diversity

MDPI

Article

Seasonal Variation of Eukaryotic Algae Community Structure in Different Soils Along Inland Lake Coasts in Central China

Qiufeng Yan [1], Zhihua Wu [2], Yachen Bai [1], Degao Wang [1], Benwen Liu [3], Jifei Hu [1] and Guoxiang Liu [3,*]

[1] College of Resources, Environment and Engineering, Anhui Technical College of Industry and Economy, Hefei 230051, China; yqfihb@163.com (Q.Y.); baiyachen1215@163.com (Y.B.)
[2] The Key Laboratory of Mariculture, Ministry of Education, College of Fisheries, Ocean University of China, Qingdao 266005, China
[3] Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
* Correspondence: liugx@ihb.ac.cn

Abstract

Eukaryotic algae are an important component of the soil microbial community. However, there is limited knowledge about the seasonal variations in the structure of soil algal communities and the correlations between different algal groups and soil environmental factors. Based on gene sequencing of the 18S rRNA V4 region, the changes in the community structure of eukaryotic algae in three different types of soil (sandy soil, forest soil and grassland soil along urban inland lake (Wuhan, China) coast) across four seasons were analyzed, and the correlations between the major taxonomic groups and environmental factors were examined. The annotated algae were ranked by abundance as follows: Chlorophyta, Bacillariophyta, Charophyta, Cryptista, Rhodophyta, Haptophyta and Glaucophyta. The top seven genera in terms of richness were: *Chlamydomonas*, *Tetracystis*, *Chlorella*, *Chlorococcum*, *Coelastrum*, *Bracteacoccus* and *Desmodesmus*. All soil samples tended to be neutral to alkaline. Environmental drivers for different groups were also explored. In terms of correlation, the environmental factors most related to Chlorophyta were total nitrogen, total phosphorus, and pH. Total nitrogen and water content were most related to Bacillariophyta and Charophyta. The seasonal differences in soil eukaryotic algal communities are more pronounced than the differences between soil types. Chlorophyta and Bacillariophyta are the two most abundant groups in different seasons and soil types. There is a significant difference in community composition between winter and other seasons.

Keywords: eukaryotic algae; community; environmental factors; 18S rDNA; sandy soil; forest soil; grassland soil

Check for updates

Academic Editor: Ipek Kurtboke

Received: 2 July 2026
Revised: 24 July 2026
Accepted: 27 July 2026
Published: 29 July 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Soil microorganisms play a crucial role in ecosystem functioning by driving biogeochemical cycles and mediating critical soil processes, yet their responses to climate change remain poorly understood [1–3]. Although algae were not formally recognized as components of soil microbial communities until the end of the 20th century [4], they are now known to inhabit a wide range of soil ecosystems, including woodlands, grasslands, farmlands, tundras, and deserts [5–7]. The inherent heterogeneity of soil environments generates diverse microhabitats that support considerable algal diversity [8,9]. Members of genera such as *Sphaerocystis* [10], *Tetracystis* [11], *Chlorococcum* [12], *Chlorosarcinopsis* [13], *Protococcus* [14], *Neochloris* [15], and *Spongiosarcinopsis* [16,17] have all been identified in

soil habitats. Beyond their ecological distribution, soil algae perform multifaceted functions that benefit both agricultural systems and soil formation. They contribute to soil quality assessment [18], accelerate soil genesis [19–22], enhance nitrogen content through biological nitrogen fixation [9,23,24], increase organic matter production to improve soil fertility [25], and maintain soil structure and water retention capacity via polysaccharide exudates [26,27]. Given these critical roles, elucidating the dynamics of soil algal communities, particularly in response to seasonal variation, is essential for understanding ecosystem functioning.

The assembly and composition of soil algal communities are shaped by the interplay between biotic and abiotic factors [28–31]. Accumulating evidence demonstrates that seasonality, precipitation, temperature, and nutrient availability collectively drive eukaryotic algal community structure [32–34]. Complementing these observations, studies have documented the effects of soil moisture and nutrient status on community composition [35–40]. Soil pH [41,42] and moisture [43] exert particularly strong influences by altering habitat conditions to which different algal groups respond distinctively: Chlorophyta tend to thrive in acidic soils; whereas, Cyanobacteria are better adapted to neutral and alkaline environments [44–48]. Methodologically, the shift from traditional microscopic identification to molecular approaches based on environmental DNA has substantially expanded our capacity to detect and characterize algal diversity [46]. Despite these advances, a critical knowledge gap persists: most investigations have examined individual environmental factors in isolation or focused on broad spatial comparisons, leaving it unclear how algal community structure across contrasting soil types within the same geographic region responds to seasonal fluctuations. Existing studies rarely capture the interactive effects of soil type and seasonality on algal community dynamics, limiting our ability to predict how these foundational microbial communities will respond to climate-driven changes in temperature and precipitation regimes.

To address this gap, the present study investigated the seasonal response of eukaryotic algal community structure in three contrasting soil types along the shoreline of East Lake in Wuhan, central China. We collected soil samples from sandy soil, forest soil, and grassland soil across all four seasons, yielding a total of 108 samples (nine sampling points with three replicates each). Using third-generation sequencing targeting the 18S rRNA V4 region, we characterized the molecular diversity of soil algae and examined their correlations with environmental variables, including total carbon (TC), total nitrogen (TN), total phosphorus (TP), pH, temperature (T), and water content (WC). This study provides several novel ecological insights that advance current understanding of soil algal ecology. First, by sampling three contrasting soil types within the same region across four seasons, we provide the first integrated analysis of how soil type and seasonality interact to shape algal community assembly—a dimension previously overlooked in studies that examined either soil type or season independently. Second, the application of third-generation sequencing enables more comprehensive detection of algal diversity than traditional microscopy-based approaches, revealing previously undocumented community patterns. Third, by quantifying the relative contributions of multiple environmental drivers simultaneously, we identify the key factors governing seasonal algal community shifts in lacustrine-adjacent ecosystems. Collectively, these findings establish a theoretical foundation for managing algal resources and addressing environmental challenges in terrestrial–aquatic ecotones under changing climate conditions.

2. Materials and Methods

2.1. Study Area and Sample Collection

The study area is located along the coast of the famous urban inland lake (30°32′34″–30°34′10″ N, 114°21′14–114°24′5″ E), East Lake, in Wuhan City, Hubei Province, China (Figure 1). We selected three different types of soils along the lake coast, which are sandy soil, forest soil, and grassland soil. Nine sample sites were selected in three different regions, respectively (as shown in Figure 1), and a total of 4 sampling sessions were conducted. The sampling periods were April 2022 (spring), August 2022 (summer), November 2022 (autumn), and January 2023 (winter), successively. A total of 108 samples were collected (4 seasons, 9 sampling points, with 3 replicates each), with the sample numbers ranging from S1-S3, F1-F3, and G1-G3, respectively. During sample collection, a sterilized was used to scrape blade (Beijing, China) the soil surface to collect 0.5–1 cm of soil. For each sample, about enough soil to fill a 50 mL centrifuge tube was collected in two portions. One portion was used for the extraction of total DNA and the determination of physicochemical indicators such as TC, TN, and TP. The other portion was used to measure WC, pH, and EC. The habitat photos and the distribution map of the sampling sites are shown in Figure 1.

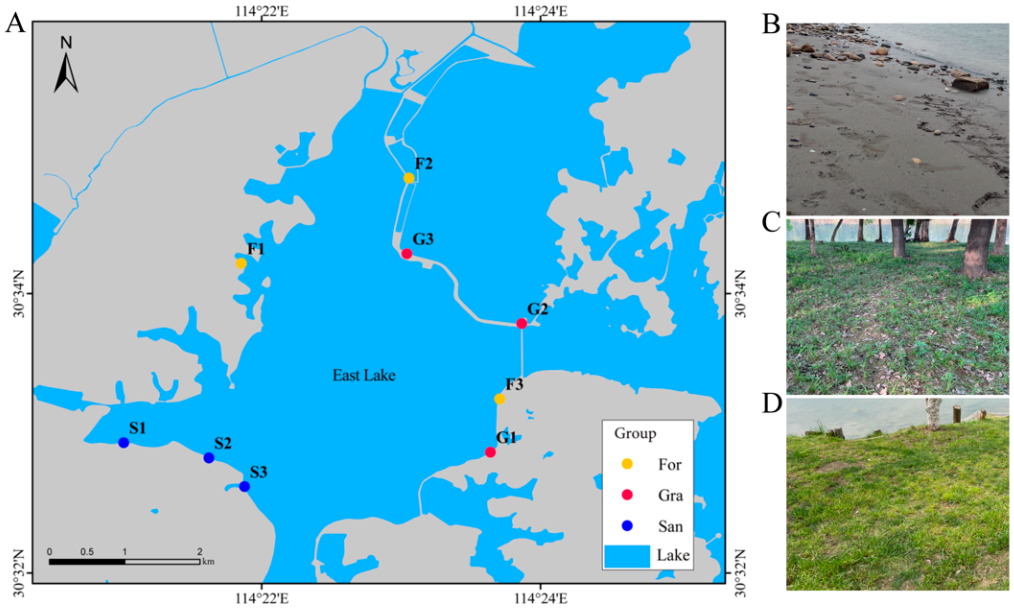


Figure 1. Distribution of sample points and habitat photos. (**A**) map of the distribution of nine sampling sites along the lake shore; (**B**) sandy soil; (**C**) forest soil; (**D**) grassland soil. San: sandy soil; For: forest land; Gra: grassland.

