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The influence of environmental factors on protistan microorganisms in grassland soils along a land-use gradient

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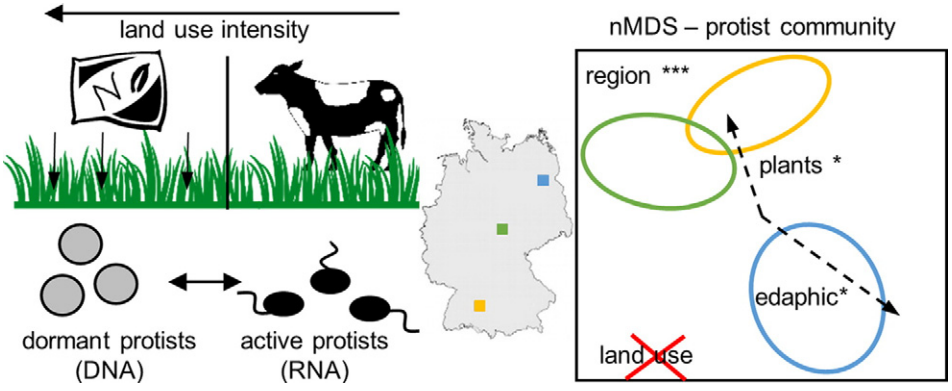
HIGHLIGHTS

- Protist communities along a land-use gradient were analysed by qPCR and T-RFLP.
- We saw different responses in the rRNA (active protists) and rDNA (all protists) pool.
- Land use did not affect the soil protist communities.
- Impact of other parameters differed with respect to distinct target groups analysed.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we investigated the effect of land use intensity, soil parameters and vegetation on protistan communities in grassland soils. We performed qualitative (T-RFLP) and quantitative (qPCR) analyses using primers specifically targeting the 18S rRNA gene for all Eukarya and for two common flagellate groups, i.e. the Chrysophyceae and the Kinetoplastea. Both approaches were applied to extracted soil DNA and RNA, in order to distinguish between the potentially active protists (i.e. RNA pool) and the total protistan communities, including potentially inactive and encysted cells (i.e. DNA pool). Several environmental determinants such as site, soil parameters and vegetation had an impact on the T-RFLP community profiles and the abundance of the quantified 18S rRNA genes. Correlating factors often differed between quantitative (qPCR) and qualitative (T-RFLP) approaches. For instance the Chrysophyceae/Eukarya 18S rDNA ratio as determined by qPCR correlated with the C/N ratio, whereas the community composition based on T-RLFP analysis was not affected indicating that both methods taken together provide a more complete picture of the parameters driving protist diversity. Moreover, distinct T-RFs were obtained, which could serve as potential indicators for either active organisms or environmental conditions like water content. While site was the main determinant across all investigated exploratories, land use seemed to be of minor importance for structuring protist communities. The impact of

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Chrysophyceae
Kinetoplastea

other parameters differed between the target groups, e.g. Kinetoplastea reacted on changes to water content on all sites, whereas Chrysophyceae were only affected in the Schorfheide. Finally, in most cases different responses were observed on RNA- and DNA-level, respectively. Vegetation for instance influenced the two flagellate groups only at the DNA-level across all sites. Future studies should thus include different protistan groups and also distinguish between active and inactive cells, in order to reveal causal shifts in community composition and abundance in agriculturally used systems.

1. Introduction

Protists are the most abundant and diverse group within the domain Eukarya. Bacterivorous protists are a key functional group within the soil communities and hold a crucial ecological position between above and below ground (Wardle, 2004) as they represent an essential driver of the microbial loop by shaping bacterial communities, enhancing nutrient recycling and stimulating microbial turnover and plant production (Bonkowski, 2004; Chatzinotas et al., 2013; Clarholm, 1985; Saleem et al., 2012). In soil studies, protists have been usually addressed as a polyphyletic assemblage with a rather artificial distinction into ciliates, amoebae and flagellates. This view has naturally left the ecology, dynamics and interactions of distinct subgroups disregarded. Although the feeding strategies and thus the top-down control of their prokaryotic prey differ significantly among protistan taxa (Boenigk and Arndt, 2000, 2002; Saleem et al., 2012, 2013; Stoeck and Stock, 2009), only a small subset of molecular studies has focused on distinct protistan taxa. Most PCR-based environmental surveys conducted have applied general eukaryotic primer sets to cover a broad diversity of eukaryotic microorganisms in soil (Bates et al., 2013; Fell et al., 2006; Lawley et al., 2004; Moon-van der Staay et al., 2006). Few studies have so far developed and applied group-specific detection tools (Bass and Cavalier-Smith, 2004; Bent et al., 2009; Glaser et al., 2014; Jousset et al., 2010; Koch and Ekelund, 2005; Lara et al., 2007; Lentendu et al., 2014) and proved their higher suitability to describe the diversity of these groups compared to studies which used universal primers. Besides the preferential amplification of certain species and the exclusion of others (Jeon et al., 2008) a major problem in environmental surveys using 18S rRNA gene primers is the lack of "protist-specific" primers (Geisen et al., 2015a). This is especially important in soil, as fungi and dead cells of metazoa are omnipresent and cannot be completely excluded by filtering. Consequently, data interpretation has always to deal with the fact that also other Eukarya than protists are detected. For this reason we additionally applied primers specific for two ubiquitous flagellate groups.

