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Ciliated Protist Communities in Soil: Contrasting Patterns in Natural Sites and Arable Lands across Italy

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Abstract: This study represents the first investigation of soil ciliate diversity and community structure in the Marche region, Italy, encompassing both natural sites and agro-ecosystems. The main aims were (i) to assess the ability of ciliates to discriminate between different types of land uses, i.e., arable lands and possible farming management practices [organic (ORG) vs. conventional (CON)], and forest (FOR) sites; and (ii) to investigate the relationships among ciliate communities and abiotic parameters at the studied sites. Soil samples were collected twice from 10 sites (5 forest (FOR) (natural soils) and 5 arable lands under different agricultural management systems (3 ORG (minimum tillage) and 2 CON (sod seeding)). Ciliate communities were studied using qualitative (non-flooded Petri dish) and quantitative methods (ciliate counts from permanent slides). Soil chemical–physical (texture, CEC, N, OM, C/N) parameters were also measured. Qualitative ciliate analysis allowed us to identify a total of 59 species representing 33 genera, 20 families, 13 orders, and 7 classes. ORG sites were the richest in species followed by CON and FOR. Multivariate analysis showed statistically significant differences between natural sites (FOR) and agricultural sites, and between ORG and CON management farming systems. CCA analysis revealed a positive correlation between the ciliate species and silt, clay, and pH in ORG sites, and sand, organic carbon, organic matter, total nitrogen, C/N ratio, and CEC (cation exchange capacity) in FOR sites, suggesting the significance of these parameters in shaping the ciliate communities. Altogether, these results showed the bioindicative potential of ciliate communities in discriminating between natural sites (FOR) and arable lands, and their capacity to discriminate, at least preliminarily, between different soil management systems (ORG vs. CON). Furthermore, this study highlights the high diversity of soil ciliates and their response to habitat variability.

Keywords: agro-ecosystem; indicator values; forest; soil biodiversity; soil health

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1. Introduction

Within the vibrant realm of soil, an intricate tapestry of life unfolds brimming with an array of organisms. Astonishingly, just one square meter of soil may cradle more than a thousand animal species [1] and over half a million species of prokaryotes [2]. Amidst this diverse community, soil protists, particularly ciliates with short generation times, rapid reproduction, and elevated respiration rates [1,3,4], emerge as pivotal players in the microbial landscape. Positioned as keystone organisms within soil ecosystems [5], they play a crucial role in shaping the intricate web of soil life. Their prevalence provides vital functions in nutrient cycling, accelerating the turnover of soil bacterial biomass, processes like ammonification and nitrification, and rapid mineralization of organic nitrogen (N) and phosphorus (P) nutrients [3,6]. These activities, in turn, increase the bioavailability of soil nutrients [7,8], thereby enhancing the growth of plants and animals [9].

It is evident that a healthy and functional soil serves as the cornerstone of agricultural production, furnishing vital resources crucial for human well-being such as sustenance, livestock feed, and raw materials [10]. While conventional farming significantly contributes towards meeting the dietary needs of a burgeoning population [11], its heavy reliance on synthetic fertilizers, pesticides, and herbicides has led to profound environmental contamination and, over time, compromised ecosystem functionality [12,13]. In contrast, studies have shown that, organic farming presents a potentially more sustainable approach [14], fostering soil biodiversity and favorable ecological interactions, thereby mitigating adverse environmental repercussions compared with conventional practices [15–17].

However, the measurement of soil health in agro-ecosystems is primarily based on in situ measurements of various chemical–physical parameters such as pH, bulk density, nutrients, and pesticide levels. While these measurements offer quantitative insights into soil health dynamics over time and space, they fail to directly capture the impacts of different agricultural practices (i.e., conventional vs. organic farming) on soil organisms, thus overlooking the crucial role that soil organisms play in functions such as plant growth and nutrient cycling [18,19]. Consequently, there is a need for bioindicators capable of reflecting the diverse abiotic stressors affecting soil biodiversity and its functions in agricultural soil management. In this context, soil protists, especially ciliates (due to their unique ecological characteristics and responsiveness to soil conditions such as pH [20], nitrogen levels [21,22], soil moisture [23], and pesticide levels [24]), suggest a significant potential for bioindication in agricultural soil ecosystems. Many ciliate taxa can serve as markers of environmental stress in the soil ecosystem [25–27], and they become invaluable for monitoring major soil pollutants, contaminants, and transformations in land use [28–33]. Despite their potential, the utilization of soil ciliates as bioindicators remains largely unexplored. Moreover, even basic information on the community structure of ciliates from agricultural fields, specifically as indicator species in agro-ecosystems, remains incomplete, with a few scattered reports targeting limited taxa [17,33,34].

This study aims to (i) assess the ability of ciliates to discriminate between different types of land uses, i.e., arable lands, possible farming management practices [organic (ORG) vs. conventional (CON)], and forest (FOR); and (ii) examine the relationships among ciliate communities and abiotic parameters at the investigated sites. Overall, the gathered data will contribute to the enrichment of the Italian ciliate checklist. This effort will help fill existing gaps in community structure studies concerning soil ciliates while simultaneously assessing their potential as bioindicators of soil health across both natural sites and arable lands in the Marche region (Italy). Finally, this study aims to provide a first baseline to discriminate between different land uses and agricultural management practices using ciliate as indicators.

2. Materials and Methods

2.1. Study Area

Soil sampling was carried out in the Marche region, Italy (Figure 1), twice in autumn (A) (October, 2011) and spring (S) (May 2012) at 10 sites representative of 5 natural forest (undisturbed virgin soils), hereafter defined as FOR; and 5 agricultural fields at 3 sites under organic management (ORG) with low soil disturbance (e.g., minimum tillage at 25 cm), and 2 sites under conventional management (CON) (e.g., sod seeding (no-tillage and chemical weed control)) (Figure 1). Forest sampling sites were represented by three beechwood and two mixed woodlands dominated by oak (*Quercus pubescens*) and chestnut (*Castanea sativa*). The agricultural (arable land) sites were cultivated with wheat in spring (hereafter S), with no crops present at the autumn (hereafter A) sampling. For each monitored season, soil samples were taken at the same topographical positions (Table 1). The sampling sites were previously selected by the soil observatory of the Marche region, which funded the MOSYSS (MOnitoring SYstem of Soils at multi Scale) project, within which part of the activities described in this study were carried out [35,36]. The Marche region has a Mediterranean-type climate in the coastal areas and in the middle of the hills, which

gradually becomes sub-Mediterranean in the interior. The mean annual temperature is 12.5 °C, and the mean annual precipitation is about 780 mm. The geological substrates and other data are summarized in Table 1.