2.2. DNA Extraction and Determination of Environmental Factors

Soil samples for total DNA extraction were thoroughly shaken and mixed, and then total DNA was extracted according to the instructions of the HP Plant DNA Kit (Omega Bio-Tek, Norcross, GA, USA). Subsequently, the extracted total DNA was placed in dry ice and mailed to the sequencing company.

The measurement of TC employed the elemental analyzer SerCon Integra 2 (Sercon Limited, Crewe, UK). Initially, a sample of 50 mg was precisely weighed and placed in a tin capsule, which was then inserted into a combustion tube operating at 1000 °C (containing silver wire, copper oxide, and chromium oxide) for combustion. The gases produced were passed through a reduction tube filled with linear copper at 600 °C for reduction, and after the removal of moisture, they were separated by a chromatography column (with a column

temperature of 60 °C). Subsequently, the gases entered the elemental analyzer detector, and the soil carbon content was determined by comparison with standard samples.

The determination of total nitrogen refers to the Agricultural Industry Standard of the People's Republic of China (NY/T 1121.24-2012). An appropriate amount of soil sample was weighed using an ML204 type one-in-ten-thousand balance (Mettler, Columbus, OH, USA) and placed at the bottom of a dry Kjeldahl flask. Then, 2 g of accelerator and 5 mL of concentrated sulfuric acid were added, and the mixture was thoroughly mixed. Placed the Kjeldahl flask on the stove and heat it with a gentle flame for about 10–15 min. When the reaction became mild, the heat was increased to keep the soil solution in a gentle boiling state. Once the liquid and soil particles had all changed to a grayish white with a hint of green, the solution continued to boil for another hour. After completion, the sample was cooled awaiting distillation. During the boiling process, three blank tests were conducted, with all operations the same except for the soil sample added. Before distillation, it was necessary to prepare sodium hydroxide, sulfuric acid standard solution, and mixed indicator. We thoroughly preheated the nitrogen determination apparatus, and cleaned the pipeline through empty distillation until the readings stabilized. We took an appropriate amount of the above-mentioned sample solution and injected it into the distillation tube of the Kjeldahl nitrogen determination apparatus. After setting the parameters, proceeded with the measurement.

The determination of total phosphorus referred to the National Environmental Protection Standard of the People's Republic of China (HJ 632-2011). Using an ML204 type analytical balance (Mettler, Columbus, OH, USA) to accurately weigh 0.2 g of air-dried sample, carefully placed it at the bottom of a nickel (or silver) crucible. We added 3–4 drops of anhydrous ethanol to moisten the sample, and then spread 2 g of sodium hydroxide evenly over the sample. We placed the crucible into a high-temperature electric furnace, heated it to about 400 °C, then cut off the power and paused for 15 min. Then we raised the temperature to 640 °C and maintained this temperature for 15 min before removing the sample to cool down. We then added approximately 10 mL of water at about 80 °C. Once dissolved, we transferred the solution, without loss, into a 50 mL volumetric flask. We rinsed the crucible multiple times with water and also transferred the washing liquid into the same volumetric flask. We cooled the solution, restored it up to the mark by adding water, and filtered it through non-phosphorus qualitative filter paper or centrifuged it for clarification. We conducted a blank test simultaneously. We pipetted 1–10 mL of the sample solution into a 25 mL volumetric flask and diluted with water to about 3/5 of the total volume. We added 2–3 drops of dinatrium phenol indicator and adjusted the solution to a faint yellow color using an acid–base solution. We accurately added 2.5 mL of the molybdenum antimony anti-color developing agent, restored it up to the mark by adding water, shook it well, allowed the color to develop for 30 min, and then performed colorimetry.

The determination of electrical conductivity and pH is carried out by mixing the soil sample with distilled water at a soil-to-water ratio of 5:1 (mL/g) and stirring thoroughly until well mixed. The mixture is then measured using a YSI multi-parameter water quality monitor (Xylem Inc., Rye Brook, NY, USA) (model 6920V2-2) [47]. The determination of water content is carried out using the dry–wet weight difference method. The weight of the wet soil sample is measured using a balance, then the sample is dried in an oven and weighed again at 105 °C. The difference in weight is the water content.

2.3. Sequencing and Data Processing Methods

Sequencing was conducted by Personalbio Technology Co., Ltd. (Shanghai, China). The target fragment (18S rDNA V4 region) was subjected to high-throughput sequencing using the PacBio sequencing platform (Pacbio, Menlo Park, CA, USA). PCR amplification

used the forward primer TAReuk454FWD1 (5′-CCAGCASCYGCGGTAATTCC-3′) and the reverse primer TAReuk454REV3 (5′-ACTTTCGTTCTTGATYRA-3′) [48]. After the sequencing data was obtained from the sequencer, the data were aligned and corrected using SMRT Link v7.0 (Pacbio, Menlo Park, CA, USA) software to achieve high-quality consensus sequences (Circular Consensus Sequences, CCS), also known as raw reads. Subsequent to the raw reads, filtering and screening were performed (retaining sequences of 1500 to 4500 bp), followed by primer removal and chimera removal, ultimately obtaining clean reads for subsequent analysis.

The sequencing depth was evaluated using QIIME2 (2022) [49], while aligning all sequence directions in the same direction, i.e., 5 'to 3' direction. Based on the minimum sequence count for each sample, all samples were rarefied. Subsequently, OTU (Operational Taxonomic Unit) clustering was performed at a similarity threshold of 97% using the software USEARCH (version 11). This clustering process resulted in the generation of an OTU abundance table and representative sequences for each OTU. The sequences were aligned against the GenBank database using Blastn, followed by annotation of the OTUs. The sequences have been deposited in the NCBI database, and the accession numbers of the sequences are shown in Table 1.

Table 1. Accession numbers of the sequences.

PZ277261	PZ277168	PZ277035	PZ276994	PZ276794	PZ276402	PZ280600
PZ277260	PZ277167	PZ277027	PZ276993	PZ276793	PZ276213	PZ280601
PZ277259	PZ277139	PZ277021	PZ276960	PZ276791	PZ276162	PZ280603
PZ277258	PZ277138	PZ277020	PZ276959	PZ276777	PZ276153	PZ280606
PZ277255	PZ277057	PZ277017	PZ276958	PZ276776	PZ276149	PZ280604
PZ277252	PZ277046	PZ277016	PZ276956	PZ276765	PZ276143	PZ280605
PZ277253	PZ277044	PZ277014	PZ276957	PZ276755	PZ276142	PZ280608
PZ277254	PZ277043	PZ277013	PZ276951	PZ276754	PZ276091	PZ280607
PZ277251	PZ277040	PZ277012	PZ276949	PZ276753	PZ276089	PZ280609
PZ277250	PZ277041	PZ277011	PZ276907	PZ276752	PZ276087	PZ280614
PZ277249	PZ277036	PZ277006	PZ276903	PZ276750	PZ276086	PZ280613
PZ277247	PZ277037	PZ277005	PZ276900	PZ276705	PZ276063	PZ280610
PZ277248	PZ277038	PZ277004	PZ276899	PZ276697	PZ276058	PZ280612
PZ277246	PZ277039	PZ277001	PZ276810	PZ276698	PZ280599	PZ280611
PZ277243	PZ277034	PZ277002	PZ276808	PZ276407	PZ280602	PZ280615

2.4. Statistical Analysis

All of the below analyses and graphical renderings were carried out using R software (4.2.2). The non-metric multidimensional scaling (NMDS) ordination [50] was achieved through the metaMDS function in the vegan package. The relationship between algal communities and environmental factors was determined by R software (4.2.2) and the vegan package [51]. The difference map of community richness was plotted using the ggplot package. The corrplot package was used to draw the correlation heatmap [52]. The random forest model was constructed by performing 1000 random samplings using the randomForest package [53].

