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Collembolans maintain a core microbiome responding to diverse soil ecosystems

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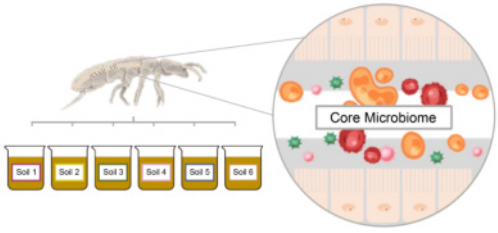
ABSTRACT

- The unique gut habitat led to a core intestinal microbiome in diverse soil ecosystems.
- The collembolan guts may help eliminate soil pathogens.
- Host-selection carried more weight on community assembly of gut microbiome.



Soil invertebrates are widely distributed in the ecosystem and are essential for soil ecological processes. Invertebrate gut microbiome plays an important role in host health and has been considered as a hidden microbial repository. However, little is known about how gut microbiome in soil invertebrates respond to diverse soil ecosystems. Based on a laboratory microcosm experiment, we characterized the assembling of microbiome of soil collembolans (*Folsomia candida*) from six representative regions of the soil ecosystem which they inhabit. Results showed that collembolan gut microbial communities differed significantly from their surrounding soil microbial communities. A dominant core gut microbiome was identified in gut habitat. Community analyses indicated that deterministic process dominated in the community assembly of collembolan gut microbiome. The results further demonstrate a dominant contribution of host selection in shaping gut microbiome. It is also worthy to mention that pathogens, such as common agricultural phytopathogenic fungi *Fusarium*, were involved in core microbiome, indicating that collembolans could act as vectors of pathogens. Our results unravelled the existence of gut core microbiome of collembolans in soil ecosystems and provided new insights for understanding the crucial role of gut microbiome of soil fauna in maintaining microbial biodiversity and stability of soil ecosystems.

Keywords gut microbiome, collembolan, core microbiome, community assembly

1 Introduction

Soil invertebrates are important contributors to soil ecosystems. Their gut microbiome was reported crucial in multiple ecological processes, including growth promotion, nutrient acquisition, energy harvest and stress tolerance (Claesson et al., 2012). For example, the hindguts of termites have been reported as microbial bioreactors in the metabolic processes and cellulose digestion (Otani et al., 2014; Brune, 2014). Moreover, researchers have demonstrated a range of antimicrobial activities in gut microbiome of soil invertebrates possibly relating to nutrition or defense against pathogens (Gibson et al., 2015; Bahrndorff et al., 2018; Agamennone et al., 2019). However, complex interactions among microorganisms have not been fully demonstrated and much less is known to disentangle factors that shape gut microbiome in soil invertebrates (Li et al., 2021).

The gut harbours diverse microorganisms comprised of bacteria, fungi, viruses and protists (David et al., 2016). The high community diversity remains ecological stability (Goyal, 2022). Recent research on the gut microbiome has mostly focused on the role of bacteria, while an increasing number of studies noticed the presence of other microorganisms such as fungi and the complex interactions among microbes. For example, yeast in the gut of fish or invertebrates can increase the diversity of microorganisms interacting with the host intestine (Raggi et al., 2014; Hannula et al., 2019). Additionally, fungi shared in the gut of honey bees are identified as pathogens though it is less diverse than bacteria (Callegari et al., 2021; Decker et al., 2022). Under natural conditions, bacteria form complex communities with fungi

together, and microbial ecology are dictated by environmental factors and microbial interactions with the host and among microbes (Ellegaard and Engel, 2019). Microorganisms form a complex gut niche together through competition and symbiosis (Otani et al., 2014; Callegari et al., 2021). As other microbiome components are not well characterized in invertebrate guts, more studies are needed to help clarify these complex interactions to deepen our understanding on ecological associations in gut microbiome.

The gut microbiome varies widely among individuals; it also varies depending on the behavior of individuals, as well as among locations and periods (Smith et al., 2015; Bourguignon et al., 2018; Zhu, 2022). Microbial community assembly mechanisms remain unclear in soil invertebrate guts, referring to the availability of microbial colonizers from the environment or the selection by host. So far, to determine the underlying mechanisms of microbial community assembly, some tools have been developed to broadly described processes as either random (stochastic) or non-random (deterministic) (Berg et al., 2016a), many of which rely on null models. A null modeling approach considers randomizing the original community structure and then compares microbiome properties between the original and randomized communities without the force of a specified assembly process (Keating et al., 2022). Previous work has applied this approach in microbial studies for some invertebrates such as nematodes (Berg et al., 2016a). However, much of the research up to now has been descriptive in microbial ecology, the assembly mechanisms in gut microbiome still need to clarify especially to model the nature community.

Identifying a core microbiome is also essential to unravelling the ecology of microbial community. An identifiable core microbiome has been defined as a set of shared genes or functional capabilities among habitats or most individuals (Turnbaugh et al., 2009). Some invertebrates such as caterpillars lack resident gut microorganisms (Hammer et al., 2017); others suggest a certain functional importance of gut microbes for their host, including honey bees (Dong et al., 2020; Almasri et al., 2022), bumblebees (Meeus et al., 2015), cockroaches (Tinker and Ottesen, 2016), mosquitoes (David et al., 2016), and mud crabs (Wei et al., 2019), leading to a stable and predictable gut microbiome. However, previous studies on host-associated microbiome have focused on variations of gut microbiome in different species or sites. Hence, laboratory tests for one species may help to identify specific functional groups in gut microbiome.

