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RESEARCH ARTICLE

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A dormant amoeba species can selectively sense and predate on different soil bacteria

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Abstract

1. Soil protists are the invisible majority of soil eukaryotes, which are essential but often forgotten parts of the soil ecosystem. They play key roles in microbial food webs by predating on other soil microbes. However, it is not clear how dormant soil protists sense, recognize and feed on diverse microbial prey.
2. In this study, we used a soil amoeba, *Dictyostelium discoideum*, to study selective discrimination and predation of 14 different bacteria. We found that discrimination and sensing of prey in *D. discoideum* started as early as resting spores. *Dictyostelium discoideum* had higher hatching rates, formed bigger amoeba plaques and preferred high nutritional value bacteria. The feeding speed of amoeba on various bacteria was constant and was not linked with sensing of prey or bacterial nutritional value. We also found that higher bacterial density decreased predation efficiency, and one species, *P. fluorescens*, induced a strong density-dependent inhibition of amoeba spore production.
3. In conclusion, we find that dormant *D. discoideum* can selectively sense and predate on different soil bacteria, a process that is likely mediated through active amoeba preference as well as bacterial inhibition. This study provides new insights into the role of protists in shaping soil bacterial communities, and future study needs to assess this in natural soil environments.

KEYWORDS

Amoeba, *Dictyostelium discoideum*, predation, protist, selective grazing, soil food web

1 | INTRODUCTION

Soil protists are a vital component of the terrestrial ecosystem, and they play an essential role in element cycling (Geisen et al., 2018), primary production (Seppey et al., 2017), absorption of nutrition (Kramer et al., 2016) and the soil food web (Xiong et al., 2018). Given the immense diversity of soil protists in the soil environment, it is surprising that only a small fraction of literature is focused on soil protists compared to bacteria, fungi and nematodes, and we are just starting to understand their diversity, functions and ecology (Geisen et al., 2017, 2018).

In soils, heterotrophic protists feed on bacteria, and they are the primary cause of bacterial mortality (Rosenberg et al., 2009; Saleem et al., 2013), which can have significant consequences for the bacterial community (Matz & Kjelleberg, 2005; Saleem et al., 2013; Shi et al., 2021). Bacteria have evolved different mechanisms to avoid predation by protists, such as escaping the internalization of protist feeding or surviving and replicating within protists after internalization (Balczun & Scheid, 2017; Matz & Kjelleberg, 2005; Paquet & Charette, 2016; Shi et al., 2021; Shu, Brock, et al., 2018). Therefore, the interactions between protists and bacteria are complicated and highly dynamic. It is generally assumed that protists can selectively affect the population dynamics of their bacterial prey (Gao et al., 2019; Geisen et al., 2018). However, the exact mechanisms of selective discrimination and predation between protists and bacteria are mostly unknown. Theoretically, protists should be able to sense and recognize their food bacteria and they should also need to distinguish and avoid pathogenic bacteria, both of which can have significant impacts on bacterial communities. However, these are hypotheses that need experimental testing (Geisen et al., 2017, 2018; Shi et al., 2021). Currently, the following aspects of soil protist–bacteria interactions remain unclear:

First, it is not clear whether soil protists can sense and discriminate against bacteria in their dormant stage. The dormant stage is a period in the amoeba's life cycle when growth, development and physical activity are temporarily stopped. Dormancy is a strategy that allows organisms to survive harsh environmental conditions, which is energetically not only costly but also evolutionarily adaptive (Bradley et al., 2019; Greening et al., 2019; Shoemaker & Lennon, 2018). There are large numbers of dormant protists in soil, ranging from 10^4 to 10^7 per gram of soil, and they will hatch under suitable environments (Adl & Coleman, 2005). Although several studies have investigated food preferences in several protists (Flues et al., 2017; Geisen et al., 2015; Glucksman et al., 2010; Massana et al., 2009; Pedersen et al., 2009, 2010, 2011; Rosenberg et al., 2009; Schulz-Bohm et al., 2017; Singh, 1941; Weekers et al., 1993; Winding et al., 2004), none of them were done in the dormant stage, which is essential because sensing of prey is the beginning of successful hunting and they need to feed soon after emerging from dormancy. Therefore, soil protists must be able to sense and recognize their food bacteria and avoid pathogens even when dormant.

Second, whether soil protists consume bacteria at different speeds is unknown. Feeding at different speeds would provide direct evidence that soil protists can affect the microbial community in a specific manner, in addition to selective feeding (Flues et al., 2017; Rosenberg et al., 2009). There is some evidence of distinct amoeba responses to different bacteria. For example, the social amoeba *Dictyostelium discoideum* had different gene expression profiles when exposed to different bacteria (Benghezal et al., 2006; Lamrabet et al., 2020). Another study found that a different set of genes was activated when *D. discoideum* fed on Gram-positive and Gram-negative bacteria (Nasser et al., 2013). However, feeding speed might also be determined by bacterial traits, such as motility, density and cell wall types, making it more complicated to assess feeding speed quantitatively.

Third, whether soil protists prefer bacteria with high nutritional values is unknown. High nutritional value of bacteria can be defined by greater production of amoeba spores after feeding on a given amount of bacteria but we currently know very little about the nutritional value of bacteria to soil protists. Limited evidence suggests that different bacterial species may have distinct nutritional values to an amoeba *Hartmannella hyalina* but only two bacterial species were tested in that study (Cutler & Crump, 1927). It is unclear whether soil protists can preferentially sense and feed on high nutritional value bacteria.

Finally, it is not clear whether some bacteria can inhibit the hatching and growth of soil protists or even kill them through density-dependent inhibition. Quorum sensing is a process that allows bacteria to produce compounds, such as virulence factors, based on cell density, which plays a crucial role in pathogen–host interactions (Dandekar et al., 2012; Papenfort & Bassler, 2016). However, we know little about the role of bacterial inhibition in soil protist–bacteria interactions. One study found that bacterial density affected amoeba growth but only one bacterial species was tested (DiSalvo et al., 2014), and more studies are needed.

The soil amoeba *D. discoideum* is an ideal model system to investigate sensing of prey, feeding efficiency and nutritional value in soil protists because it has a complex relationship with bacterial species (Brock et al., 2011, 2020; DiSalvo et al., 2015; Haselkorn et al., 2019; Shu, Brock, et al., 2018; Shu et al., 2020; Shu, Zhang, et al., 2018; Strassmann & Shu, 2017) and its life cycle can be precisely manipulated and measured. In a nutrient-rich environment, *D. discoideum* lives as independent haploid amoebae that feed on bacteria and reproduce by binary fission (Figure 1). When food is scarce, cAMP-mediated aggregation occurs, leading to the formation of multicellular slugs that move to a favourable location to develop into fruiting bodies, where 20% of the cells die to form a long thin stalk that supports a spherical structure called the sorus, whereas the remaining 80% ascend into the sorus and turn into spores (Figure 1). Total spore production is an ideal measure of amoeba fitness and bacterial nutritional value (Kessin, 2001). In addition, *D. discoideum* lives in forest soils where there are thousands of bacterial species. Therefore, it is informative to investigate whether and how *D. discoideum* sense, recognize and feed on diverse bacteria.

In this research, we aimed to systematically investigate selective discrimination and predation by mixing *D. discoideum* with diverse soil bacteria. Specifically, we wanted to test the following four hypotheses: First, we hypothesized that dormant protist cells (spores or cysts) could sense and discriminate against different soil bacteria.

