Bacterial traits and quality contribute to the diet choice and survival of bacterial-feeding nematodes

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A B S T R A C T
The dietary choices of bacterial-feeding nematodes could control the structure and ecological functions of soil bacterial communities. However, the physiological basis for the selection of particular bacterial species as food, and the consequences of these dietary choices for the survival of bacterial-feeding nematodes, is poorly understood. The objectives of this study were (1) to determine how nematode feeding preference was related to bacterial traits (cell size, gram stain and growth rate) and quality (water content, carbohydrate content, protein content and metabolite concentration), and (2) to evaluate how dietary choices affected the reproduction and lifespan of two soil-dwelling bacterial-feeding nematodes of the species Mesorhabditis and Acrobeloides. Their food sources included one model bacterium, Escherichia coli OP50, and four soil-dwelling bacterial species: Bacillus amyloliquefaciens, Bacillus megaterium, Variovorax paradoxus and Pseudomonas fluorescens. Both nematode species exhibited a similar hierarchy of diet choice, with P. fluorescens and E. coli OP50 being the most preferred food, whereas B. megaterium was the least preferred bacteria. Nematode feeding preference was strongly related to the water content, growth rate and metabolite concentration of bacterial cells, which explained 63−75% of the variation in the feeding preference index (PI, which indicates the number of nematodes attracted to specific bacteria), and the rest of the variation was attributed to bacterial cell size, gram stain, carbohydrate content and protein content. We propose two physiological mechanisms to explain dietary choices of bacterial-feeding nematodes: 1) chemical attraction to higher carbon dioxide levels around rapidly-growing bacteria or repulsion to volatile organic molecules released from bacterial cells, and 2) selective ingestion of bacterial cells with preferred characteristics (e.g., high water content in cells). Nematodes feeding on preferred bacteria always had higher reproduction, but dietary choices were not a good predictor of their lifespan. For example, Acrobeloides feeding on their preferred food P. fluorescens had the largest brood size but a moderate survival time. However, when Acrobeloides consumed their least-preferred food B. megaterium, they produced the smallest brood size and had the shortest survival time. This may be due to the fact that dietary resources are allocated first towards reproduction, and second to prolong the lifespan of bacterial-feeding nematodes. Our findings suggest that dietary choices are important for the survival of bacterial-feeding nematodes, and their ability to find and selectively ingest preferred bacterial species may have implications for soil bacterial community structure and ecological functions.

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1. Introduction

Bacterial-feeding nematodes, one of the primary grazers of soil bacteria, are often considered as a single trophic group in model soil food webs. However, nematodes evolved like other terrestrial animals to exhibit dietary choice, and they show preference for
certain soil bacterial species (Salinas et al., 2007; Shtonda and Avery, 2006; Venette and Ferris, 1998). Nematode food-seeking behavior may be related to their morphological features and nutritional requirements. For example, the buccal morphology of bacterial-feeding nematodes are always funnel-shaped (e.g., family Cephalobidae) or rod-shaped (e.g., family Rhabditidae) (Ferris et al., 1996), and the buccal shape affects their ability to capture and ingest bacterial cells of various sizes and motility (Bongers and Ferris, 1999).

Bacterial cell size is another factor contributing to the feeding preference of bacterial-feeding nematodes. Small-sized bacteria are always preferred because they are easy to swallow and nutrient-rich, allowing more efficient ingestion and nutrient acquisition, whereas large-sized bacteria are considered to be a lower quality food (Avery and Shtonda, 2003; Avery and You, 2012; Shtonda and Avery, 2006) and harder to pass through the narrow buccal cavity (Salinas et al., 2007). Bacteria with high respiration and growth rates are attractive to C. elegans, presumably because the CO₂ concentration is higher in the vicinity of their cells (Yu et al., 2015). Nematodes can also distinguish pathogenic bacteria from healthy bacteria by experience and learn to avoid feeding on the pathogenic species (Beale et al., 2006; Zhang et al., 2005). Bacterial-feeding nematodes show a preference for greater bacterial cell density than gram-positive bacteria, which is attributed to the fact that gram-negative bacteria have a thinner cell wall and thus may be easier to digest (Salinas et al., 2007; Xiao et al., 2010). Soil protists are also reported to prefer gram-negative bacteria over gram-positive bacteria (Renn et al., 2002).