Collembolans are ubiquitous members of the soil invertebrates, and they are reported as omnivores feeding on litter, vegetation, lichens, pollen, and fungi (Leinaas and Fjellberg, 1985; Aptroot and Berg, 2004; Potapov et al., 2016, 2022). Soil-dwelling collembolans occupy various microhabitats (Ferlian et al., 2015; Malcicka et al., 2017) and serve as important prey for macropredators (McNabb et al., 2001; Coleman, 2013). The gut microbiome of this non-target soil fauna has recently received increasing attention from soil ecologists (Samuel et al., 2016; Wang et al., 2020, 2022). Previous studies have identified a dominant core microbiota in a fertilization experiment, and established that the soil microbial community has an important effect on the collembolan gut microbiota (Ding et al., 2019a; Xiang et al., 2019). Therefore, few studies focus on fungal components and interactions among microbial species. Although soil physiochemical characteristics including soil moisture, temperature, pH and nutrient status have been reported to be key factors affecting the growth and reproduction of collembolans (Smit and van Gestel, 1997; Bandow et al., 2014; Holmstrup et al., 2014; Pitombeira de Figueirêdo et al., 2020), few studies have characterized how these factors act on gut microbiome. Laboratory-cultured collembolans provide a powerful model for studying host-microbiome interactions because they permit the relative contributions of the environment and the host to shaping the gut microbiome to be characterized (Burns et al., 2017; Xiao et al., 2021). Here, we conducted a controlled microcosm experiment to test the effects of different soil ecosystems on collembolan gut microbiome. The parthenogenetic *Folsomia candida*, a model organism often used in ecotoxicology and ecological risk assessment (Fountain and Hopkin, 2005; Agamennone et al., 2015; Buch et al., 2016), was applied in this test due to its sensitivity. We hypothesized that (1) the unique habitat of gut would shape distinct microbiome from soil; (2) a core microbiome with indispensable bacteria and fungi would be identified in collembolan gut; and (3) the assembly pattern of gut microbial communities would differ from that of the soil microbial communities.

2 Materials and methods

2.1 Collembolan culture and age-synchronization

Folsomia candida was originally obtained from Aarhus University in Denmark and has been cultured in our laboratory for over 5 years. Individuals were cultured in Petri dishes (90 mm × 13 mm) on a moist layer containing activated charcoal, plaster of Paris (Usg Boral, China, made from a mixture of extremely fine calcined gypsum, wood fiber, and other additives), and ultrapure water in a mass ratio of 1:8:8 in a climate chamber in the dark at (20 ± 1)°C and 70% humidity. Dried baker's yeast (Angel Yeast Co., Ltd., China) and ultrapure water were added twice a week. According to the standardized methods of the Organization for Economic Co-operation and Development (OECD) and a previous study (Zhu et al., 2016), 10–12-day-old synchronized juveniles were exposed to different types of soil.

2.2 Experiment design

Test soil was collected from six provinces in China, and these soils varied substantially in their characteristics. Black soil, red soil and latosol soil were collected from Heilongjiang (HLJ), Jiangxi (JX), and Hainan (HAN), respectively. Fluvo-aquic soil, brown soil, and sierozem soil were collected from Henan (HN), Beijing (BJ), and Ningxia (NX), respectively. All soil was collected from the plow layer (5–20 cm depth) of farmland; the samples were then air-dried, homogenized, and passed through a 2 mm sieve. Detailed physio-chemical soil properties are summarized in Table S1.

According to the protocol of reproduction tests (OECD, 2016), soil samples with excessive amounts of yeast were equilibrated for one week at 50% of soil maximum water holding capacity (WHC). Twenty synchronized *F. candida* (10–12 d) individuals were introduced into a test vessel (5.5 cm diameter, 10 cm height) containing 60 g of spiked soil to conduct a standard 28-day test in the climate chamber. Ultrapure water was added to maintain the mass balance and WHC.

2.3 DNA extraction, PCR amplification and high-throughput sequencing

After 28 d of exposure, distilled water was supplied to each container to suspend the animals and approximately 15−20 adults in each replicate were collected for gut DNA extraction. The collembolans were first washed with 2% sodium hypochlorite solution for 10 s and then rinsed four times in phosphate-buffered saline (PBS) to eliminate surface microbial contamination. The guts dissected with sterile forceps in a sterile environment were placed in a 1.5 mL centrifuge tube containing 100 μL PBS. A total of 30 gut DNA samples (6 treatments × 5 replicates) were extracted. Gut DNA extraction was carried out using the DNeasy Blood and Tissue Kit (QIAGEN, Germany) performing the manufacturer's instructions. Meanwhile, 30 soil samples (6 treatments × 5 replicates) were collected. Soil DNA was extracted using a FastDNA Spin Kit for Soil (MP Bio Laboratories, USA). The concentration of the extracted DNA was measured using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, USA).

Because of the low concentration of DNA from gut samples, nested PCR was conducted to obtain sufficient material for sequencing (Berg et al., 2016a). For bacteria, we used primers 27f and 1492r primers to amplify the full-length 16S rRNA gene, followed by amplification of the 16S V4 region using the 515f primer and barcoded versions of 806r. For fungi, the ITS5-1737f and ITS4r primers were used to amplify the full-length ITS genes, and then ITS1f and ITS2r primers were used.

2.4 Bioinformatic and statistical analysis

Purified amplification products were sequenced using an Illumina Miseq platform (Meiji Biological Medicine Co., Ltd., Shanghai, China). The raw data were analyzed by QIIME2 (version 2021.11) (Bolyen et al., 2019) following the online instructions. After removing the adapter, primer, and low-quality sequences, DADA2 (Callahan et al., 2016) was used to obtain amplicon sequence variants (ASVs). The SILVA database (version 138) (Quast et al., 2012) was used to identify bacterial taxonomic assignment. ASVs belonging to the endosymbiont *Wolbachia* genus were excluded from the data set. Fungal taxa were identified by UNITE fungal ITS database (Abarenkov et al., 2010). Uneven sampling was accounted for by rarefying data to the minimum number of reads across all samples. Soil ASVs and gut ASVs were rarefied separately.

