

An alternative approach to transfer functions? Testing the performance of a functional trait-based model for testate amoebae



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ABSTRACT

Transfer functions are now widely available to infer past environmental conditions from biotic assemblages. Existing transfer functions are based on species assemblages but an alternative is to characterize assemblages based on functional traits, characteristics of the organism which determine its fitness and performance. Here, we test the potential of trait-based transfer functions using testate amoeba functional traits to reconstruct peatland water-table depths.

A total of seven functional traits with linkages to peat moisture content were identified and quantified as community weighted-means for each of 43 taxa in a training set from Tierra del Fuego, South America. Transfer functions based on (multiple) linear regression and partial least-squares were produced, validated using cross-validation and an independent test set, and applied to three core records. Trait-based models performed remarkably well. Model performance based on cross-validation and an independent test set was only marginally weaker than for models based on species and reconstructed down-core trends were extremely similar. Trait-based models offer considerable potential for paleoecological reconstruction particularly in no-analogue situations, where no species transfer function is available and for inexperienced analysts. The approach deserves further validation and testing for both testate amoebae and other groups of microfossils.

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1. Introduction

Transfer functions are frequently applied to biostratigraphic indicators from sediments to quantify past changes in environmental variables. Frequently used combinations of indicators and predicted variables include pollen-temperature/precipitation (Klemm et al., 2013), chironomids-temperature (Massferro and Larocque-Tobler, 2013), foraminifera-sea level (Kemp et al., 2013) and testate amoebae-water table level (Amesbury et al., 2016; Lamarre et al., 2013). These transfer functions are based on the principle of uniformitarianism and assume that there is a direct relationship between the environmental variable to be reconstructed and the abundance of

individual taxa and that the relationship has remained constant through time (Birks et al., 2010). All published transfer functions have in common that the biotic assemblages are defined by their taxonomy, e.g. to family, genus or species level. However, a possible alternative may be considered, which is to base transfer functions on functional traits. Functional traits are the key characteristics of an organism which determine its fitness or performance (Green et al., 2008). Species with similar traits are likely to perform similar roles in the ecosystem and favour similar environmental conditions. Defining an assemblage based on functional traits has the advantage of providing a mechanistic link with ecosystem processes and environmental conditions which may therefore give more information on ecosystem functioning than taxonomy alone (Barnett et al., 2007; Tilman et al., 1997). The use of functional traits has attracted increasing interest in paleoecology primarily with the aim to better understand the mechanisms underlying past assemblage change (Eronen et al., 2010; Lacourse, 2009; Marcisz et al., 2016). However, the possibility of building transfer functions based on traits rather than species has not been previously explored. Trait-

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based transfer functions may have three possible advantages: 1) they may offer superior performance if the functional trait assemblage of a sample is more strongly linked to an environmental variable than the species assemblage; 2) they may be more generalizable, allowing a transfer function to be applied to species not included in the training set based on functional traits which are included, and 3) they may be more robust to problems of taxonomic resolution and error, as traits may often be easier to determine than species. Here, we evaluated and compared the performance of transfer functions based on species and trait assemblages, using testate amoebae and peatland water-table reconstructions as a case study.

Testate amoebae are a polyphyletic collection of at least three taxonomic groups of unicellular eukaryotes (Kosakyan et al., 2016a). The shells (tests) of these organisms have been studied as indicators of ecosystem health (Fournier et al., 2012), sea level change in marshes (Charman et al., 2010) and lake water quality (Neville et al., 2013; Roe et al., 2010), but most frequently to reconstruct past water-table dynamics in mid- and high-latitude, mostly ombrotrophic, peatlands (e.g. Mitchell et al., 2001; Warner and Charman, 1994). Such reconstructions may reflect past changes in hydroclimate (e.g. Booth, 2002; Charman et al., 1999; Lamentowicz et al., 2010) and may be linked to past variability in carbon sequestration rates at decadal to millennial timescales (Charman et al., 2015; Loisel and Garneau, 2010; van Bellen et al., 2011). Compared with other indicators frequently analyzed in peatlands, such as plant macrofossils, testate amoebae respond quickly to environmental change because of their short life span and high rates of reproduction (in the order of 10–27 generations per year; Charman, 2001). Transfer functions have been developed for several regions around the globe (Amesbury et al., 2013; Booth, 2008; Charman et al., 2007; Lamarre et al., 2013; Payne et al., 2008; Qin et al., 2013; van Bellen et al., 2014), motivated by regional differences in peatland ecohydrology and taxa distribution and associated ecological preferences.

Lack of consistency in the current taxonomy is considered a major issue which may affect the quality of the reconstructions from testate amoeba assemblages (Heger et al., 2009; Kosakyan et al., 2016a; Payne et al., 2011). Testate amoeba taxonomy is based largely upon ‘morphospecies’, i.e. defined by test morphology, and phylogenetic data remains sparse, despite recent efforts (e.g. Gomaa et al., 2013; Kosakyan et al., 2016b; Lahr et al., 2011). Testate amoeba studies frequently group cryptic species, which cannot be identified solely by morphology (e.g. the genus *Nebela*; Oliverio et al., 2014), and pseudo-cryptic species, which have only very subtle morphological dissimilarities (Singer et al., 2015). A range of identification keys for testate amoeba identification and quantification have been developed (e.g. <http://istar.wikidot.com/id-keys> and Charman et al., 2000) but include many inconsistencies. While this may be due to the parallel development of original taxonomies, the potential for real geographical differences in shell morphology cannot be excluded. Rare taxa with low abundance or with presence restricted to a few samples, are often excluded from the assemblage prior to the application of a species-based transfer function, because their optimal water-table depth may be uncertain. The elimination of taxa represents a loss of information for reconstructions. Low taxonomic resolution and inconsistencies in the identification of testate amoebae may lead to significant biases in quantitative environmental reconstructions (Mitchell et al., 2014; Payne et al., 2011).

Functional traits of testate amoebae have been increasingly considered since a first study by Mitchell and Gilbert (2004). Some important functional traits of testate amoebae can be readily identified and measured based on the morphological characteristics of their test. Functional traits have been investigated primarily to increase the understanding of the interactions between these organisms and their microenvironment (Fournier et al., 2016; Fournier et al., 2015; Jassey et al., 2013b; Lamentowicz et al., 2015; Marcisz et al., 2016) but the use of functional traits in transfer function development has not been addressed. These studies presume functional traits are stable per taxon and taxon

response to variations in environmental conditions is a function of their functional traits. Conversely, the behavioural and morphological characteristics of testate amoebae may provide information on ecosystem characteristics and functioning. Mechanistic relationships between some of these traits and peat surface wetness conditions have now been documented. For instance, a testate amoeba with a small and compressed test is likely advantaged, and thus more abundant, under low moisture conditions, because it will be better adapted to living in a thin water film (Fournier et al., 2012). The relative exposure of the aperture may be an indication of the resistance to drought, with a cryptostomic morphology (‘concealed’ aperture) being best adapted to such conditions (Fournier et al., 2012; Lamentowicz et al., 2015; Marcisz et al., 2016). Given the possibility that functional traits may vary within the accepted taxonomy, e.g. the number of pores in the test of *Hyalosphenia papilio* (Booth and Meyers, 2010), traits should be selected that are unlikely to show high intra-taxon variability. For instance, testate amoeba biovolume may vary by almost three orders of magnitude between taxa found within the same ecosystem (Fournier et al., 2012), so slight intra-taxon variability is likely to be insignificant compared to the large differences in biovolume among taxa.

The direct links between shell morphology and wetness, combined with the well-known inadequacies of the current morphospecies taxonomy, suggest that there is potential for an alternative approach to paleoenvironmental reconstructions. We hypothesize that better reconstructions may be produced using transfer functions based solely upon traits, which can be objectively determined, rather than using transfer functions based on uncertain and inconsistent morphospecies taxonomy. We therefore developed and tested a testate amoeba transfer function entirely based on functional traits using a previously-published training set sampled in Tierra del Fuego, southernmost South America (van Bellen et al., 2014). Here we compare the performance of trait-based and morphospecies-based transfer functions and apply both down-core to compare inferred water-table depths.

2. Methods

2.1. Fieldwork and laboratory work

The training set used for transfer function construction was the 2012 dataset from van Bellen et al. (2014). This dataset includes a total of 99 samples from four peatlands along with measurements of water-table depth (WTD), pH and electrical conductivity (EC). These peatlands are ombrotrophic with dominance of *Sphagnum magellanicum* from low lawns up to the highest hummocks. Sampling was performed along a total of 20 transects spanning the entire hollow-hummock gradient, which represented a water-table level range of 104 cm. Study region and site characteristics as well as field sampling methods have been described in van Bellen et al. (2014).

