Ciliated protists as indicators of soil health:Three case studies from Italy

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Abstract summary

This report aims to provide an overview of the outcomes and the challenges encountered in using ciliated protists as indicator of soil health in the framework of several projects conducted in Italy since 2009. Ciliates in spite of the key roles they play in the microbial loop, still constitute a neglected component of the biodiversity, which is rarely included in biomonitoring plans. In this scenario, the report highlights the potential of ciliates as bioindicators of soil health (in natural, industrial and agro- ecosystems) to evaluate the potential impact of soil disturbance such as those produced by different farming practices (organic vs. conventional) and contaminants. Overall, the results of our surveys conducted in the frame of three distinct projects (*BioPrint, Ciliates in Organic Vineyards & Soil mapping Lombardia*) confirmed the bioindicative potential of soil ciliate communities in discriminating different agricultural management systems, land uses and in detecting different levels of soil pollution.

Keywords: Bioindicators, Soil protists, Community structure, Agroecosystem, Farming practices, Soil health

Introduction, scope and main objectives

Soil biodiversity is a key component of terrestrial ecosystems, being involved in the delivery of several essential ecosystem services such as, among others, nutrient cycling, soil formation, pest and pollution control (Pascual *et al.,* 2015). Thus, soil biodiversity indicators can be used by governments and farmers to monitor soil health and ecosystem functioning under various land uses and farming practices (Turbé *et al.* 2010). In this context, the use of ciliated protists as bioindicators is less common, in spite of the fact that they play key roles in nutrient cycling by feeding on bacteria, fungal biomass and even invertebrates in the plant rhizosphere, thus promoting soil fertility and productivity (Foissner, 1997). Further, soil biomonitoring projects including ciliates offer not only the opportunity to assess the potential of ciliates as bioindicators of soil health but also to explore in detail their diversity, and allowing the discovery of new species (Bharti, Kumar and La Terza, 2015; Bharti, Kumar and La Terza, 2017) (Figure 1).

In the framework of several projects conducted in Italy since 2008, we have investigated the potential of ciliates as indicator of soil health analysing the structure of their communities in both natural, industrial and agro-ecosystems (vineyards, fodders, arable fields) under different level of soil disturbance (Bharti, Kumar and La Terza, 2015; Bharti, Kumar and La Terza, 2017; La Terza *et al.*, 2015). The main aim of all projects was to unveil as to what extent and how, ciliates might contribute to soil bioindication, as well as to generate new baseline knowledge for a more informed use of ciliates as bioindicators of soil health. In this regard, the main hypothesis that were tested through the projects, were the following: i) to evaluate the capacity of ciliate communities to discriminate between different land uses (forests and agroecosystems) with different levels of physical and/or chemical soil disturbance; and ii) farming management practices (organic *vs* conventional); iii) to assess relationships among ciliate communities and abiotic factors.

Methodology

Soil sampling, processing and ciliate communities' analysis

To investigate the diversity of soil ciliated protists, at each investigate site, ten soil samples (0–10 cm depth) were randomly collected with an Edelman auger, mixed to obtain a composite sample (weighing approximately 1 kg), then sealed in a sterile plastic bag and transferred to the laboratory. Ciliate communities were studied by means of qualitative (non-flooded *Petri* dish method) and quantitative methods (direct counting on slide) as described by Kumar *et al.* (2014).

Multivariate analysis was realized using the PAST 2.17c package (Øyvind Hammer, Natural History Museum, University of Oslo).