Differences in the abundances of ASVs in the soil and gut were determined using the Welch's *t*-test in R. Alpha-diversity was measured using community richness (the observed species). The data were presented as the mean values (n = 5) ± standard deviation. All the variables were tested for normality using Shapiro-Wilk test. Levene's test for homogeneity of variance was performed. Normally distributed data were analyzed with ANOVA while others with nonparametric Kruskal–Wallis test in the R package *nparcomp*; the threshold for the statistical significance of these tests was P = 0.05 (Konietschke et al., 2015). Principal coordinate analysis (PCoA) based on the Bray−Curtis distance matrix was used to characterize the differences in the structure of microbial communities. Alpha diversity, beta diversity and distance-based redundancy analysis (db-RDA) with fitted environmental vectors were calculated using the R package *vegan* (Oksanen et al., 2013). Adonis test was used to determine whether the composition of microbes differed among soil types and between soil and gut communities. One-way ANOVA was conducted to assess the effects of soil properties (pH, EC (electrical conductivity), OM (organic matter), TN (total nitrogen), TP (total phosphorus)) and soil alpha-diversity on the gut microbial community.

PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) (Douglas et al., 2020) was used to predict the functional composition of the metagenome using 16S rRNA data sets. Fungal ASV table was parsed against the FunGuild database (Nguyen et al., 2016) to assign putative life strategies.

Checkerboard scores (C-scores) were calculated using the R package *EcosimR* (Gotelli et al., 2015), and compared with a distribution of C-scores generated from 5000 permutations of the same data set (Stone and Roberts, 1990; Dormann et al., 2009). The C-score enables to test rules of community assembly with random species assortment as the null hypothesis. The values obtained were standardized

to allow comparisons among assemblages using the standardized effect size (SES) under the null model. The SES for the C-score was estimated as the difference between the observed index and the mean of the stimulated index over the standard deviation of the stimulated index (Crump et al., 2009). ASVs with a relative abundance of at least 0.1% were used to construct a bacterial and fungal co-occurrence network by package *edgeR* (Chen et al., 2016). The correlations between the bacterial and fungal microbiome in the collembolan gut were examined using Spearman coefficients in the *Hmisc* package in R platform. Correlations were considered statistically significant if the Spearman correlation coefficient was greater than 0.6 or less than 0.6, and if the Benjamini Hocchberg adjusted *P*-value was less than 0.05.

3 Results

3.1 Diversity of soil and gut microbiomes

Our results indicated that there were specific microbial ASVs in collembolan gut that consistently responded to different soil ecosystems. The alpha-diversity of bacterial and fungal communities was assessed in terms of ASV richness. The alpha-diversity of bacterial communities was higher in soil than gut ($P < 0.05$). Microbial alpha-diversity was highest in soil from BJ ($P < 0.05$) as measured by observed ASVs among the six treatments (Fig. S1a). No significant difference was observed in ASVs between soil bacterial communities from BJ and HN ($P > 0.05$) (Fig. S1a), which might be explained by the similarity in the soil at these two sites. Similar patterns of richness were observed in gut bacterial communities in BJ and HN ($P > 0.05$) (Fig. S1a). The richness of fungal communities in the gut samples was not lower than that of soil ($P < 0.05$). We observed no significant difference in fungal alpha diversity across all gut samples (all $P > 0.05$) (Fig. S1b).

PCoA results revealed pronounced separation in both bacterial and fungal communities between soil and gut ($P < 0.01$) (Fig. 1). Bacterial community structure of the collembolan gut was significantly affected by soil type, according to the first axis, which accounted for 62.83% of the total variability. However, fungal community structure of collembolan gut was not significantly affected by soil type ($P = 0.961$) (Fig. S2). These findings indicate that, despite various available environmental communities, gut mediates the assembly of a diverse bacterial community and a comparatively less diverse fungal community. Results of the PCoA based on the functions predicted by PICRSUSt2 and FUNGuild were consistent with the ASVs results (Fig. S3).

3.2 Composition of the soil and gut microbiome

A total of 16519 bacterial ASVs (from 883 genera) and 10 812 fungal ASVs (from 553 genera) were identified from all soil and gut samples, respectively. Prominent members of soil and gut bacteria included Actinobacteria, Gammaproteobacteria, Bacilli, and Alphaproteobacteria (Fig. 2A and 2B). The three most abundant genera in the gut samples were *Rhodococcus*, *Geobacillus* and *Bacilllus* (Fig. S4b). Ascomycota and Basidiomycota were the two most abundant fungal phyla detected in soil (Fig. 2C) and gut samples (Fig. 2D). *Hannaella*, *Cladosporium*, and *Alternaria* were the most abundant genera in all gut samples (Fig. S4d). The genera detected in soil samples differed from those detected in the gut samples, indicating that only specific fungi managed to colonize the gut from the soil.

To identify ASVs enriched in soil or the collembolan gut, we characterized differences in the abundances of dominant ASVs (with relative abundance > 0.1%) among soil and gut

