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Original article

Impact of farming practices on soil diatoms and testate amoebae: A pilot study in the DOK-trial at Therwil, Switzerland

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ABSTRACT

Testate amoebae (Arcellinida and Euglyphida) and diatoms (Bacillariophyta) respond to different ecological gradients. These protists are useful tools for biomonitoring and paleoecological studies in aquatic and terrestrial ecosystems. However, little is known about the responses of these micro-eukaryotes to soil management practices. We analyzed the testate amoeba and diatom communities from the DOK-trial (D: biodynamic, O: bio-organic, K: german "konventionell" integrated conventional) agricultural experiment at Therwil, Switzerland. Soil samples were collected from biodynamic and conventional plots and subsequently incubated for four months in a growth chamber. The diatom diversity tended to be higher in the biodynamic than in the two conventional systems. Redundancy analysis (RDA) suggested that diatom community structure differed between organic and the two conventional systems. Testate amoeba abundance was about five times higher in biodynamic than in conventional systems ($P < 0.05$) but no significant differences in diversity were reported between treatments. Altogether, these data suggest that diatoms and testate amoebae are sensitive to farming systems. As direct analyses of soil samples are time-consuming, molecular tools would be very useful for further development of the use of protists in bioindication.

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1. Introduction

Testate amoebae (Arcellinida and Euglyphida) and diatoms (Bacillariophyta) are diverse and can be found in a vast range of habitats. Although most diatom species occur in aquatic habitats, specific diatom communities occur in soil where they grow with other microalgae and cyanobacteria [1–4]. In the soil food web, algae serve as food for soil protozoa and micro- and meio-fauna such as nematodes or collembolans. Moreover, these primary producers are thought to be important contributors to the organic carbon content of soils [5] and play a crucial role in the formation and stability of soil aggregates [6,7]. Testate amoebae represent another important group of protist in soils. They feed on bacteria, plant cells, protists, fungi or small metazoans [8–10]. They are key players in food web processes as well as in nutrient cycling [11,12].

Testate amoebae are diverse in the soil environment where about 300 morphospecies were reported [13].

Testate amoebae and diatoms are valuable tools for bio-monitoring and paleoecological studies for several reasons: 1) they respond to different ecological gradients, 2) the shells of testate amoebae or the frustules of diatoms allow the identification of morphospecies more easily than in the vast majority of other protists, 3) their fossils are often preserved in sediments or soils, and 4) they have generally a large distribution range. Consequently, testate amoebae and diatoms are extensively used in several ecosystems such as lake, rivers, wetlands or peat bogs as indicators for acidification, eutrophication, toxic pollution or past moisture and pH conditions [14–16]. Several diatom indicator indices have been developed for the assessment of trophic conditions in freshwater ecosystems [17–19]. However, it remains unclear if diatoms and testate amoebae can be used to monitor agricultural soil quality. Only a few studies have investigated the effects of some abiotic and biotic parameters on diatom and testate amoeba communities in agricultural soils.

Bérard et al. [20] compared soil microalga communities between a treated field and a reference untreated organic cornfield

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and showed that atrazine application in the conventional cornfield had changed the species composition of the communities. The effect of herbicides on soil diatom communities was also reported by other algologists, who drew similar conclusions [21–24].

The response of testate amoebae to several factors related to farming practices was studied, including soil compaction [25], impact of fertilizers and lime [26], pesticides [13]. Finally, Foissner [27] reported that in certain study sites, testate amoeba biomass was significantly lower in conventional than in organic fields.

However, all of these studies were based on short-term experiments (agricultural experiment established less than a few years before the study) or on experiments where several factors such as soil properties, crop rotation, crop varieties, soil tillage, fertilization regimes and plant protection also varied between conventional and organic soil management. The long-term DOK-trial (D: biodynamic, O: bio-organic, K: German “konventionell” integrated conventional) agroecosystem experiment in Therwil, Switzerland overcomes this shortcoming. As mentioned by Esperschütz et al. [28], it is the only field experiment where the same crop varieties, crop rotations and organic fertilization intensities are applied in organic and conventional farming systems.

