

# Growth rate as a link between microbial diversity and soil biogeochemistry

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Measuring the growth rate of a microorganism is a simple yet profound way to quantify its effect on the world. The absolute growth rate of a microbial population reflects rates of resource assimilation, biomass production and element transformation—some of the many ways in which organisms affect Earth's ecosystems and climate. Microbial fitness in the environment depends on the ability to reproduce quickly when conditions are favourable and adopt a survival physiology when conditions worsen, which cells coordinate by adjusting their relative growth rate. At the population level, relative growth rate is a sensitive metric of fitness, linking survival and reproduction to the ecology and evolution of populations. Techniques combining omics and stable isotope probing enable sensitive measurements of the growth rates of microbial assemblages and individual taxa in soil. Microbial ecologists can 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, coupled with measurements of biogeochemical fluxes, 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.

Achieving growth in the face of a changing environment is a 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, the 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 that allows the cell to modify its activities depending on changes in its surrounding environment—often on a very short time scale. Over billions of years of evolution, soil microorganisms developed strategies

for growing in extreme cold and heat, in highly acidic and alkaline habitats, on the inside and outside of plant roots and on mineral surfaces. 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<sup>1</sup>.

As soil microorganisms grow, they assimilate, transform and redistribute key elements in their environment<sup>2</sup>, with far-reaching consequences for Earth's ecosystems and climate. Microorganisms aid in the process of extracting phosphorus and sulfur from their geological

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reservoirs, where they typically reside for thousands to millions of years, moving them into biological systems with much shorter residence times, typically ranging from weeks to months<sup>3,4</sup>. The assimilation and retention of nutrients such as nitrogen and phosphorus in microbial biomass can constrain plant growth and limit the capacity of ecosystems to capture carbon from the atmosphere<sup>5</sup>. Microbial redox transformations determine whether organic carbon molecules in soil reach the atmosphere as CO<sub>2</sub> or CH<sub>4</sub>, potentially amplifying the effect of the gas on Earth's climate.

Soil microorganisms exist in a range of physiological states, from dormancy to exponential growth, with profoundly different consequences for soil carbon and nutrient cycling. The relative growth rate (that is, the rate of increase in mass or abundance per unit time relative to the starting size) captures such variation and is a powerful index of how microorganisms adjust their physiology in response to the environment. As traits that confer stress tolerance can hinder the ability of cells to grow quickly<sup>6</sup>, many microbial species have developed distinct phenotypes for survival in stressful versus growth-conducive environments. These phenotypes vary profoundly, not only in their rates of growth, but also in their central carbon metabolic networks<sup>7</sup>, cell sizes and macromolecular compositions<sup>8–13</sup>. At the population level, growth concepts are intimately linked with fitness and capture the nuances that arise as microorganisms interact with each other and their surroundings (we use the term growth to refer to gross growth, rather than net growth, which is a function of both growth and mortality rates). Along with the per capita mortality rate, the per capita growth rate—a measure of the average individual growth rate in a population—reflects how well microorganisms compete for resources and respond to challenges associated with stress and predation. Measures of relative growth, such as per capita growth rate, are especially useful for understanding how growing microorganisms respond to the environment and can also be used to quantify the intensity of interactions, such as competition, predation and mutualism<sup>14</sup>.

Absolute growth rate, or the rate of change in mass or abundance per unit time, is useful for quantifying microbial contributions to element fluxes. Measurements of absolute growth rates relative to soil quantity (that is, the rate of change in mass or abundance of microorganisms per unit time per unit mass or volume of soil) reflect rates of microbial element assimilation and use. Along with the absolute mortality rate, the absolute growth rate sets the standing stock of microbial biomass and ultimately drives changes in the taxonomic makeup of entire communities. Soil microorganisms can exhibit rapid rates of turnover (the rate at which microorganisms in populations or communities are replaced via growth and mortality), often with minimal changes in population size or biomass. For this reason, microbial biomass and abundance alone are poor predictors of element flux<sup>15,16</sup>. Measurements of absolute growth, along with other quantitative metrics of physiologically active microorganisms, would provide a powerful means for testing the effects of microbial biodiversity on carbon and nutrient cycling at the ecosystem scale<sup>17</sup>.

