



Top-down predators shape soil bacterial community composition while bottom-up nutrients drive bacterial abundance

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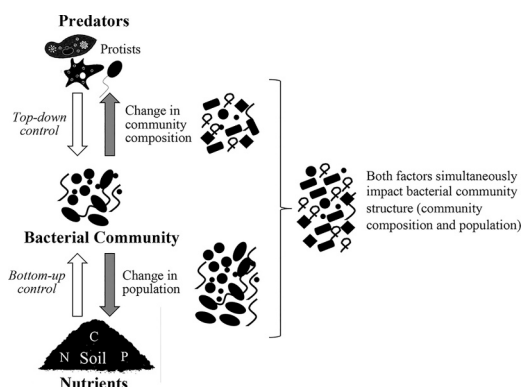
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HIGHLIGHTS

- The top-down predators modulated the bacterial community composition.
- The bacterial gene abundances were primarily controlled by the bottom-up nutrients.
- Both top-down and bottom-up factors shaped the absolute abundance of bacteria.
- Most affected families by top-down and bottom-up showed similarities.

GRAPHICAL ABSTRACT



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ABSTRACT

Although the top-down and bottom-up concept in microbial food-webs has been a primary interest in ecology, less is still known about it in soil ecosystems. Protists are the primary top-down predators of bacterial communities, altering their compositions, while the bottom-up resources are the primary factors limiting bacterial growth. Here, we hypothesized that the top-down predators modulate soil bacterial community composition, while the bottom-up nutrients control the bacterial growth and population. To precisely control nutrient levels, we used an inert soil substitute consisting of a combination of calcined clay and sand. Nutrients equivalent to the reference paddy field soil were added to microcosms as a control treatment. To investigate the effects of C, N, and P, six additional bottom-up treatments in the absence and double amounts of the nutrients were prepared. Four top-down treatments (no protist addition, *Acanthamoeba castellanii*, *Vermamoeba vermiformis*, and *Heteromita globosa*) were set up for each bottom-up treatment. A total of 252 microcosms under 28 treatments were incubated. Bacterial communities were analyzed using high-throughput sequencing and real-time PCR in the 1st, 3rd, and 5th weeks. The results revealed that the top-down predators significantly altered the bacterial community composition, and the bacterial population was predominantly controlled by the bottom-up nutrients. Analysis of absolute abundance data demonstrated that both top-down and bottom-up factors shaped the bacterial community structure (community composition and population). Random forest analysis classified the amplicon sequence variants associated with the treatments, showing that mostly similar families were affected by both bottom-up and top-down factors. In conclusion, the results of this study fully supported our hypothesis that top-down predators alter community composition, while bottom-up factors influence bacterial population dynamics.

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1. Introduction

The factors shaping communities and populations are often classified as either top-down (predator-driven) or bottom-up (nutrient-driven) factors (Hairston et al., 1960; Hunter and Price, 1992). Thus, understanding the relative importance of top-down versus bottom-up factors on food-webs has been a long-standing area of interest in ecology (Leroux and Loreau, 2015). Despite some primary factors not included in this concept, the top-down and bottom-up concept, which is well-adopted in microbial ecology in marine and freshwater ecosystems (Berdjeb et al., 2011; Chow et al., 2014; Lynam et al., 2017), answers important ecological questions. Microbial population and community composition are simultaneously affected by the top-down predators and bottom-up nutrients. The relative importance of the top-down and bottom-up factors can vary depending on the ecosystem and environmental conditions (Berdjeb et al., 2011; Chow et al., 2014; Lynam et al., 2017). For instance, bacterial communities in marine ecosystems are generally top-down regulated (Weinbauer et al., 2003, 2007; Chow et al., 2014; Teira et al., 2019), while bottom-up factors play a relatively bigger role in freshwater ecosystems (Jardillier et al., 2005; Berdjeb et al., 2011). Although the top-down and bottom-up concepts are crucial for predicting the responses of microbial communities to environmental changes, still, less is known about this concept for bacterial communities and populations in the soil ecosystem (Asiloglu et al., 2021; Liu et al., 2024).

Protists are the primary top-down predators of bacterial communities in the soil ecosystem (Gao et al., 2019). Protist predation shows species-specific patterns; therefore, the effects of protists on bacterial communities vary depending on the protist traits, such as size, feeding type, and taxonomy (Geisen et al., 2018; Gao et al., 2019). Protists selectively feed on bacterial taxa decreasing their population, while the non-targeted or survived bacterial taxa take advantage of protist predation through mainly predation-related nutrient turnover (Protists consume bacteria and release the excess of nutrients such as nitrogen, phosphorus, or micronutrients), increasing their population (Gao et al., 2019; Leander, 2020). Although the effect of protists on the bacterial community composition has been confirmed in various environmental conditions, top-down control on bacterial populations may vary depending on soil biological and physicochemical properties (Geisen et al., 2018; Gao et al., 2019; Fujino et al., 2023; Bodur et al., 2024a). Taking together, protist-predation is considered one of the most important factors controlling bacterial community composition. The bottom-up resources (organic and inorganic fertilizers, root exudates, and initial soil nutrients) are the primary factors limiting bacterial growth and controlling bacterial populations (George et al., 2024; Qiao et al., 2024). In addition to their effect on the bacterial population, the bottom-up nutrients shape the bacterial community composition as well, which is often associated with competition among bacterial species (Hibbing et al., 2010; Guo et al., 2024). In our previous study, we showed that the effect of top-down predation is stronger on the bacterial community composition than those of bottom-up factors in paddy field soil (Asiloglu et al., 2021). Considering that higher microbial biomass is often associated with increased resource availability, bottom-up nutrients rather than top-down predators are more likely to control overall bacterial growth and population size. Therefore, here we hypothesized that while both factors influence composition and population, top-down predators exert a stronger effect on soil bacterial community composition, whereas bottom-up nutrients have a relatively greater impact on bacterial growth and population size.