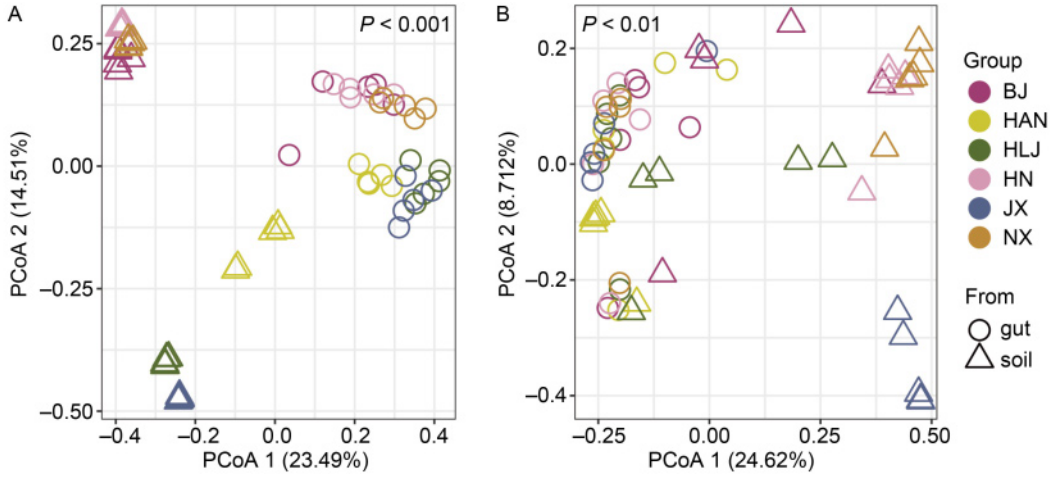


Fig. 1 The principal coordinate analysis (PCoA) of pairwise distance among bacterial (A) and fungal (B) communities between soil and gut samples based on the Bray−Curtis distance matrix. Adonis test was used to test whether microbial composition differed between soil and gut communities. BJ, Beijing; HAN, Hainan; HLJ, Heilongjiang; HN, Henan; JX, Jiangxi; NX, Ningxia.

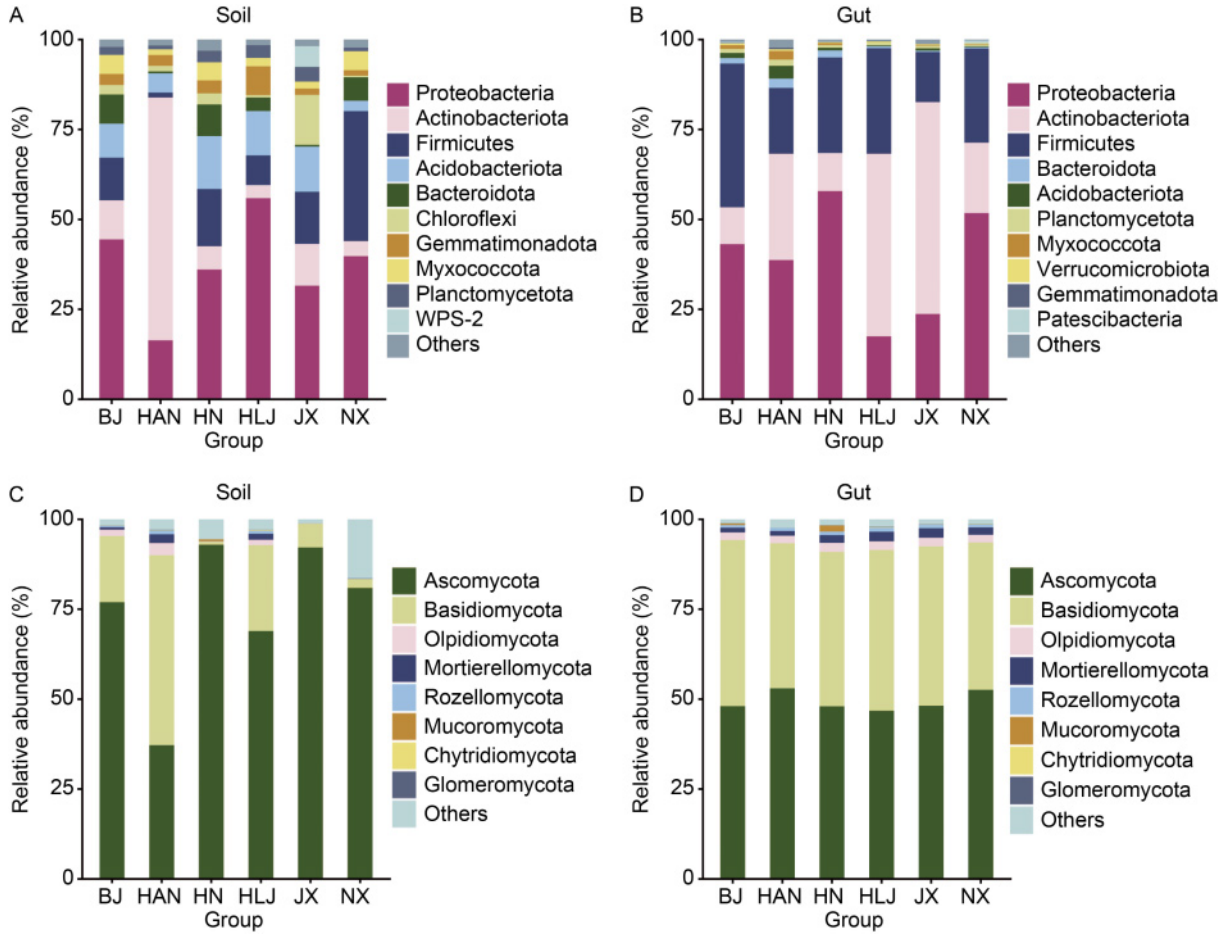


Fig. 2 Bar plot showing relative abundance of different taxa (phylum level) in soil and collembolan microbiome. The letters of BJ, HAN, HN, HLJ, JX and NX in horizontal axis represented soil and gut samples from Beijing, Hainan, Henan, Heilongjiang, Jiangxi and Ningxia, respectively.

samples. *Geobacillus* and *Pseudoxanthomonas* were significantly more abundant in gut samples than in soil samples ($P < 0.05$) (Fig. 3A). A total of 15 fungal genera including *Hannaella*, *Cladosporium*, *Alternaria*, *Fusarium*, and *Saitozyma,* were significantly more abundant in collembolan gut samples than in soil samples ($P < 0.05$) (Fig. 3B), and these were mostly within the phyla Basidiomycota and Ascomycota.

