IN pelagic ecosystems, protozoa feed on bacteria and pico- and nano-phytoplankton as well as serving as prey for zooplankton and metazoans, such as fish larva. They are therefore regarded as important link in the cycling of matter and the transfer of energy to higher trophic levels (Azam et al. 1983; Berninger et al. 1991; Calbet and Landa 2004; Sherr and Sherr 1994). Most work on protozoa has been focused on planktonic forms as they are relatively easily sampled. In contrast, the quantitative importance, community structure, and ecological functions of those in benthic environments have been studied less.

The first quantitative studies on benthic protozoa were conducted by Krogh and Sparck (1936), and Mare (1942). They used live observation or simple methods for the separation of benthic protozoa from the sediment and revealed a very large and diverse community in sediments. Later quantitative studies (Fenchel 1969; Hartwig 1973) documented various functional roles of protozoa in benthic environments. The protozoa in sediments were found to differ from pelagic forms not only in their higher abundance, but also by the many special characters they possess. Many of the protozoa living in the sediment display a convergent evolution in size, shape, and physiological adaption to the benthic environment. For instance, their cell shapes are commonly elongate, vermiform, or flat, allowing a large surface-to-volume ratio for respiration (Carey 1992). In addition, many protozoa can be allocated to a variety of nutrition types. For instance, foraminifera and ciliates feed on various trophic levels including bacteria, algae, flagellates, other protozoa, and some even prey on metazoans (Boltovskoy and Wright 1976; Fenchel 1968, 1969; Lei et al. 2010). These diverse feeding types complicate the simple assignment of a trophic position. Due to the high functional diversity and high growth rates...
of protozoa, and especially to the fact that the numbers of protozoa in sediments are higher than those in the water column by one to several orders of magnitude, they have the potential to fulfill an important ecological role in benthic ecosystems (Fenchel 1987; Garstecki et al. 2000; Hamels et al. 2004, 2005).

So far, however, relatively few studies have quantitatively addressed the structure and dynamics of protozoa from different sediment types (e.g. Bak and Nieuwland 1989; Du et al. 2012; Epstein 1997a, b; Hamels et al. 2004; Lee and Patterson 2002; Lei et al. 2010). This can partly be explained by methodological difficulties in experimental approaches, especially in the extraction of these comparatively fragile organisms from sediments, with the difficulty increasing as the sediment becomes finer grained (Alongi 1986; Epstein and Rossell 1995; Starink et al. 1994; Tso and Taghon 2006; Wickham et al. 2000).

Finlay and Esteban (1998) reviewed the biodiversity and ecological function of protozoa in freshwater and suggested that in benthic habitats, ciliate biomass could account for up to 10% of total benthic invertebrate biomass, and ciliate production may even exceed invertebrate production. In groundwater sediments, high species richness of ciliates was even observed in 20–60 cm sediment depth (Andrushchyshyn et al. 2007). However, the species composition, quantity, and distribution of protozoa are likely to differ in different benthic environments (Dietrich and Arndt 2000; Hamels et al. 2004; Madoni 2006; Meng et al. 2012). To obtain a better understanding of microbial food webs in benthic ecosystems, it is important to quantify the distribution and diversity of protozoa in diverse sediment habitats.

We carried out a spatial investigation in 15 stations representing different sediment types to compare benthic protozoa. The main goals of this study were: (1) to compare the diversity and distribution of ciliates, foraminifers, and amoebae in marine, brackish, and freshwater sediments; (2) to examine which environmental factors (temperature, salinity, silt/clay, carbon, nitrogen, and chlorophyll a) influence their distributions in sediments; and (3) to evaluate the potential ecological importance of protozoa in sediments. Further studies concerning the role of interstitial protozoa in transferring matter and energy and their contribution to benthic microbial food webs in the aquatic ecosystem can be based on these results.

MATERIALS AND METHODS

Study sites and sampling

Samples from 15 stations (St. 1–15) in shallow marine, brackish, and freshwater sediments in different climate areas were taken from August 2001 to December 2003 (Table 1). Among the 15 stations, seven sites (St. 1–3, St. 12–15) were located in temperate climate regions, three of them in the U.S., and four in northern Germany, and eight sites (St. 4–11) were from the arctic climate region in the northeast of Greenland. At each sampling, three samples were taken, and environmental factors (temperature, salinity, silt/clay, carbon, nitrogen, and chlorophyll a) were measured (Stumm 2006).

The marine and brackish sediment samples in tidal areas were taken 500 m from the waterline at low tide. The freshwater sediments and marine sediment in nontidal areas were collected in nearshore shallow waters. The sediments were sampled using round plexiglass cores (36-mm inner diam.) or by carefully removing an undisturbed section of the upper tidal flat sediment with a shovel. The samples were stored in the dark at 4 °C, transported to the laboratory immediately and processed within 4 h. In the laboratory, about 2 ml (ca. 6 cm2) of a subsample was sliced off from the top 3 mm of the sediment and transferred into a sterile tube. The subsample was diluted with 2 ml of 0.2 μm-filtered ambient water from the each sampling location, and immediately chemically fixed with ice cold glutaraldehyde solution (final concentration 2%). The fixed samples were stored at 4 °C in the dark for a maximum of 2 wk until further processing.

