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RESEARCH ARTICLE

The Functional Significance of Bacterial Predators

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ABSTRACT Predation structures food webs, influences energy flow, and alters rates and pathways of nutrient cycling through ecosystems, effects that are well documented for macroscopic predators. In the microbial world, predatory bacteria are common, yet little is known about their rates of growth and roles in energy flows through microbial food webs, in part because these are difficult to quantify. Here, we show that growth and carbon uptake were higher in predatory bacteria compared to nonpredatory bacteria, a finding across 15 sites, synthesizing 82 experiments and over 100,000 taxon-specific measurements of element flow into newly synthesized bacterial DNA. Obligate predatory bacteria grew 36% faster and assimilated carbon at rates 211% higher than nonpredatory bacteria. These differences were less pronounced for facultative predators (6% higher growth rates, 17% higher carbon assimilation rates), though high growth and carbon assimilation rates were observed for some facultative predators, such as members of the genera *Lysobacter* and *Cytophaga*, both capable of gliding motility and wolf-pack hunting behavior. Added carbon substrates disproportionately stimulated growth of obligate predators, with responses 63% higher than those of nonpredators for the *Bdellovibrionales* and 81% higher for the *Vampirovibrionales*, whereas responses of facultative predators to substrate addition were no different from those of nonpredators. This finding supports the ecological theory that higher productivity increases predator control of lower trophic levels. These findings also indicate that the functional significance of bacterial predators increases with energy flow and that predatory bacteria influence element flow through microbial food webs.

IMPORTANCE The word "predator" may conjure images of leopards killing and eating impala on the African savannah or of great white sharks attacking elephant seals off the coast of California. But microorganisms are also predators, including bacteria that kill and eat other bacteria. While predatory bacteria have been found in many environments, it has been challenging to document their importance in nature. This study quantified the growth of predatory and nonpredatory bacteria in soils (and one stream) by tracking isotopically labeled substrates into newly synthesized DNA. Predatory bacteria were more active than nonpredators, and obligate predators,

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such as *Bdellovibrionales* and *Vampirovibrionales*, increased in growth rate in response to added substrates at the base of the food chain, strong evidence of trophic control. This work provides quantitative measures of predator activity and suggests that predatory bacteria—along with protists, nematodes, and phages—are active and important in microbial food webs.

KEYWORDS ^{18}O-H_2O, *Bdellovibrio*, food webs, predator, qSIP, stable isotope probing, top-down control, trophic interactions

Bacteria that prey on other bacteria are too small to engulf their victims, yet they consume them no less ferociously. Members of the *Bdellovibrionales* attach to prey cells, penetrate the cell membrane, and then take up residence in the host cytoplasm, consuming cellular constituents while growing filaments and producing daughter cells that eventually lyse and kill the prey (1). Some bacterial predators have names that tell their mode of predation; *Vampirovibrio* (2, 3) and *Vampirococcus* (4) insert cytoskeletal protrusions, "fangs," which extract the cytoplasm from the attacked cell. Some members of the genus *Cytophaga* are "cell eaters" (5, 6), and *Lysobacter* are "lysers of bacteria" (7). These and members of the *Myxococcales* are social organisms which hunt in packs (8, 9). Many of these organisms can also subsist as saprotrophs and thus are facultative predators (10), in contrast to *Vampirovibrio* and *Bdellovibrio*, which are obligate predators (11). Most of what we know about the physiology, growth, and activity of predatory bacteria has been learned from laboratory studies because of the difficulty of measuring taxon-specific bacterial activity *in situ*.

Predators are thought to be functionally significant in microbial food webs, but quantitative estimates *in situ* have been very difficult to obtain. It is possible to use fluorescent markers and plate counts to estimate growth rates of predators in artificial media (12), but applying such approaches in the field is challenging. For example, it is known that phages prey upon cyanobacteria in rice paddy soils, but the rates of predation are unknown (13). Experimental manipulations of soil protozoa in mesocosm studies demonstrate the importance of these eukaryotic predators for nitrogen cycling (14) and for decomposition of plant litter (15), but the quantitative impacts on these ecosystem processes under field conditions are difficult to measure experimentally. Various environmental conditions also influence predator-prey interactions; changing moisture content alters soil connectivity, stabilizing or destabilizing predator-prey dynamics (16). It is important that predator activity and growth be measured under realistic and varied conditions.

Although protists (17), rotifers (18), nematodes (19), and phages (11, 20, 21) are thought to function as the dominant predators in microbiomes, predatory bacteria are common in both soil (8, 22) and aquatic (23) systems. But beyond their common occurrence in these habitats, we know little of their activity in the wild, how rapidly they grow, their functional significance in food webs, and how they respond to enrichment at the base of the food web through substrate additions.

DNA sequencing and other 'omics techniques can provide detailed information on the composition and functional potential of the microbiome (24), but most measurements of *in situ* bacterial growth rates lack taxonomic resolution and are conducted at the scale of the entire microbial assemblage (25, 26). Such aggregate measurements mask the contributions of genetically and functionally distinct populations. Even in macroscopic assemblages, taxa are known to vary in their influences on ecosystem processes (27). Techniques that combine isotopes and genetic sequencing hold promise for parsing the contributions of individual microbial taxa to interactions within microbial assemblages and to biogeochemical processes (28, 29).

