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TESTATE AMOEBAE REFLECT PRESENT ENVIRONMENTAL CONDITIONS IN RESTORED CUT-OVER BOGS

A NEW TOOL FOR EVALUATION AND MONITORING?

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SUMMARY

After thirty years of bog restoration in North-Western Germany practical methods for the evaluation of restoration success are still lacking. Here, we tested if testate amoebae (TA) can provide information on the present state of restored bog ecosystems and thus used as monitoring tools. Therefore, TA communities extracted from *Sphagnum* mosses (n=46) collected in restored sites throughout Lower Saxony were identified and related to measured water table fluctuations and biogeochemical characteristics such as acidity and water nutrient concentrations of pore water. Results of indirect ordination show that TA communities strongly reflect present hydrological conditions and to some extend pH and nutrient status. This leads us to conclude that TA has significant potential to be increasingly used in the future as a helpful tool for evaluation of restoration projects.

KEYWORDS: Bog Restoration, North-Western Germany, water level, rewetting, indicators.

INTRODUCTION

In Germany, bogs have significantly decreased in both extent and quality; more than 95 % of the original peatland area is currently degraded and changed due to their extensive use for centuries for agricultural or peat extraction areas (Couwenberg and Joosten, 2001). This loss is particularly drastic in North-Western Germany (Lower Saxony) where huge bogs were originally very common. However, since the establishment of the peatland protection programme in 1980, Lower Saxony achieved first successes in the protection of bogs by initiating restoration measures on 11,000 ha until 2005 (NLWKN, 2006). Nevertheless, in many cases information about restoration success and its constraints are not available (Sieg *et al.*, 2010). This deficit may be at least partly due to a lack of monitoring methods that can be easily used to evaluate at a larger scale to what extent the most important restoration targets such as re-establishment of favourable hydrological conditions and appropriate water quality (Gorham and Rochefort, 2003) are achieved. A promising alternative approach is testate amoebae which primarily have been used as proxies in palaeoecology (de Jong *et al.*, 2010, Lamentowicz and Mitchell 2005) but recent studies also showed their potential as bioindicators for contemporary environmental conditions in peatlands (Mitchell *et al.*, 2008).

Here, we tested if testate amoebae have the potential to be used as a feasible tool for the evaluation of large-scale bog restoration in Lower Saxony. The aims of this study are, (a) to identify which taxa are widespread in restored sites to provide a baseline for further surveys, (b) to investigate how the distribution of TA is related to water regime and water chemistry and (c) to evaluate whether TA have potential to serve as indicators for restoration success.

METHODS

In 16 bogs throughout Lower Saxony living *Sphagnum* moss samples were collected between April and September 2011 on 46 rewetted sites. Sites differed in dominating vegetation, mainly covered either by *Eriophorum vaginatum* (n =17), *E. angustifolium* (n=7), *Juncus effusus* (n=20) or *Molinia caerulea* (n=2) and restoration age (period of time since re-wetting: 2–32 years) and former agricultural land-use before the peat extraction (yes/no). In PVC tubes water table (relative to terrain surface, with standing water as positive values) as well as pH and conductivity (WTW pH 323) of pore water were measured every fifth week for six months. In April, June/July and September pore water was sampled also and stored as cool as possible until analysis in the laboratory was conducted. NH_4^+, NO_3^- were measured photometrically (FIAS Aquatec Autoanalyzer) and PO_4^{3-} with absorption at 578 nm (PERKIN ELMER Lambda). Concentrations of K^+, Na^+, Ca^{2+} and Mg^{2+} were determined by AAS. Isolation of tests from the mosses followed Charman *et al.* (2000) by washing through a 200 µm sieve. One tablet of *Lycopodium* spores was added to the residues to permit the calculation of absolute test concentration. Most likely because of squeezing all water out samples contained very low concentrations (e.g. 500 hundred *Lycopodium* spores to reach 30 shells). This made it impossible to reach the aimed minimum count of 50 tests in a reasonable time frame (Payne and Mitchell, 2009). Therefore all samples (uppermost 5-7cm) were recollected in January 2012 and similar treatments conducted. At time of re-sampling testate amoebae communities the water table depth was the only parameter measured again. Finally, species were identified and counted until a number of 60 living shells were reached per sample using a light microscope under 200x and 400x magnification. Living and encysted amoebae were distinguished from the empty shells. For the identification of taxa the keys of Charman *et al.* (2000), Clarke (2003) and Mazei and Tsyganov (2006) were used. Detrended correspondence analysis (DCA) was performed with total concentrations of living tests per gram of dried *Sphagnum* to explore distribution patterns in TA communities and underlying environmental gradients. All taxa with absolute occurrence less than 3 were excluded prior to analysis. No data transformation was applied. To characterize the water regime, we calculated mean water table depths for the year ([april–september/5 + January]/2), dry season (end of may–august/3), wet season ([september+january+april]/3) and the maximum water amplitude. Water table data and all other environmental parameters were correlated with the ordination axes. For metric and normally distributed data we used the Pearson, otherwise Spearman correlation. Additionally we calculated bootstrap optima and tolerances (using percentage data) in relation to the water table using weighted averaging model using C2 application. Ordination analyses were performed with PC-ORD 5.0, all other calculation with SPSS 19.

