




Functional traits as a new approach for interpreting testate amoeba palaeo-records in peatlands and assessing the causes and consequences of past changes in species composition

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Abstract

Subfossil remains of various groups of organisms preserved in peat and sediment archives are commonly used to infer past environmental changes using transfer functions based on species composition. However, the changes in community structure can also be explored using the functional trait approach. Investigation of functional traits in palaeoecological records can inform about the mechanisms through which abiotic variables such as temperature or moisture impact communities. Moreover, changes in functional traits provide information about changes in ecosystem functioning and can potentially lead to the reconstruction of past processes at the ecosystem scale. Here, we use five key functional traits of arcellinid testate amoebae (TAs), a group of protozoa that are key actors in the microbial foodwebs in peatlands. We apply this approach to the subfossil TA Holocene record of four geographically independent peatlands from Alaska, Switzerland, Poland and Russia. We found that species with larger shells were frequently eliminated from the communities most likely as a result of a switch towards drier conditions. However, when conditions were wetter, species with large shells and species with small shells could coexist because they differed in their trophic niche (i.e. preys). Our results show direct but site-specific links between TA trait data and the depth to water table and pH data inferred from TA species composition. This suggests that past environmental changes influenced both species composition and community function in these ecosystems. Overall, this study demonstrates that species- and trait-based approaches yield complementary information on past environmental changes. For instance, while taxonomic approaches reveal the changes in community composition over time, investigation of traits informs both on the causes and the consequences of these changes on ecosystem functioning.

Keywords

community-weighted mean of traits (CWM), long-term changes, microbial ecology, palaeoecology, protist, trait-based approach, transfer function

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Introduction

Species–environment relations (transfer functions) are commonly used in palaeoecological studies to infer past conditions from subfossil records (Birks, 1995). Transfer functions based on the hutchinsonian niche concept are generally built on correlative approaches in which modern (surface) samples are collected and a set of environmental variables measured. The variable showing the strongest correlation with the biotic community data of interest is selected to build a transfer function. The selected variable may thus not directly influence the biotic community, but one of the key assumptions of the approach is that it may be linearly related to an important determinant of the ecological system of interest (Belyea, 2007; Birks, 1995). This approach has been criticized as it largely ignores theoretical development in community ecology such as the existence of non-linear responses of communities to environmental gradients as well as the importance of biogeography and spatial autocorrelation in shaping species distribution (Belyea, 2007).

Further limitations of the transfer functions approach are that it neither considers the functional role of species in the ecosystems

nor the mechanisms driving community composition. One way to address this limitation is to use functional traits (FTs). FTs were shown to be strongly related to ecosystem processes (Cornwell

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et al., 2008) and assembly mechanisms (Mason et al., 2012; Spasojevic and Suding, 2012). However, to our knowledge, most FT-based studies have focused on living organisms and the FT framework is only starting to be considered in palaeoecology to understand past changes in ecosystem functioning (Lamentowicz et al., 2015). The FT approach can improve species-based reconstruction by providing information on the impact of environmental changes on communities in a more mechanistic way. FTs can therefore help overcome one of the main limitations of the transfer function approach by providing information about the past functioning of ecosystems.

A trait-based approach can be used for palaeo-reconstruction only if the relationships between the chosen traits and ecosystem function have been assessed and the traits can be measurable also on the subfossil remains of the organisms. Subfossil records can give access to much longer time series than ecological studies (Blois et al., 2013). However, investigations of ecological processes in subfossil records suffer from strong limitations because of poor preservation or incomplete records (Wagner and Lyons, 2010), and FTs are rarely measurable on the subfossil remains of the most commonly studied taxa in community ecology (e.g. vascular plants and macro-invertebrates). An alternative is to use the subfossils of shell-building microorganisms that are very abundant and diverse – thus providing the required statistical power – and whose short generation times make it possible to reach high temporal resolution (Lamentowicz et al., 2010; Payne, 2013).

FTs are defined as traits that impact fitness (Violle et al., 2007). Two categories of FTs can be defined: (1) response traits (RTs) that reflect species response to environmental change and (2) effect traits (ETs) that reveal species impact on ecosystem processes. Changes in RTs at the community scale are inferred as being driven by changes in environmental conditions, while changes in ETs may indicate shifts in process at the ecosystem scale. Some traits can possibly also fall in both categories. As a general example, tree growth at the taiga–tundra treeline responds to climatic factors such as growing season length (MacDonald et al., 2008) and drought stress (Ohse et al., 2012), but once established, trees feed back to climate change through change in snow cover and albedo (Euskirchen et al., 2010). Investigating RTs and ETs using subfossil records therefore provides information about past environmental conditions and ecosystem functioning, respectively. Moreover, the correlation between RTs and ETs was shown to determine how environmental change will influence ecosystem functioning (Hillebrand and Matthiessen, 2009). Investigating past changes in ecosystems using FTs therefore requires (1) defining RTs and ETs, (2) reconstructing the temporal changes of these traits using palaeo-records and (3) assessing the correlation between ETs and RTs.