3. Results

3.1. Soil Physicochemical Parameters

Overall, the soil at the sampling sites is mostly neutral to alkaline, with a pH range of 6.90 to 8.83. The electrical conductivity ranges from 15.5 to 148.9 μS/cm. Seasonally, the water content is highest in spring (212 g/kg) and lowest in autumn (89 g/kg), with summer at 144 g/kg and winter at 115 g/kg. In terms of total carbon (TC), it is highest in spring (23.1 g/kg), which is higher than in summer (15 g/kg), autumn (15.8 g/kg), and

winter (15.9 g/kg). In terms of total nitrogen (TN), the levels are higher in spring (1.6 g/kg) and winter (1.57 g/kg) than in summer (1.35 g/kg) and autumn (1.15 g/kg). In terms of phosphorus (P), the concentration in spring (0.5 g/kg) is slightly lower than in summer (0.75 g/kg), autumn (0.73 g/kg), and winter (0.64 g/kg). Viewed by soil type, the total carbon (TC) content in sandy soil (18.1 g/kg) and grassland (18.4 g/kg) is comparable and higher than that in forest land (15.8 g/kg). The content of total phosphorus (TP) shows no significant differences among the three types of soil: sandy soil (0.7 g/kg), forest land (0.64 g/kg), and grassland (0.63 g/kg). The water content in sandy soil (0.17) is higher than that in forest land (0.13) and grassland (0.12). The raw data is listed in (Tables 2–5).

Table 2. Soil physicochemical properties of site in spring.

Site	TC g/kg	TN g/kg	TP g/kg	EC us/cm	PH	T °C	WC
S1-1	40.8	0.64	0.36	67.6	8.58	28.9	0.23
S1-2	45.8	1.96	0.47	58	8.67	28.9	0.34
S1-3	79.7	5.47	1.32	46.2	7.63	28.9	0.22
S2-1	3.1	0.22	0.14	38	7.39	28.4	0.3
S2-2	5.2	0.16	0.11	45.4	7.23	28.4	0.2
S2-3	3.1	0.24	0.12	43.4	7.19	28.4	0.26
S3-1	32.1	1.49	0.48	39.3	7.33	28.7	0.13
S3-2	13.5	0.72	0.16	52.9	7.3	28.7	0.21
S3-3	10.7	0.35	0.26	34.8	7.26	28.7	0.21
L1-1	14.8	0.94	0.39	47.7	7.99	26.5	0.21
L1-2	11.6	1.02	0.36	45.9	7.28	26.5	0.15
L1-3	25.2	2.08	0.61	47.4	7.5	26.5	0.18
L2-1	19.8	1.47	0.62	61.1	7.88	26.2	0.24
L2-2	18.1	1.4	0.44	58.9	7.95	26.2	0.24
L2-3	29.9	1.85	0.55	54.3	8.51	26.2	0.25
L3-1	17.7	1.43	0.48	82.7	8.53	28.1	0.17
L3-2	17.9	0.86	0.91	64.3	8.83	28.1	0.21
L3-3	16.8	0.59	0.50	30	7.57	28.1	0.15
C1-1	15.5	2.21	0.47	45.1	7.57	27.4	0.2
C1-2	26.8	2.35	0.59	45.5	7.62	27.4	0.17
C1-3	15.4	1.49	0.42	47	7.39	27.4	0.19
C2-1	42.6	3.75	0.49	36.3	7.48	27.8	0.18
C2-2	17.3	1.74	0.45	45.5	7.79	27.8	0.17
C2-3	19.1	1.94	0.58	53	7.59	27.8	0.23
C3-1	20.9	1.74	0.77	55.4	7.75	26.9	0.2
C3-2	19.8	1.48	0.71	67.3	7.8	26.9	0.23
C3-3	40.6	3.6	0.71	57.6	7.61	26.9	0.24

Table 3. Soil physicochemical properties of site in summer.

Site	TC g/kg	TNg/kg	TPg/kg	EC	PH	T	WC
S1-1	11.8	0.58	1.50	53.6	7.12	30.5	0.15
S1-2	12.6	0.73	1.93	69.4	7.1	30.5	0.18
S1-3	17.9	0.61	2.72	40.3	7.12	30.5	0.16
S2-1	28.3	0.37	0.31	62.6	7.01	31.9	0.12
S2-2	36.1	0.31	0.15	91.3	7.23	31.9	0.09
S2-3	29.0	0.42	0.33	88.7	6.98	31.9	0.15
S3-1	14.7	0.3	0.22	76.6	7.09	32.3	0.19
S3-2	24.0	0.31	0.27	34.4	7.09	32.3	0.23
S3-3	17.5	0.32	0.21	48.3	6.9	32.3	0.07

Table 3. *Cont.*

Site	TC g/kg	TNg/kg	TPg/kg	EC	PH	T	WC
L1-1	35.1	2.4	0.69	85.3	6.97	32.4	0.1
L1-2	10.2	2.61	0.72	119	7.24	32.4	0.18
L1-3	26.6	2.22	0.74	61	7.13	32.4	0.1
L2-1	13.2	1.91	0.87	71.1	7.27	33.5	0.15
L2-2	16.3	1.89	0.53	102.7	7.22	33.5	0.1
L2-3	14.7	1.88	0.70	87.7	7.34	33.5	0.22
L3-1	2.9	1.42	0.84	84.9	7.61	31.6	0.15
L3-2	3.7	1.2	1.19	61.4	7.37	31.6	0.14
L3-3	2.6	1.77	0.96	43	7.6	31.6	0.24
C1-1	2.5	1.75	0.57	47.8	7.34	32.9	0.19
C1-2	2.9	1.79	0.60	61.7	7.45	32.9	0.14
C1-3	8.5	1.87	0.57	47.1	7.52	32.9	0.13
C2-1	5.4	2.29	0.55	56.3	7.1	33.1	0.18
C2-2	9.4	2.38	0.69	101.8	7.39	33.1	0.18
C2-3	8.1	2.31	0.68	51	7.38	33.1	0.15
C3-1	14.0	0.93	0.61	50.5	7.24	32.8	0.06
C3-2	12.0	1	0.57	62.5	7.64	32.8	0.06
C3-3	23.9	0.97	0.45	66.6	7.13	32.8	0.07

Table 4. Soil physicochemical properties of site in autumn.

Site	TC g/kg	TNg/kg	TPg/kg	EC	PH	T	WC
S1-1	49.1	2.87	2.97	109.2	7.44	16.4	0.1
S1-2	25.6	1.81	2.49	133.4	7.66	16.4	0.07
S1-3	11.0	0.79	1.01	106.2	8.16	16.4	0.04
S2-1	3.5	0.22	0.19	26	7.55	17.2	0.2
S2-2	2.9	0.17	0.16	26.2	7.27	17.2	0.2
S2-3	2.0	0.16	0.09	23.7	7.73	17.2	0.19
S3-1	2.8	0.31	0.20	36.7	7.99	18.5	0.14
S3-2	1.9	0.23	0.19	45.4	7.92	18.5	0.19
S3-3	2.6	0.2	0.25	45.1	8.12	18.5	0.17
L1-1	17.3	1.91	0.70	30.2	7.68	17.8	0.01
L1-2	17.5	1.4	0.69	40.5	7.68	17.8	0.02
L1-3	20.8	1.73	0.67	22.7	7.17	17.8	0.03
L2-1	15.4	1.27	0.69	53.5	7.74	16.5	0.14
L2-2	11.5	1.21	0.57	47	7.61	16.5	0.11
L2-3	22.9	1.81	0.67	58.8	7.71	16.5	0.11
L3-1	14.1	0.55	0.51	148.9	8.03	14.8	0.02
L3-2	14.5	0.43	0.63	53.4	7.9	14.8	0.01
L3-3	14.2	0.39	0.49	37	7.56	14.8	0.01
C1-1	18.8	1.66	0.70	36.3	7.39	17.7	0.05
C1-2	22.4	1.81	0.82	29.8	7.28	17.7	0.06
C1-3	22.2	1.7	0.82	36.6	7.83	17.7	0.09
C2-1	11.2	1.13	0.73	58.5	7.61	17.5	0.05
C2-2	16.3	1.71	0.63	68.1	8	17.5	0.03
C2-3	27.0	1.87	0.87	31	7.43	17.5	0.05
C3-1	15.7	1.26	0.68	66.9	7.94	16.3	0.13
C3-2	15.2	1.21	0.64	40.4	7.77	16.3	0.07
C3-3	28.3	1.35	0.65	31.5	7.54	16.3	0.11

Table 5. Soil physicochemical properties of site in winter.