Table 1. Basic information of the 15 sampling sites

| Sampling sites           | Stations | Habitats          | Date       | Temperature (°C) | Salinity (psu) | Location          |
|-------------------------|----------|-------------------|------------|-----------------|----------------|------------------|
| Greenlane Lake, USA     | St. 1    | Lake              | Dec 3, 2003| 0               | 0              | 40°20’N; 75°27’E |
| Nockamixon Lake, USA    | St. 2    | Lake              | Dec 3, 2003| 7               | 0              | 40°28’N; 75°13’W |
| Shark River Bay, USA    | St. 3    | Estuary           | Nov 20, 2003| 12              | 26             | 40°10’N; 74°01’E |
| Basalt Lake, Arctic     | St. 4    | Lake              | Sep 8, 2003| 9               | 5              | 72°43’N; 22°27’W |
| Duck Lake, Arctic       | St. 5    | Lake              | Aug 22, 2003| 10              | 0              | 76°25’N; 18°45’W |
| Lake near Hochstetter Fjord, Arctic | St. 6 | Pond              | Sep 6, 2003| 6               | 4              | 75°37’N; 19°44’W |
| Ice floe, Arctic        | St. 7    | Pond              | Aug 20, 2003| 1               | 5              | 77°09’N; 01°12’W |
| Koldevey Beach, Arctic  | St. 8    | Marine tidal flat | Aug 13, 2003| 2               | 36             | 76°06’N; 18°30’W |
| Melles Lake, Arctic     | St. 9    | Lake              | Aug 13, 2003| 9               | 3              | 76°07’N; 18°37’W |
| Shannon Beach, Arctic   | St. 10   | Marine tidal flat | Aug 26, 2003| 5               | 36             | 76°07’N; 18°31’W |
| Potsdam Lake, Arctic    | St. 11   | Lake              | Aug 26, 2003| 7               | 3              | 75°03’N; 18°45’W |
| Dorum, Germany          | St. 12   | Brackish tidal flat| Jun 13, 2002| 16              | 25             | 53°42’N; 08°29’E |
| Köln, Germany           | St. 13   | Lake              | Jun 2, 2003 | 24              | 0              | 50°58’N; 74°01’E |
| Pliön, Germany          | St. 14   | Lake              | Nov 13, 2001| 4               | 0              | 54°09’N; 10°26’E |
| Sytt, Germany           | St. 15   | Marine tidal flat | Sep 12, 2002| 21              | 31             | 55°02’N; 08°25’E |

The samples were taken from the upper 3-mm sediment layer. Temperature was measured within the sediment.
Extraction and staining

Benthic protozoa were separated from the sediments by using centrifugation in a silica gel (Percoll, Sigma, Deisenhofen, Germany) gradient, following a modified protocol of Wickham et al. (2000). The supernatant was gently (< 5 mmHg) filtered onto 1.2 μm pore-size cellulose nitrate filters and impregnated on glass slides according to the quantitative protargol stain (QPS) method (Montagnes and Lynn 1987; Skibbe 1994). The slides were viewed in their entirety with a compound microscope (Nikon Eclipse E800, Nikon Instruments INC Shinjuku-ku, Tokyo, Japan) equipped with a high-power oil immersion objective at 200 to 1,000X magnification.

Enumeration and classification

Protozoan communities (ciliates, foraminifera, amoebae, and a heterotrophic flagellate Stephanopogon) were enumerated under the microscope. The other heterotrophic flagellated protozoa were not included in this study due to the method used (QPS). Ciliates were studied at species level and were identified using Kahl (1930–1935), Carey (1992), Lynn and Small (2002), and related taxonomic literature.

Data analyses

For ciliates, species composition, species richness (number of species per sample), and diversity were determined. Ciliates were differentiated into 18 groups: Bursaridium, chlamydodontids, dysteriids, euplotids, Frontonia, cociliates, strombidiids, stichotrichs, and synhymeniids.

The other protozoa including amoebae, foraminifers, and Stephanopogon (a large flagellate) were analysed at the group level. The biovolumes of amoebae and foraminifers were calculated using individual cell size inside of the shell measured from protargol preparations and common geometric equations, and were converted to carbon content using appropriate conversion factors (Borsheim and Bratbak 1987; Edler 1979; Hillebrand et al. 1999; Menden-Deuer and Lessard 2000).

Statistics

Among the protozoa studied, ciliates were the most frequent group occurring in every station and were studied at species level, and as a result, statistical analyses were mainly conducted by using the ciliate data. Univariate correlations (Spearman’s r values) between biotic variables (ciliate abundance, biomass, diversity, and species richness) and abiotic variables (temperature, salinity, silt/clay, carbon, nitrogen, and chlorophyll a) were analysed to evaluate the relationships between community parameters and environmental conditions. These analyses were carried out using SAS, ver. 6.12 (SAS Institute Inc 1989).

The multivariate analyses of spatial distributions in benthic ciliate communities were analysed using PRIMER v6.1 package (Clarke and Gorley 2006). The matrices of 15 stations based on all ciliate community parameters (abundance, biomass, species richness, Margalef index, species evenness, and Shannon–Wiener diversity index) were analysed by multidimensional scaling (MDS) ordination on Bray–Curtis similarity matrices. Clustering the 15 sampling sites based on dominant ciliate species was assigned by the routine CLUSTER, with data from fourth root-transformed species-abundance data. The submodule BIOENV was used to find a subset of the abiotic parameters that most closely matched the biotic matrix to determine the comprehensive effects of environmental factors. The significance of biota–environment correlations was tested using the routine RELATE (Clarke and Gorley 2006).