Here, we test this approach using testate amoebae (TAs), a group of free-living protozoa that are abundant and diverse in *Sphagnum*-dominated peatlands. These amoeboid organisms secrete a shell (or test), which is preserved over millennia in peat (Charman, 2001). Their shells have a characteristic morphology that allows identification to (morpho)species level. Numerous ecological studies have shown strong correlations between their community composition and the dominant ecological gradients in peatlands such as depth to water table (DWT) and pH (Charman, 2001; Mitchell et al., 2008a). TAs also play a key functional role in the cycling of elements in soils (Aoki et al., 2007; Wilkinson, 2008; Wilkinson and Mitchell, 2010) and as top predators in *Sphagnum* peatlands microbial food webs (Jassey et al., 2013a). In contrast to most microbial taxa, RTs and ETs that are easily measurable on the shells have been identified (Fournier et al., 2012; Jassey et al., 2013a, 2013b), and recently used in palaeoecology (Lamentowicz et al., 2015).

We used TA palaeo-records from four *Sphagnum* peatlands for which reconstructions of DWT and pH already existed. We

compared these data with the patterns of past changes of two ETs and three RTs.

The first ET was the shell aperture size (APER), which is an indicator of the trophic position of TAs in the microbial food web (Jassey et al., 2013b). TAs with low APER mainly feed on small size bacteria, fungi, algae and heterotrophic protists and thus have a low trophic position; whereas species with large APER feed preferentially on larger protists and micro-metazoan (Gilbert et al., 2000, 2003; Jassey et al., 2012; Yeates and Foissner, 1995) and thus occupy a higher trophic position in the food web. The second ET was the ratio of mixotrophic individuals over heterotrophic ones in the community (MIXO). Mixotrophic species feed on preys through phagocytosis (heterotrophy) but also use their endosymbionts (generally green algae from genus *Chlorella* (Gomaa et al., 2014)) to acquire food and energy through their photosynthetic activity. Although most TA species are strict phagotrophs, mixotrophy is an important foraging strategy. Mixotrophs can be very numerous and outcompete phagotrophs when food sources are rare by relying on the advantage given by their endosymbiotic algae (Jassey et al., 2013b). Mixotrophy thus influences the competitive hierarchy among species and can modify the functioning of the microbial food web through a decrease of the predation pressure of TAs on lower trophic levels.

The RTs were (1) shell biovolume (BIOVOL), (2) shell compression (COMPRESS) and (3) aperture position (POSITION) that account for species response to changes in hydric conditions (Laggoun-Défarge et al., 2008). The rationale for these traits is that TAs with smaller and/or more compressed shells can remain active in thinner water films. Similarly, having a ventral cryptic (cryptostomic) rather than a terminal (acrostomic) aperture further prevents desiccation and allows the amoeba to creep over surfaces in a thinner water film. BIOVOL, COMPRESS and POSITION can thus be considered as an RT *sensu* (Violle et al., 2007). For instance, BIOVOL was shown to respond to changes in hydric conditions such as induced by flooding (Fournier et al., 2012) and to experimental warming (Jassey et al., 2013a). As climatic conditions and/or hydric regime are key factors controlling the functioning of peatland ecosystems (Davidson et al., 2000; Dorrepaal et al., 2009), these three traits should constitute good indicators of long-term environmental changes in peatlands.

Ombrotrophic peatlands are highly acidic and nutrient-poor ecosystems (Rydin and Jeglum, 2013); we thus hypothesized that (1) APER will be positively correlated to pH because the availability of prey (bacteria, fungi, other micro-eukaryotes) decreases in nutrient-poor conditions generally associated to lower pH in these ecosystems (Gilbert et al., 1998). In parallel, we expected that (2) MIXO would increase with decreasing pH as autotrophy becomes more and more advantageous when preys become rare. We further hypothesized that (3) changes in BIOVOL, COMP and POSITION would parallel changes in DWT as inferred from species compositions using the classical transfer function approach. Furthermore, we expected strong temporal changes and important differences among sites in traits as a result of the different ecological processes and environmental changes experienced by the four peatlands in the time frame encompassed by the subfossil records (ca. 3–10,000 years).

