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Protists exhibit community-level adaptation and functional redundancy under gradient soil salinity

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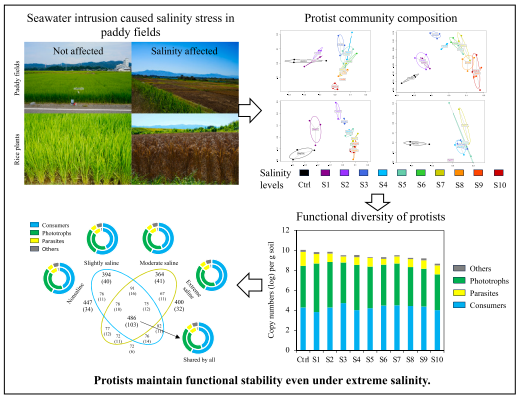
HIGHLIGHTS

- Soil salinity significantly shifts protist community composition and diversity.
- Beta diversity of protists clusters consistently along the salinity gradient.
- Protist 18S rRNA gene abundance decreases tenfold under extreme salinity.
- Protists maintained functional stability even under extreme salinity.

GRAPHICAL ABSTRACT



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ABSTRACT

One of the most important climate change-related issues that has extremely negative impacts on terrestrial life is soil salinization, especially in lowland paddy fields. Despite the enormous impact of salinity on microbial life, the majority of research focused on bacteria and fungi, neglecting the vast majority of eukaryotic diversity, the protists. Here we aimed to understand the sole impact of the soil salinity on protist communities in paddy field soil. To exclude the variations in other environmental factors that co-varies with the soil EC, we conducted a controlled in vitro experiment to study the direct effect of gradually increased salinity levels (ranging from 0.1 dS m^{-1} to 12 dS m^{-1}) on protists in three non-saline (<0.3 dS m^{-1}) paddy field soils. Then, our in vitro results were confirmed in a field study, in which seawater intrusion caused the accumulation of sea salts in paddy fields along a river. The results of the in vitro and field studies were consistent, showing that alpha and beta diversities of protists are affected by soil salinity. While protist alpha diversity exhibited inconsistent patterns across soil types, beta diversity showed strong and consistent clustering by the salinity gradient. Although salinity significantly shifted protist communities and caused a 10-fold decrease in 18S rRNA gene abundances of protists, protists

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maintained functional stability, suggesting that even with the compositional shifts, the critical ecosystem functions, such as predation and primary production, remained intact. These results underscore the importance of functional redundancy in sustaining ecosystem functions under salinity stress.

1. Introduction

Heat, flood, droughts, and storms! Global climate change increasingly threatens biodiversity and ecosystem functioning. One of the most important climate change-related issues that has extremely negative impacts on terrestrial life, especially on plants and microorganisms, is soil salinization, a process that results in excessive accumulation of soluble salts in the soil. On top of the fact that we do not have effective desalinization strategies for the currently saline agricultural lands, the salinization of soils keeps increasing, which is estimated to affect half of the arable lands in the near future (Hassani et al., 2021). The soluble salts are distributed to the soil mainly by wet (rainfall) and dry (aeolian processes) deposition of oceanic salts (Zhu and Yang, 2010; Voigt et al., 2020; Hassani et al., 2021), which makes lowlands such as paddy fields vulnerable to climate change-driven soil salinization (Gopalakrishnan and Kumar, 2021). In addition to the wet and dry deposition of oceanic salts, sea level rise, seawater intrusion, saline groundwaters, and unsustainable extraction of freshwater resources can worsen the soil salinization issue in paddy fields resulting in an increasing number of abandoned paddy fields in coastal regions (Khanom, 2016; Schneider and Asch, 2020; Gopalakrishnan and Kumar, 2021; Park et al., 2022; Yang et al., 2022). Salinity threatens not only crop production but also the microbial life in the soil ecosystem (Daliakopoulos et al., 2016). Indeed, a comprehensive study analysing global patterns in bacterial diversity showed that salinity rather than other environmental factors such as pH and extreme temperature is the major environmental determinant of bacterial community composition (Lozupone and Knight, 2007). However, despite the mounting number of studies on soil salinity-microbe interaction, the majority of research focused on bacteria and fungi (Haj-Amor et al., 2022), neglecting the vast majority of eukaryotic diversity, the protists.

The lack of research on soil protistology, which is not limited to salinity studies, is recognised as one of the major issues in microbiology research (Caron et al., 2009; Sibbald and Archibald, 2017; Geisen et al., 2018; Asiloglu, 2022). Protists are ubiquitous, predominantly microscopic, and unicellular organisms representing the vast majority of eukaryotic diversity, which makes them a major group in the soil microbiome (Geisen et al., 2018). The major functional group of protists is the predators, representing more than half of the relative abundance of protists (Singer et al., 2021). The predatory protists feed on other microorganisms, and control soil biodiversity (Bodur et al., 2024c), stimulate microbial activity (Gao et al., 2019), modulate plant microbiome (Asiloglu et al., 2024), suppress plant pathogens (Fujino et al., 2024) and substantially contribute to nutrient cycling and plant productivity (Gao et al., 2019). Decomposers (fungi-like protists) and photoautotrophic organisms play crucial roles in nutrient cycling via organic matter degradation and carbon fixation, respectively (Jassey et al., 2022). Plant pathogenic protists cause enormous negative impacts on agricultural production, whereas animal and microbial parasitic protists negatively affect their hosts' health (Caron et al., 2009; Geisen et al., 2018). Taken together, protists play essential roles in soil biodiversity, nutrient cycling, and agricultural productivity.

Despite the importance of protists and salinity, the knowledge of salinity-protist interaction almost exclusively comes from studies on marine and freshwater ecosystems, which showed that protist communities in marine and freshwater ecosystems are shaped by salinity (Telesh et al., 2015). Indeed, the salinity barrier is one of the main factors separating marine and freshwater protist communities (Logares et al., 2009; Balzano et al., 2015). For instance, a previous study comparing protist communities in the major ecosystems on Earth (soil, freshwater, and marine systems) showed that soil and freshwater protist communities are more similar to each other than marine protist communities likely due to the salinity (Singer et al., 2021). The effect of salinity on protist communities varies depending on protists' morphological and lifestyle characteristics. Photoautotrophic protists (algae) show great plasticity and adaptability to salinity, and they are the major primary producers in saline ecosystems (Shetty et al., 2019). A substantial diversity of predatory protists inhabits saline habitats (Telesh et al., 2015; Lennartz et al., 2023), and several predatory protists can grow in mediums with over 20 % salt concentration (Park and Simpson, 2015). Although the effect of salinity on soil protists has not yet been studied, soil protists are known to inhabit and maintain their activity in saline soils (Asiloglu et al., 2021b; Feng et al., 2025; Sun et al., 2025). Taken together, protists are ecophysiologically critical inhabitants of saline ecosystems, serving crucial roles as primary producers, decomposers, and predators (Massana et al., 2015; Filker et al., 2017; Feng et al., 2025; Sun et al., 2025). Here, we hypothesised that soil protists would exhibit community-level adaptation and functional redundancy in response to increased soil salinity.