The main objectives of the present study were: 1) to assess the effects of long-term agricultural practices on diatoms and testate amoeba communities, 2) to get more insights into the diversity (i.e. species richness) and relative abundance of diatoms and testate amoebae in agricultural soils, and 3) to evaluate the potential use of these protists for soil biomonitoring.

2. Methods

2.1. Study site

The DOK-trial in Therwil, Switzerland is a long-term agricultural experiment established in 1978 [29]. The goal of this experiment was to assess the agronomic and ecological effects of biodynamic (BIODYN) and conventional (CONFYM or CONMIN) farming systems. The experiment uses a split-split plot design. The plot area is 5 × 20 m in size and separated by 5 m wide strips of grass to minimize interaction between adjacent treatments [28]. The farming systems mainly differ in plant protection approach as well as fertilization. A system-specific farmyard manure is used to fertilize the BIODYN and CONFYM systems at a rate corresponding to 1.4 livestock units per hectare, roughly equivalent to 2000 kg organic carbon per hectare and per year. Supplementary fertilization was achieved with mineral fertilizers (N, P and K) in the CONFYM system following official recommendations. The CONMIN system mimics a conventional system without livestock. It is only fertilized using mineral fertilizers and this fertilization started in year 8 of the study [29]. These soils have an identical pedological origin [29,30]. Further information on the biological, chemical or physical characteristics of the different treatment plots are available in the literature [28,29,31,32].

2.2. Sampling procedure and laboratory incubation

In order to assess the long-term effect of farming management on protist communities, we sampled four replicate plots of BIODYN, CONFYM, and CONMIN treatments on April 20th 2006 (i.e., before the first fertilizer application of the season) from the grass-clover plots in their second year. All sampled plots were derived from the DOK experiment with a 7-year ley rotation [29]. Crop rotation was identical in all treatments (i.e., potato, winter wheat 1, soy bean, corn and winter wheat 2, followed by 2 years of grass-clover). In each plot we took ten 6 cm long soil cores using a 2 cm diameter soil auger at random locations within each plot, but avoiding the 50 cm marginal zone and aiming at sampling in the dominant type

of microtopography and vegetation (where relevant) in order to minimize heterogeneity. The ten soil cores were pooled for each plot to make up a composite sample. The samples were placed in plastic bags and carried to the laboratory. We analyzed diatom communities of all three sampled treatments. Testate amoebae were analyzed from the two most contrasted treatments: BIODYN and CONMIN. Our first approach was to extract the protists directly from the soil samples. However because of the texture of this soil (haplic luvisol, 70% silt, 15% sand and 15% clay [29]) most mineral particles were within the same size range as the protists we were trying to extract and a direct counting approach would be extremely time-consuming. We therefore used a second approach. The composite soil samples were placed in plastic boxes (10 × 12 × 5 cm), kept moist by regularly spraying with water and covered with a thin multiporous plastic film to limit evaporation. The presence of small drops of water under the multiporous plastic film indicated that the humidity of the air inside the mesocosms reached saturation. The culture boxes were incubated in a growth chamber (24–16 °C, 14:10 h Light:Dark) equipped with fluorescent tubes for four months. Every week, the boxes were randomly arranged. By the end of the four month incubation we found a higher concentration of protists at the surface of the soil and we were thus able to use a direct extraction approach with success. We attribute this difference to the fact that we had sampled early in the season and that the protist communities had probably not yet had time to develop completely after the winter.

2.3. Slide preparation and counting

2.3.1. Diatoms

We took three surface subsamples (about 1 g in total) within each soil culture box. The three subsamples were then pooled. Diatom frustules were treated with 50 ml of 35% hydrogen peroxide (H₂O₂) to remove organic matter. Hydrochloric acid (35%) was added to remove calcium carbonate from the samples. The supernatant was removed after 12 h of sedimentation. Each sample was then re-suspended in 100 mL distilled water, and 0.3 ml of this solution was dried on a cover glass. Clean diatom valves were mounted in synthetic resin dissolved in toluene (Naphrax[®]) and was scanned (1000× magnification) to count and identify 150 valves (75 individuals on each of two slides). In addition, we verified the identification of some morphotypes by scanning electronic microscopy (SEM). We placed 0.3 ml of the same solution as used for optical microscopy on an aluminum stub. The samples were dried 3 days in a desiccator and then SEM was performed as described in Heger et al. [33].