The results of our previous study were in agreement with top-down predators exerting a stronger effect on soil bacterial community composition (Asiloglu et al., 2021). However, our previous study lacks the data on the bacterial population that is crucial to estimating community structure (population and community composition). In addition, we previously studied fertilizer addition as a bottom-up factor, which accounts for approximately 5–10 % of the total nutrients in the soil.

Therefore, the initial soil nutrients should have a bigger bottom-up impact on the bacterial communities, which are mostly neglected due to methodological challenges. The most often used method to understand the contribution of top-down and bottom-up factors on microbial communities is conducting *in vitro* experiments in the presence and absence of predators and nutrients ((Wollrab et al., 2012; Leroux and Loreau, 2015; Lynam et al., 2017; Asiloglu et al., 2021)). However, it is challenging to obtain a nutrient-free soil. Therefore, in this study, we used an inert soil substitute, a combination of calcined clay and sand, allowing us to precisely control nutrient levels (Bai et al., 2015; Lebeis et al., 2015; Zhang et al., 2019). We first analyzed the soil nutrients of a reference paddy field soil and then obtained a protist-free indigenous bacterial community from the same soil. The corresponding soil nutrients were added to the inert soil substitute. The most important nutrients for bacteria in the soil ecosystem are carbon (C), nitrogen (N), and phosphorus (P) (Xu et al., 2024). Here we designed an *in vitro* experiment consisting of 252 microcosms to study the bottom-up effects of C, N, and P (absent, present, and in double amounts of the reference paddy field), and the top-down effects of three axenic predatory protists species. High-throughput sequencing and real-time PCR methods were employed to study the bacterial community composition and population dynamics (16S rRNA gene abundances), respectively.

2. Materials and methods

2.1. Soil and protist-free indigenous bacterial communities

Soil samples were taken from the plow layer (0–10 cm) in a paddy field at Shindori Station in the Field Centre for Sustainable Agriculture and Forestry, Niigata University, Niigata, Japan (N37.86, E138.96) on February 8, 2022. The soils were air-dried, sieved (<2 mm), and then stored at 4 °C. The protist-free bacterial community was obtained from the collected paddy field soil using a filtration method (Asiloglu et al., 2021). Briefly, 300 mL ultra-pure H₂O was added to 200 g of the soil and then filtered (1.2 µm, Advantec, Tokyo, Japan) using a vacuum filter. The absence of protists was confirmed with an inverted microscope (Nikon Eclipse TE2000-S, Tokyo, Japan).

2.2. Top-down and bottom-up factors

Three axenic cultures of protist isolates (*Veramoeba vermiformis* [50256TM], *Acanthamoeba castellanii* [30234TM], and *Heteromita globosa* LAP3-2017) were used in this study. *V. vermiformis* and *A. castellanii* were purchased from the American Type Culture Collection (ATCC) and *H. globosa* LAP3-2017 was isolated from a paddy field (Asiloglu et al., 2020). Before the experiment, each protist species was axenically grown for 2 weeks in sterile amoeba saline solution (AMS) (containing per liter: 120 mg NaCl, 4 mg MgSO₄·7H₂O, 4 mg CaCl₂·2H₂O, 142 mg Na₂HPO₄ and 136 mg KH₂PO₄) (Page, 1988) including heat-killed *Escherichia coli* MG1655 (approximately 6×10^6 cells mL⁻¹).

To determine the amount of the bottom-up nutrients, we first characterized the chemical properties of the paddy field soil. Carbon and nitrogen contents were determined after drying the soil at 105 °C for 24 h using an MT-700 Mark 2 CN analyser (Yanaco, Kyoto, Japan). Available P was extracted from 0.5 g of the soil samples with 0.002 N H₂SO₄ and then colorimetrically analyzed by a spectrophotometer (UV-160 A, Shimadzu, Kyoto, Japan) according to Truog (1930). The exchangeable cations (Ca, Mg, K, and Na) were extracted from the soil samples with 1 M ammonium acetate (pH 7.0) and measured in an atomic absorption spectrophotometer (McGeorge, 1954).

2.3. Experimental set-up

The experiment was carried out under laboratory conditions, a mixture of calcined clay (60 %) and sand (40 %) was used instead of soil to precisely control nutrient levels (Bai et al., 2015; Lebeis et al., 2015;

Zhang et al., 2019). To mimic the paddy field soil, we added the same amount of C, N, P, K, Mg, and Ca of the reference paddy field soil into the microcosms as indicated in Table 1. To understand the bottom-up effects of C, N, and P on the bacterial communities, treatments in the absence, presence, and double amounts of C, N, and P were set up, making 7 bottom-up treatments (Table 1). About 500 cells g⁻¹ soil of each axenic protist species were added into the microcosms, while Ctrl treatment received the same amount of sterile water. We had 4 top-down treatments (Ctrl; Control with no protist addition; Ac, *Acanthamoeba castellanii*; Vv, *Vermamoeba vermiformis*; Hg, *Heteromita globosa*), making in total 28 treatments ($n = 9$). The microcosms were incubated at room temperature in the dark for 5 weeks. Three replications of the microcosms for each treatment were destructively sampled at the 1st, 3rd, and 5th weeks as described previously (Asiloglu et al., 2021). Briefly, the surface water of the microcosms was removed, and the calcined clay was mixed thoroughly. Immediately, 0.5 g of calcined clay sample was placed into 2 mL DNA extraction tubes and stored at -80 °C until nucleic acid extraction. DNA was extracted using ISOIL for Bead Beating (Nippon Gene, Tokyo, Japan) according to the manufacturer's instructions and eluted in TE buffer (50 µL). The rest of the calcined clay samples were stored at 4 °C for physicochemical analysis.