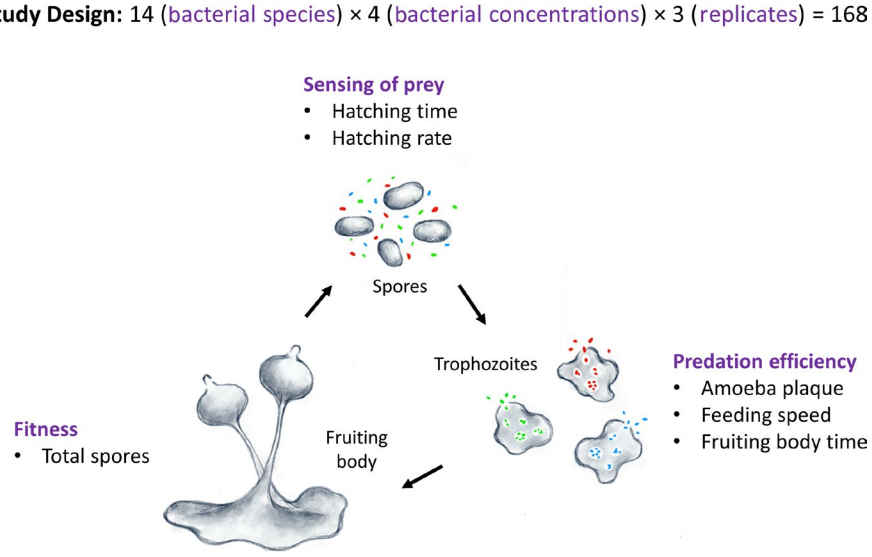

FIGURE 1 Scheme summarizing the life cycle of social amoeba *Dictyosteliumdiscoideum* as well as the design of this study

We tested this by measuring the hatching rate and hatching time of *D. discoideum* spores mixed with various soil bacteria (Figure 1). Second, we hypothesized that *D. discoideum* has different feeding speeds on diverse bacteria. We tested this by measuring the diameter of amoeba plaques (small circles on the plate when amoebae feed on the bacterial lawn; Farinholt et al., 2019), representing the speed of *D. discoideum* predation (Figure 1). We also recorded the fruiting body time (the emergence of the first fruiting body), which is also an indicator of feeding speed. Third, we hypothesized that *D. discoideum* prefers bacteria with high nutritional values. We used total spore production as a measure of amoeba fitness and bacterial nutritional value, and we explored whether there are any correlations among bacterial sensing, feeding efficiency and nutritional value (Figure 1). Finally, we hypothesized that bacterial density could affect amoeba predation through density-dependent inhibition in different soil bacterial species. We used four different bacterial densities to test the inhibition effect of 14 different bacterial species on amoeba predation and growth. We focused on species-level variations, as well as Gram-stain type and bacterial motility because existing evidence suggests that cell wall types and energy expenditure may impact protist–bacterium interactions (Matz & Jurgens, 2005; Nasser et al., 2013; Rashidi & Ostrowski, 2019).

2 | MATERIALS AND METHODS

For all assays, bacterial density was measured with a spectrophotometer at a wavelength of 600 nm (Myers et al., 2013), and this measurement will be referred to as optical density (OD) throughout.

2.1 | *Dictyostelium discoideum* clone, media and culture conditions

This study used a wild soil amoeba *D. discoideum* QS9 for all experiments (Brock et al., 2011). We grew *D. discoideum* from frozen spores on SM/5 agar plates (2 g glucose, 2 g BactoPeptone [Oxoid], 2 g yeast extract [Oxoid], 0.2 g $MgCl_2$, 1.9 g KH_2PO_4, 1 g K_2HPO_4 and 15 g agar per litre), in which we mixed 2×10^5 *D. discoideum* spores with 200 μl (OD 1.5) of food bacteria *Klebsiella pneumoniae* and cultured them at 21°C. *D. discoideum* spores were harvested 5 days after plating and were used for all subsequent experiments and analyses.

2.2 | Soil bacteria and culture conditions

We used 14 phylogenetically diverse soil bacteria that differed in their functions (biogeochemical cycle, bioremediation, pathogen, biocontrol and others), Gram-stain types (seven Gram-negative and seven Gram-positive bacteria) and motility (10 motile and 4 non-motile; Table 1). For all assays, we grew bacteria on SM/5 plates at room temperature (26 ± 1°C), collected and diluted them to a final OD of 2.0, 1.5, 0.5 and 0.1 in KK2 buffer (2.2 g KH_2PO_4 and 0.7 g K_2HPO_4 per litre).

2.3 | Experimental setup

We set up an experiment to investigate whether *D. discoideum* amoeba could discriminate diverse soil bacteria. We quantified the outcomes of their interactions by measuring hatching time, hatching rate, plaque diameter and total spore numbers of *D. discoideum* amoeba (Figure 1). To set up the experiment, we spread 100 *D. discoideum* spores with 200 μl of bacterial suspensions of different OD (2.0, 1.5, 0.5 and 0.1) on non-nutrient agar plates with Page's Amoeba Saline (NNA-PAS plates; 0.142 g Na_2HPO_4, 0.136 g KH_2PO_4, 4 mg $MgSO_4.7H_2O$, 4 mg $CaCl_2.2H_2O$, 0.12 g NaCl and 15 g agar per litre; Thomas et al., 2006) and incubated them at 21°C. We monitored each plate every 12 hr up to 180 hr.

We used NNA-PAS plates to eliminate variability due to bacterial growth, and each plate started with the same number of

TABLE 1 Soil bacteria used in this study. 14 phylogenetically diverse soil bacteria that differed in Gram-stain types (seven Gram-negative and seven Gram-positive bacteria) and motility (10 motile and 4 non-motile) were used in this study. These bacteria cover a wide range of functions, including biogeochemical cycle, bioremediation, pathogen, biocontrol and others

Gram stain	Species	Clones	Functional group	Motility	Origin
Gram-negative	*Paracoccus denitrificans*	DSM413	Nitrogen cycle	Non-motile	Guangdong Microbial Culture Collection Center (GDMCC)
	Sphingobium hydrophobicum	C1	Bioremediation	Non-motile	Guangdong Microbial Culture Collection Center (GDMCC)
	Klebsiella pneumoniae	Dictybase	Nitrogen cycle and Opportunistic pathogen	Non-motile	Dicty Stock Center
	Xanthomonas campestris	ATCC33913	Plant pathogen	Motile	ATCC
	Pseudomonas fluorescens	Pf-5	Biocontrol	Motile	ATCC
	Hyphomicrobium denitrificans	X	Nitrogen cycle	Motile	Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)
	Shewanella decolorationis	S12	Bioremediation	Motile	Guangdong Microbial Culture Collection Center (GDMCC)
Gram-positive	*Corynebacterium casei*	DSM44701	Others	Non-motile	Guangdong Microbial Culture Collection Center (GDMCC)
	Oerskovia turbata	ATCC25835	Opportunistic human pathogen	Motile	Guangdong Microbial Culture Collection Center (GDMCC)
	Bacillus thuringiensis	LCK10	Biological pesticide	Motile	Isolated from our lab
	Lysinibacillus varians	GY32	Bioremediation	Motile	Guangdong Microbial Culture Collection Center (GDMCC)
	Paenibacillus polymyxa	ATCC842	Nitrogen cycle	Motile	ATCC
	Brevibacillus laterosporus	AMC 797	Invertebrate pathogen	Motile	Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)
	Bacillus pumilus	272	Others	Motile	Guangdong Microbial Culture Collection Center (GDMCC)

D. discoideum spores and bacteria. To test how bacterial density affects *D. discoideum's* predation, we used four different bacterial concentrations (OD 2.0, 1.5, 0.5 and 0.1). Three replicates were established for each treatment, resulting in 168 experimental units (14 bacteria × 4 OD levels × 3 replicates).