There is a rich history of measuring growth rates in soil microbial ecology, including decades of measurements in culture<sup>18,19</sup> and in situ<sup>20–24</sup>. Many recent developments in soil ecology invoke microbial growth rates to conceptually link microbial physiology to ecosystem services such as climate mitigation<sup>25</sup>, pollution decrease<sup>26</sup> and food supply<sup>27</sup>. In situ measurements of growth rate, including those of specific taxonomic groups<sup>16,28,29</sup> and individual cells<sup>30</sup>, enable rigorous tests of the controls over the ecology of these organisms where they live and grow and how they connect to larger-scale ecological processes<sup>31–33</sup>.

## Measurements of microbial growth rates in soil

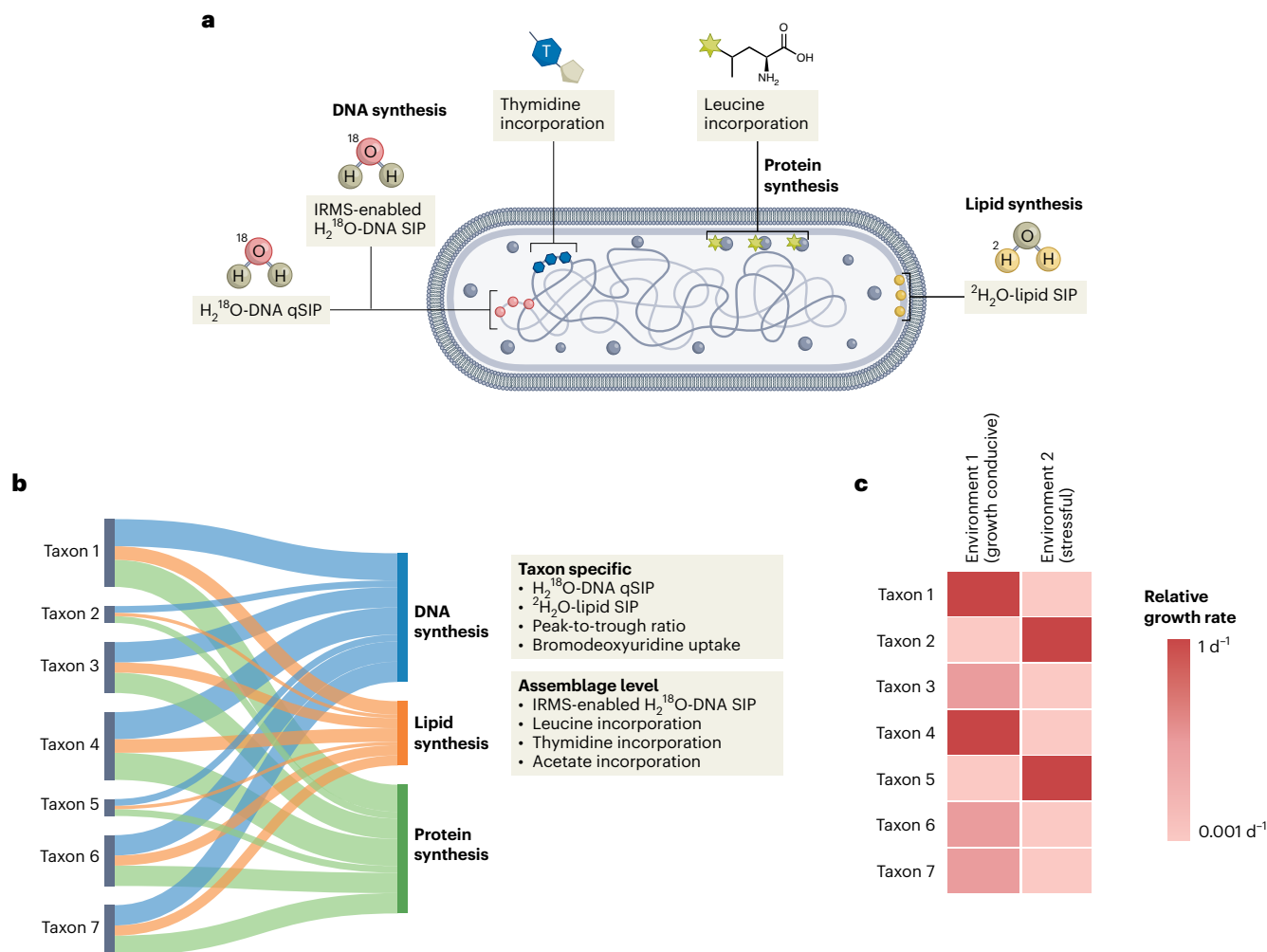
Methods measuring soil microbial growth rates in situ capture different processes, from the synthesis of biomolecules that make up individual cells to the expansion of populations and the gross production of biomass carbon at the assemblage level (Fig. 1 and Supplementary Table 2). Such methodological diversity is reflected in published estimates of