The selective feeding behavior of bacterial-feeding nematodes may be motivated by other characteristics of bacterial cells, which we refer to as ‘food quality’. Food quality could be determined by the water content, nutritional compounds (e.g., protein and carbohydrate) and metabolites released from bacterial cells as attractant or repellent compounds. Water is needed for digestion and metabolic processes, and bacterial-feeding nematodes may selectively consume bacterial cells with higher water content. While mammals discriminate between protein-free diets and a diet containing low to moderate level of protein (Fromentin et al., 2012), and soil invertebrates like earthworms showed a strong preference for litter with high soluble carbohydrate content (Curry and Schmidt, 2007), it is not known if bacterial-feeding nematodes have similar dietary cues. Soil-dwelling Pseudomonas produce metabolites such as small alcohols, ketones, diacetyl, and esters, which could act as natural chemoattractants or repellents for C. elegans (Bargmann et al., 1993). However, there is currently a lack of knowledge about how food quality is related to bacterial traits (e.g., cell size, gram stain and growth rates) and to what extent these food quality attributes influence the diet choice of bacterial-feeding nematodes.

Feeding preference has implications for the survival of bacterial-feeding nematodes. Food is a physiological need for basal metabolism throughout the lifespan of the organism and for secondary production, also referred to as reproduction, leading to trade-offs between reproduction and survival when there are food shortages (Partridge et al., 2005). The bacteria-feeder C. elegans produced more eggs after consuming its preferred food, but reduced reproduction to ensure survival when the food supply was limited (Mukhopadhyay and Tissenbaum, 2007). Yu et al. (2015) reported that C. elegans produced more offspring and had a shorter lifespan when supplied with preferred food resources (P. fluorescens and E.coli OP50), but the brood size decreased and the lifespan increased when C. elegans was given less-preferred B. megaterium. In contrast, Coolon et al. (2008) found that C. elegans preferred Pseudomonas sp. over B. megaterium and it had a shorter lifespan when feeding on B. megaterium. While dietary choices affect nematode life traits, it is unclear from the literature how bacterial-feeding nematodes make trade-offs between reproduction and lifespan when feeding on preferred and less-preferred bacterial species. It is difficult to generalize results from C. elegans to all soil-dwelling bacterial-feeding nematodes because C. elegans is a colonizer of microbe-rich habitats, in particular decaying plant matter (Felix and Braendle, 2010). Thus, we chose to study the dietary choices of two widely distributed soil-dwelling bacterial-feeding nematodes, Mesorhabditis sp. and Acrobeiloides sp., rather than C. elegans.

The objective of this study was to determine i) the relative contribution of food quality, based on bacterial traits (i.e., cell size, gram stain and growth rate) and chemical composition (i.e., water content, carbohydrate content, protein content and metabolite concentration) to the dietary preference of bacterial-feeding nematodes; and ii) if food selection will cause bacterial-feeding nematodes to make a tradeoff between reproduction and longevity. We hypothesized that nematodes feeding on preferred bacteria will produce more offspring but shorten their lifespan, whereas those consuming the less-preferred bacteria will have fewer offspring but a longer lifespan.