2.3.1 | Hatching time and rate

When *D. discoideum* hatch and feed on a plate with a bacterial lawn, they will form small circles on the plate called amoeba plaques (Figure S1). We used hatching time (the appearance of the first amoeba plaque) and hatching rate (number of plaques/100 spores) to represent how resting *D. discoideum* spores sense and respond to different soil bacteria at each density level. After plating, we examined the plate under a microscope every 12 hr to record the hatching time and the hatching rate for each plate (Figure S1).

2.3.2 | Predation speed and fitness measurement

We used plaque diameter as one of two measures of the speed of *D. discoideum* predation. We measured the diameters of random plaques on each plate every 12 hr for 180 hr in total. At least nine plaques were measured for each plate. If there were nine plaques or fewer, all of them were recorded. When bacteria are consumed, *D. discoideum* enters the aggregation stage and form a fruiting body. We recorded the time of the appearance of the first fruiting body as a second measure of predation speed.

After consuming all the bacterial food around, *D. discoideum* enters into a multicellular stage and form fruiting bodies with spores sitting on top, which gives us an excellent system to evaluate the nutritional value of each bacterium (DiSalvo et al., 2014). We measured total *D. discoideum* spore production at the end of the experiment (after 180 hr). To harvest spores from each plate, we flooded the plate with 2 ml KK2 + 0.1% NP-40 and collected the spores into a 2 ml falcon tube. We counted spores on a haemocytometer using a light microscope.

2.4 | Statistical analyses

We used one-way ANOVA to analyse the differences among the 14 bacterial species for *D. discoideum* hatching time, hatching rate (the count of plaques for each plate), plaque size, feeding speed, fruiting body time and total spore production (spore counts). We analysed the influence of Gram stain (two levels: Gram-negative and Gram-positive), bacterial motility (two levels: motile and non-motile), bacterial density (OD; four levels: 0.1, 0.5, 1.5 and 2.0) and their interactions on hatching time, hatching rate, fruiting body time and total spore production data using generalized linear models with the Poisson distribution and on plaque size and feeding speed using general linear models. Pairwise comparisons within factors (Gram stain, motility and OD) were conducted using Tukey's post-hoc tests. Finally, we used Spearman's correlations to analyse the relationships among hatching time, hatching rate, fruiting body time, plaque sizes, bacterial density, feeding speed and total spore production, applying Holm's correction to account for multiple comparisons.

3 | RESULTS

3.1 | Hatching time: *Dictyostelium discoideum* hatched earlier on non-motile bacteria

Overall, *D. discoideum* showed significant variation in hatching time when mixed with different bacteria (ANOVA, $p < 0.001$, Figure 2A). *Corynebacterium casei* (42 ± 3 hr; mean ± SE) induced the shortest hatching time, whereas *Paenibacillus polymyxa* (113 ± 2 hr) induced the longest hatching time (Figure 2A). One bacterium, a Gram-positive bacillus called *Oerskovia turbata*, induced no hatching in *D. discoideum* (Figure 2A).

We also explored whether Gram-stain types or bacterial motility affected the hatching time of amoebae. No significant difference was observed between Gram-positive and Gram-negative bacteria (Table S1; Figure 2B), and this pattern was consistent at all OD. However, bacterial motility had a significant effect on the hatching time of amoebae (Table S1; Figure 2B). *D. discoideum* hatched earlier when mixed with non-motile bacteria (46 ± 3 hr) compared to motile bacteria (68 ± 2 hr). Non-motile bacteria had shorter hatching times than motile bacteria at higher bacteria densities, including OD 0.5 (47 ± 7 hr vs. 69 ± 4 hr, $p = 0.020$), OD 1.5 (45 ± 7 hr vs. 68 ± 4 hr, $p = 0.011$) and OD 2.0 (43 ± 7 hr vs. 68 ± 4 hr, $p = 0.005$). At the lowest OD of 0.1, no significant difference in hatching times was observed between non-motile and motile bacteria (50 ± 7 hr vs. 66 ± 4 hr, $p = 0.121$, Figure 2B). Taken together, these results suggest that *D. discoideum* hatched earlier on non-motile bacteria, and amoeba spores can thus effectively sense and respond to their bacterial prey in the environment.

3.2 | Hatching rate: *Dictyostelium discoideum* had higher hatching rates on Gram-negative bacteria

Dictyostelium discoideum had different hatching rates among the 14 different bacteria (ANOVA, $p < 0.001$, Figure 3A). *Dictyostelium discoideum* had the lowest hatching rate when mixed with *Oerskovia turbata* (0%) and *P. polymyxa* (1.2 ± 0.8%), and the highest hatching rate when mixed with *Xanthomonas campestris* pv. *campestris* (39.8 ± 0.85%; Figure 3A).

Dictyostelium discoideum had higher hatching rates when mixed with Gram-negative bacteria compared to Gram-positive bacteria at higher bacterial densities, including OD 0.5 (22.7 ± 11.5% vs. 11.2 ± 12.7%, $p = 0.003$), OD 1.5 (30.4 ± 15.4% vs. 11.5 ± 10.9%, $p < 0.001$) and OD 2.0 (33.2 ± 21.4% vs. 10.6 ± 10.5%, $p < 0.001$). At the lowest OD of 0.1, we found no significant difference in hatching rates (16.7 ± 9.4% vs. 16.1 ± 17%, $p = 0.879$, Figure 3B).