RESULTS

Hydrology and water chemistry

In rewetted sites, during summer mean depth to water table (DWT) was 7.2 cm and ranged between +34 and -38 cm. During the so called "wetter" season DWT was at 21.2 cm with a range between +41 and -15 cm. In general, amplitude of water level increases with lower depth to water table. Fluctuations ranged between 17 cm as the lowest and 55 cm as the highest value. From the water chemistry data only those investigated parameters are presented here (Table 1), where a series of measurements were significant correlated (P< 0.05) with one Axis of DCA analysis. Except the non-correlated single-time measurement of PO_4^{3-} and the mean values for NO_3^- and NH_4^+ are shown nonetheless.

Table 1. Median and range of measured water chemistry parameters for restored sites (n=46) in bogs of North-Western Germany. (Conductivity in µS; Nutrients in mgl^{-1}).

	mpH	mCond.	Ca^{2+}	Mg^{2+}	Na^+	K^+	PO_4^{3-}	mNO_3^-	mNH_4^+
Time of sampling	April-Septmber	May-August	June/July	June/July	September	September	June/July	April-September	April-September
Median	4.22	96.67	1.25	1.05	6.24	1.28	0.07	0.12	0.40
Range	3.6 – 4.8	65 – 230	0.3 – 8.7	0.3 – 6.7	2.5 – 16.6	0.1 – 4.9	0.0 – 1.3	0.0 – 1.6	0.0 – 8.5

Testate Amoebae

In total, we found 31 testate amoebae taxa, whereas 6 species occurred less than 3 times. The highest species richness was observed with 11. The poorest sample simply contained 2 species. Absolute concentrations of living tests ranged from 4.257 to 76.078 individuals per dried gram of *Sphagnum*. The percentage of dead testate amoebae until 60 alive or encysted tests were counted ranged from 3-47%.

The DWT optima during the dry season (Fig. 1) ranged from -35.1 (±8.3) *Heleopera sylvatica* to 22.2 (±12.0) *Assulina seminulum* and during the wet season from -9.4 (±8.7) *Heleopera sylvatica* to 32.0 (±16.0) *A. discoides pseudovulgaris*. Species indicative of wet sites, where the water-table remains above or at least at the surface through the whole year (Fig. 1) are: *A. discoides pseudovulgaris*, *Difflugia leidyi*, *D. rubescens*, *A. vulgaris* and *A. gibbosa* type. In addition, the latter two were mostly found in combination with higher pH values as it was characteristic for *Juncus effusus* dominated sites (Fig. 2). *A. discoides*, *Cyclopyxis arcelloides*, *Arcella catinus* and *Archerella flavum* seemed to tolerate if sites temporarily dry out stronger during summer (Fig. 1). *Nebela tincta, Phryganella acropodia* and *Assulina muscorum* were common taxa in drier habitats as their optima clearly lies below surface (Fig. 1). *Heleopera sylvatica* and *Trinema complanatum* were exclusively found in non-flooded sites. Most of the other found species have intermediate distributions. *Trinema lineare* in particular occurred almost along the whole gradient from very dry to very wet sites (Fig. 1). Also worth mentioning is the high morphological variability in the group *Euglypha strigosa* type observed on sites with strong fluctuating water levels.