3.3 Core microbiome in the collembolan gut

We identified bacteria and fungi that were prominent in the collembolan guts responding to different soil ecosystems. Core bacteria or fungi were defined as the dominant ASVs (with relative abundance > 0.1%) shared among all samples within the six treatments as shown in the Venn diagrams (Fig. 4A and 4D). A total of 6 bacterial ASVs (Fig. 4A) were identified, and these accounted for 17.56%−28.56% of the total abundance of bacterial ASVs in gut samples (Fig. 4B). *Bacillus*, *Geobacillus*, *Lysobacter* and *Rhodococcus* were the four dominant genera (Fig. 4B). PICRUSt2 was used to investigate the potential functional mechanisms regulating the effect of soil on the bacterial community and fitness of collembolans (Fig. 4C). 280 pathways were predicted from 16S rRNA gene sequencing data for the gut bacterial ASVs. The main several pathways were classified at level 3 as metabolic pathways, biosynthesis of secondary metabolites, microbial metabolism in diverse environments, carbon metabolism, biosynthesis of amino acids, ribosome and ABC transporters. 44 fungal ASVs (Fig. 4D) were identified, counting for 51.50%–60.50% of the total abundance of fungal ASVs. *Hannaella*, *Cladosporium*, *Zygosporium*, *Saitozyma*, *Papiliotrema* and *Fusarium* were the four most dominant genera (Fig. 4E). The guild and trophic mode of fungal taxa were identified using FUNGuild. Notable trophic modes in the shared gut mycobionts were classified as pathotroph, saprotroph and symbiotroph. Some genera were classified as probable or possible plant pathogens, animal pathogens and parasites (Fig. 4F).

3.4 Environmental factors affecting community composition in collembolan guts

Db-RDA was used to determine the independent

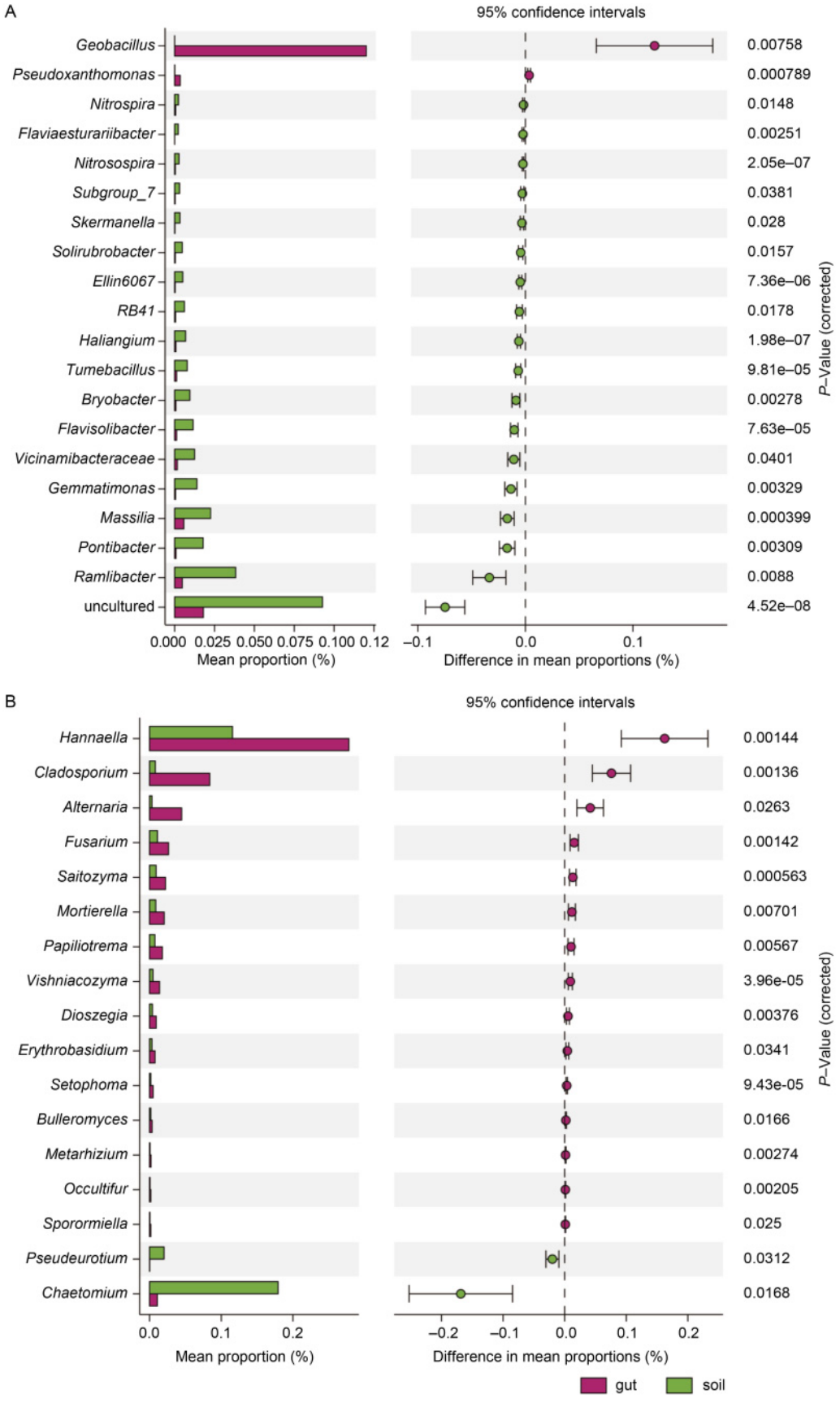


Fig. 3 The discrepant bacterial (A) and fungal (B) genera between soil and collembolan samples. Only significantly different (Student test, $P < 0.05$) genera were showed.

