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Testate amoebae as estuarine water-level indicators: modern distribution and the development of a transfer function from a freshwater tidal marsh (Scheldt estuary, Belgium)

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ABSTRACT: Little is known about the century-scale response of water levels in inland estuaries to sea-level change and human modifications to estuarine morphology. This study explored the ability of using testate amoebae (Protozoa, Rhizopoda) from sediments of a freshwater tidal marsh as indicators of water level in an inland estuary. The hypothesis was that modern testate amoeba assemblages change with surface elevation (approximately the duration of tidal flooding) within a freshwater tidal marsh. Variation in testate amoeba assemblages in relation to multiple environmental variables and sediment characteristics was studied through redundancy analysis. This demonstrated that a significant part of the variation in modern testate amoeba assemblages could be explained by flooding frequency, surface elevation, organic content and particle size of the soil. Transfer functions, partial least squares and weighted average regressions were made to show that testate amoebae can be used for reconstruction of water level (with an accuracy of 0.05 Normalized Elevation). A preliminary test of application of the transfer function to palaeo testate amoeba assemblages showed promising results. Testate amoebae from a freshwater tidal marsh provide a potentially powerful new tool for estuarine water-level reconstructions. Copyright © 2011 John Wiley & Sons, Ltd.

KEYWORDS: ecology; freshwater tidal marsh; testate amoebae; transfer function; water level.

Introduction

The ongoing and future climate change is a severe threat to densely populated areas along lowland coasts and estuaries (Solomon *et al.*, 2007; Miller and Douglas, 2004). The acceleration of sea-level rise (Church and White, 2006) and increase in frequency and intensity of storm surges (Webster *et al.*, 2005) strongly enhance the risks of flood disasters. Along estuaries, these climate-induced flood risks are often further amplified by human-induced modifications to estuarine morphology. In particular, the embankment of intertidal flats and marshes over the past centuries to millennia (e.g. Rippon, 2000) has led along many estuaries to a decrease of intertidal water storage capacity and hence to a significant additional rise of high water levels (e.g. Lane, 2004; van der Spek, 1997). For example in the Scheldt estuary (Belgium, the Netherlands), over the last century the rise of mean high water level has been up to five times faster in the inland estuary than at the coast (Temmerman *et al.*, 2004). Nevertheless, empirical reconstructions of estuarine water-level changes over longer timescales (centuries to millennia) are extremely scarce. Therefore, reconstructions of past estuarine water-level changes are a potentially important reference for our understanding of present-day and future estuarine water-level changes in response to global climate change.

The distribution of protist shells (especially foraminifera and diatoms) in saltmarsh sediments has been used extensively to quantitatively reconstruct Holocene sea-level changes (Horton *et al.*, 2006; Kemp *et al.*, 2009; Campeau *et al.*, 1999; Gehrels *et al.*, 2001; Ghosh *et al.*, 2009; Woodroffe and Long, 2009; Hassan *et al.*, 2006; Ng and Sin, 2003). The most crucial aspect of this method is to establish a transfer function, which is based on the relationship between modern species assemblages and environmental variables. Once calibrated and validated, the transfer function is used to infer past environmental changes from palaeo species assemblages that are preserved in deeper, older sediment layers. For the reconstruction of past sea-level changes, modern protist assemblages of saltmarsh surface sediments are studied in relation to the elevation gradient (i.e. tidal inundation gradient). This relationship is translated in a transfer function in which elevation is expressed as soil elevation relative to sea level. Existing transfer functions for sea-level reconstructions are very accurate with vertical errors of, for example, ±0.08 m for diatoms (Horton *et al.*, 2006) and ±0.10 m for foraminifera (Leorri *et al.*, 2009).

A third group of protists, testate amoebae, has been studied in saltmarsh sediments with the intention of using them as proxies for sea-level reconstructions. Charman *et al.* (1998) demonstrated that saltmarsh testate amoebae appear to be related to elevation and tidal inundation. Since then, multiple studies in Britain and North America have proven that saltmarsh testate amoeba assemblages can be used as accurate (±0.10 m) sea-level indicators (Gehrels *et al.*, 2001, 2006; Charman *et al.*, 2002, 2010; Riveiros *et al.*, 2007).

Despite the fact that sea-level change is often amplified further inland in estuaries, the potential of protists for inland estuarine water-level reconstructions has not yet been investigated. The most inland zone of estuaries is characterized by the presence of freshwater tidal marshes. Existing transfer functions based on saltmarsh protists may not be applied here. For freshwater tidal marshes, foraminifera cannot be used as this group of protists is bound to a marine environment. Diatoms may be expected in freshwater tidal marsh sediments, but high numbers of planktonic species may be buried in freshwater marsh sediments (e.g. Struyf *et al.*, 2007), perhaps hampering the use of diatoms as water-level indicators in a freshwater tidal marsh. Testate amoebae have not been studied so far in freshwater tidal marshes, but they have been found to be good indicators of hydrological conditions in terrestrial freshwater wetlands such as peatbogs (e.g. Charman *et al.*, 2007). Here, we hypothesize that testate amoebae are present in freshwater tidal marsh sediments in high abundances and that variations in species composition are related to variations in tidal flooding, so that a transfer function can be made and used for the reconstruction of estuarine water-level changes, assuming good preservation of tests in stratigraphic records.

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Here the spatial distribution of testate amoeba assemblages in a freshwater tidal marsh will be related to multiple environmental variables (soil elevation, flooding frequency, vegetation type, sediment particle size, organic matter content and bulk density). This study provides a transfer function between species composition and elevation relative to mean high water level (MHWL), normalized elevation and flooding frequency. The transfer functions are then applied to a limited number of palaeo testate amoeba assemblages from a sediment core, demonstrating that testate amoeba can be used in future studies for reconstructing past water-level changes in the inland freshwater tidal zone of an estuary.

Material and methods

Study site

The estuarine part of the Scheldt river is situated in south-west Netherlands and north-west Belgium (Fig. 1). The estuary is 160 km long, extending from its mouth near Vlissingen to its most upstream part at Gent. A full salinity gradient exists, mainly determined by the magnitude of the river discharge, covering a marine part (from the mouth up to Hansweert), brackish part (up to the tributary river Rupel) and freshwater part (up to Gent) (Meire *et al.*, 2005) (Fig. 1). This estuary has a semi-diurnal tidal regime with a mean tidal range that varies along the estuary from 3.85 m at the mouth, reaching a maximum value of 5.39 m at Temse and decreasing again to 2.63 m at the most inland part in Gent (Taveniers and Mostaert, 2009).

The study area, the Notelaar tidal marsh, is situated in the freshwater part (salinity 0–5 PSU – practical salinity units) of the Scheldt estuary (Fig. 1). This freshwater tidal marsh has a surface area of 27 ha, over a length of 2 km and the marsh surface is cut by small tidal channels and creeks (Temmerman *et al.*, 2003a). Fine sediments (clay, silt and fine sand) are supplied and deposited on the marsh during tidal flooding, resulting in heightening of the marsh surface at a rate of 1–2 cm a^{-1} (Temmerman *et al.*, 2003a,b).

The oldest part of the freshwater tidal marsh is visible on the Ferraris maps (1772–1779), whereas the younger part was only established by plant colonization on a mudflat after 1944 (Hoffman, 1993). The sediment stratigraphy of the old and young marsh parts has been studied in detail, based on radiometric dating, sedimentological characterization and description of macroscopic plant remains in sediment cores (Temmerman *et al.*, 2003a, 2004). These studies showed that under the old marsh surface at least 4 m of freshwater tidal marsh sediments exist (based on plant remains and silt content), which must be several hundreds of years old, as dating revealed a mean sediment deposition rate of 1.2 cm a^{-1}. The young marsh part was accreting at a faster mean rate of 4.6 cm a^{-1} between 1944 and 1960, and since then has been accreting at a mean rate of 1.8 cm a^{-1}.