2. Materials and methods

2.1. Preparation of bacterial-feeding nematodes

Two bacterial-feeding nematodes Mesorhabditis sp. and Acrobeiloides sp. were obtained from a sandy loam alluvial soil collected from Banqiao Town, Nanjing City, Jiangsu Province, China. Nematodes were extracted from soil using a modified Baermann method (Liu et al., 2008) and observed under the microscope to select the bacterial-feeding nematodes based on morphological features. Selected bacterial-feeding nematodes were rinsed with sterile water and dark-cultivated on freshly prepared nematode growth medium (NGM) inoculated with E. coli OP50 in a 20 °C incubator (see supplementary information for details). Selected organisms were transferred sequentially to new culture plates with NGM until a single bacterial-feeding nematode species was present in the culture plate. This procedure isolated two dominant nematode species, whose morphological characteristics head, tail, genitalia and excretory pore were visualized with a scanning electron microscope (Fig. S1 and Fig. S2). Finally, the dominant nematode species were sent to BGI Company (Shenzhen, China) for 18S rRNA sequencing and analysis of molecular data with BLAST in the NCBI database followed by phylogenetic analysis with the Neighbor-Joining method in MEGA software (Fig. S3). The morphological and molecular analyses confirmed that the bacterial-feeding nematodes were the species Mesorhabditis and Acrobeiloides.

2.2. Preparation of bacterial species

Five bacterial species were selected for this study, including Bacillus amyloliquefaciens (B.a), Bacillus megaterium (B.m), Variorax paradoxus (V.p), Pseudomonas fluorescens (Pf) and Escherichia coli OP50 (E.c). In a pre-feeding trial, these bacteria were confirmed to support the growth of bacterial-feeding Mesorhabditis and Acrobeiloides. As the standard food for C. elegans in laboratory studies, the E.coli OP50 was obtained from CGC (Caenorhabditis Genetics Center, USA), whereas the other four bacteria were collected from the same field as bacterial-feeding nematodes. The bacteria were obtained by first plating a serial dilution (to 10⁻⁶ to 10⁻⁴ of soil suspension onto LB medium dishes (10 g L⁻¹ tryptone, 5 g L⁻¹ yeast extract, 10 g L⁻¹ NaCl, and 17 g L⁻¹ agar, pH 7.0). Next, bacteria were purified by transferring a single colony onto LB media in a fresh dish, growing cells at 20 °C, and repeating the procedure (transfer of a single colony, growing cells at 20 °C for 7–8
generations. After the final transfer of a single colony onto fresh LB media, the dish was incubated at 20 °C to obtain sufficient bacterial biomass for morphological evaluation (Table 1) and species identification from 16S rRNA sequencing at the BGI Company (Shenzhen, China). The four field-dwelling bacteria, along with E.coli OP50 were grown in fresh liquid LB medium at a shaking speed of 180 rpm, and the bacterial OD600 values were normalized to 1.

2.3. Nematode feeding preference experiment

The experimental unit was a glass petri dish (90 mm in diameter). Each petri dish was filled with fresh NGM and divided into five equal sections, each having a dot-circle (15 mm diam.) located 20 mm from the center dot-circle (Fig. 1). The five bacterial species (20 μL) were pipetted into five designated dot-circles in each petri dish. Nematode species were concentrated in test tubes to a density of 1500 nematodes per 20 μL in M9 buffer, which was verified by direct counting. Approximately 1500 individual nematodes (Mesorhabditis or Acrobeloides) were placed on the center dot-circle. The five dot-circles were defined as “bacterial zones”, and all other locations were defined as the “non-bacterial zone”. After preparing 10 replicate dishes per nematode species, the petri dishes were placed immediately in a dark incubator at 20 °C. The number of nematodes that migrated to each designated bacterial zone was recorded at 8, 12, 24, 36 and 48 h under a stereomicroscope at 50 × magnification.

Nematode feeding preference index (PI) was calculated as PI = Ni/Nr, where Ni is the number of nematodes in each designated bacterial zone, and Nr is the total number of nematode in all bacterial zones.