Here, we synthesized measurements using quantitative stable isotope probing (qSIP), a technique that quantifies the isotopic composition of DNA after exposure to an isotope tracer (30). qSIP with ^{13}C-labeled organic matter tracks the rate of labeled carbon assimilation into DNA, and qSIP using ^{18}O-water tracks the incorporation of

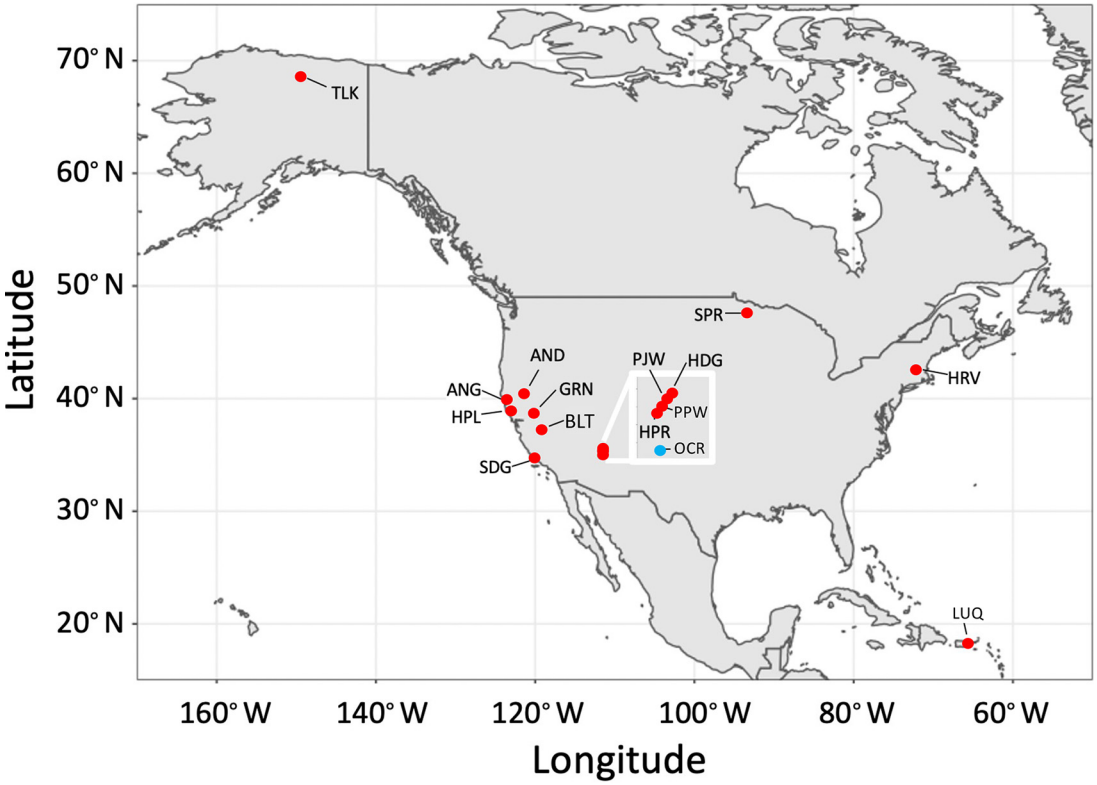

FIG 1 Location of sites included in our meta-analysis of growth rates of predatory and nonpredatory bacteria. Additional site information and abbreviations are shown in Table 1. Inset shows a cluster of sites in Arizona (box scale is 1 by 1°).

oxygen from water into DNA. Recovery of the isotope tracer in taxon-specific DNA sequences reflects rates of growth and carbon assimilation of individual microbial taxa (28, 31). The survey conducted here included qSIP measurements conducted in natural microbial assemblages from sites in North America, including 14 soils (1 arctic, 1 boreal, 11 temperate, and 1 tropical), along with one temperate stream (Fig. 1,

TABLE 1 Site description[a]

Ecosystem (abbreviation)	Lat	Long	MAT (°C)	MAP (cm)	^{13}C	^{18}O	Temp (°C)[b]	Substrates[b]	Predators (%)[b]
Moist acidic tundra (TLK)	68.63	−149.61	−7.0	30	−	+	5, 15, 25, 35	NA	6.8
Temperate conifer forest (AND)	38.63	−120.23	9.1	115	+	+	NA	glu, exu, lit, ox	7.6
Boreal forest (SPR)	47.52	−93.46	3.3	77	−	+	5, 15, 25, 35	NA	2.0
Temperate grassland (ANG)	39.73	−123.64	13.0	216	−	+	NA	NA	5.7
Temperate grassland (HPR)	35.35	−111.73	6.6	66	+	+	5, 15, 25, 35	glu, glu + NH_4^+	8.4
Temperate conifer forest (BLT)	40.59	−121.38	9.1	115	+	+	NA	glu, exu, lit, ox	7.8
Temperate conifer forest (GRN)	37.16	−119.20	9.1	115	+	+	NA	glu, exu, lit, ox	7.4
Temperate grassland (HDG)	35.58	−111.57	13.0	19	+	+	NA	glu, glu + NH_4^+	4.9
Temperate grassland (PJW)	35.50	−111.62	10.5	28	+	+	NA	glu, glu + NH_4^+	6.0
Temperate grassland (PPW)	35.42	−111.67	9.1	52	+	+	NA	glu, glu + NH_4^+	6.8
Temperate broadleaf forest (HRV)	42.53	−72.19	7.1	110	+	−	NA	gluc, aas, lip, cel	3.2
Tropical forest (LUQ)	18.31	−65.74	25.9	176	−	+	5, 15, 25, 35	NA	9.5
Temperate grassland (SDG)	34.69	−120.04	16.8	38	−	+	NA	NA	6.7
Temperate grassland (HPL)	38.97	−123.12	14.0	96	−	+	NA	NA	6.5
Temperate stream (OCR)	34.91	−111.73	8.3	NA	−	+	NA	NA	7.4

[a]Lat, latitude; Long, longitude; MAT, mean annual temperature; MAP, mean annual precipitation.

[b]The columns Temp (°C) and Substrates indicate experimental treatments applied during the qSIP assay, with temperatures in degrees Celsius and substrates compared to a control with no added substrate. Added substrates included glucose (glu), glucose with ammonium (glu + NH_4^+), a mixture of compounds simulating root exudates (exu) (62), plant litter, and oxalic acid. Temperature indicates experimental incubation temperatures. Predators (%) indicates for each site the relative abundance across all putative predator groups as defined here.