RESULTS

Distributions of protozoa

The abundance of protozoa varied between 10 cells/ml (St. 4 and 10) and 1,499 cells/ml (St. 15). The high number at St. 15 was due to the high number of comparably small amoebae. The biomass of protozoa varied from 0.01 μg - C/ml at St. 4 to a maximum of 18.98 μg C/ml at St. 12, where the high biomass was due to high number of large foraminifera (Fig. 1, 2). Protozoan communities were dominated by ciliates in terms of abundance in most stations, especially at St. 4, 10, 13, and 14. However, in term of biomass, ciliates were dominant at only six of the 15 stations, whereas amoebae were dominant in the other seven stations. Only one station (St. 12) was dominated by foraminiferal biomass.
In more detail, amoeba abundance ranged from 0 to 937 cells/ml (median: 11 cells/ml; SD = 286), and biomass from 0 to 4.71 μg C/ml (median: 0.55 μg C/ml; SD = 1.44). Highest amoeba density was observed at station 15 and contained only naked forms. In addition, naked amoebae were also dominant at St. 8, whereas at St. 1 only testaceous amoebae were observed. On an ice floe (St. 7), amoebae were also only comprised of naked forms and contributed to an average of more than 96% to total protozoan abundance or biomass. At the other stations, both naked and testate amoebae were found.

The range of foraminifer abundance was 0–155 cells/ml (median: 0 cells/ml; SD = 39.9), while the biomass range was 0–15.87 μg C/ml (median: 0 μg C/ml; SD = 4.1). Although foraminifers were not a frequent group among stations, they dominated with more than 84% of the total protozoan biomass at St. 12. Rotalid species were commonly found and Ammonia tepida was usually dominating in foraminiferal communities based on monthly samplings over a year at this station (Lei 2005). Food vacuoles of this species were observed often filled with small diatoms and cyanobacteria.

The flagellate *Stephanopogon* spp. was found only in sediments of St. 8 and St. 10. Their abundance (0.8–2.5 cells/ml) and biomass (0.0008–0.002 μg C/ml) were very low and therefore contributed a very small portion to the total protozoan abundance and biomass (Fig. 2).

**Distributions of ciliates**

Ciliate abundance ranged from 9 to 562 cells/ml (median: 31 cells/ml; SD = 198), and biomass from 0.004 to 2.78 μg C/ml (median: 0.07 μg C/ml; SD = 0.79) among stations. The lowest abundance and biomass were found at the arctic stations (St. 4 and 10), and the highest values occurred at the German St. 12 and St. 15 (Table 2). A total of 91 ciliate species representing 53 genera were identified from the 15 sampling sites (Appendix S1). Among those species, 71 were recorded from the German stations (St. 12–15), 41 were from Arctic stations (St. 4–11), and 32 were from U.S. stations (St. 1–3). Ciliate community parameters at the 15 stations are shown in Fig. 3 and Table 2. Not surprisingly, high species richness or diversity usually occurred in samples with high abundance or biomass. Ciliate species richness ranged from 5 (St. 4) to 30 (St. 14), and the Margalef index from 1.75 (St. 2) to 4.79 (St. 14). Shannon–Wiener diversity index (H) varied between 1.21 (St. 4) and 2.78 (St. 14), and community evenness (J) ranged from 0.56 (St. 11) to 0.96 (St. 5).

The composition of dominant species varied among stations (Table 3). *Chlamydonella lori* was most frequently the dominant species, reaching 59% of total ciliate abundance (at Basalt Lake freshwater sites, St. 4) and 17–36% of total abundance at three other stations (Table 3). Several *Chilodonella* species such as *Chilodonella uncinata* were also frequent (e.g. St. 1, 6, and 7). Scuticociliates were the next most frequent group (St. 1, 2, 8, and 13), some of which were bacterivorous species (e.g. *Cyclidium* sp. and *Uronema marinus*), while others were herbivorous (e.g. *Pleuromenia marinum*). Frontonia was frequently dominant in terms of biomass (on average 10% contribution across all stations), while euglenoids and *Aspidisca* were sometimes dominant in terms of abundance (on average 13% contribution across all stations). For instance, *Aspidisca fusca* occupied an average of 18% and 36% of abundance at St. 3 and St. 12 respectively.

Based on ciliate groups, the most frequent ciliate groups commonly were the chlamydodontids, scuticociliates, and prorodontids, contributing on average 22%,
Table 2. Ciliate community parameters at the 15 sampling sites

| Parameters           | St.1 | St.2  | St.3 | St.4 | St.5 | St.6 | St.7 | St.8 | St.9 | St.10 | St.11 | St.12 | St.13 | St.14 | St.15 |
|----------------------|------|-------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|
| Abundance (cells/ml) | 55   | 31    | 82   | 9    | 12   | 19   | 30   | 21   | 17   | 9     | 127   | 314   | 501   | 426   | 562   |
| Biomass (µg C/ml)    | 0.07 | 0.03  | 0.15 | 0.00 | 0.05 | 0.04 | 0.07 | 0.01 | 0.01 | 0.01  | 0.23  | 2.78  | 0.29  | 1.36  | 2.34  |
| Species richness     | 10   | 7     | 19   | 5    | 11   | 7    | 10   | 7    | 6    | 5     | 19    | 18    | 20    | 30    | 19    |
| Margalef index       | 2.25 | 1.75  | 4.09 | 1.81 | 4.02 | 2.04 | 2.64 | 1.97 | 1.78 | 1.80  | 3.72  | 2.96  | 3.06  | 4.79  | 2.84  |
| Species evenness     | 0.78 | 0.72  | 0.87 | 0.75 | 0.96 | 0.88 | 0.57 | 0.85 | 0.94 | 0.80  | 0.56  | 0.74  | 0.75  | 0.82  | 0.68  |
| Diversity (H’)       | 1.80 | 1.39  | 2.56 | 1.21 | 2.29 | 1.71 | 1.32 | 1.65 | 1.69 | 1.29  | 1.64  | 2.13  | 2.25  | 2.78  | 2.00  |

Figure 3 Relationships between ciliate abundance and diversity (A) and between ciliate biomass and species richness (B) at 15 stations.

