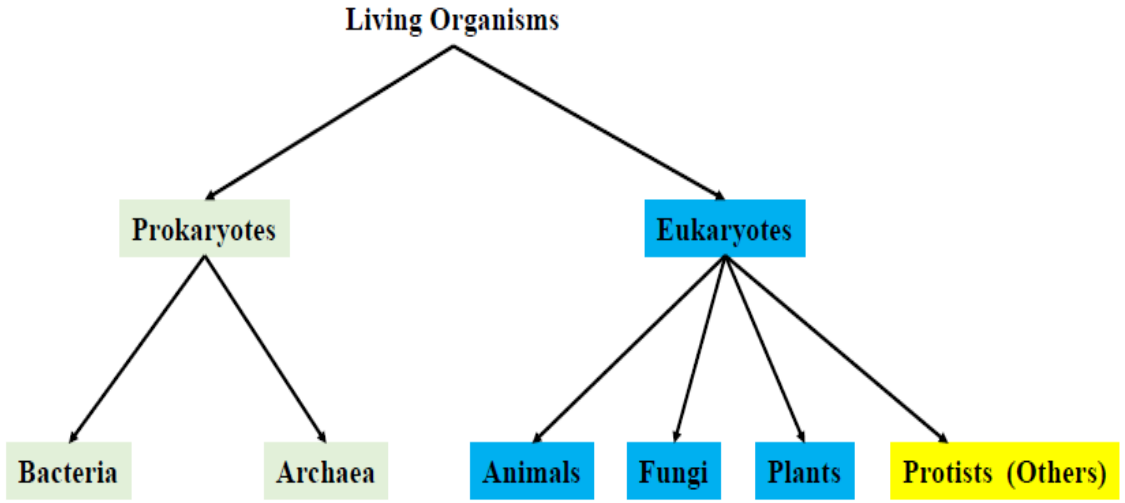
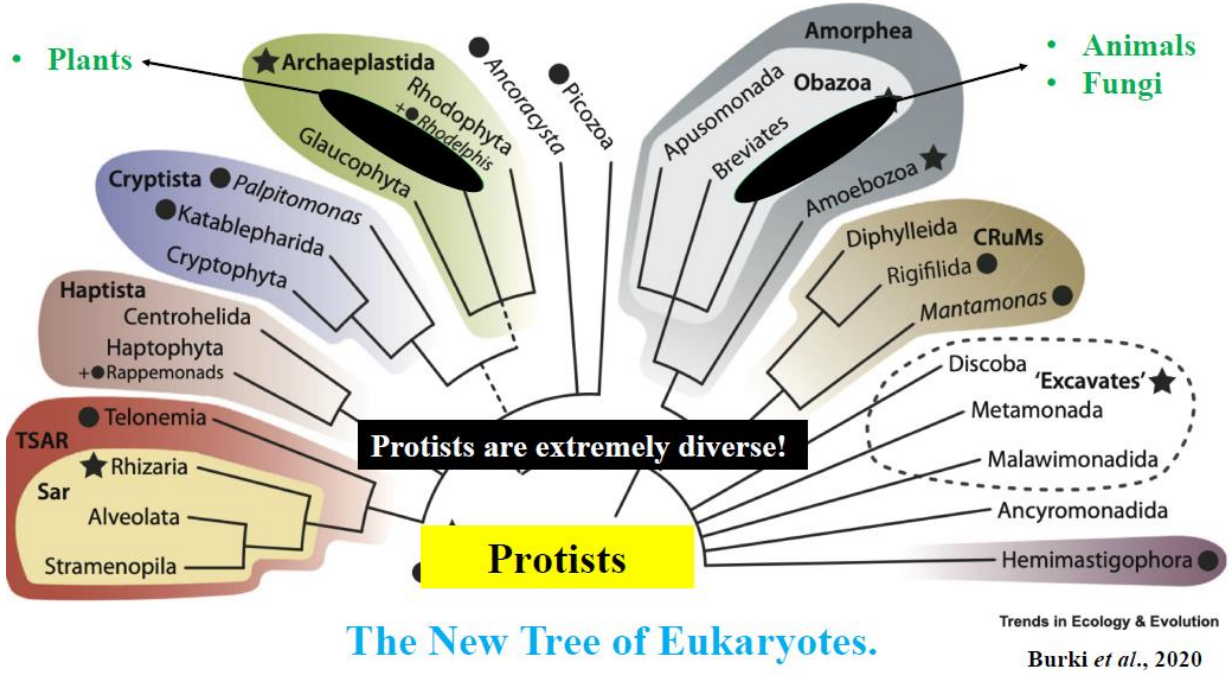
Soil properties have more significant effects on the community composition of protists than the rhizosphere effect of rice plants in alkaline paddy field soils

What are protists...?



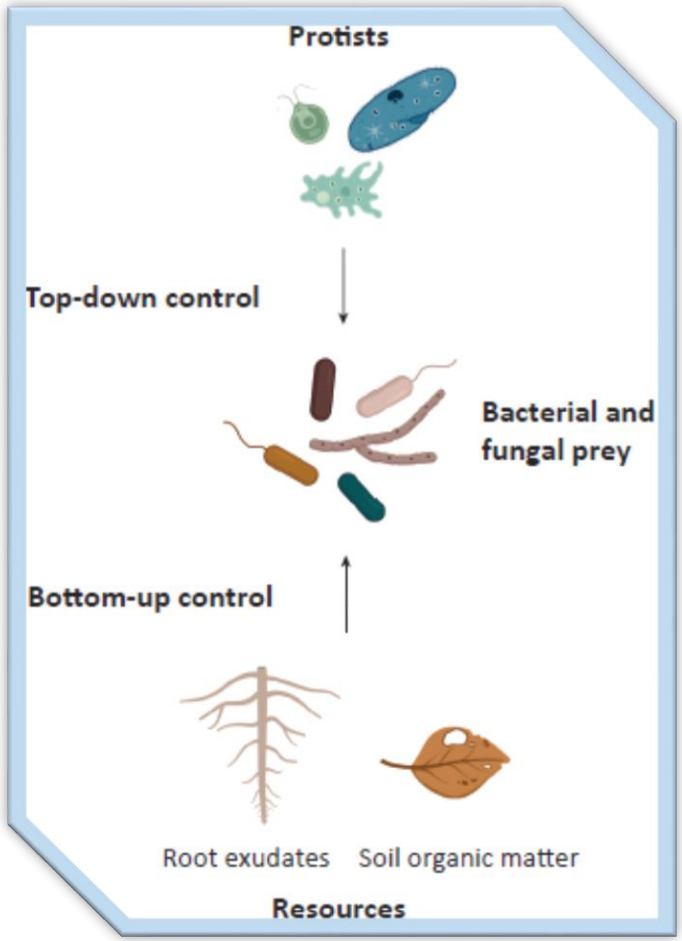


Protists are all Eukaryotes except Animals, Fungi, and Plants!

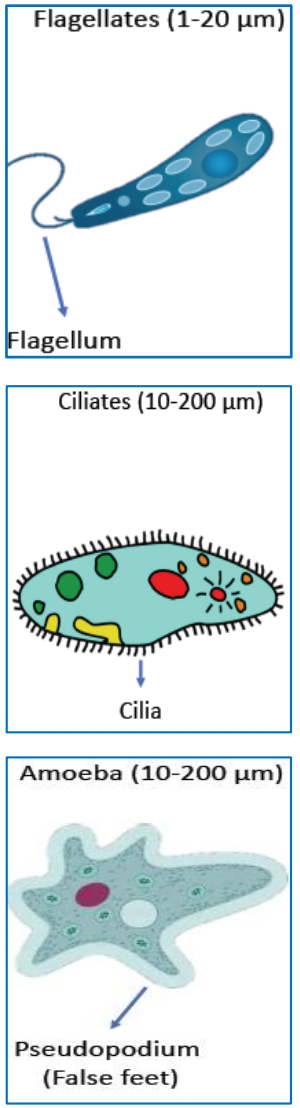
- microscopic, unicelluar (single cell) organisms with a nucleus and mitochondria
- aquatic or moist environments (i.e. rice field) are favorable for the protists
- abundant members of the soil microbiome (typically present at densities of 10^4–10^8 per gram of soil)
- found in all eukaryotic supergroups (i.e. *Amoebozoa,Obazoa, Archaeplastida, SAR, and Excavata)*.
- essential components of soil biodiversity and ecosystem functioning (vital role in the microbial food web as consumers of bacteria, fungi, and other small eukaryotes
- maintaining soil fertility and plant productivity

What do protists function ...?