2.3.2. Testate amoebae

Testate amoebae were extracted from 2 g of dry soil surface subsamples using a sieving and back-sieving method that retained all particles with a size between 15 µm and 125 µm. The back-sieving method is frequently used for extracting testate amoebae from soil samples [34]. However, we cannot rule out the possibility that very small morphotypes having a breadth thinner than 15 µm were lost through back-sieving. Two sub-fractions diluted in the same volume of water were used: 15–25 µm and 25–125 µm.

Wet mounts (100 µl per slide) were analyzed under the microscope at 200× and 400× magnifications. As a standard sampling effort, two slides were counted for each of the four replicates of the BIODYN and CONMIN farming systems.

2.4. Numerical analyses

One-way analysis of variance (ANOVA) was used to test the effect of farming practices on species richness, Shannon's diversity

(which is the exponential of Shannon entropy) and abundance of testate amoeba and diatoms. The data was log-transformed to homogenize variances. Statistical analyses of diatom dataset were followed by Tukey post hoc tests. ANOVAs tests were applied after corroboration of the assumptions of normality and variance homogeneity. To measure the relationship between diatom communities and the farming managements, we used redundancy analysis (RDA) after Hellinger transformation of the species data set [35]. Hellinger transformation is a pre-transformation of species data to allow using RDA without considering the common absence of the species as a resemblance between communities [36]. Significance was assessed using permutation tests (1000 permutations). All analyses were performed with R 2.12.1 [37].

3. Results

3.1. Diatom

3.1.1. Community composition

A total of 16 morphotypes were identified in the BIODYN, CONFYM and CONMIN samples of the second year grass–clover plots (Table 1). The species richness was on average 8.5 (SE 0.6). The minimum as well as the maximum number of species per sample (respectively 5 and 12) were reported from BIODYN samples. Shannon's diversity ranged between 5.95 and 1.33 (average 1.19 SE 0.13). *Eolimna minima* (Fig. 1a), *Nitzschia pusilla* and *Pinnularia cf. obscura* were the only three taxa to be present in all samples. Together, these three taxa made up 76% of the total count. *Hantzschia amphioxys* was observed in a high majority of samples (11/12) but in low abundance (percentage of the valves counted always lower than 4%). According to Cullimore and McCann [38], this species is resistant to different herbicides. Similarly, *Mayamaea atomus* and *Stauroneis thermicola* (Fig. 1b) were present in most samples (10/12), but were never dominant. Most of the observed morphotypes such as *Hantzschia amphioxys*, *Luticola mutica* or *Diadesmis contenta* are typical soil taxa [3,39]. Of the 16 taxa recorded, seven morphotypes could not be assigned a precise name based on their morphology: *Pinnularia cf. obscura*, *Pinnularia cf. intermedia*, *Navicula cf. goeppertiana*, *Navicula sp1* and *Surirella cf. subsalsa/ovalis*.

3.1.2. Differences among treatments

Both species richness and Shannon–Wiener diversity tended to be higher in the Biodynamic (BIODYN) samples than in the

