**Title:** Microbial growth in soil

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**Figures:** 2

**Tables:** 1

**Supplementary Material:** Tables S1-S4, Figures S1

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**Conflict of Interest:** The authors declare no conflict of interest.

## Abstract

The growth rate of a microorganism is a simple yet profound way to quantify its impact on the world. Microbial fitness in the environment depends on the ability to reproduce quickly when conditions are favorable and adopt a survival physiology when conditions worsen, which cells coordinate by adjusting their growth rate. At the population level, per capita growth rate is a sensitive metric of fitness, linking survival and reproduction to the ecology and evolution of populations. The absolute growth rate of a microbial population reflects rates of resource assimilation, biomass production, and element transformation, some of the many ways that organisms affect Earth's ecosystems and climate. For soil microorganisms, most of our understanding of growth is based on observations made in culture. This is a crucial limitation given that many soil microbes are not readily cultured and *in vitro* conditions are unlikely to reflect conditions in the wild. New approaches in 'omics and stable isotope probing make it possible to sensitively measure growth rates of microbial assemblages and individual taxa in nature, and to couple these measurements to biogeochemical fluxes. Microbial ecologists can now explore how the growth rates of taxa with known traits and evolutionary histories respond to changes in resource availability, environmental conditions, and interactions with other organisms. We anticipate that quantitative and scalable data on the growth rates of soil microorganisms will allow scientists to test and refine ecological theory and advance process-based models of carbon flux, nutrient uptake, and ecosystem productivity. Measurements of *in situ* microbial growth rates provide insights into the ecology of populations and can be used to quantitatively link microbial diversity to soil biogeochemistry.

## Introduction

Achieving growth in the face of a changing environment is the most fundamental challenge for microorganisms living in soil. Microbial growth requires the coordination of a cell's system-level physiology, including the extraction of energy and substrates from the environment, synthesis of hundreds of molecules at appropriate concentrations, and the events of cell division. All of this coordination has to be done in such a way as to allow the cell to modify its activities depending on changes in its surrounding environment – often on a very short time scale. With millions of years of evolution, soil microorganisms have developed a range of strategies for growing in diverse environments; they grow in extreme cold and heat, in highly acidic and alkaline habitats, on the inside and outside of plant roots, and in the bedrock of soil. Microorganisms have wide-ranging metabolic capabilities and can capitalize on diverse redox pairs and reactions that occur not only within but also among cells representing multiple domains of life[1].

As soil microorganisms grow, they assimilate, transform, and redistribute key elements in their environment[2], with far-reaching consequences for Earth's ecosystems and climate. Microbial acquisition of phosphorus and sulfur for growth mobilizes these elements from their geological reservoirs, where they typically reside for thousands to millions of years, and transfers them into dynamic biotic pools with much shorter residence times, often on the scale of weeks to months[3,4]. Assimilation and retention of nutrients like nitrogen (N) and phosphorus in microbial biomass can constrain plant growth and limit the capacity of ecosystems to capture carbon (C) from the atmosphere[5]. When microorganisms transform substrates through redox reactions, they can amplify the radiative forcing of greenhouse gas molecules, intensifying the impacts that these molecules have on Earth's climate[6].

Growth rate quantifies these interactions with the world. At the cellular level, microorganisms grow by synthesizing the macromolecules that make up cells and growth rate is a powerful index of how microorganisms adjust their physiology in response to a changing environment. Since stress-resistant physiological configurations hinder the ability of cells to grow quickly[7], many microbial species have developed distinct phenotypes for stressful versus growth-conducive environments. These phenotypes vary profoundly, not only in their rates of growth, but also in their central C metabolic networks[8], cell sizes, and macromolecular compositions[9]. The evolution of distinct growth phenotypes is linked to genetic mutations that affect global gene regulation[10], suggesting that growth rate may be evolutionarily related to a range of traits that impact how microbes survive in soils, including the synthesis of extracellular polymeric substances (EPS)[11], motility[12], nutrient uptake pathways[13], and even mortality rate[14].

At the population level, growth occurs when reproduction outpaces mortality. Per capita growth rate (the change in abundance relative to the starting size of the population) measures how well microorganisms compete for resources and respond to challenges associated with stress, competition, and predation. Evolutionary fitness depends on the persistence of an organism's genes in the population gene pool[15], meaning that quantitative metrics of fitness should ideally reflect both reproduction and survival[16]. If high reproductive rates are offset by high rates of mortality, the long-term persistence of lineages carrying those genes (*i.e. relative fitness)* is lower than that of lineages with the same reproductive rate in a population with little mortality, differences that are captured by a microorganism's per capita growth rate[17]. Per capita growth rates can also quantify the intensity of intraspecific interactions, such as density dependence resulting from competition[18,19], and interspecific interactions, such as competition, predation, and mutualism[20].