20%, and 12% of total abundance, respectively, and on average 13%, 14%, and 16% of total biomass across all stations (Fig. 4). Oligotrichous ciliates did not dominate at any station, and only Strombidium saebreya was relatively abundant at St. 3 (6 cells/ml). In addition, peritrichs, stichotrichs, and heterotrichs only occasionally dominated the ciliate abundance or biomass, and some groups (e.g. strombiids, plagiohyllids, and synhymeniids) were never dominant either in terms of abundance or biomass (Fig. 4).

It should be mentioned that although their abundance was not very high, the large heterotrichous ciliates Condyllostoma remanei and C. arenarium (more than 500 µm long) contributed 37% and 25% to total ciliates biomass at St. 14 and St. 15 respectively. In addition, these heterotrichs were also observed in the brackish St. 12. Moreover, a karyorelictid ciliate Trachelocerca sp. occurred only at St. 3, St. 9, and St. 12, and this large species sometimes made up a considerable proportion of total ciliate biomass. In addition, some typical benthic small-sized species (e.g. Gastronauta derouxi, Microthorax simplex, and Pseudomicrothorax dubius) were frequently observed.

The food vacuoles of benthic ciliates were observed with diatoms and flagellates in most species or ciliate groups, especially for chlamydodontids, Aspidisca, Frontonia, and Pleurostomatids. In contrast, small scuticociliates had mainly bacterial remnants in their food vacuoles. The food vacuole of some haptorids or karyorelickids contained small scuticociliates.

Linkage between ciliate community parameters and environmental factors

Spearman’s correlation analysis between ciliate community parameters (abundance, biomass, diversity, and species richness) and environmental variables were conducted. The results showed that diversity and species richness were significantly and positively correlated with abundance and biomass, and among the environmental factors, only chlorophyll a was significantly correlated with the community parameters (Table 4).

The associations between ciliate community parameters (species abundance, community abundance, biomass, species richness, Margalef index, Shannon–Weiner diversity index, and evenness) and environmental variables were established by generating bivariate and abiotic similarity matrices using MDS and then comparing them with multivariate biota–environment (BIOENV) analysis (Table 5). The results showed that the best match with individual ciliate species occurred with the combination of salinity, temperature, and silt/clay. The abiotic parameters best matching ciliate community parameters were similar, occurring with the combination of temperature, silt/clay, and carbon. It was notable that temperature and salinity were included in almost all correlations. The biotic variables significantly correlated with several combinations of environmental factors (Table 5).

The relationships among the 15 sampling sites based on environmental data and dominant ciliate species were detected by cluster analysis (Fig. 5). These dendograms were conducted using Euclidean distance from the fourth root-transformed station data. The cluster analysis resulted in 15 stations falling into four clades (1–4) at 40% similarity level: clade 1 was composed of St. 13, St. 1, St. 9, St. 5, and St. 2, all belong to temperate freshwater sites; clade 2 was made up of St. 14, St. 11, St. 6, and St. 7, mostly Arctic freshwater sites; clade 3 included St. 10, St.
Table 3. Distribution of dominant ciliate species at the 15 sampling places showing the per cent of total ciliate abundance at each station

| Dominant ciliates       | St.1 (%) | St.2 (%) | St.3 (%) | St.4 (%) | St.5 (%) | St.6 (%) | St.7 (%) | St.8 (%) | St.9 (%) | St.10 (%) | St.11 (%) | St.12 (%) | St.13 (%) | St.14 (%) | St.15 (%) |
|-------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| Aspidisca fusca         | –        | –        | 18       | –        | –        | –        | –        | –        | –        | 36       | –        | –        | 2        |
| Aspidisca lyncaster     | –        | –        | 12       | –        | –        | –        | 6        | –        | –        | 3        | –        | –        | –        |
| Aspidisca polypha      | –        | –        | –        | –        | –        | –        | –        | –        | –        | 1        | 16       | 8        | 1        |
| Bursariidum             | –        | –        | –        | –        | –        | –        | –        | –        | –        | 9        | –        | –        | –        |
| Chilodonella sp.1      | 27       | –        | –        | 5        | –        | –        | 16       | –        | –        | –        | 9        | –        | –        |
| Chilodonella sp.3      | –        | –        | –        | –        | –        | –        | 29       | –        | –        | –        | –        | –        | –        |
| Chilodonella uncinata  | –        | –        | –        | –        | 24       | –        | 5        | 9        | 3        | –        | 3        | 4        | –        |
| Chlamydonellopsis sp.1 | –        | 17       | 59       | –        | –        | 35       | –        | 36       | –        | –        | –        | 2        |
| Coleps sp.1             | –        | –        | –        | –        | –        | –        | –        | –        | –        | 1        | –        | 1        | 51       |
| Euplotes sp.1           | –        | –        | –        | 5        | –        | –        | 54       | –        | –        | –        | –        | –        | 2        |
| Gastronuta derouxi      | –        | –        | 16       | –        | –        | –        | –        | –        | –        | 6        | –        | 2        | –        |
| Microthorax simplex    | –        | –        | –        | 5        | –        | –        | –        | –        | –        | 27       | –        | –        | 2        |
| Peritrichia gen. sp.1  | –        | –        | –        | –        | 15       | –        | –        | 32       | –        | –        | –        | 1        | –        |
| Peritrichia gen. sp.2  | –        | –        | –        | –        | 15       | –        | –        | –        | –        | –        | –        | –        | –        |
| Prorodon sp.1           | 19       | 12       | –        | 10       | 16       | 3        | –        | 16       | –        | 1        | –        | 7        | –        |
| Pseudomicrothorax dubius| 10       | –        | –        | –        | 12       | –        | 12       | –        | –        | –        | 7        | 9        | –        |
| Remanella minuta       | –        | –        | –        | –        | –        | –        | –        | –        | –        | 11       | –        | –        | 1        |
| Cyclidium sp.           | –        | 5        | –        | –        | –        | –        | 29       | –        | 44       | –        | –        | –        | –        |
| Scuticociliatia         | 29       | 57       | –        | 15       | –        | –        | 22       | 8        | –        | 39       | 26       | –        | –        |

-, not found; +, less than 1%.
8, and St. 4, represented Arctic marine sediment stations; and clade 4 consisted St. 12, St. 3, and St. 15, represented temperate marine/brackish habitat.