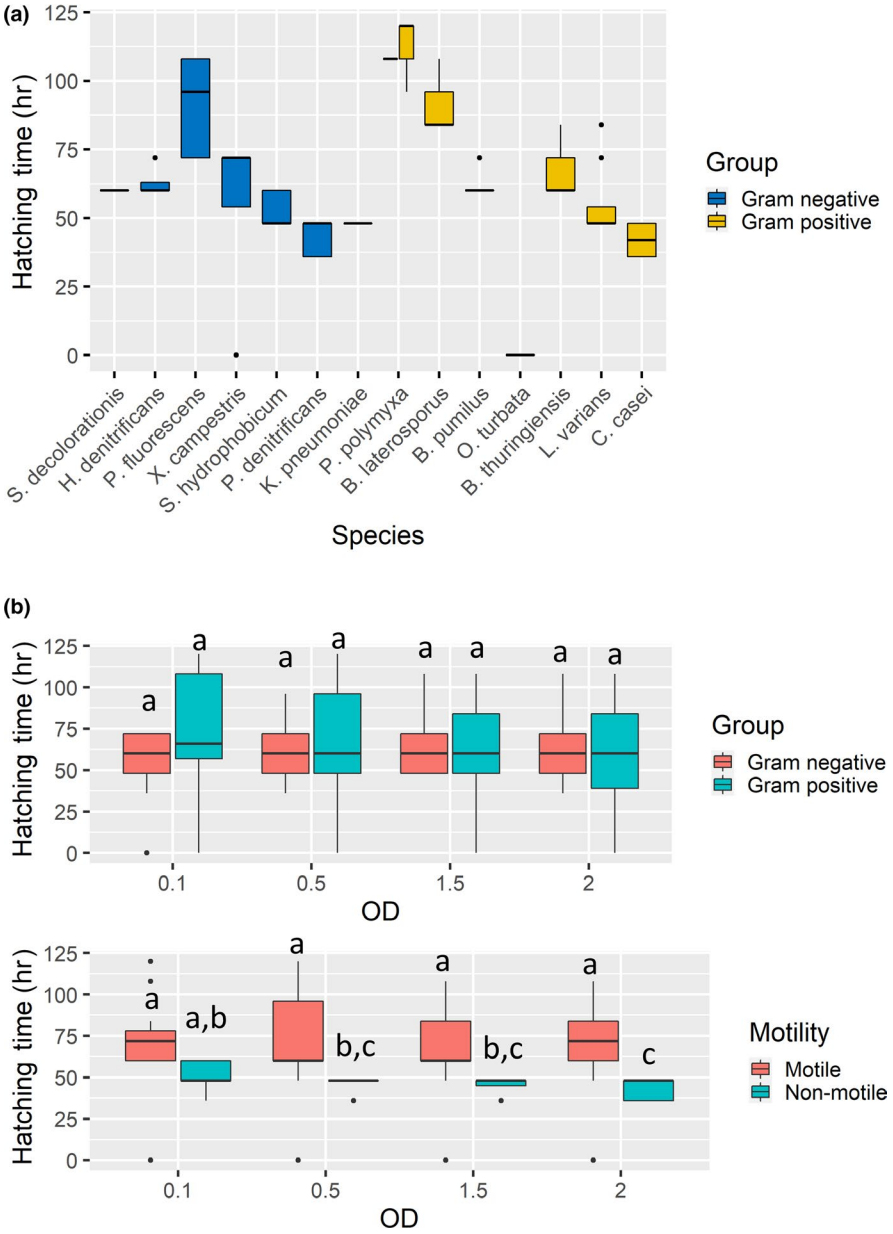

FIGURE 2 Hatching time of *Dictyosteliumdiscoideum* on different bacteria. (A) By species; (B) By Gram-stain types (Gram-positive: $n = 7$; Gram-negative: $n = 7$) and bacterial motility (motile: $n = 10$; non-motile: $n = 4$). Boxplots represent the interquartile range (25% and 75%) and whiskers represent the minimum or maximum values. A black line within the box marks the median. Different lowercase letters represent significant differences between factor levels, based on Tukey's HSD test ($p < 0.05$)

Non-motile bacteria induced a higher hatching rate than motile bacteria only at the lowest bacterial density (OD 0.1, 24.1 ± 17.6% vs. 13.4 ± 10.2%, $p = 0.011$, Figure 3B), and there were no significant differences at higher bacterial densities (OD 0.5, 1.5 and 2.0). Hence, *D. discoideum* had higher hatching rates on Gram-negative bacteria, but early hatching was not linked to a higher hatching rate.

3.3 | Predation efficiency: *Dictyostelium discoideum* consumed different bacteria with distinct patterns but at a constant speed

Using the diameter of amoeba plaques as a measure of the speed of *D. discoideum* predation, we found that consumption patterns of *D. discoideum* differed among bacteria (Figure 4). The earliest amoeba plaques appeared at 36 hr (*P. denitrificans* and *C. casei*) while others did not emerge until 120 hr (*P. polymyxa*; Figure 4). The maximal plaque sizes also varied significantly across different bacteria (ANOVA, $p < 0.001$, Figure S2). *Dictyostelium discoideum* formed the largest plaques on *K. pneumoniae* (1,186 ± 314 mm) while *O. turbata* (0 mm), *X. campestris* pv. *campestris* (211 ± 77 mm) and *P. polymyxa* (373 ± 161 mm) had the smallest plaque sizes (Figure S2). Thus, the predation efficiency of different bacteria was not related to *D. discoideum* hatching rate.

Bacterial density also affected the feeding of *D. discoideum*. On average, bigger plaques were created at a bacterial density of OD 0.1 compared to other bacterial densities, and there were no significant differences in plaque size among the higher bacterial densities (OD 0.5, 1.5 and 2; Figure S3). However, while this pattern was consistent for some species (*B. thuringiensis*, *K. pneumoniae* and *L. varians*), other bacteria, such as *C. casei* and *S. decolorationis*, exhibited an opposite pattern in which plaque size was greatest at high OD levels (Figure S4).