2.4. Molecular analysis, bioinformatic and statistics

The 0.5 g of the calcined clay and sand mixture was used for DNA extraction. The DNA was extracted using ISOIL for Bead Beating (Nippon Gene, Tokyo, Japan). The PCR by using barcoded universal 16S rRNA gene primers (515F-806R), Illumina MiSeq sequencing, and primary analyses of raw FASTQ data were performed as described previously (Asiloglu et al., 2021). Briefly, the primary analysis of raw FASTQ data was processed using DADA2 in the QIIME2 pipeline (version 2021.11, <https://qiime2.org>). DADA2 in QIIME2 has been used to denoise the paired-end sequences into amplicon sequence variants (ASVs) after random resampling. QIIME2's q2-feature-classifier plugin was used for taxonomy assignment against the latest SILVA reference database (138.1 release). The rarefied sequences were used to generate the dissimilarity matrices based on the Bray–Curtis distances using the phyloseq package. The matrices were then used to calculate the permutational multivariate

Table 1
Amount and sources of the bottom-up nutrients, and the experimental set-up.

Bottom-up treatments	Bottom-up nutrients (mg g soil ⁻¹)					
	C	N	P	K	Mg	Ca
Reference paddy field soil	26.29	2.47	0.04	0.21	0.42	1.03
Regular nutrients (RN)	26.29	2.47	0.04	0.21	0.42	1.03
Carbon high (CH)	52.58	2.47	0.04	0.21	0.42	1.03
Carbon low (CL)	0	2.47	0.04	0.21	0.42	1.03
Nitrogen high (NH)	26.29	4.94	0.04	0.21	0.42	1.03
Nitrogen low (NL)	26.29	0	0.04	0.21	0.42	1.03
Phosphorus high (PH)	26.29	2.47	0.08	0.21	0.42	1.03
Phosphorus low (PL)	26.29	2.47	0	0.21	0.42	1.03
Nutrient sources	Glucose, Glycine, Sucrose	NH ₄ NO ₃	Ca (H ₂ PO ₄) ₂	KCl	MgSO ₄	CaCl ₂

For each bottom-up treatment, four top-down treatments were prepared (Ctrl, no protist addition; Ac, *Acanthamoeba castellanii*, Hg, *Heteromita globosa*, and Vv, *Vermamoeba vermiformis* [$n = 9$]).

analysis of variance (PERMANOVA) with the adonis function in the vegan package. Principal coordinate analysis (PCoA) based on the Bray–Curtis distance matrix was used to visualize the bacterial beta diversities using Phyloseq and Vegan packages in R with the betadisper function. First, ASV table was obtained from the Phyloseq package, and then Bray–Curtis distance matrix was created using *bcdist* function in Ecodist package.

Bacterial gene abundances were measured by a quantitative real-time PCR (qPCR) using the same primers without the barcode as described previously (Fujino et al., 2023), which indicates bacterial population, while the relative abundances-based results indicate bacterial community composition. To reveal the effects of the top-down and bottom-up factors on bacterial community composition and gene abundances, we first created a scatter plot, in which the y-axis indicates PC2 of relative abundance-based PCoA as the effect of protists was best explained by PC2. The x-axis indicates bacterial gene abundance, which mainly explains the effect of bottom-up control.

The bacterial community structure that includes both community composition and population data is referred to as absolute abundance. The bioinformatic analyses for absolute abundances were done as described previously (Bodur et al., 2024b) with modifications. Briefly, the ASV table was obtained from the phyloseq object and the relative abundances were calculated. Then, the relative abundances were multiplied by the bacterial gene abundances to obtain an absolute abundance ASV table. For PERMANOVA and PCoA analyses, a log transform was applied to the absolute abundance ASV table to handle heteroscedasticity in the data (Barlow et al., 2020) using *log* function in R. For the Random Forest analysis, the ASV table in the phyloseq object was replaced with absolute abundance ASV table without transforming to log values. Random Forest analysis was conducted in the R program using the *randomForest* function in the randomForest package (version 4.7–1.1). To calculate the effect of top-down and bottom-up factors on bacterial absolute abundances, the samples were separated to compare each protist treatment with the control treatment or to compare each nutrient treatment with the control treatment. Mean decreased gini (MDG) values were obtained using a machine learning algorithm of Random Forest analysis based on the differences between top-down or bottom-up treatments and their representative controls. Random Forest provides an importance ranking of ASVs (represented by MDGs), but it doesn't assess statistical significance. The significance of random forest results was evaluated by the *rFPermute* package (v2.5.1)(Archer, 2013), which provides an additional layer of validation by calculating the significance of variable importance metrics for each ASV. Only ASVs with statistically significant MDG values ($p < 0.05$) were used in the further calculations. All of the statistical analyses were performed in R program version 4.2.2 (2022.10.31; <https://www.r-project.org/>) unless otherwise specified.

3. Results

3.1. Nutrients

The nutrient application levels in microcosms varied significantly across the three treatment conditions (High, Regular, Low) for total C, total N, and available P (Fig. 1). For C (Fig. 1A), the High treatment exhibited a significantly higher median value (approximately 10 mg g-soil⁻¹) compared to the Regular treatment (around 5 mg g-soil⁻¹) and the Low treatment (near 0 mg g-soil⁻¹). Statistical analysis confirmed that the differences among all three treatment groups were significant ($p < 0.05$). Similarly, N (Fig. 1B) levels were highest in the High treatment (median around 2.5 mg g-soil⁻¹), followed by the Regular treatment (median near 1.5 mg g-soil⁻¹) and the Low treatment (median around 0.5 mg g-soil⁻¹). Significant differences were observed among the three treatment levels ($p < 0.05$). For P (Fig. 1C), the High treatment again had the highest median value (around 0.2 mg g-soil⁻¹), with the Regular treatment showing a slightly lower median (just above 0.1 mg g-soil⁻¹),

and the Low treatment having the lowest median (approximately 0.05 mg g-soil⁻¹). The statistical analysis indicated that the High treatment was significantly different from the Low treatment, while the Regular treatment showed some overlap with both High and Low treatments. The nutrient levels were measured at the 1st (W1), 3rd (W3), and 5th (W5) weeks (Fig. S1), and there was a decrease over time. However, the nutrient levels maintained the same pattern among the High, Regular, and Low treatments. The presence of the top-down predators had no effect on the nutrient levels.