2.4. Nematode lifespan and brood size experiment

Nematode lifespan and brood size experiments were performed separately from the feeding preference experiment. The lifespan of Acrobeloides and Mesorhabditis was evaluated on petri dishes with NGM medium. A total of 10 nematodes in the L1 stage (P0) were transferred onto NGM medium inoculated with one of the five bacterial species (a single bacterial species per dish). There were 6 replicate dishes for each bacterial species. All dishes were placed in a dark incubator at 20 °C. Surviving P0 nematodes were counted and transferred to fresh dishes every day until all nematodes died. The nematode was considered dead when it stopped moving and did not respond to a gentle touch with a platinum wire.

Reproduction was measured for one reproductively mature nematode (L4 stage) that was taken from laboratory cultures. The randomly selected mature nematode was placed into a NGM medium dish inoculated with one of the five bacterial species (a single bacterial species per plates). Six replicate dishes were prepared for each bacterial species. Each individual nematode was transferred to a fresh NGM medium dish daily, until they stopped laying eggs, and the number of eggs was recorded every day. Nematode brood size was the sum of all eggs laid by a single hermaphrodite of Acrobe- loides during its lifespan. No data were collected for Mesorhabditis because it is a dioecic nematode.

2.5. Measurement of bacterial trait and quality parameters

Three bacterial traits were measured: 1) Cell size: morphology of each of the five bacterial species was observed under oil lens after simple staining. We selected 40 individual cells of each bacterial species and measured their length and width under the software Motic Image plus 2.0, and then calculated bacterial body size (V) based on length (L, in μm) and width (W, in μm), where V (μm³) = π × W² × L; 2) Gram stain; 3) Growth rate: 500 μL bacteria from liquid cultures with OD600 = 1 were added to 150 mL triangular flasks containing liquid NGM medium with 6 replicates per bacterial species. Then, flasks were cultured at 20 °C with a shaking speed of 180 rpm. The number of colony-forming units (CFUs) was measured each hour for the first 8 h and every 2 h thereafter, for 48 h. The doubling time (DT) was calculated as DT = ln2/(t2-t1)/(lnW2 - lnW1), where t1 and t2 indicate two different times during the mid-exponential phase and W1 and W2 are the number of colonies at t1 and t2, respectively. The growth rate (G) was calculated as G = ln2/DT (Schulze and Lipe, 1964).

Five bacterial species were cultured in liquid media on a shaker, and 18 mL (6 × 10⁷–5 × 10⁹ individual) bacteria with 3 replicates were respectively used to measure chemical composition: 1) Water content: the supernatant was removed by centrifugation, and the wet weight of the bacterial pellet was measured. Bacteria were then lyophilized for 48 h to measure their dry weight; 2) Carbohydrate content: the carbohydrate (i.e., total sugar) of bacteria was measured by the anthrone colorimetric method (Brooks et al.,

<table>
<thead>
<tr>
<th>Abbreviate</th>
<th>Bacteria species</th>
<th>Phylum</th>
<th>Shape</th>
<th>Gram stain</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ba</td>
<td>B.amyloliquefaciens</td>
<td>Firmicutes</td>
<td>rod</td>
<td>G+</td>
<td>JX424611</td>
</tr>
<tr>
<td>Bm</td>
<td>B.megaterium</td>
<td>Firmicutes</td>
<td>rod</td>
<td>G+</td>
<td>JX424613</td>
</tr>
<tr>
<td>Vp</td>
<td>V.paradous</td>
<td>Proteobacteria</td>
<td>rod</td>
<td>G-</td>
<td>JX424612</td>
</tr>
<tr>
<td>Pf</td>
<td>P.fluorescens</td>
<td>Proteobacteria</td>
<td>rod</td>
<td>G-</td>
<td>KC962432</td>
</tr>
<tr>
<td>Ec</td>
<td>E.coli</td>
<td>Proteobacteria</td>
<td>rod</td>
<td>G-</td>
<td>–</td>
</tr>
</tbody>
</table>
2.6. Statistical analysis

