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Caenorhabditis elegans responses to bacteria from its natural habitats

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Most *Caenorhabditis elegans* studies have used laboratory *Escherichia coli* as diet and microbial environment. Here we characterize bacteria of *C. elegans*' natural habitats of rotting fruits and vegetation to provide greater context for its physiological responses. By the use of 16S ribosomal DNA (rDNA)-based sequencing, we identified a large variety of bacteria in *C. elegans* habitats, with phyla *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* being most abundant. From laboratory assays using isolated natural bacteria, *C. elegans* is able to forage on most bacteria (robust growth on ∼80% of >550 isolates), although ∼20% also impaired growth and arrested and/or stressed animals. Bacterial community composition can predict wild *C. elegans* population states in both rotting apples and reconstructed microbiomes: alpha-Proteobacteria-rich communities promote proliferation, whereas *Bacteroidetes* or pathogens correlate with non-proliferating dauers. Combinatorial mixtures of detrimental and beneficial bacteria indicate that bacterial influence is not simply nutritional. Together, these studies provide a foundation for interrogating how bacteria naturally influence *C. elegans* physiology.

Caenorhabditis elegans | host–microbe interactions | ecology

Biological organisms constantly live in contact with other organisms in a complex web of ecological interactions, which include prey–predator, host–parasite, competitive, or positive symbiotic relationships. Bacteria are now considered key players in multiple aspects of the biology of multicellular organisms (1–3). The richness and importance of these interactions were so far neglected because laboratory biology had succeeded in simplifying and standardizing the environment of the model organisms, providing in most cases a single microbe as a food source, and not necessarily even a naturally encountered one. The many aspects of organismal biology that were shaped by evolution in natural environments are thus undetectable in the artificial laboratory environment and can only be revealed in the presence of other interacting species. Examples include feeding behavior, metabolism of diverse natural food sources, interactions with natural pathogens that have shaped the organism's immune system, behavioral traits, and regulation of development and reproduction. At the genomic level, many individual genes may not be required in a standard laboratory environment but their role may be revealed by using more diverse and relevant environments (4, 5).

The nematode *Caenorhabditis elegans* is a typical example of a model organism that has been disconnected from its natural ecology: although the species has been studied intensively in the laboratory for half a century, its habitat and natural ecology—what it naturally feeds on, its natural predators and pathogens, and its adaptive responses to its environmental and biological challenges—are only now being determined (6–11). Nematodes such as *C. elegans* have long been known to feed on bacteria that proliferate on decaying organic material, but the specific bacterial species that *C. elegans* encounters in the wild have not been comprehensively studied. In the laboratory, *C. elegans* is routinely fed the *Escherichia coli* B bacterial strain OP50, a uracil auxotroph that forms thinner lawns on plates to allow for easy microscopic visualization of this transparent worm. All other microbes are removed by routine bleach treatments of the cultures, which the nematode embryos are resistant to and survive. The original selection of *E. coli* as a nutritional source for *C. elegans* was not based on knowledge of the natural microbes associated with *C. elegans* in its natural habitat, but on the availability of *E. coli* in research laboratories and in the history of Sydney Brenner, an *E. coli* bacteriophage geneticist, as he developed *C. elegans* as a model organism (12). Since the effect of diverse pathogenic bacteria has been studied in *C. elegans* (13–16), however, little effort has been made to isolate ecologically relevant and not necessarily detrimental bacteria. A recent study has also made use of soil where *C. elegans* does not proliferate to isolate bacteria and study their community assembly (17).

Here we sought to determine the natural bacterial environment in which *C. elegans* lives. To this end, we comprehensively survey the bacterial inhabitants of a set of decaying fruits and plant material that *C. elegans* inhabit (the "microbiome" of its habitat, in the classical ecological sense used by Joshua Lederberg for the "totality of microorganisms and their genetic material occupying a given environment"). Using culture-independent 16S rRNA sequences from nucleic acids isolated from these *C. elegans*-associated bacterial ecosystems, we characterized the various taxa of bacteria that make up the rotting fruits and plant material where *C. elegans* are also found. We find that the community is composed of thousands of operational taxonomic units (∼2,400 OTUs) of bacteria, spanning bacterial diversity, but with particular bacterial phyla dominating

Significance

***Caenorhabditis elegans* is a major model organism, both from the pathogenesis dimension and also for metabolism, aging, and developmental biology perspectives. And yet, its natural ecology, most especially, its natural microbiome, is almost untouched. Here we establish the natural microbial community of *C. elegans*. Using extensive culture collections, we categorize its spectrum of responses (from antagonistic to beneficial) to a wide breadth of wild bacteria with nearly 80% of isolates supporting robust growth. In the wild, specific microbes correlate with the population state of the animals, which is supported by reconstruction experiments in the lab. Thus, a simplified natural community related to that found in the wild can now be studied in the laboratory for its impact on *C. elegans* physiology.**

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Data deposition: The sequences reported in this paper have been deposited in the Sequence Read Archive database (www.ncbi.nlm.nih.gov/sra).

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these populations: *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria*.

In addition to the culture-independent assessment of bacteria associated with *C. elegans*, we cultured 564 bacterial strains that 16S ribosomal gene sequencing classifies into a wide range of taxonomic groups. We assessed whether each of these bacterial species promotes growth of *C. elegans* and whether each of them causes induction of stress reporter genes for unfolded protein, or mitochondrial stress, or innate immune response. We find that about 80% of the bacterial species from the natural habitats of *C. elegans* in rotting fruits are equal or better than the benign *E. coli* OP50 at fostering growth of *C. elegans* and not inducing various stress and pathogen reporter genes, whereas about 20% of the bacterial isolates cause slow growth (or death) and robust stress reporter induction.

To distinguish between active systems of bacterial pathogenicity versus simply poor nutrition, we mixed beneficial bacteria pairwise with pathogenic bacterial species in a dilution series and found that in some cases even a small fraction of the pathogenic bacteria suppressed *C. elegans* growth on benign bacteria, suggesting an active system of antagonism. Similarly, a small portion of beneficial bacteria could ameliorate some (and in certain instances all) of the pathogenic impact. Our comparison of rotting fruit environments with large numbers of proliferating nematodes versus those with probable pioneer *C. elegans* dauer larvae indicated particular bacterial species that are highly correlated with and predictive for growth and reproduction of *C. elegans*. In reconstruction experiments of communities with about 20 species of bacteria, faster growth and reproduction of *C. elegans* was elicited when the cardinal bacterial species of proliferating *C. elegans*'s natural ecosystems were reconstructed compared with communities comparable to those with dauer-arrested *C. elegans* in the wild. Thus, our analyses allow a simplified microbiome related to that found in the wild to be reconstituted and interrogated in the laboratory.

Results

The Natural Bacterial Microenvironment of *C. elegans*. Field studies show that *C. elegans* is commonly found proliferating in microbe-dense rotting fruits or vegetation (7) rather than in the soil where they are more likely to persist as stress-resistant dauers. We sought to establish the microbes that *C. elegans* encounters in its natural habitat—both by culture-independent 16S rRNA gene sequencing and by culturing individual bacteria from these ecosystems, followed by subsequent taxonomic classification based on 16S sequencing. We collected and analyzed 59 decaying apples from the ground in two independent locations near Paris, France, plus a diverse set of four samples, including other decaying fruits (orange and *Opuntia* cactus fruit), vegetation (*Tamus communis* black bryony stem), and a snail, an animal vector of *C. elegans* dispersal (associated with the cactus fruit in this case), during four separate field seasons and an array of geographic locations in Europe (Fig. 1*A*, Fig. S1, and Dataset S1).

To determine the members of the community, we first asked which bacterial taxa are most common within *C. elegans* habitats, through multiplexed sequencing of small subunit rDNA amplicons (bacterial 16S rRNA gene) from bulk DNA isolated from each habitat sample with a wild *C. elegans* population (Dataset S1 *A* and *B*). Within these samples, the most prevalent bacterial divisions (phyla) were: *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Fig. 1*B*). Seven rarer divisions are also sporadically present in these environments (Dataset S1*C*). Of the over 250 bacterial genera that we identified, nearly all were present at some level in rotting apples, from the most abundant *Enterobacteriaceae* to lactic-acid bacteria (*Lactococcus* and *Lactobaccilli*) and acetic acid-producing *Acetobacteriaceae* (*Acetobacter*, *Gluconobacter*, and *Gluconoacetobacter*) to the rarer *Providencia* (Fig. 1*C* and Fig. S2*C*). Relative proportions of many of these genera were often comparable between the apples and nonapples (Dataset S1*D*), although there was no single phylotype that was found in all samples (Dataset S1*E*).