The present-day marsh vegetation exhibits a vertical zonation (Fig. 1). The lowest part, bordering the tidal flat, comprises dense reed (*Phragmites australis*) vegetation, that can reach a canopy height of up to 4 m in summer (Temmerman *et al.*, 2003b). The higher, older part is dominated by willow vegetation (*Salix* sp.), under which multiple herbaceous plants grow (e.g. *Impatiens glandulifera*, *Urtica dioica*, *Convolvulus arvensis)*. At the highest parts of the marsh *Populus canadensis* trees are found.

Sampling method

The surface sediment was collected at 54 sites during two sampling campaigns (Fig. 1C). During the first campaign (January 2008), 44 sites were sampled, six of which were situated on the mudflat in front of the marsh. The other 38 sites were sampled randomly over the elevation range within the marsh vegetation. Throughout the second campaign (September 2009), 10 extra sites were sampled to obtain a more even distribution along the elevation range. Two surface sediment samples were collected at every site. The first one, for analysis of testate amoebae, was obtained by pooling of five subsamples. These were taken in a grid of 20 × 20 cm, using a small sediment corer (diameter = 0.9 cm). The top 2 cm of the

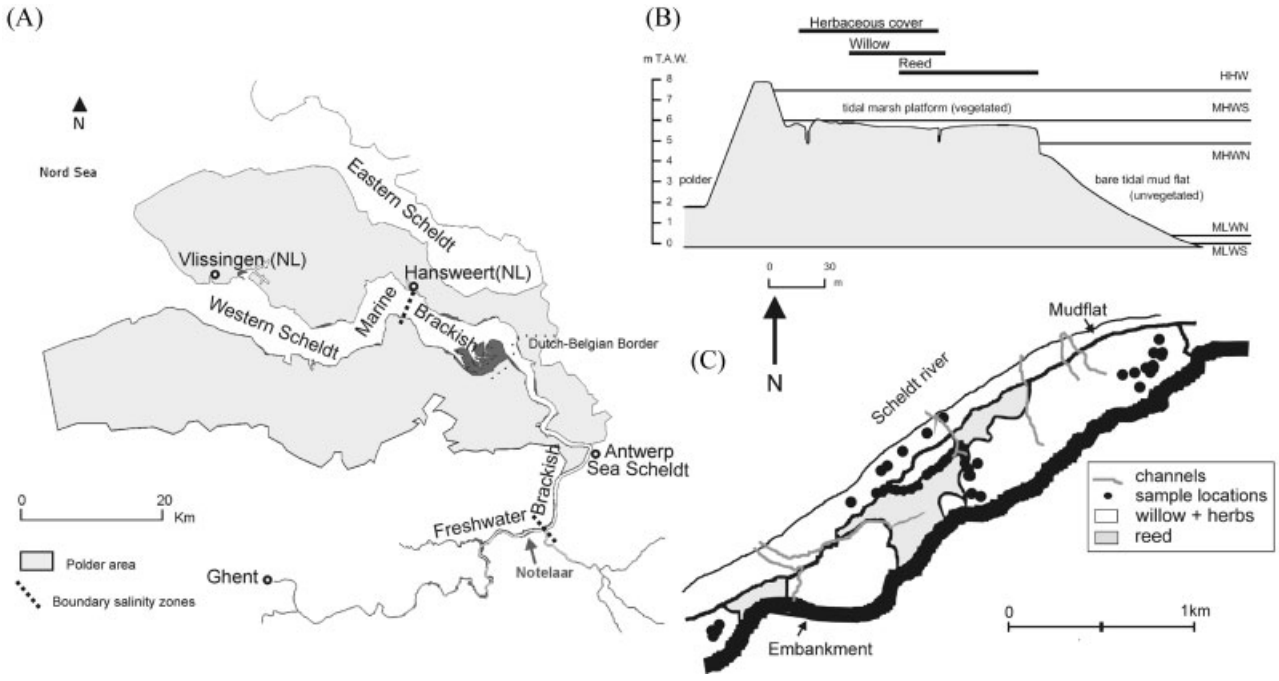

Figure 1. (A) The Scheldt estuary with indication of the Notelaar freshwater tidal marsh. (B) The marsh profile and vegetation of the Notelaar. (C) Map of the Notelaar with indication of sampling locations and indication of vegetation zones.

sediment surface was sampled, in order to recover sediment that has been deposited over about 1 year (Temmerman *et al.*, 2004) and hence to diminish potential seasonal effects in testate amoeba assemblages (Horton *et al.*, 2006). The five subsamples were mixed into one bulk sample and fixed in 5% formaldehyde solution. The second sample was collected for sediment analysis using a corer with a fixed volume of 84.5 cm^3.

Based on the most abundant plant species, each site was classified in three, non-exclusive, different vegetation types (*Phragmites*, *Salix*, herbaceous vegetation). GPS positions were recorded and sediment surface elevation was measured relative to the local Belgian Ordnance Level (m TAW) using a DGPS system and Total station (vertical accuracy of ±1 cm).

Palaeo-sediment samples were collected at the old part of the marsh, within the willow vegetation, using a gouge auger set (diameter = 2.5 cm). Every 5 cm a sample of 1 cm thick was taken for testate amoebae analysis. The selected samples for testate amoebae analysis were 5, 15, 25, 35 and 45 cm deep.

Preparation method for testate amoebae study

The preparation method, used for modern and palaeo samples, was based on Hendon and Charman (1997). Before preparation, exotic *Lycopodium* spore tablets (Stockmarr, 1971) were added to the sample to calculate testate amoeba concentrations. Aggregations of mineral particles and testate amoebae were separated while boiling the sample for 10 min. Thereafter, the sample was sieved and the material between 10 and 300 μm was retained for analysis.

On a glasslide two drops of testate amoebae solution were mixed with one droplet of glycer(ine)ol-Rose Bengal blend to stain the living tests. A coverslide was put on top and the edges were sealed with nailpolish to protect against desiccation. One hundred and fifty testate amoebae were counted per sample using an Olympus BX50 microscope with Nomarski optics. A microscopic magnification of 400 was used for species determinations.

When fewer than 150 tests were found, a maximum of 15 slides was counted. All samples included in the analysis contained more than 100 testate amoebae. Modern samples from 42 sites were included. Samples of the mudflat were omitted, because they contained too low numbers of tests. Four of five palaeo samples contained enough testate amoebae (150 tests); the sample at depth 35 cm was omitted from further analysis.

Taxonomic reference works that were used for testate amoeba species determination were Deflandre (1928, 1929), Decloitre (1962, 1974, 1981), Grospietsch (1964, 1965), Ogden (1983), Chardez (1991), Foissner and Korganova (2000), and Mazei and Tsyganov (2006).

Sediment analysis

First, the dry bulk density was measured by drying the sediment samples for 48 h at 105°C, cooling them in an exsiccator with silicagel and weighing them. Secondly, the organic matter content was determined by loss on ignition (LOI), after combusting the samples for 4 h at 550°C. Thirdly, on a separate subsample, particle size distribution of the mineral fraction was determined, using the laser diffraction technique (Malvern particle size analyser). The grain size classes were based on the Udden–Wenthworth scale (Blott and Pye, 2001): sand (>63 μm), silt (2–63 μm) and clay (<2 μm).

