Microbial Ecology (2025) 88:14
https://doi.org/10.1007/s00248-025-02510-7

RESEARCH

Effects of Geosmin on the Behavior of Soil Protists

Jamie L. Micciulla[1] · **Capucine Baubin**[1] · **Noah Fierer**[1,2]

Received: 30 October 2024 / Accepted: 4 March 2025

Abstract
Geosmin is a volatile organic compound (VOC) produced by a range of different soil microorganisms, and is most commonly recognized for its characteristic "earthy" scent evident after rainfall. Though it remains unclear why microorganisms produce geosmin, we know that exposure to geosmin can influence behaviors across a wide range of organisms, serving as both an attractant and a repellant, but geosmin effects on soil protists remain largely unstudied. We investigated how soil protists respond to geosmin exposures, focusing on representatives of three morphological groups of protists, *Colpoda* sp. (ciliate), *Cercomonas* sp. (flagellate), and *Acanthamoeba castellanii* (naked amoeba), testing the hypothesis that geosmin production by bacteria influences soil protist behavior. We conducted experiments to evaluate protist excystment (waking up) and predation responses to geosmin-producing (*Streptomyces coelicolor* M145) and non-producing (*S. coelicolor* J3003) bacteria, as well as synthetic geosmin. All three protists excysted at higher rates when exposed to geosmin-producing bacteria or synthetic geosmin, while no significant excystment occurred with the non-producing strains or in the absence of synthetic geosmin. Protist feeding preferences were also affected, with two of the three protists (*Cercomonas* sp. and *A. castellanii*) less likely to predate geosmin-producing versus non-producing bacterial strains. Our findings suggest that soil protists can detect geosmin as a signal indicating favorable soil conditions and geosmin production by bacteria may serve as a deterrent to predation by protists. More generally, our results highlight the ecological significance of geosmin in the soil food web and its role in mediating bacteria-protist interactions.

Keywords Soil protists · Geosmin · Bacterial signaling · Soil microbial interactions · Volatile organic compounds

Introduction

Geosmin, meaning "earth odor," is a volatile organic compound (VOC) produced by a variety of microorganisms in both terrestrial and aquatic environments [1]. This compound is notably associated with the distinct scent of rain, as geosmin is often released by soil bacteria, including actinobacteria and myxobacteria, during wetting events after periods of drought [2, 3]. While geosmin production is widespread across microbial taxa, its biological function remains poorly understood, despite evidence suggesting it may play a significant role in microbial life cycles and interactions within ecosystems.

The ecological importance of geosmin extends beyond its contribution to the scent of soil, acting as a chemical signal that can attract or repel various organisms. For example, geosmin serves as a warning signal for fruit flies, deterring them from potentially harmful environments [4], and acts as an anti-predation factor for bacterivorous nematodes [5]. Conversely, geosmin can serve as an attractant for animals, including fire ants, mosquitoes, honeybees, and elephants, aiding in locating nesting sites and water sources [6–9]. Geosmin production by streptomycete bacteria has been shown to serve as an attractant for springtails, which consume the bacteria and disperse its spores through their feces [10]. These examples illustrate the versatile roles of geosmin in shaping ecological interactions, but they also highlight a critical knowledge gap: the effects of geosmin on soil protists.

✉ Jamie L. Micciulla
Jamie.Micciulla@gmail.com

✉ Noah Fierer
Noah.Fierer@colorado.edu

[1] Cooperative Institute for Research in Environmental Sciences, University of Colorado Boulder, Boulder, CO, USA

[2] Ecology and Evolutionary Biology Department, University of Colorado Boulder, Boulder, CO, USA

Published online: 14 March 2025

Protists are nearly ubiquitous in soil environments [11] where they can shape nutrient cycling, the activities and composition of microbial communities, and plant health [12, 13]. Protist behaviors can be strongly influenced by plant metabolites (e.g., root exudates) and the metabolites produced by other microorganisms (e.g., exopolysaccharides), which guide processes such as foraging and excystment [14, 15]. Due to the wide range of factors which can influence protist excystment and predation preferences [16–18], it is no surprise that investigations of the role of VOCs in mediating long-distance communication between protists and prey in aquatic ecosystems have been conducted [19]. However, the potential for VOCs like geosmin to serve similar signaling functions in soil environments remains largely unstudied. Geosmin production by bacteria may influence the behavior of soil protists by serving as a cue for favorable environmental conditions or by signaling prey availability. While previous work has explored the responses of aquatic protists to geosmin [20], we do not know if similar behavioral responses occur in soil and what role geosmin might play in the soil food web and mediating interactions between soil microorganisms.

This study aimed to address the research question: What is the effect of geosmin on the excystation and predation behaviors of three morphotypes of soil protists? We hypothesized that if geosmin influences the behavior of soil protists, then exposure to this VOC will affect their rates of excystation and predation on geosmin-producing bacteria. Specifically, we assessed the effects of geosmin on three soil protists, *Colpoda* sp., *Cercomonas* sp., and *Acanthamoeba castellanii*, by studying their excystation rates following geosmin exposure as well as the potential influence of bacterial geosmin production on protist predation behavior. By exploring differences in the responses among these protist species, we sought to better understand the ecological implications of geosmin detection in soil environments.

Methods

Protist Excystment

To determine how geosmin production by bacteria may influence protist excystment rates, we grew encysted protists in a shared headspace with geosmin-producing bacteria and measured the change in the number of cysts over time.

Colpoda sp. and *Cercomonas* sp. were isolated from rhizosphere samples as described in Taerum et al. 2020 and cultured in Page's Saline Solution with heat killed *Escherichia coli* DH5α ($OD_{595} = 0.005$) as a food source [21, 22]. *Acanthamoeba castellanii* (ATCC #30010) was cultured in ATCC Medium 712 PYG with additives as recommended. All three of these protists form cysts in unfavorable conditions. Protist cultures were grown in 25cm^2 BioLite Cell Culture Treated Flasks (ThermoFisher #130192) in 10 mL of their growth medium and were maintained in the dark, at room temperature for 3 weeks prior to the excystment experiment to synchronize life cycles.