2.2. Trait data and community-weighted means

Functional trait values for each taxon, summarized in Table S1, were based on our own unpublished measurements. The functional traits tested included:

- 1) Test biovolume, believed to be positively related to peat surface wetness, because larger tests need thicker water films to maintain their position within the peat matrix (Laggoun-Défarge et al., 2008). For biovolume calculations, the formulas from Fournier et al. (2015) were used.
- 2) Aperture position, which is believed to minimize sensitivity to droughts, because more cryptic apertures are less exposed to moisture loss and thus allows for survival in low humidity conditions (Fournier et al., 2012).
- 3) Test compression, which is considered an adaptation to thin water films and dry conditions (Fournier et al., 2012; Jassey et al., 2013a).

- 4) Aperture size, which is linked to the potential size of food (Jassey et al., 2013b) and may vary with surface wetness. For instance, hollow-dwelling amoebae may have wider apertures, because they prey mainly on algae, whereas hummock-dwelling amoebae generally prey on smaller bacteria.
- 5) Mixotrophy. Some testate amoebae use symbiotic algae which perform photosynthesis and may thus provide food when prey is scarce (Jassey et al., 2013b). Such mixotrophic taxa (e.g. *Amphitrema wrightianum*, *H. papilio* and *Heleopera sphagni*) may be more abundant in wetter conditions, because these environments generally have lower vascular plant cover and therefore more light penetration to the moss layer (Marcisz et al., 2014; Payne et al., 2016).
- 6) Phylogenetic group, which indicates potential differences in survival strategies linked with disturbance (Wanner et al., 2008). Three phylogenetic groups were present in the datasets: Amphitremida, Arcellinida and Euglyphida. Many taxa within the order Euglyphida (including *Euglypha*, *Assulina*, *Corythion-Trinema* type and *Sphenoderia lenta*) are primarily bacterivorous and bacteria may be more abundant in drier sites. In contrast, Amphitremida include *A. wrightianum*, the presence of which may be associated with wet conditions.
- 7) Mineral matter presence, used by some testate amoebae for test reinforcement, may reflect the availability of dust at the surface (Fiałkiewicz-Kozieł et al., 2015).

Aperture position and test compression were expressed as ordinal values; phylogenetic group, mixotrophy and mineral matter presence were nominal and biovolume and aperture size were continuous (Table S1).

Taxon functional trait values were not independent of taxonomy, as we assumed functional traits are constant for each taxon. However, taxonomy is generally based on a range of characteristics which do not split taxa along environmental gradients, but rather allow for the most straightforward distinction of morphospecies. Therefore, the use of functional traits which have a plausible mechanistic link with environmental conditions may a priori be a better approach for the understanding of amoeba abundance under these environmental conditions. To this effect, community-weighted means (CWMs) of each functional trait were calculated for each sample as the sum of the products of the trait value of the taxon and the relative abundance (%) of the taxon within the assemblage using Canoco 5 (Ter Braak and Šmilauer, 2012). Whereas the species-based transfer function was limited to 28 taxa (van Bellen et al., 2014), the CWMs were calculated based on all 43 taxa, because exclusion of rare taxa was not relevant. CWMs were calculated for both the training set and the fossil data.

2.3. Trait-environment relationships and transfer function development

The trait values of all taxa were assessed using principal component analysis. The relationships between CWMs and the measured environmental variables WTD, pH and EC were explored using redundancy analysis to identify the main linkages. Partial redundancy analysis was used in a second step, to quantify variability explained by each environmental variable after taking into account variability explained by the other two variables. A Monte Carlo permutation test (1000 iterations) was used to test the significance of canonical axes. All ordinations were performed using Canoco 5 (Ter Braak and Šmilauer, 2012).

Relationships between trait CWMs and measured WTD were explored using stepwise multiple regression in JMP 12 (1989–2007). This procedure used forward selection of independent variables, during which variables were tested individually, adding additional variables until optimal performance was attained. In addition to multiple regression, partial least-squares (PLS) trait-based transfer functions were tested. The PLS method uses components to summarize variability within the taxon assemblages, extracted using both biological and environmental data. Both methods assume linear relationships between CWMs and environmental variables. In the selection of variables for

modelling, care was exercised to obtain good model performance, as quantified by validation methods, but to avoid over-fitting. Model construction and (cross-) validation was performed using the MR and WAPLS functions of rioja (Juggins, 2015) in R 3.3.2 (R Core Team, 2016).

Model performance was evaluated based on the strength of the relationship between observed and predicted WTD values based on cross-validation (leave-one-out and bootstrapping) and an independent test set, and visual inspection of the observed-predicted plots. In evaluating models, we considered the root mean square error of prediction (RMSEP) for both the complete WTD gradient and fixed intervals ('segment-wise' RMSEP), R^2 and average and maximum bias. Finally, the transfer function was further evaluated by a linear regression of WTD optima of the 28 taxa included in both transfer functions. The trait-based transfer function WTD optima were obtained by multiplying individual taxon trait values by the model parameters, with the WTD optimum defined as the WTD obtained when individual taxon abundance was set to 100%.

2.4. Inferring past water tables

The new trait-based transfer function was applied to three paleoecological records, TiA12, AND12 and KAR13, for which species-based reconstructions have been published previously (van Bellen et al., 2016). These trait-based reconstructions were obtained by applying the trait-based transfer function to the past assemblages, including standard errors of prediction from bootstrapping with 1000 samples.

3. Results

3.1. Training set: traits and taxonomy

Principal component analyses of the taxon-by-trait matrix show that Euglyphida, including *Assulina*, *Corythion-Trinema* type and *Euglypha* spp., are associated with plagiostomic/cryptostomic and compressed tests (Fig. 1). The other end of the main axis is characterized by *Diffflugia globulosa* type and *Cyclopyxis arcelloides* type, which are positively correlated with aperture size and mineral matter presence. Mixotrophy is poorly correlated with any of the other traits, except for Amphitremida presence, as mixotrophic taxa (e.g. *A. wrightianum*, *H. sphagni*) may be either compressed or uncompressed and have varying aperture size.

3.2. Training set traits and environmental variables

Redundancy analysis of functional trait CWMs, constrained by the observed environmental variables WTD, pH and EC (all axes $p = 0.006$; Fig. 2), showed that WTD is the main variable explaining variability in CWMs. Partial constrained ordination of training set CWMs showed WTD explained 42.7% of the variability in CWMs when pH and EC were partialled out, with pH 8.3% and EC 8.6%.

Of all functional traits, mineral matter presence CWMs showed the strongest correlation with observed WTD, increasing with higher water tables ($r^2 = 0.63$ in linear regression; Fig. 3). Euglyphida abundance ($r^2 = 0.62$) and test compression ($r^2 = 0.51$) were highly positively correlated with WTD, thus generally associated with deeper water tables. Aperture position ($r^2 = 0.27$) was also positively correlated with WTD, which implies that, as hypothesized, tests with less-exposed apertures are more abundant in drier conditions. Arcellinida abundance ($r^2 = 0.55$), biovolume ($r^2 = 0.31$), aperture size ($r^2 = 0.30$) and to a lesser extent mixotrophy and Amphitremida abundance (both $r^2 = 0.21$) were negatively correlated with WTD (all $p < 0.0001$). Regressions showed most relationships between WTD and CWMs were linear, although aperture size and mixotrophy may culminate at WTDs between 20 and 25 cm (Fig. 3).