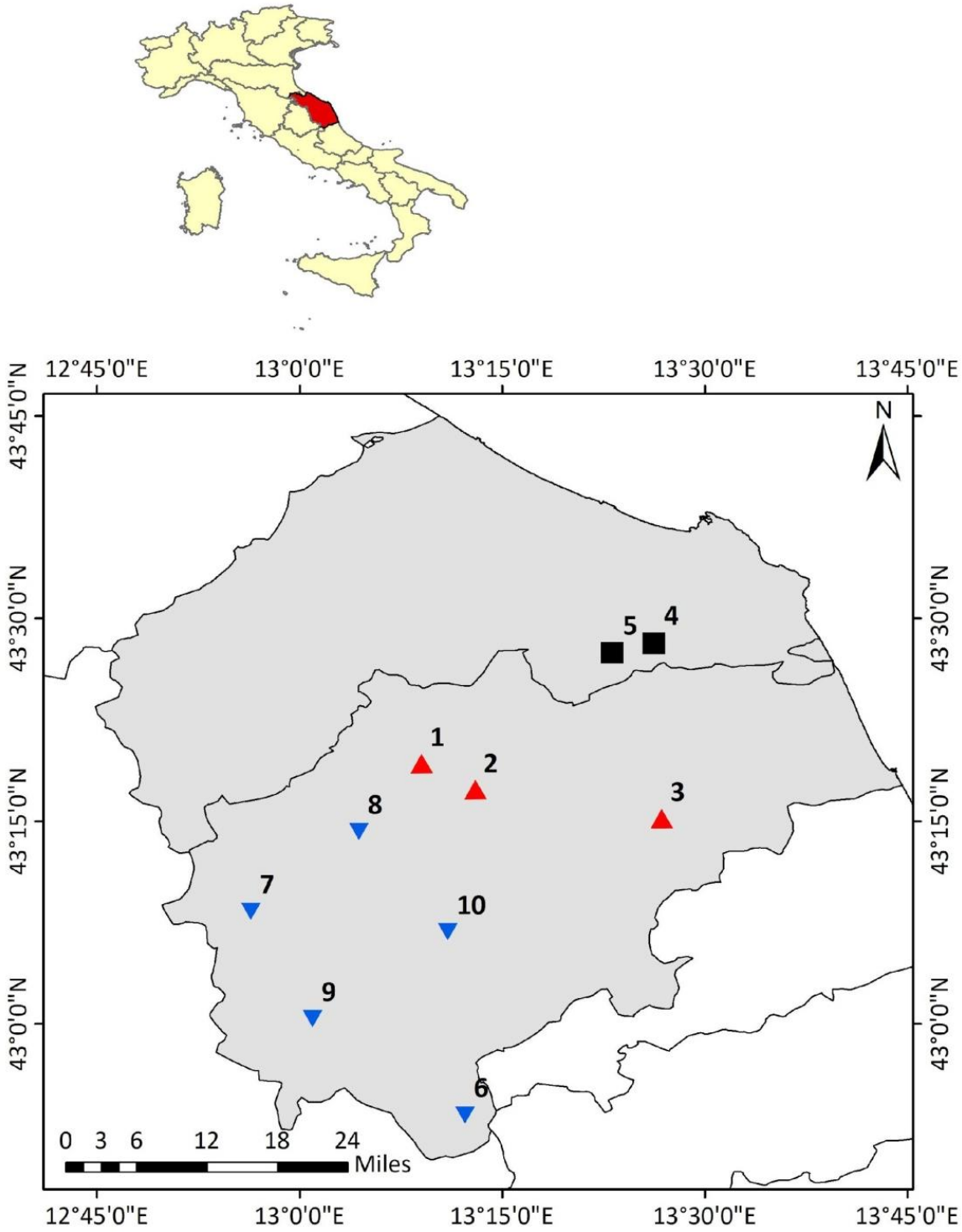

Figure 1. Study area showing the sampling locations. The highlighted region in the upper inset map shows the Marche region, while the larger map shows two of the five provinces in the Marche region, i.e., Ancona (sites 4 and 5) and Macerata (sites 1–3 and 7–10), where samplings were performed. The red triangles mark the ORG farm, the black boxes mark the CON farms, and the blue inverted triangles mark the forest sites.

Table 1. Sampling sites characteristics.

Site No.	Site Code	Habitat Type	Locality	Latitude	Longitude	Altitude (asl)	Humus Type	Geological Substrate
1	ORG_APP	Arable land	Gaglianvecchio, San Severino Marche (MC)	43°19′15.74″	13°8′59.86″	506 m	Not present	Calcareous marls
2	ORG_M	Arable land	Berta, San Severino Marche (MC)	43°17′17.53″	13°12′56.29″	187 m	Not present	Marly clay
3	ORG_CUC	Arable land	Cantagallo, Pollenza (MC)	43°15′10.75″	13°26′47.20″	265 m	Not present	Peliticcalcareous rocks
4	CON_MG30	Arable land	Passatempo, Osimo (AN)	43°28′10.26″	13°26′11.22″	46 m	Not present	Calcareous marls
5	CON_MG34	Arable land	Pian del Medico, Jesi (AN)	43°27′27.43″	13°23′7.90″	88 m	Not present	Calcareous marls
6	BF_GUA	Beech forest	Gualdo, Visso (MC)	42°53′16.71″	13°12′15.17″	1236 m	Oligomull	Marl limestones
7	BF_FIU	Beech forest	Monte Vermenone, Fiuminata (MC)	43°8′19.58″	12°56′24.34″	1126 m	Dysmoder	Flint limestones
8	BF_CAN	Beech forest	Canfaito, San Severino Marche (MC)	43°14′14.21″	13°4′24.04″	1025 m	Dysmoder	Flint limestones
9	OF_FB	Mixed forest	FossoBarronciano, Serravalle del Chienti (MC)	43°0′23.31″	13°0′58.45″	843 m	Hemimoder	Marl limestones
10	CF_TOR	Mixed forest	Torrone, Camerino (MC)	43°6′48.78″	13°10′58.08″	684 m	Hemimoder	pelitic calcareous rocks

2.2. Soil Sampling and Sample Processing

From each site, ten soil samples were randomly collected from a 100 m^2 area at a depth of 0–10 cm using an Edelman auger. These samples were then combined to form a composite sample weighing approximately 1 kg, which was sealed in a sterile plastic bag and transported to the laboratory. To prevent cross-contamination between soil samples from different locations, the Edelman auger underwent a thorough cleaning process by (1) scrubbing off adhering soil particles with a metal brush, (2) rinsing with tap water, (3) immersion in a 10% bleach solution for 5 min, and (4) a final rinse with sterile water. The cleaned auger was then stored in a sterile plastic bag until its next use [37,38].