Multidimensional scaling results plotted by using species-specific abundance of ciliates revealed a similar pattern of stations (Fig. 6A): all freshwater sites were grouped together (St. 1, St. 2, St. 5–7, St. 9, St. 11, St. 13, and St. 14), separate from marine and brackish sites which themselves were divided into Arctic (St. 4, St. 8, and St. 10) and temperate sites (St. 3, St. 15, and St. 12).

Multidimensional scaling ordination based on distribution of 18 ciliate groups is shown in Fig. 6B. The central part of the large circle comprises the most commonly seen groups both in marine and freshwater sediments including prorodontids, euplotids, pleurostonatids, chlamydodontids, scuticociliates, and haptorids. The more peripheral ciliate

Figure 4 Per cent composition of ciliate abundance (A) and biomass (B) at the 15 stations. Note that total ciliate abundance and biomass were partitioned among 18 groups, mostly at the class or order levels, occasionally at family levels.
groups such as Metacystis, Frontonia, microthoracids, plagiopylids, and heterotrichs were moderately common among stations. Several groups seen in freshwater (Bursaridium, peritrichs, and synhymeniids) were not very common among stations. Karyorelictids and heterotrichs were often observed at marine/brackish or freshwater sites with low abundance, but with high biomass due to their relative large body size. The other small circle included two groups (dysteriids and strombiidiids) of marine forms and occurred only in one or two stations.

### Distributions of cysts

Numerous cysts were observed at St. 4, St. 5, St. 7, and St. 12, with average densities of 32, 113, 7, and 43 cells/ml respectively. The maximum abundance of cysts (113 cells/ml) occurred at an Arctic lake (St. 5), where the abundance of active protozoa was 22 cells/ml. The proportion of cysts to active individuals was on average of 115.2% at Arctic stations and of 1.9% at temperate stations. Most cysts were from unknown flagellates. In addition, some ciliate and diatom cysts were observed (Fig. 7). Because our particular interest was in the ciliate communities, the identification of the ciliate cysts was mainly based on analysing a large number of protargol-stained specimens in the encystment or excystment stages. Ciliate cysts were attributed to the following species or genera:

- Aspidisca lynceus
- Peritrichmus californicus
- Fontonia
- Loxophyllum
- Chilodonella

### Table 4. Correlations (Spearman’s r values) between ciliate diversity, species richness, and environmental factors

| Ciliate abundance | Chlorophyll-a | Salinity | Temperature | Silt/clay | Carbon | Nitrogen |
|-------------------|--------------|----------|-------------|-----------|--------|----------|
| 0.6500 (0.0987)** | 0.7177 (0.0026)** | 0.7209 (0.0024)** |
| 0.5107 (0.0871) | 0.4286 (0.1676) | 0.5141 (0.0834) |

### Table 5. Summary of results from biota–environment (BIOENV) analysis showing the best matches of environmental variables with spatial variations in ciliate species-specific abundance and community parameters (species abundance, community abundance, biomass, species richness, Margalef index, Shannon–Weiner diversity, and evenness) at 15 sampling sites

| Ciliates       | Rank | R       | Environmental variables | p       |
|----------------|------|---------|-------------------------|---------|
| Species composition | 1    | 0.325   | Sal, Tem, Silt/clay     | 0.007** |
| 2              | 0.274 | Sal, Tem |                         | 0.015** |
| 3              | 0.263 | Sal, Tem, Silt/clay, N |         | 0.021** |
| 4              | 0.238 | Sal, Tem, N |                  | 0.028*  |
| 5              | 0.201 | Tem, Silt/clay |            | 0.043*  |
| Community parameters | 1    | 0.305   | Tem, Silt/clay, Carbon  | 0.008** |
| 2              | 0.285 | Sal, Tem, Silt/clay, Carbon | | 0.007** |
| 3              | 0.278 | Tem, Silt/clay |                | 0.023** |
| 4              | 0.273 | Sal, Tem, Silt/clay |            | 0.012** |
| 5              | 0.241 | Sal, Tem, Silt/clay, Carbon, Chl-a | | 0.021** |
| 6              | 0.240 | Tem, Silt/clay, Carbon, Chl-a |         | 0.018** |
| 7              | 0.238 | Tem, Silt/clay, Carbon, N |         | 0.037*  |
| 8              | 0.234 | Sal, Tem, Silt/clay, Carbon, N |   | 0.020** |
| 9              | 0.229 | Sal, Tem, Silt/clay, Chl-a |         | 0.027*  |
| 10             | 0.226 | Tem, Carbon |             | 0.037*  |

Because the same data for ciliates were used in two different analyses (species-specific abundance and community parameters), the x-level was reduced from 0.05 to 0.025. p-values in the lower line parentheses beneath 0.025 are marked with double asterisks. Effects with a p-level between 0.025 and 0.05 were considered as marginally significant trends, and are marked with an asterisk.
However, most of the cysts could not be identified due to the lack of information in the literature. Remarkably, some cysts (including ciliate cysts) were in the division stage (Fig. 7).