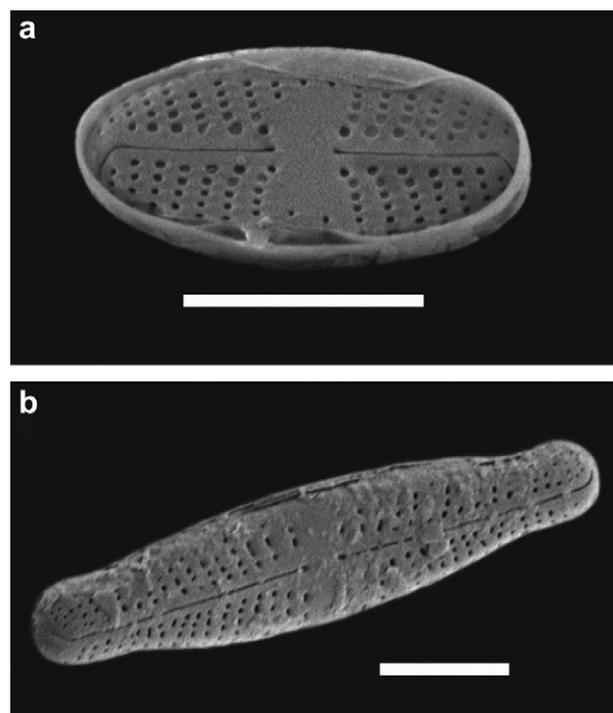


Fig. 1. Scanning electronic microscopy pictures of two diatoms observed in the incubated soil samples: (a) *Eolimna minima*, (b) *Stauroneis thermicola*. Scale bar = 3 μ m.

CONFYM and/or CONMIN samples (Fig. 2). However, these differences were not statistically significant (ANOVA, $P > 0.05$). The ANOVAs showed that relative abundance of *Pinnularia cf. obscura*, *Surirella cf. subsalsa/ovalis* and *Nitzschia capitellata* were significantly higher in the BIODYN than in the CONMIN and CONFYM samples. In contrast, the abundance of *Navicula sp1* was significantly higher in the BIODYN than in the CONFYM samples.

The relative abundance of *S. thermicola* was significantly higher in the CONFYM than in the BIODYN sample. The relative abundance of the other species did not differ significantly among treatments (Table 1).

The redundancy analysis (RDA) also suggested that farming practices had an effect on diatom communities (Fig. 3). Along the first ordination axis, explaining 26% of the variance, biodynamic samples (BIODYN) were projected on the negative side while

Table 1

Relative abundance with standard errors ($N = 4$) of diatom morphotypes encountered in the biodynamic (BIODYN) and conventional (CONFYM and CONMIN) soils of the DOK field trial. Significance of effects of farming system on the relative abundance of diatoms was determined with one-way ANOVA.

	Abbrev	CONMIN	CONFYM	BIODYN	P value		
					CONFYM-BIODYN	CONMIN-BIODYN	CONMIN-CONFYM
<i>Eolimna minima</i> (Grun.) Lange-B.	EMIN	71.2 \pm 3.8	57.0 \pm 14.9	47.0 \pm 12.3	ns	ns	ns
<i>Nitzschia pusilla</i> (Kütz.) Grun. em Lange-B.	NPUS	9.0 \pm 2.5	12.3 \pm 3.8	5.2 \pm 2.4	ns	ns	ns
<i>Pinnularia cf. obscura</i>	POBS	5.7 \pm 1.3	5.2 \pm 2.0	16.5 \pm 2.6	$P < 0.05$	$P < 0.05$	ns
<i>Mayamaea atomus</i> (Kütz.) Lange-B.	MATO	3.5 \pm 0.8	11.2 \pm 5.1	1.2 \pm 0.7	ns	ns	ns
<i>Diadesmis contenta</i> (Grun.) D.G. Mann	DCON	2.7 \pm 2.3	0.2 \pm 0.1	0.5 \pm 0.3	ns	ns	ns
<i>Stauroneis thermicola</i> Pet.	STHE	2.5 \pm 0.6	10.2 \pm 3.1	0.7 \pm 0.4	$P < 0.05$	ns	ns
<i>Surirella cf. subsalsa/ovalis</i>	SSUB	1.8 \pm 1.6	0.2 \pm 0.1	9.0 \pm 2.3	$P < 0.05$	$P < 0.05$	ns
<i>Hantzschia amphioxys</i> (Ehr.) Grun.	HAMP	1.3 \pm 0.4	1.7 \pm 0.7	1.0 \pm 0.3	ns	ns	ns
<i>Navicula sp1</i>	NSP1	1.0 \pm 0.5	0.0 \pm 0.0	0.0 \pm 0.0	ns	$P < 0.05$	$P < 0.05$
<i>Surirella angusta</i> Kütz.	SANG	0.8 \pm 0.7	0.0 \pm 0.0	1.2 \pm 0.7	ns	ns	ns
<i>Luticola mutica</i> (Kütz.) D.G. Mann.	LMUT	0.3 \pm 0.2	0.0 \pm 0.0	0.2 \pm 0.1	ns	ns	ns
<i>Nitzschia capitellata</i> Hust.	NCAP	0.2 \pm 0.1	0.0 \pm 0.0	13.7 \pm 11.8	$P < 0.05$	$P < 0.05$	ns
<i>Mayamaea asellus</i> (Weinhold) Lange-B.	MASE	0.0 \pm 0.0	0.3 \pm 0.2	2.0 \pm 1.2	ns	ns	ns
<i>Luticola goeppertiana</i> (Bleisch) D.G. Mann	LGOE	0.0 \pm 0.0	0.2 \pm 0.1	0.3 \pm 0.2	ns	ns	ns
<i>Pinnularia cf. intermedia</i>	PINT	0.0 \pm 0.0	1.5 \pm 0.6	0.7 \pm 0.4	ns	ns	ns
<i>Stauroneis anceps</i> Ehr.	SANC	0.0 \pm 0.0	0.2 \pm 0.1	1.0 \pm 0.6	ns	ns	ns