Electrical conductivity (EC) of saturated soil is used as a measure of the soil salinity, which is expressed in dS m^{-1}. Research on global salinity showed that EC values of the majority of saline soils range from 2 to 16 dS m^{-1} (Ivushkin et al., 2019). According to the globally accepted soil salinity classification, >2 dS m^{-1} represents saline soils (Hassani et al., 2021). Salinity levels higher than 8 dS m^{-1} represent excessive soil salinity where the diversity and function of soil microorganisms show a severe decrease (Rath and Rousk, 2015; Singh, 2016). Environmental variables often co-vary with salinity in field conditions making it difficult to disentangle the direct effects of salinity from the other variables in the soil ecosystem (Rath and Rousk, 2015). Therefore, field-based studies on soil salinity mostly provide correlation-based results. Indeed, we previously observed significant differences in the protist communities between saline (EC > 2 dS m^{-1}) and non-saline (EC < 1 dS m^{-1}) paddy field soils (Asiloglu et al., 2021b). However, variations in other environmental factors such as pH, soil nutrients, and soil physical properties co-varied with the soil EC, which prevented us from understanding the direct effect of soil salinity on protist communities (Asiloglu et al., 2021b). Controlled laboratory experiments, on the other hand, enable us to identify the direct effects of salinity on soil microbial communities as previously shown for bacteria (Rath et al., 2019). Previously, protist community composition in saline-alkali soils (Asiloglu et al., 2021b; Sun et al., 2025) and the positive impact of protists on plant growth under saline soils (Feng et al., 2025) have been studied. However, we still lack an understanding of how salinity shapes the taxonomic and functional community composition of protists, as well as their population dynamics. Here, we aimed to understand the response of protist communities to the increasing salinity levels. Therefore, rather than using salt-affected paddy field soil, we studied the direct effect of gradually increased salinity levels (ranging from 0.1 dS m^{-1} to 12 dS m^{-1}) on protists in three non-saline (<0.3 dS m^{-1}) paddy field soils. Then, our in vitro results were confirmed in a field study, in which seawater intrusion caused the accumulation of sea salts in paddy fields along a river. The protist community composition was studied with a high throughput sequencing method and protist abundances were estimated with a real-time PCR method (qPCR).

2. Materials and methods

2.1. Experimental setup and chemical analyses

The soil samples were collected from three paddy field soils with different physicochemical properties to check the robustness of the effect of salinity in different soils. The paddy fields are located in Niigata (N37.85.69, E138.96.22), Aichi (N34.56.47, E136.53.24), and Nagano (N36.20.08, E137.87.03) prefectures, Japan. The details of sampling sites and the physicochemical properties of the soils were published elsewhere (Suzuki et al., 2023). Briefly, the soil samples were obtained from the plow layer (0–10 cm) at five locations in each field. Then, the soil samples for each soil type were sieved (<2 mm), individually mixed to homogenize, and then stored at 4 °C.

Microcosms were established in sterile plastic tubes filled with 40 g of paddy field soils in triplicate for control (Ctrl) and ten gradiently increasing salt concentration treatments (S1–S10) giving a total of 99 microcosms (3 soil types × 11 salinity levels × 3 replications). Depending on factors related to the soil type such as initial EC, texture, and organic matter concentration, the addition of the same concentration of NaCl could result in different soil EC values (Li et al., 2006; Mavi et al., 2012). Therefore, the NaCl concentration was determined for each soil type with a pilot experiment to obtain similar EC values across all soil types. In the pilot experiment, we added gradationally increased NaCl from 0.1 to 60 $mg.g^{-1}$ soil to each soil type making a total of 300 microcosms and we measured the EC values after 24 h. Then, ten gradient salinity levels were decided and the NaCl addition was done accordingly. Briefly, Shindori (factor 1) soil had the lowest addition of NaCl, followed by Aichi (factor 1.06) and Nagano (factor 1.21) soils. The soil samples were mixed with a range of solutions of different concentrations of NaCl dissolved in sterile water to create a gradient of ten different salt concentrations. The control microcosms received the same amount of sterile water. The microcosms were incubated at room temperature (24 °C) under submerged conditions for 4 weeks. The microcosms were watered daily to keep the water level approximately 1 cm above topsoil during the incubation period to mimic the paddy field conditions. Soils were sampled for molecular and physicochemical analyses as described previously (Asiloglu et al., 2021a). Briefly, the top water was removed, and then the soil in the microcosms was mixed through. The 0.5 g of the soil was sampled for molecular analysis and immediately stored at −80 °C. Soil EC was measured in a 1:5 soil-to-water slurry. The slurry was prepared by mixing 10 g of soil with 50 mL of distilled water in a mechanical shaker at 120 rpm, then allowed to equilibrate for 1 h. Following the equilibration period, the soil EC was determined using an EC meter. Soil pH and cations were determined as described before (Asiloglu et al., 2021a).

2.2. Molecular analysis and bioinformatics

The soil DNA extraction process involved using 0.5 g of soil with the ISOIL for Bead Beating kit, adhering to the manufacturer's guidelines, and eluting the DNA in 50 μL of TE buffer. For amplification, the 18S rRNA gene's V9 region was targeted using universal eukaryotic primers (1389F/1510R) (Amaral-Zettler et al., 2009) with barcoded adapters (Caporaso et al., 2012). The initial PCR followed previously established protocols (Amaral-Zettler et al., 2009), while subsequent steps, including PCR product purification, index PCR for Illumina MiSeq, and sequencing, were conducted using standard methods (Asiloglu et al., 2021b). Briefly, data analysis utilised the QIIME2 pipeline, with sequence pre-processing performed using the DADA2 algorithm. This included error correction, primer removal, quality filtering, and elimination of singleton, doubleton, and chimeric reads. Single-end reads were truncated at 180 bp to ensure a minimum 50 bp overlap between forward and reverse sequences (corresponding to a quality score >30). Protist taxonomy assignment employed the QIIME2 q2-feature-classifier plugin with the PR^2 database (version 5.0.0). Non-protist sequences (Fungi, Metazoa, unidentified Opisthokonta, Streptophyta, Rhodophyta, and unclassified eukaryotes) were removed from the dataset using Qiime2's taxa filtering. To enable comparison between treatments and soil samples, read counts were normalised to 4000 sequences through random subsampling. Protist taxa were categorised into three functional groups: autotrophs (photosynthetic organisms), consumers (organisms obtaining nutrients through phagocytosis), and pathogens (organisms reducing host fitness, including plant pathogens and parasites).