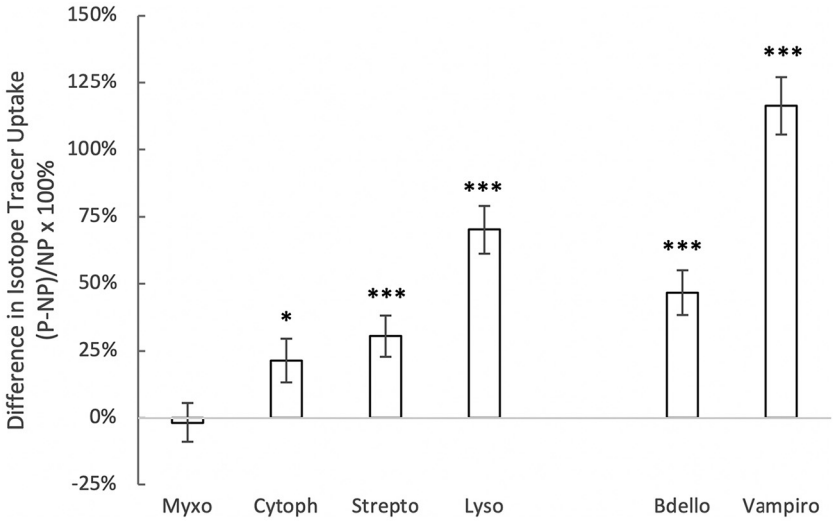

FIG 2 Difference in isotope tracer uptake (^{18}O and ^{13}C) between predatory and nonpredatory bacteria. From left to right, the first four taxa are facultative predators and the last two are obligate predators. Symbols are means ± standard errors of the mean. Predator groups (and numbers of experiments in which they occurred) were *Bdellovibrionales* ($n = 71$), *Cytophagia* ($n = 71$), *Lysobacter* ($n = 48$), *Myxococcales* ($n = 106$), *Streptomycetaceae* ($n = 86$), and *Vampirovibrionales* ($n = 25$). Asterisks indicate cases where means were significantly higher than zero (*, $P < 0.05$; ***, $P < 0.001$).

Table 1). We evaluated this data set to compare rates of growth by predatory and nonpredatory bacteria, and their responses to substrate addition.

RESULTS AND DISCUSSION

Bacterial taxa identified as potentially predatory were detected at all sites and amounted to 7.4 ± 6.0% of taxa detected at each site (median ± standard deviation). We refer to these as "predatory bacteria" henceforth, acknowledging the limitations of that designation based on 16S rRNA sequence variation (see Materials and Methods). Most of the predatory bacteria detected were facultative, with 64.7% from the order *Myxococcales*, 16% from the class *Cytophagia*, and 9.2% from the order *Streptomycetales*; 8% were obligate predatory bacteria, with 7.0% from the order *Bdellovibrionales* and 1.0% from the order *Vampirovibrionales*.

Across all sites and experiments, predatory bacteria assimilated isotope tracer into their DNA at rates 23.1 ± 7.0% higher than nonpredatory bacteria (meta-analysis, $P = 0.002$, $n = 407$; Fig. 2). Climate appeared to have little discernible influence on the differential isotope uptake between predatory and nonpredatory bacteria, with weak and nonsignificant relationships across sites for mean annual temperature ($P = 0.336$) and for precipitation ($P = 0.738$). Soil pH ($P = 0.871$) and soil water content ($P = 0.165$) also had no statistically discernible influence on the relative isotope assimilation between predators and nonpredators. Given the current design (15 sites), power may have been limited for detecting such environmental effects.

Predator identity significantly influenced isotope assimilation ($P < 0.0001$; Fig. 2); although both obligate and facultative predators assimilated the isotope tracers at rates higher than nonpredatory bacteria, the difference was larger for obligate (57.7 ± 8.4%, $P < 0.001$) than facultative (17.6 ± 7.1%, $P = 0.019$) predatory bacteria. Finer resolution revealed taxon-specific patterns, with especially high isotope uptake in the members of the obligate predator order *Vampirovibrionales* (2, 3) and in the genus *Lysobacter*, which is known to exhibit wolf-pack type predation (7–9). Isotope uptake was also higher in the *Bdellovibrionales*, *Streptomycetaceae*, and *Cytophagia*, whereas rates of isotope uptake for the *Myxococcales*, many of which are thought to function as saprotrophs (10), were similar to rates of nonpredators. The higher values

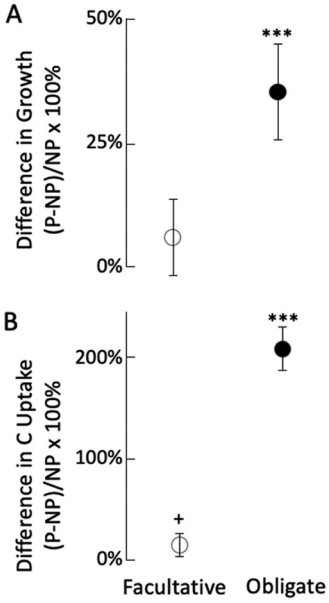

FIG 3 (A and B) Relative difference in predator growth rate (A) and ^{13}C uptake rate (B) compared to those of nonpredators. Values are shown separately for facultative (open symbols) and obligate (filled symbols) predators. Symbols are means ± standard errors of the mean. Statistical results from meta-analysis: ***, $P < 0.001$; +, $P < 0.100$.

of recovery of ^{13}C and ^{18}O in the DNA of bacterial predators indicates relatively high rates of element flux through bacterial predators in the microbial food webs represented in this 15-site survey.

Across the 15 sites, bacterial growth rates were log-normally distributed, with a median growth rate of 0.035 per day (d^{-1}), and 95% confidence from 0.003 to 0.198 d^{-1}, a range consistent with past estimates (31). The difference in growth rates between predators and nonpredators was higher for obligate predators than for facultative predators (Fig. 3A). The pattern held for rates of C uptake from ^{13}C-labeled substrates; obligate predators had significantly higher C uptake than facultative predators and nonpredatory bacteria (Fig. 3B).