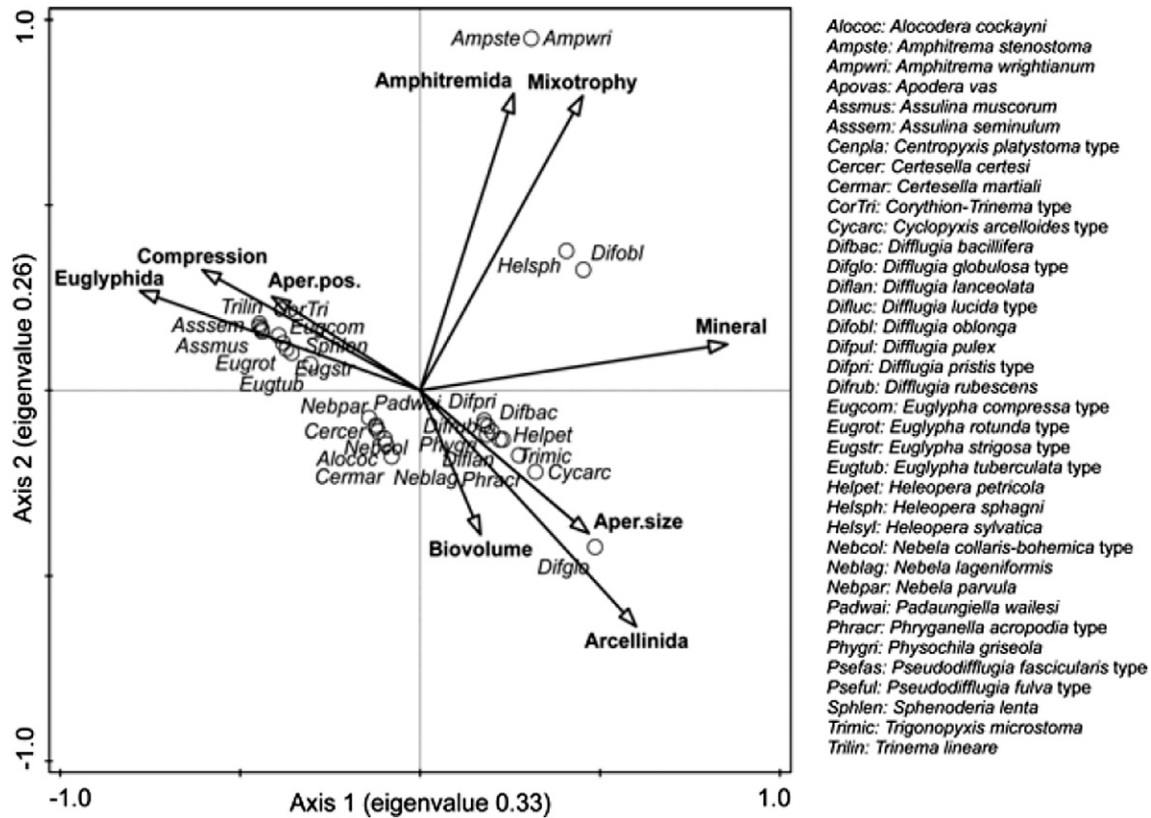


Fig. 1. Principal component analysis of functional traits for the 30 best-fitting taxa within the training set (99 samples). Complete names for taxa are identified in the list on the right (some taxa are not shown in Fig. 1, but are specified here for Fig. 6). The distribution of taxa along Axis 1 suggests an underlying WTD gradient from dry conditions (low values; left) to wet (high values; right).

3.3. Trait-based transfer function

Exploring several models, it appeared models became unstable when too many correlated variables were added, as shown by multiple parameter inversions (Legendre and Legendre, 2012). We therefore selected the best-performing model which showed parameter values of the same sign as obtained in simple regression and minimal Bayesian information criterion. Three models were retained and tested in detail. Two of these (one multiple-regression model and one PLS model) included a subset of three functional traits, mixotrophy, test compression

and mineral presence, and one included only mineral presence as independent variable.

The multiple regression model including mixotrophy, compression and mineral presence showed best performance according to all four validation methods (Table 1 and Fig. 4) although the advantage was small compared to the partial least-squares model. The multiple regression trait-based transfer function performance assessed by R^2 was similar but slightly weaker (R^2 at 0.65 for both leave-one-out and bootstrapping) than the species-based transfer function (R^2 of 0.72). Similarly, RMSEP values of the trait-based transfer function were slightly higher at 15.20 (leave-one-out) and 15.46 cm (bootstrapping) compared with 13.51 and 14.01 cm for the species-based transfer function, indicating slightly weaker performance. Using the independent test set, the trait-based transfer function showed lower performance than the species-based transfer function, with R^2 of 0.45 and 0.62 and RMSEP of 20.95 and 18.23 cm, respectively (Table 1 and Fig. S1). The trait-based transfer function model performance, measured by RMSEP on segments of the WTD gradient, was best for the 0–70 cm interval of measured WTD (RMSEP ~10 cm), with the model generally underestimating values for the deepest water tables (Fig. 5).

Regression of taxon WTD optima of the species-based and the trait-based transfer functions showed significant correlation (Fig. 6; $r^2 = 0.46$, RMSE = 19.6 cm, $p < 0.0001$). Some of the differences in optimum WTD may be explained by the fact that trait-based taxon-WTD modelling is based on the entire assemblage of taxa, in contrast to the species-based transfer function, which links individual taxa to WTD. As a result, extreme values for WTD optima for individual taxa are more likely when these have a uniform collection of traits. In addition, the trait-based approach assumed a linear relationship between traits and WTD which is more likely to result in extreme values at both ends of the WTD gradient. Major outliers may be interpreted as taxa which have traits that seem incongruent with their optimum WTD as modelled by

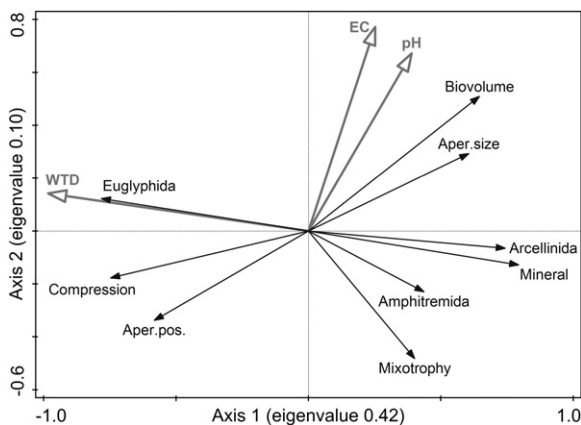


Fig. 2. Redundancy analysis of functional trait CWMs constrained by environmental variables WTD, pH and EC, showing WTD is the main variable explaining variability in functional trait CWMs.

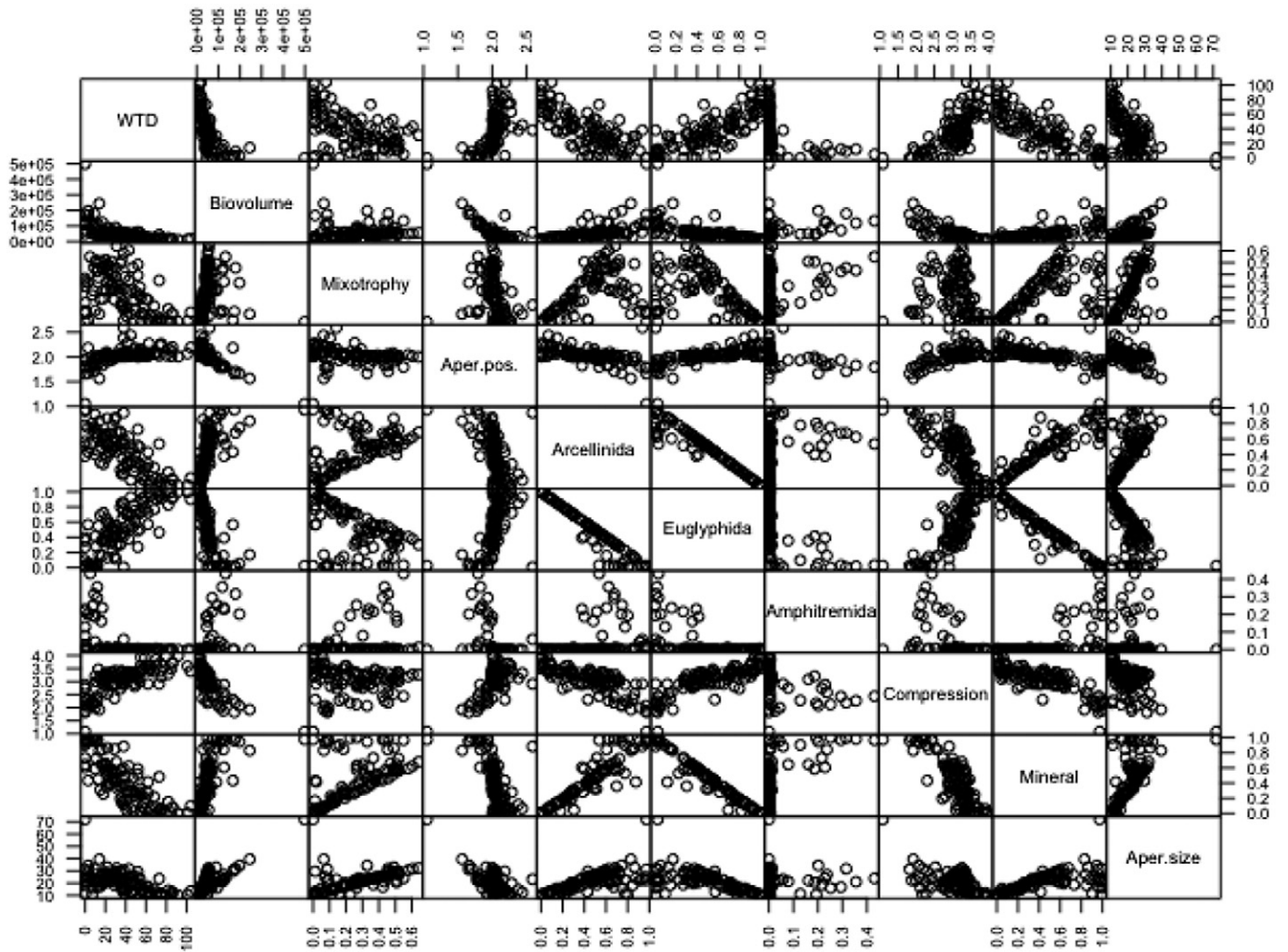


Fig. 3. Relationships among all functional trait CWMs and with WTD. Units for WTD are cm, μm^3 for biovolume and μm for aperture size. Trait CWMs are quantified by indices: 0–1 for mixotrophy, Arcellinida, Euglyphida, Amphitremida and mineral presence, 1 (central) to 3 (cryptic) for aperture position and 1 (uncompressed) to 4 (highly compressed) for compression.

the species-based transfer function. The most notable examples are *H. sphagni*, *Amphitrema stenostoma* and *Diffugia pristis* type, the traits of which suggest an optimum water table higher than obtained by the species-based transfer function, while *Assulina seminulum* and *Certesella martiali* had an optimum water table lower than modelled based on taxonomy only (Fig. 6).