Site	TC g/kg	TNg/kg	TPg/kg	EC	PH	T	WC
S1-1	21.8	1.56	2.00	26.6	7.57	8.1	0.09
S1-2	36.7	4.18	1.48	38.9	7.56	8.1	0.11
S1-3	35.5	2.59	2.30	29.4	7.45	8.1	0.08
S2-1	3.7	0.38	0.22	22.8	7.79	7.9	0.2
S2-2	1.8	0.37	0.09	18.6	7.71	7.9	0.16
S2-3	8.6	0.35	0.07	26.1	7.61	7.9	0.19
S3-1	3.2	0.45	0.10	19.9	7.25	8.8	0.18
S3-2	7.8	0.47	0.22	39.1	7.98	8.8	0.2
S3-3	6.0	0.44	0.12	33.2	7.94	8.8	0.18
L1-1	18.3	1.99	0.73	22.4	7.84	7.7	0.04
L1-2	11.7	1.83	1.26	18.7	7.8	7.7	0.04
L1-3	14.3	1.54	0.54	16.2	7.51	7.7	0.06
L2-1	10.4	1.11	0.48	18.6	7.88	7.4	0.1
L2-2	13.7	1.22	0.50	22.2	7.78	7.4	0.13
L2-3	15.0	1.12	0.53	41.7	7.88	7.4	0.14
L3-1	12.6	1.38	0.35	15.5	7.71	7.1	0.17
L3-2	16.6	1.49	0.41	20.2	8.04	7.1	0.12
L3-3	11.1	1.18	0.34	17.4	7.71	7.1	0.16
C1-1	20.6	2.2	0.73	27.4	7.95	8.1	0.07
C1-2	16.4	2.07	0.62	29	7.96	8.1	0.07
C1-3	21.2	2.08	0.63	21.7	7.91	8.1	0.07
C2-1	11.8	1.7	0.59	34.5	7.8	7.2	0.05
C2-2	18.1	2.1	0.58	105	7.4	7.2	0.04
C2-3	15.4	1.68	0.58	33	7.78	7.2	0.03
C3-1	50.1	4.83	0.81	16.1	7.96	7.6	0.22
C3-2	14.0	1.09	0.48	33.2	7.81	7.6	0.08
C3-3	13.5	1.05	0.47	20.8	7.77	7.6	0.13

3.2. Community Composition

The sequences were clustered at 97% similarity, and after sequence rarefaction, a total of 5471 Operational Taxonomic Units (OTUs) were obtained, including 640 algal OTUs. This study focuses only on the diversity of soil eukaryotic algae; therefore, only algal sequences will be analyzed in subsequent steps. Overall, they belonged to seven phyla, in order of abundance: Chlorophyta, Bacillariophyta, Charophyta, Cryptista, Rhodophyta, Haptophyta, Glaucophyta. Among them, the Chlorophyta and Bacillariophyta phyla were absolutely dominant throughout all four seasons of the year. Within the Chlorophyta phylum, there were 375 OTUs, of which 251 belonged to the Chlorophyceae class. Within the Chlorophyceae class, there were further subdivisions, including 175 OTUs belonging to the order Chlamydomonadales. A total of 53 Chlorococcaceae OTUs were annotated, involving six genera, in order of abundance: *Chlorococcum*, *Spongiochloris*, *Spongiococcum*, *Tetracystis*, *Chlorococcopsis* and *Radiosphaera*.

Algae's relative abundance in different seasons and soil types is shown in Figure S1A. Overall, Chlorophyta and Bacillariophyta held an absolute dominant position across the four seasons and three soil types. The community structure of soil eukaryotic algae exhibited significant seasonal differences, with the highest overall richness in spring, followed by summer, autumn, and winter in descending order. Additionally, the richness of the Chlorophyta phylum was higher than that of the Bacillariophyta phylum (Figure S1B), with the relative abundance peaking in spring. The relative abundance maps for different soil types revealed differences in overall richness among the three types of soil, with the highest richness in grassland and the lowest in forest land (Figure S1C). This suggests that

the community structure of soil eukaryotic algae is more influenced by seasonal changes than by soil type.

At the genus level, the top seven most abundant genera in descending order are *Chlamydomonas*, *Tetracystis*, *Chlorella*, *Chlorococcum*, *Coelastrum*, *Bracteacoccus* and *Desmodesmus*. Among them, *Chlamydomonas* was in absolute dominance across all four seasons, and its abundance varied with the seasons. The species richness was highest in spring, followed by summer, autumn, and winter, reaching the lowest in winter (Figure S1D). The species richness of *Tetracystis* was highest in spring and lowest in both autumn and winter. *Chlorella* exhibited the highest species richness in spring and the lowest in autumn. *Chlorococcum* had the highest species richness in spring and the lowest in winter (Figure S1E). *Chlamydomonas* was in absolute dominance across all three types of soil, with the highest abundance in sandy soil and the lowest in grassland. *Tetracystis* had higher abundance in forest soil and grassland than in sandy soil. *Chlorococcum* had the lowest abundance in sandy soil and the highest in grassland.

3.3. Community Difference Analysis

Based on the results of non-metric multi-dimensional scaling (NMDS) analysis, the community composition of the three soil types shows significant differences. There is a very significant distinction between sandy soil and both forest land and grassland. Despite some overlap between forest land and grassland, the difference remains significant (Figure 2A). Seasonally, the soil community composition in winter is different from the other three seasons, with the most significant difference observed between winter and spring (Figure 2B). This result is consistent with species richness.

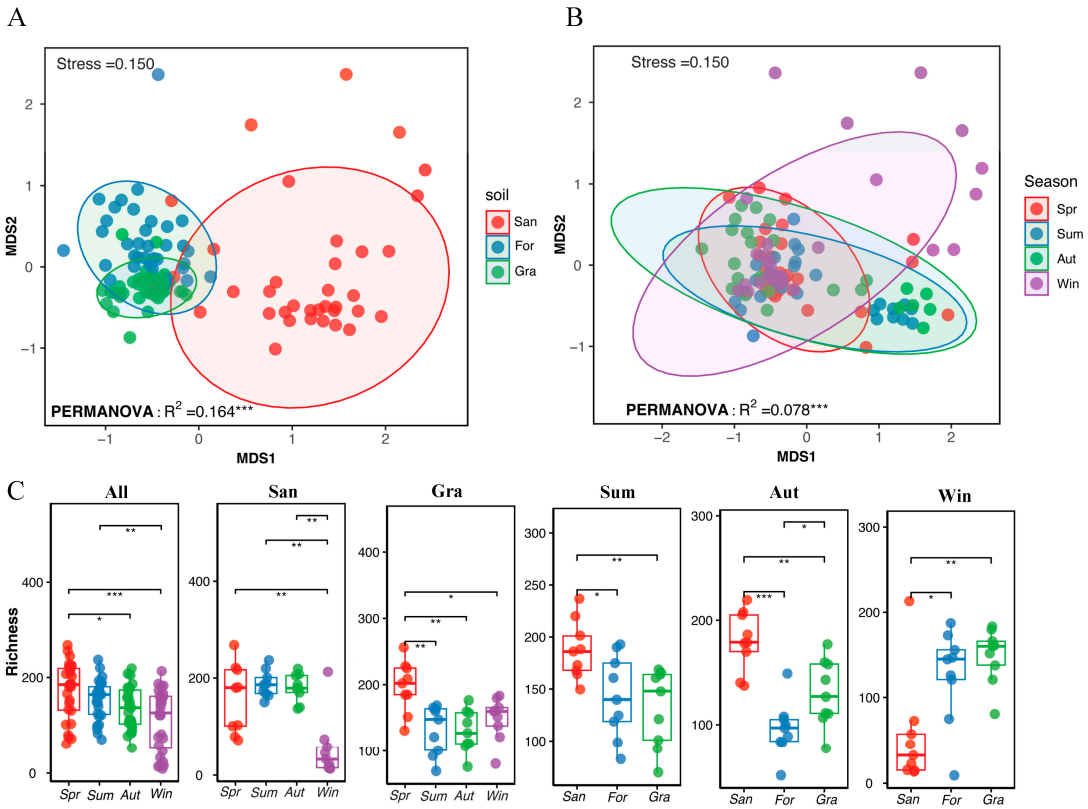


Figure 2. (**A**) analysis of inter-group differences between different soil types; (**B**) analysis of inter-group differences between different seasons; (**C**) community differences under different seasons and soil types. (Spr: spring; Sum: summer; Aut: autumn; Win: winter; San: sandy soil; For: forest land; Gra: grassland; * ($p < 0.01$): difference exists; ** ($0.01 \leq p < 0.05$): significant difference; *** ($0.05 \leq p < 0.1$): very significant difference).