Adding a source of energy for heterotrophs, in the form of carbon substrates, disproportionately stimulated growth rates of obligate predatory bacteria, whereas responses were indistinguishable between facultative predatory and nonpredatory bacteria (Fig. 4). This indicates that higher productivity increases top-down (predator-mediated) control in food webs, that added energy disproportionately flows to the predator trophic level, and that predators exhibit functional responses to shifts in prey resource availability. These findings are consistent with long-standing ecological theory that predicts the functional importance of predators increases with productivity (32–34), theory that also has support in macroscopic food webs (35, 36), and are consistent with observations in polar ocean systems where boom-bust cycles suggest viral response to increased algal productivity (37). The similar response of obligate predators from phylogenetically distant clades (i.e., protebacteria *Bdellovibrionales* and cyanobacteria *Vampirovibrionales*) implies that the mode of feeding determines the response. As such, similar results may be expected for other obligate predatory clades such as the widely distributed marine clade OM27 (*Deltaproteobacteria*) and family *Halobacteriovoraceae*. Across all predator taxa, adding nitrogen and carbon together elicited a larger ($P < 0.001$) growth response (38.6 ± 7.5%) than adding carbon alone (19.1 ± 10.4%), indicating that carbon-nitrogen stoichiometry of resources affects energy transfer to predatory bacteria (38).

Our findings indicate that predatory bacteria are highly active in microbial food webs, synthesizing DNA with elements derived from added isotope tracers at rates

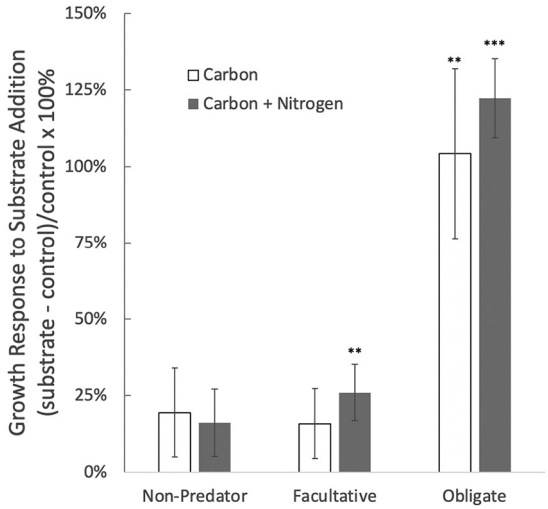

FIG 4 Growth response of predatory and nonpredatory bacteria to substrates containing organic carbon or carbon plus nitrogen. Values are means ± SE across 15 sites (Fig. 1) where *in situ* growth rates were measured using qSIP with ^{18}O-H_2O. Statistically significant differences from meta-analyses are shown with asterisks; **, $P < 0.010$; ***, $P < 0.0001$.

higher than nonpredatory bacteria, consistent with evidence from experimental microcosms (39). These results suggest that bacteria should be considered alongside eukaryotes and viruses as important predators in microbial food webs. Similarly, a recent metagenomic qSIP analysis using a ^{13}C-CO_2 tracer introduced via plant root exudates found that ^{13}C recovery in metagenomes associated with putative predator bacteria was comparable to the recovery in viruses and substantially higher than that in predatory eukaryotes (40). Slower growth might be expected if bacterial predators were inactive or dormant, as are many soil microorganisms (41). The results presented here indicate that bacterial predators grow, metabolize, and feed at higher rates than most bacteria in the soil food web and that predatory bacteria may exert top-down effects in microbial food chains. Though our analysis focused on predation, techniques that combine isotopes and gene sequencing can also quantify evidence of other ecological interactions in microbiomes and how they shape carbon flow and nutrient cycling in microbiomes. Multiple signatures of interactions among bacteria have now been identified (42–44), informing the use of qSIP, metagenomics, and traits to evaluate the functional significance of interactions in diverse microbiomes.

Element flux through the microbiome is central to its functioning, and results from macroecology show how ecological interactions—competition (45), mutualism (46), and predation (47, 48)—strongly influence those fluxes. The evidence presented here synthesizing isotope-enabled microbiome analysis couples predator identity and activity *in situ* and demonstrates that predatory bacteria are highly active in environmental microbiomes, more active than the average bacterial member. Patterns observed across the sites surveyed indicate that top-down trophic interactions are an active force that may structure the composition of element flow in microbiomes and clearly suggests the functional significance of predatory bacteria in microbial food webs.

MATERIALS AND METHODS

Atom fraction excess (AFE) values for ^{18}O and ^{13}C were extracted from qSIP measurements. AFE values were used to estimate bacterial growth rates based on ^{18}O assimilation from ^{18}O-labeled water, and the ^{13}C assimilation rate from ^{13}C-labeled organic substrates, using methods described in references 30, 49, and 50. All qSIP measurements involved parallel incubations with samples receiving either isotopically labeled (e.g., 97 atom % ^{18}O-H_2O, 99 atom % ^{13}C-glucose) or unlabeled substrates (e.g., water with natural abundance ^{18}O or glucose with natural abundance ^{13}C). Incubations lasted for 7.1 ± 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 the atom fraction excess ^{18}O or ^{13}C of each sequenced taxon (30, 31).

Across the 15 sites, multiple qSIP measurements were conducted, including experiments within each site. Across all sites and experimental treatments, there were a total of 82 qSIP data sets, and each data set contained estimates of ^{18}O or ^{13}C AFE for hundreds of bacterial taxa from a particular site and under a given experimental treatment. The identities of bacterial taxa were used to assign taxa to bacterial groups known to be capable of predation or to nonpredatory taxa. Predators were assigned based on belonging to one of six bacterial taxonomic groups known to exhibit predatory behavior: *Bdellovibrionales*, *Cytophagales*, *Lysobacter*, *Myxococcales*, *Streptomycetales*, and *Vampirovibrionales*. We recognize that assuming these taxa are unambiguously predatory based on their taxonomic assignment is uncertain. In particular, the facultative groups are known to vary in substrate utilization; the designation of "facultative" acknowledges the range of feeding behaviors exhibited by large groups, such as the *Cytophagales* (51), *Streptomycetales* (52), and *Myxococcales* (51). Not all taxa in these groups have been documented to be predatory; we use such broad groups because finer divisions are not available for the trophic behaviors of these organisms. Also, our approach relies on taxonomic assignments based on 16S rRNA gene sequences, which can be unreliable for delineating species or strain (53). In 98% of cases, we were able to assign taxa to possible predator groups based on name occurrences in class, order, or family, the higher levels of taxonomic resolution where 16S rRNA gene assignments have been found to be more robust (54).