Material and methods

Palaeoecological datasets

To develop and test the FT approach, we used existing palaeoecological data from four peatlands located respectively in Alaska, USA (Jigsaw), Poland (Tuchola), the Ural Mountains (Pechora region, Russian Federation) and the Swiss Jura Mountains (Praz-Rodet). TA community data and the associated reconstructions of DWT and pH were available for each of these peatlands (Kishaba, 2010; Klein et al., 2005; Lamentowicz et al., 2008; Lemonis, 2012; Mitchell et al., 2001).

Table 1. Description of five selected functional traits of testate amoebae.

	Short name	Unit	Description	Type of trait	Justification	References
Biovolume	BIOVOL	μm^3	Volume of the shell occupied by the living amoeba	Response	Responds to changes in DWT in response to warming, pollution or other disturbances	Lousier (1974), Tsyganov et al. (2012), Fournier et al. (2012), Jassey et al. (2013a), Laggoun-Défarge et al. (2008)
Aperture position	POSITION	1-4	Aperture terminal (1) to completely cryptic (4)	Response	Responds to changes in DWT in response to warming, pollution or other disturbances	Lousier (1974), Tsyganov et al. (2012), Fournier et al. (2012), Jassey et al. (2013a), Laggoun-Défarge et al. (2008)
Shell compression	COMPRESS	1-4	Shell compression form almost spherical (1) to strongly compressed (4)	Response	Responds to changes in DWT in response to warming, pollution or other disturbances	Lousier (1974), Tsyganov et al. (2012), Fournier et al. (2012), Jassey et al. (2013a), Laggoun-Défarge et al. (2008)
Mixotrophy	MIXO	0-1	Presence or not of photosynthetic endosymbionts	Effect	Impacts the functioning of the microbial food web by changing the mixo-/heterotrophy ratio of the community	Yeates and Foissner (1995), Gilbert et al. (2000, 2003), Jassey et al. (2012)
Aperture size	APER	μm	Width of the shell aperture	Effect	Impacts the functioning of the microbial food web by constraining the maximum size of food items	Jassey et al. (2013b), Goma et al. (2014)

DWT: depth to water table.

Jigsaw mire is located on the shore of Jigsaw Lake, in the lowlands of the Kenai Peninsula, Alaska (USA). The peatland is located at the end of a small cove. Jigsaw is a closed basin lake, and its water level has changed considerably in the past millennia (Reger et al., 2008). The peat record spans the last ca. 8000 years (Kishaba, 2010). Tuchola mire developed in a kettle hole in Northern Poland. This site experienced several wet–dry shifts over its ca. 10,000 year history and became wetter in the last ca. 500 years most likely as a result of deforestation causing increased surface runoff from the surrounding landscape (Lamentowicz et al., 2008). Pechora mire is located in the upper Pechora basin in the Russian taiga in an extremely remote region with no detectable signs of human influence on the landscape or vegetation composition (Lemonis, 2012). The peat record covers the last ca. 3200 years. Further details of this study will be published elsewhere. Praz-Rodet mire is located in the Swiss Jura Mountains (Mitchell et al., 2001). It is surrounded by pasture and by a slow-flowing river (Orbe) on the SE. Studies based on TAs, pollen and plant macrofossil records have shown that the mire developed following a classical succession from lake to fen to peatland over the ca. 8500 years of its developmental history. After a low period of wet oligotrophic conditions, it became drier ca. 150 years ago following forest clearance to create pasture which was believed to have exposed this small peatland to summer drought although further impact from river erosion and drainage could also have played a role (Mitchell et al., 2001).

One peat core was taken in each peatland. The cores were cut into 1 cm slices. Selected slices were radiocarbon-dated, and the missing dates were interpolated using simple linear models. We choose linear models instead of more complex but better performing models (e.g. weighted averages and partial least square) because the peat records were considered here as ecological time series and the exact age of individual samples was not critical for our analysis.

TAs were extracted from each slice by filtering (Booth et al., 2010) and identified at 200 \times and 400 \times magnification to morpho-species level, in some cases to genus or species-group (type) (Supplementary material, available online). The percentage data are based on a minimum count of 150 individuals per sample.

Selection of TA species and FTs

TAs include at least three phylogenetically unrelated groups, acellinids (Meisterfeld, 2002a; Nikolaev et al., 2005),

euglyphids (Meisterfeld, 2002b; Wylezich et al., 2002) and amphitrematids (Goma et al., 2013). We selected taxa with shells that are well preserved over millennia in peat in order to avoid possible biases because of the differential preservation of subfossil TAs in peat (Swindles and Roe, 2007). For instance, most euglyphids disappear rapidly in peat records (Mitchell et al., 2008b) and were thus removed from the dataset. However, we retained genus *Assulina*, which preserves very well, even in pollen slides owing to an organic coating covering the siliceous shell plates (Mitchell et al., 2008b; Payne et al., 2012). This data filtering procedure avoids potential over-estimation of water table depths because of differential preservation of tests during dry periods (Payne, 2007). The final dataset included 76 taxa in total, 29 in Jigsaw, 35 in Tuchola, 55 in Pechora and 30 in Praz-Rodet.