The community differences in soil types across different seasons and the community differences in various soils across different seasons were assessed using the richness and PD_whole_tree diversity indices. Overall, in spring, there were no significant differences in species richness and community genetic distance among the three soil types; in summer, autumn, and winter, significant differences in species richness were observed between sandy soil and grassland (**); in autumn, the genetic distance difference between sandy land and forest land was very significant (***) (Figure 2C). The difference in species richness between spring and winter is very significant (***), and the genetic distance also exhibits significant differences (***). In sandy soil, the species richness in winter shows significant differences compared to the other three seasons (**), the genetic distance also shows significant differences (**)/or very significant differences (***); in forest land, there is a difference in species richness only between summer and autumn (*). The species richness and genetic distance in the other seasons show no significant differences. In grassland, there are significant differences in species richness between spring and the other three seasons (**)/differences exist (*), and the genetic distance only shows differences between spring and winter (*) (Figure 2C).

3.4. Correlation Analysis

Overall, the factors most closely related to the Chlorophyta occurrence are TN ($R > 0.4$, $p < 0.01$), TP ($R > 0.4$, $p < 0.01$), and PH ($R > 0.2$, $p < 0.01$) (Figure 3A). The factors most closely related to Bacillariophyta are TN ($R > 0.2$, $p < 0.01$) and WC ($R > 0.2$, $p < 0.05$). The factor most closely related to Charophyta is WC ($R > 0.2$, $p < 0.01$). The factors related to Rhodophyta, in order, are: TP ($R > 0.4$, $p < 0.01$), TC ($R > 0.4$, $p < 0.01$), and TN ($R > 0.2$, $p < 0.05$). Cryptista, Haptophyta, and Glaucophyta, however, do not show significant correlation with these seven environmental factors.

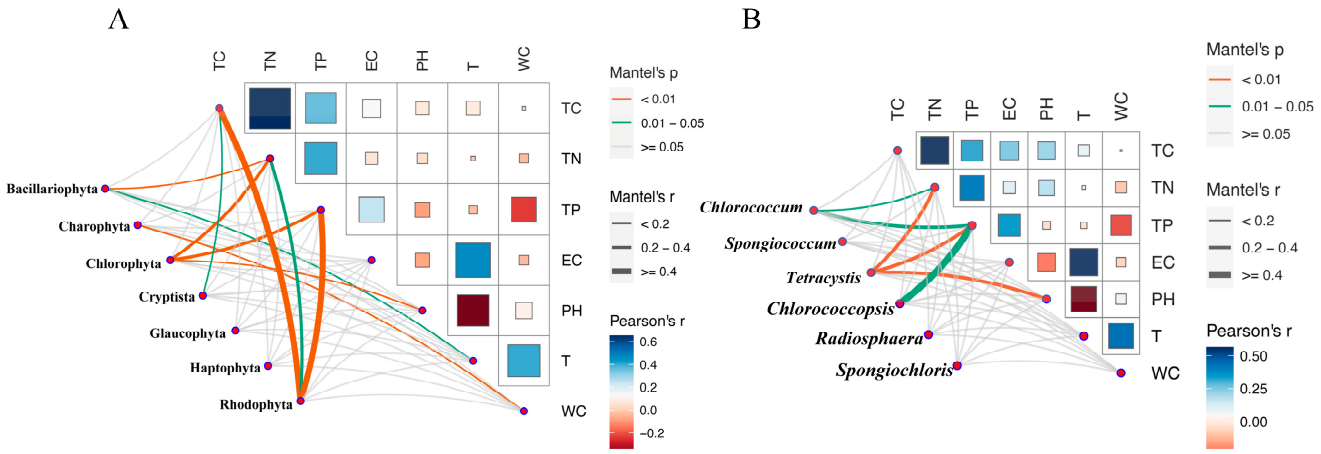


Figure 3. (**A**) correlation analysis between different phyla and environmental factors, as well as among environmental factors; (**B**) correlation analysis between different genera and environmental factors, as well as among environmental factors. The Spearman correlation coefficient reflects the pairwise correlation between nutritional factors, with the color depth representing the size of the correlation coefficient. The Mantel test is used to reflect the correlation between different groups and various environmental factors. The line width corresponds to the r value of the Mantel test, and the line color represents the statistical significance.

We also investigated the correlation between six genera within Chlorococcaceae and nutrient factors such as nitrogen and phosphorus, as shown in Figure 3. Overall, the factor most closely correlated with *Chlorococcum* members is TP ($R > 0.4$, $p < 0.05$), followed by TN ($R > 0.2$, $p < 0.05$), while there is no significant correlation with TC, EC, PH, T, and WC (Figure 3B). Abundance of *Tetracystis* is significantly correlated with TN ($R > 0.4$,

$p < 0.01$), TP ($R > 0.4$, $p < 0.01$), and PH ($R > 0.4$, $p < 0.01$), with a considerable degree of correlation among them. The factor most closely related to *Chlorococcopsis* is TP ($R > 0.4$, $p < 0.05$), while there is no significant correlation with the other five environmental factors. *Spongiococcum*, *Radiosphaera*, and *Spongiochloris* show no significant correlation with these six nutrient factors.

3.5. Community Driving Factors

Overall, the environmental factors driving changes in community diversity in the region are primarily TP, TN, and TC. Among these, TC, T, and seasonal changes have the most significant impact on the diversity of eukaryotic algae in the area (Figure 4A). From the seven phyla involved, some genera with the highest species richness were selected for random forest analysis, which are as follows: *Chlorococcum*, *Tetracystis*, *Protosiphon*, *Fasciculochloris*, *Carteria*, *Chlamydomonas*, *Chloromonas*, *Bracteacoccus*, *Coelastrella*, Cryptista. For example, *Coelastrella* are primarily driven by TN, showing a positive correlation. The correlation and importance of other genera with environmental factors are shown in Figure 4B.