3.4. Trait-based WTD reconstructions

The testate amoeba assemblages of TiA12, AND12 and KAR13 (Fig. S2; van Bellen et al., 2016) were used as input for the trait-based WTD reconstructions. The reconstructed trends in WTD as obtained by the trait-based and the species-based transfer function were similar overall for TiA12 ($r^2 = 0.86$, RMSE = 5.07 cm), AND12 ($r^2 = 0.77$, RMSE = 4.08 cm) and KAR13 ($r^2 = 0.81$, RMSE = 3.88 cm).

4. Discussion

4.1. Functional trait linkages with water-table dynamics in the training set

WTD explained 42.7% of the variability in trait CWMs as shown by partial correspondence analysis, which is higher than the proportion of the morphospecies community variability explained by canonical Axis 1 (16.6%), associated with WTD, in canonical correspondence analysis (van Bellen et al., 2014). Of all traits analyzed, mineral matter

presence and Euglyphida abundance were the best predictors of WTD (Fig. 3). The majority of Euglyphida encountered were *Assulina muscorum* (60.6%), which was also the most common taxon overall at 31.8% of all taxa. Many Euglyphida, as opposed to Arcellinida and Amphitremida, may be considered *r*-strategists (Beyens et al., 2009), i.e. they are better adapted to unstable conditions and aim to dominate through rapid recolonization rather than through persistence (Fournier et al., 2012). In addition, Euglyphida abundance may follow abundance of bacteria, as they are generally bacterivorous (Lara et al., 2007) and bacteria may be more abundant under dry conditions in temperate, ombrotrophic peatlands (Fenner et al., 2005).

The peatlands of Tierra del Fuego are characterized by extreme microtopographical gradients, with WTD attaining 104 cm in the driest hummocks during summer (van Bellen et al., 2014). These hummocks are more exposed to wind and show a greater daily variability in moisture content than nearby hollows (Loader et al., 2016). A higher abundance of tests integrating mineral matter was found in hollow communities. Mineral matter (dust) deposition may be higher in hollows because airflow is reduced after it passes over a hummock and dust may thus be concentrated into hollows. Alternatively, dust concentrations may be higher in hollows because of a dominant water flow which may redistribute dust initially deposited on the more exposed hummocks. More detailed data on within-site variability of dust deposition is essential to interpret the relative presence of mineral-matter integrating testate amoebae.

Table 1
Performance for the three trait-based models and the original species-based model. The multiple regression model with mixotrophy, test compression and mineral presence was retained for further analyses (in bold).

Performance measure	Apparent (observed-predicted)		Cross-validation	
	2012 data	Independent (2013) data	Leave-one-out	Bootstrapping (1000)
<i>Multiple regression – three traits</i>				
RMSE(P) (cm)	14.68	20.95	15.20	15.46
R²	0.67	0.45	0.65	0.65
Average bias (cm)	0	11.16	0.05	0.04
Maximum bias (cm)	38.58	26.74	39.75	39.72
<i>Simple regression – mineral matter</i>				
RMSE(P) (cm)	15.44	21.45	15.74	15.88
R ²	0.63	0.45	0.62	0.62
Average bias (cm)	0	12.08	0.04	0.07
Maximum bias (cm)	39.23	29.99	40.28	40.47
<i>PLS (two-component) – three traits</i>				
RMSE(P) (cm)	14.70	20.96	15.12	15.31
R ²	0.67	0.46	0.65	0.65
Average bias (cm)	0	11.35	0.04	0.12
Maximum bias (cm)	38.59	27.34	39.74	39.94
<i>Species-based</i>				
RMSE(P) (cm)	12.32	18.23	13.51	14.01
R ²	0.77	0.62	0.72	0.72
Average bias (cm)	0	10.39	−0.02	<0.01
Maximum bias (cm)	31.59	26.07	33.25	33.22

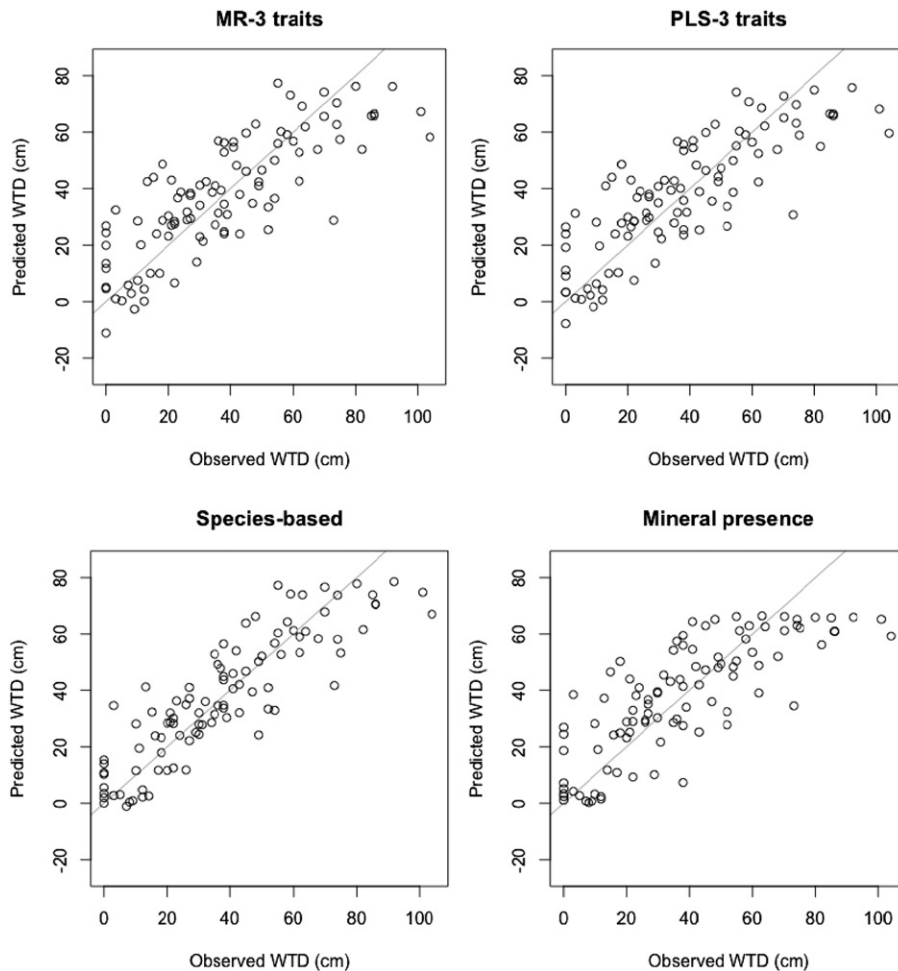


Fig. 4. Model performance as shown by the relationship between observed WTD from the training set and WTD as predicted by each model using leave-one-out cross-validation. Grey line represents $y = x$. The multiple regression model including mixotrophy, compression and mineral presence was retained as transfer function (RMSEP = 15.20 cm, $R^2 = 0.65$, average bias = 0.05 cm and maximum bias = 39.75 cm).

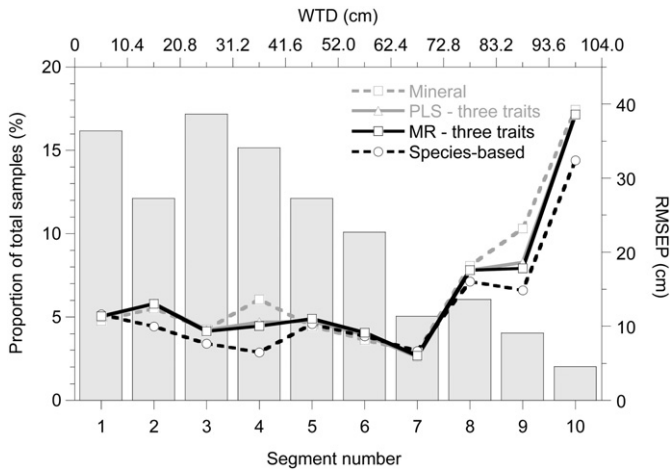


Fig. 5. Segment-wise RMSEP and corresponding proportion of samples along the water-table gradient for the three tested trait-based models and the species-based transfer function of van Bellen et al. (2014).