Sediment analyses were only carried out on the modern sediment samples, in order to investigate the possible relationships between modern testate amoeba assemblages and sediment characteristics. No sediment analyses were carried out on the palaeo samples, since they were used to check the applicability of the obtained transfer function for marsh elevation. The sediment characteristics of deeper cores are described in Temmerman *et al.* (2003a) and are similar to the characteristics of surface sediments.

Calculating flooding frequency and normalized elevation

High water level data of 2007 from the closest upstream and downstream tide gauges were used to interpolate high water levels for the Notelaar tidal marsh. Based on the distribution of high water levels, the MHWL and flooding frequency was computed for every sampling location as the percentage of high tides that flood a location.

Normalized elevation was calculated to compare data with other studies, by the use of the following formula:

$$\text{Normalized elevation} = (\text{Elevation} - \text{MTL})/(\text{MHWS} - \text{MTL})$$

where MTL stands for mean tide level and MHWS is the mean high water of spring tides (Zong and Horton, 1999, Charman, 2001). Tidal characteristics of the Notelaar tidal marsh are shown in Fig. 1.

Data analysis

Relative abundances were calculated for the modern testate amoeba species of the remaining 42 sites. Species that have ≥ 2% relative abundance for at least one sample were used for further analysis. Analysis was performed with the total testate amoeba assemblages (dead + alive).

Diversity indices (Shannon-Wiener index) and turnover rate (Sørensen dissimilarity index) were calculated in R (R Development Core Team, 2009). The Sørensen dissimilarity index quantifies the difference in species composition between two successive samples along the elevation gradient. As the distribution of the samples over the elevation gradient was not equal, a moving average of the Sørensen and Shannon-Wiener index values was calculated over a fixed but moving elevation range. The smallest elevation range was selected based on at least two samples for every calculation. The elevation range was 26 cm for the Shannon-Wiener index and 16 cm for the Sørensen dissimilarity index.

Cluster analysis was performed using CONISS in Tg view (Tilia). In this case, square root transformation (Edwards & Cavalli-Sforza's chord distance) was chosen.

Different ordination analyses were carried out in the program CANOCO 4.5. Species data were square root transformed to give rare species more weight in the analysis, because they showed more variability in abundances between the different samples. First, a detrended correspondence analysis (DCA) was performed to determine whether a unimodal or monotonic (linear) model should be used. A gradient length of less than two standard deviations (SD) should indicate the use of a linear model (ter Braak, 1987). Direct ordination analysis was applied to examine the relationship between species and the different environmental variables. Environmental variables were selected based on their variance inflation factor (VIF) values. This factor was calculated for each environmental variable. Large VIF values (>20) indicated that the variable was highly correlated with other environmental variables, while VIF values of zero showed completely multicollinearity between environmental variables (ter Braak and Smilauer, 1998). Environmental variables with VIF values of zero and more than 20 were omitted as they did not contribute to the model. Furthermore,

partial ordination analysis, using the Monte Carlo Permutation Test (999 permutations), was done to estimate the amount of species variation explained by the environmental variables.

Regression analyses were carried out in the program C2 version 1.6.3, to analyse the response of each species to the environmental variable (ter Braak, 1987). A number of different regression models were used to test the robustness of the relationships found. As a linear method, partial least squares regression (PLS) was chosen (e.g. Woodland *et al.*, 1998). Through this method multiple regressions are calculated, extracting underlying factors that explained the greatest amount of variation in the predictor (Testate amoeba assemblages) and response data (environmental variable) (SAS/STAT, 2009).

The weighted average (WA) was used as unimodal regression technique. This method was built on the assumption that a species was most abundant at sites with an environmental variable close to the species optimum (ter Braak and Juggins, 1993). Another method, WA-PLS, was used because it could calculate multiple weighted average regressions. The first calculated component was the original weighted average$_{inverse}$ of the environmental variable. The following components were the weighted averages for the residual of the environmental variable (ter Braak and Juggins, 1993).

The accuracy of the regression analyses was tested by looking at the r^2 and root mean square error of prediction (RMSEP) values. The RMSEP indicated the systematic differences in prediction errors, whereas the r^2 measured only the strength of the relationship between the observed environmental variable and predicted environmental variable values (Horton *et al.*, 2006). The jackknifing technique was applied to calculate r^2 and RMSEP values. In this technique multiple cycles were run. In every cycle the dataset was divided into a training set (for calibration of the transfer function) and a test set (for validation of the transfer function), but for every cycle one sample was omitted from the analysis (Birks *et al.*, 1990; ter Braak and Juggins, 1993).

The ultimate goal was to use the regression analyses as part of a transfer function for soil elevation relative to MHWL, normalized elevation and flooding frequency.

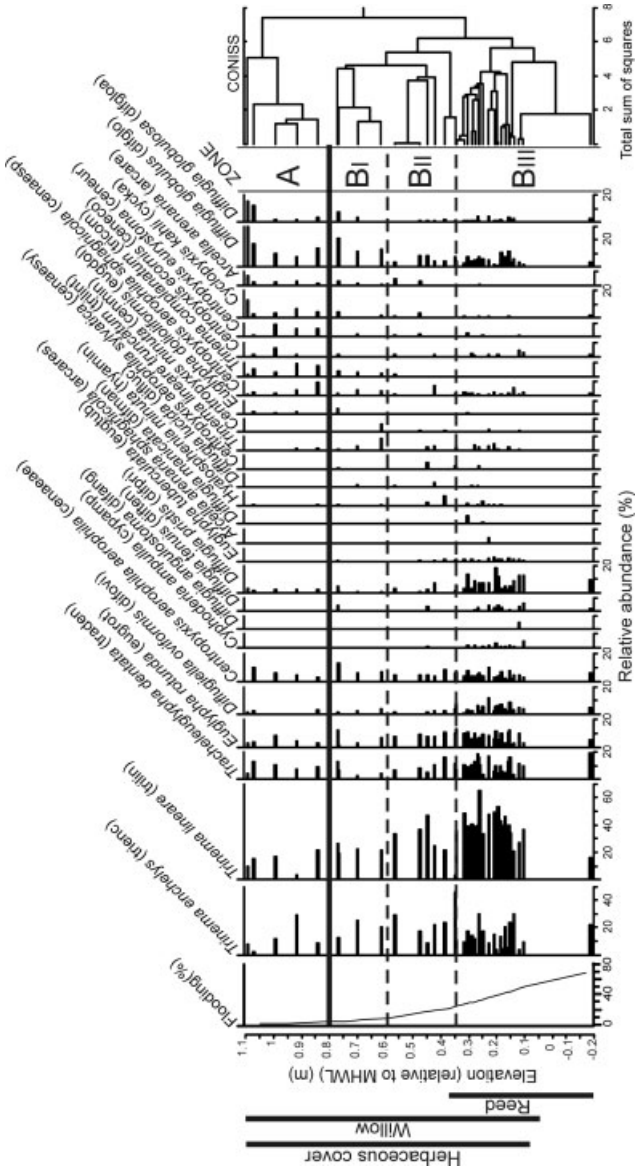

Figure 2. Testate amoeba species diagram, with indication of the four testate amoeba zones based on cluster CONISS analysis.

Results

Testate amoebae and assemblages

The modern testate amoeba assemblage of the freshwater tidal marsh was very species-rich (> 90 species; see Suporting information, Table S1), covering 18 different genera. Almost half of the found testate amoeba tests belonged to the genus *Trinema*, with *Trinema lineare* as most abundant species (32% of total counts). This taxon, together with *Euglypha rotunda*, *Trinema enchelys* and *Tracheleuglypha dentata*, occurred in all analysed samples. Apart from freshwater species, some brackish and one marine interstitial species (*Cyphoderia littoralis*) were found.