Species were classified according to their aperture position (APER) along a semi-continuous scale ranging from acrostomic (terminal position) to cryptostomic (ventral invaginated position) (Table 1) representing increasing tolerance to dry conditions (Supplementary material, available online). Similarly, we used a semi-continuous scale ranging from spherical to strongly compressed (i.e. height less than 1/4 of width) to discriminate species based on shell compression (COMPRESS). The biovolume of each taxon was approximated using different formulas depending on the general shape of the shell

$$\text{Hemisphere: } V = \text{Pi} \times r^3 \times 2/3 \quad (1)$$

$$\text{Saucer-shaped: } V = \text{Pi} / 2 \times r^2 \times h \quad (2)$$

$$\text{Cylindrical to ovoid: } V = \text{Pi} / 6 \times d^2 \times h \quad (3)$$

$$\text{Ovoid: } V = \text{Pi} / 6 \times L \times w \times h \quad (4)$$

where r is the radius, h the height, d the diameter, L the length and w the width of the shell in μm .

MIXO was measured as the proportion of mixotrophic species within a community using a ratio ranging from 0 to 1. Finally, APER was measured in μm as the smallest diameter of the shell aperture.

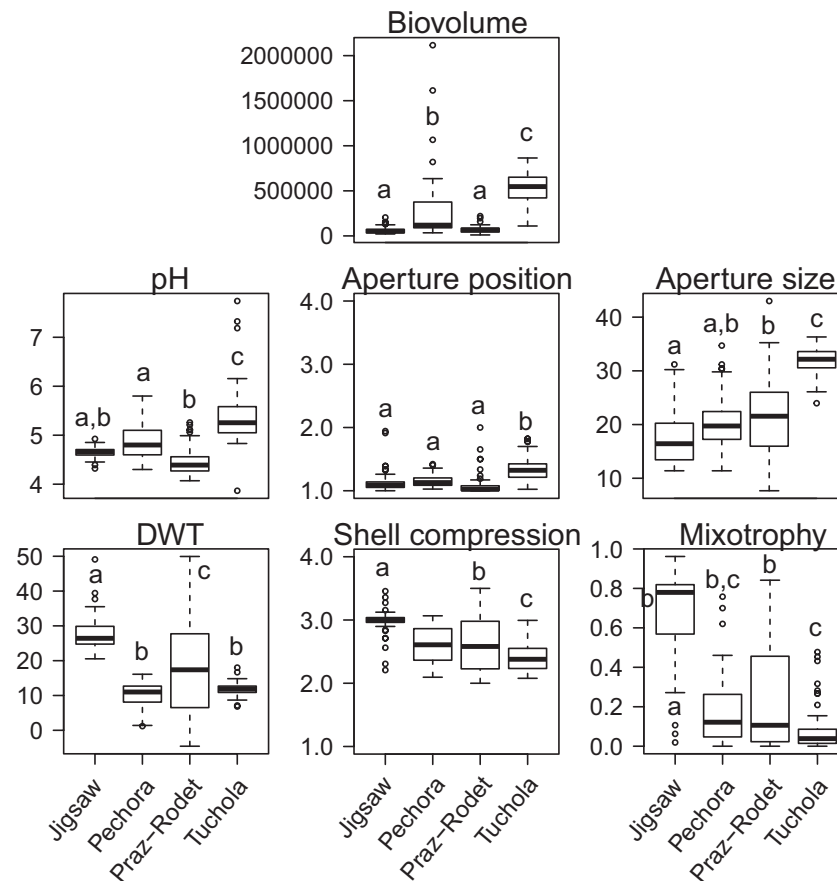


Figure 1. Boxplots showing the distribution of depth to water table (DWT, cm below soil surface) and pH as well as the community-weighted mean biovolume (BIOVOL; μm^3), aperture size (APER, μm), aperture position (POSITION), shell compression (COMPRESS) and ratio of mixotrophic species (MIXO) of testate amoebae in four peatlands. DWT and pH are inferred from species composition using transfer functions. Letters indicate significant differences among peatlands calculated using the Tukey's Honest Significant Difference method.

Numerical analyses

We investigated the changes in the community-weighted means (CWMs) of BIOVOL, COMPRESS, POSITION, APER and MIXO. CWM is the abundance-weighted mean trait values of the species within a community and was calculated as follows

$$\text{CWM} = \sum_i^S p_i \times t_i \quad (5)$$

where S = number of species within the community, p = species relative abundance, t = species trait value. CWM grants more importance to the most abundant species in agreement with the mass ratio hypothesis (Grime, 1998). We then compared the average value of traits among peatlands and fitted generalized linear model (GLM) using pH and DWT as response and the traits as explanatory variables. This allowed us to define which traits are responsible for the inferred changes in pH and DWT in each peatland.