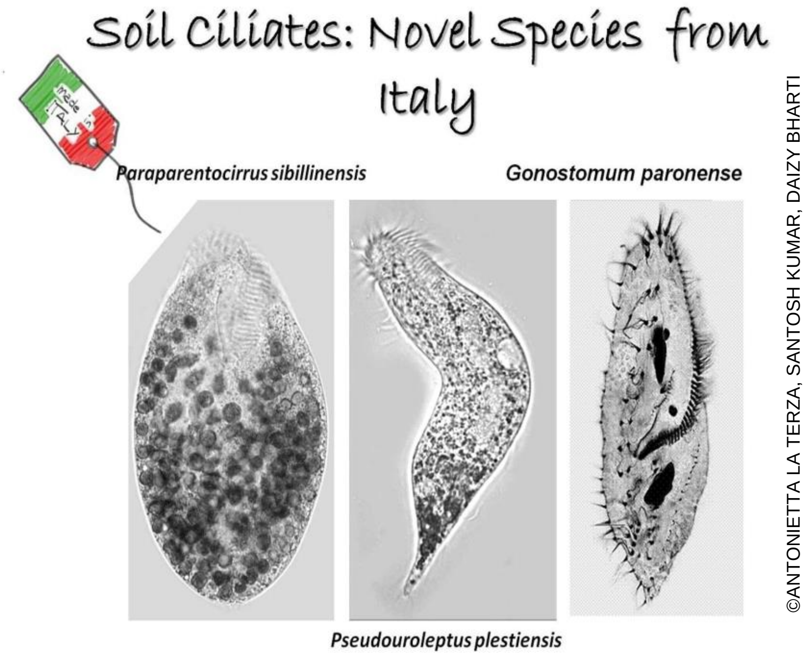

Figure 1: Novel species of soil ciliated protists from Italy

Paraparentocirrus sibillinensisn. gen., n. sp (Kumar *et al.*, 2014); Pseudouroleptus plestiensis n. sp. (Bharti, Kumar and La Terza, 2014); Gonostomum paronense n. sp. (Bharti, D., Kumar, S. & La Terza, 2015).

Results

First case study - The BioPrint project

In the 2009, in the framework of the BioPrint Project and for the first time in Italy, we investigated the biodiversity and the community structure of soil ciliates from both natural and agro-ecosystems of Marche Region. Soil samples were collected twice from 10 sites with different levels of soil disturbance (5 natural sites: FORest (virgin soils); and 5 agricultural fields: 3 ORGanic (minimum-tillage) and 2 CONventional (sod seeding). Soil chemical-physical (texture, NPK, OM, C/N ratio, soil moisture, and temperature) parameters were also measured. Qualitative analysis allowed us to identify a total of 59 ciliate species representing 29genera and 12 orders (plus 10 species new to science) (Kumar *et al.,* 2014). ORG sites were the richest in species followed by CON and FOR. The mean values for H' (2.6), d (3.4) and J (0.8) are significantly higher in ORG than in CON (H'=2.1; d=2.7; J=0.7) and FOR (H'=1.7; d=1.95; J=0.6). These results support the "Intermediate Disturbance Hypothesis" (IDH), that slightly disturbed habitats (e.g. minimum-tillage) usually have higher organism diversities than stable ones (Foissner, 1997).

Multivariate analysis show statistically significant differences between natural sites (FORest) and agricultural sites as well as between the ORGanic and CONventional management farming systems. Canonical Correlation Analysis (CCA) analysis show correlations between the distribution of species with environmental parameters; indicating the importance of these parameters in shaping the ciliate communities at different sites (Figure 2).