Growth rates were estimated using ^{18}O qSIP after accounting for potential differences in the sources of ^{18}O among organisms functioning at different trophic levels. qSIP-derived estimates of growth rate using ^{18}O-H_2O begin with the observation that some of the oxygen in DNA is derived from the oxygen in water, so the assimilation of ^{18}O from water into DNA reflects its rate of replication, a proxy for cellular growth (55). Ribose sugars, nitrogenous bases, and phosphate (56) all acquire oxygen from water (55). Therefore, the DNA of predators will likely contain oxygen both from water in their growth environment as well as from cellular constituents of prey; these two potential sources of ^{18}O in predator DNA may or may not be additive.

To distinguish between these two sources, we compared ^{18}O versus ^{13}C enrichment in predatory taxa—since many of our SIP studies included treatments with both labeled water and labeled organic C substrates (Table 1). It is standard in food web studies using isotope tracers to treat the ^{13}C isotope composition of predator taxa as a conservative indicator of the ^{13}C composition of their prey (57). The qSIP data sets we evaluated included a subset of dual-isotope measurements, where both ^{18}O and ^{13}C were determined in parallel experiments with ^{18}O-labeled H_2O and ^{13}C-labeled carbon substrates. These measurements occurred in separate incubations, with identical conditions and resource availability but with different isotope labels applied; in one case, ^{18}O water was added with a natural abundance carbon substrate, and in the other, the carbon substrate was ^{13}C-labeled, while the added water was at natural abundance ^{18}O. With these parallel measurements, we were able to estimate both the ^{13}C and ^{18}O for multiple taxa.

Across 5 sites and 12 experiments, there were 2,197 simultaneous measurements of ^{13}C and ^{18}O, including 2,060 cases of nonpredatory taxa and 137 cases of predatory taxa. We evaluated the relationships between ^{18}O and ^{13}C for both predator and nonpredator taxa, reasoning that the two sources of ^{18}O to predators (compared to one source for nonpredators) would result in predator DNA that was relatively higher in ^{18}O compared to ^{13}C, to the extent that these sources were additive. As expected, for a given value of ^{13}C, predator taxa had higher values of ^{18}O than nonpredator taxa (Fig. S1). We used the difference in the relationships (model II linear regressions) between ^{18}O versus ^{13}C for predators and prey (Fig. S1) to predict what the ^{18}O composition of predator taxa would have been based on growth on ^{18}O-labeled H_2O alone. This approach resulted in the following correction, which was applied to all predator taxa in the data set:

$$^{18}O_c = {}^{18}O_m - \left({}^{18}O_m \times 0.0383 + 0.0065\right) \quad (1)$$

where $^{18}O_m$ is the measured predator AFE value and $^{18}O_c$ is the adjusted value.

This approach allowed us to avoid overestimating growth rates of predators because of their dual ^{18}O sources and helps ensure that values of predator and prey AFE ^{18}O were comparable. For nonpredator taxa, we used the measured qSIP ^{18}O AFE value as the estimate of ^{18}O assimilation from ^{18}O-H_2O, the standard approach in ^{18}O-qSIP studies (31, 55). An additional consideration is that oxygen concentration can affect ^{18}O assimilation from labeled water (58). Although oxygen concentrations were not measured in the incubations, for the mixed conifer, ponderosa, pinyon-juniper, and grassland sites included here, median final CO_2 concentrations were 0.31% (0.81%, 95th percentile) (59), which translates to a small change in atmospheric O_2 and suggests that oxygen depletion during the incubations was unlikely to have reached levels shown to affect ^{18}O assimilation from labeled water (58).

Experiments with ^{18}O were conducted by adding 97 atom % ^{18}O-H_2O to the experimental system and incubating for several days. Because background levels of unlabeled water were present, the ^{18}O composition of water in each incubation was determined as a function of the amount of 97 atom % ^{18}O water added and the amount of background water. The relative growth rate for each taxon was estimated according to equation 7 from reference 31, using the AFE $^{18}O_m$ of individual bacterial taxa, the AFE ^{18}O of water during the incubation, and the duration of the incubation in days.

We compared AFE, growth rates, and carbon assimilation rates of predatory and nonpredatory bacteria using meta-analysis (metafor package in R [60]), using the log ratios of predator:nonpredator as the

metric of difference between trophic strategies. This analysis was tested across all sites, treatments, measurement conditions, and tracers. Because some sites included experiments with both ^{18}O and ^{13}C tracers, isotope treatment was nested within sites to preserve independence. For all analyses, site was included as a random effect, because sites included multiple effect sizes which were not independent from each other. Computing multiple estimates with the same control group induces dependency on sampling errors, requiring the use of a variance-covariance matrix in the analysis (61). We computed the covariance in log response ratios as

$$SD_C^2/(N_C * C^2) \quad (2)$$

where SD_C is the standard deviation of the control group, C is the mean, and N_C is the sample size.

We tested for the effect of predator identity on AFE, growth rate, and carbon assimilation rate. Predator identity was evaluated by taxonomic assignment and functional group—obligate predators (*Bdellovibrionales* and *Vampirovibrionales*) and facultative predators (*Cytophagales*, *Lysobacter*, *Myxococcales*, and *Streptomycetales*). The effect of predator identity was nested within experiment, because multiple predator groups occurred in the same data set, so their assimilation rates were not independent of each other.

We used a similar meta-analysis model to evaluate the influence of added carbon substrates on the relationship between growth rates of predatory and nonpredatory bacterial taxa. Twenty-four of the compiled qSIP data sets included experimental substrate additions, in which ^{18}O-H_2O qSIP was conducted in soils amended with various carbon substrates compared to a control. Substrates included glucose (6 experiments), oxalic acid (2), ground plant litter (6), a mixture of glucose and ammonium (4), and a mixture of sugars, organic acids, and amino acids simulating root exudates (6). Across all substrate addition experiments and predator taxonomic groups, there were 113 log ratios comparing predator and nonpredator growth rates with substrates added, and 187 log ratios comparing predator and nonpredator growth rates without substrates added. (The compiled data set also included experimental manipulations of temperature and of leaf litter species, but the sample sizes were too small to evaluate these as potential drivers.) We evaluated the effect of substrate addition on the growth rates of predators using models with both predator identity and substrate as moderators.