Test compression was highly positively correlated with WTD and Euglyphida abundance (Fig. 3), whereas biovolume was highly negatively correlated with both compression and WTD. The high degree of compression and low biovolume as observed within the Euglyphida may contribute to their effectiveness as *r*-strategists, as smaller tests are associated with increased mobility (Wilkinson et al., 2012). This also supports the theory that larger tests are associated with higher water tables as they need thicker water films to maintain their position.

Aperture size and position were negatively correlated, i.e. more cryptic apertures were generally smaller, and such apertures were associated with drier conditions (Fig. 3). Aperture size increased with higher water tables which is in agreement with our hypothesis of variable size of available prey linked with water-table position. Aperture size was also strongly positively correlated with biovolume and therefore the significant negative relationship between biovolume and WTD may also explain the negative relationship between aperture size and WTD. Past studies have related aperture size to the position of testate amoebae within the microbial food web. Jassey et al. (2013a) specifically linked the aperture size/body size ratio with the trophic position in

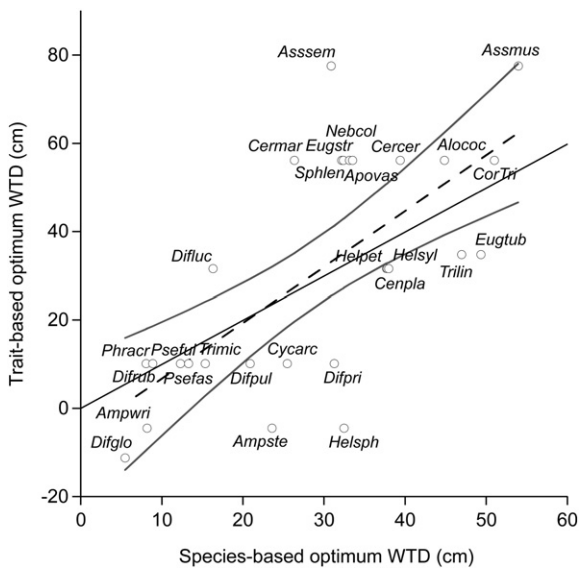


Fig. 6. Relationship between optimum WTD as obtained by the species-based and trait-based transfer functions. Solid line represents $y = x$, dashed line shows modelled linear regression and grey lines represent confidence intervals. Taxa with high residuals have traits that seem incongruent with their optimum WTD as modelled by the species-based transfer function. Taxon abbreviations are detailed in Fig. 1.

the ecosystem, with a high ratio reflecting a preference for predation on other protists and micro-metazoa and a low ratio representative for bacterivores and algivores. Fournier et al. (2015) hypothesized a relationship between aperture size as such (irrespective of body size) and trophic position. Testate amoebae with large apertures may be dominant in sites where peat surface wetness is high enough to sustain the presence of testate amoebae with high biovolume. Aperture size may be related to WTD through the higher abundance of typical prey under wetter conditions.

Mixotrophic taxa were more abundant in wetter conditions in southern Patagonia. Previous studies suggested mixotrophy is an indicator of disturbance, as mixotrophic taxa are rare under (anthropogenic) drying and enhanced dust deposition (Marcisz et al., 2016). In combination with compression and mineral presence, this trait performed well in predicting WTD in our peatlands.

4.2. Comparing trait-based and species-based transfer functions

RMSEP values of the trait-based model are much lower than the standard deviation of the training set WTD of 25.7 cm, suggesting the transfer function has predictive ability. Although performance of the trait-based transfer function was slightly weaker than the species-based transfer function (Table 1), main differences were concentrated at the wet end of the WTD gradient as shown by cross-validation and validation using the independent test set (Figs. 4 and S1). The independent test set revealed that the trait-based transfer function predicted deeper water tables for the wet end of the WTD gradient (Fig. S1). Despite this apparent difference in performance, reconstructed trends from both transfer functions were well correlated for the three cores and the main shifts in WTD were similar (e.g. low water tables at 1480 cal BP in KAR13 and high water tables at 990–940 cal BP in TiA12; Fig. 7) (van Bellen et al., 2016). This coherence between the

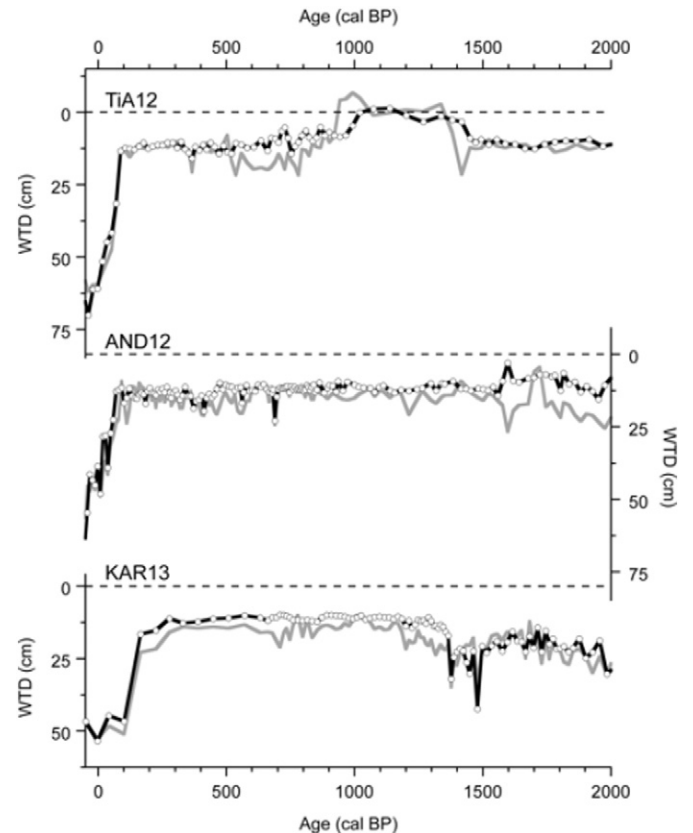
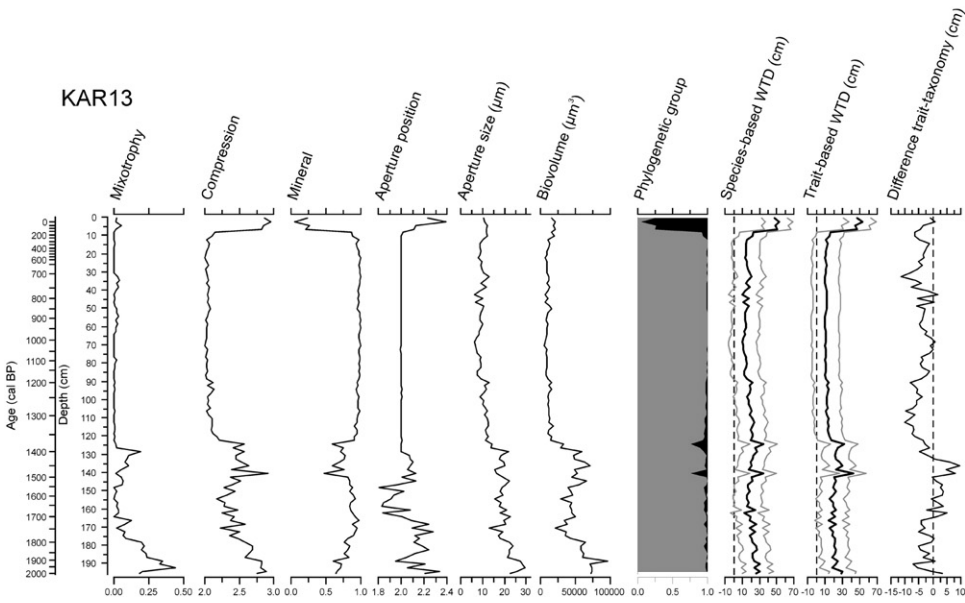
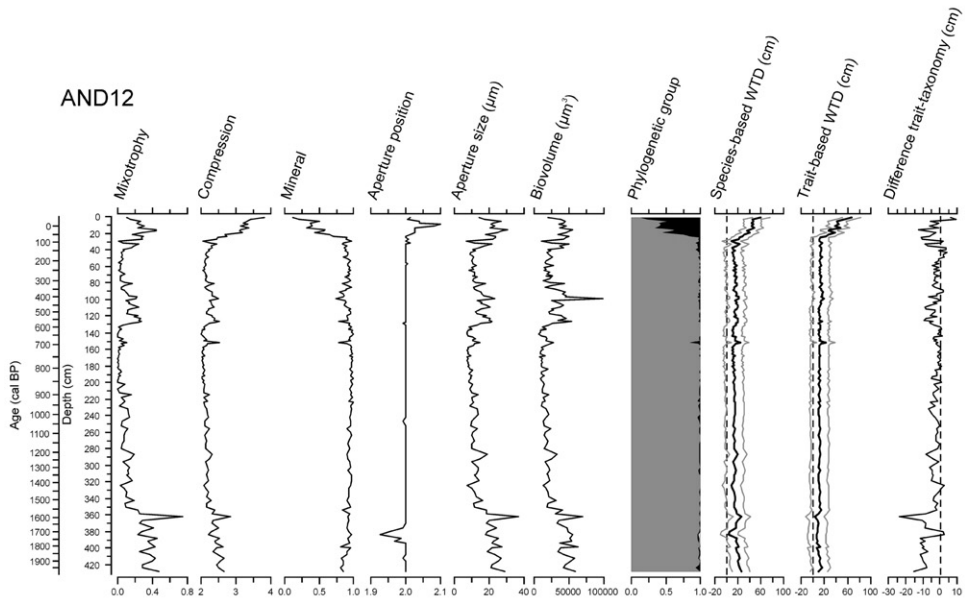
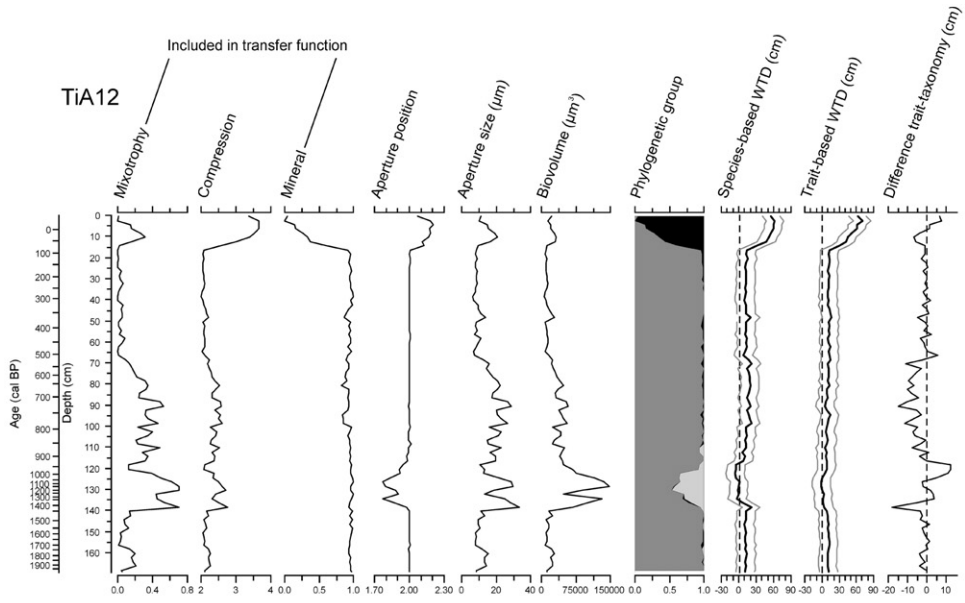