Functionality of soil protist



- **Consumers (heterotrophs)**
  - Phagotrophs (consuming other microorganisms such as bacteria)
  - Saprotrophs
- **Autotrophs (Photosynthetic protists)**
- **Parasites**

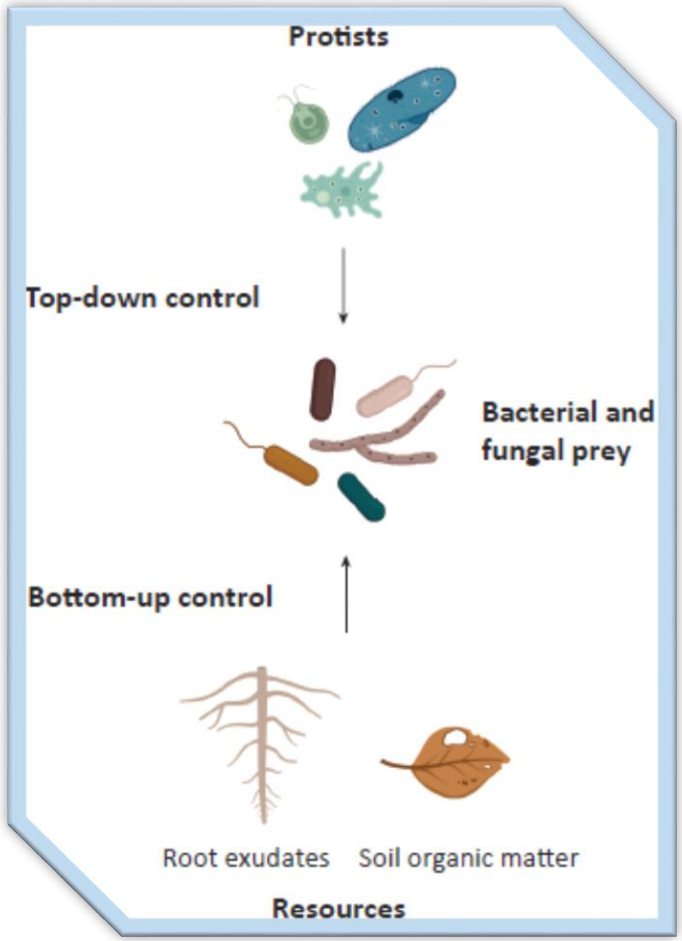


''top-down control'' refers to controlling community structure or population dynamics of the ecosystem by a top predator reflecting to lower trophic levels (through bottom of the pyramid).

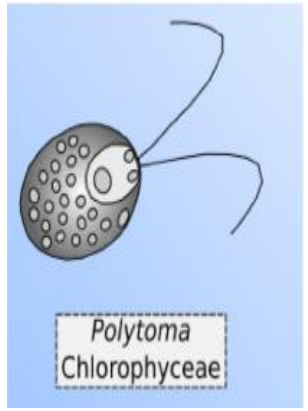
Geisen et al. 2018

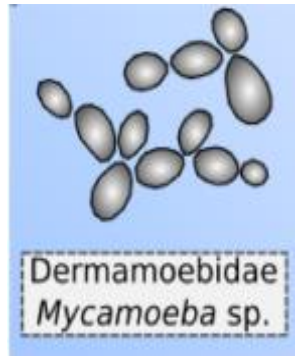
What do protists function ...?

Functionality of soil protist



- **Consumers (heterotrophs)**
  - Phagotrophs
  - Saprotrophs (consuming dead or decaying organic material) important role in the nutrient cycling via organic matter decomposition
- **Autotrophs (Photosynthetic protists)**
- **Parasites**

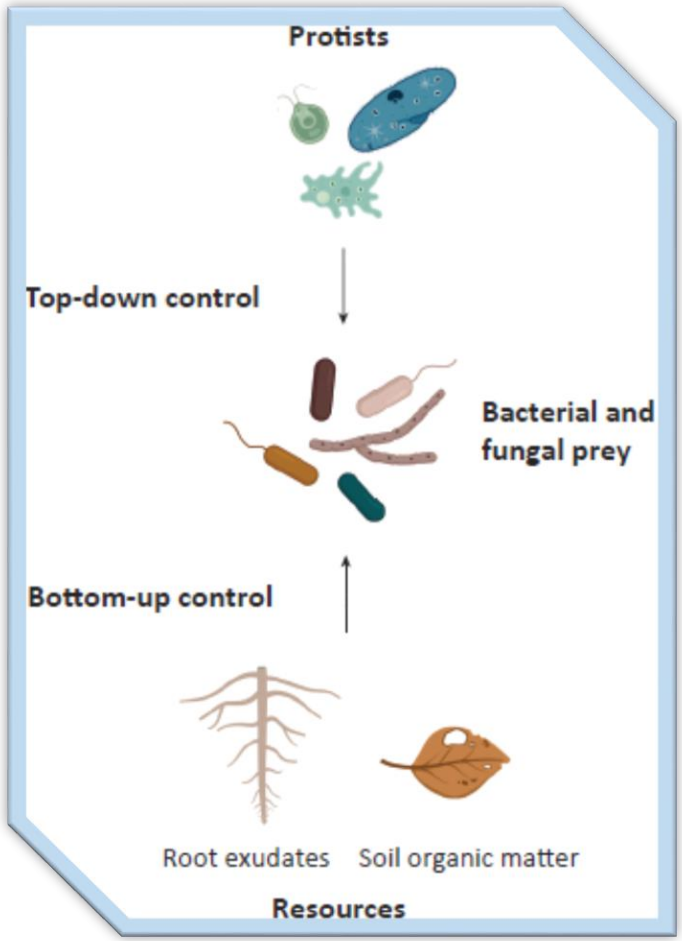




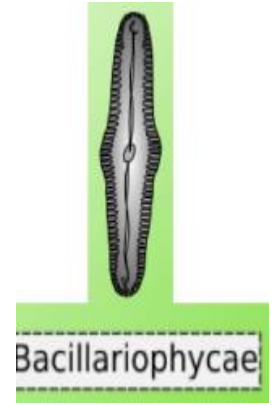
Geisen et al. 2018

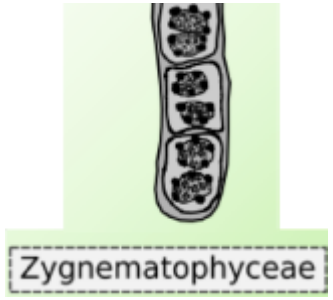
What do protists function ...?

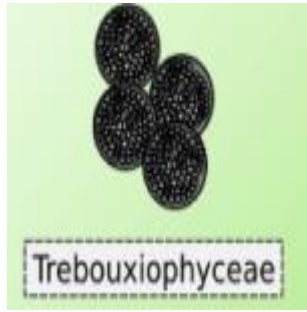
Functionality of soil protist



- **Consumers (heterotrophs)**
- **Autotrophs (photosynthetic protists)**
  - contributing SOC input and C sequestration
  - most abundant in the top soil
  - major autotrophic protist group is <u>algae</u>
- **Parasites**



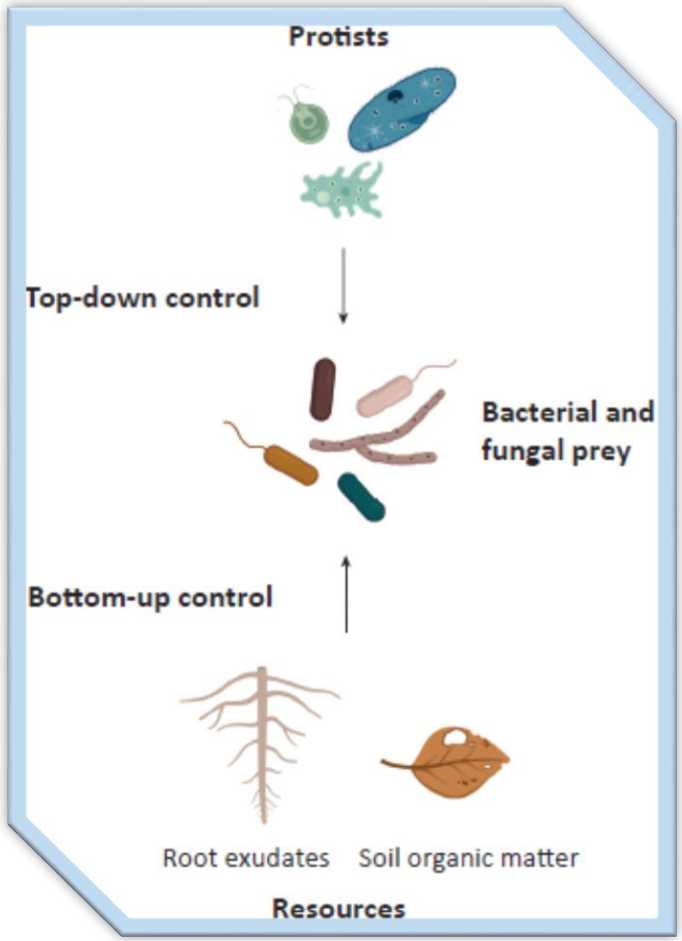




Geisen et al. 2018

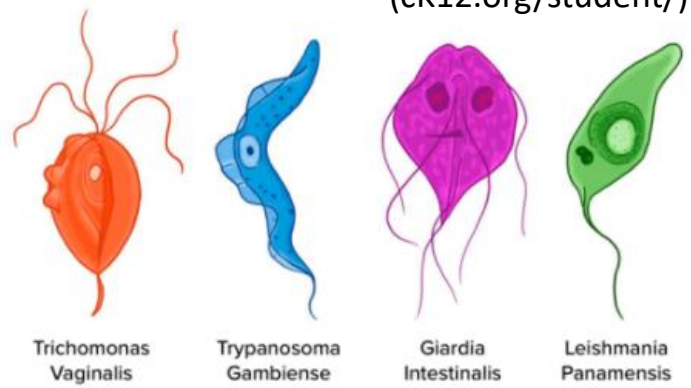
What do protists function ...?