Beta diversity dissimilarities were visualised using principal component analysis (PCoA) analysis based on Bray-Curtis distance matrices using *Phyloseq* and *Vegan* packages in R platform version 4.2.2 (https://www.r-project.org/) with *betadisper* function. Statistical differences in community composition were assessed using permutational multivariate analysis of variance (PERMANOVA with 999 random permutations) using the *Adonis* function as implemented in the *Vegan* package. Venn diagram and Random Forest analysis were conducted in R. For this, we first obtained *Phyloseq* objects using the qiime2 derived rooted-tree, table, and taxonomy files, and the metadata file. Unique and shared core ASVs among treatments were detected using the microbiome package. Random Forest analyses were conducted as described elsewhere (Bodur et al., 2024b). All of the statistical analyses were performed in the R program unless otherwise specified.

Abundances of 18S rRNA and 16S rRNA genes of protists and bacteria, respectively, were detected by a qPCR as described previously (Asiloglu et al., 2024). Briefly, the qPCR analysis was conducted with the same primers as used in amplicon sequencing (515F/806R for bacteria and 1389F/1510R for protists) without the Miseq barcode adaptor to obtain absolute abundances, which is an often-used method (Tkacz et al., 2018; Barlow et al., 2020). Copy numbers for each gene were calculated using a regression equation for each assay relating the cycle threshold (Ct) values to the known number of copies in the standards of *Escherichia coli* MG1655 (for bacteria) and *Acanthamoeba castellanii* (for protists). Since the results of 18S rRNA gene copy numbers include amplification of non-protist taxa, the obtained qPCR results were re-calculated based on the results of the taxonomic assignment to exclude the sequences identified as non-protist.

The absolute abundances of protists were calculated as follows: First, the ASV table was obtained from the *Phyloseq* object and the relative abundances were calculated. Then, the relative abundances were multiplied with the qPCR results to obtain an absolute abundance ASV table. The absolute abundance-based analyses were conducted as described above with modifications. 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 the log function in R, and then the Bray–Curtis distance matrix was prepared. The rest of the analyses for absolute abundances were conducted as described above unless otherwise stated.

2.3. Field study

The in vitro experiment was designed to assess how salinity affects protist communities in a controlled environment using three distinct paddy field soils with varying physicochemical properties. However, in vitro studies doesn't necessarily relate to the natural conditions. Therefore, to confirm our in vitro experiment results, we further analysed the protist communities in natural salinity-affected paddy fields. The paddy fields (37°52′55.3″N 139°10′02.5″E, an elevation of three meters above sea level) are located along the Agano River in Niigata City (Supplementary Fig. S1). The fields have a long history of rice production and are regularly watered from the Agano River with water pumps with no salinity history. The river water level dropped due to the drought (extreme heat combined with low rainfall) in August 2023 causing seawater intrusion, which resulted in salinity in the fields watered from the river. Due to the salinity, rice plants have died in 11 ha of the paddy fields (BSN News, 2023). The sampling fields (size of the fields was approximately 15 × 40 m) were located near each other

(<200 m apart). To determine the fields to be sampled, first, we measured EC in various fields with a probe (HydraGO, Setevens, OR, USA). Then, six fields with a salinity gradient ranging from 0.52 dS m^{-1} to 7.35 dS m^{-1} were determined. Soils are collected on September 22nd, 2023, from 5 locations in each field and mixed through. Each field was represented with 3 replications. Although a different soil type was used in the field study, the key objective was to determine whether the patterns observed in vitro were also present under natural, uncontrolled conditions. Thus, while both experiments used different specific sites and slightly different scales (three soils in vitro, six fields in situ), their designs are complementary: the laboratory microcosms allowed us to isolate the effects of salinity under controlled conditions, and the field study confirmed the ecological relevance of these patterns in an actual salinity-affected agricultural setting. Soil physicochemical analyses, including EC, molecular, and bioinformatics analyses were conducted as described above. All of the raw sequence data obtained in this study have been deposited in the NCBI database under the BioProject ID PRJNA1222634.

3. Results

3.1. Soil chemical properties

At the end of the incubation, the soil EC was gradually increased from 0.3 (Ctrl) to 12.6 dS m^{-1} as intended (Fig. 1a–b). We successfully obtained similar EC values (dS m^{-1}) among all three soil types in each treatment and ANOVA analysis showed no significant differences in soil EC between the soil types (Fig. 1b and Table S1). Soil pH was not affected by increased salinity in Aichi soil; however, the increased salinity caused a slight decrease in the soil pH in the Shindori and Nagano soils in moderate and extreme saline conditions (Fig. 1c, Table S2). The maximum decrease in the pH between the control and S10 was 0.64 in Aichi soil, 0.33 in Shindori soil, and 1.36 in Nagano soil. The correlation coefficient analyses showed a significant correlation between the soil EC and pH ($R^2 = 0.3961$) (Fig. S3). To obtain similar EC values (dS m^{-1}) in all soil samples, we added more NaCl to the Nagano soil due to its high organic matter content; thus, the soil Na concentration at the end of the incubation was significantly higher in the Nagano soil (Fig. S2, Table S3). A significant correlation coefficient was observed between soil EC and Na ($R^2 = 0.8914$) (Fig. S3). The K content was significantly higher in the Nagano soil compared to the soils of Shindori and Aichi (Fig. S2, Table S4). The increased salinity did not affect the K concentration of the three soils (Fig. S2, Table S4). Both Ca and Mg contents were different in each soil type and they were affected by the salt addition (Fig. S2, Tables S5–S6). The correlation coefficient analyses showed no significant correlation between soil EC and K ($R^2 = 0.0031$), Mg ($R^2 = 0.0134$), or Ca ($R^2 = 0.0232$) (Fig. S3).

3.2. Protist diversity and community composition

The alpha diversity that was measured with the Shannon index was higher in Nagano soil, followed by Shindori and Aichi soils (Fig. 2a–c). The Shannon index showed increased or decreased patterns with the gradually increased soil EC depending on the soil type. Compared to the control treatment, protist alpha diversity was higher in saline soils however only the S10 treatment had significantly higher results in Aichi soil (Fig. 2a). Unlike the protist alpha diversities in Aichi soil, the Shannon index showed decreased patterns in Shindori soil compared to the control treatment (Fig. 2b). The protist alpha diversity was significantly decreased in S5, S6, S8, S9, and S10 treatments (Fig. 2b). In Nagano soil, all treatments had a higher Shannon index compared to the control, and among them S2, S3, S5, S6, S7, and S9 had significantly higher results (Fig. 2c).