3.2. Bacterial community composition and 16S rRNA gene abundances

After filtering, we obtained 6,271,562 sequences (21,852 median reads per sample) with 7225 features across the 252 samples. Principal Coordinate Analysis (PCoA) based on the Bray-Curtis dissimilarity index (Fig. 2A) shows the bacterial community composition among different treatments based on relative abundance data. The principal coordinates explain 38.01 % (PC1) and 14.06 % (PC2) of the total variation, and PC2 indicated a difference in the protist treatments, while no consistent patterns were observed by the bottom-up treatments. The distinct clustering of the groups, as visualized by the ellipses, indicates variation in community composition by the top-down predators. This was confirmed by the PERMANOVA analysis (Fig. 2 and Table S1), which revealed that the top-down predators had a significant impact ($p < 0.001$) on the bacterial community composition. In contrast, the bottom-up nutrients' effect was not significant ($p > 0.05$).

The box plots (Fig. 2B–C) show the effects of top-down and bottom-up factors on the 16S rRNA gene copy numbers (bacterial abundance). Although the top-down predators slightly decreased the bacterial abundances ($p = 0.03$), only Hg treatment showed a significant decrease (Fig. 2B). Bottom-up factors, on the other hand, had a stronger impact on the bacterial abundance ($p < 10^{-16}$). Among the nutrients, C had the strongest effect, followed by N and P, respectively (Fig. 2C). Among the treatments, the lowest bacterial abundance was observed in low C treatments, while high C treatment had the highest bacterial abundance (Fig. S2).

3.3. Bacterial community structure (absolute abundances)

To check the hypothesis, the top-down predators modulate soil bacterial community composition, while the bottom-up nutrients control the bacterial population, we illustrated the interaction between bacterial community composition and bacterial gene abundances with a scatter plot (Fig. 3A). The result showed a clear separation along the x-axis (representing bacterial abundance), with nutrient-enriched treatments positioned on the right side and the regular nutrient treatments positioned in the middle (Fig. 3A). Along the y-axis (representing bacterial community composition), treatments with predators are

positioned towards the top of the plot (Fig. 3A). Although a few samples didn't follow this pattern, in general, the scatter plot (Fig. 3A) clearly illustrated our hypothesis. Then we conducted a PCoA analysis based on the absolute abundances to see the effects of top-down and bottom-up factors on the bacterial community structure (community composition and population), which revealed distinct clustering patterns driven by the top-down predators and the bottom-up nutrients (Fig. 3B). Along the first principal coordinate (PC1), a clear separation was observed between communities subjected to nutrient enrichment and those without nutrient addition (Fig. 3B). The second principal coordinate (PC2) separated communities based on the presence or absence of predators (Fig. 3B). The PERMANOVA analysis showed that both protists ($p < 0.001$) and nutrient treatments ($p < 0.001$) significantly influenced bacterial community structure. The PCoA and PERMANOVA analyses based on absolute abundances (Fig. 3B) corroborate the findings from the scatter plot analysis (Fig. 3A), further emphasizing the importance of both resource availability and predation in shaping the bacterial community structure.

Next, we performed a random forest analysis to identify bacterial ASVs that classified each treatment. The results showed that the top-down factors had higher importance (Mean Decrease Gini) for shaping bacterial community structure (Fig. 4A). Then we checked the distribution of the total absolute abundances of top-down (Ac, Hg, and Vv) or bottom-up (C, N, and P) classified ASVs in each factor (Fig. 4B). The result showed that the absolute abundance of the bacterial communities exhibited a bidirectional influence, with both bottom-up and top-down factors influenced by the other. In addition, the CH and NH treatments exhibited the highest absolute abundance of classified ASVs with contributions from both top-down and bottom-up classifiers. Then we analyzed the family-level similarities of the top-down and bottom-up classified ASVs (Fig. 4C). Of the 142 families, 45 were unique for the top-down factors, and 49 were unique for the bottom-up factors, while 48 were shared by both factors (Fig. 4C). While the Sphingomonadaceae, Nocardioidaceae, Comamonadaceae, and Xanthobacteraceae were the first four most affected bacterial family, the gini values of the shared families differed according to the type of protist and nutrient. The top-down factors especially affected specific bacterial families, where the total mean decreased gini values for the top 3 families represented more than half, while bottom-up factors exerted a more uniform effect on the shared families (Fig. 4D). The same patterns were observed not only for shared families but also all families (Fig. S3).

