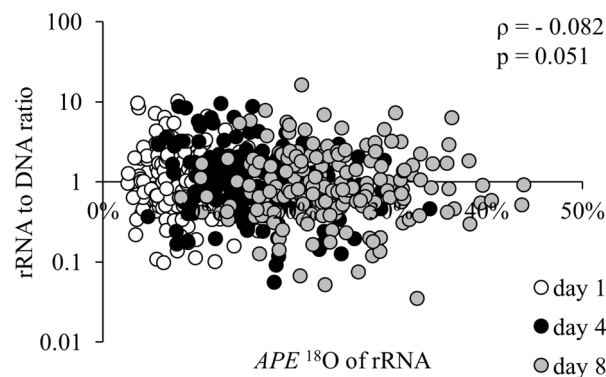


**Fig. 1** Densities of rRNA extracted from soil incubated with 95 atom %  $\text{H}_2^{18}\text{O}$  ( $\blacklozenge$ ) or natural abundance  $\text{H}_2^{18}\text{O}$  ( $\blacklozenge$ ) on three time points. **a** rRNA densities of taxa detected on day 1, **b**, **c** rRNA densities of taxa detected on day 4 and 8, respectively. Taxa are ranked by the same alphabetical order in each panel. Symbols represent means  $\pm$  standard deviations

chained workflow: <https://github.com/alk224/akutils-v1.2> [9]. To assess rRNA synthesis of individual taxa, we measured the incorporation of  $^{18}\text{O}$  into rRNA by calculating taxon-specific shift in rRNA density and by converting it to atom percent excess ( $APE$ )  $^{18}\text{O}$  using a freely available R code ([https://bitbucket.org/QuantitativeSIP/qsip\\_repo](https://bitbucket.org/QuantitativeSIP/qsip_repo)).  $APE$   $^{18}\text{O}$  indicated the excess of  $^{18}\text{O}$  in microbial rRNA relative to natural abundance of the isotope, and was used to estimate rRNA synthesis rate, a measure of microbial activity. We assessed temporal patterns and variation in rRNA synthesis rates among soil microbial populations using qSIP, and compared our results to RNA to DNA ratios.

All taxa contained  $^{18}\text{O}$ -labeled rRNA after four days of incubation with  $\text{H}_2^{18}\text{O}$ . Densities of rRNA in non-labeled incubations varied slightly around the mean ( $1.7808 \pm 0.0011$  g/ml), whereas densities of labeled rRNA significantly differed on each day (Fig. 1), which likely reflects taxonomic variation in the rate of metabolic activity [10] or differential reliance among taxa on de novo ribonucleotide synthesis [11] vs. ribonucleotide salvaging. If ribonucleotides are synthesized de novo,  $^{18}\text{O}$  will be assimilated throughout the ribonucleotide in addition to its assimilation into phosphodiester bonds [12], which will increase the  $^{18}\text{O}$  composition of rRNA more than recycling alone.

Entirely dormant soil taxa were absent in our study, which challenges the widely accepted idea that dormancy is



**Fig. 2** Relationship between rRNA to DNA ratios and  $APE$   $^{18}\text{O}$  of rRNA among soil taxa on three time points: open symbols—day 1, black symbols—day 4, and gray symbols—day 8

widespread among microbial taxa in the environment [13, 14]. We would observe many populations with non-labeled rRNA (i.e., containing  $^{18}\text{O}$  only at the natural abundance level) if dormancy were common under the incubation conditions. However, the average, positive  $^{18}\text{O}$  labeling we observed for all populations detected does not rule out individual cells within these populations that are dormant—non-labeled with  $^{18}\text{O}$  and exhibiting no rRNA synthesis. Positive  $APE$   $^{18}\text{O}$  could also result from potential interactions between RNA molecules during ultracentrifugation [15], which possibly influenced our results and deserve additional research. Our observation of a weak correlation between the rRNA to DNA ratio and the  $APE$   $^{18}\text{O}$  of rRNA across taxa (Spearman's rank-order correlation,  $\rho(574) = -0.082$ ,  $p = 0.051$ , Fig. 2) suggests that the ratio may be a poor proxy for metabolic activity, despite its positive correlation with microbial growth rate in some pure culture studies [16]. We expected that taxa with high rRNA to DNA ratios would have highly labeled rRNA, but this was not observed.

We observed a significant temporal increase in  $^{18}\text{O}$  content for total RNA ( $F_{2,4} = 15.404$ ,  $p = 0.013$ , Figure S1 and S2) and for RNA of phyla (Figure S3). RNA is thought to turn over rapidly [17], with estimates ranging from 20% per day [18] to 25% per hour [19]. In our experiment, we modeled rRNA turnover varied between 9 and 18% per day, which was slower than previously reported. The labeled RNA had  $\sim 23\%$  of its oxygen atoms replaced with  $^{18}\text{O}$ , indicating that either some of the rRNA that was formed prior to  $\text{H}_2^{18}\text{O}$  addition remained intact, and that the rRNA was newly synthesized but partly made with ribonucleotides that were more than 8 days old, or that newly synthesized ribonucleotides obtain part of their oxygen from organic substrates. Assuming that 50% of oxygen atoms come from  $\text{H}_2^{18}\text{O}$  and 50% come from organic substrates [20], the isotopic composition of rRNA would be 50% at the fast modeled turnover rate and would have increased only

minimally over time. The observed increase in <sup>18</sup>O composition of RNA over time suggested that increasingly more ribonucleotides were synthesized and that the turnover rate of ribonucleotides in soil is ~23% per week.

Our knowledge of ribosome biosynthesis and degradation derives mostly from pure culture experiments, but, based on the soil we assessed, bacterial rRNA dynamics in soil may differ from those observed in pure cultures. Specifically, synthesis of new rRNA was slower than expected and unrelated to rRNA to DNA ratios in a soil microbial community, which is thought to have many dormant members. Yet, we found that all detected taxa synthesized new RNA during the 8-day incubation. Further research, conducted over longer incubations, will help determining maximum rRNA labeling and time required to reach it. Our work illustrates how RNA-qSIP can quantify taxon-specific activity relating to synthesis of new nucleic acids, opening doors to broader tests about microbial dormancy and metabolic activity across a range of soils and environments.

### Accession numbers

All sequences have been deposited in NCBI SRA (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>.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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