At the start of the excystment experiment, protists were resuspended from the cell culture flasks, transferred to a 50-mL conical tube, centrifuged at $5000 \times g$ for 15 min, and then the supernatant was removed. The pelleted protists were resuspended with 1 mL Page's Saline Solution to wash the cells, then centrifuged again at $5000 \times g$ for 5 min. After removing the supernatant, the pelleted protists were resuspended in 500 µL Page's Saline Solution and enumerated using the average of three 1-µL spots on a compound microscope.

We used two strains of *Streptomyces coelicolor*, a wild-type geosmin producer *S. coelicolor* strain M145 [23] and the complimentary geosmin synthase mutant *S. coelicolor* J3003 (*S. coelicolor* M145 Δcyc2scar) [24]. These strains are phenotypically identical other than the production of geosmin [10]. This pairing of the two *Streptomyces* strains allows for the specific study of the effect of geosmin on protist behavior while controlling for the release of other VOCs by the wild-type strain.

S. coelicolor M145 (+ geosmin) and *S. coelicolor* J3003 (− geosmin) were separately streaked onto R2A + $Cip_{0.05}$ agar plates and incubated at 25 °C for 4 days. A single colony from each plate was used to individually inoculate 5 mL R2A + $Cip_{0.05}$ liquid media, which was incubated at 25 °C for 6 days with constant shaking at 200 rpm. The concentration of bacteria was measured using a spectrophotometer.

To create an environment where protists shared the same headspace as *S. coelicolor*, we created protist-geosmin sandwiches using two 24-well plates. R2A agar media was solidified into each well of three 24-well black walled, glass bottom plates. All of the wells in a plate were then streaked with either *S. coelicolor* M145 (+ geosmin), *S. coelicolor* J3003 (− geosmin), or left uninoculated (− geosmin, negative control). The 24-well plates were incubated at 25 °C for 6 days to capture the stationary phase of growth as *S. coelicolor* is known to produce the highest concentration of geosmin in stationary phase [25].

A total of 1000 protists per well were cultured in 500 µL of Page's Saline Solution (without food) in three 24-well plates (8 wells per protist type). Well plates containing protists were incubated at room temperature in the dark for 3 weeks to ensure the entire protist population encysted. Under these conditions, the protists are experiencing starvation.

After incubation of the protists and bacteria, lids were removed from both plates and the bacteria-containing well plate was inverted to lay flat on top of the open protist plate. This allowed for the protists and bacteria to remain

physically separated while still sharing the same headspace, and allowed for VOCs produced by the bacteria to diffuse into the protist liquid media (Fig. 1A). This experiment was replicated in triplicate plates.

As *S. coelicolor* produces a large number of VOCs in addition to geosmin [26], we next sought to determine whether the protist excystment responses observed for the *S. coelicolor* experiments (described above) are similar when exposing protists to synthetic geosmin, to confirm that geosmin, not other VOCs, are responsible for the observed responses. For this experiment, *Colpoda* sp., *Cercomonas* sp., and *A. castellanii* were cultured into three 24-well plates as described above. Synthetic geosmin was diluted 1:8000 in methanol to a final volume of 100 mL in a 250-mL open beaker. The beaker and lid-less 24-well plate were placed into a closed container, allowing for the synthetic geosmin to diffuse into the entire headspace as well as the protist culture media (Fig. 1B). To control for the effect of methanol on the protists, a methanol only control was set up with 100 mL of methanol in a 250-mL beaker. A true negative control, 100 mL of water, was also run concurrently. This experiment was replicated in triplicate plates.

Protists in the 24-well plates were imaged daily for 5 days using an Olympus IX81 widefield microscope, where day 0 captured the number of protist cysts prior to incubation with either bacteria or synthetic geosmin. The average number of cysts were measured from three representative fields of view from each well per day. Images were captured using Olympus cellSens Dimension V3.2 64 bit software (EVIDENT by Olympus).

Protist Predation

To determine how geosmin production by bacteria may influence protist predation, we fed protists meals of *S. coelicolor* M145 (+ geosmin) and *S. coelicolor* J3003 (− geosmin) and measured the change in colony-forming units (CFUs) of the bacteria over time.

Due to their identical colony morphology, *S. coelicolor* M145 (+ geosmin) and *S. coelicolor* J3003 (− geosmin) could not be co-fed and enumerated to determine protist predation behavior. Therefore, to assess protist feeding preferences, we used *E. coli* as baseline food source. *E. coli* was selected not because of an interest in its direct interaction with the protists, but because it is a familiar and desirable food source for the protists used in this study. By providing *E. coli*, we aimed to establish a baseline feeding behavior and determine whether protists would choose to consume *E. coli* over another non-geosmin producing bacteria (*S. coelicolor* J3003), or over a geosmin-producing bacteria (*S. coelicolor* M145). Prior to the co-feeding experiments, it was confirmed that *Colpoda* sp., *Cercomonas* sp., and *A. castellanii* will consume all three bacterial strains individually (data not shown.)