relative growth rates of soil microbial assemblages, which span at least four orders of magnitude from 0.0009–1.9800 d<sup>−1</sup>. Syntheses of in situ growth rate measurements can be used to identify sources of variation within and between methods. Linear model analysis of published estimates of assemblage-level growth rates indicates that method, ecosystem type and soil depth can be significant predictors of in situ growth (Fig. 2a; whole-model  $R^2 = 0.24$ ; ecosystem:  $F_{3,276} = 7.10$  ( $P < 0.001$ ); method:  $F_{5,276} = 71.90$  ( $P < 0.001$ ); depth:  $F_{2,276} = 9.75$  ( $P < 0.001$ ); Supplementary Methods). Environmental factors such as carbon availability<sup>24</sup>, soil moisture<sup>34</sup>, temperature, pH and seasonality<sup>35</sup> are important determinants of soil microbial growth too. Systematic reviews and meta-analyses are needed to comprehensively synthesize growth rate measurements and quantify the relative importance of environmental and methodological factors across ecosystems and under future climate scenarios.

Methodological variation may arise from multiple sources. For example, incubations may be biased if temperatures are held below or above those typical of the organisms' natural habitat and shorter incubations are less sensitive at detecting, and may thus exclude, taxa with slower growth rates compared with longer incubations (but see ref. 16). Methodological variation may also be a product of methods targeting different biomolecules, such as DNA, proteins or lipids, which may have variable rates of synthesis and degradation that are contingent on the cell's physiological state. During exponential growth, cells synthesize macromolecules at near-constant differential rates and divide at a particular cell mass or size. Under these conditions of balanced growth, the relative growth rate sets key cellular phenotypes such as cell size and the mass fractions of nucleic acids, proteins and lipids. In nature, relationships between replicative growth and rates of macromolecular synthesis may not always be so tightly coupled. Applying multiple methods could help to identify the physiological adjustments that allow microorganisms to strike a balance between survival and proliferation in soil. For example, in response to carbon limitation, microorganisms may undergo reductive division<sup>36</sup>, simultaneously catabolizing lipids for energy<sup>37</sup> while synthesizing DNA and protein in order to divide into smaller and more stress-resistant cells, which could be explored using stable isotope probing (SIP) approaches to target lipid<sup>16</sup>, DNA<sup>28,29,38</sup> and protein<sup>39</sup> synthesis concurrently. A wide range of methods are needed to capture the many strategies microorganisms may use to grow in soil.

Most measurements quantify the relative growth rate, which is useful for understanding how microorganisms respond to the environment. Measurements of absolute growth rate are needed to understand how microorganisms move elements through ecosystems. Converting metrics of relative growth rate (for example, based on rates of tracer uptake per unit time) to absolute growth rates (in units of mass or the number of microorganisms per unit time) can be challenging because direct measurements of biomass and abundance are difficult to obtain and validate<sup>40</sup>. Estimates of absolute growth rate may also require known extraction efficiencies of biomolecules from soils. For example, SIP-based methods measure growth based on rates of isotope incorporation into target biomolecules, which require biomolecule extractions from soils. Extractions of DNA, lipids or proteins from soil seldom yield complete recovery. Extraction efficiencies may be low or variable or—in the case of DNA—may not typically be evaluated. Including recovery standards<sup>41</sup> and developing better constraints on the recovery of necromass-derived biomolecules would improve the accuracy of absolute growth rate measurements in soil.