Data availability. The 16S rRNA amplicon sequence data synthesized here have been deposited at NCBI under the accession numbers PRJNA649787, PRJNA649546, PRJNA649571, PRJNA649802, PRJNA669516, PRJNA701328, PRJNA718849, and PRJNA702085.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, PDF file, 0.3 MB.

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REFERENCES

1. Makowski Ł, Trojanowski D, Till R, Lambert C, Lowry R, Sockett RE, Zakrzewska-Czerwińska J. 2019. Dynamics of chromosome replication and its relationship to predatory attack lifestyles in Bdellovibrio bacteriovorus. Appl Environ Microbiol 85:e00730-19. https://doi.org/10.1128/AEM.00730-19.
2. Soo RM, Woodcroft BJ, Parks DH, Tyson GW, Hugenholtz P. 2015. Back from the dead; the curious tale of the predatory cyanobacterium Vampirovibrio chlorellavorus. PeerJ 3:e968. https://doi.org/10.7717/peerj.968.
3. Steichen SA, Brown JK. 2019. Real-time quantitative detection of Vampirovibrio chlorellavorus, an obligate bacterial pathogen of Chlorella sorokiniana. J Appl Phycol 31:1117–1129. https://doi.org/10.1007/s10811-018-1659-z.
4. Jurkevitch E, Davidov Y. 2007. Phylogenetic diversity and evolution of predatory prokaryotes, p 11–56. *In* Jurkevitch E (ed), Predatory prokaryotes. Microbiology monographs, vol 4. Springer, Berlin, Germany.
5. Gumbo RJ, Ross G, Cloete ET. 2008. Biological control of Microcystis dominated harmful algal blooms. Afr J Biotechnol 7:4765–4773.
6. Gerphagnon M, Macarthur DJ, Latour D, Gachon CMM, Van Ogtrop F, Gleason FH, Sime-Ngando T. 2015. Microbial players involved in the decline of filamentous and colonial cyanobacterial blooms with a focus on fungal parasitism. Environ Microbiol 17:2573–2587. https://doi.org/10.1111/1462-2920.12860.
7. Seccareccia I, Kost C, Nett M. 2015. Quantitative analysis of Lysobacter predation. Appl Environ Microbiol 81:7098–7105. https://doi.org/10.1128/AEM.01781-15.
8. Wang WH, Luo X, Ye XF, Chen Y, Wang H, Wang L, Wang YB, Yang YY, Li ZK, Cao H, Cui ZL. 2020. Predatory Myxococcales are widely distributed in and closely correlated with the bacterial community structure of agricultural land. Appl Soil Ecol 146:103365. https://doi.org/10.1016/j.apsoil.2019.103365.