Absolute growth rate, or the actual change in mass or abundance per unit time, reflects rates of microbial element assimilation and use. Along with absolute mortality rate, absolute growth rate determines the standing stock of microbial biomass and the interactions with other microorganisms and the environment, ultimately driving changes in the taxonomic makeup of entire communities. Given that soil microorganisms can exist in a range of physiological states and exhibit rapid turnover, often with minimal changes in the standing stock of biomass, assessing microbial abundance alone is a poor predictor of element flux[21–25]. Rates of absolute growth and mortality are needed to quantify the turnover of elements through microorganisms per unit time. Such metrics provide a powerful means for mapping element flux through entire assemblages and testing the impacts of microbial biodiversity on C and nutrient cycling at the ecosystem scale[22].

While plant and animal growth in nature is routinely measured, microbial growth is typically studied in the laboratory under highly artificial conditions. Most often, the maximum growth rates of culturable organisms are assessed during exponential phase in resource-rich media. Although many recent developments in soil ecology invoke microbial growth rates[26–38], growth rates of soil microorganisms *in situ* are difficult to measure and interpret. New approaches[24,39–41] leveraging ‘omics technologies and stable isotope probing (SIP) make it possible to measure microbial growth rates *in situ*, capturing the phylogenetic and metabolic diversity of actively growing populations in soil and making it possible to better understand the microbial contributions to soil biogeochemical processes.

## Measurements of microbial growth rates in soil

Current estimates of soil microbial generation times vary enormously, spanning at least four orders of magnitude from ~43 minutes to ~ 2 years (Figure 1). Such variation may be a product of SIP-based methods that target different biomolecules, such as DNA, proteins, or lipids with varying turnover rates, contingent on the cell's physiological state. During exponential growth, cells synthesize macromolecules at near-constant differential rates and divide at a particular mass. Under these conditions of balanced growth, growth rate sets key cellular phenotypes like cell size and the mass fractions of nucleic acids, proteins, and lipids. In nature, where microorganisms exist in a range of states from exponential growth to dormancy, relationships between replicative growth and rates of macromolecular synthesis may not always be so tightly coupled. Applying multiple methods that measure synthesis rates of various macromolecules would be an excellent way to explore the physiological adjustments that allow microorganisms to strike a balance between survival and proliferation in soil. For example, in response to extreme C limitation, microorganisms may undergo reductive division[42], simultaneously catabolizing lipids for energy[43] and replicating other cellular constituents in order to divide into smaller and more stress resistant cells, which could be explored using SIP approaches targeting lipid[24] and DNA[39,44] synthesis. Entirely different networks of regulatory molecules are responsible for coordinating cell growth and division during different phases of growth, demonstrating that the strategies microorganisms employ to grow and survive in nature may differ profoundly from those used during exponential growth in culture.

Most measurements quantify relative growth rate, useful for understanding how microorganisms respond to challenges in the environment. However, measurements of absolute growth rate – which quantify the actual change in mass or abundance of microorganisms over time – are needed to understand how microorganisms transform and redistribute elements through ecosystems. New approaches that convert rates of tracer uptake to growth in terms of C units are major advances[22,45], but it is still a major challenge to accurately estimate absolute changes in mass and abundance using these approaches. Adopting and developing protocols that quantify the efficiency of DNA[46], protein, and lipid[47] extractions would advance these efforts.

Most measurements of soil microbial growth quantify the growth rates of whole microbial assemblages, which result in a single estimate for a soil sample, an aggregate of thousands of microbial populations. A single population of plants can suppress N availability[48], and a single population of animals can modify soil disturbance[49]. The tremendous biodiversity of soil microorganisms means that individual microbial populations should also have profound influence on ecosystems [50,51]. New methods that quantify the growth rates of individual microbial taxa[24,39–41] are promising avenues for developing quantitative links between specific microbial taxa and soil processes. Estimates of growth from over 46,000 measurements of rates of DNA synthesis show tremendous variation in growth rates among bacterial groups in soil and indicate that most microorganisms exhibit low to intermediate growth rates (Figure 2). The growth rate of an individual taxon is not clearly related to its abundance, a finding that aligns with previous observations from LH-SIP[24] demonstrating that faster growing taxa are not necessarily more abundant in soil since population growth can be matched or outpaced by mortality[19].