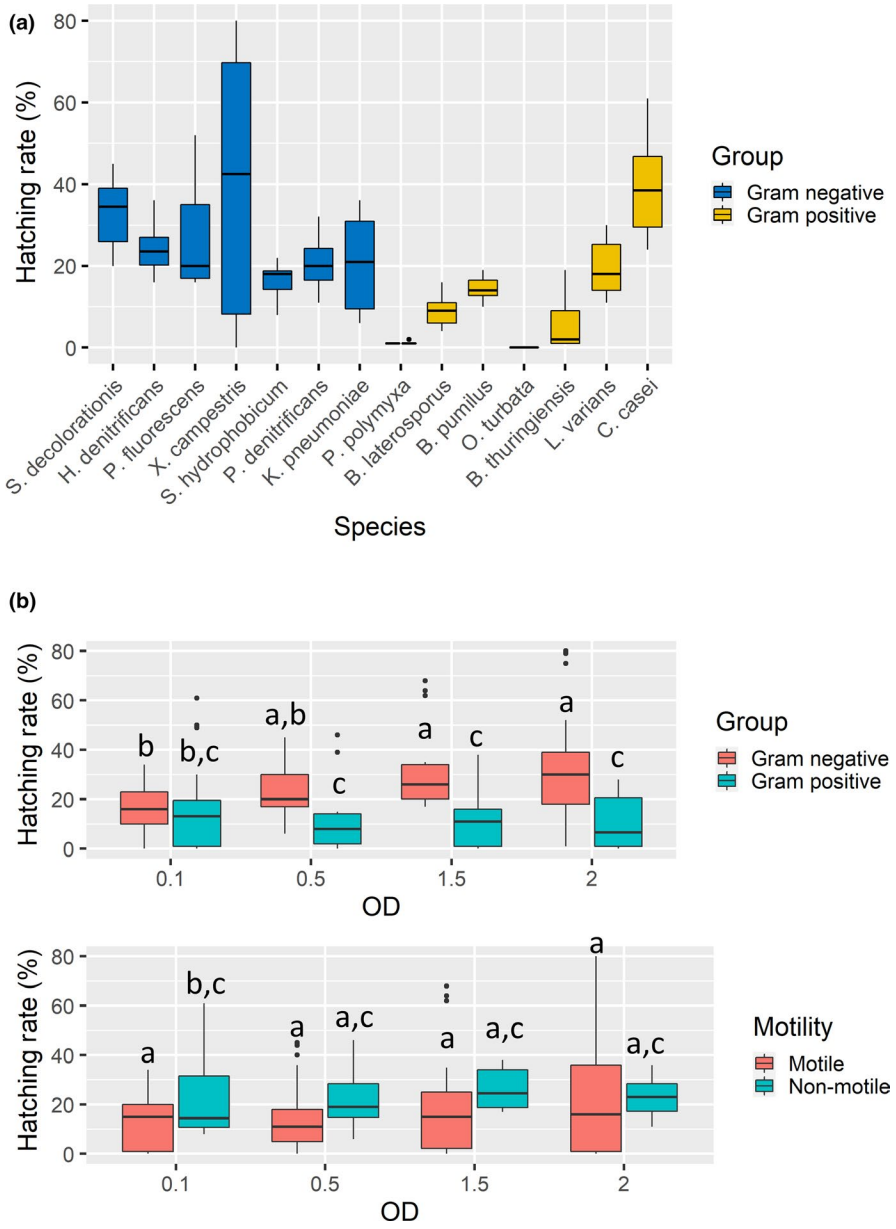

FIGURE 3 Hatching rate of *Dictyosteliumdiscoideum* on different bacteria. (A) By species; (B) By Gram-stain types (Gram-positive: $n = 7$; Gram-negative: $n = 7$) and bacterial motility (motile: $n = 10$; non-motile: $n = 4$). Boxplots represent the interquartile range (25% and 75%) and whiskers represent the minimum or maximum values. A black line within the box marks the median. Different lowercase letters represent significant differences between treatments based on Tukey's HSD test ($p < 0.05$)

Finally, overall feeding speed did not differ among the 14 bacteria (ANOVA, $p = 0.067$, Figure 5A) and neither Gram-stain type nor bacterial motility significantly affected feeding speed (Table S2; Figure 5B), indicating that feeding speed is not determined by bacterial motility or cell walls.

3.4 | Fitness: *Dictyostelium discoideum* produced more spores on Gram-negative and non-motile bacteria

Dictyostelium discoideum produced significantly different amounts of spores after feeding on different bacteria (ANOVA, $p < 0.001$, Figure 6A). Feeding on *K. pneumoniae* (5,717,083 ± 716,052) produced the highest amount of spores while feeding on *O. turbata* (0) and *P. polymyxa* (147,666 ± 640,456) yielded the lowest (Figure 6A), which indicates that *K. pneumoniae* has nearly 40 times more nutritional value than *P. polymyxa* to *D. discoideum*. Among the 14 bacterial species, *B. laterosporus*, *O. turbata* and *P. polymyxa* barely supported any spore production, whereas *B. thuringiensis* and *L. varians* only poorly supported spore production (Figure S5). We also compared the effect of bacterial densities on *D. discoideum* spore production (Figure S5). Spore production generally increased with the increasing densities of most bacteria (Figure S5). However, there was a strong density-dependent inhibition of spore production with *P. fluorescens* at OD 2.0 compared to OD 1.5.

We also explored whether Gram-stain types or bacterial motility affected total spore production and found that both of them had significant effects on spore production (Table S1; Figure 6B). *D. discoideum* produced more spores when mixed with Gram-negative bacteria (4,143,625 ± 159,444) compared to Gram-positive bacteria (1,731,641 ± 229,985). This pattern was consistent

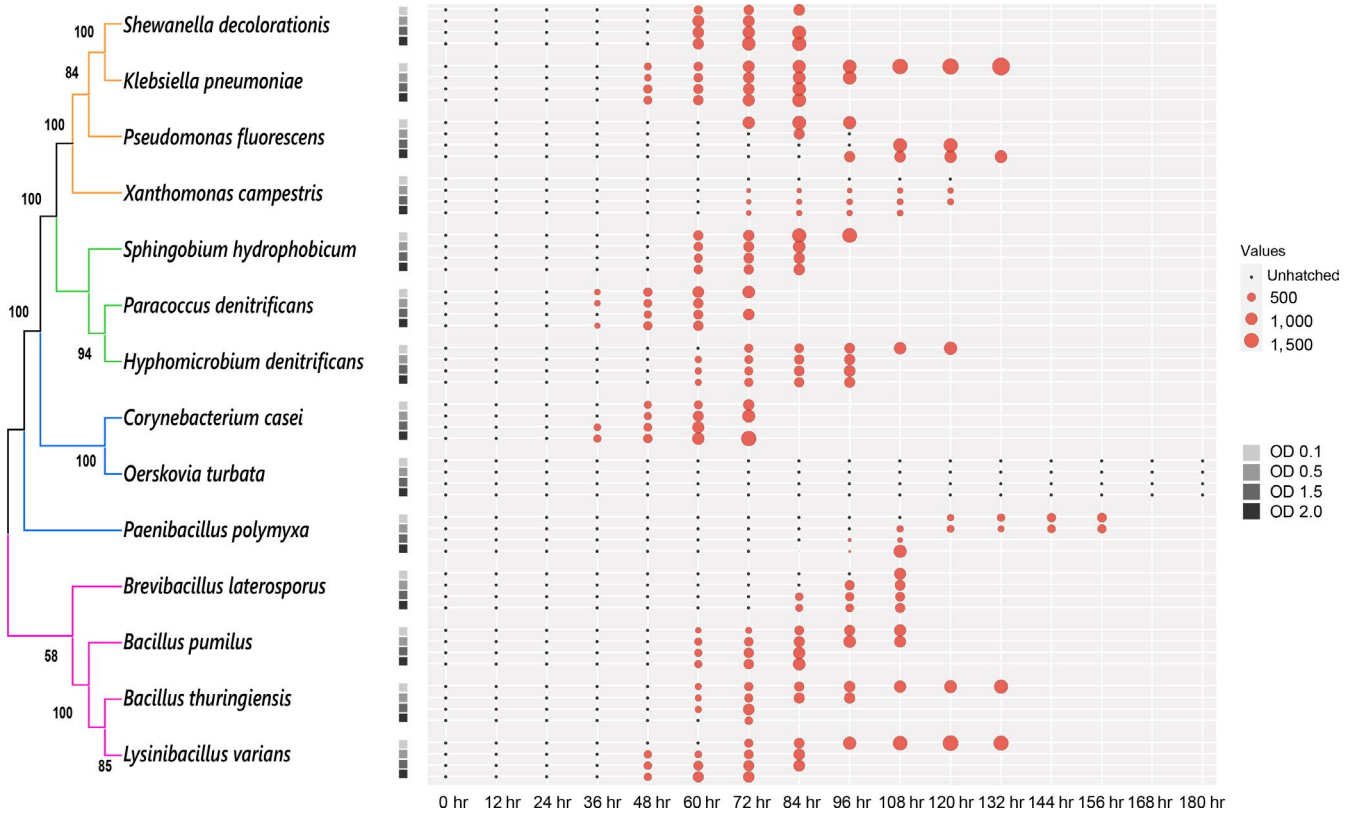

FIGURE 4 Heatmap showing the development of *Dictyosteliumdiscoideum* plaques on different bacteria. The maximum-likelihood phylogenetic tree of bacterial 16S rRNA gene sequences was constructed using MEGA6. Bootstrap support values were calculated from 1,000 replicates, and values ≥50 were shown in the phylogenetic tree

at all bacterial densities, including OD 0.1 (756,700 ± 414,783 vs. 405,625 ± 439,380, $p = 0.005$), OD 0.5 (1,617,857 ± 804,408 vs. 418,645 ± 364,654, $p < 0.001$), OD 1.5 (6,286,785 ± 2,759,864 vs. 1,415,250 ± 1,099,998, $p < 0.001$) and OD 2.0 (7,259,673 ± 3,574,904 vs. 2,005,795 ± 1,903,354, $p < 0.001$, Figure 6B).