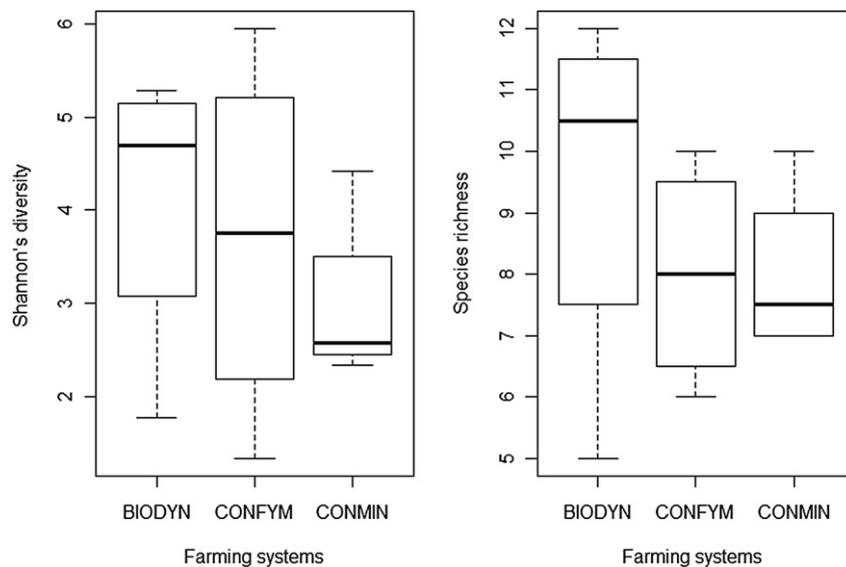


Fig. 2. Boxplot showing the relationship between farming systems (BIODYN, CONFYM and CONMIN) and diatom diversity (Shannon's diversity) or species richness. No significant differences were reported among treatments ($P > 0.05$).

conventional samples (CONFYM and CONMIN) had positive scores. *S. thermicola* and *Navicula atomus* were positively correlated with the first axis while *Surirella cf. subsalsajovalis*, *Pinnularia cf. obscura* and *Nitzschia capitellata* were negatively correlated with this axis. The variance explained by the second axis was not significant for explaining differences in the communities. The RDA further revealed that one BIODYN and one CONFYM samples are clearly different from all other samples (Fig. 3). These samples contain respectively a high abundance of *Nitzschia capitellata* and *Mayamaea atomus*. We tentatively attribute this result to the high micro-heterogeneity of the soil within samples.