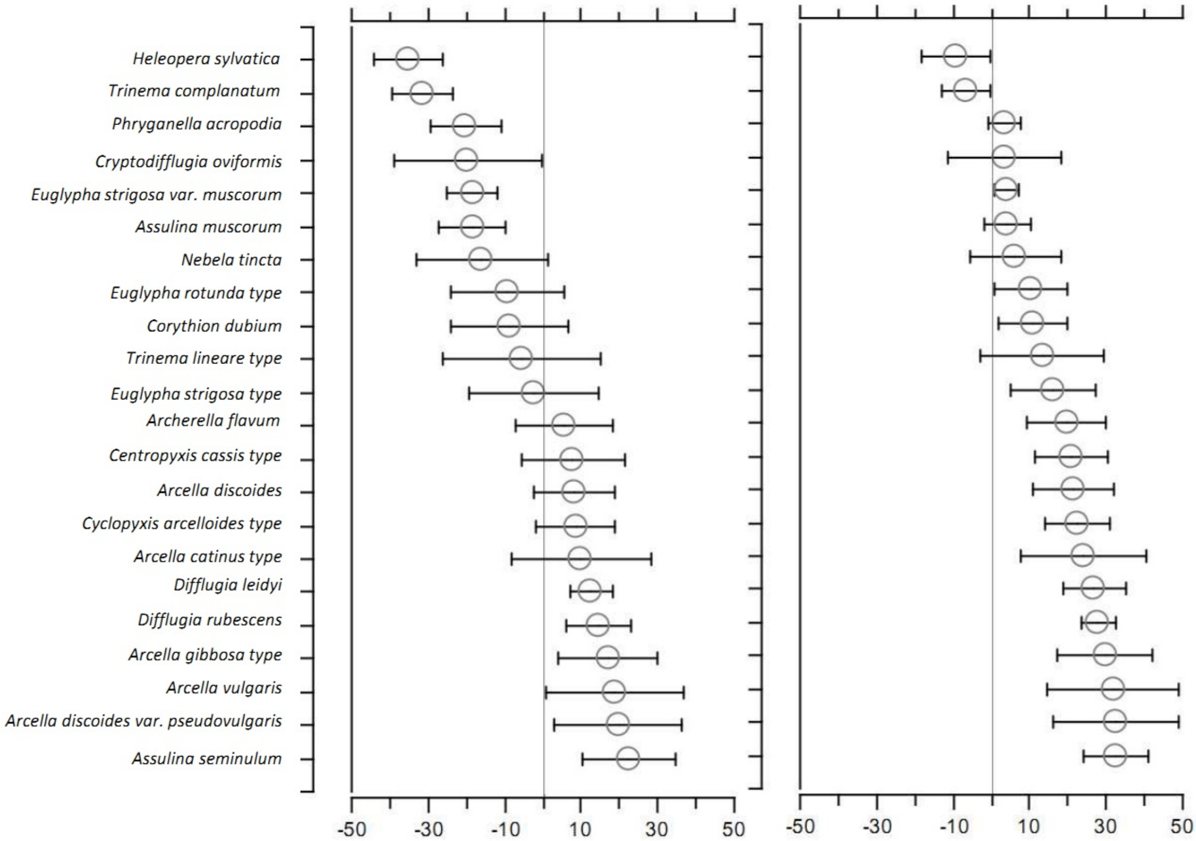

Fig. 1. Bootstrap optima of testate amoebae inhabiting restored sites in North-Western Germany for mean depth of water table [cm] in dry/summer (left) and wet/winter seasonal conditions (right). Line indicates terrain surface.

Testate amoebae, hydrology and water chemistry: multivariate analyses

In accordance to all previous studies (Booth, 2002; de Jong, 2010) DCA ordination clearly showed a strong correlation with the water level gradient. Further correlations with environmental data revealed strong relationships between testate amoebae communities and pH as well as restoration age in opposing directions (Fig.2). Correlations with conductivity, Potassium, Sodium, Calcium and Magnesium indicated water chemistry as a secondary environmental control on species composition. The percentage of dead shells seemed to be interrelated with the age of the sites.

Additionally, DCA ordination implied that samples on the wet side of the hydrological gradient were more variable in species compositions than samples on the dry side of the gradient. This suggests that the composition of testate amoeba in hollows and pools are more strongly affected by pH along the second gradient. Such differences may also be reflected by vegetation, either characterized by dominance of *Juncus effusus* or *Eriophorum* species.