E. coli DH5α was streaked from freezer stock onto R2A + $Kan_{2.5}$ agar plates and incubated at 37 °C for 24 h.

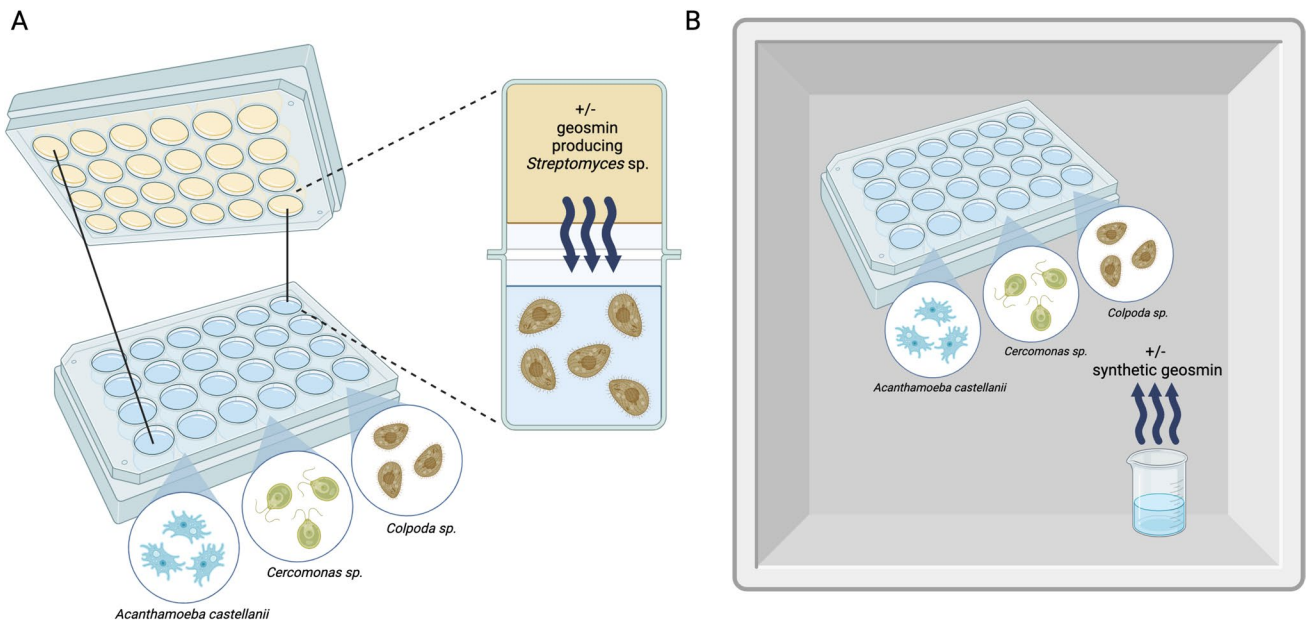

Fig. 1 Illustration of the set-up for the protist excystment experiments with **A** bacterial-produced geosmin and **B** synthetic geosmin. **A** *Colpoda* sp., *Cercomonas* sp., and *A. castellanii* were individually cultured into eight wells of a 24-well plate and "sandwiched" with an open 24-well plate with cultured + / − geosmin-producing strains of *Streptomyces coelicolor* so VOCs could diffuse into protist media. **B** *Colpoda* sp., *Cercomonas* sp., and *A. castellanii* were individually cultured into 8 wells of an open 24-well plate and placed into a container with a beaker containing synthetic geosmin (or a corresponding "no geosmin" control)

A single colony was used to inoculate 5 mL R2A + $Kan_{2.5}$ liquid media, which was incubated at 37 °C for 24 h with constant shaking at 200 rpm. *S. coelicolor* M145 (+ geosmin) and *S. coelicolor* J3003 (− geosmin) were prepared as described above. The concentration of bacteria was measured using a spectrophotometer.

Colpoda sp., *Cercomonas* sp., and *A. castellanii* were separately cultured into two 25-cm^2 BioLite Cell Culture Treated Flasks (ThermoFisher #130,192) in 10 mL of Page's Saline Solution and diluted to 1000 cells per flask prior to the start of the experiment. Protist cultures were fed with a combination of either *E. coli* DH5α and *S. coelicolor* M145 (+ geosmin) or *E. coli* DH5α and *S. coelicolor* J3003 (− geosmin). To not overwhelm the environment, all bacteria were fed at a final concentration of $OD_{595} = 0.015$. For colony counting, a subset of the culture was diluted 1:800, and 10µL from each culture dilution was lawn plated onto both an R2A + $Cip_{2.5}$ agar plate and an R2A + $Kan_{2.5}$ agar plate, to allow for the growth of *S. coelicolor* and *E. coli*, respectively. *E. coli* plates were incubated at 37 °C for 24 h and *S. coelicolor* plates were incubated at 25 °C for 3 days. After incubation, CFUs were recorded from each plate. Plating was repeated once a day for 5 days, with day 0 occurring immediately after inoculation of the protist culture with the *E. coli* DH5α and *S. coelicolor* meals. This experiment was replicated in triplicate.

Statistical Analysis

Statistical analyses were performed using R v4.2.3. To evaluate the protist excystment data, we used a grouped two-tailed *t*-test where the differences in the number of cysts were compared between conditions for each day. Conditions for the experiments using bacterial-produced geosmin included *S. coelicolor* M145 (+ geosmin), *S. coelicolor* J3003 (− geosmin), and R2A only control (− geosmin). Conditions for the experiments using synthetic geosmin included synthetic geosmin, methanol-only control and water. Eight replicates were included in each test.

Results

Protist Excystment

Colpoda sp., *Cercomonas* sp., and *A. castellanii* were cultured in axenic media and exposed to VOCs produced by *S. coelicolor* M145 (+ geosmin) or *S. coelicolor* J3003 (− geosmin) (Fig. 2). All three protists excysted within 24 h of exposure to *S. coelicolor* M145 (+ geosmin), while the same protists exposed to *S. coelicolor* J3003 (− geosmin) and R2A media only remained in their encysted state. Excystment was significantly increased in the presence of *S. coelicolor* M145 (+ geosmin) for all protists at all time points ($p < 0.05$). Excystment was not significantly increased for any of the protists upon exposure to *S. coelicolor* J3003 (− geosmin) and the R2A media only control when compared to exposure to *S. coelicolor* M145 (+ geosmin) ($p > 0.05$). *Colpoda* sp. excysted at a faster rate upon exposure to bacterial-produced geosmin than both *Cercomonas* sp. and *A. castellanii*. On average, *Colpoda* sp. had 6 fewer cysts per field of view after 24 h of exposure compared to 3 *Cercomonas* sp. cysts and 1.25 *A. castellanii* cysts in the same time. The effects of the *Streptomyces*-produced geosmin were consistent for the duration of the experiment, with excystment rates being higher when the protists were exposed to geosmin compared to the two 'no geosmin' controls across all time points (Fig. 2).