Although alpha diversity showed inconsistent patterns depending on the soil type (Fig. 2a–c), the protist beta diversity that was visualised with PCoA based on the Bray-Curtis distance matrix showed highly

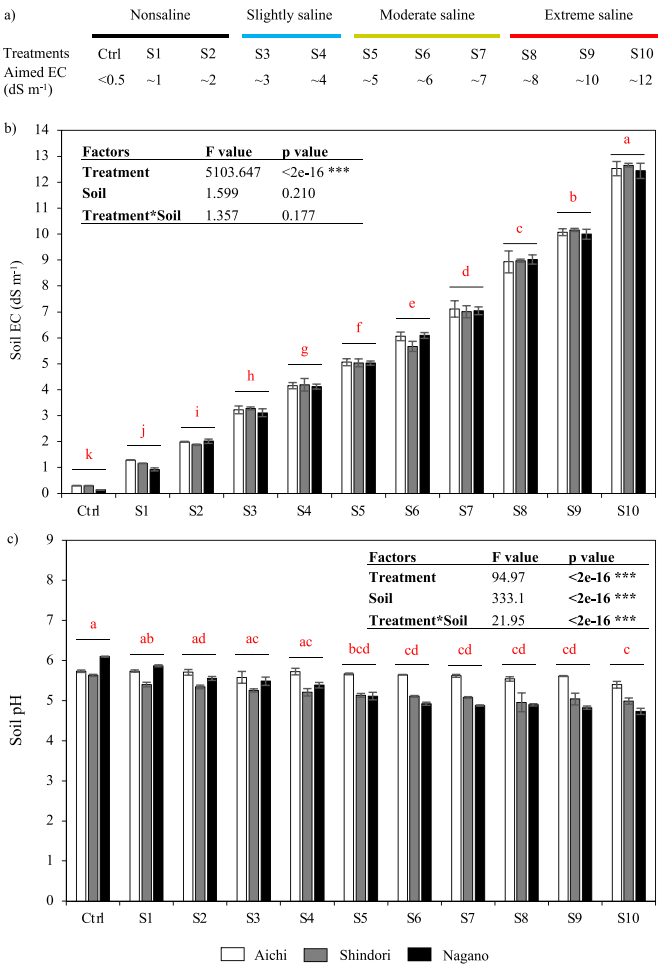

Fig. 1. Treatments and salinity groups (a) and soil EC (b) and pH (c). Ctrl, control with no NaCl addition, S1 to S10 represents gradually increased salinity treatments. Error bars represent standard deviation within treatments. Different letters indicate significant differences within treatments ($p < 0.05$). White bars, Aichi soil; grey bars, Shindori soil; black bars, Nagano soil. See Tables S1–S2 for the results of the statistical analysis. Nonsaline, <2 dS m^{-1}; Slightly saline 2–4 dS m^{-1}; Moderate saline, 4–8 dS m^{-1}; Extreme saline, >8 dS m^{-1}.

consistent patterns in all soil types. The effects of soil salinity on the protist beta diversities were significant in all soil types (PERMANOVA, Fig. 2d–f). The effect of soil salinity was associated with both PC1 and PC2. While PC1 separated nonsaline treatments (<2 dS m^{-1}) and saline treatments (>2 dS m^{-1}), in general, PC2 separated saline treatments where slightly saline treatments (2–4 dS m^{-1}) were placed on top, moderate saline treatments (4–8 dS m^{-1}) were placed in the middle, and the extreme saline treatments (>8 dS m^{-1}) were placed in the bottom (Fig. 2d–f).

The copy numbers of both 18S and 16S rRNA genes were decreased with gradually increased salinity (Figs. S4–S5). Although protist copy numbers tended to decrease in treatments with EC below 4 dS m^{-1}, in general, the decrease was not significant. A significant decrease was observed in the protist population for moderate saline and extreme saline treatments (>4 dS m^{-1}) compared to the control (Fig. S4). A similar trend was observed in bacterial copy numbers; however, the bacterial population showed a significant decrease in treatments with soil EC higher than 2 dS m^{-1} (slightly, moderate, and extreme salinity) consistently in all soil types. By using the protist copy numbers, PCoA illustrated the differences in protist community structure based on the absolute abundances (Fig. S6), which were highly consistent with relative abundance-based PCoA results, showing that protist community structure is strongly affected by the gradually increased salinity levels