Two major modern testate amoeba assemblages were distinguished based on CONISS cluster analysis (Fig. 2). The first major species assemblage (zone A), occurring on the highest elevations, was characterized by *Cyclopyxis kahli*, *Arcella arenaria*, *Difflugia globulus* and *Difflugia globulosa*. The second major species assemblage (zone B) was located in the lower part of the marsh and was characterized by *Difflugia tenuis* and *Euglypha tuberculata*. Three sub-assemblages were recognized in the lower marsh assemblage. The highest of the three (zone BI) contained highest abundances of *Trinema lineare* var. *truncatum* and *Centropyxis minuta*. The middle sub-assemblage (zone BII) was defined by *Hyalosphenia minuta*, *Difflugia lucida* and *Centropyxis aerophila* var. *sylvatica*. Species such as *Difflugia pristis* and *Cyphoderia ampulla* defined the lowest sub-assemblage (zone BIII).

The short unconstrained DCA gradient length (1.702 SD) indicated that a linear model was appropriate. Therefore, redundancy analysis (RDA) was performed to investigate the relationship between the testate amoeba assemblages and environmental variables (flooding frequency, *Salix, Phragmites*, herbaceous cover, LOI, sand, silt, clay, elevation, bulk density). First, two environmental variables, elevation (VIF > 20) and sand (VIF = 0.00), were omitted from the model.

The RDA graph (Fig. 3) showed that *Arcella arenaria*, *Trinema complanatum*, *Cyclopyxis kahli*, *Difflugia globulus* and *Difflugia globulosa* formed a separate group, in agreement with the CONISS analysis (Fig. 2). These species were negatively correlated with flooding frequency (Fig. 3). Partial RDA revealed that the environmental variables together, without sand and elevation, explained 39.3% ($P = 0.0010$) of the species variance. The significant variables were flooding frequency ($P = 0.0330$), LOI ($P = 0.0040$) and clay ($P = 0.0030$). Together, they made up a significant part of the total explained species variance (Table 1).

The four palaeo samples contained 29 testate amoeba species (10 genera), of which two were not found in

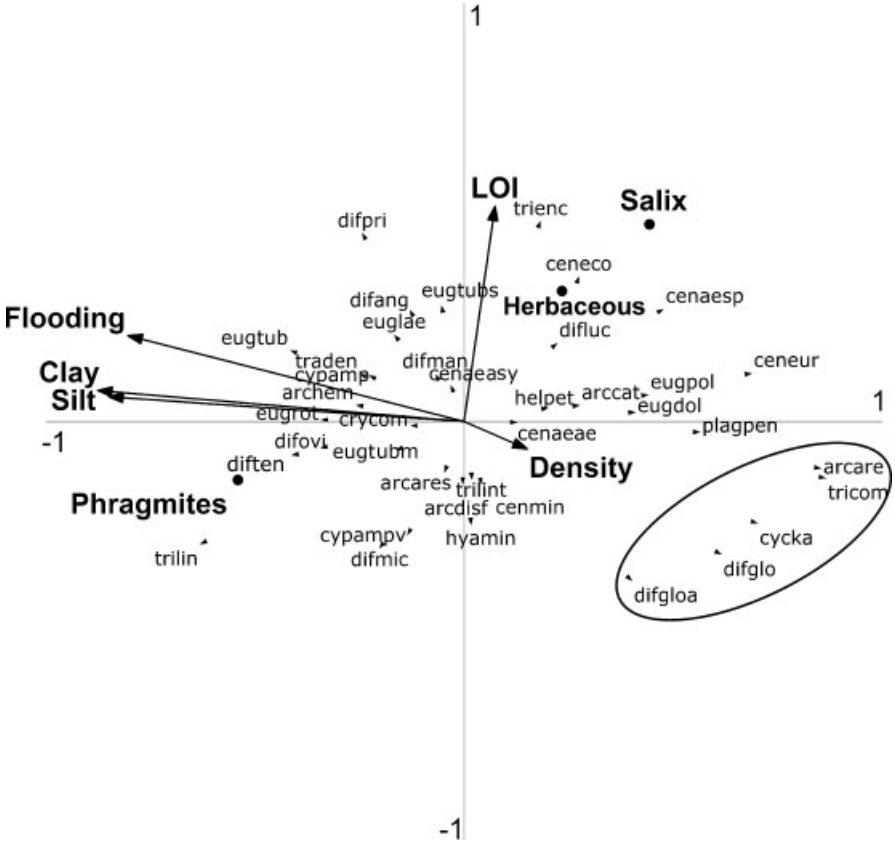

Figure 3. RDA (species were square root transformed). The ellipse shows species that are negatively correlated with flooding frequency and characteristic for zone A (Fig. 2). Abbreviations of species names are given in Fig. 2. Continuous variables are indicated with arrows, categorical variables with centroids.

modern testate amoeba assemblages of this study area (*Difflugia minuta*, *Euglypha strigosa* var. *glabra*; see supporting Table S2). In the palaeo testate amoeba assemblages, *Trinema* was the dominant genera (42%) and *Trinema enchelys* the most abundant species (19% of total counts).

Diversity analysis

The moving average trend line fitted through the Shannon-Wiener species diversity data increased gradually with increasing marsh elevation (Fig. 4). The species diversity tended to be rather constant as long as the flooding frequency was higher than 8%, while the diversity increased with decreasing flooding frequencies smaller than 8% (Fig. 4). For the Sørensen dissimilarity index, there was an overall trend of decreasing dissimilarity with increasing elevation on the marsh. Hence on the low marsh there tended to be larger changes in species composition between successive samples along the elevation gradient, while the higher on the marsh, the smaller changes in species composition between successive samples became. At low flooding frequencies (< 2%) the dissimilarity decreased strongly.

Table 1. Total and partial RDA variation.

	Variation (%)
Total	
Explained variation	39
Unexplained variation	61
Partial	
Loss on ignition	18
Clay	17
Flooding	13
Intercorrelation	52

Transfer function

A transfer function was developed for flooding frequency, elevation (relative to MHWL) and normalized elevation. As the gradient length of the unconstrained DCA (1.702 SD) was close to 2 SD, both linear and unimodal regression methods were applied. The WA-PLS regression was not useful for this dataset as the best component was component 1.

For flooding frequency, the r^2 and RMSEP values of $WA\text{-}TOL_{classic}$ (Table 2) seem to show the best results, but this method tended to overestimate low flooding frequencies and underestimate high flooding frequencies (Fig. 5). On the whole (Table 2 and Fig. 5), $WA_{inverse}$ showed the best fit for flooding frequency.

For the elevation, the cross validation plots were more or less comparable, apart from $WA\text{-}TOL_{inverse}$, which underestimated the elevation of samples high on the marsh, and $WA\text{-}TOL_{classic,}$ which showed a larger error range. As there were no apparent differences between the other regression methods in cross validation, we chose PLS (component 2) as the best regression method for elevation. It had the lowest RMSEP (0.15m).

For the normalized elevation, the cross validation plots were similar to those of elevation, but in this case $WA_{classic}$ was chosen as it showed a slightly better r^2 value (0.70).