**DISCUSSION**

**Abundance and biomass of protozoa in sediments**

In sediment environments, ciliates, amoebae, and foraminifers are three conspicuous protozoan groups due to their relatively large body size and wide distribution. *Stephanopogon* is usually a rarely occurring group and with low abundance and biomass (Fig. 1, 2, 6). In this study, the high latitude stations in Arctic regions harboured lower standing stocks of benthic protozoa, both in freshwater and marine stations (freshwater St. 4–6, St. 9, St. 11; marine St. 8, St. 10). An exception was St. 7, a pond on an Arctic ice flow, where the protozoan abundance and biomass was as high as in the European samples (St. 12, St. 15). Abundance and biomass as low as in the Arctic samples were detected in the freshwater and marine sites in the U.S. (St. 1–3). The abundance and biomass of protozoa were at least five times and up to more than 10 times higher in the European samples than in the Arctic or in samples from temperate U.S. A general trend was for lower abundance or biomass of protozoa in high latitude Arctic sediments or cold seasons in temperate regions (St. 1–3 were sampled during winter).

In this study, all the protists were sampled by sediment cores and extracted by Percoll and stained by the QPS method. This method was developed by Wickham et al. (2000) and was shown an effective method of obtaining quantitative information about both benthic protozoa and meiofauna (Lei et al. 2010). Percoll extraction has been shown to recover more than 94% of protists from sediments (Starink et al. 1994). In addition, the QPS method does not result in lower pelagic ciliate abundance than live counting (Pfister et al. 1999). Using live counting parallel to the QPS method would potentially raise the estimated richness in the samples, but there are both logistical and conceptual problems with this approach. The enormous quantity of benthic protozoa inhabiting the sand/clay interstitial (10−1,499 cells/ml in this study) precluded the short handling time necessary to conduct live counts before there are handling-induced changes in community structure. This was particularly true with the Arctic samples, where transfer of samples from the field required awaiting the arrival of a helicopter. Moreover, in a comparative study such as ours, live counting would require not only short handling times, but the handling times of the same duration to minimise differences between stations arising from handling artefacts. The conceptual problem with including data from live counts is that the data would be qualitative or at best semi-quantitative. While this is acceptable when calculating species richness, it would not be possible to use live count data with QPS-derived data when calculating diversity measures such as Shannon’s $H$. It is however important to note that our diversity measures are conservative and almost certainly underestimate the true diversity of the stations we sampled.

While our study sampled a moderately large number of stations from a variety of habitats, the sampling was inevitably limited in several dimensions. Among the 15 sta-
Figure 6  Multidimensional scaling (MDS) ordination on Euclidean distance for 15 sampling sites plotted using abundance of ciliate dominant species (A), and MDS plotting for distribution of ciliate groups (B). Each plot is based on Spearman’s rank correlations and from fourth root-transformed data.
tions, seven sites (St. 1–3, St. 12–15) were located in temperate regions, and eight sites (St. 4–11) were from the Arctic. Of these, nine were from lake sediments and six from brackish or marine sites. Clearly, a larger number of sites in each habitat type, or more habitat types, or repeatedly sampling the same sites in different seasons would have revealed finer scaled differences in protozoan distributions than could be found in the present study. Moreover, sampling the core-top sediments likely led to overlooking some endobenthic amoebae and foraminifers. The goal of the current study, however, was to determine broad patterns in benthic protozoan distribution. Furthermore, molecular techniques have been utilised in the studies of protists and depicted new diversity and

Figure 7 Microphotographs of ciliates (A–F), sediment cysts (G–K), amoebae (L, M), Stephanopogon (N), foraminifera (O, P) after protargol impregnation. A. Ventral view of Chlamydomonas cyclops; arrow indicates a large diatom ingested by the cell. B. Food vacuoles of Frontonia sp. contained cyanobacteria (arrows). C. Left view of Loxophyllum uninucleatum. D. Ventral view of Gastronota derouxi. E. Microdysteria decora. F. Aspidisca polypoda. G. Cyst of Aspidisca lynceus was covered with mucous layer colonised by bacteria (arrow). I. Division stage of a ciliate cyst. F. Ammonia tepida. BC = buccal cirrus; Ma = macronucleus; Mi = micronucleus. Scale bar = 30 μm.

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geographical distributions (Engel et al. 2012). Molecular methods, however, still often lack quantitative information such as abundance or biomass distribution, making difficult direct comparisons to our results. There is clearly ample room for subsequent studies to refine the patterns revealed in our data.

**Distribution of protozoan groups in sediments**

In this study, the abundance and biomass across all stations of ciliates were in the range of 9–562 cells/ml (biomass: 0.004–2.34 µg C/ml); amoebae were in the range of 0–937 cells/ml (biomass: 0–4.71 µg C/ml); and foraminifera were in the range of 0–155 cells/ml (biomass: 0–15.87 µg C/ml). Due to the different distributions and proportions of the three taxonomic groups among stations, the abundance of total protozoa was in the range of 10–1,499 cells/ml, with the highest value due to occurrence of amoebae in the Arctic ice floe pond. Protozoan biomass ranged from 0.01 to 18.98 µg C/ml, where the highest value was due to the occurrence of foraminifera. *Stephanopogon* could only be detected in the two marine arctic stations (St. 8 and St. 10).

Generally at all stations, protozoan communities were dominated by ciliates in term of abundance, while amoebae usually dominated biomass. Most conspicuous in this pattern was station 7, the ice floe pond, where naked amoebae contributed to an average of more than 96% of total abundance and 87% of biomass respectively.