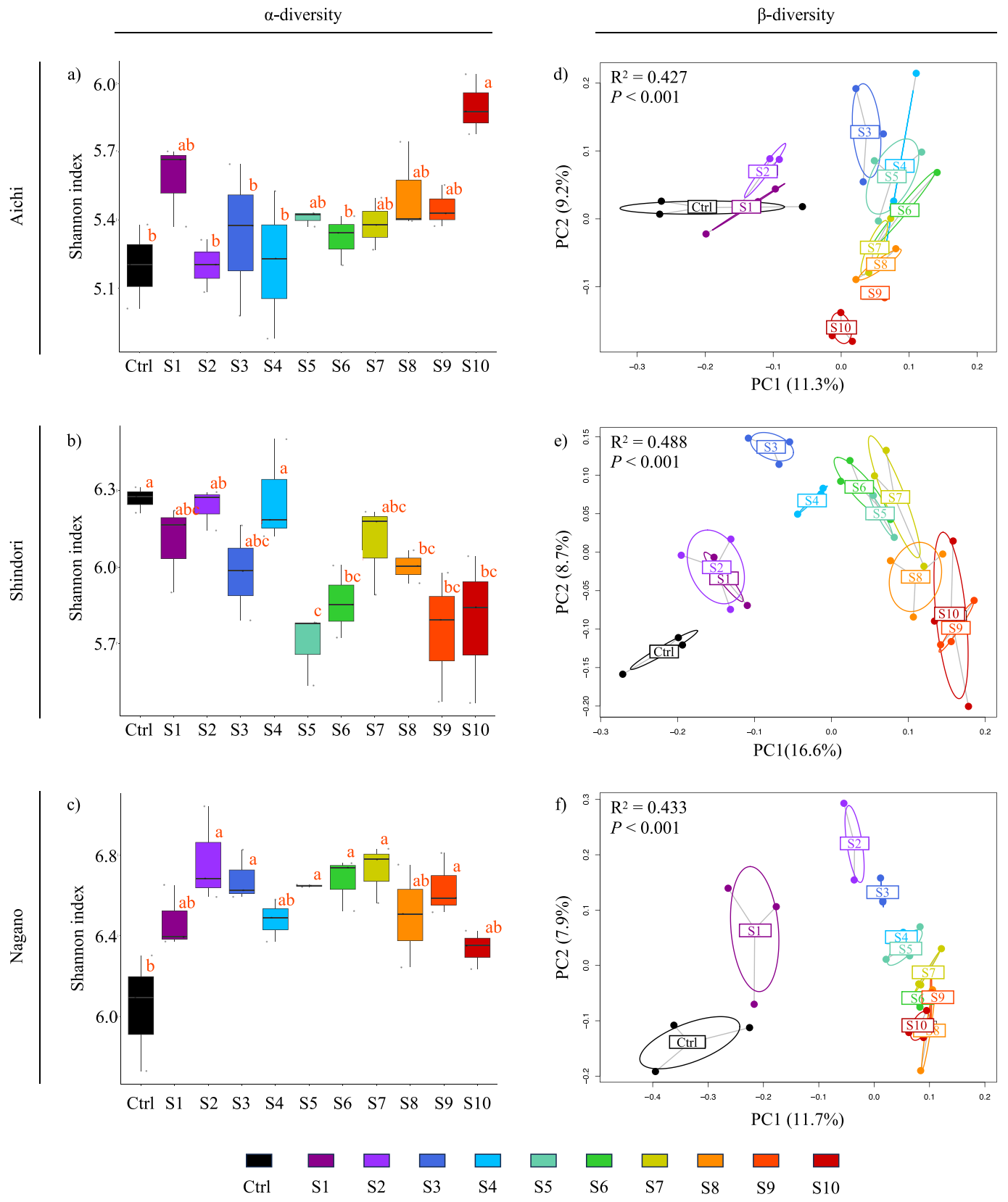

Fig. 2. Effect of gradiently increased salinity on α (a–c) and β (d–f) diversities of protists in Aichi soil (a and d), Shindori soil (b and e), and Nagano soil (c and f). The central line in the boxplot (a–c) represents the median, box hinges represent first and third quartiles. Lines indicate minimum and maximum values. Different letters represent significant differences ($P < 0.05$, ANOVA with Tukey's post hoc test). Principal coordinate analysis (PCoA) based on the Bray–Curtis dissimilarities of protists taxa in Aichi soil (d), Shindori soil (e), and Nagano soil (f) showing the effect of salinity on protists communities with confidence ellipses of the eigenvalues of the covariance matrix. Ctrl, control with no NaCl addition, S1 to S10 represents gradually increased salinity treatments.

(Fig. S6).

Archaeplastida, Rhizaria, and Amoebozoa dominated in all paddy field soils followed by Stramenopiles and Alveolata (Fig. 3a–c). The distribution of the protist supergroups was different depending on the soil type. Archaeplastida was the most dominant supergroup in Aichi and Shindori soils, while Rhizaria was the most dominant group in Nagano soil (Fig. 3a–c). Although the total population of protists decreased by the increased salinity levels, Archaeplastida tended to increase with the salinity gradient, especially in Aichi and Nagano soils. A slight decrease was observed in the absolute abundance of Rhizaria in all soils. Other supergroups had no consistency among the soil types. Consumers including predatory protists were the most dominant supergroup in Shindori and Nagano soils, while phototrophs dominated in Aichi soil (Figs. 3d–f and S7). Although a slight decrease was observed in the absolute abundances of the protist functional groups, protists showed functional redundancy in the saline treatments in all soil types (Fig. 3d–f).

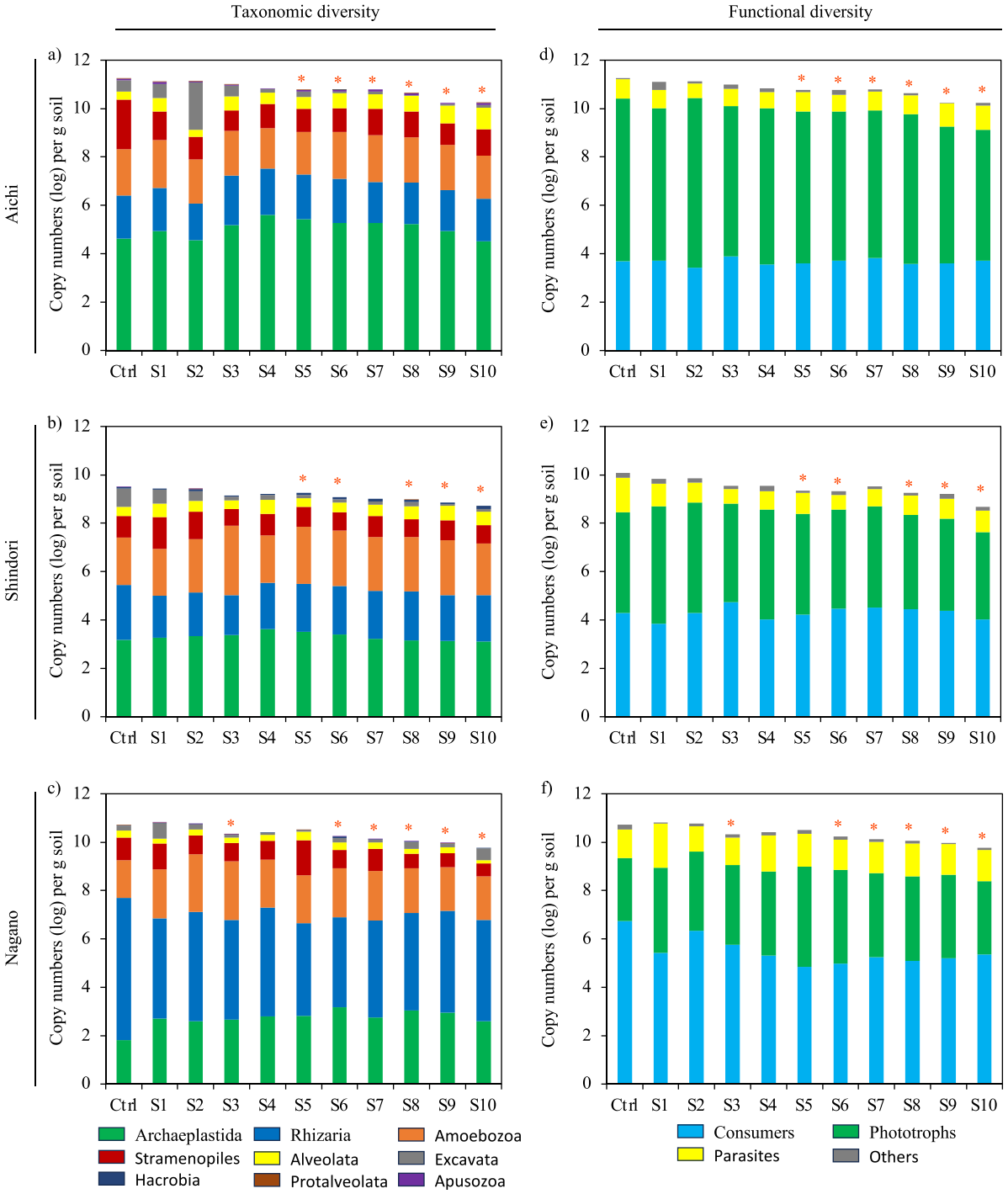

Fig. 3. Absolute abundances of the taxonomic (a–c) and functional (d–f) composition of protists. Bars shows the total 18S rRNA gene abundances (log) per gram soil. The colors show percentage abundance of each taxon or functional group. Asterisk indicates significantly lower total 18S rRNA gene copy numbers compared to the control treatment.