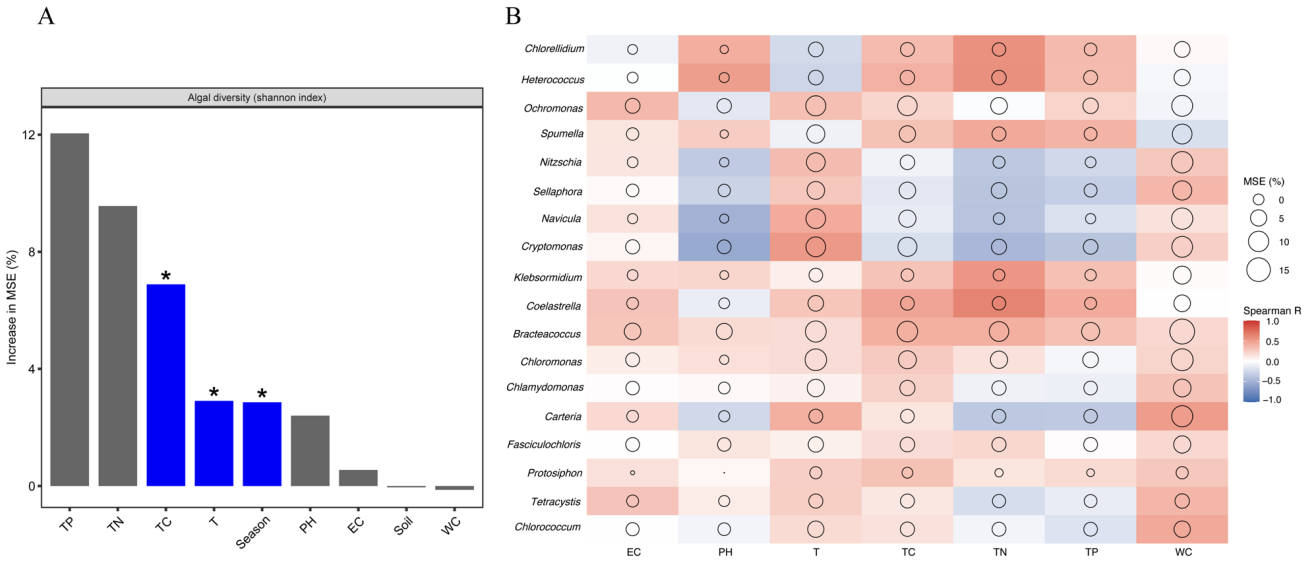


Figure 4. The contribution of soil properties to the differences in soil eukaryotic algal communities and the differences in relative abundance at the genus level are based on correlation and the best multivariate regression model. (**A**) the main driving factors of soil eukaryotic algal communities; (**B**) the main environmental driving factors at the genus level. The size of the circles represents the relative importance of the variables (i.e., the proportion of variance explained calculated through multivariate regression modeling and variance partitioning analysis), and the color represents the correlation. *: significantly correlated.

4. Discussion

Soil microorganisms are key participants in biogeochemical cycles [54], and their community composition is influenced by factors such as temperature, total nitrogen, soil type, and pH [55–59], with the extent of the impact varying depending on the biological community [56,60]. In the late 20th century, algae were considered to be a component of the soil microbial community [4]. The soil algal community is influenced by factors such as water content, pH, organic carbon, and nitrogen [61–63]. Nitrogen and phosphorus are essential nutrients for algal growth and can affect the composition and diversity of algae [64–66]. While earlier investigations primarily documented static snapshots of soil algal composition across limited environmental gradients, our work offers a comprehen-

sive seasonal resolution along inland lake shorelines—a habitat interface that has been substantially underrepresented in soil algae literature.

We demonstrate that seasonal dynamics, rather than soil type alone, are the dominant driver of soil eukaryotic algal community structure. Although significant differences in community composition among sandy soil, forest land, and grassland were observed—with the most pronounced divergence between sandy soil and both forest land and grassland—seasonal variation exerted a stronger overall influence. This finding refines the prevailing view [62] by quantifying the relative magnitude of seasonal versus edaphic effects, revealing that seasonal shifts can partially override soil-type signatures in shaping algal assemblages. Importantly, we identified that winter communities are distinctly different from those in the other three seasons, with the greatest dissimilarity occurring between winter and spring. This seasonal decoupling suggests that overwintering strategies or dormancy mechanisms play a critical role in community assembly, a pattern not previously resolved at comparable temporal detail. Prior studies consistently reported Chlorophyta as the most taxonomically rich algal division in soils [67]. Within the Chlorophyta, a majority of our Chlorococcaceae sequences shared less than 99% similarity with known sequences in the NCBI database, pointing to substantial unrecognized diversity in inland lake shore ecosystems. Such hidden diversity likely contributes to the well-documented high environmental tolerance of Chlorophyta [68], though the specific adaptive mechanisms remain to be elucidated. This confirms and extends the early presence reports of Chlorococcaceae in soil algal studies [69–72] by providing molecular evidence that many lineages may represent undescribed species.

Then, we uncovered striking taxa-specific responses to soil type and season within Chlorococcaceae. Species richness peaked in summer but was lowest in winter, creating a strong seasonal amplitude inconsistent with the traditionally viewed stability of soil microbial communities. Within sandy soil, richness reached its maximum in autumn—a seasonal pattern opposite to that observed in grassland during winter. At the genus level, the abundance trajectories of Chlorococcum and Chlamydomonas were markedly inverse across the three soil types, suggesting niche partitioning or competitive interactions that have not been previously described in soil algal ecology. These genus-level patterns warrant targeted investigation but already indicate that functional roles within the broadly classified Chlorophyta are far more differentiated than earlier surveys suggested.

At last, we quantitatively disentangled the environmental drivers of algal diversity using random forest analysis. Consistent with established correlations between Chlorophyta and nutrient availability (TN and TP, both $R > 0.4$, $p < 0.01$), our machine learning approach identified total phosphorus, total nitrogen, and total carbon—along with temperature and seasonal changes—as the primary factors structuring eukaryotic algal diversity in this region. Notably, TC emerged alongside temperature as the most important predictors, highlighting the combined influence of carbon substrate and thermal regime on algal community assembly. We further demonstrated genus-specific environmental sensitivities; for example, Coelastrella abundance correlated positively with TN ($R > 0.4$, $p < 0.01$). Soil moisture also proved indispensable, reinforcing its established role in algal growth and reproduction [28]. These results collectively provide a mechanistic framework linking environmental variables to taxon-specific responses, offering theoretical support for interpreting soil algal community shifts as bioindicators of ecological change.

In sum, this study advances soil algal ecology by (i) quantifying the hierarchical importance of seasonal over edaphic drivers, (ii) revealing cryptic diversity within dominant chlorophyte lineages, (iii) exposing intra-family and inter-genus contrasts in seasonal and edaphic responses, and (iv) integrating statistical correlation with machine-learning-based feature importance to identify the key environmental axes governing algal diversity. To-

gether, these contributions position soil eukaryotic algae along inland lake shorelines as a responsive and informative model system for understanding how microbial eukaryotes assemble and persist in terrestrial environments.

5. Conclusions

This study explored the seasonal variations in the community structure and species richness of eukaryotic algae in different soils along the inland lake shores of a central Chinese city, initially revealing the molecular diversity of eukaryotic soil algae in the region. The study also found that the community composition varied significantly between different seasons and soil types, and was more significantly influenced by seasonal changes. Overall, Chlorophyta and Bacillariophyta had the highest richness. Additionally, Chlorococcaceae exhibited a very high cryptic diversity. In terms of environmental factor correlations, the soil at the sampling sites was mostly neutral to alkaline. Water content (WC), total carbon (TC), and total nitrogen (TN) were all at their highest in spring, with TN and TP being most closely related to Chlorophyta. At the genus level, the environmental factors most closely related are usually TN and TP. Through random forest analysis, it was found that the environmental factors driving changes in community diversity in the region are mainly TP (total phosphorus), TN (total nitrogen), and TC (total carbon), with TC, temperature (T), and seasonal changes having the most significant impact on the diversity of eukaryotic algae in the area. This study provides new insights into understanding the community structure of soil eukaryotic algae and the correlation between specific groups and environmental factors, offering reference materials for the utilization and control of algae, as well as for solving ecological environmental issues.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18080454/s1. Figure S1: Seasonal changes in the relative abundance of samples in different soils; (A) Relative abundance at the phylum level across the 36 sampling sites; (B) Seasonal variations in relative abundance at the phylum level; (C) Relative abundance at the phylum level in different soils; (D) Seasonal variations in relative abundance at the genus level; (E) Relative abundance at the genus level in different soils. ABCD represent the four seasons respectively, SFG represents three types of soil (Spr: spring; Sum: summer; Aut: autumn; Win: winter; San: sandy soil; For: forest land; Gra: grassland; NS: no significant difference; *: difference exists; **: significant difference; ***: very significant difference).

Author Contributions: Q.Y.: sample collection, experimental design, data analysis, manuscript writing. Z.W.: data analysis. Y.B.: sample collection, data analysis. D.W.: manuscript polishing. B.L.: sample collection. J.H.: manuscript polishing. G.L.: funding acquisition, experimental guidance. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Major Natural Science Research Project of Anhui Province (Grant No. 2025AHGXZK20007), by Qiufeng Yan.

Data Availability Statement: The sequence data have been deposited in the NCBI database with accession numbers (Table 5), which can be accessed through the following URL: https://www.ncbi.nlm.nih.gov/ (accessed on 24 July 2025). And other data have been presented in the manuscript.

Acknowledgments: We are grateful to the Institute of Hydrobiology (IHB) of the Chinese Academy of Sciences for generously providing the equipment used in this study. Additionally, we express our appreciation to all scientific researchers who participated in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

Water content (WC), total carbon (TC), and total nitrogen (TN); TP (total phosphorus); temperature (T).