The convergence or divergence of BIOVOL, APER and MIXO was tested by comparing the observed CWM values with a random distribution obtained using a null model. The null model consisted in shuffling abundance values among the species present in a community before computing CWM. This approach preserves community abundance and species richness but breaks the link between abundance and traits (Bernard-Verdier et al., 2012). This method was preferred over other null models because it does not require shuffling species among time steps. Convergence of FTs has been explained

either by abiotic condition filtering (Cornwell et al., 2006; Weiher et al., 1998) or by competitive filtering that equalizes fitness among species according to another view (Chesson, 2000; Grime, 2006). Conversely, divergence of FTs has been explained by the limiting similarity hypothesis (MacArthur and Levins, 1967; Wilson, 2007; Wilson and Stubbs, 2012) where superior competitors exclude other species, thus reducing niche overlap. Strong divergences are more likely in more competitive and productive habitats. All computations were done with R3.0.1 (R Development Core Team, 2011) using packages 'FD' (Laliberté and Shipley, 2010) and 'vegan' (Oksanen et al., 2012).

Results

Average species richness per sample was highest in Pechora (15 species), lowest in Tuchola (8 species) and intermediate in Jigsaw and Praz-Rodet (10 species). The average span from one sample to the next was of 140 years across all peatlands, 137 in Praz-Rodet, 155 in Jigsaw, 197 in Tuchola and 67 in Pechora with strong differences among time steps within peatland (typically increasing with depth owing to peat compaction and partial decomposition). In order to assess whether these differences caused a sampling bias when estimating biodiversity, we calculated the correlations between species richness and the duration in years of a time step. As no significant correlation was observed, we did not correct the data.

Average inferred environmental variables (DWT and pH) and FTs (BIOVOL, COMPRESS, POSITION, APER and MIXO) differed among sites (Figure 1). Average DWT was highest (i.e. driest

Table 2. Summary statistics of GLM for testate amoeba community-weighted means (CWMs) of traits and testate amoeba-inferred DWT and pH conditions in each peatland.

	Site	% Explained deviance	AIC	Biovolume (BIOVOL)	Aperture position (POSITION)	Shell compression (COMPRESS)	Mixotrophy (MIXO)	Aperture size (APER)
pH	Praz-Rodet	31.1	18.7	ns	ns	ns	0.001	0.016
	Tuchola	18.1	99.9	ns	0.033	ns	ns	ns
	Pechora	65.4	-5.9	ns	ns	0.001	ns	0.008
	Jigsaw	28.9	-90.3	ns	ns	0.009	ns	ns
DWT	Praz-Rodet	82.6	364.7	<0.001	0.004	<0.001	<0.001	0.001
	Tuchola	17.1	215.9	0.035	ns	ns	0.033	0.009
	Pechora	23.3	266.1	ns	ns	0.027	0.005	ns
	Jigsaw	72.5	266.2	0.036	ns	ns	<0.001	0.003

Table 3. Summary statistics of GLM for the pooled data including site as explanatory variable.

	% Explained deviance	AIC	Site	Biovolume (BIOVOL)	Aperture position (POSITION)	Shell compression (COMPRESS)	Mixotrophy (MIXO)	Aperture size (APER)
DWT	26.6	1508	<0.001	ns	0.003	0.039	ns	ns
pH	46.1	211.23	0.013	<0.001	<0.001	ns	0.001	0.042

conditions) in Jigsaw while average pH was highest in Tuchola. DWT and pH were more variable in the two human-impacted sites (Praz-Rodet and Tuchola) than in the pristine sites (Jigsaw and Pechora). Averaged CWM of traits also differed among peatlands. More specifically, average BIOVOL was highest, POSITION most cryptic and APER largest in Tuchola, whereas COMPRESS and MIXO were highest in Jigsaw (Figure 1).

GLMs revealed that changes in pH were explained by CWM of traits, but differed between sites (Tables 2 and 3; Figure 2). Specifically, pH was negatively correlated to MIXO in Praz-Rodet, positively to APER in Praz-Rodet and Pechora, negatively to COMPRESS in Pechora and positively in Jigsaw and negatively to POSITION in Tuchola. Changes in DWT mirrored those in MIXO in each peatland (Table 2) but not overall (Table 3), while other traits were correlated to DWT depending of the sites: APER in Praz-Rodet, Jigsaw and Tuchola; BIOVOL in Praz-Rodet, Jigsaw and Tuchola; COMPRESS in Praz-Rodet and Pechora; and POSITION in Praz-Rodet. Moreover, traits explained a larger amount of the variance in inferred DWT values in Praz-Rodet and Jigsaw. By contrast, traits explained a higher proportion of the variance of pH as compared with that of DWT in Pechora. Finally, traits explained a limited amount of the variance in pH and DWT in Tuchola.