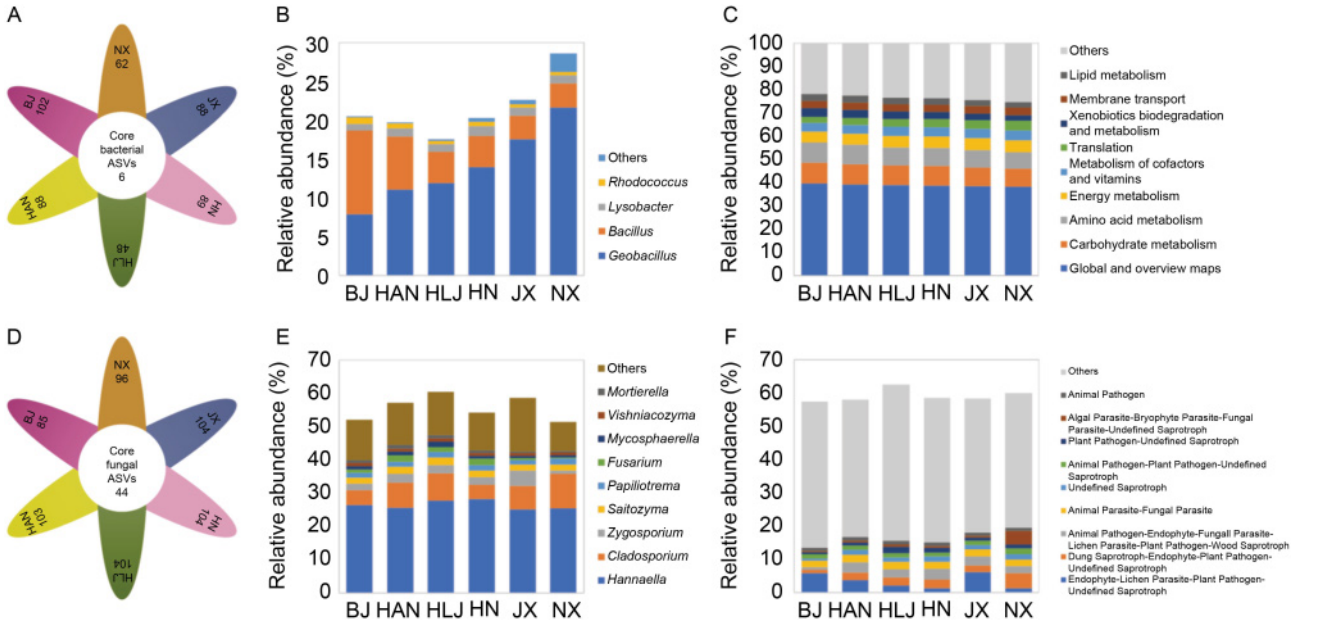


Fig. 4 Shared bacterial ASVs (A) and genera (B) in collembolan gut were displayed in flower plot, and their composition was represented by bar plot (C). Shared fungal ASVs (D) and genera (E) in collembolan gut were displayed in flower plot, and their composition was represented by bar plot (F). The letters of BJ, HAN, HN, HLJ, JX and NX in horizontal axis represented soil and gut samples from Beijing, Hainan, Henan, Heilongjiang, Jiangxi and Ningxia, respectively.

contributions of selected variables to the variation of community composition at the ASV level. Our results revealed the main driver of the composition of microbes in the collembolan gut. pH, EC, OM, TN, and TP significantly affected the bacterial composition in gut samples (Fig. S5a), and none of these properties significantly affected the fungal composition (Fig. S5b). The total variance in bacterial composition explained by the first axis and the second axis was 12.06% and 6.54%, respectively (Fig. S5a). Most of the variations remained unexplained.

3.5 Community analysis

C-scores were calculated for the gut microbiome, and the average number of cases of mutual exclusion in a set of communities was determined (Fig. 5). The null hypothesis was rejected on the basis of comparison with a distribution of scores generated through random permutations. Observed C-score ($C\text{-score}_{obs}$) values were higher than simulated C-score ($C\text{-score}_{sim}$) values, which reflected non-random co-occurrence patterns in both bacterial and fungal communities. SES were calculated to assess the strength of the effect of deterministic processes on the microbial assemblages. The SES was high for the gut microbiome, indicating the increased relative importance of deterministic processes in shaping gut community assemblages (Fig. 5). Interaction networks were further generated for the soil (Fig. 6A) and gut taxa (Fig. 6B). In the soil network, 24 bacterial ASVs and 34 fungal ASVs were presented; 229 edges among 263 (87.07%) showed positive links. In the gut network, 2 bacterial ASVs and 47 fungal ASVs were presented; 66 edges among 71 (92.96%) showed positive links. Generally, more fungal nodes were observed in gut than soil; positive interactions were dominant in both soil and gut networks. Additionally, comparisons of ASV pairs with negative interactions (representing mutual exclusion) in gut and soil revealed negligible to non-existing overlap between interactions in soil and gut (Fig. 6C).

4 Discussion

4.1 Collembolan gut harboured different microbiome from soil

Our study provided evidences that soil ecosystems may regulate the gut microbiome of collembolan *Folsomia candida*. A previous study suggested that diet can regulate the gut bacteria of *Folsomia candida* in a Petri dish microcosm (Xiang et al., 2019), and Actinobacteria have been reported to be the most common phyla. However, Proteobacteria prevailed in our soil and gut samples, which were also the most abundant phyla in another soil-dwelling collembolan (Pathiraja et al., 2022) and other soil-dwelling invertebrates (Berg et al., 2016b, 2016a). Ascomycota, Basidiomycota, and Olpidiomycota were the three most common fungal phyla in soil mycobiota, and they prevailed in gut samples as well. Besides the similarity in microbial composition, comparisons of gut microbiome with microbiome in their soil habitats revealed that the assembly of the gut microbiome is