Functionality of soil protist



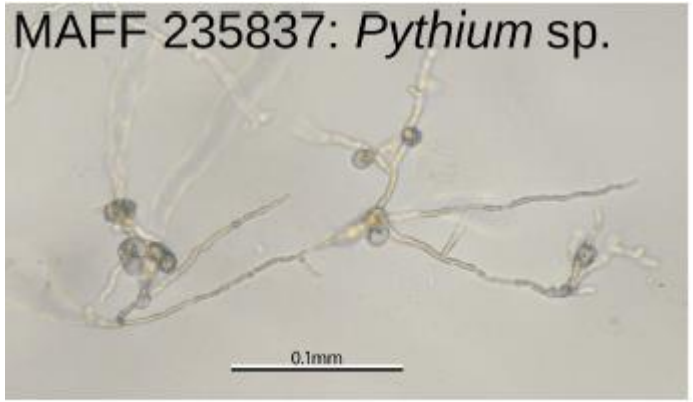
- **Consumers (heterotrophs)**
- **Autotrophs (photosynthetic protists)**

(cK12.org/student/)



- **Parasites**

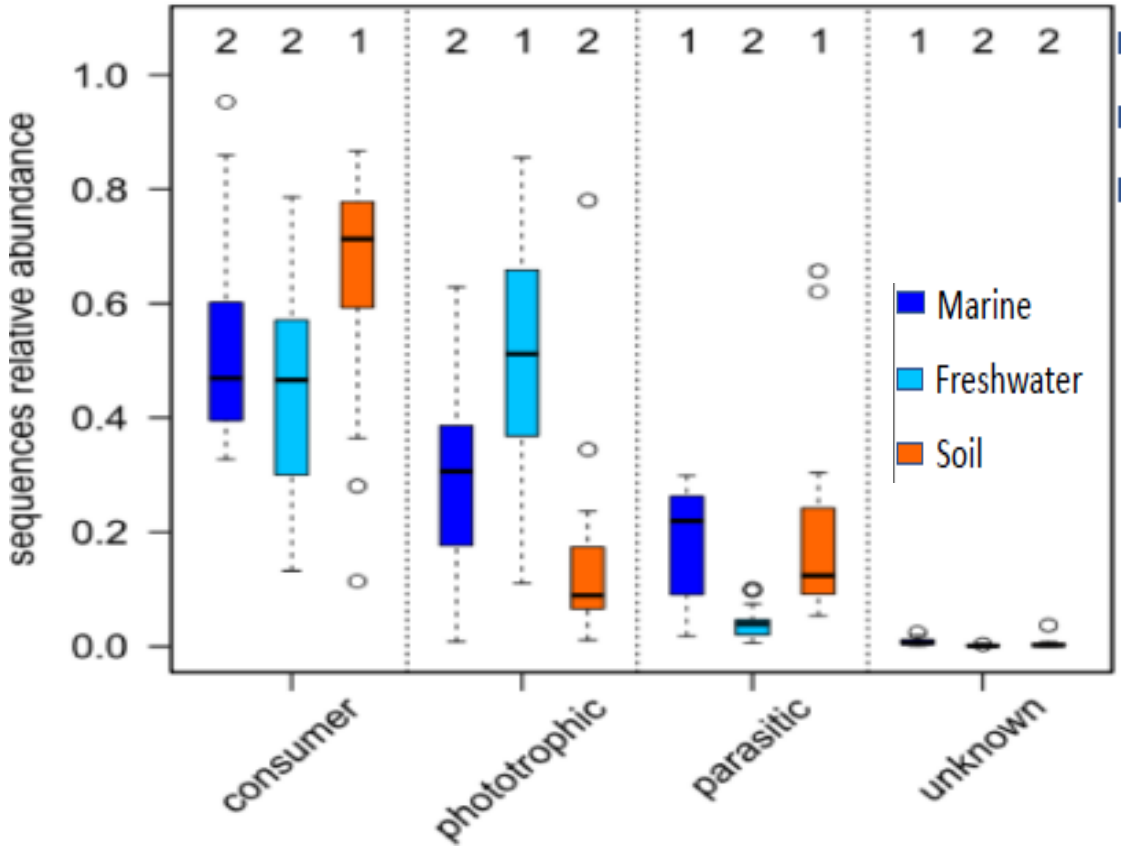
  - ➢ <u>Animal / microbial parasites</u> (negatively effect the host health and existing in soil)
  - ➢ <u>Plant pathogens</u> (i.e. Oomycetes, Peronosporomycetes, Stramenopiles) effecting plant health and cause enormous yield loss.



Nara Gene Bank: Microbial Images

What do protists function ...?

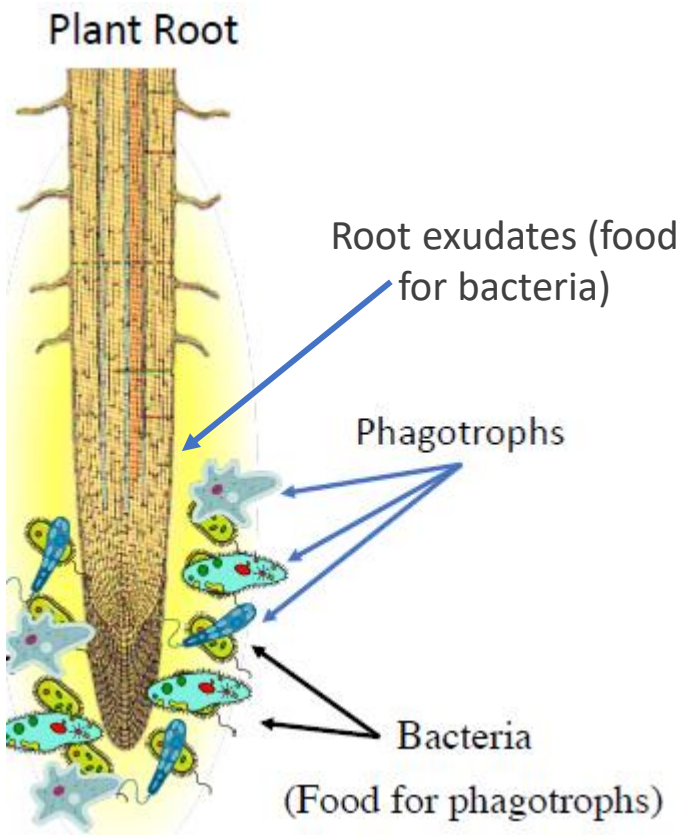
Nature wide distribution of soil protist



Numbers at the top of the boxplot indicate significantly different distributions within a functional groups (Nem-enyi test P <0.05), "1'' representing the highest distribution and "2" the lowest. (Singer et al., 2021)

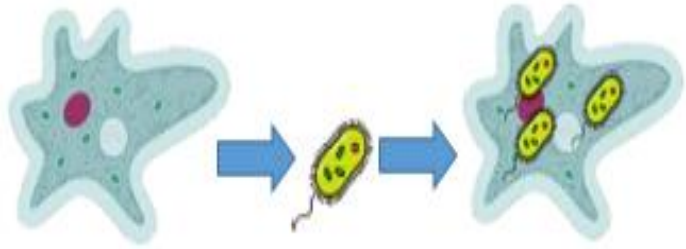
- Consumers dominates relative abundance in soil.
- Phototrophs are the second most abundant functional group and abundant in freshwater systems.
- Parasitic taxa represented roughly 15 to 20% of all sequences in marine and soil systems, but only around 5% in freshwater systems.

Why phagotrophs are populated in rhizosphere ...?



Root exudates → Bacteria → Protists

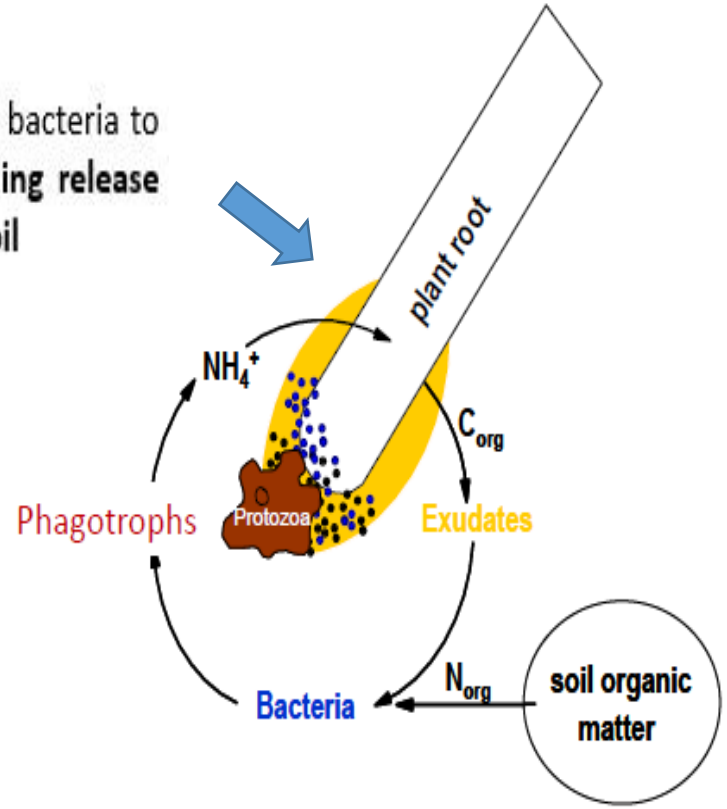
Mystery of phagotrops is a simple arithmetic !



