Title: Comparing In-Situ, Individual Bacterial Growth Rates in Cropped and Successional Soils Using a 16S rRNA Internal Standard

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Project goals:
We hypothesize that carbon fate in soil is governed by microbial growth dynamics, which determine consumption and production of soil organic matter, and that these dynamics are constrained and determined by soil properties. Project goals include:

- **Develop a method for determining in-situ bacterial growth rates using 16S rRNA amplicon sequencing.**
- **Determine how carbon inputs to soil alter growth rate frequency distributions of bacterial communities.**
- **Investigate the impacts of soil management history and resource availability on bacterial community growth dynamics.**

Abstract text: Soil bacteria drive biogeochemical cycles via metabolic activities that break down, assimilate, and transform compounds containing carbon (C), nitrogen, and other elements. Bacterial growth dynamics are inherently connected to bacterial metabolism and C transformation. Only recently have individual in-situ growth rates been measured, and little is known about bacterial community growth dynamics in nature. Here we used an internal standard (16S V4 rRNA modified oligo of *Aquifex aolicus*) with 16S rRNA amplicon sequencing to estimate in-situ growth rates from cropped and successional soils, with water or C amendment (3.6 mg/g dry soil, various soluble and insoluble compounds). We hypothesized that soil habitat and C availability would impact growth dynamics within the bacterial community, specifically that cropped and C-amended soils would harbor more fast growing taxa compared to successional or water-amended soils, reflecting differences in soil disturbance and resource availability. We also hypothesized that in-situ growth rates would correlate positively with 16S rRNA copy number. The internal standard comprised an average of 1.6% of the sequenced reads per sample and displayed a strong negative correlation with DNA yield (Spearman, rho = -0.66, $p < 0.001$). Overall, we were able to estimate in-situ growth rates for 453 taxa across all soils after filtering for sparsity and controlling for false positives. We observed a weak, positive correlation between in-situ growth rates and 16S rRNA copy number (Spearman, rho = 0.166, $p < 0.001$). There was a significant difference in the growth rate frequency distributions between the cropped and successional soils in the water-amended treatment but not in the C-amended treatment (Fisher's exact test, $p = 0.004$ and $p > 0.05$). Although overall growth rate frequency distributions differed between water-amended soils, the number of "fast" and "slow" taxa were not significantly different in either the cropped or successional, water-amended soils (Welch's paired t-tests, $p > 0.05$). Overall, these results demonstrate (i) the utility of using internal standards for estimating individual bacterial growth rates in soils, (ii) that 16S rRNA copy number explains significant variation in observed bacterial growth rates in soils, and (iii) soil habitat and resource availability have large and complex impacts on growth within bacterial communities. Going forward, we will use this method to investigate how differences in bacterial community growth dynamics impact C cycling in soils.

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