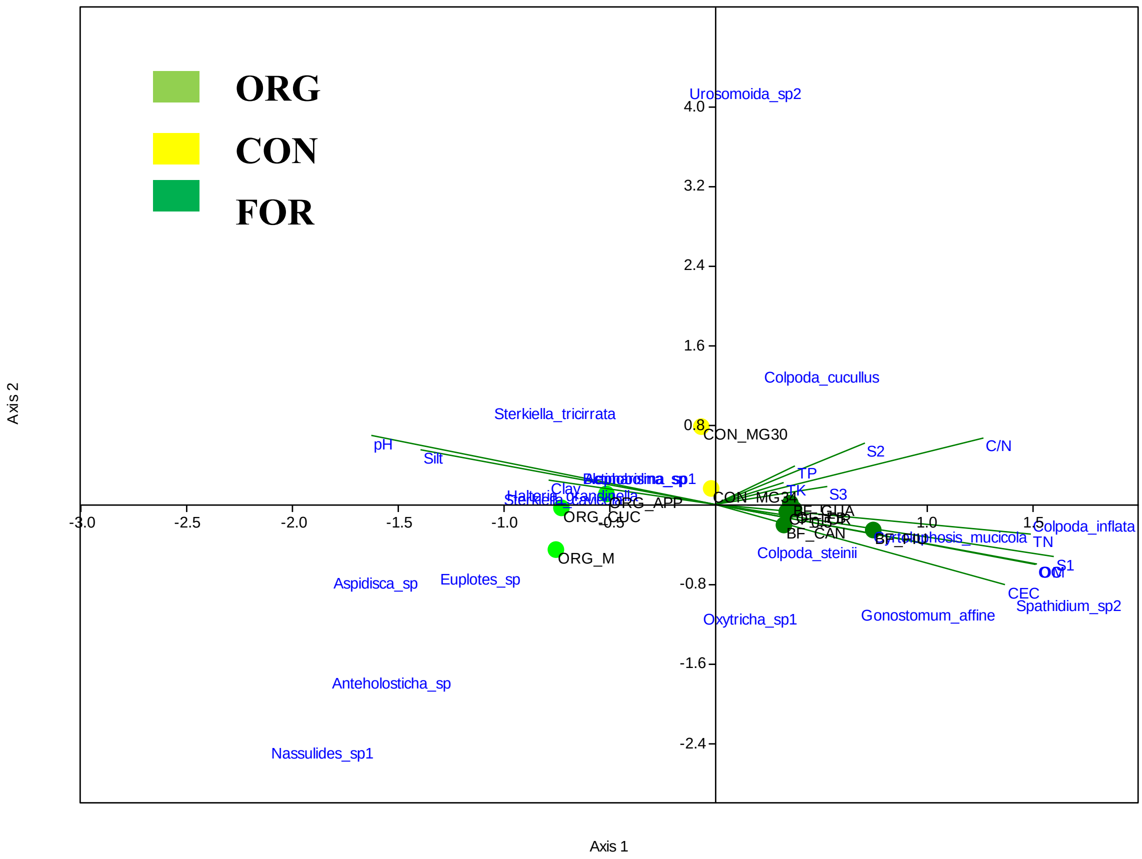

Figure 2: Canonical Correspondence Analysis (CCA)

Relationships between individual ciliate species in the community and abiotic factors at all sites CCA of log-transformed abiotic and species-abundance data.

Second case study -Ciliates in the Organic Vineyards Project

Organic farming employs a set of farming practices that contribute towards preserving soil quality. In this scenario, the aim of this study was to assess the long term effect of organic floor management on soil health by mean of ciliate communities analysis in 3 vineyards which were organically managed (at the time of sampling) for 19 years (V92), 13 years (V98) and 2 years (V09) respectively. The study was realized in a commercial vineyard in the *terroir* of "Verdicchio di Matelica" (Italy), in 2011 with a total of three samplings for each vineyard. Qualitative ciliate analysis allowed us to identify a total of 27 ciliate species belonging to 6 classes, 11 orders, 19 genera. The results of diversity indices (*H'*, J, d) and the multivariate data analysis (nMDS), indicated that the most stable habitat for ciliate communities was the "oldest" (V92) followed by the V98 and the "youngest" V09 vineyards (Figure 3). This effect may be due to the greater soil resilience, possible achieved due to the long-term organic management, of the vineyards V92 and V98 (La Terza *et al.*, 2014).