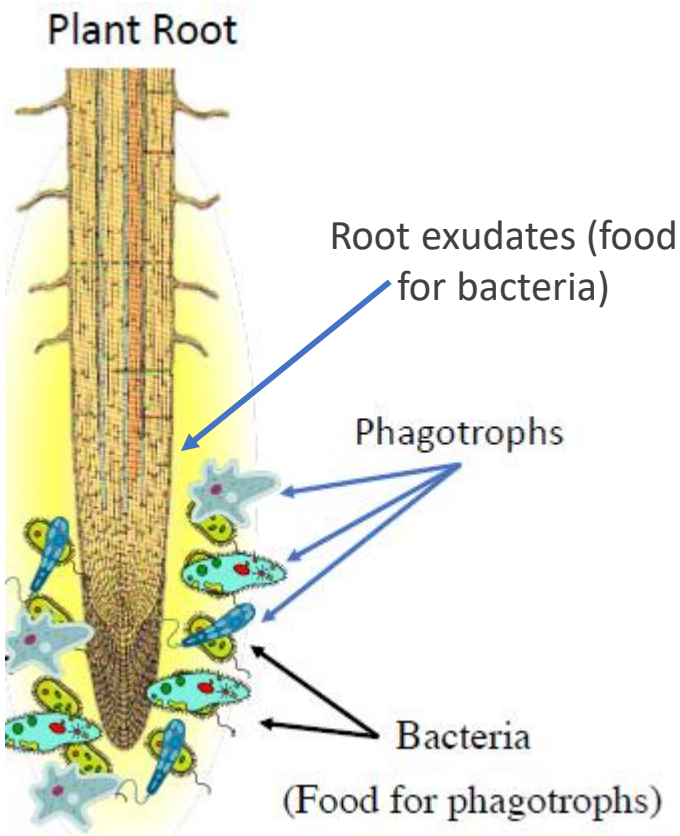
Phagotrophs C/N ratio: 9/1

Bacteria C/N ratio: 3/1

Phagotrophs needs to eat 3 bacteria to reach C/N ratio: 9/1, **meaning release of redundant nitrogen to soil**

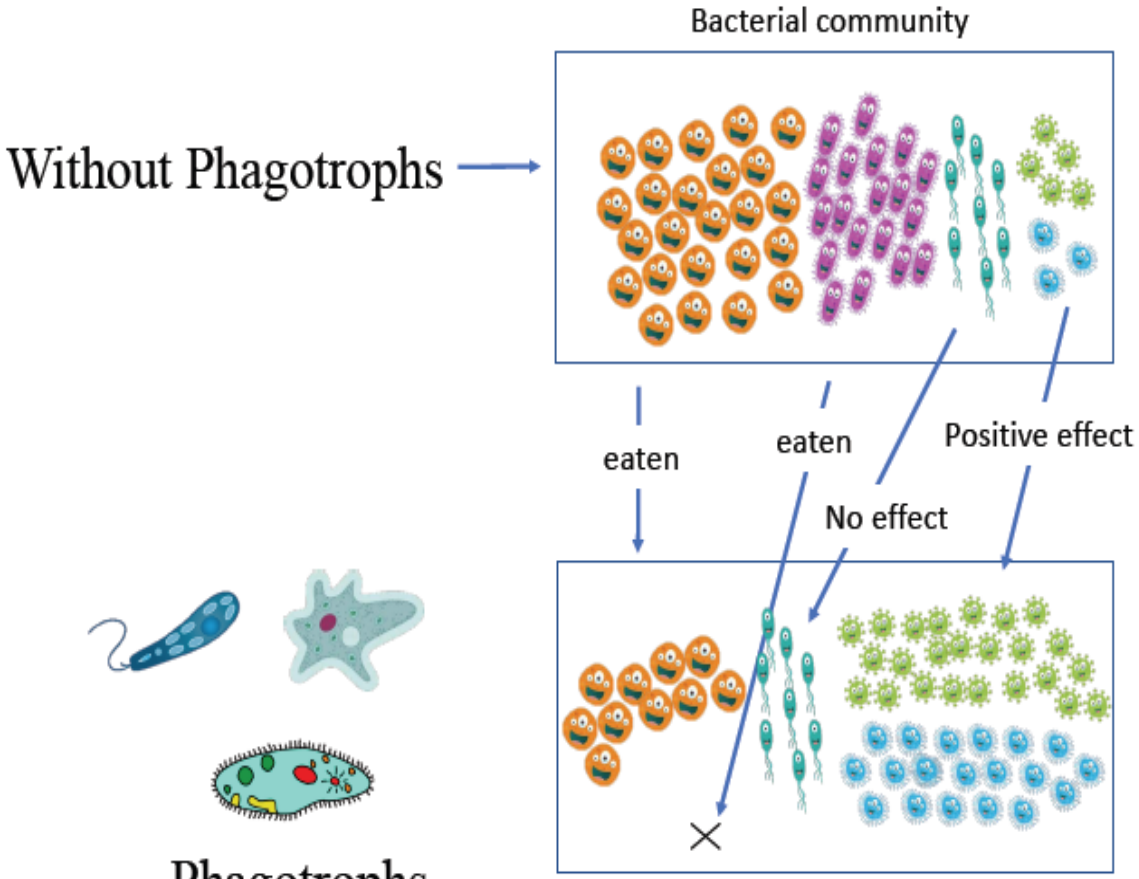


Why phagotrophs are populated in rhizosphere ...?