Bacterial motility also had a significant effect on the spore production of amoebae. *Dictyostelium discoideum* produced more spores when mixed with non-motile bacteria (4,391,666 ± 260,783) compared to motile bacteria (1,841,216 ± 158,488). Non-motile bacteria produced more spores than motile bacteria at all bacterial densities, including OD 0.1 (872,916 ± 495,687 vs. 459,568 ± 311,431, $p = 0.002$), OD 0.5 (1,589,791 ± 529,912 vs. 766,355 ± 319,696, $p = 0.003$), OD 1.5 (6,168,958 ± 529,912 vs. 2,944,462 ± 317,016, $p = 0.001$) and OD 2.0 (8,935,000 ± 529,912 vs. 3,194,480 ± 319,696, $p < 0.001$). These results suggest that soil bacteria differ in their nutritional value, and *D. discoideum* produced more spores on Gram-negative and non-motile bacteria.

3.5 | Hatching rate, bacterial density and plaque sizes are related to spore production

We explore the relationships among sensing of prey, feeding efficiency and nutritional value in *D. discoideum*. We found that the hatching rate, OD and plaque sizes were positively related to final spore production (Figure 7), which suggests that *D. discoideum* prefer bacteria with high nutritional value because they hatched earlier and formed bigger plaques when mixed with high nutritional value bacteria. Surprisingly, hatching time and feeding speed were not related to final spore production, indicating that early hatching or faster intracellular killing is not linked to bacterial nutritional value. Finally, bacterial density was positively related to spore production but not to any other factors (Figure 7).

4 | DISCUSSION

4.1 | Discrimination and sensing of prey start in the dormant stage

Our results show that *D. discoideum* had a higher hatching rate on Gram-negative bacteria and hatched earlier on non-motile bacteria, but they did not hatch when mixed with one of three potential pathogens (*O. turbata*), which provides robust evidence that dormant soil protists can sense and discriminate their soil bacterial prey. Dormancy is a strategy that allows organisms to survive harsh environmental conditions under reduced energy cost, which is evolutionarily adaptive (Shoemaker & Lennon, 2018). Although the energetic cost of dormancy in *D. discoideum* is unknown, studies from other microbial systems suggest that dormant microbes still require

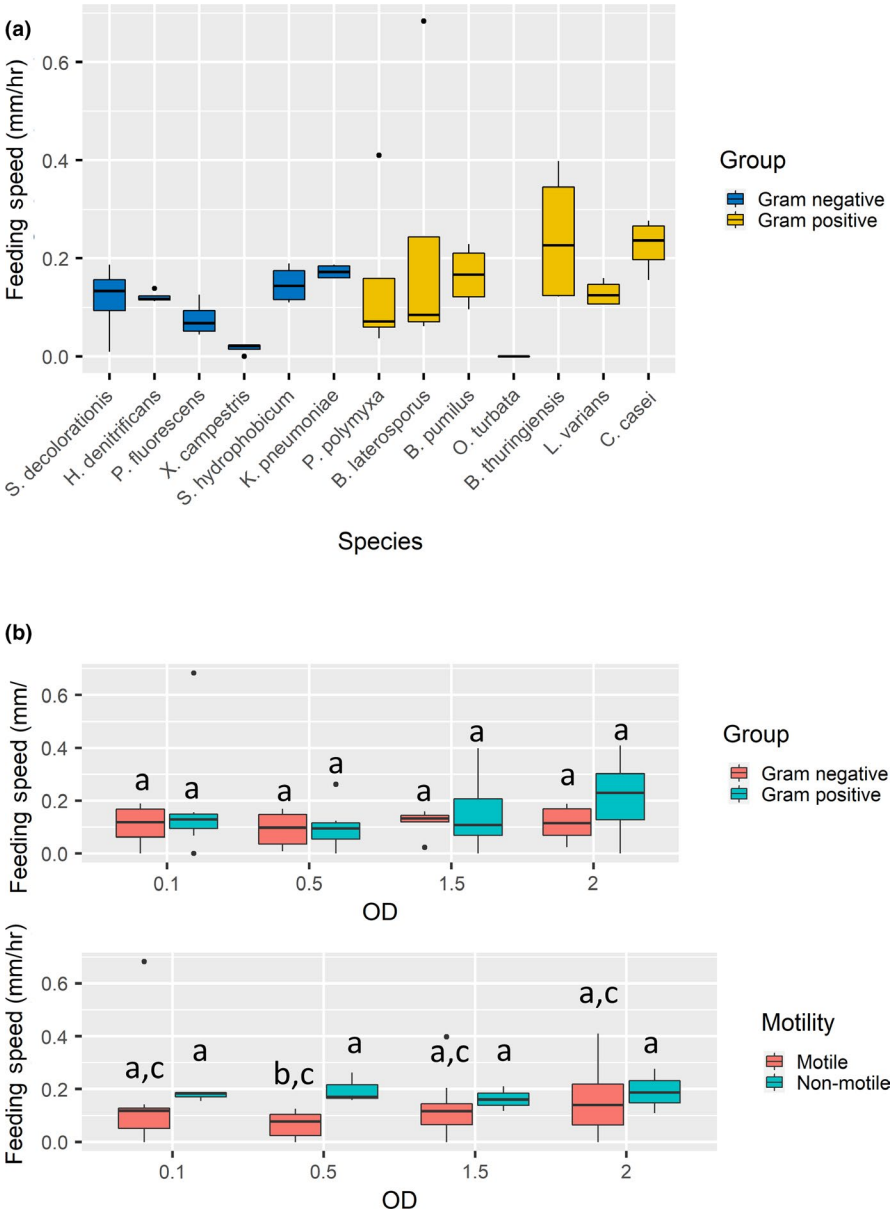

FIGURE 5 Feeding speed of *Dictyosteliumdiscoideum* on different bacteria. (A) By species; (B) By Gram-stain types (Gram-positive: $n = 7$; Gram-negative: $n = 7$) and bacterial motility (motile: $n = 10$; non-motile: $n = 4$). Boxplots represent the interquartile range (25% and 75%) and whiskers represent the minimum or maximum values. A black line within the box marks the median. Different lowercase letters represent significant differences between treatments based on Tukey's HSD test ($p < 0.05$)

energy for maintenance (Bradley et al., 2019; Greening et al., 2019), which could explain why *D. discoideum* needs to sense and distinguish their bacterial prey because they need to feed soon after emerging from dormancy. To our knowledge, ours is the first study that directly provides empirical evidence that dormant protists can sense and discriminate against different soil bacterial prey.

4.2 | *Dictyostelium discoideum* prefers bacteria with high nutritional values

We show that high nutritional value bacteria induce higher hatching rates in *D. discoideum*. Some protists are known to have selective grazing behaviours, and understanding the particular grazing impact of such protists is essential to evaluate their contribution to ecosystem processes (Gonzalez et al., 1990; Matz & Kjelleberg, 2005; Montagnes et al., 2008; Murase & Frenzel, 2008; Rosenberg et al., 2009). Several studies reported that *D. discoideum* had different gene expression profiles when exposed to different bacteria (Benghezal et al., 2006; Lamrabet et al., 2020; Nasser et al., 2013). They were also more attracted to Gram-negative bacteria in a chemotaxis assay (Rashidi & Ostrowski, 2019). However, it is not clear whether such food preference is determined by bacterial nutrition (greater production of amoeba spores after feeding on a given amount of bacteria). Our results show that *D. discoideum* has a higher hatching rate and form bigger amoeba plaques when mixed with high-value bacteria, which provides direct evidence that soil protists can discriminate and prefer high-value bacteria.