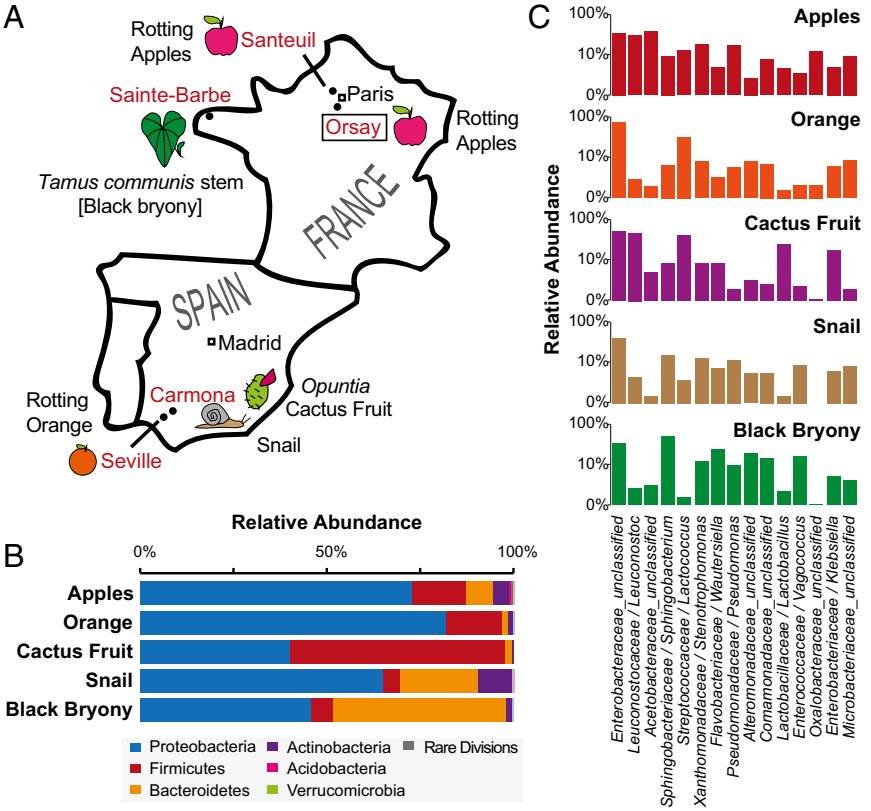

Fig. 1. Diverse phyla of bacteria found in *C. elegans* natural habitats. (*A*) Regional map of approximate locations for the sampling sites. (*B*) Division (phylum)-level composition of bacterial diversity in rotting fruit and vegetation harboring *C. elegans* populations. The specific locations of the habitats are noted in Fig. S1, briefly: bulk DNA was isolated, PCR amplified using bacterial small subunit (SSU) primers, and sequenced in a multiplexed fashion from rotting apples from Santeuil, France and Orsay, France; a rotting orange from Carmona, Spain; a rotting *Opuntia* cactus fruit and associated snail from Seville, Spain; and rotting *T. communis* (black bryony) stems and leaves from Sainte-Barbe, France, all of which also contained *C. elegans* nematodes (various population states). (*C*) Most abundant bacterial genera in *C. elegans* habitats (15 of the top 25 with greater than 1% mean abundance shown, ordered from highest (*Left*) to lowest (*Right*) mean abundance among all habitat types; detailed in Dataset S1 *C*–*E*).

Although likely an underestimate of the total diversity in these samples (*Experimental Procedures*), ~2,400 observed OTUs represent four- to fivefold fewer than that observed in bulk soil samples and comparable to the levels that are observed in specialized niches like the rhizosphere (18, 19). The specificity of the bacterial genera observed is supported by previous studies of analyses of fruit surfaces (20) (especially the apple phyllosphere) (21) and also microbiome studies of other fruit-associated animals like *Drosophila* (22, 23). Particular bacterial phylotypes were identified from quite disparate habitats (e.g., a snail, a rotting apple, and a rotting orange), which could indicate their close association with *C. elegans* animals, but further studies of more habitats and *C. elegans* populations are needed to test this link.

In a subset of samples, *Escherichia* sequences were also identified, which could either indicate PCR contamination (reagent OTUs removed from analysis) or more likely, deposition by defecation from other animals. However, no isolates from our extensive culture-based analyses have been classified as *Escherichia*, so those present may be quite divergent from the laboratory strains. Regardless, the abundance of *Enterobacteriaceae* in these habitats indicates that the use of *E. coli* in laboratory *C. elegans* experiments may not be entirely unnatural, although these bacteria are always only a fraction of the diversity of bacterial species present in a given habitat.

Impact of Natural Bacteria on *C. elegans* Physiology. To assess how individual bacterial species from natural habitats of *C. elegans* may impact its growth and physiology, we established a culture collection of wild bacterial strains isolated from *C. elegans* habitats. Specifically, we isolated bacteria on simple bacterial growth media from samples where *C. elegans* was found. Some bacteria were isolated from the *C. elegans* animals themselves after isolation using diacetyl attraction (*Experimental Procedures* and Dataset S2). The collection comprises 437 bacteria from rotting Orsay apples (or other habitats from sites around Paris) harboring large populations of *C. elegans* and 128 isolates from a variety of sample types and locations, including rotting fruits, stems, compost, and live snails/slugs where *C. elegans* (and/or *Caenorhabditis briggsae*) animals were identified (Dataset S2). Together, the isolates represent nearly 80% of the genera found by culture-independent means in rotting apples.

C. elegans was grown on each individual cultivated bacterial strain from our collection (565 isolates included), and we assayed rates of growth and induction of stress and immune reporter genes (Dataset S3). These reporters were chosen to monitor whether *C. elegans* may respond to any of the bacterial species as a pathogenic stress (24–27). We expected that natural pathogens or competitors isolated from the rotting fruit would induce expression of these stress or immunity reporter genes, whereas relatively benign or even beneficial bacteria would not. We found that several stress reporter genes were up-regulated by about 20% of the bacterial strains compared with *E. coli* OP50: endoplasmic reticulum (ER) stress reporter promoter *hsp-4::GFP* (112 strains induce it, 22 strongly), the mitochondrial stress reporter promoter *hsp-6::GFP* (101 strains induce it, 6 strongly), the bacterial virulence reporter gene promoter *irg-1::GFP* (123 strains induce it, 2 strongly), the *pmk-1* dependent pathogen reporter gene promoter *F35E2.5::GFP* [133 strains induce it, 47 strongly (*Pseudomonas* spp. were the most robust inducers.)] (Dataset S3). Additionally, two of the reporters were more selectively induced, including the oxidative stress response gene promoter *gst-4::GFP* (8 strains induce it, 2 strongly), and the antimicrobial C-type lectin promoter *clec-60::GFP* (11 strains induce it, none strongly) (Dataset S3). Activation of multiple reporters was notably rare (mean 13 ± 13%, 0–50% range for any pair) and strong induction of a particular reporter gene was generally not correlated with strong activation of another reporter gene (Dataset S3C); one exception is 8 gamma-Proteobacteria (17–33% of each set of positives; e.g., *Pseudomonas*, *Erwinia*, and *Aeromonas*) that strongly induce both *hsp-4::GFP* and *F35E2.5::GFP*, which suggests that ER stress and activation of innate immune pathways by *Pseudomonas aeruginosa* (28) in the laboratory may be more broadly used to resist pathogenic gamma-Proteobacteria in the wild. Activation of multiple reporters was also stronger after treatment with culture supernatants from a subset of bacteria (Fig. S3), suggesting that toxin concentrations may not have reached appropriate thresholds for activation or that there are widespread programs for active suppression of the worm's response by bacteria (as was shown for natural *Pseudomonas* spp. suppression of *hsp-6*::GFP activation following mitochondrial stress) (25).

We used these *C. elegans* responses to categorize each bacterial strain as generally "beneficial" (faster growth, little to no induction of pathogen reporters), "detrimental" (slow growth or active killing, activation of pathogen reporters), or "intermediate" (mixed responses). *C. elegans* grows well on a wide range of bacterial genera: nearly twice as many strains were classified as beneficial compared with detrimental (Fig. 2*A* and Dataset S3). Specific genera tend to be predominantly beneficial or detrimental. For example, several Proteobacteria (i.e., *Gluconobacter*, *Enterobacter*, *Providencia*, and *Enterobacteriaceae*) and most *Lactococcus* strains were beneficial to *C. elegans* (Fig. 2*A*); note that many of these genera are also common commensals of other animal hosts (22, 29). Similarly, the more detrimental Bacteroidetes (e.g., *Chryseobacterium* and *Sphingobacterium*) and potentially pathogenic gamma-Proteobacteria (e.g., *Xanthomonas* and *Stenotrophomonas*) impaired *C. elegans* physiology in a number of ways and are often pathogens of *C. elegans* and other animals (14). *Pseudomonas* sp. GRb0427 was a particularly pathogenic member of the pseudomonads (dramatic reduction of growth rates and survival, among the highest inducers of the immune reporter F35E2.5::GFP; Dataset S3), which were generally detrimental to *C. elegans*, consistent with the animal's responses to human pathogenic *P. aeruginosa* PA14 (30, 31). Strikingly, with the exception of the *Gluconobacter* sp., different members of every bacterial genus exhibited a range of effects, indicating that there is likely to be variable genomic and metabolic determinants within a genus (e.g., toxins, metabolites, etc.) that affect its impact on *C. elegans* physiology.

Overall these assays of growth and stress response gene expression suggested that 78% of the bacterial species that we isolated from *C. elegans* habitats support robust *C. elegans* growth (beneficial or intermediate combined), in that they do not induce stress or immunity reporter genes and are compatible with reproductive growth on that one bacterium alone. The other 22% of individually cultured bacterial strains induce pathogen responses in *C. elegans* or cause slow animal growth, suggesting that these bacterial coinhabitants with *C. elegans* in rotting fruit are antagonistic. Using a representative set of 12 natural microbes (representing the major genera present in at least three habitat types), we found that this profile of reporter-based responses can also be observed with shorter exposures to the bacteria alone or treatment with a culture supernatant, perhaps bearing secreted toxins (Fig. S3).