The resulting soil samples were divided into approximately two halves. One half was used to measure the main chemical–physical parameters, while the other half was used for the qualitative and quantitative assessment of the ciliate communities (Table 2). Soil texture analyses and further chemical analyses were performed at the laboratory of Agrochemistry of AMAP (Marche, Agricoltura, Pesca, https://www.amap.marche.it/, accessed on 12 January 2024), as described by Kumar et al. [39].

For ciliate analysis, soil samples were dried for two weeks at room temperature in the laboratory to promote cyst maturation and subsequent ciliate excystment, following the non-flooded Petri dish method [29]. To avoid potential contamination from airborne ciliate cysts, soil samples were covered with straw paper, as also indicated by Ning et al. [4]. Live observations were conducted under a microscope with bright field illumination at magnifications ranging from 100× to 1000×. The cells' body shape and flexibility were observed without applying coverslip pressure, and protargol staining [40] was utilized to reveal their ciliature for species identification.

Table 2. Chemical–physical soil parameters at the sampling sites.

Soil Parameters	ORG (Organic)	CON (Conventional)	FOR (Forest)	*p*-Value
pH	8–8.2	8.2–8.3	6.5–7	0.0001 ***
	(8.1 ± 0.2)	(8.2 ± 0.1)	(6.8 ± 0.2)	
Organic carbon (OC) (g/Kg)	7.4–8.3	9.1–10.8	17.7–206.3	ns
	(7.7 ± 0.6)	(10 ± 1.3)	(80.3 ± 85.6)	
Organic matter (OM) (g/Kg)	12.6–14.3	15.6–18.2	30.6–355.6	ns
	(13.3 ± 1)	(16.9 ± 1.9)	(138.5 ± 147.5)	
Total nitrogen (TN) (g/Kg)	0.9–1.1	1.1–1.4	1.6–1.9	ns
	(1 ± 0.2)	(1.3 ± 0.2)	(1.8 ± 0.2)	
Carbon/nitrogen (C/N)	6.8–8.6	8–8.2	10.1–16.4	0.03 *
	(7.8 ± 1)	(8.1 ± 0.2)	(12.2 ± 2.7)	
Cation exchange capacity (CEC) meq/100 g	16.4–22.7	18.8–19.8	17.6–71.7	ns
	(19.3 ± 3.2)	(19.3 ± 0.8)	(41 ± 19.7)	
S1 (g/Kg)	4–71	7–10	68–380	ns
	(26.7 ± 38.4)	(8.5 ± 2.2)	(201.2 ± 130.1)	
S2 (g/Kg)	13–57	50–64	20–145	ns
	(33.7 ± 22.2)	(57 ± 9.9)	(58 ± 51.1)	
S3 (g/Kg)	95–171	162–215	116–484	ns
	(142.4 ± 41.3)	(188.5 ± 37.5)	(234.4 ± 153.9)	
Silt (g/Kg)	424–471	479–480	162–314	0.0001 ***
	(443.7 ± 24.5)	(479.5 ± 0.8)	(228.6 ± 67.5)	
Clay (g/Kg)	277–452	232–301	90–526	ns
	(353.7 ± 89.5)	(266.5 ± 48.8)	(277.8 ± 226.4)	

Note: ns—not significant. Asterisks indicate level of statistical significance: * $p \leq 0.05$, *** $p \leq 0.001$.

To conduct qualitative ciliate abundance analyses, we utilized a counting method based on the non-flooded Petri dish approach, wherein about 5 mL of soil runoffs was collected on the 2nd, 5th, and 7th days. Subsequently, three permanent protargol-stained slides were prepared from each of the collected runoffs for the 2nd, 5th, and 7th days. These slides were then utilized for the direct identification and counting of species. Average values were calculated by summing the specimens of each species present on the slides made from runoffs of the 2nd, 5th, and 7th days and dividing accordingly. The number of specimens varied widely, ranging from as few as 2 to over 1500 for species identified.

2.3. Statistical Analysis

The spatial distribution of ciliate species in the study areas was analyzed through multivariate methods. Using Bray–Curtis similarity-based cluster analysis, ciliate abundance data (which accounted for up to 92% of the total abundance) were transformed using the square root and classified into assemblages following standard protocols [41–43]. The contribution of each species and spatial differences in ciliate assemblages were assessed using SIMPROF (similarity percentage analysis) [43]. To study biotic–abiotic interactions,

square-root-transformed ciliate abundance data were analyzed using unimodal canonical correspondence analysis (CCA) to elucidate variations in ciliate composition across the different investigated sites [44]. Monte Carlo randomization tests (499 permutations under the reduced model with inter-species distance matrix) were conducted to evaluate the probability of observed patterns [45]. Additionally, alpha diversity metrics, including species richness (S), Margalef's richness index (*d*'), Shannon–Weiner diversity (*H*'), and Pielou's evenness index (*J*'), were computed to highlight patterns of ciliate diversity across the different site groups. Differences between group sites in relation to diversity indices and abundance data were evaluated through one-way analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$). Before ANOVA, the normality and homogeneity of the data were tested. The PRIMER v6.1 software package (PRIMER-E, Plymouth, UK, 2001), developed by Clarke and Gorley [43], was utilized for all the univariate and multivariate procedures described earlier. For canonical correspondence analysis (CCA), CANOCO v4.5 [46] was employed. The box plots were obtained using Origin v8. Indicator species analysis (ISA) [47,48] was carried out using software developed by Prof. Michele Scardi (available on request at http://ecologia.uniroma2.it/, accessed on 15 January 2024).