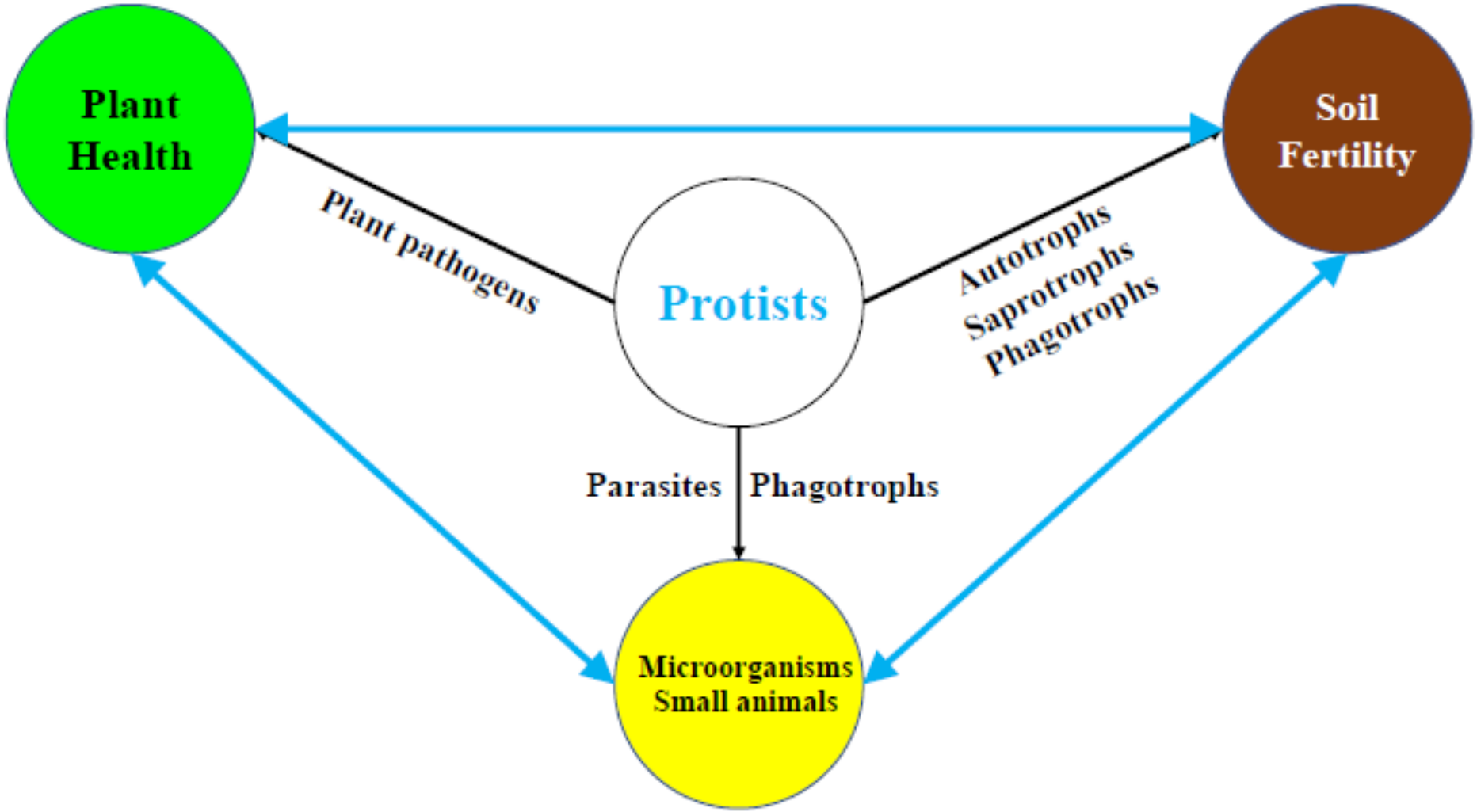


Root exudates → Bacteria → Protists

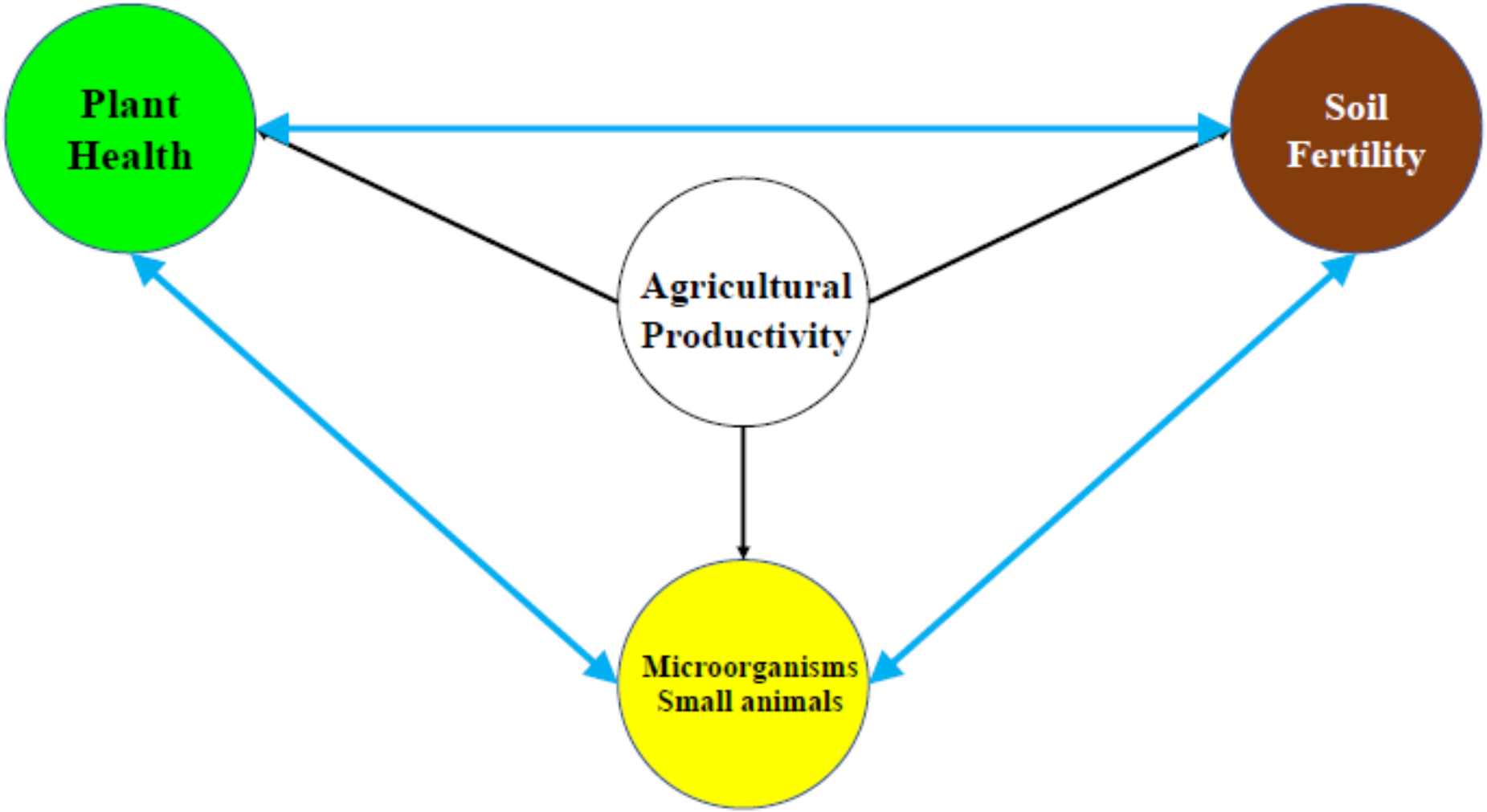
Changing bacterial community composition due to feeding activity of Phagotrophs



In Summary...


Plant Health
Soil Fertility
Protists
Plant pathogens
Autotrophs
Saprotrophs
Phagotrophs
Parasites
Phagotrophs
Microorganisms
Small animals

In Summary...


Plant Health
Soil Fertility
Agricultural Productivity
Microorganisms Small animals

Research priorities on soil protist ...

1. Factors shaping protist communities

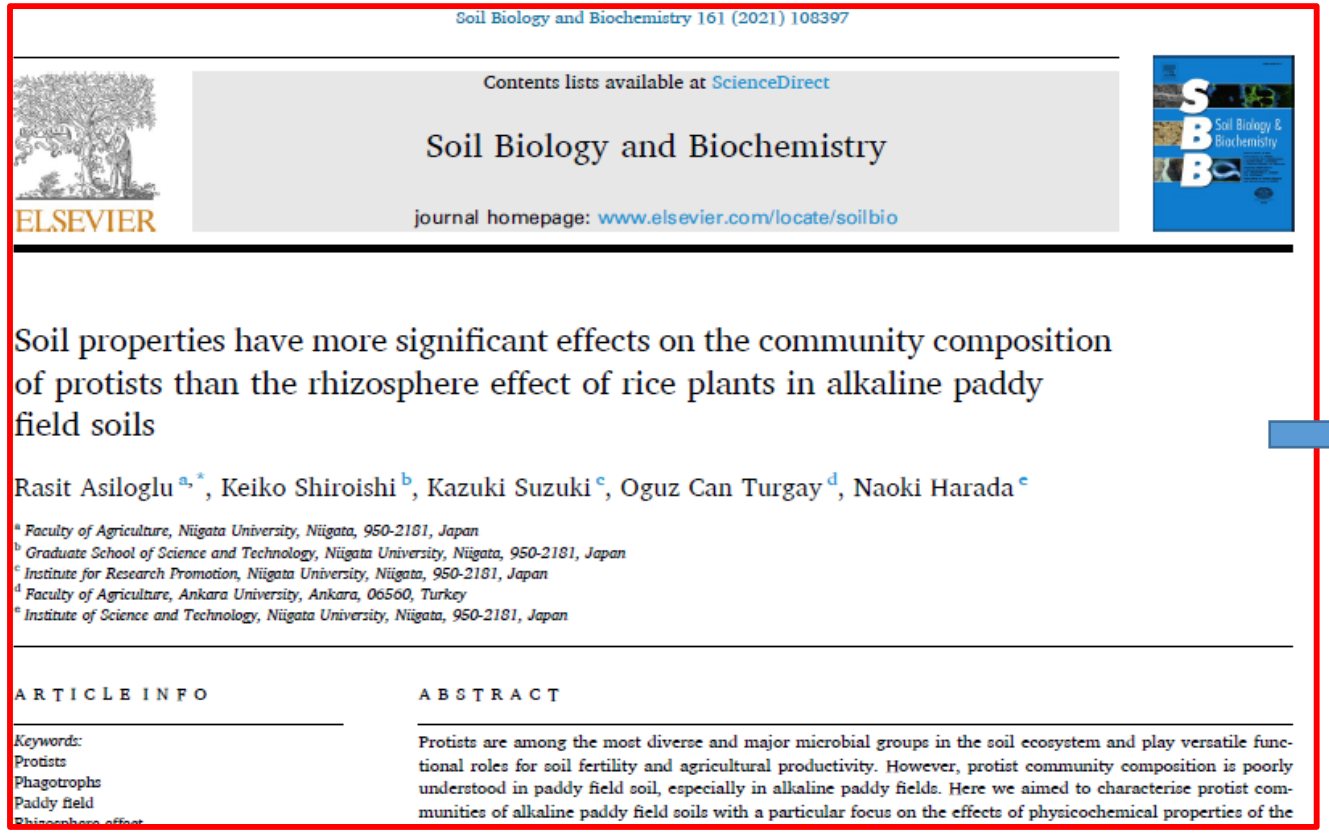
2. Prey – Predator interaction between Bacteria – Phagotrophs

3. Effects of phagotrophs on plant growth

Culture-dependent methods
Isolation, cultivation *etc*.

Culture-independent methods
High Throughput Sequencing, qPCR *etc*.