Measurements of growth rate in soil indicate that bacterial groups also vary in their rates of resource use and responses to changes in nutrient availability[52–54], temperature[55–58], disturbance[18,59,60], mineralogy[61], and climate gradients[62,63]. Microbial contributions to C assimilation and respiration[22] and N assimilation[64] appear to be highly taxon-specific, and this variation appears to be meaningful when scaled to the ecosystem level[22]. Such measurements offer a new set of data for testing and developing representations of C and N cycling that include representations of microbial diversity. Measurements of growth rate have also shown how interactions among soil microorganisms – including competition[65], mutualism[23], and predation[66,67] – can influence element flux through the soil microbiome too, just as interactions between plants and animals influence ecosystem processes.

Measurements of growth rate have a clear place in testing the role of ecological theory in soil microbial ecology, too. Like macroscopic organisms, microbial phenotypes in soil are constrained by their evolutionary histories[68–71]. Phenomena such as negative density dependence and r/K selection theory are key for understanding population growth of larger organisms, but these concepts have failed to explain patterns in the growth of microbes *in situ* [19,72]. As such there is a great need for evidence-based ecological frameworks that are built on direct observations of soil microbiomes[73]. Moving forward, quantitative data on soil microbial growth rates should be integrated into tests of microbial ecological theory and used to refine process-based models of element flux and ecosystem productivity.

## Relevance to soil ecology

The diversity, physiology, and ecology of microorganisms influence biogeochemical cycling, soil organic matter (SOM) formation and loss, and plant productivity, with implications for pollution, food supply, and climate. Soil biogeochemical process rates are rarely measured simultaneously with microbial growth, but doing so could offer powerful insight into how microbes contribute to these processes and could help discover new tools for managing the soil microbiome.

*Microbial physiology and soil organic C cycling*

The physiological properties of microorganisms play a key role in governing the formation and loss of SOM stocks[74,75] that are vital for mitigating C emissions and enhancing the sustainability of agricultural systems[76]. Measurements of *in situ* soil microbial growth could be used to inform and test emerging hypotheses on soil organic C (SOC) cycling. For example, low molecular weight C substrates are hypothesized to increase the accrual of mineral-associated organic matter derived from microbial necromass, a large and slowly-cycling C reservoir, by promoting fast and efficient microbial growth and turnover at the assemblage level[77–80]. However, the relationships between substrate quality, microbial growth rate, and physiological traits are complex. The growth rates of soil microbes have been both positively[81,82] and negatively[77,83] linked to growth efficiency, and taxa are known to vary in their rates of growth on low versus high molecular weight C substrates[70,84]. Establishing quantitative relationships between the taxonomic composition, growth rate, and growth efficiency of microbial assemblages is needed to advance our conceptual understanding of SOC cycling.

Microbial processes affecting soil C accrual and persistence, including growth rate, are represented in some numerical models of SOC cycling[26–30,33]. These microbially explicit biogeochemical models can be used to integrate measurements of microbial growth with mechanistic understanding of SOC responses to environmental changes. For example, formulations of microbial dormancy[29] and density dependent growth[85] can improve predictions of SOC dynamics at the ecosystem scale. At the global scale, modeling growth efficiency in soil is key to predicting soil C stocks[86], suggesting growth rate may be an important factor to consider in these large-scale geochemical models. Additional measurements of soil microbial growth rates in nature will provide the data needed to test conceptual and quantitative models of how microbes influence the soil C cycle. There is a clear need for direct measurements of *in situ* growth rates to better understand the roles of the microbial community – and of individual microbial genes, metabolic pathways, and taxa – as conduits of energy and element cycling through soils.

*Microbial diversity and ecological strategies concepts*

Amidst a wealth of archived genomic, transcriptomic, and proteomic data, frameworks categorizing the ecological strategies of soil microorganisms have emerged to integrate these data with biogeochemical concepts and mechanistic models[87–89]. Such frameworks are valuable given that they can effectively reduce complex microbial assemblages into a manageable number of functional groups and provide a basis for generating effective, hypothesis-driven insights into soil microbial ecology[90]. Collectively, these frameworks represent diverse hypotheses about interactions between microbial community structure and soil processes.

Many microbial frameworks have been derived from classic ecological theory (i.e., theory primarily developed from conceptual models of plant life history strategies) and these microbial frameworks often lack experimental validation. For example, ecological strategies are commonly assigned based on taxonomy[88] but tests of whether microorganisms use their assigned strategies in nature are rare[72]. Alternatively, broad ecological strategies can be identified based on genomic features[87] and gene expression[91], but our ability to translate microbial genes to function is nascent.