3. Results

3.1. Ciliate Community Structure and Indicator Species Analysis in Organic (ORG), Conventional (CON), and Forest (FOR) Sites

A total of 59 species representing 33 genera, 20 families, 13 orders, and 7 classes were identified. The ciliate abundance data were subjected to cluster analysis based on the Bray–Curtis similarity index using the group average similarity (Figure 2). Seventeen ciliate taxa were included in the analysis, collectively contributing 92% of the total ciliate population. These taxa comprised *Colpoda inflata*, *Colpoda cucullus*, *Colpoda steinii*, *Sterkiella tricirrata*, *Gonostomum affine*, *Halteria grandinella*, *Actinobolina* sp., *Cyrtolophosis mucicola*, *Oxytricha* sp1., *Blepharisma* sp1., *Nassulides* sp1., *Euplotes* sp., *Spathidium* sp2., *Aspidisca* sp., *Sterkiella cavicola*, *Urosomoida* sp2., and *Anteholosticha* sp. It is interesting to note that the cluster analysis revealed the following three distinct clusters representing different study areas: ORG (arable land under organic management; 1A, 1S, 2A, 2S, 3A, 3S), CON (arable land under conventional management; 4A, 4S, 5A, 5S), and FOR (forest; 6A, 6S, 7A, 7S, 8A, 8S, 9A, 9S, 10A, and 10Aa) (Figure 2, Table 1). The analysis of similarity (ANOSIM) between these ciliate assemblages indicated statistically significant results, with a global R-value of 0.850 at $p < 0.001$.

Based on the results of the cluster analysis, the community structures of soil ciliates from the three identified study areas were treated separately and the contribution of each taxonomic group to the total population was measured (Figure 3). Notably, class *Colpodea* was found to be more abundant than the other groups, contributing up to 48%, 63%, and 59% in ORG (organic), CON (conventional), and FOR (forest), respectively, followed by *Spirotrichea* (38%, 26%, and 25%), *Litostomatea* (11%, 9%, and 10%), and others (3%, 2%, and 6%).

Indicator species analyses (ISA) were performed for ciliates across the ORG, CON, and FOR sites, considering significant indicator values (IndVal) as a percentage of perfect indication based on relative abundance and frequency [47,48]. A total of nine indicator species were identified across the three group sites. Four indicator species were identified in ORG (*Actinobolina* sp. (IndV = 50.3, $p = 0.0495$), *Anteholosticha* sp. (IndV = 83.3, $p = 0.0013$), *Aspidisca* sp. (IndV = 100, $p = 0.0001$), and *Halteria grandinella* (IndV = 62.6, $p = 0.024$)); two in FOR (*Colpoda inflata* (IndV = 80.2, $p = 0.0001$) and *Gonostomum affine* (IndV = 62.9, $p = 0.0024$)); three in CON (*Colpoda cucullus* (IndV = 74.5, $p = 0.0005$), *Frontonia* sp., (IndV = 47.05, $p = 0.0192$), and *Rigidocortex octanucleatus* (IndV = 50, $p = 0.0001$)) (Table 3).