Heterotrophic flagellates represent an important subgroup of bacterivorous protists and are crucial in transferring nutrients from bacterial biomass to higher trophic levels (Adl et al., 2006; Bonkowski, 2004; Ekelund and Rønn, 1994). In particular flagellates smaller than 20 μm show very high metabolic activity (Fenchel, 1987) and contribute significantly to overall respiration (Foissner, 1992). Heterotrophic nanoflagellates belonging to the Kinetoplastea and the Chrysophyceae occur in a wide range of different environments (Boenigk et al., 2005, 2006; Lara et al., 2007; Lentendu et al., 2014; Patterson and Lee, 2000; Pfandl et al., 2009; von der Heyden, 2005) and are also among the most common and abundant groups in soil (Domonell et al., 2013; Ekelund et al., 2001). Although they possibly hold a critical role in the nutrient network, information on their diversity and ecology in soil is rare. Recently, PCR-primers targeting specifically the Chrysophyceae and the Kinetoplastea have been developed (Glaser et al., 2014) opening the possibility to specifically target these organisms groups in environmental surveys.

Protists inhabiting dynamic ecosystems like soil require strategies such as cyst formation to survive extreme or unfavourable conditions (Ekelund et al., 2002; Findenig et al., 2010; Gutiérrez et al., 2001; Verni and Rosati, 2011). However, detection and analysis of encysted populations is still a major obstacle in environmental surveys. A possible way to distinguish encysted, i.e. largely inactive protist cells from active protist cells is to separately analyse the ribosomal RNA and the ribosomal DNA pools (Jones and Lennon, 2010; Stoeck et al., 2007). Although this comparison suffers from some limitations, like extracellular DNA which still can be amplified (Frostegård et al., 1999) or cysts that contain a remarkable high amount of rRNA (Glaser, 2008; Sukenik et al., 2012), it is still a powerful tool to provide a more complete picture of the protist community.

Most of the molecular biological environmental surveys on protists carried out to date focus on the ribosomal DNA level only. As a consequence, there is a lack of knowledge about how inactive and active protist populations, respectively, respond to changing environmental conditions and how this affects their role in maintaining soil quality and ecosystem performance in agriculturally managed systems. Although protists have been proposed as bioindicators for land use impact due to their sensitive response to chemical treatments (Foissner, 1987), the influence of land use type and management type on soil protists in contrast to soil prokaryotes has only rarely been addressed. Significant negative correlations between disturbance and both testate amoebae and flagellate abundances were reported in a Canadian study (Mills and Adl, 2006), whereas recently no clear effect of the land use gradient on cultivable protist communities was observed for grassland and forest soils (Domonell et al., 2013). These approaches are often limited to the cultivable part of the total protistan community and suffer from difficulties in morphological identification (Boenigk, 2008; Boenigk et al., 2005), which explains the lower resolution of culture-dependent methods in comparison to culture-independent approaches. It is thus adequate to assume that only a minor part of the entire protist community or of distinct taxa is revealed by these approaches.

In this study we applied both quantitative and qualitative cultivation-independent methods to investigate the influence of land use types on protist communities in grasslands. The terminal restriction fragment length polymorphism (T-RFLP) method has been repeatedly shown to reliably detect and compare shifts in communities similar to sequencing approaches (van Dorst et al., 2014) and is thus suitable for fast and cost-effective analyses of many samples, while quantitative PCR allows quantifying phylogenetic marker genes for estimating shifts in the sequence abundances of distinct taxa. Absolute quantification, nevertheless, remains a difficult issue in environmental studies (Kim et al., 2013; Smith and Osborn, 2009). Copy numbers can only be considered as a rough estimation of the cell numbers as the amount of 18S rRNA-genes differ between species and also within cell cycles (Gong et al., 2013; Weber and Pawlowski, 2013); group-specific primers could potentially reduce the bias by analysing species with similar 18S rRNA-gene copy numbers (Medinger et al., 2010). Even so comparison between qPCR and microscopic techniques (e.g. FISH) proved a high robustness of qPCR (Baptista et al., 2014; Drenovsky et al., 2008) and most importantly, several studies have shown the usefulness of qPCR data to compare 18S rRNA pools along environmental gradients and in response to environmental changes (Liu and Gong, 2012; Marie et al., 2006; Zhu et al., 2005).

We hypothesized that targeting (i) different taxonomic levels with universal and group-specific protistan primers, respectively, and (ii) both the DNA and the RNA-level would be more appropriate to reveal significant correlations between environmental factors and compositions of protist communities, their richness and abundance. In particular, we expected that distinguishing between the potentially active members (as described by the rRNA pool) and the whole protistan community including also inactive resting-forms (as described by the rDNA-pool)