3.3. Field survey

Natural salinity-affected paddy fields (Fig. S1) allowed us to validate our in vitro experiment results. Although we had 11 in vitro treatments, only six salinity levels with EC ranging from 0.53 to 7.15 dS m^{-1} were represented in the natural field conditions: control, S1, S3, S4, S5, and S7 (Fig. 4a–b). Soil pH was significantly decreased by one factor in the saline treatments (S3, S4, S5, and S7) compared to the non-saline treatments (Control and S1) (Fig. 4c). Compared to the control soil, the alpha diversity of protists decreased in all treatments, however, only S1, S5, and S7 had significantly lower Shannon index (Fig. 4d). The beta diversity of protists exhibited a highly similar distribution between the field soil and the in vitro experiment (Fig. 4e). The effect of soil salinity associated with both PC1 and PC2. While PC1 separated the control treatment from the others, PC2 separated saline treatments, where slightly saline treatments were placed on the bottom, and the moderate saline treatments (>4 dS m^{-1}) were placed in the top (Fig. 4e).

The decrease in the protists' 18S rRNA gene copy numbers due to soil salinity was confirmed in the field study (Fig. S8a–b), which had highly similar patterns to the in vitro experiment's results. The distribution of absolute abundance-based beta diversity of protists also confirmed our in vitro results (Fig. S8c). Taxonomic distribution of protists in the field conditions was similar to the in vitro results with one exception of Stramenopiles being dominant (Figs. 4f and S7d). Similar to the in vitro experiment, the absolute abundance of Archaeplastida was increased, and Rhizaria's absolute abundance was decreased. Although Hacrobia was not affected by the salinity in the in vitro experiment, they showed a consistent positive correlation with the increased salinity levels under field conditions. Phototrophs, which were the dominant functional group in the field conditions, showed increased absolute abundance while consumers and parasites were decreased (Figs. 4g and S8d). Nevertheless, predators showed functional redundancy even under high soil salinity (>7 dS m^{-1}).

3.4. Functional redundancy and soil salinity

Protists showed functional redundancy at the community level, meaning that although protist diversity is affected by soil salinity, key ecological functions, predators and phototrophs, were maintained through the overlapping roles of different protist taxa (Figs. 2–4). Protists that were associated with increased soil salinity were illustrated using a Venn diagram (Fig. 5a). All of the functional groups are represented as unique and shared core protist ASVs in the four salinity groups, even at extreme saline conditions with soil EC reaching over 12 dS m^{-1} (Fig. 5a). Among the functional groups, consumers had the highest number of unique and shared core ASVs, followed by phototrophs and parasites, respectively. Furthermore, random forest analysis showed the protist ASVs that were characterised by the salinity treatments (Fig. 5b). Similar to the Venn diagram results, ASVs belonging to the consumers always had the highest number of ASVs (Fig. 5b). A regression analysis showed a significant ($P < 0.05$, $R^2 = 0.081$) negative correlation between soil EC and protist populations (Fig. 5c). We performed another regression analysis to show the correlation between soil EC and the population of predatory protists (Fig. 5d), which showed a significant negative correlation with a slightly lower R^2 value ($P < 0.05$, $R^2 = 0.058$) than that of all protists. We observed a significant correlation between the population of predatory protists and their prey (bacteria) population ($P < 0.001$, $R^2 = 0.611$) (Fig. 5e).

4. Discussion

4.1. Community level adaptation of protists to soil salinity

The salinity gradients in this study covered most of the ranges of soil salinities observed globally from nonsaline to extreme saline environments (Thompson et al., 2017). We hypothesised that soil protists would exhibit community-level adaptation and functional redundancy to the increased soil salinity levels. We observed that protists were well adapted to the saline conditions at the community level. Although salinity significantly shifted protist communities and caused a 10-fold decrease in protist copy numbers, protists maintained functional stability, suggesting that even with the compositional shifts, the critical ecosystem functions remained intact. Although our study does not provide functionality assays, the functional classification of protists by taxonomic information is an often-used method providing valuable information on protist functionality (Geisen et al., 2018; Asiloglu et al., 2021b). Our results are in line with the previous studies on protists in saline soils that showed that protists, especially predators, can maintain their activity, thus, positively impact soil health and plant growth (Asiloglu et al., 2021b; Feng et al., 2025; Sun et al., 2025). Environmental variables often co-vary with salinity in field conditions making it difficult to disentangle the direct effects of salinity from the other variables in the soil ecosystem (Lozupone and Knight, 2007; Rath and Rousk, 2015). Here, our in vitro approach successfully eliminated the influence of other soil-derived factors, allowing us to observe for the first time the direct impact of salinity on protist community composition. Our field validation results demonstrated the reliability of the in vitro approach.

4.2. Functional redundancy of protists to soil salinity

Although protist alpha diversities were significantly affected by salinity in all soils, the results revealed variable patterns, with some soils showing increased trends while others exhibited decreases. Previous studies showed that while some protist taxa cannot grow under increased salinity levels, several protists can adopt and even prefer relatively higher salinity (Balzano et al., 2015; Telesh et al., 2015; Zhao and Xu, 2016; Sun et al., 2025). Therefore, the increase or decrease in the alpha diversity of protists is likely to depend on the initial protist community composition of a given soil sample. We observed community-level adaptation of protists to soil salinity. Unlike individual-level adaptation, which involves genetic or phenotypic changes in a single microorganism, community-level adaptation focuses on how the entire community evolves or reorganizes to maintain functionality and resilience. The significant shift in protist community composition with the resiliency of the functional groups in each salinity level in both in vitro and field studies support our hypothesis that soil protists exhibit community-level adaptation and functional redundancy to increased soil salinity levels.