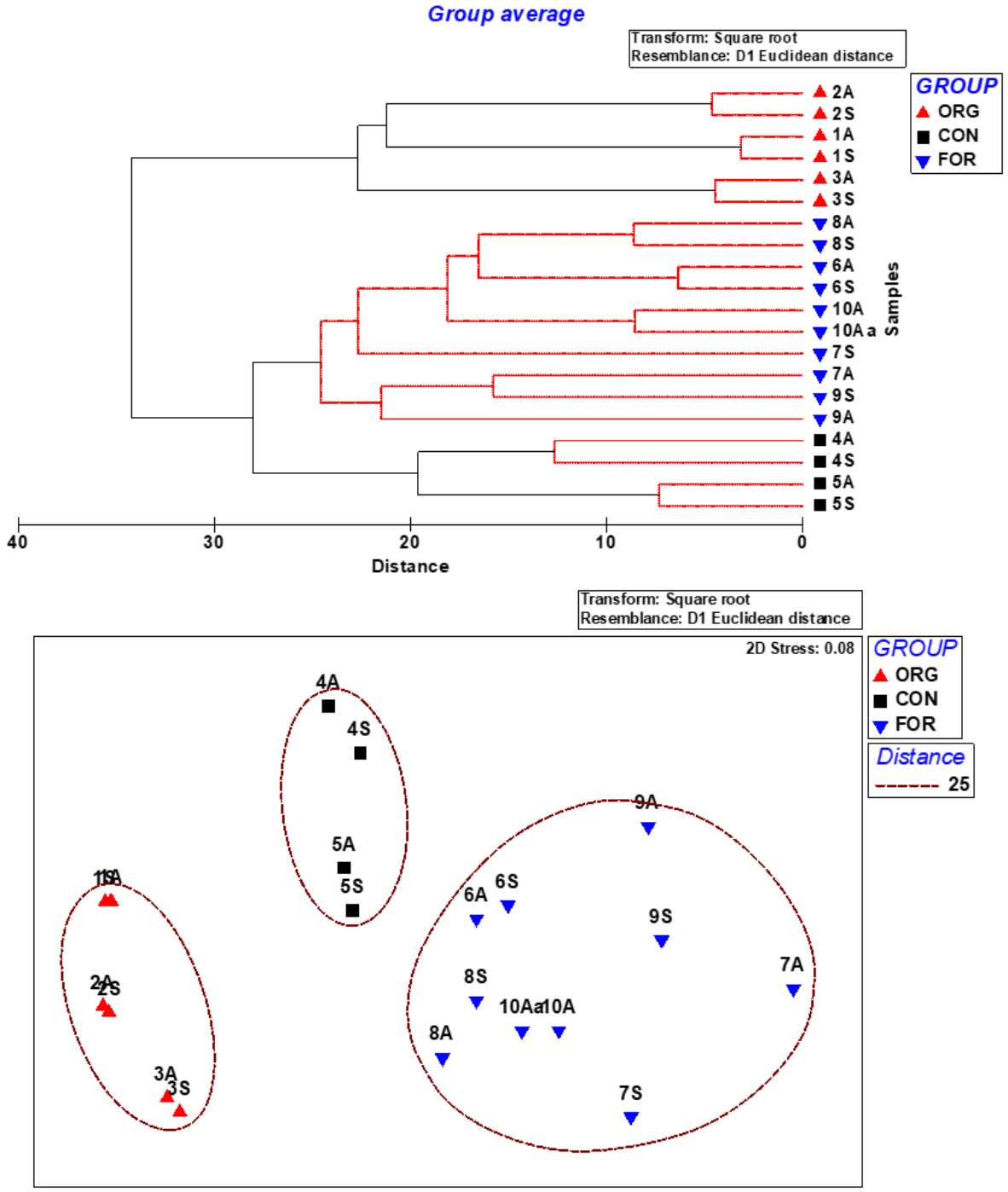

Figure 2. Bray–Curtis similarity based cluster analysis was performed on the square-root-transformed dominant ciliate taxa abundance data that contributed 92% of the total population through group average linking for classifying the ciliate assemblages. ANOSIM global R = 0.85; $p \leq 0.001$. Red triangles mark the ORG farms, black boxes mark the CON farms, and blue inverted triangles mark the forest sites.

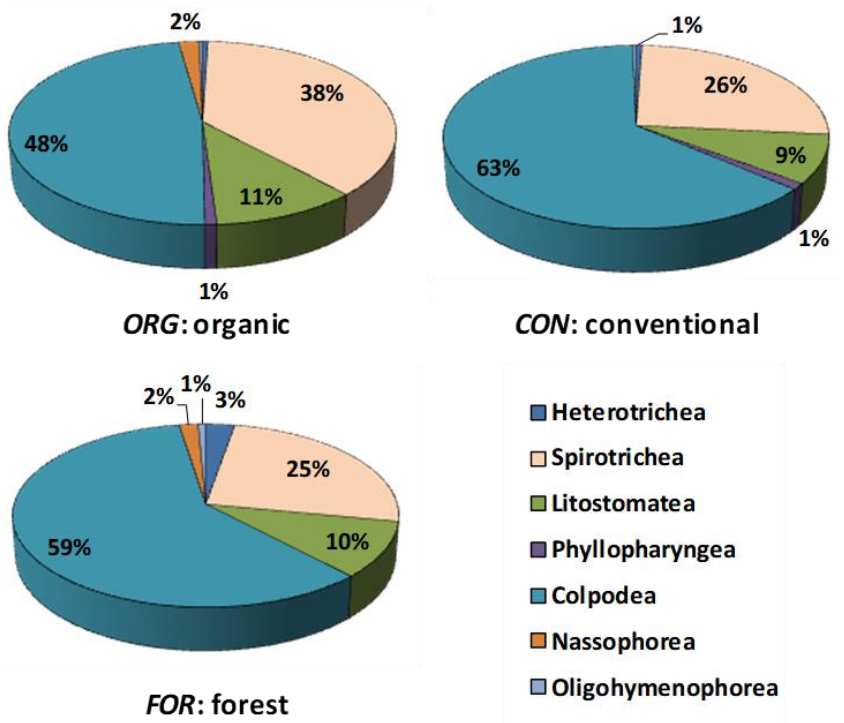

Figure 3. Composition of ciliates at the three study areas.

Table 3. Indicator species analysis for ciliates across the ORG, CON, and FOR sites. Indicator values (IndV) for the ciliate species most characteristic for a specific site are in bold. Only shown are significant indicator values as a percentage of perfect indication based on relative abundance and frequency. Asterisks indicate level of statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Species	ORG (IndV)	FOR (IndV)	CON (IndV)	*p*-Value
Actinobolina sp.	**50.3**	12.6	28.6	0.0495 *
Anteholosticha sp.	**83.3**	0.0	0.0	0.0013 **
Aspidisca sp.	**100**	0.0	0.0	0.0001 ***
Colpoda inflata	0.0	**80.2**	19.7	0.0001 ***
Colpoda cucullus	6.9	16.6	**74.5**	0.0005 ***
Frontonia sp.	0.0	0.5	**47.05**	0.0192 *
Gonostomum affine	13.6	**62.9**	11.7	0.0024 **
Halteria grandinella	**62.6**	4.339	22.8	0.024 *
Rigidocortex octanucleatus	0.0	0.0	**50**	0.0001 ***

3.2. Diversity and Abundances of Ciliates

Ciliate diversity indices were measured using the algorithms developed in the Primer 6 software package, and it was found that the ORG sites had higher diversity indices, except for abundance value, which was higher in the FOR sites. Species richness (S) ranged from 11 to 27, with an average of 17.25. The lowest value (11) was recorded at 8S located in FOR and the highest at 3A in ORG (Table 4). Statistical analysis using ANOVA applied to the species richness data of three different sites showed a significant difference between them at $p < 0.05$. Similarly, the alpha diversity indices measured based on the abundance data for the three group sites are presented in the form of box plots (Figure 4). Abundance (N), Margalef's index (*d*'), Pielou's (*J*'), and Shannon Weiner (*H*'), were also subjected to ANOVA, and it was found that, except for abundance (N), the analyzed diversity indices showed statistically significant variation between the three group sites (Figure 4, Table 4).