Evidence-based tests of ecological frameworks are now possible. As an essential property of an organism's life history and metric of competitive ability, *in situ* growth rate has a direct role in validating frameworks that build on classical ecological theory. As a metric of fitness, growth rate could be assayed in multiple environments to determine whether evolutionary adaptation to a selective environment has been accompanied by a loss of reproductive potential in nonselective environments – in other words, whether a tradeoff has occurred. Quantifying the growth of organisms where they actually live and grow also provides access to a broader suite of trait dimensions than can be extrapolated from pure culture studies. Direct, *in situ* growth rate measurements could thus provide powerful, empirical means to develop alternative ways of organizing soil microbial diversity into ecologically meaningful units. Coupling these with measures of nutrient and energy fluxes will help test links between community composition and ecosystem dynamics.

### *Ecological interactions and soil food webs*

Microorganisms influence energy flow and alter rates of nutrient cycling through their interactions with other microorganisms[92]. For example, predation in the rhizosphere changes the taxonomic structure of prokaryotic communities and alters rates of N mineralization, influencing productivity[93]. Mutualistic interactions between microbial taxa can drive depolymerization of complex C compounds[94] and antagonistic interactions can influence growth and mortality rates through negative density dependence[18], altering rates of C turnover from microbial biomass[85]. Taxon specific growth rates are a valuable tool for assessing microbial interactions in which one soil microorganism influences another by altering its growth, reproduction, or any trait impacting fitness.

Growth rates of microbial taxa could help construct accurate food webs, as opposed to static measurements of microbial biomass[95] which are a poor surrogate for growth (Figure 3b). Food webs are an excellent tool for modelling the connectivity of microorganisms and quantifying how energy and elements are transferred between microbial taxa[96]. Consistent with observations that top-down control of food webs increases with productivity, obligate microbial predators respond to shifts in prey resource availability by disproportionately increasing their rates of growth (compared to non-predator taxa) when C substrates, a common source of energy for their heterotrophic prey, are added to soil[66]. Food web structure is widely recognized to be a major determinant of productivity and element flux in marine and freshwater ecosystems, and may play an equally important role in soil ecosystems too. In particular, the CUE of microbial assemblages is important for modelling SOC cycling at the global scale[86] and microbial turnover may be significant sources of variation in this parameter[97]. Measurements of growth, along with mortality, can be used to quantify turnover and taxon-specific measurements of growth can identify factors, like predation and density-dependent effects, that contribute to its variation.

## Conclusion

There is an urgent need to improve our quantitative understanding of how microorganisms contribute to soil processes, given their central role in ecosystem C storage, nutrient cycling, and productivity. Growth rate integrates the many ways that microbes affect soil processes, and is a sensitive metric for studying cell and population-level responses to challenges that microorganisms encounter in nature, including challenges from changes in environmental conditions and biotic interactions. New approaches for measuring *in situ* microbial growth are important for accurately estimating the full range of growth rates in soils and offer a promising avenue to advance soil ecology. Understanding how microbial growth rates vary in the environment will enable greater cohesion between emerging ecological concepts and microbiological data. As soil ecological concepts and models are developed, it is critical that quantitative and sensitive measurements of *in situ* microbial growth be used alongside measurements of biogeochemical fluxes to understand how individual microbial taxa and aggregate microbial communities influence soil processes.

## Methods

### *Relative growth rates of soil microbial assemblages*

We compiled published estimates of relative growth rates of soil microbial assemblages, measured using seven common techniques: $H_2{}^{18}O$ SIP with IRMS, $H_2{}^{18}O$ qSIP, thymidine incorporation, leucine incorporation, acetate incorporation, lipidomic hydrogen SIP, and soil C mass balance modelling. We obtained estimates of relative growth rate from secondary sources[24,40] for the thymidine incorporation, leucine incorporation, acetate incorporation, lipidomic hydrogen SIP, and mass balance modelling methods. For $H_2{}^{18}O$ SIP with IRMS method, we searched papers citing Spohn et al. 2016 (the study that developed the method) and included measurements from papers that clearly reported growth rate or turnover time, sample preparation techniques, and mean and errors values. For the $H_2{}^{18}O$ qSIP method, we computed estimates of relative growth rate as the average of population relative growth rates across taxa. For all methods, we did not include soils that were contaminated with metals or soils that received additions of fertilizer, glucose, biochar, or microbial growth inhibitors in our dataset. In total we collected data from 30 studies and 287 measurements of relative growth rate of microbial assemblages (Supplementary Table 1).