Natural Bacterial Influence on *C. elegans* Physiology Is More Than Simply Nutritional. As our taxonomic survey of *C. elegans* habitats shows, nematodes encounter complex communities of microbes—some beneficial, some detrimental, rather than a single species. Antagonistic bacteria that do not support optimal *C. elegans* growth rates or cause induction of stress reporters could produce a toxin or virulence factor that actively and perhaps potently suppresses *C. elegans* growth, or could fail to supply a key micro- or macronutrient [e.g., iron (32) or vitamins (33, 34)]. Beneficial bacteria could either supply key nutrients or actively suppress *C. elegans* stress and immune responses. Binary mixtures of detrimental and beneficial bacteria, in various ratios, allowed us to explore this question. In the nutritional scenario, detrimental bacteria would also serve as a poor nutritional source for *C. elegans* and limit growth/survival. Equal or higher levels of beneficial bacteria, providing better nutrition and promoting growth, could then neutralize this impact of detrimental, poor nutritional bacteria. In an active bacterial system of antagonism or

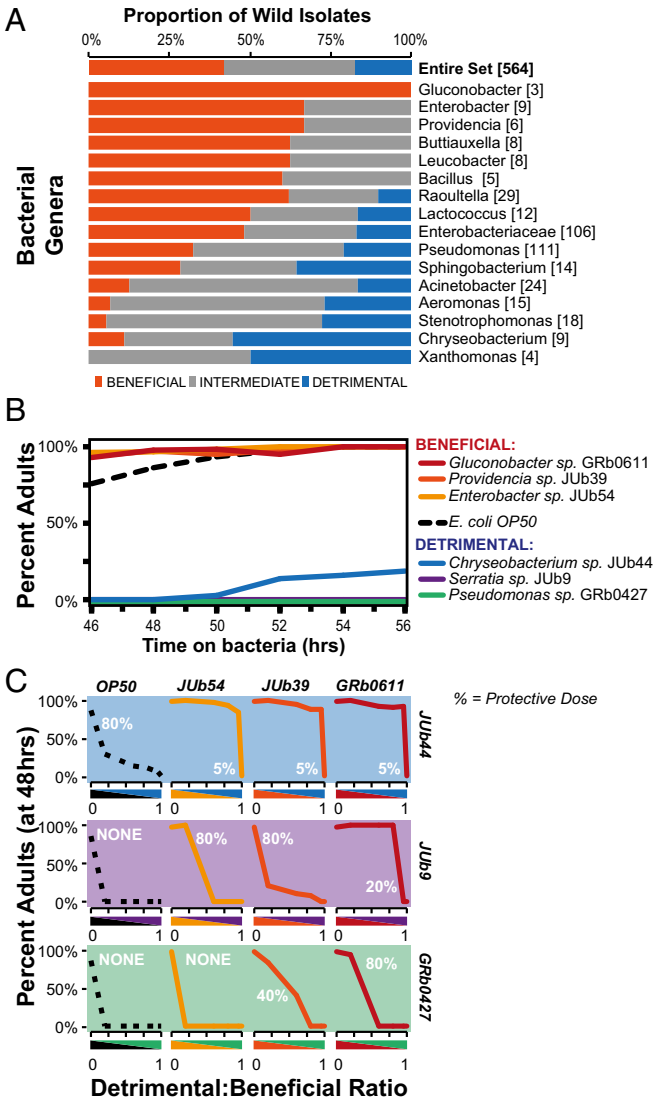

Fig. 2. Impact of natural bacterial isolates on *C. elegans* growth and activation of stress reporters identifies active pathogenic and beneficial influence beyond nutritional. (*A*) Categorization of the cultured isolates (564 in total) and select genera (isolate numbers in brackets) that have a beneficial (improve growth, fail to activate stress reporters), intermediate (activate stress reporters weakly, minimal effect on growth), or detrimental (activate stress reporters, decrease growth or kill) impact on *C. elegans* physiology. (*B*) Proportion of animals that have reached adulthood when grown on a subset of natural beneficial or detrimental bacteria. (*C*) Assessment of the proportion of animals that have reached adulthood after 48 h (22 °C) after growth on a one-to-one dilution series of beneficial: detrimental bacteria mixtures (from *Right* to *Left* 0%:100%, 5%:95%, 20%:80%, 40%:60%, 80%:20%, 0%:100%; $n > 100$ animals assayed per condition per experiment). Minimum proportion of beneficial bacteria needed to promote growth of 25% of the *C. elegans* animals to adulthood is noted.

benefit, we would expect small fractions of one bacterial type to be able to have dominant influences on the host response to that mixture— that is, the chemical toxin or virulence factor would be expected to be potent.

To distinguish between an active system of bacterial influence (detrimental or beneficial) versus simple differences in quality of nutrition, we examined binary mixtures of some of the most beneficial (*Gluconobacter* sp. GRb0611, *Enterobacter* sp. JUb54, and *Providencia* sp. JUb39) and detrimental bacteria (*Serratia* sp. JUb9, *Pseudomonas* sp. GRb0427, and *Chryseobacterium* sp. JUb44) from our collection of bacterial coinhabitants of rotting fruit with *C. elegans*. After 48 h on each pure bacterial strain, *C. elegans* grown on just the beneficial bacteria were healthy adults (8–10 h faster growth than on *E. coli* OP50 for comparison), whereas the animals grown on just the detrimental bacterial strains were either dead and/or arrested on the *Serratia* or *Pseudomonas* strains and significantly delayed (10–12 h) on the *Chryseobacterium* strain (Fig. 2*C*). Dilution of each of the natural detrimental bacteria with benign *E. coli* OP50 was not able to mitigate the pathogenicity of either *Serratia* or *Pseudomonas* and only at 20–1 excess over the more benign *Chryseobacterium* (Fig. 2*C*). This indicates that these detrimental bacteria are not merely a poor nutritional source, but rather produce potent antagonistic activities, for example, virulence factors or toxins that are not neutralized by excess *E. coli* OP50. We also diluted each of the detrimental bacteria with increasing proportions of each of the beneficial bacteria (ranging from 0% detrimental/100% beneficial to 100% detrimental/0% beneficial) and assayed the impact on *C. elegans* growth rates. The natural beneficial bacteria of *C. elegans* provided more protection from the natural pathogens than *E. coli*. For example, the beneficial *Gluconobacter* sp. GRb0611 was the most effective at mitigating the *C. elegans* growth inhibition on all three pathogenic bacterial strains tested, although at different proportions (protection observed when at least 5%, 20%, and 80% of the mixture with *Chryseobacteria*, *Serratia*, and *Pseudomonas*, respectively; Fig. 2*C*). The other two beneficial bacterial species, *Enterobacter* sp. JUb54 and *Providencia* sp. JUb39, also both protected against *Chryseobacterium* effectively at low relative proportions (5%), but each provided only partial protection for *Serratia* (only in excess by a factor of 20). Further, *Enterobacter* sp. JUb54 did not provide any protection against *Pseudomonas* at any mixture tested, whereas *Providencia* sp. JUb39 provided modest protection (40%). Whether these beneficial effects represent a direct impact on the worms (e.g., immune-boosting), antimicrobial impact on the pathogen (microbe–microbe interactions), or a related mechanism remains to be elucidated in future studies.

Overall, these data indicate that *C. elegans* physiology is influenced beyond just nutritional content of the microbes through both pathogenic and beneficial modalities. Antagonistic bacteria mixed at a very low ratios with *E. coli* can still have harmful effects on *C. elegans* growth, consistent with a possible secreted or potent pathogenesis mechanism, whereas the wild beneficial bacteria from the natural habitat of *C. elegans* actively promote *C. elegans* growth by mitigating the influence of potential pathogens. The ability of the natural beneficial bacteria from the *C. elegans* natural habitat to mitigate pathogenic effects of bacteria more effectively than *E. coli* suggests that the naturally beneficial bacteria may possess active programs of antivirulence that are absent in *E. coli*. High proportions of beneficial bacteria to detrimental bacteria in a rotting fruit may have direct consequences on whether or not *C. elegans* is either able to or decides to grow and proliferate.

Natural Microbiome Composition Influences Wild *C. elegans* Growth in Rotting Apples. To examine whether the different bacterial community composition may have similar influences on *C. elegans* physiology in the wild, we expanded our collection efforts to allow examination of bacterial abundance and composition as a function of *C. elegans* population state. To do this, we collected 26 apples from the Orsay orchard (different trees) and two groups of five apples that were subsequently subsampled (as slices) from under the same tree (Fig. S1 *B* and *C*). By focusing our sampling efforts on one location and one year, we sought to minimize the influences of other types of variation that may influence bacterial community composition and *C. elegans* response to microbes (7, 9). For each sample, we recorded: (*i*) presence or absence of *C. elegans*; (*ii*) the population size (number of nematodes); and (*iii*) the population state (i.e., whether they were larvae and reproductive adults associated with proliferation or growth-arrested dauer larvae (the nonproliferating dispersal stage for many nematodes) (7, 9). Dauers are likely

to play a role at the initial inoculation of a rotting apple (7, 9), as well as at the time of exit and dispersal in search of a more optimal bacterial milieu, once the population had proliferated and exhausted the resource. The assessment of nematode population in each sample was used to assign a nematode population score (log scale bins) to correlate bacterial composition with nematode population growth.