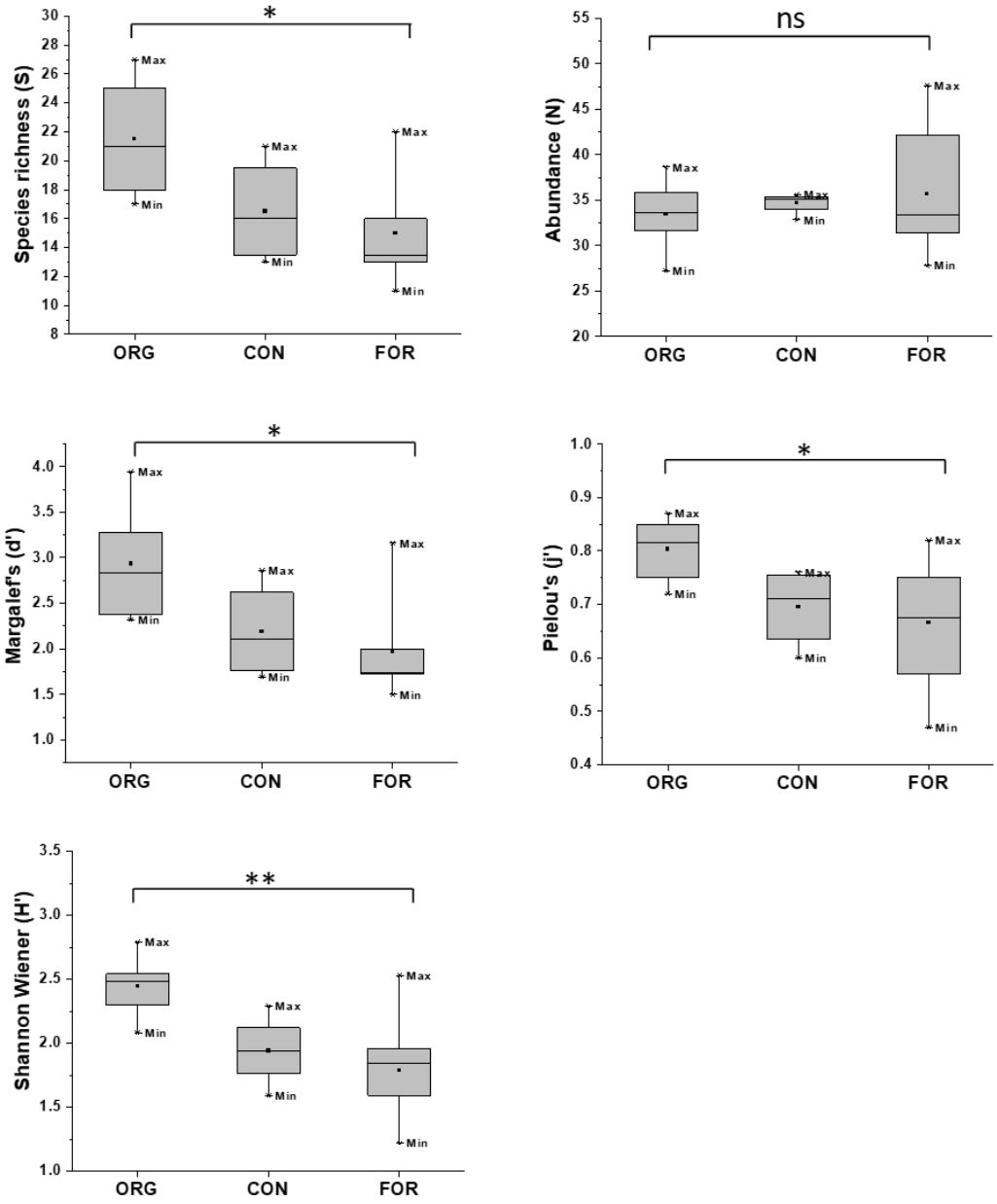

Figure 4. Variations in diversity indices between the study areas (box represents quartile deviation, whisker represents minimum and maximum; symbols inside the box represent mean values and horizontal line). Asterisks indicate level of statistical significance: * $p \leq 0.05$, ** $p \leq 0.01$, ns, not significant.

Table 4. Summary of ciliate diversity indices.

Diversity Indices	ORG (Organic)		CON (Conventional)		FOR (Forest)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Speciesrichness (S)	17–27	21.5 ± 3.94	13–21	16.5 ± 3.7	11–22	15 ± 3.5
Abundance (N)	740–1492	1131 ± 269	1079–1267	1203 ± 84.1	773–2267	1313 ± 506
Margalef's index (*d'*)	2.32–3.9	2.9 ± 0.6	1.69–2.86	2.2 ± 0.5	1.5–3.16	2 ± 0.5
Pielou's index (*J'*)	0.72–0.9	0.8 ± 0.1	0.60–0.76	0.7 ± 0.1	0.47–0.82	0.7 ± 0.1
Shannon–Weiner (*H'*)	2.08–2.79	2.45 ± 0.24	1.59–2.29	1.94 ± 0.28	1.22–2.53	1.8 ± 0.4

3.3. Ciliate Communities and Correlation with Abiotic Parameters across ORG, CON, and FOR Sites

In order to identify the abiotic parameters most involved in the structuring of the ciliated protist communities in the different areas studied, a canonical correspondence analysis (CCA) was applied. The square-root-transformed ciliate abundance data were projected for canonical correspondence analysis (CCA) along with the soil characteristics to understand the biotic and abiotic relationships (Figure 5, Table 5). The first canonical axis explained with the highest eigenvalue (0.326) delineated the main environmental gradient within the study area. This axis showed positive correlations with soil variables such as silt, pH, and clay, characterizing the ORG sites. Conversely, variables including sand, organic carbon, organic matter, total nitrogen, C/N ratio, and CEC (cation exchange capacity) displayed negative correlations, distinguishing the FOR sites from the others. Total phosphorus (TP) displayed homogeneous distribution across both FOR and CON sites. The species–environment relationship variance was notably high along axis 1 (42.9%), highlighting ciliate species such as *Colpoda inflata*, *Colpoda steinii*, *Spathidium* sp2., *Cyrtolophosis mucicola*, *Gonostomum affine*, and *Oxytricha* sp1., signifying the ORG sites. In contrast, *Sterkiella tricirrata*, *Halteria grandinella*, *Actinobolina* sp., *Blepharisma* sp1., *Nassulides* sp1., *Euplotes* sp., *Aspidisca* sp., and *Anteholosticha* sp. were highly correlated with the FOR sites. *Colpoda cucullus* and *Urosomoida* sp2. were the only species positively correlated with the CON sites. Three site groups were again identified in CCA by considering the correlation values of soil characteristics and Bray–Curtis analysis superimposition. Collectively, the first two axes explained over two-thirds of the spatial variation in the ciliate community, accounting for 62.6% of the total 88.2% constrained variability by the measured variables. The influence of canonical axis 1 was particularly pronounced ($F = 0.661$, $p = 0.028$), as validated by Monte Carlo permutation tests (499 permutations under the full model).

Table 5. Canonical correspondence analysis (CCA) generated eigen and correlation values between the ciliates and the soil characteristics.