### *Relative growth rates of soil microbial amplicon sequence variants*

We extracted values of excess atom fraction (EAF) $^{18}O$ from qSIP measurements compiled across 15 different sites (Supplementary Table 2) and estimated bacterial growth rates based on the rate $^{18}O$ assimilation from $^{18}O$-labeled water. All qSIP measurements involved parallel incubations, with samples receiving either isotopically labeled (e.g., 97 atom % $^{18}O$-$H_2O$) or unlabeled substrates (e.g., water with natural abundance $^{18}O$). The incubations lasted for 7.4 ± 1.8 days (average ± SD). After each incubation, DNA was extracted and subjected to density separation via isopycnic centrifugation. Density fractions were collected, the 16S rRNA gene was sequenced, and the total abundance of 16S rRNA gene copies in each fraction was quantified using qPCR. Quantitative stable isotope probing calculations were then applied to estimate EAF $^{18}O$[39,40].

Values of EAF $^{18}O$ that were negative or above the theoretical maximum enrichment of microbial DNA ($EAF_{max}$) are physically impossible and were considered outliers if variation among technical replicates was high (defined here as $SD > 0.15$) or the estimate was more than 1.5 standard deviations away from that taxon's average EAF $^{18}O$ across all replicates in all experiments. $EAF_{max}$ is computed as the product of the isotopic composition of soil water in each incubation (determined as a function of the amount of 97 atom % $^{18}O$ water added and total soil water content) and the fraction of oxygen atoms in newly synthesized DNA that are derived from environmental water, which was set to 0.6[40]. Out of 47,580 observations of EAF $^{18}O$, 492 observations were identified as outliers and removed. A density correction was performed to account for slight differences in the preparation of the CsCl density gradient solution of each replicate[69] and any remaining negative estimates of EAF $^{18}O$ (a total of 4,358) were corrected to zero. A total of 3,719 estimates of EAF $^{18}O$ remained above $EAF_{max}$, likely reflecting rapid microbial growth and assimilation of $^{18}$O from additional sources like organic matter or prey biomass. These values were corrected to EAFmax-0.002[98].

The relative growth rate (RGR) for each taxon was estimated according to using the EAF $^{18}$O of individual bacterial taxa (EAF) and the duration of the incubation (*t*) in days as:

$$\mathrm{RGR}\left(\mathrm{day}^{-1}\right)=\frac{\mathrm{EAF}\ {}^{18}\mathrm{O}}{\mathrm{EAF}_{\mathrm{max}}\ {}^{18}\mathrm{O}}\times\frac{1}{\mathrm{t}}$$

We applied a lower threshold of 0.002 EAF $^{18}$O when computing relative growth rates[98] meaning that if an ASV was enriched less than 0.002 EAF $^{18}$O it was considered to have an EAF $^{18}$O value of 0. Multiple qSIP measurements were conducted across the 15 sites, including experiments within some sites (Supplementary Table 2).

### *Statistical analyses*

We analyzed our database of growth rates of microbial assemblages to assess the influence of different ecosystems and methodological characteristics. To understand the extent that growth rate may not be independent from each other within studies, we compared a linear model against a mixed effects model, where study (i.e., paper) was coded as a random effect, using log-likelihood ratio testing and Akaike Information Criterion corrected for finite sample size (AICc) and Bayesian Information Criterion (BIC). Study was coded as a random effect (allowing for independent intercepts) in the mixed model (lme4 R package[99]). In both models, all methodological details (ecosystem, method, depth, incubation length in days, and whether soils were prepared as slurries) were included as fixed effects. Comparing the difference in model fit between the linear model and mixed model, we found a slight increase in model performance due to adding the random term ($X^2 = 4.88$, $p = 0.027$; $\Delta\mathrm{AICc} = -10.66$) but BIC suggested that the increase in model complexity may not be justified ($\Delta\mathrm{BIC} = 14.32$). For this analysis we chose to prioritize model simplicity and therefore report on linear model outputs. To determine the most important methodological variables driving relative growth rates, we use AICc and BIC to select the best, most parsimonious, statistical model from a set of candidate models (Supplementary Table 3). We considered all combinations of main effects as well as the interaction between ecosystem and method. The best model included ecosystem, method, and depth as significant predictors of microbial assemblage relative growth rates ($R^2 = 0.24$, ecosystem: $F_{3,273} = 2.94$ $p=0.03$, method: $F_{4,273} = 22.13$, $p < 0.001$, depth: $F_{2,273} = 12.39$ $p < 0.001$).