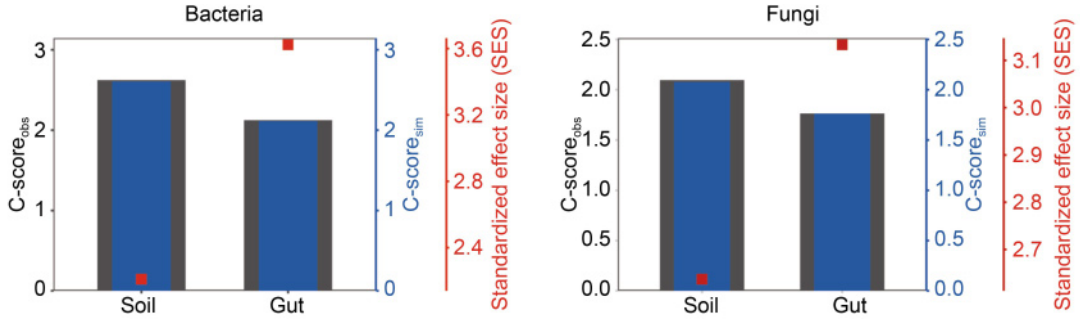


Fig. 5 Gut microbiome were assembled in a non-random fashion. C-score for cooccurrence patterns observed among soil and gut ASVs, compared with a score distribution generated from 5000 random permutations of the same data set. The values of observed C-score (C-score$_{obs}$) > simulated C-score (C-score$_{sim}$) indicate non-random co-occurrence patterns. Standardized effect sizes (SES) represented aggregation (< −2) or segregation (> 2) respectively.

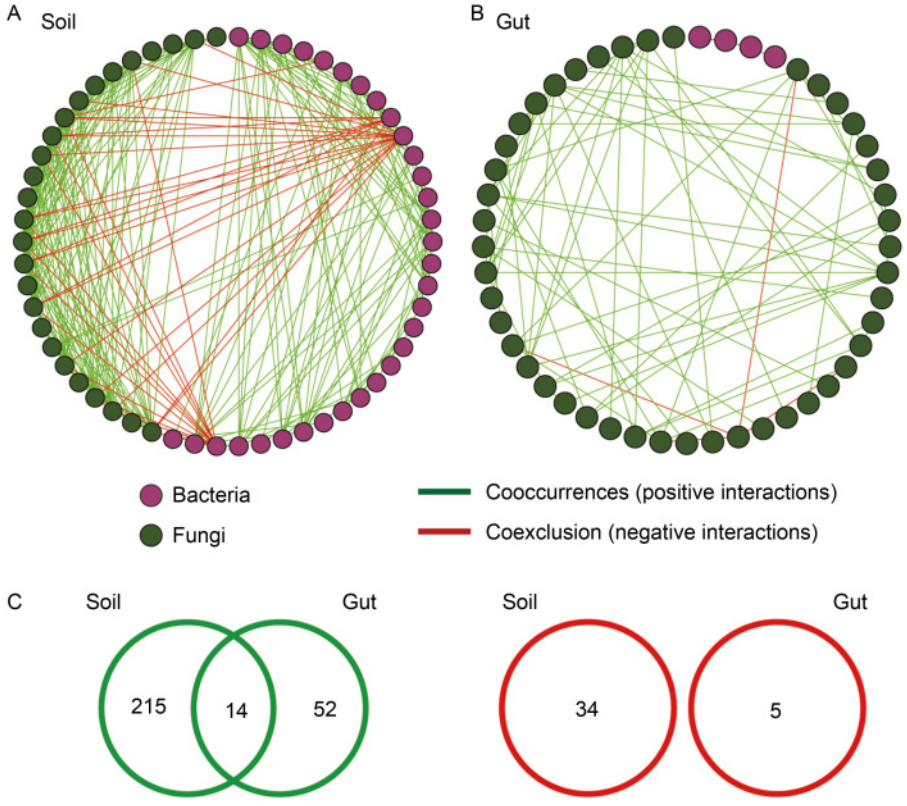


Fig. 6 Interaction networks between ASVs in soils (A) or guts (B) as designated with abundance data. Venn plots (C) showed positive and negative interactions between ASVs in soil and gut samples. Green lines or circles represented co-occurrences (positive interactions); red lines or circles represented co-exclusion (negative interactions).

essentially a non-neutral process, such that similar gut communities can be formed from different soil communities. These findings are supported by previous research (Ding et al., 2019a). In general, collembolan gut can obtain bacteria and fungi from soil ecosystems, and soil microbiome may have significant impact to gut microbiome.

Notably, our results revealed that there was a distinct difference between gut microbiome and soil microbiome. Community structure of the gut microbiome was different from that of the soil microbial community (Fig. 1); the predicted functions in the soil microbial community and the gut microbiome also differed (Fig. S3). Consistently, considerable differences in community structure and microbial functions between gut microbiome and surrounding microbiome have been noted in some species (Knapp et al., 2008; Xiang et al., 2019). Further, the results of this study showed that the collembolan gut microbiome comprised a diverse set of microbial taxa with diverse functions from the surrounding soil. On one hand, this may owe to the feeding preference for fungi, including saprotrophic and pathogenic fungi of collembolans (Broza et al., 2001; Jonas et al., 2007; Anslan et al., 2018). *Cladosporium* and *Alternaria* have been reported as preferred food for collembolans (Lavy and Verhoef, 1996; Mills and Sinha, 1971; Pfeffer et al., 2010;

Staaden et al., 2011). They may be located and digested by collembolans from diverse soil ecosystems. On the other hand, the special anaerobic gut environment may provide habitats for certain microorganisms, which may establish a unique niche within the collembolan gut. In the context of host–microbiome associations, prior studies have noted host as the environment imposing ecological filters, limiting the microbial strains that associate with it (Stagaman et al., 2017). Owing to the feeding preference and selective filtering, gut microbiome may originate from but are distinct from soil microbiome according to our results.