Axes	1	2	3	4	Total Inertia
Eigenvalues	0.326	0.149	0.108	0.086	0.818
Species–environment correlations	1	0.993	0.971	0.997	
Cumulative percentage variance					
of species data	39.8	58.1	71.3	81.9	
of species–environment relation	42.9	62.6	76.9	88.2	
Sum of all eigenvalues					0.818
Sum of all canonical eigenvalues					0.759
Sand	−0.713	−0.224	−0.028	−0.106	
Silt	0.697	0.384	0.060	0.116	
Clay	0.251	−0.057	−0.017	0.027	
pH	0.783	0.357	−0.052	0.048	
OC	−0.592	−0.283	−0.004	0.054	
OM	−0.592	−0.283	−0.004	0.054	
TN	−0.560	−0.226	0.092	−0.045	
C/N	−0.727	0.070	0.167	−0.482	
TP	−0.213	0.293	−0.040	−0.092	
CEC	−0.694	−0.392	0.126	0.177	

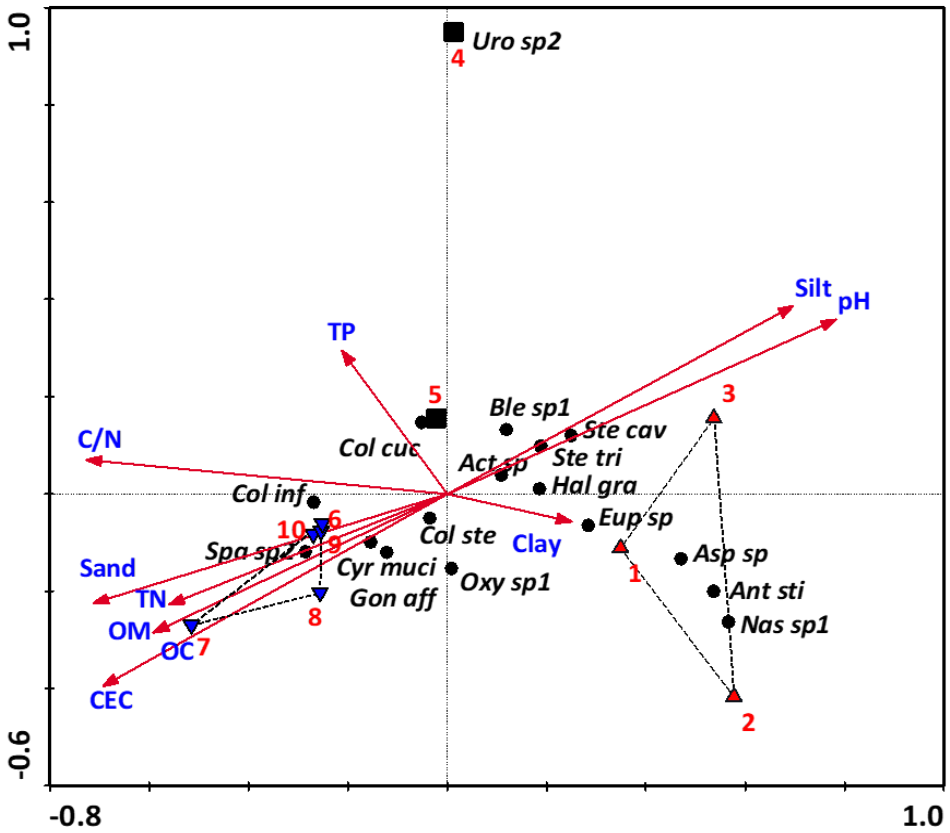

Figure 5. Canonical correspondence analysis (CCA) triplet with biotic and abiotic variables that forms the threestudy areas. Abbreviations of the ciliate species used for the CCA: *Col inf*—*Colpoda inflata*; *Col cuc*—*Colpoda cucullus*; *Col ste*—*Colpoda steinii*; *Ste tri*—*Sterkiella tricirrata*; *Gon aff*—*Gonostomum affine*; *Hal gra*—*Halteria grandinella*; *Act* sp.—*Actinobolina* sp.; *Cyr muci*—*Cyrtolophosis mucicola*; *Oxy* sp1.—*Oxytricha* sp1.; *Ble* sp1.—*Blepharisma* sp1.; *Nas* sp1.—*Nassulides* sp1.; *Eup* sp.—*Euplotes* sp.; *Spa* sp2.—*Spathidium* sp2.; *Asp* sp.—*Aspidisca* sp.; *Ste cav*—*Sterkiella cavicola*; *Uro* sp2.—*Urosomoida* sp2.; *Ant sti*—*Anteholosticha* sp.).

4. Discussion

The abundance and species compositions of soil organisms are closely associated with the type and physical characteristics of the soil and agricultural management practices and land uses. In this regard, the habitats of ciliated protists also exhibit temporal and spatial heterogeneity, leading to an uneven distribution of their communities in soils [49–51].

Multivariate analysis has been shown to be best at characterizing changes in the community structure and explaining how they vary along environmental gradients [52]. In our study, Bray–Curtis similarity was used to plot the canonical correspondence analysis of principal coordinates (CAP) and, interestingly, it showed three distinct ciliate community assemblages. In this respect, the first canonical axis separated the ciliate community present in the organic farming sites (ORG: 1A, 1S, 2A, 2S, 3A, 3S) from that present in the conventional farming sites (CON: 4A, 4S, 5A, 5S), even though these sites were included in the arable land category. The third group was represented by the community living in the forest sites (FOR: 6A, 6S, 7A, 7S, 8A, 8S, 9A, 9S, 10A, 10Aa), with global ANOSIM R = 0.85; $p \leq 0.001$.

Our results showed that the composition of ciliates significantly varied between the three sites, namely ORG, CON, and FOR, with the predominant groups being Colpodea and Spirotrichea. According to Foissner [29], the taxonomic composition of ciliates is related to the morphological and ecological characteristics of each ciliate taxa. The dominance of these two groups can be attributed to their ecological traits, which enable them to readily adapt to soil habitats. For instance, species of Colpodea can encyst during periods of decreased soil

moisture and excyst promptly when soil moisture increases [53,54]. Conversely, Spirotrichs have the ability to maneuver into adjacent soil granules or litter in search of food due to their dorsoventrally flattened bodies [54,55].