To confirm that the increases in excystment rates were specifically associated with geosmin exposures, we conducted a similar experiment, but instead of using *S. coelicolor* as a source of geosmin (Fig. 2), the protists were exposed to synthetic geosmin (Fig. 3). The patterns were nearly identical with synthetic geosmin as the observed excystment rates were significantly higher ($p < 0.001$) for all three protists upon exposure to synthetic geosmin compared to the corresponding "no geosmin" control treatments ($p > 0.05$) where minimal excystment was observed (Fig. 3). It is noted that the increase in excystment with the geosmin-producing bacteria was slightly different when the same protists were exposed to synthetic geosmin. This could be due to the differences in geosmin concentrations or because the synthetic geosmin is a mixture of the two stereoisomers of the molecule while the biologically produced geosmin has only one stereoisomer [27].

Protist Predation

We next sought to determine how geosmin production by bacteria may influence protist predation rates. We did so by growing each protist in the presence of *S. coelicolor* M145 (+ geosmin) or *S. coelicolor* J3003 (− geosmin) and tracking predation (decrease in CFU), over time. Bacterial meals of equal concentrations of *E. coli* and *S. coelicolor* M145 (+ geosmin) or *E. coli* and *S. coelicolor* J3003 (− geosmin) were delivered to individual cultures of *Colpoda* sp., *Cercomonas* sp., and *A. castellanii*, comparing predation rates with either of the *Streptomyces* strains to baseline predation rates of *E. coli*. Daily CFU counts were recorded to track the decrease (predation) or constant (no predation) measurement of bacterial cells over a 5-day period.

When fed meals of *E. coli* and *S. coelicolor* J3003 (− geosmin), the bacteria were consumed at similar rates by all three protists (Fig. 4 top). All three protists displayed a slight preference for *E. coli* over *S. coelicolor*

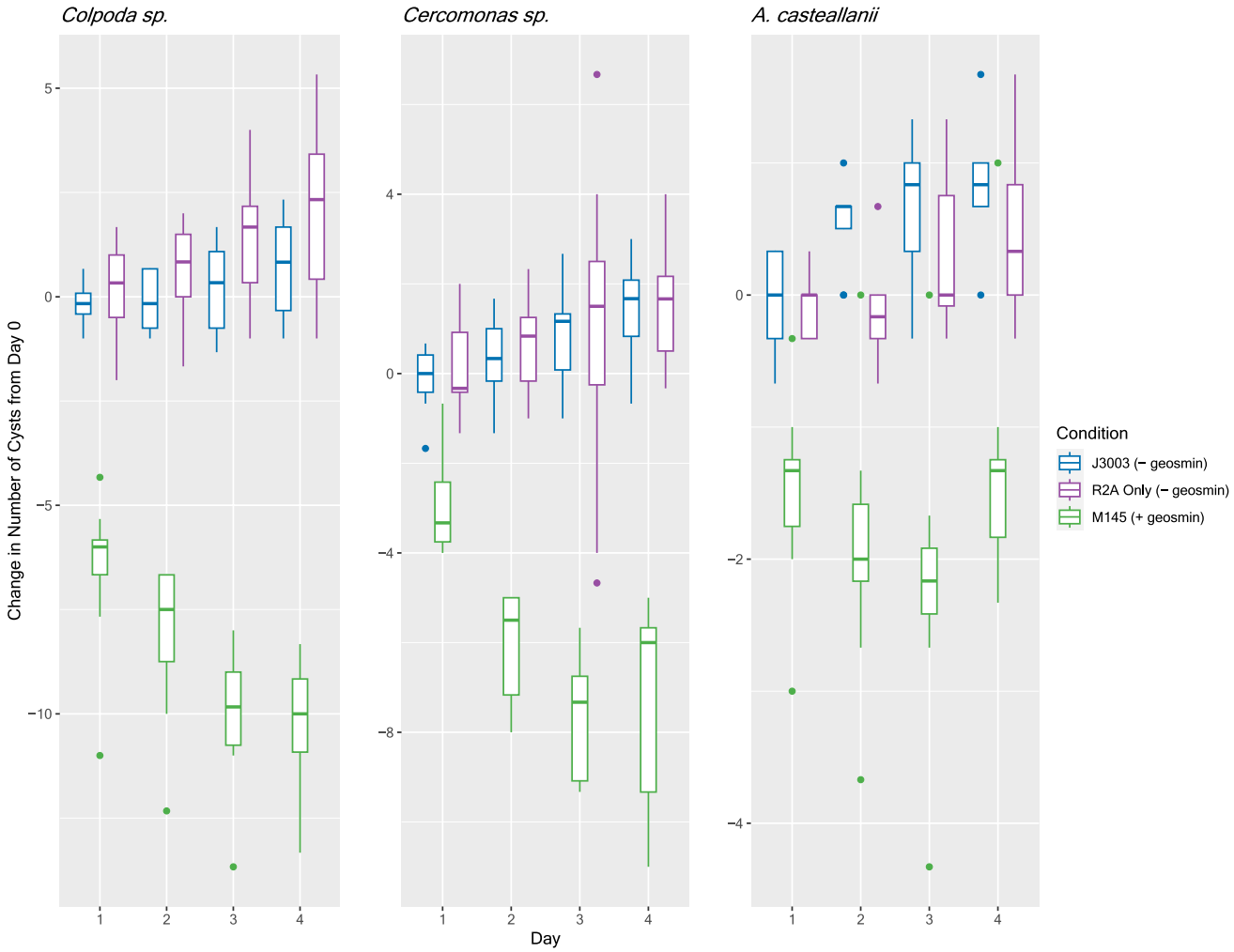

Fig. 2 Change in in the number of protist cysts per field of view from day 0 when grown in the presence of bacterial-produced geosmin. Protists were grown in the presence of *S. coelicolor* J3003 (− geosmin, blue), R2A media only (purple), or *S. coelicolor* M145 (+ geosmin, green). The vertical axis represents the change in the number of cysts relative to day 0, where negative values indicate a decrease in the number of cysts, corresponding to greater excystment and an increase in the number of active protists. A total of 1000 protists were cultured into each well at the start of the experiment. For all protists at all time points, except *A. castellanii* day 2 "no geosmin" controls ($p = 0.01$), there were no significant difference in the change in number of cysts from day 0 between *S. coelicolor* J3003 (− geosmin) and the "R2A Only" control (− geosmin) ($p > 0.05$). For all protists at all time points, the number of cysts from day 0 significantly decreased (greater excystment) when protists were exposed to *S. coelicolor* M145 (+ geosmin) compared to *S. coelicolor* J3003 (− geosmin) ($p < 0.01$)

J3003 (− geosmin), as demonstrated by the consumption of 60–90% of *E.coli* and 40–60% of *S. coelicolor* J3003 (− geosmin) by the end of the experiment (Fig. 4 top insets).