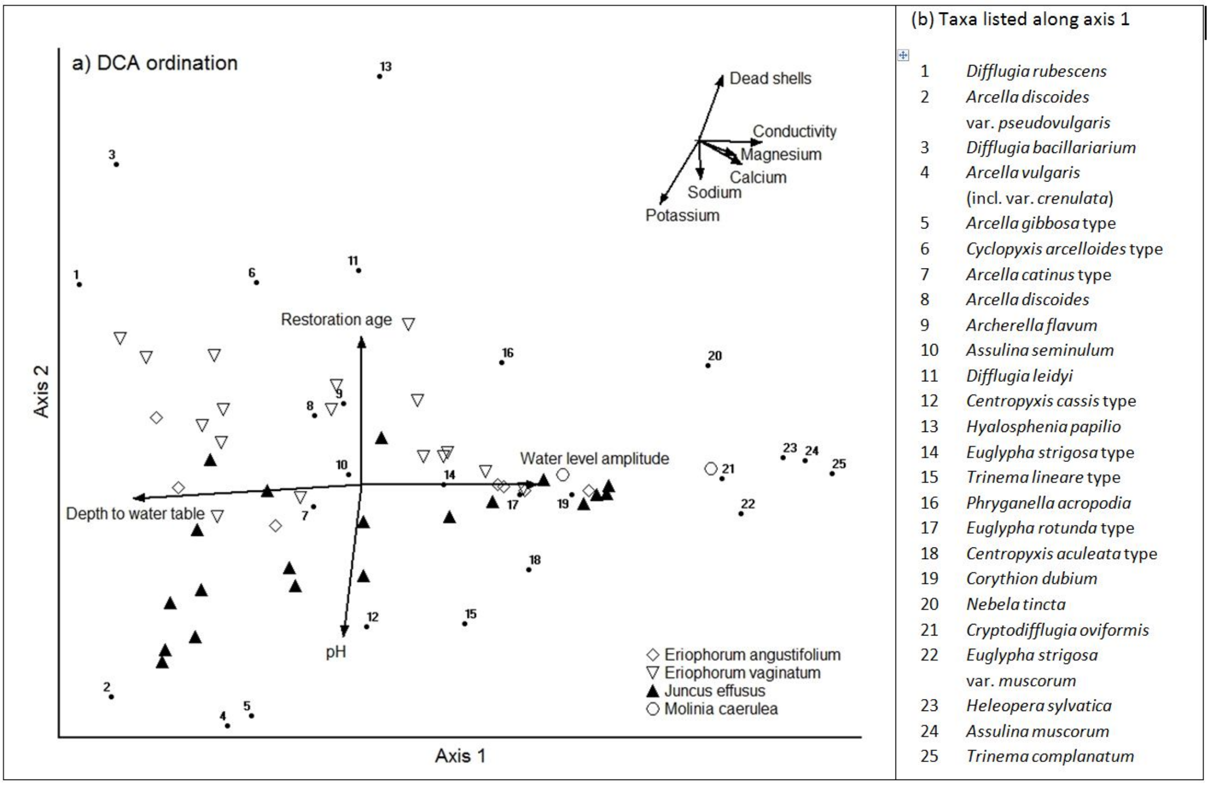

Fig. 2. DCA biplot of testate amoebae communities inhabiting restored bogs in North-Western Germany. Symbols represent dominating vegetation on sampling sites. Axis 1 and 2 represent 30% of the total variance (eigenvalues 0.736 and 0.267, respectively, total inertia = 3.388). Taxa are numbered according to their position along axis 1. Direction and strength of environmental correlations (r) are indicated by the direction and length of arrows. (b) Significant correlations lower than 0.5 but greater than 0.3 are shown separately. (b) List of taxa with numbers corresponding to those in (a), arranged along the axis 1 gradient from wet to dry (depth to water table).

CONCLUSIONS

For the first time, we tested the applicability of testate amoebae as bioindicators to evaluate restoration success in cut-over bogs of North-Western Germany. Although leaving considerable room for further investigations, the obtained results disclose the significant potential of TA by integrating environmental conditions to reveal positive as well as negative developments and their underlying causes.

In particular, the issue of strong water-table fluctuations on restored sites between the dry and wet season which is well reflected by species composition. Besides this well-known hydrological indication capacity (Mitchell *et al.*, 2008) TA also revealed clear species composition patterns in terms of pH which was negatively related to restoration age. In combination with other methods (e.g. vegetation) TA can contribute valuable information to describe the present state of restored sites and thus might have the potential to replace labour- and cost-intensive hydrological and biogeochemical measurements for large-scale monitoring of bog restoration.

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