Lastly, we used linear regression to test the relationship between an ASV's growth rate measured with $H_2{}^{18}O$ qSIP and its abundance in soil. The output of the linear model was $y=1.5e^{-7}+3.6e^{-2}$ ($p = <0.001$, $r^2= 0.06$). All statistical analyses were performed in R version 4.2.2.

## Acknowledgments

The authors thank the Center for Ecosystem Science and Society (ECOSS) and the Lawrence Livermore National Laboratory (LLNL) Soil Microbiome Scientific Focus Area team for helpful discussions. This work was supported by the U.S. Department of Energy (DOE), Office of Biological and Environmental Research, Genomic Science Program (GSP) LLNL 'Microbes Persist' Soil Microbiome Scientific Focus Area SCW1632. Work conducted at LLNL was conducted under the auspices of the US Department of Energy under Contract DE-AC52-07NA27344. BWGS is grateful for support from the Linus Pauling Postdoctoral Distinguished Fellowship program through Pacific Northwest National Laboratory.

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**Table 1**. Descriptions, characteristics, published estimates of average generation times of soil microbial assemblages, and challenges associated with methods for measuring growth rate in soil. References are provided in Supplementary Table 3. “NA” indicates average generation time is not calculable.

***Microbial biomass carbon production rate***

| Approach | Description | Generation times | Characteristics | Challenges |
|---|---|---|---|---|
| $H_2{}^{18}O$ SIP with IRMS | Growth rates of soil microbial assemblages are estimated by incubating soil with $^{18}O$-labelled water or water vapor for several days. The rate of $^{18}O$ incorporation into microbial DNA is quantified using a thermochemical elemental analyzer (TC/EA) coupled to an Isotope Ratio Mass Spectrometer (IRMS) which approximates microbial DNA biomass synthesis[1,2]. | 2.67-761 days[3,4] | Captures the gross growth of microbial assemblages through biosynthesis over relatively short (days) periods of time before significant biomass turnover. Valuable for measuring gross growth of microbial assemblages. | Soil environment may interfere with microbial biomass and DNA extractions[5,6]. Assumption that water is the only source of oxygen for growth may underestimate growth rate[6]. Approaches that use additions of $^{18}O$-water may overestimate growth due to rewetting effects[2]. |

***DNA synthesis rate***

| Approach | Description | Generation times | Characteristics | Challenges |
|---|---|---|---|---|
| $H_2{}^{18}O$ quantitative SIP (qSIP) | Growth rates of soil microbial taxa are estimated by incubating soil with $^{18}O$-labelled water or water vapor for several days. The rate of $^{18}O$ incorporation into taxon-specific DNA is quantified using a combination DNA density fractionation via isopycnic centrifugation, DNA sequencing, and modeling of isotope substitution in DNA. $^{18}O$ incorporation rate approximates microbial DNA biomass synthesis and is used to model population growth and mortality rates[7,8,9]. | 2.99-34.7 days[10,11] | Measures the gross growth of microbial populations through biosynthesis over relatively short (days) periods of time before significant biomass turnover. Valuable for measuring gross growth and mortality rates of individual microbial amplicon sequence variants (ASVs) or metagenome-assembled genomes (MAGs). | Soil environment may interfere with DNA extractions. Approaches that use additions of $^{18}O$-water may overestimate growth due to rewetting effects[2]. |