Data on the distribution of live amoebae in nearshore sediments are rare. Wall et al. (2010) investigated the abundance of testate amoebae in lake sediments in west central Europe. The abundance was in the range of 1,403–10,870 shells/cm³, but with a large influence of the mesh size to take the samples. The method used by Wall et al. (2010) included recent and fossil and dead shells. This explains the high abundance in contrast to abundance of amoeba found in this study. We used Percoll density gradient solutions to extract the live cells of protozoa and amoebae with the advantage to also extract and count naked amoeba and fragile cells. In this study, high densities of amoeba (both naked and testaceous) were found in most stations (Fig. 2). In contrast, at St. 15 and St. 8, two marine sediments in arctic and temperate regions, as well as in the sediment of an ice floe pond (St. 7), naked amoebae dominated the amoeba community. The actual density, especially of naked amoeba, would have been strongly underestimated if only meshes were used for sampling and extraction of these fragile organisms from the sediments. The importance of naked amoeba seems to be underestimated at least in the study of Wall et al. (2010).

Previous work has found a wide range in the abundance of foraminifera in sediments. For example, in Skagerrak surface sediments, a density of 5–120 cells/cm³ were found, while a range of 10–150 cells/ml were observed in the offshore sediments near Gulf of Mannar between the Arabian Sea and Indian Ocean (Alve and Murray 1995; Gandhi et al. 2007). In the offshore sediments from the Yellow Sea of China, total foraminiferal abundance was in a range of 5–20 cells/cm³ in the >150 µm size fraction, and of 90–340 cells/cm³ in the 63–150 µm size fraction. However, the live foraminiferal abundance was only in a range of 2–10 cells/cm³ in >150 µm size fraction, and 3–40 cells/cm³ in 63–150 µm size fraction (Lei., unpubl. data). Foraminiferal abundance in our data was in the range of those studies. Although in the present study foraminifera were not a common group, they occupied up to 84% of the total protozoan biomass in the brackish tidal flat sediment at St. 12. If present, their communities were dominated by Rotaliidae species such as *A. tepida* (Fig. 7). Ammonia species were often the dominant foraminiferal species in intertidal flats or tidal estuary communities (Berkeley et al. 2008; Ghosh et al. 2009; de Nooijer et al. 2009). This foraminifera is known as a benthic, omnivorous species. However in this study, the food vacuoles of the observed individuals were filled with diatoms, algae, and cyanobacteria, implying they played a significant role in consuming benthic microalgae at the brackish tidal flat at St. 12.

Our results follow common trends in sediment microbial communities. For example, ciliate abundance in sediments of Adriatic Sea lagoons was from 2 to 759 cells/cm³ and biomass was in the range of 1.1–30.3 µg C/ml (Madoni 2006); in the sandy hyporheic zone of a lowland stream sediment, ciliate abundance varied between 0 and 895 cells/ml, and the biomass ranged between 0 and 5.3 µg C/ml (Cleven 2004). In our study, ciliate abundance varied between 9 and 562 cells/ml, and biomass between 0.004 and 2.34 µg C/ml, similar to the above-cited studies.

**Potential ecological significance of cysts**

Many protists were observed to have formed resting cysts. Cysts are assumed to be the major dispersal agents of unicellular organisms because they are more stable than the live stage. During this study, we observed many cysts at St. 4, St. 5, St. 7, and St. 12 (Fig. 7). The maximum density of cysts was observed at an Arctic site (St. 5; 113 cells/ml), where the abundance of protozoa was very low (22 cells/ml). The mean proportion of cysts to active individuals was higher at the Arctic stations (115.2%) than that at the temperate stations (1.9%), indicating potentially higher abundance and diversity when conditions are favourable. It is now well established that many protozoa may pass through a benthic resting stage during their life cycle (e.g. Kremp 2001; Müller et al. 2002). Resting cysts are usually regarded as the most important factor for species’ dispersal, facilitating a cosmopolitan distribution (Foissner 2011). However, the marked difference in their occurrence in temperate and arctic sites underlines the importance of cysts in maintaining within-site diversity, particularly in harsh environments. The cysts in the Arctic sediment (St. 4, St. 5, and St. 7) presumably gave the species producing them an ecological advantage, allowing them to overcome the unfavourable conditions of the arctic winter and assuring the persistence of the population. In this study, the density of cysts was probably underestimated because many could not be reliably identified, due to our limited knowledge
and available information on cysts. Future studies of resting stage dynamics in sediments would lead to a better understanding of the dynamics of microbial food webs.

Community compositions of ciliates in sediments

Compared to protozoa commonly found in the plankton, those from sediments tend to be substrate-associated and well adapted for life in the benthic environment. As benthic protists live in the interstitial of sand grains, most of them possess some common characters such as being more slender or flatter, or more contractile or adherable to facilitate swimming among sand grains or crawling on the substrate and against the tidal current etc. (Carey 1992). In pelagic environments, oligotrichs are usually dominant in protozoan communities (e.g. Finlay and Esteban 1998; Wickham et al. 2011). Their body shapes are usually globular or ellipsoidal as an adaptation to the planktonic life in the water column. In contrast, in our sediment samples, oligotrichs were never dominant at any station. Instead, diatom-consuming species such as *Chlamydomenopsis* and *Chilodonella* spp. were most often dominant in ciliate communities. From the contents of food vacuoles of ciliates indicating that herbivorous feeding were most common across sediments, this was also indicated by the occurrence of the dominant species and ciliate groups.

The microscopic observation showed that there were different ciliate fauna among the three sediment types: in freshwater sediment, *Prorodon*, *Euplotes*, *Chilodonella*, peritrichs, and scuticociliates were commonly seen; in estuary sediment hypotrichous ciliates such as *A. fusca* and *Chlamydomenopsis* sp1. were most commonly observed; in marine sediment *Chlamydomenopsis* sp1, scuticociliates, and *Coleps* occurred more frequently (Table 3; Fig. 4). This partitioning of species between freshwater and marine/brackish sediments holds true for the MDS results using ciliate species/groups and biota-environment analysis (Table 5; Fig. 5, 6).