4. Discussion

Understanding the relative importance of top-down and bottom-up factors contributes to ecosystem structure and dynamics and is crucial for developing effective management strategies to maintain biodiversity and ecosystem health (Leroux and Loreau, 2015). Although the factors

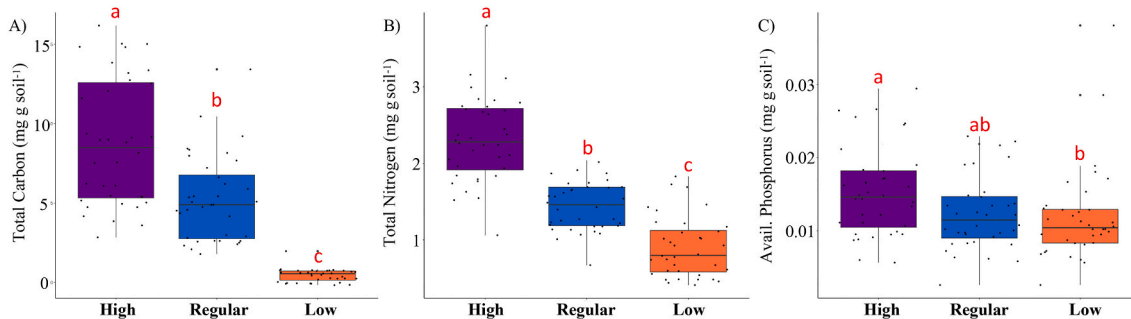


Fig. 1. Nutrient levels in microcosms at each sampling time. Box plots showing the concentration of nutrient levels across the treatments (High, Regular, Low). A, Total Carbon (mg g soil⁻¹); B, Total Nitrogen (mg g soil⁻¹), C, Available Phosphorus (mg g soil⁻¹). The purple, blue, and orange colors represent High, Regular, and Low treatments, respectively. The central line in the boxplot represents the median, box hinges represent the first and third quartiles. Lines indicate minimum and maximum values. Different letters represent significant differences ($p < 0.05$, ANOVA with Tukey's *post hoc* test).

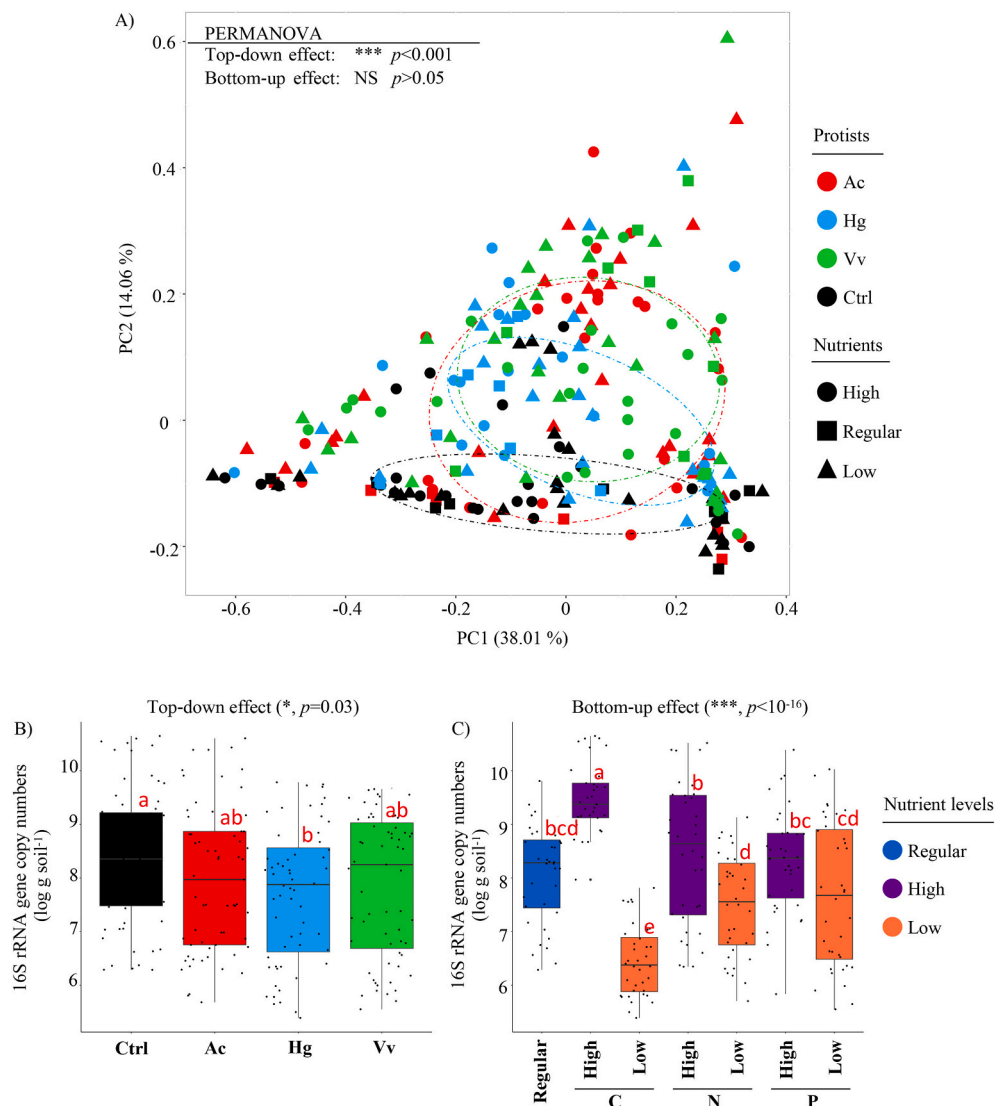


Fig. 2. Effects of the top-down and bottom-up factors on bacterial community composition and gene abundances. A, Principal Coordinate Analysis (PCoA) with confidence ellipses based on the eigenvalues of the covariance matrix. The box plots show the effects of top-down (B) and bottom-up (C) factors on the bacterial population (16S rRNA gene copies). The central line in the boxplot represents the median, box hinges represent the first and third quartiles. Lines indicate minimum and maximum values. Different letters represent significant differences ($p < 0.05$, ANOVA with Tukey's *post hoc* test).

that are not considered in the top-down and bottom-up concept, such as pH, soil organic matter, and soil moisture, have a profound impact on bacterial communities and populations, our results showed that the top-down predators shape the bacterial community composition, while the bottom-up nutrients maintain bacterial growth. In this study, we used an inert soil substitute (calcined clay and sand), which may have some limitations due to its simplified composition that is lack of soil complexity and microporosity. As the soil complexity and microporosity enhance bacterial survival from predation, the top-down effect of predators may have been overestimated in the calcined clay system. On the other hand, due to the lack of surface charge, nutrients are freely available in the calcined clay and sand combination, which could influence the bottom-up effect. However, the inert soil substitute is often used in confirmation experiments in microbiology, which provides consistent results with field experiments (Bai et al., 2015; Lebeis et al., 2015; Zhang et al., 2019). Therefore, while acknowledging the potential limitations, our results provide valuable insights into the relative importance of top-down and bottom-up forces in shaping bacterial community structure in the soil ecosystem.