In the arable land sites under organic management practices, 48% of the ciliates were represented by the Colpodea family, compared with 63% and 59% in CON and FOR sites, respectively. This result is consistent with the Colpodea and Polyhymenophorean (C/P) quotient (ratio of r-selected Colpodean and k-selected Polyhymenophorean ciliates), a commonly used metric to assess habitat preference/suitability for ciliate species [29]. Generally, values below 1 indicate that the habitat is "ordinary" and predictable, whereas a value above 1 indicates that the habitat is harsher and unpredictable. In our study, ORG sites showed the lowest values, with C/P = 1.24, indicating a comparatively more suitable soil habitat for ciliates (at least for some ciliate taxa) than CON and FOR sites, with C/P = 2.33 and C/P = 2.10, respectively. In this respect, colpodids are known to be opportunistic r-selected generalists, tolerating fluctuations in humidity and temperature by rapidly encysting and excysting, and rapidly transitioning between active and dormant states [28,29]. In addition, species of the genus *Colpoda* contribute to increased abundance due to their short generation time and unique reproductive strategy, i.e., cyst division, which often produces more than two daughter cells. On the contrary, Polyhymenophorean ciliates are more abundant in less stressed "ordinary" soils. Consequently, the C/P quotient is often used to differentiate stressed soil systems from less stressed ones. Thus, the higher value observed in CON and FOR sites in our study indicates that these sites are less suitable for Polyhymenophorean species.

Indicator species analysis identified nine ciliate species that were significantly associated with the different investigated land uses (see Table 2). Notably, the relative abundance and frequency of ciliate species characteristic to each site were identified: *Actinobolina* sp., *Anteholosticha* sp., and *Halteria grandinella* were prominent for the ORG site; *Colpoda cucullus*, *Frontonia* sp., and *Rigidocortex octonucleatus* for the CON site; and *Colpoda inflata* and *Gonostomum affine* for the FOR site, based on their significant indicator values as a percentage of perfect indication (see Table 3).

Comparing the diversity indices (H', d', J') measured at the three sites ORG, CON, and FOR showed that ORG had significantly higher values than CON and FOR for all the indices studied. In this respect, the ORG sites showed higher mean values for Margalef's index (d' = 2.9), Pielou's index (J' = 0.8), and Shannon–Weiner (H' = 2.45) than for CON (d' = 2.2; J' = 0.7; H' = 1.9) and FOR (d' = 2; J' = 0.7; H' = 1.8). In addition, species richness (S) was higher in ORG than in the other sites, ranging from 17 to 27 (mean = 21), followed by 13 to 21 (mean = 16) and 11 to 22 (mean = 15) in CON and FOR, respectively. This indicates that ORG sites were the most diverse and richest in species compared with the CON and FOR sites. Overall, these results support the "intermediate disturbance hypothesis" (IDH), which states that slightly disturbed habitats (such as arable lands under minimumtillage farming practices) usually have higher organism diversities than stable ones [56,57]. In this regard, organically managed arable lands (ORG) are subject to frequent tillage, albeit at a shallow depth (minimum tillage, 15–20 cm). This light but constant mechanical treatment over the seasons helps to create ecological niches suitable for maintaining a rich and diverse soil ciliate community. In contrast, the CON sites were subjected to a different treatment at soil level, namely sod seeding, a conservative agronomic soil management technique that involves no-tillage of the herbaceous cover crops to reduce soil erosion and maintain a physical fertility comparable to that of natural soils. From this point of view (e.g., lack of mechanical disturbance at soil level), the CON sites were more similar to the FOR sites in terms of ciliate community structure, as also highlighted by the cluster and CCA analyses and the diversity data (Figures 2 and 4).

Previous studies of biotic–abiotic relationships have revealed the significant role of variations in physicochemical parameters in influencing the abundance, distribution, and diversity of ciliates [58–60]. In this study, canonical correspondence analysis (CCA) was carried out in order to elucidate the multiple linear relationships between ciliate species

and soil parameters (refer to Table 5, Figure 5). In this regard, CCA explained up to 42.9% of the total variance in ciliate community structure. The presence of species from the genera *Colpodea* and *Spathidium* indicated that factors like silt and clay content, along with soil pH, play pivotal roles in determining the distribution of ciliate communities. For instance, *Spathidium* species are typically found in alkaline environments with pH ranging from 7.9 to 8.5 [61], while Colpodida, a dominant group, thrives in moisture-rich conditions, particularly in silt and clay soils [53,54]. Factors such as the texture and moisture of the soil content are recognized as key regulators of soil ciliates populations. Previous research has highlighted the significant effects of soil moisture, texture, and structure on ciliate abundances [62,63]. Additionally, various soil parameters like pH, organic matter content, and nutrient levels (such as total nitrogen and phosphorus) also impact ciliate communities [62,63]. While there is extensive research on the relationships between ciliate abundances and factors like soil texture, moisture, and pH, there is limited information on their associations with specific nutrients like ammonia-nitrogen and nitrate-nitrogen. However, studies have shown that total nitrogen levels can influence the abundance of soil protists, suggesting a potential link between nutrient availability and ciliate populations.

5. Conclusions

Overall, the results of this study confirm the suitability of the soil protist community and its diversity as a bioindicator of different land uses. Moreover, the approach utilized herein successfully discerned differences in the community structure of soil ciliates under different agricultural management practices, with organic practices showing the most favorable outcomes. The correlations between observed and predicted values were all significant, highlighting the association of species distribution with abiotic parameters (such as pH, soil texture, and organic matter) and the distinction between different agricultural practices (ORG vs. CON), underscoring the importance of these parameters in shaping ciliate communities at the investigated sites (Figure 5). Altogether, these results showed the bioindicative potential of ciliate communities in discriminating between natural sites (FOR) and arable lands, and their capacity to discriminate, at least preliminarily, between different soil management systems (ORG vs. CON).

Brief Notes on the Soil Ciliate Diversity from Italy

In the current study, out of 59 species representing 33 genera, 20 families, 13 orders, and 7 classes, 5 species were identified as new to science (Daizy Bharti, Santosh Kumar, and Antonietta La Terza, unpublished results). During the course of this study and other projects particularly focused on soil ciliates from various ecosystems in Italy carried out at "Soil Biodiversity and Monitoring Laboratory" of the University of Camerino, an unexpectedly high number of new species/genera (more than 15) of ciliates have been identified, some of which have already been published [38]. Additionally, many of the species identified (over 30) were new records for the Italian ciliate fauna. These findings are consistent with earlier reports [39–65] and support observations by other authors [9–67], underscoring the inadequate understanding of soil ciliate diversity. Given the importance of soil protists in the soils, there is a necessity for internationally coordinated research efforts to explore ciliate diversity and their functional roles in soils across various land use systems.

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