The chosen regression methods were applied as a transfer function on the palaeo testate amoeba assemblages. An overview of the results is provided in Table 3. Taking the error range (RMSEP) into account, there were no considerable changes in flooding frequency, elevation or normalized

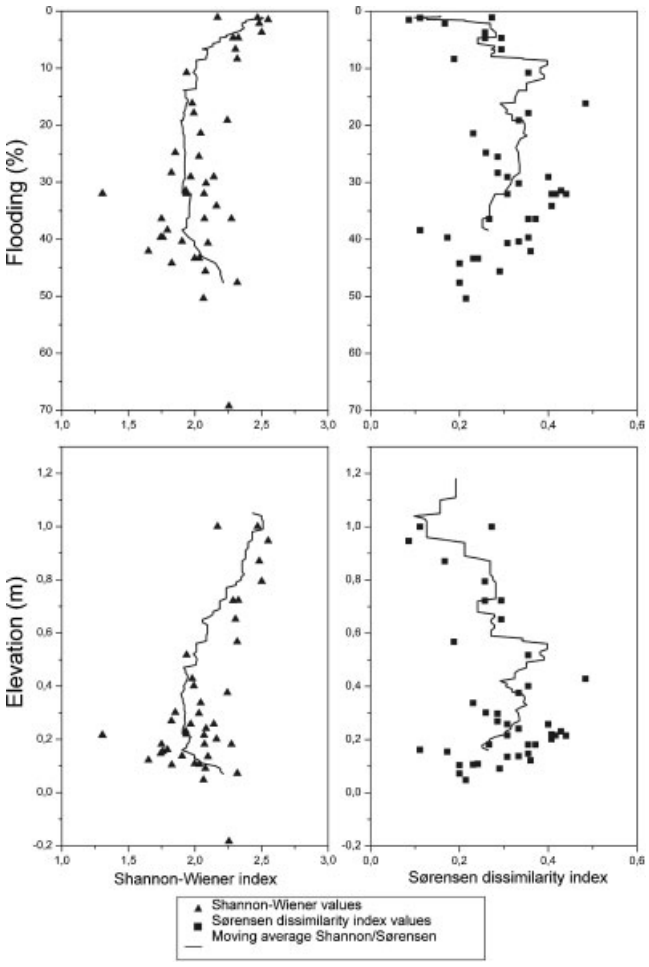

Figure 4. Shannon-Wiener diversity index and Sørensen dissimilarity index plotted against flooding frequency and elevation (m). The line shows the moving average of Shannon-Wiener diversity index and Sørensen dissimilarity index

elevation between the different depths. The reconstructed values were close to the present values, suggesting that the transfer function works.

Discussion

Modern data

This study demonstrates that testate amoebae from freshwater tidal marshes can be used as indicators for estuarine water levels with relatively high accuracy. The precision of the presented transfer function for normalized elevation is similar to published sea-level transfer functions based on foraminifera, diatoms and testate amoebae from salt marshes (Table 4). Both r^2 and RMSEP of our transfer function lay within the range of the salt marsh transfer function values.

The different regression models that were used for the transfer functions for (normalized) elevation and flooding frequency all resulted in r^2 and RMSEP values that were in the same order of magnitude (Table 2). This demonstrates the robustness of the relationships that were found between testate amoeba assemblages and the environmental variables (normalized) elevation and flooding frequency. Although these results are comparable, the precision of the reconstructions of the different regression methods is not. Some regression methods tend to under- or overestimate the lowest and/or highest observed values of variables (Fig. 5). It is not surprisingly that for every environmental variable a different regression method was found to give the best fit; because there is no linear relationship between the environmental variables, flooding frequency decreases exponentially with increasing elevation (Fig. 2).

Although the obtained transfer functions appear to be robust and of similar precision as previously published transfer functions, they must be used cautiously. The relationship between testate amoeba assemblages and elevation or tidal flooding may differ greatly between marshes. Therefore, this study must be considered as a first step towards the use of testate amoebae as indicators of estuarine water levels, and data from more sites along the estuary should follow to enable the construction of a transfer function that is applicable on a more regional scale (for the whole estuary). Furthermore, variation in species assemblages is not related solely to the variation in elevation or flooding frequency. Other environmental variables such as sediment particle size and organic matter content play an important role in our freshwater tidal marsh (Fig. 3), as was also demonstrated for salt marshes (e.g. Charman *et al.*, 2002).

Although freshwater tidal marsh and salt marsh testate amoebae respond to the same environmental variables, there are a number of considerable differences. First, testate amoeba concentrations of salt marshes (up to 65 600 per cm^3) (Charman *et al.*, 1998) are much lower than freshwater tidal marsh testate amoeba concentrations (max. ~300 000 per cm^3). Secondly, testate amoebae inhabit a much smaller vertical range in salt marshes than in freshwater tidal marshes (Table 4). Finally, testate amoebae species might act differently in the two marsh types. For example, *Tracheleuglypha dentata* is a good indicator species for sea-level changes in salt marshes (narrow vertical range) (Gehrels *et al.*, 2006), whereas the species is found over the entire freshwater tidal marsh elevation range.

Four different testate amoeba zones have been distinguished. In zone A (Fig. 2), the species assemblage consists of terrestrial soil testate amoebae (e.g. *Cyclopyxis kahli*, *Difflugia globulus*) (Chardez and Lambert, 1981). The soil testate amoebae *Cyclopyxis kahli* is also found in the highest, and therefore

Table 2. Jack-knifed cross-validation results for partial least squares, weighted average and weighted average-partial least squares regressions. Asterisks (*) indicate values for the chosen regression methods.

	Flooding frequency		Elevation (m)		Normalized elevation	
Regression model	r^2	RMSEP (%)	r^2	RMSEP (m)	r^2	RMSEP
PLS (component 2)	0.54	11.0	0.69*	0.15*	0.69	0.05
$WA_{classic}$	0.60	11.8	0.70	0.17	0.70*	0.05*
$WA_{inverse}$	0.59*	10.3*	0.69	0.16	0.69	0.05
$WA\text{-}TOL_{classic}$	0.64	9.8	0.53	0.23	0.53	0.07
$WA\text{-}TOL_{inverse}$	0.63	11.8	0.51	0.20	0.51	0.06
WA-PLS (component 1)	0.59	10.3	0.69	0.16	0.69	0.05