When fed meals of *E. coli* and *S. coelicolor* M145 (+ geosmin), the bacteria were consumed at different rates, depending on the protist (Fig. 4 bottom). *Colpoda* sp. consumed *E. coli* (75%) and *S. coelicolor* M145 (+ geosmin) (65%) at almost equal rates, while *Cercomonas* sp. and *A castellanii* had a strong preference for *E. coli* (80% and 70%, respectively) over *S. coelicolor* M145 (+ geosmin) (30% and 25%, respectively).

Discussion

Soil microorganisms, including *Streptomyces* species, release geosmin in response to wetting events, and geosmin may serve as a signaling molecule broadly influencing the behavior and activities of the soil community [2]. Our hypothesis was that soil protists could detect geosmin and alter their behavior accordingly. Confirming our hypothesis, we observed that protists excyst in response to geosmin, potentially using geosmin as a signal indicating favorable conditions for proliferation following rainfall,

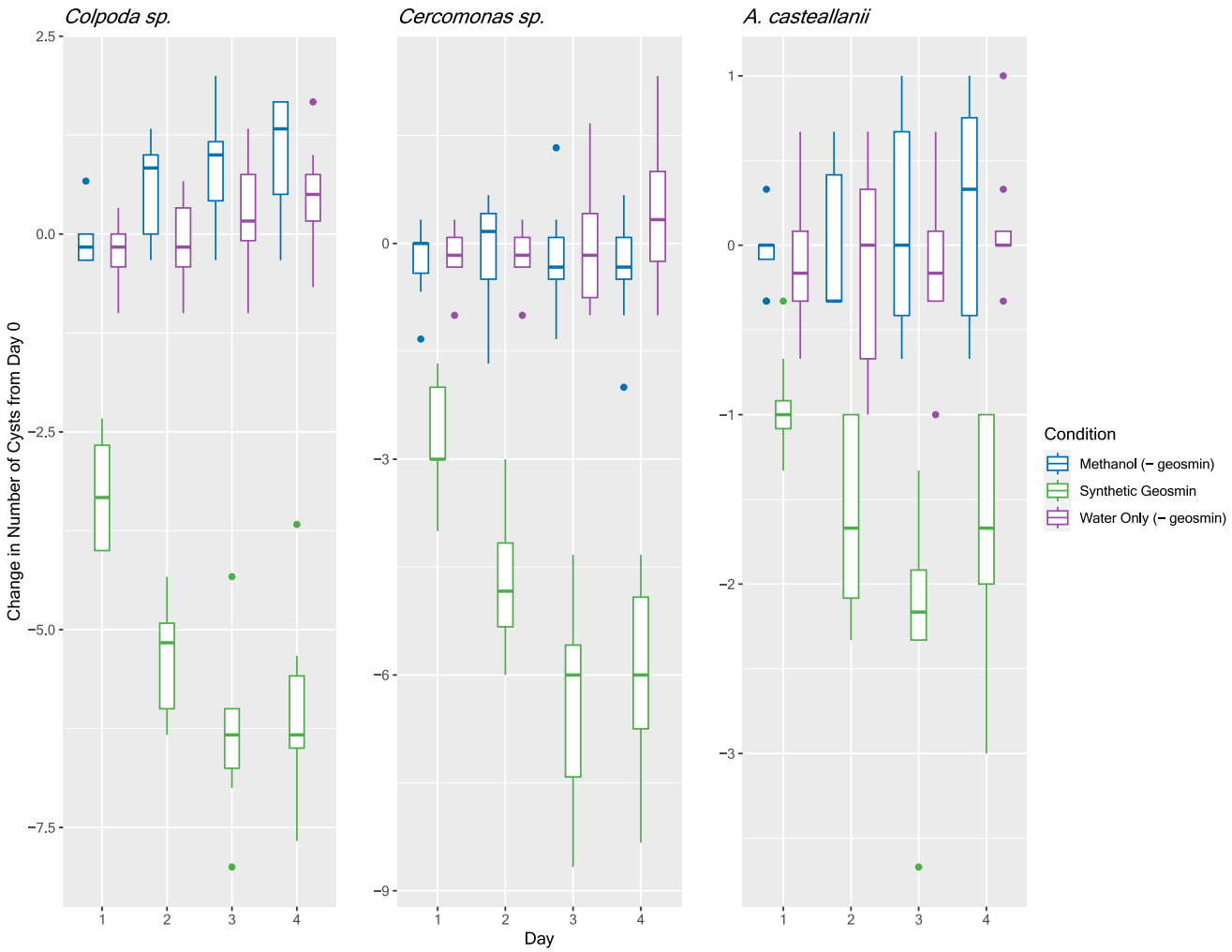

Fig. 3 Change in number of protist cysts per field of view from day 0 when grown in the presence of synthetic geosmin. Protists were grown in the presence of synthetic geosmin (green) or the "no geosmin" controls: water only (purple) and methanol only (blue). The vertical axis represents the change in the number of cysts relative to day 0, where negative values indicate a decrease in the number of cysts, corresponding to greater excystment and an increase in the number of active protists. A total of 1000 protists were cultured into each well at the start of the experiment. As shown with exposure to bacterial-produced geosmin (Fig. 2), exposure to synthetic geosmin also increases excystment rates compared to the "no geosmin" control treatments. For all protists at all time points, except *Colpoda* sp. day 2 "no geosmin" controls ($p = 0.03$), there were no significant differences in the number of cysts from day 0 between methanol only (− geosmin) and water only control (− geosmin) ($p > 0.05$). For all protists at all time points, the number of cysts from day 0 significantly decreased (greater excystment) when protists were exposed to synthetic geosmin compared to the methanol-only control (− geosmin) ($p < 0.001$)

such as increased moisture and resource availability. The specificity of this behavior towards geosmin was evident as there was a significant increase in excystment regardless of whether the protists were exposed to bacterial-produced geosmin or synthetic geosmin as compared to the corresponding "no geosmin" controls. Protists play a critical role in regulating bacterial populations and nutrient cycling in soil ecosystems, and their ability to detect geosmin may be an adaptive strategy for exploiting these transient resource-rich conditions [12, 16]. The excystment behavior observed here suggests that geosmin may act as an early environmental cue, prompting protists to become active before other microorganisms fully exploit the available resources, thus conferring an ecological advantage.