9. Munoz-Dorado J, Marcos-Torres FJ, Garcia-Bravo E, Moraleda-Munoz A, Perez J. 2016. Myxobacteria: moving, killing, feeding, and surviving together. Front Microbiol 7:781. https://doi.org/10.3389/fmicb.2016.00781.
10. Jurkevitch E. 2007. Predatory behaviors in bacteria: diversity and transitions. Microbe 2:67–73. https://doi.org/10.1128/microbe.2.67.1.
11. Johnke J, Cohen Y, de Leeuw M, Kushmaro A, Jurkevitch E, Chatzinotas A. 2014. Multiple micro-predators controlling bacterial communities in the environment. Curr Opin Biotechnol 27:185–190. https://doi.org/10.1016/j.copbio.2014.02.003.
12. Sathyamoorthy R, Maoz A, Pasternak Z, Im H, Huppert A, Kadouri D, Jurkevitch E. 2019. Bacterial predation under changing viscosities. Environ Microbiol 21:2997–3010. https://doi.org/10.1111/1462-2920.14696.
13. Lee CG, Watanabe T, Fujita Y, Asakawa S, Kimura M. 2012. Heterotrophic growth of cyanobacteria and phage-mediated microbial loop in soil: examination by stable isotope probing (SIP) method. Soil Sci Plant Nutr 58:161–168. https://doi.org/10.1080/00380768.2012.658739.
14. Clarholm M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biol Biochem 17:181–187. https://doi.org/10.1016/0038-0717(85)90113-0.
15. Geisen S, Hu S, Dela Cruz TEE, Veen GF. 2020. Protists as catalyzers of microbial litter breakdown and carbon cycling at different temperature regimes. ISME J 15:618–621. https://doi.org/10.1038/s41396-020-00792-y.
16. Petrenko M, Friedman SP, Fluss R, Pasternak Z, Huppert A, Jurkevitch E. 2020. Spatial heterogeneity stabilizes predator-prey interactions at the microscale while patch connectivity controls their outcome. Environ Microbiol 22:694–704. https://doi.org/10.1111/1462-2920.14887.
17. Hahn MW, Hofle MG. 2001. Grazing of protozoa and its effect on populations of aquatic bacteria. FEMS Microbiol Ecol 35:113–121. https://doi.org/10.1111/j.1574-6941.2001.tb00794.x.
18. Arndt H. 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates): a review. Hydrobiologia 255:231–246.
19. Moens T, Vincx M. 1997. Observations on the feeding ecology of estuarine nematodes. J Mar Biol Assoc U K 77:211–227. https://doi.org/10.1017/S0025315400033889.
20. Starr EP, Shi SJ, Blazewicz SJ, Probst AJ, Herman DJ, Firestone MK, Banfield JF. 2018. Stable isotope informed genome-resolved metagenomics reveals that Saccharibacteria utilize microbially-processed plant-derived carbon. Microbiome 6:122. https://doi.org/10.1186/s40168-018-0499-z.
21. Al-Shayeb B, Basem RS, Chen LX, Ward F, Munk P, Devoto A, Castelle CJ, Olm MR, Bouma-Gregson K, Amano Y, He C, Méheust R. l, Brooks B, Thomas A, Lavy A, Carnevali PM, Sun C, Goltsman DSA, Borton MA, Nelson TC, Kantor R, Jaffe AL, Keren R, Farag IF, Lei S, Finstad K, Amundson R, Anantharaman K, Zhou J, Probst AJ, Power ME, Tringe SG, Li WJ, Wrighton K, Harrison S, Morowitz M, Relman DA, Doudna JA, Lehours AC, Warren L, Cate JHD, Santini JM, Banfield JF. 2020. Clades of huge phage from across Earth's ecosystems. Nature 578:425–431. https://doi.org/10.1038/s41586-020-2007-4.
22. Wang WH, Wang N, Dang KK, Dai W, Guan L, Wang BR, Gao JS, Cui ZL, Dong YH, Wang H. 2020. Long-term nitrogen application decreases the abundance and copy number of predatory myxobacteria and alters the myxobacterial community structure in the soil. Sci Total Environ 708:135114. https://doi.org/10.1016/j.scitotenv.2019.135114.
23. Paix B, Ezzedine JA, Jacquet S. 2019. Diversity, dynamics, and distribution of Bdellovibrio and like organisms in perialpine lakes. Appl Environ Microbiol 85:e02494-18. https://doi.org/10.1128/AEM.02494-18.
24. Diamond S, Andeer PF, Li Z, Crits-Christoph A, Burstein D, Anantharaman K, Lane KR, Thomas BC, Pan CL, Northen TR, Banfield JF. 2019. Mediterranean grassland soil C-N compound turnover is dependent on rainfall and depth, and is mediated by genomically divergent microorganisms. Nat Microbiol 4:1356–1367. https://doi.org/10.1038/s41564-019-0449-y.
25. Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, Gonzalez A, Kosciolek T, McCall LI, McDonald D, Melnik AV, Morton JT, Navas J, Quinn RA, Sanders JG, Swafford AD, Thompson LR, Tripathi A, Xu ZJZ, Zaneveld JR, Zhu QY, Caporaso JG, Dorrestein PC. 2018. Best practices for analysing microbiomes. Nat Rev Microbiol 16:410–422. https://doi.org/10.1038/s41579-018-0029-9.
26. Schimel JP, Gulledge J. 1998. Microbial community structure and global trace gases. Global Change Biol 4:745–758. https://doi.org/10.1046/j.1365-2486.1998.00195.x.
27. Chapin FS, Shaver GR. 1985. Individualistic growth response of tundra plant species to environmental manipulations in the field. Ecology 66:564–576. https://doi.org/10.2307/1940405.
28. Blazewicz SJ, Hungate BA, Koch BJ, Nuccio EE, Morrissey E, Brodie EL, Schwartz E, Pett-Ridge J, Firestone MK. 2020. Taxon-specific microbial growth and mortality patterns reveal distinct temporal population responses to rewetting in a California grassland soil. ISME J 14:1520–1532. https://doi.org/10.1038/s41396-020-0617-3.
29. Morrissey EM, Mau RL, Hayer M, Liu XA, Schwartz E, Dijkstra P, Koch BJ, Allen K, Blazewicz SJ, Hofmockel K, Pett-Ridge J, Hungate BA. 2019. Evolutionary history constrains microbial traits across environmental variation. Nat Ecol Evol 3:1064–1069. https://doi.org/10.1038/s41559-019-0918-y.
30. Hungate BA, Mau RL, Schwartz E, Caporaso JG, Dijkstra P, Van Gestel N, Koch BJ, Liu CM, McHugh TA, Marks JC, Morrissey E, Price LB. 2015. Quantitative microbial ecology through stable isotope probing. Appl Environ Microbiol 81:7570–7581. https://doi.org/10.1128/AEM.02280-15.
31. Koch BJ, McHugh TA, Hayer M, Schwartz E, Blazewicz SJ, Dijkstra P, Gestel N, Marks JC, Mau RL, Morrissey EM, Pett-Ridge J, Hungate BA. 2018. Estimating taxon-specific bacterial growth rates in intact soil communities. Ecosphere 9:e02090. https://doi.org/10.1002/ecs2.2090.
32. Fretwell SD, Barach AL. 1977. The regulation of plant communities by the food chains exploiting them. Perspect Biol Med 20:169–185. https://doi.org/10.1353/pbm.1977.0087.
33. Oksanen L, Fretwell SD, Arruda J, Niemela P. 1981. Exploitation ecosystems in gradients of primary productivity. Am Nat 118:240–261. https://doi.org/10.1086/283817.
34. Oksanen T, Power ME, Oksanen L. 1995. Ideal free habitat selection and consumer-resource dynamics. Am Nat 146:565–585. https://doi.org/10.1086/285815.
35. Wootton JT, Power ME. 1993. Productivity, consumers, and the structure of a river food-chain. Proc Natl Acad Sci U S A 90:1384–1387. https://doi.org/10.1073/pnas.90.4.1384.
36. Marks JC, Power ME, Parker MS. 2000. Flood disturbance, algal productivity, and interannual variation in food chain length. Oikos 90:20–27. https://doi.org/10.1034/j.1600-0706.2000.900103.x.
37. Behrenfeld MJ, Hu Y, O'Malley RT, Boss ES, Hostetler CA, Siegel DA, Sarmiento JL, Schulien J, Hair JW, Lu X, Rodier S, Scarino AJ. 2017. Annual boom-bust cycles of polar phytoplankton biomass revealed by space-based lidar. Nature Geosci 10:118–122. https://doi.org/10.1038/ngeo2861.
38. Finkel ZV, Beardall J, Flynn KJ, Quigg A, Rees TAV, Raven JA. 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. J Plankton Res 32:119–137. https://doi.org/10.1093/plankt/fbp098.
39. Williams HN, Lymperopoulou DS, Athar R, Chauhan A, Dickerson TL, Chen H, Laws E, Berhane T-K, Flowers AR, Bradley N, Young S, Blackwood D, Murray J, Mustapha O, Blackwell C, Tung Y, Noble RT. 2016. Halobacteriovorax, an underestimated predator on bacteria: potential impact relative to viruses on bacterial mortality. ISME J 10:491–499. https://doi.org/10.1038/ismej.2015.129.
40. Starr EP, Shi S, Blazewicz SJ, Koch BJ, Probst AJ, Hungate BA, Pett-Ridge J, Firestone MK, Banfield JF. 2020. Stable isotope informed genome-resolved metagenomics uncovers potential trophic interactions in rhizosphere soil. bioRxiv 2020.2008.2021.262063.
41. Blagodatskaya E, Kuzyakov Y. 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem 67:192–211. https://doi.org/10.1016/j.soilbio.2013.08.024.
42. Green ER, Mecsas J. 2016. Bacterial secretion systems: an overview. Microbiol Spectr 4 https://doi.org/10.1128/microbiolspec.VMBF-0012-2015.
43. Pasternak Z, Pietrokovski S, Rotem O, Gophna U, Lurie-Weinberger MN, Jurkevitch E. 2013. By their genes ye shall know them: genomic signatures of predatory bacteria. ISME J 7:756–769. https://doi.org/10.1038/ismej.2012.149.
44. Sutton D, Livingstone PG, Furness E, Swain MT, Whitworth DE. 2019. Genome-wide identification of myxobacterial predation genes and demonstration of formaldehyde secretion as a potentially predation-resistant trait of Pseudomonas aeruginosa. Front Microbiol 10:2650. https://doi.org/10.3389/fmicb.2019.02650.
45. Sitch S, Smith B, Prentice IC, Arneth A, Bondeau A, Cramer W, Kaplan JO, Levis S, Lucht W, Sykes MT, Thonicke K, Venevsky S. 2003. Evaluation of ecosystem dynamics, plant geography and terrestrial carbon cycling in the LPJ dynamic global vegetation model. Global Change Biol 9:161–185. https://doi.org/10.1046/j.1365-2486.2003.00569.x.
46. Seymour JR, Amin SA, Raina JB, Stocker R. 2017. Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. Nat Microbiol 2:17065 https://doi.org/10.1038/nmicrobiol.2017.65.
47. Webb AP, Eyre BD. 2004. The effect of natural populations of the burrowing and grazing soldier crab (Mictyris longicarpus) on sediment irrigation,