4.1. Top-down control

The primary factor shaping the bacterial community composition was the top-down predators, while no significant effect was observed by the bottom-up nutrients, confirming our hypothesis. Our results are also consistent with our previous study (Asiloglu et al., 2021), in which the top-down effect of predators was bigger than the bottom-up effect of fertilizers in paddy field soil. Furthermore, a previous long-term field study showed that rather than the bottom-up nutrients, top-down predators were the major factor controlling bacterial community composition (Liu et al., 2024). It is well known that predatory protists shape the bacterial community composition in the soil ecosystem (Gao et al., 2019; Leander, 2020), including paddy fields (Murase and Asiloglu, 2023). The primary mechanism behind the change in the bacterial communities is predation, which decreases, or even completely extinct the target bacterial taxa, while an abundance of non-target bacteria increases (Gao et al., 2019; Leander, 2020). This shift in bacterial taxa results in changes in overall bacterial community composition (Gao et al., 2019). The same mechanism is also likely to affect the abundance of enhanced and depleted taxa, balancing the overall bacterial

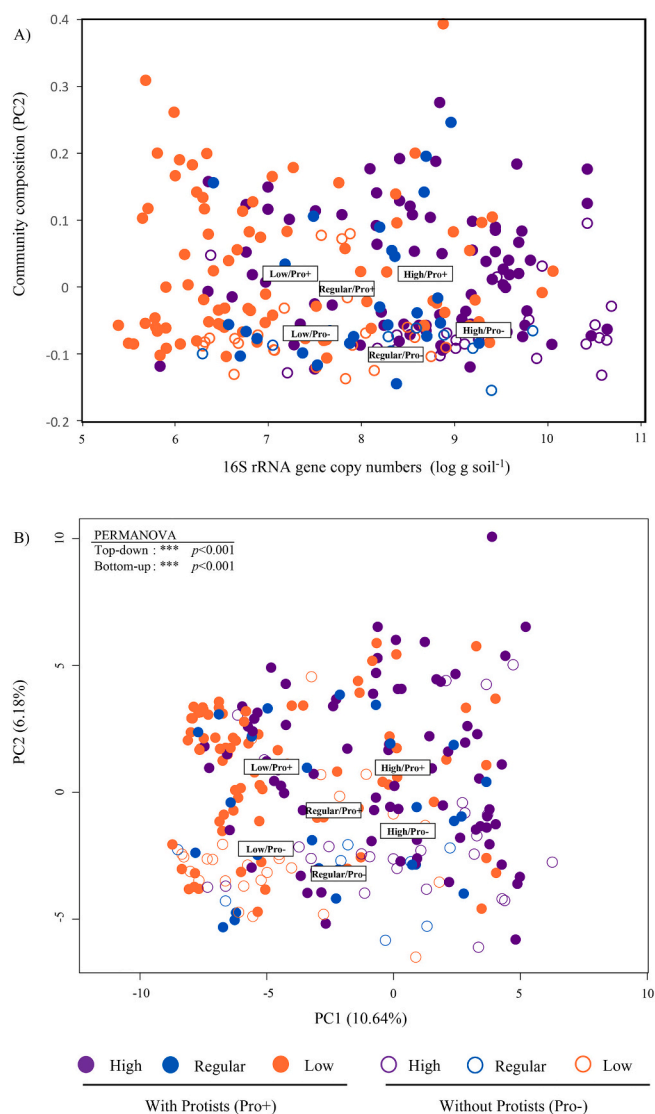


Fig. 3. The effect of the top-down and bottom-up factors on bacterial community structure (absolute abundances). A, The scatter plot shows the interaction between Principal Component 2 (PC2) of the PCoA shown in Fig. 2A, which exhibited the effect of protists, and the 16S rRNA gene copy numbers (log). B, Principal Coordinate Analysis (PCoA) calculated from the log transformed absolute abundances of bacterial taxa showing the impact of top-down predators and bottom-up nutrient levels on bacterial community structure. Colors indicate nutrient level, the filled shapes indicate the presence of protists, and the hollow shapes indicate the absence of protists. Names of the categories are placed at the median values.

population (Flues et al., 2017; Fujino et al., 2023).

The results of random forest analysis showed that each protist species affected different ASVs. In addition, all protist treatments slightly affected the bacterial population; however, only the Hg treatment had a significant decrease in the bacterial population. Protists show prey-selection patterns that are mainly species-specific; therefore, predator's traits such as feeding mode and cell size play important roles in the outcome of the protist predation on bacterial community composition (Asiloglu and Murase, 2017; Gao et al., 2019; Leander, 2020). In our study, the protist traits (size, taxonomy, and feeding modes) were different from each other, which could explain the differences in their impact on bacterial ASVs and their abundances.