The simplest explanation of whether *C. elegans* grows in a rotting apple could be the amount of bacteria present. Dauer arrest by the dauer pheromone of *C. elegans* is specifically modulated in the laboratory by abundance of *E. coli* food (35). To examine whether this holds true in the wild, we collected apples at various stages of decay. Bacteria proliferate as the apple decays via breakdown and utilization of plant cell wall components and sugars released by the decomposing plant cells. With the known limited dispersal of *C. elegans* within an orchard (only ~20% of apples harbor identifiable *C. elegans*) (7, 9), large proliferating populations of *C. elegans* were more likely to be observed in more rotten apples (Fig. 3), which supports the notion that a certain threshold of bacterial density is required before *C. elegans* will proliferate (35). However, we also observed many well-rotted apples with small nonproliferating populations (Fig. 3*C*). So even with access to microbes of presumably sufficient levels, the dauers either have not yet or may choose not to proliferate. This suggests that the microbial community composition of rotting apples may also affect the growth of *C. elegans*. Although fungi are indeed present and also contribute to decay of the apples, their shear size of fungal cells in relation to the pharyngeal opening, especially the hyphael form, may limit significant consumption by *C. elegans* (36). This notion together with our microscopy-based analyses of apples that indicate correlation between bacterial cells and apple decay (Fig. S4) led us to postulate that the bacterial constituents of rotting apples may be the primary drivers of *C. elegans* population growth.

To examine this question directly, we explored the bacterial diversity using 16S ribosomal gene sequencing in these rotting apples, as a function of whether the apple is supportive (proliferating) or refractory (nonproliferating with few/mostly dauers) to *C. elegans* growth. Bacterial abundance-weighted comparisons of shared phylogeny (UniFrac) in each rotting apple showed distinct clustering by population state of the *C. elegans* at time of collection (Fig. 4*A*). The distinct clustering reflected striking differences in complexity among these communities, with proliferating *C. elegans* populations more common in rotting apples with simpler microbiomes—i.e., 30% fewer bacterial species and lower phylogenetic diversity (PD) (Faith's PD and Shannon index) compared with those with nonproliferating populations (Fig. 4 *B* and *C*). Furthermore, examination of the microbial community structure of the two closely localized groups of rotting apples revealed that pieces of similarly rotten apples with proliferating populations of *C. elegans* shared highly similar microbiomes (~75% of bacterial OTUs; Fig. S5), whereas this was not true for pieces of apples with small nonproliferating populations of *C. elegans*. This indicates that there may be specific mixtures of microbes that influence the "habitability" (potential to support growth) of an apple for *C. elegans* and that perhaps there are many more types of refractory communities (e.g., different dominant pathogens) than supportive communities.

Proliferating *C. elegans* populations were observed predominantly in simple communities enriched in alpha-Proteobacteria (e.g., several *Acetobacteriaceae*) and missing many potential pathogenic gamma-Proteobacteria (e.g., *Pseudomonas*, *Xanthomonas*, *Stenotrophomonas*, etc.) and Bacteroidetes (e.g., *Chryseobacterium* and *Flavobacteria*) (Figs. 5*A* and 6). These results were further supported by a supervised learning-based approach to determine how discriminative a given bacterial genus is for predicting *C. elegans* population state. Members of the *Enterobacteriaceae* and *Acetobacteraceae* families were the most diagnostic for rotting apples with large proliferating populations of *C. elegans*, whereas the converse was true for members of *Pseudomonadaceae*, *Xanthomodaceae*, *Flavobacteriaceae*, and *Microbacteriaceae* (Fig. S6). These results support our findings of *C. elegans* responses to our collection of natural isolates representing these genera (Fig. 2*A*), especially that among the isolates tested certain bacterial taxa like the *Acetobacteraceae* (i.e., *Gluconobacter* spp.) are exclusively beneficial for *C. elegans*.

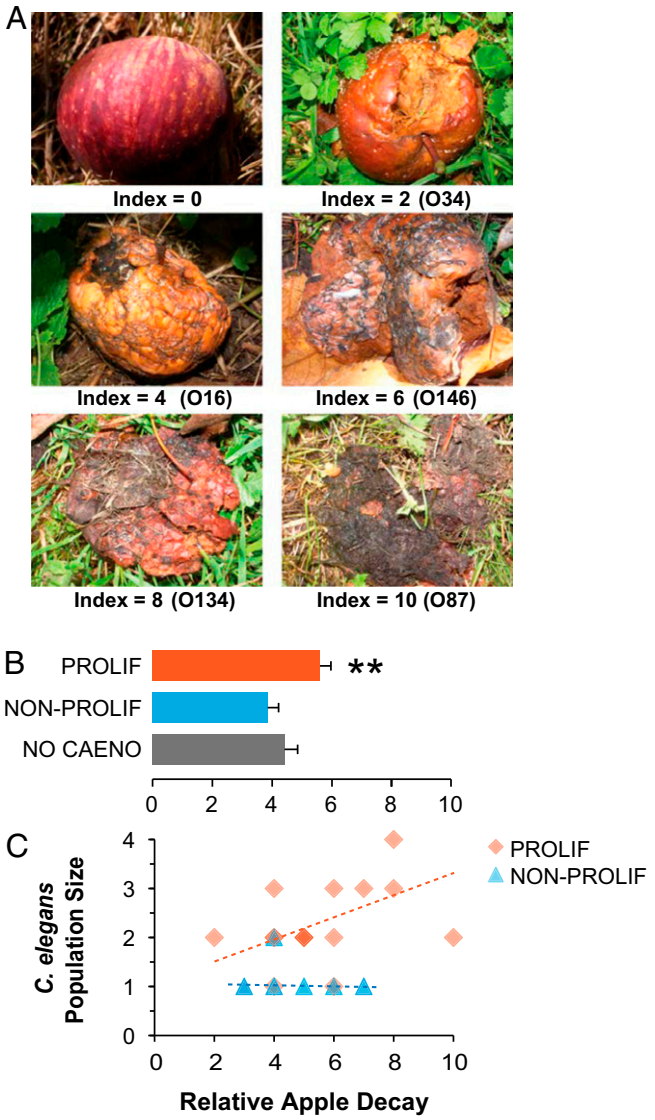

Fig. 3. *C. elegans* population sizes are greater in more rotten apples. (*A*) Different apples at different relative degrees of decomposition are shown. A rottenness index of 0 corresponds to an apple without apparent decomposition. An index of 10 corresponds to the last stage of decomposition, when it has reduced and flattened to the point of disappearing and has become very dark. Representative intermediate indices are shown. The course of decomposition depends on the apple. In scoring the index, the shape and the internal tissue resistance and color were taken into account. The picture of apple O34 has been used previously in ref. 52. (*B*) Mean rottenness in Orsay apples with proliferating (PROLIF) or nonproliferating dauer (NON-PROLIF) *C. elegans* populations compared with those without observed *Caenorhabditis* populations (NO CAENO). n = 13–20 per group (not all sequenced); means ± SEM presented; $^{**}P < 0.01$. (*C*) Plot of apple decay compared with estimated population size of *C. elegans* [based on subsampling of the rotting apples (size of bins: 1 = 1–10, 2 = 10–100, 3 = 100–1,000, and 4 = 1,000+)]. Color intensity indicates more apples at a given point.

Natural Proteobacteria Levels Promote *C. elegans* Growth in the Laboratory. We sought to reconstruct a microbiome from bacteria that are naturally associated with *C. elegans* and examine how they may influence *C. elegans* growth and metabolism. We exposed

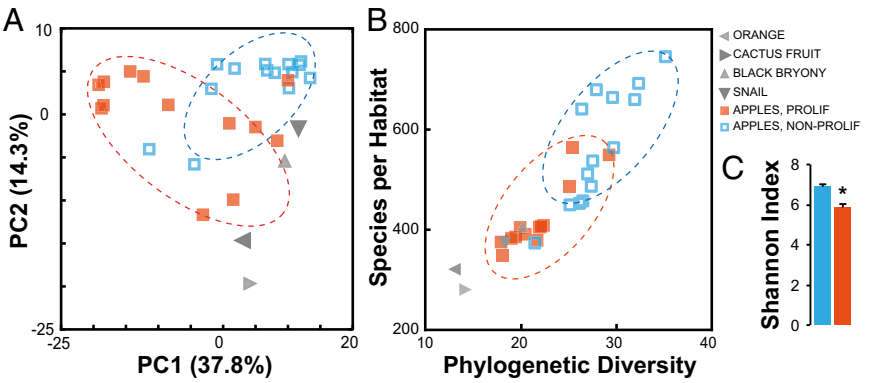

Fig. 4. *C. elegans* proliferates in rotting apples with simpler microbiomes. (*A*) Clustering of samples by phylogenetic beta-diversity (weighted normalized UniFrac) found in rotting apples from Orsay with proliferating (PROLIF) or nonproliferating dauer (NON-PROLIF) *C. elegans* populations. Subsampled apples were pooled evenly and represented as a single apple in these comparisons; other habitats are included for reference. Alpha-diversity measures for bacterial diversity with each habitat: (*B*) Comparison plot of species-level OTU (97% identity) number (richness) based on a Chao1 estimates versus phylogenetic diversity (Faith's PD); and (*C*) Shannon index of diversity. Means ± SEM are presented; $*P < 0.01$ (Student's *t* test). $n = 650$ sequences per sample.