Overall, we found repeated convergences of BIOVOL and APER that occurred preferentially when the community mean aperture size and shell biovolume were low (Figure 2). On the contrary, we observed repeated divergences of MIXO (Praz-Rodet and Jigsaw) when the proportion of mixotrophic species was high. Tuchola stood out with repeated divergences of BIOVOL but not APER and no case of divergence of MIXO. Convergences towards low values of POSITION and COMPRESS occurred repeatedly while divergence occurred only once in Praz-Rodet (COMPRESS) and Jigsaw (POSITION). Within peatlands, BIOVOL was relatively stable and low over time in Jigsaw and Praz-Rodet, while it was higher and more variable in Pechora and Tuchola. MIXO and APER were the most variable traits while COMPRESS and POSITION were the most stable traits.

In Jigsaw, significant convergences of BIOVOL occurred from 6000 years BP onwards. APER was generally low but peaks occurred repeatedly. As for BIOVOL, significant convergences

were observed from 2000 years BP onward. MIXO was generally high but declined sharply three times (i.e. at ca. 500, 3000 and 8000 years BP) and repeated divergences were observed. POSITION was relatively stable and convergences were observed six times while divergence was recorded only once. COMPRESS was relatively high and stable and no convergence or divergence was observed.

In Pechora, BIOVOL was low but peaked repeatedly. Periods of significant convergence occurred during the intervals among peaks. APER oscillated around 20 μ m with repeated periods of convergence. MIXO was generally low but increased between 1500 and 2600 years BP and towards present. The only observed case of significant divergence corresponded to the maximal value of MIXO. POSITION was relatively stable with only four convergences and no divergence. COMPRESS was relatively high but less stable than in Jigsaw and Praz-Rodet and repeated convergences were observed.

In Praz-Rodet, BIOVOL was low and stable over time. Periods of significant convergence occurred around 7000 years BP and after 2000 years BP. APER was relatively high and unstable. Cases of significant convergences were observed around 1000 and 7000 years BP. MIXO varied strongly during the considered period with sharp increases and decreases and both negative and positive peaks. We observed cases of significant divergence around 1000 and 6000 years BP and one case of convergence towards present. APER was first unstable but did not change much after 6000 years BP. Repeated convergences were observed from 100 years BP onward. POSITION was relatively stable and convergences were observed six times and divergence only once. As for POSITION, COMPRESS was first unstable but did not change much after 6000 years BP.

In Tuchola, BIOVOL was high and relatively unstable over time. Periods of significant divergence occurred repeatedly while one case of convergence was observed around 1000 years BP. APER was generally high but changed strongly during the considered period. Significant convergences occurred simultaneously as negative peaks. MIXO was generally low but repeated positive peaks occurred. POSITION was relatively stable and convergences were observed six times against only one divergence. COMPRESS was relatively high and stable and no convergence or divergence was observed.