Factors influencing ciliate distributions in sediment

For better understanding of the role of ciliates in benthic microbial food webs, it is necessary to know which factors influence their diversity and distribution in sediments (Lee and Patterson 2002). Previous studies showed that many factors, including temperature, salinity, silt and clay content/grain size, content of dissolved oxygen, or chlorophyll *a* can affect the distribution of protozoa and meiofauna in sediments (e.g. Burgess 2001; Finlay et al. 1997; Patterson et al. 1989). In the intertidal, ciliates generally are more abundant in medium and fine sand than in silt and mud, presumably due to the restricted pore size and permeability (Patterson et al. 1989). However, karyorelictids have been found to be more common in silty than sandy subtidal sediments (Garstecki et al. 2000).

Ciliate biomass is usually driven by chlorophyll *a* in typical marine systems (e.g. Wickham et al. 2011). While the univariate analysis and feeding types of ciliates also supported this view (Table 4), the multivariate analyses showed that other factors also drive ciliate community composition. The dendrograms from the cluster analysis (Fig. 5, 6) showed the 15 stations falling into four clades. The primary two branches were freshwater (clades 1, 2) vs. marine/brackish water sediment (clades 3, 4), and each of them was secondarily divided into temperate vs. Arctic sediment. The MDS analysis also separated freshwater, brackish, and marine sites, as well as dividing the Arctic sites and temperate sites, with larger differences between freshwater to marine/brackish than the difference between temperate and arctic. The multivariate biota-environment (BIOENV) analysis confirmed this view, with salinity and temperature patterns most closely matching the pattern in ciliate community parameters, with sediment type also playing a role (Tables 5, 6).

Potential importance of protozoa in benthic metabolism

In this study, total protozoan communities were usually dominated by ciliates in term of abundance, while amoeboae were often dominant in term of biomass. Lei (2005) investigated entire micro- and meiofauna communities in the same stations and calculated the abundance and biomass contributions. Applying the biomass-metabolic rate equation \( R = a W^{0.248} \) (Fenchel 1974), where weight-specific metabolic rate \( (R) \) is associated to body weight \( (W) \), and \( a \) is a constant with a value of 10\(^{-1.34}\) for protozoa and 10\(^{-1.64}\) for metazoas. On the basis of the formula by Fenchel (1974) and the biomass of each group, we calculated the relative metabolic rates of benthic ciliates, amoeboae, and foraminifera, and meiofauna in the present sample sites (Table 6). Based on these calculations, ciliates, amoeboae, and foraminifera together contributed 66% of the abundance and 33% of the biomass, but up to 55% of the combined metabolic rate to the micro- and meiobenthos at 15 stations. This is in accordance with some earlier studies suggesting that meiofauna biomass was much higher than heterotrophic protists in sediments (Arndt et al. 1990; Garstecki et al. 2000). Nevertheless, the contribution of protozoa to benthic energetics is disproportional to their biomass due to the higher turnover of smaller cells. Although these ratios are rough estimates, they distinctly suggest that protozoa are disproportionately important in sediment respiration and nutrient cycling. However, these values are expected to be different

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**Table 6.** Average values and ranges (in parentheses) of relative abundance and biomass (%), and relative metabolic rate (%) of three organismal groups, viz., ciliates, amoeboae, and foraminifera, and meiofauna in sediments investigated

|                  | Ciliates | Amoebae and Foraminifera | Meiofauna |
|------------------|---------|--------------------------|-----------|
| Abundance        | 46 (8–96) | 20 (0–96)                | 34 (0.1–87) |
| Biomass          | 15 (1–89) | 18 (0–98)                | 67 (0.4–98) |
| Metabolism       | 34 (12–41) | 21 (0–28)                | 46 (40–78) |

Note that the data on the meiofauna are from Lei (2005).
among different sediment habitats such as nearshore vs. offshore sediments. In offshore sediments of the Yellow Sea, ciliates contributed very little to the collective metabolic rate of the micro- and meio-benthos (Meng et al. 2012). Although the present study encompassed a wide range of habitats, more sampling on a seasonal basis and in less-known biotopes is necessary before it can confidently said that the trend found in our study is truly universal to benthic habitats.

In summary, when the quantitative importance and species composition of ciliates, foraminifera, and amoebae were investigated across marine, brackish, and freshwater sediments, ciliates were the most abundant and frequent group, but amoeba contributed most of the biomass. Applying the biomass-metabolic rate equation, ciliate, amoeba, and foraminifera were estimated to contribute 66% of the total abundance and 33% of the biomass, but up to 55% of the combined metabolic rate to the micro- and meio-benthos at the 15 sediments.

Sediment environments, unlike the water column, are highly heterogeneous. Therefore the community composition and ecological characters of protozoa and the factors regulating their distribution are likely to be complex. While chlorophyll a, as in pelagic systems, was clearly a driving factor, ciliate community composition was also driven by more comprehensive factors or their combination, such as salinity, temperature, and silt & clay contents. Given their heterogeneity and steep gradients, single factors are unlikely to control the benthic communities. In this study, ciliate distribution in sediments differed primarily by freshwater to marine/brackish sediments, and to a lesser degree, from arctic to temperate sediment. There are, however, too few benthic protozoa studies worldwide to know whether the trends we found are universal. Further studies particularly in lesser-known habitats, are needed to compare the differences in the biological distribution.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The occurrence of the ciliate taxa with respective abundance (cells/ml) at the 15 sampling sites.

Wickham, S., Steinmair, U. & Kamennaya, N. 2011. Ciliate distributions and forcing factors in the Amundsen and Bellingshausen Seas (Antarctic). *Aquat. Microb. Ecol.*, 62:215–230.

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