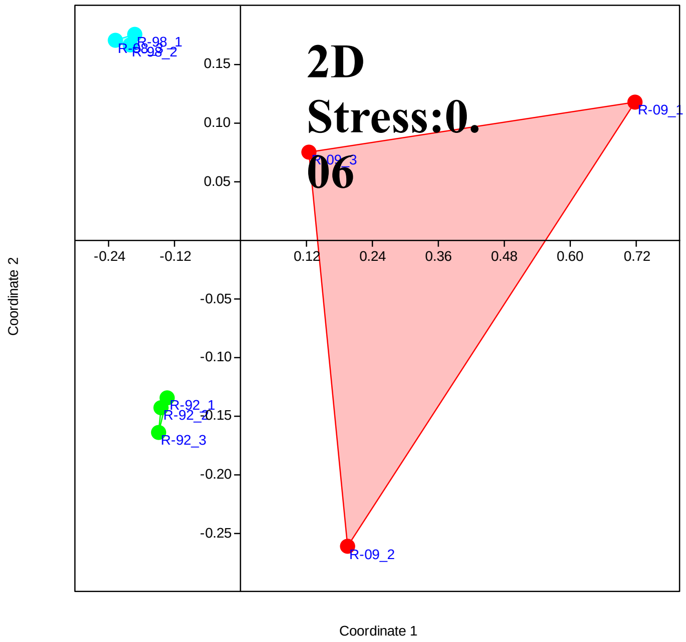

Figure 3: *Non-metric Multidimensional scaling (nMDS)* for spatial taxonomic patterns of soil ciliates for log transformed species-abundance data on Euclidean Distances, for the 3 vineyard, V92, V98 & V09

The *nMDS* analysis evidences a pronounced fluctuation of ciliate community structure in the "younger" V09 vineyard in the 3 sampling periods and, the absence of variations in community structures in the V92 and V98 vineyards.

Third case study- SoilMapping LombardiaProject

In the framework of the *Soil Mapping Project*, the ciliate communities were investigated in four industrial sites (Incinerator of Parona;Site of National Interest, SIN Brescia; Plant of regeneration of exhausted oils, Viscolube; Cement factory of Broni) of the region Lombardia with the main aims: i) to evaluate their potential in discriminating different levels of soil contamination; and ii) to assess relationships among ciliate communities and abiotic factors. Qualitative ciliate analysis allowed us to identify a total of 73 species belonging to 7 classes, 12 orders, and 30 genera, including 12 species new to the science (Bharti, Kumar and La Terza 2015; Bharti, Kumar and La Terza, 2016). The results of diversity indices (*H'*,J,d) and multivariate data analysis, show that the communities associated at the four sites are significantly different; except at the sites of Parona and Viscolube, which were similar for species composition and abundances. Furthermore, multivariate analysis showed correlation between the distribution of species with environmental parameters (and contaminants), indicating the importance of these factors in shaping the ciliate communities at the four investigated sites (La Terza *et al.*, 2015).

Discussion

Each one of the above-described case studies shed light on how ciliate communities can contribute as bioindicators of soil health (Figure 1). In this regard, the *BioPrint project* show the bioindicative potential of ciliate communities in discriminating between natural (FORest) and agro-ecosystems, and different management systems (ORGanic vs CONventional) (Figure 2). In the project: *Ciliates in Organic Vineyards,* the ciliate communities act as a measurable proxy of soil resilience in agroecosystems (and thus, as indicators of sustainable land management) (Figure 3). In final, the *Soil Mapping* project highlight the capacity of ciliate communities in discriminating between different levels of soil contamination in polluted sites.

Conclusions

In summary, these outcomes generated new knowledge and provided baselines for a more informed use of ciliates as bioindicators of soil health. However, further surveys should be conducted in order to better refine and possible standardize sampling protocols, to develop less time consuming methods for soil ciliates analysis and possibly associate the classical methods to the molecular one. Furthermore, these surveys have contributed to "reboot" soil ciliate diversity studies in Italy by updating Italian checklist, identifying more than 25 novel species, and dwelling deeper into the "soil ciliate diversity black box" in natural, contaminated and agro-ecosystems.

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