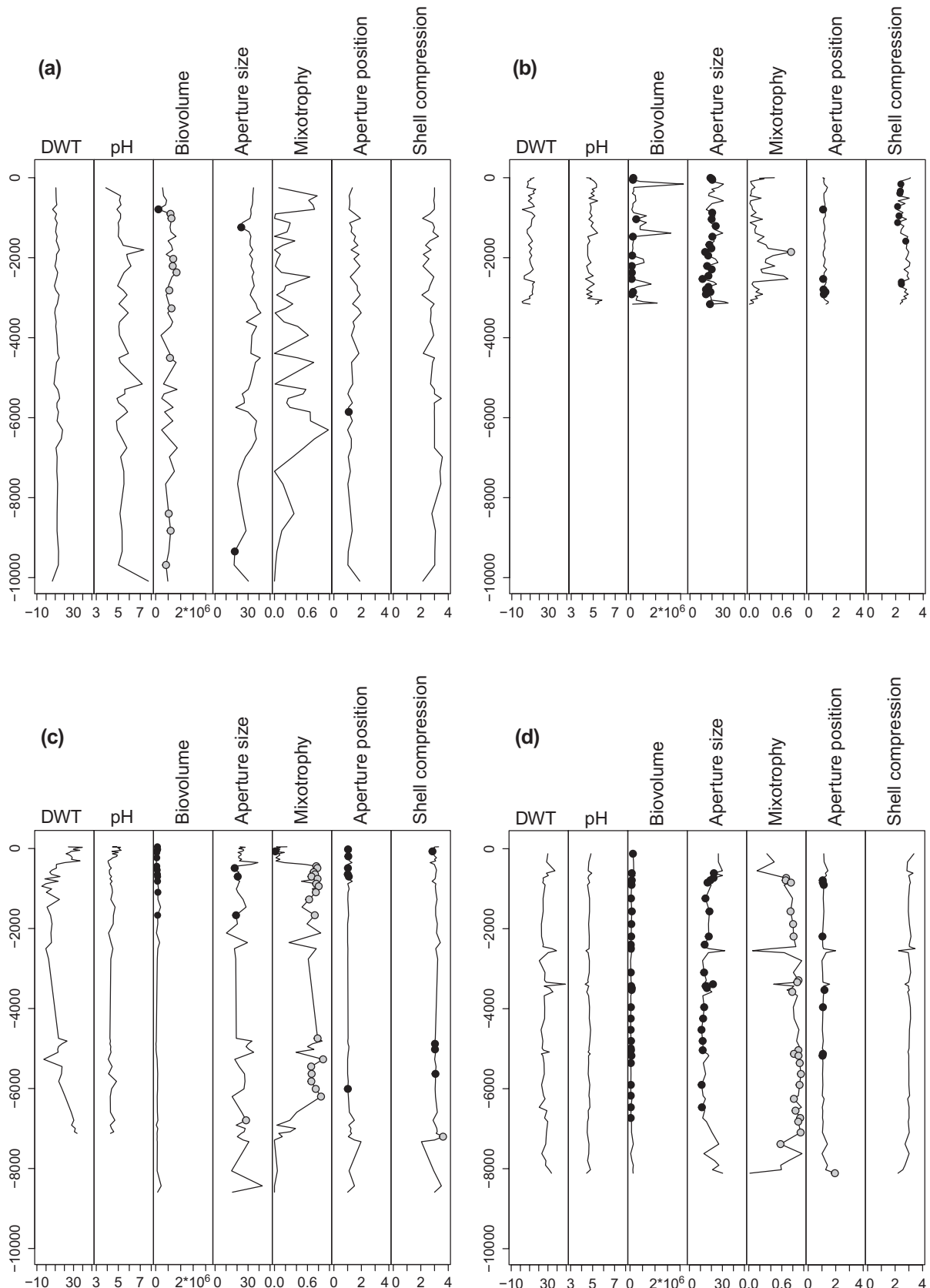


Figure 2. Temporal changes of depth to water table (DWT, cm below soil surface) and pH as well as community-weighted biovolume (BIOVOL, μm^3), aperture position, shell compression, aperture size (APER, μm) and ratio of mixotrophic species (MIXO) of arcellinid testate amoebae in four peatlands: (a) Tuchola, (b) Pechora, (c) Praz-Rodet and (d) Jigsaw. Black circles indicate periods of significant convergence of traits and grey circles significant divergences.

Discussion

This study explores the use of FTs in a palaeoecological context. The results show that FTs provide complementary information to species-based transfer functions by documenting functional

processes related to community changes over time. In other words, the analysis of RTs and ETs reflects the causes and the consequences of the changes in species composition, respectively. It represents, therefore, an approach that tackles directly the

ongoing processes at the ecosystem level by summarizing the information to the part that is directly related to these processes. TA FTs thus constitute a promising new approach for interpreting subfossil records and reconstructing past peatland functioning.

The four studied peatlands differed in the patterns of inferred DWT and pH, both in average values and variability. As hypothesized, the distribution of TA subfossil FTs differed strongly among the four studied peatlands. However, the correlations between traits and inferred DWT and pH also varied among peatlands. These differences suggest that the four peatlands differed markedly not only in their history of changes in DWT and pH changes but also in the way these ecological drivers affected the TA communities through TA RTs (i.e. biovolume, aperture position and shell compression) and in the effect of these community changes for the functioning of the microbial food web through TA ETs (i.e. aperture size and mixotrophy).

In Tuchola, on average, environmental conditions were wettest, TA shell compression lowest and biovolume highest, suggesting abundant prey availability (Figure 1). Wetter conditions and higher pH than in other sites is also in line with the low percentage of mixotrophic species as higher pH values generally indicate more minerotrophic conditions (Rydin and Jeglum, 2013) in which mixotrophs are likely to be less competitive than in more oligotrophic conditions (Douglas, 1994). By contrast, average trait values suggest that conditions were driest and prey least abundant in Jigsaw where biovolume was low, pH low and the community was strongly dominated (>75%) by mixotrophic species – in this case, mainly *Archerella flavum*, the smallest mixotrophic species. In Pechora and Praz-Rodet, the situation was intermediate between Jigsaw and Tuchola.