References

1. Leff, J.W.; Bardgett, R.D.; Wilkinson, A.; Jackson, B.G.; Pritchard, W.J.; De Long, J.R. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. *ISME J.* **2018**, *12*, 1794–1805. [CrossRef] [PubMed]
2. Domeignoz-Horta, L.A.; Pold, G.; Liu, X.A.; Frey, S.D.; Melillo, J.M.; DeAngelis, K.M. Microbial diversity drives carbon use efficiency in a model soil. *Nat. Commun.* **2020**, *11*, 3684. [CrossRef] [PubMed]
3. Sweeney, C.J.; de Vries, F.T.; van Dongen, B.E.; Bardgett, R.D. Root traits explain rhizosphere fungal community composition among temperate grassland plant species. *New Phytol.* **2021**, *229*, 1492–1507. [PubMed]
4. Starks, T.L.; Shubert, L.E.; Trainor, F.R. Ecology of soil algae: A review. *Phycologia* **1981**, *20*, 65–80. [CrossRef]
5. Kabirov, R.R.; Gaisina, L.A. Parameters of the productivity of soil algae in terrestrial ecosystems. *Eurasian Soil Sci.* **2009**, *42*, 1374–1379. [CrossRef]
6. Lin, C.S.; Chou, T.L.; Wu, J.T. Biodiversity of soil algae in the farmlands of mid-Taiwan. *Bot. Stud.* **2013**, *54*, 41. [CrossRef] [PubMed]
7. Perera, I.; Subashchandrabose, S.R.; Venkateswarlu, K.; Naidu, R.; Megharaj, M. Consortia of cyanobacteria/microalgae and bacteria in desert soils: An underexplored microbiota. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 7351–7363. [CrossRef] [PubMed]
8. Hammel, W.; Steubing, L.; Debus, R. Assessment of the ecotoxic potential of soil contaminants by using a soil-algae test. *Ecotoxicol. Environ. Saf.* **1998**, *40*, 173–176. [CrossRef] [PubMed]
9. Metting, B. The systematics and ecology of soil algae. *Bot. Rev.* **1981**, *47*, 195–312. [CrossRef]
10. Chodat, R. Algues pélagiques nouvelles. *Bull. l'Herbier Boissier* **1897**, *5*, 119–120.
11. Brown, R.; Bold, H.C. Comparative studies of the algal genera *Tetracystis* and *Chlorococcum*. *Univ. Tex. Publ.* **1964**, *6417*, 1–213.
12. Meneghini, G. Monographia Nostochinearum Italicarum addito specimine de Rivulariis. *Augustae Taur.* **1842**, *24*, 143.
13. Herndon, W. Studies on chlorosphaeracean algae from soil. *Am. J. Bot.* **1958**, *45*, 298–308. [CrossRef]
14. Agardh, C. *Systema Algarum*; Literis Berlingianis: Lund, Sweden, 1824.
15. Starr, R.C. A comparative study of *Chlorococcum* meneghini and other spherical, zoospore-producing genera of the Chlorococcales. *Indiana Univ. Publ. Sci. Ser.* **1955**, *20*, 1–111.
16. Temraleeva, A.; Moskalenko, S.; Mincheva, E.; Bukin, Y.; Sinetova, M. *Spongiosarcinopsis terrestris* gen. et sp. nov. (chlorophyta, chlorophyceae): A new genus of green algae from gray forest soil, russia. *Phytotaxa* **2018**, *6*, 376.
17. Temraleeva, A. Validation of *Spongiosarcinopsis terrestris gen. et sp. nov.* (*Protosiphonaceae*, Chlorophyta). *Not. Algarum* **2019**, *98*, 1–2.
18. Trofim, A.; Donţu, N.; Şalaru, V.; Gheorhiţa, C. Taxonomic and ecological structure of the edaphic algae flora from certain agrocenoses of the Republic of Moldova. *Stud. Univ. Mold.* **2013**, *61*, 83–87.
19. Yang, J.; Jiang, H.; Wu, G.; Liu, W.; Zhang, G. Distinct factors shape aquatic and sedimentary microbial community structures in the lakes of Western China. *Front. Microbiol.* **2016**, *7*, 1782. [CrossRef] [PubMed]
20. Maltsev, Y.I.; Didovich, S.V.; Maltseva, I.A. Seasonal changes in the communities of microorganisms and algae in the litters of tree plantations in the steppe zone. *Eurasian Soil Sci.* **2017**, *50*, 935–942. [CrossRef]
21. Maltsev, Y.I.; Pakhomov, A.Y.; Maltseva, I.A. Specific features of algal communities in forest litter of forest biogeocenoses of the steppe zone. *Contemp. Probl. Ecol.* **2017**, *10*, 71–76. [CrossRef]
22. Abinandan, S.; Subashchandrabose, S.R.; Venkateswarlu, K.; Megharaj, M. Soil microalgae and cyanobacteria: The biotechnological potential in the maintenance of soil fertility and health. *Crit. Rev. Biotechnol.* **2019**, *39*, 981–998. [CrossRef] [PubMed]
23. Kuzyakhmetov, G.G. Productivity of algocenoses in zonal arable soils of steppe and forest-steppe. *Eurasian Soil* **1998**, *31*, 406–410.
24. Goyal, S.K. Algae and the soil environment. *Phykos* **1997**, *36*, 1–13.
25. Mishra, U.; Pabbi, S. Cyanobacteria: A potential biofertilizer for rice. *Resonance* **2004**, *9*, 6–10. [CrossRef]
26. Roger, P.A.; Reynaud, P.A. Free—Living blue—Green algae in tropical soils. In *Microbiology of Tropical Soils and Plant Productivity*; Springer: Dordrecht, The Netherlands, 1982; pp. 147–168.
27. Choudhary, K.K.; Singh, S.S.; Mishra, A.K. Nitrogen fixing cyanobacteria and their potential applications. In *Advances in Applied Phycology*; Gupta & Pandey: New Delhi, India, 2007; pp. 142–154.
28. Nisha, R.; Kaushik, A.; Kaushik, C.P. Effect of indigenous cyanobacterial application on structural stability and productivity of an organically poor semi-arid soil. *Geoderma* **2007**, *138*, 49–56. [CrossRef]
29. Kostryukova, A.; Mashkova, I.; Shchelkanova, E.; Trofimenko, V. Retrospective analysis of phytoplankton community structure in water bodies of South Ural (Russia). *IOP Conf. Ser. Earth Environ. Sci.* **2021**, *626*, 012008. [CrossRef]
30. Sawestri, S.; Rais, A.H. Abundance and composition of phytoplankton communities in middle stream of citanduy river, West Java. *J. Phys. Conf. Ser.* **2021**, *1943*, 012069. [CrossRef]