4.2. Bottom-up control

The effects of bottom-up nutrients on bacterial community composition were relatively lower compared to the top-down predators. For the sake of the aim of this study, we selected the nutrients as they would affect a wide range of bacterial species and we simply studied the presence and absence of nutrients. For instance, we used a combination of three carbon sources (glucose, glycine, and sucrose), which can affect a wide range of bacteria (Xu et al., 2024). Therefore, rather than having specific effects on certain bacteria, the bottom-up nutrients used in this study are likely to affect a wide range of bacterial species. Predators, on the other hand, are known to have species-specific effects, explaining why the effect of top-down predators is more important for shaping bacterial community composition rather than bottom-up nutrients (Asiloglu et al., 2021). It should be noted that bacterial community composition can be affected by differences in the source of the bottom-up nutrients (Guo et al., 2024).

C, N, and P are essential macronutrients that control bacterial growth and population dynamics in soil environments (Demoling et al., 2007). C serves as the primary energy source, while N is a critical component of proteins, nucleic acids, and other biomolecules (Demoling et al., 2007; Xu et al., 2013). P plays a vital role in bacterial proliferation through energy transfer reactions (Tezuka, 1990; Xu et al., 2013). Our study confirmed the effects of C, N, and P on the growth of soil bacteria. The availability and ratios of these nutrients in soil significantly impact bacterial abundance, especially heterotrophs (Kirchman, 1994; Demoling et al., 2007). Indeed, the top 5 families affected by the bottom-up nutrients (Sphingomonadaceae, Nocardiodaceae, Comamonadaceae, Xanthobacteraceae, and Isosphaeraceae) were all heterotrophic bacteria. Our experiments were conducted under dark conditions; therefore, our study unintentionally neglected the autotrophic bacteria. The response of autotrophic bacteria to top-down and bottom-up factors should be further studied. Understanding the intricate relationships between macronutrients and soil bacteria is crucial for managing soil fertility, plant health, and ecosystem functioning.

4.3. Relative importance of top-down versus bottom-up factors

The majority of the research in soil microbial studies reached conclusions based on relative abundance analysis, which is effective in determining the major microbial taxa in a given environment. However, in relative-abundance-based analysis, an increase in one taxon's abundance causes an equivalent decrease across the remaining taxa and *vice-versa*. Furthermore, relative abundance-based results do not include information on the real abundance or population of microbes. Therefore, absolute-abundance-based results provide better information to understand the effect of environmental factors on both bacterial population and species composition (Tkacz et al., 2018; Barlow et al., 2020). Indeed, our results based on the relative abundance data showed that the bottom-up nutrients had no effect on bacterial communities. The absolute-abundance-based results, on the other hand, provided more realistic results, showing that the bacterial community structure is influenced by both top-down and bottom-up factors. This is also true for higher-level organisms in agricultural systems. The two factors could have mixed effects on both arthropod abundance and composition in terrestrial agroecosystems, indicating that there may be no clear patterns for this subject (Dyer and Letourneau, 2003; Han et al., 2022). Under specific environmental conditions or for particular organisms, contradictory results may also emerge. For instance, a study on soybean aphids across a gradient of agricultural management systems found that the overall population growth of soybean aphids was almost exclusively controlled by the top-down factors. Therefore, understanding the relative influences of top-down and bottom-up factors in various environmental conditions is crucial for predicting population dynamics in agricultural systems.