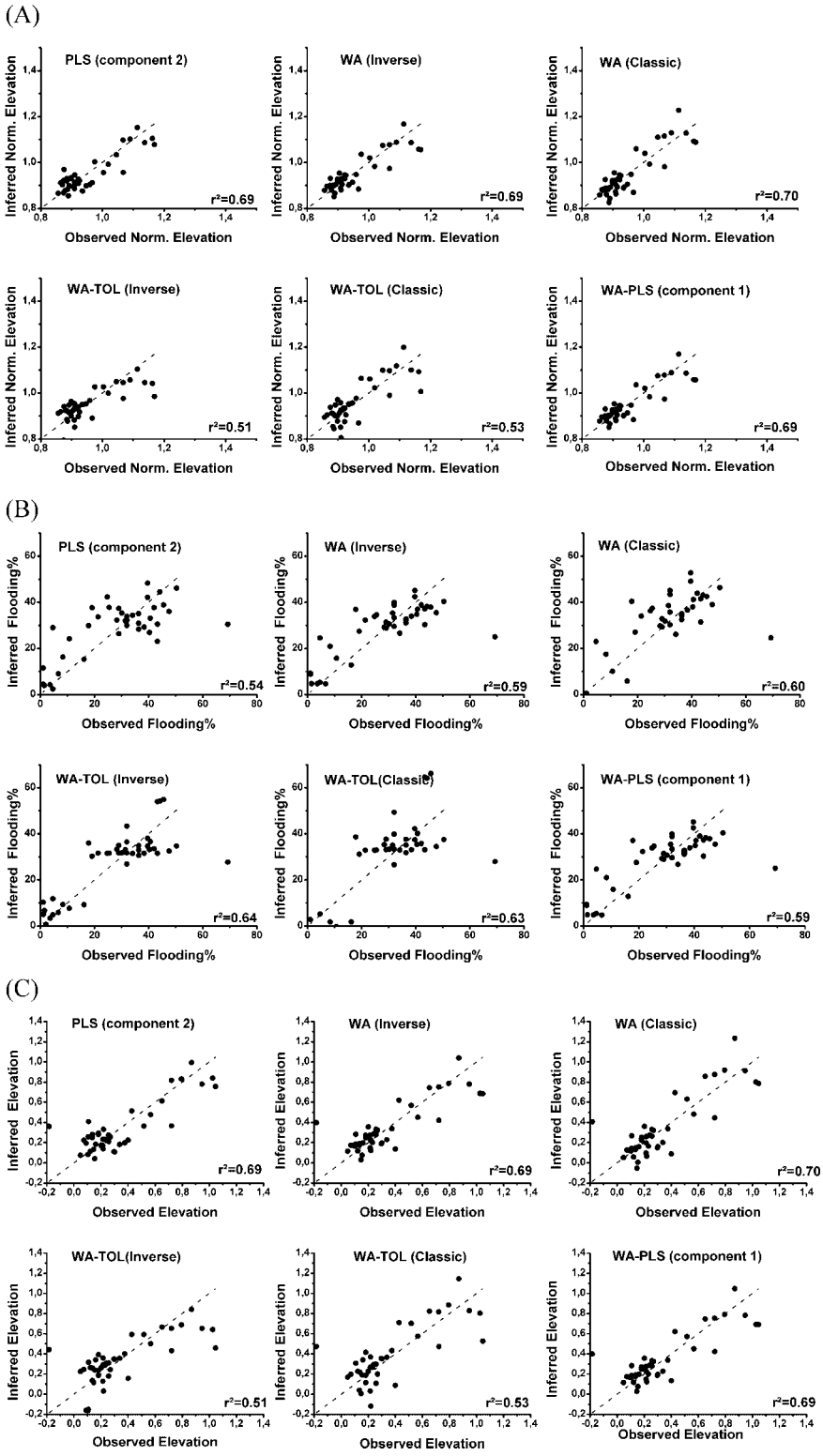

Figure 5. Cross validation plots of PLS, WA and WA-PLS regression methods for normalized elevation (A), flooding frequency (B) and elevation (relative to MHWL) (C).

Table 3. Application of the transfer functions on palaeo-samples.

Transfer function	Method	Modern values	RMSEP (error)	D05	D15	D25	D45
Flooding frequency (%)	$WA_{inverse}$	32	10.3	34.6	37.5	35.75	38.78
Elevation (m)	PLS (comp. 2)	0.22	0.15	0.2	0.18	0.16	0.25
Normalized Elevation	$WA_{classic}$	0.9	0.05	0.9	0.92	0.91	0.96

Table 4. Comparison of our study with studies on salt marshes of the UK (based on Gehrels *et al.*, 2001).

	Training set	Samples (*n*)	Normalized sampled range	Regression model	r^2	Normalized RMSEP	References
Salt marshes (UK)	Testate amoebae	52	1.01–1.36	WA-TOL	0.438	0.076	Gehrels *et al.* (2001)
Freshwater tidal marsh (Belgium)	Testate amoebae	42	0.78–1.17	WA	0.70	0.05	This study
Salt marshes (North America)	Testate amoebae	29	~0.65–1.18	$WA_{classic}$	0.85	0.054	Gehrels *et al.* (2001)
Salt marshes (UK)	Diatoms	94	0.73–1.33	WA-PLS	0.78	0.054	Gehrels *et al.* (2001)
Salt marshes (UK)	Diatoms	88	~0.00–1.40	WA-TOL	0.72	0.214	Zong and Horton (1999) (as in Gehrels *et al.*, 2001)
Salt marshes (UK)	Foraminifera	92	0.73–1.21	PLS	0.38	0.08	Gehrels *et al.* (2001)
Salt marshes (UK)	Foraminifera	131	~0.4–1.2	WA	0.67	0.116	Horton *et al.* (1999) (as in Gehrels *et al.*, 2001)

freshwater, part of the salt marsh in the Seymour-Belize Inlet Complex (Riveiros *et al.*, 2007).

Although a rather high number of testate amoeba species are found, Shannon-Wiener diversity numbers (Fig. 4) are predominantly quite low ($\leq$ 2.5). As the interpretation of Shannon-Wiener diversity index is difficult, the Shannon-Wiener categories following Patterson and Kumar (2002) and Riveiros *et al.* (2007) are applied. They state that the index indicates a stressed environment (Shannon-Wiener index 0.1–1.5), a transition environment (1.5–2.5) or a stable environment (2.5–3.5). This method shows that the whole sampled elevation gradient (zone A – BIII) lies within the category of an 'environment in transition'. In the highest zone (zone A), the Shannon-Wiener index progressively approaches (but does not reach) a value characteristic for a 'stable environment'. Here, less than 2% flooding is required to find a rather stable (Sørensen dissimilarity <0.1; Shannon-Wiener =2.5; Fig. 4) terrestrial soil assemblage (zone A). Flooding frequencies of more than 2% result in a 'transition environment' characterized by lower species diversity and a rise in dissimilarity between adjacent testate amoeba assemblages (zone B; Fig. 2). In zone B, two boundaries separate the three different sub-assemblages. The first boundary, between zone BI and BII, lies exactly on the MHWS level (i.e. mean high water level during spring tides), dividing testate amoebae in a supratidal zone (above MHWS) and an intertidal zone (between MHWS and MTL). In salt marshes, the MHWS forms the lower boundary of testate amoebae occurrence (MHWS = 1.0 Normalized Elevation; Table 4). The reason for the disappearance of salt marsh testate amoebae below MHWS is still not known with certainty, but it is hypothesized that the testate amoebae distribution in salt marshes may be limited by salinity (Charman *et al.*, 1998).

In the freshwater tidal marsh a flooding frequency of 8% can be seen as a threshold value between a supratidal (zone A + BI; Fig. 2) and intertidal (zone BII + BIII; Fig. 2) environment. *Trinema complanatum* can be called a supratidal species, while *Cyphoderia ampulla* is an intertidal species.

The intertidal testate amoebae zone contains two sub-assemblages (zones BII and BIII) which can be separated by the presence and absence of *Phragmites australis*. The boundary matches approximately (height difference of 0.05 m) with the natural upper boundary of the *Phragmites australis* vegetation. Thus, high abundances of *Difflugia pristis* and *Cyphoderia ampulla*, indicator species of zone BIII, might be seen as indicators for the presence of *Phragmites australis*. The fact that *Cyphoderia ampulla* was also only found within the range of *Phragmites australis* at the UK salt marshes of the Erme (transects 1 and 2) and in Brancaster (Charman, 2001) supports this finding.

The lower elevation limit of the appearance of testate amoebae seems to be determined by the presence or absence of marsh vegetation. Within the marsh vegetation, testate amoebae were always found in high abundances (max. ~300 000 per cm^3). At the bare mudflat, however, testate amoebae were only present in much lower concentrations (max. ~3700 per cm^3). This is probably due to the fact that the sediment surface on the bare mudflat is much more mobile during flood tides compared with the marsh sediment surface that is stabilized and protected from erosion by the marsh vegetation (e.g. Temmerman *et al.*, 2003b; Bouma *et al.*, 2005).

Application of the transfer function to palaeo samples

Application of the transfer functions of normalized elevation, elevation and flooding frequency to palaeo samples show the robustness of the function, as all reconstructed values lie close to the modern values. The fact that the reconstructed values do not show important differences in (normalized) elevation or flooding frequency can be explained by the fact that the vertical sediment accretion in the marsh is keeping pace with the rising water level, as reported by Temmerman *et al.* (2003a, 2004). As the marsh has been rising equally with water level, the relative position of our samples to the water level should have stayed the same.