C. elegans to simplified mixtures of cultured wild microbes (18–24 strains, pregrown and then mixed in specific amounts) that supply similar proportions of abundant cardinal bacterial species, representative of either proliferating *C. elegans* (80% Proteobacteria, alpha-Proteobacteria-rich) or nonproliferating dauer-arrested *C. elegans* (40% Proteobacteria, higher levels of gamma-Proteobacteria and Bacteroidetes). As assessed by the median body size of the nematodes after a particular time (a surrogate measure for larval or adult stages of development), *C. elegans* growth rates were significantly slower on the "nonproliferation" mixture of natural bacterial strains (Fig. 5*B*).

Discussion

Our survey of the bacterial inhabitants of decaying fruits and plant material where *C. elegans* are found has shown that four main bacterial phyla (Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria) are common in these natural habitats of *C. elegans*. It is also apparent that *C. elegans* is able to at least persist in a wide range of different microbial communities, revealing its ability to physiologically accommodate large differences in microbial diversity. However, despite the complexity of bacterial strains in each habitat, in those rotting fruits where *C. elegans* determines that the "conditions are right," abundant specific genera of Proteobacteria correlate with population growth. Not only was this observed in the natural *C. elegans* habitats, but also when we reconstructed a simplified community of cultured bacteria from that habitat we could recapitulate the Proteobacteria support of *C. elegans* growth in the laboratory. These simplified *C. elegans* microbiomes that more accurately represent the microbial environment in the wild will enable a dissection of how these microbes engage *C. elegans* physiology and innate immune responses.

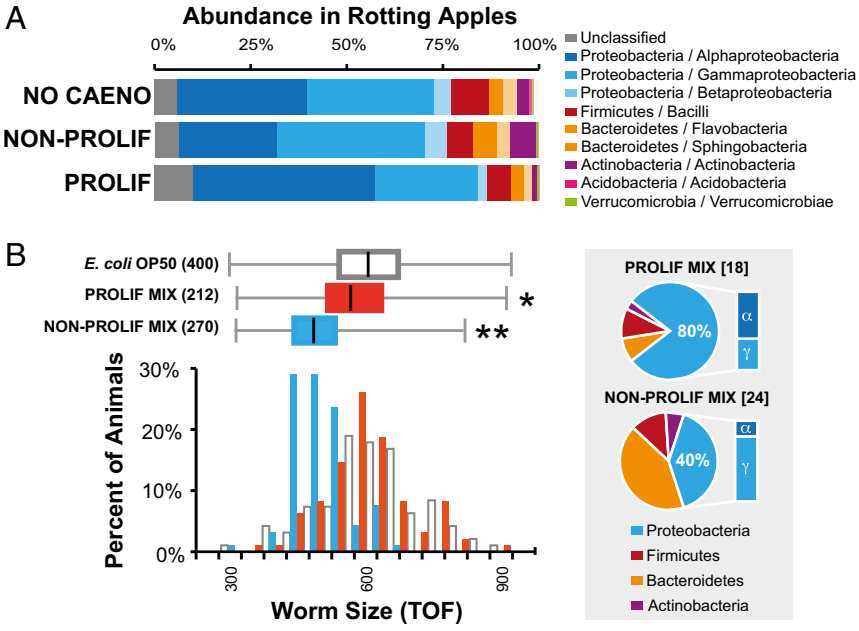

Fig. 5. Proteobacteria promote *C. elegans* growth in rotting apples and in the laboratory. (*A*) Proportion of bacterial divisions and classes in rotting apples by population state of *C. elegans*. Apples without detectable *Caenorhabditis* spp. (NO CAENO) are included for reference. (*B*) A size distribution plot of *C. elegans* animal length (COPAS Biosorter; proxy for growth rates) following exposure to either a Proteobacteria-rich "proliferation" mixture (80%, 18 isolates of bacteria) or a Proteobacteria-poor "non-proliferation" mixture (40%, 24 isolates of bacteria) (F1 assay; $n = 92–95$ per group per experiment). Kruskal–Wallis test: $**P < 0.0001$.

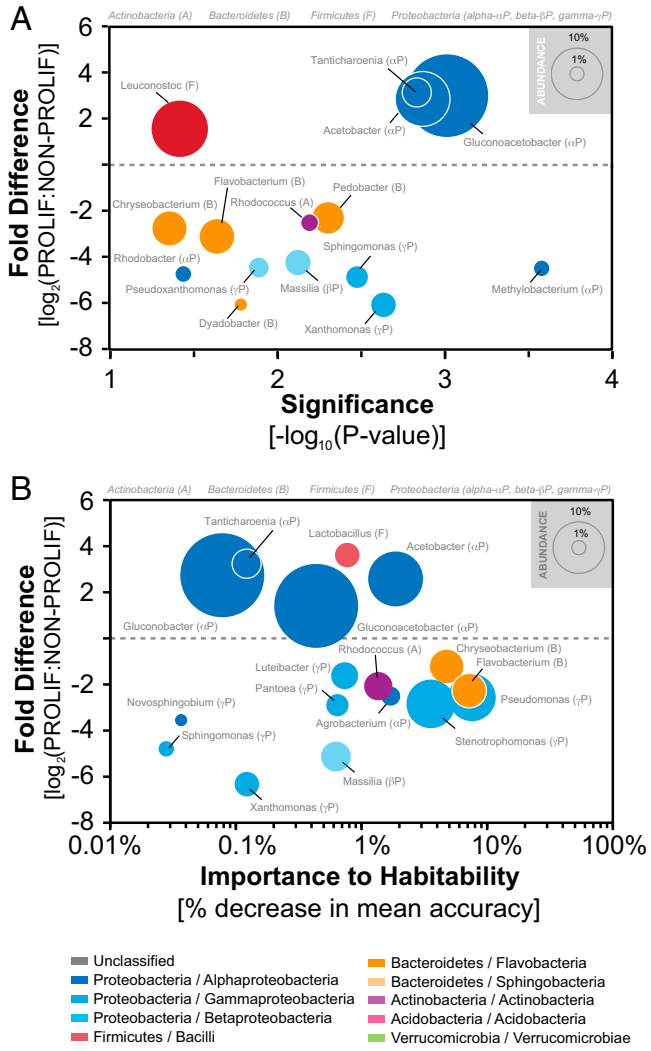

Fig. 6. Bacteria that determine whether *C. elegans* proliferates in rotting apples. (*A* and *B*) Fold difference in representation of bacterial genera by population state versus the degree of their significance (weighted ANOVA) or a supervised learning-model–based determination of its importance to habitability classification (Random Forest; "out-of-bag" error model, 10,000 trees). Circle size represents the maximal mean abundance between the two groups of apples.

Using individual cultured bacterial species, we demonstrate that nearly 80% of the bacterial isolates individually support growth of *C. elegans* with half doing so without induction of stress or pathogen reporter genes. Some of these bacterial taxa are the most abundant types of bacteria in the metagenomic samples (e.g., *Enterobacteriaceae*), suggesting that they may constitute a main bacterial nutritional source of *C. elegans* in rotting fruits and vegetation. Additional studies of vegetation and additional locales may provide a clearer picture of the microbes that *C. elegans* encounters and feeds on. Although beyond the scope of this study, it will also be enlightening to examine the fungal constituents of *C. elegans* natural habitats. Fungi may not be easily consumed as food by *C. elegans* based on their size (36) yet they may affect *C. elegans* physiology, directly or indirectly via their interplay with bacteria.

Whether these beneficial bacteria may be inoculated in the fruit by *C. elegans* or its invertebrate host for transport remains to be explored. Nondauer *C. elegans* animals often harbor associated bacteria, in the gut or on their cuticle (7, 10), and may be transported by molluscs such as slugs and snails (7, 9). It will be interesting to discern whether pioneering dauer larvae may disperse bacteria as well, and if so, how they carry the bacteria. As introduced bacteria potentially multiply with the typical bacterial 20-min doubling time when nutrients are plentiful, the dauer larvae may resume development and the progeny of the pioneering animals may also consume the bacteria their parents introduced. It is also possible that the mere presence of *C. elegans* can also shape the composition of these microbial communities and make them more hospitable.

By contrast, about 20% of the bacterial species when cocultured with *C. elegans* cause slow growth and stress reporter induction. Mixing experiments with beneficial versus detrimental bacteria showed that some of the beneficial bacteria could actively suppress the pathogenicity of particular detrimental bacteria. These data are suggestive of secreted toxins or virulence factors, which would be expected to allow the antagonistic bacteria to dominate in a community. The data are less supportive of a nutritional or micronutrient model of bacterial/*C. elegans* antagonism, whereby the microbe sequesters key nutrients or produces antagonistic compounds of low specific activity. The 20% hit rate for induction of *C. elegans* reporters of stress and innate immune response also suggests that these bacterial communities possess active systems of interaction with their nematode coinhabitants.

Altogether, this study provides a foundation for interrogating the natural influences that microbes have on the multifaceted physiology of this otherwise well-studied organism, so far mostly studied using *E. coli* as environment and food. Our characterization of bacteria to which *C. elegans* is naturally exposed will allow identification of new features of *C. elegans* biology, including metabolism, behavior, immunity, and even development. In addition, it not only pioneers a simple system with a fast-growing animal that can be used for experimental community ecology in a standardized environment, but also joins other model systems (19, 22, 23, 37, 38) to facilitate greater examination of the conserved pathways that mediate influence of natural microbes on host physiology and vice versa.