In this study, high-value bacteria are characterized as Gram-negative and non-motile bacteria. The exact mechanisms need further exploration, but one possible explanation could be energy expenditure. Gram-positive bacteria generally have a thick peptidoglycan cell wall, whereas Gram-negative bacteria have a thin layer of peptidoglycan. It may be energetically costly for *D. discoideum*

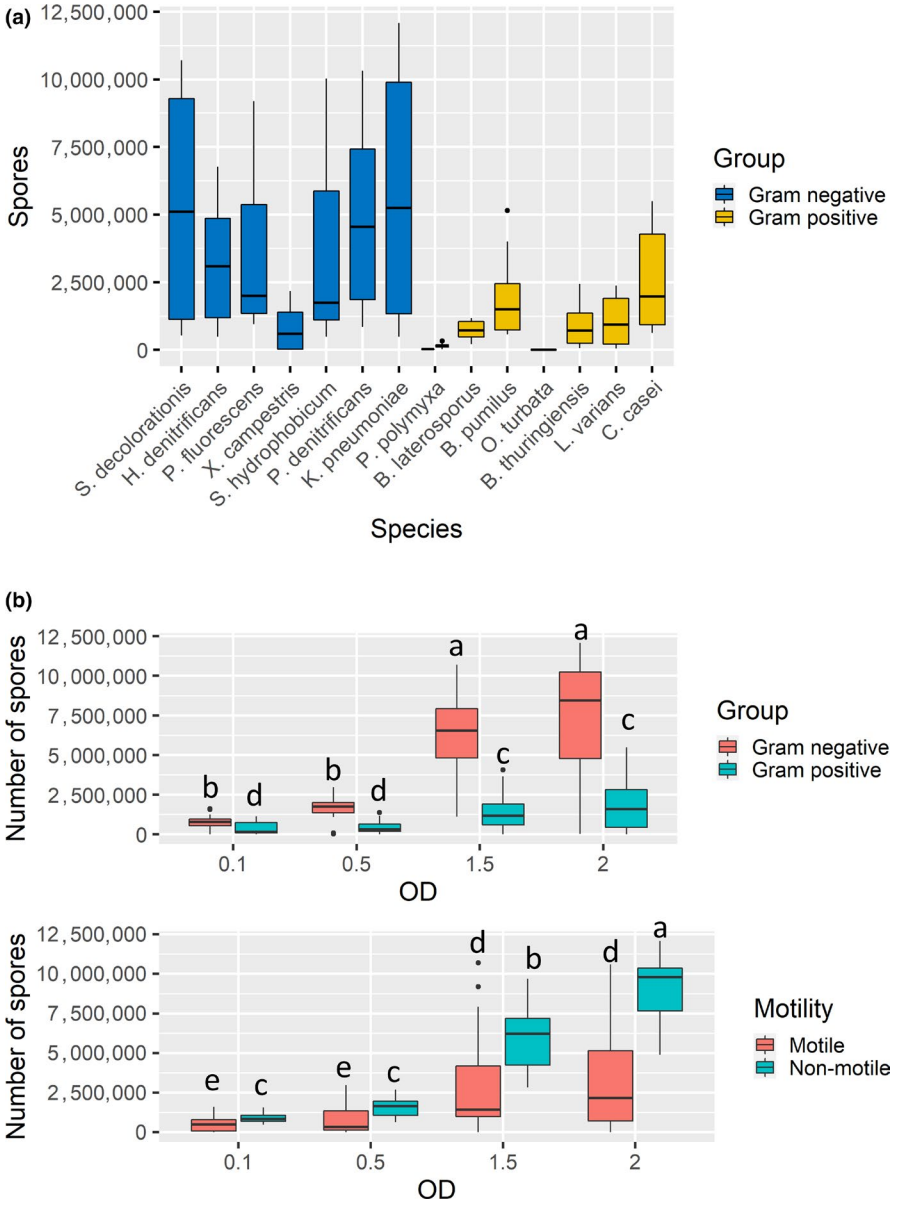

FIGURE 6 Spore production of *Dictyosteliumdiscoideum* on different bacteria. (A) By species; (B) By Gram-stain types (Gram-positive: $n = 7$; Gram-negative: $n = 7$) and bacterial motility (motile: $n = 10$; non-motile: $n = 4$). Boxplots represent the interquartile range (25% and 75%) and whiskers represent the minimum or maximum values. A black line within the box marks the median. Different lowercase letters represent significant differences between treatments based on Tukey's HSD test ($p < 0.05$)

to digest the thick peptidoglycan layer, and there is some evidence for that. For instance, a different set of genes were activated when exposed to Gram-negative and Gram-positive bacteria (Benghezal et al., 2006; Lamrabet et al., 2020; Nasser et al., 2013). It is also costly to chase motile bacterial prey, as shown by studies from the aquatic system in which highly motile bacteria have better survival due to lower flagellate ingestion rates (Matz & Jurgens, 2005; Pernthaler, 2005). Therefore, *D. discoideum* prefers Gram-negative and non-motile bacteria maybe because less energy is required to predate on them.

4.3 | Feeding speed is independent of sensing of prey or bacterial nutritional value

Surprisingly, we found that feeding efficiency was not linked with sensing of prey or bacterial nutritional value. Amoebae use sophisticated phagosome machinery to kill and digest bacteria effectively. The phagosome first becomes acidic, in which V-ATPase plays a central role (Kissing et al., 2015; Lelong et al., 2011). In addition, the phagosome also contains proteases, hydrolases, lysozymes, antimicrobial peptides, certain metals, and ROS (reactive oxygen species), which combine to kill and breakdown bacteria (Cosson & Lima, 2014; German et al., 2013). As we found that neither Gram-stain types nor bacterial motility significantly affected the feeding speed of *D. discoideum* (Figure 5b), feeding speed is likely determined by the phagosome machinery rather than bacterial motility or cell walls. Our study provides further evidence to support this hypothesis: we recorded fruiting body time to represent the emergence of the first fruiting body (which is when the amoeba enters the social life cycle after eating all prey). We found that neither Gram-stain type nor bacterial motility significantly affected fruiting body time (Table S1; Figure S6), suggesting that intracellular killing is independent of bacterial sensing or bacterial nutritional value.