Although the transfer functions seem to work up to a depth of 45 cm (i.e. sediments about 40 years old; Temmerman *et al.*, 2003a), it remains to be seen whether samples from deeper sediments contain enough testate amoebae. Testate amoeba concentrations decreased considerably within the 45-cm depth interval (±73 000 to 3200 tests g^{-1}), which may hamper the counting of tests in deeper sections of the sediment profile. The same problems of decreasing test concentrations with depth were found by Charman *et al.* (2010). A possible explanation for the poor preservation of testate amoebae in coastal settings is given by Roe *et al.* (2002), suggesting that partial dehydration of the fossil tests may influence testate amoebae preservation. This explanation is very unlikely for our freshwater tidal marsh, where the sediment is always saturated. We suggest another possible reason for the poor preservation of testate amoeba fossils in estuarine settings. Freshwater tidal marshes play an important role in estuarine silica cycling (Struyf *et al.*, 2005a,b, 2006). Freshwater tidal marshes contain high amounts of biogenic silica in sediment and vegetation, which dissolves in the pore water and functions as an important source of dissolved silica to the river (Struyf *et al.*, 2005a). The constant export of dissolved silica to the river with every tidal cycle

exponentially decreases the amount of biogenic silica stored in the marsh sediments (Struyf *et al.*, 2007), retaining only 40% of the deposited biogenic silica at a depth of 50 cm. Testate amoebae (together with diatoms) can be the source of biogenic silica in the freshwater tidal marsh sediments. Testate amoebae have shells (partly) made up by silica. This would also imply that there is a selective dissolution of testate amoebae and that testate amoeba assemblages might alter with depth.

Conclusions

This study has demonstrated that testate amoebae from a freshwater tidal marsh can be used as indicators of water level in an inland estuary. The main conclusions are as follows.

1. Freshwater tidal marsh testate amoebae can be used to reconstruct estuarine inland water levels with an accuracy of 0.05 Normalized Elevation. The accuracy is similar to sea-level transfer functions based on salt marsh diatoms, foraminifera and testate amoebae.
2. Testate amoeba assemblages of freshwater tidal marshes are controlled by the same environmental variables as testate amoeba assemblages of salt marshes, being flooding frequency, surface elevation, organic content and particle size of the soil. There are considerable differences between the two marsh types regarding testate amoeba concentrations, behaviour of testate amoeba species and the elevation range over which testate amoeba species occur.
3. The testate amoebae assemblage of the freshwater tidal marsh responds in the first place to flooding frequency, but in second place they seem to react to the presence (*Phragmites australis*) or absence (mudflat) of marsh vegetation.
4. The different transfer functions based on the modern testate amoeba assemblages give good preliminary results when applied to fossil testate amoeba assemblages. However, we cannot exclude that testate amoeba assemblages might be altered over time, because of selective preservation.

The transfer functions presented offer a potentially powerful new tool to reconstruct and investigate Holocene estuarine water-level changes in response to sea-level change and human modifications of estuarine morphologies, assuming good preservation of testate amoebae in the sediments.

Supporting information

Additional supporting information can be found in the online version of this article:

Table S1. Modern counts.

Table S2. Palaeo counts.

Please note: This supporting information is supplied by the authors, and may be re-organized for online delivery, but is not copy-edited or typeset by Wiley-Blackwell. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

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Abbreviations. DCA, detrended correspondence analysis; LOI, loss on ignition; MHWL, mean high water level; MHWS, mean high water of spring tides; MTL, mean tide level; PLS, partial least squares regression; PSU, practical salinity units; RDA, redundancy analysis; RMSEP, root mean square error of prediction; VIF, variance inflation factor; WA, weighted average.

References

Birks HJB, Line JM, Stevenson AC, *et al.* 1990. Diatoms and pH reconstruction. *Philosophical Transactions of the Royal Society of London B* **327**: 263–278.

Blott SJ, Pye K. 2001. Gradistat: a grain size distribution and statistics package for the analysis of unconsolidated sediments. *Earth Surface Processes and Landforms* **26**: 1237–1248.

Bouma TJ, De Vries MB, Low E, *et al.* 2005. Flow hydrodynamics on a mudflat and in salt marsh vegetation: identifying general relationships for habitat characterisations. *Hydrobiologia* **540**: 259–274.

Campeau S, Pienitz R, Hequette A. 1999. Diatoms as quantitative paleodepth indicators in coastal areas of the southeastern Beaufort Sea, Arctic Ocean. *Palaeogeography Palaeoclimatology Palaeoecology* **146**: 67–97.

Chardez D. 1991. Le genre *Cyphoderina* Schlumberger, 1845 (Protozoa: Rhizopoda: Testacea). *Acta Protozoologica* **30**: 49–53.

Chardez D, Lambert J. 1981. Thécamoebiens indicateurs biologiques (Protozoa: Rhizopoda testacea). *Bulletin des Recherches agronomiques de Gembloux* **16**: 181–204.

Charman DJ. 2001. Biostratigraphic and palaeoenvironmental applications of testate amoebae. *Quaternary Science Reviews* **20**: 1753–1764.

Charman DJ, Blundell A, Members A. 2007. A new European testate amoebae transfer function on ombrotrophic peatlands. *Journal of Quaternary Science* **22**: 209–221.

Charman DJ, Gehrels WR, Manning C, *et al.* 2010. Reconstruction of recent sea-level change using testate amoebae. *Quaternary Research* **73**: 208–219.

Charman DJ, Roe HM, Gehrels WR. 1998. The use of testate amoebae in studies of sea-level change: a case study from the Taf Estuary, south Wales, UK. *Holocene* **8**: 209–218.

Charman DJ, Roe HM, Gehrels WR. 2002. Modern distribution of saltmarsh testate amoebae: regional variability of zonation and response to environmental variables. *Journal of Quaternary Science* **17**: 387–409.

Church JA, White NJ. 2006. A 20th century acceleration in global sea-level rise. *Geophysical Research Letters* **33**: L01602 (4 pp.) [doi: 10.1029/2005GL024826]

Decloitre L. 1962. Le genre *Euglypha* Dujardin. *Archiv für Protistenkunde* **106**: 51–100.

Decloitre L. 1974. Le genre Euglypha. Compléments à jour ou 31 décembre 1974 de la monographie du genre parue en 1962. *Archiv für Protistenkunde* **118**: 18–33.

Decloitre L. 1981. Le Genre *Trinema* DUJARDIN, 1841. Révision à jour au 31 XII. 1979. *Archiv für Protistenkunde* **124**: 193–218.

Deflandre G. 1928. Le genre *Arcella* Ehrenberg. Morphologie-Biologie. Essai phylogénetique et systématique. *Archiv für Protistenkunde* **64** (1,2): 152–287.

Deflandre G. 1929. Le genre *Centropyxis* Stein. *Archiv für Protistenkunde* **67**: 322–375.

Foissner W, Korganova GA. 2000. The *Centropyxis aerophila* Complex (Protozoa: Testacea). *Acta Protozoologica* **39**: 257–273.

Gehrels WR, Hendon D, Charman DJ. 2006. Distribution of testate amoebae in salt marshes along the North American East Coast. *Journal of Foraminiferal Research* **36**: 201–214.

Gehrels WR, Roe HM, Charman DJ. 2001. Foraminifera, testate amoebae and diatoms as sea-level indicators in UK saltmarshes: a quantitative multiproxy approach. *Journal of Quaternary Science* **16**: 201–220.