GLOCAL AGE 2020
(Protist Research Group)



Soil Biology and Biochemistry 161 (2021) 108397

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Soil properties have more significant effects on the community composition of protists than the rhizosphere effect of rice plants in alkaline paddy field soils

Rasit Asiloglu [a,*], Keiko Shiroishi [b], Kazuki Suzuki [c], Oguz Can Turgay [d], Naoki Harada [e]

[a] Faculty of Agriculture, Niigata University, Niigata, 950-2181, Japan
[b] Graduate School of Science and Technology, Niigata University, Niigata, 950-2181, Japan
[c] Institute for Research Promotion, Niigata University, Niigata, 950-2181, Japan
[d] Faculty of Agriculture, Ankara University, Ankara, 06560, Turkey
[e] Institute of Science and Technology, Niigata University, Niigata, 950-2181, Japan

ARTICLE INFO

Keywords:
Protists
Phagotrophs
Paddy field
Rhizosphere effect

ABSTRACT

Protists are among the most diverse and major microbial groups in the soil ecosystem and play versatile functional roles for soil fertility and agricultural productivity. However, protist community composition is poorly understood in paddy field soil, especially in alkaline paddy fields. Here we aimed to characterise protist communities of alkaline paddy field soils with a particular focus on the effects of physicochemical properties of the

Aims of the research

To investigate protist communities in a series of Turkish paddy fields to answer the following questions:

- **Who are they? (**Taxonomical characterization by high-throughput sequencing**)**
- **What do they do?** (Their potential functionalities)
- **Factors affecting them?** (Soil properties vs. the rhizosphere effect)
- **Do they interact with each other** (Pathogens & Predators)

Materials and Methods

Soil samples were obtained from 11 paddy fields from 3 regions in Turkey. (Kizilirmak, 3; Osmancik, 3; Samsun, 5)

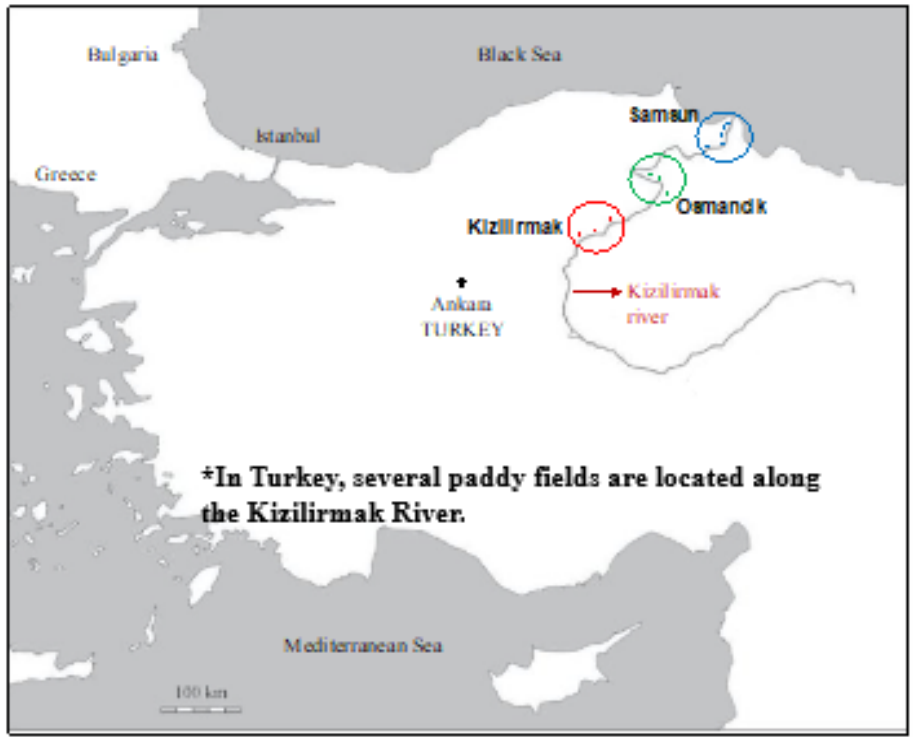


Fig. 1. Map showing the sampled paddy fields in Kizilirmak region (red colour), Osmancik region (green colour), and Samsun region (blue colour) along the Kizilirmak river (the green line).

The soil samples obtained from each region exhibited differential physicochemical characteristics, and the regions were grouped separately by the PCA analysis.

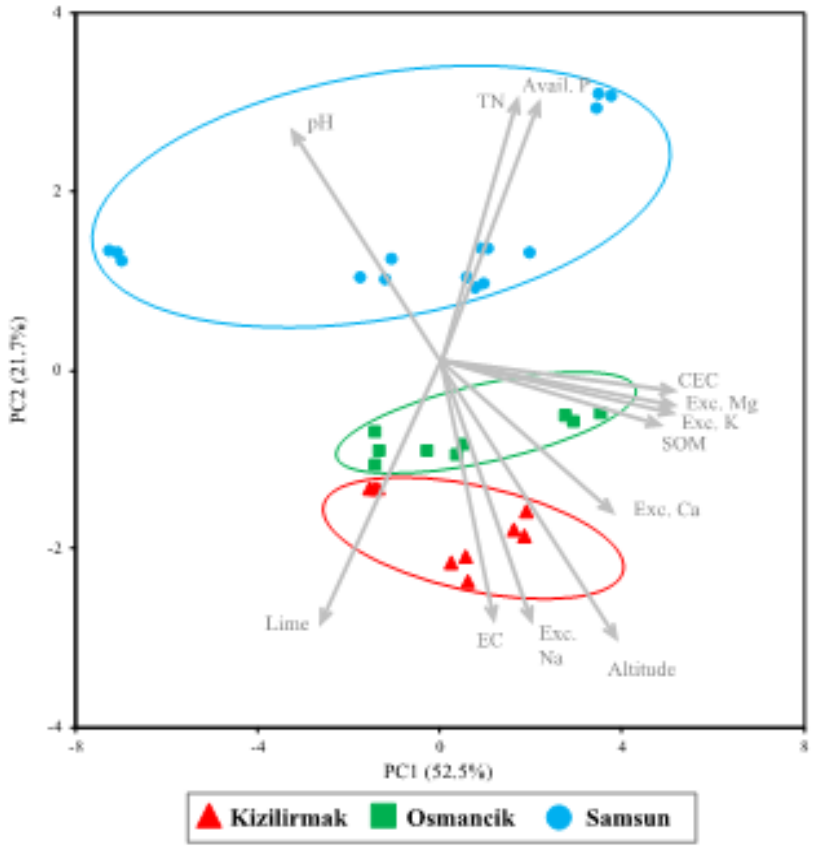
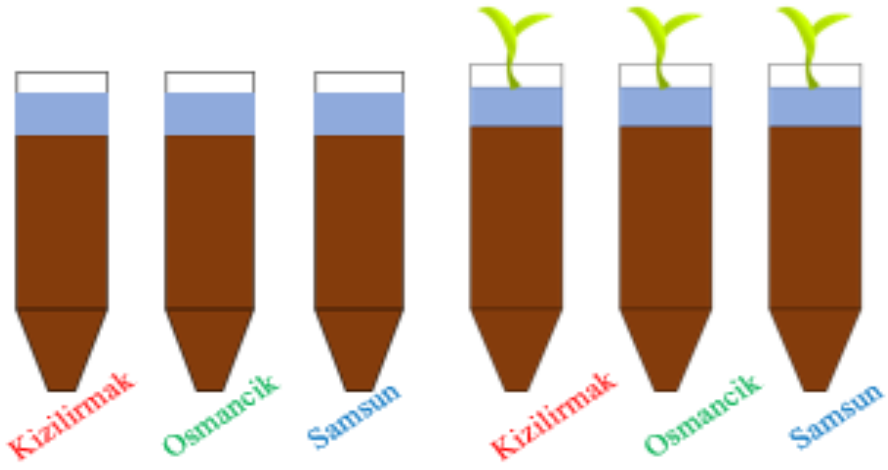


Fig. 2. Principal Component Analysis (PCA) based on the soil physicochemical properties. The soil samples were obtained in the Kizilirmak region (red triangle), Osmancik region (green square), and Samsun region (blue circle). Exc., exchangeable; avail., available.

Materials and Methods

The microcosms were established in sterile 50 ml plastic tubes filled with the paddy field soils.



Incubation in a growth chamber under submerged conditions at 25 °C with a day length of 15 h (250 µmol m− 2 s− 1) for 21 days.

Sampling of bulk soil (non-planted) and the rhizosphere soil (planted).

Molecular Analysis

- **DNA Extraction** (0.5 g soil samples using ISOIL for Bead Beating)
- **PCR** (The V9 region of the 18S rRNA gene with the the universal eukaryotic primers [1389F/1510R])
- **Illumina MiSeq Sequencing**

Bioinformatics

- The bioinformatics were done to **assign taxonomies** to the obtained sequences.
- The protist taxonomies were assigned into three functional groups (**consumers, autotrophs, and pathogens**).
- **NMDS** analysis was conducted to visualize the protist diversities.
- **LEfSe** analysis was conducted to identify the significantly different ($p < 0.05$, Kruskal–Wallis test, LDA score >2.0) protist groups which were affected by the soil physicochemical properties and the rhizosphere effect.
- A network analysis was used to understand the interaction among the functional groups of protists.

Results: Taxonomic and functional groups of protists.