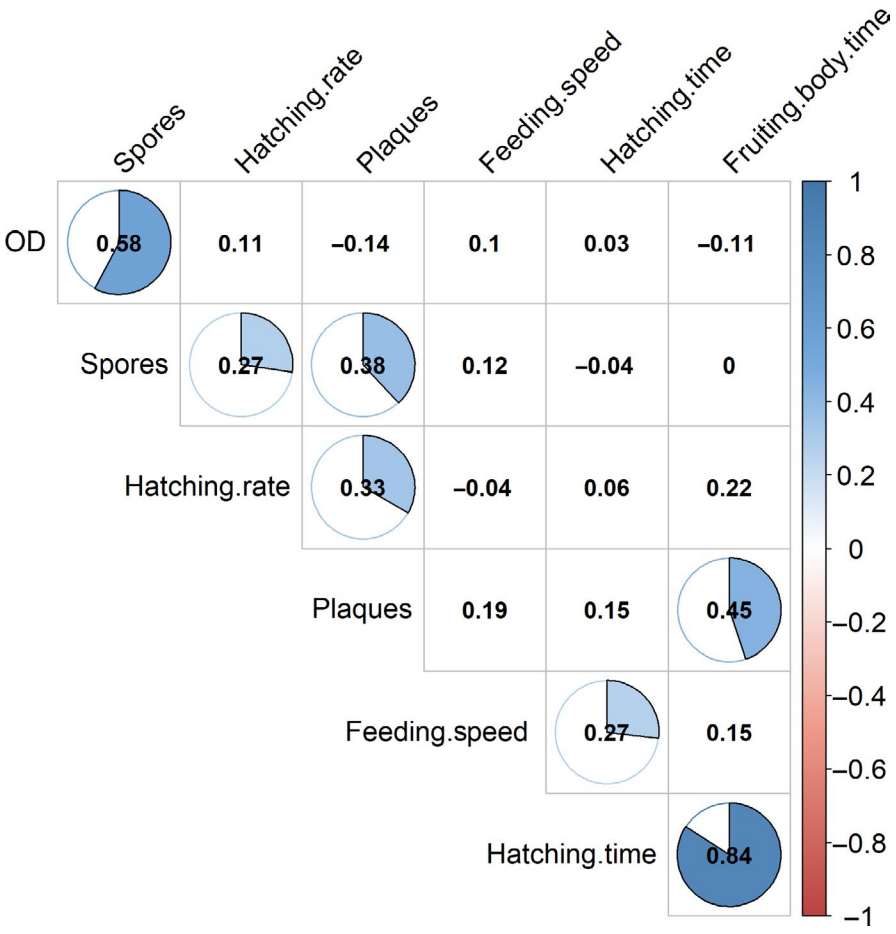

FIGURE 7 Correlogram showing the correlation among hatching rate, hatching time, bacterial density, feeding speed, fruiting body time and spore production by pie charts. The variance explained by significant correlations is shown as pie charts. The scale gives the coefficient of correlation from Spearman's Rank Correlations, where blue is a positive correlation and red is a negative correlation

4.4 | Mechanisms of discrimination: Active amoeba sensing or bacterial inhibition

Our results show that *D. discoideum* did not hatch when mixed *O. turbata*, which can be explained by two alternative mechanisms. First, dormant *D. discoideum* actively senses the potential pathogen and chooses not to hatch. Second, *O. turbata* inhibits or even kills *D. discoideum*, so the spores cannot hatch because they are dead. We performed additional experiments and confirmed that *O. turbata* did not kill these amoeba spores. We plated *D. discoideum* spores on a mixture of *O. turbata* and *K. pneumoniae* (50%:50% vol) and found that these amoeba spores were not dead. On the contrary, they had a higher hatching rate and produced more spores than *O. turbata* or *K. pneumoniae* alone (Figure S7). These results confirm that dormant *D. discoideum* can indeed actively sense and discriminate prey.

Our study also finds evidence that bacterial inhibition plays a role in soil protist–bacteria interactions. Density-dependent inhibition, such as quorum sensing, is a typical process that allows bacteria to produce compounds such as virulence factors (Dandekar et al., 2012; Papenfort & Bassler, 2016). Our study showed that bigger amoeba plaques were formed at a bacterial density of OD 0.1 compared to other higher bacterial densities, indicating predation efficiency was lower at higher bacterial densities. In addition, there was a strong density-dependent inhibition of spore production with *P. fluorescens* at OD 2.0 compared to OD 1.5. Since the genus *Pseudomonas* is known to be equipped with quorum sensing systems (Papenfort & Bassler, 2016), this indicates a potential quorum sensing mediated defence over amoeba predation. Taken together, these results suggest that sensing and discrimination are mediated through active amoeba preference as well as bacterial inhibition.

4.5 | Implications for the bacterial community and soil ecosystem

In soil, many protists are bacterivorous and are the primary cause of bacterial mortality, with significant consequences for the bacterial community (Matz & Kjelleberg, 2005; Rosenberg et al., 2009; Saleem et al., 2013). This study provides three new insights into how soil protists might affect the bacterial community and the soil ecosystem.

First, soil protists can sense and recognize their bacteria prey even at the dormant stage, a process that is likely mediated through soluble or volatile molecules (Schulz-Bohm et al., 2017; Shu, Zhang, et al., 2018). Besides, one bacterium, *O. turbata*, completely inhibited *D. discoideum* spore hatching, indicating that the presence of potentially pathogenic bacteria can inhibit the hatching of dormant cells. If this also happens in natural environments, it means certain bacteria can significantly affect the abundance of soil protists and bacterial communities. Since our assay was done on agar plates,

future studies should investigate this in soil because the sensing and predation of amoebae on bacteria might be negatively affected by the spatial heterogeneity of soil (Petrenko et al., 2020). However, we believe the overall pattern in soils will be consistent with the current study. In addition, we only studied *D. discoideum* by feeding them on individual bacterial taxa, and it is not clear how *D. discoideum* will respond to a mixture of bacteria consisting of both food and pathogenic bacteria. Future studies should also address this point in a soil environment.

Second, this study shows that *D. discoideum* preferentially feeds on Gram-negative and non-motile bacteria, which will decrease their abundance in soil environments. A previous study found that *D. discoideum* prefers Gram-negative bacteria in a chemotaxis assay (Rashidi & Ostrowski, 2019). This study further shows that *D. discoideum* has a higher hatching rate, forms bigger amoeba plaques and produced more spores when mixed with Gram-negative bacteria. In addition, we found that *D. discoideum* hatches earlier and produces more spores on non-motile bacteria. It has been reported that bacterial motility affects the predator–prey interactions in the aquatic system (Matz & Jurgens, 2005; Pernthaler, 2005). To our knowledge, this is the first evidence that bacterial motility also plays a role in soil systems, and it could be energetically costly for the amoeba to predate on motile bacteria compared to non-motile ones. Since phylogenetically closely related protist species can still have different food preferences (Glucksman et al., 2010; Pedersen et al., 2011), more studies are needed to access soil protists' feeding preferences systematically, which will help to disentangle the contribution of biotic and abiotic factors to the population dynamics and community assembly of bacterial communities.

Finally, we found that despite the high variation in motility and physiology among the 14 different bacteria, *D. discoideum* consumed bacteria at the same speed. Neither Gram stain nor bacterial motility significantly affected the feeding speed (Figure 5B). These results suggest that *D. discoideum* may not significantly alter a given bacterial community because they consume all bacteria at the same speed, despite differences in the sensing of prey and bacterial nutritional value. These contrasting results suggest that soil protist–bacteria interactions are complicated, and their contribution to the bacterial community needs to be assessed carefully.

5 | CONCLUSIONS

In conclusion, this study shows that dormant *D. discoideum* can selectively sense and predate on different soil bacteria, and they can also distinguish and avoid pathogenic bacteria. Our results also suggest that the selective interaction between amoebae and their prey is mediated through active amoeba preference and bacterial inhibition. Future research should explore protist feeding in natural soil environments, which have significant spatial heterogeneity. It will be informative to investigate how soil protists sense, recognize and feed on diverse bacteria within soils.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data deposited in the Dryad Digital Repository https://doi.org/10.5061/dryad.2v6wwpzn7, (Shu et al., 2021).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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