Many approaches quantify growth rate at the scale of whole microbial assemblages, which result in a single estimate of growth for a soil sample—an aggregate of thousands of microbial populations. Methods that quantify the growth rates of microbial taxa<sup>16,28,29,42</sup> and single cells<sup>30</sup> are promising avenues for developing quantitative links between specific microorganisms and soil processes. Estimates of growth from over 46,000 measurements of rates of DNA synthesis



**Fig. 1 | Methods for measuring soil microbial growth rates in situ. a,** Isotope tracing approaches capture the synthesis of various biomolecules, such as DNA, lipids and proteins, providing an approximation of gross microbial growth. **b,** Biomolecules have variable rates of synthesis depending on a cell's physiological state and biochemical composition, which may contribute to variation among growth rate estimates in soil. Many methods capture the growth rate of entire assemblages of microorganisms in a sample. These measurements are useful for understanding how microorganisms, in aggregate, affect element fluxes but cannot capture the growth dynamics of individual

populations. **c,** Some methods leverage omics technologies to pair growth rate measurements with taxonomic information. These methods quantify the growth rates of individual taxa, allowing researchers to test hypotheses in microbial ecology. Shown is a hypothetical heat map of taxon-specific growth rates in two distinct environments—an approach that can be used to explore whether microbial adaptations to a selective environment are accompanied by a loss of reproductive potential in non-selective environments (that is, whether a trade-off has occurred). IRMS, isotope ratio mass spectrometry; qSIP, quantitative SIP.

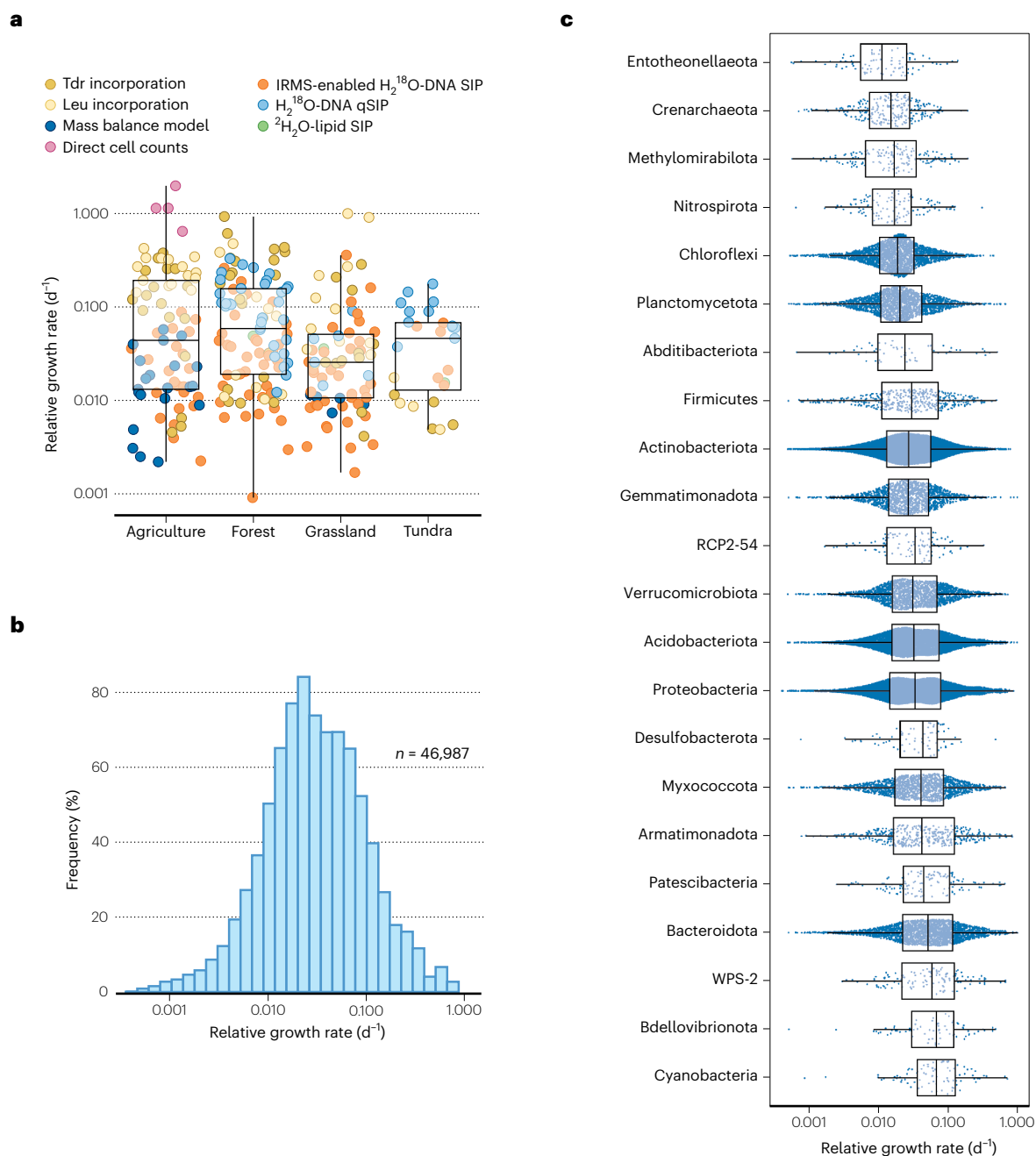
show tremendous variation in relative growth rates among bacterial groups in soil (Fig. 2b) and indicate that relative growth rates of soil bacteria are comparable to those of marine bacteria<sup>43</sup>, both of which are slow compared with growth rates in culture<sup>44</sup>. Future comparative studies applying multiple approaches are needed to critically compare the growth rates of phylogenetically related microorganisms in nature.