Our further analyses based on absolute abundance data revealed the

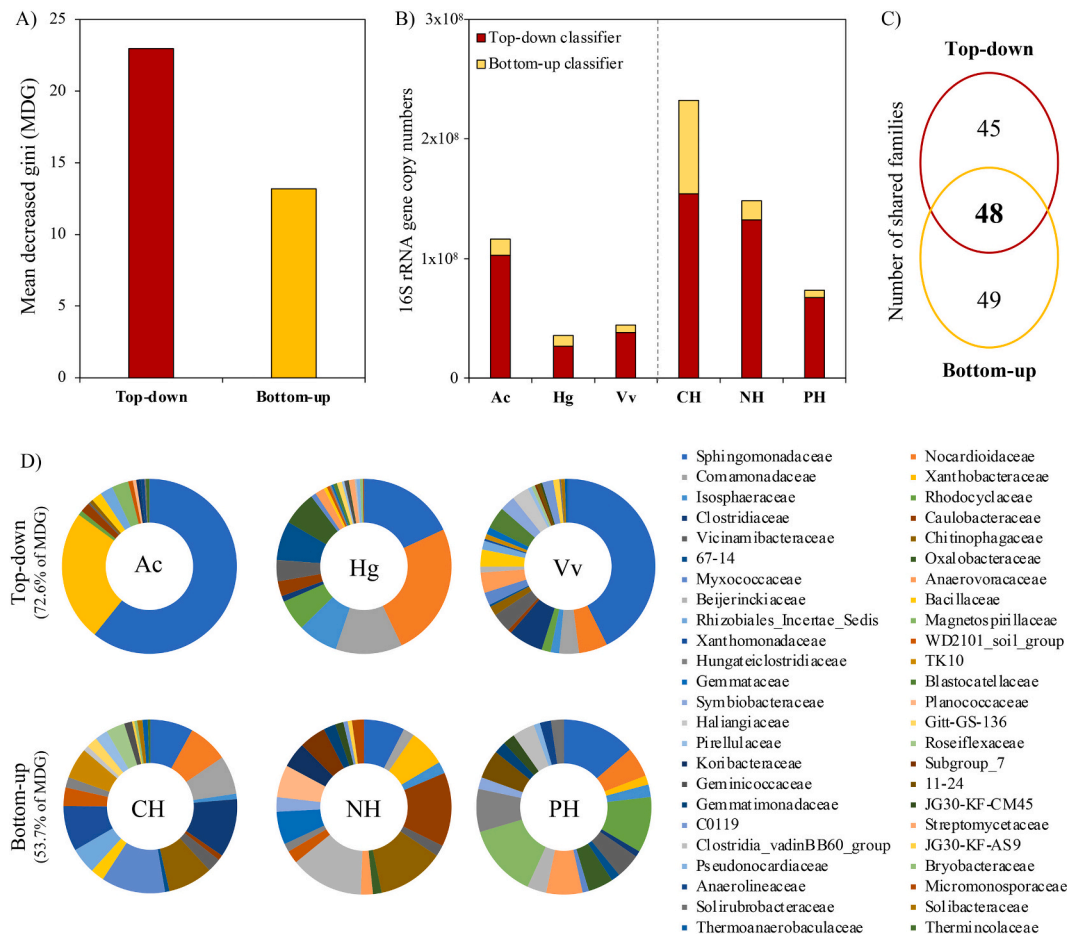


Fig. 4. Random-forest model identifies bacterial ASVs that classified each treatment. A, Bar plot displaying the mean decrease gini (MDG) values. The red bars represent top-down factors (total of Ac, Hg, and Vv), while the yellow bars represent bottom-up factors (total of CH, NH, PH). B, Bar plot shows the absolute abundance (in log scale) of bacterial populations affected by top-down and bottom-up classifiers indicating each protist treatment and nutrient levels. C, The Venn diagram shows the overlap of the number of affected families by top-down and bottom-up factors. D, The pie charts comparing the similarities in family level distribution of top-down affected (top row) and bottom-up affected (bottom row) families. The values represent MDG. The colors in the pie charts represent families shared by both bottom-up and top-down factors. For all families, see Fig. S3. Top-down (red) and bottom-up (yellow) classifiers were built by merging the Ac, Hg, and Vv and CH, NH, and PH, respectively.

effects of top-down predators and bottom-up nutrients on bacterial ASVs. Interestingly, over 40 families were affected by both factors, showing that both top-down and bottom-up factors interact to shape natural communities, which is in line with previous studies (Moore et al., 2003; Leroux and Loreau, 2015; Liu et al., 2024). Response of bacterial communities to top-down and bottom-up factors can be divided into two main groups in this context: survival from the top-down (predator-prey interactions) (Matz and Kjelleberg, 2005) and competition for bottom-up (prey-prey interactions) (Hibbing et al., 2010). The success or failure of predators is determined by the prey's ability to evade or fend off their predators, which is called vulnerability traits. Vulnerability traits include but are not limited to motility (a higher speed than its predators), chemical defense (e.g., bioactive metabolites, antibiotics, and toxin release), cell-to-cell communication (e.g., quorum sensing), microcolony formation (e.g., biofilms), change in body size (e.g., filamentous morphology), and rapid colonisation (that is, growth rate) (Matz and Kjelleberg, 2005). This is also true for the response of species to the bottom-up limitation; only competition traits apply instead of vulnerability traits (Hibbing et al., 2010). Competition traits are, indeed, identical to vulnerability traits. For instance, a higher speed that is used to fend off predators also benefits microorganisms to reach nutrient-rich niches faster than their competitors (Hibbing et al., 2010).

Most of the vulnerability traits such as chemical defense, quorum sensing, microcolony formation, body size, and rapid colonisation are well-known effective strategies for bottom-up competition as well (Hibbing et al., 2010), which provides an excellent chance for bacteria to survive top-down predators and outcompete in the presence of bottom-up nutrients with the same strategy; *killing two birds with one stone*. This could explain the similarities between the top-down and bottom-up affected families observed in our study (Fig. 5).

5. Conclusion

Factors determining communities and populations have been debated with vigour in the past centuries. Here, we studied one of the most important concepts in ecology to understand how bacterial communities and populations are shaped, which is overlooked in soil ecology. Although here we ignored the abiotic factors that are not counted in the top-down and bottom-up concept such as soil moisture and pH, our results showed that both bottom-up and top-down factors are crucially important in bacterial community structure (Fig. 5) and it is difficult to estimate which factors affect which bacterial taxa under natural conditions. Indeed, predation-related nutrient turnover can influence bacterial communities like the bottom-up factor. The majority of studies to

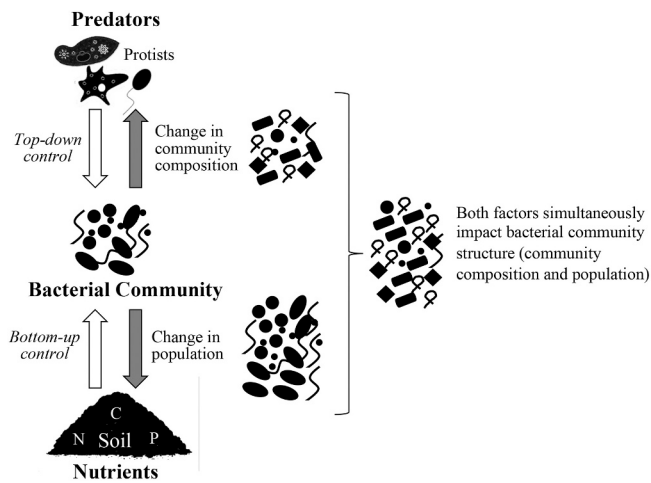


Fig. 5. A conceptual model explaining the top-down and bottom-up concept for soil bacterial communities. White arrows indicate the factors influencing bacterial communities, while grey arrows indicate the response of bacterial communities to the top-down and bottom-up factors.

understand how bacterial communities are shaped focused on nutrient-oriented factors. However, given the pivotal role of predators in regulating soil microbiome, it is crucial to seriously consider top-down predators' ecological significance on soil microbiome structure and functioning.

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CRedit authorship contribution statement

Seda Ozer Bodur: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Kazuki Suzuki:** Writing – review & editing, Resources. **Naoki Harada:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition. **Rasit Asiloglu:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.177634>.

Data availability

The raw sequence data obtained in this study have been deposited in the NCBI database under the BioProject ID PRJNA1117895.

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