Experimental Procedures

***C. elegans* Strains and Maintenance.** N2-Bristol, *Phsp-4::GFP*, *Phsp-6::GFP*, *Pgst-4::GFP* (acquired from the *Caenorhabditis* Genetics Center); *PF35E2.5*, *Pclec-60::GFP*, *Pirg-1::GFP* (gifts from F. Ausubel, Massachusetts General Hospital, Boston) (39–41). All strains were maintained on NGM plates (no antibiotics) spotted with *E. coli* OP50 at 20 °C–22 °C using standard protocols (42) unless otherwise noted. All assays began with exposure of bleach sterilized ("germ-free") and synchronized L1 animals to microbes.

Sample Collection, Nematode Identification, and Isolation of Wild Bacteria. Rotting fruits and vegetation were collected and assessed for the presence of *C. elegans* nematodes as previously described (7, 9). Briefly, the samples were collected in plastic bags and transported back to the laboratory. For the Orsay orchard apples, in the same day, half of the sample was frozen for further processing and 16S rRNA sequencing (see below), whereas the other half was used to determine the abundance of each *Caenorhabditis* larval and adult stage—those with large populations devoid of dauers were classified as "proliferating," whereas those with small populations or only dauers were classified as nonproliferating (7). The identity of the *Caenorhabditis* species was determined using mating or PCR tests (43). The degree of apple decay was expressed by a "rotteness score," from fresh (0) to nearly completely decayed (rotting score of 10), corresponding to the progression from a solid spherical to a thin and flat object, respectively (Fig. 3). A subset of apples were also sliced and processed independently to determine where in the rotting apples *C. elegans* animals are most likely to be found (7). The Orsay apples are a subset of those in refs. 7 and 9. *C. elegans* isolates from all positive samples have been kept and frozen.

For isolation of wild bacteria, a subset of the samples was homogenized and resuspended in sterile PBS, and aliquots were spread on LB, YPD, and/or mannitol agar plates and then grown for 1–2 d at room temperature (~25 °C). Single colonies were isolated from the plates, grown in the same media overnight (shaking at 200 rpm, 28 °C) or until significant growth was achieved, and frozen and stored at –80 °C in 15% glycerol stocks. An analogous approach was also used for culturing bacteria directly from *C. elegans* worms in Orsay apples (noted

in Dataset S2) by either allowing the freshly isolated animals (odorant method) to explore a sterile media plate or by plating worm homogenates. For each bacterial clone, a 16S rRNA fragment was amplified and sequenced (Dataset S2). The remainder of the fruit was frozen at −80 °C for DNA isolation at a later time.

DNA Isolation, PCR Amplification, and Multiplexed 16S Sequencing. Bulk metagenomic DNA was extracted from frozen samples using a bead-beating–based approach according to established methods (44). Samples were homogenized with a mortar and pestle under liquid nitrogen. Approximately 0.25 g from each sample was used for isolation of DNA. A total of 500 μL of filter-sterilized buffer A 2× (200 mM NaCl, 200 mM Tris, 20 mM EDTA, pH 8.0, 210 μL of 10% SDS and 500 mL of phenol:chloroform:IAA, 25:24:1) (Ambion AM9730) was added, in a 2-mL screw-cap tube. The sample was bead beaten with 500 μL zirconium beads (Biospec 11079101z; acid washed and UV irradiated) for 2 min in a Bispec MiniBeadbeater-8, followed by 5,000 × *g* centrifugation at 4 °C for 3 min. The aqueous phase was transferred to a new tube and further extracted with 500 μL phenol-chloroform followed by isopropanol precipitation. We normalized isolated samples to a concentration of 10 ng/μL (nanodrop and/or Picogreen) by dilution with PCR-grade water.

Bacterial diversity was targeted by PCR-based amplication of 16S rRNA gene sequences in duplicate from the extracted DNA. Barcoded PCR primers based on those previously described (45) that amplify the 16S rRNA gene variable region 4 (515F/806R) in bacteria/archaea were modified for use on the Ion Torrent sequencing system for downstream multiplexed sequencing (Dataset S4). We pooled duplicate amplified samples and purified them with the desalting protocol of the Qiagen QiaQuick 96-well PCR cleanup kit (Qiagen). PCR amplicons were quantified using Picogreen and equimolar pooled. Libraries were constructed at a 1× concentration with the Ion OneTouch System. Beads were loaded onto Ion 314 chips (with 1.26 million wells) and the library sequenced on the Ion Personal Genome Machine Sequencer. Flows were analyzed postrun to produce sequences for each well, eliminating those with poor signal or signatures of polyclonality and trimming 3′ ends to remove adaptor sequences or low-quality bases.

Sequence Analyses. Processing of the fastq files of sequencing reads was completed using the QIIME software package (45). Briefly, sequences were truncated at the first low-quality base and quality filtered to remove reads with an average quality score below 25, shorter than 175 nt, or longer than 500 nt, more than 1 ambiguous base, primer mismatches, and erroneous barcodes that could not be corrected using the Golay barcodes. The resulting dataset (497,380 reads total, with a mean of 6,377 and median of 4,344 reads per sample) of 16S rRNA gene sequences were further analyzed using QIIME. De novo OTU picking was performed with the uclust option in QIIME (46). Representative OTU sequences were aligned using the PyNAST algorithm with a minimum percent identity of 80% (47). Taxonomic assignment of representative OTUs was completed using the Ribosomal Database Project classifier (48). Sequences matching plant chloroplast, mitochondrial, or archaeal 16S rRNA as well as those identified in reagent controls were filtered from the dataset, as were those OTUs that were not identified in at least three independent samples with at least 15 total reads. Samples with only *C. briggsae* nematodes were also excluded from the analyses.

For estimates of alpha-diversity (within sample), samples were rarefied to 1,000 sequences (unless otherwise noted), and those with fewer reads were removed. For comparisons between rotting apples, slices from the same apple were pooled with the highest equivalent number of reads for all of the slices, and then rarefied to 1,000 sequences. Alpha diversity metrics (chao1, PD, observed species) were also computed within QIIME using default parameters. Distance matrices were generated using weighted normalized UniFrac (49) methods to facilitate comparisons of the relative abundance and presence/absence patterns between groups of rotting apples. Our beta diversity (sample to sample) measures were computed with QIIME and jackknifed 100 times by repeatedly sampling at 750 sequences per sample and plotted with confidence ellipses. The Random Forests package (50) in QIIME was used to determine the taxa that are most discriminative for *C. elegans* population states.

Genera or species abundance in rotting apples ("APPLES.ALL" in Dataset S1 *B–E*; all collected in Orsay plus 3 in Santeuil) versus nonapples (snail, orange, black bryony, and cactus fruit) comparisons were completed by collapsing OTU-level abundance data to the appropriate level using QIIME, merging the sequencing reads by habitat type, and rarefying the resultant tables to 150,000 sequences per habitat type. Rarefaction curves indicate that even at this depth it is likely that not all of the microbial OTUs have been identified, and thus "true" habitat diversity is likely to be underestimated.

We note that any PCR-based analysis may have technical issues and biases that cannot completely be eliminated. The design of PCR primers cannot account for every possible change in even the well-conserved regions that we targeted in the 16S ribosomal genes. The primers we chose have served as benchmarks for the Earth Microbiome Project (51) and have effectively been used across a wide range of terrestrial and host-associated samples.

The sample structure used in the different analyses is detailed in Dataset S1*A*.

Effect of Wild Bacteria on Activation of GFP Reporters and on *C. elegans* Growth Rate. Bacteria isolated from various habitats harboring wild *C. elegans* populations (Dataset S2) were grown in LB (or mannitol broth for the Acetobacteria) at 28 °C shaking for 16–18 h; *E. coli* OP50 was also assayed for comparison and was grown similarly but at 37 °C. The microbes were concentrated 3× and seeded (30 μL) into 24-well NGM plates in duplicate. Plates were dried and allowed to grow overnight at room temperature before ∼40 synchronized L1 worms were added to the wells containing a single bacterial strain. Animals were scored after 48 h at 20 °C.

The impact of individual bacterial strains on *C. elegans* growth rates was also measured in a similar manner using N2 animals as noted. Delays are noted when a majority of the population is much younger (and often smaller) at a time when animals on *E. coli* OP50 are much more developed (e.g., 48 h at 22 °C as in Fig. 2); conversely, growth promotion is noted when animals reach adulthood faster than on *E. coli* OP50.

For bacterial codilution series, all strains were grown alone in their appropriate media [LB for everything but *Gluconobacter* (mannitol broth), alongside uninoculated controls for contamination] and mixed using culture OD_{600} values in a range of dilutions (same approximate total cfus of bacteria in every condition) in 24-well plates: 100% beneficial, 5% beneficial/95% pathogen, 20% beneficial/80% pathogen, 40% beneficial/60% pathogen, 80% beneficial/20% pathogen, 100% pathogen. Animals were assayed after 48 h of growth at 22 °C for how many of the animals reached early adulthood.

For complex mixtures of wild microbes, again each strain was grown independently, concentrated, and then mixed in appropriate ratios based on culture OD_{600} values to mimic the composition found in rotting apples, either with proliferating or nonproliferating populations of *C. elegans*, then spotted (50 μL) onto 10-cm NGM plates. Synchronized L1 animals were added to the plates, allowed to grow to adulthood, then transferred to new plates with the same bacterial mixtures, allowed to lay eggs (2 h at 22 °C) and then removed. Using the COPAS worm sorter, we quantified the impact of these mixtures on *C. elegans* growth rates by measuring the body size of these F1 animals.