One-way ANOVA with LSD multiple range test for post-hoc comparisons was used to analyze the brood size of Acrobeoloides response to five bacterial species. Generalized linear model (GLM) was used to analyze the pairwise correlations between nematode feeding preference index (PI) and bacterial parameters including cell size, gram stain, growth rate, water content, carbohydrate content, protein content, metabolite concentration, metabolite concentration was the sum of alcohol, aldehyde, ketone, ester, acid and phenol concentrations, which is appropriate since the individual variables were present in similar concentrations and all were negatively and significantly correlated with PI (Table S1). Relationships among PI and bacterial parameters (traits and quality) were evaluated with principal component analysis (PCA). The relationship between PI and bacterial parameters (traits and chemical composition) were evaluated with multiple regression model analysis. In the multiple regression model, PI was the dependent variable and bacterial parameters were considered as independent variables (predictor variables). The relative weights of selected predictor variables was evaluated under the relweights function (Johnson, 2000). Statistical analyses were conducted in R (R Core Team, 2015). Statistical analyses were conducted in R (R Core Team, 2015).

3. Results

3.1. Nematode feeding preference

Bacterial-feeding nematode species Mesorhabditis and Acrobeoloides showed strong preference among the five bacterial species tested, especially the Mesorhabditis. They exhibited a similar hierarchy of diet choice, with P. fluorescens being the most preferred food, followed by E. coli OP50, whereas B. megaterium was the least preferred bacteria (Fig. 2). At the 8 h measurement time, more Mesorhabditis were present in the bacterial zones of P. fluorescens and E. coli OP50 than other bacterial zones (P < 0.05, Fig. 2, Table S2). There was a significant (P < 0.05) increase in Mesorhabditis presence in the P. fluorescens than E. coli OP50 and other bacterial zones after 24 h, and this feeding preference behavior was maintained after 48 h (Fig. 2, Table S2). In contrast, the Acrobeoloides population was evenly dispersed in five bacterial zones after 12 h, although there were more Acrobeoloides at the P. fluorescens zone than elsewhere on the petri dish from 24 h to 48 h (Fig. 2).

3.2. Nematode feeding preference affected by bacterial trait and quality parameters

Bacterial traits (indicated by cell size, gram stain and growth rate) and chemical composition (indicated by water content, carbohydrate content, protein content and metabolite concentration) differed significantly between five bacterial species (Table 2, Fig. 3). PI were negatively correlated (P < 0.05) with bacterial cell size, carbohydrate content and all metabolites components, but positively correlated (P < 0.001) with water content and growth rate (Fig. 3, Table S1). However, PI of Acrobeoloides was not significantly correlated with the protein content of bacteria (Table S1). Both nematodes preferred gram-negative bacteria over gram-positive bacteria (Table S1). A multiple regression model evaluated the contribution of bacterial traits and chemical composition to PI. Together, bacterial traits and chemical composition explained 94-79% of the variation in the PI of Mesorhabditis and Acrobeoloides. The most important bacterial parameter was the water content (26-28% of the variation in PI of Mesorhabditis and Acrobeoloides), followed by growth rate (24-20%), metabolite concentration (25-15%), gram stain (6-17%), cell size (7-12%), protein content (5-6%) and carbohydrate content (5-3%) (Fig. 4).

3.3. Nematode reproduction and lifespan

Acrobeoloides had a longer survival time (61-99 d) than Mesorhabditis (28-57 d) when feeding on the five bacterial species of this study (Fig. 5A). Both nematodes survived longer when feeding on B. amyloliquefaciens than other bacterial species (Fig. 5A). Nematodes supplied with P. fluorescens and E. coli OP50 had the largest brood size, whereas nematodes that fed on B. megaterium and B. amyloliquefaciens had the smallest brood size (Fig. 5B). The PI correlated positively with nematode brood size (P < 0.05, Fig. 6A), indicating that nematodes feeding on preferred food had more offspring. However, PI was not correlated with nematode survival time (Fig. 6B), suggesting that the provision of preferred food or less-preferred food had no effect on the lifespan of these bacterial-feeding nematode species.