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<sup>45–47</sup>, temperature<sup>48–51</sup>, disturbance<sup>52–54</sup>, mineral composition<sup>55</sup> and climate<sup>56,57</sup>. Microbial contributions to respiration and carbon and nitrogen assimilation appear to be highly taxon specific, and variation in microbial contributions to element fluxes can be meaningful when scaled to the ecosystem level<sup>17,58</sup>. Such measurements offer a new set of data for testing and developing microbe-explicit representations of carbon and nitrogen cycling. Measurements of relative growth rate have shown how interactions among soil microorganisms—including competition<sup>59</sup>, mutualism<sup>15</sup> and predation<sup>60,61</sup>—can influence element flux, just as interactions between plants and animals can influence ecosystem processes.

Growth rate measurements have a clear place in testing the role of ecological theory in soil microbial ecology. Like macroscopic organisms, microbial phenotypes in soil are constrained by their evolutionary histories<sup>62,63</sup>. Phenomena such as negative density dependence and r/K selection theory are key for understanding the population growth of larger organisms, but these concepts have failed to be strong predictors of growth patterns of microorganisms in situ<sup>64,65</sup>. As such, there is a great need for evidence-based ecological frameworks that are built on direct observations of soil microbiomes<sup>66</sup>. Below, we describe how quantitative data on soil microbial growth rates can 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<sup>67</sup>, soil organic carbon (SOC) formation and loss<sup>68</sup> and plant productivity<sup>69</sup>, with implications for pollution<sup>26</sup>, food supply<sup>27</sup> and climate<sup>25</sup>. Quantitative in situ measurements of microbial growth could offer powerful insight into how microorganisms contribute to



**Fig. 2 | Relative growth rates of soil microbial assemblages and individual taxa in multiple ecosystems.** **a**, Published estimates of relative growth rates of soil microbial assemblages in agricultural, forest, grassland and tundra ecosystems. Measurements were made using IRMS-enabled  $\text{H}_2^{18}\text{O}$ -DNA SIP,  $\text{H}_2^{18}\text{O}$ -DNA qSIP, thymidine (Tdr) incorporation, leucine (Leu) incorporation,  $^2\text{H}_2\text{O}$ -lipid SIP or soil carbon mass balance modelling. The y axis is log transformed. Study information is provided in Supplementary Data 1. **b**, Distribution of relative growth rates of amplicon sequence variants measured by  $\text{H}_2^{18}\text{O}$ -DNA qSIP in five

ecosystems: tropical forest, temperate grassland, temperate conifer forest, boreal forest and moist acidic tundra. The x axis is log transformed. **c**, Distribution of relative growth rates of bacterial and archaeal phyla. The x axis is log transformed. Study information is provided in Supplementary Data 2. In **a** and **c**, the middle lines correspond to median values, the lower and upper edges correspond to the first and third quartiles and the whiskers extend to the highest and lowest points within 150% of the interquartile range.

ecosystem processes and could help in the discovery of new tools for managing the soil microbiome to promote ecosystem services.