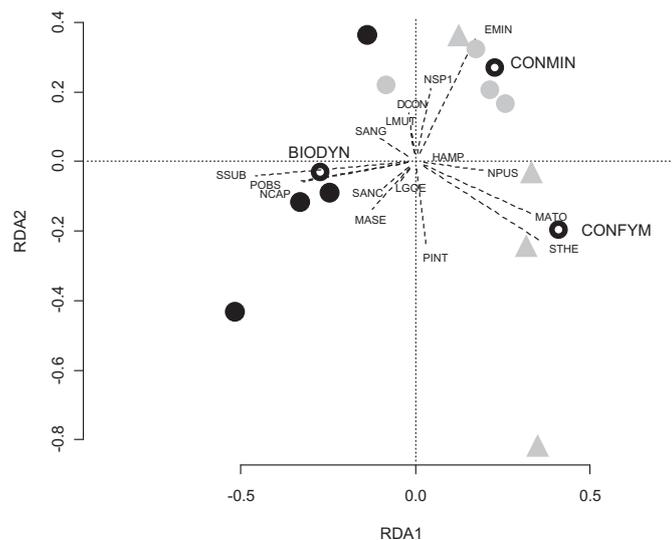


Fig. 3. Ordination diagram (redundancy analysis; RDA) of the diatom community composition from BIODYN (black circles), CONMIN (grey circles) and CONFYM (grey triangles) samples. Farming treatments (qualitative variable) were used as explanatory variables. Blue triangle and circles represent centroids of each farming treatments. Axes 1 and 2 hold, respectively, 26% and 5% of the explained variance but only the first axis is significant.

3.2. Testate amoebae

3.2.1. Community composition

The total number of testate amoeba observed on two slides per sample ranged from 14 to 210. In the samples, a total of seven morphotypes were identified (Fig. 4), all of which are typical soil taxa [40,41]. The three most dominant morphotypes *Plagiopyxis callida*, *Plagiopyxis declivis* and *Phryganella acropodia* together made up 73% of the testate amoebae count and were reported in all investigated samples. Lobose testate amoebae (Arcellinida) dominated the testate amoeba communities. Only two filose testate amoebae (Euglyphida) individuals (two *Euglypha laevis*) were reported in this study.

3.2.2. Differences between the BIODYN and CONMIN farming treatments

As for diatoms, both the species richness and Shannon's diversity of testate amoebae tended to be higher, but not significantly so ($P > 0.05$) in the BIODYN samples than in the CONMIN samples (Species richness: 5.8 SE 0.6 and 4.8 SE 0.3; Shannon's diversity 4.41

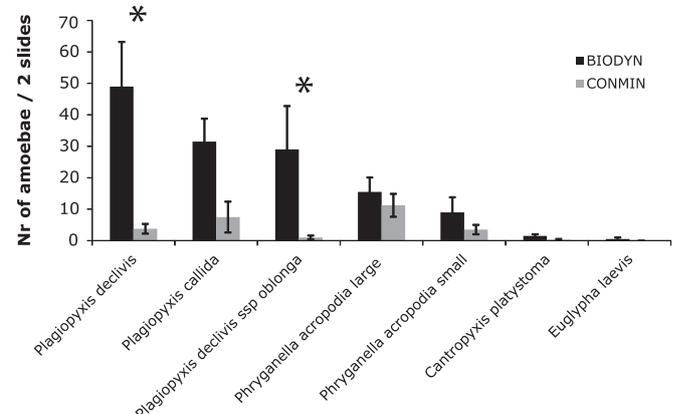


Fig. 4. Testate amoebae morphotypes counted in the BIODYN (black bars) and CONMIN (grey bars) samples. Standard error bars are presented for $N = 4$. Asterisk (*) indicates significant difference between treatments ($P < 0.05$).

SE 0.38 and 3.68 SE 0.29, respectively in the BIODYN and CONMIN samples). By contrast a significant difference was recorded in apparent density, estimated based on the total number of amoebae counted on two microscope slides. The total number of amoebae recorded was on average five times higher in the BIODYN than in the CONMIN samples (respectively 136.0 SE 33.3 and 27.3 SE 7.8; $P < 0.05$). All seven recorded morphotypes were on average more abundant in the BIODYN than in the CONMIN samples but significant differences were detected only for two morphospecies: *P. declivis* and *P. declivis* ssp *oblonga* (Fig. 4).

4. Discussion

The DOK experiment has already shown that organic practices enhanced soil fertility, biological activity, microbial biomass and activity, the diversity of arbuscular mycorrhizal fungi, the biomass and abundance of earthworms, and the activity density of three groups of arthropods: carabids, staphylinids, and spiders [29,32].