4. Discussion

4.1. Feeding preference of bacterial-feeding nematodes

The bacterial-feeding nematode species Mesorhabditis and Acrobeoloides exhibited a similar hierarchy of diet choice, with P. fluorescens and E. coli OP50 being the preferred food, whereas B. megaterium was the least-preferred bacteria (Fig. 2). These results are similar to Grewal and Wright (1992) and Salinas et al. (2007), who found that C. elegans and Cephalobus brevicauda preferred to eat E. coli rather than B. megaterium. We surmise that B. megaterium, a gram-positive bacteria with a large cell size, is not easily ingested and harder to digest due to its cell wall composition, making gram-negative bacteria with a small cell size the preferred food of bacterial-feeding nematodes. Both Salinas et al. (2007) and Yu et al. (2015) suggested that gram-positive bacteria are a non-preferred food because large-celled bacteria do not pass easily through the small buccal cavities, plus the thick cell wall of gram-positive bacteria makes them difficult to digest.

However, bacterial cell size and gram stain were not the most important factors explaining the feeding preference of Mesorhabditis and Acrobeoloides. Instead, the water content, growth rate and metabolites concentration were stronger explanatory variables of feeding preference. We suppose two physiological mechanisms can explain the preference for bacteria with greater water content, faster growth rates and higher concentration of metabolic...
byproducts. The first mechanism relates to the metabolic water requirements of bacterial-feeding nematodes. These nematodes live in water films and depend on external water for mobility, but they must ingest water with their food or obtain water from the external environment to support metabolic functions and maintain their osmolytic balance (Bongers and Ferris, 1999). Therefore, bacteria that are hydrated and have higher water content are likely a good source of water for bacterial-feeding nematodes. The water requirements of bacterial-feeding nematodes could be governed by an internal physiological mechanism that is based on hormonal responses related to the nutritional and hydration status of the organism, but this remains to be determined.

The second mechanism controlling the food preference of bacterial-feeding nematodes is based on attraction to certain bacteria. Nematodes can detect and discriminate the presence of many volatile organic molecules via chemotaxis (Bargmann et al., 1993), thus they could be attracted or repelled by chemical signals released from bacterial cells in respiration and other metabolic byproducts. High CO2 concentrations around fast-growing bacteria could be a signal that attracts the nematodes to this food source (Yu et al., 2015). It seems likely that nematodes are sensitive to CO2 concentration, as the higher CO2 level in the rhizosphere is believed to help plant-feeding nematodes locate roots, their preferred food source (Rasmann et al., 2012). The negative correlation between the PI and bacterial metabolite concentration suggests that alcohol, phenol and other substances that diffused through the agar substrate were detected and repulsed these bacterial-feeding nematodes. As far as we know, this is the first report linking bacterial metabolite concentrations to the feeding preference of nematodes. Finally, nematodes could exhibit a food preference according to their physiological requirements. Feeding behavior of C. elegans is characterized by directional movement to food sources. When C. elegans encounters a non-preferred food, it moves away rapidly, but when it finds a preferred food, it moves slowly around the food with frequent reversals and stops (Shtonda and Avery, 2006). In this