In additional support of our hypothesis, we observed that two of the three protists (*Cercomonas* sp. and *A. castellanii*) may leverage geosmin as a cue for selective feeding, favoring prey that do not produce the VOC. When given the option to prey on *S. coelicolor* M145 (+ geosmin) or *S. coelicolor* J3003 (− geosmin), compared to a palatable prey of *E. coli* DH5α, *Cercomonas* sp. and *A. castellanii* consumed less *S. coelicolor* M145 (+ geosmin). A previous study observed a similar behavioral response in nematodes, showing that the number of interactions between *C. elegans*

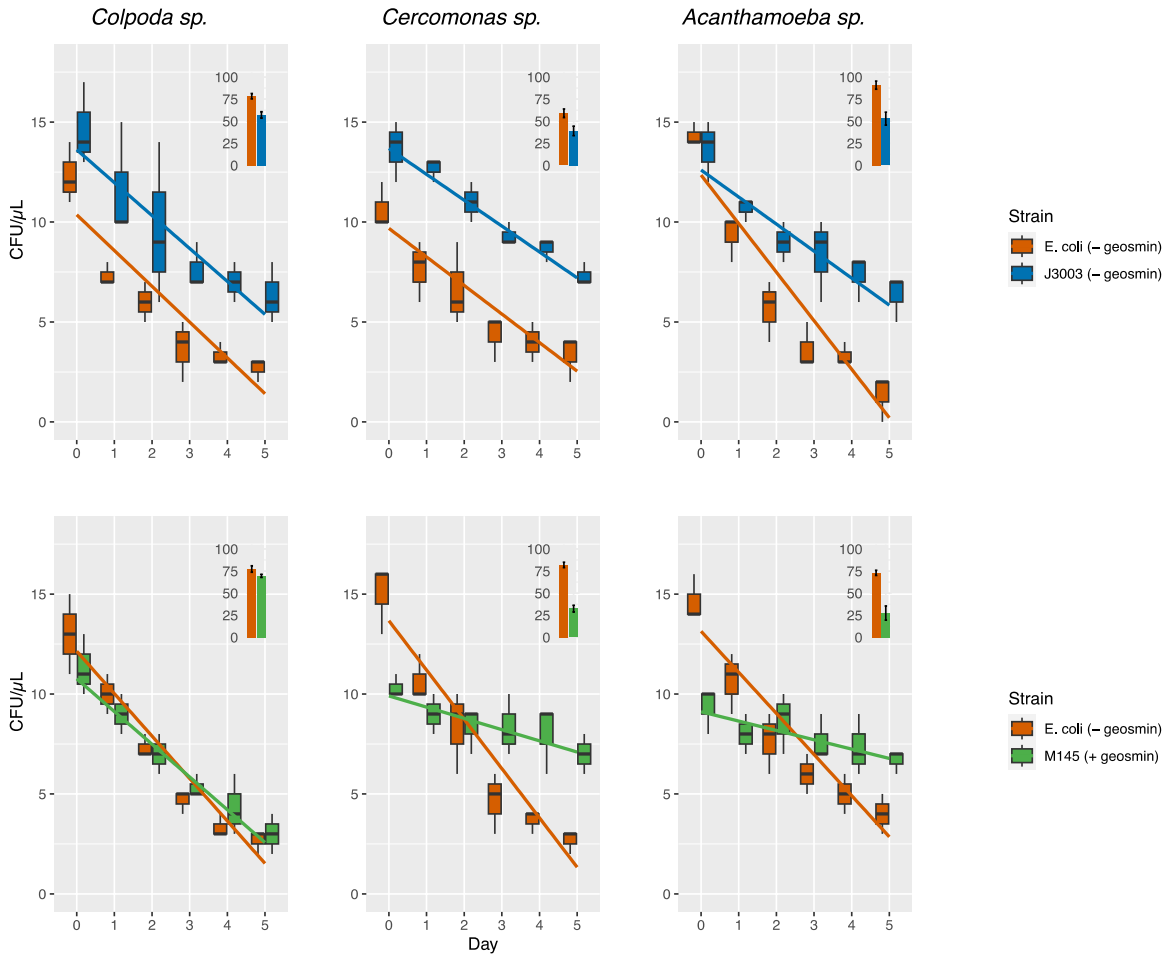

Fig. 4 Predation of bacteria when *Colpoda* sp. (left), *Cercomonas* sp. (middle), and *A. castellanii* (right) were fed equal meals of (top) *E. coli* DH5α (orange) and *S. coelicolor* J3003 (− geosmin, blue) or (bottom) *E. coli* DH5α and *S. coelicolor* M145 (+ geosmin, green). Inset: Percent of bacteria consumed by day 5 with each of the different bacteria. We note that we included the *E. coli* results as a baseline across the experiments to compare predation rates to either the geosmin-producing *S. coelicolor* wild-type strain (M145) or the mutant strain of *S coelicolor* (J3003) that does not produce geosmin

and geosmin-producing bacteria was reduced compared to bacteria which did not produce geosmin [5]. Together these data suggest that geosmin is used by bacterial producers as an anti-predation mechanism.