4.2 Core microbiome

Our analysis of the shared ASVs among gut samples in different soil ecosystems revealed a stable core microbiome in the collembolan intestine. Core microbiome is essential to unravelling the ecology of microbial consortia because these commonly occurring microorganisms that appear in most assemblages associated with a particular niche are likely critical to the function of gut communities (Shade and Handelsman, 2012). Function prediction results in this study supported the view as well that core microbiome plays key roles in energy acquisition and ensuring the welfare of the host. Specifically, the results of this study showed that the 6 core bacterial ASVs were identified potential pathways of material and energy metabolism, as well as translation and biodegradation. Genetic studies of strains in the genus *Rhodococcus* have revealed that they can degrade polyethylene, polychlorinated biphenyl, and polystyrene (Ohkuma, 2003; Yang et al., 2014, 2015). Gammaproteobacteria was the most abundant class of bacteria and some ASVs in Gammaproteobacteria shared in most of our collembolan gut samples. Gammaproteobacteria are facultatively anaerobic and can alter the gut environment by colonizing anaerobic bacteria and disturbing the stability of the microbial community (Moon et al., 2018; Bhattacharyya et al., 2021). In addition, Bacteroidetes might contribute to the decomposition process. Members of this phylum have been reported to be capable of breaking down polysaccharides (Bernardet et al., 2002). These bacterial results suggested the core bacteria may maintain the stability of gut communities and may have critically important functions. Moreover, our network results suggested that fungal taxa took more account and shared more connections in the network than bacterial taxa (Fig. 6B). Previous studies have characterized bacterial changes in the collembolan gut when exposed to microplastics (Zhu et al., 2018), liming (Ding et al., 2019b), and fertilization (Ding et al., 2019a), dedicatedly neglecting fungal shifts. Additionally, some fungal taxa were accumulated in collembolan guts, which was further supported by the high similarity in fungal community structure among samples (Fig. S2d). Hence, it could conceivably be hypothesized that certain gut microorganisms may supposed to be dwellers rather than transient passengers in our study. Specifically, Ascomycota and Basidiomycota are the two most common fungal phyla in collembolan gut, who prevail in neotropical termite guts (Vikram et al., 2021). *Hannaella* and *Cladosporium* were dominant in all samples. This result agrees with a previous work in silk worm gut (Chen et al., 2018) and many species of *Cladosporium* were reported to produce antibiotics that can inhibit the growth of *Bacillus subtilis* and *Escherichia coli* (Torres et al., 2017). In contrast to some other species, however, honey bee or caterpillars were reported lacking microbial residents in the gut (Hammer et al., 2017; Decker et al., 2022). This discrepancy could be attributed to feeding or development strategies and may further support soil invertebrates as repositories for maintaining microbial diversity.

As collembolan represent one of the most species-rich invertebrate radiations, a conspicuous question is whether dependence on microbes may benefit host welfare. It has been argued that while gut microbiome can provide novel ecological functions, they may also increase the risk of food-borne pathogen transmission (Zhu et al., 2019). Notably, soil-borne phytopathogenic fungi *Fusarium* were found in the core microbiome, which were probably located and acquired by collembolans from soil as described in previous studies (Wolfarth et al., 2013, 2017). Nearly all *Fusarium* species have been reported to produce mycotoxins, many of which are under international regulation, and these can be collected by collembolans (Wolfarth et al., 2017). Additional work is needed to clarify the relationships between the gut microbiome and pathogens. Our study indicated that *Folsomia candida* could help to modulate the plant-associated disease complex due to their feeding preference in presence of fungi. Susceptibility tests with germ-free collembolans may provide insights into on pathogen defense (Agamennone et al., 2018). A further mesocosm study including plant microbiome is therefore suggested. Accordingly, we further wonder if this feeding preference is a win-win situation for both collembolans and soil ecosystems.

4.3 Community analysis

Soil may influence the assembly of the collembolan gut microbial community in two ways: by colonizing microorganisms in collembolan gut and by changing host status with physio-chemical characters. In this study, soil varying in physio-chemical properties and microbial community structures resulted in the formation of different gut microbiome. pH, EC, OM, TN and TP were the main factors driving microbial changes in the gut. This indicated that gut microbiome may be susceptible to the soil environment. However, much of the variation in microbial communities remained

unexplained. Thus, to look into how microorganisms colonize in gut, we conducted community assembly and network analysis to explore the complex interactions between collembolans and their gut microbiome. Although C-score results suggested that both soil and gut microbial communities were driven by non-neutral assortment, SES results indicated that deterministic processes contributed more to the assembly of the gut microbiome (Fig. 5), which supported the role of host selection in community assembly. Our results also demonstrated that interactions between ASVs in the collembolan gut differed from those between the same taxa in the soil, suggesting that the host selection process may filter microorganisms. This is also supported by a study of temporal changes in microbial composition among developmental stages in collembolans (Pathiraja et al., 2022). Strong competitors with a flexible metabolism and rapid growth, such as Xanthomonadaceae, Burkholderiaceae, and Pseudomonadaceae, are more common in the collembolan gut than in soil. These microbes might increase the strength of competition and increase the magnitude of differences between soil and gut microbial communities (Compant et al., 2008), which further supported the vital role of host selection in community assembly.

5 Conclusion

This study set out to explore the assembling of gut microbiome of soil collembolans in different soil ecosystems. A diverse and stable core microbiome was maintained in the collembolan gut, and the host had a dominant role in the community assembly of the intestinal microbiome. Our results revealed the existence and the potential function of core gut microbiome and provide useful information for understanding the assembly mechanisms and host-microbiome interactions of soil-dwelling collembolans. The findings highlight the importance of gut microbiome of soil invertebrates as repositories of microbial biodiversity.

Data availability

The raw dust sequencing data are available at the NCBI Sequence Read Archive with the bioproject ID PRJNA915927. The authors declare that the other main data supporting the findings of this study are available within this Article and in the Supplementary materials files. Extra data supporting the findings of this study are available from the corresponding author upon reasonable request.

Code availability

Custom codes for all analyses are available from the corresponding authors upon request.

Conflict of interest

We declare no conflict of interest.

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Electronic supplementary material

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