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**Table 2**

Bacterial traits (cell size, growth rate) and quality (water content, protein content, carbohydrate content and metabolites concentration) parameters measured in the nematode feeding preference experiment. Values are the mean ± standard error. Data within a column followed by the same letter are not significantly different (P < 0.05, LSD test). Bacterial species are abbreviated as: B.a, B. amyloliquefaciens; B.m, B. megaterium; V.p, V. paradoxus; P.f, P. fluorescens; E.c, E. coli OP50.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Cell size (μm²) (n = 40)</th>
<th>Growth rate (n = 6)</th>
<th>Water content (%) (n = 3)</th>
<th>Carbohydrate content (mg g⁻¹) (n = 3)</th>
<th>Protein content (mg g⁻¹) (n = 3)</th>
<th>Metabolites (%) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.a</td>
<td>5.1 ± 1.9 b 0.39 ± 0.0 b 66 ± 0.7 d 9.9 ± 0.6 a</td>
<td>1.9 ± 0.3 b</td>
<td>11.1 ± 0.2 b</td>
<td></td>
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</tr>
<tr>
<td>B.m</td>
<td>13.0 ± 3.7 a 0.28 ± 0.0 c 67.6 ± 0.2 c 2.4 ± 0.6 c</td>
<td>0.7 ± 0.0 d</td>
<td>7.9 ± 1.2 c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V.p</td>
<td>1.4 ± 0.6 d 0.51 ± 0.1 b 64.4 ± 0.3 e 9.7 ± 1.3 a</td>
<td>1.8 ± 0.6 b</td>
<td>16.0 ± 1.3 a</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P.f</td>
<td>1.5 ± 0.5 d 1.65 ± 0.1 a 78.8 ± 3.4 a 2.6 ± 0.2 c</td>
<td>1.2 ± 0.2 c</td>
<td>2.1 ± 0.3 d</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>E.c</td>
<td>2.2 ± 0.7 c 0.48 ± 0.1 b 74.3 ± 2.7 b 4.2 ± 0.2 b</td>
<td>2.6 ± 0.3 a</td>
<td>0.8 ± 0.2 e</td>
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**Fig. 2.** Nematode number observed in the bacterial zone from 8 to 48 h after beginning the nematode feeding preference experiment. Bacterial species (x-axis) are abbreviated as: B.a, B. amyloliquefaciens; B.m, B. megaterium; V.p, V. paradoxus; P.f, P. fluorescens; E.c, E. coli OP50.

**Fig. 3.** Principle component analysis (PCA) showing relationships among nematode feeding preference index (PI), bacterial traits (cell size, gram stain, growth rate) and quality (water content, carbohydrate content, protein content and metabolite concentration).
study, the diet choice developed with time (Fig. 2), suggesting that nematodes may move randomly to taste each of the five different bacteria in the dot-circles, until they encounter a preferred food that supplies the water and nutritive substances that meet their needs.

4.2. Trade-off between reproduction and lifespan as related to feeding preference

Increased reproduction is frequently associated with a shorter lifespan in vertebrates and invertebrates (Barnes et al., 2006;
Partridge et al., 2005), due to the consequences of limited resource availability on survival (Mukhopadhyay and Tissenbaum, 2007). As hypothesized, nematodes feeding on preferred bacteria had larger brood size. However, feeding preference did not shorten the nematode lifespan, because Acrobeloides consuming the preferred P. fluorescens had a moderate survival time and those feeding on the least-preferred food B. megaterium had the shortest lifespan. This phenomenon indicates that nematodes inhabiting a microenvironment with their favorite food could experience population growth due to more offspring during a longer lifespan. Within the soil foodweb, this outcome could yield more nematodes as food for higher trophic groups and accelerate the turnover of soil bacteria that are a preferred food source (Jarvis et al., 2007; Rønn et al., 2002). According to Walker et al. (2005), nematodes always decrease their reproduction to allocate more resources towards a longer lifespan when food resources are limited. However, our data do not support this assertion, possibly because the food resources are not limiting when non-preferred food is present, rather the food quality is less desirable to the nematode species.