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Supporting Information

Samuel et al. 10.1073/pnas.1607183113

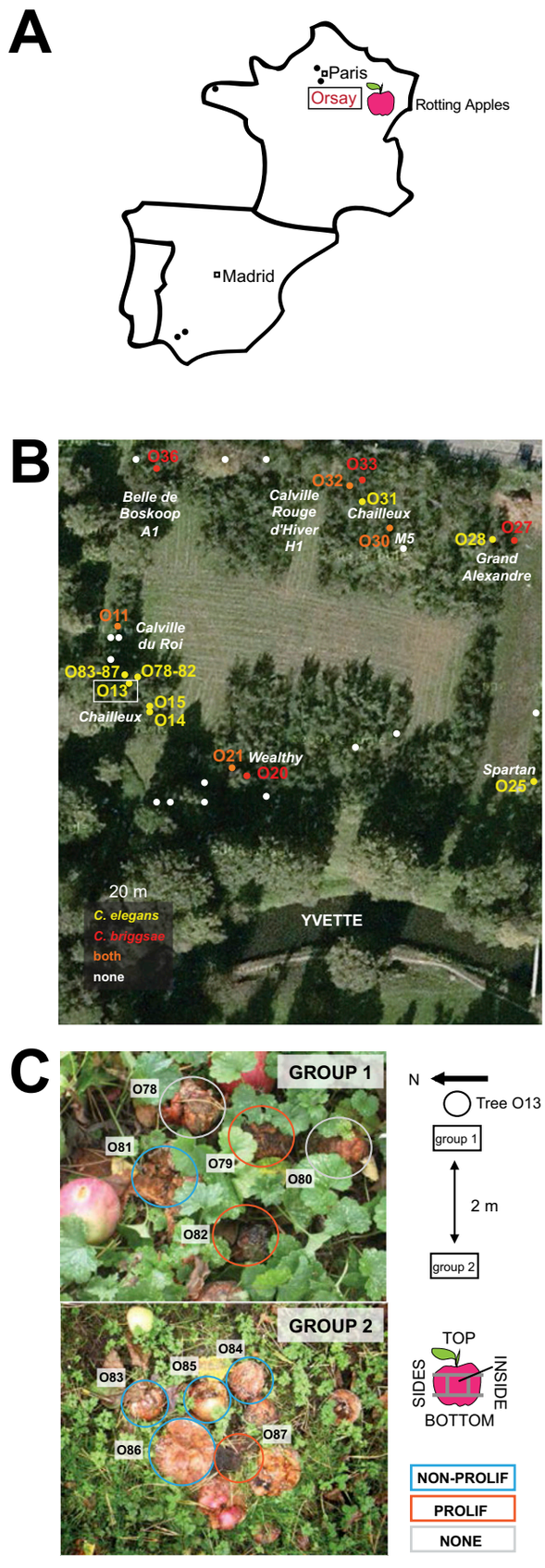

Fig. S1. Sampling of *C. elegans* natural habitats at different scales. (*A*) Regional map of approximate locations for the sampling sites. (*B*) Example of a sampling day at an Orsay orchard where 22 rotting apples were collected and analyzed, and (*C*) pictures of the two groups of five rotting apples from the same tree collected at a later date (see Dataset S1 for details). The apples from the same tree were also sliced (when possible) into several slices as indicated (*Inset*).

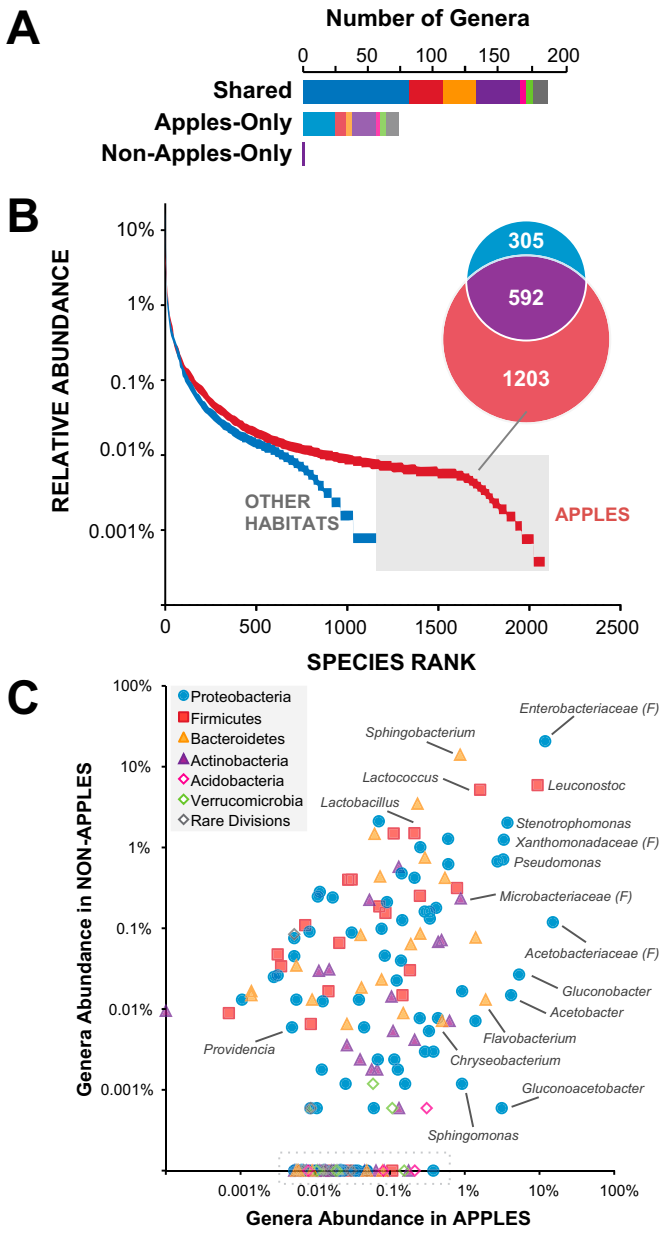

Fig. S2. Frequency and distribution of bacterial genera in the different habitats. (*A*) Number of genera identified that were found in multiple habitat types (shared) versus those that were specific for apples or all nonapples (orange, cactus fruit, snail, and black bryony stem). (*B*) Rank abundance plot of species in apple and nonapple habitats (merged as class, rarefied to 2,000 sequences per group). Venn diagram represents overlap in species present in each of the groups. (*C*) Plot of bacterial genera abundance in apple and nonapple habitats (detailed in Dataset S1 *C*–*E*). Subsampled apples were treated as a pool of slices for a given apple. Note that the number of apples is much higher than that of the nonapples. A detailed analysis of the apple samples is shown in Figs. 4 and 5.

	pGST-4::GFP (Oxid)				pHSP-6::GFP (Mt UPR)				pHSP-4::GFP (ER UPR)				
	L1-72HR	L1-96HR	L4-36HR	L4-SUP 24HR	L1-72HR	L1-96HR	L4-36HR	L4-SUP 24HR	L1-72HR	L1-96HR	L4-36HR	L4-SUP 24HR	GROWTH (VS. OP50)
E. coli OP50	0	0	0	0	0	0	0	0	0	0	0	0	
E. coli HB101	0	0	1	0	0	0	0	2	0	0	0	1	FASTER
Acinetobacter sp. JUb2	0	1	1	2	1	1	1	1	1	0	0	1	FASTER
Bacillus sp. JUb13	0	0	1	1	1	1	2	2	1	0	1	2	FASTER
Providencia sp. JUb39	1	1	1	1	2	2	2	2	1	0	1	2	FASTER
Pseudomonas sp. JUb12	0	0	1	1	1	0	1	2	1	1	3	2	
Paenibaccillus sp. JUb50	2	1	1	1	1	1	2	1	1	1	2	1	
Rhizobium sp. JUb45	3	2	3	0	1	1	1	2	0	0	1	2	FASTER
Raoultella sp. JUb54	3	3	2	1	1	1	2	2	0	0	1	2	FASTER
Delftia sp. JUb8	3	3	3	4	1	0	1	1	1	0	2	1	
Raoultella sp. JUb38	0	2	2	1	2	2	2	2	5	3	3	1	SLOWER
Stenotrophomonas sp. JUb19	0	0	1	1	3	4	4	3	3	2	4	3	SLOWER
Sphingobacterium sp. JUb56	2	1	5	2	3	3	3	2	3	2	1	2	SLOWER
Chryseobacterium sp. JUb44	3	2	3	5	3	2	3	2	3	2	2	3	SLOWER

Fig. S3. *C. elegans* reporter strains induced after short exposures to whole bacteria and culture supernatants. Impact of representative cultured wild isolates on growth rates (relative to *E. coli* OP50) and activation of stress reporters [0–5 scale, high = more activation of reporter; *Phsp-4::GFP* [ER unfolded protein response (UPR)], *Phsp-6::GFP* (mitochondrial UPR) and *Pgst-4::GFP* (oxidative stress)] under various conditions: transfer to the wild microbe as an L1 or L4 larvae or on *E. coli* OP50 plus bacteria-free supernatant (L4-SUP). Exposure times to the bacteria at 22 °C are noted; n = >100 animals per condition per experiment.