4.3. Adaptive strategies and trophic interactions in saline soils

The observed changes in protist community composition in response to salinity provide further evidence of the critical role of taxonomic shifts in shaping soil ecosystems. The dominance of Archaeplastida and Rhizaria in non-saline soils shifted as salinity increased, with Archaeplastida becoming more abundant in the higher salinity treatments, particularly in Aichi and Nagano soils. Although this study did not analyse individual-level adaptation, autotrophic protists (mostly belonging to Archaeplastida) may show phenotypic plasticity, including adaptations to osmotic stress, under saline conditions (Shetty et al., 2019). Conversely, the slight decrease in predatory protists at higher salinity levels was not accompanied by a collapse in their functional role, suggesting that predators belonging to other taxa may have functionally replaced them. These shifts highlight how environmental stress, such as salinity, can induce taxonomic reorganization within functional groups.

The differential responses of autotrophic and predatory protists to increased salinity levels can be attributed to their distinct energy acquisition strategies. Our findings indicate that while the absolute abundance of autotrophs remained stable or even increased depending on the soil type, predatory protists experienced a slight decline. This

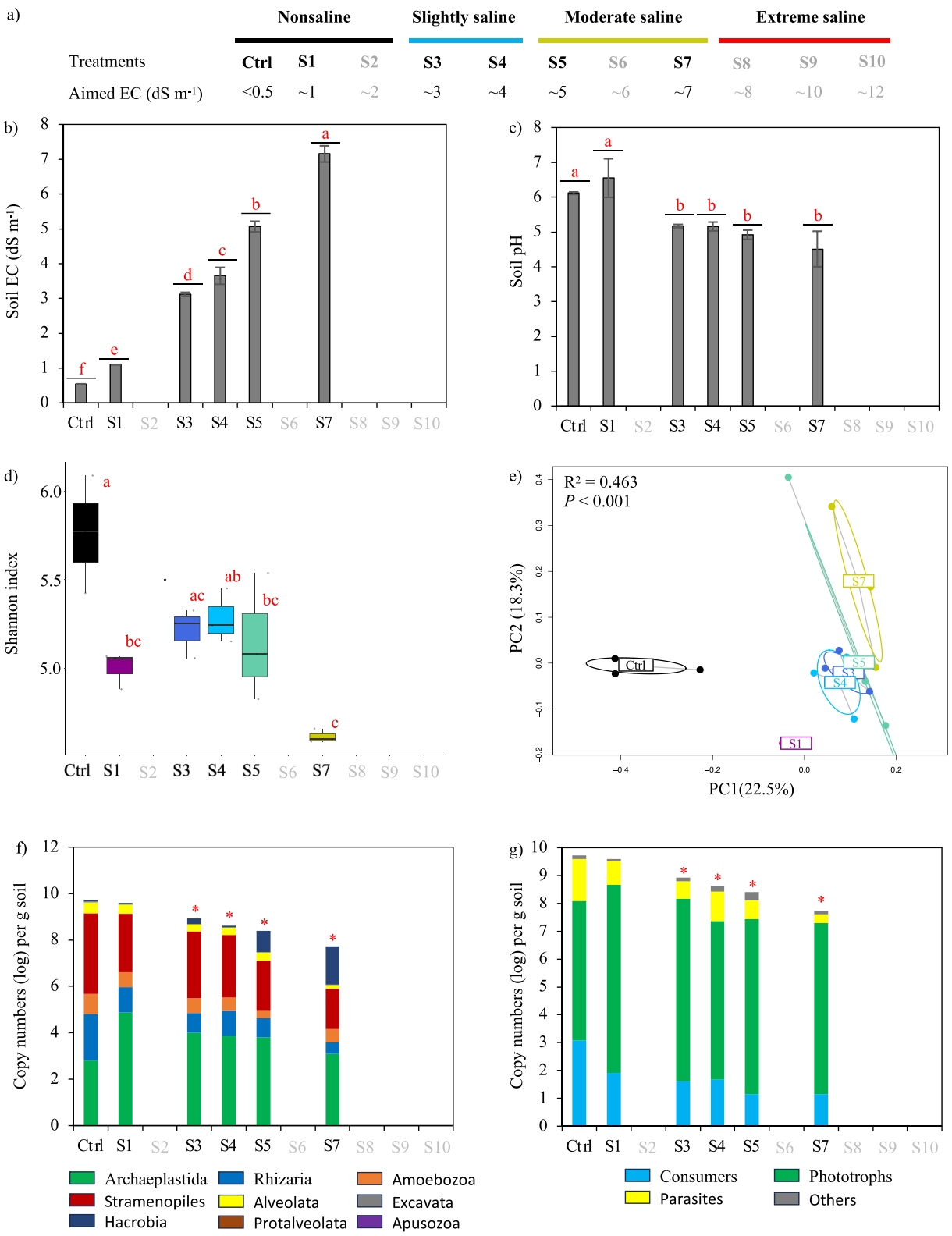

Fig. 4. Results of the field experiment. Treatments and salinity groups (a) and soil EC (b) and pH (c). Ctrl, control with no NaCl addition, S1 to S10 represent gradually increased salinity treatments. Effect of gradiently increased salinity on α (d) and β (e) diversities of protists. The central line in the boxplot (d) represents the median, box hinges represent first and third quartiles. Lines indicate minimum and maximum values. Principal coordinate analysis (PCoA) based on the Bray–Curtis dissimilarities of protists taxa (e) showing the effect of salinity on protists communities with confidence ellipses of the eigenvalues of the covariance matrix. Absolute abundances of the taxonomic (f) and functional (g) composition of protists. Bars show the total 18S rRNA gene abundances (log). The colors show percentage abundance of each taxon or functional group. Asterix indicates significantly lower total 18S rRNA gene copy numbers compared to the control treatment. Error bars represent standard deviation within treatments. Different letters represent significant differences ($P < 0.05$, ANOVA with Tukey's post hoc test). EC values that matched with those of S2, S6, S8, S9, and S10 treatments were not found in the field survey (shown in grey).