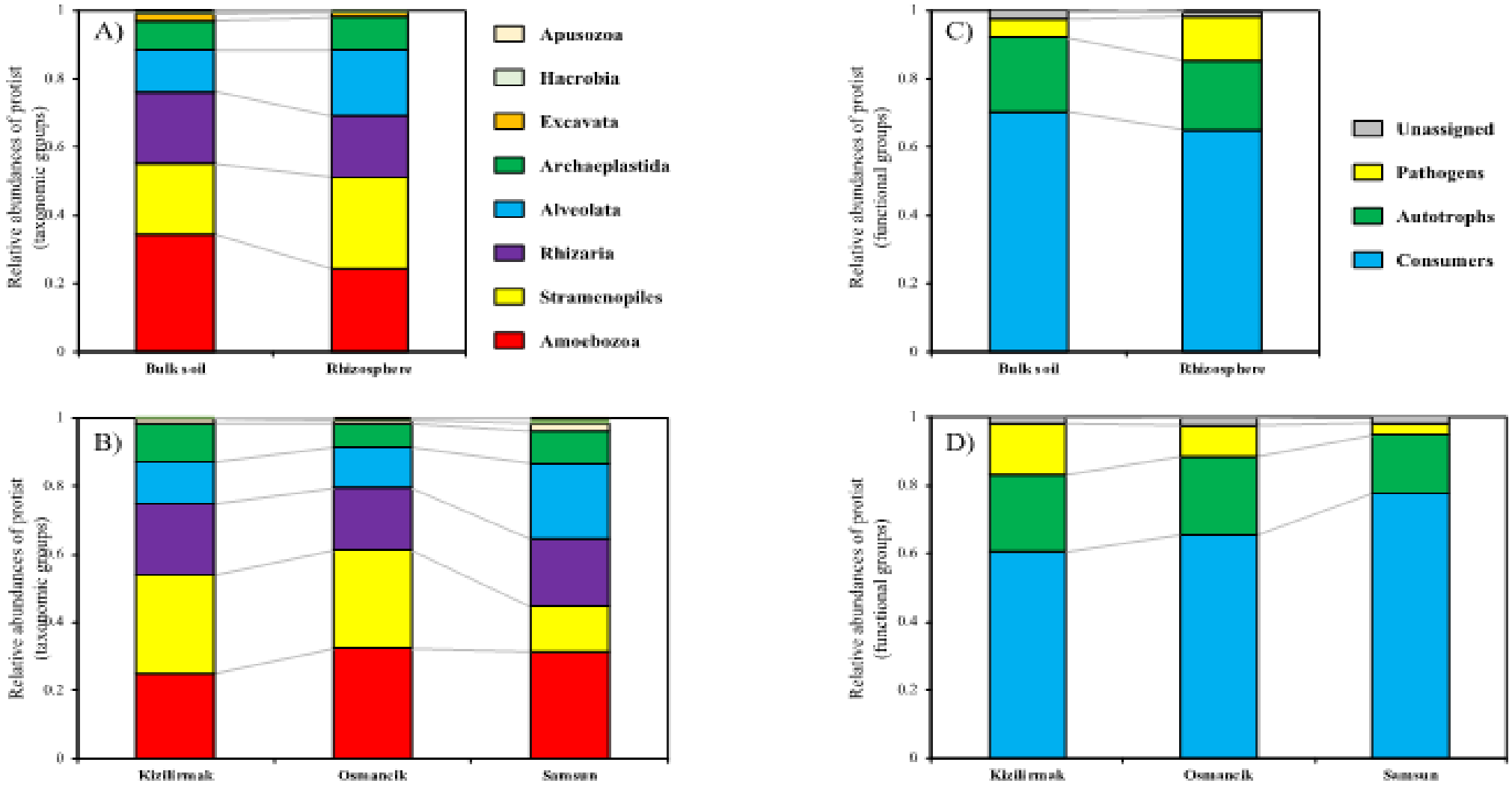


Fig. 3. Taxonomic (A and B) and functional (C and D) community compositions of protists affected by rhizosphere (A and C) and regions (B and D).

Results: Comparison of factors effecting protists

Table 1
Permutational multivariate analysis of variance (PERMANOVA) results based on Bray-Curtis dissimilarities for the effects of region (soil properties) and plant roots.

Factors	Df	SumsOfSqs	MeanSqs	F. Model	R^2	p
Region	2	2.8495	1.4247	3.8307	0.1025	0.001 ***
Rhizosphere effect	1	1.0101	1.0101	2.7158	0.0363	0.001 ***
Region: Rhizosphere effect	2	1.6256	0.8128	2.1854	0.0585	0.001 ***
Residuals	60	22.3156	0.3719	0.8027		
Total	65	27.8008	1.0000			

Soil properties > Rhizosphere effect

- The differences in the soil physicochemical properties in each region were the major controlling factors on the beta diversity of protist communities.
- The rhizosphere effect of rice significantly affected the protist community composition.
- However, it was less prominent than the effects of the soil properties.

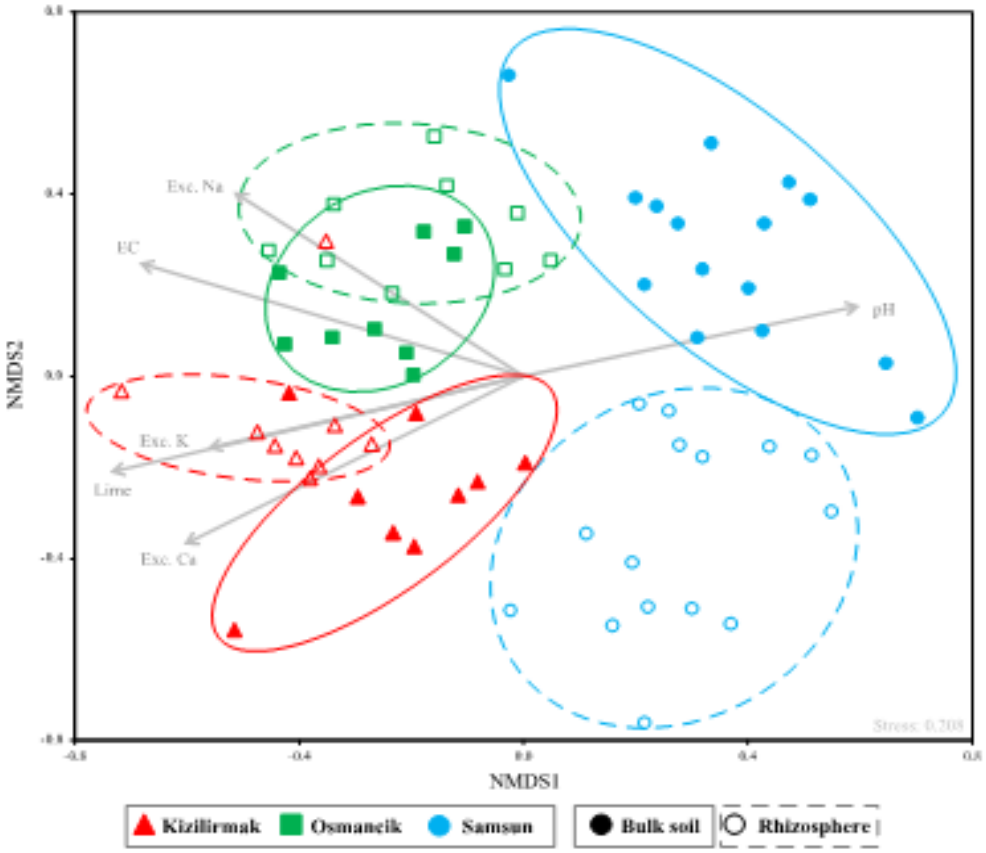
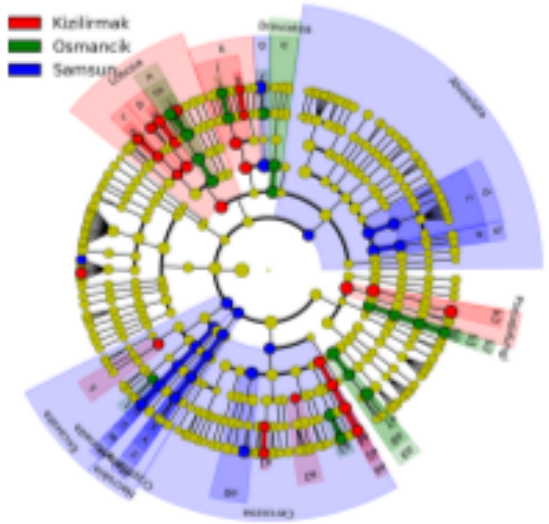


Fig. 2. Non-metric multidimensional scaling (NMDS) plots of protist communities with significant correlations ($p < 0.01$) with the soil physicochemical properties. Red triangles, Kizilirmak region; Green square, Osmancik region; Blue circle, Samsun region; Solid marks, Bulk soil; Hallow marks, Rhizosphere soil. The arrows indicate significant correlations among protist communities and environmental parameters. Exc., exchangeable.