Repeated convergences towards low biovolume and aperture size suggest that TAs with larger biovolume and aperture size were filtered out most likely following shifts towards drier conditions (Figure 2). However, when conditions were wet as in Tuchola, species with small and large biovolume could coexist most likely because they share a different trophic niche. Indeed, according to their foraging traits (e.g. biovolume and aperture size), small and large TA species are specialized on different prey items such as bacteria and ciliates, respectively, (Gilbert et al., 2000, 2003; Jassey et al., 2012) and thus have different trophic functions. This further suggests that TA species can occupy a high trophic position only when environmental conditions are sufficiently wet to allow species with large biovolume to occur. Whether other organisms (i.e. micro-invertebrates, other protists or the non-aquatic soil meso-fauna) endorse the role of top predators during dry phases remains to be assessed.

The observed divergences of the ratio of mixotrophic species suggest that species possessing photosynthetic endosymbionts can survive in the presence of superior heterotrophic competitors by switching to autotrophy following the principle of limiting similarity (MacArthur and Levins, 1967). Mixotrophy, as a feeding strategy, allows species to survive in both light- and nutrient-limited habitats (Sanders, 1991). It has been shown to be common in TAs that live in Northern ombrotrophic (and oligotrophic) peatlands, where species that harbour phototrophic symbionts can make up a substantial proportion of the total number of individuals (Gilbert and Mitchell, 2006). In contrast, only one mixotrophic species, *Diffflugia pyriformis venusta* has been reported from mesotrophic lakes. Protists in general tend to use mixotrophy in oligotrophic environments, as shown in many planktonic groups in the ocean (Christaki et al., 1999; Hartmann et al., 2012). Our results suggest that TAs can also use mixotrophy to survive in the presence of species that are better competitors for preys. Moreover, communities dominated by mixotrophic species had a low average biovolume and compressed shells, suggesting that in drier conditions, small mixotrophs such as *Archerella flavum*

were very successful and reached overall higher abundance than larger mixotrophic species (e.g. *Hyalosphenia papilio*, *Amphitrema wrightianum*).

Our results showed that inferred pH and DWT were correlated to TA FTs, but to various degrees depending on the site and the variable considered. Indeed, different traits were related to pH and DWT in the four peatlands, suggesting that no single mechanism can explain the changes in community structure following changes in pH and/or DWT. The mechanisms through which DWT and pH influence TA communities were site-specific, as shown by inter-site differences in the correlations between FTs and inferred DWT and pH (Supplementary Table 1, available online) and in the patterns of trait convergence and divergence (Figure 2). A likely explanation for this result is the different context experienced by TAs in the peatlands. For instance, the capacity of a species to occupy its most favourable environmental conditions depends on dispersal, competitive, demographic and stochastic (neutral) limitations (Ai et al., 2013). These limitations are likely to differ among sites but are never considered in palaeo-environmental reconstructions potentially causing bias as stressed by Belyea (2007).

Our study demonstrates the strong relations between TA trait data and the DWT and pH data inferred from species composition, and brings therefore a new tool to palaeoecologists. This suggests that past environmental changes influenced both species composition and community function in the ecosystem. Investigating both response and ET therefore informs about the functioning of microbial communities and the mechanism driving TA community changes over millennia. Trait-based approaches could thus complement or perhaps replace inference based only on species composition data to reconstruct past peatland environmental conditions and functioning (Lamentowicz et al., 2015). Similar approaches can be applied to other palaeo-indicators such as diatoms or pollen. This can provide a more mechanistic understanding of past environmental changes in general. However, trait-based approaches will not solve the bias inherent to the different taxa. For instance, as for the different decomposition rate of TA shells, diatom species are likely to differ in their preservation in palaeo-records. Furthermore, because of its long distance dispersal pollen informs on local to regional vegetation changes. This complexity needs to be understood when investigating assembly mechanisms or site-specific questions. Moreover, trait-based approaches are also a pertinent way to overcome the current limitations of morphology-based taxonomy. For instance, as for most groups of microorganisms, the taxonomy of TAs is not perfect and ongoing studies combining molecular phylogeny and morphometry are changing substantially their classification, both for deep phylogenetic relationships (Gomaa et al., 2012) and among closely related species (Kosakyan et al., 2012; Singer et al., 2015). In addition, they bring a methodology that can be used regardless of the geographic location of the study sites, an important advantage given the fact that TAs have often geographically restricted distributions (Smith et al., 2008).

Further observational and experimental studies are required to understand how site-specific conditions influence the trait-environment relationship. With such work, it will be possible to assess the full potential of FTs as a new method for future palaeoecological studies.

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