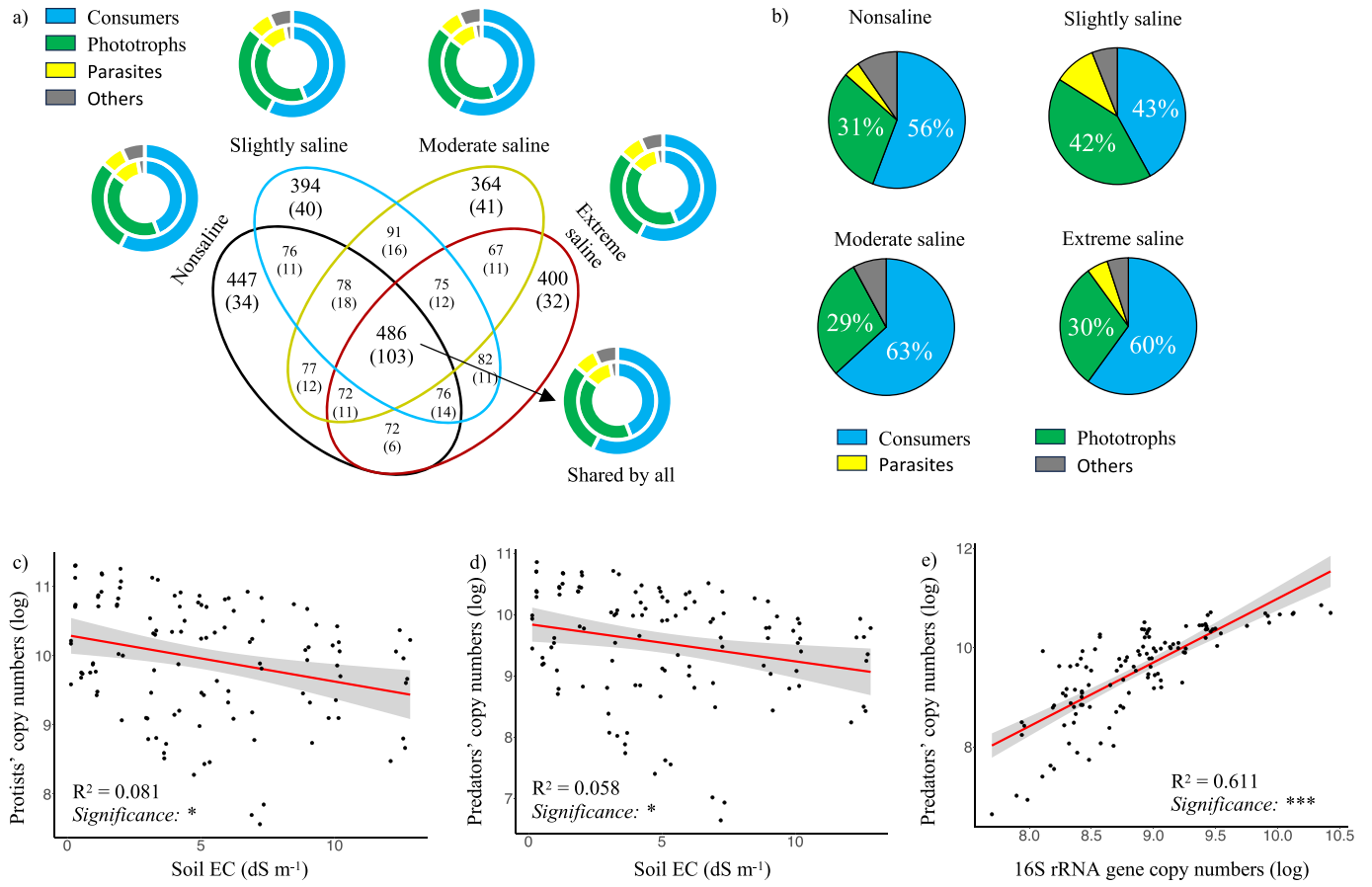

Fig. 5. (a) Venn diagram showing the number of unique and shared core (in parenthesis) ASVs between the four salinity groups. Shared core ASVs represent at least 90 % detection rate among different samples and soil types. Distribution of unique and shared core ASVs to functional groups (number of ASVs) was represented with pie charts (Outer chart, unique ASVs; Inner chart, shared core ASVs). Nonsaline, <2 dS m^{-1}; Slightly saline 2–4 dS m^{-1}; Moderate saline, 4–8 dS m^{-1}; Extreme saline, >8 dS m^{-1}. (b) Pie graphs showing the functional groups of random forest predictions for the most significant ASVs that characterize the salinity groups. Regression analyses conducted with all samples show the correlation of the soil EC with 18S rRNA gene copy numbers of protists (c) and predatory protists (d), and 18S rRNA gene copy numbers of predatory protists and 16S rRNA gene copy numbers of bacteria (e). Significance: *, $P < 0.05$; ***, $P < 0.001$.

contrast may be partially explained by the reduced availability of bacterial prey under saline conditions, which likely constrained the growth of predatory protists (Leander, 2020). Indeed, we found a higher correlation between the population of predatory protists and bacteria, compared to the predatory protists and soil EC. In contrast, autotrophs, which rely on sunlight as their primary energy source, are less dependent on trophic interactions and thus likely to maintain their populations despite salinity-induced changes in the microbial food-web (Balzano et al., 2015; Singer et al., 2021). These findings underscore the pivotal role of energy source and trophic positioning in mediating protist community responses to soil salinity. Therefore, predator-prey interaction and its outcome for microbial community composition should be further studied for a better understanding of how salinity-induced shifts in microbial food webs influence soil ecosystem functioning and resilience. As predatory protists enhance soil fertility and plant productivity (Gao et al., 2019; Murase and Asiloglu, 2023; Bodur et al., 2024a), predation can be a strong driver for the management of saline soils.

5. Conclusion

This study elucidates the complex relationships between soil salinity and protist community composition, providing significant insights into the resilience of soil microbial ecosystems under salinity stress. The findings reveal that, while soil salinity impacts protist diversity, it does not necessarily result in the loss of key functional groups. The consistency between laboratory results and field surveys reinforces the validity of the findings and provides a robust framework for future research. While controlled laboratory conditions allow for precise manipulations of salinity levels, the field validation in natural paddy fields confirms that the observed patterns in protist community composition and abundance hold under real-world conditions. This is crucial for understanding the broader ecological implications of salinization, particularly in coastal agricultural regions affected by seawater intrusion. Our results not only enhance the understanding of protist ecology in saline soils but also provide a basis for developing sustainable soil management practices that account for the functional roles of protists in maintaining soil health and agricultural productivity amidst increasing salinity. As predatory protists enhance soil fertility and plant productivity, by identifying resilient predatory protists, this research can inform strategies for microbial community engineering aimed at promoting nutrient cycling and plant health in saline environments. Ultimately, such knowledge may contribute to improving crop yields and ensuring food security in salt-affected farmlands.

CRediT authorship contribution statement

Seda Ozer Bodur: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Solomon Oloruntoba Samuel:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Muhammet Fatih Polat:** Writing – review & editing, Visualization, Methodology, Formal

analysis. **Murat Aycan:** Writing – review & editing, Visualization, Supervision, Formal analysis, Conceptualization. **Rasit Asiloglu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation.

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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.2025.179606.

Data availability

The raw sequence data obtained in this study have been deposited in the NCBI database under the BioProject ID PRJNA1222634.

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