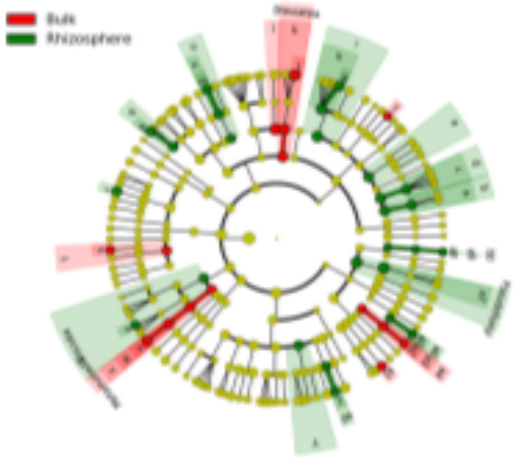
Results: Comparison of factors effecting protists

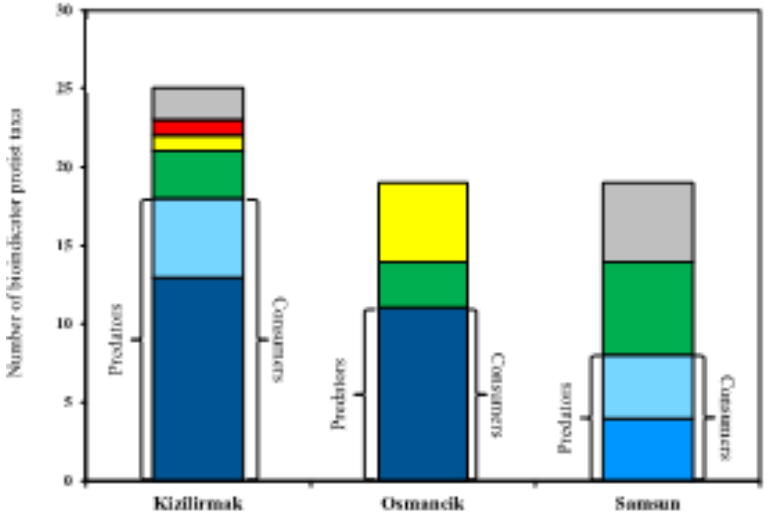
Effect of soil properties

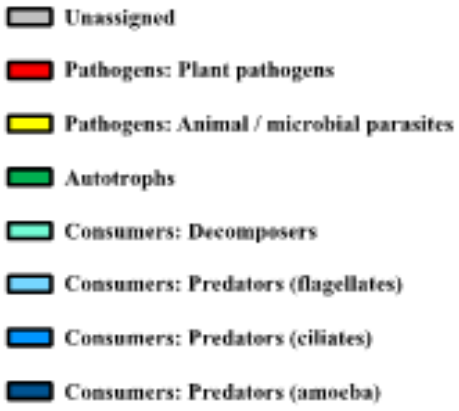


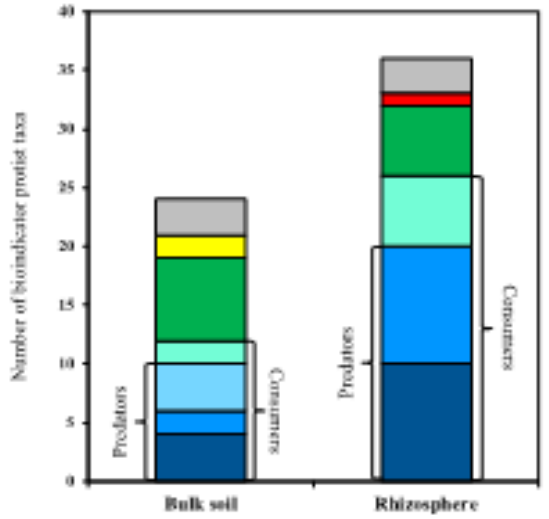
LEfSe cladograms are illustrating the taxonomic groups that explain the most variation among protist communities.

The rhizosphere effect



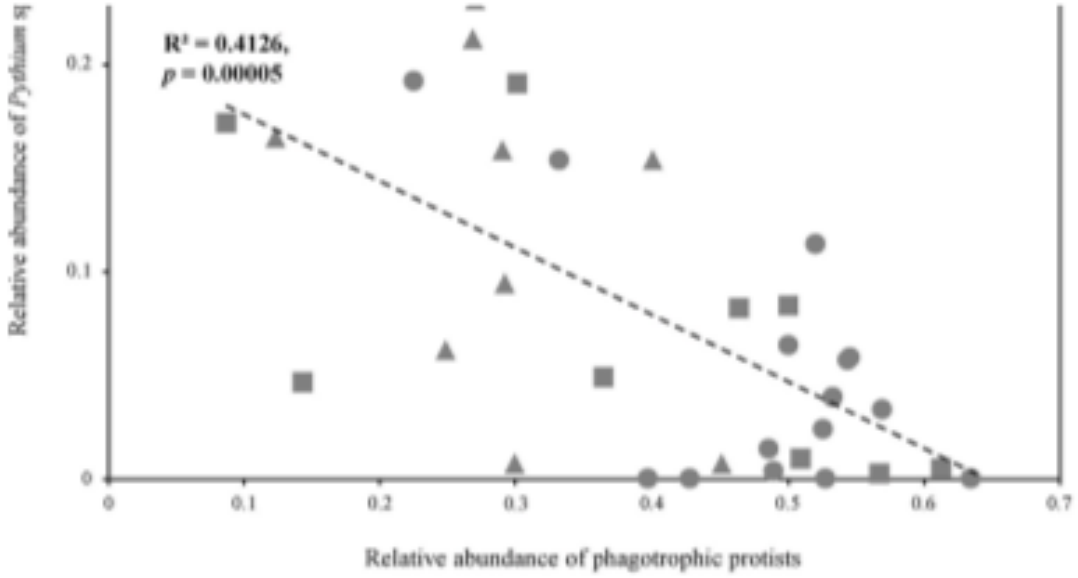


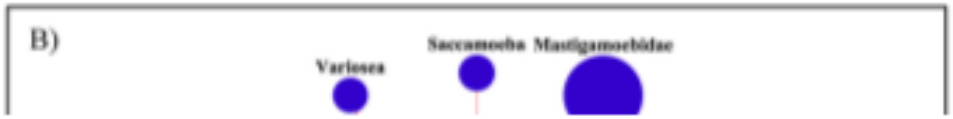




Results: Comparison of factors effecting protists

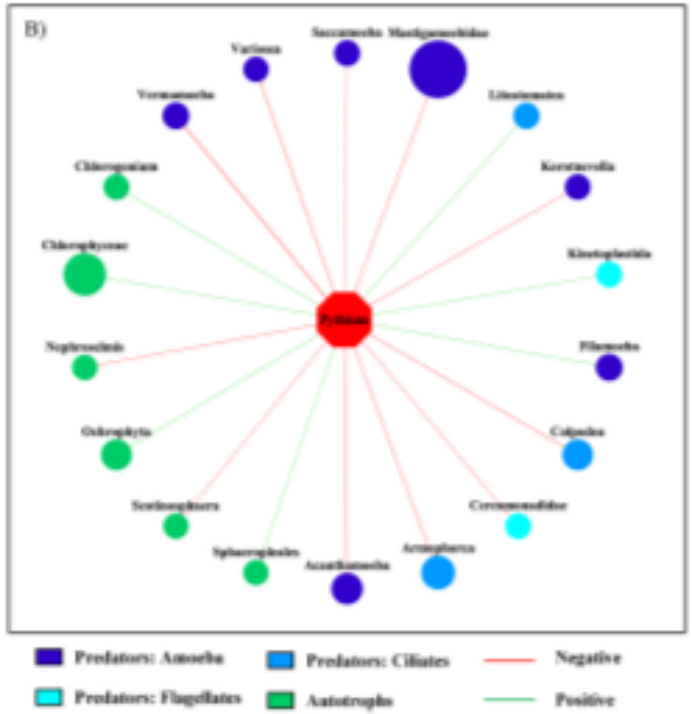
The relative abundance of *Pythium* sp. was negatively correlated (Regression analysis, R2 = 0.4126, $p = 0.00005$) with the relative abundance of phagotrophs in the rhizosphere samples.





A) The regression analysis showing the significant correlations between the relative abundance of phagotrophic protists and *Pythium* sp. in the rhizosphere of rice plants.

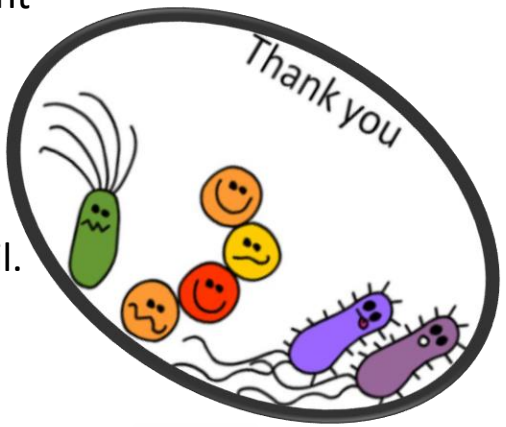
Over 75% of the interactions between phagotrophs (predators) and *Pythium* sp. were negative ($r < -0.75$).



B) Microbial co-occurrence network analysis of the protists that are directly associated with the *Pythium* sp. in all samples of the rice rhizosphere. The node sizes indicate the mean taxonomic abundances.

Summary & Conclusions

- Here, we revealed that the protist communities were driven by the soil physicochemical properties and the rhizosphere effect of rice roots in alkaline paddy field soils of Turkey.
- The soil physicochemical properties have a bigger effect determining protist communities than those of the rice roots.
- The phagotrophic protists (predators) are the dominant protist groups in the bulk and rhizosphere soils of alkaline paddy fields.
- As protist communities, especially phagotrophs, were differed depending on the soil properties, they may have different the top-down control on bacterial communities in soil food-web of paddy fields.
- In addition, we showed a significant negative correlation between phagotrophic protists and plant pathogens, which indicates that the plant pathogens could be top-down controlled by the phagotrophs.
- Further research on the whole microbiome (not only protists but also bacterial, archaeal, and fungal communities) should provide a better picture of the microbial food-web in paddy field soil.



Feel free to read the published manuscript for more details:
Soil Biology and Biochemistry 161 (2021) 108397
DOI: 10.1016/j.soilbio.2021.108397