The differential responses to geosmin across the three protist species studied, *Colpoda* sp., *Cercomonas* sp., and *A. castellanii*, indicate varying abilities to detect and respond to this signal. Protists are generally characterized into four morphological groups: ciliates, flagellates, naked amoeba, and testate amoeba. Previous work has shown that the "area of influence" protists have on their surrounding environment can be directly associated with morphological type [28]. As a ciliate, *Colpoda* sp. has numerous cilia that facilitate rapid movement and environmental processing, allowing it to come into contact with geosmin more quickly than the slower-moving flagellates (*Cercomonas* sp.) and amoebae (*A. castellanii*). However, while encysted protists are still able to detect signals from their environment [17], further research is needed to determine how *Colpoda* sp. was able to excyst in the presence of geosmin at a faster rate than *Cercomonas* sp. and *A. castellanii* (Figs. 2 and 3).

Morphological type also has direct effects on feeding behavior [29]. Ciliates often quickly filter their environment through oral funnels, while flagellates and amoeba have more selective feeding strategies, typically employing flagella and pseudopodia [30–34]. This enhanced motility and ability to filter-feed may enable *Colpoda* sp. to detect chemical cues in the environment more efficiently. *Colpoda* sp. feeding behavior, which does not show strong selectivity between prey of similar sizes, such as *E. coli* and *S. coelicolor*, supports the idea that it uses rapid processing of the environment to capitalize on available resources (Fig. 4). In contrast, the slower excystment and selective feeding observed in *Cercomonas* sp. and *A. castellanii* may reflect

their different ecological niches and foraging strategies, specifically more selective feeding, suggesting a more conservative strategy for resource exploitation.

While this study focused on soil protists, it is important to consider the implications for freshwater systems. Although geosmin can be produced in both soil and aquatic ecosystems [19], the concentration and distribution patterns of geosmin may vary, potentially leading to different rates of response from aquatic protists as compared to those observed with soil protists here. It has been shown that aquatic protists are able to respond to geosmin [17], but given that signaling molecules may disperse differently in water than in soil, it is possible that aquatic protists have evolved different mechanisms for detecting and responding to geosmin. Further studies are needed to explore whether the mechanisms responsible for the observed behaviors in soil protists are conserved in aquatic protists, and how factors such as water flow, oxygen availability, temperature, and microbial community composition influence both the concentration of geosmin in these environments and protist behavioral responses [35, 36].

To build on these findings, future research should investigate the effects of geosmin on protists and other soil microorganisms under different environmental conditions, such as varying moisture levels, geosmin concentrations, and the presence of other VOCs. It would be particularly valuable to determine the minimum geosmin concentration required to elicit excystment and other behavioral changes in protists, and how these thresholds might vary across different taxa. Additionally, examining a broader range of protist species, including representatives from other morphological groups (including testate amoebae and additional ciliate species), could provide insight into the generality of geosmin effects. Controlled experiments simulating natural soil and aquatic environments could further elucidate how complex ecological factors interact to modulate the responses of protists to VOCs. Understanding these dynamics would have significant implications for predicting how protist-mediated processes, such as bacterial predation and nutrient cycling, respond to the microbial production of geosmin.

Acknowledgements Support for this work was provided by the United States National Science Foundation (DEB 2126106). We also want to thank collaborators who helped with the design and implementation of this project including Michael Hoffert, Osnat Gillor, Haik Gevorgyan, Ben Poodiack, and Hagar Siebner.

Author Contributions Study conception and design was performed by J.M and N.F. Material preparation and data collection were performed by J.M. and C.B. Data analysis and figure preparation was performed by J.M. The first draft of the manuscript was written by J.M. and all authors read and approved the final manuscript.

Funding This work was supported by the United States National Science Foundation (DEB 2126106).

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

References

1. Gerber NN, Lechevalier HA (1965) Geosmin, an earthy-smelling substance isolated from actinomycetes. Appl Microbiol 13:935–938. https://doi.org/10.1128/am.13.6.935-938.1965
2. Garbeva P, Avalos M, Ulanova D, van Wezel GP, Dickschat JS (2023) Volatile sensation: the chemical ecology of the earthy odorant geosmin. Environ Microbiol 25:1565–1574. https://doi.org/10.1111/1462-2920.16381
3. Gerber NN (1968) Geosmin, from microorganisms, is trans-1, 10-dimethyl-trans-9-decalol. Tetrahedron Lett 9:2971–2974. https://doi.org/10.1016/S0040-4039(00)89625-2
4. Stensmyr MC, Dweck HKM, Farhan A, Ibba I, Strutz A, Mukunda L et al (2012) A conserved dedicated olfactory circuit for detecting harmful microbes in Drosophila. Cell 151:1345–1357. https://doi.org/10.1016/j.cell.2012.09.046
5. Zaroubi L, Ozugergin I, Mastronardi K, Imfeld A, Law C, Gélinas Y et al (2022) The ubiquitous soil terpene geosmin acts as a warning chemical. Appl Environ Microbiol 88:e00093-e122. https://doi.org/10.1128/aem.00093-22
6. Huang H, Ren L, Li H, Schmidt A, Gershenzon J, Lu Y et al (2020) The nesting preference of an invasive ant is associated with the cues produced by actinobacteria in soil. PLoS Pathog 16:e1008800. https://doi.org/10.1371/journal.ppat.1008800
7. Melo N, Wolff GH, Costa-da-Silva AL, Arribas R, Triana MF, Gugger M et al (2020) Geosmin attracts Aedes aegypti mosquitoes to oviposition sites. Curr Biol CB 30:127-134.e5. https://doi.org/10.1016/j.cub.2019.11.002
8. Scarano F, Deivarajan Suresh M, Tiraboschi E, Cabirol A, Nouvian M, Nowotny T et al (2023) Geosmin suppresses defensive behaviour and elicits unusual neural responses in honey bees. Sci Rep 13:3851. https://doi.org/10.1038/s41598-023-30796-5
9. Wood M, Chamaillé-Jammes S, Hammerbacher A, Shrader AM (2022) African elephants can detect water from natural and artificial sources via olfactory cues. Anim Cogn 25:53–61. https://doi.org/10.1007/s10071-021-01531-2