The results of this present study further suggest that testate amoebae and diatoms are also affected by farming systems. In diatoms, the multivariate analyses showed that the community structure differed between organic and conventional systems. However the trend for higher diatom species richness and diversity in the biodynamic than in the two conventional systems were not significant. To our knowledge, this is the first report of the long-term effect of farming management on diatom communities.

Testate amoebae species richness and diversity did not differ between treatments. However, testate amoeba abundance was about five times higher in the BIODYN than in CONMYN samples. In agreement with these results and as reported above, Foissner [27,42] found a higher testate amoeba biomass in organic fields than in conventional fields. These differences were significant for wheat crop and vineyard but not for the grassland. Similarly, a recent study in the same field experiment in Therwil, based on the determination of phospholipid fatty acids (PLFA) and phospholipid ether lipids (PLEL) reported a higher protozoan biomass in the organic than in the conventional system. The differentiation of community structures among the management regimes were however very low [28]. Furthermore given the fact that protozoa do not constitute a monophyletic group but are rather distributed in many different groups of eukaryotes [43], it is unclear which groups of protozoa are effectively recorded with PLFA and PLEL analyses. Flagellates and naked amoebae were also recently analyzed in the DOK-trial experiment. No differences in abundances were detected among farming systems [31].

4.1. Testate amoeba and diatom diversity in samples from the DOK-trial experiment

The overall diatom and testate amoeba diversity is much lower than what can be expected in undisturbed terrestrial or aquatic environments [15,44,45]. However, this low diversity is not surprising. Testate amoebae are considered to be mostly K strategists and therefore would be expected to be especially sensitive to repeated perturbation such as plowing, cutting or grazing and diatoms are very sensitive to desiccation because they live at the surface of the soil where moisture content is frequently very low. Therefore, the different degree of soil disturbance [29] and potential differences in soil moisture between the conventional and organic fields might explain at least one part of the differences observed among treatments. The number of species reported in this study is quite likely underestimated. Indeed, our morphology-based approach does not allow us to rule out the presence of cryptic species within diatom and testate amoeba morphotaxa [46–48]. Moreover, several morphotaxa in this study as well as in

other comparable works could not be assigned a precise name and might therefore represent new species [1,20,23]. Molecular studies may allow for a better resolution for both species identification and diversity estimates. Generally, soil protists remain poorly known and many species are still undescribed [13,49,50].

4.2. Limitations of the study and perspectives

The incubation method we used corresponds in fact to a culture method. Such an approach is commonly used in comparable studies [23,51,52]. Nevertheless, culture methods have the disadvantage that they may favor the development of taxa that are adapted to the artificial experimental conditions rather than the natural field conditions [53]. Recent studies have shown that the use of modern molecular approaches such as high-throughput molecular techniques can be extremely powerful and effective methods to assess protist communities from fresh environmental samples. Bérard et al. [54] demonstrated that microalgae rDNA can be amplified in some extent by PCR from soil samples. However molecular ecological studies are still too often restricted to bacterial, fungi or to aquatic eukaryotes communities. Clearly, further work is urgently needed to develop such methods and open up new biomonitoring possibilities in soils [55–57].

With respect to methodology, another aspect that would deserve more attention is the seasonal and spatial variability in soil protist density, diversity and community structure. In this study, we did only one sampling campaign in early spring but Warner et al. [58] and Lousier [59] reported important seasonal variation in testate amoeba communities in an open bog/fen and Lund [4] indicated that soil diatom abundance can decrease considerably during summer droughts. Clearly, more data are required to better understand the relationship between protist communities and seasonal changes and to assess if the differences observed among treatments are comparable through the year. Such information is crucial to develop biotic indices based on diatoms and testate amoebae.

5. Conclusions

Despite the above-mentioned limitations, our results seem to confirm our hypothesis that both diatoms and testate amoebae respond to agricultural practices. Nevertheless, further studies are needed before any conclusions can be drawn regarding potential use of diatom and testate amoebae as indicators of agricultural soils. We anticipate that molecular-genetic approaches such as next-generation sequencing, could open up new biomonitoring possibilities.

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