31. Rahman, M.M. Dynamics of epibenthic copepods in relation to environmental factors and phytoplankton abundance in tropical river, estuarine and coastal environments. *Estuar. Coast. Shelf Sci.* **2022**, *266*, 107748. [CrossRef]
32. Kammann, S.; Schiefelbein, U.; Dolnik, C.; Mikhailyuk, T.; Demchenko, E.; Karsten, U.; Glaser, K. Successional development of the phototrophic community in biological soil crusts on coastal and inland dunes. *Biology* **2023**, *12*, 58.
33. Rippin, M.; Borchhardt, N.; Williams, L.; Colesie, C.; Jung, P.; Budel, B. Genus richness of microalgae and cyanobacteria in biological soil crusts from Svalbard and Livingston Island: Morphological versus molecular approaches. *Polar Biol.* **2018**, *41*, 909–923. [CrossRef]
34. Samolov, E.; Baumann, K.; Budel, B.; Jung, P.; Leinweber, P.; Mikhailyuk, T. Biodiversity of algae and cyanobacteria in biological soil crusts collected along a climatic gradient in Chile using an integrative approach. *Microorganisms* **2020**, *8*, 1047. [CrossRef]
35. Bohlen, P.J.; Groffman, P.M.; Driscoll, C.T.; Fahey, T.J.; Siccama, T.G. Plant–soil–microbial interactions in a Northern Hardwood forest. *Ecology* **2001**, *82*, 965–978. [CrossRef]
36. Prasanna, N.R. Soil pH and its role in cyanobacterial abundance and diversity in rice field soil. *Appl. Ecol. Environ. Res.* **2007**, *5*, 102–113. [CrossRef]
37. Zhang, H.; Zong, R.; He, H.; Liu, K.; Yan, M.; Miao, Y. Biogeographic distribution patterns of algal community in different urban lakes in China: Insights into the dynamics and co-existence. *J. Environ. Sci.* **2021**, *100*, 216–227. [CrossRef]
38. Zhao, F.; Xu, H.; Kang, L.; Zhao, X. Spatial and seasonal change in algal community structure and its interaction with nutrient dynamics in a gravel-bed urban river. *J. Hazard. Mater.* **2022**, *425*, 127775. [CrossRef] [PubMed]
39. Gabriel, A.; Venancio, C.; Sousa, J.P.; Leston, S.; Ramos, F.; Soares, A. Soil pH matters in the ecotoxicity of Basamid(R) to freshwater microalgae and macrophytes. *Sci. Total Environ.* **2023**, *859*, 160165. [CrossRef] [PubMed]
40. Graham, E.B.; Knelman, J.E. Implications of soil microbial community assembly for ecosystem restoration: Patterns, process, and potential. *Microb. Ecol.* **2023**, *85*, 809–819. [CrossRef] [PubMed]
41. Wang, Z.Y.; Cottlein, A.G.; Bartonek, G. Effects of growing roots of Norway spruce (*Picea abies* [L.] Karst.) and European beech (*Fagus sylvatica* L.) on rhizosphere soil solution chemistry. *J. Plant Nutr. Soil Sci.* **2001**, *164*, 35–41. [CrossRef]
42. Thoms, C.; Gattinger, A.; Jacob, M.; Thomas, F.M.; Gleixner, G. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. *Soil Biol. Biochem.* **2010**, *42*, 1558–1565. [CrossRef]
43. Brussaard, L.; de Ruiter, P.C.; Brown, G.G. Soil biodiversity for agricultural sustainability. *Agric. Ecosyst. Environ.* **2007**, *121*, 233–244. [CrossRef]
44. Baldrian, P.; Kolarik, M.; Stursova, M.; Kopecky, J.; Valaskova, V.; Vetrovsky, T. Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *ISME J.* **2012**, *6*, 248–258. [PubMed]
45. Bailey, D.; Mazurak, A.P.; Rosowski, J.R. Aggregation of soil particles by algae. *J. Phycol.* **2010**, *9*, 99–101.
46. Satjarak, A.; Graham, L.E.; Piotrowski, M.J.; Trest, M.T.; Wilcox, L.W.; Cook, M.E. Shotgun metagenomics and microscopy indicate diverse cyanophytes, other bacteria, and microeukaryotes in the epimicrobiota of a northern Chilean wetland *Nostoc* (cyanobacteria). *J. Phycol.* **2021**, *57*, 39–50. [PubMed]
47. Cao, M.; Tang, Z.H.; Zhao, L.; Zhang, Z.H. Relationship between soil pH and glomalin-related soil protein content in aggregates of soda saline-alkali soil. *Soil* **2018**, *50*, 319–325.
48. Stoeck, T.; Bass, D.; Nebel, M. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. *Mol. Ecol.* **2010**, *19*, 21–31. [CrossRef] [PubMed]
49. Caporaso, J.G.; Kuczynski, J.; Stombaugh, J. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* **2010**, *7*, 335–336. [CrossRef] [PubMed]
50. Bakker, J.D. *Applied Multivariate Statistics in R*; Springer International Publishing: Basel, Switzerland, 2024; p. 393.
51. DeSarbo, W.S.; Hwang, H.; Jedidi, K. Redundancy analysis. In *Wiley StatsRef: Statistics Reference Online*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2016.
52. Wei, T.; Simko, V.; Levy, M.; Xie, Y.; Jin, Y.; Zemla, J. Package 'corrplot'. *Statistician* **2017**, *56*, e24.
53. Rigatti, S.J. Random forest. *J. Insur. Med.* **2017**, *47*, 31–39. [CrossRef] [PubMed]
54. Fierer, N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [CrossRef] [PubMed]
55. Bragazza, L.; Robroek, B.J.M.; Jassey, V.E.J.; Arif, M.S.; Marchesini, R.; Guglielmin, M.; Cannone, N. Soil microbial community structure and enzymatic activity along a plant cover gradient in Victoria Land (continental Antarctica). *Geoderma* **2019**, *353*, 144–151. [CrossRef]
56. He, L.; Rodrigues, J.L.M.; Soudzilovskaia, N.A.; Barceló, M.; Olsson, P.A.; Song, C.; Tedersoo, L.; Yuan, F.; Yuan, F.; Lipson, D.A.; et al. Global biogeography of fungal and bacterial biomass carbon in topsoil. *Soil Biol. Biochem.* **2020**, *151*, 108024. [CrossRef]
57. Kang, H.; Yu, W.; Dutta, S.; Gao, H. Soil microbial community composition and function are closely associated with soil organic matter chemistry along a latitudinal gradient. *Geoderma* **2021**, *383*, 114744. [CrossRef]
58. Xu, Z.; Yu, G.; Zhang, X.; He, N.; Wang, Q.; Wang, S. Biogeographical patterns of soil microbial community as influenced by soil characteristics and climate across Chinese forest biomes. *Appl. Soil Ecol.* **2018**, *124*, 298–305. [CrossRef]

59. Xue, P.P.; Carrillo, Y.; Pino, V.; Minasny, B.; McBratney, A.B. Soil properties drive microbial community structure in a large scale transect in South Eastern Australia. *Sci. Rep.* **2018**, *8*, 11725. [CrossRef] [PubMed]
60. Tu, Q.; Deng, Y.E.; Yan, Q.; Shen, L.; Lin, L.U.; He, Z. Biogeographic patterns of soil diazotrophic communities across six forests in the North America. *Mol. Ecol.* **2016**, *25*, 2937–2948. [CrossRef] [PubMed]
61. Zancan, S.; Trevisan, R.; Paoletti, M.G. Soil algae composition under different agro-ecosystems in North-Eastern Italy. *Agric. Ecosyst. Environ.* **2006**, *112*, 1–12. [CrossRef]
62. Dirborne, C.M.; Ramanujam, P. Diversity and ecology of soil algae in broadleaf sacred grove and pine forest in East khasi hills, meghalaya. *Nelumbo* **2017**, *59*, 195. [CrossRef]
63. Agha, Q.; Asrar, M.; Leghari, S.K.; Somalani, M.A. Algae, soil fertility and physicochemical properties in agricultural fields of balochistan, Pakistan. *Pak. J. Bot.* **2020**, *52*, 1491–1495. [CrossRef]
64. Schulz, K.; Mikhailyuk, T.; Dressler, M.; Leinweber, P.; Karsten, U. Biological soil crusts from coastal dunes at the Baltic Sea: Cyanobacterial and algal biodiversity and related soil properties. *Microb. Ecol.* **2016**, *71*, 178–193. [PubMed]
65. Liu, Y.; Zang, H.; Ge, T.; Bai, J.; Lu, S.; Zhou, P. Intensive fertilization (N, p, K, Ca, and s) decreases organic matter decomposition in paddy soil. *Appl. Soil Ecol.* **2018**, *127*, 51–57. [CrossRef]
66. Delgado, M.; Reich, P.B.; Khachane, A.N.; Campbell, C.D.; Thomas, N.; Freitag, T.E. It is elemental: Soil nutrient stoichiometry drives bacterial diversity. *Environ. Microbiol.* **2017**, *19*, 1176–1188. [CrossRef]
67. Calijuri, M.C.; Santos, A.C.; Jati, S. Temporal changes in the phytoplankton community structure in a tropical and eutrophic reservoir (Barra Bonita, SP—Brazil). *J. Plankton Res.* **2002**, *24*, 617–634. [CrossRef]
68. Karsten, U.; Holzinger, A. Green algae in alpine biological soil crust communities: Acclimation strategies against ultraviolet radiation and dehydration. *Biodivers. Conserv.* **2014**, *23*, 1845–1858. [CrossRef] [PubMed]
69. John, R.P. An ecological and taxonomic study of the algae of British soils: I. The distribution of the surface-growing algae. *Ann. Bot.* **1942**, *6*, 323–349. [CrossRef]
70. Arce, G.; Bold, H.C. Some chlorophyceae from cuban soils. *Am. J. Bot.* **1958**, *49*, 2–503.
71. Cain, J. A preliminary survey of the algal flora of soils of certain areas of Texas. *Southwest. Nat.* **1964**, *9*, 166–170. [CrossRef]
72. Trainor, F.R.; Verses, P.A. A new *Chlorococcum* from a Connecticut cornfield soil. *Phycologia* **1967**, *6*, 237–239. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.