| | | | | |
|---|---|---|---|---|
| Thymidine incorporation | Growth rates of soil bacterial assemblages are estimated by incubating bacterial cell extracts with radiolabeled thymidine, a precursor for DNA synthesis, for several hours. Incorporation rate of thymidine into cells is quantified using liquid scintillation which approximates bacterial DNA biomass synthesis[12]. | 0.75 – 168 days[13,14] | Captures the gross growth of bacterial assemblages through biosynthesis over short (hours) periods of time before significant biomass turnover. Valuable for measuring gross growth of bacterial assemblages. | Not all bacteria are able to incorporate extracellular thymidine into DNA[15]. Growth rates in bacterial suspension may not reflect growth rates of the assemblage in the original soil environment[13]. Amending soil with carbon sources and nutrients may stimulate microbial growth. |
| Peak to trough ratio (iREP, GRiD, DEMIC) | Relative growth rates of bacterial taxa are estimated based on patterns of read coverage in metagenomic sequence data. Read coverage reflects the growth rate of bacterial since more genome copies accumulate at the origin of replication compared to the terminus in circular bacterial genomes during growth[16-19]. | NA | Infers the relative growth rates of bacterial taxa through genome replication. Estimates represent an inference of instantaneous growth rate at the time of sample collection before DNA extraction. Valuable for interpreting the growth status of bacteria in a wide range of metagenomic datasets. | There has been limited validation of this approach in naturally occurring soil microbial assemblages. Current PTR methods may not reliably predict in situ growth rates in naturally occurring bacterial assemblages[20]. |
| BrdU uptake | Relative growth rates of soil bacterial taxa are estimated by incubating bacterial cell extracts with 5-bromo-2′-deoxyuridine (BrdU), an analog of the DNA precursor thymidine, for a few days. Cell that are synthesizing new DNA and incorporate BrdU are isolated by immunocapture and can be characterized using amplicon or metagenomic sequencing[21,22]. | NA | Measures the relative growth rates of soil microbial taxa through biosynthesis over relatively short (days) periods of time before significant biomass turnover. Valuable for identifying growing microbial taxa and their responses to environmental perturbations. | Many microbial taxa do not incorporate exogenous thymidine analogs into their DNA[23] and there can be up to 10-fold variation among taxa in BrdU incorporation rates leading to skewed or incomplete representations of active populations[24]. Approaches that use additions of water may overestimate growth due to rewetting effects[2]. |

### *Lipid synthesis rate*

| Approach | Description | Generation times | Characteristics | Challenges |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Lipidomic hydrogen stable isotope probing (LH-SIP) | Growth rates of soil microbial taxa are estimated by incubating soil with $^{2}$H-labelled water for several days. The rate of $^{2}$H incorporation into compound-specific membrane lipids is quantified using gas chromatography/pyrolysis/isotope ratio mass spectrometry (GC/P/IRMS). $^{2}$H incorporation rate approximates lipid membrane biomass synthesis and growth rate of broad taxonomic groups[25]. | 20-64 days[25] | Captures the growth of microorganisms over relatively short (days) periods of time before significant biomass turnover. Valuable for sensitively measuring gross growth of microbial assemblages, especially those exhibiting slow growth, as IRMS captures minute levels of $^{2}$H incorporation. Growth rates can be distinguished at the phylum level as well as by functional groups (e.g., methanotrophs, methanogens, fungi, AMF, anaerobes, cyanobacteria, etc.). | Approaches that use additions of water may overestimate growth due to rewetting effects[2,26]. Lipid biomarkers are only taxonomically resolved at the phylum level or among various functional groups[27]. |
| Acetate incorporation | Growth rates of soil fungal assemblages are estimated by incubating soil slurries with radiolabeled acetate for several hours and measuring the incorporation of acetate into the fungal-specific lipid ergosterol. Incorporation rate of acetate into ergosterol is quantified using liquid scintillation which approximates fungal lipid biomass synthesis[28]. | 0.94-468 days[29,30] | Captures the gross growth of fungal assemblages through biosynthesis over short (hours) periods of time before significant biomass turnover. Valuable for measuring gross growth of fungal assemblages. | Growth rates in soil slurries may not reflect growth rates of the assemblage in the original soil environment[26]. Amending soil with carbon sources and nutrients may stimulate microbial growth. |

## *Protein synthesis rate*

| **Approach** | **Description** | **Generation times** | **Characteristics** | **Challenges** |
|---|---|---|---|---|
| Leucine incorporation | Growth rates of soil bacterial assemblages are estimated by incubating bacterial cell extracts with radiolabeled leucine, a precursor for protein synthesis, for several hours. Incorporation rate of leucine into cells is quantified using liquid scintillation approximates bacterial protein biomass synthesis[31,32]. | 0.70-142 days[13,33] | Captures the gross growth of bacterial assemblages through biosynthesis over short (hours) periods of time before significant biomass turnover. Valuable for measuring gross growth of bacterial assemblages. | Growth rates in bacterial suspension may not reflect growth rates of the assemblage in the original soil environment[13]. Amending soil with carbon sources and nutrients may stimulate microbial growth. |

## *Maximum potential growth rate*

| Approach | Description | Generation times | Characteristics | Challenges |
|---|---|---|---|---|
| Codon usage bias | Maximum potential growth rates of soil microbial taxa are estimated from genomes based on the degree to which a genome or MAG favors one set of codons to encode an amino acid. Codon usage bias and growth are correlated due to selection pressure for highly expressed genes to use the optimal codons for translation, resulting in some codons being used more than others[34]. | 0.03-0.19 days[34,35] | Infers the maximum potential growth rate of microbial taxa. In contrast to in situ growth rate, maximum potential growth rate is an inferred physiological strategy rather than a field observation. The value in this approach is as a starting point for modeling population dynamics and evaluating the growth potential of organisms represented by MAGs in sequencing datasets. | There has been limited validation of this approach in naturally occurring microbial assemblages. CUB appears to works reasonably well for fast growing prokaryotes that can be binned into a high-quality MAG[34]. |