Fig. 7. Comparisons of WTD reconstructions from species-based (grey) and trait-based (black) transfer functions for TiA12, AND12 and KAR13 records for the last 2000 years.



methods at centennial to millennial timescales suggests a degree of signal stability. As a result, interpretations of the species-based and trait-based reconstructions would not be substantively different.

An important recent dry shift was reconstructed using the species-based transfer function (van Bellen et al., 2016) and was reproduced by the trait-based transfer function in all cores (Fig. 8). These sections were characterized by Euglyphida, more compressed tests, a decrease in mineral matter presence and, to a lesser extent, more cryptic apertures (Fig. 8). Whether this sharp dry shift represents a real recent drying or perhaps the effect of increased UV-B affecting microbial life at the surface of the peatland (Robson et al., 2005; van Bellen et al., 2016) remains unclear. The high test compression and low biovolume of *A. muscorum*, the taxon associated with the drying, suggest this taxon is indeed adapted to dry conditions, which thus provides mechanistic evidence for its low water-table optimum.

4.3. Use of trait-based transfer functions for reconstructions

The trait-based transfer function did not outperform the species-based transfer function but reconstructions for three records showed similar trends. Many functional trait CWMs, such as phylogenetic group and mineral presence, and aperture size, aperture position and biovolume were strongly correlated with each other and with WTD. This suggests several combinations of functional traits may be suitable for the reconstruction of water-table depths. However, we warn against the inclusion of too many mutually correlated predictors as there is a risk of over-fitting (Legendre and Legendre, 2012). Such over-fitted models are likely to poorly predict new samples for reconstructions. The different types of cross-validation may not manage to detect over-fitting in multiple regression modelling, the occurrence of which may be assessed only using independent test sets (Hawkins, 2004). Weighted-averaging methods, frequently applied to species-based transfer functions, are less sensitive to such bias from over-fitting because of the implicit unimodal relationship between individual species abundance and the environmental variable to reconstruct. However, the use of independent test sets has been recommended as well for species-based transfer functions using the modern analogue technique to detect possible important autocorrelation between training set sites (Telford and Birks, 2005).

4.4. Value of trait-based transfer functions

Our results provide strong support to the value of transfer functions based on functional traits. Despite the trait datasets containing far less information than the species dataset the trait-based transfer function produced results which very closely matched and showed broadly similar performance with an independent test set. This method will require further testing and validation when applied to other regions and for other types of indicators, but our results suggest that this will be worthwhile. There are several situations where transfer functions based on traits rather than species may be particularly valuable. Trait-based transfer functions may be useful in areas with poorly documented taxonomy, or when fossil samples contain atypical assemblages, for instance including taxa absent from training sets. Trait-based transfer functions may be particularly useful when conducting paleoecological research in regions for which no transfer function is available. Trait-based approaches also offer potential for simplified identification. The accurate identification of testate amoebae to species level requires a level of skill and experience with considerable potential for error (Payne et al., 2011). In contrast, the determination of traits such as mineral material presence and aperture position requires little or no prior experience. It is plausible to imagine that identification of individuals

solely based on traits could 'open up' identification of testate amoebae to many non-specialists such as land managers and inexperienced students (cf. Koenig et al., 2015). This could make the application of testate amoeba bioindication much more widely viable.

While our study has focussed on testate amoebae there is little reason to believe that this approach is only of value in testate amoeba studies. Other microorganism groups also include considerable (morpho) trait variability which is likely to be similarly linked to functional role and environmental conditions (Bremner, 2008; Cleary and Renema, 2007; Pacheco et al., 2010; van der Linden et al., 2012), although this has not been explored yet in paleoecology. Trait-based transfer functions represent a promising new direction in paleoecological research.

5. Conclusion

We hypothesized that transfer functions based on traits may result in more accurate reconstructions, because the relationship between traits and environmental variables is likely to be more direct than the relationship between taxa and environmental variables. Trait-based transfer functions have the advantage that no information is excluded because the elimination of rare taxa is irrelevant. Most functional traits included in this study were indeed primarily related to the water-table position, which explained 42.7% of the variability in trait CWMs. The best-performing multiple regression model, which included a subset of three functional traits, had slightly lower performance than the species-based transfer function. Both transfer functions result in similar reconstructions for three records from Tierra del Fuego, which suggests that for these peatlands the trait-based transfer function is not able to invalidate the WTD reconstructions based on morphospecies. However, this also implies that the identification of only a few functional traits, generally easily identifiable, may be sufficient for reconstructing past trends in water-table depth. As linkages between functional traits and ecosystem functioning have been documented for an increasing range of indicators and environments, we have no reason to believe this method is only valid in testate amoeba research. The trait-based approach may be particularly useful for paleoecological research in regions where transfer functions are currently unavailable. The creation of future transfer functions in other regions may benefit from this novel method.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.palaeo.2016.12.005>.

References

- Amesbury, M.J., Mallon, G., Charman, D.J., Hughes, P.D.M., Booth, R.K., Daley, T.J., Garneau, M., 2013. Statistical testing of a new testate amoeba-based transfer function for water-table depth reconstruction on ombrotrophic peatlands in north-eastern Canada and Maine, United States. *J. Quat. Sci.* 28, 27–39.
- Amesbury, M.J., Swindles, G.T., Bobrov, A., Charman, D.J., Holden, J., Lamentowicz, M., Mallon, G., Mazei, Y., Mitchell, E.A.D., Payne, R.J., Roland, T.P., Turner, T.E., Warner, B.G., 2016. Development of a new pan-European testate amoeba transfer function for reconstructing peatland palaeohydrology. *Quat. Sci. Rev.* 152, 132–151.

Fig. 8. Individual functional trait CWMs and inferred WTD for all records as a function of depth below the surface. Phylogenetic group includes Arcellinida (dark grey), Euglyphida (black) and Amphitremida (light grey). WTD records are shown with standard errors from bootstrapping.