### Microbial physiology and soil organic carbon cycling

The physiological properties of microorganisms have a key role in governing the formation and loss of SOC stocks<sup>70</sup> that are vital for mitigating greenhouse gas emissions and enhancing the sustainability of agricultural systems<sup>71</sup>. Measurements of in situ soil microbial growth could be used to inform and test emerging hypotheses on SOC

cycling. For example, microbial necromass may constitute as much as 50% of the mineral-associated organic matter pool—the largest and slowest-cycling reservoir of SOC<sup>72–74</sup>. Thus, fast and efficient microbial growth and turnover should increase the production of microbial residues and the accrual of microbial-derived, mineral-associated organic matter<sup>68,75</sup>. In situ growth rate measurements that capture absolute growth at the assemblage scale (for example, isotope ratio mass spectrometry-enabled  $\text{H}_2^{18}\text{O}$ -DNA SIP<sup>38</sup> and  $^2\text{H}_2\text{O}$ -lipid SIP<sup>16</sup>) could be used to identify relationships between growth rate, growth



efficiency and SOC formation at the ecosystem scale. Relationships between growth rate and growth efficiency, defined as the portion of consumed substrate that is converted into biomass, are critical for such conceptualizations of SOC formation but poorly defined for soil microorganisms. The mechanisms theorized to underpin relationships between growth efficiency and relative growth rate, such as maintenance requirements, overflow metabolism and protein synthesis costs, are physiological and may therefore be most apparent at the level of individual microbial cells, species or populations. Whether population-scale physiology drives emergent relationships between microbial growth and SOC formation at the ecosystem scale could then be tested, for example, using individual-based modelling to couple observations of relative growth rate and growth efficiency at the population level (for example, via soil isolates<sup>76</sup> or genome-informed, trait-based modelling<sup>77</sup>) to assemblage-level measurements of growth rate and rates of SOC formation.

Microbial processes affecting soil carbon accrual and persistence are represented in some numerical models of SOC cycling<sup>78–81</sup>. Measurements of microbial growth can be used to parameterize microorganism-explicit biogeochemical models and test how microbial physiology modulates SOC responses to environmental changes. For example, growth rate measurements could be used to parameterize formulations of microbial dormancy<sup>79</sup> and density-dependent growth<sup>82</sup> in ecosystem-scale models. At the global scale, modelling growth efficiency is key to predicting the dynamics of soil carbon stocks<sup>83</sup>, and growth rate may be an important factor to consider in these large-scale geochemical models too. Additional measurements of soil microbial growth rates will provide the data needed to test conceptual and quantitative models of how microorganisms influence the soil carbon cycle. There is a clear need for direct measurements of in situ growth rates using existing approaches 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

Amid 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<sup>84–86</sup>. 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<sup>87</sup>. Collectively, these frameworks represent diverse hypotheses about interactions between microbial community structure and soil processes. Soil microbiologists are well positioned to begin experimentally testing these frameworks by coupling in situ measurements of growth with omics data.

Many microbial frameworks have been derived from classic ecological theory (that is, 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<sup>85</sup>, but tests of whether microorganisms use their assigned strategies in nature are rare<sup>65</sup>. Alternatively, broad ecological strategies can be identified based on genomic features<sup>84</sup> and gene expression<sup>88</sup>, but our ability to translate microbial genes to function and rate of function is nascent.

As an essential property of an organism's life history and metric of competitive ability, in situ relative growth rate has a direct role in testing frameworks that build on classical ecological theory. Relative 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 non-selective environments—in other words, whether a trade-off has occurred. For example, the relative growth rate of an organism with a stress tolerator

strategy<sup>84</sup> would be expected to be above average under stressful conditions and below average in the absence of environmental stressors. Quantifying the growth of microorganisms where they live and grow in nature also provides access to a broader suite of trait dimensions than can be extrapolated from pure culture studies. Direct, in situ relative 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 to 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<sup>89</sup>. Predation in the rhizosphere changes the taxonomic structure of prokaryotic communities and alters rates of nitrogen mineralization, influencing vegetation productivity<sup>90</sup>. Mutualistic interactions between microbial taxa stimulate the depolymerization of complex carbon compounds<sup>91</sup> and antagonistic interactions influence growth rates through negative density dependence<sup>52</sup>, altering rates of carbon flux from microbial biomass to soil<sup>82</sup>. Taxon-specific estimates of relative growth rate would be valuable for assessing microbial interactions in which one soil microorganism influences another by altering its growth, reproduction or any trait affecting fitness.