benthic metabolism and nitrogen fluxes. J Exp Mar Biol Ecol 309:1–19. https://doi.org/10.1016/j.jembe.2004.05.003.
48. Kristensen E. 2008. Mangrove crabs as ecosystem engineers; with emphasis on sediment processes. J Sea Res 59:30–43. https://doi.org/10.1016/j.seares.2007.05.004.
49. Purcell AM, Dijkstra P, Finley B, Hayer M, Koch BJ, Mau RL, Morrissey E, Papp K, Schwartz E, Stone BW, Hungate BA. 2019. Quantitative stable isotope probing with H218O to measure taxon-specific microbial growth. Methods Soil Anal 84:1503–1518. https://doi.org/10.2136/msa2018.0083.
50. Finley BK, Hayer M, Mau RL, Purcell AM, Koch BJ, van Gestel NC, Schwartz E, Hungate BA. 2019. Microbial taxon-specific isotope incorporation with DNA quantitative stable isotope probing, p 137–149. *In* Dumont MG, Garcia MH (ed), Stable isotope probing: methods and protocols, vol 2046. Springer, New York, NY.
51. Imai I, Ishida Y, Hata Y. 1993. Killing of marine phytoplankton by a gliding bacterium Cytophaga sp., isolated from the coastal Sea of Japan. Marine Biol 116:527–532. https://doi.org/10.1007/BF00355470.
52. Kumbhar C, Mudliar P, Bhatia L, Kshirsagar A, Watve M. 2014. Widespread predatory abilities in the genus Streptomyces. Arch Microbiol 196:235–248. https://doi.org/10.1007/s00203-014-0961-7.
53. Johnson JS, Spakowicz DJ, Hong B-Y, Petersen LM, Demkowicz P, Chen L, Leopold SR, Hanson BM, Agresta HO, Gerstein M, Sodergren E, Weinstock GM. 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat Commun 10:5029. https://doi.org/10.1038/s41467-019-13036-1.
54. Mizrahi-Man O, Davenport ER, Gilad Y. 2013. Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs. PLoS One 8:e53608. https://doi.org/10.1371/journal.pone.0053608.
55. Schwartz E. 2007. Characterization of growing microorganisms in soil by stable isotope probing with H218O. Appl Environ Microbiol 73:2541–2546. https://doi.org/10.1128/AEM.02021-06.
56. Cohn M, Hu A. 1978. Isotopic (18O) shift in 31P nuclear magnetic resonance applied to a study of enzyme-catalyzed phosphate–phosphate exchange and phosphate (oxygen)–water exchange reactions. Proc Natl Acad Sci U S A 75:200–203. https://doi.org/10.1073/pnas.75.1.200.
57. Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320. https://doi.org/10.1146/annurev.es.18.110187.001453.
58. Coskun OK, Ozen V, Wankel SD, Orsi WD. 2019. Quantifying population-specific growth in benthic bacterial communities under low oxygen using (H2O)-O-18. ISME J 13:1546–1559. https://doi.org/10.1038/s41396-019-0373-4.
59. Liu XJA, Finley BK, Mau RL, Schwartz E, Dijkstra P, Bowker MA, Hungate BA. 2020. The soil priming effect: consistent across ecosystems, elusive mechanisms. Soil Biol Biochem 140. https://doi.org/10.1016/j.soilbio.2019.107617.
60. Viechtbauer W. 2010. Conducting meta-analyses in R with the metafor package. J Stat Soft 36:1–48. https://doi.org/10.18637/jss.v036.i03.
61. Lajeunesse MJ. 2011. On the meta-analysis of response ratios for studies with correlated and multi-group designs. Ecology 92:2049–2055. https://doi.org/10.1890/11-0423.1.
62. Finley BK, Dijkstra P, Rasmussen C, Schwartz E, Mau RL, Liu XJA, Van Gestel N, Hungate BA. 2018. Soil mineral assemblage and substrate quality effects on microbial priming. Geoderma 322:38–47. https://doi.org/10.1016/j.geoderma.2018.01.039.