- Barnett, A.J., Finlay, K., Beisner, B.E., 2007. Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshw. Biol.* 52, 796–813.
- Beyens, L., Ledeganck, P., Graae, B.J., Nijls, I., 2009. Are soil biota buffered against climatic extremes? An experimental test on testate amoebae in arctic tundra (Qeqertarsuaq, West Greenland). *Polar Biol.* 32, 453–462.
- Birks, H.J.B., Heiri, O., Seppä, H., Björne, A.E., 2010. Strengths and weaknesses of quantitative climate reconstructions based on Late-Quaternary biological proxies. *Open Ecol. J.* 3, 68–110.
- Booth, R.K., 2002. Testate amoebae as paleoindicators of surface-moisture changes on Michigan peatlands: modern ecology and hydrological calibration. *J. Paleolimnol.* 28, 329–348.
- Booth, R.K., 2008. Testate amoebae as proxies for mean annual water-table depth in *Sphagnum*-dominated peatlands of North America. *J. Quat. Sci.* 23, 43–57.
- Booth, R.K., Meyers, B., 2010. Environmental controls on pore number in *Hyalosphenia papilio*: implications for paleoenvironmental reconstruction. *Acta Protozool.* 49, 29–35.
- Bremner, J., 2008. Species' traits and ecological functioning in marine conservation and management. *J. Exp. Mar. Biol. Ecol.* 366, 37–47.
- Charman, D.J., 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. *Quat. Sci. Rev.* 20, 1753–1764.
- Charman, D.J., Amesbury, M.J., Hinchliffe, W., Hughes, P.D.M., Mallon, G., Blake, W.H., Daley, T.J., Gallego-Sala, A.V., Mauquoy, D., 2015. Drivers of Holocene peatland carbon accumulation across a climate gradient in northeastern North America. *Quat. Sci. Rev.* 121, 110–119.
- Charman, D.J., Blundell, A., Members, A., 2007. A new European testate amoeba transfer function for palaeohydrological reconstruction on ombrotrophic peatlands. *J. Quat. Sci.* 22, 209–221.
- Charman, D.J., Gehrels, W.R., Manning, C., Sharma, C., 2010. Reconstruction of recent sea-level change using testate amoebae. *Quat. Res.* 73, 208–219.
- Charman, D.J., Hendon, D., Packman, S., 1999. Multiproxy surface wetness records from replicate cores on an ombrotrophic mire: implications for Holocene palaeoclimate records. *J. Quat. Sci.* 14, 451–463.
- Charman, D.J., Hendon, D., Woodland, W.A., 2000. The Identification of Testate Amoebae (Protozoa: Rhizopoda) in Peats. Quaternary Research Association, London.
- Cleary, D.F., Renema, W., 2007. Relating species traits of foraminifera to environmental variables in the Spermonde Archipelago, Indonesia. *Mar. Ecol. Prog. Ser.* 334, 73–82.
- Eronen, J.T., Polly, P.D., Fred, M., Damuth, J., Frank, D.C., Mosbrugger, V., Scheidegger, C., Stenseth, N.C., Fortelius, M., 2010. Ecometrics: the traits that bind the past and present together. *Integr. Zool.* 5, 88–101.
- Fenner, N., Freeman, C., Reynolds, B., 2005. Hydrological effects on the diversity of phenolic degrading bacteria in a peatland: implications for carbon cycling. *Soil Biol. Biochem.* 37, 1277–1287.
- Fiakiewicz-Kozieł, B., Smieja-Król, B., Ostrovskaya, T.M., Frontasyeva, M., Siemińska, A., Lamentowicz, M., 2015. Peatland microbial communities as indicators of the extreme atmospheric dust deposition. *Water Air Soil Pollut.* 226, 1–7.
- Fournier, B., Coffey, E.E.D., van der Knaap, W.O., Fernández, L.D., Bobrov, A., Mitchell, E.A.D., 2016. A legacy of human-induced ecosystem changes: spatial processes drive the taxonomic and functional diversities of testate amoebae in *Sphagnum* peatlands of the Galápagos. *J. Biogeogr.* 43, 533–543.
- Fournier, B., Lara, E., Jassey, V.E., Mitchell, E.A., 2015. 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. *The Holocene* 25, 1375–1383.
- Fournier, B., Malysheva, E., Mazei, Y., Moretti, M., Mitchell, E.A.D., 2012. Toward the use of testate amoeba functional traits as indicator of floodplain restoration success. *Eur. J. Soil Biol.* 49, 85–91.
- Gomaa, F., Mitchell, E.A.D., Lara, E., 2013. Amphitremida (Poche, 1913) is a new major, ubiquitous labyrinthulomycete clade. *PLoS ONE* 8, e53046.
- Green, J.L., Bohannon, B.J.M., Whitaker, R.J., 2008. Microbial biogeography: from taxonomy to traits. *Science* 320, 1039–1043.
- Hawkins, D.M., 2004. The problem of overfitting. *J. Chem. Inf. Comput. Sci.* 44, 1–12.
- Heger, T.J., Mitchell, E.A.D., Ledeganck, P., Vincke, S., Van De Vijver, B., Beyens, L., 2009. The curse of taxonomic uncertainty in biogeographical studies of free-living terrestrial protists: a case study of testate amoebae from Amsterdam Island. *J. Biogeogr.* 36, 1551–1560.
- Jassey, V.E.J., Chiapusio, G., Binet, P., Buttler, A., Laggoun-Défarge, F., Delarue, F., Bernard, N., Mitchell, E.A.D., Toussaint, M.-L., Francez, A.-J., Gilbert, D., 2013a. Above- and belowground linkages in *Sphagnum* peatland: climate warming affects plant-microbial interactions. *Glob. Chang. Biol.* 19, 811–823.
- Jassey, V.E.J., Meyer, C., Dupuy, C., Bernard, N., Mitchell, E.A.D., Toussaint, M.L., Metian, M., Chatelain, A.P., Gilbert, D., 2013b. To what extent do food preferences explain the trophic position of heterotrophic and mixotrophic microbial consumers in a *Sphagnum* peatland? *Microb. Ecol.* 66, 571–580.
- JMP, 1989–2007. Version 12. SAS Institute Inc., Cary, NC.
- Juggins, S., 2015. Rioja: Analysis of Quaternary Science Data, R Package Version 0.9–7. <http://cran.r-project.org/package=rioja>.
- Kemp, A.C., Telford, R.J., Horton, B.P., Anisfeld, S.C., Sommerfeld, C.K., 2013. Reconstructing Holocene sea level using salt-marsh foraminifera and transfer functions: lessons from New Jersey, USA. *J. Quat. Sci.* 28, 617–629.
- Klemm, J., Herzschuh, U., Pisarcic, M.F.J., Telford, R.J., Heim, B., Pstryakova, L.A., 2013. A pollen-climate transfer function from the tundra and taiga vegetation in Arctic Siberia and its applicability to a Holocene record. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 386, 702–713.
- Koenig, I., Feldmeyer-Christe, E., Mitchell, E.A.D., 2015. Comparative ecology of vascular plant, bryophyte and testate amoeba communities in four *Sphagnum* peatlands along an altitudinal gradient in Switzerland. *Ecol. Indic.* 54, 48–59.
- Kosakyan, A., Gomaa, F., Lara, E., Lahr, D.J.G., 2016a. Current and future perspectives on the systematics, taxonomy and nomenclature of testate amoebae. *Eur. J. Protistol.* 55 (Part B), 105–117.
- Kosakyan, A., Lahr, D.J.G., Mulot, M., Meisterfeld, R., Mitchell, E.A.D., Lara, E., 2016b. Phylogenetic reconstruction based on COI reshuffles the taxonomy of hyalosphenid shelled (testate) amoebae and reveals the convoluted evolution of shell plate shapes. *Cladistics* 32, 606–623.
- Lacourse, T., 2009. Environmental change controls postglacial forest dynamics through interspecific differences in life-history traits. *Ecology* 90, 2149–2160.
- Laggoun-Défarge, F., Mitchell, E., Gilbert, D., Disnar, J.-R., Comont, L., Warner, B.G., Buttler, A., 2008. Cut-over peatland regeneration assessment using organic matter and microbial indicators (bacteria and testate amoebae). *J. Appl. Ecol.* 45, 716–727.
- Lahr, D.J.G., Grant, J., Nguyen, T., Lin, J.H., Katz, L.A., 2011. Comprehensive phylogenetic reconstruction of *Amoebozoa* based on concatenated analyses of SSU-rDNA and actin genes. *PLoS ONE* 6, e22780.
- Lamarre, A., Magnan, G., Garneau, M., Boucher, É., 2013. A testate amoeba-based transfer function for paleohydrological reconstruction from boreal and subarctic peatlands in northeastern Canada. *Quat. Int.* 306, 88–96.
- Lamentowicz, M., Galka, M., Lamentowicz, Ł., Obremska, M., Köhl, N., Lücke, A., Jassey, V.E.J., 2015. Reconstructing climate change and ombrotrophic bog development during the last 4000 years in northern Poland using biotic proxies, stable isotopes and trait-based approach. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 418, 261–277.
- Lamentowicz, M., van der Knaap, W., Lamentowicz, L., van Leeuwen, J.F.N., Mitchell, E.A.D., Goslar, T., Kamenik, C., 2010. A near-annual palaeohydrological study based on testate amoebae from a sub-alpine mire: surface wetness and the role of climate during the instrumental period. *J. Quat. Sci.* 25, 190–202.
- Lara, E., Heger, T.J., Mitchell, E.A.D., Meisterfeld, R., Ekelund, F., 2007. SSU rDNA reveals a sequential increase in shell complexity among the euglyphid testate amoebae (Rhizaria: Euglyphida). *Protist* 158, 229–237.
- Legendre, P., Legendre, L., 2012. *Numerical Ecology*. Elsevier.
- Loader, N.J., Street-Perrott, F.A., Mauquoy, D., Roland, T.P., van Bellen, S., Daley, T.J., Davies, D., Hughes, P.D.M., Pancotto, V.O., Young, G.H.F., Amesbury, M.J., Charman, D.J., Mallon, G., Yu, Z.C., 2016. Measurements of hydrogen, oxygen and carbon isotope variability in *Sphagnum* moss along a micro-topographical gradient in a southern Patagonian peatland. *J. Quat. Sci.* 31, 426–435.
- Loisel, J., Garneau, M., 2010. Late Holocene paleoecohydrology and carbon accumulation estimates from two boreal peat bogs in eastern Canada: potential and limits of multi-proxy archives. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 291, 493–533.
- Marcisz, K., Colombaroli, D., Jassey, V.E.J., Tinner, W., Kolaczek, P., Galka, M., Karpińska-Kolaczek, M., Słowiński, M., Lamentowicz, M., 2016. A novel testate amoeba trait-based approach to infer environmental disturbance in *Sphagnum* peatlands. *Sci. Rep.* 6, 33907.
- Marcisz, K., Lamentowicz, Ł., Słowińska, S., Słowiński, M., Muszak, W., Lamentowicz, M., 2014. Seasonal changes in *Sphagnum* peatland testate amoeba communities along a hydrological gradient. *Eur. J. Protistol.* 50, 445–455.
- Massaferro, J., Laroque-Tobler, I., 2013. Using a newly developed chironomid transfer function for reconstructing mean annual air temperature at Lake Potrok Aike, Patagonia, Argentina. *Ecol. Indic.* 24, 201–210.
- Mitchell, E.A.D., Gilbert, D., 2004. Vertical micro-distribution and response to nitrogen deposition of testate amoebae in *Sphagnum*. *J. Eukaryot. Microbiol.* 51, 480–490.
- Mitchell, E.A.D., Lamentowicz, M., Payne, R.J., Mazei, Y., 2014. Effect of taxonomic resolution on ecological and palaeoecological inference – a test using testate amoeba water table depth transfer functions. *Quat. Sci. Rev.* 91, 62–69.
- Mitchell, E.A.D., van der Knaap, W.O., van Leeuwen, J.F.N., Buttler, A., Warner, B.G., Gobat, J.M., 2001. The palaeoecological history of the Praz-Rodet bog (Swiss Jura) based on pollen, plant macrofossils and testate amoebae (Protozoa). *The Holocene* 11, 65–80.
- Neville, L.A., Patterson, R.T., Gammon, P., Macumber, A.L., 2013. Relationship between ecological indicators (*Arcellaceae*), total mercury concentrations and grain size in lakes within the Athabasca oil sands region, Alberta. *Environ. Earth Sci.* 72, 577–588.
- Oliverio, A.M., Lahr, D.J.G., Nguyen, T., Katz, L.A., 2014. Cryptic diversity within morpho-species of testate amoebae (Amoebozoa: Arcellinida) in New England bogs and fens. *Protist* 165, 196–207.
- Pacheco, A.S., González, M.T., Bremner, J., Oliva, M., Heilmayer, O., Laudien, J., Riasco, J.M., 2010. Functional diversity of marine macrobenthic communities from sublittoral soft-sediment habitats off northern Chile. *Helgol. Mar. Res.* 65, 413.
- Payne, R.J., Charman, D.J., Matthews, S., Eastwood, W.J., 2008. Testate amoebae as palaeohydrological proxies in Sürmene Agaçbasi Yaylasi peatland (Northeast Turkey). *Wetlands* 28, 311–323.
- Payne, R.J., Creevy, A., Malysheva, E., Ratcliffe, J., Andersen, R., Tsyganov, A.N., Rowson, J.G., Marcisz, K., Zielińska, M., Lamentowicz, M., Lapshina, E.D., Mazei, Y., 2016. Tree encroachment may lead to functionally-significant changes in peatland testate amoeba communities. *Soil Biol. Biochem.* 98, 18–21.
- Payne, R.J., Lamentowicz, M., Mitchell, E.A.D., 2011. The perils of taxonomic inconsistency in quantitative palaeoecology: experiments with testate amoeba data. *Boreas* 40, 15–27.
- Qin, Y., Mitchell, E.A.D., Lamentowicz, M., Payne, R.J., Lara, E., Gu, Y., Huang, X., Wang, H., 2013. Ecology of testate amoebae in peatlands of central China and development of a transfer function for paleohydrological reconstruction. *J. Paleolimnol.* 50, 319–330.
- R Core Team, 2016. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Robson, T.M., Pancotto, V.A., Scopel, A.L., Flint, S.D., Caldwell, M.M., 2005. Solar UV-B influences microfaunal community composition in a Tierra del Fuego peatland. *Soil Biol. Biochem.* 37, 2205–2215.
- Roe, H.M., Patterson, R.T., Swindles, G.T., 2010. Controls on the contemporary distribution of lake thecamoebians (testate amoebae) within the Greater Toronto Area and their potential as water quality indicators. *J. Paleolimnol.* 43, 955–975.