10. Becher PG, Verschut V, Bibb MJ, Bush MJ, Molnár BP, Barane E et al (2020) Developmentally regulated volatiles geosmin and 2-methylisoborneol attract a soil arthropod to Streptomyces bacteria promoting spore dispersal. Nat Microbiol 5:821–829. https://doi.org/10.1038/s41564-020-0697-x
11. Oliverio AM, Geisen S, Delgado-Baquerizo M, Maestre FT, Turner BL, Fierer N (2020) The global-scale distributions of soil protists and their contributions to belowground systems. Sci Adv 6. https://doi.org/10.1126/sciadv.aax8787
12. Bonkowski M (2004) Protozoa and plant growth: the microbial loop in soil revisited. New Phytol 162:617–631. https://doi.org/10.1111/j.1469-8137.2004.01066.x
13. Rønn R, McCaig AE, Griffiths BS, Prosser JI (2002) Impact of protozoan grazing on bacterial community structure in soil microcosms. Appl Environ Microbiol 68:6094–6105. https://doi.org/10.1128/AEM.68.12.6094-6105.2002
14. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol Online Plantannualreviewsorg 57:233–266. https://doi.org/10.1146/annurev.arplant.57.032905.105159
15. Hawxhurst CJ, Micciulla JL, Bridges CM, Shor M, Gage DJ, Shor LM (2023) Soil protists can actively redistribute beneficial bacteria along Medicago truncatula roots. Appl Environ Microbiol 89:e01819-e1822. https://doi.org/10.1128/aem.01819-22
16. Geisen S, Mitchell EAD, Adl S, Bonkowski M, Dunthorn M, Ekelund F et al (2018) Soil protists: a fertile frontier in soil biology research. FEMS Microbiol Rev 42:293–323. https://doi.org/10.1093/femsre/fuy006
17. Li Y, Wang Y, Zhang S, Maurer-Alcalá XX, Yan Y (2022) How ciliated protists survive by cysts: some key points during encystment and excystment. Front Microbiol 13. https://doi.org/10.3389/fmicb.2022.785502
18. Roberts EC, Legrand C, Steinke M, Wootton EC (2011) Mechanisms underlying chemical interactions between predatory planktonic protists and their prey. J Plankton Res 33:833–841. https://doi.org/10.1093/plankt/fbr005
19. Schulz-Bohm K, Geisen S, Wubs ERJ, Song C, De Boer W, Garbeva P (2017) The prey's scent – Volatile organic compound mediated interactions between soil bacteria and their protist predators. ISME J 11:817–820. https://doi.org/10.1038/ismej.2016.144
20. Avalos M (2019) Volatile compounds from Actinobacteria as mediators of microbial interactions. Doctoral dissertation, Leiden University
21. Corliss JO, Page FC (1977) An illustrated key to freshwater and soil amoebae with notes on cultivation and ecology. Trans Am Microsc Soc. https://doi.org/10.2307/3226125
22. Taerum SJ, Steven B, Gage DJ, Triplett LR (2020) Validation of a PNA clamping method for reducing host DNA amplification and increasing eukaryotic diversity in Rhizosphere microbiome studies. Phytobiomes J 4:291–302. https://doi.org/10.1094/PBIOMES-05-20-0040-TA
23. Bentley SD, Chater KF, Harper D, Bateman A, Brown S, Chandra G et al (2002) Complete genome sequence of the model actinomycete Streptomyces coelicolor A3(2). Nature 417:141–147
24. Gust B, Challis GL, Fowler K, Kieser T, Chater KF (2003) PCR-targeted Streptomyces gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. PNAS 100:1541–1546. https://doi.org/10.1073/pnas.0337542100
25. Jiang J, He X, Cane DE (2006) Geosmin biosynthesis. Streptomyces coelicolor Germacradienol/Germacrene D synthase converts farnesyl diphosphate to geosmin. J Am Chem Soc 128:8128–9. https://doi.org/10.1021/ja062669x
26. Jones SE, Elliot MA (2017) Streptomyces exploration: competition, volatile communication and new bacterial behaviours. Trends Microbiol 25:522–531. https://doi.org/10.1016/j.tim.2017.02.001
27. Jüttner F, Watson SB (2007) Biochemical and ecological control of geosmin and 2-methylisoborneol in source waters. Appl Environ Microbiol 73:4395–4406. https://doi.org/10.1128/AEM.02250-06
28. Micciulla JL, Shor LM, Gage DJ (2024) Enhanced transport of bacteria along root systems by protists can impact plant health. Appl Environ Microbiol 90:e02011-e2023. https://doi.org/10.1128/aem.02011-23
29. Esteban GF, Fenchel TM (2020) Ecology of protozoa. 2020. https://doi.org/10.1007/978-3-030-59979-9
30. Boenigk J, Matz C, Jürgens K, Arndt H (2001) Confusing selective feeding with differential digestion in bacterivorous nanoflagellates. J Eukaryot Microbiol 48:425–432. https://doi.org/10.1111/j.1550-7408.2001.tb00175.x
31. Boenigk J, Arndt H (2002) Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Antonie Van Leeuwenhoek 81:465–480. https://doi.org/10.1023/A:1020509305868
32. Böhme A, Risse-Buhl U, Küsel K (2009) Protists with different feeding modes change biofilm morphology. FEMS Microbiol Ecol 69:158–169. https://doi.org/10.1111/j.1574-6941.2009.00710.x
33. Shi Y, Queller DC, Tian Y, Zhang S, Yan Q, He Z et al (2021) The ecology and evolution of amoeba-bacterium interactions. Appl Environ Microbiol 87:e01866-e1920. https://doi.org/10.1128/AEM.01866-20
34. Verni F, Gualtieri P (1997) Feeding behaviour in ciliated protists. Micron. https://doi.org/10.1016/S0968-4328(97)00028-0
35. Schrader KK, Blevins WT (2001) Effects of carbon source, phosphorus concentration, and several micronutrients on biomass and geosmin production by Streptomyces halstedii. J Ind Microbiol Biotechnol 26:241–247. https://doi.org/10.1038/sj.jim.7000121
36. Dionigi CP, Ingram DA (1994) Effects of temperature and oxygen concentration on geosmin production by Streptomyces tendae and Penicillium expansum. J Agric Food Chem 42:143–145. https://doi.org/10.1021/jf00037a025