In soil microbial ecology, network analyses of co-occurrence patterns in molecular abundance datasets are used to infer in situ interactions between microorganisms. These analyses are based on the premise that microorganisms must co-occur to interact and that interactions affecting demography will drive patterns in co-occurrence data. Environmental variability in time and space, dynamic species distributions and other ecological complexities weaken and may obscure relationships between co-occurrence and interactions<sup>92</sup>. Assessing how the growth rate of one organism affects the growth of another could constrain inferences about interactions. Multilayer network analyses could combine independent data streams such as growth rate and co-occurrence, which would allow inferences to be cross-validated, potentially improving the accuracy of interaction studies.

Growth rates of microbial taxa, along with growth efficiencies, could be used to construct accurate food webs to quantify how energy and elements are transferred between microbial taxa<sup>93</sup>. Compound-specific growth estimates (via SIP of <sup>13</sup>C and <sup>15</sup>N nutrient sources) trace the flow of soil nutrients through microorganisms and quantify their rates of transformation<sup>94–96</sup>. These approaches identify syntrophic interactions in soil by tracing biogeochemical fluxes between organisms and nutrient pools<sup>97,98</sup> and identifying rates of biomass production resulting from specific metabolic strategies<sup>99,100</sup>. There are several key limitations to such approaches, such as the ability to resolve cross-feeding. Experimental designs that explicitly account for these limitations are especially useful. For example, Hungate et al.<sup>60</sup> correct for differences in potential sources of <sup>18</sup>O between predatory and non-predatory soil bacteria (predators derive <sup>18</sup>O from labelled prey biomass and soil water, whereas non-predators derive <sup>18</sup>O from soil water alone) in computations of growth rate, finding that obligate predators respond to increases in prey resource availability by disproportionately increasing their relative growth rates (compared with non-predator taxa) when carbon substrates—a common source of energy for their heterotrophic prey—are added to soil. Taxon-specific growth rates also provide a means for assessing the importance of interactions in structuring individual populations. For example, density-dependent population growth (typically measured as net growth) reflects direct interactions among individuals within a population, such as competition for resources, which can be assessed by quantifying relationships between population density and relative growth rate in situ<sup>52</sup>. Growth rate is a clear and promising metric for defining ecological interactions, offering a way to quantitatively link

interactions between individual taxa to the trajectory of entire populations and the flow of elements within the soil microbiome.

## Conclusion

There is an urgent need to improve our quantitative understanding of how microorganisms contribute to soil processes, given their central role in ecosystem carbon storage, nutrient cycling and productivity. Growth rate integrates the many ways that microorganisms affect soil processes and is a sensitive metric for studying cell- and population-level responses to challenges in nature, including challenges from biotic interactions and changes in environmental conditions. Moving forward, diverse approaches are needed to accurately estimate the full range of microbial growth rates in soil, and comprehensive reviews, meta-analyses and comparative studies will be critical for quantifying biological and methodological sources of variation. Understanding how microbial growth rates vary in soil will enable greater cohesion between emerging ecological concepts, microbial identity and biogeochemistry. 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 whole assemblages influence soil processes.

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## Author contributions

M.M.F., B.J.K., T.A.C., N.F. and B.A.H. conceived of the study idea. M.M.F., B.W.G.S., B.J.K. and B.A.H. designed the study methodology and performed the formal analysis. M.M.F., B.W.G.S., B.J.K., S.J.B., P.D., M.H., K.H., B.K.F., J.M., R.L.M., V.M.-Q., E.M., J.P., A.P. and N.F. performed the investigation. M.M.F., B.W.G.S. and B.A.H. curated the data. M.M.F. and B.A.H. visualized the data. S.J.B., K.H., E.M., E.S., J.P.-R. and B.A.H. acquired the funding. S.J.B., K.H., E.M., E.S., J.P.-R. and B.A.H. provided resources. J.P.-R. and B.A.H. supervised the project and performed project administration. M.M.F., B.J.K., T.A.C., N.W.S., N.F. and B.A.H. wrote the manuscript. All authors reviewed and edited the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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