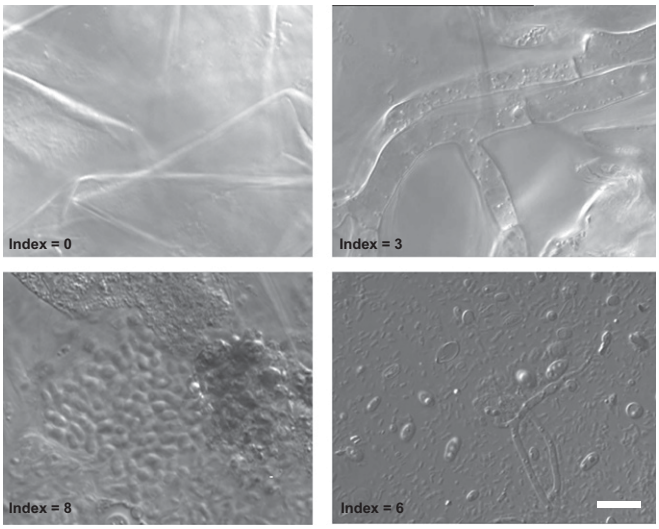

Fig. S4. Microbial populations proliferate as apples decay. Nomarski pictures of apples in diverse stages of decomposition, representing the general progression of microbial proliferation associated with apple decay. Apple tissue was directly placed on a microscope slide for examination. (*Top Left*) Nonrotten apple (index 0). (*Top Right*) Fungal hyphae proliferate and loosen the apple tissues. (*Bottom Left*) Eukaryotic (*Center*) and bacterial (*Top*) colonies (apple O151). (*Bottom Right*) Diverse bacteria and some fungal cells (apple O134). The two latter apples harbored proliferating *C. elegans* populations (Dataset S1). (Scale bar: 10 μm.).

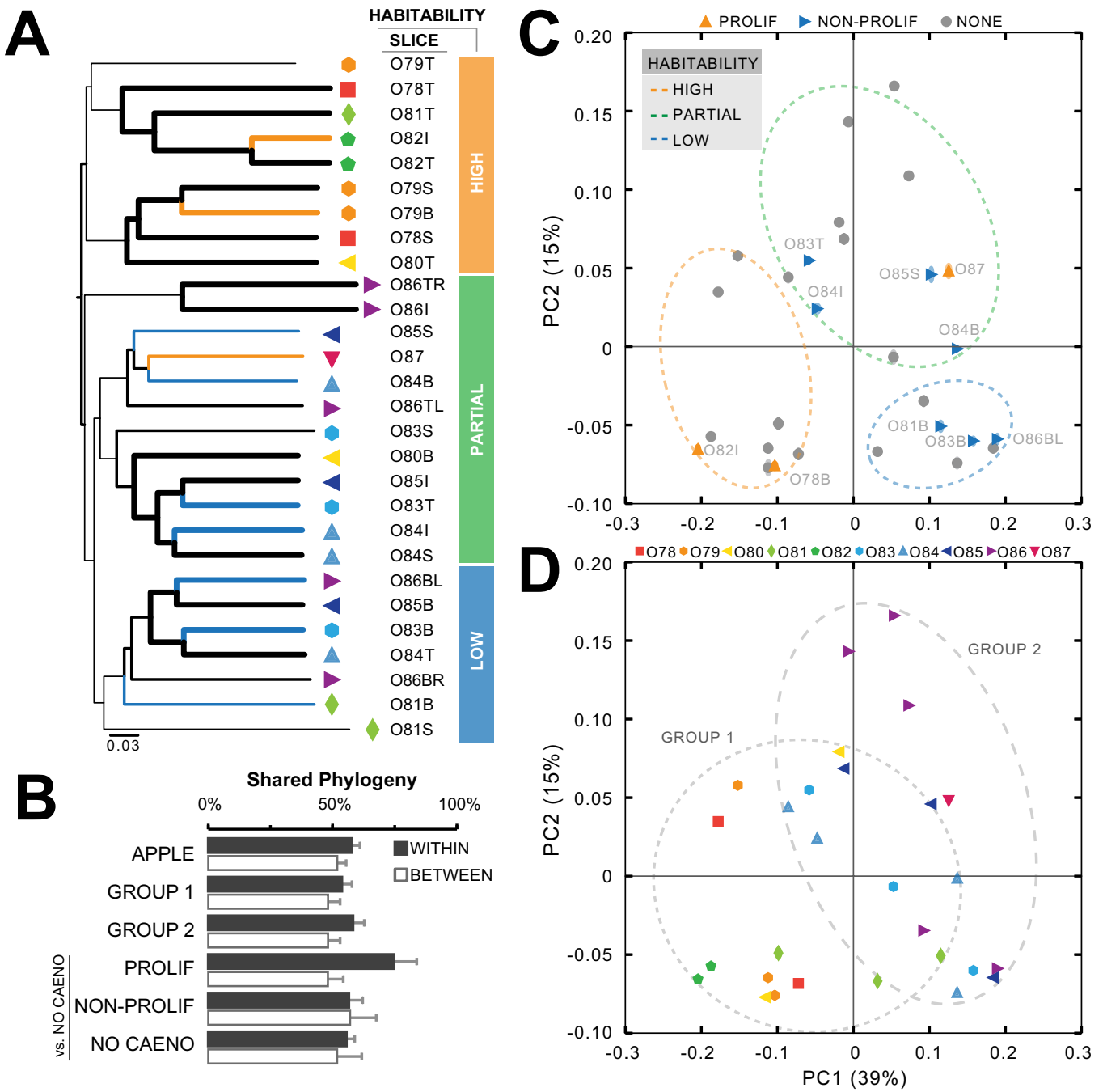

Fig. S5. Phylogeny-based clustering of subsampled rotting apples from the same tree. Apples collected in two groups from under the same tree were further subsampled into slices (Fig. S1*C*) and assessed for their phylogenic beta-diversity (normalized abundance-weighted UniFrac distance). (*A*) Clustering of the samples reveals distinct groups based on habitability rather than apple of origin (jackknifed 100 times; greater line thickness means more support for a branch). (*B*) Shared phylogeny (branch length) among different habitat characteristics. (*C* and *D*) PCoA analyses (weighted normalized UniFRAC) of the samples based on habitability and apple group, respectively. The most habitable apples are in group 1, but the nonhabitable apples of group 1 cluster with the other nonhabitable apples. Further sampling would be required to determine whether this trend holds true in general. $n = 650$ sequences per sample.

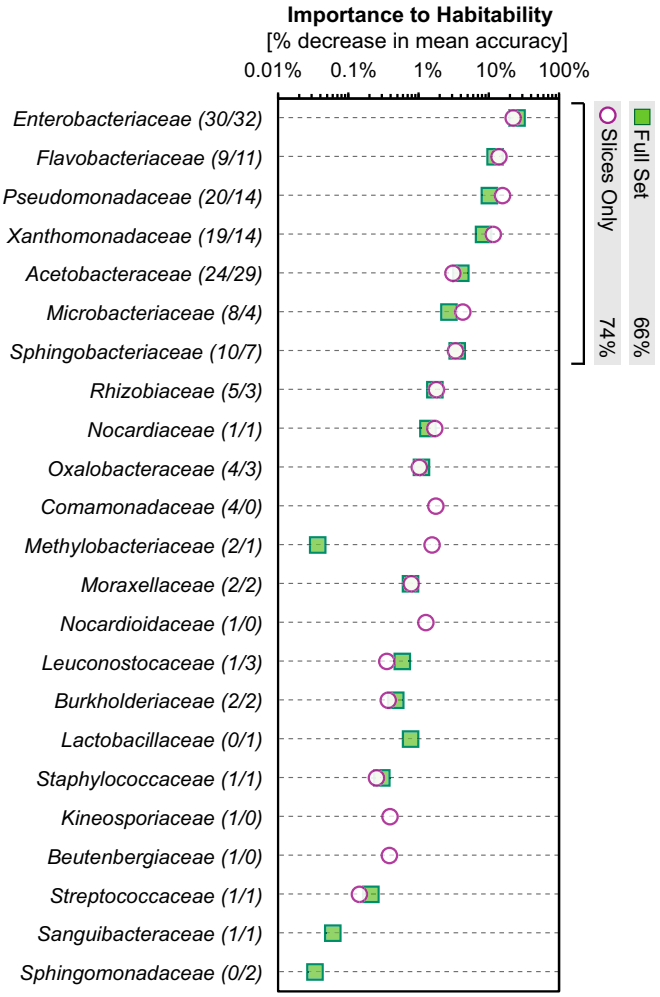

Fig. S6. Supervised learning determination of bacterial families important to classification of habitability. Determination of the most discriminative families of microbes for population state of *C. elegans*. Values are presented as a summation of phylotypes determined to be important (>0.01% decrease in error plotted) within a bacterial family for the subsampled apples from the same tree ("Slices Only") or the full set of rotting apples (with subsampled apple pools). Estimated error for the model was 0.20 compared with a baseline error of 0.58 (random guessing).

Dataset S1. Sample metadata and rank abundance of bacteria at varying phylogenic levels in habitats with or without wild *C. elegans*

Dataset S1

Dataset S2. Wild microbes used in this study: the JUb and BIGb collections

Dataset S2

Dataset S3. Detailed responses of *C. elegans* to exposure to each wild microbe of the collection, using growth assay and reporter strains

Dataset S3

Dataset S4. Sequencing primers used in this study

Dataset S4