We propose a post-hoc hypothesis to explain the trade-off between reproduction and lifespan in bacterial-feeding nematodes, assuming that energy and nutrition derived from food is allocated towards basal metabolism first, reproduction second and lifespan third. Considering that bacterial-feeding Acrobeloides is an r-strategist that produces a large number of eggs and has a short life cycle (Bongers and Bongers, 1998), we postulated that they would first reproduce and then allocate residual nutrients to prolong the lifespan. Acrobeloides consuming the moderately-preferred bacteria B. amyloliquefaciens had a brood size with 200 offspring on average and they lived for nearly 100 d, suggesting that this food source gave them the energy and nutrition to balance reproduction and survival. Similarly, Acrobeloides produced nearly 193 offspring, on average, when feeding on the non-preferred bacteria B. megaterium, meaning that they derived enough resources from this to meet their basic physiological needs and achieve reproduction, but they lacked the energy and nutrients to prolong their lifespan beyond 78 days. The trade-off between reproduction and lifespan is clear for Acrobeloides consuming other bacterial species, since these preferred food resources increased brood size to about 250–425 offspring, at the expense of survival since they only lived for 79–84 d (Fig. 4). Our results suggest that the bacterial-feeding Acrobeloides will produce a minimum brood size, regardless of the food supply, and that resources in excess of the minimum requirements will be allocated for reproduction before survival, thus it fits the classic definition of an r-strategist (Pianka, 1970). This trade-off strategy between reproduction and lifespan had important implications for the population dynamics and maintenance of nematode diversity, as well as other trophic interactions in soil foodwebs.

4.3. Implications of nematode feeding preference for the structure and functions of soil bacterial communities in a natural soil environment

As our study was conducted in a controlled laboratory environment (petri dishes), it is challenging to extrapolate these findings to natural soil environments. However we assert that findings from the petri dish reveal fundamental biological truths about nematode feeding preference that may be difficult to detect in complex soil environments where bacteria are distributed heterogeneously, affecting the nematodes’ ability to find, capture and ingest food resources. We present a conceptual model (Fig. S4) and short discussion about how the diet choice of bacterial-feeding nematodes regulates the structure and functions of soil bacterial communities in a natural soil environment, to advance the future research on this topic. Nematode’s selective grazing can modify bacterial populations and their community structure by reducing the number of older bacterial cells, thereby stimulating the growth and activity of new bacteria (Djigal et al., 2004; Fu et al., 2005). According to our results, nematodes prefer Pseudomonas (i.e., P. fluorescens) over Bacillus (i.e., B. amyloliquefaciens and B. megaterium) (Fig. 2), which may stimulate high growth and population turnover rates of Pseudomonas. Because P. fluorescens strains are dominant plant growth-promoting rhizobacteria of importance for many crops (Beneduzi et al., 2012), bacterial-feeding nematodes attracted to feed upon P. fluorescens in the rhizosphere may contribute indirectly to crop protection against abiotic and biotic stressors (Blanc et al., 2006).

5. Conclusions

We conclude that dietary choice of bacterial-feeding nematodes was related strongly to bacterial traits (growth rate) and chemical composition (water content, metabolite concentration), which explains why small-celled and gram-negative bacteria are often reported to be the preferred food of bacterial-feeding nematodes. It is

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**Fig. 6.** Relationships between nematode feeding preference index (PI), nematode reproduction (brood size) and lifespan (survival time) of the hermaphrodite Acrobeloides. Bacterial species are abbreviated as: B.a, B. amyloliquefaciens; B.m, B. megaterium; V.p, V. paradoxus; Pf, P. fluorescens; E.c, E. colI OP50.
likely that bacterial traits and chemical composition act as chemical signals for chemotaxis, which helps soil-dwelling nematodes to locate preferred food in natural environments. Feeding preference was influenced by the water content of bacteria, suggesting that ingested food is a source of water for bacterial-feeding nematodes. Our results also confirm that bacterial-feeding Acrobeloides is a classic r-strategist that will produce a minimum brood size, regardless of the food supply, and that additional energy and resources will be allocated for reproduction first and survival second.

We postulate that nematode dietary choices affect the size and activity of bacterial populations, with consequences for the structure and ecological functions of bacterial assemblages in soil-plant systems. Future work on the diet choice of bacterial-feeding nematodes is needed, and we suggest that rhizosphere-based microcosms could be a good representation of natural soil environments to determine how nematode feeding preference affects the structure and functions of soil bacterial communities in the context of rhizobacteria-nematode-plant interactions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2017.09.014.

References