### *Carbon flux through microbial biomass rate*

| Approach | Description | Generation times | Characteristics | Challenges |
|---|---|---|---|---|
| Soil C mass balance modeling | Growth rates of soil microbial assemblages are modelled from soil microbial biomass and annual litter input rates as:<br>$u = [Y/x * dL/Dt - a]/(1-y)$<br>where u is growth rate, dL/dt is the rate of litter inputs, x is microbial biomass, Y is the yield coefficient, and a is the specific maintenance rate[36,37]. | 3.3-632 days[37,38] | Integrates drivers from population and assemblage scales as well as time-dependent factors such as biomass turnover and the recycling of necromass in estimates of growth rate. Provides a measure of assemblage-level growth via biosynthesis plus mortality and necromass recycling across generations of cells. Valuable as an ecosystem-level measure of microbial biomass turnover over longer time scales. | Parameters for maintenance and growth yields are challenging to quantify for soil microbial assemblages. |

**Figure 1: Published estimates of growth rates of soil microbial assemblages in agricultural, forest, grassland, and tundra ecosystems**. Estimates span four orders of magnitude (0.0009-1.98 day$^{-1}$) in studies using $H_2{}^{18}O$ SIP with IRMS, $H_2{}^{18}O$ qSIP, thymidine incorporation, leucine incorporation, acetate incorporation, lipidomic hydrogen SIP (LH-SIP), and soil C mass balance modelling. The y-axis is log$_{10}$ transformed. Additional study information is shown in Supplementary Table 1.

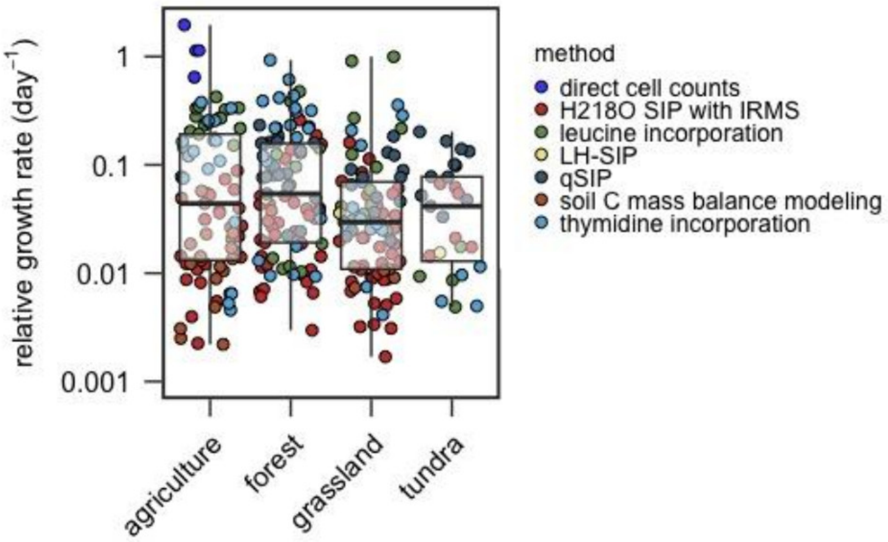

**Figure 2: Relative growth rates of soil bacterial and archaeal taxa measured by $H_2{}^{18}O$ qSIP across 13 sites and five ecosystems: tropical forest, temperate grassland, temperate conifer forest, boreal forest, and moist acidic tundra.** a) Distribution of *in situ* relative growth rates of amplicon sequence variants (ASVs). Most ASVs exhibit low to intermediate rates of growth. b) Average relative growth rates of bacterial and archaeal ASVs against their abundances (linear model; $p < 0.001$, $r^2 = 0.0001$). ASV sequencing abundances were converted to absolute abundance based on the number of 16S rRNA gene copies per gram of dry soil. C) Distribution of relative growth rates of bacterial and archaeal phyla. The middle line corresponds to the median, lower and upper edges correspond to the first and third quartiles, and whiskers extend to the highest and lowest point within 150% of the interquartile range. All estimates from growth were measured at approximately room temperature. Additional study information shown in Supplementary Table 2.