Ghosh A, Saha S, Saraswati PK, *et al.* 2009. Intertidal foraminifera in the macro-tidal estuaries of the Gulf of Cambay: implications for interpreting sea-level change in palaeo-estuaries. *Marine and Petroleum Geology* **26**: 1592–1599.

Grospietsch T. 1964. Die Gattungen Cryptodifflugia and Difflugiella (Rhizopoda testacea). *Zoologische Anzeiger* **172**: 243–257.

Grospietsch T. 1965. Monographische Studie der Gattung Hyalosphenia Stein (Rhizopoda, Testacea). *Hydrobiologia* **26**: 211–241.

Hassan GS, Espinosa MA, Isla FI. 2006. Modern diatom assemblages in surface sediments from estuarine systems in the southeastern Buenos Aires province, Argentina. *Journal of Paleolimnology* **35**: 39–53.

Hendon D, Charman DJ. 1997. The preparation of testate amoebae (Protozoa: Rhizopoda) samples from peat. *Holocene* **7**: 199–205.

Hoffman M. 1993. Vegetatiekundig-ecologisch onderzoek van de buitendijkse gebieden langs de Zeeschelde met vegetatiekartering. *Laboratorium voor Morfologie, Systematiek en Ecologie van de Planten* 214.

Horton BP, Corbett R, Culver SJ, *et al*. 2006. Modern saltmarsh diatom distributions of the Outer Banks, North Carolina, and the development of a transfer function for high resolution reconstructions of sea level. *Estuarine Coastal and Shelf Science* **69**: 381–394.

Kemp AC, Horton BR, Corbett DR, *et al*. 2009. The relative utility of foraminifera and diatoms for reconstructing late Holocene sea-level change in North Carolina, USA. *Quaternary Research* **71**: 9–21.

Lane A. 2004. Bathymetric evolution of the Mersey Estuary, UK, 1906–1997: causes and effects. *Estuarine Coastal and Shelf Science* **59**: 294–293.

Leorri E, Gehrels WR, Horton BP, *et al*. 2009. Distribution of foraminifera in salt marshes along the Atlantic coast of SW Europe: tools to reconstruct past sea-level variations. *Quaternary International* **221**: 104–115.

Mazei YA, Tsyganov AN. 2006. *Freshwater Testate Amoebae*. KMK: Moscow.

Meire P, Ysebaert T, Van Damme S, *et al*. 2005. The Scheldt estuary: a description of a changing ecosystem. *Hydrobiologia* **540**: 1–11.

Miller L, Douglas BC. 2004. Mass and volume contributions to twentieth-century global sea level rise. *Nature* **428**: 406–409.

Ng SL, Sin FS. 2003. A diatom model for inferring sea level change in the coastal waters of Hong Kong. *Journal of Paleolimnology* **30**: 427–440.

Ogden CG. 1983. Observations on the systematics of the genus *Difflugia* in Britain (Rhizopoda, Protozoa). *Bulletin of the British Museum of Natural History (Zoology)* **44**: 1–73.

Patterson RT, Kumar A. 2002. A review of current testate rhizopod (thecamoebian) research in Canada. *Palaeogeography Palaeoclimatology Palaeoecology* **180**: 225–251.

R Development Core Team. 2009. *R: A Language and Environment for Statistical Computing*.

Rippon S. 2000. *The transformation of coastal wetlands. Exploitation and management of marshland landscapes in North West Europe during the Roman and medieval periods*. University Press: Oxford.

Riveiros NV, Babalola AO, Boudreau REA, *et al*. 2007. Modern distribution of salt marsh foraminifera and thecamoebians in the Seymour-Belize Inlet Complex, British Columbia, Canada. *Marine Geology* **242**: 39–63.

Roe HM, Charman DJ, Gehrels WR. 2002. Fossil testate amoebae in coastal deposits in the UK: implications for studies of sea-level change. *Journal of Quaternary Science* **17**: 411–429.

SAS/STAT. 2009. *SAS/STAT 9.2 User's Guide*, 2nd edn. SAS Inc.: Cary, NC.

Solomon S, Qin D, Manning M, *et al*. 2007. *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007*. Cambridge University Press: Cambridge, UK.

Stockmarr J. 1971. Tablets with spores used in absolute pollen analysis. *Pollen Spores* **13**: 615–621.

Struyf E, Dausse A, Van Damme S, *et al*. 2006. Tidal marshes and biogenic silica recycling at the land-sea interface. *Limnology and Oceanography* **51**: 838–846.

Struyf E, Temmerman S, Meire P. 2007. Dynamics of biogenic Si in freshwater tidal marshes: Si regeneration and retention in marsh sediments (Scheldt estuary). *Biogeochemistry* **82**: 41–53.

Struyf E, Van Damme S, Gribsholt B, *et al*. 2005a. Freshwater marshes as dissolved silica recyclers in an estuarine environment (Schelde estuary, Belgium). *Hydrobiologia* **540**: 69–77.

Struyf E, Van Damme S, Gribsholt B, *et al*. 2005b. Biogenic silica in tidal freshwater marsh sediments and vegetation (Schelde estuary, Belgium). *Marine Ecology-Progress Series* **303**: 51–60.

Taveniers E, Mostaert F. 2009. MONEOS-jaarboek monitoring WL 2008: Overzicht monitoring hydrodynamiek en fysische parameters zoals door WL in 2008 in het Zeescheldebekken gemeten. Versie 4.0 WL rapporten, projectnr 883_07. 183.

Temmerman S, Govers G, Meire P, *et al*. 2003a. Modelling long-term tidal marsh growth under changing tidal conditions and suspended sediment concentrations, Scheldt estuary, Belgium. *Marine Geology* **193**: 151–169.

Temmerman S, Govers G, Wartel S, *et al*. 2003b. Spatial and temporal factors controlling short-term sedimentation in a salt and freshwater tidal marsh, Scheldt estuary, Belgium, SW Netherlands. *Earth Surface Processes and Landforms* **28**: 739–755.

Temmerman S, Govers G, Wartel S, *et al*. 2004. Modelling estuarine variations in tidal marsh sedimentation: response to changing sea level and suspended sediment concentrations. *Marine Geology* **212**: 1–19.

ter Braak CJF. 1987. Calibration. In *Data Analysis in Community and Landscape Ecology*, Jongman RHG, ter Braak CJF, van Tongeren OFR (eds.). Pudoc, Wageningen; 299.

ter Braak CJF, Juggins S. 1993. Weighted averaging partial least squares regression (WA-PLS): an improved method for reconstructing environmental variables from species assemblages. *Hydrobiologia* **269–270** (1): 485–502.

ter Braak CJF, Smilauer P. 1998. *CANOCO Reference Manual and User's Guide to Canoco for Windows: Software for Canonical Community Ordination (version 4)*. Microcomputer Power: Ithaca, NY.

van der Spek AJF. 1997. Tidal asymmetry and long term evolution of Holocene tidal basins in The Netherlands: simulation of palaeo-tides in the Schelde estuary. *Marine Geology* **141**: 71–90.

Webster PJ, Holland GJ, Curry JA, *et al*. 2005. Changes in tropical cyclone number, duration, and intensity in a warming environment. *Science* **309**: 1844–1846.

Woodland WA, Charman DJ, Sims PC. 1998. Quantitative estimates of water tables and soil moisture in Holocene peatlands from testate amoebae. *Holocene* **8**: 261–273.

Woodroffe SA, Long AJ. 2009. Salt marshes as archives of recent relative sea level change in West Greenland. *Quaternary Science Reviews* **28**: 1750–1761.

Zong YQ, Horton BP. 1999. Diatom-based tidal-level transfer functions as an aid in reconstructing Quaternary history of sea-level movements in the UK. *Journal of Quaternary Science* **14**: 153–167.