- Singer, D., Kosakyan, A., Pilonel, A., Mitchell, E.A., Lara, E., 2015. Eight species in the *Nebela collaris* complex: *Nebela gimlii* (Arcellinida, Hyalospheniidae), a new species described from a Swiss raised bog. *Eur. J. Protistol.* 51, 79–85.
- Telford, R.J., Birks, H.J.B., 2005. The secret assumption of transfer functions: problems with spatial autocorrelation in evaluating model performance. *Quat. Sci. Rev.* 24, 2173–2179.
- Ter Braak, C.J.F., Šmilauer, P., 2012. *Canoco Reference Manual and User's Guide: Software for Ordination (Version 5.0)*. Microcomputer Power, Ithaca, NY, USA.
- Tilman, D., Knops, J., Wedin, D., Reich, P., Ritchie, M., Siemann, E., 1997. The influence of functional diversity and composition on ecosystem processes. *Science* 277, 1300–1302.
- van Bellen, S., Garneau, M., Booth, R.K., 2011. Holocene carbon accumulation rates from three ombrotrophic peatlands in boreal Quebec, Canada: impact of climate-driven ecohydrological change. *The Holocene* 21, 1217–1231.
- van Bellen, S., Mauquoy, D., Hughes, P.D., Roland, T.P., Daley, T.J., Loader, N.J., Street-Perrott, F.A., Rice, E.M., Pancotto, V.A., Payne, R.J., 2016. Late-Holocene climate dynamics recorded in the peat bogs of Tierra del Fuego, South America. *The Holocene* 26, 489–501.
- van Bellen, S., Mauquoy, D., Payne, R.J., Roland, T.P., Daley, T.J., Hughes, P.D.M., Loader, N.J., Street-Perrott, F.A., Rice, E.M., Pancotto, V.A., 2014. Testate amoebae as a proxy for reconstructing Holocene water table dynamics in southern Patagonian peat bogs. *J. Quat. Sci.* 29, 463–474.
- van der Linden, P., Patrício, J., Marchini, A., Cid, N., Neto, J.M., Marques, J.C., 2012. A biological trait approach to assess the functional composition of subtidal benthic communities in an estuarine ecosystem. *Ecol. Indic.* 20, 121–133.
- Wanner, M., Elmer, M., Kazda, M., Xylander, W.E.R., 2008. Community assembly of terrestrial testate amoebae: how is the very first beginning characterized? *Microb. Ecol.* 56, 43–54.
- Warner, B.G., Charman, D.J., 1994. Holocene changes on a peatland in northwestern Ontario interpreted from testate amoebae (Protozoa) analysis. *Boreas* 23, 270–279.
- Wilkinson, D.M., Koumoutsaris, S., Mitchell, E.A.D., Bey, I., 2012. Modelling the effect of size on the aerial dispersal of microorganisms. *J. Biogeogr.* 39, 89–97.